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AUDITORY RESPONSES IN THE COCHLEAR NUCLEUS OF AWAKE MUSTACHED BATS: PRECURSORS TO SPECTRAL INTEGRATION IN THE AUDITORY MIDBRAIN

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Abstract

In the cochlear nucleus (CN) of awake mustached bats, single and two-tone stimuli were used to examine how responses in major CN subdivisions contribute to spectro-temporal integrative features in the inferior colliculus (IC). Across CN subdivisions, the proportional representation of frequencies differed. A striking result was the substantial number of units tuned to frequencies below 23 kHz. Across frequency bands, temporal response patterns, latency, and spontaneous discharge differed. For example, the 23–30 kHz representation, which analyzes the fundamental of the sonar call, had an unusually high proportion of units with onset responses (39%) and low spontaneous rates (53%). Units tuned to 58–59 kHz, corresponding to the sharply tuned cochlear resonance, had slightly but significantly longer latencies than other bands.

In units tuned to frequencies above 30 kHz, 31% displayed a secondary excitatory peak, usually between 10 and 22 kHz. The secondary peak may originate in cochlear mechanisms for some units, but in others it may result from convergent input onto CN neurons. In 20% of units tested with two-tone stimuli, suppression of BF responses was tuned at least an octave below BF. These properties may underlie similar IC responses. However, other forms of spectral interaction present in IC were absent in CN: we found no facilitatory combination-sensitive interactions, and very few combination-sensitive inhibitory interactions of the dominant IC type, in which inhibition was tuned to 23–30 kHz. Such interactions arise above CN. Distinct forms of spectral integration thus originate at different levels of the ascending auditory pathway.

Keywords

cochlear nucleus; combination sensitivity; inferior colliculus; *Pteronotus parnellii*; spectral integration

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INTRODUCTION

Neurons of the cochlear nucleus (CN) transform the relatively homogeneous input from the auditory nerve into distinctive temporal and spectral response properties. In turn, these response properties serve as the bases for diverse auditory analyses within CN itself or the auditory nuclei that receive its projections. A major focus of auditory research has been to understand how the output of the cochlear nucleus contributes to parallel analyses of sounds that form a prominent feature of the ascending auditory pathway. For example, numerous studies have related the properties of bushy cells in the anteroventral cochlear nucleus (AV) to binaural responses in the superior olivary complex and higher levels (reviews: Rhode and Greenberg, 1992; Irvine, 1992). Likewise, several studies have described how the spectral analyses performed by DCN neurons contribute to responses in the inferior colliculus (IC) and higher levels (Oertel and Young, 2004, Ramachandran et al., 1999). The present study focuses on CN response properties that may contribute to several types of spectral integration in the IC of mustached bats and other species.

We consider three types of spectral integration displayed by neurons at higher levels of auditory systems, including multi-peaked excitatory tuning, combination-sensitive facilitation, and combination-sensitive inhibition. Multi-peaked tuning, characterized by two or more distinct frequency response areas, is widespread in vertebrate auditory systems although the frequency of occurrence varies (Allon et al., 1981; Cohen and Knudsen, 1996; Ehret and Moffat, 1985; Fuzessery and Feng, 1983; Sutter and Schreiner, 1991). In the mustached bat, recordings in the IC (Portfors and Wenstrup, 1999, 2002), medial geniculate body (Wenstrup, 1999), and auditory cortex (Fitzpatrick et al., 1998) suggest that many neurons display multiple distinct frequency response areas. Multi-peaked excitatory tuning may result from cochlear mechanisms that create “tails” for high frequency tuning curves, neural integration of excitatory or inhibitory inputs, or both (Foeller et al., 2001; Snyder and Sinex, 2002; Sutter et al., 1999). Does multi-peaked excitatory tuning occur in the mustached bat’s CN? If so, what are the underlying mechanisms?

Facilitatory combination-sensitive interactions create selective responses to timedependent combinations of two or more spectral elements in complex vocal signals. In the mustached bat IC and forebrain, most facilitated combination-sensitive neurons respond to spectral combinations that occur in both sonar signals and social vocalizations (Portfors and Wenstrup, 1999, 2002; Olsen and Suga, 1991b; Fitzpatrick et al., 1998a), while others respond to spectral combinations present in social vocalizations but not in sonar signals (Leroy and Wenstrup, 2000; Portfors and Wenstrup, 2002; Wenstrup, 1999). Facilitated combinationsensitive responses thus extract information from pulse-echo combinations of sonar signals (O’Neill and Suga, 1979; Suga et al., 1983) or respond selectively to one of several social vocalizations (Ohlemiller et al., 1996; Esser et al., 1997; Portfors, 2004). In other species, facilitatory combination-sensitive responses have been related to processing of social vocalizations (frog: Fuzessery and Feng, 1983; bird: Margoliash and Fortune, 1992; monkey, Rauschecker et al., 1995). More generally, facilitatory interactions activated by widely separated spectral elements may contribute to auditory cortical analyses of complex sounds (Brosch et al., 1999; Kadia and Wang, 2003). In the mustached bat, however, facilitated combination sensitivity appear to originate in IC, since few such interactions are found in nuclei of the lateral lemniscus (NLL) (Portfors and Wenstrup, 2001) and since glycine receptor blockade in IC eliminates the facilitation (Wenstrup and Leroy, 2001; Nataraj and Wenstrup, 2005). This facilitatory spectral integration appears to occur as the result of a lower frequency input into higher frequency tonotopic representations of the IC. Tract-tracing experiments suggest that the lower frequency input most likely originates from the ventral CN or from NLL (Wenstrup et al., 1999). How do CN response properties contribute to the spectral or temporal features of combination-sensitive facilitation in the IC?

In the third type of spectral integration, referred to here as combination-sensitive inhibition, a neuron's response to one spectral element in vocal signals is suppressed by the presence of a second spectral element in vocalizations (O'Neill, 1985; Mittmann and Wenstrup, 1995; Portfors and Wenstrup, 1999). The inhibition, typically tuned to a frequency at least an octave away from the neuron's best excitatory frequency, is thought to arise from a specifically tuned inhibitory input rather than from either broadly tuned inhibitory inputs or cochlear suppressive phenomena (Mittmann and Wenstrup, 1995). It is thus distinct from sideband or lateral inhibition that has been documented extensively. Similar combinatorial inhibitory properties apparently occur in the forebrain in other vertebrate species (monkey: Rauschecker et al., 1995; cat, Sutter and Schreiner, 1999). One function of combination-sensitive inhibitory neurons is to increase response selectivity for vocalizations by limiting a neuron's response to those signals with little energy in the inhibitory frequency band. When combined with facilitatory interactions in IC neurons, another function may be to enhance the temporally selective response to signal combinations (Mittmann and Wenstrup, 1995; Portfors and Wenstrup, 1999; Nataraj and Wenstrup, 2005). In the mustached bat, inhibitory interactions are common in the IC, but may be formed below the IC, since they have been reported in the NLL (Portfors and Wenstrup, 2001) and since blockade of glycine and GABA-A receptors in the IC usually fails to eliminate the inhibitory interaction (Nataraj and Wenstrup, 2005, in preparation). Do inhibitory combination-sensitive interactions originate in CN?

The questions raised here were examined by recording single unit activity in the CN of awake mustached bats. We characterized basic features of CN responses to tones as well as responses to combinations of tones similar to our studies in NLL and IC. The goal was to relate basic and two-tone responses to the major CN divisions, to functionally defined frequency bands in the mustached bat's audible range, and to the responses observed in the IC. Preliminary reports have appeared (Marsh et al., 2001; Marsh and Wenstrup, 2002).

MATERIAL AND METHODS

Neural activity evoked by acoustic stimuli was recorded from the CN in 17 awake mustached bats (*Pteronotus parnellii rubiginosa*) captured in Trinidad, West Indies. The Institutional Animal Care and Use Committee of Northeastern Ohio Universities College of Medicine approved all animal procedures.

Surgical Procedures

After bats were anesthetized with methoxyflurane (Metofane, Schering-Plough Animal Health, Omaha, NE) in combination with sodium pentobarbital (5 mg/kg, i. p.; Nembutal, Abbot Laboratories, North Chicago, IL) and acepromazine (2 mg/kg, i. p.; Med-Tech, Inc., Buffalo, NY), the dorsal surface of the skull was exposed. A tungsten reference electrode was cemented into the right cerebral cortex, and a metal pin was cemented to the skull so that the head could be maintained in a uniform position during physiological experiments. After surgery, a topical antibiotic (Neosporin, Pfizer, Morris Plains, NJ) was applied to the wound margins. Bats recovered for at least two days prior to physiological recording.

Acoustic Stimulation and Recording

Acoustic stimulation and data acquisition were computer-controlled. Acoustic signals (11–61 ms total duration, 0.5 ms rise/fall times, 2–4/sec) consisted of tone bursts or combinations of tone bursts. Acoustic signals were digitally synthesized, converted to analog signals (sampling rates of 300,000 or 500,000 Hz) and attenuated (Tucker-Davis Technologies, System II components). The signals were then amplified (Parasound model HCA-800II) and routed to a speaker (Technics leaf tweeter) placed 25° into the sound field ipsilateral to the CN under study. The performance of the entire acoustic system was tested with a calibrated microphone

(Brüel and Kjaer, model 4135). There was a smooth decrease of approximately 2.7 dB per 10 kHz from 10 to 120 kHz. Distortion components were not detectable 60 dB below the signal level, as analyzed by a fast Fourier transform of the digitized microphone signal.

