

Facilitatory and Inhibitory Frequency Tuning of Combination-Sensitive Neurons in the Primary Auditory Cortex of Mustached Bats

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Kanwal, J. S., D. C. Fitzpatrick, and N. Suga. Facilitatory and inhibitory frequency tuning of combination-sensitive neurons in the primary auditory cortex of mustached bats. *J. Neurophysiol.* 82: 2327–2345, 1999. Mustached bats, *Pteronotus parnellii parnellii*, emit echolocation pulses that consist of four harmonics with a fundamental consisting of a constant frequency (CF_{1-4}) component followed by a short, frequency-modulated (FM_{1-4}) component. During flight, the pulse fundamental frequency is systematically lowered by an amount proportional to the velocity of the bat relative to the background so that the Doppler-shifted echo CF_2 is maintained within a narrowband centered at ~ 61 kHz. In the primary auditory cortex, there is an expanded representation of 60.6- to 63.0-kHz frequencies in the “Doppler-shifted CF processing” (DSCF) area where neurons show sharp, level-tolerant frequency tuning. More than 80% of DSCF neurons are facilitated by specific frequency combinations of ~ 25 kHz (BF_{low}) and ~ 61 kHz (BF_{high}). To examine the role of these neurons for fine frequency discrimination during echolocation, we measured the basic response parameters for facilitation to synthesized echolocation signals varied in frequency, intensity, and in their temporal structure. Excitatory response areas were determined by presenting single CF tones, facilitative curves were obtained by presenting paired CF tones. All neurons showing facilitation exhibit at least two facilitative response areas, one of broad spectral tuning to frequencies centered at BF_{low} corresponding to a frequency in the lower half of the echolocation pulse FM_1 sweep and another of sharp tuning to frequencies centered at BF_{high} corresponding to the CF_2 in the echo. Facilitative response areas for BF_{high} are broadened by ~ 0.38 kHz at both the best amplitude and 50 dB above threshold response and show lower thresholds compared with the single-tone excitatory BF_{high} response areas. An increase in the sensitivity of DSCF neurons would lead to target detection from farther away and/or for smaller targets than previously estimated on the basis of single-tone responses to BF_{high} . About 15% of DSCF neurons show oblique excitatory and facilitatory response areas at BF_{high} so that the center frequency of the frequency-response function at any amplitude decreases with increasing stimulus amplitudes. DSCF neurons also have inhibitory response areas that either skirt or overlap both the excitatory and facilitatory response areas for BF_{high} and sometimes for BF_{low} . Inhibition by a broad range of frequencies contributes to the observed sharpness of frequency tuning in these neurons. Recordings from orthogonal penetrations show that the best frequencies for facilitation as well as excitation do not change within a cortical column. There does not appear to be any systematic representation of facilitation ratios across the cortical surface of the DSCF area.

INTRODUCTION

To understand how acoustic stimuli are processed in the mammalian auditory cortex, several studies have focused on

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the basic response properties of cortical neurons, such as the sharpness of frequency tuning, response thresholds, dynamic response range, amplitude-response functions, etc., and how they are spatially and temporally organized (deCharms and Merzenich 1996; Merzenich et al. 1975; Schreiner 1995; Schreiner and Cynader 1984; Schreiner and Mendelson 1990; Schreiner and Sutter 1992; Schreiner et al. 1992; Shamma et al. 1993; Sutter and Schreiner 1991, 1995; Takahashi 1995). These studies provide important clues to the functional organization of the auditory cortex in mammals without necessarily attributing single-cell response properties to species-specific behaviors. A few recent studies, however, have made a special attempt to link neural responses and mechanisms with the specific auditory functions such as communication (Ehret and Schreiner 1997; Rauschecker 1998; Rauschecker et al. 1995; Wang et al. 1995).

In mustached bats, the functional organization of the auditory cortex has been explored with great success using species-specific stimuli relevant to their echolocation behavior (Fitzpatrick et al. 1998; Suga 1965, 1984; Suga and Kanwal 1995; Suga et al. 1983, 1997) and communication behavior (Esser et al. 1997; Kanwal 1999; Ohlemiller et al. 1996). These stimuli consist of combinations of information-bearing elements in a bat’s echolocation pulse and echo (Fig. 1A). A mustached bat’s echolocation pulse consists primarily of four harmonics (H_1-H_4) each including a constant frequency (CF_{1-4}) tone followed by a downward sweeping FM (FM_{1-4}). The fundamental frequency of the FM_1 component sweeps downward from 30 to 24 kHz at a rate of 2 kHz/ms. Typically, studies on the functional organization of the bat’s auditory cortex and of neural specializations for echolocation have been coupled with analysis of the excitatory, facilitatory, and inhibitory tuning properties of neurons to stimulus parameters that are behaviorally relevant. On the basis of these studies, the bat’s auditory cortex has been divided into several distinct functional areas that contain computational maps of behaviorally relevant combinations of stimulus parameters (e.g., maps of FM-FM delays for measuring target distance and CF/CF combinations for measuring relative target velocities) (Suga and O’Neill 1979) (see Fig. 1, B and C). This organization of the auditory cortex is based strictly on the specialized behavior of echolocation, which is essential to the bat’s ecological niche.

The Doppler-shifted CF processing (DSCF) area lies within the main tonotopic representation in the auditory cortex. The resting CF_2 in the echolocation pulse of mustached bats varies in different individuals, and this variation is reflected in the neural tuning of DSCF neurons (Henson et al. 1980, 1987; Suga and O’Neill 1980; Suga and Tsuzuki 1985). The DSCF

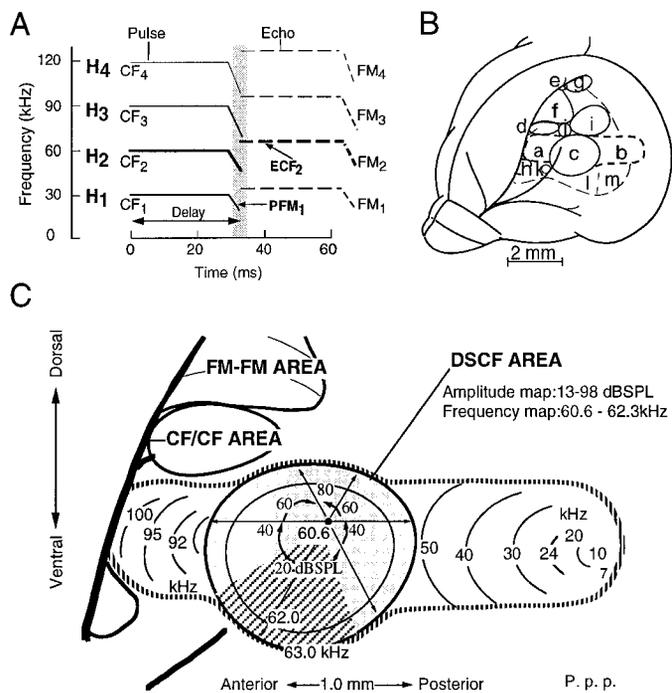


FIG. 1. A: schematized spectrogram of the mustached bat's echolocation signal. H_{1-4} refer to harmonics 1-4 of the echolocation pulse or echo. Shaded region shows the time and frequency components (indicated by arrows) that lead to a facilitative response in the Doppler-shifted constant frequency processing (DSCF) area. B: lateral view of the bat's brain showing the organization of functionally defined subdivisions of the auditory cortex. a, A1-anterior; b, A1-posterior; c, DSCF area; d, CF/CF area; e, DIF area; f, FM-FM area; g, dorsal fringe (DF) area; h, ventral fringe (VF) area; i, dorsomedial (DM) area; j, tiny enclosed (TE) area; k, H_1 - H_2 area; l, ventroanterior (VA) area; and m, ventroposterior (VP) area. C: schematic showing the location of the DSCF area in the auditory cortex. DSCF area occupies nearly 30% of the area of the tonotopically organized primary auditory cortex and represents a small range (60.6-62.3 kHz) of frequencies (adapted from Suga 1984).

area receives projections from the ventral division of the medial geniculate body (MGB) (Olsen 1986; Suga 1982). This area is considered to be homologous to a part of the primary auditory cortex of other mammals. Specifically, this area over-represents a single iso-frequency contour ranging from ~60 to 62 kHz (normalized to the average CF_2 resting frequency of 61.0 kHz) within a radial frequency axis and a circular amplitopic axis (Suga 1977; Suga and Manabe 1982; Suga et al. 1987) (Fig. 1C). On the basis of previous studies, neurons in this area have been defined as having sharp, "level tolerant" tuning curves and their response is restricted to Doppler-shifted CF_2 frequencies in the echo (Suga and Jen 1976; Suga and Manabe 1982). Focal inactivation of the DSCF area in mustached bats disrupts specifically the ability to make fine frequency discriminations. This finding is consistent with the postulated role of the DSCF area in target detection and characterization (Riquimaroux et al. 1991).

The response of DSCF neurons is facilitated when a 30-ms-long CF tone burst of 60-62 kHz is paired with another equally long CF tone burst ranging from 22 to 28 kHz or when a 3-ms-long pulse FM_1 sweeping from 29 to 23 kHz precedes the onset of the tone burst (Fitzpatrick et al. 1993; Kanwal et al. 1991). Furthermore, for 20-ms-long CF tones, the facilitation is broadly tuned to echo delays between 11 and 26 ms where pulse FM_1 overlaps temporally with echo CF_2 . This range of tuning is quite different and complementary to the

range of tuning to echo delays (<10 ms) in the FM-FM specialized, delay tuned areas [FM-FM, dorsal fringe (DF), and ventral fringe (VF) areas] in the auditory cortex (Edamatsu et al. 1989; O'Neill and Suga 1982; Suga and Horikawa 1986; Suga and O'Neill 1979). Although the delay tuning of DSCF neurons has been studied in some detail, the effect of combination-sensitive aspects of frequency and amplitude tuning to CF tones at ~61 kHz frequencies is unclear. We also do not know how facilitation affects the sharpness of frequency tuning, thresholds and best amplitude-response levels or whether the magnitude of facilitation varies with depth or position in the DSCF area.

Knowledge of these response properties and the associated facilitative mechanisms is critical for understanding the behavioral role of the specializations described for neurons in this cortical area. In this study, we describe the excitatory, facilitatory, and inhibitory frequency tuning properties of DSCF neurons and also address the question of how the various stimulus parameters for facilitation are represented within the DSCF area in the primary auditory cortex of the mustached bat.

METHODS

The materials and methods of the present experiments are generally similar to those described previously (Suga and Tsuzuki 1985). Therefore the common procedures are described briefly, whereas those that have been modified or are unique to this experiment are described in detail.

Surgery and recording of neural activity

Fourteen Jamaican mustached bats, *Pteronotus parnellii parnellii*, weighing between 11 and 13 g were used in this study. Before surgery, animals were placed in a Styrofoam body mold and injected intramuscularly with 0.08 mg/kg body wt fentanyl and 4 mg/kg droperidol mixture (Innovar), and 2 mg/kg body wt prednisolone (methyl derivative). Innovar causes neuroleptic analgesia, while Prednisolone, a corticosteroid hormone, reduces the level of metabolic stress. After surgical exposure of the skull, a metal post (length = 1.5 cm) was mounted vertically on the midline of the skull with cyanoacrylate glue (Eastman 910). The two flaps of skin were sutured loosely around the metal post on the skull, and the bat was allowed to recover for 3 days before the first recording session. Neural activity was recorded biweekly from each bat for ≤ 12 wk per bat.

All recordings were made from unanesthetized, restrained animals. All experiments were carried out using protocols approved by the Animal Care and Use Committee at Washington University. The bat was placed in a Styrofoam body mold to restrain body movements. The mold was designed to provide airspace around the bat's body and move with any body movements of the bat. The mold was suspended by elastic bands in a heated (31°C), sound-proofed and echo-attenuated chamber (IAC 400A). The walls of the room were covered with echo-attenuating foam (Sonex). The head of the bat was immobilized by clamping the affixed metal post within a hollow rod clamped vertically above the recording table. Lidocaine was applied topically before manipulation of the surgical wounds. The animal was observed carefully for signs of discomfort. Drinking water was offered two to three times during the recording session.

Acoustic stimulation

Sound stimuli were presented from two condenser loudspeakers mounted on a vertical hoop and positioned 95 cm directly in front of the bat. The two loudspeakers were positioned adjacent to each other in the same azimuth in front of the bat to avoid binaural effects. The

stimulus generation and delivery system consisted of three channels such that two sounds were presented from one speaker and the third was presented from a different speaker. However, when two sounds came from the same speaker, they were separated in time. The three sounds could be controlled independently in frequency, amplitude, and duration and could be delivered simultaneously or successively. These sounds could be triggered either manually or via a computer. Sounds consisted of CF tones and/or FM sweeps. The condenser loudspeakers were calibrated by placing a B&K microphone at the position of the ear and were reasonably flat between 20 and 100 kHz with a significant roll off at 120 kHz. The maximum amplitude level that could be delivered for *speaker A* was 98 dB SPL (re 20 μ Pa, RMS) at 90 kHz and that for *speaker B* was 95 dB SPL at 85 kHz.

