Frequency Organization and Responses to Complex Sounds in the Medial Geniculate Body of the Mustached Bat

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Wenstrup, Jeffrey J. Frequency organization and responses to complex sounds in the medial geniculate body of the mustached bat. J. Neurophysiol. 82: 2528–2544, 1999. The auditory cortex of the mustached bat (Pteronotus parnellii) displays some of the most highly developed physiological and organizational features described in mammalian auditory cortex. This study examines response properties and organization in the medial geniculate body (MGB) that may contribute to these features of auditory cortex. About 25% of 427 auditory responses had simple frequency tuning with single excitatory tuning curves. The remainder displayed more complex frequency tuning using two-tone or noise stimuli. Most of these were combination-sensitive, responsive to combinations of different frequency bands within sonar or social vocalizations. They included FM-FM neurons, responsive to different harmonic elements of the frequency modulated (FM) sweep in the sonar signal, and H1-CF neurons, responsive to combinations of the bat’s first sonar harmonic (H1) and a higher harmonic of the constant frequency (CF) sonar signal. Most combination-sensitive neurons (86%) showed facilitatory interactions. Neurons tuned to frequencies outside the biosonar range also displayed combination-sensitive responses, perhaps related to analyses of social vocalizations. Complex spectral responses were distributed throughout dorsal and ventral divisions of the MGB, forming a major feature of this bat’s analysis of complex sounds. The auditory sector of the thalamic reticular nucleus also was dominated by complex spectral responses to sounds. The ventral division was organized tonotopically, based on best frequencies of singly tuned neurons and higher best frequencies of combination-sensitive neurons. Best frequencies were lowest ventrolaterally, increasing dorsally and then ventromedially. However, representations of frequencies associated with higher harmonics of the FM sonar signal were reduced greatly. Frequency organization in the dorsal division was not tonotopic; within the middle one-third of MGB, combination-sensitive responses to second and third harmonic CF sonar signals (60–63 and 90–94 kHz) occurred in adjacent regions. In the rostral one-third, combination-sensitive responses to second, third, and fourth harmonic FM frequency bands predominated. These FM-FM neurons, thought to be selective for delay between an emitted pulse and echo, showed some organization of delay selectivity. The organization of frequency sensitivity in the MGB suggests a major rewiring of the output of the central nucleus of the inferior colliculus, by which collicular neurons tuned to the bat’s FM sonar signals mostly project to the dorsal, not the ventral, division. Because physiological differences between collicular and MGB neurons are minor, a major role of the tecto-thalamic projection in the mustached bat may be the reorganization of responses to provide for cortical representations of sonar target features.

The auditory cortex of the mustached bat (Pteronotus parnellii) displays some of the most highly developed features described in mammalian auditory cortex. Many of these features are based on a fundamental neuronal response property called combination sensitivity, characterized by neural integration of inputs from distinct frequency bands in the bat’s audible range (Suga et al. 1978, 1983). The neural comparisons facilitate the analysis of spectrally and temporally complex vocalizations. Although these properties occur in the forebrains of many vertebrates, from frogs to birds to primates (Fuzessery and Feng 1983; Margoliash and Fortune 1992; Rauschecker 1997), their physiological and organizational features have been described best in the mustached bat (Fitzpatrick et al. 1998b; Ohlemiller et al. 1996; O’Neill and Suga 1982; Suga et al. 1983). The focus of this paper is on physiological and organizational properties in the medial geniculate body (MGB) that may contribute to this specialized auditory cortex.

The mustached bat displays two highly developed acoustic behaviors—biosonar and social communication—that may rely on specialized, combination-sensitive responses of auditory cortical neurons. In sonar behavior, the mustached bat is believed to use combination-sensitive responses to its multimodal sonar call (Fig. 1) to extract information about a target’s distance and movement. The mapping of sonar-related response properties across subregions of auditory cortex represents one of the most extensive studies of behaviorally relevant functional organization in mammalian auditory cortex (Fitzpatrick et al. 1998b; O’Neill and Suga 1982; Suga and O’Neill 1979; Suga et al. 1983). The mustached bat’s highly developed acoustic communication (Kanwal et al. 1994) also uses combination-sensitive responses. Neurons in auditory cortex appear to respond selectively to certain combinations within or between syllables (Esser et al. 1997; Ohlemiller et al. 1996). The types and organization of such combination-sensitive responses provides insight to the species-specific processing of complex sounds in auditory cortex.

Because distinct frequency bands used in sonar or social communication signals may provide different information to a bat, the neural analyses and representations of these frequency bands may likewise differ. One example concerns frequency bands associated with the mustached bat’s analysis of the distance of objects using sonar. These bands correspond to the harmonics of the frequency modulated (FM) downswEEP in the bat’s sonar signal (Fig. 1). In auditory cortex, neurons sensitive to combinations of FM signals display physiological properties and organization that are not shared by neurons tuned to the constant frequency (CF) component in sonar signals or by neurons tuned to frequencies outside the sonar bands (Fitzpatrick et al. 1999b; Suga et al. 1983). One physiological property of the neurons responding to FM bands is delay
tuning, which can be related to the bat’s analysis of target distance in echolocation (O’Neill and Suga 1982; Suga and O’Neill 1979). Neurons tuned to the FM frequency bands are virtually absent from the main tonotopic axis of primary auditory cortex but are instead located in other cortical areas and appear to be organized according to delay tuning (Fitzpatrick et al. 1998b; O’Neill and Suga 1982; Suga and Horikawa 1986). Although these neurons also may analyze social communication signals or other sounds, their responses and organization appear strongly related to the analysis of sonar echoes. Thus a key to understanding auditory cortical or thalamic organization is an understanding of how information within specific frequency bands is analyzed and represented. This study focuses on the representation and physiological properties in the auditory thalamus of nine frequency bands defined by their inclusion in sonar and social vocalizations (Fig. 1).

Combination-sensitive responses require temporally sensitive neural integration of inputs having distinct frequency receptive fields, in much the same way that visual motion-selective cells require temporally sensitive integration of inputs with distinct spatial receptive fields (Albright and Stoner 1995; Newsome and Salzman 1993). The site(s) and features of this integration in the auditory system are not certain, but they clearly occur at levels of the ascending auditory pathway below the MGB (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999b; Wenstrup and Grose 1995). Two different tone or noise burst stimuli (3–30 ms duration, 1- or 0.5-ms rise-fall times, 3–4/s) were separately generated, switched, and attenuated. The digitally-generated sinusoids from the signal generators were accurate to 1 Hz. Signals from the two channels were added, amplified, and sent to a speaker placed 10 cm (30 cm in earlier experiments) away from the bat and 25° into the sound field contralateral to the recording electrode. The acoustic properties of the entire system were tested using a calibrated microphone placed in the position normally occupied by the bat’s head. There was a smooth, gradual decrease in the sound pressure from 10 to 120 kHz of ~2.7 dB per 10 kHz. Distortion components in the speaker output were ~60 dB below the sound level of the signal, as measured by a fast Fourier analysis of the digitized microphone signal (1-MHz sampling rate).

Beginning 1–2 days after surgery, physiological recordings were obtained from awake animals placed in a plexiglas restraining apparatus in a heated and humidified experimental chamber. Between electrode penetrations, the bat was offered water from a medicine dropper. Recording sessions generally lasted 4–6 h. The number of sessions for any bat ranged from one to seven.

Acoustic stimulation and recording

Acoustic responses and their topographic distribution were examined in the auditory thalamus of 21 greater mustached bats (Pteronotus parnellii) captured in Jamaica, West Indies. Anatomic data from some experiments were reported elsewhere (Wenstrup and Grose 1995). All animal procedures were approved by the Institutional Animal Care and Use Committee.

