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Single Unit Responses to Linear Frequency-Modulations in the Inferior Colliculus of the Greater Horseshoe Bat, *Rhinolophus ferrumequinum*

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Summary. 1. Recordings were made from 135 single inferior colliculus neurons of the Greater horseshoe bat, *Rhinolophus ferrumequinum*. These bats emit sonar signals which are characterized by a long constant frequency (CF-) component and a terminal frequency modulated (FM-) component. The responses of the units to CF- and FM-stimuli with different modulation heights, durations and directions were studied.

2. The majority of single units studied had minimum thresholds that were either equal for CF- and FM-signals of the same duration or lower for the CF-stimulus. Only rarely was the sensitivity for FM-signals higher than for a CF-signal.

3. With FM-signals the response properties of units with best frequencies between 65–81 kHz, i.e. in the frequency range of the FM-component of the echolocation call, did not differ significantly from units with best frequencies in lower frequency ranges. Relatively many units with best frequencies in the filter region of the audiogram (81–88 kHz) required FM-signals with particular modulation heights and rates to elicit excitatory responses.

4. Response patterns and spike count functions were found to differ with CF- vs. FM-signals. The most common difference was a smaller number of impulses per stimulus and a temporally more restricted ('phasic') discharge pattern to FM-signals than to CF-signals. Greater discharge activity to FM-signals occurred in only a few units. 'Latency constant' and 'FM-specialized' neurons, as reported for other species were also found.

5. Inhibition was frequently observed with FM-stimuli in spontaneously active neurons. An accurate prediction of a neuron's response to FM-signals from knowledge of the excitatory and inhibitory response areas to CF-signals was not always possible.

6. The responses of 'FM-specialized' neurons to the FM-component of a CF-FM-stimulus was not significantly altered by the presence of a CF-component in the filter region of the audiogram.

7. Responses of single units to signal and noise combinations are described.

8. Data are discussed in relation to results on the processing of FM-signals in other bats.

Introduction

The Greater horseshoe bat, *Rhinolophus ferrumequinum*, uses echolocation for orientation and hunting insects. Its orientation call consists of a 10 to 100 ms long constant frequency (CF-) component with frequencies around 83 kHz followed by a brief frequency modulated (FM-) component sweeping downward to about 65 kHz. The duration of the FM-component is approximately 1/11th to 1/14th of the call length. Sometimes, the echolocation calls start with an upward sweeping FM (Schnitzler, 1968).

The two signal components carry different information (Novick, 1971; Simmons et al., 1975). The long CF-component is well suited for measurement of Doppler shifts introduced by relative velocities of bat and target and for detection of a target. In response to upward frequency shifts of the echo, *Rhinolophus* lowers the frequency of the emitted call so that the frequency of the echo CF-component is kept constantly within a narrow frequency band around 83 kHz ('Doppler shift-compensation'; Schnitzler, 1968; Schuller et al., 1974). Echolocation theory suggests that the broadband FM-component is well suit-

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Abbreviations: BF best frequency; *CF* constant frequency; *FM* frequency modulated; *FTC* frequency-threshold-curve; *IC* inferior colliculus; *SNR* signal/noise ratio; *SPL* sound pressure level re. 0.00002 $N \cdot m^{-2}$

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ed for the determination of target size, shape, surface structure, spatial location and in particular for measurment of the distance between bat and target. It is furthermore stated that only the FM-component of CF-FM-echolocation sounds is used for range determination (Simmons, 1973; Schnitzler, 1968).

Behavioral (Long and Schnitzler, 1975) and neurophysiological (Neuweiler, 1970) investigations have shown that the auditory system of horseshoe bats is sharply tuned to the frequency range of the CF-component. Single units with best frequencies (BFs; the frequency in the frequency-threshold-curve (FTC) where the minimum threshold is reached) in the frequency band between 81 to 88 kHz typically possess tuning sharpness expressed in $Q_{10\ \mathrm{dB}}$ (BF divided by the bandwidth of the FTC at 10 dB above minimum threshold) greater than 20 and even up to 500 (Suga et al., 1976; Möller et al., 1978). They are largely overrepresented throughout the ascending auditory pathway (Suga et al., 1976; Schuller and Pollak, 1979) as compared to neurons with BFs in other frequency ranges.

The processing of linear FM-stimuli in the auditory system of *Rhinolophus* has only been studied in recordings of gross evoked potentials in the inferior colliculus (IC; Schuller et al., 1971; Schuller, 1972). On the other hand, a considerable amount of neurophysiological data exists about single unit responses of the IC to linear FM-stimuli in other species of bats emitting FM-orientation calls (*Myotis*; Suga, 1973), short CF-FM-calls (*Molossus*; Vater and Schlegel, 1979) and FM-, CF-, and short CF-FM-calls depending on orientation situation (*Tadarida*; Pollak et al., 1978).

Bats employing different echolocation signals can be distinguished by the properties of their peripheral and central auditory nervous system (Grinnell, 1970, 1973; for review see Neuweiler, 1977). Such differences mainly concern the tuning sharpness of the audiogram and the tuning properties of single units. The best known examples of sharply tuned neurons are those concerned with the processing of the long CF-component of the orientation calls in *Rhinolophus* (Suga et al., 1976; Möller et al., 1978) and *Pteronotus* (Suga et al., 1975). The neurons of bats using short CF-FM-calls (*Molossus*; Vater et al., 1979) or FM-calls (*Myotis*; Suga, 1973) show only moderate degrees of tuning sharpness. The echolocation calls of most bats contain FM-sweeps and it has been shown in behavioral experiments that the performance in range discrimination tasks of bats with long CF-FM-, short CF-FM- and FM-signals closely follows the predicted performance of a receiver using the cross-correlation function of the FM-sonar signal. It was proposed that the central processing of FM-signals should follow similar principles in different bat species (Simmons et al., 1975). Furthermore, since attachment of a long CF-signal to a FM-signal reduces the target range resolving power of the whole signal, it was speculated that in *Rhinolophus* a separate processing of CF- and FM-components takes place (Simmons et al., 1975).

The present study investigates single unit responses to linear upward and downward FM-sweeps in the IC of *Rhinolophus*. The same signals (i.e., upward and downward sweeps of two different durations) as in the IC-study on *Molossus* (Vater and Schlegel, 1979) were used to allow for direct comparison between bats belonging to different genera and emitting structurally different echolocation calls. Combined CF-FM-stimuli simulating the echolocation call of *Rhinolophus*, were also used.

Methods

5 male *R. ferrumequinum* (weight 20–27 g) were anaesthetized for surgery with intraperitoneal injections of Nembutal (2.5–3.5 mg/100 g). Recordings from the IC of the awake bats started at the earliest 24 h after surgery to allow for full recovery from anaesthesia. The same bat was studied on consecutive days. In some sessions a neuroleptic agent (Droperidol, 4 mg/kg) was administered.

Recording and stimulation equipment has previously been described by Neuweiler and Vater (1977). CF- and FM-pulses with rise-decay times of 0.5 ms and different durations (mostly 2 ms and 20 ms or longer) were delivered to the contralateral ear at repetition rates of 2–4 Hz under freefield conditions. The condenser loud speaker (Machmerth et al., 1975) had a flat frequency response (±2 dB between 15 and 90 kHz). The linear upward and downward sweeping signals had modulation heights of 40, 20, 10, 5 and 2.5 kHz. Each sweep lasted throughout the pulse and was centered at each neuron's BF. The orientation call of *Rhinolophus* was simulated by a combined CF-FM-stimulus. The duration of the CF-component was varied between 5 and 45 ms, the modulation started at a frequency between 80–85 kHz and swept down 20 kHz in 2 ms. Pseudorandom noise of 20 kHz bandwidth centered at a neuron's BF was shaped into pulses of 80 ms duration with rise-fall times of 1 ms and added to the signals (CF or FM) which were delayed 20 ms relative to noise onset.

Single unit activity was recorded with 3 M KCl micropipettes (resistance between 8 and 12 MOhm) in electrode penetrations preferentially made through medial portions of the IC directed rostrolaterally.

Action potentials converted into unitary pulses were fed 'on-line' to a PDP 11/40 computer for Post-Stimulus-Time (PST)-histogram acquisition. Histograms were constructed from 40 or 50 stimulus presentations with 0.1 to 0.5 ms binwidth in 200 bins. Data were stored on magnetic discs for "off-line" processing (Plot-programs PLOHIS, PERHIS, Zöller, H.)