A single stimulus (tone burst) was used for studying neural excitation and paired stimuli were used for studying facilitation. When time permitted, three types of response areas were obtained for each neuron. 1) Excitatory response areas were generated from neural responses to single tones varied in frequency and amplitude. The responsiveness of a neuron was scanned for frequencies from 10 to 100 kHz, and response areas were typically measured at \sim 25 and 61 kHz. 2) Facilitatory response areas were generated by keeping the frequency and amplitude of one tone constant and varying both of these parameters for the other tone. The amplitude of the fixed tone was generally adjusted 10 dB above its excitatory response threshold so that when presented separately, it produced a minimal response. The excitatory response was not subtracted when estimating the facilitatory response areas centered at either 25 or 61 kHz frequencies. The 30-ms-long CF tone bursts were paired so that they had simultaneous onsets. 3) Inhibition was studied with pairs or triplets of tone bursts for excitatory and facilitatory responses, respectively. The inhibitory test tone of 4-ms duration was delivered just before, without overlap, with a second excitatory tone or facilitatory tone pair. For obtaining inhibitory response areas, the excitatory and facilitatory tones were presented at the best frequency and at \sim 10 dB above threshold level and the inhibitory tone was varied systematically between 5 and 100 kHz. This minimized the effects of two-tone suppression. The amplitude of the first tone presented at different frequencies that caused inhibition of the response to the second was recorded. The boundaries of the inhibitory response areas were detected by audiovisually monitoring suppression of the facilitated neural response to its threshold level. The time course of the inhibition was measured by increasing the time delay between the onset of the test tone and the fixed excitatory/facilitatory tone/s.

The minimum threshold for facilitation was determined by presenting the fixed tone at its best frequency and at its minimum threshold level while simultaneously presenting the test tone at just above threshold level and attenuating it further until the response was nearly extinguished. Similarly, the upper threshold level for the facilitatory tone was determined by increasing the amplitude of the test tone until the response to the tone pair was once again minimal. The best low frequency for excitation (BF_{low}) was determined after establishing the best high frequency (BF_{high}). Once BF_{low} was determined, BF_{high} was checked once again and adjusted if necessary. This iterative process was repeated a few times until a stable value for both BF_{low} and BF_{high} was obtained. To minimize experimenter bias, the frequency counter was momentarily turned off when evaluating the best response audiovisually.

To test the effect of harmonics in the pulse on the facilitative response, five neurons were tested with different combinations of harmonics in the pulse with an echo stimulus consisting of multiple-harmonics (ECF_{2-4}) based on the best ECF_2 frequency. These harmonics were generated using a custom-built harmonic generator. The fundamental frequency in the bat's own pulse is relatively weak so that the frequencies corresponding to the fundamental frequency in the echo are unlikely to contribute to a facilitative response. The first harmonic (H_1) of the pulse was simulated by generating a 6-kHz FM

sweep with the neuron's best frequency at its center and preceding it with a 27-ms-long CF_1 at the appropriate frequency.

Data acquisition and analysis

The resting frequency of the CF_2 component of orientation sounds ("CF₂ resting frequency") emitted by each animal was measured at the beginning of each experiment. This averaged [60.43 ± 0.83 (mean \pm SD) kHz; $n = 14$, males = 8, females = 6]. The CF_2 resting frequency differs among individual bats, ranging from \sim 60 to \sim 63 kHz (Suga et al. 1987). Neurons in the DSCF area are extremely sharply tuned to frequencies within this range. This sharp tuning also varies according to the bat's own CF_2 resting frequency, so the best frequencies measured for neurons in the DSCF area of different animals were normalized to 61.0 kHz (the population average) according to the method of Suga and Tsuzuki (1985). This allows comparison and pooling of tuning curve data across different animals.

The activity of single neurons was recorded with sharpened, vinyl-coated tungsten-wire electrodes with tip diameters of \sim 10 μ m and impedance of \sim 2 M Ω . An indifferent tungsten-wire electrode was placed in the nonauditory frontal cortex. Before insertion of the electrode, holes of \sim 50 μ m in diameter were made in the skull with a sharpened needle. The recording electrode was inserted orthogonally in 1.0- to 2.5- μ m steps using a Kopf hydraulic microdrive controlled by a stepping motor. Orthogonality of electrode penetrations was based primarily on visual inspection with a dissection microscope. In addition to visual inspection, the accuracy of an orthogonal penetration was confirmed by the fact that in these penetrations the BF_{high} did not change with depth as suggested by the excitatory tuning data in a previous study (Suga and Manabe 1982). Further confirmation was based on imaging a frontal view of the bat's skull on a video monitor and measuring the angle of the electrode against the tangent of the skull curvature at the penetration site on the skull. The actual depth of the recording site was estimated by noting the reading for the cortical surface during the initial insertion and final withdrawal of the electrode and taking the average value. This adjusted for any effects of indentation or drying of the cortical surface. Recordings were made from well-resolved single units isolated from the background activity with a BAK window discriminator (BAK DIS-1) including an analog delay (BAK AD-3). Signal-to-noise ratios generally ranged from 3:1 to 5:1 and were occasionally higher. Action potentials the waveform of which was restricted to the preset time-amplitude window generated an acceptance pulse that triggered an oscilloscope display of the shape of the delayed action potential. A stored waveform was used as a template-match to monitor any changes in the shape of the ongoing spike waveform and therefore ensure that the activity of the same neuron was being recorded.

Acquisition of neural activity was controlled by Modular Instruments (MI²) software such that trigger pulse generation was synchronized with data acquisition. An electronic module was used to control stimulus amplitude by varying the level of attenuation at the output of an electronic switch. CF tones were 30 ms long and were delivered at a rate of 4/s, and neural activity was acquired for 200 ms from stimulus onset. Response was quantified as the number of impulses per 200 stimulus presentations in a 60-ms time window, although for phasic responses a 40-ms window covered the whole response (these spike count windows included a 10-ms prestimulus duration). Computer-controlled frequency scans consisted of at least five repetitions at a rate of 5/s of 22 blocks of recorded neural activity with the last block acting as a control to record spontaneous activity. The frequency of the 13th block was designated as the center-frequency and decreased/increased by specified frequency steps of 100–250 Hz in the blocks preceding/succeeding the center-frequency block. This procedure using the customized MI² software generated a dot raster and either a cumulative or a running histogram display of the frequency-tuning of the neuron. For off-line analyses, spike times were

stored on the hard drive of the computer and responses were plotted using custom software.

RESULTS

Facilitation of responses by two CF tones

The response of many DSCF neurons is facilitated by the presentation of CF sounds that are contained in at least two discrete, nonoverlapping frequency ranges. A facilitative response is described as a response to two or more stimuli delivered together that is greater than the sum of the response to each stimulus delivered alone. A neuron displaying such a response is considered to be combination sensitive. The best high frequency (BF_{high}) that produces a facilitative response in DSCF neurons typically ranges from 61 to 63 kHz and lies within the range of the constant frequency of the second harmonic of the Doppler-shifted echo (ECF_2 ; see Fig. 1A). The best low frequency for facilitation (BF_{low}) ranges from 22 to 28 kHz and overlaps with the range of the downward frequency sweep in an echolocation pulse (PFM₁ in Fig. 1). Occasionally, a third, middle frequency (BF_{mid}) in the neighborhood of 50 kHz also triggers a facilitative response when paired with BF_{low} .

The majority of DSCF neurons are excited poorly or not at all when BF_{low} is delivered alone and strongly when it is paired with a BF_{high} such that they exhibit facilitation. The simultaneous delivery of two tones may lead to a relative increase in sound pressure level (SPL) of the sound, although at each individual frequency there will be no increase. In the DSCF area, an increase in SPL beyond a particular value reduces the magnitude of facilitation because of the upper thresholds in the response areas of these neurons, particularly to BF_{high} frequencies when delivered alone or when paired with a BF_{low} . Upper thresholds of DSCF neurons when stimulated with a single tone burst at ~61 kHz are documented in a previous study (Suga and Tsuzuki 1985).

As reported earlier (Fitzpatrick et al. 1993), facilitation also is observed in DSCF neurons when a CF/FM signal corresponding to the pulse H₁ (1st harmonic in the pulse) is paired at a best delay with a CF corresponding to the echo CF₂ in an echolocation signal (Fig. 2, A–C). However, this response is generally smaller than or equal to the same neuron's response to two CF tones delivered simultaneously. Notice that the response to two tones at 0 ms delay is larger than the response at 31 ms “pulse H₁-echo CF₂” delay (Fig. 2D). These data justify our use of paired tone bursts with a simultaneous onset for studying the tuning properties of DSCF neurons. Use of paired tone bursts is also desirable because frequency tuning for facilitation is obtained more easily with tones than by FM sweeps.

Classification of DSCF neurons based on excitatory and facilitatory responses

For each DSCF neuron, we calculated the mean response from three to six measurements of the response to BF_{low} and to BF_{high} frequencies alone and to combinations of these frequencies. From these data, a facilitation ratio (FR) was calculated for each neuron by dividing the facilitated response by the sum of the responses to each component alone. FRs typically were calculated from the peak response magnitude of a neuron to

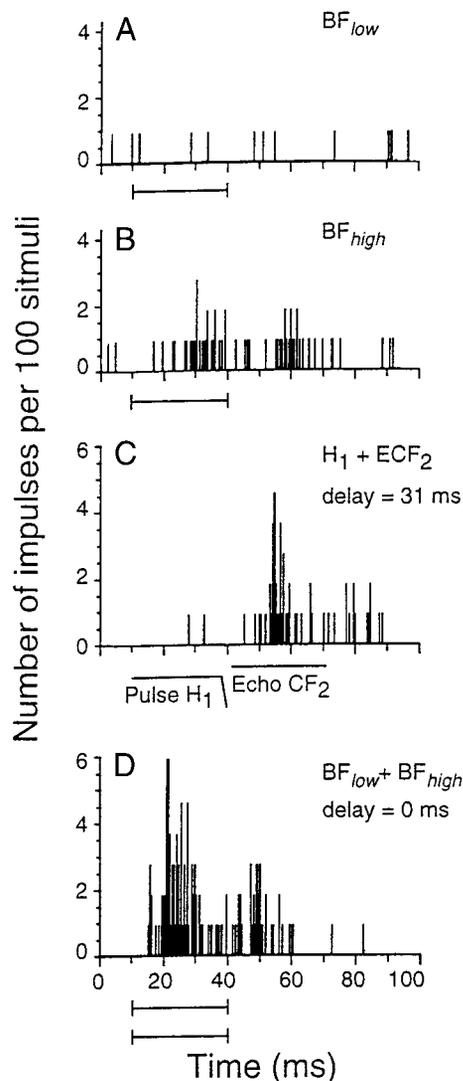


FIG. 2. A–D: series of poststimulus time (PST) histograms obtained from a single neuron to compare the facilitative response to paired tone bursts (BF_{low} / BF_{high}) with the combination of pulse H₁ and echo CF₂ presented at their best delay in a DSCF neuron.

tones delivered at a SPL corresponding to 10 dB above its minimum excitatory threshold. Each tone in a pair was delivered at the same SPL as when it was delivered alone. The spontaneous level of activity was subtracted from all responses. A neuron was said to be facilitated if the mean value of its FR was >1.1. The majority (66%) of neurons show FRs from 1.1 to 2.0 (Fig. 3A). The mean value of FR for DSCF neurons was 2.16 (n = 226). About 2% of the neurons had a FR >5. The mean response to BF_{low} frequencies alone is about one-sixth of that to the BF_{high} frequencies alone at their respective best amplitudes (BAs).

The line plotted in Fig. 3A shows the distribution of DSCF neurons based on different FRs, and the histograms show the average relative magnitude of responses to BF_{low} and BF_{high} when presented alone for different values of facilitation. Except for some neurons with values of FR ranging from four to five, neurons respond relatively little to BF_{low} frequencies. When the response to each component is normalized to FR and expressed as a percentage, the majority of neurons tend to respond either equally or better to BF_{high} than to BF_{low} .

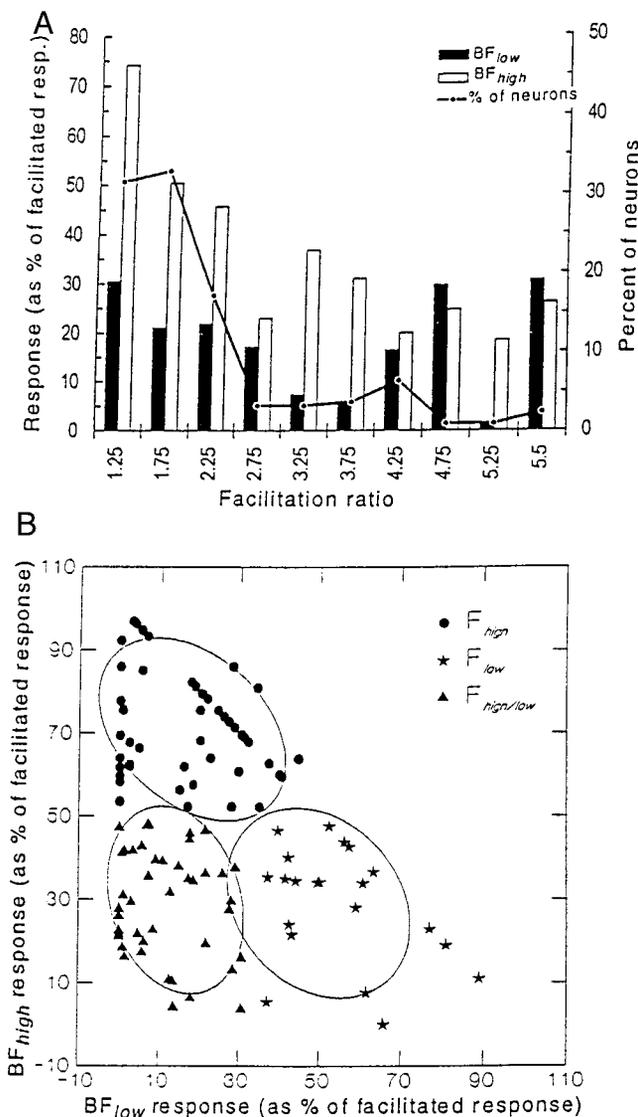


FIG. 3. A: bar graphs showing the magnitude of the neural response to BF_{low} (■) and BF_{high} (□) frequency components alone, relative to that obtained when both stimuli are paired. Each pair of bars covers a FR range of 0.5 with the center value labeled on the horizontal axis. Responses are normalized to 100% of the facilitated response and categorized according to the facilitation ratio (FR), where $FR = (P + E) - S / (P - S) + (E - S)$, where P = response at BF_{low} , E = response at BF_{high} , and S = level of spontaneous activity. Superimposed line plot refers to the percentage of the neurons obtained for each range of FR. B: scatterplot of the relative response to the low- and high-frequency tones in each tone pair. Three types of neurons are identified on the basis of a 3-way classification as described in the text. Neurons showing marginal facilitation in their response are arranged along a diagonal, indicating that in these neurons the response to stimulus combinations is simply a sum of the responses to the individual components.

