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Auditory Cortex of the Rufous Horseshoe Bat: 1. Physiological Response Properties to Acoustic Stimuli and Vocalizations and the Topographical Distribution of Neurons

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Abstract

The extent and functional subdivisions of the auditory cortex in the echolocating horseshoe bat, *Rhinolophus rouxi*, were neurophysiologically investigated and compared to neuroarchitectural boundaries and projection fields from connectional investigations. The primary auditory field shows clear tonotopic organization with best frequencies increasing in the caudorostral direction. The frequencies near the bat's resting frequency are largely over-represented, occupying six to 12 times more neural space per kHz than in the lower frequency range. Adjacent to the rostral high-frequency portion of the primary cortical field, a second tonotopically organized field extends dorsally with decreasing best frequencies. Because of the reversed tonotopic gradient and the consistent responses of the neurons, the field is comparable to the anterior auditory field in other mammals. A third tonotopic trend for medium and low best frequencies is found dorsal to the caudal primary field. This area is considered to correspond to the dorsoposterior field in other mammals. Cortical neurons had different response properties and often preferences for distinct stimulus types. Narrowly tuned neurons ($Q_{10dB} > 20$) were found in the rostral portion of the primary field, the anterior auditory field and in the posterior dorsal field. Neurons with double-peaked tuning curves were absent in the primary area, but occurred throughout the dorsal fields. Vocalization elicited most effectively neurons in the anterior auditory field. Exclusive response to pure tones was found in neurons of the rostral dorsal field. Neurons preferring sinusoidal frequency modulations were located in the primary field and the anterior and posterior dorsal fields adjacent to the primary area. Linear frequency modulations optimally activated only neurons of the dorsal part of the dorsal field. Noise-selective neurons were found in the dorsal fields bordering the primary area and the extreme caudal edge of the primary field. The data provide a survey of the functional organization of the horseshoe bat's auditory cortex in real coordinates with the support of cytoarchitectural boundaries and connectional data.

Introduction

Within the borders of the mammalian auditory cortex, subdivisions can be distinguished following physiological and anatomical criteria (for review see Clarey *et al.*, 1992). In non-primate mammals, the most extensive data are available for the cat, in which at least five cortical fields with an approximately complete cochleotopic (tonotopic) organization can be defined. In most other non-primate animals mainly the primary auditory cortex has been specified, and detailed maps have been described in rabbits, grey squirrels, rats, ferrets, guinea-pigs, hedgehogs, mice, marsupial native cats, gerbils (for the gerbil see Thomas *et al.*, 1993; for the other species see Clarey *et al.*, 1992) and bats (detailed references will be cited below).

The investigation of the functional significance of different cortical fields has gained increasing importance and extends beyond tonotopic organization and processing properties for simple stimuli to more complex and temporally structured stimuli in a variety of species (for review see e.g. Clarey *et al.*, 1992).

Especially in some species of echolocating bats, the auditory cortex has been explored extensively in recent years with the aim of defining and describing specific functional 'processing areas'. Investigations on the cortical neuroarchitecture and connectivity in close conjunction with neurophysiological recordings are, however, rather sparse.

The species in which functional cortical differentiation has been explored most exhaustively is the moustached bat, *Pteronotus parnellii* (New World family Mormoopidae). The moustached bat belongs to the so-called long CF/FM bats, which use a long-duration constant-frequency (CF) component terminated by a downward-sweeping frequency modulation (FM) as its echolocation call. The moustached bat's echolocation pulse is composed of four harmonics, the second, of ~60–62 kHz, being most prominent.

The phylogenetically unrelated horseshoe bat, *Rhinolophus rouxi* (Old World family Rhinolophidae), which is described in this study, uses a similar kind of echolocation call composed of a long CF

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component followed by a short FM sweep. Two harmonically related components with CF at ~38 and 76 kHz are present in the call, and the upper harmonic carries most of the call energy.

The two species show strong convergence in their acoustical behaviour. Both perform Doppler shift compensation (DSC) (Schnitzler, 1970; Schuller *et al.*, 1974) to stabilize the CF portion of the echoes at their particular 'reference frequency', irrespective of their own flight speed. Their hearing system is extraordinarily sharply tuned to this reference frequency and a small frequency range above it. Both species analyse periodical modulations of the long CF portion induced by insect wing-beats to forage for flying insects in strongly cluttered environments, i.e. around dense vegetation (Neumann and Schuller, 1991).

The tonotopically organized auditory cortex in the moustached bat shows a pattern similar to that in other mammals, high frequencies being represented rostrally and low frequencies caudally. As a special feature, neurons within two narrow frequency bands at the second and third harmonics of the echolocation call are largely over-represented (Suga and Jen, 1976; Suga and Manabe, 1982; Asanuma *et al.*, 1983; Suga *et al.*, 1979, 1983a).

There are a number of functionally specialized 'processing areas' outside the tonotopic area characterized by neurons encoding and extracting different acoustic features of the species' complex echolocation signal and echoes. The single-unit response properties exhibit facilitation for echolocation-relevant parameters, and the units display orderly arrangements of stimulus parameters in the cortical plane (O'Neill and Suga, 1979, 1982; Suga and O'Neill, 1979; Suga *et al.*, 1983b; Suga, 1984; Suga and Horikawa, 1986; Edamatsu and Suga, 1993). Such specialization of neurons in cortical fields is not limited to bats using long CF/FM calls, but similar neurons have been found also in the cortex of *Myotis lucifugus*, a bat using sonar signals composed only of FM sweeps (Sullivan, 1982; Wong and Shannon, 1988; Berkowitz and Suga, 1989).

Physiologically, the horseshoe bat shows a comparable organization of the auditory cortex into a tonotopically organized auditory field (Schweizer and Radtke, 1980; Ostwald, 1984) surrounded by acoustical areas involved in more complex auditory processing. Neurons in a part of the dorsal auditory cortex of this bat exhibit specializations for stimulus combinations comparable to those in the moustached bat (Schuller *et al.*, 1991).

In most bats, however, apart from gross functional localizations, no correlation between functionally defined processing areas and neuroarchitectonic features or connectional attributes has been established.

Bats, with their unique special features of flight and echolocation, have often been considered as 'exotic' representatives of the mammalian order compared to common laboratory animals like for example cats or rats. In the search for a 'basic plan of the mammalian neocortex', the cortical neuroanatomy of bats has been of interest since the beginning of this century. Opinion on the functional importance of the auditory cortex for echolocation in bats has developed from being of relative insignificance (e.g. Konstantinov, 1965; Suga, 1969) to representing a highly advanced system for auditory imaging (Suga, 1990; Dear *et al.*, 1993).

Our combined investigations of physiological properties, neuroarchitecture and connectivity of cortical fields in the horseshoe bat aim to contribute to the discussion of how similar or how specialized the auditory cortex of this species is compared to that of other mammals.

In this paper we describe the basic physiological properties of cortical fields in the horseshoe bat against the background of cytoarchitectonic and connectional boundaries. The detailed cytoarchitectonic characterization of cortical fields and the description of thalamocortical connections will be subject of subsequent papers.

Materials and methods

Animals

Twenty-three Indian or Sri Lankan rufous horseshoe bats, *R. rouxi*, were used in this study. Bats were kept in captivity under seminatural conditions for under a year.

Preparation

The animals were surgically prepared under halothane anaesthesia. The skin overlying the skull was additionally infused with local anaesthetic (lidocaine 2%). The skin was cut along the midline and reflected to the sides to affix a tube that was attached to the stereotaxic device during experiments. The tube was glued with cyanoacrylate glue and dental cement to the caudal part of the skull overlying the inferior colliculi and the cerebellum. Rostral to the fixation tube, the tissue was carefully cleaned from an area of skull ~1.5 mm left and right of the midline. This area was used to determine the position of the skull surface in stereotaxic coordinates and to place holes to introduce the recording electrodes. After surgery, the animals were allowed to recover through the following day. The recording experiments started typically on the second postoperative day, with daily sessions no longer than 6 h, which were typically repeated over 3 weeks. Throughout the experiments, the wound margins were treated with local anaesthetic (lidocaine 2%), but the animals were otherwise unanaesthetized.

Stereotaxic procedure

The experiments were conducted in an acoustic chamber lined with convoluted foam that reduced acoustical interference from the environment and minimized the reflections of ultrasonic signals. The bats were placed in an animal holder that prevented gross body movements, and the head was immobilized by attaching the surgically affixed tube to a head-holder that guaranteed accurate repositioning of the animal in the stereotaxic coordinates throughout recording series, which lasted for several weeks. The actual skull position in the stereotaxic coordinate system was determined during a short (~1 h) session on the first postoperative day by scanning the profile of the exposed skull in both the parasagittal and transverse directions relative to a fixed reference point. Details of the stereotaxic procedure have been described elsewhere (Schuller *et al.*, 1986). The method yields a typical accuracy for the localization of recording sites of 100 μm in all three dimensions. Localization of the recording sites within the brain was further verified by injection of tracer substances, such as horseradish peroxidase or wheatgerm agglutinin conjugated with horseradish peroxidase, or by making small electrolytic lesions.

Recording

For single-unit recording, a small hole was cut into the skull over the target area and the dura was perforated under local anaesthesia. The holes had diameters <500 μm and several electrode penetrations were made through each hole with different mediolateral inclinations in planes corresponding to the frontal section plane of the stereotaxic atlas. For single-unit recording, 3 M KCl-filled glass microelectrodes with impedance between 4 and 15 MΩ were lowered from the surface of the brain in steps of 2 μm using a piezoelectric micropositioner (Burleigh Inchworm). In the last five experiments of this series Parylene-coated tungsten electrodes (Micro Probe) with impedance

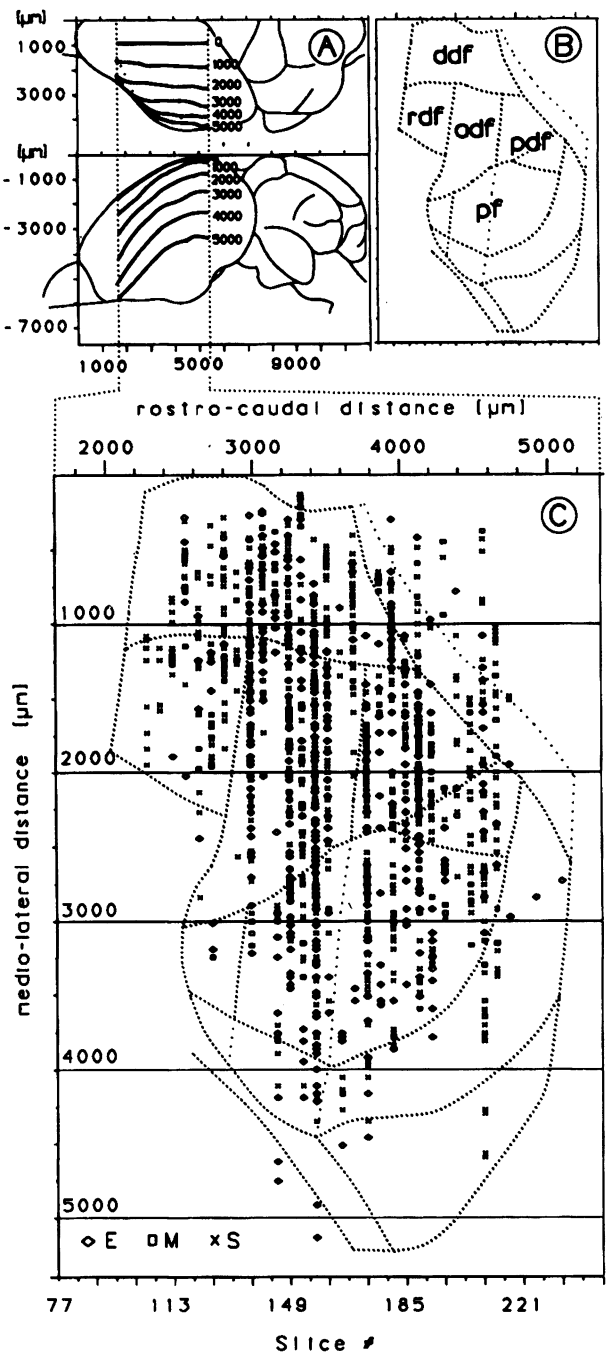


FIG. 1. Recording sites in the cortex of the horseshoe bat yielding responses to acoustic stimulation. The locations of auditory neurons (crosses for single units, squares for multiunits) and evoked potential recordings (diamonds) are represented in rostrocaudal and mediolateral coordinates on a flattened cortical surface projection (C). The position of the flattened area is represented in graph A in a lateral and dorsal view of the brain. The heavy lines correspond to the horizontal lines (1000–5000 µm) in C, and give the distance to the reference line defined as the parasagittal rim of the cortical surface 1000 µm lateral to the midline. Dotted lines in graph C indicate cytoarchitectonic borders. The naming of the fields according to their relative position is given in B. The recording sites have been pooled over rostrocaudal distances of 88 µm on a central atlas slice. The penetrations were usually carried beyond the acoustically responsive area until neurons were no longer drivable by the available acoustic stimuli. The recordings cover the entire auditory neocortex, as anticipated from cytoarchitectonic and connectional evidence. Recordings are sparse only in the most rostral and most ventral parts, where access is surgically difficult and risky.

between 1.8 and 2.5 MΩ were used. The exposed electrode tips of the metal electrodes had diameters of ~1–2 µm and lengths of 5–10 µm. The indifferent electrode (sharpened tungsten wire) was chronically implanted under the most anterior part of the skull during the initial surgery.

The action potentials were amplified, filtered and amplitude-discriminated with conventional methods. The temporal occurrence of spikes was recorded relative to the onset of the acoustic stimuli and could be displayed either as a dot raster or as peristimulus time histograms. Acoustic stimuli with a fixed or a single, stepwise-varying parameter were presented. Each frame, or 'segment', consisted of either 32 or 64 presentations. Recording and processing programs were run on a DEC LSI11/23 computer. All programs were written by M. Betz.

Acoustic stimulation

Acoustic stimuli were generated by passing sine waves from function generators (Wavetek) through custom-made electronic switches

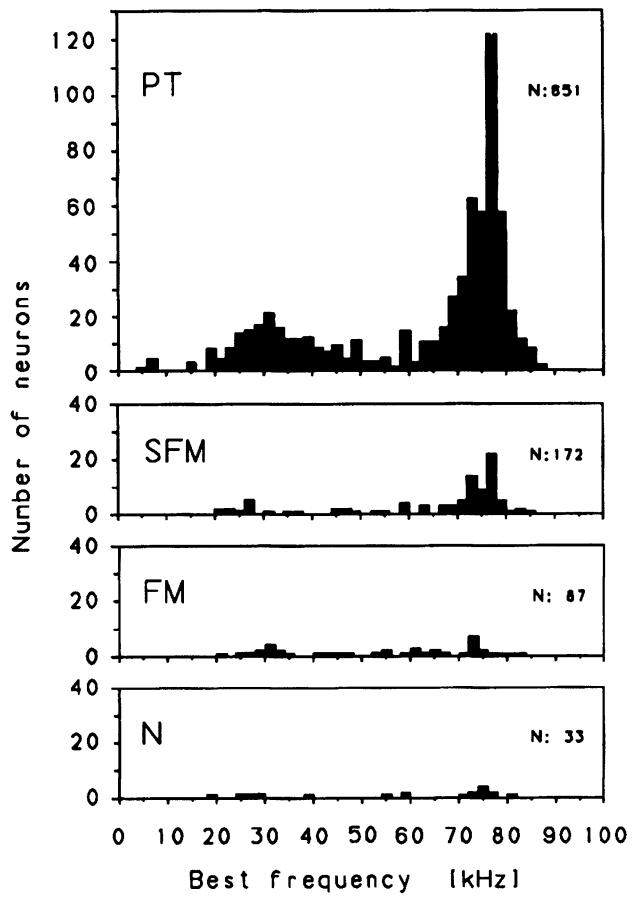


FIG. 2. Distribution of best frequencies in the auditory cortex of the horseshoe bat. The neurons have been divided into a large class of neurons that did not show any special preference for a stimulus type (PT neurons) and three classes of neurons that responded best either to sinusoidal (SFM) or linear frequency-modulated (FM) stimuli or to narrow-band noise signals (N). In the latter three response classes the centre frequency of the frequency modulations or the noise band at lowest threshold is taken as best frequency. All four classes show a bimodal distribution of best frequencies around the prominent spectral components of the echolocation calls, i.e. at the first and second harmonics of the constant frequency and frequency-modulated portions. The best frequencies at and a few kHz above the resting frequency are largely over-represented.

shaping the stimuli into bursts with a 1 ms rise–fall time. Typically, pure-tone stimuli, linearly or sinusoidally frequency-modulated stimuli or narrow-band noise stimuli were used. Stimulus duration was 50 ms (occasionally 40 or 60 ms), and linearly frequency-modulated components lasted between 1 and 3 ms. Stimuli from two channels could be broadcast together at adjustable interstimulus delays and with independently controlled amplitudes and frequencies. The acoustic stimuli were broadcast by an electrostatic loudspeaker (2 cm diameter) located 30° contralateral to the recording site and 15 cm away from the bat's ears. The elevation of the loudspeaker was adjusted perpendicularly to the plane of the nose leaf (the position of greatest sensitivity of the ear at the reference frequency).

