Medial Superior Olive of the Big Brown Bat: Neuronal Responses to Pure Tones, Amplitude Modulations, and Pulse Trains

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INTRODUCTION

The superior olivary complex is the first stage of the mammalian ascending auditory system at which binaural processing takes place. In the traditional view, the two principal nuclei of the superior olive are thought to function exclusively in the context of azimuthal sound localization. According to this scheme, the lateral superior olive (LSO) processes interaural level differences (ILDs) of high-frequency sounds while the medial superior olive (MSO) processes interaural time differences (ITDs).

The LSO appears to be anatomically and physiologically similar in all mammals that have been investigated (see Covey and Casseday 1995; Irvine 1992; Schwartz 1992 for reviews). In contrast, both the structure and the function of the MSO are highly variable across mammals. In mammals with good low-frequency hearing and sufficient interaural distance to generate significant ITDs, the MSO is large, the low frequency representation is greatly expanded, and most neurons are responsive to sound at either ear, with the maximal response occurring when both ears are stimulated with a specific ITD (dogs: Goldberg and Brown 1969; cats: Galambos et al. 1959; Yin and Chan 1990; gerbils: Spitzer and Semple 1995; rabbits: Batra et al. 1997). In some small mammals with high-frequency hearing, such as mice, the MSO is very small. However, despite the fact that echolocating bats are some of the smallest mammals and have the highest frequency hearing range of any terrestrial mammals, the MSO of many bat species (e.g., mustached bats) is well differentiated. Moreover, its relative size exceeds that of the MSO in cats or dogs. Apparently, this increase in size is due to an increase in the number of cells receiving monaural inputs only (for review, see Grothe 2000; Grothe and Park 2000).

Examination of the MSO in different bat species has revealed pronounced species-specific differences. The MSO, more than any other part of the brain except the ventral nucleus of the lateral lemniscus, varies in both structure and function from one species of bat to another. It is therefore reasonable to suppose that the variations in the MSO of different species of bats have come about as an adaptation for processing the different patterns of auditory information that are commonly used by each species. For example, in the mustached bat, Pteronotus parnellii, the MSO is a large homogeneous-appearing structure that receives mainly contralateral input. Most MSO neurons in this species are monaural, responding to sound at the contralateral ear (Covey et al. 1991; Grothe 1994). In the mustached bat, MSO neurons receive both excitatory and inhibitory input from neurons driven by sound at the contralateral ear. The excitatory and inhibitory inputs are both

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sustained for the sound’s duration and slightly offset in time relative to one another. In neurons in which the excitatory input arrives first, inhibition cancels the latter part of the excitation, leaving only a transient onset response. In neurons in which the inhibitory input arrives first, the inhibition cancels the early part of the excitation, leaving only a transient offset response (Grothe 1994; Grothe et al. 1992). In addition to creating ON and OFF responses, this pattern of time-delayed excitatory and inhibitory inputs produces low-pass filtering for periodic stimuli, including amplitude modulations (AM). The mustached bat’s MSO appears to be specialized in that it contains predominantly neurons that process temporal information.

A different functional arrangement is found in the MSO of the free-tailed bat, Tadarida brasiliensis. This bat’s MSO is also large and well developed. However, in addition to monaural cells with ON and OFF discharge patterns like those in the mustached bat, it has a considerable population of binarual cells. These cells also show filter properties for the temporal structure of sounds but are heavily influenced by spatial cues including IIDs (Grothe et al. 1997).

It seems likely that the differences between the MSOs of these two species of bats are related in some way to the different environments in which they feed and the different echolocation strategies that they employ. The mustached bat’s echolocation call consists of a relatively long (20–30 ms) constant frequency (CF) component followed by a short (1–3 ms) downward frequency modulated (FM) component. This type of call, commonly referred to as a “CF-FM” call, is useful for detecting fluttering targets within the clutter of foliage in which these bats forage for insects. A fluttering insect would impose a characteristic pattern of periodic AM on the CF component of the echo, generally within the range of modulation frequencies to which MSO cells in the mustached bat are sensitive. The free-tailed bat is considered a pure “FM” bat because it emits a short downward FM sweep that can be varied in shape and duration depending on the bat’s hunting strategy. These calls are not well suited for the detection of flutter (for review, see Neuweiler 1990). Its MSO shows low-pass properties for modulation rate, but the filter cutoffs in most neurons depend on the binaural context (Grothe et al. 1997). Thus the MSO of the free-tailed bat has considerably fewer neurons with stable filter cutoffs independent of other stimulus parameters than does the mustached bat’s MSO.