In an attempt to identify functionally different subpopulations of DSCF neurons, we used a nonhierarchical splitting algorithm for a three-way classification of DSCF neurons based on their facilitative responses to two tones (Hartigan 1975). The resultant clusters were based on the FR versus the relative contribution of single tone BF_{low} and BF_{high} components to the facilitated response and accordingly designated as “ F_{high} ,” “ F_{low} ,” and “ $F_{high/low}$ ” (Fig. 3B). Although the response properties exhibit an apparent continuum of variation, we generated a three-way classification scheme that repre-

sented a discrete orthogonal split into two groups and a third intermediate group. The distribution of neurons between the three groups is shown as a scatterplot. The ellipses indicate the boundaries of the three distributions for a 50% confidence level (Wilkinson et al. 1998). This information cannot be illustrated in the bar graph, which only shows the mean response ratios for each frequency relative to the magnitude of facilitation for different values of FR. F_{high} class consisted of 37%, F_{low} class consisted of 18%, and the $F_{high/low}$ class consisted of the remaining 45% of the total number of neurons. The F_{low} group of neurons was missed completely in previous studies of the DSCF area because BF_{low} frequencies were not tested. Additionally, F_{low} neurons exhibited lower thresholds to BF_{low} than to BF_{high} stimuli, whereas F_{high} and $F_{high/low}$ neurons exhibited lower thresholds to BF_{high} than to BF_{low} stimuli. Also, $F_{high/low}$ neurons had significantly ($P < 0.001$) higher values of FR (2.67 ± 1.1) than that for F_{high} ($FR = 1.82 \pm 1.06$) and F_{low} ($FR = 1.78 \pm 1.38$) neurons.

Examples of PST histograms obtained for the three types of DSCF neurons are shown in Fig. 4. The neuron shown in Fig. 4B is classified as a $F_{high/low}$ neuron with an unusually high FR. The neuron shown in Fig. 4C is a typical F_{high} neuron with a much better response to BF_{high} alone than to the BF_{low} stimulus but has an atypically high rate of spontaneous activity and a more tonic component in its response than the other two neuron types. Note that the facilitated response in Fig. 4C is preceded by a short inhibitory period. This is observed frequently for facilitated response in DSCF neurons.

To study the time course of inhibition for a single neuron, a 4-ms-long CF prepulse at the best amplitude for inhibition

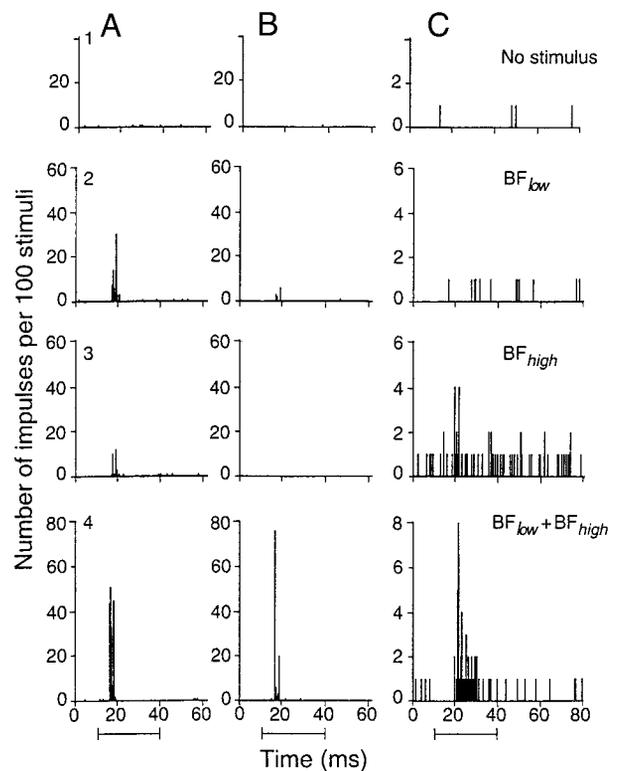


FIG. 4. PSTHs obtained to single tone and paired tone stimuli for 3 different neurons. A: F_{low} type of neuron where response to BF_{low} is greater than to BF_{high} . B: $F_{high/low}$ type of neuron where response to both BF_{low} and BF_{high} is equally small, whereas the response to the simultaneous presentation of both frequencies is large. C: response to BF_{high} is greater than BF_{low} .

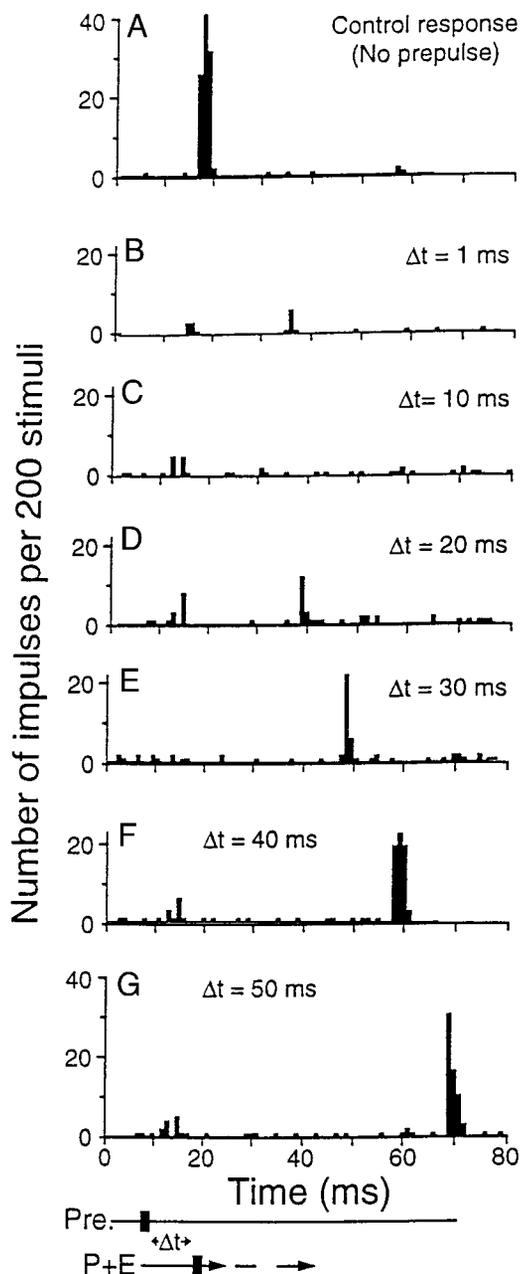


FIG. 5. Series of PST histograms (A–G) to show the time course of inhibition of a facilitative response in single neurons. Time interval (Δt) between the prepulse (pre) and the combination of two tones (P + E) is varied from 1 to 50 ms. Four-ms-long prepulse had a frequency of 62.4 kHz and was delivered at 78 dB SPL. Excitatory frequencies of PFM₁ and ECF₂ are 24.0 and 60.4 kHz, respectively. Data were acquired at a resolution of 0.2 ms and plotted with a binwidth of 1 ms.

precedes a two-tone, BF_{low}/BF_{high} combination and the delay between the prepulse and the two-tone combination is varied systematically (Fig. 5, A–G). Although only three neurons were tested systematically in this way, the data clearly show that a short, single frequency tone burst can completely suppress the facilitative response for nearly 20 ms and the response is still reduced at ~50 ms. The inhibitory frequency most effective for long-lasting inhibition is usually close to the best excitatory frequency of the neuron (e.g., see Figs. 6, 8, and 15). Thus in the example shown, the prepulse was delivered at an amplitude of 78 dB SPL had a frequency of 62.4 kHz

compared with the BF_{high} at 60.4 kHz. The BF_{low} frequency for facilitation was fixed at 24 kHz.

Excitatory, facilitatory, and inhibitory response areas

Excitatory and/or facilitatory response areas were measured for 109 DSCF neurons and inhibitory response areas were measured for 26 neurons. Response areas obtained for three representative neurons are shown in Figs. 6, 7, and 8. The facilitative response evoked by two tones can be inhibited if a nonoverlapping third tone is presented before the pair of tones causing the facilitation. Inhibitory response areas measured for 37 DSCF neurons were commonly very broad and overlapped with or “skirted” the facilitative areas. Minimum thresholds for inhibition were usually found at both sides of the BF_{high} facilitative tuning (Figs. 6–8). Typically, closed excitatory and facilitatory response areas resulted from the presence of inhibitory response areas at high stimulus levels for frequencies neighboring the BF. In Fig. 6, a broad, continuous inhibitory zone is present between 10 and 45 kHz. This and other inhibitory response areas were obtained by using a relatively high-intensity (>85 dB SPL) CF tone or “prepulse” just before and nonoverlapping with the facilitatory tone pair.

The neuron shown in Fig. 6 is tuned to 25- and 61-kHz tone bursts. The excitatory response of this neuron to BF_{low} exhibits a relatively broad, closed response area. In contrast, the response area for BF_{high} is very sharp and closed with no response being elicited to sound intensities >60 dB SPL. The narrow and closed BF_{high} response area is replotted in the expanded frequency axis. Shaded areas indicate inhibitory response areas that typically flank the excitatory and facilitatory response areas. The shape of the facilitative response areas (see Fig. 6B) is basically the same as that of the excitatory areas shown in Fig. 6A. However, the response magnitude to both BF_{low} and BF_{high} is enhanced. The most significant effect is on the response width of the two areas; BF_{low} facilitatory response area is widened in both the frequency and amplitude domains (Fig. 6B). The threshold of the BF_{high} facilitatory response area is lowered by ~10 dB, and the width of the response area increases from 0.5 to 1 kHz at BA. This translates into a change in Q_{BA} (Q value at best amplitude; typically at 35 dB above minimum threshold) for BF_{high} from 120.3 to 58.3.

A second type of response area is shown in Fig. 7. In this neuron, the excitatory response area to BF_{low} (Fig. 7A) is relatively small and the excitatory response to BF_{high} is less sharply tuned than for the neuron shown in Fig. 6A. The most significant differences are a lower sensitivity (higher minimum threshold) to BF_{high} and an open response area for BF_{low} as well as BF_{high}. At 50 dB above threshold the width of the facilitative response area increases from 0.75 to 1.25 kHz. The facilitative response areas once again show both lower thresholds and an increased width compared with the excitatory response areas (Fig. 7, A and B). An unexpected result, obtained for the facilitative tuning of this neuron is the presence of an additional facilitatory response area in the range of 50 kHz (BF_{mid}) that corresponds to the second harmonic of BF_{low}. As for BF_{low} response areas, this area was revealed by presenting a second fixed frequency at BF_{high} at ~10 dB above its threshold response level. An excitatory response area at BF_{mid} is present in only a few neurons. The magnitude of the facilitatory response to BF_{mid} is much less than that to BF_{high} but

