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Zoltan M. Fuzessery, Marlin D. Richardson, and Michael S. Coburn
Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming

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Fuzessery, Zoltan M., Marlin D. Richardson, and Michael S. Coburn. Neural mechanisms underlying selectivity for the rate and direction of frequency-modulated sweeps in the inferior colliculus of the pallid bat. *J Neurophysiol* 96: 1320–1336, 2006. First published June 21, 2006; doi:10.1152/jn.00021.2006. This study describes mechanisms that underlie neuronal selectivity for the direction and rate of frequency-modulated sweeps in the central nucleus of the inferior colliculus (ICC) of the pallid bat (*Antrozous pallidus*). This ICC contains a high percentage of neurons (66%) that respond selectively to the downward sweep direction of the bat's echolocation pulse. Some (19%) are specialists that respond only to downward sweeps. Most neurons (83%) are also tuned to sweep rates. A two-tone inhibition paradigm was used to describe inhibitory mechanisms that shape selectivity for sweep direction and rate. Two different mechanisms can create similar rate tuning. The first is an early on-best frequency inhibition that shapes duration tuning, which in turn determines rate tuning. In most neurons that are not duration tuned, a delayed high-frequency inhibition creates rate tuning. These neurons respond to fast sweep rates, but are inhibited as rate slows, and delayed inhibition overlaps excitation. In these neurons, starting a downward sweep within the excitatory tuning curve eliminates rate tuning. However, if rate tuning is shaped by duration tuning, this manipulation has no effect. Selectivity for the downward sweep direction is created by an early low-frequency inhibition that prevents responses to upward sweeps. In addition to this asymmetry in arrival times of low- and high-frequency inhibitions, the bandwidth of the low-frequency sideband was broader. Bandwidth influences the arrival time of inhibition during an FM sweep because a broader sideband will be encountered sooner. These findings show that similar spectrotemporal filters can be created by different mechanisms.

INTRODUCTION

The vocalizations of most animals contain prominent frequency modulations (FM). Much of the information contained in human speech resides in FM sweeps (formant transitions). They are also indispensable components of bat echolocation pulses. Given this biological significance, the neural mechanisms that underlie the processing of FM signals have received considerable attention. Studies of the auditory systems of a variety of species report that neurons at the level of the cochlear nucleus and higher respond preferentially to the direction or rate (or velocity) of FM (e.g., Britt and Starr 1976; Erulkar et al. 1968; Gordon and O'Neill 1998; Heil et al. 1992a,b; Mendelson and Cynader 1985; Møller 1974; Nelson et al. 1966; O'Neill and Brimijoin 2002; Phillips et al. 1985; Poon et al. 1991; Shore et al. 1987; Vartanian 1974). Echolocating bats have taken such functional specializations a step further in that a significant percentage of auditory neurons responds selectively to FM signals (e.g., Casseday and Covey 1992; Casseday et al. 1997; Fuzessery 1994; Suga 1969; Vater and Schlegel 1979). Their auditory systems thus provide useful models for examining the mechanisms that shape neuronal selectivity for these important signal components.

The present study examines the mechanisms that shape response selectivity for the direction and rate of FM sweeps in the inferior colliculus (IC) of the pallid bat. The IC of this bat has an unusually high degree of response selectivity for the downward FM sweep of the echolocation pulse (Bell 1982; Brown 1976; Fuzessery et al. 1993), with over 50% of neurons responding selectively to the downward sweep direction and nearly 30% of these responding only to downward FM sweeps and not to individual tones within the sweep (Fuzessery 1994; Fuzessery and Hall 1996). The goals of this study are to determine the mechanisms that shape response selectivity for FM sweep direction and rate, to compare these mechanisms with those that create the same forms of selectivity in auditory cortex, and to create a reference for examining the ontogeny of these response properties.

The mechanisms proposed to shape neuronal selectivity for the direction of an FM sweep are similar to those suggested to shape directional selectivity for motion across visual and somatosensory receptor surfaces—that is, that there is an asymmetry in excitation and/or inhibition created in one direction that is not created in the other (Barlow and Levick 1964; Movshon et al. 1978; Reid et al. 1991; Sillito 1977). The asymmetry has been modeled as the result of interactions between networks of neurons at multiple levels of the auditory system (Gordon and O'Neill 1998; Suga 1965, 1973) or as the spatiotemporal integration of inhibitory or excitatory subthreshold postsynaptic events at single neurons (Rall 1964; Segev 1992). Phillips et al. (1985) suggested an excitatory summation that occurs when the tonal response area is approached from a given direction. An inhibitory mechanism often proposed is an asymmetry in the inhibitory sidebands flanking excitatory tuning curves (Britt and Starr 1976; Gordon and O'Neill 1998; Heil et al. 1992a; Shannon-Hartman et al. 1992; Suga 1965). This mechanism has recently received additional support from studies that used either patch clamping to measure the timing of inhibitory and excitatory inputs (Zhang et al. 2003) or two-tone inhibition paradigms designed to reveal the arrival times of these inputs (Gordon and O'Neill 1998). It has also been suggested that excitatory and inhibitory events work in concert to produce a directional asymmetry (Casseday et al. 1997); a neuron may receive sufficient excit-

Address for reprint requests and other correspondence: Z. M. Fuzessery, Department of Zoology and Physiology, University of Wyoming, 1000 E. University Ave. Dept. 3166, Laramie, WY 82071 (E-mail: zmf@uwyo.edu).

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atory drive only if excitatory inputs combine with a rebound from an earlier inhibitory input and this coincidence may occur only in one sweep direction.

Some of the mechanisms that shape direction selectivity may also contribute to a selectivity for FM sweep rate because auditory neurons are reported to display direction selectivity only at certain sweep rates (Gordon and O'Neill 1998; Heil and Irvine 1998; Tian and Rauschecher 1994). Gordon and O'Neill (1998) noted that it is important to consider both temporal and spectral differences in sideband inhibition to understand their roles in shaping selectivity for various FM sweep dimensions. A low-frequency inhibitory sideband, for example, would prevent a neuron from responding to an upward sweep if the inhibition arrived earlier than excitation, and thus create direction selectivity. However, if this inhibition were delayed relative to excitation, it would allow the neuron to respond to an upward sweep, but only until the sweep rate slowed to the extent that the delayed inhibition caught up with the excitation. Thus it is the relative timing of excitation and inhibition, as they occur during the course of an FM sweep, that determines the form of the resulting spectrotemporal filter.

The main focus of the present study is to understand the mechanisms that shape selectivity for FM sweep direction and rate in the region of pallid bat IC tuned to the echolocation pulse. We previously described the direction selectivity of this neuronal population (Fuzessery 1994); here we describe fast-pass and band-pass tuning for FM sweep rates that approximate those of the bat's echolocation pulse. A two-tone inhibition paradigm similar to that used by Gordon and O'Neill (1998), Faure et al. (2003), and Brimijoin and O'Neill (2005) is used to estimate the timing of excitatory and inhibitory inputs during the course of an FM sweep. The method was found to be effective in predicting how spectrotemporal asymmetries in low- and high-frequency inhibitions shape selectivity for sweep direction and rate.

An unexpected finding is that essentially identical forms of rate tuning are created by two different mechanisms: duration tuning and a delayed high-frequency inhibition. Over 50% of neurons in the high-frequency region of the pallid bat IC have been found to respond maximally to short-tone durations of $\leq$5 ms (Fuzessery and Hall 1999). This duration tuning is reflected in their sweep rate selectivity; they prefer rates in which FM sweeps traverse their excitatory tuning curves in a time equal to their best duration. Neurons that lack duration tuning derive their rate selectivity for downward sweeps from a high-frequency inhibition that is delayed relative to excitation, imposing a fast-pass rate filter. Auditory systems can create similar spectrotemporal filters through different mechanisms.

METHODS

Pallid bats, captured in New Mexico and Arizona, were housed in a 16 $\times$ 11-ft^2 room in which they flew freely. They were maintained on a reversed 12 h:12 h light:dark cycle and fed mealworms raised on a vitamin-rich diet of Purina rat chow. Before surgery, bats were placed in a cage and fed additional mealworms to increase body weight. All experimental procedures used in this study followed the guidelines of the National Institutes of Health and were approved by the University of Wyoming Institutional Animal Care and Use Committee.

Surgical procedures

Bats were prepared for surgery by first anesthetizing them with methoxyflurane (Metofane) inhalation, followed by an intraperitoneal injection of sodium pentobarbital (Nembutal, 30 μg/g body weight) and acepromazine (2 μg/g body weight). To expose the IC, the head was held in a bite bar and a midline incision was made in the scalp; the muscles over the dorsal surface of the skull were reflected. In preparation for securing an aluminum head pin to the skull, the skull over the neocortex was scraped clean and covered with a layer of glass microbeads in cyanoacrylate cement, followed by a layer of dental cement. Using skull and brain surface landmarks (the skull is semi-transparent), a small hole (diameter 0.5 mm) was made over the IC.

Recording procedures

Experiments were conducted in a heated (85–90°F), soundproof chamber lined with anechoic foam. Bats were kept lightly anesthetized throughout the course of the experiments with sedation maintained by inhalation of Metofane and additional pentobarbital sodium (one third of presurgical dose) injections. Stimuli were generated using Modular Instruments and Tucker Davis Technologies digital hardware and custom software. The waveforms were amplified with a stereo amplifier and presented as closed-field stimuli through Infinity emit-K ribbon tweeters fitted with funnels that were inserted into the pinnae and sealed with petroleum jelly. This procedure attenuated speaker intensity level at the opposite ear by $\geq$30 dB. The speaker–funnel frequency–response curve showed a gradual increase of 20 dB from 6 to 70 kHz, as measured with a Brüel & Kjær 1/8-in. microphone placed at the tip of the funnel.

Recordings were made with glass microelectrodes filled with 1 M NaCl, with an impedance of 3–7 MΩ. Electrodes were advanced remotely from outside the recording chamber using a Kopf Model 660 Micropositioner. Electrodes were advanced vertically down into the center one third of the dorsal surface of the IC and recording began when neurons with best frequencies (BFs) of $>$30 kHz were encountered. Based on the initial positioning of the electrode, it is assumed that all recordings were made in the central nucleus of the IC (ICC). The echolocation pulse of the pallid bat typically sweeps downward from 60 to 30 kHz (maximum range 80–25 kHz; Brown 1976) and previous studies (Fuzessery 1994; Fuzessery and Hall 1996) showed that the great majority of neurons that respond selectively to the echolocation pulse have BFs of $\geq$35 kHz. Response magnitudes and poststimulus time histograms were acquired and stored with the use of a Modular Instruments high-speed clock controlled by custom software. A response to a given stimulus was quantified as the total number of spikes elicited over 30 stimulus presentations. Because neurons in the pallid bat IC typically show little or no spontaneous activity, the isolation of neurons depended on responses to search stimuli consisting of tones, and downward and upward FM sweeps presented over a range of durations, from 1 to 10 ms.

Data collection

Sounds were presented at intervals of 400 ms. Envelope rise/fall times were set at 1 ms. When sound durations were $<$2 ms, the rise/fall times were each 50% of the duration. For each neuron, all sounds were presented at a single intensity 5–20 dB above the response threshold. This strategy allowed us time to conduct most of required stimulus manipulations before contact with the neuron was lost, but did not allow us to determine whether response properties changed with sound pressure level.

Data collection proceeded in the following sequence. If a neuron responded to tones, its excitatory tonal response area was mapped audiovisually as the combinations of sound pressure levels and tone frequencies evoking a response to each of ten consecutive stimulus presentations. Responses to the signals described below were quanti-

fied as the total number of spikes elicited by 30 stimulus presentations, collected 0–50 ms after stimulus onset. The great majority of responses were phasic and time-locked to stimulus onset.