Data processing

For the determination of the individual resting frequency (RF) of the bats, vocalizations were monitored with a Bruel & Kjaer 1/4" ultrasonic microphone (type 4135), amplified and fed to a frequency-to-voltage converter with a resolution better than 50 Hz. The bats had RFs between 72.52 and 78.64 kHz with a mean value of 76.28 ± 1.86 (SD) kHz; $n = 23$. The individual RF was used to normalize all frequency data obtained in an individual bat to a standard RF of 76 kHz with the formula $f_{\text{normalized}} = (f - \text{RF}_{\text{individual}} + 76)$ [kHz].

The locations of the recording sites were transformed into coordinates of the stereotaxic brain atlas of *R. rouxi* (Radtke-Schuller, unpublished). To provide a uniform representation of the topographical organization of stimulus parameters, the recording sites were projected to a flattened version of the cortical surface after a standardized projection procedure. Details of the surface projection method have been described in Schuller *et al.* (1991). All positional data in the cortex, i.e. recording positions, cyto- and myeloarchitectonic boundaries and results of tracer injection, were processed using the same reconstruction procedure and are therefore mutually comparable. Unit positions were entered together with their neurophysiological properties in a commercially available database management programme (Reflex, Borland) and were sorted and graphed using various criteria. Since data from all experiments were entered into the database, the results could be analysed either individually or pooled over selected cases.

Results

A total of 942 neurons was recorded in the auditory cortex in 23 bats. The activity in 673 recordings could be characterized as single-unit responses, whereas in 269 cases the isolation was not perfect and the activity of more than one unit was monitored simultaneously (multiunit recording). Additional information from 424 local evoked potential recordings was used as supporting background information.

Physiological definition of auditory cortex

The penetrations were guided parallel to the standard frontal section plane defined by the brain atlas (Radtke-Schuller, unpublished; Schuller *et al.*, 1986) and with a mediolateral tilt so that the penetration path was locally parallel to the surface of the auditory cortex. The recordings covered a large area of the neocortex of the bat, and Figure 1 shows the location of all recordings that yielded responses to acoustical stimuli. The representation of recording sites is given in coordinates of the unrolled, i.e. flattened, cortical surface (Fig. 1A, B).

The outermost stippled lines delineate the borders of the auditory neocortex as expected from cytoarchitectonic and connectional evidence, which will be presented in detail in separate papers. It is obvious that the physiological recordings fit well into these borders. No, or only very few, acoustically responsive neurons have been recorded in the ventrocaudal and most rostral parts of this area, although they are the target of auditory thalamocortical projections. The reason may be insufficient refinement of the tested acoustical stimuli.

No difference or bias concerning the type of recordings (single unit, multiunit and evoked potential) except for some recording sites at very ventral positions (only evoked potentials) is apparent at the borders of the acoustically responsive area.

General properties of neurons in the auditory cortex

Out of 942 recordings of single units or multiunits, 143 units (15%) showed spontaneous activity, 296 units (31%) exhibited a clear preference for one of the stimulus types presented (pure tones, linear FM, sinusoidally modulated FM, band-limited noise and spontaneously emitted vocalizations), whereas the remaining neurons (503, or 54%) had no distinct preference to any stimulus type as long as the spectral components of the stimulus fell within the response area of the unit.

In neurons having a stimulus preference, the response activity was either distinctively accentuated or more consistent to a specific stimulus when compared to other stimulus types. More than half of the units (52%) preferred pure tones and did not respond, or responded very poorly, to more complex stimuli. Fifteen percent of the neurons showed best responses to linear frequency modulations (LFM) and 18% were optimally driven by sinusoidally frequency-modulated (SFM) signals. Narrow-band noise stimuli elicited best responses in 8% of the units and 7% of the neurons preferentially processed the bat's own vocalizations.

Spontaneously active units were drivable by acoustic stimuli in only 61% of the cases (87 neurons) and 24 of these neurons showed a preference for a distinct stimulus class.

Best frequencies of the recorded units were unequally distributed along the frequency axis, as shown in Figure 2. The graph for pure-tone (PT) neurons shows the distribution of neurons that had a well determined best frequency and did not have a preference for one of the other stimulus types. Most prominent is the peak at and above the bat's resting frequency (normalized to 76 kHz). The number of neurons tuned to frequencies of the FM sweep of the echolocation call rapidly declines from <76 to ~58 kHz. A second peak builds up

TABLE 1. Response patterns of neurons in the auditory cortex of the horseshoe bat, *R. rouxi*, ordered following the preferred stimulus type

	PT (N)	PT (%)	SFM (N)	SFM (%)	FM (N)	FM (%)	Noise (N)	Noise (%)
Phasic-on	218	57	63	42	44	47	11	23
Phasic-off	12	3	1	1	5	5	1	2
Phasic-on/off	58	15	17	11	23	25	6	12
Tonic	78	20	53	35	16	17	10	21
Inhibitory	19	5	17	11	5	5	20	42
Class	385		151		93		48	
Class	385	84	151	74	93	81	48	62
Rejection	46	10	18	9	16	14	23	30
No-class	25	6	36	17	6	5	6	8
Total number	456		205		115		77	
Inconsistent	101	22	57	28	11	10	11	14
Adapting	21	5	4	2	2	2	0	0

PT, pure tone; SFM, sinusoidal frequency modulation; FM, linear frequency modulation.

at lower frequencies around half the resting frequency (38 kHz) and reaches a peak at 30 kHz. This second peak of the distribution is located within the band of the lower harmonic FM sweep.

Whereas SFM-preferring neurons have a distribution of best frequencies very much like that of PT neurons, the neurons showing preferences for LFM or noise have best frequencies mostly in the frequency bands matching the FM portion.

The overall distribution of best frequencies in the auditory cortex of the horseshoe bat reflects the same bimodal frequency pattern as found in lower auditory brain structures.

Response types and stimulus preferences

Table 1 compiles the response types of 677 cortical neurons classified as phasic, tonic or inhibitory and subdivided within the phasic class as on, off and on/off neurons. In neurons showing a combination of response patterns the most prominent pattern was used for classification. Units showing response patterns that varied considerably with the stimulus parameters were labelled as not classifiable ('no-class'). The neurons were further ordered after their stimulus type preference (PT, SFM or FM and noise). The phasic response type was by far the most common (68%), and 50% of the neurons showed on, 3% exclusively off and 15% combined on/off responses. Tonic units represented 23% of the population and 9% of the neurons were inhibited by acoustic stimulation.

The response types were unevenly distributed among optimal stimulus types. The ratio of phasic to tonic pattern was 3.75 in PT, 4.5 in FM, only 1.5 in SFM and 1.8 in noise-driven neurons. Inhibition occurred most often in noise-driven neurons (42%) whereas all other types showed inhibition in only 5% (PT and FM) or 11% (SFM) of cases.

Neurons that responded to all stimulus types but one, i.e. showed rejection to a specific stimulus type, were also classified as stimulus-specific to that stimulus type. Between 9 and 14% of the PT, SFM and FM neurons showed rejection. Only the class of neurons with particular responses to noise comprised a higher number of neurons rejecting noise stimuli (30%).

Cortical neurons did not always respond in a strict one-to-one relationship to the stimulus. Activity often showed temporal fluctuations, manifested in a temporal shut-down or reduction of response followed by a sudden restart at full or reduced strength. About one-fifth of the neurons showed such an inconsistent behaviour. A smaller fraction of auditory neurons (between 2 and 5%) adapted with repetitive stimulation and turned silent after a few stimulus presentations.

Apart from neurons that responded to sound in a temporally

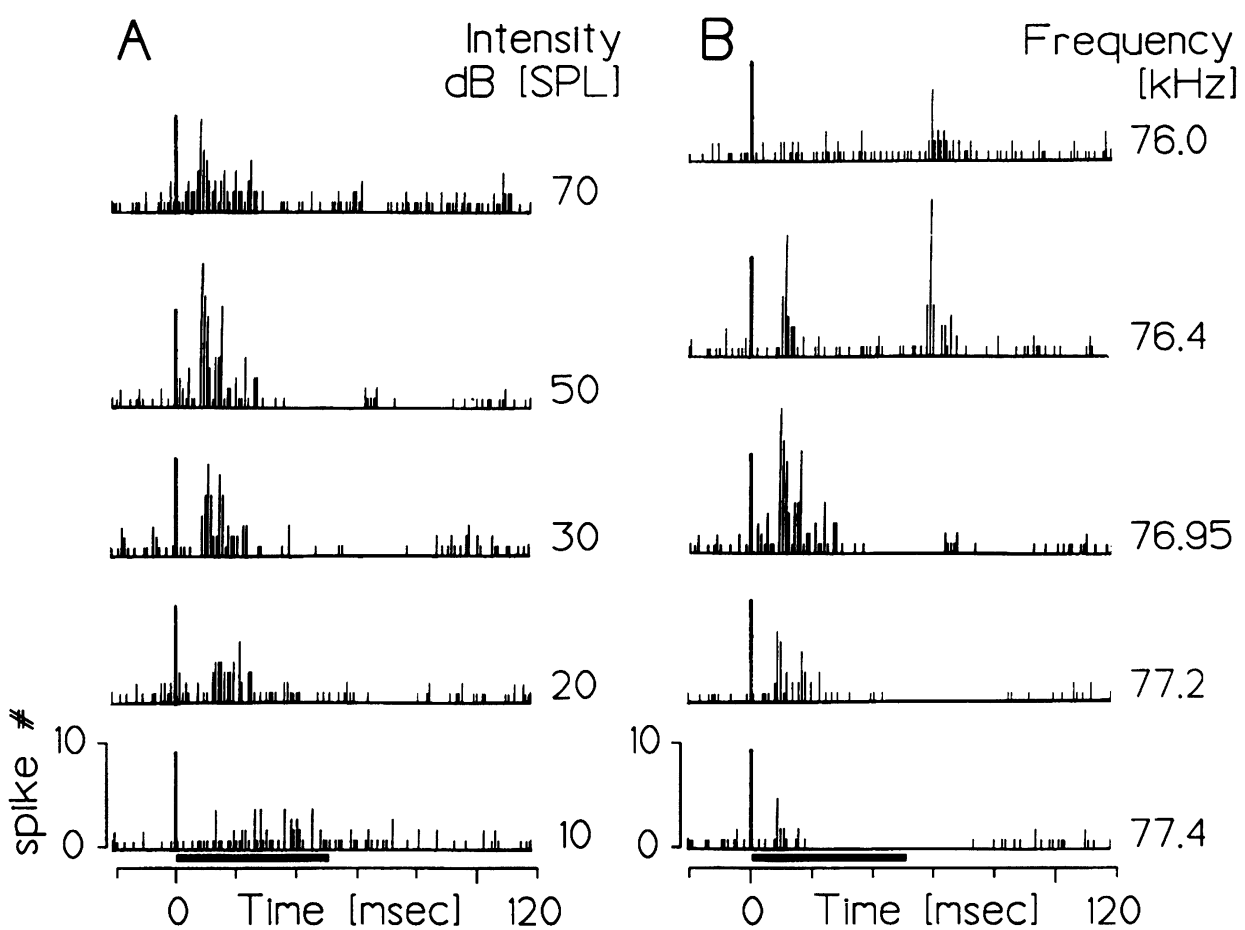


FIG. 3. Responses of a primary field (pf) neuron with phasic response pattern at its best frequency of 76.95 kHz. Column A shows histograms for different stimulus sound pressure levels at the best frequency; column B displays pattern changes with changing frequency at a constant SPL of 50 dB. The neuron is sharply tuned, has a non-monotonic response with best amplitude at 50 dB SPL, and changes its response pattern with varying frequency. (Note that the vertical line at time zero in each histogram indicates the start of the stimulus, and its length signifies the scale for the spike numbers). Stimulus repetitions 32, bin width 1 ms.

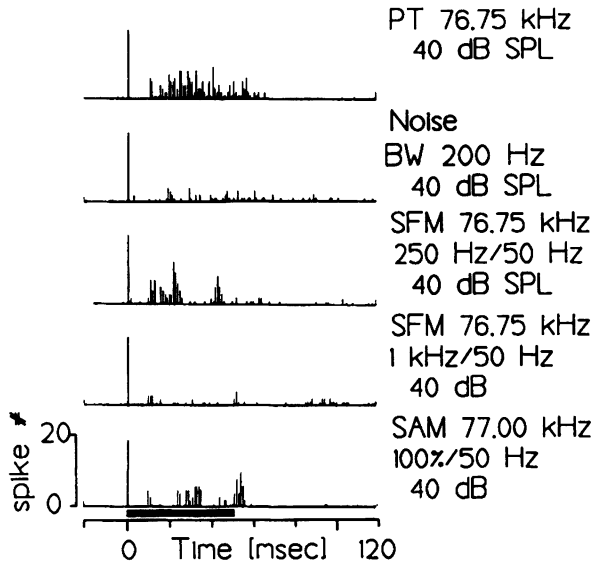


FIG. 4. Tonic response pattern of a neuron of the primary cortex (pf) and responses to noise and sinusoidal modulations. The neuron is tuned to the 'RF' frequency range (best frequency 76.75 kHz) and shows a tonic response pattern (upper histogram). The responses are suppressed by narrow-band noise (bandwidth ±100 Hz, 2nd row); frequency-modulated stimuli (SFM) synchronize or suppress responses depending on the modulation depth (250 Hz and 1 kHz, 3rd and 4th row; cycle duration is 20 ms, corresponding to 50 Hz modulation frequency). Sinusoidal amplitude modulation induces a synchronized response pattern. PT, pure tone; SAM, sinusoidal amplitude modulation; BW, bandwidth; frequency following stimulus type is the carrier frequency. Figures separated by a slash indicate the amplitude\frequency of modulation. Stimulus repetitions 32, bin width 1 ms. Sinusoidal modulations (SFM and SAM) always start at zero phase and with rising slope.

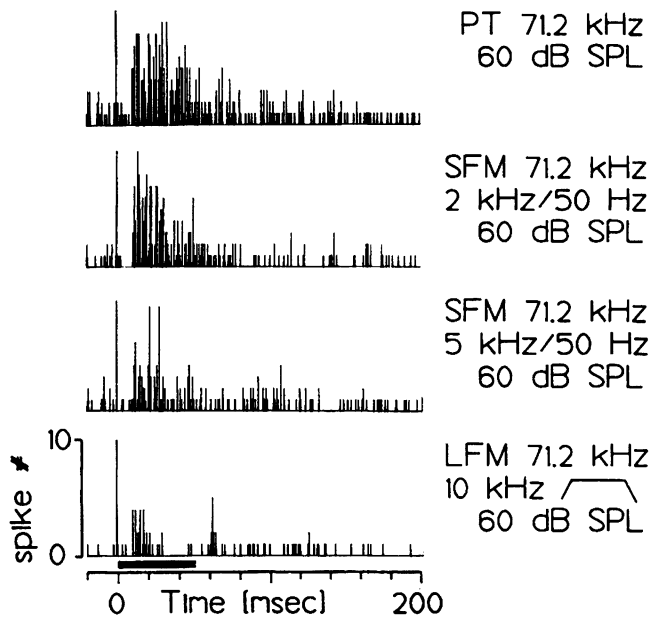


FIG. 5. Response of a neuron with best frequency (71.2 kHz) in the 'FM' range. Frequency modulations reduce the response activity (SFM or LFM) with increasing modulation depth. LFM, linear frequency modulation; other abbreviations and labelling as in previous figures. Stimulus repetitions 32, bin width 1 ms.

correlated manner, units were observed that changed their activity in a way that could not consistently be described. They appeared to be influenced but not driven by acoustical stimulation. Such neurons and the adapting neurons were predominantly recorded at caudal or far rostral edges of the acoustical cortex.