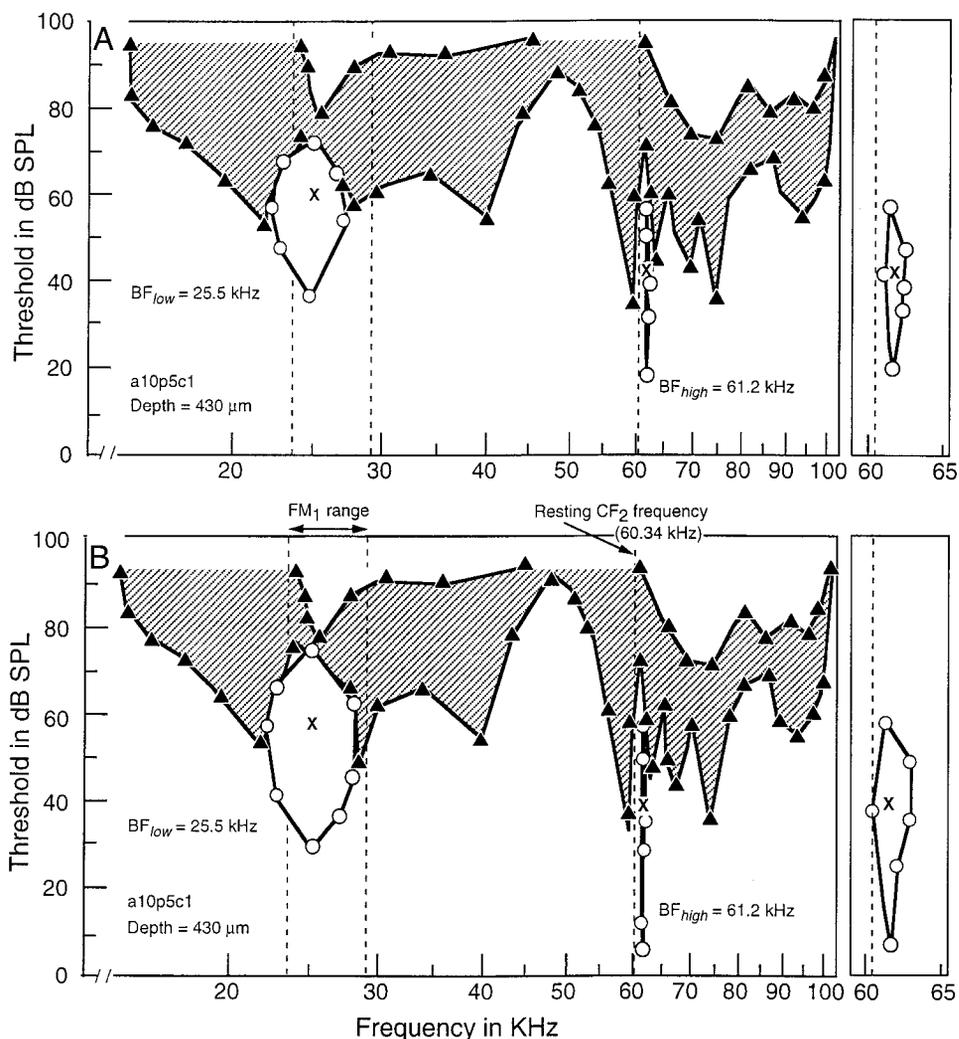


FIG. 6. Response areas for excitation and inhibition (A) and facilitation and inhibition (B) in a single neuron. Note the upper and lower thresholds for both the excitatory and facilitatory response areas. Sharp tuning at BF_{high} corresponds to the ECF_2 frequency in an echo. Excitatory and facilitatory areas (unshaded and open circles) were obtained by computer controlled scanning of frequencies at 100-Hz intervals and decreasing intensity by attenuating in 5-dB steps. x, best amplitude (BA) for each frequency component. Inhibitory response areas (hatched and filled triangles) were obtained by delivering a 2nd or a 3rd (for facilitation) tone preceding the CF sound that is varied in frequency and amplitude (see text for further explanation). In both panels, the pair of dashed lines at BF_{low} indicate the typical range of a downward sweeping FM_1 in the pulse (after accounting for “Doppler-shift compensation”) of a flying bat. Dashed line at BF_{high} indicates the location of the resting CF_2 on the frequency axis. Boundaries of the inhibitory area for excitatory tuning were more difficult to determine than those for facilitation because of a lack of a robust excitatory response. Small panels on the right show the respective excitatory and facilitatory response areas for BF_{high} on an expanded scale.

may be greater than that to BF_{low} . A second peak of the inhibitory response area is seen in the neighborhood of BF_{low} .

Figure 8A shows excitatory response areas for BF_{low} and BF_{high} that are closed as in Fig. 6A. The excitatory response area for BF_{low} is relatively small compared with the facilitatory response area at the same BF. Facilitation, in this case, results in opening of both the closed excitatory response areas so that high stimulus intensities of either BF_{low} or BF_{high} can produce a response when presented together with the other frequency at threshold or 10 dB above threshold levels. Facilitation increases the BF_{low} response area by a greater percentage than the BF_{high} response area, although both show an “outward” shift in the upper and lower thresholds (Fig. 8B). For this neuron, the single lobed inhibitory response area overlapped the excitatory and facilitatory response area for BF_{low} that extends from 22 to 28 kHz. The excitatory response area at 62 kHz also is embedded within an essentially single inhibitory response area for tuning to BF_{high} . These types of excitatory and facilitatory response areas are quite commonly observed, although in this case, the inhibitory response shows an unexpected additional low threshold (55 dB SPL) peak at 93 kHz. This inhibitory response area is fairly wide, ranging from 85 to 95 kHz at 30 dB above threshold.

In the preceding three examples (Figs. 6–8), the BF_{high} excitatory response areas are very sharp, whereas the BF_{low}

response areas are fairly broad with high thresholds and show a good response only to high sound intensities. In many neurons, BF_{low} alone does not produce any measurable excitatory response (see Fig. 3B). Also, a neuron generally exhibits a lower threshold (sometimes by >60 dB) to BF_{high} than to BF_{low} for both excitatory and facilitatory response areas. BF_{low} facilitatory response areas generally show multiple peaks of sensitivity varying by >10 dB within the range of a few kilohertz. This is a common finding for many of the neurons studied. Table 1 summarizes the tuning parameters for those neurons that showed clear facilitation (only neurons showing a $FR > 1.1$ were used for this analysis).

Typically, the inhibitory response areas show multiple peaks of sensitivity. The best frequency for inhibition (BFI) is defined as the frequency at which a threshold response can be obtained at the lowest SPL relative to other frequencies. BFIs are identified separately for the low- and high-frequency regions defined on the basis of excitatory and facilitatory tuning. As for the facilitatory response areas, inhibitory response areas always have lowest thresholds at frequencies neighboring the BF_{high} compared to those near BF_{low} . BFIs are slightly higher or slightly lower than the BFs for the corresponding excitatory and facilitatory areas. For example, the BFIs for the neuron shown in Fig. 6 are 22 and 27 kHz for BF_{low} and 60 and 62 kHz for BF_{high} . The mean difference between BFIs and BFs for

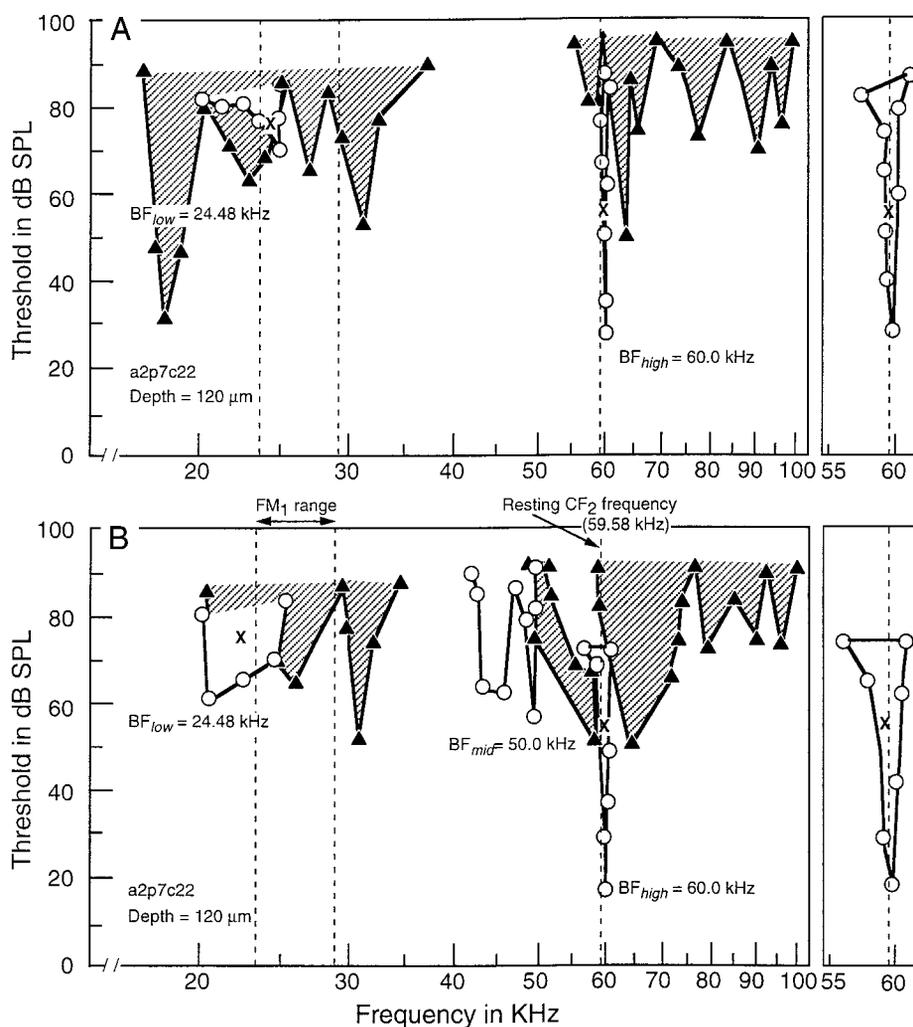


FIG. 7. Response areas for excitation and inhibition (A) and facilitation and inhibition (B) in a single neuron. Facilitative response area in B shows 3 separate BFs for facilitation corresponding to BF_{low} at 24.5-kHz frequencies, BF_{mid} at 50-kHz frequencies, and BF_{high} at 60-kHz frequencies. Facilitative response area for the BF_{high} is relatively broad, with a Q_{BA} of 59.9 and a Q_{50} of 48.2 compared with a Q_{BA} of 120.3 and a Q_{50} of 240.1 for the excitatory tuning. Note that facilitative and inhibitory areas overlap at high stimulus levels. Small panels on the right show the respective excitatory and facilitative response areas for the BF_{high} frequency on an expanded scale. For further explanation, see legend for Fig. 6.

each excitatory and facilitatory area, obtained for seven neurons, are listed in Table 2. For BF_{high} , these neurons have BFIs that are on average 1.61 kHz lower and 0.58 kHz higher than the BFs of DSCF neurons and contribute to the sharp tuning of the facilitative response.

The difference in sensitivity for inhibition around the two best excitatory frequencies is more pronounced in the example shown in Fig. 8 than those shown in Figs. 6 and 7. Also, inhibitory response areas are generally asymmetrical around the excitatory and facilitatory response areas at BF_{high} so that the inhibitory response areas on the high-frequency side of BF_{high} are generally larger than those on the low-frequency side of BF_{high} because of differences in width and/or sensitivity of tuning (Figs. 6–8). Thus in Fig. 6, the inhibitory response area on the low-frequency side of BF_{high} is <2 kHz wide at 30 dB above threshold, and that on the high-frequency side is >10 kHz wide at the same SPL.

“Oblique” threshold-response curves

For $\sim 15\%$ of the neurons, the BF_{high} excitatory threshold-response curves exhibited a distinctly “oblique” shape, where the BF increased with a decrease in sound pressure level of the single tone. An example of this is shown in Fig. 9A where frequency-response functions are plotted on a linear scale and

stacked along an increasing amplitude scale. These types of neurons showed a similar facilitatory pattern of tuning for a combination of the BF_{low} and BF_{high} (Fig. 9B). As explained above, the facilitatory response area examined at high resolution shows a slightly larger amplitude and width of frequency tuning than the excitatory response area at several amplitude levels (see also Table 1). In this case, this spread is toward the left (lower frequencies) for high amplitudes (>70 dB SPL) and toward the right (high frequencies) for low-stimulus amplitudes (<65 dB SPL), thus enhancing the “obliqueness” of this curve.

To get a population estimate of the change in center frequency with amplitude, the frequencies were plotted for both excitatory and facilitatory tuning at 5-dB steps for four different neurons (Fig. 9, C–F). The best frequencies for excitation and facilitation of these neurons are normalized to the values of their resting CF_2 as explained above. A line of best fit for excitation and facilitation is plotted for each neuron. On the basis of these four plots, the mean slope for changes in the center frequency for excitatory and facilitatory tuning is -0.03 dB SPL/kHz. Within a range of 50 dB SPL, these correspond to an average net change in the Doppler-shifted frequency of ~ 1.8 and 1.3 kHz for facilitatory and excitatory tuning, respectively.