For electrophysiological recordings, the bat was placed in a custom-made stereotaxic holder, housed in a heated and humidified soundproof chamber lined with sound attenuating foam. On the first day of recording, bats were lightly anesthetized with methoxyflurane, and a small hole was opened in the skull overlying the cerebellum. Recording on the first day began after the bat had recovered from the anesthesia. In subsequent recording sessions, the bat was not anesthetized. If a bat struggled or showed other signs of discomfort during any recording session, it was returned to its holding cage. Between electrode penetrations, the bat was given water as needed. Recording sessions typically lasted four hours. Between recording sessions, the hole in the skull was covered with bone wax.

Stereotaxic coordinates developed in our laboratory for this species guided electrode penetrations through CN. Responses were recorded from a CN area of interest in several successive penetrations, one of which was marked by tracer deposits. Thus, nearly all electrode penetrations were placed very close to electrode tracks marked by deposits. Penetrations placed within the rostral 5% of CN and caudal 5–10% of CN did not receive tracer deposits. We assumed that penetrations in the most rostral CN were exclusively from AV and penetrations through the caudalmost CN were in DCN. Recordings from both right and left CN are displayed on the left side.

The evoked activity of single units or clusters of a few units (multi-units) was recorded using micropipette electrodes (tip diameters 1–3 μm , resistances of 8–30 $\text{M}\Omega$) filled with 1 M NaCl or 0.9% NaCl. In addition, electrodes were often filled with a neural tracer to mark recording sites (see Histological Procedures). Dorso-ventral electrode penetrations were angled 10–15° medial-to-lateral and advanced by a hydraulic micropositioner (David Kopf Instruments, model 650) through the overlying cerebellum into the CN, typically encountered 3700–4200 μm below the dorsal cerebellar surface. Extracellular action potentials were amplified and filtered (bandpass, 500–6000 Hz), then routed to a spike discriminator and event timer (Tucker-Davis Technologies, models SD1 and ET1, respectively) that recorded the time of occurrence of spikes with one microsecond resolution. Peristimulus time histograms (PSTH), interspike interval histograms (ISIH), rasters, and statistics on the neural responses were displayed in real time. Quantitative data were obtained only from well-isolated single units with high signal/noise ratios. Histograms, raster displays, and associated response functions were based on 20–50 stimulus repetitions.

When a single unit was isolated, single tone bursts (10–120 kHz, usually 61 ms total duration) were used to determine best frequency (BF) and minimum threshold (MT). BF was defined as the frequency requiring the lowest sound level to elicit stimulus-locked spikes. (BF in the neuroethological literature corresponds to characteristic frequency elsewhere.) MT was defined as the lowest sound level required to elicit stimulus-locked spikes to consecutive BF stimuli. Some units were excited by sounds in more than one frequency band. In multi-peaked units, the frequency separation between the higher and lower BFs was an octave or more, and tuning curves remained separate except at the highest levels tested. After BF(s) and MT(s) were obtained, temporal response pattern and median first spike latency were assessed using BF signals, 61 ms duration, 20–30 dB above MT. In some neurons with strongly non-monotonic rate-level functions, response latency and discharge pattern were obtained at 10–15 dB above MT.

Responses of CN single units to combinations of tones were examined under conditions similar to studies of the lateral lemniscal nuclei (Portfors and Wenstrup, 2001), IC (Mittmann and

Wenstrup, 1995; Leroy and Wenstrup, 2000; Portfors and Wenstrup, 1999; Portfors and Wenstrup, 2002) and medial geniculate body (Wenstrup, 1999). However, sound duration, typically 4 ms in previous studies, was changed to 11 ms due to better visualize response enhancement or suppression in the presence of higher spontaneous rates typical of many CN neurons. Rate-level functions for all units were acquired at BF in 5–10 dB steps, from below MT to a maximum of 80–100 dB SPL. A curve was considered to be non-monotonic if the response to higher sound levels was at least one-third below the maximum response rate. These rate-level functions were used to set the level of BF sounds when testing for combinatorial interactions in the CN, so that both response enhancement and suppression could be observed in the presence of a second signal. Spontaneous discharge rates are calculated based on 20 samples of a 100 ms recording window.

Sensitivity to combinations of tone bursts was first assessed audiovisually using a twotone paradigm: a BF tone was presented at a fixed sound level (typically 10 dB above MT) and a second tone was presented over a range of frequencies, timing, and sound levels. If response enhancement or suppression was observed, parameters to the second tone (typically the low-frequency signal) were adjusted to maximize the interaction and quantitative tests of timing and/or frequency tuning were obtained. Sensitivity to the relative timing (delay) between the BF and other signal was assessed in quantitative tests by varying the delay in steps of 1–4 ms, including delays in which the BF signal led and followed the other signal. The delay between the two signals that elicited the greatest response (or the least response) was termed the unit's best delay. Spectral interactions were examined in quantitative tests by varying the frequency of the second tone (typically in 1 kHz steps) in the presence of the BF signal. The frequency that showed the greatest response (or the least response) was defined as the neuron's best facilitatory (or inhibitory) frequency. Both audiovisual and quantitative tests were integral to evaluating spectral interactions: the audiovisual testing spanned a broad range of possible frequencies, delays, and levels of interacting tone bursts, while the quantitative tests documented particular interactions. Since our interest was in interactions of distant frequency bands, we did not usually examine interactions within or near a unit's excitatory tuning curve.

For comparison to combination-sensitive neurons in the mustached bat's IC (Portfors and Wenstrup, 1999; Leroy and Wenstrup, 2000), we examined spectral interactions that met several criteria: a) a unit's response to the combination of signals was 25% greater than (for facilitation) or 25% less than (for inhibition) the sum of the responses to the signals presented separately, b) the facilitatory or inhibitory interactions were tuned to frequencies separated by an octave or more from the BF, and c) they displayed temporal sensitivity demonstrated in delay tests.

Histological Procedures

Electrode penetrations were marked by one of the following tracers dissolved in 0.9% NaCl: Fluoro-Gold (Fluorochrome, Inc., Englewood, CO; 1–2%), Fluororuby (Molecular Probes, Eugene, OR; 5–10%), Rhodamine green (Molecular Probes; 5–10%), or biotinylated dextran amine (BDA, Molecular Probes; 5–10%). After physiological recordings were completed and 2–20 days after tracer deposits, an animal was deeply anesthetized (sodium pentobarbital, >75 mg/kg, i. p.) and then perfused with saline followed by 4% paraformaldehyde fixative. Histological procedures followed those described previously (Wenstrup et al., 1999; Portfors and Wenstrup, 2002; Marsh et al., 2002).

Data analysis

Photomicrographic images of the CN were taken with brightfield or fluorescence illumination using an Olympus Provis AX microscope and SPOT digital camera (Diagnostic Instruments Inc.). Grayscale images were imported into Photoshop (Adobe Systems, Inc.), where

adjustments in brightness and contrast were made globally to each photograph. Composite plates were sized and lettered in Canvas (ACD Systems).

Tracer deposits were drawn under brightfield or fluorescence illumination using a drawing tube. Drawings were scanned and imported into a graphic application (Corel Draw 8). Cytoarchitectonic boundaries were drawn from adjacent Nissl-stained sections, based on previous work in the CN (Zook and Casseday, 1982b; Kössl and Vater, 1990a). Each single unit was assigned to one of the CN subdivisions (AV, PV, DCN) or the marginal zone (M). In penetrations marked by tracer deposits, matching units to subdivisions was straightforward. In penetrations not marked by tracer deposits, we used stereotaxic criteria, comparison to nearby marked penetrations, and physiological criteria to assign units to particular subdivisions. Previous descriptions of the tonotopy and functional properties of the mustached bat's CN (Zook and Casseday, 1982b; Kössl and Vater, 1990a, b) facilitated the match between unit responses and subdivisions.

RESULTS

The results describe responses of 480 single units that were localized to one of the major divisions of the mustached bat's cochlear nucleus: the anteroventral cochlear nucleus (AV), posteroventral cochlear nucleus (PV), and dorsal cochlear nucleus (DCN) as in other species, but also a specialized marginal zone (M) that is distinctive in mustached bats (Zook and Casseday, 1982b). The marginal zone is sometimes considered to be a part of AV (Zook and Casseday 1985; Zook and Leake, 1989). In the first section, we focus on the distribution of basic response properties (best frequency, temporal response patterns, latency, and spontaneous rate) across the major CN divisions. In a subset of recorded units ($n = 64$), we characterized more completely the frequency tuning and sensitivity to combinations of stimuli. The second section describes the occurrence of more complex responses among these units.