Responses to different stimuli

Pure-tone responses

The responses to pure-tone stimulation could be very different in pattern, tuning properties and consistency, mainly as a function of the location of the neuron. Most reliable responses, e.g. a one-to-one relationship between neural activity and stimulus, were found in neurons of the primary field, the anterior and the posterior dorsal fields (adf and pdf respectively; Fig. 1).

Figure 3A represents the phasic responses of a neuron at a best frequency of 76.95 kHz (resting frequency 76 kHz) consistent over the entire dynamic range from 10 to 70 dB sound pressure level (SPL), with an optimum response at 50 dB SPL. The latency of the response increases with decreasing SPL. The response pattern of the neuron is, however, dependent on the frequency of the stimulus (Fig. 3B) and changes from a phasic-on/off response below its best frequency to a phasic-on response with increasing inhibition for frequencies above the best frequency. Frequency-dependent response pattern changes are common in cortical neurons. The frequencies between the resting frequency of 76 and 80 kHz will be called 'RF' frequencies and are relevant for the processing of the constant-frequency portion of the echolocation call.

The tonic response of an 'RF' neuron (best frequency 76.75 kHz) in the primary field is exemplified in Figure 4 together with responses to other stimulus types. The tonic pure-tone activity is almost completely suppressed by superimposing narrow band noise to the pure tone or by sinusoidal frequency modulation at higher modulation depth (1 kHz). Sinusoidal frequency modulations with low modulation depth (250 Hz), however, evoke correlated responses. Sinusoidal amplitude modulation induces some synchronization of the response to the modulation cycles. The suppression of the response by narrow-band signals is a feature often found in neurons with best frequencies near the reference frequency, whereas their response properties to modulations are more varied.

Frequencies between 45 and 75.5 kHz have been labelled 'FM' frequencies as they cover the frequency-modulated portion of the echolocation call. Their response properties can be divided into two principle classes: neurons that showed a preference to pure-tone stimuli and neurons that were also activated by modulated stimuli. The 'FM' neuron in Figure 5 displays strong tonic response to pure tones, whereas the activity to frequency-modulated stimuli (SFM, LFM) is reduced at high modulation depth.

Various features of low-frequency neurons (10–45 kHz, 'LO') are exemplified in Figure 6. The activity is a vigorous phasic-on response to pure tones at the two best frequencies of this double-tuned neuron, which are not harmonically related. The response to 21.3 kHz is non-monotonic with a best amplitude at 40 dB SPL. Sinusoidal frequency modulation enhances the response and results in a longer duration of activity without synchronization of the response. Modulations of the amplitude reduce the responses significantly. If linear frequency transitions are added to the beginning and end of the pure tone, the neuron reacts to both modulations with a vigorous response. Addition of noise with narrow bandwidth to the linear modulations diminishes the responses considerably.

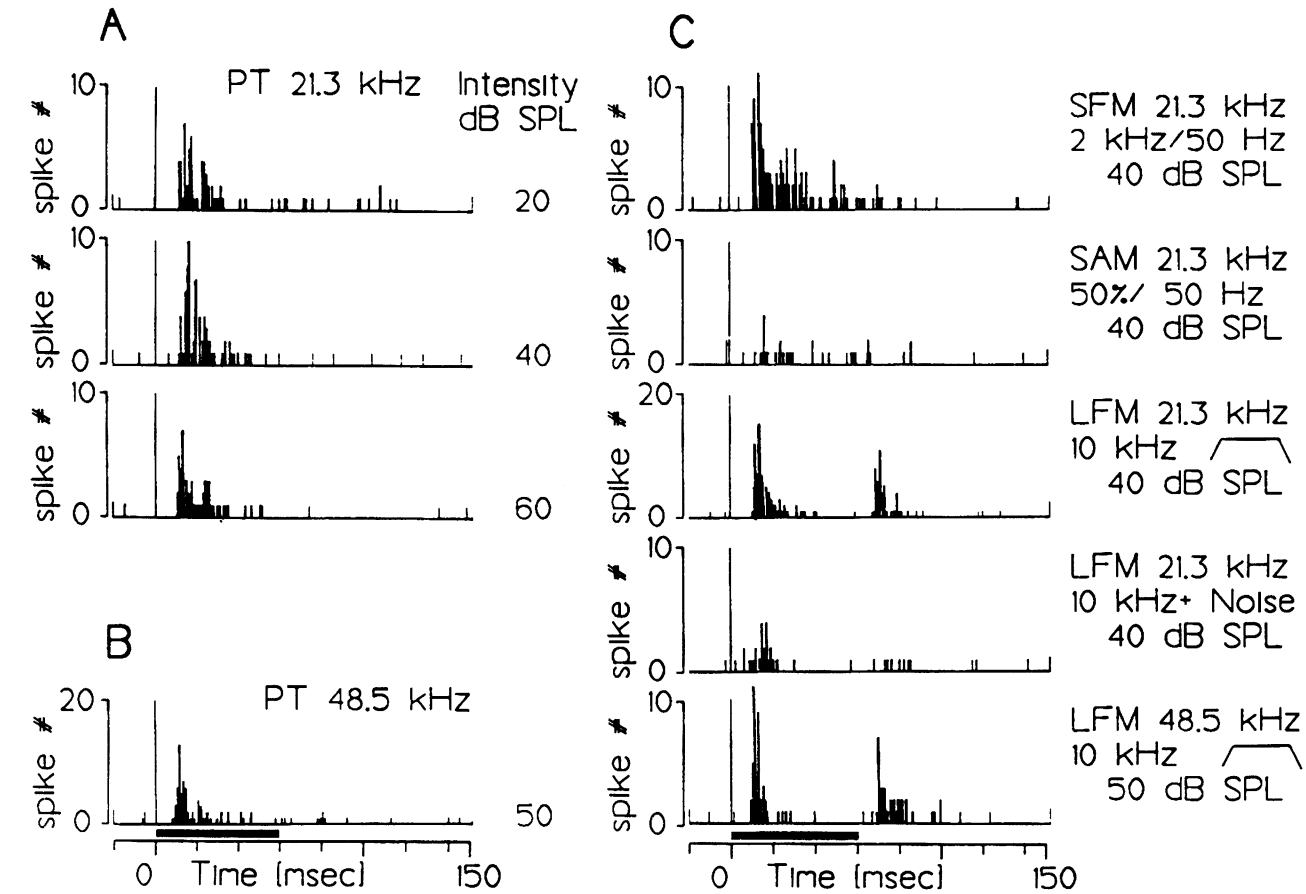


FIG. 6. Low-frequency neuron [double-tuned: best frequency 1 = 21.3 kHz (A) and best frequency 2 = 48.5 kHz (B)]. SFM and LFM enhance the response (rows 1, 3 and 5 in C), whereas SAM and addition of noise reduce the response activity (2nd and 4th rows in C). The two characteristic frequencies are not harmonically related. Abbreviations and labelling as in previous figures. Stimulus repetitions 32, bin width 1 ms.

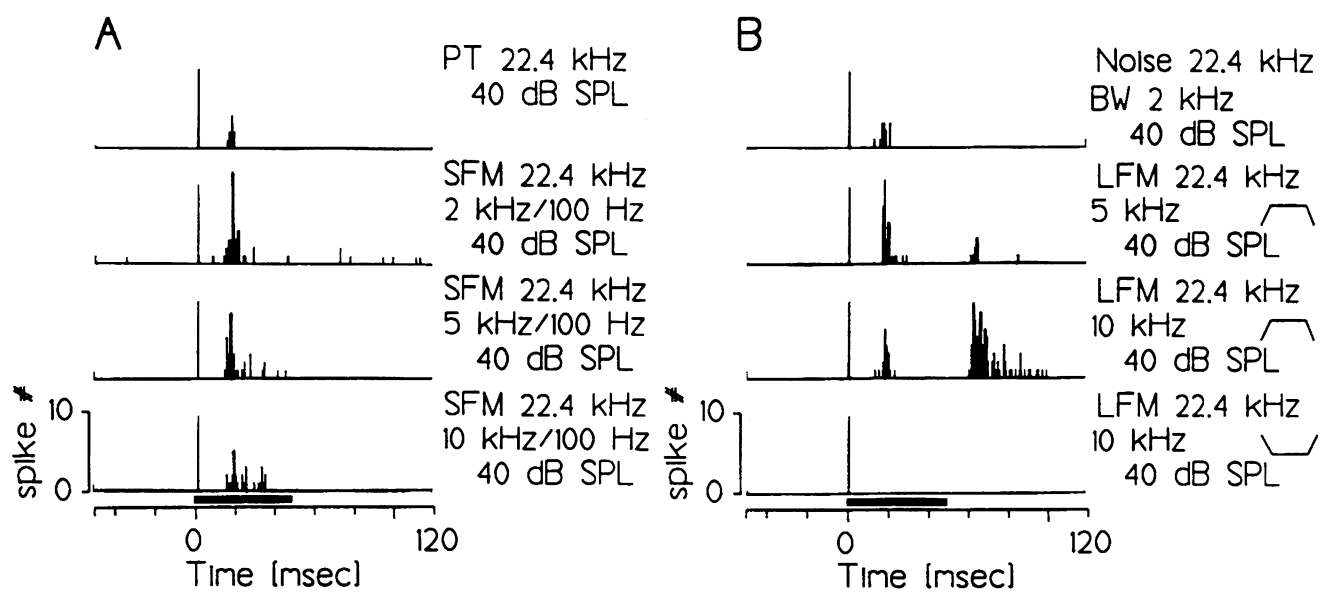


FIG. 7. Low-frequency neuron responding preferentially to frequency-modulated stimuli. Responses to SFM (A) and LFM (B) are dependent on depth and/or direction of the modulation. Depths of 2 and 5 kHz in SFM (A, 2nd and 3rd rows) were answered by a phasic-on response, whereas the 10 kHz modulation yielded less activity with a slightly different pattern (4th row). Responses to upward linear frequency modulations decreased with increasing depth, whereas the responses to the falling slope increased (B, 2nd and 3rd rows). The modulation pattern inverted relative to the starting frequency does not activate the neuron (B, 4th row). Abbreviations and labelling as in previous figures. Stimulus repetitions 32, bin width 1 ms.

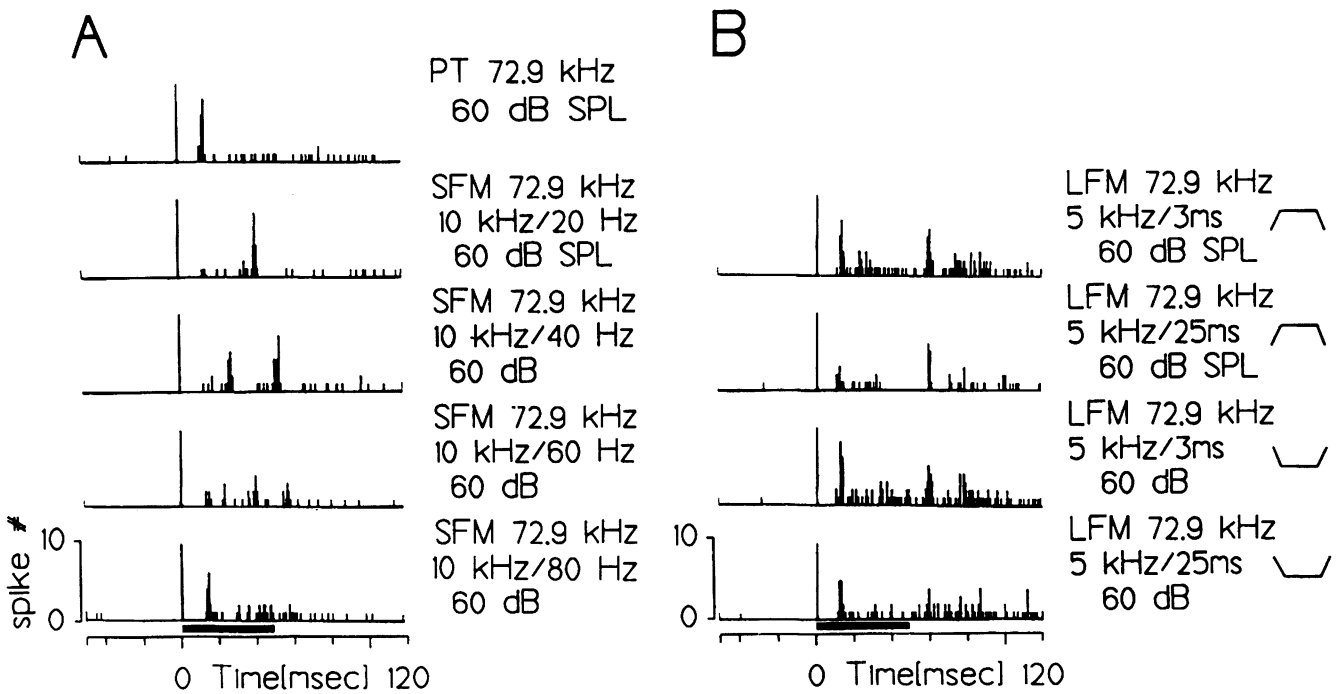


FIG. 8. 'FM' neuron synchronously activated by sinusoidal frequency modulation (SFM) at low modulation frequencies (<80 Hz) (A) and by LFM (B). The response to the upward-modulated components in the normal and reversed modulation pattern is slope-dependent (5 kHz within 3 or 25 ms), whereas the downward-modulated component provokes relatively stable response peaks. Abbreviations and labelling as in previous figures. Stimulus repetitions 32, bin width 1 ms.

Complex stimuli

Responses to frequency-modulated stimuli. Frequency modulations proved to be very effective stimuli for many neurons and evoked differentiated response patterns as a function of modulation depth and slope and location of the neurons. In many cases pure tones had little effect compared to sinusoidal or linear frequency modulations (Fig. 7). The depth of frequency modulation determined the strength and the pattern of the response. The spike pattern elicited by LFM depended on the modulation span or slope and on the direction of frequency transition, as illustrated in Figure 7B. Only modulations crossing the frequency band above the best frequency were effective.

Synchronized responses to the SFM cycles and differentiated responses to FM parameters are illustrated in Figures 8 and 9. The neuron in Figure 8 displayed synchronized response patterns only for low modulation frequencies, whereas at a modulation frequency of 80 Hz the evoked activity was similar to a pure-tone response with some increase of the tonic component. The activity to LFM stimuli is dependent on the direction and the slope of the modulation. The neuron in Figure 9 is tuned to higher modulation rates and synchronizes up to 140 Hz with well correlated response peaks, whereas the pure tone itself is not very effective in eliciting responses. The frequency transition of the LFM is also more efficient in eliciting responses than the pure tone and the activity is dependent both on the modulation depth or slope and on the direction of the sweep.

Some neurons encoded the modulation frequency of SFM stimuli by the total spike number. The total spike number of the neuron in Figure 10 peaked at a modulation frequency around 75 Hz and decreased to lower and higher modulation frequencies. The strong tonic unsynchronized response to SFM differed markedly from the needle-like phasic-on response to pure tones (uppermost histogram). The latency to SFM stimuli exceeded that for pure tones by 18–26 ms. As the neuron's best frequency is in the 'RF' class, it can contribute to the analysis of modulations superimposed to the constant frequency portion of the echolocation call.

Responses to narrow-bandwidth noise. The three main effects of narrow-bandwidth noise on the activity of cortical neurons were complete inhibition of the response, increase of response activity compared to the pure-tone response at the carrier frequency and changes of the response pattern.