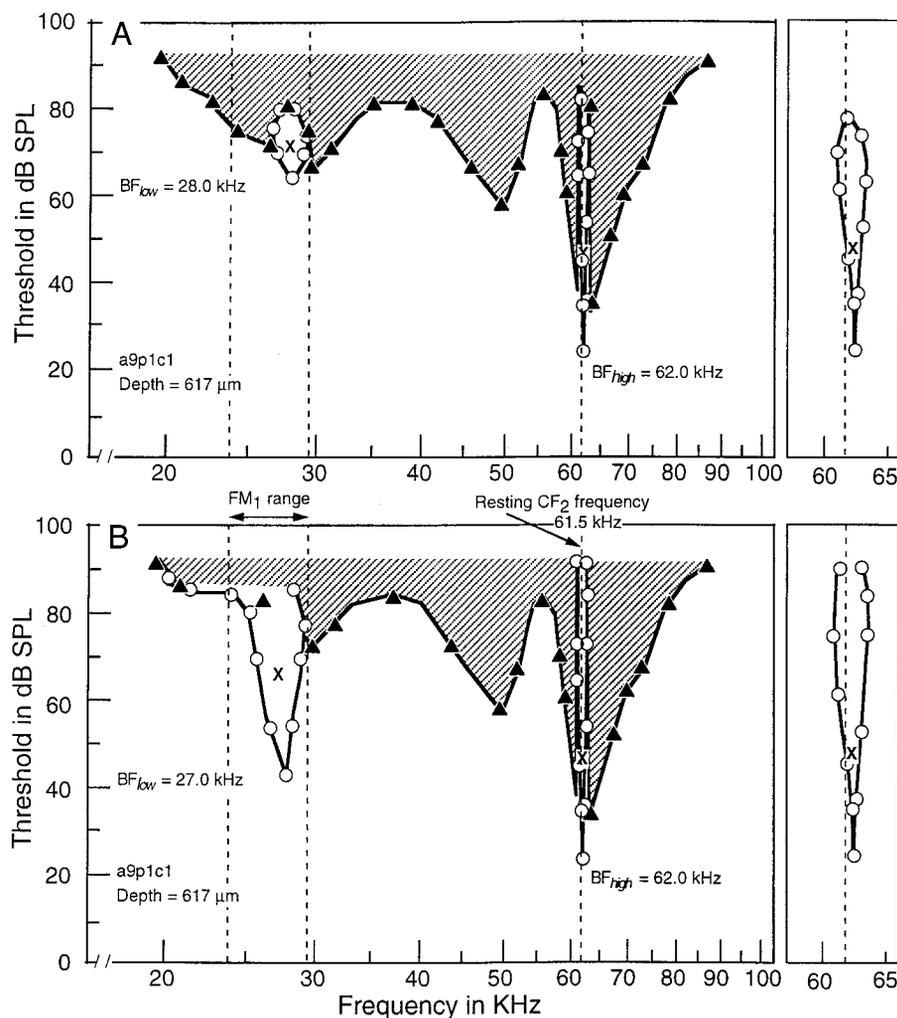


FIG. 8. *A*: excitatory and inhibitory response areas at BF_{low} and BF_{high} frequencies. *B*: facilitative and inhibitory tuning of the same neuron as in *A*. This neuron has an unusually low threshold level of <50 dB SPL for the BF_{low} frequency. Facilitatory response area for BF_{high} is relatively broad and does not exhibit upper thresholds. Inhibitory surrounds for the facilitatory frequencies are asymmetrical and extend down to 10 dB above facilitatory response thresholds. This neuron also showed relatively low thresholds for inhibition in the 30- and 50-kHz region. Small panels on the right show the respective excitatory and facilitatory response areas for BF_{high} on an expanded scale. For further explanation, see legend for Fig. 6.

Facilitation versus excitation: rate-level functions, minimum thresholds and best amplitudes

The overall effect of facilitation on the tuning of a neuron is best seen in a three-dimensional, frequency-amplitude-response plot (Fig. 10, *A* and *B*) and a cross-sectional profile of the frequency-response tuning at 30 dB above minimum threshold for excitation.

The effects of facilitation include increased response magnitude, especially at amplitude levels that range from 10 dB above to 10 dB below minimum threshold for excitatory tuning, lowered threshold, and an overall greater width of tuning. All of these contribute toward a greater response volume and an abrupt plateau shape of the facilitatory tuning relative to the single tone excitatory tuning. Figure 10C shows a profile of the response magnitude for frequencies at 30 dB above minimum threshold for facilitatory and excitatory frequency tuning. The data shown in Table 1 were calculated for 30 dB above minimum threshold for excitatory tuning. Data for facilitatory tuning were calculated from response at the same dB SPL level as that for excitatory tuning. The curves based on a weighted moving average (3-point smoothing; 1:4:1) of the data are plotted on a linear frequency scale. Response widths were calculated from frequencies corresponding to a 20% level of the peak response. Responses were normalized by equating the peak response to 100 and calculating the 20% separately for

curve. These data were used to calculate mean values of some of the parameters shown in Table 1.

Nearly all DSCF neurons show nonmonotonic amplitude tuning to single and paired tones, i.e., the number of impulses per tone-burst increases and then declines with higher amplitudes. The nonmonotonic tuning results from the interaction between excitation and inhibition within overlapping frequency ranges (see Figs. 6–8). For both excitation and facilitation, there is a broad spectrum of rate-level or impulse-count functions that are usually unimodal and occasionally show multi-peaked amplitude tuning. The width of amplitude tuning may span the entire range (100 dB SPL) of amplitude levels tested or may be confined to <30 dB SPL. The pattern of amplitude tuning for the best excitatory and facilitatory frequencies for four of the neurons that showed distinct facilitation are presented in Fig. 11, *A–D*. Spontaneous levels of activity have been subtracted and total number of impulses are counted over a 100-ms window for a 30-ms tone burst. Facilitation by two tones did not result in any significant modification in the shape of the rate-level function at the best frequency for BF_{high} , although the location of the peak response may shift by 20 dB (Fig. 11C).

Facilitation usually is associated with a decrease in the minimum thresholds for excitation. The scatterplot in Fig. 12A compares the minimal amplitude levels (in dB SPL) required to

TABLE 1. Mean response parameters for excitatory and facilitatory tuning

Response Parameter	BF _{low} Frequency Response	BF _{high} Frequency Response
Facilitation ratio at BF	—	2.16 ± 1.25 (n = 226)
Minimum threshold for excitation	75 ± 3.60 dB SPL (n = 7)	7 ± 0.36 dB SPL (n = 317)
Minimum threshold for facilitation	52 ± 2.50 dB SPL (n = 16)	0 ± 0.02 dB SPL** (n = 323)
Shift in minimum threshold	9 ± 0.42 dB SPL (n = 7)	7 ± 0.35 dB SPL (n = 317)
Excitatory frequency-tuning width at BA	—	1.35 ± 0.18 kHz (n = 28)
Facilitatory frequency-tuning width at BA	4.58 ± 0.54 kHz (n = 16)	1.71 ± 0.25 kHz (n = 28)
Change in frequency-tuning width at BA	—	0.38 ± 0.24 kHz (n = 28)
Q _{BA} value for excitation	—	89.5 ± 20.3 (n = 30)
Q _{BA} value for facilitation	9.3 ± 3.6 (n = 16)	60.1 ± 9.1* (n = 30)
Q ₅₀ value for excitation	—	80.1 ± 14.3 (n = 29)
Q ₅₀ value for facilitation	—	52.8 ± 6.3** (n = 29)

Mean values (means ± SD) of relevant parameters of excitatory and facilitatory response areas for a subset of the neurons studied to show how facilitation modifies basic tuning properties of Doppler-shifted constant frequency (DSCF) neurons. Q₅₀ values were calculated for an amplitude level corresponding to 50 dB above minimum threshold for excitation. Statistical analysis included a paired *t*-test for a comparison of similar parameters for excitation versus facilitation. BF, best frequency; BA, best amplitude. **P* value of <0.05, ***P* value of <0.01. *P* value for excitatory vs facilitatory frequency tuning width = 0.065.

obtain a threshold response for excitation and facilitation at BF_{high} in 350 neurons recorded from the DSCF area. Many of the data points in this plot overlap with each other. Points lying below the diagonal indicate a lowering of minimum thresholds. In the case of neurons with oblique excitatory and facilitatory response areas, minimum and maximum thresholds shift with frequency so that the usual effect of facilitation on minimum thresholds may appear to be reversed. For the neuron shown in Fig. 11C, the facilitatory tuning at 60.15 kHz exhibits a higher minimum threshold than that for excitatory tuning. In fact, for this neuron, the minimum thresholds for excitation and facilitation were 10 and 0 dB SPL, respectively. Figure 12B is a similar comparison for seven neurons at BF_{low} frequencies. Minimum thresholds for BF_{high} tuning may be lowered by as much as 65 dB, whereas for BF_{low} frequencies they may

TABLE 2. Best frequencies for inhibition

Neuron	BFF	BFI _{low}	BFI _{high}	BFF-BFI _{low}	BFI _{high} -BFF
<i>p1u1</i>	62.0	61.0	62.5	1	0.5
<i>p3u1</i>	62.0	61.9	62.5	0.1	0.5
<i>p3u2</i>	62.5	61.5	63.0	1	0.5
<i>p3u3</i>	62.5	60.0	62.6	2.5	0.1
<i>p8u2</i>	62.5	60.0	63.8	2.5	1.3
<i>p2u2</i>	63.0	60.0	63.8	3	0.8
<i>p5u1</i>	61.2	60.0	63.0	1.2	1.8
Mean				1.61 ± 0.98	0.79 ± 0.54

The best frequencies (in kHz) for inhibition skirting the facilitatory ECF₂ frequencies in 7 DSCF neurons. Means expressed as ±SD. BFF, best frequency for facilitation; BFI, best frequency for inhibition.

decrease by as much as 25 dB. Only seven neurons provided data for threshold comparison with BF_{low} tuning because single-tone BF_{low} stimuli alone rarely produce a measurable response spanning several dB. The best amplitude (BA) for facilitation at BF_{high} also can change significantly compared with the BA for excitation for the same neuron. The data in Fig. 12C represent a subset of the neurons included in Fig. 12A and show that BAs for facilitation may be lowered by as much as 50 dB compared with the BAs for excitation at BF_{high}. How-

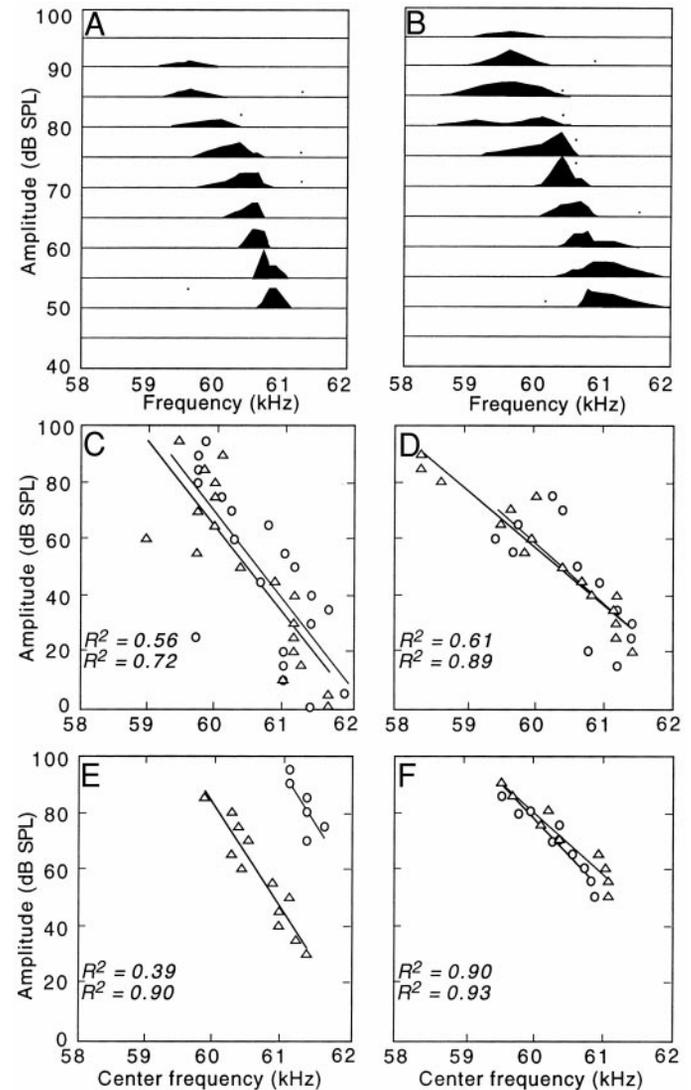


FIG. 9. A: graphic representation of a computer-controlled frequency-amplitude scan to show the excitatory tuning of a neuron. Response is shown by the filled area under the smoothed curve. Variation in response magnitude of a neuron as a function of frequency is shown along a horizontal frequency scale. Vertical stack of these plots (amplitude scan) indicates the variation in response magnitude and frequency range with amplitude to a single tone stimulus. Spontaneous level of activity is subtracted from this plot. B: similar plot as shown in A to show the facilitatory tuning of the neuron by the BF_{low} and BF_{high} frequency components. Frequencies are plotted on a linear scale in both plots. C–F: plots of the relationship between the tuned (center) frequencies and stimulus amplitude of response areas obtained from 4 neurons. A line of best fit is drawn for each neuron to show the negative slope of the relationship between the center frequency for each response area and the stimulus amplitude. ○, center frequencies of excitatory tuning; △, center frequencies of facilitatory tuning. Values of regression coefficients (*R*²) also are shown for the excitatory (top) and facilitatory (bottom) curves of each neuron.

