Auditory Responses in the Cochlear Nucleus of Awake Mustached Bats: Precursors to Spectral Integration in the Auditory Midbrain

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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: Irvine 1992; Rhode and Greenberg 1992). Likewise, several studies have described how the spectral analyses performed by dorsal co-

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cortical analyses of complex sounds (Brosch et al. 1999; Kadia and Wang 2003). In the mustached bat, however, facilitated combination sensitivity appears to originate in IC because few such interactions are found in nuclei of the lateral lemniscus (NLL) (Portfors and Wenstrup 2001) and because glycine receptor blockade in IC eliminates the facilitation (Nataraj and Wenstrup 2005; Wenstrup and Leroy 2001). 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 (Mittmann and Wenstrup 1995; O’Neill 1985; 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 et al. 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; Nataraj and Wenstrup 2005; Portfors and Wenstrup 1999). In the mustached bat, these inhibitory interactions are common in the IC but may be formed below the IC because they have been reported in the NLL (Portfors and Wenstrup 2001) and because blockade of glycine and GABA<sub>A</sub> receptors in the IC usually fails to eliminate the inhibitory interaction (Nataraj and Wenstrup 2005; K. Nataraj and J. J. Wenstrup, unpublished data). 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 and Wenstrup 2002; Marsh et al. 2001).

**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 (1 mg/kg ip; Nembutal, Abbot Laboratories, North Chicago, IL) and acepromazine (2 mg/kg ip; Med-Tech, 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 ≥2 days prior to physiological recording.

**Acoustic stimulation and recording**

Acoustic stimulation and data acquisition were computer-controlled. Acoustic signals (11 to 61 ms total duration, 0.5-ms rise/fall times, 2–4 kHz) 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 (Bruel & Kjaer, model 4135). There was a smooth decrease of ~2.7 dB/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 stereotactic holder housed in a heated and humidified sound-proof 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 4 h. 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 (multitunits) was recorded using micropipette electrodes (tip diameters: 1–3 μm, resistances of 8–30 MΩ) 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). Dorsoventral 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 3,700–4,200 μm below the dorsal cerebellar surface. Extracellular action potentials were amplified and filtered (band-pass, 500–6,000 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 1-μs resolution. Peristimulus time histograms (PSTHs), interspike interval histograms (ISIHs), 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 (equivalent to characteristic frequency in the non-bat literature). 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 or near separate 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 nonmonotonic 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 (Leroy and Wenstrup 2000; Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999, 2002), and medial geniculate body (Wenstrup 1999). However, sound duration, typically 4 ms in previous studies, was changed to 11 ms 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- to 10-dB steps from below MT to a maximum of 80–100 dB SPL. A curve was considered to be nonmonotonic 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 two-tone 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. Because 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 (Leroy and Wenstrup 2000; Portfors and Wenstrup 1999), we examined spectral interactions that met several criteria: 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; the facilitatory or inhibitory interactions were tuned to frequencies separated by an octave or more from the BF; and 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, 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 killed (sodium pentobarbital, >75 mg/kg ip) and then perfused with saline followed by 4% paraformaldehyde fixative. Histological procedures followed those described previously (Marsh et al. 2002; Portfors and Wenstrup 2002; Wenstrup et al. 1999).

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). Grayscale images were imported into Photoshop (Adobe Systems), 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 (Kössl and Vater 1990a; Zook and Casseday 1982a). Each single unit was assigned to one of the CN subdivisions [anteroventral cochlear nucleus (AV), posteroventral cochlear nucleus (PV), dorsal cochlear nucleus (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 (Kössl and Vater 1990a,b; Zook and Casseday 1982b) 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 AV, PV, and DCN as in other species but also a specialized marginal zone (M) that is distinctive in mustached bats (Zook and Casseday 1982a). 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 (BF, temporal response patterns, and latency) 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- and multiunit 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 (Kössl and Vater 1990a; Ross et al. 1988; Zook and Leake 1989). Thus BFs generally increased from rostral to caudal in AV (Fig. 1, A and B). The marginal subdivision (M), mostly representing frequencies <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 (Fig. 1C and D). In DCN, we observed increasing BFs from caudal to rostral and from ventral to dorsal (compare Fig. 1E and F). Commonly, discontinuities in the frequency representation were apparent between divisions (Fig. 1C) or subdivisions (Fig. 1A) within a single penetration.