The big brown bat, Eptesicus fuscus, employs an echolocation strategy that incorporates elements of the calls used by both the mustached bat and the free-tailed bat. When searching for prey, the big brown bat emits a long (∼20 ms) “quasi-CF” call that is in the range between 23 and 28 kHz (Simmons and Stein 1980). When pursuing prey, it uses a short (<1–10 ms) FM call, the main harmonic of which sweeps from ∼50 to ∼20 kHz. Given that the big brown bat must sometimes process information similar to that generated by the CF component of the mustached bat’s echolocation call, it would be reasonable to hypothesize that its MSO should contain a significant population of monaural neurons that act as low-pass filters for sinusoidal AM (SAM) sounds. Because the big brown bat also processes FM information similar to that used by the free-tailed bat, it might be supposed that its MSO would contain a large number of binarual neurons. The aim of this study was to test these hypotheses by recording responses of MSO neurons in the big brown bat to monaurally and binaurally presented SAM sounds.

Parts of this study have been presented in abstract form (Grothe et al. 2001).

METHODS

Surgery and stereotaxic setup

We recorded from the MSO of three North American big brown bats, E. fuscus. Two days before recording began, each bat was anesthetized with methoxyflurane (Metofane, Pittman-Moore) and a subcutaneous injection (1 ml per 100 g body wt) of the neuroleptanalgetic, Thalamonal (Janssen; 0.05 mg Fentanyl plus 2.5 mg Droperidol per ml). After deflating the skin and muscles overlying the dorsal part of the skull, a small hollow metal post was mounted on the surface of the skull using cyanoacrylate adhesive and dental cement. The post was used to fix the head in a standard position in a custom-made stereotaxic apparatus during multiple recording sessions. To determine the stereotaxic coordinates and angle of electrode penetration, we used a procedure described in detail by Schuller et al. (1986).

Briefly, the sagittal profile of the skull was reconstructed in 50-μm steps along the midline and 100 μm lateral to the midline on both sides. Additionally, the transverse profile of the skull was reconstructed in 50-μm steps at two different rostrocaudal positions. The reconstructed profiles were compared with a standard profile for the big brown bat’s skull and brain derived from earlier studies. The alignment of the experimental bat’s skull profile with the standard profile allowed us to calculate the stereotaxic coordinates of the MSO relative to a fixed reference point on the stereotaxic apparatus with an error of ±100 μm or less. The histological analysis performed at the end of the experiments allowed us to confirm that our recordings were from MSO and to reconstruct the recording sites.

On the first day of recording, a small opening, <1 mm diam, was made in the skull overlying the inferior colliculus (IC) for insertion of the recording electrode. Recording sessions were conducted in a sound-attenuating chamber, heated to ∼28°C. Prior to each recording session, the bat was tranquilized with 0.15–0.2 ml Thalamonal injected subcutaneously. Incisions and pressure points were treated with a local anesthetic (Xylocaine). Each recording session lasted four hours or less. Drinking water was offered at periodic intervals during the recording session. The recording session was terminated if the bat showed signs of restlessness.

Stimuli

Sounds were generated digitally using instrumentation from Tucker-Davis Technologies and delivered using custom-made earphones (Schlegel 1977) fitted to the ears with tubes 5 mm in diameter. The earphone system was calibrated using a 18-in Brüel and Kjaer microphone and a Brüel and Kjaer measuring amplifier (type 2606). The variability in the output of the system was less than ±4 dB over the frequency range used (10–80 kHz). Acoustic isolation between the ears was 40 dB for all frequencies presented. The output levels of the two loudspeakers differed from one another by less than ±3 dB.

Stimuli were single pure tone bursts, trains of pure tone bursts, and sinusoidally amplitude modulated tones (SAM). SAM tones were modulated at frequencies (Fmod) from 20 Hz up to 1 kHz with 100% modulation depth. The duration of SAM stimuli was 100 ms. Pure tone durations ranged from 3 to 100 ms. Stimuli were presented at repetition rates ranging from 1 to 4/s and had rise-fall times of 0.5 ms.

Recording and analysis

Each stimulus was presented 20 times unless otherwise stated in the text or figure legends. Action potentials were recorded extracellularly using glass micropipettes filled with 2 M NaCl. The resistance of the
The electrodes were advanced by a hydraulic motorized microdrive (Wells) operated by remote control from outside the recording chamber. Action potentials from single neurons with a constant, biphasic waveform and stable amplitude were fed into a PC via a recording amplifier, a band-pass filter (0.3–5 kHz), and a window discriminator.

To quantify the degree to which neuronal discharges evoked by SAM stimuli were correlated with the phase of \( F_{\text{stim}} \), the vector strength (VS) was calculated as described by Goldberg and Brown (1969). For the analysis of modulation transfer functions based on spike count or VS, the response to the first cycle was excluded. If the first cycle was \(< 10 \text{ ms} \), the first 10 ms of the response were excluded. Only statistically significant VS values that fulfilled the \( P < 0.001 \) criterion in the Rayleigh test following Batchelet (1991) are presented in this paper or used for population statistics.