Duration selectivity was tested at a neuron's BF. A neuron was considered duration selective if it exhibited a short-pass or band-pass duration function in which responses dropped to ≤50% of maximum response as tone duration increased or decreased relative to the best duration. The best duration was defined as the arithmetic center of the durations evoking a ≥80% maximum response. The shortest duration that was always tested was 0.5 ms, but in some neurons, durations as short as 0.1 ms were presented. If a neuron responded maximally to the shortest duration tested, this was assigned as its best duration. Duration tuning was defined only in response to tones and not in response to FM sweeps because response changes with FM sweep duration (with FM spectrum fixed) could be the result of a selectivity for sweep rate rather than sweep duration.

Responses to linear upward and downward FM sweeps of identical spectra were then tested to determine direction selectivity. The spectra of FM sweeps were centered on the neuron's BF. A neuron was termed selective for sweep direction if the maximum response in one direction had a directional selectivity index (DSI) of ≥0.6 (at which the response to the nonpreferred direction was 25% of the response to the preferred direction). The DSI = (D − U)/(D + U), where D and U are the maximum responses to downward and upward sweeps, respectively, regardless of the sweep rates at which they occurred (Britt and Starr 1976; Heil et al. 1992a; O'Neill and Brimijoin 2002). All direction-selective neurons preferred the downward direction; therefore preference for this direction was assigned a positive value, unlike the convention used in previous studies.

Downward FM–selective neurons were defined as responding similarly to both downward FM sweeps and tones, but giving ≤20% of maximum response to upward sweeps and noise. Downward FM specialists were more selective. They responded maximally only to downward sweeps and gave ≤20% to tones, upward sweeps, and noise.

Although the biosonar pulse of the pallid bat is an exponential downward FM sweep, linear sweeps were used in this study to maintain a constant rate of frequency change. This allowed us to more easily relate time and frequency within a sweep, which in turn allowed us to assign a single best sweep rate to rate-selective neurons and to more easily predict rate and direction selectivity (as described in RESULTS).

Band-pass noise with the same bandwidths as those of excitatory FM sweeps were presented as a control to determine whether a neuron actually required the orderly progression of frequencies present in a sweep or whether the simultaneous presentation of a spectrum that encompassed both the excitatory and inhibitory tonal response areas was also excitatory. Noise was presented at durations of 1 to 10 ms or, if the neuron was duration tuned, at the neuron's best duration. The band-pass filter was digitally generated and had a rolloff of >40 dB/octave.

Selectivity for FM sweep rate was then tested by presenting FM sweeps of at least three different bandwidths, centered on the neuron's BF and located, to the extent possible, within the bandwidth of bat's echolocation range of 25–80 kHz. A neuron was considered sweep rate selective if it exhibited a preferred rate when presented with a downward FM sweep that approximated the bat's echolocation pulse of 60 to 30 kHz. Some sweep bandwidths fell outside of this range, as low as 20 kHz, to generate a full range of sweep rates. The rates of each of these sweeps were varied by changing sweep duration. Sweep rates were calculated by dividing the bandwidth by the duration (kHz/ms). The sweep rate functions were then normalized to percentage of maximum response to compare the degree of overlap. If these normalized functions overlapped by within 1 kHz/ms along the slow-rate flank of the rate function peak, the neuron was considered rate selective. The upper limit of rate tuning was not reached in some neurons because, as a result of the decision to keep the sweep spectrum within the bat's biosonar range, the durations of the sweeps became so short (0.1 ms) as the sweep rate was increased that signal distortion became an issue. The best sweep rate was calculated by taking the arithmetic center of ≥80% maximum response for each rate function and averaging them.

Two-tone inhibition over time

The bandwidths and arrival times of low- and high-frequency inhibitions both play a role in shaping direction and rate selectivity. To estimate both the bandwidth and relative arrival times of inhibition and excitation using extracellular recording, we used a "two-tone inhibition over time" (TTI over time) paradigm in which intensity was held constant, and two tones, one excitatory and one inhibitory, were delayed with respect to one another. An example is shown in Fig. 6.

First the BF and excitatory bandwidth at the single test intensity level were determined. The BF tone was presented in combination with a second tone with a frequency that was higher or lower than that of the excitatory band. The duration of the excitatory tone was either 5 ms or set to the neuron's best duration, if it was duration tuned. The duration of the second tone was at least twice as long as that of the excitatory tone so that the latter remained temporally overlapped by the second tone when the onset of the excitatory tone was delayed. The second inhibitory tone was fixed in time and the onset of the shorter excitatory tone delayed or advanced relative to the onset of the inhibitory tone. At each onset difference, the frequency of the second tone was varied to audiovisually determine the frequencies that completely suppressed the response to the BF tone. This manipulation served to map the inhibitory sidebands and to estimate the arrival times of inhibitory input generated by these inhibitory frequencies, relative to the arrival time of excitatory input triggered by the BF tone.

If the onset of the excitatory tone was advanced (negative delay, e.g., Fig. 3) relative to that of the inhibitory tone, and the response could still be suppressed, then inhibitory input was assumed to arrive before excitation. If the excitatory tone had to be delayed before the second tone suppressed the response, then it was assumed that inhibitory input arrived after excitation.

The relative arrival times of excitatory and inhibitory inputs were then more accurately quantified by repeating the above process with a BF tone and a second tone from the low- or high-frequency inhibitory flank (e.g., Fig. 6*B*). The arrival time of inhibition, relative to excitation, was defined as the delay at which the response was suppressed to 10% of maximum response or, if the response was not completely suppressed, the delay at which maximum suppression occurred.

The duration of the inhibitory input—more specifically, whether it at least lasted the duration of the inhibitory tone—was determined by delaying the excitatory input until it no longer completely overlapped in time with the inhibitory tone (e.g., Fig. 12). The equation used to calculate whether the inhibitory input persisted for the duration of the inhibitory tone was: excitatory tone delay = (duration of the inhibitory tone) − (duration of the excitatory tone) + (minimum duration required for maximum response). If the result was equal to 0, inhibition persisted for this duration: if the value was <0 ms, inhibition did not last the duration of the inhibitory tone; if >0 ms, it persisted beyond this duration.

RESULTS

A prominent feature of the high-frequency region of pallid bat ICC is the large percentage of neurons that respond selectively to the downward FM sweep direction of the bat's echolocation pulse (Fuzessery 1994; Fuzessery and Hall 1996). We exploited this feature to determine the mechanisms that shape response selectivity for the direction and rate of downward FM sweeps. The results are based on the responses of 64

neurons recorded in 20 bats. In all of these neurons, at least rate or direction selectivity was tested. All neurons had BFs within the spectrum of the bat's echolocation pulse (30–80 kHz). The majority had BFs of 35–50 kHz, near the spectral energy peak of the pulse.

To determine selectivity for the spectrotemporal dimensions of FM sweeps, neurons were presented with four types of sounds: pure tones, upward and downward FM sweeps, and band-pass noise. Tones were used to define excitatory tuning curves (if present) and to determine whether neurons required a full FM sweep to be excited or whether a single excitatory tone within the sweep would suffice. FM sweeps of identical spectra, but different directions, were used to assess directional selectivity. Band-pass noise with a spectrum identical to that of an excitatory sweep was used to determine whether an orderly sequence of frequencies was required for excitation.

Response selectivity for FM sweep direction

Neurons were considered selective for FM sweep direction if their direction selectivity index (DSI) was ≥0.6 for the preferred direction, which corresponds to the nonpreferred direction evoking ≤20% of the response to the preferred direction. Of the 64 neurons, 42 (66%) were selective for the downward sweep direction. None responded selectively to the upward direction. Of the 42 direction-selective neurons, 30 (71%) of these (47% of the recorded population) responded to both downward sweeps and tones; these are termed downward FM (dFM) selective. The remaining 12 of the 42 (29%) direction-selective neurons (19% of the recorded population) responded only to downward FM; tones evoked ≤20% of the maximum response. These are termed downward FM specialists.

The remaining 22 of 64 recorded neurons (34%) were not selective for FM sweep direction. Tones and upward and downward sweeps all evoked ≥20% of the maximum response. None of the 64 recorded neurons responded to band-pass noise, indicating that simultaneous presentations of excitatory and inhibitory frequencies in the FM sweep spectra were either inhibitory and/or not excitatory. The percentages of downward FM selective and downward FM specialist neurons found in the present study are similar to those previously reported (53 and 31%, respectively; Fuzessery 1994).

Response selectivity for downward FM sweep rate

Most neurons (35 of 40 neurons tested, 88%) were selective for the rates (kHz/ms) of downward FM sweeps. Rate-tuned neurons are defined as showing a preference for the same sweep rate when presented with at least three different sweeps that varied in bandwidths ranging from 10 to 50 kHz. The rates of these sweeps were varied by changing their durations, from 0.25 to 50 ms. As seen in Fig. 1*A*, if a neuron is rate tuned, the sweep duration eliciting a maximum response will systematically increase with sweep bandwidth. The duration functions were converted to rate (kHz/ms) by dividing the bandwidth of the sweep (kHz) by the duration (ms) of the sweep (Fig. 1*B*). A neuron was considered rate tuned if the three rate functions fell within a 1 kHz/ms range along the "slow" flanks of the rate functions. Rate-tuned neurons exhibited either a fast-pass selectivity, in which responses dropped to ≤50% of maximum response at slower rates, or a band-pass selectivity, in which

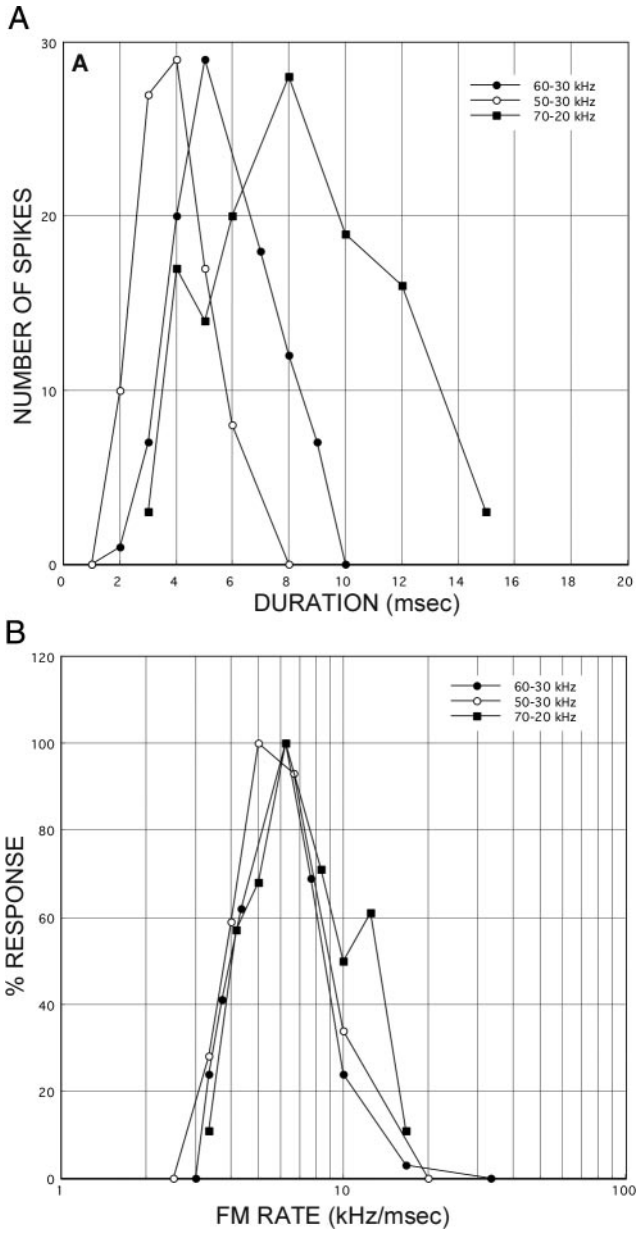


FIG. 1. Determining the frequency-modulated (FM) sweep rate tuning of a neuron. *A*: response to downward FM sweeps of 3 different bandwidths, 50 to 30 kHz (open circles), 60 to 30 kHz (filled circles), and 70 to 20 kHz (filled squares), over a range of sweep durations. Note that the preferred duration increases with bandwidth. *B*: conversion of response functions in *A* to the rate metric of kHz/ms = bandwidth of FM sweep/sound duration.

the responses dropped to ≤50% of maximum of response at faster and slower nonoptimal rates. In most cases, responses dropped to zero as the sweep rate decreased. There was most likely an upper rate limit for all neurons, but sweep rate was increased by shortening sweep duration. Once FM sweep durations were decreased to ≤0.25 ms to test responses to the highest rates, the fidelity of speaker output became suspect and thus higher rates were not tested. However, even at these very short FM sweep durations, these neurons were able to discriminate FM sweeps from band-pass noise with the same spectrum because none responded to the noise.

