Response Properties and Location of Neurons Selective for Sinusoidal Frequency Modulations in the Inferior Colliculus of the Big Brown Bat

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Yue Q, Casseday JH, Covey E. Response properties and location of neurons selective for sinusoidal frequency modulations in the inferior colliculus of the big brown bat. J Neurophysiol 98: 1364–1373, 2007. First published July 18, 2007; doi:10.1152/jn.00432.2007. Most animals vocalizations, including echolocation signals used by bats, contain frequency-modulated (FM) components. Previous studies have described a class of neurons in the inferior colliculus (IC) of the big brown bat that respond exclusively to sinusoidally frequency modulated (SFM) signals and fail to respond to pure tones, noise, amplitude-modulated tones, or single FM sweeps. The aim of this study was to further characterize these neurons’ response properties and to determine whether they are localized within a specific area of the IC. We recorded extracellularly from 214 neurons throughout the IC. Of these, 47 (22%) responded exclusively to SFM. SFM-selective cells were tuned to relatively low carrier frequencies (9–50 kHz), low modulation rates (20–210 Hz), and shallow modulation depths (3–10% modulation). Most had extremely low thresholds, with an average of 16.5 ± 7.6 dB SPL, and 89% had upper thresholds and closed response areas. For SFM-selective cells with spontaneous activity, the spontaneous activity was eliminated when sound amplitude exceeded their upper threshold and resumed after the stimulus was over. These findings suggest that SFM-selective cells receive low-threshold excitatory inputs and high-threshold inhibitory inputs. SFM-selective cells were clustered in the rostrodorsal part of the IC. Within this area, best modulation rate appeared to be correlated with best carrier frequency and depth within the IC.

INTRODUCTION

Previous studies in bats have described a class of neurons in the inferior colliculus (IC) that respond exclusively to sinusoidally frequency modulated (SFM) signals (Casseday et al. 1997). In nature, the big brown bat, Eptesicus fuscus, encounters several sources of repetitive FM signals that in many respects resemble SFM. The echolocation calls that the bats emit while foraging consist of repetitive downward FM sweeps. During the different stages of hunting and prey pursuit, bats vary the frequency range of the FM sweeps that they emit, as well as their duration and repetition rate. During the searching phase of foraging, bats emit low-duration (~20 ms), low-frequency FM sweeps with a relatively narrow bandwidth (~28 to ~23 kHz), at a rate of ~30 Hz. As the bat approaches its prey, it increases the bandwidth of its call (~50 to ~23 kHz), decreases the duration (from ~20 to ~5 ms), and increases the repetition rate (from ~25 to 100 Hz) (Simmons 1989). When the bat is searching for prey and using long-duration, narrow-bandwidth signals, the wing beats of a flying insect could impose a pattern of periodic amplitude and frequency modulations on the reflections of these signals. The modulation pattern would correspond to the rate at which the insect beats its wings and would thus provide important information to help identify the insect species.

Another natural source of repetitive FM signals is the bats’ social communication calls. In Eptesicus, these signals contain both upward and downward FM sweeps. In the species of bats that have been studied, communication calls also include SFM-like signals (e.g., Gould 1971; Kanwal et al. 1994; Ma et al. 2006; Monroy et al. 2006). Studies in two different species of bats, Eptesicus fuscus (Gould 1971) and Pteronotus parnellii (Kanwal et al. 1994), show that the modulation rates of periodic FM in their communication signals are <200 Hz, suggesting that relatively slow rates of SFM are a general characteristic of bat communication calls.

In Eptesicus, SFM-selective neurons exhibit two forms of specialization (Casseday et al. 1997). First, they respond selectively to SFM, with little or no response to other types of stimuli including pure tones, amplitude-modulated signals, noise bursts, or single FM sweeps. Second, they are tuned to multiple parameters of SFM signals, including modulation rate and modulation depth. There is also some evidence suggesting that some SFM-selective neurons may be selective to low-amplitude sound. The finding of SFM-selective neurons is consistent with the finding of other forms of selectivity in IC neurons, including tuning to duration, the delay between two sounds, and FM direction (Casseday and Covey 1992; Casseday et al. 1994; Feng et al. 1978; Fuzessery 1994; Suga 1969). Here we address several interesting issues that were not examined in the previous study of SFM-selective neurons (Casseday et al. 1997).

