Medial Superior Olive in the Free-Tailed Bat: Response to Pure Tones and Amplitude-Modulated Tones

BENEDIKT GROTHE, THOMAS J. PARK, AND GERD SCHULLER
1Zoologisches Institut, Universität München, D-80333 Munich, Germany; and 2Neurobiology Group, Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60607-7060

INTRODUCTION

The superior olivary complex (SOC) is the first station of binaural interaction within the mammalian ascending auditory pathway. The medial superior olive (MSO), one of several subdivisions within the SOC, is traditionally thought to function in the context of sound localization (Goldberg and Brown 1968, 1969; Langford 1984; Masterton and Diamond 1967; Spitzer and Semple 1995; Yin and Chan 1990). Moreover, it has been suggested that the MSO is not involved in processing other stimulus parameters commonly related to pattern recognition, such as coding of the temporal structure of a stimulus envelope (Langner 1992; Masterton 1992). Although alternative functions such as processing of temporally coded spectral information (Loeb et al. 1983, on the basis of a concept of Licklider 1951) have been suggested, experimental evidence has not been available. There is strong evidence that the MSO in mammals with low-frequency hearing and with sufficient interaural distance acts as an interaural time difference (ITD) detector by comparing the exact arrival times of the binaural inputs. Several studies showed that MSO neurons may function as coincidence detectors because they respond maximally if the phase-locked low-frequency inputs from the two ears arrive simultaneously (“in phase”) and minimally if the inputs arrive asynchronously (“out of phase”; Goldberg and Brown 1969; Langford 1984; Spitzer and Semple 1995; Yin and Chan 1990).

Recent results, however, call into question the general applicability of this concept for all mammals. In particular, many bats do not hear low frequencies well and have small interaural distances. Nevertheless, there is strong anatomic evidence for the existence of an MSO in all bats investigated so far (Grothe et al. 1994; Poljak 1926; Schweizer 1981; Schweizer and Radke 1980; Zook and Casseday 1982a, b). These results suggest that MSO neurons, at least in small mammals, serve functions additional to or other than coding ITDs. To date, there are two lines of evidence that MSO neurons are involved in the processing of other stimulus parameters instead or in addition to ITDs. First, it has been shown that the MSO in the mustached bat is particularly well suited for filtering amplitude modulations (Grothe 1994). However, the MSO in this bat is unique (as far as we know) in that it is a functionally monaural nucleus lacking significant ipsilateral inputs (Covey et al. 1991; Grothe et al. 1992). Second, intracellular recordings from a gerbil brain slice preparation revealed that MSO units are not only sensitive to the timing of the bilateral inputs, but also exhibit filter characteristics for the repetition rate of pulse trains (Grothe and Sanes 1994). However, it has not yet been tested in vivo whether these filter characteristics are functional in a binaural MSO. These results raise three questions.

Grothe, Benedikt, Thomas J. Park, and Gerd Schuller. Medial superior olive in the free-tailed bat: response to pure tones and amplitude-modulated tones. J. Neurophysiol. 77: 1553–1565, and Brown 1968, 1969; Langford 1984; Masterton and Diamond 1967; Spitzer and Semple 1995; Yin and Chan 1990). Moreover, it has been suggested that the MSO is not involved in processing other stimulus parameters commonly related to pattern recognition, such as coding of the temporal structure of a stimulus envelope (Langner 1992; Masterton 1992). Although alternative functions such as processing of temporally coded spectral information (Loeb et al. 1983, on the basis of a concept of Licklider 1951) have been suggested, experimental evidence has not been available. There is strong evidence that the MSO in mammals with low-frequency hearing and with sufficient interaural distance acts as an interaural time difference (ITD) detector by comparing the exact arrival times of the binaural inputs. Several studies showed that MSO neurons may function as coincidence detectors because they respond maximally if the phase-locked low-frequency inputs from the two ears arrive simultaneously (“in phase”) and minimally if the inputs arrive asynchronously (“out of phase”; Goldberg and Brown 1969; Langford 1984; Spitzer and Semple 1995; Yin and Chan 1990).

Recent results, however, call into question the general applicability of this concept for all mammals. In particular, many bats do not hear low frequencies well and have small interaural distances. Nevertheless, there is strong anatomic evidence for the existence of an MSO in all bats investigated so far (Grothe et al. 1994; Poljak 1926; Schweizer 1981; Schweizer and Radke 1980; Zook and Casseday 1982a, b). These results suggest that MSO neurons, at least in small mammals, serve functions additional to or other than coding ITDs. To date, there are two lines of evidence that MSO neurons are involved in the processing of other stimulus parameters instead or in addition to ITDs. First, it has been shown that the MSO in the mustached bat is particularly well suited for filtering amplitude modulations (Grothe 1994). However, the MSO in this bat is unique (as far as we know) in that it is a functionally monaural nucleus lacking significant ipsilateral inputs (Covey et al. 1991; Grothe et al. 1992). Second, intracellular recordings from a gerbil brain slice preparation revealed that MSO units are not only sensitive to the timing of the bilateral inputs, but also exhibit filter characteristics for the repetition rate of pulse trains (Grothe and Sanes 1994). However, it has not yet been tested in vivo whether these filter characteristics are functional in a binaural MSO. These results raise three questions.
1) Do binaural MSO units in small mammals with mainly high-frequency hearing also act in the context of pattern recognition (e.g., filtering of amplitude modulations), as shown for the mustached bat and suggested for the gerbil?

2) Are binaural MSO neurons in small mammals sensitive to ITDs or interaural intensity differences (IIDs), and thus, do they play a role in coding azimuthal position? And if so, are the main features of ITD sensitivity of these units comparable with those of cat MSO neurons?