Thresholds of single units were measured with audiovisual criteria or judged from PST-histograms. For measurement of the FTCs, 20 ms long CF-signals were used. For determination of threshold-signal/noise-ratios (Threshold-SNRs), noise of increasing intensity was added to the CF- or FM-signals (equal intensity, 20 to 40 dB above minimum threshold) until in PST-histograms the response to the signal just disappeared.

The amplitude of the acoustic signals is expressed in dB SPL (sound pressure level referred to 0.00002 N/m^2).

Frozen sections of brains fixed in formaldehyde were cut in frontal plane at 60 to 80 µm and stained with cresylviolet for crude histological verification of electrode tracts.

Results

135 neurons of the IC of *R. ferrumequinum* were tested with linear FM-signals. Particular attention was paid to neurons with BFs in the FM-frequency range of the audiogram (65–81 kHz; $N=53$). The sample furthermore comprises 53 low frequency neurons (BF < 65 kHz), 24 filter neurons (BF 81–88 kHz) and one high frequency neuron (BF 89.5 kHz). The classification of neurons according to frequency range follows the one given by Neuweiler and Vater (1977). All neurons, with the exception of 5, which had BFs in the filter region, responded to linear FM-signals of modulation heights of 10 kHz or larger. Four neurons did not respond to CF-signals.

Comparison of Threshold Values for Different Signals

At a unit's BF, threshold values obtained with CF-signals were compared with those obtained with FM-signals of equal duration. For CF- and FM-signals with similar duration there was a strong dependence of minimum thresholds on the type of signal used, as also reported by Vater and Schlegel (1979) for molossids.

Table 1 summarizes the findings for *R. ferrumequinum*. For signal durations of 20 ms, the majority of neurons responded with lower thresholds to CF-signals (36%) or with equal thresholds (within ± 5 dB) to both FM- and CF-signals (60%). Only 4% of the neurons possessed lower thresholds for the FM-stimulus. This distribution did not significantly change, when the signal durations were shortened to 2 ms. Twentynine percent and 65%, respectively, reached lower thresholds for CF-signals or equal threshold values for both signal types, 6% were more sensitive to FM-stimuli.

By comparing the threshold values obtained for FM-signals of different modulation rates, it was found that 50% had equal threshold for both rates, 42% had lower thresholds for 20 ms FM-signals and 8% had lower values for 2 ms signals.

Threshold values to CF-signals of 20 ms and 2 ms duration were equal (57%) or lower for the longer duration (42%), only one neuron possessed lower thresholds for short CF-stimuli.

Furthermore, the sensitivities of single units to downward and upward sweeping FM-signals were investigated for 20 ms and 2 ms stimuli and the data are summarized in Table 2. Most neurons reach equal thresholds (± 5 dB) for both sweep directions. Asymmetries in the responses to upward and downward modulations became apparent in only few units, where threshold differences of up to 50 dB were found.

In addition to the units described so far, which in terms of threshold either did not distinguish between signal types or showed different sensitivities to the stimuli, a small population of 'selective' neurons was encountered that did not respond with excitatory discharge activity to a particular signal (see Tables 1B, 2). For example, some units only responded to one sweep direction or to one sweep rate, similar to the findings of Suga (1973) and Vater and

Table 1. *A* Comparison of minimum thresholds to constant-frequency (CF-) and downward sweeping frequency-modulated (FM-) stimuli of equal duration; downward sweeping FM-stimuli of different modulation rates (constant modulation height, but different duration) and CF-stimuli of different durations for single inferior colliculus neurons. *B* List of neurons not responding to particular signals. *N* total number of neurons. CF-signals were at best frequency, all FM-signals were centered at best frequency

Stimulus comparisons	A	Equal (±5 dB)	Lower for FM 20 ms	Lower for CF 20 ms	B	No response to FM	No response to CF	No response to both
Minimum thresholds								
CF 20 ms vs. FM 20 ms	$N=97$	58 (60%)	4 (4%)	35 (36%)	$N=16$	12	–	4
		Equal (±5 dB)	Lower for FM 2 ms	Lower for CF 2 ms		No response to FM	No response to CF	No response to both
CF 2 ms vs. FM 2 ms	$N=82$	53 (65%)	5 (6%)	24 (29%)	$N=23$	15	4	4
		Equal (±5 dB)	Lower for FM 20 ms	Lower for FM 2 ms		No response to FM 20 ms	No response to FM 2 ms	No response to both
FM 2 ms vs. FM 20 ms	$N=66$	33 (50%)	28 (42%)	5 (8%)	$N=9$	1	3	5
		Equal (±5 dB)	Lower for CF 20 ms	Lower for CF 2 ms		No response to CF 20 ms	No response to CF 2 ms	No response to both
CF 20 ms vs. CF 2 ms	$N=84$	48 (57%)	35 (42%)	1 (1%)	$N=8$	–	4	4

Table 2. Comparison of minimum thresholds to upward and downward sweeping FM-stimuli of equal duration (20 ms, 2 ms, respectively) for single IC-neurons

Response of units	FM-pulse duration	
	20 ms	2 ms
Equal threshold (±5 dB)	33	24
More sensitive to downward FM	3	4
More sensitive to upward FM	3	4
No response to downward FM	1	3
No response to upward FM	6	5
No response to upward and downward FM	9	5
N	55	45

Table 3. Comparison of response patterns of single inferior colliculus neurons to CF- and FM-signals of equal duration (Total $N=74$ for 20 ms duration, 85 for 2 ms duration). For classification of response patterns as judged by PST-histograms see text

Response of units			Response of units to FM		
to CF		N	Burst	On	Negative
Tonic	20 ms	31	17	5	9
	2 ms	–	–	–	–
Burst	20 ms	19	17	2	–
	2 ms	26	10	14	2
On	20 ms	19	3	15	1
	2 ms	51	2	42	7
Negative	20 ms	5	–	–	5
	2 ms	8	–	4	4

Schlegel (1979). In general, units not responding to FM-stimuli were more common than units not responsive to CF-signals (Table 1). However, 4 units were found which appeared to be selective for fast sweeping FM-signals; they were neither excited by CF-signals of any duration nor by slowly sweeping FM-signals. These neurons shall be described in more detail later.

The BFs of neurons not responding to particular FM-signals were scattered throughout the audiogram of *R. ferrumequinum*. However, in the sample of filterneurons, the proportion of units only responding to a limited set of FM-signals was relatively high: 9 out of 24 filterneurons required a particular sweep direction or sweep rate to be excited by FM. Furthermore, all 5 neurons not excited by any FM-signal of modulation heights of 10, 20 or 40 kHz had BFs in the filter region. The term 'CF-specialized' (Suga, 1973) is not appropriate for these neurons, since by limiting the modulation height to values of 5 kHz or below, firings could be elicited. Therefore, it is more appropriate to call these neurons selective for narrowband signals.

Response Patterns and Spike Count Functions

In order to investigate the response behavior of single units to suprathreshold CF- and FM-stimuli, the response patterns were determined as PST-histograms to stimulus intensities between 10 to 30 dB above minimum thresholds.

The units' response patterns to CF-signals of 20 ms or longer were classified into tonic and phasic. A further distinction of the phasic neurons was made (see also Pollak et al., 1978; Vater and Schlegel, 1979). Based on the number of spikes per stimulus, 'burst' units, discharging 3 or more impulses were distinguished from 'phasic-on' units having only one or two impulses per stimulus. The 'negative' pattern refers to a suppression of spontaneous discharge activity (Neuweiler and Vater, 1977) or to no response to the signal in non-spontaneous or weakly spontaneous active neurons.

The response patterns to CF- and FM-signals are compared in Table 3 for signal durations of 20 ms and 2 ms, selected examples of response patterns to CF-, downward and upward sweeping FM-signals are illustrated in Fig. 1.

The temporal pattern of activity to CF- and FM-signals can differ, some unit types show systematic changes in discharge activity. Neurons with tonic responses (Fig. 1 A) to 20 ms CF-signals most commonly possessed a 'burst' pattern to FM-signals of equal duration. Rarely, a transition to 'phasic-on' activity was seen. The alteration from excitatory response pattern to CF-signals to 'negative' pattern to FM-signals is shown for a tonic neuron in Fig. 1 B. The majority of 'burst' neurons and 'phasic-on' neurons displayed similar patterns to FM-signals (Fig. 1 C, D). It was often observed that the response latency relative to stimulus onset was longer for 20 ms FM- than for CF-signals (Fig. 1 D), due to the delayed occurrence of effective frequencies in the long FM-signals.