One issue of particular interest is whether SFM-selective neurons are segregated within a particular part of the IC and whether one or more SFM parameters might be mapped in neural space. There is some evidence that other types of specialized neurons are segregated within the IC. Duration-tuned neurons in Eptesicus appear to be confined primarily to the caudal part of the IC (Ehrlich et al. 1997). In the rat, neurons selective for novel stimuli are mostly located in the dorsal and pericentral regions of the IC (Perez-Gonzalez et al. 2005). Moreover, there is some evidence that specific stimulus parameters are mapped in a more or less systematic fashion throughout a given dimension of the IC. These include best modulation rate for sinusoidally amplitude modulated (SAM) tones (Schreiner and Langer 1988) and response latency (Park and Pollak 1993). In addition to functional gradients, there are...
also corresponding connectional and biochemical gradients (Covey 2007). For example, terminals containing γ-aminobutyric acid (GABA) and glycine, as well as receptors for those neurotransmitters, have opposite gradient-like distributions within the IC (Fubara et al. 1996; Winer et al. 1995). Certain ion channels also have a gradient-like distribution within the IC (Rosenberger et al. 2003). The main goals of this study were to determine the tuning properties of SFM-selective neurons, especially their amplitude sensitivity, to compare their distribution to that of nonselective neurons, and to look for evidence of gradients in tuning to one or more parameters of SFM signals.

METHODS

Surgical procedures

The animals used in this study were 15 big brown bats (Eptesicus fuscus) of both sexes. Surgery was performed 1 day before recording. During the surgery, the bat was anesthetized with isoflurane. The bat’s head was held in a specially designed bite bar attached to manipulators that allowed the head to be rotated in three dimensions. Fine adjustments were made in the orientation of the skull so that it conformed to a standard stereotaxic position; then a metal post was attached to the skull with cyanoacrylate adhesive. The post was constructed so that the placement of the bat in the stereotaxic apparatus could be replicated precisely from one day’s recording session to the next. Each bat was used in one to six recording sessions, each lasting about 6 h, with one session per day. Between recording sessions, bats were housed in individual cages with unrestricted access to food and water. The cages were located in a room with controlled temperature and humidity.

Electrophysiological recording

The first recording session took place at least 1 day after attachment of the post. Before placement in the stereotaxic device, the bat was lightly tranquilized with a mixture of fentanyl (0.05 mg/ml) and droperidol (2.5 mg/ml), 0.0125 ml/g. During recording, local anesthesia was applied to the scalp incision. During the recording session the bat was restrained in a foam-lined holder molded to the shape of the body, to hold it firmly but comfortably. The holder was suspended in an elastic sling to damp movements. Water was offered at regular intervals during the recording session. If the bat showed signs of distress was applied to the scalp incision. During the recording session the bat could be replicated precisely from one day’s recording session to the next. Each bat was used in one to six recording sessions, each lasting about 6 h, with one session per day. Between recording sessions, bats were housed in individual cages with unrestricted access to food and water. The cages were located in a room with controlled temperature and humidity.

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Neural responses were recorded with glass micropipettes filled with 5% biotinylated dextran amine (BDA) in 0.9% saline solution. Pipette tip diameters were typically 5% biotinylated dextran amine (BDA) in 0.9% saline solution. Pipette tip diameters were typically 5% biotinylated dextran amine (BDA) in 0.9% saline solution. Pipette tip diameters were typically 5% biotinylated dextran amine (BDA) in 0.9% saline solution. Pipette tip diameters were typically 5% biotinylated dextran amine (BDA) in 0.9% saline solution. Pipette tip diameters were typically 5% biotinylated dextran amine (BDA) in 0.9% saline solution.