3) To what extent are ITD coding and amplitude-modulation filtering (or filtering of other features of a sound’s temporal structure) interdependent? In other words, is it possible to decide whether MSO neurons in small mammals are specialized for localization or pattern recognition?

Here we present data from in vivo recordings in the free-tailed bat, which is known to possess—a “common,” binaural MSO (Grothe et al. 1994). The data illustrate how the complex interaction of excitation and inhibition that occurs in the MSO influences the response to pure tones and to amplitude-modulated tones. We particularly used amplitude modulations because comparable data exist for the mustached bat (Grothe 1994). Also, amplitude modulation rate is an important feature of complex sounds, including human speech. In a subsequent paper we will address the question of how MSO neurons in the free-tailed bat respond to ITDs, relating our data to the common theories of coincidence detection and discussing the question of the evolution of ITD coding in mammals.

METHODS

Five free-tailed bats were used in this study. During surgery the bats were anesthetized with pentobarbital sodium (15 mg/kg) and methoxyflurane inhalation. Skin and muscles were deflected from the upper part of the skull and a metal rod that was later used to secure the bat’s head during recordings was mounted to the skull with the use of cyanoacrylate and dental cement. A small hole (0.5–1 mm diam) was cut over the inferior colliculus on one side. The stereotaxic procedure described by Schuller et al. (1986) was used to define the position of the skull and the position of the recording electrode.

Recording started after full recovery of the bat in a sound-attenuated and heated room (27–30°C). Water was offered to the bat repeatedly during recording sessions and in case of apparent discomfort small injections of pentobarbital sodium were given (<1.5 mg/kg).

Action potentials were recorded extracellularly with the use of glass pipettes filled with 1 M NaCl. Impedance of the recording electrodes ranged from 5 to 20 MΩ. The electrodes were advanced with a piezoelectric drive (Burleigh) from outside the recording chamber. Spikes from single units were fed via a recording amplifier, a band-pass filter (0.3–5 kHz), and a window discriminator into a computer. The software used for controlling stimulus presentation and recording was programmed by M. Baumann and S. Kieslich.

Acoustic stimuli were presented via custom-made earphones fitted to the ears with probe tubes (5 mm diam). The earphones were calibrated with the use of a 1/4-in. Bruel & Kjaer microphone, and showed a variability of less than ±3 dB over the frequency range used (15–80 kHz). Acoustic isolation between the two ears was >40 dB for all frequencies used in our experiments. Intensities between the two earphones did not vary more than ±3 dB.

Pure tones and sinusoidally amplitude-modulated (SAM) stimuli (100% modulation depth) were used as search stimuli. The stimuli were presented at a rate of four per second. A unit’s best (characteristic) frequency (BF) and threshold for both ears was determined to set stimulus parameters for subsequent control by computer. Rate-level functions were measured for both sides independently as well as for binaural stimulation (increasing SPL in 10-dB steps).

A rate-level function was defined as nonmonotonic if there was a decrease of >20% of spike rate for SPLs above the best SPL (the SPL that elicited the maximal response). Binaural characteristics were then tested by keeping the intensity at one ear 20 dB above threshold and changing the intensity of the opposite ear (10-dB steps) and vice versa. Both tests were performed with the use of pure tones (40 ms in duration) as well as with the use of SAM stimuli at 100- and 200-Hz modulation rates (duration 100 ms). Next, the responses to various SAM frequencies ranging from 20 to 1,000 Hz were tested for both ears independently and under various binaural conditions. Modulation transfer functions (MTFs) were determined in three ways: 1) by the entire late response (without the onset; see below) for each modulation frequency; 2) by calculating the mean number of spikes per cycle for each modulation frequency tested; and 3) by calculating the vector strength (formulas from Goldberg and Brown 1969) on the basis of peristimulus time histograms (PSTHs) with 64 bins per SAM cycle. ON responses (5 ms beginning with the 1st correlated spike) or the discharge to the first cycle (if 1 cycle was >5 ms) were excluded before the MTFs were calculated. Because of the limited time we were able to hold the cells in the awake animals and for reasons of comparison with the SAM data from the mustached bat MSO (Grothe 1994), we only focused on the upper cutoffs of the MTFs and ignored lower cutoffs that were visible in some neurons at <40-Hz modulation frequency. In some neurons the responses to different carrier frequencies for SAM and pure tones were tested to determine inhibitory sidebands, with the use of a suppression of spontaneous activity as criterion (20 dB above threshold at BF). In some units we measured relative delay and relative timing of inhibitory inputs with the use of brief (2 ms) downward frequency-modulated (FM) sweeps ranging from 5 kHz above a neuron’s BF down to 5 kHz below BF. These sweeps cause the shortest response we could elicit (only 1, sometimes 2 spikes) and presumably also the shortest inhibitory impact. FM sweeps were presented at a variety of electronically induced ITDs. Each test signal was presented either 10 or 20 times.

Recording sites were confirmed by small horseradish peroxidase (HRP) injections at the end of each experiment. Perfusion and histology procedures followed Vater and Feng (1985).