Surgical procedures

The dorsal surface of the cerebral cortex was exposed in bats anesthetized with methoxyflurane (Metofane, Pitman-Moore, Inc., Mundelein, IL) in combination with pentobarbital sodium (5 mg/kg ip; Nembutal, Abbott Laboratories, North Chicago, IL) and acepromazine (2 mg/kg ip; Med-Tech, Buffalo, NY). A midline incision was made in the skin overlying the skull, and the muscles were reflected laterally. A tungsten ground electrode was cemented into the right cerebellar cortex, and a small hole (usually <0.5 mm) was placed in the skull over the cerebral cortex. A metal pin, cemented to the skull and secured to a restraining apparatus, maintained the head in a uniform position during physiological experiments. After application of a local anesthetic (lidocaine, Elkins-Sinn, Cherry Hill, NJ) and a topical antibiotic, the animal was placed in a holding cage to recover from the surgery.

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Acoustic stimulation and data acquisition equipment have been described in detail elsewhere (Portfors and Wenstrup 1999b; Wenstrup and Grose 1995). Two different tone or noise burst stimuli (3–30 ms duration, 1- or 0.5-ms rise-fall times, 3–4/s) were separately generated, switched, and attenuated. The digitally-generated sinusoids from the signal generators were accurate to 1 Hz. Signals from the two channels were added, amplified, and sent to a speaker placed 10 cm (30 cm in earlier experiments) away from the bat and 25° into the sound field contralateral to the recording electrode. The acoustic properties of the entire system were tested using a calibrated microphone placed in the position normally occupied by the bat’s head. There was a smooth, gradual decrease in the sound pressure from 10 to 120 kHz of ~2.7 dB per 10 kHz. Distortion components in the speaker output were ~60 dB below the sound level of the signal, as measured by a fast Fourier analysis of the digitized microphone signal (1-MHz sampling rate).

The evoked activity of single unit and multiunit responses was recorded with micropipettes having tip diameters of 5–10 μm (resistances of 1–15 MΩ) and filled with a tracer to mark recording sites (described later). Because tracers in most experiments were dissolved in 0.9% saline, electrode resistance usually exceeded 10 MΩ. Electrodes were advanced by a hydraulic micropositioner. Extracellular action potentials were amplified, filtered (band-pass, 500–6000 Hz), and sent through a window discriminator. The pulse output of the window discriminator was digitized at 10 kHz. Peristimulus time (PST) histograms, raster displays, and statistics on the neural responses were generated by the computer. Multiunit responses that were analyzed consisted of stimulus-locked clusters of clearly defined spikes; the threshold of the window discriminator was adjusted to detect spike activity exceeding the baseline level. The window discriminator output also was displayed audiovisually.

Using a stereotaxic procedure as a guide, electrodes were placed to record neural activity within the MGB or adjacent areas. Multiunit
responses usually were sampled at 100- to 150-μm intervals; single units were examined whenever they could be isolated. Response properties were evaluated using tone or noise bursts. The best frequency (requiring the lowest intensity to elicit stimulus-locked spikes) and threshold at best frequency (the lowest intensity required to elicit ≥1 spike to each of 5 consecutive stimuli) were measured. In most cases, the Q10dB measure of tuning sharpness was obtained (best frequency divided by the bandwidth 10 dB above threshold) because very sharp tuning distinguishes MGB neurons analyzing some components of the biosonar signal (Olsen and Suga 1991a). Although frequency tuning was measured to the nearest 0.1 or 0.01 kHz, Figs. 7–9 express tuning to the nearest kilohertz to save space. At some recording sites, there was more than one peak in the excitatory tuning curve. The threshold, best frequency, and usually the Q10dB value were obtained for each excitatory tuning curve. Tone bursts were used because nearly all sonar-related combination-sensitive MGB neurons are reported to respond to these (Olsen and Suga 1991a,b). Band-pass noise was used because some neurons tuned to frequency bands outside the sonar range were found to respond preferentially to such stimuli.

Using a two-tone stimulus paradigm, neurons then were tested for sensitivity to combinations of tones or noise bands across the bat’s audible range, including frequency bands used in sonar and communication (Fig. 1). If a facilitatory or inhibitory interaction was obtained, the frequencies, intensities, and timing of the two signals were adjusted to obtain a strong combination-sensitive interaction. Neurons were considered to be combination-sensitive if, for clearly distinct frequency bands, the response to the two signals presented together was 20% more (for facilitation) or 20% less (for inhibition) than the sum of responses to the signals presented separately. The degree of facilitation or inhibition was quantified as the index of interaction (I), according to the following formula: $I = (R_h - R_l - R_o)/(R_h + R_l + R_o)$ where $R_h$, $R_l$, and $R_o$ are, respectively, the neuron’s responses to the combination of the low- and high-frequency signals, low-frequency signal alone, and high-frequency signal alone. An interaction index value of 0.09 corresponds to 20% facilitation. The neurons’ responses to the combination of the low- and high-frequency signals, low-frequency signal alone, and high-frequency signal alone. An interaction index value of 0.09 corresponds to 20% facilitation, the criterion for combination-sensitive facilitation. Negative numbers indicate combination-sensitive inhibition in which the excitatory response to one signal is suppressed by the other signal. Interaction index values of 1 and −1 indication maximum facilitation and inhibition, respectively. If a combination-sensitive response was observed, additional measures of frequency- and/or delay-dependent facilitation (or inhibition) often were obtained.

One or two tracer deposits were used to mark electrode penetrations. At the end of a penetration, response properties again were characterized at tracer deposit sites. Iontophoretic deposits were made using a constant current source (Midgard model CS4).

Tracer and histological techniques

Tracers included wheat germ agglutinin conjugated to horseradish peroxidase (Sigma Chemical, St. Louis, MO), biocytin (Sigma Chemical), cholera toxin B-subunit (List Biologicals, Campbell, CA), Fluoro-Gold (Fluorochrome, Englewood CO), or dextran-conjugated rhodamine and fluorescein (Molecular Probes, Eugene, OR). Techniques used to deposit and visualize the nonfluorescent tracers have been described in detail elsewhere (Wenstrup and Grose 1995). Electrodes containing fluorescent markers were filled with: 10% dextran-conjugated rhodamine or fluorescein in 0.9% NaCl, or 1% Fluoro-Gold in 0.1 M acetate buffer. Iontophoretic deposits used 5.0 μA (10 min, 7 s on/7 s off) for the dextran conjugates (positive current for rhodamine, negative current for fluorescein) and +1.0 μA (5 min, 7 s on/7 s off) for Fluoro-Gold. Fluorescent labeling was viewed with an Olympus BH-2 microscope and appropriate filter combinations.

After the last recording session, the animal was anesthetized deeply with Nembutal (>60 mg/kg ip). Once nociceptive reflexes were eliminated, the chest cavity was opened, and phosphate buffered saline (pH 7.4) and an aldehyde fixative were perfused through the heart. The fixed brain was blocked in the plane of the electrode penetrations, inclined ~15° from dorsal and caudal to ventral and rostral, consistent with previous anatomic and physiological studies of the MGB (Wenstrup and Grose 1995; Wenstrup et al. 1994a). Each brain was refrigerated overnight in a 30% sucrose-phosphate buffer solution before sectioning. Brains were sectioned transversely on a freezing microtome at 30- to 40-μm thickness, then collected into cold 0.1 M phosphate buffer or phosphate-buffered saline. Every third section was processed by a different protocol; one of the three series was stained with cresyl violet. Cytoarchitectonic boundaries were drawn based on previous descriptions (Wenstrup 1995; Winer and Wenstrup 1994a,b).

To test qualitative observations regarding topographic organization of a response property (delay sensitivity), a nondirectional Pearson correlation analysis examined the relationship between best delay and recording site. Each recording site was plotted within the coordinates of the MGB in that animal. Caudorostral location was expressed relative to the caudal-to-rostral distance in that MGB, mediolateral location was expressed as the fraction of the maximal midline-to-lateral-edge distance of the MGB for that animal. Dorsoventral position was expressed relative to the ventralmost extension of the MGB, a reliable measure of dorsoventral position.