BFs 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. 2, C–F). AV demonstrated the most complete distribution of BFs, ranging from 9 to 100 kHz (Fig. 2C). However, few BFs were recorded between 30 and 47 kHz, frequencies typically associated with social but not sonar vocalizations. The largest peaks, as in the overall CN distribution, were at 57–62 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 to 93 kHz (Fig. 2E), but these frequencies were distributed differently than in AV. Almost half of BFs (48%) were in the 57- to 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 with 14% of the units in AV. The PV also demonstrated a smaller percentage of BFs <30 kHz: 7% compared with 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- to 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- to 62-kHz frequency range (15%), a finding in sharp contrast to PV. The range of BFs extended from 17 to 105 kHz, but few units were tuned to the 26- to 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, average threshold was elevated by ≥20 dB, whereas thresholds of units tuned to ≤20 kHz were generally ≥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 BFs 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 <20 kHz had good sensitivity, with thresholds <30 dB SPL.

Temporal response patterns of CN units in the mustached bat (Fig. 3) were categorized by PSTHs and ISIHs 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. PL sustained responses (PLS) show similar ISIHs (Fig. 3B) but lacked the precisely timed initial spike of the PL pattern. This response pattern occurred in 20% of units. The notched PL response pattern (PLN) 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). Build-up 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 build-up 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.

PL 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 PL subtypes varied as a function of division. For instance, PLs 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 build-up 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 BFs (Fig. 4). The major exception was in the 23- to 30-kHz band, which corresponds to the first sonar harmonic. In this band, the proportions of PL (48%) and onset (39%) patterns were similar. This distribution was particularly striking in comparison to units tuned to the 9- to 22-kHz band, in which onset patterns were scarce (3%). Onset responses tuned to 23–30 kHz were only observed in the AV or M subdivisions.

First-spike latencies among CN single units ranged from 1.7 to 11.6 ms and averaged 3.6 ± 1.1 (SD) ms (Fig. 5A). Most response latencies (90%) were between 2.1 and 5.0 ms. There were no significant differences in latency among divisions \[F(3,273) = 1.44, P = 0.05\]. However, there was a significant effect of frequency \[F(6,224) = 5.17, P < 0.0001; \text{Fig. 5B}\] . Units tuned in the range 58–59 kHz [mean: 4.2 ± 0.9 ms] had slightly but significantly longer latencies compared with six other frequency bands in the mustached bats audible range (multiple t-test with Bonferroni correction; \(P < 0.01\)). The longer latency of 58- to 59-kHz units is consistent with a pronounced cochlear resonance occurring in this range (Pollak et al. 1979; Suga et al. 1975). 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. Because 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 and 90 kHz, frequencies also associated with sharp cochlear tuning (Kössl 1992; Pollak et al. 1979), did not have significantly different latencies from the remainder of the sample (t-test with Bonferroni correction, \( P > 0.05 \)).

Spontaneous rate among CN neurons ranged from 0 to 214 spikes/s (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 with 22% for the entire population. The proportion of low SR units was also significantly different across frequency bands; the 10- to 22-kHz band had substantially fewer low SR units (3%), whereas the 23- to 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 Fig. 6, which displayed an asymmetric excitatory frequency tuning curve tuned to 24.6 kHz and a PL temporal response pattern (Fig. 6A). There was no spontaneous discharge.