Short (2 ms) CF-stimuli led to a 'burst' or 'phasic-on' activity in all tonic neurons. Most 'phasic-on' neurons did not change their pattern to 2 ms FM-signals. 'Burst' neurons could either stay in the 'burst' category or change to 'phasic-on'. Most interestingly, out of the 'negative' responders to 2 ms CF-signals, 4 neurons responded with 'phasic-on' activity to short FM-signals (Fig. 1 E).

Two 'phasic-on' neurons with BFs in the FM-frequency range of the audiogram were found which discharged one spike per stimulus with a latency variation of only ±200 µs for CF- and/or FM-signals

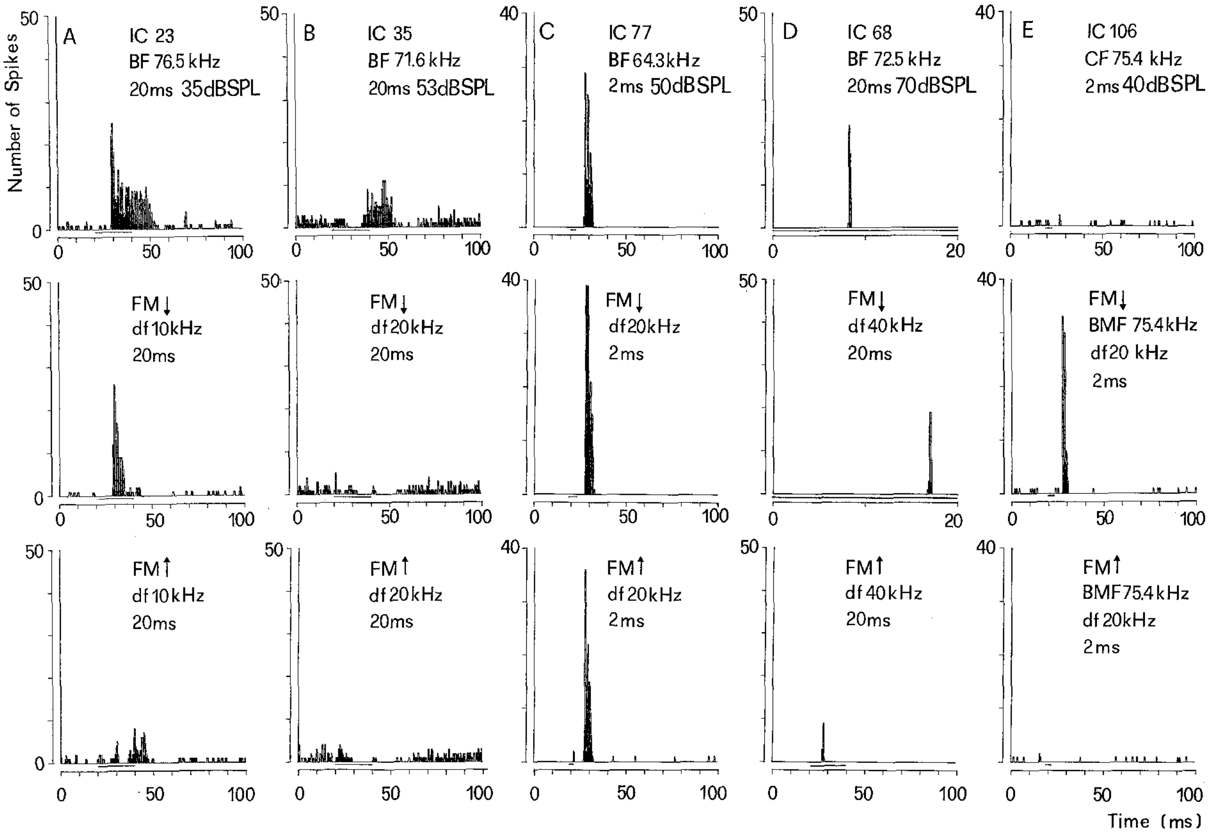

Fig. 1 A–E. Response patterns (PST-histograms) of single IC-neurons (**A–E**) to constant-frequency (*CF*-) stimuli at best frequency (*BF*) (upper row), downward sweeping frequency-modulated (*FM*-) stimuli (middle row) and upward sweeping FM-stimuli (lower row) centered around BF. Intensity is equal for CF- and FM-stimuli for the particular unit as indicated. Stimulus duration is marked by bars. *df* modulation height of the FM-signal; *BMF* 'best center frequency' of the FM-signal. Histograms were taken with a binwidth of 500 μs, except for unit IC 68 (**D**, upper, middle histograms, 100 μs) in 200 bins to 40 or 50 stimulus presentations

Table 4. Comparison of discharge activity (number of spikes per 50 or 40 stimuli) at 10 to 30 dB above minimum threshold of single inferior colliculus neurons

	Response of units			
	Equal (±20%)	Greater discharge activity to CF	Greater discharge activity to FM	*N*
20 ms	21	31	7	59
2 ms	27	35	10	72

(Fig. 1D), they thus display characteristics similar to the 'phasic constant latency responders' found in other species of bats, *Myotis* (Suga, 1970), *Tadarida* (Pollak et al., 1977a, b, 1978) and *Molossus* (Vater and Schlegel, 1979).

The discharge activity measured in terms of number of spikes per stimulus at intensities of 10 dB to 30 dB above minimum threshold was compared for CF- and FM-signals (Table 4). Activity to both signals was called equal if the number of spikes per stimulus to FM-signals was within ±20% of the activity elicited by CF-signals. For both short and long signal durations most units showed equal discharge activity to CF- and FM-stimuli or possessed greater activity to CF-pulses. However, some units were more strongly activated by long FM-signals ($N=7$) or by short FM-signals ($N=10$) as compared to CF-signals.

Therefore, there is a tendency for most neurons to have a smaller discharge activity per FM-stimulus and to fire in a more temporally restricted pattern (i.e., tonic to phasic, 'burst' to 'phasic-on') in response to FM-stimuli than to CF-stimuli.

Inhibitory influences became more obvious with FM-stimulation than with CF-stimulation as shown by the increased number of 'negative' response patterns (Table 3) or by strong suppression of discharges during or after the stimulus. These inhibitory processes as a reaction to FM-stimulation become especially clear when the neurons' responses over a larger intensity range were observed. Taking into account the intensity parameter, it is clear that the classification of neurons into tonic and phasic is an oversimplification. Depending on intensity, complex

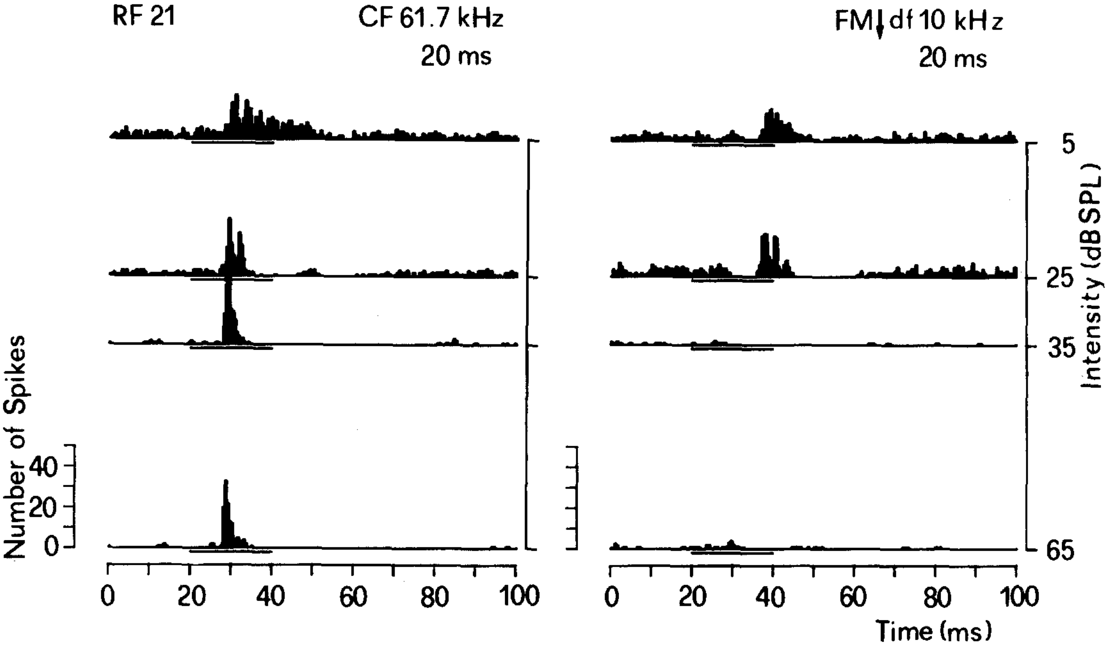

Fig. 2. Response patterns of a single IC-unit to CF-stimuli (left) and downward sweeping FM-stimuli (right) of 20 ms duration and different intensities

changes in discharge patterns can be observed in many units ('complex' responders, Möller et al., 1978); see for example the neuron in Fig. 2 which responds to low CF-stimulus intensities with tonic activity, but the discharge activity is restricted to a 'burst' with increasing stimulus intensity; at 25 dB SPL a 'triphasic' pattern occurred with inhibition of spontaneous activity at stimulus onset, followed by a 'bell-shaped' excitation pattern, terminated by inhibition. With further increase in stimulus intensity, an 'upper threshold' is reached for the excitatory response component.