At the end of each experiment, horseradish peroxidase (HRP) was iontophoresitically injected from the recording electrode to mark recording sites. Methods for iontophoretic HRP injection, perfusion, and histochemistry have been described in detail by Feng and Vater (1985).

RESULTS

We recorded from 97 neurons in the medial regions of the superior olivary complex in three animals (\( \text{Ept01}: n = 24; \text{Ept02}: n = 48; \text{Ept03}: n = 25 \)). In two animals (\( \text{Ept01} \) and \( \text{Ept02} \)), the stereotaxic coordinates, visible electrode penetrations and HRP injections together confirmed that the recording sites were within the MSO. In the third animal (\( \text{Ept03} \)), no HRP histology was available, so we cannot rule out the possibility that in this animal some recording sites may have been in areas adjacent to MSO such as the superior paraolivary nucleus. However, visible electrode tracks indicated that the recording sites were within the MSO, and the response properties of all neurons were consistent with their being in the MSO.

With the exception of tonotopy, there did not appear to be any topographic distribution of response properties within MSO. For example, neurons that were excited by sound at the ipsilateral ear and unaffected by sound at the contralateral ear (EO) were intermingled with those excited by sound at either ear (EE) and those excited by sound at the contralateral ear and unaffected by sound at the ipsilateral ear (OE). Similarly, \( \text{on} \), \( \text{off} \), and primary-like discharge patterns were intermingled throughout the MSO of all three animals.

General response characteristics

FREQUENCY TUNING. The best frequencies (BFs) of the 97 neurons from which we recorded were distributed throughout most of the audible range of the big brown bat (Koay et al. 1997). The lowest BF encountered was 11 kHz and the highest 79 kHz. The distribution of BFs (Fig. 1A) shows that the majority of neurons were tuned to frequencies between 20 and 40 kHz. This expanded frequency representation, particularly between 20 and 30 kHz, matches the BF distribution seen in other brain stem nuclei in the big brown bat (IC: Casseday and Covey 1992; Jen et al. 1989; Poon et al. 1990; nuclei of the lateral lemniscus: Covey and Casseday 1991; cochlear nucleus: Haplea et al. 1994). The expanded frequency range corresponds to the range of frequencies within the first and most intense harmonic of the big brown bat’s echolocation call (Simmons 1979). In single-electrode penetrations, BFs tended to increase with depth, indicating that in the big brown bat, as in other mammals, the MSO has a tonotopic arrangement in which neurons with low BFs are located dorsally and those with high BFs are located ventrally (e.g., Irvine 1992). This tonotopic arrangement of the MSO has been seen in all bats so far studied (\( \text{Molossus ater} \): Harnischfeger et al. 1985; mustached bat: Covey et al. 1991; Grothe et al. 1994; free-tailed bat: Grothe et al. 1994, 1997; for review, see Grothe and Park 2000).

DISCHARGE PATTERNS. For 93 neurons we obtained discharge patterns from PSTHs that were recorded using test tones at BF, 20 dB above threshold (Fig. 1B). Primary-like responses were seen in 33% of the neurons (Fig. 2), transient \( \text{on} \) in 37% (Fig. 2), and transient \( \text{off} \) in 23% (Fig. 3). About half of the \( \text{on} \) responders had a weak sustained component. Four neurons responded with a chopper-type discharge that lasted only a few milliseconds regardless of the stimulus duration, and two neurons responded with pauser-build-up response patterns. We tested 70 MSO neurons with pure tones at BF at different sound pressure levels. Of these, 81% had monotonic rate-level functions. Their discharge patterns remained consistent across all stimulus levels tested.

OFF responses. As mentioned in the preceding text, 21 neurons responded with a strong \( \text{off} \) discharge that followed the end of the stimulus. As shown in Fig. 3, the response latency relative to stimulus onset varied as a function of the stimulus duration. About half of these offset neurons (12/21) exhibited a short response consisting of one or two spikes per stimulus that was independent of amplitude or duration (Fig. 3A). The spike counts and response period of these neurons remained constant or decreased slightly when stimulus duration was increased, but they never increased. The second type of neurons (9/21) with \( \text{off} \) responses discharged with \( \leq 10 \) spikes per stimulus. In these neurons, increases in amplitude but not duration caused an increase in discharge rate (Fig. 3B).

All of the 21 \( \text{off} \) neurons were excited by the contralateral
ear, and only 5 of them were influenced by ipsilateral stimulation, with 4 being inhibited and 1 weakly excited.