Shorter tone bursts (11 ms) were used in tests shown in Fig. 6, B and C. 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

![Fig. 5. Timing of initial spike among CN units expressed as median 1st spike latency. A: median 1st spike latency, obtained at each unit’s BF, 20–30 dB above MT. Latencies extended from 1.7 to 11.9 ms with most occurring between 2 and 5 ms. B: latencies displayed as a function of frequency. Latencies of units tuned near 60 kHz were significantly longer (see text).](http://jn.physiology.org/)

**TABLE 2. Spontaneous discharge in CN units**

<table>
<thead>
<tr>
<th>CN Subdivision</th>
<th>AV</th>
<th>M</th>
<th>PV</th>
<th>DCN</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Best frequency, kHz; Temporal pattern</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sample</td>
<td>57</td>
<td>20</td>
<td>77</td>
<td>44</td>
<td>198</td>
</tr>
<tr>
<td>Average rate ± SD, spikes/s</td>
<td>37 ± 52</td>
<td>21 ± 37</td>
<td>31 ± 34</td>
<td>36 ± 36</td>
<td>33 ± 41</td>
</tr>
<tr>
<td>% Low SR, &lt;2.5 spikes/s*</td>
<td>30</td>
<td>50</td>
<td>13</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>Best Frequency, kHz</td>
<td>9–22</td>
<td>23–30</td>
<td>57–60</td>
<td>31–56, 61–110</td>
<td>Total</td>
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<tr>
<td>Sample</td>
<td>29</td>
<td>19</td>
<td>57</td>
<td>93</td>
<td>198</td>
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<tr>
<td>Average rate ± SD, spikes/s</td>
<td>41 ± 34</td>
<td>18 ± 49</td>
<td>32 ± 37</td>
<td>33 ± 42</td>
<td></td>
</tr>
<tr>
<td>% Low SR, &lt;2.5 spikes/s†</td>
<td>3</td>
<td>53</td>
<td>19</td>
<td>24</td>
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<tr>
<td>Temporal Pattern</td>
<td>Prim-Like</td>
<td>Chopper</td>
<td>Onset</td>
<td>Pause/Build</td>
<td>Total</td>
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<td>Sample</td>
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<td>38</td>
<td>14</td>
<td>12</td>
<td>186</td>
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<td>Average rate ± SD, spikes/s‡</td>
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<td>24 ± 25</td>
<td>11 ± 21</td>
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<tr>
<td>% Low SR, &lt;2.5 spikes/s</td>
<td>22</td>
<td>11</td>
<td>43</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

*, \( \chi^2 \) test, df = 3, \( P < 0.005 \); †, \( \chi^2 \) test, df = 3, \( P < 0.001 \); ‡ANOVA, \( f(3,182) = 3.12, P < 0.05 \), Primary-like vs. onset: t-test, df = 29, \( P < 0.001 \).
which the second, simultaneous tone was varied from 30 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 (Palmer and Winter 1996; Rhode and Greenberg 1994; Spirou et al. 1999). Two such units are shown in Fig. 7. The DCN unit in Fig. 7, A–C, displayed a single frequency tuning curve with a BF of 96.8 kHz (Fig. 7A) and a PL1 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 of a second tone at a range of frequencies (Fig. 7C) suggested that broadly tuned suppression may occur in the 10- to 30-kHz band, and revealed clear suppression in the 75- to 85-kHz range. The unit in Fig. 7, D–F, from 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 below the BF (11–25 kHz) and signs of an inhibitory input tuned to 46–47 kHz (Fig. 7F). Because the inhibition was within an octave of the BF and because 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 with all other frequency bands. Units tuned to the 10–22 kHz band displayed the broadest tuning (mean Q10dB, 2.1). Their tuning sharpness 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 Fig. 9 had two distinct frequency tuning curves that remained separate at the highest sound levels tested (Fig. 9A, □). The insensitivity just below the sharply tuned ~60 kHz BF is consistent with a region of cochlear insensitivity (Kössl and Vater 1990b; Pollak et al. 1979; Russell and Kössl 1999; Suga et al. 1975). Nearly every response feature differed in the two excitatory regions (Fig. 9, A–D). Thus the higher frequency response had much sharper tuning (Q10dB of 114.5 vs. 0.6) and a lower threshold (44 vs. 64 dB SPL). When the sound level was set to 20 dB above their respective thresholds, the higher and lower BFs evoked different temporal response patterns (chopper vs. pauser pattern), different rates (5.1 vs. 3.6 ms), and different discharge rates. Two-tone interactions were investigated with a second tone varied from 10 to 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, because 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 Fig. 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 (Fig. 10, A and D). Among the sample of multi-peaked units, the lower BF ranged from 12.4 to 41.2 kHz, whereas the higher BF varied between 30.5 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 with 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 <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 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- to 30-kHz suppressive sideband and the excitatory tuning curve at the highest intensities tested. This low frequency inhibition was time-sensitive (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 Fig. 11, the frequency- and time-dependent inhibition in the 15- to 23-kHz band satisfied the criteria we used for combinatorial-sensitive 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 BF 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 primary-like and monotonic, having a first-spike latency of 3.4 ms at 30 dB above threshold (Fig. 12).
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 nonmonotonic 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 ≤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 Fig. 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 BFs 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