The best rate of a neuron for downward FM sweeps was calculated as the arithmetic center of the range of rates evoking

an 80% maximum response, averaged across responses to FM sweeps of at least three different bandwidths. Best rates ranged from 1 to 13 kHz/ms (average = 3.85 kHz/ms; median = 3.33 kHz/ms), with the great majority of neurons preferring rates of ≤5 kHz/ms.

The majority of neurons tested that were selective for the downward FM (dFM) sweep direction were also tuned to sweep rate. Of 11 dFM specialists tested for both direction and rate selectivity, 82% (n = 9) were rate tuned. Of 25 dFM-selective neurons tested for both, 76% (n = 19) were rate tuned. Thus of 36 direction-selective neurons tested, 78% were also selective for rate. Among neurons that were not direction selective, a greater percentage were also not rate tuned. Of 19 such neurons, only 42% were rate tuned.

Two different mechanisms create similar rate tuning

The mechanisms underlying rate tuning for downward FM sweeps were tested in 32 of the 40 rate-tuned neurons. Similar rate tuning appears to be created by two different mechanisms. The first is an early on-BF inhibition that creates duration tuning for excitatory tones, which is applicable to the 56% (n = 18) of rate-tuned neurons that were also selective for tone duration (Fig. 2, *A* and *B*). However, 44% (n = 14) of

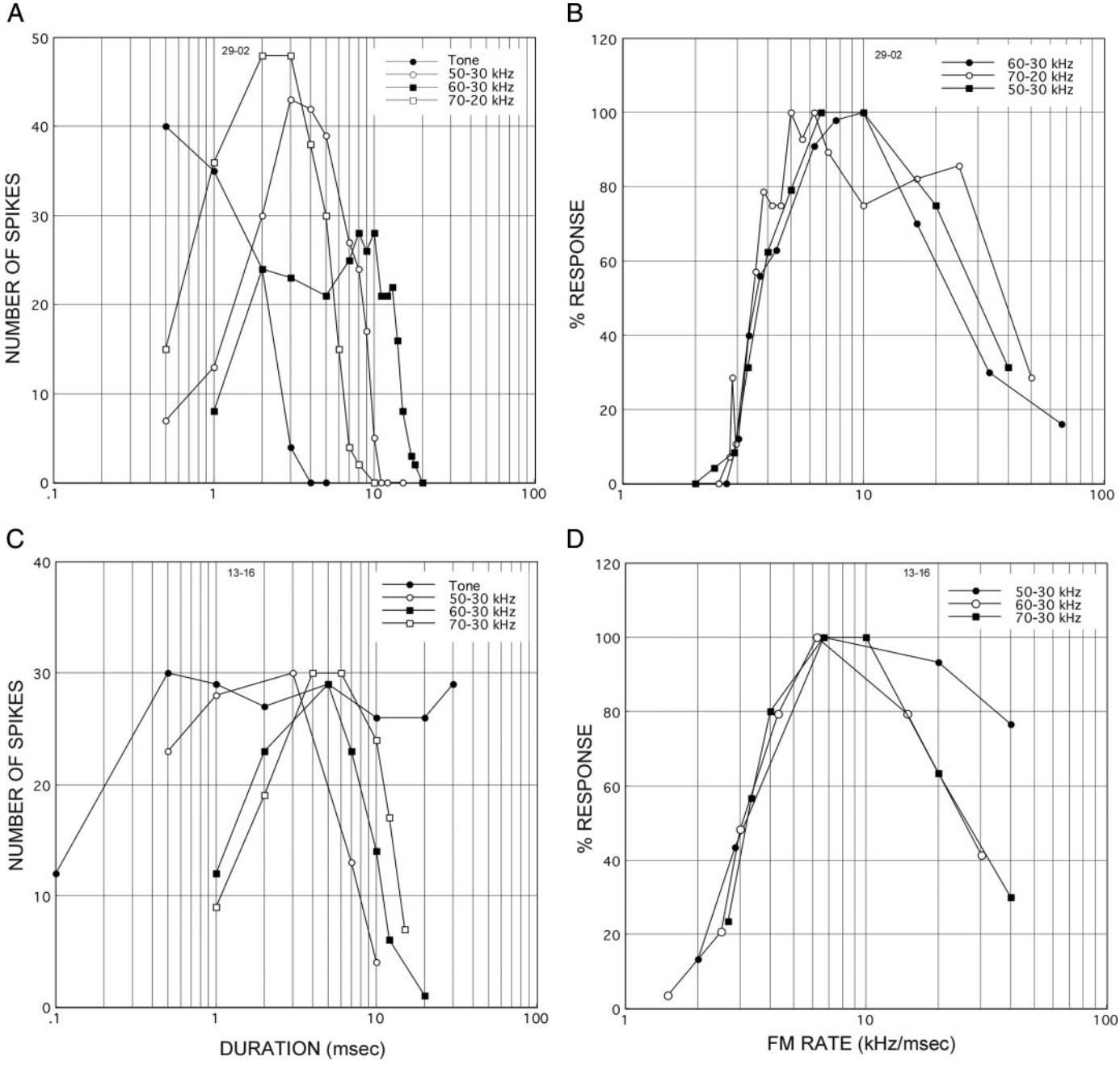


FIG. 2. Examples of similar FM sweep rate tuning in 2 neurons created by different mechanisms. *A*: this neuron is duration tuned for tones, which shapes its rate tuning, shown in *B*. *C*: this neuron is not duration tuned for tones, but has a similar rate tuning, shown in *D*. Note that in some neurons, the number of spikes varied with sweep bandwidth (e.g., *A*), whereas in the majority of neurons, the number of spikes remained quite consistent (e.g., *C*).

rate-tuned neurons were not duration tuned for tones (Fig. 2, *C* and *D*), so another mechanism must shape rate tuning. This appears to be a late-arriving, high-frequency inhibition, initiated by a dFM passing through frequencies higher than the excitatory frequency. This is termed delayed high-frequency inhibition. Because some duration-tuned neurons also have high-frequency inhibitory flanks, it is also possible that both mechanisms may work in concert.

Figure 3 provides an explanation of how these two mechanisms may shape rate tuning. Figure 3*A*, a plot of frequency versus the arrival time of inhibitory input relative to excitatory input, illustrates the inhibitory components that we assume to create FM sweep rate and direction selectivity. Figure 3*A* shows the excitatory frequency band (light gray) at the single test intensity and three inhibitory domains (dark gray). The low- and high-frequency inhibitory flanks (denoted 1 and 2,

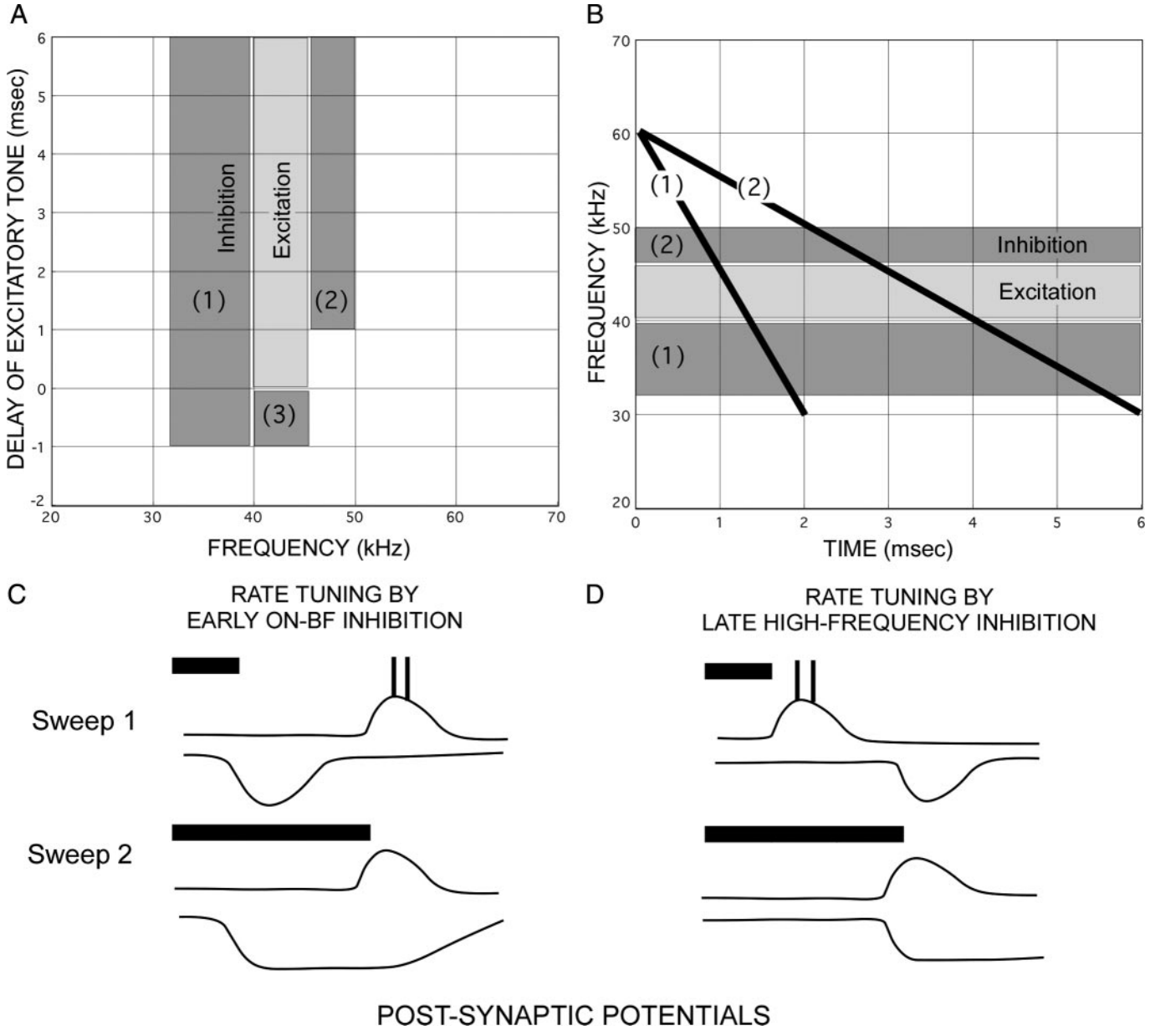


FIG. 3. Two working models for creating FM sweep-rate tuning. See text for full explanation. *A*: excitatory (light gray) and inhibitory (dark gray) domains of a neuron. Excitatory bandwidth is that obtained at the single test intensity. Inhibitory domains were obtained by presenting a tone at the best frequency (BF) and changing its onset time relative to a second, inhibitory tone of higher or lower frequency. Onset of the excitatory tone is arbitrarily set to 0 ms. Negative delay values mean that the excitatory tone was advanced relative to the inhibitory tone. If inhibition occurred under this condition, this means that inhibitory input arrived before excitation, as is the case for the low-frequency inhibitory sideband labeled (1). If inhibition was present only if the excitatory tone was delayed, this means the inhibition arrived after excitation, as is the case for the high-frequency inhibitory sideband labeled (2). *B*: same excitatory band, and inhibitory bands (1) and (2) shown on a time–frequency plot, illustrating the time that two 60- to 30-kHz sweeps of 2- and 6-ms durations (labeled 1 and 2) will spend within these bands. *C*: postsynaptic events expected in response to FM sweeps of the 2 durations if rate tuning is created by an early on-BF inhibition, labeled (3) in plot *A*. Black bars represent the durations of FM sweeps 1 (2 ms) and 2 (6 ms) shown in *B*. Below the bars, the *top line*, upward delection, represents the excitatory postsynaptic potential (EPSP) and action potentials (vertical lines); *bottom line*, downward deflection represents the inhibitory postsynaptic potential (IPSP). If the 2 overlap in time, it is assumed that there is no response. *D*: postsynaptic events expected if rate tuning is created by late high-frequency inhibitory band, labeled (2) in plots *A* and *B*.

respectively) are obtained with the TTI over time paradigm described above. The third inhibitory domain 3 is an on-BF inhibition that arrives before excitation. The presence of this third inhibitory component is inferred from a previous study (Fuzessery and Hall 1999), suggesting that this early inhibition creates a neuronal selectivity for short durations. This model for creating short-pass duration tuning has two components: an early inhibitory input and a delayed excitatory input, both generated by the same excitatory tone. The excitatory response is assumed to be a transient onset spiking of short duration and independent of sound duration. The inhibitory input lasts for the duration of the sound and, if the sound is long enough, the inhibition temporally overlaps with excitation and suppresses the response (Fig. 3*C*).