RESULTS

This study is based on 551 physiological recording sites in the MGB and surrounding regions. Sensitivity to complex sounds was examined at 427 acoustically responsive recording sites. Because a major goal was to map the distribution of basic response properties, both single-unit and multiunit responses were included. Combination-sensitive response properties in the MGB are clearly observable in multiunit recordings (Wenstrup and Grose 1995). About 70% of the responses were multiunits, and similar percentages were obtained for most subgroups of the population (Table 1). This finding supports the reliability of multiunits for documenting most aspects of the complex responses examined here.

About one-quarter of the responses (112 of 427) had simple frequency tuning, with a single excitatory tuning curve (Table 1). These responses were tested for sensitivity to tone bursts outside their tuning curves, in which the frequency, amplitude, and relative timing of the second sound were varied. Additional tests were conducted with band-pass noise bursts. Although many of these responses could be inhibited by sounds on the flanks of the tuning curve or by lower frequency sounds at high sound levels, they were unaffected (neither facilitated or inhibited) by low-to-medium level sounds presented well outside their excitatory tuning curves.

<table>
<thead>
<tr>
<th>Spectral Response Properties</th>
<th>Single Units</th>
<th>Multiunits</th>
<th>Totals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total acoustic responses*</td>
<td>121</td>
<td>306</td>
<td>427 (100)</td>
</tr>
<tr>
<td>Single tuning curve</td>
<td>31</td>
<td>81</td>
<td>112 (26.2)</td>
</tr>
<tr>
<td>Sonar combination-sensitive:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>facilitated</td>
<td>63</td>
<td>159</td>
<td>222 (52.0)</td>
</tr>
<tr>
<td>Sonar combination-sensitive:</td>
<td>15</td>
<td>18</td>
<td>33 (7.7)</td>
</tr>
<tr>
<td>Other complex frequency tuning</td>
<td>12</td>
<td>48</td>
<td>60 (14.1)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. *Values only include neurons that were tested for combination sensitivity or multiple tuning.
Neurons with complex frequency tuning

The majority of auditory recording sites displayed complex responses to sound frequency (Table 1). These include multiple tuning, better sensitivity to noise than to tones, and combination-sensitive facilitation or inhibition as defined in the METHODS. Facilitatory combination-sensitive responses were the most common, comprising more than half of auditory responses tested for complex spectral sensitivity. Inhibitory combination-sensitive interactions, in which the excitatory response to a higher frequency signal was suppressed by a lower frequency signal, were uncommon in the auditory thalamus (10% of auditory responses). As a group, the responses that showed multiple tuning or combination-sensitive inhibition responded well to single tone bursts within at least one frequency band. Among recording sites showing better sensitivity to noise or combination-sensitive facilitation, however, the responses to single tone bursts were weaker and more variable, never eliciting the maximum discharge.

Three major categories of combination-sensitive facilitation displayed different functional properties and topographic distribution in the MGB. The three categories are based partly on the frequency tuning of the responses. In two categories, responses were to frequency ranges used by specific components of the mustached bat’s sonar signal (Fig. 1). In the third category, responses were to signals outside of the sonar frequency range but within the range of acoustic signals used in social communication (Kanwal et al. 1994) (Fig. 1). This section describes distinguishing response properties of these categories, including population measures of combination-sensitive interactions that have been compared with similar data from neurons in the inferior colliculus (Portfors and Wenstrup 1999b).

FM-FM RESPONSES. These comprised 34% (n = 145) of all auditory responses. FM-FM neurons display temporally sensitive facilitation (or inhibition) that occurs when signals in two frequency bands are combined. One frequency band is associated with the first harmonic, FM sweep in the bat’s sonar signal (FM1, 24–29 kHz), and the other associated with higher FM sweep harmonics (FM2, 48–59 kHz; FM3, 72–89 kHz; FM4, 96–119 kHz; Fig. 1). Figure 2A shows a facilitated FM-FM single unit, which responded weakly to a best frequency tone burst (74.8 kHz) or a lower frequency tone burst (27.6 kHz). It responded very strongly to combinations of the tone bursts but only when the higher frequency tone was delayed by 3–6 ms (Fig. 2A, delay tuning). The range of frequencies that evoked the facilitating effect (Fig. 2A, frequency tuning) corresponds to the FM1 and FM3 components of the bat’s sonar signal. Tuning to the lower frequency signal among FM-FM responses ranged from 24.1–29.1 kHz, with 80% in the range 25.5–27.9 kHz (Fig. 3A). In no case did responses tuned to the
higher harmonic FM frequency ranges show facilitation (or inhibition) by lower frequency signals tuned outside the frequency range of the first FM harmonic. This suggests that FM-FM neurons represent a functionally distinct class of combination-sensitive response designed to analyze complex signals within the frequency ranges of the first and higher harmonic FM sonar signals.

It has been hypothesized that the low-frequency response is to the FM1 signal in the emitted pulse, whereas the higher frequency response is to an FM harmonic in the delayed echo. Consequently, the selectivity for delay of the higher harmonic FM signal after an FM1 signal would correspond to selectivity for pulse-echo delay (O’Neill and Suga 1979, 1982), the main cue used to estimate the distance of sonar targets. FM-FM responses in the MGB showed well-defined selectivity to the timing of the two facilitating signals, characterized by the “best delay,” the delay at which the facilitated response was greatest (4 ms in Fig. 2A). Best delays ranged from 1 to 24 ms, with 80% in the range 1–9 ms (Fig. 3B). This broad range of best delays distinguished the population of FM-FM responses from other combination-sensitive responses. Sharpness of tuning to delay was measured in 23 single units as the width of the delay curve at response rates that were 50% of maximum. Delay width varied from 1.8 to 14.5 ms. There was a significant correlation ($r = 0.52$, $P < 0.05$) between delay width and a neuron’s best delay (Fig. 4A). Neurons with shorter best delays ($\leq 5$ ms) generally had the narrowest delay tuning curves, but some short best-delay neurons had wide delay tuning curves and some longer best-delay neurons had relatively narrow delay tuning curves.

The single unit in Fig. 2A was extreme in its poor response to single tone bursts at the lower or higher frequency. Under the conditions used to test facilitated responses (3-ms tone burst, best facilitative frequencies, sound level 10–20 dB above the neuron’s threshold for facilitation), responses to single tones were generally weak. Among 23 FM-FM single units, the response to combination stimuli averaged 2.4 ± 1.5 (SD) spikes/stimulus (range 0.5–7.1 spikes/stimulus). The response to the higher frequency tone burst averaged 0.7 ± 0.7 spikes/stimulus (range 0–2.9 spikes/stimulus), with five units (23%) showing no response to the higher frequency stimulus (≤0.1 spikes/stimulus). Responses were usually smaller to the lower frequency signal, averaging 0.5 ± 0.5 spikes/stimulus (range 0–1.6 spikes/stimulus). Nine units (41%) did not respond to the lower frequency signal.

The single unit in Fig. 2A was correspondingly extreme in the degree of facilitation. The response to the combination stimulus at the best delay (4 ms) was 700% of the sum of the responses to the two components presented separately, yielding a facilitation index of 0.75. Other single units showed less facilitation between the two signals, whereas only a few showed a purely inhibitory influence of the FM1 signal (Fig. 4B). The average facilitation index for facilitated FM-FM single units was 0.40 ± 0.34, equivalent to 235% facilitation.
H1-CF RESPONSES. These responses (26% of acoustic responses) were sensitive to the combination of sounds in frequency ranges of the first sonar harmonic (H1, 24–31 kHz) and a higher harmonic of the CF sonar component in echoes (CF2, 60–63 kHz; CF3, 90–94 kHz). Figure 2B shows a facilitated H1-CF single unit. It responded weakly to a higher frequency tone burst (60.53 kHz) and even less to a lower frequency (26.2 kHz) tone burst (Fig. 2A, delay tuning). The neuron responded strongly to the combination of tone bursts when the higher frequency tone was present at times from 5 ms before to 5 ms after the lower frequency signal. The facilitating effect of the higher frequency signal had very sharp frequency tuning (Q10dB of 87), a characteristic of neurons tuned in the 60- to 63-kHz range throughout the mustached bat’s auditory system (Fig. 2B, frequency tuning). In the presence of the facilitating 60.53 kHz signal, the neuron was broadly tuned to the FM1 frequency range (Q10dB of 6).