Frequency Tuning and Temporal Properties in CN Divisions

Single unit and multi-unit responses were sampled from the AV to more caudal parts of the DCN (Fig. 1). Penetrations throughout the CN revealed a tonotopic organization in general agreement with previous reports (Zook and Leake, 1989; Kössl and Vater, 1990a; Ross et al., 1988). Thus, BFs generally increased from rostral to caudal in AV (Fig. 1A, B). The marginal subdivision (M), mostly representing frequencies below 30 kHz, showed no clear gradient of BF. However, in the part of M that extends laterally, a higher frequency BF was recorded (103.8 kHz, Fig. 1D). In PV, BFs increased from ventromedial to dorsal and lateral locations (Figs. 1C, D). In DCN, we observed increasing BFs from caudal to rostral and from ventral to dorsal (compare Fig. 1E, F). Commonly, discontinuities in the frequency representation were apparent between divisions (Fig. 1C) or subdivisions (Fig. 1A) within a single penetration.

Best frequencies ranged between 9 and 106 kHz, with three peaks in the distribution (Fig. 2A). The lowest peak, at 17–32 kHz, is associated with mustached bat social vocalizations (Kanwal et. al., 1994) and the first sonar harmonic (23–30 kHz). The largest peak, 57–62 kHz, is associated with the second harmonic, constant frequency (CF2) component of the sonar signal and is disproportionately represented throughout the mustached bat's auditory system. The third peak, at 87–92 kHz, corresponds to frequencies of the third harmonic, constant frequency component of the sonar signal (CF3).

The distribution of BFs was not uniform across divisions (Fig. 2C–F). AV demonstrated the most complete distribution of BFs, ranging from 9 to 100 kHz (Fig. 2C). However, few BFs were recorded between 30 kHz and 47 kHz, frequencies associated with many social vocalizations but not with sonar signals. The largest peaks, as in the overall CN distribution, were at 57–62 kHz and 87–92 kHz. AV contrasted sharply with M, which displayed a distinct

BF distribution (Fig. 2D). With the exception of one unit tuned near 104 kHz, all BFs were in the range 16–38 kHz.

PV displayed a wide range of BFs, from 17–93 kHz (Fig. 2E), but these frequencies were distributed differently than in AV. Almost half of BFs (48%) were in the 57–62 kHz frequency range associated with the CF2 portion of the mustached bat sonar call. Only 2% of PV units were tuned between 87 and 92 kHz, compared to 14% of the units in AV. The PV also demonstrated a smaller percentage of BFs below 30 kHz: 7% compared to 22% in AV.

The distribution of BFs in DCN varied considerably from the overall CN distribution (Fig. 2F). Some 34% were recorded within the 68–87 kHz frequency range associated with the frequency modulated component of the third harmonic sonar call. This compares with 16% in AV and 11% in PV. Few DCN responses were tuned within the 57–62 kHz frequency range (15%), a finding in sharp contrast to PV. The range of BFs extended from 17 kHz to 105 kHz, but few units were tuned to the 26–55 kHz range.

Figure 2B displays a CN audiogram for the mustached bat based on the thresholds of the most sensitive CN single units in each of several frequency bands. There were two frequency regions, near 40 and 60 kHz, with the best sensitivity near 0 dB SPL. At 70 kHz and above, average threshold was elevated by 20 dB or more, while thresholds of units tuned to 20 kHz or below were generally at least 40 dB higher than near 60 kHz. This reduction in sensitivity at lower frequencies was surprisingly sharp, apparently occurring at 22–23 kHz. Neurons with best frequencies of 23–25 kHz ($n = 10$) were on average 18 dB more sensitive than neurons tuned to 20–22 kHz ($n = 12$; $p < 0.01$, t -test). However, a few units below 20 kHz had good sensitivity, with thresholds below 30 dB SPL.

Temporal response patterns of CN units in the mustached bat (Fig. 3) were categorized by peristimulus time and interspike interval histograms (PSTHs and ISIHS, respectively) based on established criteria (Pfeiffer, 1966; Rhode and Smith, 1986a,b). Primary-like response patterns were most common, occurring in 68% of the units. The largest subgroup of these units, designated PL, was characterized by an initial response tightly locked to stimulus onset, followed by sustained firing (Fig. 3A). The ISIH for PL responses showed an asymmetric distribution that declined hyperbolically. Primary-like sustained responses (PL_S) show similar ISIHS (Figs. 3B), but lacked the precisely timed initial spike of the PL pattern. This response pattern occurred in 20% of units. The notched primary-like response pattern (PL_N) was distinguished by a brief period of inactivity (<2 ms) following the initial stimulus-locked spike. (Fig. 3C). This pattern was uncommon, occurring in 8% of units.

Choppers were the second most common temporal response pattern (20% of units). Chopper units showed distinct, regularly spaced peaks in the PSTH that persisted through the early part of the response (Fig. 3D). The ISIH showed a large, narrow, symmetrical peak corresponding to the regular interval. Onset responses, consisting of one or a few precisely timed spikes at stimulus onset (Fig. 3E), were relatively uncommon, occurring in 8% of units. The PSTH patterns of pauser units were characterized by onset spikes followed by a period of reduced discharge, in turn followed by a resumption of spikes in response to the stimulus (Fig. 3F). Buildup response units (not shown) showed similar PSTHs, except for the lack of onset spikes. The ISIH for the pauser response had broad, symmetrical envelope. Pauser and buildup units were rare (4% of units). Different subgroups were observed for chopper, onset, and pauser/buildup patterns, but these are not separated due to their low numbers.

Primary-like response patterns formed the majority in each CN division (Table 1). AV had the largest percentage (77%), followed by M (70%), PV (69%), and DCN (54%). The distribution of the primary-like subtypes varied as a function of division. For instance, primarylike sustained responses were most abundant in PV. Choppers formed the second largest category

in AV, PV, and DCN, but were most common in DCN (30%). Only one chopper unit was recorded in M (4%). Onset responses were uncommon in all divisions except M, where 22% of units were of the onset type. Finally, pauser or buildup responses were rare outside of DCN, where they formed 16% of the population.

In general, the major PSTH patterns were distributed similarly across units with different best frequencies (Fig. 4). The major exception was in the 23–30 kHz band, which corresponds to the first sonar harmonic. In this band, the proportions of primary-like (48%) and onset (39%) patterns were similar. This distribution was particularly striking in comparison to units tuned to the 9–22 kHz band, in which onset patterns were scarce (3%). Onset responses tuned to 23–30 kHz were only observed in AV or M subdivisions.

First-spike latencies among CN single units ranged from 1.7 ms to 11.6 ms and averaged 3.6 ms (s.d. = 1.1 ms) (Fig. 5A). Most response latencies (90%) were between 2.1 and 5.0 ms. There were no significant differences in latency among divisions ($(f[3,273] = 1.44, p > .05)$). However, there was a significant effect of frequency ($(f[6,224] = 5.17, p < 0.0001)$) (Fig. 5B). Units tuned in the range 58–59 kHz (mean $\pm$ standard deviation: 4.2 ± 1.2 ms) had slightly but significantly longer latencies compared to six other frequency bands in the mustached bats audible range (multiple t-tests with Bonferroni correction; $p < 0.01$). The longer latency of 58–59 kHz units is consistent with a pronounced cochlear resonance occurring in this range (Suga et al., 1975; Pollak et al., 1979). Judging from latencies of units tuned below this range (32–56 kHz, 3.4 ± 0.9 ms), or above it (62–85 kHz, 3.2 ± 1.1 ms), the ~59 kHz resonance increased latency by nearly 1 ms for BF sounds ~30 dB above threshold. Since units were not normalized to the frequency of the cochlear resonance in individual bats, this figure may underestimate the latency difference. Units tuned just below 30 kHz and 90 kHz, frequencies also associated with sharp cochlear tuning (Pollak et al., 1979; Kössl, 1992), did not have significantly different latencies from the remainder of the sample (t-tests with Bonferroni correction, $p > 0.05$).

Spontaneous rate among CN neurons ranged from 0 to 214 spikes per second (mean: 32.6 ± 40.7 spikes/s). We found no significant relationship between spontaneous rate and either absolute threshold or threshold relative to the average of most sensitive units in 5 kHz-wide frequency bands (see Fig. 2B). For example, spontaneous rate and relative threshold were uncorrelated (Pearson correlation, $r = 0.004$, $df = 196$, $p > 0.5$). Further, when units were separated into low (low SR, < 2.5 spikes/s) and high (high SR, ≥ 2.5 spikes/s) spontaneous rates, the proportions of units having thresholds within 10 dB of the most sensitive units were similar (low SR = 28%, high SR = 30%).

In contrast, spontaneous rate was related to subdivision, frequency bands, and temporal response pattern (see Table 2). The proportion of low SR units was significantly different across subdivisions; 50% of units in M were low SR, compared to 22% for the entire population. The proportion of low SR units was also significantly different across frequency bands; the 10–22 kHz band had substantially fewer low SR units (3%), while the 23–30 kHz band had substantially more low SR units (53%) than the entire population. Finally, average spontaneous rate varied significantly with temporal response pattern; units with onset patterns had the lowest spontaneous activity and were significantly different from primary-like units.

