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RESEARCH ARTICLE

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Frequency response areas of neurons in the mouse inferior colliculus. I. Threshold and tuning characteristics

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Abstract Excitatory and inhibitory frequency response areas of 130 neurons of the central nucleus of the mouse inferior colliculus (ICC) were mapped by extracellular single-unit recordings and quantitatively evaluated with regard to thresholds, steepness of slopes of excitatory tuning, characteristic frequencies of excitation (CF_E), inhibition (CF_I), and bandwidths of response areas (sharpness of tuning). Two-tone stimuli were used to determine the shapes of inhibitory response areas. Class I neurons (n=54) had asymmetrical (with regard to the CF_E) excitatory and inhibitory response areas, with inhibition above CF_E having lower thresholds and covering larger areas than inhibition below CF_E. Quantitative relationships between CF_E and CF_I thresholds, and sharpness of tuning showed that the receptive fields of about two-thirds of these neurons had properties similar to auditory nerve fibers. Class II neurons (n=36) had small symmetrical or tilted excitatory areas of rather constant bandwidths and broad inhibitory areas reaching far into and often through the excitatory area, leading to closed excitatory areas in ten neurons. Class III neurons (n=32) had higher excitatory thresholds and the highest proportions of unilateral inhibitory areas compared with neurons of the other classes. Their excitatory area often widened symmetrically with increasing sound level. Their inhibitory areas did not overlap with the excitatory area. Class IV neurons (n=8) had two branches of excitatory areas (two-CFs_E) and six of the neurons had a central inhibitory area in addition to the low- and high-frequency inhibitory areas. In most neurons, the shapes of excitatory response areas predicted the shapes of inhibitory areas. Altogether, 15 neurons from all 4 classes had areas of facilitation in addition to inhibitory areas. Facilitation in six class IV neurons occurred between the two branches of the excitatory area. All 130 neurons had large inhibitory areas, 106 of them on both sides of the excitatory area. That is, sound processing in the ICC shows strong inhibitory components. The close relationships between excitatory and inhibitory CFs found here indicate that inhibitory projections to and interactions within the ICC are tonotopically organized comparable to the excitatory ones.

Keywords Auditory midbrain · Frequency tuning · Inhibitory areas · Inferior colliculus · Receptive fields

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Introduction

The receptive fields of most neurons of the auditory pathway from the auditory nerve to the auditory cortex divide up into areas of excitatory responses and areas where responses are suppressed or inhibited. Due to mechanical interactions in the cochlea, all auditory nerve fibers possess areas of two-tone suppression at the low- and high-frequency side of the excitatory tuning curve (e.g., Arthur et al. 1971; Sachs and Kiang 1968; Delgutte 1990; Hill and Geisler 1992). From the cochlear nucleus upwards, synaptic inhibition comes into play, and a variety of shapes of excitatory and inhibitory response areas have already been observed in neurons of the cochlear nucleus (e.g., Evans and Nelson 1973; Young and Brownell 1976; Young et al. 1988). At the level of the auditory midbrain, the inferior colliculus (IC), more than 50% of the neurons in the central nucleus (ICC) (e.g., Vater et al. 1979; Ehret and Moffat 1985; Ehret and Merzenich 1988a) have excitatory receptive fields deviating substantially in shape from those of primary neurons in the auditory nerve (e.g., Kiang et al. 1965; Kiang and Moxon 1974; Liberman 1978).

The high incidence of closed and tilted excitatory tuning curves and those with steep slopes on both sides (level-tolerant units) suggest that these response areas

are shaped by lateral inhibition. In fact, there are multiple inhibitory pathways from various auditory brainstem nuclei to the ICC (e.g., Glendenning and Baker 1988; Saint Marie et al. 1989; Shneiderman et al. 1988, 1993; Shneiderman and Oliver 1989; Vater et al. 1992; González-Herández et al. 1996) and intrinsic inhibitory (GABAergic) neurons of the ICC (Thompson et al. 1985; Nagai et al. 1985; Roberts and Ribak 1987; Moore and Moore 1987; Oliver et al. 1994) that all may mediate inhibitory effects on frequency response areas of ICC neurons. Auditory processing that has been shown to be influenced by inhibition in the ICC includes binaural response properties (Park and Pollak 1993, 1994; Klug et al. 1995; Gooler et al. 1996), and temporal (Casseday et al. 1994; Saitoh and Suga 1995; Le Beau et al. 1996; Lu et al. 1997, 1998) and intensity-dependent processing (Pollak and Park 1993; Faingold et al. 1991; Jen et al. 1998).