It has been shown by blocking inhibition that OFF discharges of neurons in the mustached bat’s MSO result from sustained inhibition that coincides with and cancels sustained excitation (Grothe 1994). To determine whether the generation of OFF responses in the MSO of the big brown bat might also involve sustained inhibition, we tested 10 OFF neurons with two sequential pulses at BF, 20 dB above threshold and varied the interpulse interval (IPI), i.e., the silent period between the two stimuli. If inhibition begins soon after the onset of the stimulus, then, using this stimulus paradigm, there should be some noncontiguous, nonoverlapping IPI at which the early inhibition from the second pulse inhibits the offset excitation evoked by the first. Figure 4 shows a typical example of the results obtained using the two-tone paradigm. At IPIs >10 ms, the neuron responded with two OFF discharges, one correlated with the first pulse and one correlated with the second. As IPI decreased <10 ms, the late part of the response to the first pulse progressively disappeared until at IPIs of ≤4 ms, the response to the first stimulus was completely suppressed. This finding is consistent with the proposal that sustained inhibition is involved in generating OFF responses as shown in the MSO of the mustached bat.

Most of the neurons with OFF discharges responded to all frequencies with this discharge pattern. However, in 4 of the 21 neurons tested, there was a small frequency range that evoked ON responses. The frequency range for ON responses was always smaller and higher than that for OFF responses. Figure 5 shows an example of a neuron that responded with a robust OFF discharge to frequencies from 23 to 29 kHz and a weak ON discharge from 31 to 35 kHz. In between the two response areas, at 30 kHz, there was virtually no response.

A possible explanation for this phenomenon is that the neuron received two independent inputs, an inhibitory one tuned to lower frequencies and an excitatory input with higher or broader tuning particularly in the high-frequency region. Pharmacological results from the mustached bat MSO (Grothe 1994) indicate that MSO neurons receive excitatory and inhibitory inputs that partly but not entirely overlap in frequency, suggesting that perhaps one of the inputs was more broadly tuned than the other. To investigate these possibilities, we used a two-tone paradigm to test three of the neurons that exhibited OFF and ON responses at different frequencies. The frequency of the first pulse was at BF, evoking a robust OFF response, and the frequency of the second pulse was varied throughout the response range of the neuron. The delay of the second pulse was chosen to inhibit all but the first spike of the response to the first tone when both stimuli were presented at BF. The remaining spike could be suppressed by advancing the second stimulus by 1 ms. Figure 6 shows one example of the results obtained (same neuron as in Fig. 5). In this case, the two pulses

FIG. 2. Poststimulus time histograms (PSTHs) of 2 neurons at 3 different stimulus levels relative to threshold. Left: the neuron responded with a sharp ON discharge consisting of 1 spike per stimulus presentation. Right: the neuron responded with a sustained “primary-like” discharge. The ON neuron showed a plateau in spike counts 10 dB above threshold (a.T.), while the primary-like neuron showed a monotonic increase in spike count with increasing stimulus level. Bin width, 1 ms. PSTHs derived from 20 stimulus repetitions. Stimulus frequency was at the neurons’ BFs.

FIG. 3. PSTHs of 2 neurons with OFF responses at different stimulus levels (A and B, left) and durations (A and B, right). The neuron in A responded with a sharp OFF discharge consisting of 1, or occasionally 2, spikes per stimulus. The response pattern did not vary as a function of stimulus level or duration. The neuron in B responded with a long-lasting OFF discharge consisting of several spikes per stimulus. The response pattern did not vary as a function of sound duration or stimulus level, but spike count monotonically increased with stimulus level. Bin width, 1 ms. PSTHs derived from 20 stimulus repetitions. Stimulus frequency at BF.
were 20 ms in duration, the frequency of the first pulse was 26 kHz (BF), and the IPI was 5 ms. Both stimuli were presented at the same sound level (20 dB above BF threshold). When the frequency of the second stimulus was far above BF (e.g., 36 kHz), a robust OFF response was evoked by the first pulse but there was no response to the second pulse. At 32 and 34 kHz, a weak ON response to the second tone (compare Fig. 5) added to the OFF response, resulting in the strongest discharges. When the frequency of the second stimulus was <32 kHz, the response to the first stimulus was suppressed except for the first spike. The late response to the first stimulus only reappeared when the frequency of the second pulse was ≥20 kHz. Thus there was a very effective suppression of the response to the first tone by the second tone at frequencies between 20 and 31 kHz. Even though the second tone did not elicit a strong response itself at some of the frequencies tested (30, 22, and 20 kHz), it was quite effective in suppressing the response to the first stimulus. Between 32 and 34 kHz, a frequency that elicited a weak ON response, the second tone facilitated the OFF response to the first tone. This finding indicates that there was a considerable mismatch in the frequency tuning of the excitatory and inhibitory inputs with the excitatory input extending farther into the high-frequency range. Similar results were found in the two other “ON-OFF” neurons tested with tone pairs.