The higher frequency tuning of most H1-CF responses (86%) was to frequencies in the second harmonic CF signal, 60–63 kHz. For the lower frequency response, 83 of 85 H1-CF neurons showed low-frequency facilitation or inhibition tuned between 25.0 and 31.1 kHz (Fig. 3C). Eighty percent were in the 25.6- to 29.2-kHz range. This distribution is broader than for FM-FM neurons. It is noteworthy that the best low facilitative frequency for many H1-CF neurons was in the 27.5- to 28.5-kHz range (Fig. 3C), which served in other studies to distinguish the FM1 and CF1 frequency bands (Fitzpatrick et al. 1993). Because it was difficult to use the first harmonic best facilitating frequency as a consistent criterion to distinguish between FM1-CF and CF1-CF neurons, I use a more general term, H1-CF. The distinction between FM1-CF and CF1-CF responses is nonetheless important and is addressed elsewhere concerning topographic organization.

H1-CF responses were sensitive to the timing of the two signals. Like the single unit in Fig. 2B, most facilitated H1-CF responses were best when the two signals were presented simultaneously, at 0-ms delay (Fig. 3D). In this respect they differed sharply from FM-FM responses (Fig. 3B). However, like FM-FM responses, there was considerable variation in the width of delay tuning curves (Fig. 4A). Because these responses were nearly all tuned to the same best delay, there was no correlation between best delay and width of delay curves.

Most H1-CF responses to single tone bursts were better to the higher frequency signal (15 of 18 single units). Under conditions used to test facilitated responses (30-ms tone burst, best facilitative frequencies, sound level 10–20 dB above the neuron’s threshold for facilitation), the responses of 18 single units to the higher frequency tone burst averaged 1.1 ± 0.8 spikes/stimulus (range 0–2.8 spikes/stimulus), with one unit showing no response to the higher frequency stimulus (≤0.1 spikes/stimulus). Responses to the lower frequency signal averaged 0.5 ± 0.5 spikes/stimulus (range 0–1.6 spikes/stimulus). Six units did not respond to the lower frequency signal. In contrast, the response to combination stimuli averaged 3.1 ± 1.6 spikes/stimulus (range 0.5–6.2 spikes/stimulus). For the unit in Fig. 2B, the facilitation index was 0.64. For 21 single units, the interaction index ranged from −0.65 to 0.76 (Fig. 4B). For 18 facilitation neurons, the mean value was 0.33 ± 0.33.

NONSONAR COMBINATIONS. Other neurons showed facilitatory or inhibitory interactions between distinct spectral components in sounds. These also are called combination-sensitive, because their responses were in some ways similar to the FM-FM and H1-CF responses outlined in the preceding text. The single unit in Fig. 2C responded very weakly to single tone bursts but somewhat better to combinations of tones in the 12- to 23-kHz and 30- to 50-kHz range. Best frequencies were 23 and 41 kHz, respectively. There was strong facilitation (facilitation index of 0.64) between the two frequency inputs that occurred only at simultaneous presentation. However, the unit’s overall response to combinations of tone bursts was not strong, remaining <1 spike/stimulus. Wideband noise (10–150 kHz) at the same attenuation setting yielded a response twice as strong.

The defining features of these responses were their tuning to sounds in the 10- to 23-kHz and 32- to 47-kHz ranges and the occurrence of the strongest combination-sensitive interactions when the two signals were presented simultaneously. Most other response features varied. Some units responded best to tone bursts, whereas others responded best to noise or band-
limited noise (e.g., Fig. 2C). The low-frequency input could be either facilitatory or inhibitory. The tuning of these neurons to frequency ranges just below and above the first sonar harmonic (Fig. 1) suggests that they respond best to certain types of communication signals used by the mustached bat (Kanwal et al. 1994).

Functional organization

Study of the topographic distribution of auditory thalamic responses is based on 418 anatomically localized recording sites from 46 electrode penetrations. In the following text four aspects of organization are discussed: frequency organization in the MGB, distribution of combination-sensitive or other spectrally complex response properties in the MGB, distribution of sensitivity to delay among FM-FM responses, and responses of neurons in the thalamic reticular nucleus. Results are illustrated in Figs. 7–9 by penetrations throughout much the caudal to rostral extent of the MGB.

The MGB contains ventral, dorsal, and medial divisions (Fig. 5) as described previously (Wenstrup 1995; Winer and Wenstrup 1994a,b). The ventral division is composed of lateral (Vl) and medial (Vm) parts. The dorsal division is large. Its major subdivisions, the dorsal and rostral pole nuclei, dominate the rostral half of the MGB. The medial division is small and few auditory responses were recorded from it in this study. The description below focuses on the distribution of responses in the ventral, dorsal, and rostral pole nuclei of the MGB and in the thalamic reticular nucleus (Table 2).

FREQUENCY ORGANIZATION IN THE MGB. Because many neurons in the MGB displayed two frequency tuning curves, it is necessary to specify what is meant by frequency organization. Considered here is the distribution of best frequencies among singly tuned responses and the best high frequencies of combination-sensitive or multiply-tuned responses. For these complex responses, the best high-frequency response is used for two reasons: most recording sites displayed some responsiveness to the best high-frequency signal when it was presented separately, and combination-sensitive MGB neurons receive their ascending input from frequency representations of the central nucleus of the inferior colliculus (ICC) corresponding to their best high-frequency response (Wenstrup and Grose 1995). Consequently, this frequency organization in MGB is comparable with the topographic pattern of inputs from the tonotopically organized ICC.

Ventral division. There is a modified tonotopic sequence in the ventral division that includes Vl and Vm. Vl represents frequencies in the lower half of the audible range, 10–59 kHz. Responses with the lowest best frequencies, near 10 kHz, were located most ventrally, whereas responses tuned near 59 kHz occurred most dorsally (Figs. 7–9). Within this range, frequencies were represented unevenly (Fig. 6). Best frequencies were least common in the 24–31-kHz range, the range of the fundamental of the sonar signal. Only 4 of 39 responses in Vl (10%) were tuned in this range, all located along the lateral surface of the MGB. In agreement, several dorsoventral penetrations through the medial part of Vl displayed a gap in best frequencies from ~33 to 23 kHz (Figs. 7, A and C, and 8A). Surprisingly, the largest number of responses in Vl (44%) was tuned in the 32- to 47-kHz range, between the first and second sonar harmonics. Responses tuned to 48–59 kHz, frequencies within the second FM harmonic, comprised 26% of Vl responses.