Despite the demonstration of GABAergic inhibition modifying shapes of receptive fields and response properties within these fields (Yang et al. 1992; Palombi and Caspary 1996; Fuzessery and Hall 1996), a comprehensive picture is lacking about what kinds and shapes of excitatory and inhibitory response areas in the ICC do exist and how they relate to each other. In a recent study on receptive field properties of cat ICC neurons (Ramachandran et al. 1999), inhibitory response areas were characterized by inhibition of spontaneous activity. This approach is built upon the assumption that spontaneous activity is an intrinsic property of ICC neurons, which, however, is rather unlikely in the mouse (Wagner 1994, 1996; Reetz and Ehret 1999). Here, we demonstrate inhibition within and around excitatory areas by a quantitative mapping of excitatory and inhibitory frequency response areas with one- and two-tone stimuli. Our main goal is to measure and compare key parameters of excitatory and inhibitory response areas in order to determine: (a) their relationship to two-tone suppression in auditory nerve fibers, (b) whether characteristics of excitatory response areas (excitatory tuning curves) are predictive for properties of inhibition in the same neurons, and (c) how differences between response areas of neurons may reflect differences in excitatory and inhibitory innervation.

Materials and methods

Subjects and surgery

Recordings were taken from 45 female house mice (*Mus domesticus*, hybrids of outbred strain NMRI and feral mice), aged 8–15 weeks. For surgery, animals were anesthetized by inhalation narcosis (oxygen with 1.5–1.8% halothane), which was administered through a small respiratory mask. The skin over the dorsal surface of the skull was removed and a 1.5-cm-long brass bar fixed to the frontal-parietal bones with cyanoacrylate glue and cement (Technovit 3040). The bar was locked into a metal holder to immobilize the mouse head. A craniotomy was made over the left side inferior colliculus (IC) and the dura overlaying the IC carefully removed. Warm silicon oil was applied to the brain after the surgery. The animal was placed on a feedback-controlled heating pad which maintained its rectal temperature at 37±1°C.

During the recording session, a light anesthetic state was maintained by intraperitoneal injection of ketamine (Ketavet, 35 mg/kg body wt.) and xylazine (Rompun, 1.0 mg/kg) when necessary (about every 20–45 min). The animals remained quietly and motionless without indication of pain or distress, but responded with a light withdrawal reflex in response to tail or toe pinch. Similar dosages of ketamine and xylazine for maintenance of anesthesia have been used effectively in recording auditory brainstem responses of mice before (Zhou et al. 1995).

Electrophysiological recordings

Glass pipettes filled with 3 M KCl (impedances 4–8 MΩ) served as recording electrodes. They were placed stereotaxically with reference to the λ-point of the skull on the visible surface of the IC and were advanced in a dorsoventral orientation into the central part of the IC by a remote-controlled microdrive (SPI Nanostepper). Sampling did not include the anterior, posterior, medial and lateral parts of the IC, and was restricted to 1.0–1.5 mm caudal and 0.8–1.5 mm lateral of the λ-point. This area of recording has been shown to correspond to the central nucleus of the IC of the mouse (Sidman et al. 1971; Ehret and Moffat 1985; Stiebler and Ehret 1985; Romand and Ehret 1990).

The neural responses were amplified 10,000 times and band-pass-filtered (0.3–10 kHz; WPI, DAM80) and fed in parallel to an oscilloscope, audiomonitor and window discriminator to be transformed into standard TTL pulses fed into a computer for online control and stored for offline analysis (PC 386 with BOTIM3 board, software: RESPMAP and MAPX_AN2 by Dr. G.J. Dörrscheidt, Bochum).