Spectral Sensitivity and Complex Response Properties of CN Units

The majority of CN units tested with two-tone stimuli (55% of 64 units) displayed a single excitatory peak in the tuning curve and no evidence of tuned spectral interactions similar to combination-sensitive responses in the IC. A typical example is the AV unit shown in Figure 6, which displayed an asymmetric excitatory frequency tuning curve tuned to 24.6 kHz and a primary-like temporal response pattern (Fig. 6A). There was no spontaneous discharge.

Shorter tone bursts (11 ms) were used in tests shown in Figures 6B and 6C. With increasing sound level, the discharge rate increased rapidly over a 20 dB range, then remained nearly constant over a 40 dB range to the highest level tested, 92 dB SPL (Fig. 6B). To test for spectral interactions, the BF tone was presented 10 dB above threshold and a second tone was introduced over a broad range of frequencies, timing, and sound levels. Audiovisual tests indicated no such interactions. This was confirmed in a quantitative two-tone paradigm in which the second, simultaneous tone was varied from 30 kHz to 90 kHz in 1 kHz steps (Fig. 6C). The unit's discharge was unaffected by the second tone, indicating a lack of tuned spectral interactions under these test conditions.

Some singly tuned units (11%) showed evidence of response suppression consistent with previous studies in CN (Rhode and Greenberg, 1994; Palmer and Winter, 1996; Spirou et al., 1999). Two such units are shown in Figure 7. The DCN unit in Figure 7A–C displayed a single frequency tuning curve with a BF of 96.8 kHz (Fig. 7A) and a primary-like with notch temporal response pattern (Fig. 7A, inset). The rate-level function with 11 ms tones was strongly nonmonotonic (Fig. 7B). Responses to the BF tone in the presence a second tone at a range of frequencies (Fig. 7C) suggested that broadly tuned suppression may occur in the 10–30 kHz band, and revealed clear suppression in the 75–85 kHz range. The unit in Figure 7D–F from the marginal zone (M) displayed a single excitatory tuning curve with a BF of 28.7 kHz, an onset temporal pattern, and a monotonic response to increasing sound level. The simultaneous presentation of the BF and second tones revealed strong suppression 2 kHz or more below the BF (11–25 kHz) and signs of an inhibitory input tuned to 46–47 kHz (Fig. 8F). Since the inhibition was within an octave of the BF and since no delay sensitivity tests were completed, the unit was considered to be singly tuned, with only local spectral interactions.

Among singly tuned CN units, the sharpness of frequency tuning varied considerably (Fig 8). Q10dB values ranged from 1 to 246, with the highest Q values and sharpest tuning occurring for units tuned to 56–60 kHz (mean Q10dB, 116.5). Units tuned to 87–90 kHz also showed elevated Q10dB values (mean Q10dB, 44) compared to all other frequency bands. Units tuned to the 10–22 kHz band displayed the broadest tuning (mean Q10dB, 2.1). Their tuning was significantly broader even than neurons tuned to 23–30 kHz (mean Q10dB, 8.4; (t-test, $p < 0.05$), and their ranges did not overlap.

Most CN neurons responded monotonically to increasing sound level. The percentages of units in AV, PV, and M were similar, 80–84%. Fewer units in DCN were monotonic (68%), but this percentage was not significantly different from any of the divisions in the ventral CN.

Several units displayed multiple excitatory response areas when stimulated by single tonal stimuli, but showed an absence of spectral interactions consistent with combination sensitivity using two-tone stimuli. The PV unit in Figure 9 had two distinct frequency tuning curves that remained separate at the highest sound levels tested (Fig. 9A, gray bar). The insensitivity just below the sharply tuned ~60 kHz BF is consistent with a region of cochlear insensitivity (Suga et al., 1975; Pollak et al., 1979; Kössl and Vater, 1990b; Russell and Kössl, 1999). Nearly every response feature differed in the two excitatory regions (Figs. 9A–D). Thus, the higher frequency response had much sharper tuning (Q10dB of 114.5, vs. 0.6) and a lower threshold (44 dB SPL vs. 64 dB SPL). When the sound level was set to 20 dB above their respective thresholds, the higher and lower best frequencies evoked different temporal response patterns (chopper vs. pauser pattern), different latencies (5.1 ms vs. 3.6 ms), and different discharge rates. Two-tone interactions were investigated with a second tone varied from 10–47 kHz (Fig. 9D). There was evidence of suppression in two bands below and above the lower excitatory frequency band (11–15 kHz, 42–47 kHz), suggestive of inhibitory sidebands of the lower excitatory tuning curve. However, since there was no indication that the lower BF signal suppressed or enhanced

the unit's response to the higher BF signal, this multi-peaked unit was not considered to show combination sensitive interactions similar to IC units.

Multiple excitatory peaks were observed in 20 of the 64 tested units (31%). Frequency tuning curves for other multi-peaked CN units appear in Figure 10. The two curves obtained from each unit did not overlap at low and moderate sound levels, and most did not overlap at all. In some cases, the two complete tuning curves were separated by an octave or more (Figs. 10A, D). Among the sample of multi-peaked units, the lower BF ranged from 12.4 kHz to 41.2 kHz, while the higher BF varied between 30.5 kHz and 89.95 kHz (Fig. 10F). On average, higher BFs were 2.2 octaves above lower BFs, but there was no systematic relationship between the lower and higher BFs. Most lower BFs (79%) were below the lowest frequencies in the sonar signal (~23 kHz), but most higher BFs (84%) were tuned within the sonar frequency ranges. Lower frequency tuning curves were typically much broader, with average Q10dB values of 3.2 vs. 60 for higher frequency tuning curves. In part, this occurred because many multi-peaked neurons had higher BFs just below 60 kHz, where cochlear specializations create very sharp tuning. Thresholds for higher BFs (mean = 27 dB SPL) were significantly lower compared to thresholds for low BFs (mean = 66 dB SPL; paired t test; df = 13; $p < 0.05$). All multi-peaked units were recorded from tonotopic representations in CN corresponding to the higher BF.

We examined whether the lower frequency responses in units with multi-peaked tuning differed in basic properties from units that were singly tuned to these same frequencies. For responses tuned below 23 kHz, there were no significant differences (t-tests, $p > 0.1$) in threshold (single, 64.6 ± 9.2 dB, $n = 34$; multi, 63.4 ± 5.2 dB, $n = 45$), sharpness of tuning (Q10dB: single, 2.1 ± 1.3 , $n = 8$; multi, 1.4 ± 0.9 , $n = 5$), or response latency (single 3.3 ± 0.9 , $n = 34$; multi, 3.4 ± 1.4 , $n = 31$). The similarity of these measures for the two populations suggests the same cochlear mechanism generates both.

Inhibition from widely separated spectral elements ("distant inhibition") was distinct from the suppressive sidebands showed by singly tuned or multi-peaked units. Some units showed inhibition that was tuned an octave away from the BF. The unit from AV (Fig. 11) displayed properties of this spectral inhibition. This unit had a single excitatory tuning curve centered at 49.7 kHz with a MT of 27 dB SPL (Fig. 11A). At 20 dB above MT, the neuron showed a primary-like temporal response pattern with a first-spike latency of 2.5 ms (Fig. 11B). Its response increased monotonically with increasing sound level (Fig. 11C). In tests using combinations of tones, a fixed signal was presented at the BF, 10 dB above MT, and a second tone (at 30 dB attenuation and simultaneous presentation) was varied from 10.1 kHz to 30.1 kHz (Fig. 11D). The second tone strongly suppressed the response to BF in the range 15.1–23.1 kHz, with the best inhibition occurring at 18.1 kHz. There was also suppression at 27.1–30.1 kHz, which may correspond to a suppressive sideband of the BF excitatory peak. The suppression tuned to 15–23 kHz remained separate from both the 27–30 kHz suppressive sideband and the excitatory tuning curve at the highest intensities tested. This low frequency inhibition was timesensitive (Fig. 11E), strongest when the BF signal was presented simultaneously with the lower inhibitory tone. A change in the timing of the two signals by as little as 2 ms resulted in a decrease in combinatorial inhibition. For the unit in Figure 11, the frequency- and timedependent inhibition in the 15–23 kHz band satisfied the criteria we used for combinationsensitive inhibition in our IC studies.

Inhibitory spectral interactions could occur even when there was an excitatory response to the inhibiting frequency. A single unit in M (Fig. 12) with best frequency of 30.5 kHz and MT of 17 dB SPL had a second excitatory peak tuned to 14.9 kHz (MT, 61 dB SPL). The tuning (not shown) remained separate at the highest levels tested, and several features of the lower and higher frequency excitatory responses were distinct. At the higher BF, responses were primarilylike and monotonic, having a first-spike latency of 3.4 ms at 30 dB above threshold

(Fig. 12A, B). In contrast, responses at the lower BF were fewer and had an unusual temporal pattern (sustained activity with an offset), a longer latency (6.3 ms), and a non-monotonic response with increasing level (Fig. 12C).