Best frequencies of 60 kHz and above were in Vm (Figs. 7A, 8B, and 9A). The 60- to 63-kHz representation, which analyzes the CF2 sonar component, was very large (Fig. 6). It filled nearly all of Vm except for the ventromedial region, where a few responses were tuned to 90–94 kHz. This latter part of Vm was explored least, but penetrations like that in Fig. 9A suggest that frequencies between 64–89 kHz were poorly represented. No responses were found to frequencies in the 96- to 119-kHz range. These results suggest that Vm contains very few neurons tuned to frequencies in the ranges of the third and fourth FM harmonics (72–89, 96–119

### Table 2. Proportions of complex auditory responses by subdivision

<table>
<thead>
<tr>
<th>Subdivision</th>
<th>Complex Spectral Responses</th>
<th>Single Tuning</th>
<th>Total Neurons* (n)</th>
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<tr>
<td>BIC</td>
<td>100.0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>74.2</td>
<td>25.8</td>
<td>97</td>
</tr>
<tr>
<td>DS</td>
<td>60.0</td>
<td>40.0</td>
<td>5</td>
</tr>
<tr>
<td>M</td>
<td>100.0</td>
<td>0.0</td>
<td>6</td>
</tr>
<tr>
<td>RP</td>
<td>84.7</td>
<td>15.3</td>
<td>144</td>
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<tr>
<td>Rt</td>
<td>71.4</td>
<td>28.6</td>
<td>28</td>
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<tr>
<td>Sg</td>
<td>25.0</td>
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<tr>
<td>Vl</td>
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<tr>
<td>Vm</td>
<td>70.3</td>
<td>29.7</td>
<td>37</td>
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</table>

Complex spectral responses and single tuning were expressed in percentages. BIC, brachium of the inferior colliculus; D, dorsal nucleus of the medial geniculate body (MGB); DS, superficial dorsal nucleus of the MGB; M, medial division of the MGB; RP, rostral pole nucleus of the MGB; Rt, thalamic reticular nucleus; Sg, suprageniculate nucleus of the MGB; Vl, lateral part of the ventral division of the MGB; Vm, medial part of the ventral division of the MGB. *Values only include neurons that were tested for combination sensitivity or multiple tuning.
kHz). Figure 10A presents a schematic summary of the frequency organization in the ventral division.

**Dorsal nucleus.** Eighty-five percent of responses in the dorsal nucleus were tuned to one of three frequency bands: 24–31 kHz, 48–59 kHz, and 60–63 kHz (Fig. 6). Unlike the ventral division, in which 28% of responses were tuned to nonsonar frequency bands, only 4% of dorsal nucleus responses were similarly tuned. There was no apparent tonotopic representation of frequency, although the distribution of frequencies was consistent across animals (Fig. 7–9). Responses to 60–63 kHz occurred throughout most of the dorsal nucleus except the extreme rostral part, adjoining similarly tuned responses. There was no apparent tonotopic representation of frequency, although the distribution of frequencies was consistent across animals (Fig. 7–9). Responses to 60–63 kHz were always located in the caudal part of the nucleus where responses to each FM harmonic were regularly found, although it was not certain if these areas were separate. Responses with best frequencies of 48–59 kHz were located dorsomedially in the caudal part of the rostral pole nucleus (Fig. 8, C and D), whereas in the lateral part they were found both dorsally and ventrally (Figs. 7F, 8F, and 9F). Responses to 72–89 kHz were generally located ventrally in the caudal part of the nucleus (Figs. 7B and 8C) and both dorsally and ventrally in the rostral part of the nucleus (Figs. 7E, 8E, and 9E). Caudally, responses to 96–119 kHz were located between 90–94 kHz neurons (dorsally) and 72–89 kHz neurons (ventrally) (Figs. 7B and 8C). More rostrally, they dominated the lateral part of the nucleus (Figs. 7E and 8E) and extended medially (Figs. 7F and 9F). As in the dorsal nucleus, best-frequency responses to 24–31 kHz were scattered throughout. Thus there was no indication of a tonotopic representation in the rostral pole nucleus, but there was a segregation of best frequencies associated with CF sonar signals from best frequencies associated with FM sonar signals. Figure 10A schematically summarizes the most consistent features of frequency organization throughout the dorsal division.

**Rostral pole nucleus.** Ninety-seven percent of responses were tuned to frequency bands associated with the bat’s sonar signal. Because only a few (8%) had best frequencies in the 24- to 31-kHz range (1st sonar harmonic), the region was dominated by neurons tuned to the frequencies corresponding to the higher sonar harmonics (Fig. 6).

The rostral pole nucleus had a complex arrangement of best frequencies, with several features consistent from animal to animal. Responses to frequency bands of the CF sonar signals, 60–63 kHz and 90–94 kHz, were located caudally and dorsally. The 60- to 63-kHz responses were always located in the caudal, dorsal, and lateral extremes of the nucleus (Figs. 7B and 8C), with only one response located in the rostral 30% of the MGB. Responses to 90–94 kHz, located just ventral and/or medial to the 60- to 63-kHz responses, were restricted similarly caudo-rostrally (Figs. 7B, 8C, and 9C). No responses tuned to 90–94 kHz were located in the rostral 30% of the MGB.

In the remainder of the rostral pole nucleus, almost all responses were tuned to the three frequency bands of the higher harmonic sonar FM signals: 48–59 kHz, 72–89 kHz, 96–119 kHz. These responses were clearly separated from the 60- to 63- or 90- to 94-kHz responses, but their arrangement relative to each other was complex and changed with caudorostral location. There appeared to be at least two regions in the nucleus where responses to each FM harmonic were regularly found, although it was not certain if these areas were separate.
FIG. 7. Physiological responses obtained from seven reconstructed electrode penetrations through the auditory thalamus in one bat. All penetrations are from the left side. The following also applies to Figs. 8, 9. Tracer deposit sites for each penetration are shown as blackened areas, while responses at deposit sites are shown in bold. Headings (in bold) above physiological parameters indicate: BF, best frequency of singly-tuned responses or higher best frequency of a combination-sensitive response; LF, best low frequency of a combination-sensitive response; Effect, the interaction between the two frequency components (facilitation, inhibition, or none); Type, combination-sensitive response as described in first section of the Results; Delay, best delay for facilitative combination-sensitive neurons. Decimal values at the bottom of each thalamic section indicate the location of the section as a fraction of the caudal-to-rostral dimension of the MGB. For abbreviations of brain structures, see the legend for Fig. 5.
the middle one-third of the MGB. Nonsonar combination-sensitive neurons were most common in V1.

**Distribution of H1-CF responses.** The data were examined for possible segregation of FM-CF and CF-CF neurons within different MGB nuclei, similar to what occurs in the mustached bat's auditory cortex (Fitzpatrick et al. 1998b). The best frequencies of facilitation for the fundamental sonar component (H1) were compared for H1-CF neurons in Vm, the dorsal nucleus, and the rostral pole nucleus. In all three nuclei, the mean H1 frequency was nearly identical to the overall population mean (27.8 kHz). Although there was a slightly broader distribution of H1 frequencies in the dorsal nucleus (25.0–31.1 kHz) compared to the other two nuclei, there was no significant difference in the mean H1 frequencies among the three nuclei.

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**Fig. 8.** Physiological responses obtained from six electrode penetrations through the auditory thalamus of one bat. All penetrations are from the left side. Penetrations in A–C were made in nearly the same transverse plane; penetrations in D–F were made in nearly the same transverse plane. Blackened regions indicate tracer deposit sites for the characterized penetration. Shaded areas indicate deposit sites for the other penetrations visible in the illustrated transverse section. For abbreviations, see Fig. 5. See Fig. 7 legend for protocol.
kHz), all nuclei contained first harmonic facilitating frequencies in the range 26–30 kHz. These findings indicate that both FM-CF and CF-CF neurons were present in all three subdivisions.

**Distribution of delay sensitivity among FM-FM neurons.** In two areas of auditory cortex, delay-tuned FM-FM neurons are reported to be mapped according to best delay (O’Neill and Suga 1982; Suga and Horikawa 1986). This study examined whether FM-FM responses in the dorsal division of the MGB were similarly organized. The most consistent qualitative observations were that the shortest and longest best delays occurred in particular parts of the dorsal division. Thus FM-FM responses with best delays <4 ms were recorded in the caudal and medial parts of the dorsal division (Fig. 8C and 9, C–E) and never extended into the rostral 25% of the MGB. Of the 18 responses with the longest delays (9–24 ms), all but 2 occurred in the rostral 25% of the MGB, and all were located laterally or dorsally in the dorsal division (Figs. 7, E and F, 8E, and 9F).
To test the qualitative observations, a correlation analysis between best delay and response location in each dimension was performed for 78 FM-FM responses. There were highly significant correlations between best delay and caudorostral location ($r = 0.49$, $df = 76$, $P < 0.001$) and best delay and mediolateral location ($r = 0.51$, $df = 69$, $P < 0.001$). Thus short best delays were located more caudally and medially, whereas longer best delays were located more rostrally and laterally, in agreement with the qualitative observations. Best delay and dorsoventral location were not significantly correlated ($r = 0.16$, $df = 76$, $P = 0.1$). Although 3 penetrations suggested a descending dorsoventral gradient of best delay (Figs. 7, E and F, and 8 C), 11 others with three or more FM-FM responses did not (e.g., Figs. 7 B, 8, E and F, and 9 F).