Stimulus generation

Bursts of single tones and two-tone complexes were used as acoustic stimuli. Tones (50 ms duration, 5 ms trapezoidal rise and fall times) were delivered at 300-ms intervals. Sine waves in tone bursts were initiated at zero phase. Single tones and two-tone complexes were generated by a two-channel D/A converter system (Dörrscheidt, Bochum). Each channel had a conversion rate of 250 kHz, 12 bit amplitude resolution, an anti-aliasing filter, and a computer-controlled attenuator for setting the desired sound intensity. The analog output signals were electronically added, monitored on an oscilloscope and fed either to a dynamic speaker (Thiel, C2 33/8) through an amplifier (Denon, PMA-1060) or to an electrostatic speaker (Machmerth et al. 1975) through a custom-made amplifier and voltage supply. The dynamic speaker was used for frequencies between 1 and 30 kHz. Its response was flat within ±6.5 dB in a range of 1–30 kHz at the site of the animal's pinna. The electrostatic speaker was used for stimulus frequencies between 12 and 75 kHz. It had a flat ±2 dB frequency response. The frequency response characteristics of the loudspeakers were stored in the computer and automatically compensated. Thus, the final deviation from a flat spectrum at the ear of the animal was only ±1.5 dB.

The mouse was placed in a sound-proof and anechoic room. Sounds were presented free-field from anterior, 45° to the right of the mouse sagittal plane and thus contralateral to the recorded IC. The distance between the animal and the dynamic speaker (electrostatic speaker) was 60 cm (30 cm). The sound pressure levels (SPLs) of tones used in the experiments were measured at the right pinna (Brüel and Kjaer, 6.5 mm calibrated microphone 4135, preamplifier 2633, measuring amplifier 2606). At the maximum SPLs used (90 dB), harmonic distortions were at least 35 dB (mostly more than 50 dB) below the level of the fundamental frequency.

Experimental procedure

Tone bursts of variable frequencies and intensities of up to 75 dB SPL were used as search stimuli. When a single unit producing spikes of defined shape and amplitude was isolated, its excitatory characteristic frequency (CF_E) and lowest excitatory threshold to tones (the threshold at CF_E) were determined audiovisually in order to center the following computer-controlled measurements at the approximate CF_E. Then, the conventional single-tone excitatory tuning curve and two-tone response areas were measured automatically by computer-controlled programs over a broad frequency range (1–75 kHz) and sound levels from the unit's threshold up to 110 dB above it (from –20 dB SPL up to 90 dB SPL). In the two-tone paradigm, one tone (60 ms duration including 5 ms rise and fall times) was presented at the neuron's CF_E 10 dB above threshold, while the second tone started 5 ms earlier than but ended with the CF_E tone and varied across a maximum frequency range of two octaves below and one octave above CF_E. Stimulus presentation was performed in pseudo-random sequences of 16×16=256 different frequency-intensity combinations. Each frequency-intensity combination was presented 3 times. The recorded data were used for offline analysis of the exact CF_E and receptive field properties.

Data analysis

Spike data were taken as averages from the three repetitions of all single and two-tone stimuli presented and calculated relative to the average spontaneous activity (0–67 spikes/s) when the shape of the excitatory response area was established, and relative to the average response rate generated by the CF_E tone 10 dB above threshold when the inhibitory response areas were determined. The criterion for a response was an average 10% deviation (increase or decrease) from these average basic rates. In the case of no or low activity (less than 5 spikes/s), the criterion for an excitatory response was one spike per three stimulus repetitions. Facilitation was noted if the average response to the two tones presented together was 10% higher than the sum of the average responses to the single tones.