RESULTS

Anatomic connections of MSO neurons in the free-tailed bat

The main connections of the free-tailed bat MSO have been described earlier (Grothe et al. 1994) by injections of HRP and fluorescent beads into the central nucleus of the inferior colliculus, the medial nucleus of the trapezoid body (MNTB), and different subdivisions of the cochlear nucleus. Our HRP injections into the MSO confirm these earlier results that exhibited projections to the MSO originating in both anteroventral cochlear nuclei (AVCNs) and in the ipsilateral MNTB. They also confirmed the bilateral projections to the central nucleus of the inferior colliculus. Additionally, the HRP injections into the MSO revealed a fourth MSO input from the ipsilateral lateral nucleus of the trapezoid body (LNTB) to the MSO. Figure 1 presents the connectional pattern of the free-tailed bat MSO receiving two excitatory inputs from the AVCNs and two putative inhibitory
Distribution of BF

We recorded from 60 MSO units. Their BFs covered the range from 9 to 81 kHz, with a bias to BFs between 20 and 30 kHz (21 = 35%). This corresponds well with the audiograms and the biosonar signal used by the free-tailed bat. Its echolocation call consists of a multiharmonic downward FM sweep that ends in a constant-frequency component (Fenton and Bell 1981) with the fundamental at ~25 kHz (Simmons et al. 1978). The distribution of BFs is shown in Fig. 2A.

Binaural characteristics

Consistent with the bilateral inputs to the MSO, the majority of neurons (55%, 33 of 60) could be stimulated by presentation of pure tones or amplitude-modulated tones to either ear alone, but (except for 2 neurons) these cells also exhibited indirect evidence for inhibitory inputs from both ears (EI/EI). Evidence of inhibition included phasic response patterns, nonmonotonic rate-level functions, low SAM cutoffs, and IID functions with decreasing response for certain IIDs. This, of course, only suggests inhibitory influences and will be discussed in detail in the DISCUSSION. About 40% of MSO units (24 of 60) could not be driven by the ipsilateral ear and for these units our data did not provide any evidence for facilitatory effects from the ipsilateral ear either. However, most of these units (17 of 24) exhibited prominent inhibition elicited by stimulation of the ipsilateral as well as by stimulation of the contralateral ear (I/EI neurons). In five of these I/EI-type units, ipsilateral induced inhibitory effects could only be seen when the signal at the ipsilateral ear was advanced or delayed a few milliseconds. The remaining neurons showed no effects from ipsilateral stimulation but could be excited from contralateral stimulation with signs of inhibitory effects (O/EI neurons). Three units were excited by ipsilateral stimulation but without apparent contralateral influences (EI/O units). The distribution of binaural types is summarized in Fig. 2B.

Discharge patterns

The majority of MSO neurons (64%, 38 of 60) responded with a phasic discharge pattern when stimulated with a pure tone. About 1/3 showed a sustained discharge throughout the duration of the pure tone.
tone 20 dB above threshold. Most of these units (35) exhibited a phasic ON response; three showed a phasic OFF response. Sustained discharge patterns were found in 21 (36%) of units, mostly in the form of a primary-like discharge including a prominent ON response (19%), or as a tonic response without a prominent ON component (14%). Two neurons exhibited a chopper and two other neurons a pauser discharge pattern. The distribution of response patterns is shown in Fig. 2C.

Rate-level functions: pure tones

We measured rate-level functions from 43 MSO neurons with the use of ipsilateral, contralateral, or binaural stimulation. The rate-level functions were measured with the use of 100-ms pure tones, with the stimulus intensity increasing in 10-dB steps from threshold up to 40 dB above threshold. Because of the small interaural distance in this small animal, there is an acoustic cross talk from one to the other ear that occurs at ~45–50 dB. Therefore purely monaural rate-level functions can only be measured over a range of ~40 dB. For higher levels, binaural effects complicate the interpretation. In general, we observed a similar proportion of monotonic and nonmonotonic functions in response to pure tones. Of 41 neurons tested with the use of contralateral stimulation, 17 exhibited monotonic rate-level functions whereas 24 had nonmonotonic rate-level functions. Of 23 neurons tested for ipsilateral stimulation alone, 12 cells revealed monotonic rate-level functions and 11 neurons showed nonmonotonic rate-level functions.

Twenty-two neurons were tested with the use of ipsilateral, contralateral, and binaural stimulation (Fig. 3A). Roughly one third (7 of 23) exhibited monotonic rate-level functions for monaural stimulation of each side as well as for binaural stimulation. Another third (9 of 23) had a monotonic function for one side and a nonmonotonic function for the other. All but one of these units showed nonmonotonic functions for binaural stimulation. The remaining third of the neurons (7 of 23) revealed nonmonotonic rate-level functions for both sides alone as well as for binaural stimulation. Figure 3B shows representative rate-level functions of a single cell having a monotonic rate-level function for ipsilateral and for contralateral stimulation.

Rate-level functions: SAM stimuli

As shown above, a substantial population of cells had monotonic rate-level functions for stimulation with pure tones. The situation changed dramatically when MSO neurons were tested with the use of SAM stimuli (N = 23). Only 1 of 9 neurons for ipsilateral stimulation, and 1 of 14 neurons for contralateral stimulation, had a monotonic rate-level function for SAM tones with a 200-Hz modulation rate. All others had nonmonotonic rate-level functions for SAM stimuli, independent of their rate-level function for pure tones. Figure 4A shows the distribution of rate-level functions for SAM stimuli. Figure 4B shows rate-level functions from the same unit as in Fig. 3B but now for SAM stimuli. Whereas this neuron exhibited monotonic rate-level functions for pure tones, it revealed nonmonotonic rate-level functions for SAM stimuli.