Binaural response types

To obtain the simplest and most general assessment of binaural response characteristics, we presented pure tones and varied the ILD for 87 neurons. The resulting distribution of binaural characteristics is shown in Fig. 7. About half (54%) of the neurons were driven by pure tones presented to the contralateral ear and showed no signs of ipsilateral excitation, inhibition, facilitation, or suppression. Following the terminology of earlier studies in the bat MSO, we designated these neurons as OE. Another 21% of the neurons could be driven by sound presented to either ear alone and/or showed facilitatory effects indicating that both contralateral and ipsilateral inputs were excitatory. We defined these neurons as EE. Seventeen percent of the neurons were excited by sound at the contralateral ear and inhibited by sound at the ipsilateral ear (IE), whereas 5% of the neurons were excited by a sound at the ipsilateral ear and inhibited by one at the contralateral ear (EI). Only 3% of the neurons were excited by a sound at the ipsilateral ear and unaffected by one at the contralateral ear (EO).

Responses to SAM tones

We tested 64 cells with SAM tones in which the carrier frequency was the cell’s BF, and the modulation depth was...
unmodulated tones with a sustained discharge had a mean VS of 0.52. The difference between the two means was statistically significant ($P < 0.05$; Mann-Whitney test). Interestingly, the highest precision was reached by the neurons responding with an OFF discharge (VS = 0.95 in 1 neuron; average VS = 0.79 ± 1.6, $n = 8$). Only one of nine OFF neurons failed to phaselock to SAM stimuli.

RESPONSE TO DIFFERENT MODULATION FREQUENCIES. We tested 55 neurons with SAM stimuli at different $F_{\text{mods}}$. For these tests we used the binaural stimulation conditions that evoked the highest discharge rate. As would be expected, the responses of MSO neurons to SAM varied as a function of $F_{\text{mod}}$.

Figure 8 shows representative responses of a neuron to SAM tones with different $F_{\text{mods}}$. This neuron responded to pure tones with a precise ON response (not shown). At modulation rates of 50 and 100 Hz, it responded to each modulation cycle with one or more spikes that were correlated in time with the stimulus envelope. At $F_{\text{mods}} > 100$ Hz (e.g., 500 Hz in Fig. 8), the neuron responded mainly or exclusively to the onset of the sound.

As in the case of the ON neurons (e.g., Fig. 8), neurons that responded with OFF discharges to pure tones had low-pass-filter characteristics. Figure 9 shows an example of the responses of an OFF neuron to different $F_{\text{mods}}$. Up to a $F_{\text{mod}}$ of ~250 Hz, this neuron responded with a phase-locked discharge. At higher modulation rates (e.g., 500 Hz), there was only a large OFF response at the end of the entire SAM stimulus. Based on spike counts per SAM cycle, the 50% filter cutoff of the MTF was ~130 Hz. Based on VS, it was ~235 Hz.

Using spike count as the criterion for responding, 34 (62%; $n = 55$) neurons showed low-pass filter characteristics; the remaining 21 (38%) showed all-pass filter characteristics. Figure 10A gives the distribution of 50% cutoffs taken from MTFs based on spikes/cycle for the neurons with low-pass filter characteristics. The mean 50% cutoff for the 34 low-pass neurons was 224 ± 116 (SD) Hz. Twenty-two (64%) of the low-pass neurons responded to unmodulated pure tones with transient OR OFF discharge patterns. The remainder re-
sponded with a sustained pattern. The average 50% cutoff of the ON neurons did not differ significantly from that of the entire population. However, the OFF neurons showed significantly lower cutoffs than the rest of the low-pass neurons (131 ± 66.8 Hz; n = 9; P < 0.05; Mann-Whitney test).

Based on spike count, 21 neurons (38%) showed all-pass characteristics because their response rate never dropped to <50% of the maximal response. Of these neurons, 15 (71%) responded to unmodulated pure tones with a primary-like discharge pattern, 1 with an OFF response, and 4 with ON responses. Five of the all-pass neurons did not phase lock at any $F_{\text{mod}}$. The remaining 16 neurons with all-pass characteristics showed significant phaselocking ($P < 0.001$) at a modulation frequency of 100 Hz (VS = 0.49 ± 0.13), but VS dropped <0.3 or was not significant (<0.001) at an average cutoff frequency of 177 ± 60.1 Hz (n = 16). Therefore using VS as a criterion for responding, the average cutoff of these neurons was 231 ± 96.8 (Hz; n = 31). Again, neurons with OFF discharge patterns had significantly lower cutoffs (145 ± 76.4 Hz; n = 8; $P < 0.05$; Mann-Whitney test) than the rest of the neurons.