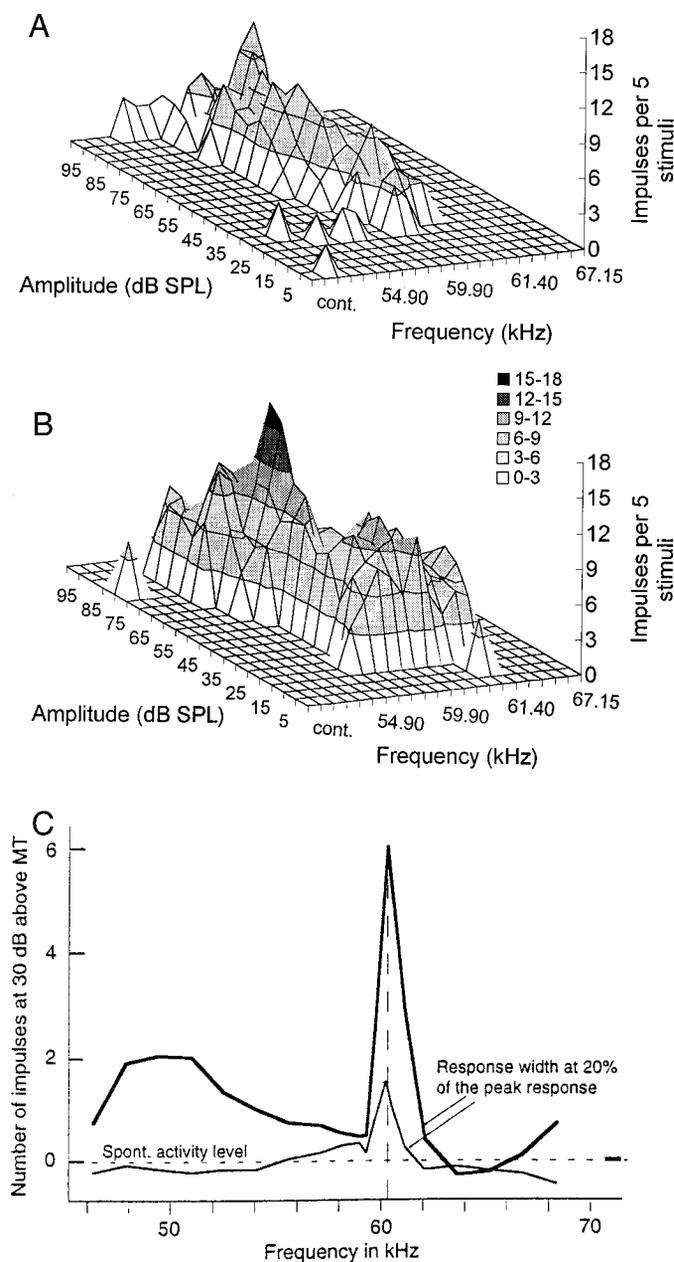


FIG. 10. Graphic representation of computer-generated 3-dimensional plots for frequency-amplitude-response functions for a neuron's excitatory (A) and facilitatory (B) tuning to BF_{high} . Each response represents a sum of 5 stimulus repetitions. C: diagram of a slice of the facilitative (thick line) and excitatory (thin line) frequency-response profile for another neuron. This shows the magnitude of the response for the tuned frequencies at 30 dB above minimum threshold for excitatory tuning. Curves are based on a weighted moving average (3-point smoothing) of the data and plotted on a linear frequency scale. Response widths are calculated at a 20% level of the peak response.

ever, for BF_{low} the facilitation-induced changes in best amplitude are less dramatic and are more difficult to measure as mentioned in the preceding text.

Response to combinations of multiple harmonics

Figure 13 shows a neuron that responds well to the combination of two tones corresponding to BF_{low} and BF_{high} when these are presented together (Fig. 13A). To examine the

relevance of CF/CF tuning of DSCF neurons to echolocation, we assumed that BF_{low} is a frequency within the frequency range of pulse FM_1 , and BF_{high} corresponds to the Doppler-shifted echo CF_2 of a pulse-echo pair (see Fig. 1A). To test the effects of additional harmonics that are normally present in the echolocation signal, we fixed the amplitude of the pulse H_1 at its BA for facilitation, which for the neuron shown in Fig. 13A corresponded to 72 dB SPL. Additional harmonics were generated at amplitudes corresponding to the relative attenuations of each harmonic present in the natural pulse (Griffin 1971; Kobler et al. 1985). A second set of harmonics was generated based on the neuron's BF_{high} and BA at this frequency. Because the FM-CF type of facilitative response of DSCF neurons is delay-tuned, these stimuli were tested at the neuron's best delay, which corresponded to 31 ms. A comparison of Fig. 13A, 2 and 3 with 1, shows that the third harmonic in the pulse caused a greater suppression of response than the second harmonic, and the inclusion of all three harmonics in the pulse results in a very small response to the pulse-echo stimulus.

In Fig. 13B, 1 and 2, the same phenomenon is shown in the form of cumulative poststimulus time histograms (PSTH) for the same neuron. From the two cumulative PSTHs shown in Fig. 13B2, it is evident that the addition of harmonics to the pulse decreases the latency of spike generation. These results are somewhat intriguing and are explained in greater detail in the discussion. Figure 13B also shows that, similar to the FM-FM area, the time of arrival of the pulse and echo CF_{2-4} frequencies is critical for facilitation to occur in the DSCF area.

Distribution of response latencies to BF_{low} and BF_{high}

We measured response latencies to the BF_{low} and BF_{high} frequencies delivered alone as well as together for 43 neurons. The latency of the response to the BF_{low} stimulus is usually greater than the response latency to the BF_{high} stimulus (Fig. 14). The latency of the facilitated response to the BF_{low}/BF_{high} combination is typically equal to the latency to the response to the BF_{high} stimulus alone. The facilitation versus latency data illustrates three important properties of DSCF neurons. First, facilitation occurs when the latency to the BF_{low} frequency is greater than or equal to the latency to the BF_{high} frequency. Second, highest FRs are obtained for neurons in which latency differences in the response to BF_{low} and BF_{high} are small. Third, neurons showing differences in response latencies to BF_{low} versus BF_{high} of >20 ms do not show any facilitation; these neurons generally are not tuned to the typical Doppler-shift-compensated frequencies in the BF_{low} , but rather show tuning to frequencies at ~ 30 kHz like most CF/CF neurons. In one of these cases, the neuron did not show an ON response, and the measured latency corresponded to an OFF response for BF_{low} . Fitting these data to a bivariate kernel plot (not shown) indicates that a majority of DSCF neurons have an average FR of 2, which corresponds to a mean latency difference ($BF_{low} - BF_{high}$) of ~ 2 ms. A minority of neurons have an FR of ~ 4.2 for a slightly shorter latency difference. A few neurons exhibit large $BF_{low} - BF_{high}$ latency differences at ~ 21 ms and are not significantly facilitated. Interestingly, for several neurons, different latency differences also showed suppression to the combination of two tones.

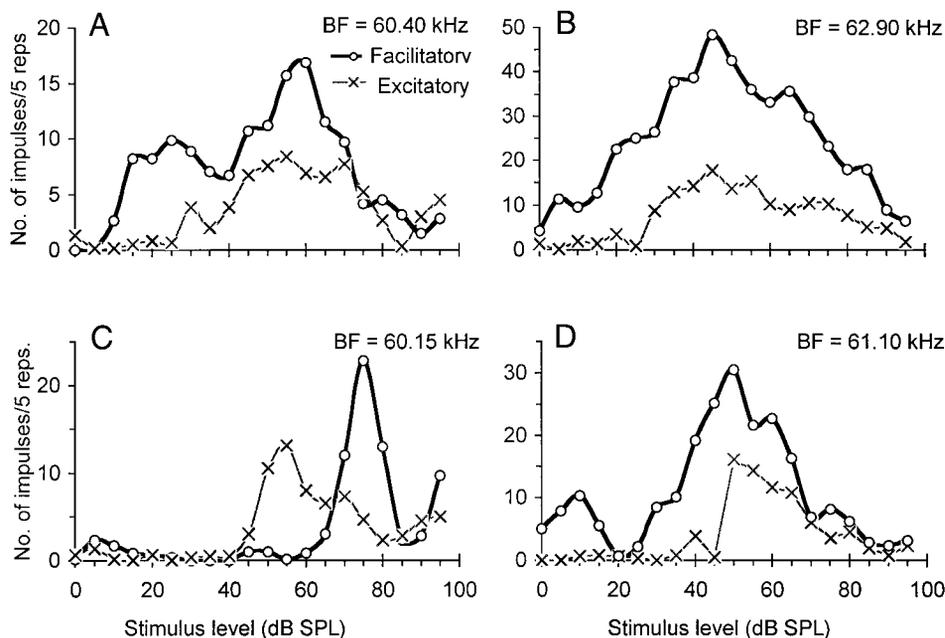


FIG. 11. A–D: plots showing nonmonotonic rate-level functions for single-tone excitation and facilitation to stimulus combinations in 4 DSCF neurons. Rate-level functions are obtained for frequencies showing the best response to single- or 2-tone stimuli. Peak response to 2-tone stimuli is invariably higher because of facilitation. Each data point is based on 5 repetitions of the stimulus and are connected by smoothed lines. Neuron shown in C shows suppression at near “minimum excitatory threshold” levels and facilitation at high sound pressure levels.

Spatial organization of response parameters

In the DSCF area, as in the CF/CF and FM-FM areas, neurons with similar physiological properties are arranged in columns (Taniguchi et al. 1988). There is also an anatomic basis of this columnar organization in terms of thalamocortical projections and corticocortical connectivity so that small, localized injections of horseradish peroxidase in one auditory cortical area label discrete columns in other auditory cortical areas (Fitzpatrick et al. 1998). We were curious as to whether the excitatory and facilitatory tuning to BF_{low} and the facilitatory tuning to BF_{high} also is organized columnarly. Figure 15 shows BAs and BFs marked on inhibitory response areas obtained from an orthogonal penetration in the DSCF area. These data provide estimates of several parameters of the neural response within a column and clearly show that all of the response parameters including the shape of inhibitory areas do not show dramatic changes with depth. For example, at all depths ranging from 317 to 610 μm , the inhibitory response areas show a similar peak at near ~ 90 kHz, although not all neurons in the DSCF area exhibit inhibitory tuning in this frequency range. The lowest thresholds for inhibition in the BF_{high} region may vary, however, by as much as 20 dB. Within a column, the shape of inhibitory tuning is significantly variable only in the BF_{low} region, where neurons show relatively broad excitatory and facilitatory tuning.

The bar graph in Fig. 16A is a density plot of the distribution of FRs within the DSCF areas in a single hemisphere. The distribution is unimodal, although there is a large spread of FRs, and it is heavily skewed to the left of a normal distribution. The source of the differences in the FRs among DSCF neurons is unknown. The magnitude of FR is based on three to four measurements of the response to 200 repetitions of each stimulus. These data show that within a single hemisphere there can be a large range of FRs represented. Several types of analyses were performed to estimate the variation in FR between neurons and to identify the parameters that may show either a causal or a coincidental relationship. However, no clear relationship was observed between FR and the normalized

BF_{high} for excitation/facilitation of a neuron. Similarly, as shown in Fig. 16B, there is a lack of any systematic relationship between the cortical depth at which a neuron is located and the value of FR for that neuron. This is an important result considering the frequency-specific columnar organization within the auditory cortex. Thus neurons within a column that are tuned to similar frequencies may also exhibit similar FRs.

DISCUSSION

Our previous study showed that neural specializations, such as combination sensitivity and delay tuning, co-exist within an expanded representation of systematically mapped best frequencies and amplitudes in the DSCF area (Fitzpatrick et al. 1993; see also Suga 1977; Suga and Manabe 1982). We describe here several basic response parameters associated with spectral facilitation of the neural response observed in the DSCF area and interpret the data in light of this area’s behavioral role in echolocation. Our analysis in concert with previous studies on the auditory cortex of the mustached bat suggests that neurons in an area designated as an expanded representation of one stimulus parameter, in fact, are specialized to respond to those combinations of stimulus parameters in the frequency and time domain that naturally occur during a relevant behavior such as echolocation during insect pursuit. Furthermore DSCF neurons appear to be specialized simultaneously to process communication as well as echolocation sounds (Kanwal et al. 1992). However, this study focuses on the functional significance of excitatory, facilitatory and inhibitory tuning of DSCF neurons for echolocation.

Combination sensitivity in the primary auditory cortex

The DSCF area in the mustached bat contains radial frequency and circular amplitopic maps for frequencies in the 61- to 63-kHz range (Suga 1977; Suga and Jen 1976). These maps, however, are based on frequency tuning of neurons to single-tone stimuli (Suga 1982). Although detailed mapping was not

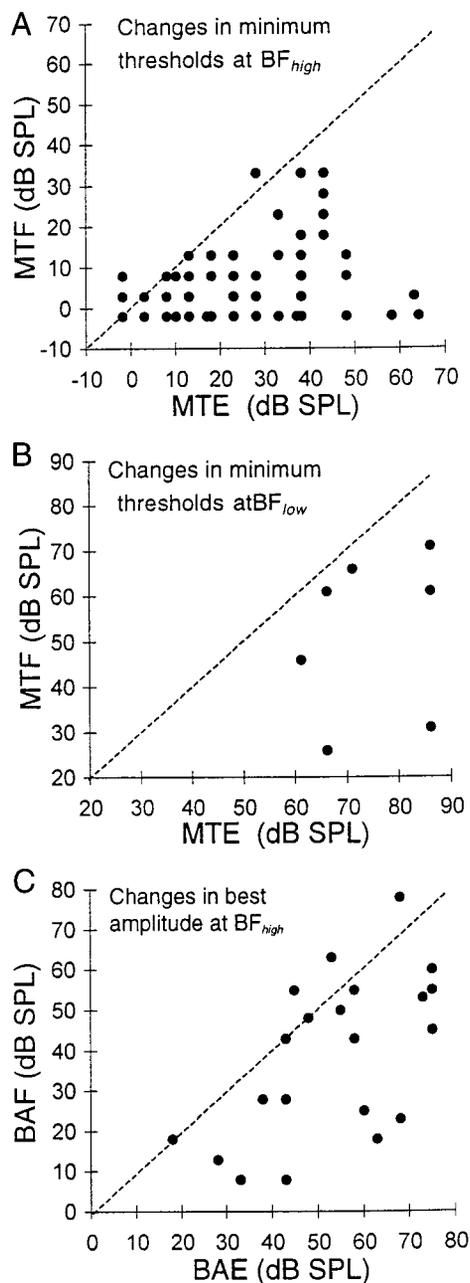


FIG. 12. A: scatterplot to compare the amplitude levels (in dB SPL) at which a minimal response is obtained for excitation vs. facilitation at BF_{high} in 46 neurons in the DSCF area. B: similar scatterplot as in A for comparison of excitatory and facilitatory responses to BF_{low} frequencies. Only 7 neurons were responsive to single BF_{low} tones. C: scatterplot to show the effect of facilitation on best amplitudes of excitation (BAE) for BF_{high} amplitude tuning.

the focus of this study, the data on response parameters of facilitatory tuning to BF_{high} frequencies indicate that the BFs are unaffected by the presentation of a second tone. Minimum thresholds, however, are lowered by ≤ 17 dB. BAs for facilitation also may change by as much as 25 dB (see Fig. 11). Responses obtained from neurons at different depths within a single orthogonal penetration also confirm the columnar organization for tuning to BF_{high} in the DSCF area (Suga and Manabe 1982). However, no clear map of FRs or correlation with BFs was observed.