In this hypothetical example (Fig. 3*A*, inhibitory domain 1), the low-frequency inhibition arrives 1 ms before excitation and has a bandwidth of 8 kHz. The high-frequency inhibition (inhibitory domain 2) arrives 1 ms after excitation and has a bandwidth of 5 kHz. The excitatory bandwidth is 5 kHz, as is the bandwidth of the on-BF inhibition, which arrives 1 ms before excitation. Figure 3*B* shows the excitatory band and inhibitory bands 1 and 2 on a time–frequency plot, with two downward FM sweeps from 60 to 30 kHz traversing these bands. Sweep 1 has a duration of 2 ms and a rate of 15 kHz/ms; sweep 2 has a duration of 6 ms and a rate of 5 kHz/ms. Given these parameters, sweep 1 will excite the neuron, whereas sweep 2 will inhibit it, regardless of whether the neuron's rate selectivity is created by early on-BF inhibition (Fig. 3*C*) or late high-frequency inhibition (Fig. 3*D*).

If rate selectivity were created by early on-BF inhibition (Fig. 3*C*), the following events would occur during the course of these FM sweeps. Sweep 1 (Fig. 3*B*) would traverse the excitatory band in 0.33 ms because it is traveling at a rate of 15 kHz/ms through a 5-kHz band. This would generate a short inhibitory postsynaptic potential (IPSP) of about 0.33-ms duration and, 1 ms later, a transient excitatory postsynaptic potential (EPSP) (Fig. 3*C*). The two events would not overlap temporally and the neuron would respond. Sweep 2 would traverse the excitatory band in 1 ms because it is traveling through the 5-kHz band at a rate of 5 kHz/ms. The early IPSP would now last about 1 ms and overlap with the EPSP, thus countering depolarization and suppressing the response. This mechanism thus creates a short-pass filter for tone duration and a fast-pass filter for FM sweep rate.

If rate selectivity were created by late high-frequency inhibition (Fig. 3*D*), the neuron would respond to sweep 1 (Fig. 3*B*) because excitation arrives before inhibition. The sweep, traveling at 15 kHz/ms, would take 0.33 ms to traverse the 5-kHz high-frequency inhibitory band and reach the excitatory band. This rapid traversing of the inhibitory band is not long enough to offset the 1-ms delay in the arrival of high-frequency inhibition. Consequently, there would be a brief EPSP followed 1 ms later by a brief IPSP (Fig. 3*D*). The two would not overlap in time and the neuron would respond. In sweep 2 (Fig. 3*B*), the sweep is traveling a 5 kHz/ms and requires 1 ms to cross the 5-kHz inhibitory band. The excitatory input is now triggered 1 ms after inhibition, offsetting the delay in the arrival of inhibition. The EPSP and IPSP now overlap in time, depolarization does not occur, and the neuron would not respond. Like early on-BF inhibition, this mechanism also creates a fast-pass filter for FM sweep rate.

Selectivity for a downward sweep direction is created by the low-frequency inhibitory flank (Fig. 3*A*, inhibitory domain 1), which has a broader inhibitory flank than that of high-frequency inhibition, and arrives earlier than or approximately the same time as excitation. An upward FM sweep will therefore cause inhibition to arrive before excitation.

Duration tuning and rate tuning

Duration tuning is defined here as a short-pass or band-pass selectivity for the duration of a tone (e.g., Figs. 2*A* and 4*B*). We previously reported (Fuzessery and Hall 1999) that the high-frequency region of the pallid bat IC contains a large percentage of neurons (59%) that respond preferentially or exclusively to sounds with durations of ≤5 ms. A similar percentage (60%) was obtained in the present study.

The majority of the 18 neurons that were duration tuned for tones had very short best durations of ≤2 ms. The longest best duration was 5 ms. All neurons that were selective for tone duration were also selective for FM sweep rate and the following evidence suggests that, in the majority of these neurons, duration tuning shapes rate tuning. In 11 of the 18 neurons for which sufficient data were collected, a neuron's best rate could be predicted from its best duration and the bandwidth of its excitatory tuning curve. The underlying assumption is that a neuron will prefer a sweep rate in which the time spent within the excitatory bandwidth equals the best duration. The predicted best rate therefore equals the excitatory bandwidth divided by the best duration (kHz/ms). Figure 4 provides an example. The excitatory bandwidth (Fig. 4*A*) is 4 kHz at the test intensity (50 dB) and best duration is 0.5 ms (Fig. 4*B*), leading to a predicted best rate of 8 kHz/ms. The actual best rate is 12 kHz/ms (Fig. 4*C*). Figure 5 summarizes the predicted and actual best rates for 11 neurons. This high correlation ($r^2 = 0.797$) suggests that duration tuning contributes to rate tuning in these neurons.

The second possible mechanism—delayed high-frequency inhibition—might also contribute to the rate tuning of the 18 duration-tuned neurons studied. Of the 18 neurons, 11 tuned to both tone duration and sweep rate lacked high-frequency inhibition, so this possibility could be eliminated. However, the remaining seven neurons did have high-frequency inhibition. To directly test whether this inhibition contributed to rate tuning, the high-frequency inhibition was eliminated by starting the downward sweeps at the high-frequency flank of the excitatory tuning curve. If high-frequency inhibition contributed to rate tuning, then rate tuning should be eliminated. However, if duration tuning shapes rate tuning, eliminating the high-frequency inhibition from the sweep should have no effect on rate tuning. In six of the seven duration-tuned neurons, eliminating high-frequency inhibition did not eliminate rate tuning, as would be expected if duration tuning, and not high-frequency inhibition, were shaping rate tuning. However, in the one remaining neuron, rate tuning was also eliminated when high-frequency inhibition was removed, indicating that an unknown mechanism(s) shaped the rate tuning of this cell.

Delayed high-frequency inhibitory sidebands and rate tuning

Fourteen neurons that were selective for dFM rate were not selective for tone duration (e.g., Fig. 2*C*). Delayed high-

frequency inhibition appears to be responsible for shaping their rate tuning. As noted above, the premise is that as the dFM rate decreases, the temporal advantage enjoyed by the faster excitatory input is lost and the response is suppressed. Delayed high-frequency inhibition thus creates a fast-pass rate filter.

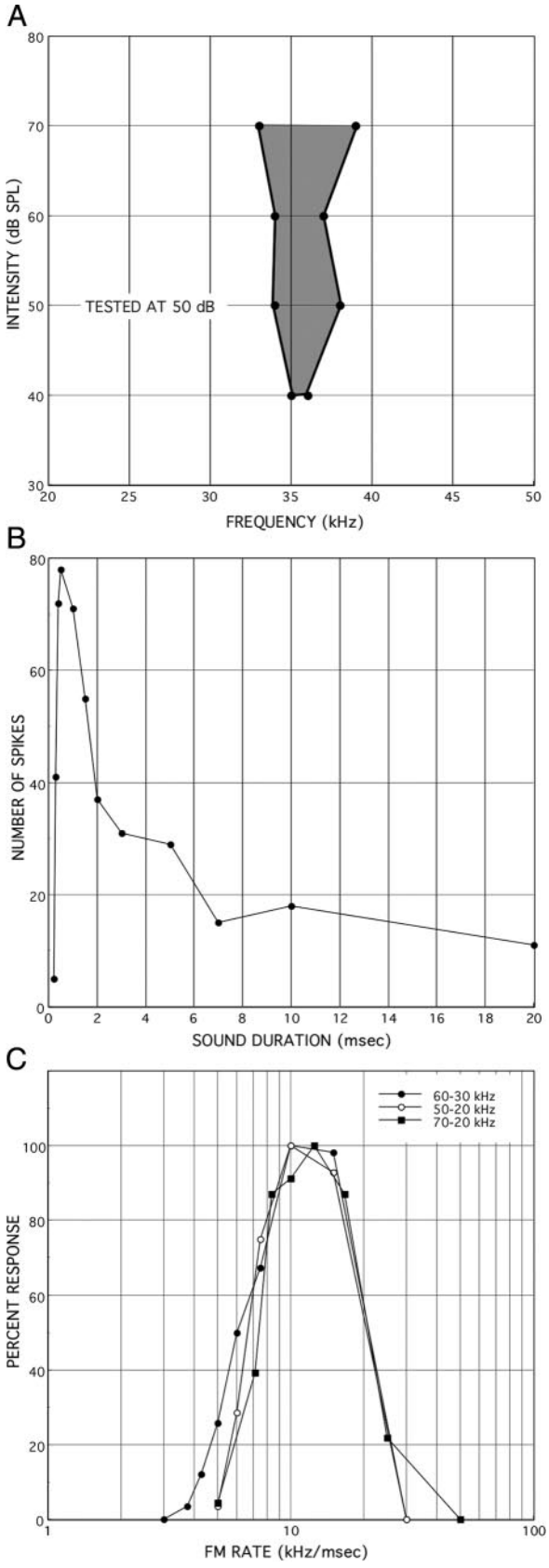


FIG. 4. Information used to predict the best FM sweep rate of a duration-tuned neuron, by the formula of rate = kHz/ms = excitatory bandwidth/best duration. *A*: bandwidth of the excitatory tuning curve at the test intensity of 50 dB is 4 kHz. *B*: best duration of the neuron is 0.5 ms. Predicted best rate is therefore 4/0.5 = 8 kHz/ms. *C*: actual best rate of this neuron, tested with 3 downward FM sweeps of different bandwidths, is 12 kHz.