For the cell shown in Fig. 4B, the peak of the nonmonotonic rate-level functions was at 20 dB above threshold for ipsilateral stimulation and at 30 dB above threshold for contralateral stimulation. For higher SPLs, spike counts decreased rapidly for both conditions. Thus the best SPL could differ significantly between the ipsilateral and contralateral side. For another neuron this is demonstrated by PSTHs and rate-level functions in Fig. 5. For contralateral stimulation the neuron responded best at 43 dB SPL to the 200-Hz SAM stimulus. At this SPL it exhibited a phase-locked response. For higher SPLs only an ON response persisted, whereas the phase-locked late component vanished. For ipsilateral stimulation there was only a weak excitation at 33 and 43 dB SPL and no response to higher amplitudes. However, for binaural stimulation (both ears at the same level) the maximal response was shifted to higher levels at ~53 dB SPL, including a strong phase-locked late component.

**IDs affect the response to SAM**

The example in Fig. 5 shows how stimulating both ears separately with the same intensity could generate very different spike rates. The rate-level functions revealed that high intensities at either ear evoked a strong suppression, most
MSO neurons phase lock to SAM at low modulation rates

We tested the response of 53 MSO neurons to 100-ms SAM stimuli with the use of the units’ BFs as the carrier frequency and varying the modulation rate between 20 and 1,000 Hz. We used 100% modulation depth only. The vast majority of neurons (45) exhibited a discharge that was phase locked to the envelope of low-frequency amplitude modulations with a minimum of 0.5 spikes/cycle, mostly with ≥1 spikes/cycle for certain modulation rates (Fig. 7, for example). All these units exhibited a selectivity for the modulation rate that will be discussed in detail below. Only eight units showed no phase locking at all. These units showed an ON response followed by a weak sustained response. In six of these eight neurons the response pattern as well as the number of spikes elicited by the SAM stimuli did not change when the modulation rate was varied. Thus these neurons were unselective for the modulation rate. In two of these units the response decreased for higher modulation rates.

MSO units exhibit low-pass filter characteristics for SAM rate

The main feature of the SAM selectivity of the 45 neurons that exhibited phase-locked responses to SAM was a low-pass filter characteristic for the modulation rate. Figure 7 shows typical examples of responses from an EI/EI neuron (Fig. 7, left) and an I/EI neuron (Fig. 7, right) in response to binaural SAM stimulation with different modulation rates (equal intensities at both ears). The PSTHs, derived from 20 presentations, illustrate the precise phase locking of these neurons to modulation rates <200 Hz. To each SAM cycle the unit responded with one spike, sometimes with two spikes. At 200-Hz modulation rate the units still phase locked but with <1 spike/cycle. Only the ON response (2–3 spikes to the 1st modulation cycle) was not affected by the increasing modulation rate. In contrast, the response to the following cycles (“late response”) decreased progressively and at >500 Hz no substantial response remained.

The MTFs calculated from the number of spikes per SAM cycle, and the firing synchronization to the stimulus (vector strength; Goldberg and Brown 1969) for the two neurons from Fig. 7 are shown in Fig. 8. Both neurons exhibited clear low-pass filter characteristics that were typical for the vast majority of MSO neurons. In contrast, not a single neuron we recorded in LNTB, MNTB, and lateral superior olive (LSO) exhibited such low-pass filter functions (data not shown).

We determined the 75% and 50% cutoff in the MTFs, on the basis of spike counts per SAM cycle, from a total of 45 MSO neurons (41 for contralateral, 21 for ipsilateral, and 22 for binaural stimulation). Because the majority of MSO neurons responded with a phasic response to pure tones (see above) as well as to each individual SAM cycle (as long as there was a response), spike count per cycle was the most straightforward and meaningful measurement. Counting total spikes per stimulus often did not reveal the decay in the temporal information, because a decreasing response per cycle can be to some extent compensated for by the increasing number of amplitude-modulated cycles per 100 ms, par-

FIG. 4. Rate-level functions of MSO neurons in response to sinusoidally amplitude-modulated (SAM) tones. For this type of stimulation the vast majority showed nonmonotonic rate-level functions for stimulation at either ear alone (n/n). Only a few neurons showed monotonic rate-level functions for both ears (m/m) or for 1 ear (m/n). B: rate-level functions in response to SAM stimuli for the same neuron shown in Fig. 3B. In contrast to pure tone stimulation, SAM stimuli evoked nonmonotonic functions for either ear.

likely due to inhibition (see DISCUSSION). To test whether the inhibition evoked from stimulating one ear can suppress spikes evoked from stimulating the other ear, we presented binaural stimuli and varied the IID. In 22 MSO neurons with EI/EI response characteristics, we tested the response to SAM stimuli at different IIDs. In 17 of these units, IIDs in either direction (favoring the ipsilateral or the contralateral ear) of ≥10 dB resulted in a decrease of spike rate of ≥25% (up to 90%). In all cases the response dropped below that to monaural stimulation at the side with the fixed intensity, as shown for the EI/EI neuron in Fig. 6. Thus a large majority of MSO neurons exhibited a clear sensitivity for IIDs, probably—at least partly—as a consequence of the different strength of inhibition that was also apparent in the rate-level functions.

Rather predictable were the effects of different IIDs on the response to SAM stimuli in I/EI neurons. The response was stable until the ipsilateral stimulus was 10 dB more intense than the contralateral stimulus, resulting in a reduction of spikes. For even higher levels at the ipsilateral side the response to the contralateral stimulus was completely inhibited. Thus I/EI MSO units exhibited IID functions comparable with those seen in many other excitatory/inhibitory cells in other auditory nuclei.
particularly in neurons with a maximum of 1 or 2 spikes/cycle. In ~50% of the neurons, vector strength followed the MTF on the basis of spikes per cycle; in the other half of the neurons the vector strength stayed high (>0.5) until the calculated value was no longer significant, because of the strong decrease in the spike count (Rayleigh test; Mardia 1972).