Histology

To mark recording sites, very small deposits (<50 μm diameter) of BDA were made iontophoretically using pulsed current, positive at the electrode tip, 1–7 μA, applied for 2 min. After the final recording session, animals were administered a lethal dose of Nembutal (pentobarbital) and perfused through the heart with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The brain was then removed and stored overnight in 30% sucrose in PBS. Sections were cut 40 μm thick on a freezing microtome. To visualize BDA, sections were reacted with avidin–biotin complex (Vector Laboratories; see Hsu et al. 1981).

Auditory simulation

To generate auditory stimuli, two digital signal processor-controlled D/A converters (Tucker-Davis Technologies) were used. These were controlled by custom software run on a PC. Sounds were presented by Bruel & Kjaer 1/4-in. condenser microphones, modified for use as loudspeakers and placed within the cone of the bat’s pinna, as close as possible to the external ear canal. SFM and pure tones were used as search stimuli. Once a unit was identified, its responses to different signals, including pure tones, single FM sweeps, SAM, and noise bursts were tested. A unit was characterized as SFM-selective if it responded to SFM, but not to any of the other test stimuli. The response properties of SFM-selective units were examined as follows. The SFM stimulus consisted of a 100-ms tone, the frequency of which was continuously modulated around a center (carrier) frequency according to a sine function. The amount by which the frequency changed around the carrier frequency during one modulation period is referred to here as “frequency deviation.” The frequency of the modulation waveform is referred to here as “modulation rate.” Testing of a neuron’s responses to SFM began by determining through audiovisual monitoring of responses its best carrier frequency, modulation rate, frequency deviation, and amplitude. Then with all other parameters set to give the optimal response, each parameter in turn was systematically varied. During data collection, the values of the variable parameter were randomized. A coefficient of synchronization (CS) was used to quantify the correlation between time of discharge and the modulation phase angle of the SFM. It was calculated as in Huffman et al. (1998) using the formula:

\[
CS = \left( \frac{\left( \sum \sin (a_i) \right)^2 + \left( \sum \cos (a_i) \right)^2}{N} \right)^{1/2}
\]

where \(a_i = \text{(spike time)} \times \text{(modulation rate)} \times 360^\circ \times 2 \) and \(N \) is the total number of spikes. Phase was arbitrarily referenced to stimulus onset (\(t_i = 0 \) ms). Note that a was calculated by treating the downward and upward components of the SFM cycle as two periods to avoid phase cancellation of responses to the downward and upward frequency sweeps.

The 50% modulation tuning width was defined as the difference between the higher and lower modulation rates at which the response was reduced to 50% of maximal value.

RESULTS

We recorded from 214 neurons in the IC of 15 bats. Of these neurons, 47 responded exclusively to SFM signals and did not respond to pure tones, single FM sweeps, SAM tones, or noise bursts. Of the 47 neurons that responded exclusively to SFM, 42 responded with a sustained discharge pattern and 5 responded with transient onset or offset patterns. Here we consider only those neurons with sustained responses because they represent the majority of SFM-selective neurons in the IC. SFM-selective neurons, by definition, do not respond to pure tones. It is likely that the sustained responses seen are not comparable to sustained responses to pure tones, but rather to onset responses each time a specific pattern of frequency change occurs.
Location in the IC

To visualize the positions of the recorded cells, the coordinates of 42 SFM-selective units and 115 nonselective units that were localized were marked on collapsed views of the IC in frontal (Fig. 1A) and parasagittal (Fig. 1B) dimensions. SFM-selective cells (red circles) were found in only the anterior and dorsal part of the IC; none was found in the more posterior and ventral part. In contrast, nonselective cells (blue circles) were located throughout the entire IC.

Overview of SFM selectivity

The SFM-selective cells with sustained responses could be divided into two distinct classes, depending on whether their spikes were phase locked to the SFM cycle, as quantified by the CS. Neural activity was considered phase locked if the CS was >0.6. Among the 42 SFM-selective cells, 24 responded with phase-locked discharge patterns and 18 did not. The dot rasters in Fig. 2 illustrate examples of phase-locked responses and those in Fig. 3 illustrate non-phase-locked responses. Figure 4 shows phase histograms for the same two neurons.