When the lower and higher frequency signals were presented together, temporally sensitive combinatorial inhibition was apparent (Fig. 12D and PSTHs). The inhibition was strongest at simultaneous presentation (0 ms delay), where the unit's response was much less than the response to either of the two tones. This indicates that each signal suppressed the response to the other signal. At negative delays (high BF signal presented first), the total response was reduced primarily because the higher frequency signal suppressed the excitatory response to the lower frequency signal, but also because, at shorter delays, early inhibition by the lower frequency signal suppressed the later part of the high frequency excitatory response. At positive delays up to 14 ms, the spike count remained near the level evoked by the lower frequency signal, indicating that the lower frequency signal suppressed the later higher frequency response. At long positive delays, the response to the combination matched that of two individual responses. These results suggest that inhibition activated by the low frequency signal preceded its excitatory response, perhaps accounting for the longer low frequency latency, but also extended for a few milliseconds beyond the duration of the signal. The inhibition evoked by the higher frequency signal was only apparent following its excitatory response. For the unit in Figure 12, this frequency- and time-dependent inhibition satisfies the criteria for combinatorial inhibition. Four units displayed an excitatory response to tones at or near the best inhibitory low frequency.

Tuned inhibition by spectral elements far from BF was observed in 13 of the 64 tested units (20%). Best excitatory frequencies were almost always higher than best inhibitory frequencies, and the average separation was 2.4 octaves (Fig. 12E). While the best excitatory frequencies were widely distributed throughout the bat's audible range, the best inhibitory frequencies were limited to the range 12–48 kHz (Fig. 12E). These inhibitory best frequencies were typically tuned below the fundamental of the sonar signal (<23 kHz). In fact, only one unit demonstrated spectral inhibitory properties in which both the BF and inhibitory responses were tuned to frequencies within the biosonar call (Fig. 12E).

Figure 13 summarizes the distribution of the general categories of spectral sensitivity. Neurons with a single excitatory tuning curve and no distant frequency-tuned inhibition were the most common throughout the CN (55%) and occurred in each CN division. One-quarter of the units had multi-peaked excitatory responses with no inhibition; these were observed in all divisions except M. Distant spectral inhibition was observed in one-fifth of the units, distributed across all CN divisions. Among the 64 units, we found no evidence of facilitatory combinatorial interactions similar to those observed in the IC (Mittmann and Wenstrup, 1995; Portfors and Wenstrup, 1999; Portfors and Wenstrup, 2002; Leroy and Wenstrup, 2000).

DISCUSSION

This study describes responses to single and two-tone stimuli among single units in the four major subdivisions of the mustached bat's CN. The results show that distributions of best frequency differ across CN subdivisions, and that distributions of temporal response features vary across frequency and across CN subdivisions. These frequency and subdivisional differences provide the bases for distinct analyses of acoustic information. Our findings corroborate previous reports of frequency tuning and temporal response features in AV and M (Kössl and Vater, 1990a,b). In addition, the present study compares responses in DCN and PV to those in AV and M, and documents widespread responsiveness to sounds below ~23 kHz, i.e., below the lowest frequencies in the sonar signal. Moreover, the results show that some forms of spectral integration observed in the IC are present in CN, but others are absent. From

this work emerges a clearer picture of the serial and parallel processing that is responsible for the abundant and diverse types of spectral integration that characterize the mustached bat's IC.

Spectral features of CN responses

BF distribution and threshold sensitivity—Frequencies across the mustached bat's audible range are not represented equally in CN. Nearly one-third of units are tuned to a narrow band just below 60 kHz, with other peaks centered on 20 and 90 kHz. Few units were recorded in bands centered at 35–45 kHz. Although our sampling throughout CN may have biased the BF distribution, it is remarkably similar to those recorded in the ventral CN (Kössl and Vater, 1990b) and lateral lemniscal nuclei (Portfors and Wenstrup, 2001). A further observation is that frequencies across the mustached bat's audible range are represented differently in CN divisions. For instance, nearly half of PV units are tuned near 60 kHz, while less than one-sixth of DCN units are tuned to the same band. In M, nearly all units are tuned below 40 kHz. We observed many neurons in M tuned in the 10–22 kHz range, in addition to the tuning to 24–32 kHz described previously (Kössl and Vater, 1990a). By comparison with cochlear frequency maps in the mustached bat (Kössl and Vater, 1985; Zook and Leake, 1989), these results indicate that the representation of sound frequencies established in the cochlea is significantly modified by auditory nerve projections to CN subdivisions.

Some aspects of frequency representation in CN are clearly related to the bat's biosonar behavior. For example, the high sensitivity, extremely sharp tuning, and disproportionately large representation of frequencies just below 60 kHz (Kössl and Vater, 1990b; this study) contribute to analyses of faint echoes of the mustached bat's second harmonic, constant frequency (CF) component of its sonar signal. The specialized features of this neural representation have their origins in the cochlea (Henson, 1973; Henson and Henson, 1991; Kössl and Vater, 1985, 1996; Pollak et al., 1972; Suga et al., 1975; Suga and Jen, 1977).

The substantial representation below 30 kHz includes two populations distinguished by multiple features. Neurons tuned in the 23–30 kHz range have moderate thresholds, moderately broad frequency tuning, low spontaneous rates, and often display onset responses to tonal stimuli. This frequency band defines the extent of the first sonar harmonic, and responses to these frequencies form an essential element of combination-sensitive response properties among neurons in NLL (Portfors and Wenstrup, 2001), IC (Mittmann and Wenstrup, 1995; Portfors and Wenstrup, 1999), medial geniculate body (Olsen and Suga, 1991a,b; Wenstrup, 1999), and auditory cortex (Suga et al., 1978, 1983; Fitzpatrick et al., 1993). While neurons responsive to this band may also analyze other signals including social vocalizations, their special features seem particularly well suited for analyses of sonar pulse-echo combinations.

In contrast, neurons tuned below 23 kHz have significantly higher thresholds, broader tuning, higher spontaneous rates, and sustained responses to tonal stimuli. Moreover, there is an abrupt transition in each of these properties for neurons tuned above or below 23 kHz. Cochlear microphonic (CM) thresholds do not change in a similar way, (Suga et al., 1975; Pollak et al., 1979; Kössl and Vater, 1990b), probably due to the low frequency sensitivity of high frequency hair cells. However, the onset response of the whole nerve action potential (N1-ON) and distortion-product threshold curves suggest a decrease in sensitivity to tones below ~25 kHz (Kössl and Vater, 1990b; Kössl, 1994; Kössl and Vater, 1996).

Neural tuning to frequencies below the mustached bat's first sonar harmonic, especially below 20 kHz, has been reported infrequently in the mustached bat (Wenstrup, 1999; Hattori and Suga, 1997; Fitzpatrick et al., 1998). The present study suggests more abundant responses to this frequency band than has been recognized. We propose that these neurons play a fundamental role in analyzing many social vocalizations. If so, there is need of a good representation within CN, but less need for high sensitivity since most social vocalizations are

produced and received at high sound levels (Kanwal et al., 1994; Kanwal et al., 1999). Thus, neurons tuned to the two bands below 30 kHz play very different roles in auditory perception, and that is reflected in their auditory response properties.

Features of spectral integration in the CN—A major goal here was to examine whether forms of spectral integration present in the mustached bat's IC (multiple tuning, combination sensitivity) also occur in CN. In particular, we examined low frequency responsiveness of neurons with high BFs. We therefore emphasized responses and combinatorial interactions at frequencies distant from a unit's BF, rather than those in the vicinity of the BF, such as sideband inhibition. Consequently, our results are not directly comparable to studies characterizing near-BF responses in the mustached bat (Kössl and Vater, 1990b) or receptive fields of CN units in several species (Evans and Nelson, 1973a,b; Young and Brownell, 1976; Shofner and Young, 1985).

Features of excitatory tuning curves: Among 64 well-studied CN single units, 55% displayed an excitatory frequency tuning curve with a single sensitivity peak. While other forms of spectral interactions were observed in some of these neurons, such as sideband and broadband inhibition (Shofner and Young, 1985; Rhode and Greenberg, 1994; Palmer and Winter, 1996; Spirou et al., 1999), they showed no evidence of spectral interactions that characterize combination-sensitive neurons in the mustached bat's IC.

The presence of a second relative threshold minimum characterized many CN neurons in the mustached bat. In our more thoroughly characterized sample, 31% showed a second excitatory peak, usually in the 10–22 kHz range. The lower frequency excitatory peaks were generally separate from the more sensitive peaks that characterized unit BFs. These lower frequency peaks may represent the most sensitive response in the tails of frequency tuning curves. Consistent with tails, they have threshold sensitivities in the 60–70 dB SPL range and much broader tuning than near the unit's BF. However, there are some unusual features that may require an alternate explanation.

First, if these low frequency responses are tails of tuning curves, they should be a consistent feature of inner hair cell, auditory nerve, and CN response properties. No data exist for inner hair cells or for anatomically verified auditory nerve fibers in mustached bats. However, over half of CN neurons in this study do not display a second excitatory peak. While interactions within CN could inhibit the excitatory response in the tail, the present study found evidence of such low frequency inhibition in only 20% of CN neurons. Such results are reasonably consistent with a study in IC that closely examined multiple tuning (Portfors and Wenstrup, 2002). There, 41% of neurons displayed multiple tuning, but 43% had neither multiple tuning nor evidence of interactions between low frequency and high frequency signals.