Neural responses in Figure 11 illustrate how narrow-band noise abolishes the phasic response elicited by pure tones (Fig. 11A), how the response activity changes with different noise bandwidths (Fig. 11B) and how the response pattern is altered upon the superposition of a small-band noise signal (Fig. 11C). In many noise-sensitive neurons inhibitory effects are apparent as reduced activity, periods of inhibition or rebound activity.

Responses to spontaneous vocalizations. As recordings were performed under semichronic conditions in awake animals, the bats often uttered vocalizations spontaneously or during acoustical stimulation. Among the neurons activated during vocalization, two classes could be distinguished: (i) neurons that also responded to acoustical stimulation, and (ii) neurons that could not be activated by artificial acoustic stimuli. Vocalization-driven neurons are shown in Figure 12. The neurons in Figure 12A and B display responses which are more consistent to the end of the vocalization than to the beginning. The neurons did not show any well correlated response to pure tones.

The neurons represented in Figure 12C and D respond to vocalizations and to pure-tone stimulation. The neuron in panel C is well activated at the resting frequency of 76 kHz and displays a tonic response pattern. At higher sound pressure levels (80 dB) a burst-

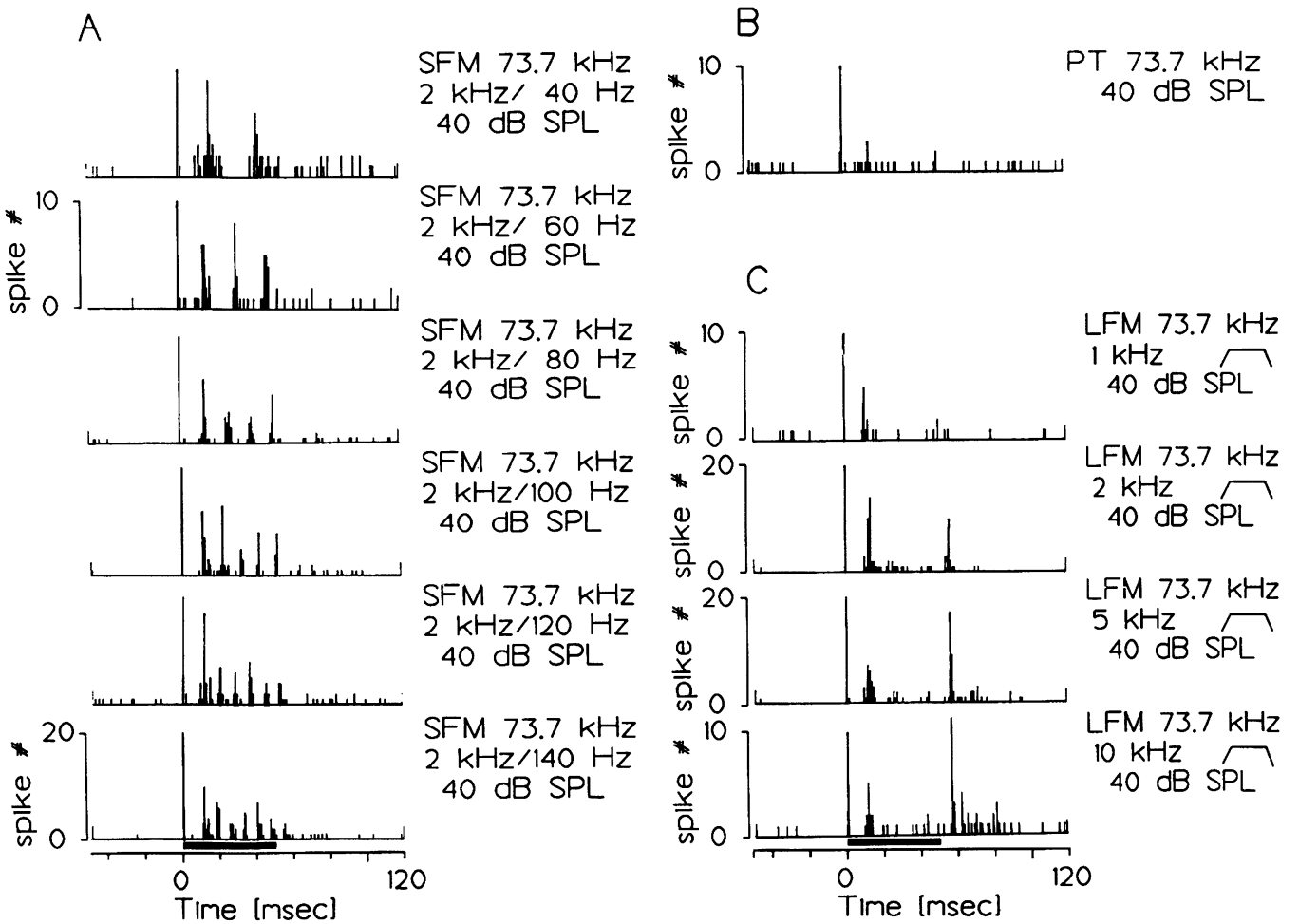


FIG. 9. 'FM' neuron with synchronized responses to SFM stimuli up to modulation frequency of 140 Hz (A). Pure tones evoke very poor spike discharges (B). The activity elicited by LFM is slope- and direction-sensitive (C). The best upward modulation is a 2 kHz sweep within 3 ms, whereas the downward sweep is most effective at the highest modulation depth and a slope of 10 kHz within 3 ms. Abbreviations and labelling as in previous figures. Stimulus repetitions 32, bin width 1 ms.

like response pattern appears during repetitive stimulation. The activity elicited by vocalizations is more consistent and exhibits a pronounced phasic component. Playback of the vocalization at different delays does not influence the neuron's response to vocalization.

The neuron depicted in Figure 12D is activated by stimuli around the resting frequency and shows a slightly scattered tonic response pattern. The vocalizations elicit phasic responses to the start of the vocalization and some sporadic responses to the end. When the vocalization is played back to the bat with a 30 ms delay, the activity to the end of the vocalization gets more consistent. The playback signal itself induces an off-activity that shifts in time with the termination of the playback vocalization. The playback response for a 50 ms delay is less consistent, but induces responses after the end of vocalization that are not present without the playback. Usually, the stability of recordings is strongly impaired when the bat is vocalizing and therefore the sample of well studied vocalization-activated neurons is limited.

Physiological properties and topographical distribution

Several physiological properties of auditory neurons in the cortex showed distinct topographical arrangements. These could be manifested in topographical gradients of a parameter or in the preferential representation of one parameter in a distinct cortical region. Topographical arrangements could be found for the best frequency of neurons, for tuning characteristics, preferences for distinct stimulus types and for neurons responding to vocalizations of the bat.

Tonotopic distribution of best frequencies

The cortical fields as defined neuroanatomically differed substantially in their frequency representation. In turn the presence of topographical arrangements of best frequencies helped in the delineation of certain cortical fields. Figure 13B shows the distribution of neurons classified after frequency ranges ('LO', 'FM', 'RF', 'HI') in a representation of the flattened cortical surface. The clearest tonotopy was found in the primary auditory field, with increasing best frequencies from caudal to rostral levels. The position of the major tonotopically organized area is given schematically in Figure 13A. The proportion of cortical area per kHz occupied by resting frequencies ('RF'), frequency-modulated frequencies ('FM') and low frequencies ('LO') is 12:2:1 for the tonotopically organized area and quantifies the important over-representation of the frequencies at and above the resting frequency.

A less distinctive topographical trend of frequencies is found in the caudal part of the posterior dorsal field next to the caudal primary

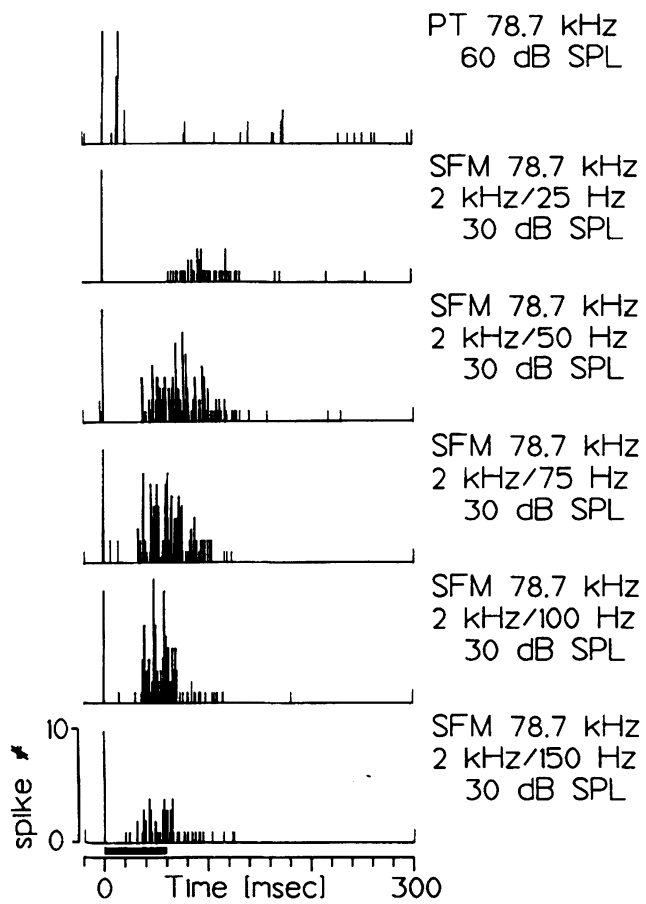


FIG. 10. Neuron in the 'RF' range showing dissimilar response patterns to pure tone (PT) and sinusoidally frequency-modulated (SFM) stimuli. The duration and the latency of the tonic responses to SFM change with increasing modulation frequency and the responses outlast the stimulus duration by several tens of milliseconds. The latencies of SFM responses (40–32 ms for modulation frequencies above 25 Hz) are considerably longer than for pure tones (14 ms) (best frequency 78.7 kHz). Abbreviations and labelling as in previous figures. Stimulus repetitions 32, bin width 1 ms.

area. Most apparent is the topographical transition between 'FM' and 'LO' frequencies. Few neurons with best frequencies in the resting frequency range were found in this field.

An additional area exhibiting tonotopic organization, although not as clear as in the primary auditory area, is the anterior dorsal field close to the high-frequency region of the primary auditory cortex. Figure 14A delineates the area in an overall view of the flattened cortical surface and shows the spatial distribution of best frequencies with different symbols for the three major frequency classes. The enlarged view of the area (Fig. 14B) contains the local focuses for each frequency class indicated at five equidistant slices. At the dorsal transition from the 'RF' range to the 'FM' range and generally in the 'FM' and 'LO' range, the overlap of frequency classes was important. However, the tonotopic gradient is clear over a rostrocaudal distance of 700 μm (slices 133–165) and extends from a medial position of 1200 to 2400 μm more laterally. The tonotopically organized area fits well into the cytoarchitectonic boundaries. The ratio of cortical surface per kHz occupied by the different frequency classes (13.8:1.6:1 for 'RF':'FM':'LO') indicates the distinct over-representation of neurons tuned to the 'RF' frequency range also in the anterior dorsal field. It is important to note that there is no discontinuity of best frequencies between the 'RF' region in the primary field and the 'RF' area of the dorsally adjacent tonotopic area, and the gradient of the topographical order reverses its direction there.

Topography of double-tuned neurons

Whereas the majority of neurons showed tuning curves with a single threshold minimum, 134 neurons exhibited two tuning peaks. Double-tuned neurons were rarely found in the primary acoustic field (four neurons), but occurred throughout the dorsal cortical fields (Fig. 15A) without showing focal concentrations. The relationship between the two best frequencies is plotted in Figure 15B. The low best frequency (abscissa) of 90% of the double-tuned neurons was between 27 and 45 kHz; the upper best frequency (ordinate) of 95% of the neurons was between 62 and 80 kHz. Neurons that showed a harmonic relationship of the two best frequencies cluster around the diagonal line and are relatively few. The best frequencies of the nearly harmonically related double-tuned neurons most often lie in the frequency band of the frequency-modulated portion of the echolocation call (30–35 and 60–70 kHz respectively). Sixty-eight percent of the neurons are located above the diagonal line, indicating that the lower best frequency is lower than a harmonic relationship would require.

Tuning properties

The best frequency was determined in 642 neurons, of which 203 were tested in more detail by establishing a complete frequency tuning curve (90 neurons) or by determining the Q values for 10 or 40 dB above absolute threshold. Generally, tuning properties were measured in neurons that exhibited stable and consistent responses over the recording time. Tuning curves could often not be evaluated in neurons that had very complicated and variable response characteristics. In 35 neurons that preferentially responded to SFM stimuli, the tuning properties were determined with a modulation depth ≤ 1 kHz and the best carrier frequency was taken as the tuning frequency.

The width of tuning was typically dependent on the best frequency of the neurons, and the form of the tuning curves could vary widely from a normal V-shaped tuning curve to a closed or obliquely oriented curve.

Figure 16A gives the dependence of the Q_{10dB} values on the best frequency of the neurons. The graph shows a pronounced peak of extremely high Q_{10dB} values at and within a few kHz above the resting frequency (76 kHz, normalized) of the bats. A second, but lower, peak is formed by the Q_{10dB} values at frequencies between 70 kHz and the RF covering the upper half of the final FM sweep in the echolocation call. The peaks of the quality factor are clearly separated for the RF frequencies and the FM frequencies by a minimum of between 74.5 and 75.5 kHz (Fig. 16B). Only a very few neurons were found at these best frequencies, and the Q values were lower than those at adjacent frequencies.

Neurons with best frequencies below 70 kHz had Q_{10dB} values rarely exceeding 20, which is the maximum Q_{10dB} value commonly found in other mammals.

The Q_{10dB} values of neurons that predominantly reacted to SFM stimuli showed a similar distribution along the best frequency axis (Fig. 16C), although the tuning was generally broader, i.e. the Q_{10dB} values were smaller than for pure tones.

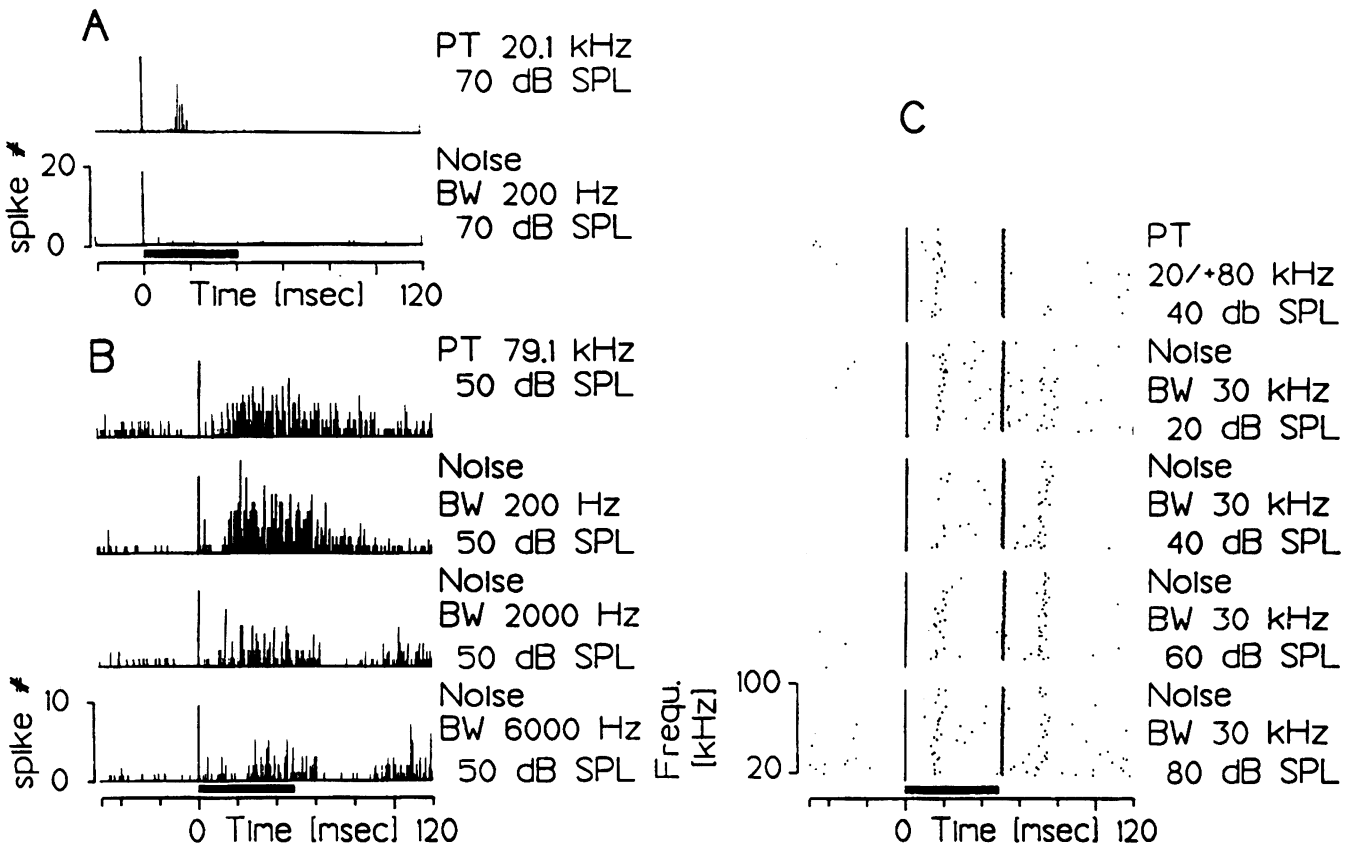


FIG. 11. Neural responses influenced by band-limited noise stimuli. (A) Neuron briskly activated by pure tone (PT) but completely silenced by a superimposed ±100 Hz narrow-band noise signal. (B) In another neuron a very small bandwidth of noise (±100 Hz) raises spike activity compared to pure tones, but leads to a reduction in tonic activity with subsequent inhibition for higher bandwidth of ±1 and ±3 kHz. In the third example (C) the spike raster displays show that noise of a bandwidth of ±15 kHz induces an additional off-response compared to the response to a pure tone (vertical axis represents the stepwise raised frequency between 20 and 100 kHz). The neuron is extremely broadly tuned and is activated within the entire 80 kHz broad frequency band. Abbreviations and labelling as in previous figures. Stimulus repetitions 32, bin width 1 ms.