![Figure 8](image.png)

**FIG. 8.** Frequency tuning properties of singly tuned units. A: frequency tuning curves. B: Q10 dB values across frequency and CN division.

![Figure 9](image.png)

**FIG. 9.** Responses of PV single unit with multi-peaked tuning. A: frequency tuning curves revealed sensitivity peaks at 58.42 and 18.9 kHz for the high- and low-frequency signals. Top line: 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. Q, the separation of low- and high-frequency tuning curves. X, frequency and sound level combinations for PSTHs. B, top: chopper pattern in response to BF signal, 20 dB above minimum threshold (MT). Bottom: 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- 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 2nd 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 and 43 kHz, but possible side band inhibition at frequencies <16 kHz and >43 kHz.
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 (Leroy and Wenstrup 2000; Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999, 2002).

**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 BF 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, whereas less than one-sixth of DCN units are tuned to the same band. In M, nearly all units are tuned <40 kHz. We observed many neurons in M tuned in the 10- to 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 and Jen 1977; Suga et
al. 1975).

The substantial representation <30 kHz includes two pop-
ulations distinguished by multiple features. Neurons tuned in
the 23- to 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; Port-
for and Wenstrup 1999), medial geniculate body (Olsen and
Suga 1991a,b; Wenstrup 1999), and auditory cortex (Fitz-
patrick et al. 1993; Suga et al. 1978, 1983). Although neurons
responsive to this band may also analyze other signals includ-
ing social vocalizations, their special features seem particularly
well suited for analyses of sonar pulse-echo combinations.

In contrast, neurons tuned <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) thresh-
holds do not change in a similar way, (Kössl and Vater 1990b;
Pollak et al. 1979; Suga et al. 1975), probably due to the
low-frequency sensitivity of high-frequency hair cells. How-
ever, 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 1994;

Neural tuning to frequencies below the mustached bat’s first
sonar harmonic, especially <20 kHz, has been reported infre-
quently in the mustached bat (Fitzpatrick et al. 1998; Hattori
and Suga 1997; Wenstrup 1999). 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 because most social vocalizations are produced and
received at high sound levels (Kanwal et al. 1994, 1999). Thus
neurons tuned to the two bands <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 stud-
ies 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; Shofner and
Young 1985; Young and Brownell 1976).
FEATURES OF EXCITATORY TUNING CURVES. Among 64 well-studied CN single units, 55% displayed an excitatory frequency tuning curve with a single sensitivity peak. Although other forms of spectral interactions were observed in some of these neurons, such as sideband and broadband inhibition (Palmer and Winter 1996; Rhode and Greenberg 1994; Shofner and Young 1985; Spirou et al. 1999), there was no evidence of spectral interactions that characterize many 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- to 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, more than half of CN neurons in this study do not display a second excitatory peak. Although 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- 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