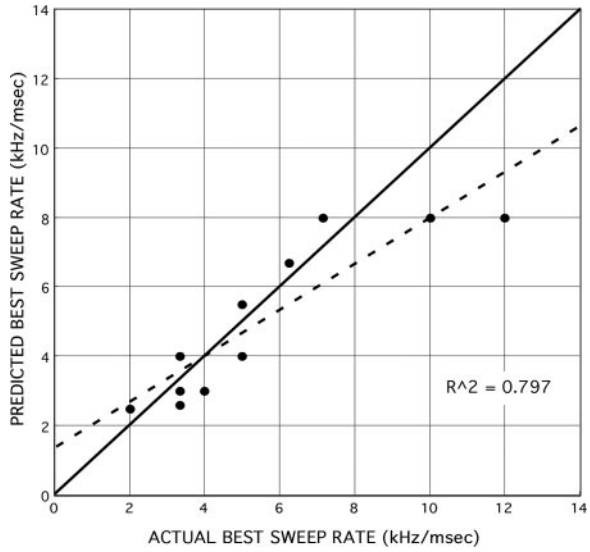
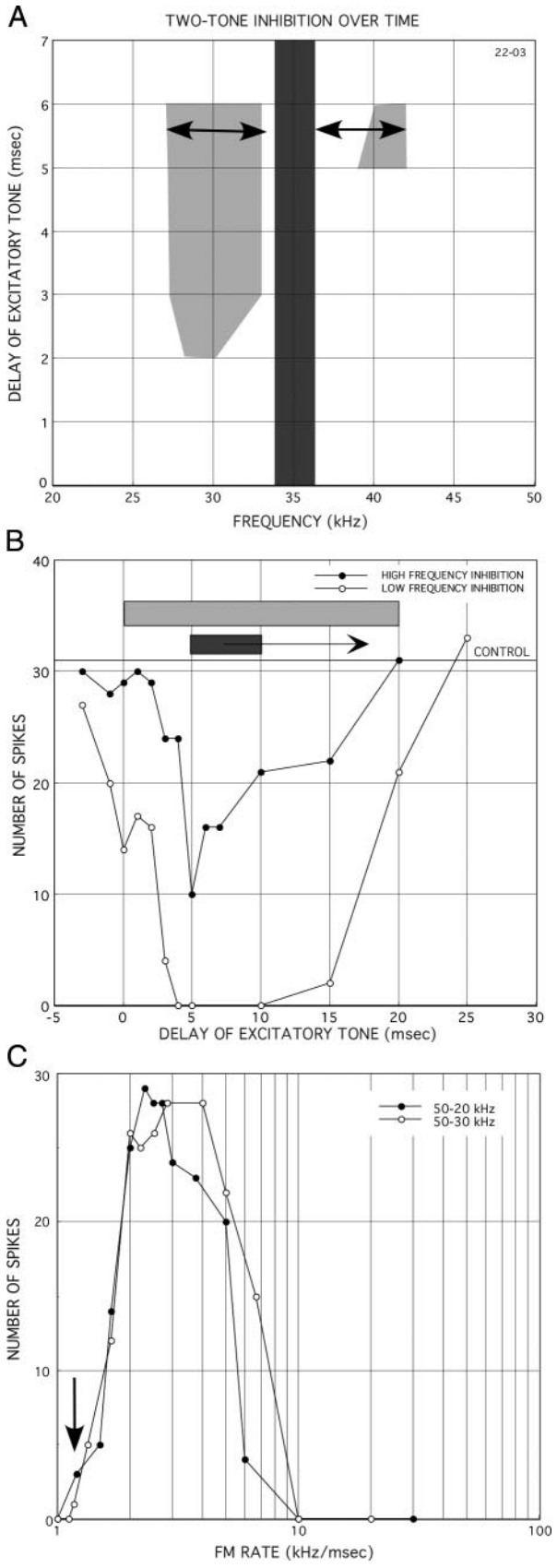


FIG. 5. Actual and predicted best FM sweep rates for downward FM sweeps of 11 neurons with duration tuning. Solid diagonal line indicates a perfect prediction; the dashed line is a simple linear regression; $r^2 = 0.797$.

Within the context of a spectrotemporally dynamic sound like an FM sweep, time and frequency are interchangeable. The arrival time of inhibition during a sweep depends on two factors: *1*) its arrival time relative to excitation when tested using a two-tone inhibition paradigm and *2*) the bandwidth of the inhibitory flank. The broader the bandwidth, the sooner the inhibitory flank will be encountered and the sooner inhibitory input will be triggered during a sweep.

The arrival times and bandwidths of inhibition were determined as follows. After the tonal response area was mapped, the TTI over time method (see METHODS) was used to quickly map inhibitory flanks in frequency and time. In the example shown (Fig. 6*A*), the excitatory tone had to be delayed 5 ms relative to a high-frequency tone before a high-frequency inhibitory sideband was apparent, indicating that high-frequency inhibition arrived 5 ms after excitation. A more detailed quantification of the arrival time of inhibition, relative to excitation, was obtained by measuring responses to pairs of tones, one from the excitatory tuning curve and one from the high-frequency inhibitory sideband (Fig. 6*B*). The timing of the excitatory tone (dark bar with arrow, Fig. 6*B*) had to be delayed 5 ms relative to the longer inhibitory tone (light bar, Fig. 6*B*) before the neuron's response was maximally suppressed. Note also that the inhibitory input triggered by low-frequency inhibition arrived earlier than that triggered by high-frequency inhibition (Fig. 6, *A* and *B*); this was typical of most neurons with both low- and high-frequency inhibitions.

The second feature of high-frequency inhibition needed to predict rate tuning is the bandwidth of the high-frequency inhibitory flank. In most neurons, the inhibitory and excitatory frequency domains were immediately adjacent to one another,



but in some there was a small gap between them. To more accurately quantify the separation in frequencies at which inhibitory and excitatory inputs would be activated during an FM sweep, the *spectral distance* was measured in kilohertz (Fig. 6*A*, arrows). For high-frequency inhibition, the spectral distance is the bandwidth between the highest frequency in the inhibitory flank and the highest frequency in the excitatory band. For low-frequency inhibition, it was the bandwidth between the lowest inhibitory frequency and the lowest excitatory frequency.

The differences in arrival time and spectral distance seen for this neuron (Fig. 6) are typical of the recorded population (Fig. 7). As shown in Fig. 7*A*, the arrival times of low- and high-frequency inhibitions of all neurons tested ($n = 36$) were significantly different (t-test, $P < 0.001$). The average arrival time of low-frequency inhibition was 0.00 ms, for high-frequency inhibition, 2.37 ms. Note that while all 36 neurons in Fig. 7*A* had low-frequency inhibition, only 22 also had high-frequency inhibition. Figure 7*C* shows the arrival times of low- and high-frequency inhibitions in these 22 neurons. The trend is for the low-frequency inhibition to arrive earlier and the two populations are significantly different (paired t-test, $P < 0.001$).

The average spectral distances for low- and high-frequency inhibitions were significantly different (t-test, $P < 0.001$), with 15.0 kHz for low-frequency and 4.2 kHz for high-frequency inhibition (Fig. 7*B*). Figure 7*D* compares the spectral distances of the 22 neurons that had both low- and high-frequency inhibitions. The trend is toward a greater spectral distance for low-frequency inhibition and the two populations are significantly different (paired t-test, $P < 0.001$).

The role of high-frequency inhibition in shaping rate tuning was tested in two ways. The first method is predictive, and calculates the sweep rate at which the delayed high-frequency inhibition will arrive at the same time as the excitatory input and suppress the response by 90% of maximum response. Note that this is different from predicting rate tuning created by duration tuning, where it is the best rate that is predicted. To calculate this 90% cutoff rate (kHz/ms), the "kHz" value used is the spectral distance and the "ms" value is the relative arrival time of inhibition that causes the response to drop to 10% of maximum response, when measured statically against an excitatory tone (e.g., Fig. 6*B*). In the example shown (Fig. 6), the

FIG. 6. Information needed to predict 90% cutoff rate for downward sweeps in a neuron that is not duration tuned. Rate tuning is assumed to be shaped by a delayed high-frequency inhibitory flank. This method predicts when the response of the neuron is reduced by 90% as sweep rate slows (see text for formula, predicted 90% inhibition occurs at 1.2 kHz/ms). *A*: 2-tone inhibition over time (see Fig. 3), showing the excitatory bandwidth at the single test intensity (dark gray) and the inhibitory bandwidths for 100% inhibition (light gray) obtained when inhibitory and excitatory tones are delayed with respect to one another. In this example, low-frequency inhibition arrives 2 ms later than excitation and high-frequency inhibition 5 ms later than excitation. Arrows indicate the spectral distances (see text) of low- and high-frequency inhibitions. *B*: a higher resolution measure of the arrival times of inhibition. A 20-ms inhibitory tone (light gray bar) from the low- or high-frequency inhibitory sideband in *A* is fixed in time and a shorter 5-ms excitatory tone (dark gray bar) is delayed or advanced relative to the inhibitory tones. Control is the response to the excitatory tone alone. Low-frequency inhibition (open circles) arrives 3 ms after excitation; high-frequency inhibition (filled circles) arrives 5 ms after excitation and does not completely inhibit. *C*: actual rate tuning, where 90% inhibition occurs at 1.25 kHz/ms (arrow).

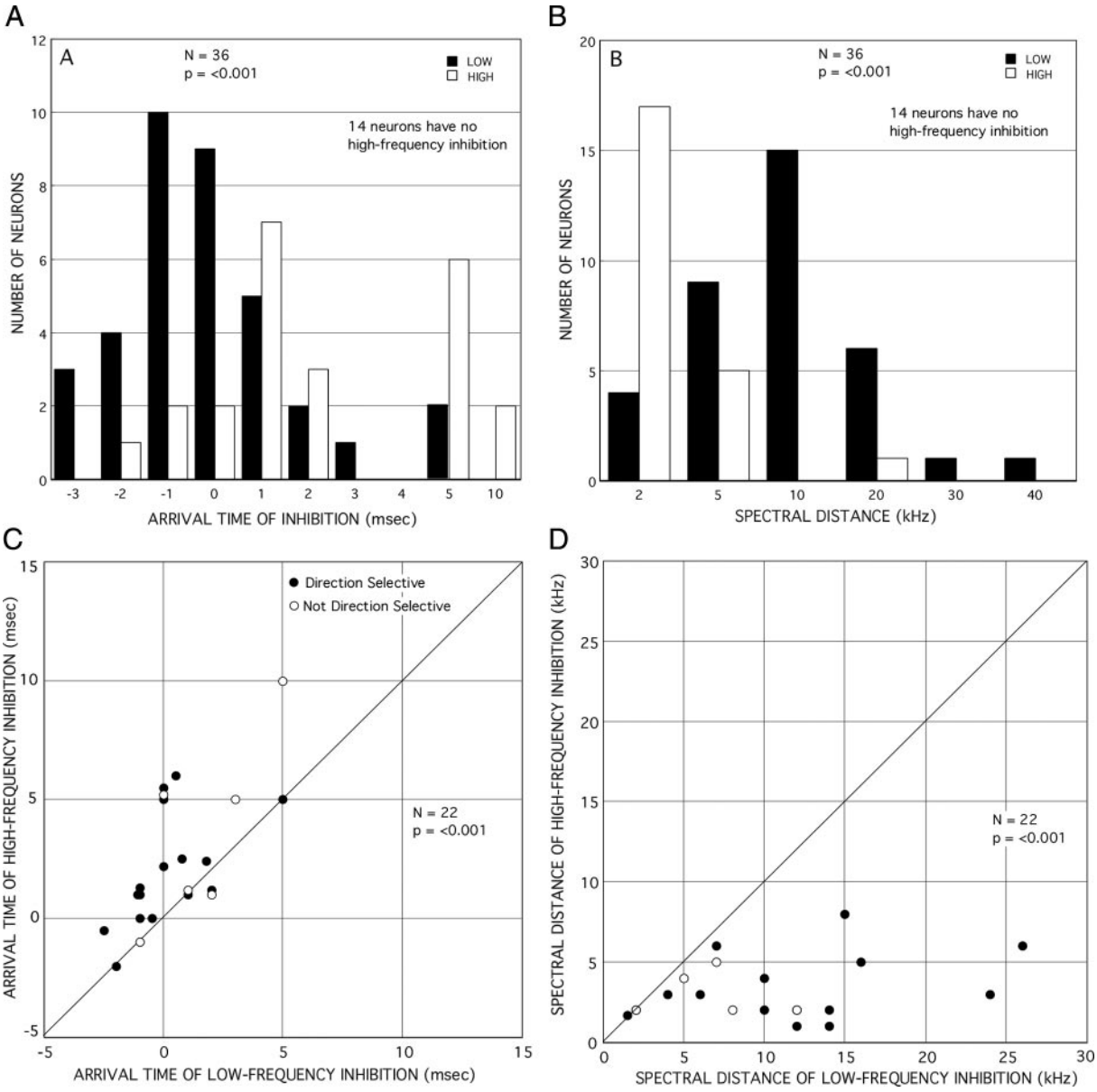


FIG. 7. Distributions of arrival time of inhibition relative to excitation (*A*) and spectral distance (*B*) of low-frequency (filled bars) and high-frequency (open bars) inhibition. Note that all neurons ($n = 36$) had low-frequency inhibition, but only 22 (61%) had high-frequency inhibition. High- and low-frequency populations are significantly different (Student's *t*-test, $P < 0.001$). *C*: distributions of arrival times (relative to excitation) of low- and high-frequency inhibitions in the 22 neurons that had both inhibitory sidebands. Low-frequency inhibition arrived before high-frequency inhibition in neurons above the diagonal line. Filled circles represent neurons that were selective for the downward sweep direction, whereas open circles represent neurons that were not selective for sweep direction. *D*: spectral distances of low- and high-frequency inhibitory sidebands in the same 22 neurons. Circles as in *C*. Spectral distances were broader for low-frequency inhibition in neurons below the diagonal line. In both *C* and *D*, low- and high-frequency populations were significantly different (paired Student's *t*-test, $P < 0.001$).

spectral distance for high-frequency inhibition is 6 kHz (36–42 kHz, Fig. 6*A*) and the inhibition arrives 5 ms after excitation (Fig. 6*B*). The neuron is predicted to respond to downward FM sweeps until the rate slows to 6 kHz/5 ms = 1.2 kHz/ms. The actual rate at which ≥90% inhibition occurs is about 1.25 kHz/ms (Fig. 6*C*, arrow). Note that although the inhibition generated by a representative tone from the high-frequency sideband did not generate complete inhibition, the neuron is nonetheless completely inhibited at slower downward sweep velocities. This may indicate that the inhibition generated during a downward sweep is stronger than that generated by a single tone within the sweep.