The neuron in Fig. 2 is a typical example of an SFM-selective neuron with a tightly phase locked discharge pattern (CS = 0.99). This neuron was representative of all phase-locking SFM-selective neurons in that it responded with only one spike per SFM cycle. As a result a single peak was present in the phase histogram, indicating that the spikes were correlated in time with a specific direction of frequency change. The neuron in Fig. 2 was tuned to multiple parameters of SFM. It responded best to a signal with a carrier frequency of about 39 kHz (Fig. 2, A and B) and a best amplitude of about 30 dB SPL (Fig. 2, C and D). This neuron was typical in that it responded to only a narrow range of amplitudes, with a lower threshold of about 20 dB SPL and an upper threshold of about 50 dB SPL. Also typical was the finding that it responded to a narrow range of modulation rates and depths of modulation. Its best modulation rate was about 80 Hz (Fig. 2, E and F) and its best frequency deviation was ±7 kHz (Fig. 2, G and H). This neuron’s responses were highly phase locked under all conditions, with the CS remaining virtually unchanged across the different values of SFM parameters to which it responded.

The neuron in Fig. 3 is a typical example of one that responded exclusively to SFM but had a poorly phase locked response. The CS changed across the different values of parameters that were varied, but never exceeded 0.5. This neuron was tuned to multiple stimulus parameters, including carrier frequency, amplitude, modulation rate, and frequency deviation. Its best carrier frequency was about 9 kHz (Fig. 3, A and B). This neuron, like the one shown in Fig. 2, was amplitude selective, with a lower threshold of about 15 dB SPL, an upper threshold of about 45 dB SPL, and a best amplitude of about 25 dB SPL (Fig. 3, C and D). The best modulation rate was about 120 Hz (Fig. 3, E and F) and the best frequency deviation was ±5 kHz (Fig. 3, G and H).

To further illustrate the difference between phase-locked and non-phase-locked neurons, Fig. 4 shows phase histograms for the two neurons shown earlier. For the highly phase-locked cell in Fig. 2, all spikes occurred within a narrow peak at about 250° (Fig. 4A). For the non-phase-locked cell in Fig. 3, spikes were distributed over the entire period of the SFM signal, with two broad and poorly defined peaks at 40 and 340° (Fig. 4B).

Carrier frequency

In *Eptesicus*, the main frequency range of the fundamental harmonic of echolocation sounds is from 23 to 50 kHz, whereas the fundamental frequencies of communication calls extend into a lower-frequency range, <20 kHz (Carter et al. 2004; Farrar et al. 2005; Monroy et al. 2006; Simmons 1989). Knowing whether SFM-selective neurons process sounds in one or the other of these ranges could help determine their role in processing natural sounds.

Figure 5 illustrates that the best carrier frequencies of SFM-selective neurons were generally lower than best frequencies of IC neurons that responded to pure tones. The mean carrier frequency for the SFM-selective cells was 27 kHz, whereas the mean best frequency for cells that responded to pure tones was 35 kHz. All but four SFM-selective cells (91%) had carrier frequencies <40 kHz, and none had carrier frequencies >50 kHz. For nonselective cells, about 75% had best frequencies <40 kHz. The carrier frequencies of 28 SFM-selective cells (67%) fell within the spectral range of the FM sweeps that *Eptesicus* uses during echolocation (50–23 kHz; Simmons 1989). However, the best carrier frequencies of the remaining 14 cells (33%) were between 9 and 22 kHz, below the range of echolocation calls. About 30 (17%) nonselective cells had best frequencies within this range.