For 14 EI/EI units we could obtain MTFs under all three conditions (ipsilateral, contralateral, and binaural). For all three conditions the majority of cells revealed cutoffs at <300-Hz modulation rate.

Figure 9, top, presents a group analysis of the MTF cutoffs for all neurons tested. The distributions of neurons in this statistic were similar for the three test situations (ipsilateral, contralateral, and binaural stimulation), suggesting that ipsilateral and contralateral cutoffs are matched. However, for individual neurons the cutoffs for the three stimulus conditions varied. In fact, the MTF cutoffs were matched (less than ±25% of the contralateral cutoff frequency) only in one neuron (Fig. 9, bottom; examples in Fig. 10). Of 17 neurons that were tested for both ipsilateral and contralateral stimulation, 6 units had higher cutoffs for ipsilateral stimulation, 10 neurons for contralateral stimulation. Thus there was a considerable mismatch in the ipsilateral and contralateral MTF cutoff frequency that is obscured in the population statistic.

There was no straightforward way to predict the cutoffs on the basis of the MTFs for binaural stimulation by analyzing the MTFs for monaural stimulation. Figure 10 shows two examples of ipsilateral, contralateral, and binaural MTFs. In the first example (Fig. 10A) the MTF for binaural stimulation is close to that of contralateral stimulation with similar cutoffs. In the second example (Fig. 10B) the cutoff frequency is much higher for ipsilateral than for contralateral and binaural stimulation. In 8 of 14 EI/EI cells that were tested with ipsilateral, contralateral, and binaural stimulation, the binaural filter cutoff frequency was identical or at least much closer to that from contralateral stimulation (±50 Hz) compared with that from ipsilateral stimulation, whereas in 5 neurons it was closer to the ipsilateral MTF cutoff, and in 1 neuron it differed from both monaural tests by >50 Hz. In three of five I/EI units the binaural SAM cutoff was
MSO IN THE FREE-TAILED BAT 1559

In the mustached bat MSO, a scenario of monaural interaction of excitation and inhibition was proposed (Grothe 1994) that implies a temporal mismatch of excitation and inhibition that caused an overlap of the inhibition elicited by one SAM cycle with the excitation evoked by the following cycle (Fig. 12).

To test whether this explanation holds for the free-tailed bat’s binaural MSO neurons, we measured the relative delay below that for contralateral stimulation; in two neurons it was identical to the MTF for contralateral stimulation.

In a previous section (IIDs affect the response to SAM) we described how changing the IID changed the spike rate to a fixed SAM rate. We did not investigate the impact of IIDs on SAM cutoffs systematically. However, in four of five EI/EI units that were tested the MTF cutoffs changed if the IID was varied. One example is shown in Fig. 11. At an IID of +30 dB (favoring the contralateral ear) the cutoff was at ~95 Hz; for 0 dB IID ~ 75 Hz; and for ~30 dB IID (favoring the ipsilateral ear) ~ 65-Hz modulation rate. Thus in this neuron there was a systematic decrease of MTF cutoff with increasing ipsilateral intensity. However, the five neurons tested did not respond in a uniform way. One other unit shifted like the neuron shown in Fig. 11, one neuron exhibited MTF shifts in the opposite direction, one neuron responded in a nonlinear way, and one did not show any shifts in the MTF cutoff.

Summarizing, our data show that 1) most units exhibited different MTFs for ipsilateral, contralateral, and binaural stimulation; 2) IIDs strongly influenced the SAM stimuli usually in an unpredictable way; and 3) in the few units we tested for MTF cutoffs at different IIDs the filter characteristics differed for different IIDs. Because IIDs are a function of azimuthal position, these results indicate that the SAM filter characteristic of MSO cells in the free-tailed bat is not robust against changes in the spatial location of a sound source. Therefore the response of these units is an integrated result of both the temporal structure of a stimulus (e.g., amplitude modulations) and the spatial location of a sound.

SAM filter: interaction of excitation and inhibition

So far we have presented data indicating that most MSO units have low-pass filter characteristics for SAM stimuli that are comparable with those shown for monaural MSO neurons in the mustached bat. To explain these filter characteristics in the mustached bat MSO, a scenario of monaural interaction of excitation and inhibition was proposed (Grothe 1994) that implies a temporal mismatch of excitation and inhibition that caused an overlap of the inhibition elicited by one SAM cycle with the excitation evoked by the following cycle (Fig. 12).

To test whether this explanation holds for the free-tailed bat’s binaural MSO neurons, we measured the relative delay...
and duration of inhibition that one ear imposed on the excitation from the other ear. We then compared these results with the SAM rate cutoff for the same cell. Because we were interested in the relative timing of the inputs from the two ears, we chose brief (2 ms) frequency sweeps as a stimulus that generates very precisely time-locked responses. Even though the absolute time course of inhibition in response to an FM sweep is most likely different from that to an SAM stimulus, we reasoned that the FM sweep was appropriate for examining the relative timing of binaural excitatory and inhibitory inputs. Sweeps descended from 5 kHz above to 5 kHz below a unit’s BF, usually evoking a single spike per presentation within a very narrow time window. We then introduced ITDs, delaying first one ear and then the other ear by different amounts. This allowed us to measure the time period during which contralaterally induced inhibition was able to suppress ipsilateral evoked action potentials and vice versa.