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Thirty-one of the neurons with low-pass filter characteristics for spike-count based MTFs phase-locked at least at some $F_{\text{mod}}$. Using VS as the criterion for responding, the average cutoff of these neurons was 231 ± 96.8 (Hz; n = 31). Again, neurons with OFF discharge patterns had significantly lower cutoffs (145 ± 76.4 Hz; n = 8; $P < 0.05$; Mann-Whitney test) than the rest of the neurons.

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**FIG. 8.** The top 4 panels show responses of an ON neuron to sinusoidally amplitude modulated (SAM) stimuli at different modulation frequencies. At modulation frequencies of 50 and 100 Hz, the neuron responded with ≥1 spikes per SAM cycle phase-locked to the modulation frequency. At higher modulation frequencies, the ongoing phase-locked response disappeared and only a response to the first few cycles remained. Insets: phase histograms for the ongoing response. The 2 bottom graphs show the modulation transfer functions based on total spike count (number of spikes; ●), spikes per cycle (●), and vector strength (VS, •). For all the phase histograms and VS analysis, the response to the 1st cycle or the 1st 10 ms of the response was excluded. Carrier frequency at BF, 20 dB above threshold. Histograms derived from responses to 20 stimulus repetitions, binwidth, 1 ms.

**FIG. 9.** The top 4 panels show the responses of an OFF neuron to SAM stimuli at different modulation frequencies. The bottom panels show the modulation transfer functions (MTFs). The format of the MTFs and PSTHs are similar to those in Fig. 8.
a measure of sensitivity to SAM, these neurons showed low-pass characteristics.

In all there were 47 neurons that phase locked to SAM, 31 low-pass based on spike counts and 16 all-pass based on spike counts. For all of these neurons, 50% cutoffs could be calculated. Figure 10B shows the distribution of cutoffs for this population of neurons.

BINAURAL STIMULATION. We tested five EE neurons to see whether there was any difference in SAM sensitivity when stimuli were presented contralaterally, ipsilaterally, or binaurally. For all of these neurons, 50% cutoffs could be calculated. Figure 10B shows the distribution of cutoffs for this population of neurons.

Responses to trains of unmodulated pure tone bursts

Our observation that neurons with low-pass filter characteristics tended to have transient ON or OFF discharge patterns is consistent with data from other bats. For the mustached bat, it has been shown that the transient ON and OFF discharge patterns of MSO neurons are created through the interaction of excitation and inhibition slightly offset in time (Grothe 1994; Grothe et al. 1992). When the $F_{\text{mod}}$ of SAM tones is varied, the time each cycle is above the neuron’s threshold, the interstimulus interval (IPI; time each cycle is below the neuron’s threshold), and the rise time all vary interactively, so there is no way to evaluate the relative importance of these variables in determining the neuron’s filter characteristics. For this reason, we used trains of unmodulated pure tones to independently vary duty cycle and repetition rate, keeping rise time constant. We tested seven neurons with transient ON responses to pure tones and low-pass characteristics for SAM stimuli with trains of pure tone pulses, systematically varying pulse duration and IPI. The results for all seven neurons were similar. A representative example of one neuron’s responses to trains of tone pulses is shown in Fig. 11. This neuron responded to a single unmodulated tone with a transient ON response. When the IPI was 2.5 ms, the neuron’s response to the first pulse of the train was equal to or only slightly larger than the responses to subsequent pulses in the train (top row of PSTHs). The slight reduction in the magnitude of responses to pulses after the initial one was virtually identical regardless of pulse duration. At an IPI of 1.5 ms (middle row of PSTHs), the responses to all but the first pulse were clearly reduced. When the IPI was 0.5 ms (bottom row of PSTHs), the responses to all but the first one or two pulses were almost completely suppressed for all three pulse durations. The bottom diagrams show the analysis of the responses to the different stimulus durations as functions of IPI, duty cycle, and repetition rate (which would correspond to $F_{\text{mod}}$ of a SAM stimulus). The response to the first pulse was excluded from the analysis. When plotted as a function of IPI,
the response functions for the three different durations are virtually identical. In contrast, when plotted as a function of duty cycle or repetition rate, the functions are widely divergent. These observations indicate that the IPI is the main factor that contributes to establishing low-pass cutoffs for AM.

**Discussion**

Our results show that the MSO of the big brown bat, like other brain stem nuclei (review see: Covey and Casseday 1995), has a full representation of this animal’s hearing range, with a bias for BFs corresponding to the dominant frequency range of the echolocation call. About half of the neurons studied appeared to be monaural, while the other half showed clear indications of binaural interactions among multiple excitatory inputs or excitatory and inhibitory inputs. The cells that had binaural excitatory inputs (EE) made up 20% of the total.