Of the 14 neurons whose cutoff rate was assumed to be created by delayed high-frequency inhibition, sufficient data for prediction were collected in 12 and the rate was predicted in nine neurons whose high-frequency inhibition arrived later than excitation (Fig. 8). The correlation ($r^2 = 0.996$) suggests that the properties of high-frequency inhibition do shape the 90% cutoff rates. However, in the remaining three of the 12 neurons, high-frequency inhibition arrived earlier than excitation. The predicted sweep rate cutoffs of these neurons could not be calculated because, according to our model, these neurons should not have responded to downward sweeps at all. Additional unknown

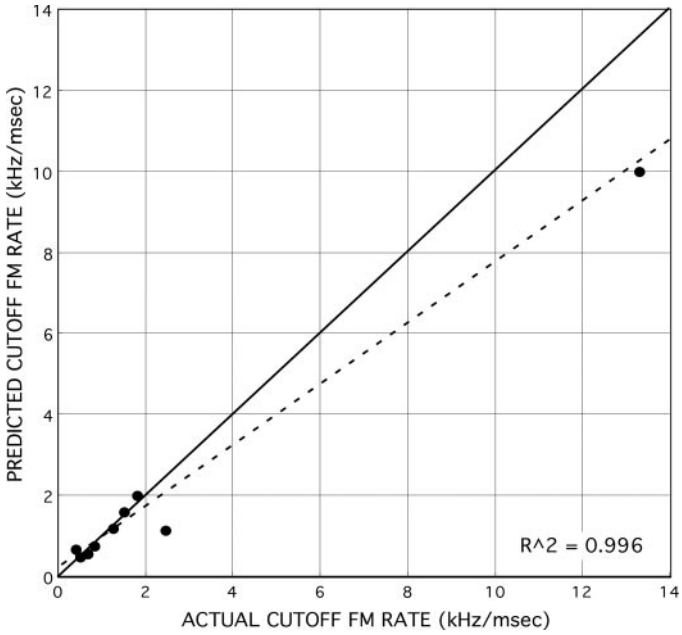


FIG. 8. Actual and predicted cutoff rates of 9 neurons, predicting the slowing rate at which their response would be reduced by 90% of maximum response. Rate tuning is assumed to be shaped by delayed high-frequency inhibition. Solid line indicates a perfect prediction; dashed line shows a linear regression; $r^2 = 0.996$.

mechanisms must therefore shape both the rate and direction selectivity in these three neurons.

The second method directly tested the role of delayed high-frequency inhibition in shaping rate tuning by eliminating the high-frequency flank from the downward sweep. This was accomplished by starting the downward sweep at the high-frequency side of the excitatory tuning curve. An example is shown in Fig. 9. This neuron was not duration tuned (Fig. 9*B*), but sensitive to sweep duration (and thus rate) if the downward sweep included the high-frequency inhibitory sideband (40- to 20-kHz sweep; Fig. 9, *A* and *B*). However, if the downward sweep was started within the excitatory tuning curve (35- to 20-kHz sweep; Fig. 9, *A* and *B*), eliminating high-frequency inhibition, rate tuning was also eliminated. As noted above, this is in contrast to neurons whose rate sensitivity was shaped by duration tuning, for which starting the downward sweep within the excitatory tuning curve did not eliminate rate tuning. Eight of the 14 neurons were tested in this fashion. In seven of eight, rate tuning was eliminated, indicating that high-frequency inhibition was necessary for creating rate tuning. The exception suggests that additional mechanisms must contribute to its rate tuning because the high-frequency inhibitory sideband was not necessary for rate tuning; moreover, duration tuning, the second mechanism proposed to shape rate tuning, was not present in this neuron.

Shaping direction selectivity

Selectivity for the downward sweep direction observed in 66% of the recorded population was correlated with the finding (Fig. 7) that low-frequency inhibition in most neurons arrived earlier than or at the same time as excitation and that low-frequency inhibitory flanks were, on average, broader than the high-frequency inhibitory flanks (15 vs. 4.2 kHz). The role of low-frequency inhibition in shaping direction selectivity was tested directly by eliminating it from upward FM sweeps by starting the sweeps at the lowest excitatory frequency. As shown in Fig. 10, this neuron had a low-frequency inhibitory sideband that was broader than that of high-frequency inhibition and also arrived earlier than high-frequency inhibition (1 vs. 5 ms). It responded well to a downward FM sweep from 40 to 20 kHz, but barely responded to a spectrally identical upward sweep of 20–40 kHz (Fig. 10*B*). However, if the upward sweep started at 27 kHz, eliminating the low-frequency inhibitory sideband from the sweep, the neuron responded nearly as strongly as it did when presented with an excitatory tone, at short durations of $\leq$5 ms. Note also that the neuron is rate tuned for downward sweeps arising from the delayed high-frequency inhibition (note that its response to an excitatory tone indicates that it is not duration tuned), but that it was not rate tuned for the upward 27- to 40-kHz sweep because, in this sweep, the delayed high-frequency inhibition would be triggered after excitation. In 10 of 12 neurons tested, this manipulation eliminated direction selectivity. It did not do so in the remaining two, suggesting that additional mechanisms are shaping their direction selectivity.

Direction selectivities of two other neurons were also not created by an early, broadband low-frequency inhibition. Figure 7 shows one extreme case in which the low-frequency inhibition of a neuron arrived 5 ms late relative to excitation (Fig. 7*D*, filled circle). Moreover, the bandwidth of the low-frequency sideband was among the most narrow observed (1 kHz). In fact, this neuron had similar late arrival times and narrow spectral distances for low- and high-frequency inhibitions. Because it had no apparent spectral or temporal asymmetries in its inhibitory flanks, it would have been predicted to respond to both upward and downward sweeps, although it responded only to downward sweeps. We also observed a second exception that had no low-frequency inhibition at all; it was selective for downward sweeps, but would have been predicted to also respond to upward sweeps.

One neuron (Fig. 11) whose low-frequency inhibition arrived very early, 3 ms before excitation, is of particular interest in terms of its direction selectivity. This neuron, as expected, did not respond to upward sweeps, but it also did not respond to downward sweeps if the downward sweeps entered the low-frequency inhibitory flank, whose highest frequency was 36 kHz (Fig. 11*A*). This neuron did not respond to standard downward FM sweeps of 60–30 and 70–20 kHz, but did respond to a 45- to 36-kHz sweep, which stopped just before entering the low-frequency inhibitory flank, with the same response magnitude evoked by an excitatory tone (Fig. 11*B*). However, if the downward sweeps were extended into the inhibitory flank (sweeps 45–34 and 45–32 kHz, Fig. 11*B*), the responses progressively decreased.

This unusual neuron highlights two points. First, low-frequency inhibition must arrive early to create direction selectivity for downward sweeps, but it must not arrive too early or it will also suppress responses to downward sweeps. Second, the sequence of synaptic inputs activated by an FM sweep does not necessarily reflect the sequence of frequencies in the sweep. In this case, frequencies in the low-frequency sideband occur last in downward sweeps, but apparently trigger input that arrives before excitation.

Duration of inhibition

Finally, the models of the mechanisms underlying rate and direction selectivity require that the inhibition flanking the excitatory band, once triggered during a sweep, lasts long enough to prevent a neuron from responding as the sweep enters the excitatory band. The two-tone paradigm provides information about whether inhibition lasts the duration of the inhibitory tone (tonicity) and how long it persists after that duration before the response returns to 90% of maximum response.

Using the equation described in METHODS, the tonicity and persistence of low-frequency inhibition were calculated for 34 neurons. In the great majority (94%, $n = 32$), inhibition lasted the duration of the 5- to 30-ms inhibitory tones used for two-tone inhibition, i.e., they had complete tonicity. In 73% ($n = 25$), inhibition persisted 1–10 ms beyond the duration of the inhibitory tone and in 23% ($n = 8$) persisted >10 ms, ≤43 ms. In one case, a range of inhibitory tone durations was tested (Fig. 12) and inhibition lasted the duration of the inhibitory tone as its duration was increased from 20 to 100 ms. In the remaining two neurons that lacked tonicity, inhibition was short-lived, returning to 90% maximum response in 3 and 7 ms when 20-ms inhibitory-tone durations were used.

High-frequency inhibition was measured in 15 of these 34 neurons. The majority (87%, $n = 13$) had inhibition that lasted for the duration of the inhibitory tone. Of these, seven had inhibition that persisted ≤5 ms beyond the duration of the inhibitory tone. Inhibition in the remaining six neurons persisted for >5 ms; the longest persistence observed was 23 ms. In the two neurons that did not display tonic high-frequency inhibition, inhibition lasted only 1 and 6 ms when tested with 20-ms inhibitory tones. The conclusion we draw is that the majority of neurons have inhibitory inputs that are sufficiently tonic and persistent so that, once activated, they will suppress responses during the brief 1.5- to 5-ms FM sweeps produced by an echolocating pallid bat.