The results of one of the cells tested with frequency sweeps are shown in Fig. 13, A and B. These graphs show the relative spike rates elicited by the FM sweeps as a function of ITD. Figure 13A shows the relative spike rate evoked by stimulation of the ipsilateral ear, whereas Fig. 13B shows the relative spike rate evoked by stimulation of the contralateral ear. We were able to distinguish spikes evoked by ipsilateral stimulation from spikes evoked by contralateral stimulation on the basis of the time of occurrence of the spikes. For example, when the ipsilateral signal was advanced in 0.25-s steps relative to the contralateral signal, the spikes evoked from the ipsilateral ear also advanced in 0.25-ms steps relative to the spikes evoked from the contralateral ear. The only condition in which we could not distinguish ipsilaterally driven from contralaterally driven spikes was when the signals were presented simultaneously (ITD less than \( \pm 0.5 \) ms). The curve in Fig. 13A shows that ipsilaterally evoked spikes were inhibited by contralateral stimulation when the ipsilateral signal was delayed by up to 2.5 ms relative to the contralateral signal. When the ipsilateral signal was delayed by \( >2.5 \) ms, the spike rate increased to \( >50\% \) of maximum, indicating that inhibition was arriving too far ahead of the excitation to effectively suppress spikes.

From these data, we can estimate that the relative timing and duration of the inhibition from the contralateral ear last from 0.5 to 2.5 ms relative to the ipsilateral evoked spike. Figure 13B shows the inhibitory effects of ipsilateral stimulation on contralaterally evoked spikes for the same cell. For nine EI/EI neurons inhibitory durations were calculated as described above, with the use of the 50% point of the functions. The end of the period of contralateral inhibition varied

---

**Fig. 8.** Normalized modulation transfer functions (MTFs) of the 2 neurons shown in Fig. 7 for spikes per SAM cycle (---) and vector strength (---). Maximum spikes per cycle were 0.95 (A) and 2.15 (B), respectively. The neurons exhibited low-pass filter characteristics for both measurements.

**Fig. 9.** Top: distribution of upper 50% cutoffs in the MTFs (spikes/cycle) for ipsilateral, contralateral, and binaural stimulation. For all 3 conditions, the majority of cells had cutoffs at \( <200 \) Hz SAM. The plot shows similar distributions for all 3 conditions with no obvious dependency on BF. Bottom: despite the similarity in the distribution of cutoffs, there is a considerable mismatch for the ipsilateral and contralateral cutoffs in individual neurons. Only 1 of 17 neurons tested for ipsilateral and contralateral cutoffs matched. Dotted lines: range of \( \pm 25\% \) of the contralateral cutoff.
FIG. 10. Normalized MTFs for spikes per SAM cycle (---) for ipsilateral, contralateral, and binaural stimulation (0 IID) for 2 EI/EI neurons. The neuron shown in A had similar cutoffs for all 3 conditions. In contrast, the neuron shown in B showed similar MTFs for binaural and contralateral stimulation, but a very different function for ipsilateral stimulation. Shaded areas: range of 50% cutoffs.

FIG. 11. Normalized MTFs for spikes per SAM cycle (---) for binaural stimulation at 3 different IIDs (+30, 0, -30; contralateral ear held constant). Positive IIDs: higher intensities at the ipsilateral ear. For more positive IIDs, the MTF systematically shifted to higher cutoffs. Shaded area: range of 50% cutoffs.

FIG. 12. SAM filter mechanism as proposed for monaural neurons in areas: range of 50% cutoffs. The mustached bat’s MSO. Temporal interplay of excitation and delayed inhibition will allow a phasic response to each SAM cycle, as long as the SAM rate is low (top). At higher amplitude modulation rates, the delayed inhibition evoked by 1 cycle overlaps with the excitation evoked by the next cycle, thereby suppressing the response to all but the 1st amplitude modulation cycle. Top line in each panel: hypothetical output of an MSO cell (schematic PSTH). Flat bars: excitatory (excitatory postsynaptic potential [EPSP]) and inhibitory (inhibitory postsynaptic potential [IPSP]) inputs from the contralateral ear. Bottom of each panel: envelope of the SAM stimulus and assumed threshold for eliciting excitatory and inhibitory inputs to the MSO neuron (adapted from Grothe 1994).

between neurons from 1.35 up to 7 ms. The range for ipsilateral induced inhibition was similar.

Because of the different nature of the stimuli, we could not directly predict the SAM cutoff for contralateral MTFs from the data collected with sweeps. However, the relative timing of the inputs (not the absolute) should be similar for different stimuli in a given neuron. According to the scenario described above (Fig. 12), greater relative delays of inhibition (relative to other neurons) are expected to correlate with lower SAM cutoffs. Figure 13C shows such a correlation of the ITD 50% points measured as described for seven EI/EI neurons and the corresponding 50% SAM cutoffs for contralateral stimuli.

**Phase locking to SAM depends on the carrier frequency**

In all recordings described so far, the BF of each neuron was used as the carrier frequency for the SAM stimuli. We tested the response to favorable SAM rates for different carrier frequencies in four neurons with clear phase locking. Three of these four units exhibited a strong dependency of
shown in Fig. 14 the discharge rate was reduced ~50% below spontaneous activity from 3.12 to 1.5 spikes/s). In all three units, best phase locking could be seen at frequencies close to the inhibitory border. Further away from the

the late response to SAM stimuli on the carrier frequency. Systematic variation of the carrier frequency revealed four major response types to different frequency ranges: 1) phasic responses (ON or OFF discharge patterns), 2) phase-locked responses, 3) sustained responses but without phase locking, and 4) complete inhibition of any response and spontaneous activity. Typically (as in Fig. 14), a sharp inhibitory area was at the high (2 of 3 cells) or the low (1 of 3 cells) end of the response area, creating a sharp border (in the neuron

FIG. 13. Response of an EI/EI neuron to short (2 ms) FM sweeps as a function of interaural time difference (ITD). The neuron responded with only 1–2 spikes per sweep to contralateral or ipsilateral stimulation. A: relative spike rate evoked by ipsilateral stimulation for a variety of electronically induced ITDs. B: relative spike rate evoked by contralateral stimulation for the same cell. Gray area: range of ITDs that caused a reduction of 50% (or more) in the normalized spike rate. See text for a more detailed explanation. C: correlation of duration of contralaterally driven inhibition derived from graphs like that in A and the 50% cutoff for the cell’s contralateral MTF. Asterisk: data point corresponding to the cell shown in A.