Spike count functions of single units ($N=43$ for 20 ms; $N=44$ for 2 ms CF- and FM-signals) were measured and termed according to Rose et al. (1963) monotonic, non-monotonic and 'upper threshold', which is an extreme case of a non-monotonic function. The intensity where maximal discharge activity occurs in non-monotonic neurons is termed 'best intensity' (BI; Suga, 1978). Selected examples of spike count functions to CF- and FM-stimuli showing the observed changes in response properties are presented in Fig. 3, data are summarized in Table 5.

Non-monotonic neurons when stimulated with long CF-signals most commonly had a similar spike count function to FM-signals or restricted their dynamic range by reaching an 'upper threshold' or responded only with 'negative' pattern to FM. One non-monotonic neuron altered to a monotonic function with FM-signals, but without increasing the discharge activity to values higher than at BI. Two 'upper threshold' neurons extended their dynamic range and became non-monotonic with FM-signals, but again without significantly increasing their discharge activity to values higher than at the BI. All monotonic neurons remained monotonic with FM-stimulation. When using short signal durations, there were similar alterations in the spike count functions with the two signal types. However, out of 4 neurons which did not show excitatory discharge activity to CF-signals, two responded with 'upper threshold' functions to FM-signals and two responded with non-monotonic functions.

Influence of Inhibitory Response Areas on the Response to FM-Signals

In some spontaneously active IC-neurons of *R. ferrumequinum*, it was possible to measure inhibitory side areas of the excitatory FTC with one-tone-stimulation. The closer examination of these neurons is especially interesting, because inhibitory side areas were shown to influence the processing of FM-signals and furthermore, their arrangement was used to predict the neurons' response to FM-signals (Suga, 1973).

Figure 4 shows a 'complex' neuron with a BF of 70.7 kHz, in the FM-frequency range of the audiogram. The excitatory FTC is flanked on both sides by inhibitory areas (Fig. 4A). Downward and upward

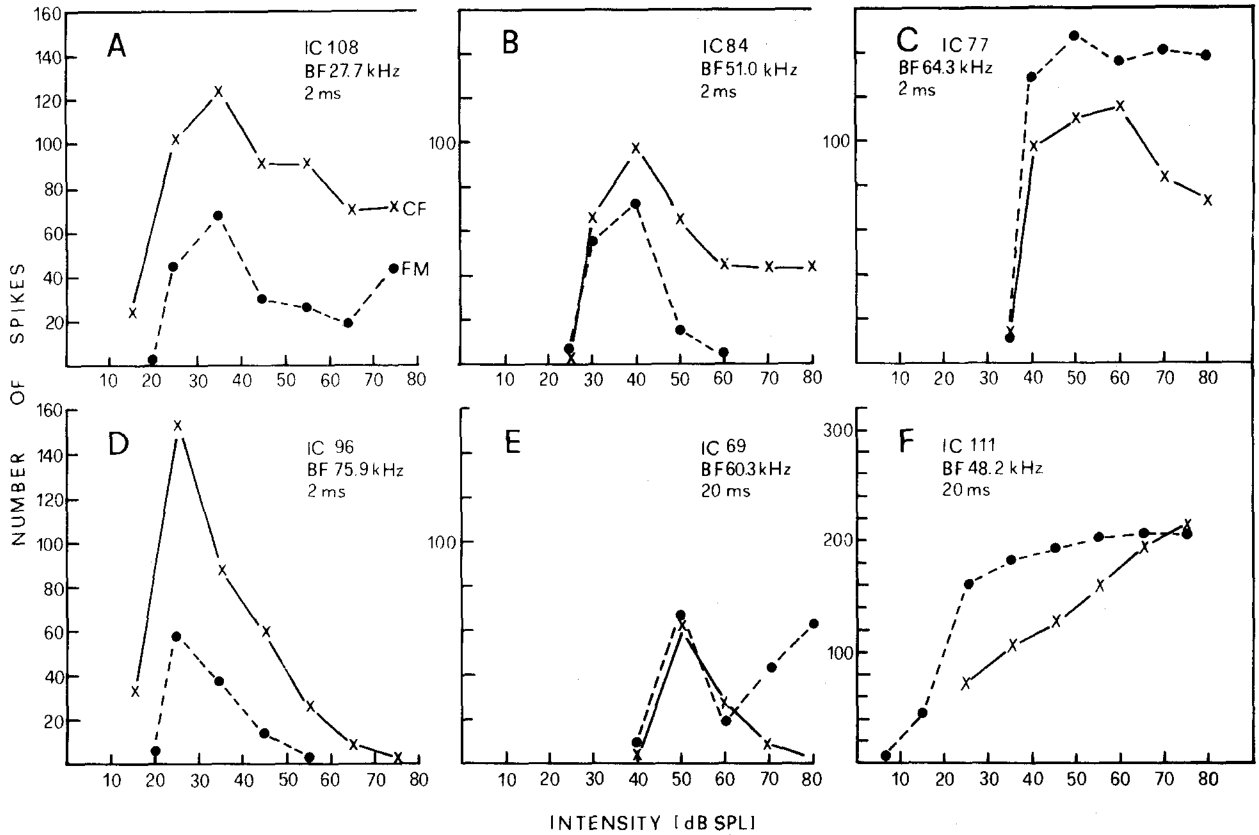

Fig. 3A–F. Spike count functions of single IC-neurons (A–F) to CF-signals (crosses) and downward sweeping FM-signals of 20 kHz modulation height (circles, stippled line). All CF-signals corresponded to BF, all FM-signals were centered around BF

Table 5. Comparison of spike count functions of single IC-neurons to CF- and FM-stimuli of equal duration

Spike count functions to CF		Spike count functions to FM				
		'upper threshold'	Non-monotonic	Monotonic	Negative	*N*
Monotonic	20 ms	–	–	7	–	7
	2 ms	–	–	12	3	15
Non-monotonic	20 ms	8	13	1	2	24
	2 ms	5	13	2	2	22
'Upper threshold'	20 ms	5	2	–	1	8
	2 ms	2	–	–	1	3
Negative	20 ms	–	–	–	4	4
	2 ms	2	2	–	–	4

sweeping FM-signals of 20 kHz modulation height and 20 ms duration evoked excitation with thresholds similar to CF-signals. The excitatory discharge activity to FM-signals is followed by a clear inhibition of spontaneous activity noticible also with CF-signals (Fig. 4C left, right). The unit responds with a phasic discharge to short CF-signals (Fig. 4D left), the suppression of spontaneous activity is equally as long as that to long CF-signals. Spike count functions are illustrated in Fig. 4B. Short FM-signals, however, were not able to evoke excitatory discharge activity: the response only consisted of a suppression of spontaneous activity (Fig. 4D, right). The effect of the inhibitory side areas of this unit therefore depends on modulation rate, a behavior not predictable by the FTC.