FIG. 12. Low-frequency inhibitory tones also evoke excitation in unit recorded from M. A: response to 30.5-kHz BF tone was PL (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 1st-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 short-latency low-frequency inhibition and longer latency inhibition by the BF tone. →, the response of the unit to each tone presented separately. PSTHs and response functions based on 50 stimulus repetitions in A and B and 20 repetitions in C and 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 < 23 kHz.
nucleus (VCN) (reviews: Rhode and Greenberg 1992; Ruggiero 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 (Kössl and Vater 1985, 1990b; Pollak et al. 1979; Russell and Kössl 1999; Suga et al. 1975). For units with BF at other frequencies or for broader insensitivity regions, however, the insensitivity is not easily explained by cochlear mechanisms (e.g., Fig. 10, A and 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- to 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 (Javel 1994; Kiang and Moxon 1974). 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- to 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 byproduct of the multiple mechanical specializations that create very sharp tuning near 60 and 90 kHz (Hensen 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. Whereas 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- to 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; Shemykholeslami et al. 2004), as Kiang and Moxon (1974) had shown in cats. The second feature is that there is less excitation evoked by 23- to 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- to 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- to 30-kHz range. The limited responsiveness of high-BF neurons to 23- to 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 in the preceding text, low-frequency responsiveness tuned <23 kHz is common among high BF units in IC, particularly among units tuned to 60 kHz. These response properties may arise directly from the CN or via numerous intermediate nuclei of the auditory brain stem.

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 subspecies 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 (Mittmann and Wenstrup 1995; Olsen and Suga 1991a,b; 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 ~25% of neurons show facilitated combination sensitivity (Nataraj and Wenstrup 2005; Portfors and Wenstrup 2001; Wenstrup and Leroy 2001).

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- to 30-kHz range, corresponding to the first sonar harmonic. These account for ~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; K. Nataraj...
and J. J. Wenstrup, unpublished results). In these units, suppression is tuned <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 (Gans et al. 2004; K. Nataraj and J. J. Wenstrup, unpublished data). The presence of similar units within CN suggests an origin in either CN or the cochlea.

Suppression of BF responses by a simultaneous low-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. PL responses formed the dominant pattern in each CN division, chopper patterns were the second most common (except in M), and pauser/build-up 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 PL patterns form the majority in both VCN and DCN (Feng and Vater 1985).

The predominance of PL 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 for less than one-fourth of units, while choppers form ~50% (Parham et al. 1998; Shofer and Young 1985; Young et al. 1988). In DCN, pauser, build-up, onset, and chopper temporal patterns are much more common than PL patterns in cat (Rhode and Smith 1986a; Shofer and Young 1985), 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 and Casseday 1982a) may explain the smaller proportion of chopper patterns in VCN because 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 (Kemmer and Vater 1997; Zook and Casseday 1982a).

First-spike latencies throughout the mustached bat’s CN were between 2 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 (Ghoshal and Kim 1997; Rhode and Smith 1986b; Young et al. 1988). Latencies in the mustached bat’s DCN ranged from 2 to 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- to 8-ms range (Joris and Smith 1998; Rhode and Smith 1986a; Rhode et al. 1983b). The shorter latency values in the mustached bat DCN may relate to the larger proportion of PL 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 <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- to 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 noradrenaline 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 (Olsen and Suga 1991b; O’Neill and Suga 1982; 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 (Sheykholeslami 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 (Nataraj and Wenstrup 2005; Wenstrup and Leroi 2001). 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- to 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 ≤30 ms after a lower-frequency signal, (e.g., in the 1st harmonic of the emitted sonar signal). For this to occur, the facilitatory effect of the 23- to 30-kHz signal must be delayed ≤30 ms within the brain. Because 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 with the low-frequency excitation latencies needed in facilitated, delay-
A combination of low-frequency excitation and inhibition facilitates combination-sensitive responses in the IC. 

**FIG. 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. C: latencies of units in lateral lemniscal nuclei (data from Portfors and Wenstrup 2001).

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. Although the temporal and spectral features that characterize these neurons may differ from the mustached bat’s similar multi-level, spectrot temporal integrative processes are likely to occur in other species. Moreover, although 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, brain stem, and midbrain.

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**FIG. 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. C: latencies of units in lateral lemniscal nuclei (data from Portfors and Wenstrup 2001).

**Spectrot 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


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