FIG. 14. PSTHs of an EI/EI neuron in response to 5 repetitions of a 200-Hz SAM stimulus (binaural stimulation, 0 IID) at different carrier frequencies. The response area was limited at both high and low frequencies by inhibitory sidebands, resulting in a discharge at ~50% below spontaneous activity (1.5 and 3.12 spikes/s, not shown). The neuron showed a weak sustained response at ~20 kHz, an ON-dominated response at 22 and 24 kHz, and phase locking (vector strength > 0.3) to the envelope only for carrier frequencies at the high portion of the frequency response area close to the high-frequency inhibitory sideband (26 kHz).
inhibitory sideband, the dominating responses were either sustained, but not phase-locked, or phasic responses. The fourth neuron responded with an OFF discharge to pure tones. This neuron phase locked to SAM stimuli over the entire frequency response range.

The data described above suggest that not only the spatial context but also the spectral composition of a sound will determine the discharge of an MSO neuron to amplitude modulations.

**DISCUSSION**

The main results from this study are as follows. 1) The majority of MSO cells we tested in the free-tailed bat receive excitatory and inhibitory projections from both ears, creating EI/EI response characteristics. Cells that varied from this pattern were usually lacking ipsilateral excitation and/or ipsilateral inhibition. 2) MSO neurons exhibit a clear low-pass filter characteristic for amplitude-modulated sounds. 3) Because of the binaural interaction in these units, the filter characteristics for SAM rate are IID dependent and thus should change with azimuthal position of a sound source.

**General response patterns**

In this study we recorded from neurons that basically covered the entire range of hearing of the free-tailed bat, ranging from 10 to 100 kHz (Schmidt et al. 1990). There was a bias toward BFs in the range of 20–30 kHz, which corresponds to the fundamental of the echolocation call of these animals (Simmons et al. 1978). This result seems to be consistent with the BF representation in the MSO of the mustached bat, which is also biased to the frequencies dominating the biosonar signal (Grothe 1994). In this respect, the MSO in bats appears to differ from the MSO in other mammals, where mainly low frequencies are represented and only a small proportion of cells have BFs >4 kHz (Guinan et al. 1972a,b). These species differences may suggest that the MSO in bats (and maybe in other small mammals) is serving a function other than ITD detection of low-frequency sounds, the major function suggested for the MSO in larger mammals.

The distribution of response binaural types in the free-tailed bat’s MSO also suggests functions other than ITD coding. Even though the majority of cells is excited from both ears, there was a high proportion (40%) that exhibited no excitation or no effect at all from the ipsilateral ear. The latter type of neuron is typical for the MSO of the mustached bat. For the mustached bat roughly 90% of MSO neurons are driven only by the contralateral ear and exhibit no signs of ipsilateral inputs. Cells of this type make up a very small proportion of MSO neurons in the cat (Guinan et al. 1972b; Yin and Chan 1990) and the gerbil (Grothe and Sanes 1993, 1994), and ~11% in the dog (Goldberg and Brown 1969), but ~45% in the rat (Indbod and Feng 1981). In terms of response types, the MSO in the free-tailed bat appears to represent a mixed nucleus positioned somewhere between the mustached bat and the cat or gerbil MSO.

**Inhibitory inputs to MSO neurons**

We did not test for the presence of inhibitory projections to the free-tailed bat’s MSO in a direct way, i.e., with the use of neuropharmacological techniques. However, there are four lines of evidence that inhibition is involved in processing acoustic stimuli in the free-tailed bat MSO, and all neurons tested showed at least one response feature indicating inhibitory influences.

First, AVCN spherical bushy cells are the source of excitatory inputs to MSO neurons in all mammals investigated so far (for review, see Schwartz 1992). These neurons are known to exhibit sustained discharge for the duration of a stimulus. In contrast, the majority of MSO neurons responded with a phasic response pattern. Thus phasic response patterns in the MSO are most likely explained by additional inhibitory inputs. In support of this idea, it has been shown that glycine inhibitory shapes phasic ON or OFF response patterns in the mustached bat’s MSO. These discharge patterns could be reversibly altered to primary-like discharge patterns when glycine inhibition was blocked by iontophoretically applying strychnine, thus revealing the pattern of the excitatory input (Grothe 1994).

Second, the late, phase-locked component of the response to SAM stimuli reacted in a nonmonotonic way when stimulus amplitude was increased, a response characteristic due to the interaction of excitation and inhibition above the level of the auditory nerve and more commonly observed in higher level neurons compared with more peripherally located neurons (for review, see Rhode and Greenberg 1992). Support for this idea comes from gerbil brain slice experiments. Increasing electric current by shocking fiber bundles projecting to the SOC caused glycine inhibition to block action potentials (Grothe and Sanes 1993).