The majority of MSO cells exhibited transient discharge patterns correlated with either the beginning or the end of the stimulus. Most MSO neurons responded with a phase-locked discharge to the envelope of SAM sounds, but only at $F_{\text{mods}} < 300$ Hz. Tests with trains of pulses showed that it is not modulation rate per se that is responsible for low-pass filtering but rather the IPI.

As we will point out in the following text, these characteristics place the MSO of the big brown bat midway between that of the free-tailed bat and that of the mustached bat, adding evidence to the notion that the structure and function of the MSO in bats is correlated with the characteristics of each species’ echolocation signal.

**Comparisons with MSO in other bats**

The MSO of the big brown bat, like that of the mustached bat and the free-tailed bat, differs somewhat from the MSO of cats or dogs with respect to morphology, connections, discharge patterns, binaural characteristics, and the representation of BFs. In dogs (Goldberg and Brown 1968) and cats (Guinan et al. 1972), the tonotopic representation in MSO encompasses the animal’s entire frequency range but is clearly biased to low frequencies. As in mustached bats (Covey et al. 1991; Grothe 1994) and free-tailed bats (Grothe et al. 1997), the BFs in the MSO of the big brown bat encompass the entire hearing range with a bias to the main harmonics of the echolocation call.

While all three species of bats have a large number of neurons with binaural properties other than EE, and dogs and cats have some, the distribution of the different binaural types varies among species. Figure 12 compares the binaural types found in the MSO of the dog and cat with those in the three species of bats. The predominant binaural type in the dog (1st \(\text{bar, top graph}\) (Goldberg and Brown 1968) as well as in the free-tailed bat (3rd \text{bar, top graph}) is EE, with only a small proportion of IE or OE cells (middle and bottom graphs). In the rat, there are approximately equal numbers of EE and IE cells and no OE (Inbody and Feng 1981). The predominant type in the MSO of the mustached bat is OE, with a very small proportion of IE or EE cells (right bars in Fig. 12). As evident from these graphs, the MSO in the big brown bat is intermediate between the two other bat species, consistent with its use, under some conditions, of a quasi-CF signal resembling that of the mustached bat and its use of a purely FM signal resembling that of the free-tailed bat under other conditions (Simmons and Stein 1980) (see following text).

**Comparison of bat MSO neurons’ discharge patterns with those in animals that have low-frequency hearing, such as the dog, cat, and gerbil, is difficult because published data from these latter species were obtained almost exclusively from low-frequency neurons that phase-lock to pure tones.**

Comparing discharge patterns among bat species, however (Fig. 13), the MSO of the big brown bat and the free-tailed bat contain nearly 40% sustained responders whereas in the mustached bat these make up <20% of the total. In this respect, the MSO of the big brown bat resembles that of the free-tailed bat. However, transient OFF responders are virtually absent in the free-tailed bat (Grothe et al. 1997) but are rather common in the big brown bat and mustached bat MSO (Covey et al. 1991; Grothe 1994). Therefore the big brown bat again occupies an intermediate position between the mustached bat and the free-tailed bat.

**Possible function of MSO in bats**

From this and other studies (Grothe 2000; Grothe and Park 1998), it becomes clear that the bat MSO did not evolve into a processor of ITDs as did that of mammals with well-developed...
The interaction of excitatory and inhibitory inputs temporally offset from one another (Grothe 1994; Grothe et al. 1997). Interactions of excitatory and inhibitory inputs with different time courses have also been reported to shape response properties in auditory midbrain neurons (Burger and Pollak 1998; Covey et al. 1996; Klug et al. 1999) and, therefore, seem to represent a fundamental principle in temporal auditory processing. One consequence of this interaction in the MSO is low-pass filtering for modulated sounds. Low-pass filtering has been described in the mustached bat, the freetailed bat and, in the present study, the big brown bat. Thus temporal processing of the stimulus envelope seems to be an important function of MSO neurons in bats. This is most obvious in the mustached bat MSO where the vast majority of cells are monaural and therefore function independently of binaural cues. Mustached bats emit echolocation calls that contain a long CF component. This CF component represents a special adaptation for hunting in the foliage. Echoes from stationary objects will contain a more or less unchanged CF component, but wing beats from fluttering insects will impose sinusoidal amplitude and frequency modulations on the CF component (Henson et al. 1987). Thus the temporal structure of echoes from branches or foliage differs significantly from that of echoes from fluttering prey, allowing the bat to hunt flying insects even in cluttered environments. Filtering AM at an early stage of auditory processing could have been part of the evolutionary process that led to the bat’s ability to utilize nocturnal flying insects as a food resource. The big brown bat hunts in cluttered spaces as well as in the open, so it is not surprising that its echolocation signals incorporate both CF and FM features. In contrast, free-tailed bats do not hunt in foliage and thus have no special need to recognize insects by their wing beats. For this reason, they almost exclusively use FM sweeps as echolocation calls.