Tuning to amplitude

Selectivity to amplitude was a prominent feature of SFM-selective cells. Figure 6 shows that the lower thresholds of
SFM-selective neurons were generally very low. The average threshold was 16.5 dB SPL, which is considerably lower than the average threshold for a population of IC neurons that responded to pure tones (about 32 dB SPL; Casseday and Covey 1992). Although there was a trend for neurons with higher best carrier frequencies to have higher thresholds, only one SFM-selective cell had a threshold above the average value for IC cells. Moreover, the majority (89%) of SFM-selective neurons had upper thresholds and closed response areas.

Neurons with a high rate of spontaneous activity provided indirect evidence that the lack of responsiveness to high sound intensity was caused by inhibition. For all seven SFM-selective neurons that had spontaneous activity, the spontaneous activity was eliminated during the time when the sound level was above their upper thresholds, and resumed after the stimulus was over. Figure 7 shows an example of such a cell. This neuron had a best amplitude of 22 dB SPL; however, when the sound intensity was >32 dB SPL, the cell stopped firing during the presentation of the stimulus and resumed after the stimulus was over. The higher the sound amplitude, the longer the period of suppression lasted.

Tuning to modulation rate

Of the 42 SFM-selective cells, 27 (64%) were selective for a particular range of modulation rate. That is, the number of spikes per cycle was reduced to <50% of the maximal value at modulation rates both higher and lower than the best rate, so that they had band-pass characteristics for modulation rate. The remaining 15 cells (36%) did not satisfy this criterion even though they were selective for SFM. Best modulation rates of neurons with band-pass tuning ranged from 20 to 120 Hz, with
an average of 70 Hz. Of 27 cells with band-pass tuning to SFM rate, 26 (96.2%) had best rates <100 Hz.

Figure 8 shows that best modulation rate was positively correlated with best carrier frequency ($r = 0.382, P = 0.045$; Fig. 8A) and with depth within the IC ($r = 0.462; P = 0.013$; Fig. 8B). The tonotopic progression from low to high frequency in the IC of *Eptesicus* is roughly dorsolateral to ventromedial, so electrode penetrations from dorsal to ventral may remain within the same general frequency range for a considerable distance. Because the region of 30–40 kHz was one in which recordings were obtained at a wide range of depths, we plotted the relationship between best modulation rate and depth within the IC for 12 neurons whose carrier frequencies fell within this range, which represents about 0.5 octave. Figure 8D shows that in this case there was a strong positive correlation between best modulation rate and depth ($r = 0.591; P = 0.043$; Fig. 8D). However, there was only a low correlation between best modulation rate and best carrier frequency within this range ($r = 0.160; P = 0.623$; Fig. 8C).

**Synchronization of discharge to SFM stimuli**

To investigate the relationship between phase-locking properties and frequency tuning, we plotted synchronization coefficient versus best carrier frequency for the population of SFM-selective cells (Fig. 9). Linear regression analysis showed that the CS was positively correlated with best modulation frequency ($r = 0.528, P < 0.001$). In addition, it appears that SFM-selective neurons were loosely organized into two clusters such that those with high CS values were found throughout the entire frequency range, but those with lower CS values (<0.6) were found only for neurons with best modulation...
frequencies < 35 kHz. Thus cells in the dorsolateral, low-frequency part of the IC displayed, on average, less-precise phase-locking properties than cells in the more ventromedial, higher-frequency regions of the IC.

For 20 cells that had band-pass selectivity to modulation rate, and for which we obtained a complete set of measurements of their response characteristics, we plotted the 50% width of modulation rate tuning against CS (Fig. 10). There was a strong negative relationship ($r = -0.945$, $P < 0.001$) between these two measures, indicating that cells with high CS values were more narrowly selective to modulation rate. There was no obvious relationship between best modulation rate and CS. These results showed that cells with higher temporal precision also displayed higher selectivity for modulation rate.

**DISCUSSION**

This study provides new information about how SFM-selective cells process sound amplitude and how they are distributed within the IC. In addition, it confirms the findings of Casseday et al. (1997) that many neurons in the inferior colliculus of the big brown bat respond best, or exclusively, to SFM, that these neurons are tuned to carrier frequency, modulation rate and depth, and that they have relatively low best modulation rates.