In auditory cortex, best delay is reported to be mapped among FM-FM neurons tuned to each higher FM harmonic, i.e., for FM1-FM2 neurons, for FM1-FM3 neurons, and for FM1-FM4 neurons (O’Neill and Suga 1982; Suga and Horikawa 1986). If the dorsal division of the MGB is organized similarly, best delay gradients should exist separately for FM-FM neurons tuned to each higher FM harmonic. Moreover, these gradients should follow the spatially complex representations of the higher harmonic FM frequency bands. These could not be mapped by the limited number of reconstructed penetrations available in any MGB studied here. Instead, correlations of best delay and MGB location were examined for FM-FM responses tuned to each higher FM harmonic. FM1-FM2 recording sites showed significant best delay-location correlations in the mediolateral ($r = 0.57$, $df = 25$, $P < 0.001$) and caudorostral ($r = 0.50$, $df = 28$, $P < 0.001$) dimensions; short best delays are medial and caudal. FM1-FM3 responses showed only a significant mediolateral correlation ($r = 0.44$, $df = 23$, $P < 0.05$). FM1-FM4 responses showed only a significant caudorostral correlation ($r = 0.69$, $df = 17$, $P < 0.002$). In no group was there an indication of a significant dorsoventral gradient. This analysis suggests that delay-tuned neurons responsive to each higher FM harmonic are at least roughly organized by best delay within the complex representation of higher harmonic FM frequency bands that occurs in the rostral part of the dorsal division.

**DISCUSSION**

Emphasizing responses to complex sounds, this study examined physiological properties and functional organization in the
mustached bat’s auditory thalamus. The study described types of combination-sensitive responses not previously reported in the MGB and showed that spectrally complex responses are common in and distributed widely throughout the MGB. The study also described distinct organizational features in different MGB divisions: the ventral division displays a modified tonotopic organization, while organization in the dorsal division appears to be related to the functional roles of the neurons in echolocation behavior.

Together with studies of the inferior colliculus and auditory cortex, the present results help to identify the nature and site of transformations in the representation of complex acoustic stimuli that occur in the ascending auditory pathway of the mustached bat. Because most complex response properties in the MGB and auditory cortex are created in auditory centers below the MGB (Mittmann and Wenstrup 1995; Wenstrup and Grose 1995) and show little alteration between the ICC and MGB (Portfors and Wenstrup 1999b), the major tecto-thalamic transformation appears to be a reorganization of response properties, laying the groundwork for much of the physiological organization within and across the different areas of auditory cortex.

Physiological responses in the auditory thalamus

A major finding of this study is that combination sensitivity, i.e., facilitated responses to (or inhibitory interactions between) different spectral components of vocalizations, occurs throughout the MGB. Combination-sensitive neurons are abundant in the rostral and dorsal MGB, as reported previously (Olsen and Suga 1991a,b), but also occur commonly in the ventral division, where more than half of the neurons are combination-sensitive. In addition, more diverse types of combination-sensitive responses were found, including responses to combinations of nonsonar frequency bands that had not been reported previously. Finally, the thalamic reticular nucleus showed a range of response properties similar to those recorded in the MGB.

FM-FM NEURONS. FM-FM neurons were recorded in both the dorsal division, as reported previously (Olsen and Suga 1991b), and in the ventral division. Interactions between responses to FM1 and higher harmonic FM signals were mostly facilitatory with few showing purely inhibitory responses to the FM1 signal. This predominance of facilitatory interactions appears similar to FM-FM regions of auditory cortex, where inhibitory combination-sensitive interactions have not described, but is distinguished from the inferior colliculus, where inhibitory interactions are more common (Portfors and Wenstrup 1999b). Similar to a previous report (Olsen and Suga 1991b), there was a broad distribution of best delays of 1–24 ms, with emphasis on delays of 1–10 ms. If best delays of these neurons code for target distance, the population in the MGB represents distances ≤4.1 m, with most neurons coding for distances of ≤1.7 m. This range of best delays closely corresponds to the range of best delays among FM-FM neurons in the inferior colliculus (Portfors and Wenstrup 1999b; Yan and Suga 1996) and auditory cortex (O’Neill and Suga 1982; Suga and Horikawa 1986) of the mustached bat. This similarity indicates that neural processing to create delay tuning is largely complete by the level of the inferior colliculus.

HI-CF NEURONS. Olsen and Suga (1991a) previously reported the presence of facilitating CF-CF neurons in the dorsal division of the MGB. This study found that combination-sensitive responses to higher harmonic CF signals (CF2, 60–63 kHz; CF3, 90–94 kHz) may be either facilitated or inhibited by signals in the first sonar harmonic, and they are present in both the ventral and dorsal divisions. Moreover, they showed a broad range of tuning to the first harmonic frequencies, from 25 to 31 kHz, indicating that both FM-CF and CF-CF neurons occur. Importantly, the peak of the distribution was near 27.5 kHz, the frequency used in earlier studies to distinguish between FM1-CF2 and CF1-CF2 responses in auditory cortex (Fitzpatrick et al. 1993). This suggests that the distinction between these types, at least in the MGB, is not clear cut, and it was for this reason that the present study used the more general term HI-CF to designate all of these. The lack of clear physiological distinctions suggests that the functional roles of many HI-CF neurons in sonar are not well understood and that further study is required.

This study showed that each of the major types of HI-CF responses described in auditory cortex, e.g., FM1-CF2, CF1-CF2, and CF1-CF3 responses (Fitzpatrick et al. 1993; Suga et al. 1983), occur in significant numbers in the MGB. These response types are also present in the ICC. For example, ~50% of the neurons in the 60- to 63-kHz representation display facilitating H1-CF responses (Portfors and Wenstrup 1999b). This comparison suggests that the basic HI-CF response properties that comprise several areas of auditory cortex are established at or below the level of the inferior colliculus.

NONSONAR COMBINATION-SENSITIVE RESPONSES. MGB neurons tuned to combinations of frequency bands outside the sonar range display facilitative or inhibitory interactions similar to sonar combination-sensitive neurons. Most are tuned to both the 10- to 23- and 33- to 47-kHz frequency bands and are located in the ventral division. They include distinct response types, one responding best to combinations of tones, another responding better to noise bands than to tones or their combinations. These nonsonar combination-sensitive neurons are common in the inferior colliculus representation of 33–47 kHz, where they comprise 64% of a sample of 81 neurons (Leroy and Wenstrup 1996; unpublished observations). These neurons may analyze social vocalizations used by the mustached bat because many signals include energy in both the 10- to 23- and 33- to 47-kHz bands and can have either tonal or noise-like elements (Kanwal et al. 1994). Such neurons have not been described previously in either the MGB or auditory cortex, although neurons tuned to the 33- to 47-kHz band in AI are reported to display broad frequency tuning (Fitzpatrick et al. 1998b). These cortical neurons are likely to show similar combination-sensitive responses as those in the ICC or MGB. Thus basic features of complex responses to nonsonar frequency bands, like those to sonar frequency bands, appear to originate by the level of the ICC.

Even more complex responses to social vocalizations have been recorded in auditory cortex (Esser et al. 1997; Ohlemiller et al. 1996). For example, Ohlemiller and colleagues recorded FM-FM neurons that also respond to social communication signals having similar frequency content but different temporal structure compared with sonar signals. These neurons may respond to what Ohlemiller and colleagues called combinations
of syllables. No such stimuli were employed in the present study, and it remains to be determined whether they occur in the MGB or ICC.