In plots of response areas, isoresponse rates corresponding to average increases or decreases of 20%, 30%, etc., from the basic rate were calculated and plotted as isoresponse curves. Excitatory and inhibitory response areas for neurons were characterized by the following measures:

1. The CF_E, the threshold level at the CF_E, "characteristic frequencies of inhibition" of inhibitory response areas below (CF_{IL}) and above (CF_{IH}) the CF_E, and their threshold levels.
2. The steepness of the slopes of the excitatory tuning curves (except for neurons with closed or tilted curves or complex shapes of excitatory response areas) measured between 10 dB and 60 dB (or 50 dB or 40 dB if the threshold levels were high) above threshold at CF_E.
3. The sharpness of tuning was estimated: (a) by calculating *Q* values for excitatory and inhibitory areas (CF_E, CF_{IL} or CF_{IH} divided by the bandwidths of the corresponding excitatory or inhibitory response areas taken at 10 dB, 20 dB,..., 80 dB above threshold of excitation or inhibition, respectively).
4. The percentage frequency bandwidths within one octave or two octaves around the CF_E covered by excitatory and inhibitory frequency response areas at 10 dB, 20 dB,..., 80 dB tone level above the threshold at CF_E.

The measures described under items 2–4 above are complementary in characterizing the shapes of receptive fields. *Q* values provide a local measure of the change of the receptive field size with increasing sound level; the steepness of tuning curve slopes provides an average measure. The relationship between the relative sizes of excitatory and inhibitory response areas is demonstrated by the percentage frequency bandwidths of the receptive field expressing excitation or inhibition. All statistical comparisons were two tailed (Sachs 1999).

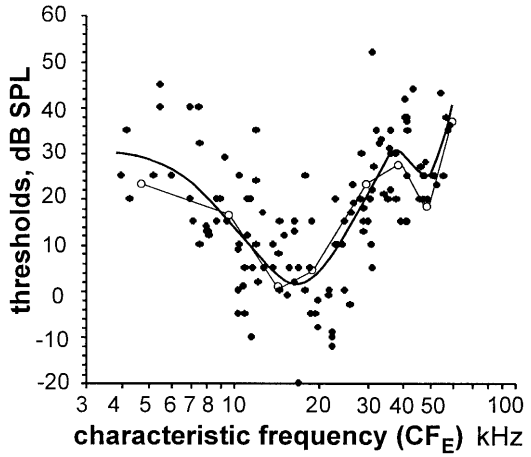

Fig. 1 Tone response thresholds of ICC neurons as a function of their characteristic frequency (CF_E). *Solid line* is a polynomial fit of the average neural thresholds. *Thin line with open circles* connects average behavioral thresholds (Ehret 1974)

Results

The results are based on a sample of 130 single units from the ICC recorded in 45 mice. All units responded to tone bursts and had CFs_E between 4.3 and 60 kHz. Tone-response thresholds as a function of CF_E are shown in Fig. 1 in comparison with the behavioral audiogram of the mouse (Ehret 1974). Lowest thresholds were obtained for neurons with CFs_E between about 10 and 25 kHz.

Classes of frequency response areas

Neurons were divided into four classes (class I, class II, class III, class IV). Examples of original recordings from which the classes have been determined are shown in Figs. 2 and 3. The objective and quantitative criteria for classification have been derived from the shape parameters of the excitatory frequency response areas as shown in Figs. 4 and 5 and from the degree of overlap of excitatory and inhibitory frequency response areas as explained below.

1. Class I neurons (n=54≙41.5%; Fig. 2a, b) were characterized by a steep slope on the high-frequency side of the excitatory response area and broadening of the frequency response area towards the low-frequency side, which may or may not include a low-frequency tail (first criterion, see Figs. 4, 5). The majority of class I neurons (n=45) had inhibitory areas flanking the excitatory area on both sides. The inhibitory areas always reached into the excitatory area, usually on both sides, but never expanded completely through it (second criterion). Other units with class I excitatory response areas (n=9) had inhibitory areas only on the low- (n=3) or high- (n=6) frequency side. Three units had areas of facilitation either below or above CF_E.