In this section, we will consider three major properties of SFM-selective cells. The first is their topographic distribution in the IC; the second is the mechanisms underlying SFM selectivity, including tuning to different SFM parameters; and the third is the role of SFM-selective neurons in the bat’s behavior.

**Topographic distribution of SFM-selective neurons in the IC**

It is a well-established fact that sound frequency is topographically organized at each stage of the auditory pathway. However, it is unclear to what extent other parameters of sound are also mapped or how such mapping relates to the tonotopic map. Our study provides evidence that SFM-selective cells are confined to the rostro-dorsal part of the IC. In another study, it was observed that band-pass duration-tuned neurons are confined to the caudal half of the IC (Ehrlich et al. 1997), in a region separate from that of the SFM-selective neurons. Together these findings suggest that within the IC there is at least a coarse spatial segregation of different types of specialized neurons. Unlike duration-tuned cells, which were found throughout the entire frequency range of the IC, SFM-selective cells had relatively low carrier frequencies compared with those of nonselective cells. This bias to low frequencies is consistent with the finding that SFM-selective neurons were located mainly in the dorsal half of the IC where low frequencies are represented, and none was found in the more ventral...
and medial regions where the highest frequencies are represented.

In addition to segregation of SFM-selective neurons within a specific part of the IC, there appears to be a relationship between best modulation rate, best carrier frequency, and depth within the IC. However, for any carrier frequency, neurons were tuned to a wide range of best modulation rates. A similar relationship between best frequency and best modulation rate for SAM has been reported in the cat, where neurons with higher best frequencies also tend to have higher best modulation rates (Langner 1992; Schreiner and Langner 1988). These authors also observed that in the cat best modulation rate for SAM varies somewhat systematically across an isofrequency contour. A systematic ordering of modulation rate for SAM has also been seen in the auditory cortex of the gerbil (Schulze and Langner 1997). Our results are not directly comparable to those of Langner and colleagues because they were performed in a different species using a different form of signal modulation. The animals in the studies by Langner and colleagues were anesthetized with pentobarbital, whereas ours were awake. Moreover, the neurons studied by Langner and colleagues did not respond exclusively to SAM, whereas ours responded exclusively to SFM. Nevertheless, despite these differences in experimental preparation and methodology, there are some parallels. For both SAM and SFM signals modulation rate appears to be organized so that each progressively higher frequency lamina contains a slightly higher range of best modulation rates. These ranges appear to be relatively broad and overlapping (see Fig. 8). Within the range of modulation rates characteristic of a narrow range of best carrier frequencies, there also appears to be some degree of ordering from low to high rates.

Mechanism of SFM selectivity

ROLE OF INHIBITION IN AMPLITUDE TUNING. Most SFM-selective neurons had closed frequency response areas. Because the majority of IC neurons in Eptesicus have V-shaped or other types of open response areas (Casseday and Covey 1992),

![FIG. 8. Scatterplots of best modulation rate against carrier frequency and depth in the IC for the 28 SFM-selective neurons that had band-pass selectivity for modulation rate. A: best modulation rate is positively correlated with carrier frequency ($r = 0.382$, $P = 0.045$). B: best modulation rate is positively correlated with depth in the IC ($r = 0.462$, $P = 0.013$). C: for the 12 SFM-selective neurons whose carrier frequencies fell between 30 and 40 kHz, the best modulation rate was poorly correlated with their best carrier frequency ($r = 0.160$, $P = 0.623$). D: for the same 12 neurons described in C, the best modulation rate was positively correlated with depth in the IC ($r = 0.591$, $P = 0.043$).](http://jn.physiology.org/)

![FIG. 9. Scatterplot of CS against carrier frequency for SFM-selective neurons. There appear to be 2 clusters of neurons, one composed of cells with lower CS and lower carrier frequencies, and another with high CS, distributed across the entire frequency range. There is a positive relationship between the coefficient of synchronization and carrier frequency ($r = 0.528$, $P < 0.001$).](http://jn.physiology.org/)