Another possible function of SAM filtering that has not yet been mentioned is processing communication calls. The mustached bat uses a surprisingly wide repertoire of communication calls (Kanwal et al. 1994), and many of these sounds contain rather complex temporal patterns of amplitude and frequency modulations. Temporal filters for repetitive components as well as markers for beginning or end of certain components of these calls might be important for processing these sounds and might have their origin in the MSO.

A possible function of MSO neurons in the big brown bat that we have not mentioned yet is related to the fact that some neurons in the inferior colliculus are tuned to narrow ranges of sound duration (Casseday et al. 1994, 2000; Ehrlich et al. 1997). Interactions of excitatory and inhibitory inputs temporally offset from one another (Grothe 1994; Grothe et al. 1997). One interesting finding is that neurons with phasic responses to pure tones show better phase-locking to SAM stimuli than do neurons with sustained responses to pure tones. Most strik-
ingly, off responding neurons phase-lock best to SAM. A similar difference has been shown previously for sustained and off responding neurons in the medial superior olivary complex of the rabbit (Kuwada and Batra 1999). In the rabbit, off neurons show low-pass filter characteristics similar to those seen in the MSO of bats (Grothe 1994; Grothe et al. 1997; this study). The only difference is that bat MSO sustained responders also exhibit low-pass filter characteristics to SAM stimuli whereas those in the medial area of the SOC in the rabbit do not (Kuwada and Batra 1999).

The finding that neurons in the medial area of the SOC of rabbits and bats show specific filter characteristics for the temporal structure of sounds indicates that the SOC plays a major role in temporal processing in all mammals.

**Role of modulation frequency and interpulse-interval in AM-filtering**

In terms of understanding both the function of MSO and the mechanisms that determine MSO neurons’ responses to sound, it is important to stress that our data indicate that the major component that defines the cutoff frequency for modulated stimuli or trains of tone bursts is not the modulation frequency, the repetition rate, or the duty cycle, but rather the IPI. The cutoffs for SAM modulation frequency simply represent a byproduct of filtering for IPI. This finding is consistent with the model of temporal interactions in MSO neurons proposed earlier (Grothe 1994) as well as the model explaining the ITD sensitivity of MSO cells in the free-tailed bat to the envelope of SAM stimuli (Grothe and Park 1998). One question that arises is whether the SAM filtering reported for other auditory structures including the auditory midbrain and cortical areas (review: Langner 1992) is \( F_{\text{mod}} \) specific or is a byproduct of processing other temporal aspects of complex sounds. For example, neurons with band-pass filter properties for sound duration in the auditory midbrain of various tetrapod species (frog: Gooler and Feng 1992; bat: Ehrlich et al. 1997; guinea pig: Chen 1998; mouse: Brand et al. 2000) might respond to only a small range of SAM frequencies if excitation from MSO provides the temporal sequence of inputs that yields suprathreshold depolarization. Neuropharmacological studies of neurons in the inferior colliculus suggest that the low-pass filter characteristics of these neurons to SAM are not created through the interaction of excitatory and inhibitory inputs but rather are due either to intrinsic properties of the neurons or low-pass filter characteristics of an excitatory input (Burger and Pollak 1998). Our data suggest that the source of such an input could be the MSO.

If it is not the modulation frequency per se but rather the IPI that determines the ability of MSO neurons to follow SAM, does the notion hold that the large, monaural MSO in mustached bats and big brown bats is suited for identifying fluttering targets? Echoes coming from wing-beating insects are modulated in a quasi-sinusoidal fashion so IPI and modulation frequency are linked, with IPI decreasing as modulation rate increases. Thus cells in the bat MSO could act as a first stage of neuronal filtering that helps extract temporal information contained in echoes from wing beating insects. The fact that the MSO in bats is large, compared with that of other mammals with high-frequency hearing, might reflect the behavioral need of these animals to encode signals that are amplitude modulated in the range of up to a few hundred Hz (Moss and Schnitzler 1995).

**Conclusions**

Across mammalian species, whatever the function or set of functions performed by the MSO may be, this function is invariably related to some form of temporal processing. The specific rule of processing that MSO neurons apply to the incoming signal appears to be comparable across species, the rule being that MSO neurons act as coincidence detectors for at least two separate inputs, the temporal relationship of which varies as a function of one or more stimulus parameters. Processing by MSO neurons creates filter characteristics for the temporal structure of a sound, with ITD sensitivity being only one consequence of this rule. In small mammals, temporal filtering by MSO neurons might be more relevant for sound recognition than for sound localization. In dogs, cats, and other large mammals with well-developed low-frequency hearing, the MSO might well be involved in both functions.

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