A second unusual feature of these lower frequency responses is the complete or near complete separation of BF and lower frequency tuning curves. Although tails of higher BF tuning curves in other species often display an insensitivity peak between the BF and the most sensitive frequencies in the tail response, distinct tuning curves appear to be unusual among neurons in the auditory nerve or ventral cochlear nucleus (VCN) (reviews: Ruggero, 1992; Rhode and Greenberg, 1992). For sharply tuned units near 60 kHz, the separation is likely due to mechanical features of the mustached bat cochlea that create an insensitivity peak just below the sharply tuned, ~60 kHz cochlear resonance (Suga et al., 1975; Pollak et al., 1979; Kössl and Vater, 1985; Kössl and Vater, 1990b; Russell and Kössl, 1999). For units with BFs at other frequencies, or for broader insensitivity regions, however, the insensitivity is not easily explained by cochlear mechanisms (e.g., Figs. 10A,C–E).

A third unusual feature of low frequency sensitivity peaks reported here is that their thresholds and tuning sharpness are indistinguishable from the population of singly tuned CN neurons with BFs in the 10–22 kHz range. Typically, neurons with low frequency BFs are more sensitive and more sharply tuned than the tail responses of neurons with high BFs (Kiang and Moxon, 1974; Javel, 1994). If this low frequency sensitivity does in fact represent the tails of hair cell and auditory nerve fiber tuning curves, then the mechanics of the mustached bat cochlea must create a distinctive pattern of basilar membrane motion resulting from low frequency stimuli. Specifically, 10–22 kHz signals that exceed ~60 dB SPL must evoke nearly the same response amplitude in the basal turn of the cochlea as they do at a tonotopically appropriate place near the apex. Moreover, the basal cochlea would not respond similarly to 23–30 kHz, to which the apical cochlea is more sensitive than to 10–22 kHz. We do not know what mechanical features could be responsible for this, but perhaps it is a by-product of the multiple mechanical specializations that create very sharp tuning near 60 and 90 kHz ((Henson and Henson, 1991; Kössl and Vater, 1985, 1996; Pollak et al., 1979; Suga et al., 1975; Vater and Kössl, 1996).

Alternate to explanations that invoke low frequency tails, multi-peaked tuning may result from convergence of separately tuned excitatory inputs. While cochlear frequency maps based on frequency-specific tracer deposits in CN suggest no such convergence (Kössl and Vater, 1985; Zook and Leake, 1989), it may result from projections within CN. Recording of auditory nerve fibers are needed to resolve the origin of the low frequency response of high BF neurons.

Whatever the origin, the result creates in many CN neurons a response to low frequency signals that has two major features. The first is excitation that is mainly limited to the 10–22 kHz range, a frequency band that contains the dominant harmonics of many social vocalizations. High-BF neurons are thus endowed with the ability to respond to low frequency vocalizations (Portfors and Wenstrup, 2002; Portfors et al., 2002; Sheykhholeslami et al., 2004), as Kiang and Moxon (1974) had shown in cats. The second feature is that there is less excitation evoked by 23–30 kHz signals. In the mustached bat, this is an essential prerequisite of facilitated, combination-sensitive neurons that are abundant in the auditory midbrain and forebrain. These neurons display a facilitatory response tuned in the 23–30 kHz range (corresponding to the first sonar harmonic) that must occur simultaneous with or prior to a BF signal. The resulting temporally sensitive facilitation creates selectivity for a narrow range of pulse-echo delays or other acoustic cues associated with sonar targets. The selectivity would be greatly diminished if high BF neurons displayed typical broad tails of tuning curves, with delay-insensitive excitation by sounds in the 23–30 kHz range. The limited responsiveness of high-BF neurons to 23–30 kHz signals may constitute another specialized feature of the mustached bat cochlea.

These low frequency excitatory responses contribute heavily to responses among IC neurons. As noted above, low frequency responsiveness tuned below 23 kHz is common among high BF units in IC, particularly among units tuned to 60 kHz. These response properties may arrive directly from the CN or via numerous intermediate nuclei of the auditory brainstem.

Combination-sensitive spectral interactions in CN and IC: The term “combination sensitivity” originally designated neural responses that were strongest when specific elements of complex acoustic stimuli were presented together (Suga et al., 1978, 1983). In mustached bats and other species, these responses are based on facilitatory interactions activated by inputs with either distinct frequency tuning and/or inputs occurring at distinct times. In the mustached bat, most such neurons receive a facilitatory input tuned within the frequency range of the bat’s first sonar harmonic (23–30 kHz in the sub-species used here) that terminates on high BF neurons, usually in the frequency range of one of the higher harmonics of the sonar signal. Combinations of tonal stimuli have been effective in documenting such responses in the IC and MGB (Olsen and Suga, 1991a,b; Mittmann and Wenstrup, 1995, Portfors and Wenstrup,

1999), and even in auditory cortex (Taniguchi et al., 1986), but we found no evidence of combination-sensitive facilitation in the mustached bat's CN. This result indicates that the facilitated combination-sensitive responses originate above the level of the CN, and further that the interaction is of neural, not cochlear, origin. The findings provide additional support for the view that these interactions are created in the mustached bat's IC, where about 25% of neurons show facilitated combination sensitivity (Wenstrup and Leroy, 2001; Portfors and Wenstrup, 2001; Nataraj and Wenstrup, 2005).

Twenty percent of the neurons in our sample displayed a form of spectral integration characterized by tuned low frequency suppression of a neuron's response to its much higher BF. In the IC, we have called this low frequency suppression "combination-sensitive inhibition" (Mittmann and Wenstrup, 1995; Portfors and Wenstrup, 1999), reflecting our view that it, like facilitatory combination-sensitive response properties, results from integration of inputs tuned to distinct frequency bands. It is not clear, however, that CN units showing low frequency "inhibition" are combination-sensitive in the same manner as described for IC neurons.

In the majority of inhibitory combination-sensitive units in the IC, low frequency inhibition is tuned in the 23–30 kHz range, corresponding to the first sonar harmonic. These account for about 25% of all units sampled in IC (Mittmann and Wenstrup, 1995; Portfors and Wenstrup, 1999). Similar units occur in the intermediate nucleus of the lateral lemniscus (INLL) (Portfors and Wenstrup, 2001), but the present study shows that these are almost entirely lacking in CN. These observations suggest that this inhibitory combination sensitivity is of neural, not cochlear, origin. Preliminary evidence based on iontophoretic studies supports a conclusion that these responses are created in INLL (Nataraj and Wenstrup, 2004).

The CN neurons that display low frequency suppression correspond to a different type of response observed in ~15–20% of neurons in the mustached bat's IC (Portfors and Wenstrup, 1999; Nataraj and Wenstrup, in preparation). In these units, suppression is tuned below 23 kHz and differs from inhibition tuned to 23–30 kHz in several features: threshold of inhibition, duration of inhibition, strength of inhibition, and presence of low frequency excitation (Nataraj and Wenstrup, in preparation; Gans et al, 2004). The presence of similar units within CN suggests an origin in either CN or the cochlea.

Suppression of BF responses by a simultaneous lower frequency tone is suggestive of two-tone suppression originating in the cochlea. At lower levels, a second tone suppresses BF responses but does not evoke excitation by itself. At higher levels, i.e., within the tails of excitatory tuning curves, low frequency signals evoke excitation but also suppress responses to relatively low level BF tones. In the present study, such effects were not a pervasive feature of CN responses. As with low frequency excitatory responses, we question how a cochlear effect such as tails or two-tone suppression could be expressed in only a small proportion of CN neurons. This suggests that some features of the low frequency response originate within CN. Recordings of auditory nerve fibers are needed to test this possibility.

Temporal Properties of CN Neurons

We observed each of the major temporal response patterns described for CN neurons in other species. Primary-like responses formed the dominant pattern in each CN division, chopper patterns were the second most common (except in M), and pauser/buildup responses were the least common (except in DCN). These observations support a previous study of AV and M in mustached bats (Kössl and Vater, 1990a). They are similar to results in the horseshoe bat CN, where primary-like patterns form the majority in both VCN and DCN (Feng and Vater, 1985).

The predominance of primary-like responses in these bats differs substantially from another bat (big brown bat, Haplea et al., 1994) and from species in other mammalian taxa. In the cat VCN, for example, primary-like units account less than one-fourth of units, while choppers form about 50% (Parham et al., 1998; Shofner and Young, 1985; Young et al., 1988).

In DCN, pauser, build-up, onset, and chopper temporal patterns are much more common than primary-like patterns in cat (Shofner and Young, 1985; Rhode and Smith, 1986a), guinea pig (Stabler et al., 1996), rabbit (Perry and Webster, 1981), and chinchilla (Kaltenbach and Saunders, 1987). These species differences in temporal patterns may result from differences in CN cell types or synaptic organization. Thus, the smaller proportion of stellate cells in the mustached bat (Zook et al., 1982) may explain the smaller proportion of chopper patterns in VCN, since stellate cells are correlated with chopper response patterns (Rhode et al., 1983a). Similarly, the mustached bat's DCN shows less distinct lamination than in cats, due in part to a lack of a uniform orientation of fusiform cells and a reduced number of granule cells (Zook and Casseday, 1982; Kemmer and Vater, 1997).