![FIG. 10. Scatterplot of CS against 50% width of modulation rate ($F_{mod}$) for the 20 cells for which we were able to calculate the width of modulation rate tuning. There is a strong negative correlation between CS and 50% width of modulation rate tuning ($r = -0.945$, $P < 0.001$).](http://jn.physiology.org/)
closed frequency response areas appear to be a distinguishing feature of SFM-selective cells. The fact that spontaneous activity was eliminated when sound amplitude was above the upper threshold of SFM-selective neurons strongly suggests that the upper thresholds were created through the action of neural inhibition. Previous studies on the role of inhibitory neurotransmitters on amplitude tuning in the IC have shown that blocking GABA or glycine receptors in IC neurons changes nonmonotonic rate-intensity functions to monotonic, and closed frequency response areas to open ones (Faingold et al. 1991; LeBeau et al. 2001; Yang et al. 1992). Although these studies did not include SFM-selective neurons, it is likely that a similar mechanism operates in both classes of cells.

In SFM-selective cells with upper thresholds, inhibition would necessarily have to grow at a faster rate than excitation as sound level was increased, to eventually become dominant and cancel the excitation. An alternative explanation would be that SFM-selective cells receive their excitatory input from a source with a closed frequency response area. The finding that at high sound levels spontaneous activity was suppressed during the stimulus argues against this idea. Furthermore, preliminary neuropharmacological evidence shows that the inhibition that creates the upper thresholds occurs at the SFM-selective cells themselves (Yue et al. 2006).

This imbalance between inhibition and excitation as amplitude increases is very different from the effects of amplitude on duration-tuned cells, which maintain their duration selectivity across a wide range of amplitude (Fremouw et al. 2005). This indicates that the excitation and inhibition that together produce duration tuning (Casseday et al. 1994; Covey et al. 1996; Faure et al. 2003) remain in balance with each other across a wide range of sound amplitude.

ROLE OF INHIBITION AND COINCIDENCE DETECTION IN RATE TUNING. Studies on other specialized types of auditory neurons, especially those that are selective for specific sound patterns, have shown that inhibition plays an important role in shaping their selectivity to temporal features. For example, blocking inhibitory inputs to IC neurons affects response latency (Johnson 1993; Park and Pollak 1993), selectivity for the direction of FM sweeps (Fuzessery and Hall 1996), and tuning to sound duration (Casseday et al. 2000). Both extracellular and intracellular recordings have shown that for duration-tuned IC neurons, the relative timing of inhibitory and excitatory inputs is one of the factors that creates duration selectivity (Casseday et al. 1994; Covey et al. 1996; Faure et al. 2003). Given the important role of inhibition in other forms of temporal selectivity, it seems likely that the relative timing of excitation and inhibition might also be involved in tuning to SFM rate.

The area in which SFM-selective neurons are found is rich in GABA_α and GABA_β receptors and terminals in *Eptesicus* (Fubara et al. 1996) and other mammals (Glendening and Baker 1988; Winer et al. 1995). One previous study (Koch and Grothe 1998) indicated that GABAergic inhibition plays a role in creating SFM rate selectivity in a general population of IC cells. However, in that study the authors did not determine whether the neurons they tested responded exclusively to SFM or whether they responded to all types of stimuli. Thus it is likely that the population studied by Koch and Grothe was far less specific than that reported here. These authors proposed that SFM rate determines the relative timing of excitation and inhibition from two sources, so that at some modulation rates the two inputs cancel each other. For example, such a model might include an excitatory input that is phase locked to SFM, followed by an inhibitory input that is also phase locked, but delayed by a certain amount of time. If the rate of SFM is such that excitation evoked by one cycle coincides with the inhibition from the preceding cycle, the neuron will not fire.

Casseday et al. (1994) proposed a similar model using coincidence detection to account for duration selectivity. In that model, a duration-tuned cell receives two principal synaptic inputs. One is subthreshold, transient excitation elicited by the onset of the sound; the other is sustained inhibition with a shorter latency than the excitation, followed by an excitatory rebound at the offset of the sound. For the cell to respond, the duration of the sound has to be such that the excitation coincides with the rebound, bringing the cell to threshold.