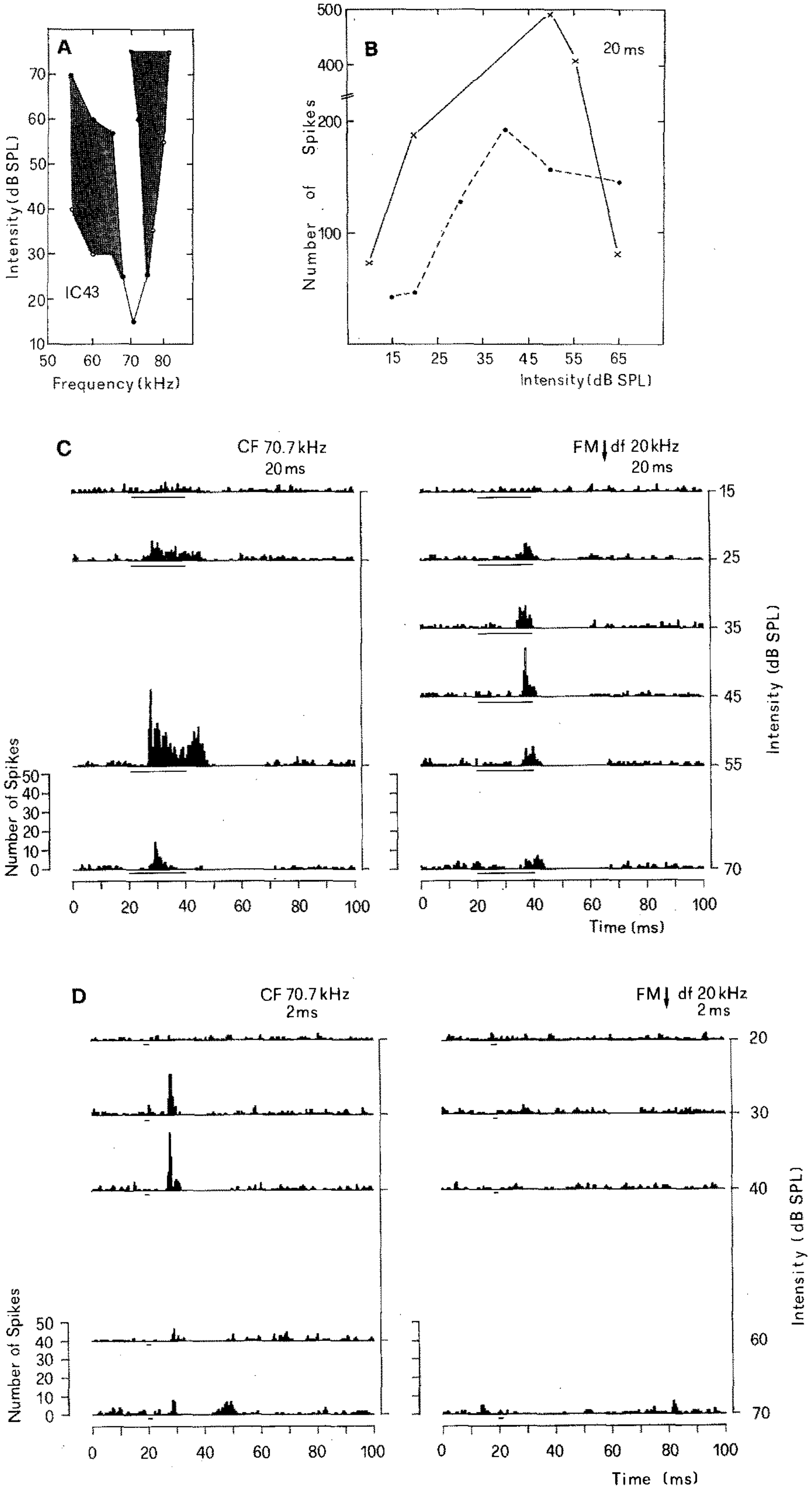

Fig. 4A–D. Tuning curve (**A**), spike count functions (**B**) and response patterns (**C, D**) of a single IC-neuron. **A** Excitatory (unshaded) and inhibitory (shaded) response area; **B** spike count functions to CF-signals of 20 ms duration (crosses) and to downward sweeping FM-signals of equal duration and modulation height of 20 kHz (circles); **C** PST-histograms to 20 ms CF-signals (left) and downward sweeping FM-signals (right) at different stimulus intensities; **D** PST-histograms to 2 ms signals

The neuron shown in Fig. 5 possessed asymmetrical inhibitory areas, the threshold of the high frequency area was 35 dB lower than the threshold of the low frequency inhibitory region and partly covered the excitatory field at high stimulus intensities (Fig. 5A). The spike count functions to CF-signals (Fig. 5B) therefore followed a non-monotonic course. The response behavior of this unit to upward and downward sweeping FM-signals of 20 ms duration can be predicted from the arrangement of the inhibitory fields: downward modulations were only effective in evoking discharge activity in intensity ranges of 0 to 5 dB SPL, higher intensities inhibited the neuron, because the modulation height of 10 kHz reached into the inhibitory FTC-area, an 'upper threshold' was reached (Fig. 5C, B). Upward modulations gave rise to a 'burst' activity (Fig. 5C), which in contrast to CF-stimuli follows only a weak non-monotonic function (Fig. 5B). The duration of the 'silent' period after stimulus offset became longer with increasing stimulus intensity. However, in contrast to the unit described before (see Fig. 4), the high frequency inhibitory area did not cancel the response to short downward sweeping modulations: the neuron responded with a phasic pattern (Fig. 5D) following a non-monotonic spike count function (Fig. 5B). Short upward modulations were also excitatory. Again, this response behavior to FM-signals is not predictable from the FTC.

Other neurons with inhibitory areas on both sides of the excitatory response area responded to both long and short FM-signals of both sweep directions with excitation.

'FM-Selective' Neurons

As already mentioned, IC-neurons were found that did not respond to CF-signals, but responded well to short FM-signals. These neurons will now be described in more detail.

By changing the center frequency of the FM-stimulus, the frequency range of the best response (minimal threshold at the 'best center frequency') was determined, or the excitatory response area of the neuron was measured. Two of these units had minimum thresholds in the FM-frequency range of the audiogram (i.e. 77.7 kHz and 75.4 kHz). The 'best center frequencies' of the other two units were 81.9 kHz and 47.2 kHz.

None of these units was excited by long upward or downward FM-signals. Only one unit responded to short upward sweeps. Three units were tested with noise bursts of 20 kHz bandwidth, only one unit was excited by this stimulus.

The excitatory response area of unit 106 is shown in Fig. 6A, measured by changing the center frequency and intensity of a short downward sweeping FM-signal of 20 kHz modulation height. The response area was closed, i.e. high stimulus intensities were unable to elicit excitatory discharge activity ('upper threshold'). The response patterns to an FM-stimulus around 'best center frequency' are shown in Fig. 6B.

In these units it is of particular interest to investigate the effect of a long CF-component preceding the FM-component, since this signal mimicks the orientation call of *R. ferrumequinum*. The responses to a combined CF-FM-signal of variable intensity, with the CF-component at 83.0 kHz, sweeping down to 63 kHz are shown in Fig. 6C. As demonstrated by the corresponding spike count functions (Fig. 6B), the addition of a long CF-component (43 ms, 18 ms) prior to the 2 ms downward sweep does not change the unit's response to the FM-component, the functions possess the same minimum thresholds, BIs and 'upper thresholds'.

Signal to Noise Experiments

Masked thresholds (threshold-SNR) for the excitatory response component to CF- and FM-signals were measured for 21 neurons with long signal durations and for 25 neurons with short signal durations by adding noise (20 kHz bandwidth, centered at BF) of variable intensity to the fixed suprathreshold signal.

Most units possessed equal threshold-SNRs for both FM- and CF-signals ($N=15$ for 20 ms, $N=13$ for 2 ms signals). In other neurons, threshold-SNRs could be lower for the FM-stimuli ($N=5$ for 20 ms, $N=5$ for 2 ms signals) or for CF-stimuli ($N=1$ for 20 ms, $N=7$ for 2 ms signals).

The sample is too small to indicate a general trend. However, in some neurons interesting mechanisms for processing a signal in noise interference were seen, which will be described more thoroughly by the example of two neurons.

As described in previous sections, the response patterns to FM-signals may contain strong inhibitory components. This FM-evoked inhibition proved to be an effective 'marker' of the signal in the noise-evoked tonic discharge activity. Figure 7 illustrates an example by comparing the response patterns of a neuron to combinations of CF-signal (Fig. 7, left) and FM-signal (Fig. 7, right) with noise of different intensities. Increase in noise intensity reduced the excitatory response to the CF-signal until the masked threshold was reached at an intensity combination of 35/40 dB. The excitatory response component to