The properties of the tuning curves and the local distribution of Q_{10dB} values on the cortical surface were specific for different classes of best frequencies.

Low-frequency neurons ('LO') processing the lower harmonic component of the echolocation calls had Q_{10dB} values below 20 except for three of 47 neurons. The sample of neurons represented here comprises only non-facilitated neurons, which responded to presentation of the low-frequency signal alone. The tuning curves ranged from V-shaped to oblique or closed forms, but no further classification of attributes could be made (Fig. 17A).

Neurons in the 'FM' frequency range with best frequencies <70 kHz had Q_{10dB} values smaller than 20, whereas Q_{10dB} values higher than 20 (up to 240) were found only for best frequencies of >70 kHz.

Tuning curves of low-Q neurons (Fig. 18A) had patterns very similar to those seen in 'LO' neurons. Most of the neurons with high Q_{10dB} values had V-shaped tuning characteristics and only in rare cases the tuning curves were oblique or closed (Fig. 19A). In a number of neurons with high Q_{10dB} values the tuning curve widened considerably at higher sound pressure levels (Fig. 19B).

Most neurons with best frequencies at and a few kHz above the resting frequency (75.5–80 kHz, 'RF') had Q_{10dB} values of >20 and reached maximum values of 400.

The tuning curves were either very narrow, V-shaped and open at high sound pressure levels (Fig. 20A) or were relatively broad and showed more complex outlines, as demonstrated in Figure 20B.

Within the group of best frequencies >80 kHz ('HI') only one of eight neurons had a Q_{10dB} value exceeding 20. The tuning curve pattern was comparable to that of other low-Q neurons.

Topography of Q_{10dB} values

Only neurons between 70 and 80 kHz, i.e. within the upper frequency-modulated band (i.e. the 'FM' band) and in the resting frequency band ('RF') had Q_{10dB} values >20. The locations of neurons with Q_{10dB} values >20 are shown in Figures 19C and 20C. Sharply tuned neurons with 'RF' frequencies were predominantly found in the rostral primary auditory cortex and the anterior dorsal field (Fig. 20C). The second aggregation of high-Q neurons had best frequencies in the upper 'FM' band and is situated in the posterior dorsal field (Fig. 19C). Surprisingly, the primary auditory field itself includes almost no 'FM' neurons with Q_{10dB} values >20. Some neurons with

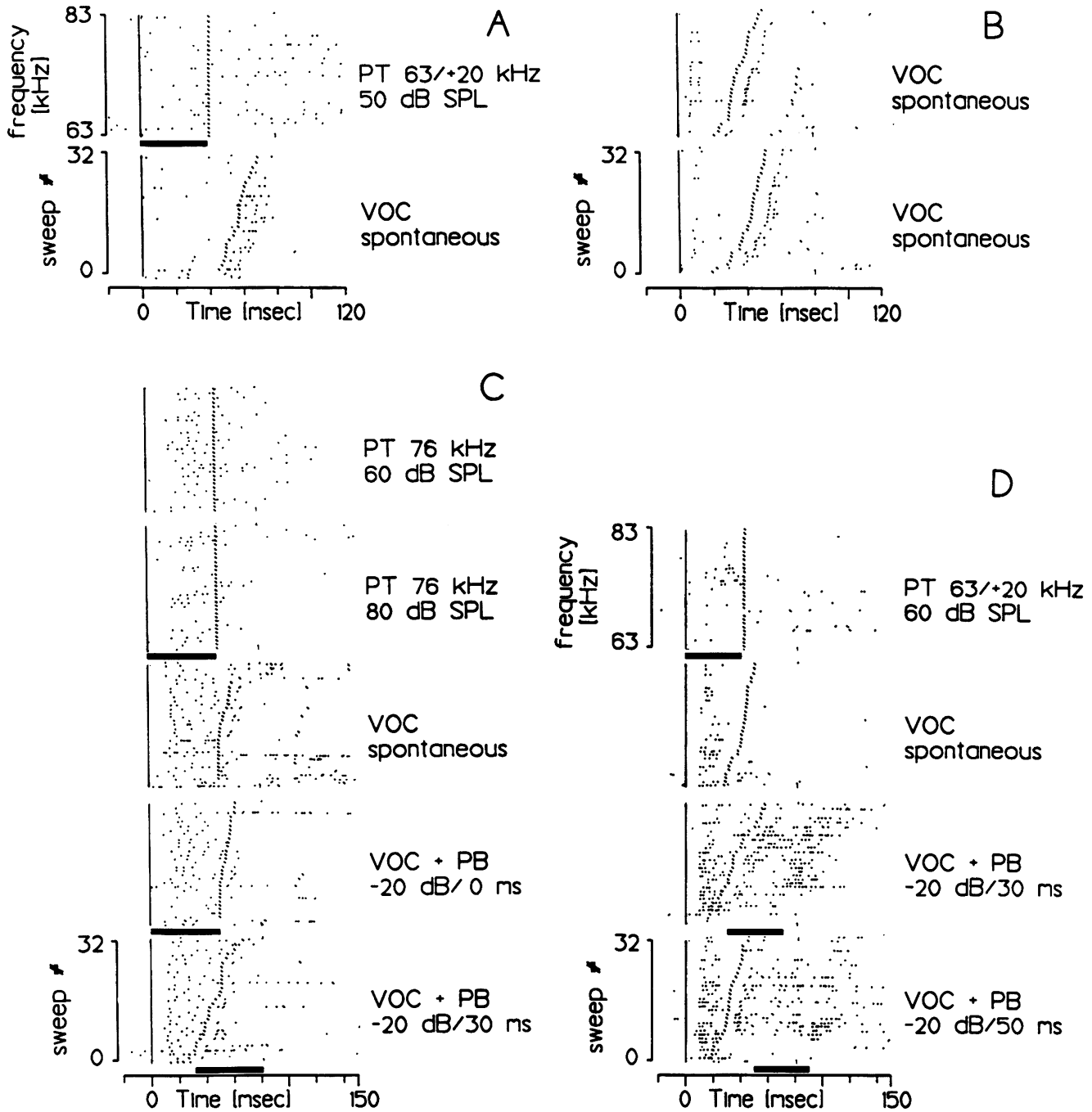


FIG. 12. Neurons activated by vocalizations (VOC). The two examples in A and B show neurons that only responded during spontaneous vocalization and could not be stimulated by artificial acoustic signals simulating vocalizations. The neurons represented in C and D were activated by vocalizations and by artificial stimuli or playback of the vocalization (PB). Each dot display shows the spike trains to 32 vocalizations or stimulus sweeps. The start of vocalizations coincides with the vertical line at time zero, whereas the end occurs after variable duration and is tagged by a backslash. The vocalizations have been sorted according to duration (bottom, low duration; top, longest duration). Artificial stimuli are indicated by bars. The vertical scale is either the number of sweeps or the frequency (from 63 to 83 kHz). The level of the playback was −20 dB with respect to the level of the emitted vocalization.

Q10dB values >20 were also found in the most dorsal part of the dorsal field (FM/FM field). In this sample only neurons activated by single stimuli were considered. However, the combination-sensitive neurons mostly showed high Q_{10dB} values for the high-frequency component of the effective stimulus pair (Schuller *et al.*, 1991).

Rate-intensity functions

The large majority (65%) of the neurons recorded in the auditory cortex had non-monotonic rate-intensity functions, with a best sound pressure level and declining spike rates for high levels. About one-third (35%) of the units displayed monotonic characteristics up to

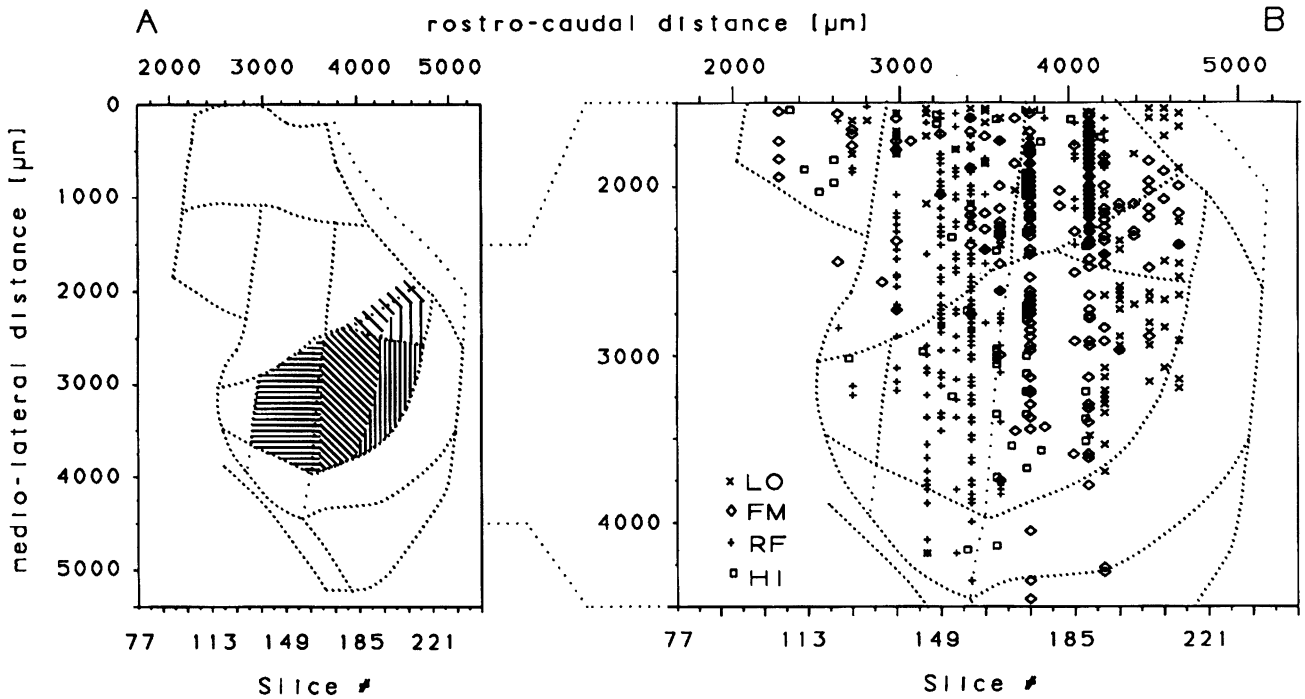


FIG. 13. Tonotopic arrangement of neurons in the primary auditory field and correspondence to cytoarchitectonic borders. Panel B shows the position of neurons and their best frequencies classified as 'LO', 'FM', 'RF' and 'HI'. Within the primary auditory field the neurons show a clear tonotopic arrangement with low frequencies at caudal and high frequencies at rostral locations. The hatching in A gives a simplified representation of the different frequency ranges within the cytoarchitectonic borders. ('LO', vertical; 'FM', diagonal; 'RF', horizontal hatching). Light hatching in dorsocaudal locations indicates a separate field with a tonotopic gradient. The strong over-representation of the narrow 'RF' frequency range is evident.

levels of typically 100 dB SPL. Monotonic neurons were found in the primary auditory field (3267–4323 µm) and the adjacent dorsal fields (anterior dorsal and posterior dorsal fields) (64%), and scattered throughout the dorsal dorsal field at the same rostrocaudal levels (36%). The relationship of monotonic to non-monotonic units in the primary auditory cortex was ~0.8.

Latency distribution

It is not possible to give a distinctive picture of latency distribution within the cortical fields, and only trends can be described. Short latencies up to 10 ms were found in 18% of the neurons. The large majority (47%) had latencies between 10 and 15 ms, 32% had latencies between 15 and 30 ms, and only 3% had latencies >30 ms. The shortest latencies (<10 ms) were found in the high-frequency portion of the primary auditory field and the dorsally adjacent dorsal fields (anterior dorsal fields, rostral dorsal field and anterior part of dorsal dorsal field), and in the dorsal part of the primary auditory field containing 'FM' frequencies. Neurons with latencies >10 ms were recorded in virtually all areas, with a trend of increasing latency to more dorsal or more caudal positions within individual fields.

Topography of vocalization-sensitive units

Neurons that responded consistently during spontaneous vocalizations, or that exhibited modified activity to acoustic stimuli when concurrent vocalization occurred, were concentrated in the tonotopically organized anterior dorsal field (Fig. 21). Fewer vocalization-sensitive neurons were located in the posterior dorsal field and in the dorsal part of the dorsal field. Neurons in the latter two locations were mainly influenced by vocalization when processing acoustic stimuli, for example by causing inhibition of the response to the artificial auditory stimuli, rather then responding to vocalizations themselves.

Topography of response patterns

Response patterns showed no distinct topography in the sense that specific patterns occurred in well defined cortical fields, but even so certain concentrations of response patterns or the almost complete absence of certain regions were apparent. Phasic-on responses and tonic responses were found in virtually all cortical fields without any striking concentration or lack in specific regions. Phasic-off responses on the other hand were not found in the dorsal dorsal field. Inhibitory responses were concentrated in the posterior dorsal field with the exception of a few (six) neurons in the high-frequency portion of the primary auditory field. The inhibitory response pattern distribution overlaps with the distribution of noise driven units, which in turn represent about half of all neurons that show inhibitory responses. Neurons exhibiting high spontaneous discharge rates were found in the anterior and posterior dorsal fields between slices 153 and 209. Spontaneously active neurons were rarely recorded in the primary auditory field, the dorsal dorsal field and the rostral dorsal field.