Based on their experimental results and the preceding two models, Casseday et al. (1997) proposed a model in which SFM rate tuning is created by the summation of subthreshold excitation and an excitatory rebound from inhibition. In this model, the excitation is elicited by a certain sequence of frequencies that occurs in one portion of the SFM cycle and the inhibition that produces the rebound is elicited by a sequence of frequencies in the opposite direction. For summation to occur, the modulation rate has to be such that the two subthreshold excitatory events coincide.

According to this model, for an SFM-selective cell to respond to only a certain range of modulation rate, both inputs would need to have precise timing and occur at a consistent phase from cycle to cycle. Depending on their time constants, the range of modulation rates to which the neuron responded would be broader or narrower. It seems reasonable to assume that those SFM-selective cells with high synchronizaton coefficients had small time constants. This idea is consistent with our finding that the higher the synchronizaton coefficient, the more narrowly tuned the cell was to modulation rate.

**Biological relevance**

SFM or SFM-like components are present in many natural sounds that are important for bats’ behavior. As mentioned in the *INTRODUCTION*, periodic frequency modulations may be present in echoes, communication signals, and sounds generated by insect wing beats. The frequency ranges of these natural signals overlap, but communication sounds and sounds made by insects include lower frequencies <20 kHz that are not present in echolocation calls. In our study, we found that the carrier frequencies of SFM-selective cells spanned a broad range (9–50 kHz), suggesting that these cells are involved in processing multiple types of signals, one of which could be a role in passive listening for insect-generated sounds.

The echoes that are reflected from objects are much lower in intensity than the calls emitted by the bat, which are around 100 dB SPL (Griffin 1958). For example, the echo reflected from an insect wing 1.5 cm in length ranges from around 0 dB SPL for a target distance of 6.4 m, to 50 dB SPL for a target distance of 0.4 m (Kober and Schnitzler 1990). Most SFM-selective cells had upper thresholds and were narrowly selective to very low amplitude sounds. The best amplitude of these cells fell within the range of typical echo amplitudes. These
two findings together indicate that these cells would not respond to the loud pulses emitted by the bat, but would very likely respond to the echoes of these sounds.

Furthermore, the inhibition that produces the upper thresholds of SFM-selective neurons could play a role in enhancing their responses to echoes. As indicated in the RESULTS, SFM-selective cells are inhibited when sound level is above their upper threshold. If loud sounds provide inhibitory input to SFM neurons, it is possible that the rebound from the inhibition elicited by the pulses might actually enhance their responses to echoes at certain delays.

Another source of SFM-like signals is the communication sounds used by bats. Studies show that some communication calls, including the chitter and courtship sounds of *Eptesicus*, contain SFM-like components, with carrier frequencies ranging from 10 to 60 kHz and modulation rates from 20 to 200 Hz (Behr and von Helversen 2004; Davidson and Wilkinson 2004; Monroy et al. 2006). Those SFM-selective cells with best carrier frequencies <23 kHz might be involved in processing these communication signals. However, this seems unlikely given that most communication sounds are relatively loud and SFM-selective neurons were all tuned to fairly low amplitudes. It seems more likely that SFM-selective neurons with low best carrier frequencies would respond to sounds made by insects, possibly attracting bats to insects calling in the distance.

The findings reported herein indicate that the IC of *Eptesicus* contains a spatially segregated population of SFM-selective neurons; across this population, best modulation rate appears to be correlated with best carrier frequency and depth within the IC. These neurons are specialized to process low-amplitude signals that contain periodic frequency modulations in the frequency ranges of both echolocation signals, communication sounds, and sounds produced by insect prey. Because they are uniformly sensitive to low-intensity sounds, it seems likely that their primary roles are processing information contained in echoes and detecting insect-generated sounds from a distance.

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**References**