First spike latencies throughout the mustached bat's CN were between 2 ms and 5 ms. Latencies in the ventral CN are similar to those reported in the big brown bat (Haplea et al., 1994) and other mammals (Rhode and Smith, 1986b; Young et al., 1988; Ghoshal and Kim, 1997). Latencies in the mustached bat's DCN ranged from 2–12 ms, but did not differ significantly from other CN divisions. No comparable data have been reported in other bats. In cats, the range of latencies is similar, but the majority of units had latencies in the 5–8 ms range (Rhode et al., 1983b; Rhode and Smith, 1986a; Joris and Smith, 1998). The shorter latency values in the mustached bat DCN may relate to the larger proportion of primary-like responses.

A major finding concerns the predominance of onset or phasic response properties among ventral CN neurons tuned to 23–30 kHz. Kössl and Vater (1990a) had reported that about half of AV and M neurons tuned to the first sonar harmonic had phasic patterns, and the present study agrees closely. Our results extend their findings in two ways. First, in contrast to AV and M, DCN and PV neurons tuned to the first sonar harmonic (23–30 kHz) do not show similar large proportions of onset patterns. Second, neurons in M or AV that are tuned below 23 kHz, a sizeable number, do not show onset response patterns. These results strongly suggest that there is specialized processing in the mustached bat's CN for the 23–30 kHz band. This is supported by studies showing a predominance of noradrenergic terminals in M and the anterior part of AV (Kössl et al., 1988), and further work demonstrating that application of noradrenalin minimizes first spike jitter in CN neurons (Kössl and Vater, 1989). This same population of neurons, (onset, in M, tuned to 23–30 kHz) also had lower rates of spontaneous activity.

Phasic, temporally precise responses tuned to 23–30 kHz provide a reliable marker for the timing of the first harmonic, frequency-modulated sweep (FM1) at the end of the bat's sonar pulse. Large numbers of combination-sensitive neurons in the IC, MGB, and auditory cortex show facilitated responses to the occurrence of the FM1 prior to a higher harmonic FM sweep in biosonar echoes, providing a code for pulse-echo delay and, ultimately, the distance of sonar targets (O'Neill and Suga, 1982; Olsen and Suga, 1991b; Portfors and Wenstrup, 1999). Preliminary evidence suggests that much of the FM1-tuned input to combination-sensitive neurons in the IC has a phasic effect (Sheykholslami et al., 2002). Moreover, some neurons in M and the anterior part of AV project to regions of the high frequency IC (Wenstrup et al., 1999), where delay-tuned, combination-sensitive neurons are created (Wenstrup and Leroy, 2001; Nataraj and Wenstrup, 2005). Together, these studies suggest that M and AV neurons tuned to 23–30 kHz contribute to the combination-sensitive response of midbrain, thalamic, and cortical auditory neurons.

One additional feature of 23–30 kHz CN neurons is relevant to combination-sensitive responses in the IC: the timing of their input at sites generating combination-sensitive spectral integration. Combination-sensitive neurons are maximally facilitated when a higher frequency signal (e.g., in higher harmonics of sonar echoes) occurs up to 30 ms after a lower frequency signal, (e.g., in the first harmonic of the emitted sonar signal). For this to occur, the facilitatory effect of the 23–30 kHz signal must be delayed up to 30 ms within the brain. Since facilitation depends on the coincidence of excitatory effects of the low and high frequency inputs (Olsen and Suga, 1991b, Portfors and Wenstrup, 1999), one can infer the range of latencies of low frequency excitation that are required (Fig. 14A), based on known best delays and high frequency excitatory latencies of the facilitated neurons (Portfors and Wenstrup, 1999). CN neurons, including those tuned to 23–30 kHz, have response latencies that are too short compared to the low frequency excitation latencies needed in facilitated, delay-tuned IC neurons (cf 14A, B), even when axonal conduction time and synaptic delays are factored in. Thus, these longer latencies must be generated by synaptic relays above CN, but even brainstem synaptic delays revealed in the latencies of NLL neurons do not seem to be sufficient (cf Fig 14A, C). It is likely, then, that synaptic mechanisms in the IC, perhaps related to post-inhibitory rebound (Nataraj and Wenstrup, 2005) or NMDA-mediated delayed excitation (Sanchez et al., 2005), complete the temporally sensitive integration of distinct spectral inputs within the IC.

Spectro-temporal integration in the ascending auditory system

The results of this study, with previous work, identify multiple sites within the ascending auditory pathway where information from distinct frequency bands in sounds is integrated to form complex response properties. The present study shows that the output of the CN reflects integration in some high BF neurons of responsiveness to the lowest frequency band in the bat's audible range, and in other neurons a low frequency suppression/inhibition of neuronal responses to higher frequency signals. Furthermore, low frequency response properties in AV and M appear to form key elements of the spectral integration operating in the nuclei of the lateral lemniscus (combination-sensitive inhibition) and the inferior colliculus (combination-sensitive facilitation). As the result of these distinct spectral integrative events, auditory responses of single IC neurons may include multiple response selectivities, for particular pulse-echo combinations, social vocalizations, and complex environmental signals.

The response properties documented in the mustached bat's IC, including facilitative or inhibitory interactions across frequency bands and multi-peaked tuning, are features of the auditory forebrain of frogs, birds, and a wide range of mammals. While the temporal and spectral features that characterize these neurons may differ from the mustached bat, similar multi-level, spectro-temporal integrative processes are likely occur in other species. Moreover, while some of these features may originate in the forebrain, as is often proposed, we believe that the more likely scenario is that the forebrain responses form the culmination of many spectral integration events, originating in the cochlea, brainstem, and midbrain.

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Figure 1.

Progression of best frequencies through CN subdivisions. Photomicrograph of Nissl-stained horizontal section through CN depicts subdivisions and rostro-caudal level of penetrations displayed in line drawings (A–F). A–F. Physiological responses obtained in six electrode penetrations through the CN of different bats. Tracer deposits for each penetration are shown as grey areas. Rostro-to-caudal position is indicated as percentage above each section.

Headings above physiological parameters indicate *type* of response encountered (*s*, single unit; *m*, multiple unit; *cap*, compound action potential) and best frequency (*BF*). Protocol for photomicrograph: plan apochromat, N.A. 0.08. All scale bars: 500 μ m.

Figure 2.

Distribution of best frequency (BF) and minimum threshold (MT) of single units across the CN. **A.** BFs are binned (5 kHz) across the audible frequency range of the mustached bat, ~5–120 kHz. Note large representations between 56–60 kHz and 86–90 kHz, the ranges associated with constant frequency sonar signals, and small representations between 30–50 kHz, bands mostly associated with non-sonar frequencies. **B.** CN audiogram based on the three most sensitive single units in 5 kHz-wide frequency bands. Dashes indicate range of the three lowest MT values in each band, line indicates average of three lowest MT values. *Inset* shows CN audiogram in 1 kHz increments for range 54–65 kHz. This CN audiogram smears the very sharp tuning near 30, 60 and 90 kHz, due to increment size and individual differences in frequencies of sharp tuning. **C–F.** Distribution of BF vs MT for single units across major divisions of CN. The distributions of best frequencies across AV, PV, and DCN span most of the mustached bat's audible range. Representation in M was mostly restricted to frequencies below 40 kHz.

Figure 3.

Temporal response patterns defined in peristimulus time histograms (PSTH) and interstimulus interval histograms (ISIH). **A.** Primary-like (PL) response in M (88 dB SPL, 20 stimulus presentations). **B.** Primarily-like sustained (PL_S) response in AV (49 dB SPL, 20 stimulus presentations). **C.** Primary-like with notch (PL_N) response in AV (77 dB SPL, 50 stimulus presentations). **D.** Chopper response in M (82 dB SPL; 50 stimulus presentations). **E.** Onset response in AV (88 dB SPL, 20 stimulus presentations). **F.** Pauser response in PV (89 dB SPL, 50 stimulus presentations). BF and median first spike latency are shown in PSTH. Bin size: 100 μ s.

Figure 4.

Distribution of temporal response patterns across the entire sample (*All*) and for units with best frequencies in particular bands. A larger proportion of onset responses distinguished the sample with BFs in the 23–30 kHz band. Neurons tuned to the 57–60 kHz band are specialized to analyze the bat's second harmonic, constant frequency echo. The 48–110 kHz band includes all higher harmonics of the sonar signal.

Figure 5.

Timing of initial spike among CN units expressed as median first spike latency. **A.** Median first spike latency, obtained at each unit's BF, 20 to 30 dB above MT. Latencies extended from 1.7 ms to 11.9 ms with most occurring between 2 and 5 ms. **B.** Latencies as a function of frequency. Latencies of units tuned near 60 kHz were significantly longer (see text).