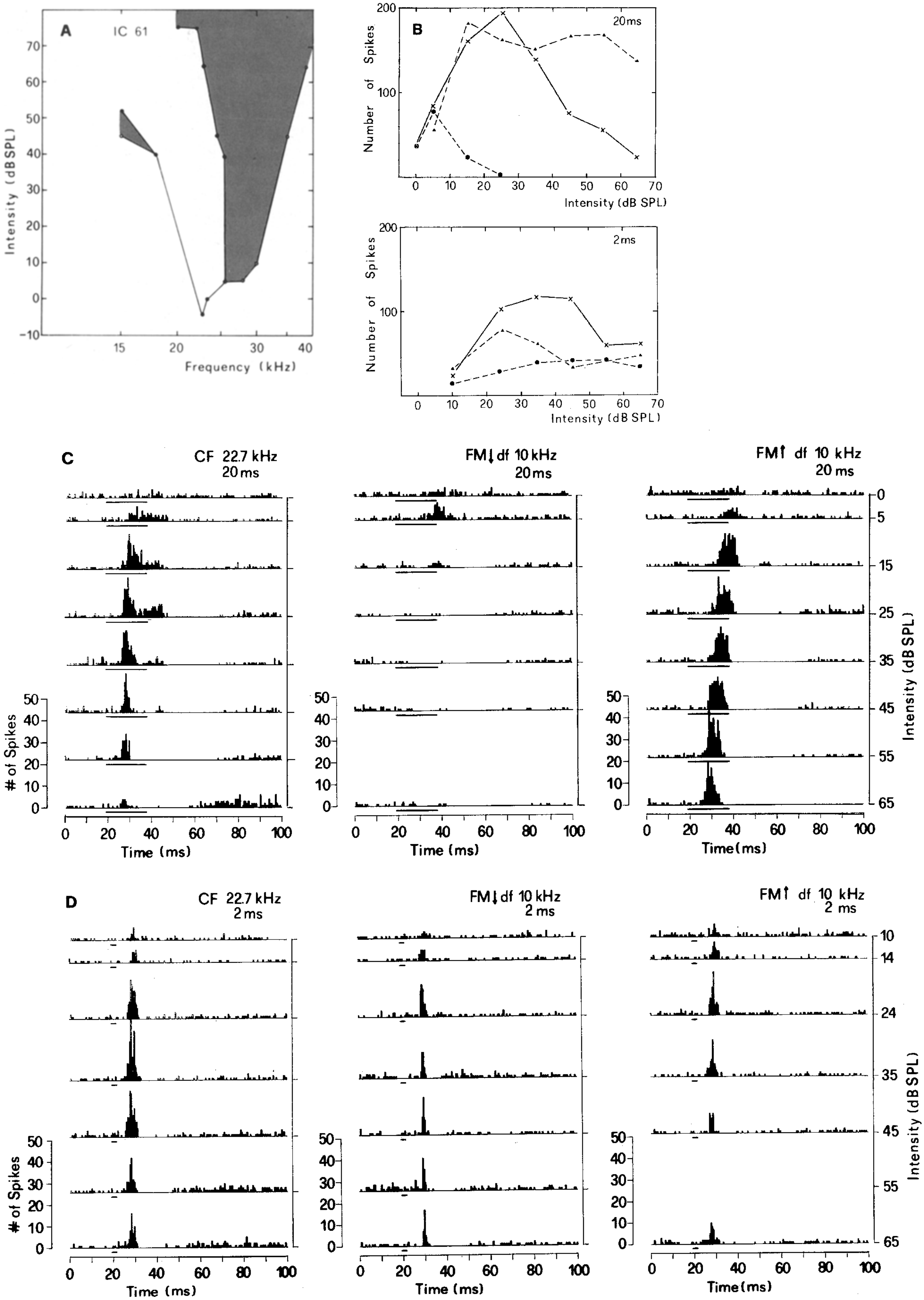

Fig. 5A–D. Tuning curve (**A**), spike count functions (**B**) and response patterns (**C**, **D**) of a single IC-neuron. **A** Excitatory (unshaded) and inhibitory (shaded) response areas. **B** Upper graph, spike count functions to 20 ms CF-signals (crosses) and to upward and downward sweeping FM-signals (triangles; circles); lower graph, spike count functions to 2 ms CF- and FM-signals. **C** PST-histograms to 20 ms CF-signals (left), downward sweeping FM-signals (middle) and upward sweeping FM-signals (right) at different stimulus intensities. **D** PST-histograms to 2 ms signals

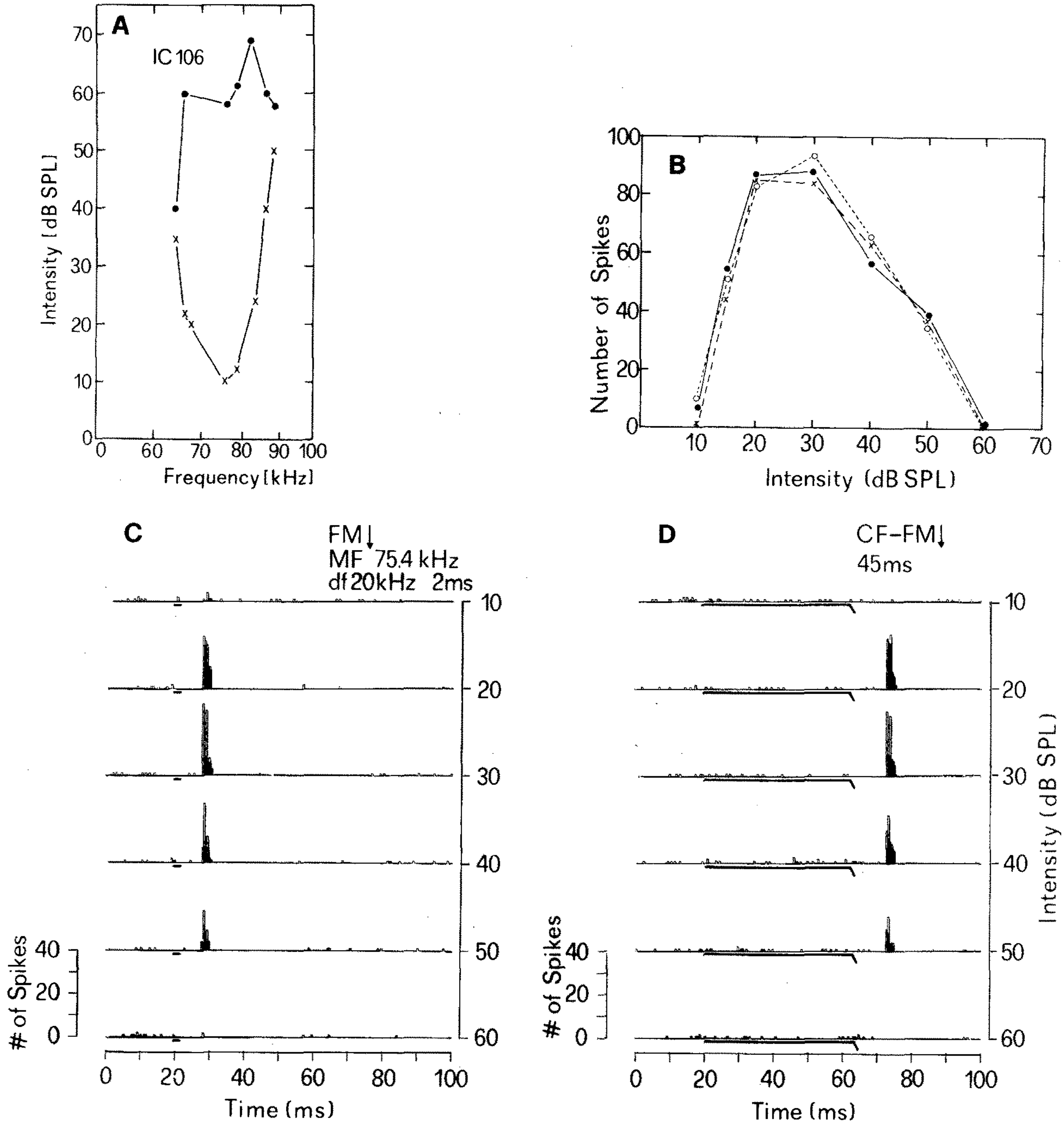

Fig. 6A–D. Excitatory response area to FM-signals (**A**), spike count functions (**B**) and response patterns (**C**, **D**) of a single IC-neuron 'specialized' for FM-signals. **A** Crosses, minimum thresholds to downward sweeping FM-signals at different center frequencies and 20 kHz modulation height; dots, 'upper thresholds'; **B** spike count functions to 2 ms downward sweeping FM-signals at 'best center frequency' of 75.4 kHz (filled circles), and to CF-FM-stimuli (frequency of the CF-component at 83 kHz, 20 kHz downward sweep, 2 ms) of two different durations of the CF-component (18 ms, crosses; 43 ms, open circles). **C** PST-histograms to downward sweeping FM-signals of 20 kHz modulation height, 2 ms duration at 'best center frequency' at different stimulus intensities. **D** PST-histograms to CF-FM-stimuli

the FM-signal was already masked at a signal to noise ratio of 35/25 dB. However, the FM-evoked inhibition marked this signal in noise to a much lower SNR, reaching the same value as the CF-signal.