In several vertebrate species, combinations of stimulus pa-

rameters have been shown to correspond to two or more components of a behaviorally relevant complex stimulus, e.g., unique frequency-modulated patterns or combinations of harmonics in sounds generated by a prey or a predator or within species-specific communication sounds (frogs: Gerhardt and Doherty 1988; Lopez and Narins 1991; birds: Margoliash and Fortune 1992; bats: Esser 1994). However, combination sensitivity within the primary auditory cortex has not been observed in a nonbat species even though other mammalian species have been studied extensively to map various response parameters (Schreiner 1992). In one study in the cat, a few multipeaked neurons exhibited decreased response latencies and thresholds and a relative enhancement of response by two-tone combinations, but, as stated, these were not facilitatory by the classic definition (Sutter and Schreiner 1991). Recent studies in the nonprimary auditory cortex of macaques also have shown facilitation of the neural response to narrow frequency bands extracted from species-specific communication calls (Rauschecker et al. 1995)

Spectral convergence in the primary auditory cortex

As a consequence of combination sensitivity, the DSCF area may be considered to overrepresent 22- to 28-kHz (BF_{low}) as well as 61- to 63-kHz (BF_{high}) frequencies. Most DSCF neurons do not show good responses to a BF_{low} presented alone, although as shown in the response area data a good response is obtained when a BF_{high} is simultaneously presented at or just above its threshold level (see Fig. 4). The intra- versus inter-columnar variation in BF_{low} frequency tuning is difficult to measure because of the absence of a single BF within the irregular threshold boundary of the response areas.

In addition to the excitatory bands, DSCF neurons also exhibit broad and/or notched inhibitory response areas as seen in Figs. 5 and 6. This suggests that DSCF neurons receive a wide range of high-frequency inhibitory inputs. These data indicate that a substantial spectrum of the bats' audiogram, including frequencies not contained in echolocation signals, is represented within a single region of the primary auditory cortex. These results mean that many DSCF neurons will respond in some way to most frequencies within its audiogram and argues against a simple cochleotopic/tonotopic representation of frequencies within the primary auditory cortex. This is an important conclusion as most previous studies have focused only on the tonotopic representation and have largely ignored representation of inhibitory frequencies. (Merzenich et al. 1975; Reale and Imig 1980; Romani et al. 1982; Tunturi 1952). The convergence of the excitatory and various inhibitory frequency components in a mustached bat's auditory system most likely takes place at subcortical levels as in the case of FM-FM and CF/CF neurons (Olsen 1986; Olsen and Suga 1991). In that case, the cortical processing of the frequency information may have to do with the sharpening of frequency tuning as suggested by our observation of large inhibitory side bands. The site of this convergence can be clarified further on the basis of neuropharmacological studies (Zhang and Suga 1997).

The sensitivity of auditory neurons to a wide range of frequencies has been noted in the auditory cortex of other species as well as in other parts of the auditory pathway.

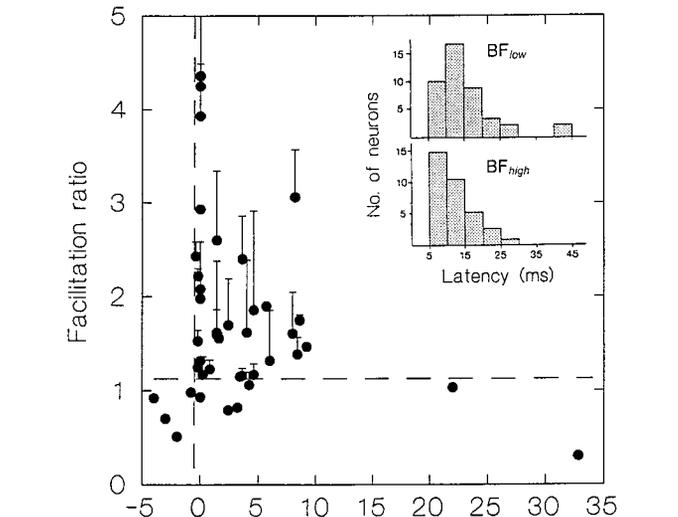
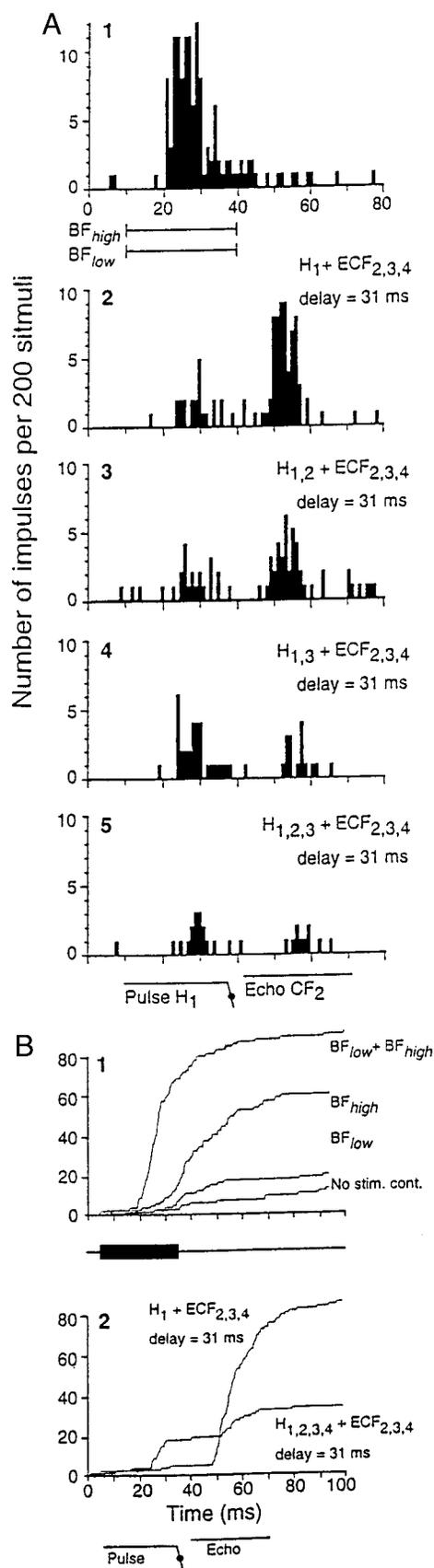


FIG. 14. Scatterplot to show the relationship between the magnitude of facilitation ratio and the difference in latency of response onset between BF_{low} and BF_{high} . Horizontal dashed line at an FR value of 1.1 indicates an arbitrary value above which the response is considered to be facilitated. No facilitation is observed on the left side of the vertical dashed line, i.e., if the BF_{low} latencies are less than the BF_{high} latency by >0.5 ms, facilitation is unlikely to occur. Very large differences (>10 ms) in response latency are observed for OFF-responses to the pulse stimulus. Inset: Distribution of response latencies in ms for BF_{low} and BF_{high} frequencies.

Several types of neurons in the dorsal cochlear nucleus of mammals have narrow excitatory regions with inhibitory surrounds (Evans and Nelson 1973; Shofner and Young 1985; Young and Brownell 1976). Some of these show nonmonotonic rate-level functions similar to those of neurons in the DSCF area. In the cat primary auditory cortex, nonmonotonic neurons are segregated at least partially from monotonic neurons and generally are suppressed by noise (Phillips and Cynader 1985; Phillips et al. 1985, 1994; Reale et al. 1979). In general, in the auditory cortex, neurons that show nonmonotonic rate-level functions do not respond to wideband noise that includes their excitatory as well as inhibitory receptive fields. Although DSCF neurons have not been tested with noise, their rate-level functions are typically nonmonotonic and generally have wide inhibitory areas.

In the auditory cortex of ferrets, an organized distribution of inhibitory response areas has been implicated in complex sound recognition (Shamma et al. 1993). The shapes of these

FIG. 13. PSTH and cumulative PST (PSTC) plots to show the effect of various harmonics in a synthesized pulse-echo pair on the response of a single neuron. *A1*: PSTHs obtained to the presentation of a paired-tone stimulus consisting of BF_{low} and BF_{high} frequencies delivered at their BAs (72 dB SPL and 45 dB SPL, respectively) for this neuron. *A, 2-5*: neural responses to different combinations of echo CF_{2-4} with the 1st, 2nd, and 3rd harmonics in the pulse H_1 ($CF_{1-2} + FM_{1-2}$). To generate an ideal pulse H_1 stimulus for this neuron, its BF_{low} of 26 kHz was considered to be at the midpoint of the pulse FM_1 sweep (●) and the echo CFs were presented at its best pulse-echo delay of 31 ms. *B1*: superimposed PSTCs to compare the onset latency and magnitude of response with the BF_{low} alone, with the BF_{high} alone, and with the facilitated response to the combination of BF_{low} and BF_{high} . Notice that the latency of the facilitated response is slightly shorter than that to the pulse and echo alone frequencies. *B2*: superimposed PSTCs comparing the response to the combination of H_1 and ECF_{2-4} with a pulse-echo delay of 31 ms and to the combination of $H_{1,2,3,4}$ and $ECF_{2,3,4}$ with a pulse-echo delay of 31 ms. Plots are based on acquisition time bins of 0.2 ms. Only pulse H_1 and echo CF_2 are represented schematically on the x axis to indicate the onset and duration of the pulse and echo components.

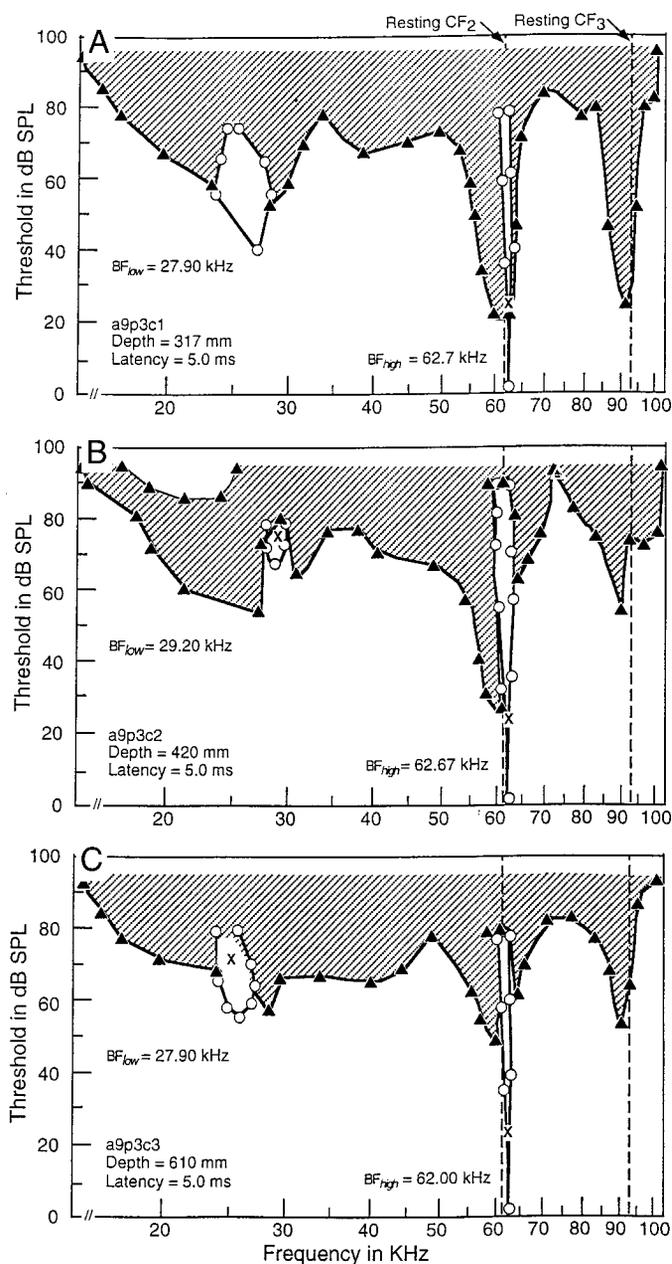


FIG. 15. Frequency response areas for facilitation and inhibition obtained from 3 neurons at increasing (A-C) depths in an orthogonal electrode penetration. BFs for both the BF_{low} and BF_{high} frequencies are generally similar. Crosses indicate best amplitudes for facilitation for each curve. Vertical dashed line indicates the resting CF_2 frequency at 61.50 kHz as measured before recording single-unit activity. Note the third peak of inhibition in the 91- to 93-kHz region.

response areas show a columnar organization as in the DSCF area of the mustached bat. It is noteworthy that inhibitory areas in the dorsal cochlear nucleus and in auditory cortical neurons in the ferret, although generally wider than the excitatory regions, are not as broad as in the DSCF area of the mustached bat. We have not yet determined if inhibitory regions show systematic changes across the surface of the DSCF area. Inhibitory response areas do vary among spatially separated DSCF neurons in spite of the similarity in the basic patterns of excitatory and facilitatory response areas. The exceptionally broad inhibition may be necessary to prevent DSCF neurons

from responding to high frequency ambient noise and to echolocation signals emitted by conspecifics; the latter have energy in restricted frequency regions but at widely spaced intervals according to the harmonic structure of the signal. This relationship of the inhibitory regions to bands within the echolocation signal will be more fully described in a later section.