Topography of optimal stimulus types

Neurons preferring special stimulus types were commonly distributed over several fields and could often be better characterized by the fact that they were absent in certain cortical fields. Preference to pure-tone stimulation was found in the rostral dorsal field, where CF–CF-facilitated neurons are common (Schuller *et al.*, 1991) and in the

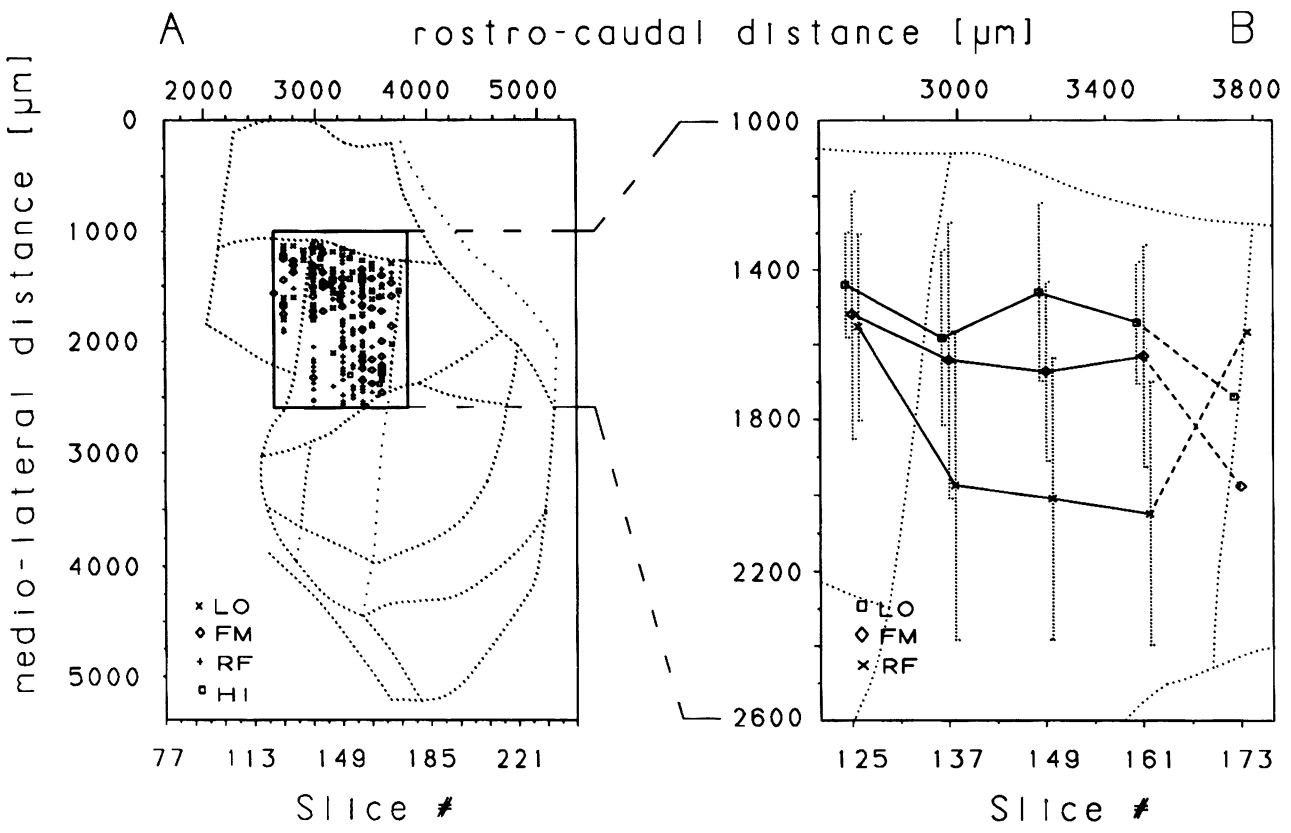


FIG. 14. Tonotopic arrangement of neurons in the anterior dorsal field bordering the primary auditory field. Graph A shows the position of neurons and their best frequencies classified as 'LO', 'FM' and 'RF' within the cytoarchitectonic boundaries dorsal to the primary auditory field. Panel B represents an enlarged subdivision of the anterior dorsal field showing the focal distribution of frequency classes. Each value was evaluated by calculating the mean mediolateral coordinate for the centre slice and its two neighbouring slices (88 µm apart) within the respective frequency class. The bars delimit the mediolateral range within which 68% of the neurons of the respective frequency class are located (standard deviation).

anterior dorsal cortical field. No such pure-tone preference was present in the primary auditory field and in the dorsal dorsal field. Neurons that responded preferentially to linear FM were exclusively recorded in the dorsal dorsal field along its full rostrocaudal extent, whereas SFM stimuli were ineffective in this area.

SFM as the optimal stimulus was found in neurons located in the primary auditory field and the adjacent dorsal fields (anterior and posterior dorsal fields).

Neurons preferring noise to all other tested stimulus forms were found in the dorsal fields bordering the primary area and at the extreme caudal edge of the primary auditory field.

Discussion

In this study we present neurophysiological data for a functional definition of the auditory cortex and its subdivisions in the insectivorous bat *R. rouxi*. The concurrent evaluation of neuroarchitectonic and connectional properties, together with the neurophysiological mapping of the acoustical cortex, considerably strengthens the definition of cortical subdivisions. The neuroarchitectonic and connectional data will be the subject of separate papers.

Insectivorous bats are highly acoustically oriented mammals in that they use ultrasonic echolocation for orienting in space and for capturing their food. The frequency range that is processed in the auditory system of bats ranges from <1 to 160 kHz. The spectral complexity of echoes impinging on bat's ears extends from pure-tone signals to noise-like broadband signals, covering a whole range of temporally structured signals (e.g. frequency and amplitude modulations). Characterization of neurons with complex signals relevant for echolocation might therefore improve the possibilities for cortical field classification. In bats this approach is widely used for the investigation of cortical as well as subcortical auditory regions, but it is also applied in other mammals (for review see Clarey *et al.*, 1992). The most elaborate investigations on cortical responses to complex acoustical signals have been done in the moustached bat, *P. p. parnellii*, by Suga and co-workers (for review see Suga, 1990).

Physiological properties and topographical distribution of cortical neurons

The frequency band used by the species tested in these experiments is especially small. The calls consist of a long constant-frequency portion at 76 kHz followed by a short frequency-modulated sweep decreasing by 16 kHz and a corresponding lower harmonic at 38 kHz. Comparing the distribution of best frequencies (Fig. 2) of cortical neurons with the spectrum of the calls demonstrates the close adaptation of the auditory system to the needs of echolocation in this species.

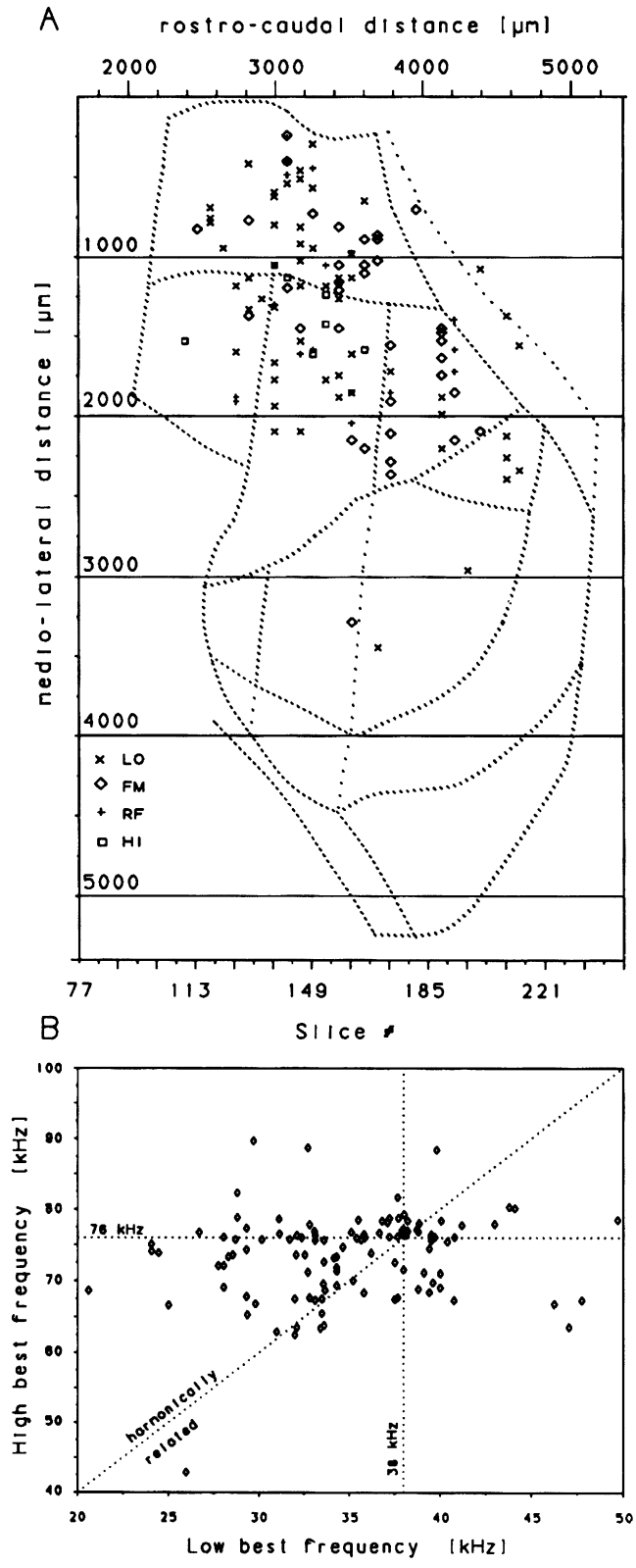


FIG. 15. Distribution of double-tuned neurons (A) and relationship of the two tuning frequencies (B). Double-tuned neurons are rarely encountered in the primary field, but are found throughout the dorsal fields. In the large majority of neurons the two tuning frequencies are not harmonically related. In most cases the frequency ratio is >2 (B; diagonal line corresponds to harmonic relationship).

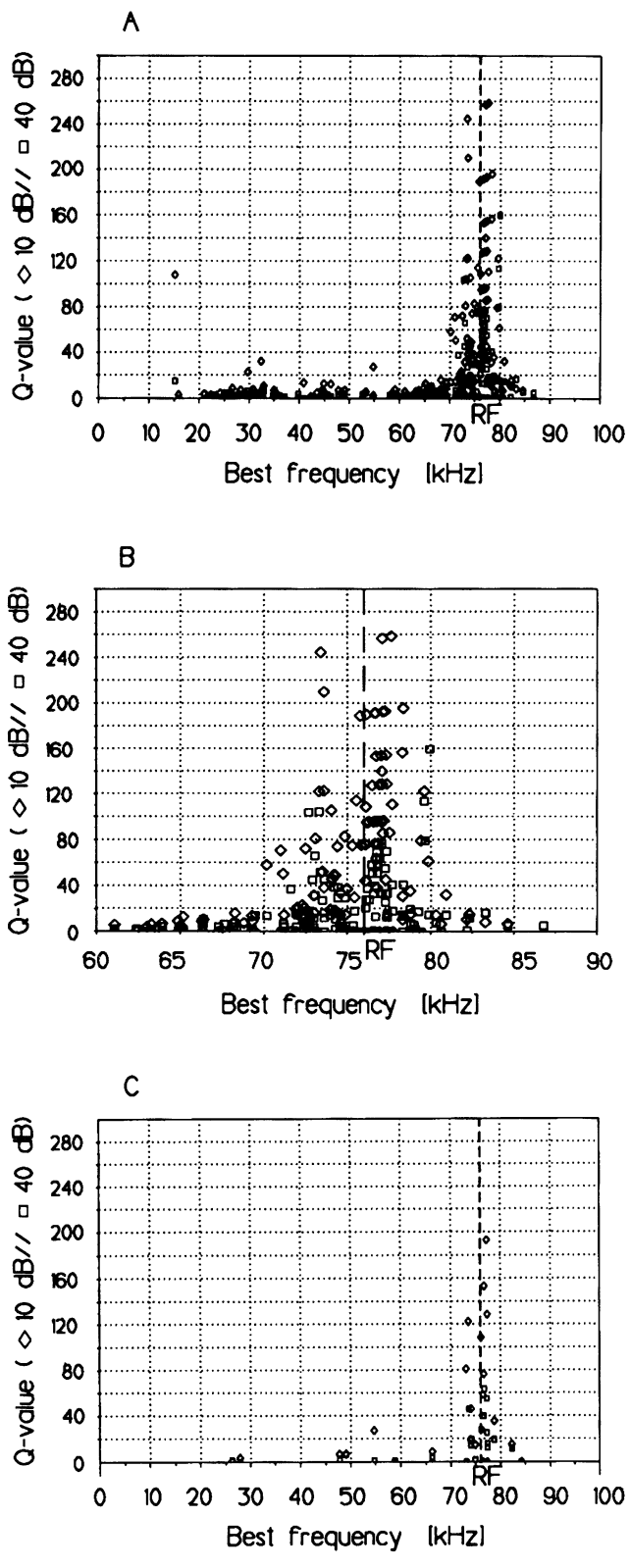
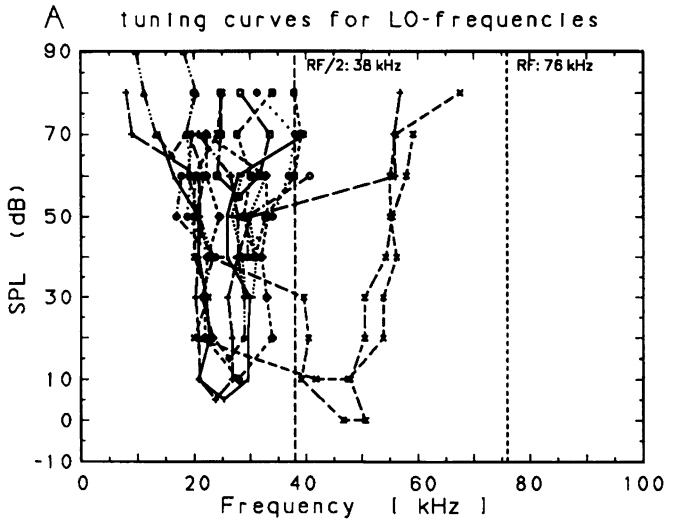


FIG. 16. Q values (10 and 40 dB above threshold) as a function of the best frequencies. (A) Neurons drivable by pure tones. (B) Expanded frequency range of 60–90 kHz (subset of A). (C) Neurons drivable by small sinusoidally frequency-modulated stimuli, but poorly by pure tones; dashed lines signify resting frequency (RF = 76 kHz). The highest Q values are found in neurons with best frequencies in the RF range.



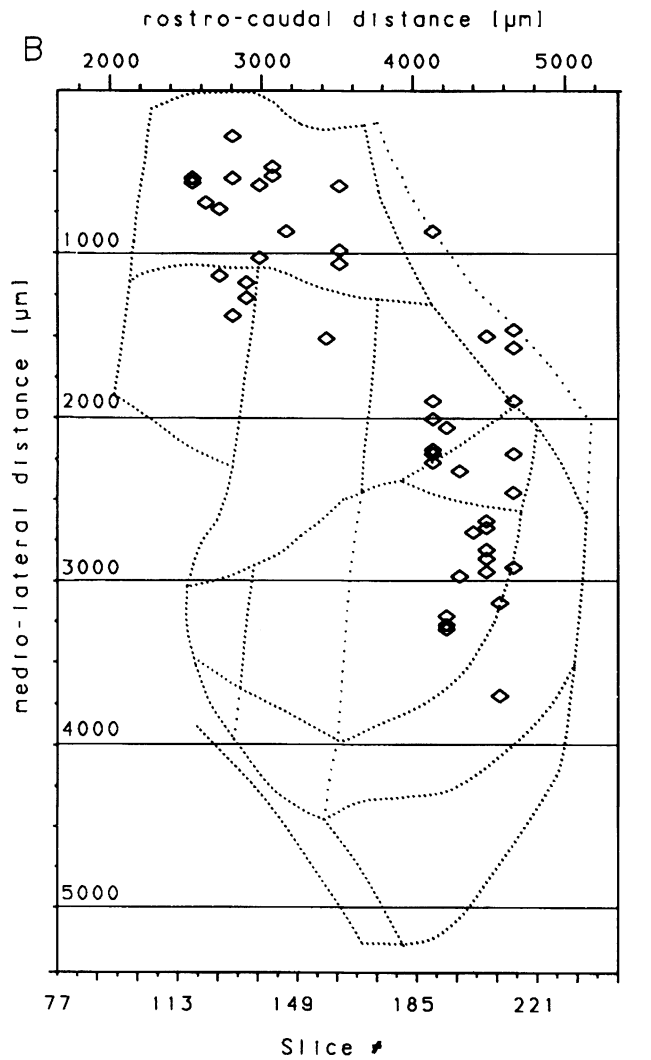
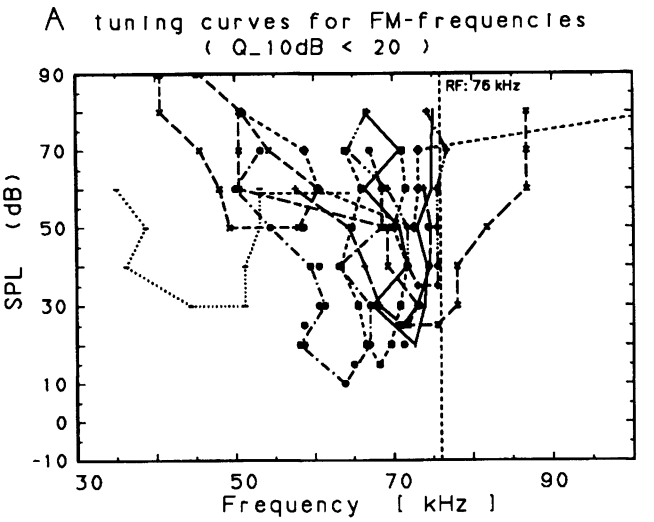


FIG. 17. Tuning properties and location of low-frequency ('LO') neurons. Panel A shows typical tuning curves; the map in B shows the location of the 'LO' neurons in which tuning parameters were evaluated. Parameters of the map are as in Figure 1.