There are two major conclusions to be drawn from this comparison of response types in the ICC, MGB, and auditory cortex of the mustached bat. First, basic features of many complex spectral responses in the auditory thalamus and cortex are formed by neural interactions that occur in the inferior colliculus or lower levels of the ascending auditory pathway. This conclusion does not suggest that no signal processing occurs within the MGB or auditory cortex, but emphasizes the qualitative and, in many cases, the quantitative similarity among complex response properties in the ICC, MGB, and auditory cortex.

The second conclusion is that these complex spectral responses form an integral property of the major ascending pathways to auditory cortex in the mustached bat, based on their abundance and widespread distribution throughout the dorsal and ventral divisions of the MGB. These are not features limited to a few specialized pathways related to the processing of sonar echoes. Instead, they form a basic strategy for the analyses of multiharmonic and/or temporally complex vocalizations (Olsen 1992; Suga 1988; Sussman et al. 1998), one that is present in both lemniscal (tonotopically organized) and nonlemniscal thalamo-cortical pathways.

Physiological organization of the mustached bat's MGB

VENTRAL DIVISION. The ventral division contains a tonotopic organization based on the best frequencies of singly tuned neurons and the higher best frequencies of combination-sensitive neurons (Fig. 10A). Three features of this organization are noteworthy. First, few neurons are tuned to 24–31 kHz, the range of the first sonar harmonic. Second, the 60–63 kHz (CF2 sonar component) representation is hypertrophied. Third, the representations of 48–59, 72–89, and 96–119 kHz (higher harmonic FM sonar components) are very small. The first two features are similar to what has been observed in the ICC (O'Neill et al. 1989; Zook et al. 1985). However, the third feature differs strikingly from what occurs in the ICC, where frequencies in the ranges of the higher harmonic FM signals enjoy substantial representations (O'Neill et al. 1989).

This frequency organization agrees well with both architectural and connectional studies. Isofrequency contours in Vl and the medial part of Vm form a good match to the orientation of dendritic (Winer and Wenstrup 1994b) and axonal (Wenstrup et al. 1994) laminae. There is little evidence of such lamination in the dorsal part of Vm, which contains the expanded 60- to 63-kHz representation. This is similar to the 60- to 63-kHz representation in ICC, which shows less evidence of fibrodendritic lamination than other parts of the ICC (Zook et al. 1985).

The pattern of frequency-specific projections from the ICC to the ventral division also agrees with the physiological organization described here (Wenstrup et al. 1994). These projections furthermore explain the difference in the tonotopic representations of the two nuclei (Frisina et al. 1989; Wenstrup et al. 1994). Specifically, each ICC frequency band representing a higher FM harmonic sends little input to the ventral division, instead projecting heavily to the dorsal division. This reorganization of ICC outputs tuned to the FM frequency bands constitutes a major feature of the tecto-thalamic projection. Because a large percentage of neurons within these frequency bands is combination-sensitive (Portfors and Wenstrup 1999b), the presumed function of this re-wiring is to create a region of the MGB, the rostral part of the dorsal division, in which specialized neural responses represent specific types of biosonar information. This in no way suggests that the region exclusively analyzes biosonar information but holds that the organization reflects sonar-related function.

Virtually every aspect of the tonotopy in the ventral division corresponds closely to what occurs in primary auditory cortex (AI) of this bat (Fitzpatrick et al. 1998b; Suga and Jen 1976). This correspondence suggests a straightforward topographic pattern of projections from the ventral division to AI, but there are no published data on this.

This study specifically examined the organization of combination-sensitive neurons within the 60- to 63-kHz (CF2) representation of the ventral division. Responses that were clearly CF1-CF2 were intermixed with those that were clearly FM1-CF2. Thus no organization was found comparable with the cortical DSCF area (containing only FM1-CF2 neurons) and the CF-CF area (containing only CF1-CF2 or CF1-CF3 neurons). This suggests that the cortical segregation of H1-CF neurons into CF-CF and FM-CF areas may be established by a reorganization of the thalamo-cortical projection. Alternatively, there may be more heterogeneity in the responses of cortical CF-sensitive areas than has been reported.

DORSAL DIVISION: FREQUENCY ORGANIZATION. The frequency representation in the dorsal division is different from the ventral division. First, nearly all neurons (~90%) are tuned to frequencies within various harmonics of the sonar pulse, whereas ~66% of neurons in the ventral division are similarly tuned. Second, there is no tonotopic organization in the dorsal division. Third, there is a frequency organization apparently related to the functional role of frequency bands in biosonar. Thus neurons tuned to the frequency bands of the higher harmonic CF sonar components, at 60–63 or 90–94 kHz, are separated from neurons tuned to the bands of the higher harmonic FM components, at 48–59, 72–89, or 96–119 kHz, with little interdigitation between the CF- and FM-tuned populations.

For best frequencies tuned to the CF bands, the 60- to 63-kHz responses were consistently placed more dorsally and laterally than the 90- to 94-kHz responses. For FM-tuned best frequencies, the topographic distribution is quite complex. Responses to each of the FM frequency bands appeared to be distributed in at least two areas of the dorsal division, as described in RESULTS (Fig. 10A).

The dorsal division of the MGB receives strong input from the ICC (Frisina et al. 1989; Wenstrup et al. 1994), and the pattern of frequency specific projections is in close agreement with the distribution of best frequencies reported here. ICC neurons from the 60- to 63-kHz representation terminated most heavily in the caudal and dorsal parts of the dorsal division, with the 90- to 94-kHz ICC input located just ventral and medial to the 60- to 63-kHz input (Wenstrup et al. 1994). The projections from ICC representations of the FM frequency bands terminated heavily in the rostral and medial part of the dorsal division (Frisina et al. 1989; Wenstrup et al. 1994).
Moreover, the pattern of inputs from each FM frequency band in the ICC was as complex as the corresponding physiological arrangement observed here. In Fig. 10B, the pattern of termination in the dorsal division resulting from tracer deposits in the 48- to 59-kHz representation of the ICC (Wenstrup and Grose 1995) shows multiple foci, patchiness, and a complex shape that correspond reasonably well to the distribution of 48- to 59-kHz responses described here.

There is less correspondence between the distribution of best frequencies and the architectonic subdivisions of the dorsal division (Winer and Wenstrup 1994a,b). In particular, populations of neurons tuned to several frequency bands span the border between the rostral pole and dorsal nuclei, and the same is true of the ICC inputs for these bands (Fig. 10B). The architectonic border may require reconsideration based on the organization of inputs and outputs of the two nuclei. However, the organization of outputs is not known.

Despite the lack of information about connections between the dorsal division and auditory cortex in the mustached bat, it is possible to draw inferences about the connections by comparing the physiological organizations of frequency in the dorsal division and in auditory cortical areas outside AI. Consider first the areas responsive to the CF frequency bands. The CF-CF area of auditory cortex contains neurons tuned to 60–63 kHz in a strip adjacent to neurons tuned to 90–94 kHz (Fitzpatrick et al. 1998b; Suga et al. 1983), much like the arrangement of neurons tuned to CF frequencies in the rostral pole nucleus. This correspondence suggests that the rostral pole nucleus may project to the CF-CF area. Another 60- to 63-kHz area of auditory cortex, located ventral to AI, may receive input from the dorsal nucleus of the MGB, which in its caudal part is dominated by neurons tuned to 60–63 kHz.

There are two consistently observed cortical areas outside AI that are dominated by neurons responsive to the FM frequency bands, the FM-FM area and the dorsal fringe area (Fitzpatrick et al. 1998b; O’Neill and Suga 1982; Suga and Horikawa 1986). Both contain neurons tuned to the three higher FM harmonics of the sonar signal. Because responses to each FM frequency band appear to occur in two different foci in the dorsal division, their complex arrangement may be related to the presence of two topographically separate neuronal populations in MGB projecting to the two cortical areas (see next section).