Figure 6.

Singly tuned unit in the AV. **A.** Frequency tuning curve with BF of 24.6 kHz and MT of 42 dB SPL. X indicates frequency and sound level combination used to obtain PSTH pattern (*inset*). **B.** Rate-level function obtained at BF from 10 dB below to 50 dB above MT. This unit showed a monotonic rate-level function (see Methods and Materials). Stimulus duration was 11 ms. **C.** Response to BF tone at 10 dB above MT as function of the frequency of a simultaneous second tone (30–90 kHz) presented at 30 dB attenuation from maximum speaker output (*upper line in graph*). Arrow shows magnitude of response to the BF tone alone.

Figure 7.

Singly tuned responses in DCN (A–C) and M (D–E) show more complex spectral interactions.

A. In this DCN unit, frequency tuning curve reveals BF at 96.8 kHz and MT of 32 dB SPL. Inset shows primary-like with notch pattern obtained at BF, 20 dB above threshold. **B.** Response to 11 ms BF tone shows strong non-monotonic rate-level function and onset response at higher sound levels. **C.** Response to BF tone at 10 dB above MT as function of the frequency of a simultaneous second tone (10–110 kHz) presented at 30 dB attenuation. Note suppression at frequencies below the BF. **D.** In this M unit, BF was 28.7 kHz and MT of 34 dB SPL. At 20 dB above MT, temporal pattern was onset locker. **E.** Responses to 30 ms signal at BF show monotonically increasing rate-level function. **F.** Response to BF tone at 10 dB above MT as function of the frequency of a simultaneous second tone (10–100 kHz) presented at 30 dB attenuation. Note suppression at frequencies below the BF. For further explanation, see legend for Fig. 6.

Figure 8.

Frequency tuning properties of singly tuned units. **A.** Frequency tuning curves. **B.** Q10 dB values across frequency and CN division.

Figure 9.

Responses of PV single unit with multi-peaked tuning. **A.** Frequency tuning curves revealed sensitivity peaks at 58.42 kHz and 18.9 kHz for the high- and low-frequency signals. *Upper line* indicates the maximum sound level across frequency produced by the acoustic stimulation system during most experiments. Tuning curves do not intersect at the highest intensities tested. *Gray bar* indicates the separation of low- and high-frequency tuning curves. "X"s indicate frequency and sound level combinations for PSTHs. **B.** *Top*, chopper pattern in response to BF signal, 20 dB above minimum threshold (MT). *Below*, pauser-like pattern in response to 18.9 kHz tone, 20 dB above MT. PSTHs are based on 50 stimulus repetitions. **C.** Rate-level functions for the high-frequency and low-frequency signals. Due to the high background discharge, response functions in C and D are based on a 25 ms window beginning with the onset of the 11 ms tones. **D.** Response to BF tone at 10 dB above MT as function of the frequency of a simultaneous second tone (10–47 kHz) presented at 30 dB attenuation. There was little effect of the scanning tone on the BF response for frequencies between 16 kHz and 43 kHz, but possible side band inhibition at frequencies below 16 kHz and above 43 kHz.

Figure 10.

Multi-peaked tuning curves among CN single units. Each unit responded to two frequency bands, usually with no overlap between tuning curves. **A.** Distinct tuning curves from unit in AV with best excitatory frequencies of 60.85 kHz and 17.4 kHz. The high frequency tuning curve was not tested with sounds above 60 dB SPL. **B.** Unit in PV with best excitatory frequencies of 59.77 kHz and 12.4 kHz. Gray bar indicates the separation of tuning curves between the high- and low-frequency peaks. **C.** AV unit with separate tuning curves centered at 88.55 kHz and 22.9 kHz. **D.** DCN unit shows two closed tuning curves with best excitatory frequencies of 71.2 kHz and 26.4 kHz. **E.** Unit in DCN with tuning to 89.05 kHz and 41.2 kHz. **F.** Comparison of best high and low frequencies among all units with multi-peaked tuning. Vertical lines indicate frequency range of the first harmonic of the echolocation call of the mustached bat.

Figure 11.

Low frequency inhibition in an AV unit. **A.** Frequency tuning curve shows BF of 49.7 kHz with no low frequency excitation. *X* indicates the frequency and sound level combination for PSTH in **B.** **B.** Primary-like response pattern recorded at BF, 20 dB above threshold. **C.** Rate-level function shows monotonically increasing response at BF with 11 ms tones. **D.** Response to BF tone at 10 dB above MT as function of the frequency of a simultaneous second tone (10–30 kHz) presented at 30 dB attenuation. The unit's response to the BF tone was completely suppressed by a second tone at 20 kHz. **E.** Temporal sensitivity of 20 kHz inhibition. The strongest inhibition occurred when the BF and 20 kHz tones were presented simultaneously (0 ms delay). In associated PSTHs (right), signal timing and duration are indicated by black bars (BF tone) and unfilled bars (best inhibitory tone). PSTHs based on 20 stimulus repetitions.

Figure 12.

Low frequency inhibitory tones also evoke excitation in unit recorded from M. **A.** Response to 30.5 kHz BF tone was primary-like (30 dB above MT). **B.** Weak sustained response pattern evoked by best inhibitory tone of 14.9 kHz tone, 10 dB above its MT. Note offset response and longer first-spike latency than in A. **C.** Rate-level function for BF tones increased monotonically (11 ms tones), while rate-level function for 14.9 kHz tones was nonmonotonic. **D.** Response as function of timing of BF and 14.9 kHz tones. Worst response was obtained with simultaneous tones, but function and PSTHs (below) show evidence of shortlatency low frequency inhibition and longer latency inhibition by the BF tone. *Arrows* indicate the response of the unit to each tone presented separately. PSTHs and response functions based on 50 stimulus repetitions in **A, B** and 20 repetitions in **C, D**. **E.** Comparison of best excitatory frequency and best inhibitory frequency among all units with showing combinatorial inhibitory interactions. In most cases, the inhibitory interactions were tuned to frequencies below 23 kHz.

Figure 13.

Distribution of types of spectral integration among single units in CN. Units in the “Single Tuning” and “Multiple Peaks” categories displayed **no** distant inhibition. In the “Distant Inhibition” category, *filled column* indicates units with a single excitatory peak, while the *unfilled column* indicates units displaying dual excitatory peaks.

Figure 14.

CN latencies are too short to generate delayed low frequency excitation in facilitated combination-sensitive neurons in the IC. **A.** *Predicted* latencies of low frequency excitation among a population of IC facilitated neurons (data from Nataraj and Wenstrup, 2005, obtained by adding high frequency latency and best delay of each neuron). There is a broad distribution of excitatory latencies in response to low frequency signals among facilitated combination-sensitive neurons. **B.** Latencies of CN units in this study. Even with axonal conduction and synaptic delays, these latencies are too short to directly generate delayed low frequency excitation in facilitated combination-sensitive IC units. **F.** Latencies of units in lateral lemniscal nuclei (data from Portfors and Wenstrup, 2001).

Table 1

Temporal response patterns in CN subdivisions

Response Pattern	AV n(%)	M n(%)	PV n(%)	DCN n(%)	Total
All Response Patterns	84	27	108	57	276
All Primary-Like Responses	64(77)	19(70)	74(69)	30(53)	187(68)
Primary Like (PL)	44(52)	12(44)	38(35)	18(32)	112(41)
Primary Like with Notch (PLN)	8(11)	2(7)	5(5)	6(11)	21(8)
Primary Like Sustained (PLS)	12(14)	5(19)	31(29)	6(11)	54(20)
Chopper	12(14)	1(4)	24(22)	17(30)	54(20)
Onset	7(8)	6(22)	9(8)	1(2)	23(8)
Pauser (P), Buildup (B), or P/B	1(1)	1(4)	1(1)	9(16)	12(4)

Table 2

Spontaneous discharge in CN units

CN SUBDIVISION	AV	M	PV	DCN	Total
Sample	57	20	77	44	19
Average rate $\pm$ S.D. (spikes/s)	37 ± 52	21 ± 37	31 ± 34	36 ± 36	33 ± 41
% Low SR (< 2.5 spikes/s)*	30	50	13	16	22
BEST FREQUENCY (kHz)	9–22	23–30	57–60	Other ^I	Total
Sample	29	19	57	93	198
Average rate $\pm$ S.D. (spikes/s)	41 ± 34	18 ± 49	32 ± 37	33 ± 42	
% Low SR (< 2.5 spikes/s)**	3	53	19	24	
TEMPORAL PATTERN	Prim-Like	Chopper	Onset	Pause/Build	Total
Sample	122	38	14	12	186
Average rate $\pm$ S.D. (spikes/s)***	39 ± 46	24 ± 25	11 ± 21	24 ± 25	
% Low SR (< 2.5 spikes/s)	22	11	43	25	

^I 31–56 kHz, 61–110 kHz

* χ^2 test, df = 3, $p < 0.005$

** χ^2 test, df = 3, $p < 0.001$

*** ANOVA, ($f[3,182] = 3.12$, $p < 0.05$). Primary-like vs Onset: t-test, df = 29, $p < 0.001$