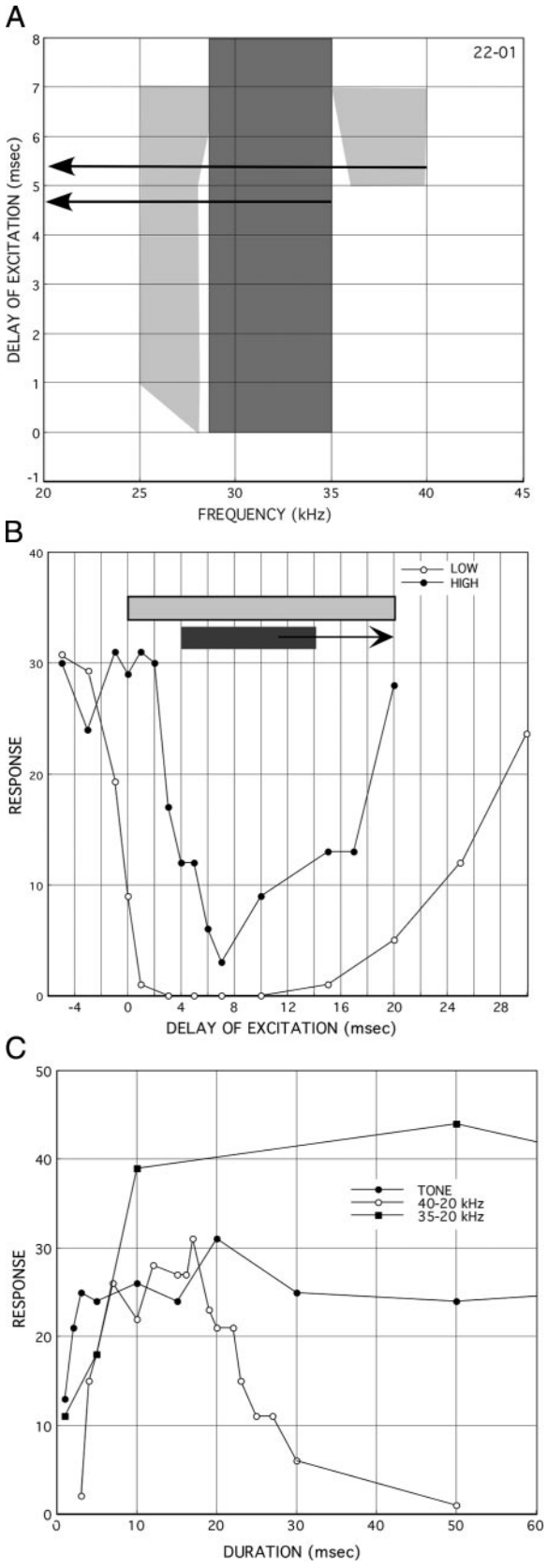


FIG. 9. An example of a neuron whose sweep rate tuning is shaped by delayed high-frequency inhibition. Sweep rate tuning is eliminated if the high-frequency inhibition is excluded from the downward FM sweep. See Figs. 3 and 6 for explanations of symbols in *A*. *A*: this neuron has high-frequency inhibition with a spectral distance of 5 kHz and arrives 5 ms after excitation. Arrows represent 2 downward FM sweeps of 40–20 kHz, which includes the inhibitory sideband, and 35–20 kHz, which starts at a frequency lower than the inhibitory sideband. *C*: neuron is not duration tuned for the tone (filled circle). Therefore duration tuning cannot contribute to the neuron's rate tuning, which is apparent in its response to the 40- to 20-kHz sweep (open circles). When the high-frequency inhibitory sideband is eliminated from the sweep in the 35- to 20-kHz sweep (filled squares), rate selectivity is lost.

DISCUSSION

The main conclusion drawn from present results is that the response selectivity of most neurons for the direction and rate of FM sweeps could be explained by relatively simple rules describing the timing of excitatory and inhibitory inputs as they occur during the course of an FM sweep. At the least, the responses of most neurons to FM sweep dimensions correlated well with responses to combinations of excitatory and inhibitory tones present in the sweeps. This suggests that the responses of these neurons behave in a largely linear fashion, in the sense that their responses to FM sweeps can be predicted from responses to constituent tones. In other words, their response selectivity is not dependent on conditions created only during FM sweeps. However, this conclusion must be tempered by the finding that there were neurons whose re-

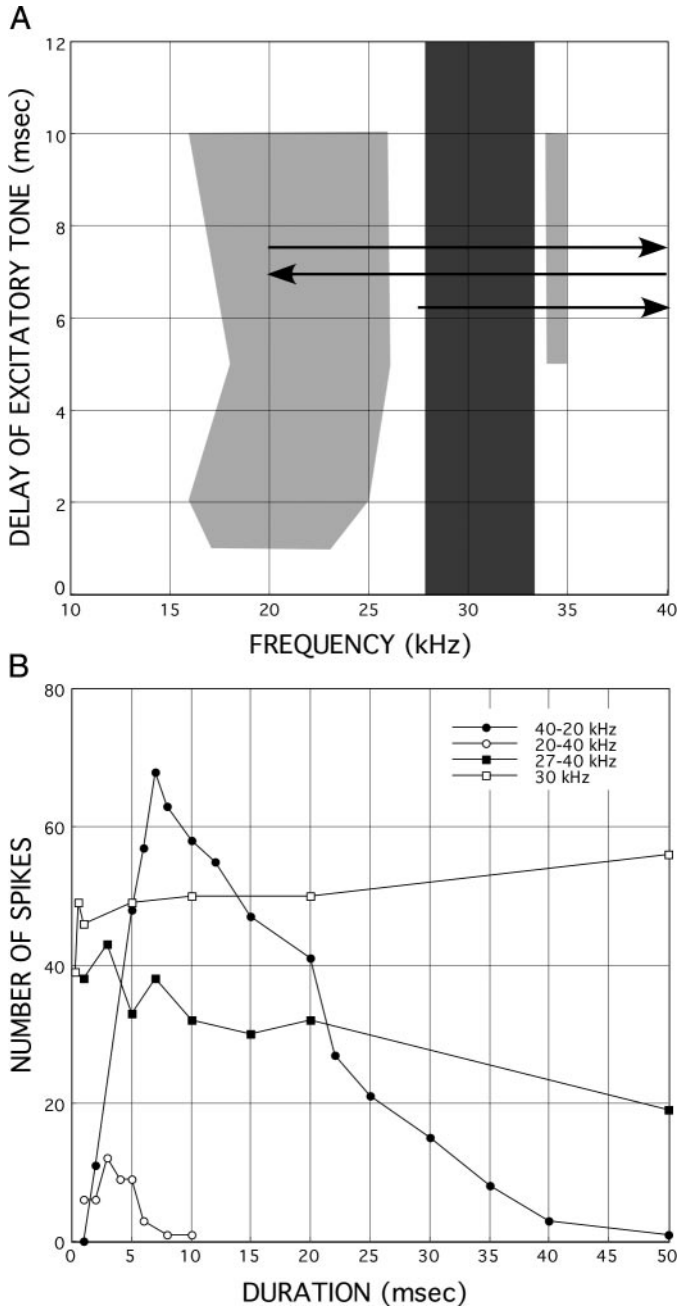


FIG. 10. An example demonstrating that eliminating the low-frequency inhibition from an upward FM sweep eliminates direction selectivity. *A*: 2-tone inhibitory flanks over time, where dark gray depicts the excitatory band and light gray indicates the inhibitory flanks. Arrows show the FM sweeps generating the responses shown in *B*. *B*: neuron responds much more strongly to a downward sweep of 40 to 20 kHz (filled circles) than to an upward sweep of 20 to 40 kHz (open circles). If the upward sweep starts at 27 kHz (filled squares), which excludes the low-frequency inhibitory flank, the response is nearly as large as a response to an excitatory tone of 30 kHz (open squares), at least at shorter durations.

sponse properties could not be predicted by these rules, suggesting that *1*) a single mechanism may not always dominate election of the response selectivity, *2*) the synaptic inputs inferred from responses to representative excitatory and inhibitory tones are not always accurate in predicting those generated in the course of a spectrotemporally complex signal like an FM sweep, and *3*) additional mechanisms not incorporated into predictions play an important role. These caveats may apply particularly to the FM specialists, which we were unable to drive with tone combinations. Their responses are likely dependent on facilitatory events generated only during downward FM sweeps (Fuzessery and Hall 1996).

These caveats aside, the response selectivity for both sweep direction and rate in most neurons could be explained by inhibitory mechanisms. All neurons selective for sweep direction were selective for the downward direction, as was also found in a previous study of the pallid bat IC (Fuzessery 1994). This selectivity is created by two types of asymmetry in

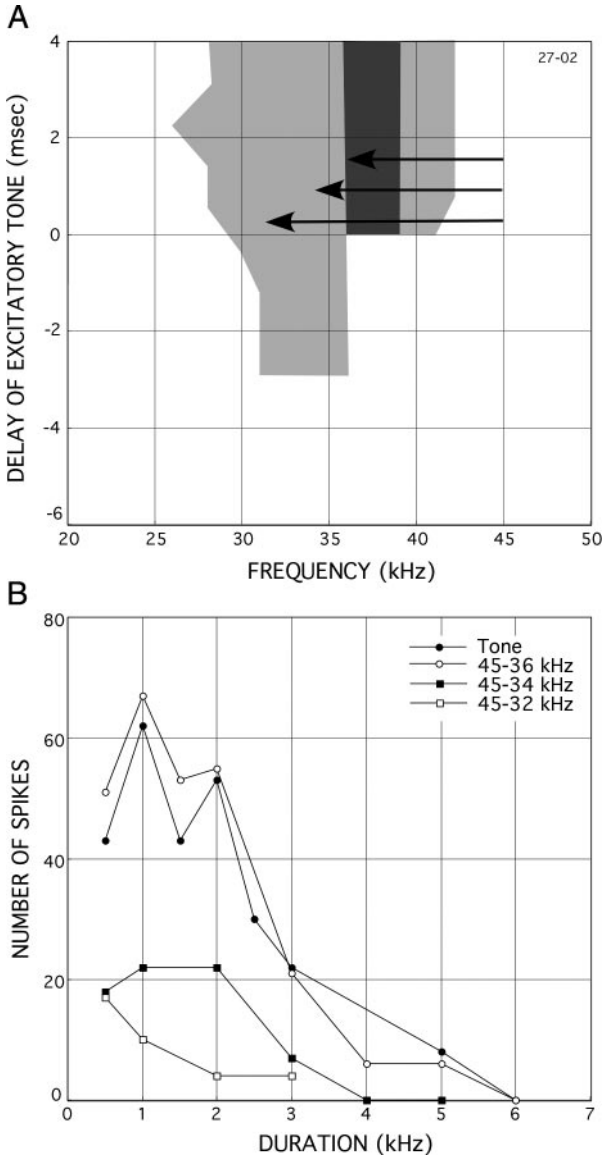


FIG. 11. Response properties of a neuron whose low-frequency inhibition arrived very early and prevented responses to upward FM sweeps, and also to downward FM sweeps that entered the low-frequency inhibitory flank (see text). *A*: 2-tone inhibition over time function shows that the low-frequency inhibition (light gray) arrives 3 ms before excitation (dark gray). Arrows indicate the spectra of the 3 FM sweeps whose duration functions are shown in *B*. A downward FM sweep of 45–36 kHz does not enter the low-frequency inhibitory sideband and evokes a response (open circles) similar to that of an excitatory tone (filled circles). As the downward sweeps extend further into the inhibitory sideband (45- to 34-kHz sweep, filled squares; 45- to 32-kHz sweep, open squares), the responses decrease.

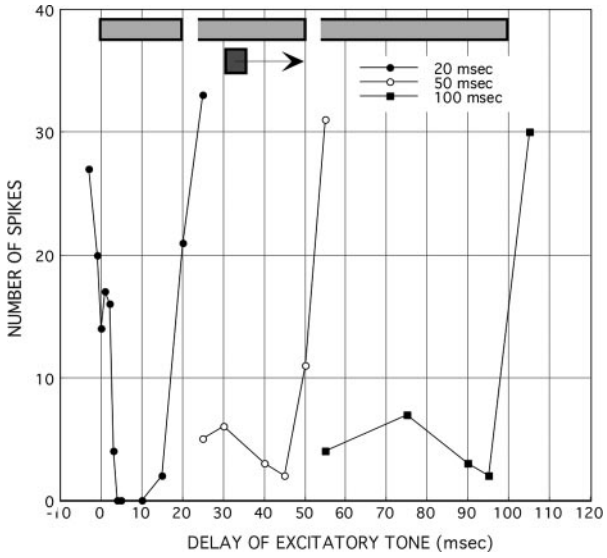


FIG. 12. Example of a neuron in which 2-tone inhibition was tested with an excitatory tone of 5-ms duration and inhibitory tones of 3 different durations of 20, 50, and 100 ms, illustrating that the inhibition can last for the duration of the inhibitory tone.

sideband inhibition. Low-frequency inhibition arrived earlier than high-frequency inhibition when tested with two-tone inhibition and the average bandwidth of low-frequency inhibition was significantly broader. The broader bandwidth further advances the arrival of low-frequency inhibition during the course of an upward FM sweep, assuming that the latency of an inhibitory tone (relative to the latency of an excitatory tone) accurately predicted its latency within the context of an FM sweep. Moreover, low-frequency inhibition was always present in downward direction–selective neurons (with one exception), whereas high-frequency inhibition was absent in 40% of the neurons tested.