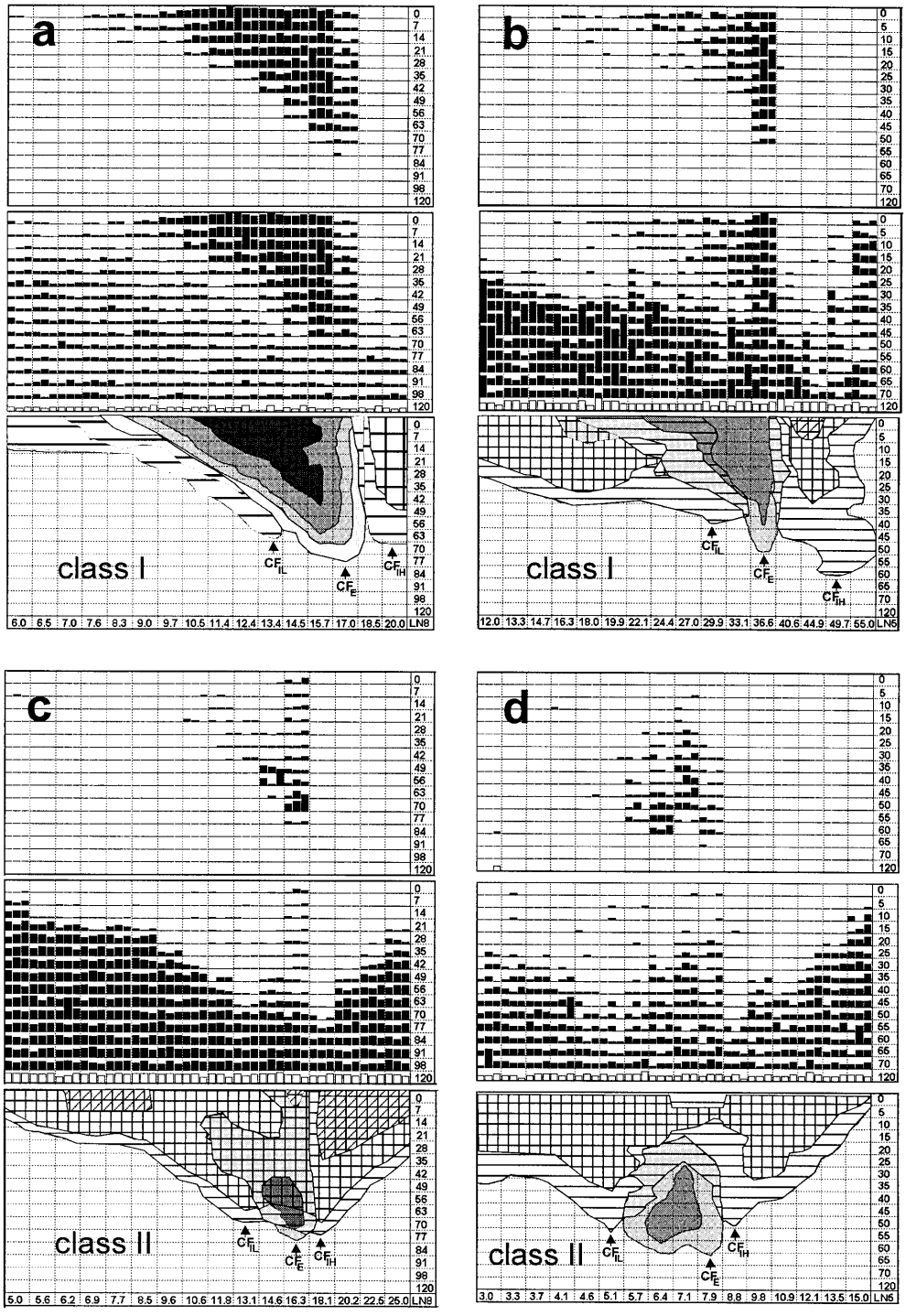

Fig. 2 Examples of frequency response areas of class I **(a, b)** and class II **(c, d)** neurons. Each subfigure consists of the same three parts: *the upper part* shows the excitatory response area determined with a single tone at 256 frequency- (*x*-axis, kHz) intensity (*y*-axis, dB attenuation values) combinations, all presented 3 times. *Three columns in each box* indicate the relative spike rate to a given frequency-intensity combination. *The central part of each figure* shows the excitatory and possible inhibitory response areas determined with the two-tone combination. One tone, 10 dB above threshold at CF_E, always elicits an excitatory response which is seen at all frequency-intensity combinations at which the variable second tone had no influence on the response of the neuron. The spike rate due to the CF_E tone is also shown in the responses corresponding to 120-dB attenuation. The second tone could increase the spike rate (e.g., in the excitatory response area), decrease it due to inhibitory effects (e.g., in the environment of the excitatory area) or have no effect. *The lower part of each figure* shows the excitatory and inhibitory response areas together with isoresponse contours (*grades of shading*) in the excitatory area and isoinhibitory contours (*hatched patterns*) in inhibitory areas. 0 dB (*y*-axis) corresponds to 85 dB SPL (CF_E excitatory characteristic frequency, CF_{IL} characteristic frequency of the low-frequency inhibitory area, CF_{IH} characteristic frequency of the high-frequency inhibitory area)

2. Class II neurons (n=36≙27.7%; Fig. 2c, d) had an excitatory response area that was restricted to a narrow bandpass with steep slopes (first criterion, see Figs. 4, 5). Areas of strong inhibition flanked both sides. Invariably, inhibition widely covered and reached through the excitatory area (second criterion) and led to a substantial reduction of the response, especially at higher sound levels resulting in tilted (3 neurons≙2.3%) and closed (10 neurons≙7.7%) excitatory areas (Fig. 2d). Two neurons had facilitation areas.

3. Class III neurons (n=32≙24.6%; Fig. 3a) showed a rather symmetrical widening of the excitatory frequency response area (i.e., V-shaped) with increasing SPL (first criterion, see Figs. 4, 5). Suppression in the inhibitory areas was weak, and often did not reach the excitatory response area (second criterion). Five units had no inhibitory areas below the CFs_E and 10 units had no such areas above the CFs_E. Four neurons had areas of facilitation either below or above CF_E.