Third, the low-pass filter characteristics for SAM rate appear to be mediated by inhibition. Glycine inhibition has been shown to create low-pass filter characteristics in MSO neurons for repetition rate of stimuli (gerbil) or SAM tones (mustached bat). MSO neurons in the mustached bat showed SAM filter characteristics similar to those in the free-tailed bat. In the mustached bat MSO, the presence of the glycine receptor blocker strychnine shifted or eliminated the low-pass filter characteristic. Neurons could then follow much higher modulation rates with a phase-locked discharge (Grothe 1994). Intracellular in vitro recordings revealed a limited ability of gerbil MSO neurons to follow trains of electric stimulation applied to the fibers entering the SOC. Most neurons exhibited, again, cutoffs between 100- and 300-Hz repetition rates that could be shifted to higher levels by addition of strychnine into the artificial cerebrospinal fluid (Grothe and Sanes 1994). In the present study, the results from the use of FM sweeps are consistent with the idea that inhibition shapes SAM filter characteristics.

Fourth, the MSO is innervated by two nuclei that are predominantly inhibitory. In all mammals investigated so far, there are prominent projections from the ipsilateral MNTP (Kiss and Majorosy 1983; Spangler et al. 1985; rodents: Kuwabara and Zook 1992; bats: Casseday et al. 1988; Covey et al. 1991). Additionally for the cat, several rodents, and several bats, a projection from the LNTB has been found (Cant 1991; Cant and Hyson 1992; Kuwabara and Zook 1992). These inputs are also found in the free-tailed bat (Grothe et al. 1994; this study). Both LNTB and MNTP neurons are glycineergic, and the MNTP is considered as one of the regions with the highest concentration of gly-
cine in the entire mammalian brain (Wenthold and Hunter 1990).

Therefore it is not surprising that MSO neurons exhibit numerous effects that can be easily explained by inhibition. However, the results from MSO in vivo recordings in cats (Yin and Chan 1990) and gerbils (Spitzer and Semple 1995) show little evidence of a significant role for inhibition in the MSO. But the available data are more or less limited to those from recordings in which pure tones or ongoing noise with ITDs were used. It might be that it is more difficult to assay inhibitory effects when pure tone stimuli are used. This is apparent from the data presented here (more inhibitory effects were observed for SAM stimuli compared with pure tones) and has also been suggested recently by Brughera et al. (1996). A final solution to the questions of the role of inhibition in the MSO requires direct evidence by neuropharmacological experiments in vivo.

Effect of IIDs

All of the binaural neurons we tested were sensitive to IIDs. The effect of changing IIDs was straightforward for neurons without ipsilateral excitatory inputs (I/EI neurons). I/EI neurons revealed IID functions comparable in many respects with those of LSO neurons in the same animal (compare Grothe and Park 1995): increasing SPL at the ipsilateral ear progressively inhibits the neuron’s response. IID sensitivity in LSO neurons is due to a subtraction of excitation from one ear and inhibition from the other ear (for review, see Irvine 1992). The difference is that whereas the inhibitory ear for LSO neurons is the contralateral ear, in MSO I/EI neurons there is excitation from the contralateral ear and inhibition from the ipsilateral ear. Thus these neurons prefer contralateral stimuli (opposite to LSO neurons), as do some MSO neurons in the dog (Goldberg and Brown 1969) and a substantial number of neurons in the rat MSO (Indbo and Feng 1981).

The response of EI/EI neurons to IIDs was more complex. The reason is that in these neurons an interplay of four inputs defines the neuron’s response: one excitatory and one inhibitory input from each ear. Presumably, differences in the strengths of these inputs could cause very different response patterns that can only be predicted on the basis of quantitative measurement of inputs that we did not perform.

Our data showed that not only the spike count, but also the filter characteristics for SAM stimuli, is influenced by IIDs. As a consequence, the discharge of an MSO neuron in the free-tailed bat will always be an integrated response to both azimuthal position and temporal structure of the stimulus.

What is the function of a binaural MSO in bats?

According to the duplex theory of hearing (Raleigh 1907; Stevens and Newman 1934), ITDs are the main cue for localizing low-frequency sounds. Free-tailed bats only hear high-frequency sounds and have an interaural distance most likely too small to generate resolvable ITDs. The question of how MSO neurons in the free-tailed bat respond to ITDs will be addressed in a subsequent paper. However, the data presented here indicate that MSO neurons in the free-tailed bat are sensitive to other features than ITDs. They act as filters for the temporal structure of a stimulus, i.e., for amplitude modulations. Amplitude modulations are a common feature in abiotic and biotic sounds (including communication signals and in the echoes of biosonar signals) and are thought to play an important role in pattern recognition (Langner 1992). In the context of hunting fluttering insects, bats might particularly benefit from analyzing the periodic components of a sound at an early stage of auditory processing (Casseday and Covey 1996). However, in contrast to the mustached bat’s MSO, the output of the free-tailed bat’s MSO appears to be an integrated one defined by stimulus pattern as well as stimulus position.

It remains an open question whether the interdependence of stimulus position and stimulus pattern also applies to the MSO output of other mammals. The only data available do not derive from in vivo studies but from a gerbil brain slice preparation. The conclusions drawn from these experiments predicted such an interdependence (Grothe and Sanes 1994). Therefore the results presented here might well represent common properties of the MSO in all mammals, and thus ITD coding might not be the exclusive function of the MSO in general.

We thank C. Schulte, S. Kieslich, and H. König for technical support. We thank M. Reed and N. Suga for detailed discussion of our data. We particularly thank G. Neuweiler, D. R. F. Irvine, G. D. Pollak, and J. J. Blum for the special interest that strongly influenced our experiments. This work was supported by SFB 204 and the Alexander-von-Humboldt Foundation.

Received 16 April 1996; accepted in final form 20 November 1996.

REFERENCES


GROTHE, B. Interaction of excitation and inhibition in processing of pure