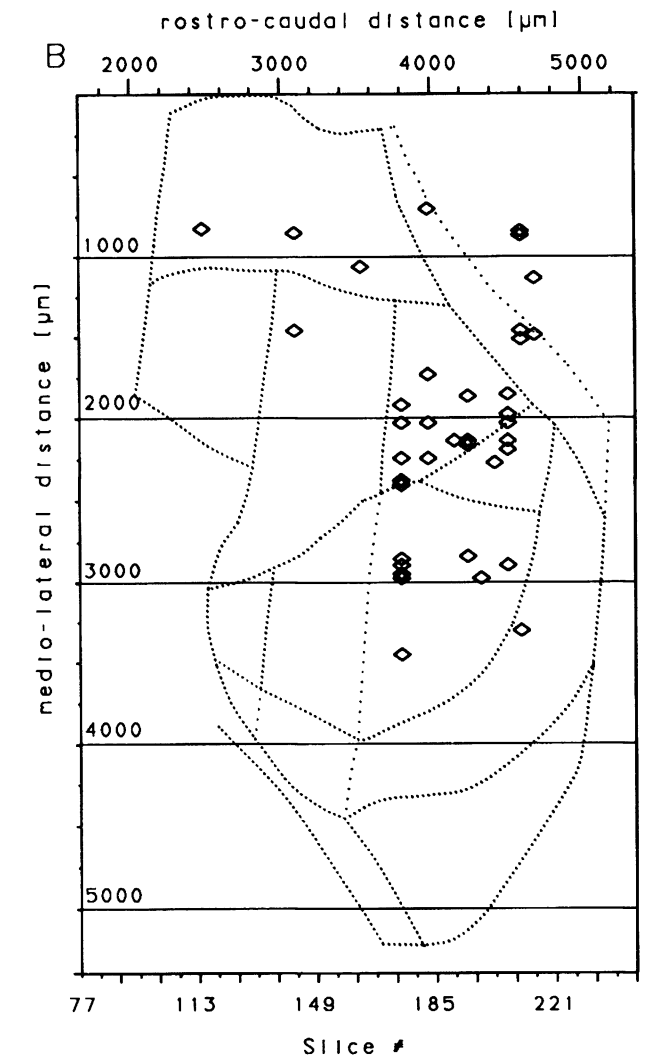


FIG. 18. Tuning properties and location of broadly tuned ($Q_{10dB} < 20$) neurons having best frequencies in the frequency-modulated ('FM') range. Panel A shows typical tuning curves; the map in B shows the location of such neurons. Parameters of the map are as in Figure 1.

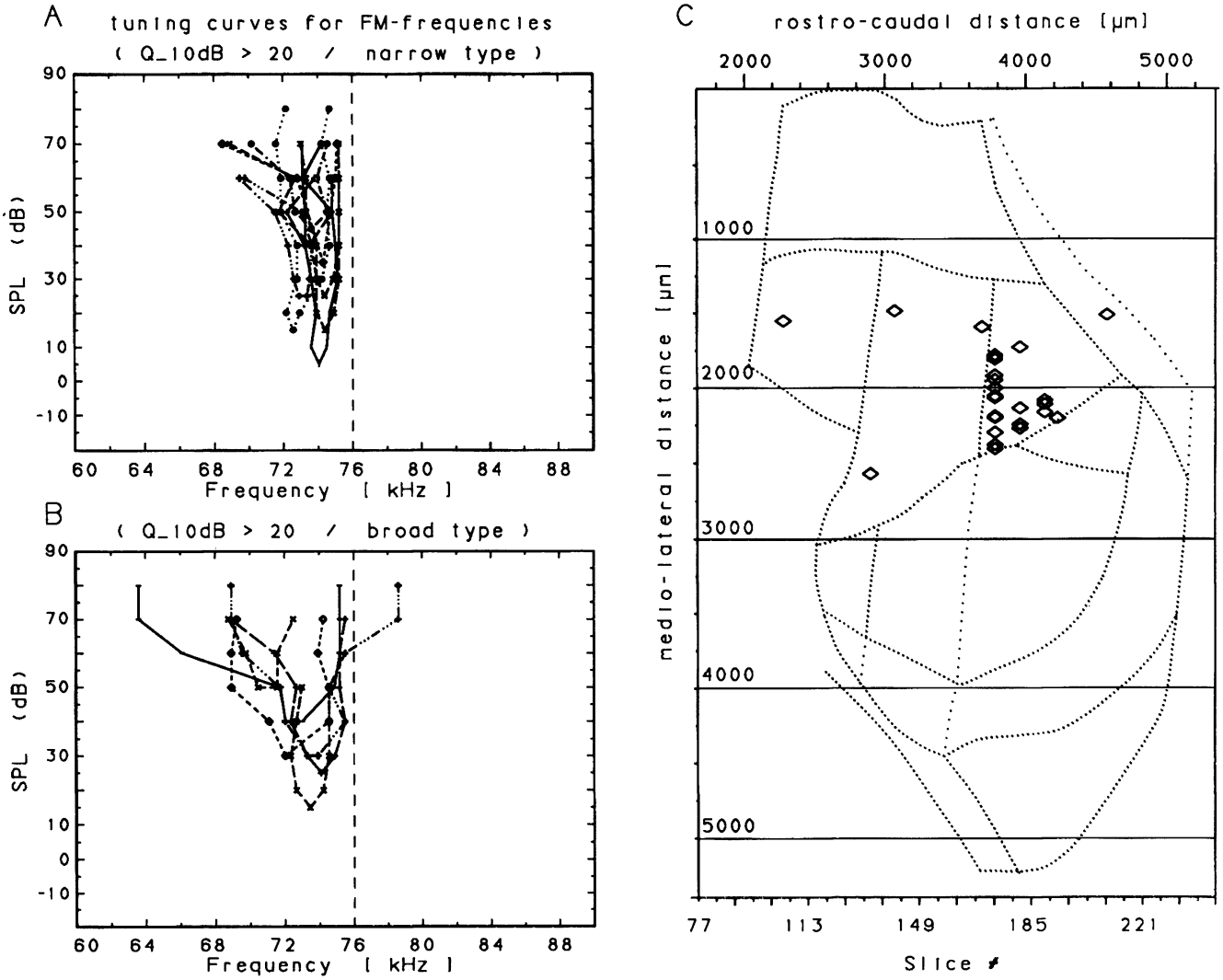


FIG. 19. Tuning properties and location of narrowly tuned ('FM') neurons. The graphs to the left show typical tuning curves of two types (A, tuning curves stay narrow over the intensity range; B, tuning curves widen with increasing intensity). The map in C shows the location of the neurons tuned to 'FM' frequencies with Q values >20. Parameters of the map are as in Figure 1.

Neurons with best frequencies in the range from several kHz below to a few kHz above 76 kHz, which perform the analysis of echo information superimposed on the constant-frequency portion and the FM sweep, are over-represented in number and neuronal space in the acoustic cortex. Neurons with best frequencies around the lower harmonic components ≤38 kHz form a second peak in the distribution. The echoes carry only very reduced energy at these low frequencies as the acoustical transmission loss is high, and neurons with best frequencies around 38 kHz are predominantly stimulated by the emitted vocalizations.

The unequal distribution of best frequencies in the acoustic cortex reflects the pattern found in virtually all subcortical structures of this species. A similar over-representation of specific frequency bands has been demonstrated in the neotropical moustached bat, *P. p. parnelli* (Suga and Jen, 1976; Asanuma *et al.*, 1983). The echolocation calls (CF/FM calls with several harmonics) and the echolocation behaviour (especially the Doppler shift compensation) of *P. p. parnellii* are very similar to those of *R. rouxi*, although these species are members of totally unrelated bat families. The over-representation of best frequencies around the resting frequency is predominantly found in the primary auditory field, the anterior auditory field and the specialized dorsal FM/FM field. In the moustached bat the over-representation of call frequencies has also been demonstrated in the DSCF (Doppler shift compensation frequency) area, the CF–CF facilitated area and the FM/FM fields, which are functionally comparable with the respective areas in *R. rouxi*. In the horseshoe bat the functional topography is supported by the neuroarchitectonically apparent boundaries, which are not available for the moustached bat.

It is remarkable that the over-representation of best frequencies within the CF or FM bands is more pronounced in cortical auditory fields than in subcortical structures. With increasing processing level within the auditory system the analysis of acoustical signals evidently narrows down to biologically relevant frequency ranges.

Tuning properties

The frequency-tuning curves in the horseshoe bat's auditory cortex show features similar to those of subcortical structures. Quality factors can reach values of up to 400 for best frequencies at and above the

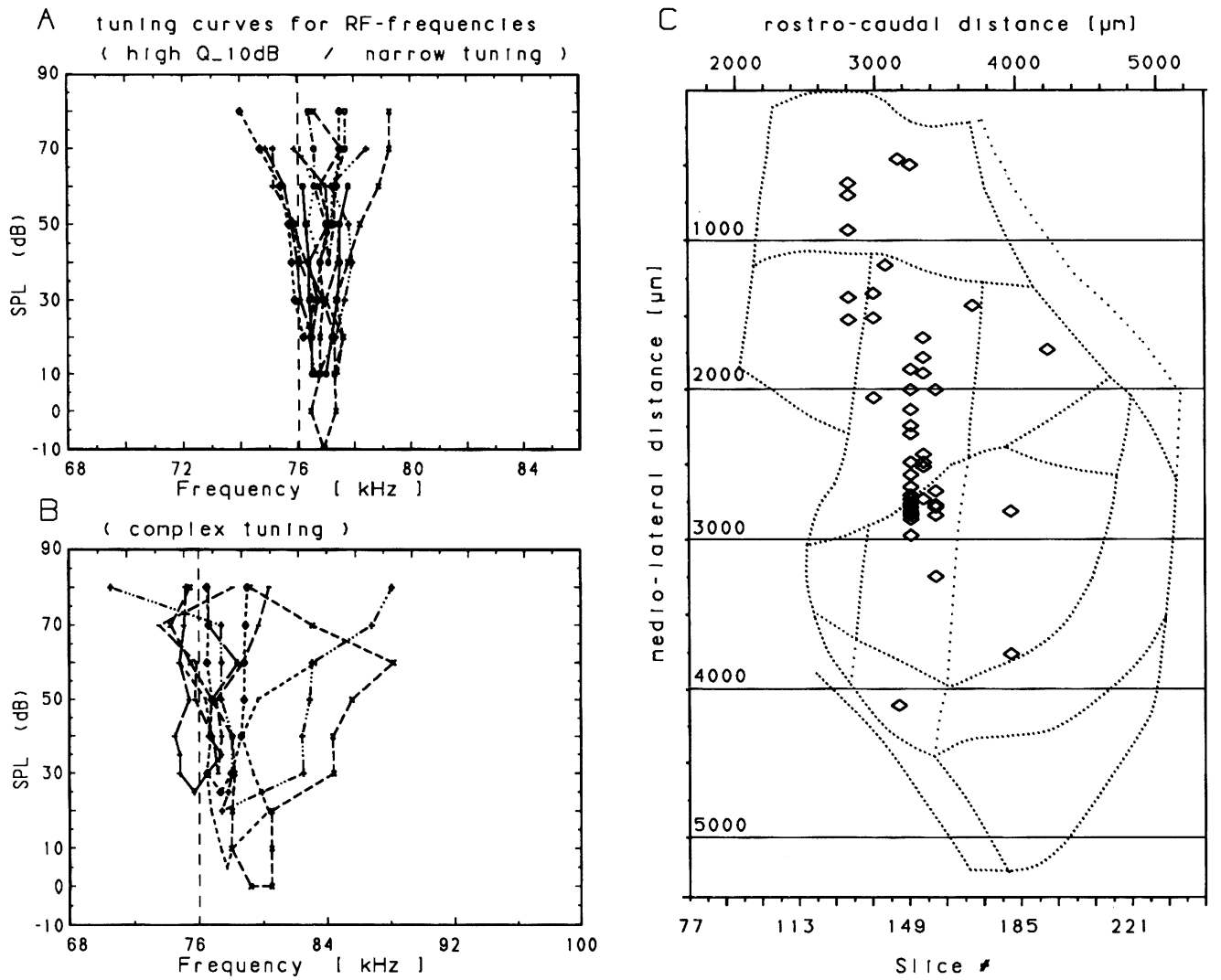


FIG. 20. Tuning properties and location of neurons with best frequencies at and above the resting frequency (RF). The graphs to the left show typical tuning curves of two types (A, tuning curves have typical V-shape; B, tuning curves have complex forms—closed, oblique or irregular). The map in C shows the location of the neurons tuned to 'RF' frequencies with Q values >20. Parameters of the map are as in Figure 1.

resting frequency, i.e. 20 times higher than the maximum Q_{10dB} value typically found for other mammals. In the moustached bat the tuning properties of auditory cortical neurons also show pronounced peaks of the Q_{10dB} values up to 500 for the strongest harmonic component of the echolocation calls at 61 kHz (Suga and Manabe, 1982; Asanuma *et al.*, 1983). The extreme sharpness of tuning can be interpreted as a consequence of peripheral fine tuning processes in this frequency band (e.g. Bruns, 1976 for *R. rouxi*), but is further accentuated by inhibitory processes at neighbouring frequencies. Inhibitory mechanisms are certainly active in those sharply tuned neurons that are muted by a slight broadening of the frequency band beyond the tuning limits, for example by the presentation of a narrow band noise or frequency-modulated stimulus.

Quality factors of >20 are also found between 70 and 75 kHz, which corresponds to the upper portion of the frequency-modulated sweep in the echolocation call. Neurons with best frequencies of <70 kHz (down to 61 kHz), matching the lower portion of the FM sweep, are not very sharply tuned. The sharp tuning of neurons processing an FM sweep translates into a high temporal resolution in the sequential activation of neurons. Sound pressure level is generally high during the initial part of FM sweeps and additionally improves the temporal locking of the neural response. The upper portion of the FM sweep may therefore be the most important part for echolocation tasks requiring high temporal resolution.