Unit 91 with a BF of 80,9 kHz (Fig. 8) showed facilitation of the response to the signal, when noise was added. The response patterns to CF and FM-signals alone are shown in the upper histograms. At the particular signal intensity (60 dB SPL) used to measure masked thresholds, this unit exhibited a weak

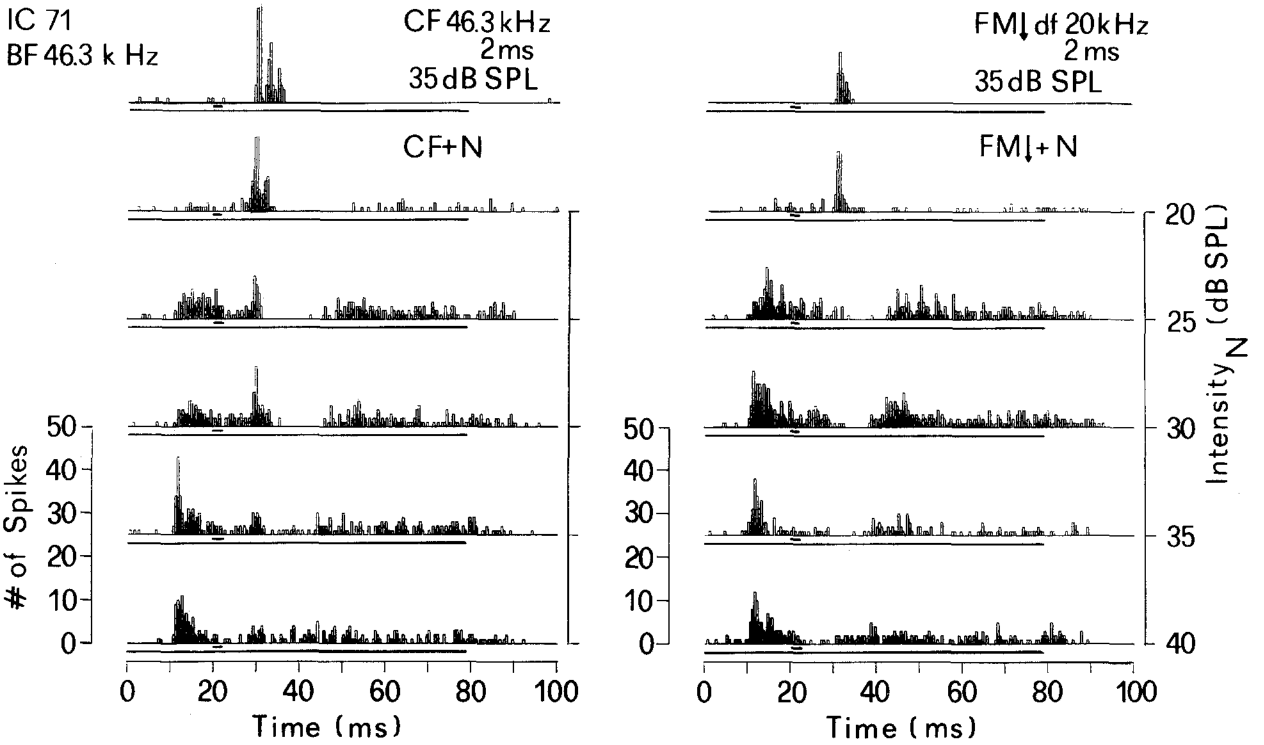

Fig. 7. Response patterns of a single IC-neuron to CF- (left) and FM-stimuli (right) with stimulus parameters as indicated (upper histograms) and to the combination of these signals with noise (*N*) of different intensities (noise duration 80 ms, bandwidth 20 kHz, centered at BF

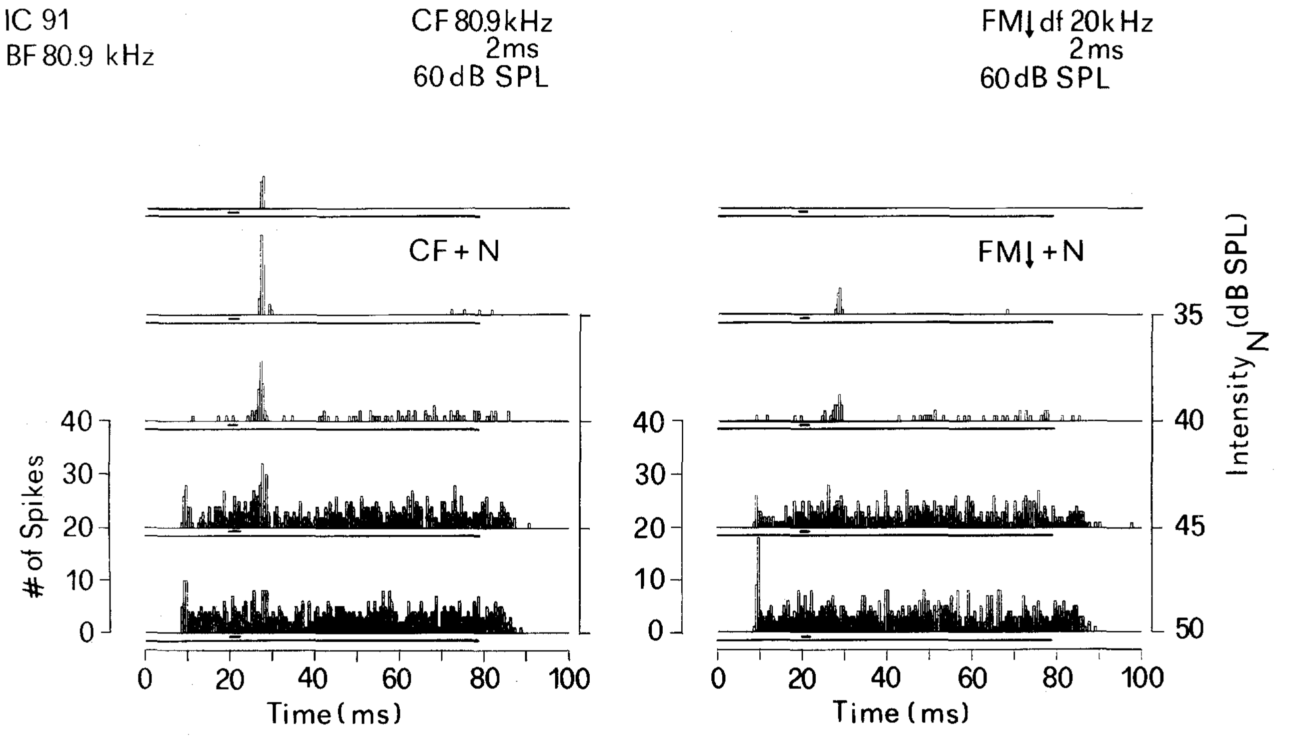

Fig. 8. Response patterns of a single IC-neuron to CF- and FM-signals and to the combination of these signals with noise. Arrangement and abbreviations as in Fig. 7

phasic response to CF-signals alone and no response to the FM-stimulus alone. Addition of a low intensity noise (35 dB SPL) lead to a more than 50% increase in discharge activity to the CF-signal, and also facilitated an excitatory response to the FM-signal. The excitatory responses to the signals can be seen up to intensity combinations of 60/45 dB for CF-signals and 60/40 dB for FM-signals.

Discussion

This report on single unit responses in the IC of *R. ferrumequinum* to linear FM-signals is mainly based on recordings from FM-frequency and low frequency neurons. The population of filterneurons, expressing the adaptations of horseshoe bats' auditory system to CF-signals, in this sample is relatively small, because preferentially medial, anteriorly directed electrode penetrations were made, aiming for neurons with BFs below 80 kHz. This intentionally introduced bias necessarily leaves some open questions concerning the role of filterneurons in the processing of the FM-component of emitted call or echo, which are raised in the reports on gross evoked potentials in the IC of *Rhinolophus* to linear FM-stimuli (Schuller et al., 1971; Schuller, 1972).

In the following, the data from IC-neurons of *Rhinolophus* will be discussed in relation to the findings in *Molossus* (Vater and Schlegel, 1979) and *Tadarida* (Pollak et al., 1978). An attempt is made to work out differences in the processing of FM-signals among bats employing different orientation calls. However, one has to keep in mind that restricted samples of single units are compared, so that conclusions can only be drawn tentatively.