Neural mechanisms for facilitation and inhibition

To further appreciate the functional significance of the combination sensitivity of DSCF neurons to BF_{low} and BF_{high} and the complex patterns of inhibitory tuning, one has to consider

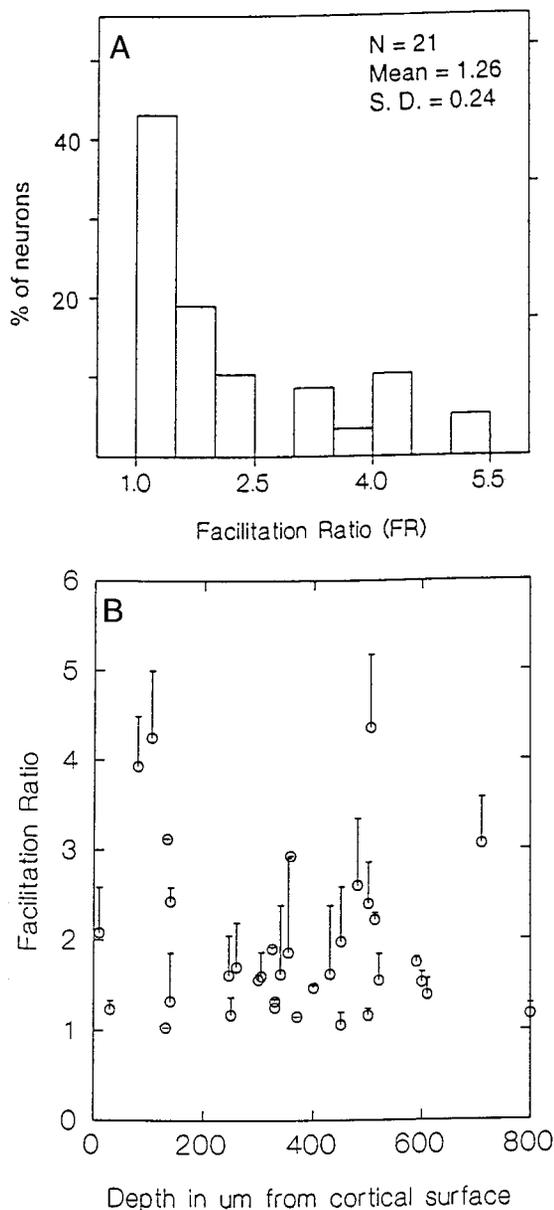


FIG. 16. A: normalized frequency distributions of neurons to show the spread of FRs in the DSCF area within a single hemisphere. B: scatterplot of the variation in magnitude of FR with respect to absolute depth from the cortical surface for 45 neurons. A mean error of 50 μm is possible because of surface indentation and collection of cerebrospinal fluid at the penetration site. Data for this plot were pooled from multiple sites within a single orthogonal penetration and from single sites within separate penetrations. Maximum thickness of the cortex is reported to be 900 μm (Suga and Jen 1976).

the behavioral role/s of the DSCF area. Inactivation of the DSCF area leads to deterioration of perception of small differences in the frequencies of tone bursts in the BF_{high} region (Riquimaroux et al. 1991). As previously alluded to, we also know that BF_{low} and BF_{high} match the frequencies within the echolocation pulse FM_1 and echo CF_2 , respectively (see Fig. 1A). Therefore it is imperative to discuss the facilitation and inhibition at least in reference to the mustached bat's echolocation behavior.

Latency differences to pulse and echo components constitute an important underlying mechanism for determining the best delay of DSCF neurons. In this study, CF tones were used to measure response latencies to pulse and echo components. We examined the distribution of response onset latencies of each component triggering the facilitation response. These data show that to evoke facilitation in DSCF neurons the latency difference between pulse and echo can be as long as 10 ms, although a latency difference of 0 to 2 ms shows the maximum facilitation (see Fig. 13). The latency difference for the response to the minimal pulse and echo components corresponds to the duration of the pulse CF_1 in an echolocation signal. Thus during insect pursuit, where pulse duration changes from 30 to 20 ms during the search and early approach phases, DSCF neurons will show facilitation to pulse-echo delays of 35 ± 5 ms (for 30 ms pulses) or to 25 ± 5 ms (for 20 ms pulses), respectively. These data are consistent with our previous study of delay tuning of DSCF neurons, which showed that delay tuning changes with pulse duration and that long discriminable delays calculated from receiver operating characteristic (ROC) curves of neural responses to pulse-echo pairs are ~ 32 ms (Fitzpatrick et al. 1993). These data together support the idea that in contrast to CF/CF and FM-FM neurons, a few DSCF neurons can produce a facilitative response with maximal FRs for objects that are relatively far off; most likely from objects that are ≤ 6 m away. This takes into consideration a relative velocity of approach of 2–3 m/s. Because the echo reflected from a small (~ 2 cm sphere) target at 6 m would be attenuated by >90 dB but from a reflective background by <40 dB (Lawrence and Simmons 1982; Moss and Schnitzler 1995), these long delays presumably correspond to echoes returning from background objects rather than an insect.

Pulse response latencies shorter than those for echoes by ~ 0.5 ms may also show facilitation (see Fig. 14). This may represent jitter in the system for very short pulse-echo delays, i.e., when the target is very close and the CF duration is very small to nonexistent. The negative (pulse-echo), latency characteristic of some DSCF neurons suggests that the echo CF_2 must produce an excitatory postsynaptic potential (EPSP) that lasts for ~ 1 ms to account for this phenomenon. In contrast, the positive (pulse-echo) latency difference of other neurons suggests that stimulation by a pulse FM_1 component (corresponding to BF_{low}) results in the generation of a long duration (≤ 10 ms) or a delayed subthreshold EPSP in either cortical or subcortical neurons. This means that the facilitative response can track an approaching target independent of the duration of the emitted pulse.

Facilitatory tuning for insect pursuit

The insect-hunting behavior of mustached bats is grossly divisible into three phases, namely search, approach, and ter-

minal (Novick 1963; Novick and Vaisnys 1964). The stimulus parameters causing facilitation in the response of DSCF neurons suggest additional adaptations, besides target detection and characterization, of these neurons for echolocation, e.g., some DSCF neurons also can track a closing-in target during insect pursuit. The evidence for this is twofold. Our previous data on delay tuning indicated that in some neurons tuned to long delays, the best delay changes (decreases) with a decrease in CF duration (Fitzpatrick et al. 1993). Furthermore, as an inevitable consequence of target approach, the echo intensity gradually increases. A profound echo-intensity compensation is observed for fixed targets/backgrounds when bats are swung on a pendulum (Gaioni et al. 1990; Kobler et al. 1985). In a natural situation, however, echo-intensity compensation, like Doppler-shift compensation, is probably based on echoes from the background vegetation so that echoes from approaching targets may continue to increase in intensity, especially at close range (Trappe and Schnitzler 1982). Our data on frequency tuning indicate that the BFs in some of the F_{high} type of neurons decrease as echo intensity increases. Furthermore because of the broad tuning to BF_{low} , some DSCF neurons can track changes in echo CF_2 frequencies independent of changes in H_1 frequencies owing to Doppler-shift compensation. Although it is not clear that the duration versus delay tracking and the frequency versus intensity tracking occur in the same neuron, the available data indicate that both types of effects can be observed in DSCF neurons.

In *Rhinolophus*, the flying speed of a bat is reduced from 6.1 m/s in the search phase to ~ 2.5 m/s during the terminal phase of insect capture (Kick and Simmons 1984). Mustached bats normally fly at a speed 4.5 to 9 m/s during the search phase but may slow down considerably when trying to capture insects in their tail wing (Griffin 1958). The net change in frequency tuning of a single neuron (1.70 ± 0.64 kHz) suggests that these tracking neurons operate within relative velocities decreasing from, e.g., 9 to ~ 3 meters/s as the echo intensity increases (The frequency of the CF_2 in the returning echo from the target presumably is lowered down to the Doppler-shift compensated pulse CF_1 frequency as the bat reorients itself to intercept a target or slows its flying speed for target capture).

Other major effects of facilitation are a lowering of the minimum thresholds and a widening of the tuning of the neural response. Both of these effects increase the probability of target detection by extending the acoustic boundaries at which a target can be perceived in terms of relative distance and motion domains. On the basis of these data and those reported previously (Suga and Manabe 1982; Suga et al. 1983), it appears that DSCF neurons signal target detection and may track targets during the characterization process.

Inhibitory tuning for noise reduction during roosting and foraging

Mustached bats are gregarious, and several hundred or even thousands of bats may roost in a single cave in the same or adjacent colonies during the nonflying and nonhunting periods of their daily life. Roosting bats routinely emit echolocation pulses and communication sounds at >100 dB SPL so that the sound intensity for the echolocation frequencies heard all around by any bat is easily 100 dB SPL (Kanwal et al. 1994). Echolocation pulse-echo pairs, however, carry information that

is useful only to the emitter. For other bats, the high sound intensities of pulse and sometimes echo frequencies that vary only by a few hundred hertz represent a noisy environment that may interfere with the processing of the bat's own pulse-echo pairs. Thus the normal spontaneous noise level of ~2 to 3 spikes/s per DSCF neuron could more than double for a bat roosting inside a cave, leading to the possibility of energy and information loss. Instead, the mustached bat has evolved a set of adaptive mechanisms that regulate the noise-driven neural activity in a behaviorally relevant signal-specific manner. These adaptations are based on the patterns of inhibitory as well as excitatory and facilitatory tuning of DSCF neurons.

At the auditory periphery, sharply tuned neurons may respond to various components of echolocation and communication sounds emitted by other bats. If these sounds coincide or precede the bat's own pulse or echo, then a cortical neuron on which several frequency channels converge may be in an absolute or relative refractory phase and therefore unable to respond adequately to the bat's own pulse-echo frequencies. Consequently several mechanisms have been enumerated that either reduce or prevent masking and/or jamming of a neuron's response (Henson et al. 1980; Suga et al. 1987). For example, small individual variations among the bats' resting CF_2 and sharp tuning to the corresponding Doppler-shifted frequencies allow the bat's auditory cortex to be personalized to listen only to its own signals.

The data obtained in this study suggest that the neural properties (described in the preceding text) that prevent masking basically are unmodified by facilitation although stimulus combinations do lead to a slight broadening of the excitatory response area. Our data further show that additional specializations exist for rejecting echolocation pulses emitted by other bats. These specializations supplement the proposed "personalization" mechanisms and are related to the inhibition of cortical auditory neurons. They allow the auditory system to adjust to a noisy environment by rejecting/attenuating noise without losing information contained in their own echolocation signals. We label these adaptations for rejecting sounds produced by conspecifics as "privatization" mechanisms to contrast them from those adaptations that allow a bat to "tune-in" to their personalized echolocation signals (Suga et al. 1987).

One important privatization mechanism is related to the broadening of inhibitory response areas at high intensities. According to the inhibitory-tuning data, high sound pressure levels of inhibitory frequencies in the H_1 and H_2 of the pulse and/or echo may lead to inhibition of most DSCF neurons. This additional broadening of the inhibitory tuning at higher intensities suggests that inhibition may serve as a mechanism to protect against overstimulation of neurons by some communication sounds and high-intensity echoes from large background objects or by echolocation pulses emitted by other bats in a small enclosed space as during roosting in caves. This problem of overstimulation does not exist for high intensities of sounds in the bat's own pulse because of the attenuation by middle ear muscles alluded to earlier (Suga and Shlegel 1972).

A second privatization mechanism is related to the presence of a low threshold of inhibition to frequencies near ~90 kHz in some DSCF neurons. This explains the intriguing observation that 90-kHz frequencies are exclusively inhibitory for some DSCF neurons when presented from an external source (Figs. 13 and 15). Interestingly, the minimum threshold for inhibition

for these frequencies is several decibels higher and the BFI is generally lower than that present in ECF_3 . These data suggest that the 90-kHz inhibition is to the pulse CF_3 component of other bats echolocating in the vicinity. Once again, pulse CF_3 frequencies in a bat's own pulse may be attenuated specifically by vocalization triggered neural feedback or by contraction of middle-ear muscles when a bat emits the echolocation pulse (Suga and Shlegel 1972). Weaker ECF_3 components in the echoes are probably ineffective in causing inhibition. Any pulse CF_3 -triggered neural activity arriving at cortical neurons just prior to a bat's own pulse FM_1 -echo CF_2 combination are ineffective in causing inhibition in the presence of the robust facilitatory response. On the contrary, the pulse CF_3 may trigger transient suppression (resetting) of ongoing neural activity, thus preparing a DSCF neuron for processing of the incoming, information-bearing echo. Thus, the different patterns of inhibitory response areas in DSCF neurons may improve signal to noise ratios for echolocation during foraging as well as communal roosting in spite of an acoustically cluttered environment.

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