There is, of course, nothing novel about the finding that sideband inhibition creates direction selectivity. This inhibitory mechanism has often been suggested since the earliest studies of directional selectivity for spectrotemporally dynamic sounds (e.g., Britt and Starr 1976; Heil et al. 1992a,b; Suga 1973). We can emphasize, however, that the pallid bat IC appears to regulate the arrival time of inhibition through a modulation of the bandwidth of inhibition. Because time and frequency are interchangeable during the course of a spectrotemporally dynamic signal like an FM sweep, this is a simple and effective mechanism for modulating the arrival time of inhibition. Inhibition can actually arrive later than excitation when tested with tone pairs, but still arrive earlier than excitation during the course of a sweep if the inhibitory bandwidth is appropriate. A practical implication, as noted by Gordon and O'Neill (1998), is that if the temporal relationship of the tones in a two-tone inhibition paradigm is not varied, the inhibitory flanks that shape direction and rate selectivity may not be apparent.

Different mechanisms, same rate selectivity

The great majority of neurons were tuned to sweep rate. An unexpected finding was that essentially identical rate tuning could be created by two different mechanisms: *1*) an early on-BF inhibition, which creates duration tuning; and *2*) a delayed high-frequency inhibition. Duration tuning is defined with tonal stimulation rather than FM sweeps because tones are of invariant frequency and the sensitivity cannot be confused with a sensitivity to FM sweep rate. Previous studies (Casseday et al. 1997; Fuzessery and Hall 1999; Gordon and O'Neill 1998) of rate or duration selectivity have noted that these two forms of sensitivity can be readily confused.

In a previous study of the pallid bat IC (Fuzessery and Hall 1999), roughly 50% of the neurons in the high-frequency region were found to have short-pass or band-pass selectivity for tone durations. In the present study, 60% of rate-tuned neurons exhibited this selectivity. The rate tuning of some duration-tuned neurons could be predicted through calculation of the time that FM sweeps spent within a neuron's excitatory tuning curve. In addition to this prediction, we also eliminated the possibility that high-frequency inhibition contributed to rate tuning by starting the downward sweeps in the excitatory tuning curve. In the majority of cases, this had no effect on rate tuning, indicating that it was indeed duration tuning that shaped rate tuning, and not the inhibitory sidebands.

Duration tuning—and the resultant sweep rate tuning—has been suggested (Fuzessery and Hall 1999) to be created by an on-BF inhibition in which excitatory frequencies first evoke an early inhibition that lasts the duration of the sound, followed by a later excitation with a fixed response latency. If the sound lasts long enough, inhibition and excitation will overlap in time and the response will be suppressed. In effect, the excitatory receptive fields of these neurons last only a few milliseconds. The mechanism underlying duration tuning has also been modeled as a coincidence mechanism, in which a rebound from the early inhibition contributes to excitation (Casseday et al. 1994). Present experimental design does not allow us to lend support to one model over the other. Faure et al. (2003) and present results provide evidence that this early inhibition typically lasts the duration of the sound, as required of these models, so both models are plausible.

These findings suggest that the purpose of early on-BF inhibition in the high-frequency region of the pallid bat IC is to shape neuronal selectivity for the rate of the bat's echolocation pulse and not a selectivity for durations of tonal stimuli. The great majority of duration-tuned neurons had best durations of $\leq$2 ms and some did not respond to tone durations $>$0.5 ms. It is unlikely that the role of these neurons is to process very short duration tones because the communication sounds used by this bat are largely of lower frequency and/or longer duration than the echolocation pulse and are also frequency modulated (Brown 1976).

Rate tuning for downward FM sweeps is also created by an "off-best frequency," delayed high-frequency inhibitory sideband. In addition to a delay in the arrival of this inhibition relative to excitation, the bandwidth of this inhibitory flank is typically narrower than the low-frequency flank. This ensures that high-frequency inhibition will not be activated too early during a downward FM sweep and thus suppress the response. This mechanism creates a "fast-pass" rate tuning in which the neuron responds to downward FM sweeps until the rate slows to the extent that the difference in arrival times of excitation and high-frequency inhibition is negated. In actuality, neurons influenced by this mechanism typically show a band-pass, rather than fast-pass, rate tuning and it is likely that the high-rate flanks of their rate functions are shaped simply by an

inability to detect and respond to extremely short duration FM sweeps.

It is of interest that an early on-BF inhibition and a late high-frequency inhibition both create fast-pass filters for sweep rate. The fact that most of the rate-tuned neurons described in this study exhibited band-pass rate functions therefore needs explanation. At present, we can only suggest that the drop in response at faster sweep rates may be the result of a neuron's integration time or detection threshold.

Comparison of IC and auditory cortex

A comparison of present results and those of a parallel study in the pallid bat auditory cortex (Razak and Fuzessery 2006) is of general interest with regard to hierarchial processing in sensory systems. The rate selectivity at both levels is similar. Neurons with band-pass rate tuning do not have significantly different best rates, although the sharpness of tuning is significantly more narrow in the IC. Although the selectivity is generally similar, however, an unexpected finding is that one of the mechanisms that creates rate tuning in the IC seems reduced or absent at the cortical level. All rate tuning in the auditory cortex is shaped by delayed high-frequency inhibition. Duration tuning is rare and, when present, does not predict rate tuning. This apparent loss of an underlying mechanism is perhaps the result of a convergence of input onto thalamic and/or cortical neurons from IC neurons that are duration tuned and others that are not. The result would appear as a lack of duration tuning. This finding has an important implication. The presence of similar forms of selectivity at the midbrain and cortical levels does not necessarily imply that the auditory cortex simply inherits the selectivity. The auditory cortex may have to re-create, at least in part, forms of response selectivity created at lower levels of the system. This notion is supported by a recent patch-clamp study of FM sweep processing in the rat auditory cortex (Zhang et al. 2003), suggesting that intracortical processes sharpen neuronal selectivity for FM sweep direction.

Regarding behavioral relevance, the rate tuning of IC and cortical neurons is appropriate for processing the exponential FM sweep of the bat's echolocation pulse. The average best rate at both levels of the system was 3–4 kHz/ms, and ranged from 1 to 12 kHz/ms. The instantaneous rates of 2-ms echolocation pulses at 40–45 kHz (the best frequencies of most recorded neurons) is 7–8 kHz/ms. The average rate tuning is thus a little lower than might be expected. It is possible that if exponential, rather than linear, FM sweeps had been used as test stimuli, the sweeps may have traversed the high-frequency inhibitory flanks more rapidly (in neurons possessing this inhibition), resulting in a range of best sweep rates more in line with what the bat normally experiences.

Facilitatory mechanisms

The use of two or more tones that evoke excitatory and inhibitory events has proven a simple and useful tool for delineating the timing of these inputs and predicting responses to more complex sounds (e.g., Brosch and Schreiner 2000; Faure et al. 2003; Fuzessery and Feng 1983; Gordon and O'Neill 1998; Nataraj and Wenstrup 2005). Its use in the present study proved successful in revealing the properties of inhibitory mechanisms that contribute selectivity for dimensions of FM sweeps. However, it is likely that excitatory mechanisms, in the form of summation or facilitation, also contribute to response selectivity. This inference derives from several sources, perhaps the most obvious being the FM specialists described here and in previous studies (e.g., Casseday and Covey 1992; Casseday et al. 1997; Fuzessery 1994; Shannon-Hartman et al. 1992; Suga 1965, 1969). Such neurons do not respond to individual tones in an excitatory sweep, suggesting a facilitation of subthreshold excitatory events that occurs only during the course of an FM sweep. Moreover, a neuron need not respond exclusively to FM sweeps to infer facilitation (O'Neill and Brimijoin 2002). Additional mechanisms not revealed here are also suggested by neurons whose response selectivity could not be predicted, notably neurons with delayed low-frequency inhibition (or none at all) that should not have been selective for downward sweeps, or with early high-frequency inhibition that should not have responded to downward sweeps. The direction selectivity of such neurons may have been shaped by strong facilitatory mechanisms that dominated inhibitory events. In a study similar in its use of "two-tone stimulation over time," Brimijoin and O'Neill (2005) were able to measure both the facilitatory and inhibitory events that occurred as the intervals between the two tones were varied. Of particular interest is that, in addition to predicting response selectivity based on spectrally asymmetrical inhibition, they found that spectrally asymmetrical facilitatory events that would be generated during the course of an FM sweep moving in one direction could also be used to predict directionality. Similar facilitatory events observed with two-tone paradigms have been reported in auditory cortex by Brosch and Schreiner (1997, 2000), but these interactions typically occurred between tones with much greater spectral and temporal disparities than those tested in the present study. Thus a potential shortcoming of the present study is that, although our methods were effective at delineating the timing of inhibitory events triggered by sidebands adjacent to excitatory tuning curves, more spectrally distant, subthreshold events that might participate in shaping rate and direction selectivity during an FM sweep were not revealed.

Temporal summation or facilitation may occur through the buildup of temporally overlapping excitatory events, as proposed by the cable models of Rall (1964) or network models of Suga (1965), or with a contribution from inhibition in which a rebound from inhibition coincides with excitation (Covey and Casseday 1999; Nataraj and Wenstrup 2005). These excitatory mechanisms can shape both direction and rate selectivity (Heil et al. 1992a; Phillips et al. 1985). If, for example, the response latency of a neuron to excitatory tones is shortest for tones at the center of its excitatory tuning curve, then the neuron would experience temporal summation of excitatory inputs, through coincident arrival, when presented with FM sweeps directed toward the center of the tuning curve over a certain sweep rate range. Although not described in this report, we did attempt to test for the presence of such mechanisms by using two- and three-tone combinations in sequences simulating their occurrence in downward FM sweeps, as performed by Gordon and O'Neill (1998). Durations of these tones were reduced to the extent practical (0.1 ms) to simulate their time course within a sweep. Combinations of inhibitory and excitatory frequencies, presented in the sequence in which they would occur in a

downward FM sweep, were also tested to determine whether inhibitory rebound played a role in exciting the FM specialists. These attempts were unsuccessful, suggesting that these FM specialists may be highly tuned to events generated during the course of downward FM sweeps—events that this study did not successfully simulate with pure tones.

In a previous study of FM specialists (Fuzessery and Hall 1996), we found that all excitatory frequencies in a downward sweep, when presented as single tones, were able to completely suppress responses to downward sweeps when both signals were presented simultaneously. We modeled this phenomenon as indicating that these neurons receive strong inhibitory input that can be overcome only by excitatory inputs created during the course of a downward sweep. At this point, we cannot provide further insight into the nature of these excitatory inputs in adult bats. We have, however, observed facilitatory events in the IC of immature bats that occur in response only to downward FM sweeps over a specific range of rates (Fuzessery and Richardson 2006).

In conclusion, the main point we wish to emphasize is that auditory systems have more than one way of creating the same form of neuronal selectivity for behaviorally relevant sounds. A natural extension of this point is that, during development, these systems may avail themselves of more than one pathway to achieve the same endpoint. We have attempted to demonstrate that small millisecond shifts in the arrival times of low- and high-frequency inhibitions, combined with changes in the bandwidths of these inhibitory sidebands, can determine whether these inhibitory flanks elect sensitivity to FM sweep direction or rate. The neuron in Fig. 11 highlights the fact that these timing changes must not be overdone. Advancing the arrival time of low-frequency inhibition is necessary for creating a direction selectivity for downward FM sweeps, although too much advancement will suppress responses to sweeps in either direction. Rate tuning can be achieved through inhibitory inputs activated by either a flanking "off-best frequency" inhibition or by an early "on-best frequency" inhibition that was initially implicated in the creation of duration tuning (Casseday et al. 1994; Fuzessery and Hall 1999). We have inferred that this duration selectivity is created to shape FM sweep rate tuning, a view supported by the fact that the resulting rate tuning approximates rates of the bat's echolocation pulse.

We have also suggested that there are additional mechanisms that shape these forms of response selectivity. The significant percentage of neurons that do not respond at all to tones within an excitatory FM sweep indicates that there are facilitatory events triggered during a sweep that remain to be described, as do the neurons whose responses could not be predicted from the inhibitory rules that were shown to be reasonably effective in predicting the behavior of others. Future studies will attempt to elucidate these mechanisms and to describe how they emerge during ontogeny.

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