The shape of the tuning curves of cortical neurons is generally complex, except for many neurons in the primary and anterior auditory fields, where V-shaped tuning curves are common. In 'RF' neurons with high Q values, the tuning curves generally had extremely steep slopes. Accordingly, the Q value changed only slightly over the dynamic range of the unit and the tuning acuity was largely independent of the stimulus level. This type of 'level-tolerant' tuning is also common in neurons of the DSCF area of *Pteronotus* (Suga and Manabe, 1982). Thus the DSCF area in this species functionally corresponds at least to the 'RF' area in the primary auditory field of *Rhinolophus*, but may also embody the anterior auditory field, as defined in *Rhinolophus*, which has been distinguished for neuroarchi-

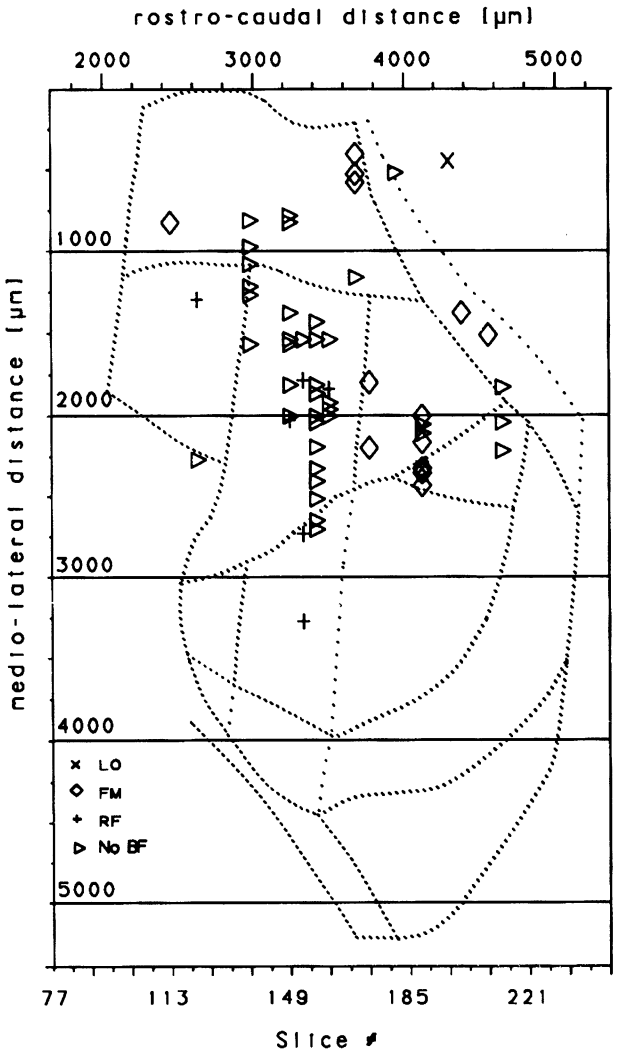


FIG. 21. Location of vocalization-sensitive neurons. Neurons active during vocalization have been found mainly in the tonotopically organized anterior dorsal field, some in the posterior and dorsal (FM/FM) portions of the dorsal field. Neurons only driven by vocalization are represented by triangles; neurons active during vocalization but also drivable by artificial stimuli are indicated by the symbols corresponding to their best frequency range.

tectonic reasons from the primary auditory field. Corresponding neuroarchitectonic data in *Pteronotus* are not available.

Multiple tuning

Multiple tuning was found in about one-fifth of the neurons throughout the dorsal fields but rarely in the primary auditory cortex (only four of 134 neurons). The multiple characteristic frequencies were in most cases not harmonically related. Neurons tuned to two frequencies and located in the dorsal part of the dorsal field might contribute to the driving of combination-sensitive FM/FM neurons, which have not been included in this report and have been discussed elsewhere (Schuller *et al.*, 1991). Since facilitated combination-sensitive neurons show in most cases a reduced activity to the single components, they belong in a broader sense to the class of double-tuned neurons. Neurons with such properties have also been described in the dorsal fields but not the DSCF area of the moustached bat (e.g. Suga *et al.*, 1983b). The proportion of double-tuned neurons in the dorsal auditory cortex was higher than at lower levels of the auditory pathway (Schuller and Pollak, 1981). The almost complete lack of double tuning in the primary auditory field of the bat is in contrast to the findings in the cat by Sutter and Schreiner (1991).

Response patterns

The response properties of cortical neurons are highly dependent on the use of anaesthetics, and barbiturates for example reduce the spontaneous activity in the auditory cortex and favour the occurrence of the transient onset response pattern. As the semichronic preparation in the bat (see Materials and methods) circumvented such influences, the number of spontaneously active cortical neurons was high (15%) and the response patterns were as varied as in subcortical auditory structures. The phasic pattern (68%) was more frequently encountered in the auditory cortex than in the medial geniculate body (53%) or inferior colliculus (47%) of horseshoe bats. Tonic response patterns occurred in the auditory cortex (23%) nearly as often as in the medial geniculate body (25%) (Engelstätter, 1981), but considerably less frequently than in the inferior colliculus (42%) (Pollak and Schuller, 1981). Response patterns were often not stable when the frequency or intensity parameters were changed and showed alterations similar to those found in subcortical structures. Presumably inhibitory influences, which are highly dependent on frequency and level, are responsible for such changes. Response patterns in the DSCF area of the moustached bat show essentially the same diversity, but numerical comparison for response patterns is not possible due to different classification schemes (Suga and Manabe, 1982).

Inhibition

Inhibitory responses have been observed in ~9% of the cortical units, which is comparable to the 12% found in the medial geniculate body and 11% in the inferior colliculus of horseshoe bats (MGB: Engelstätter, 1981, IC: Pollak and Schuller, 1981). Many pure-tone-driven neurons in the primary auditory field with extremely narrow tuning curves were entirely inhibited by narrow-band noise. The activation of the inhibitory side bands of such neurons by narrow-band signals could explain their specificity for pure tones.

Rate-level functions

Rate-level functions of cortical neurons have been described for a large variety of mammals (for review see Clarey *et al.*, 1992). In this bat, monotonic neurons were predominantly found in the primary auditory field and the anterior dorsal field, presumably homologous to the anterior auditory field in the cat. In all other cortical fields non-monotonic behaviour prevailed. This is consistent with findings in the cat and the moustached bat. The non-monotonic response behaviour is reflected in closed or complex tuning curves, deviating from the common V-shape. Non-monotonic rate-level functions are characterized by a best intensity and there is evidence that the best amplitudes of non-monotonic neurons in the moustached bat are arranged in an orderly manner (ampliotopic; Suga and Manabe, 1982). No topographic order for best sound pressure levels, however, could be detected in the primary auditory field of the rufous horseshoe bat, *R. rouxi*.

Tonotopic organization of auditory cortical fields

Frequency mapping of the auditory cortex has been performed in many mammals and has yielded primarily the location and tonotopic organization of the primary auditory field. In bats, tonotopic gradients within the primary auditory field have been found in the greater horseshoe bat, *Rhinolophus ferrumequinum* (Schweizer and Radtke, 1980; Ostwald, 1984), the moustached bat, *P. p. parnellii* (Suga and

Jen, 1976), the little brown bat, *Myotis lucifugus* (Wong and Shannon, 1988) and the big brown bat, *Eptesicus fuscus* (Dear *et al.*, 1993). The results of this investigation are in accordance with the findings in other bats.

The tonotopic organization of the horseshoe bat's primary area corresponds in its caudorostral trend of increasing frequencies from low to high frequencies to that of the cat and other mammals. The small frequency band at and above the resting frequency is largely hypertrophied by space and by number and is similar to that in the neotropical bat *P. p. parnelli* (Suga and Jen, 1976; Asanuma *et al.*, 1983). We could not, however, demonstrate in *Rhinolophus* a concentric arrangement of isofrequency lines in this over-represented frequency domain. Moderate accentuation of the higher frequencies is also found in other mammals (e.g. rat: Sally and Kelly, 1988; cat: Merzenich *et al.*, 1975).

A second tonotopically organized subdivision has been observed in the anterior dorsal field of the bat. There is a gradient of decreasing frequencies in the ventrodorsal direction sharing high frequencies ('RF' range) with the tonotopic gradient of the primary field. The tonotopic organization, the frequency reversal, the discharge properties of the neurons and connectional characteristics are reasons to compare this field to the anterior auditory field of the cat (Reale and Imig, 1980). A tonotopically organized field anterior to the primary auditory field that showed reversed tonotopic order with high frequencies at caudal locations has also been demonstrated in the mouse (Stiebler, 1987), rat (Horikawa *et al.*, 1988) and gerbil (Thomas *et al.*, 1993). The difference in orientation of the anterior auditory field in the bat could be a consequence of the disproportional expansion of the over-represented frequency range in the primary area. On the other hand, the orientation seems to be of secondary importance, as e.g. McMullen and Glaser (1982) also describe a similar anterodorsal orientation of a tonotopically organized field in the rabbit.

No anterior auditory field showing the reversal of topographical frequency arrangement has been demonstrated in the moustached bat, but the dorsal portion of the DSCF area could well correspond to the high-frequency part of the anterior auditory field. In the horseshoe bat, the distinction between the high-frequency areas in the primary auditory field and the anterior auditory field is supported by cytoarchitectural data and the boundary cannot been drawn on the basis of functional data alone.

A further tonotopic gradient was detected in the caudal part of the posterior dorsal field, with frequencies increasing from low to high in the ventrodorsal direction sharing a low-frequency border with the primary field. Neurophysiological and cytoarchitectonic reasons argue for a comparison of this area to the dorsoposterior region in other mammals. No physiological or anatomical data on this region are available for comparison in the moustached bat. In the cat two caudally situated tonotopic fields have been found (ventroposterior and posterior fields; Reale and Imig, 1980), which share the low-frequency transition with the primary area. Neurophysiologically the neurons are distinguishable from those of the primary field by e.g. longer latencies (Reale and Imig, 1980; Phillips and Orman, 1984). In some mammals the posterior area has a concentric tonotopic arrangement (rat: Horikawa *et al*, 1988; gerbil: Thomas *et al.*, 1993); in others it appears to have no tonotopy (mouse: Stiebler, 1987).

Stimulus type preferences and responses to complex sound

Detailed response peculiarities of cortical neurons were revealed by the use of complex stimuli, such as frequency modulations (sinusoidal and linear), noise and natural stimuli, in addition to pure tones. Some of the neurons responded to complex stimuli in a way that could be predicted from the pure-tone behaviour; other neurons had distinctive preferences to particular stimulus types and their responses could not be derived from pure-tone response behaviour, if pure tones were effective at all. The majority of neurons with particular stimulus preferences were found in cortical fields outside the primary field.

Neurons that were specialized for the processing of pure tones and rejected any kind of frequency-modulated stimulus or noise band were recorded in the rostral dorsal field and could contribute to the facilitated responses of the neurons in this area, which are specialized for the processing of pure tone combinations (Schuller *et al.*, 1991). Suga and Tsuzuki (1985) describe neurons with extremely narrow level-tolerant tuning curves at the major harmonic components of the call in the corresponding area in the moustached bat, *Pteronotus*. The response areas were sandwiched between broadband inhibitory areas, so that it can be suspected that these neurons would reject stimuli spectrally broader than pure tones. A preference for pure-tone stimuli in this area is further supported by the finding that 65% of the neurons tested with sinusoidally frequency-modulated stimuli showed no or very poor synchronized responses (Suga *et al.*, 1983a).

Synchronized responses to sinusoidally modulated stimuli resembled those of subcortical neurons of the same species, but the maximum modulation frequency for synchronization was lower in the cortex (up to 200 Hz) than in the inferior colliculus (350 Hz; Pollak and Schuller, 1981) or the cochlear nucleus (800 Hz; Vater, 1982). The reduction of the capability to follow higher modulation frequencies with increasing level within the auditory system is a general finding (e.g. MGB cortex in the guinea-pig; Creutzfeld *et al.*, 1980). The depth of sinusoidal frequency modulation influenced the synchronized responses of neurons in the DSCF and CF–CF areas of the moustached bats most profoundly, whereas modulation frequency was of minor importance (Suga *et al.*, 1983a). Small to moderate modulation depths (0.16–1.6%) were preferred by most neurons, indicating that bordering inhibitory fields in cortical neurons probably influence decisively the response to sinusoidal modulations.

Another variety of modulation-specific neurons reacted with unsynchronized responses and displayed maximum discharge rates for a 'best' modulation frequency. The latter neurons provide an advanced degree of signal processing, as instead of reproducing aspects of the modulation they encode the modulation frequency by their activity level. Both types of sinusoidal modulation-preferring neurons were located in the primary area and the dorsally adjacent anterior and posterior dorsal fields. Sinusoidally frequency modulation-driven neurons in the moustached bat are reported for the DSCF and CF–CF area, which correspond roughly to the primary field and rostral dorsal field in the horseshoe bat.

The activity elicited by linear frequency modulation was generally dependent on the slope, amplitude, duration and direction of the sweep. This finding matches results from other bats and other mammals (e.g. cat: Phillips *et al.*, 1985). Neurons that answered preferentially to linear modulations were not present in the primary auditory field or the anterior dorsal field, but were restricted to the dorsal part of the dorsal field, which is characterized by neurons sensitive to combinations of two frequency-modulated stimuli (Schuller *et al.*, 1991). This corresponds functionally to the dorsal FM/FM-sensitive fields as defined also in the moustached bat (Suga *et al.*, 1983b).

Noise stimuli could be either inhibitory at bandwidths as low as ± 100 Hz, or could be excitatory and elicit stronger responses than other stimulus types. Noise-preferring neurons occurred throughout the dorsal fields, but never in the primary auditory field.

Responses during spontaneous vocalizations

Most investigations on the processing of species-specific vocalizations use stimuli broadcast to the passively listening animal. In this study, bats uttered vocalizations by themselves and the cortical activity might not be the response to the auditory self-stimulation alone but may also be influenced by internal pathways and internal feedback during active vocalization.

Many neurons in our sample that were active during spontaneous emission of vocalizations could not be driven by artificial replicas of the echolocation calls, or showed very different response patterns to artificial stimuli. Such neurons were concentrated in the anterior auditory field and scattered throughout the caudal portion of the dorsal fields. On the other hand, vocal neurons of the primary auditory field were in general well driven by stimuli mimicking echolocation calls. Internal feedback seems to influence vocal neurons in the anterior auditory field and the caudal dorsal field more than vocal neurons encountered in the primary auditory field.

Support for such internal, vocalization-coupled processes comes from the observation that during vocalization the processing of acoustical stimuli can be altered (e.g. bat: Schuller, 1979; monkey: Manley and Müller-Preuss, 1978; Müller-Preuss, 1986). Spontaneous vocalization or the presentation of biologically significant signals can be expected to produce an alerted state or arousal in awake animals. Newman and Symmes (1974) have shown in the squirrel monkey that the responses to vocalizations were enhanced in a considerable number of cortical cells if the level of arousal was concurrently increased by electrical stimulation in the reticular formation. An elevated level of attention coupled to vocalization may therefore be the reason for the specific responses of cortical neurons to vocalizations in the bat.

Conclusions

The basic local differentiation of the horseshoe bat's auditory cortex fits well into a general mammalian scheme. At least three tonotopically organized fields can be distinguished, which most probably correspond to the primary, anterior and posterior auditory fields recognized in other species. In addition, the dorsal FM/FM field and the rostrodorsal CF–CF field can be functionally differentiated (Schuller *et al.*, 1991), having so far only an equivalent in the cortical organization of the moustached bat (Suga, 1990). The findings in the horseshoe bat generally fit well into the functional framework as elaborated in the moustached bat by Suga and co-workers. In the anterior and posterior dorsal fields as defined in *Rhinolophus*, no direct correspondences can be established for the two species as no comparable samples of data are available. The functionally determined subdivisions of the cortical fields in *Rhinolophus* are strongly supported by our cyto- and myeloarchitectonic data, which are not available for *Pteronotus*. The functionally segregated fields also fit well into the target range of auditory thalamic afferents. It remains to be determined whether the outer fringe areas can be regarded as mere auditory cortex, or whether they constitute instead composite areas responsive to different sensory modalities. In particular, the mutual support of cytoarchitectural, connectional and neurophysiological data of the horseshoe bat cortex has yielded highly consistent results which could not have been obtained with the same reliability through separate investigations.

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Abbreviations

CF	constant frequency
CF–CF	field containing CF–CF-facilitated neurons
CF/FM	constant frequency\frequency modulation (call type)
DSC	Doppler shift compensation
DSCF	Doppler shift compensation frequency
FM	frequency modulation
'FM'	frequency-modulated frequency range (45–75.5 kHz)
FM/FM	field containing FM/FM facilitated neurons
'HI'	high-frequency range (80–100 kHz)
LFM	linear frequency modulation (modulated)
'LO'	low-frequency range (10–45 kHz)
pf	primary field
RF	resting frequency
'RF'	resting frequency range (75.5–80 kHz)
SAM	sinusoidal amplitude modulation
SFM	sinusoidal frequency modulation
SPL	sound pressure level

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