DORSAL DIVISION: ORGANIZATION OF COMBINATION-SENSITIVE RESPONSES. Because some areas of auditory cortex are reported to be organized by specific response properties of combination-sensitive neurons, this section considers whether similar arrangements occur in the dorsal division. Among neurons sensitive to CF components in sonar signals, no further organization of combination-sensitive responses was found. In the dorsal and rostral pole nuclei, as in the ventral division, CF1-CF responses were intermixed with FM1-CF responses. Thus there is no correspondence in the MGB to the reported segregation of FM1-CF responses (in AI and a ventral cortical area) and CF1-CF responses (in the CF-CF area and another ventral cortical area) (Fitzpatrick et al. 1993, 1998b; Suga et al. 1983). Furthermore there was no indication that CF-CF neurons in MGB are organized along the axis of relative (Doppler) frequency shifts, as has been described in the CF-CF area of auditory cortex (Suga et al. 1983). Such an organization in the MGB may well be too fine-grained to be detected by the methods of this study, yet the lack of segregation of CF1-CF neurons from FM1-CF neurons suggests that a comparable thalamic organization is unlikely. To create the topographic features described in auditory cortex, the output of the MGB would require reorganization.

In the FM-FM and dorsal fringe areas of auditory cortex, FM-FM neurons are organized by best delay and by harmonic sensitivity (O’Neill and Suga 1982; Suga and Horikawa 1986; Suga and O’Neill 1979). Thus in each of these cortical areas, and for neurons responding to each higher FM harmonic, there is a strip of neurons along the cortical surface for which the best delay increases from rostral to caudal. In the dorsal division, present results indicate some organization of best delays. For FM-FM neurons tuned to each higher FM harmonic, as well as for each sample, there were significant correlations between MGB location and best delay. These correlations may depend most on the consistent locations of neurons with the shortest and the longest best delays, in the caudomedial and rostrolateral parts of the dorsal division, respectively. The study did not show within individual animals the main feature of the cortical organization: i.e., a gradient of best delays for neurons tuned to a specific FM harmonic. If there is more organization than what was described here, there are two probable reasons for missing it. First, the complex arrangement of frequencies representing any FM frequency band would make it very difficult to identify such a gradient within an individual animal. Second, the observed distribution of FM-FM neurons tuned to each FM harmonic in the dorsal division actually may consist of two representations (Fig. 10B), with two corresponding representations of best delays. This second possibility is consistent with the present results concerning the distribution of best frequency and best delay in the dorsal division, with the pattern of inputs from ICC neurons tuned to each FM frequency band (Frisina et al. 1989; Wenstrup et al. 1994), and with the occurrence of the dorsal fringe and FM-FM areas, which each contain FM-FM neurons and receive ascending input from the MGB (Fitzpatrick et al. 1998a; Olsen 1986). It is proposed here that the distributions of frequency sensitivity and best delay among FM-FM neurons of the dorsal division contain basic elements of the organization in the two cortical areas containing FM-FM responses.

Whether the arrangement of best delays in MGB originates there or in the ICC is unclear. A published report has described an organization of best delays in FM-sensitive representations of the ICC (Yan and Suga 1996), concluding that the cortical map of best delays is created in the ICC. However, because no electrode penetrations in that study were reconstructed histologically, the conclusion is based largely on four penetrations showing a delay-depth gradient. Preliminary results from this laboratory, based on a detailed topographic study of delay tuning in the 72- to 89-kHz (FM3) representation of the ICC, indicate no organization of best delay (Portfors and Wenstrup 1999a). Those results, in combination with the present finding of some organization of best delay in the MGB, suggest that the tecto-thalamic projection provides the initial organization of this response property. Thus this study concludes that features of the cortical organization of FM-FM responses are established within the MGB but emphasizes that the MGB...
Auditory thalamic organization: species comparisons

A major feature of MGB organization among mammals concerns differences between the ventral division and the dorsal and medial divisions. In the ventral division, the laminar arrangement of principal cell dendrites (Morest 1965; Winer 1992) is thought to provide a substrate for the tonotopic organization that has been observed in several species (Aitkin and Webster 1972; Clarey et al. 1992). Physiologically, ventral division neurons typically display sharp frequency tuning, relatively short latencies, good temporal precision, and consistent responses to repetitive sounds. In contrast, the dorsal and medial divisions do not typically display similar clear tonotopic organizations, and their responses are on average more broadly tuned in frequency, longer in latency, less temporally precise, and less consistent (Aitkin 1973; Aitkin and Webster 1972; Calford 1983; Clarey et al. 1992; Lennartz and Weinberger 1992).

In the mustached bat, the clearest similarity to this general mammalian plan is the different functional organizations of the ventral and dorsal divisions. The present study shows that the ventral division is tonotopically organized, whereas the dorsal division contains no similar physiological organization. However, even these similarities to the general mammalian plan have a strong species-specific character. The tonotopic organization in the ventral division, with its lack of neurons tuned to sonar FM frequency bands, is changed markedly from the ICC (O’Neill et al. 1989; Zook et al. 1985). Moreover, the non-tonotopic frequency organization in the dorsal division segregates neurons on the basis of the sonar components they analyze and appears to organize a population of these (FM-FM neurons) according to a response property related to the analysis of sonar targets (delay tuning).

A distinctive feature of the mustached bat’s MGB is the prevalence of complex spectral responses in both the ventral and dorsal divisions (58 and 79%, respectively). Most of these are facilitated, combination-sensitive responses to the frequency range of biosonar signals. Although such responses are more common in the dorsal division, both divisions have a majority of complex spectral responses. In other mammals, the majority of ventral division neurons display a single, relatively narrow, excitatory tuning curve, whereas multipeaked tuning curves are more common in the dorsal and medial divisions (Aitkin 1973; Aitkin and Webster 1972; Calford 1983; Calford and Webster 1981; Imig and Morel 1985; Lennartz and Weinberger 1992). Some of these may be combination-sensitive. In the squirrel monkey MGB, neurons responsive to combinations of different temporal and spectral elements in social vocalizations have been recorded in preliminary studies (Olsen 1994). However, even in the dorsal division of other mammals, stimulus specificity for complex sounds and overall numbers of neurons with multipeaked tuning curves do not approach the proportions found here in the mustached bat dorsal division.

This difference may result from the mustached bat’s reliance on multiharmonic vocalizations for both orientation and communication, but it may result in part from the use of barbiturate anesthetics in many studies on other species. In the MGB of cats and monkeys, the complexity of both single-unit frequency tuning and tonotopic organization is greater when obtained from unanesthetized or lightly anesthetized animals (Allon et al. 1981; Morel et al. 1987). For example, in lightly anesthetized cats, nearly 20% of ventral division neurons displayed multipeaked tuning curves and ~40% had “broad” frequency tuning. For medial division neurons in the same study, nearly 80% had either broad or multipeaked tuning curves.

The species-specific patterns in the mustached bat appear closely related to the physiology and projection patterns of neurons in the inferior colliculus. The clear majority of neurons in the mustached bat’s ICC, whether in the regions analyzing sonar calls (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999) or in nonsonar regions (Leroy and Wenstrup 1996), contain combination-sensitive or other spectrally complex (e.g., multipeaked tuning curves) responses. Furthermore, these ICC neurons provide the major projection to both the ventral and dorsal divisions, and their topographic patterns of input determine the frequency organization of both the ventral and dorsal divisions (Wenstrup and Grose 1995; Wenstrup et al. 1994). In contrast, the dorsal division in other species typically receives stronger input from other parts of the inferior colliculus, such as the dorsal cortex and external nucleus (Oliver and Huerta 1992; Winer 1992). It is unclear whether the physiological properties of these regions confer the broader frequency tuning and less precise temporal response features onto dorsal and medial division neurons or whether these are created by integration of inputs in the MGB.

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