A comparison of minimum thresholds of single units to different signal types revealed similar tendencies in both *Rhinolophus* and *Molossus*. Minimum thresholds to CF-signals of different durations were either equal or lower for the longer CF-pulse which reflects the effect of temporal integration. The majority of neurons was either equally sensitive to CF- and FM-signals of 20 ms duration or more sensitive to the CF-signal. But in contrast to the findings in *Molossus* and *Tadarida*, where more than 20% of the neurons were found to possess lower thresholds for short (2 ms) FM-signals than to CF-signals of equal duration, only 6% of the IC-neurons of *Rhinolophus* showed this response behavior. The BFs of these neurons were not restricted to the FM-frequency range of the audiogram. Again, in the comparison of threshold values to different modulation rates of the FM-signal, only 8% in *Rhinolophus* had lower thresholds for fast sweeping FM-stimuli which mimic the FM-component of the orientation call than to slow modulation rates, while about 25% of the IC-neurons in *Molossus* were more sensitive to the fast sweeps.

The response patterns and the course of spike count functions to CF- and FM-signals differed in many neurons. Similar alterations were found in *Rhinolophus* as in IC- neurons of *Molossus*. However, since the sample from *Rhinolophus* contains more tonic cells (42%) than that of *Molossus* (4–5%), the most commonly observed transition in response patterns was a temporally more restricted pattern to FM-pulses ('phasic') with a smaller number of spikes per stimulus. A greater discharge activity or an enlarged dynamic range of the spike count function to FM-signals than to CF-signals was only seen in a few units with 'burst' or 'phasic-on' patterns to CF-signals and BFs not restricted to the FM-frequency range. It was not as common as in *Molossus*, where changes from a 'phasic-on' pattern to CF-signals to a 'burst' pattern to FM-signals with a drastic increase in discharge rate were frequently found.

'Latency-constant' neurons to both CF- and FM-signals as described in the IC of *Myotis* (Suga, 1970), *Tadarida* (Pollak et al., 1977a, b; 1978) and *Molossus* (Vater and Schlegel, 1979) which can provide sharp time marks for distance measurement were also observed in *Rhinolophus*.

These results suggest that there are quantitative differences between *Rhinolophus* on the one hand and *Tadarida* and *Molossus* on the other hand in the relative proportions of units especially sensitive or with greater discharge activity to FM-signals which simulate the FM-component of the orientation calls. Differences among species would probably become more obvious if the sample from *Rhinolophus* contained more filterneurons. In the IC of *Rhinolophus*, as in the auditory system of other bats (Suga, 1973; Pollak et al., 1978) and cats (Whitfield and Evans, 1965), neurons were found that did not respond to FM-signals of particular directions and sweep rates. But interestingly, many of the filterneurons of *Rhinolophus* tested, required particular FM-signals to elicit excitation. Furthermore, all neurons that did not respond to modulation heights greater than 5 kHz had BFs in the filter range, they expressed a selectivity for narrowband signals, like FM-signals of small modulation height and CF-stimuli.

The response properties of neurons with higher sensitivity and greater discharge activity to FM-signals are advantageous, if the bat is confronted with a detection problem. For theoretical reasons, the long CF-component of the orientation call of *Rhinolophus* is better suited for target detection than the FM-component, which is probably used for measuring target range (Simmons et al., 1975; Schnitzler, 1968).

Rhinolophus regulates the duration of the FM-component according to the orientation situation: it is shortened when the bat closes in on a target and its intensity is increased (Schnitzler, 1968). In this situation, where a precise measurement of distance is required, there is no detection problem since the echoes are strong. Therefore, the relative lack of an increased sensitivity to FM-signals is not necessarily a disadvantage. Furthermore, the thresholds to FM-stimuli of units equally sensitive to both CF- and FM-signals and even the thresholds of most units more sensitive to CF-signals than to FM-signals were low enough to be of value for echolocation.

A higher sensitivity and a greater discharge activity of single units to FM-sweeps similar to those occurring in the species specific orientation call can be considered to represent adaptations between sender and receiver system. In *Rhinolophus*, one would expect that especially neurons with BFs in the frequency range of the FM-component of the echolocation call would show this response behavior. However, this was not the case: these neurons did not appear to be specifically adapted for the processing of FM-signals, in the sense that the above mentioned response properties were more often found than among neurons in other frequency ranges. Neurons displaying a high selectivity for FM-stimuli, as reported in cat's auditory cortex (Whitfield and Evans, 1965) and IC and cortex of *Myotis* (Suga, 1973) were also found in the IC of *Rhinolophus*. These neurons did not respond to CF-signals and required particular sweep rates and directions of the FM-signal to respond. But again, these neurons were not confined to the FM-frequency range of the audiogram. Furthermore, neurons not responding to fast sweeping FM-stimuli were also found in the frequency range between 65 and 81 kHz. With the stimulus paradigm used in this study, no positive evidence for a specific adaptation of units in the relevant frequency range to FM-signals mimicking the FM-component of the echolocation call of *Rhinolophus* could be found at the integration level of the IC. Such a correlation might exist at higher levels of the auditory system, since it is known that the selectivity of single units for particular signals increases from peripheral to central stations and that in the auditory cortex of the bat *Pteronotus parnellii rubiginosus* these neurons are functionally organized (for review see Suga, 1978).

Neuronal mechanisms responsible for the response to FM-signals, like temporal integration, inhibition and summation/facilitation have been discussed by several workers (e.g. Suga, 1973). The influence of inhibitory processes on the response behavior of single IC-neurons of *Rhinolophus* to FM-stimuli will now be described in more detail, since due to the more frequent occurrence of high spontaneous activity as compared to *Molossus*, inhibition could be observed during single tone stimulation. Stimulation of inhibitory side areas of the FTC prior to the excitatory area by the FM-sweep may be made responsible for the observed suppression of spontaneous activity or for the excitation to only one sweep direction in some units.

However, the action of the inhibitory surround turned out to be complex and particularly dependent on the rate of modulation, so that a straightforward prediction of a neuron's response to FM-signals on the basis of the arrangement of excitatory and inhibitory areas of the 'static' FTC measured with CF-pulses was not possible. For example, a change to faster modulation rates keeping modulation height constant could lead to phasic responses to downward sweeping FM-signals in a neuron with a high frequency inhibitory area, which only responded with a 'negative' pattern to slowly sweeping FM-signals. This could be due to the measurement procedure, which by mapping the inhibitory areas with 20 ms CF-stimuli neglects temporal aspects, like sequential stimulation at different frequencies or time constants of excitation and inhibition. For example, if the integration time of inhibition is longer than that of excitation, it can be expected that no inhibition occurs at fast sweep rates; and vice versa, due to a long integration time of excitation, fast sweep rates will not lead to excitatory responses. It is known that the extent of inhibitory areas can change depending on the measurement paradigm used. Inhibitory areas measured with single tone stimulation are not always congruent with those measured with two tone stimulation in the same unit (Möller, 1977). In two tone stimulation experiments, the extent of inhibitory areas depends on the intensity of the test tone (Suga, 1973).

Inhibitory processes turned on by FM-stimulation became also very clear in the combined stimulation with signal and noise. The FM-evoked suppression of discharge activity led to a sharp 'contrast' between the inhibitory response to this signal and the tonic response to noise. Threshold-SNRs were found to be smaller for the inhibitory response component than for the excitatory response component to FM-signals in some units.

The stimulation with CF-FM-stimuli derserves further comment. From the work of Suga (1973) it is known that an additional component in a complex stimulus can modify or even cancel the response of 'FM-specialized' units; for example, inhibitory response areas to CF-components could be measured using the 'best' FM-component as test signal. However, if the frequency of the CF-component corresponded to the initial frequency of the FM-sweep, as in a CF-FM-orientation call, the response to the modulation was not affected, i.e. the initial frequencies of the FM were always outside of the inhibitory area. A similar mechanism might also be responsible for the response of the 'FM-specialized' neurons in the IC of *Rhinolophus*. It was found that CF-components of different durations with frequencies in the filter range of the audiogram did not alter the response to the final FM-sweep of the CF-FM-signal. The processing of the FM-component seemed to be separated and independent of the CF-component in these units.

In summary, no qualitative differences in the response properties to FM-signals of IC-neurons of *Rhinolophus* and other bat species like *Molossus* (Vater and Schlegel, 1979), *Tadarida* (Pollak et al., 1978) and *Myotis* (Suga, 1973) have been found. 'FM-selective' neurons and latency-constant neurons, which due to their temporal precision of firing can serve as time markers of emitted pulse and echo and thereby provide a means of measuring target distance have now also been reported for the long CF-FM-bat *Rhinolophus*. Except for the differences in the relative proportions of units with higher sensitivity and greater discharge activity to FM-sweeps similar to those occurring in the species specific orientation calls between *Rhinolophus* and *Molossus* or *Tadarida*, the processing of FM-signals at the integration level of the IC seems to be basically similar in all the bats studied.

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