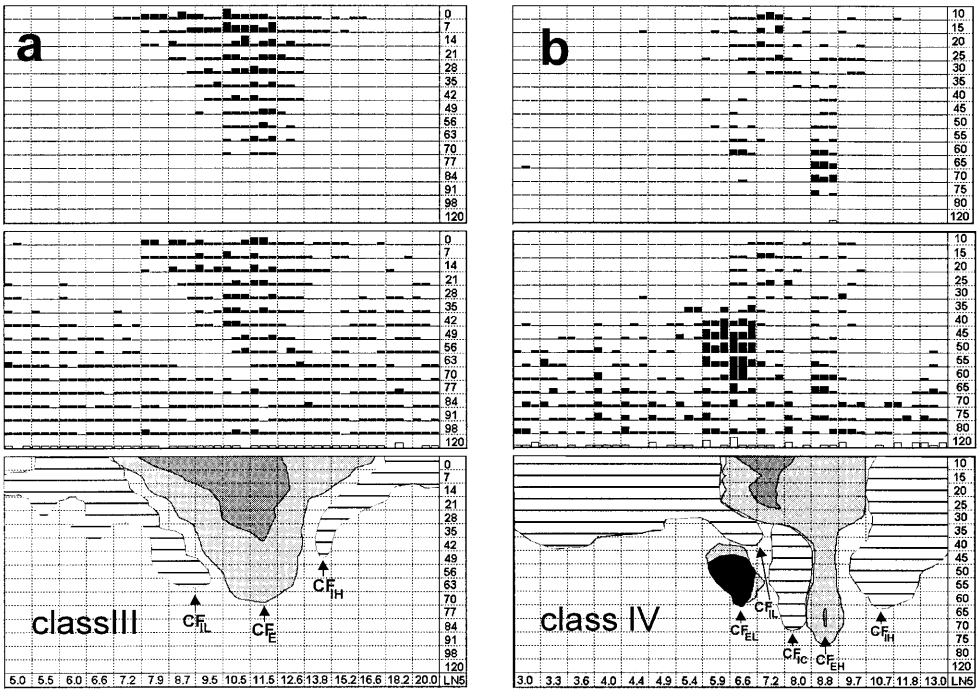

Fig. 3 Examples of frequency response areas of class III **(a)** and class IV **(b)** neurons. In **b** 10 dB (*y*-axis) corresponds to 90 dB SPL. The class IV neuron **(b)** has two excitatory characteristic frequencies (CF_{EL}, CF_{EH}) and three inhibitory characteristic frequencies (CF_{IL}, CF_{IC}, CF_{IH}). In the two-tone paradigm (*central part of* **b**), the CF_E tone was at CF_{EH} and the second tone disclosed a small facilitatory area near CF_{EL}. For further explanation see legend to Fig. 2

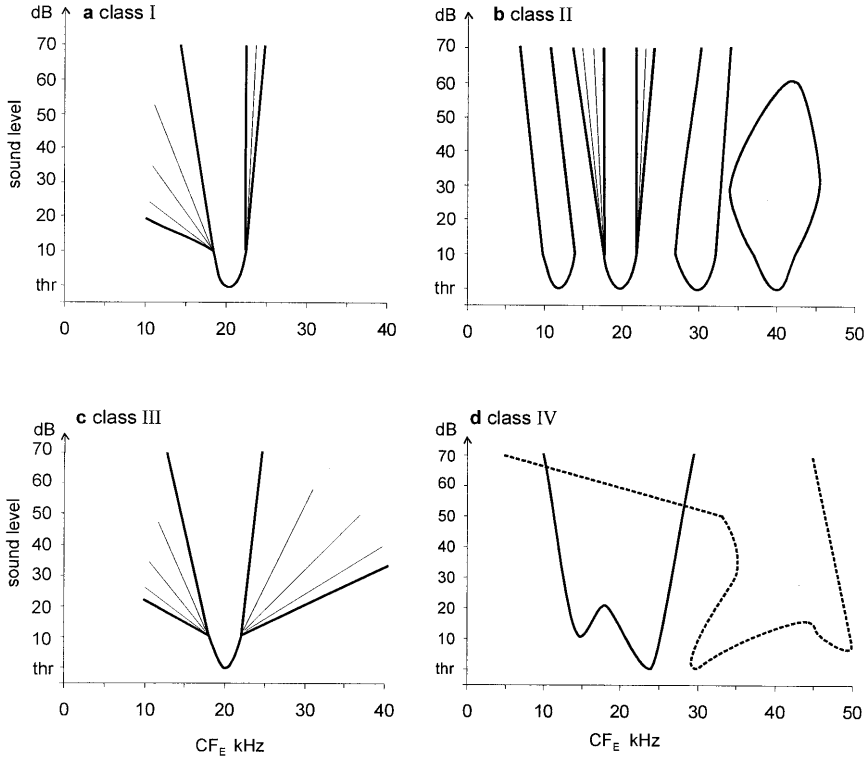

Fig. 4a–d Schemes of excitatory response areas of neurons in the four classes. The curves are placed on arbitrary positions on the CF_E axis. In **a–c**, the ranges of slopes (dB/octave) of excitatory tuning-curves found in classes I–III are shown as *thin lines bordered by thick lines*

4. Class IV neurons (n=8≙6.2%; Fig. 3b) had complex forms of excitatory and inhibitory receptive fields with more than one CF_E occurring (criterion, see Fig. 4). Inhibitory areas flanked the excitatory response area on both low- and high-frequency sides. Six neurons had inhibitory areas between the CFs_E of the excitatory area. In six of the eight neurons, facilitation of the response to one of the CFs_E occurred by a second tone placed in the second excitatory peak area (Fig. 3b).

We found that 121 (93%) of the 130 neurons studied could be classified in the same way by using only the shape of the excitatory response area (as shown in

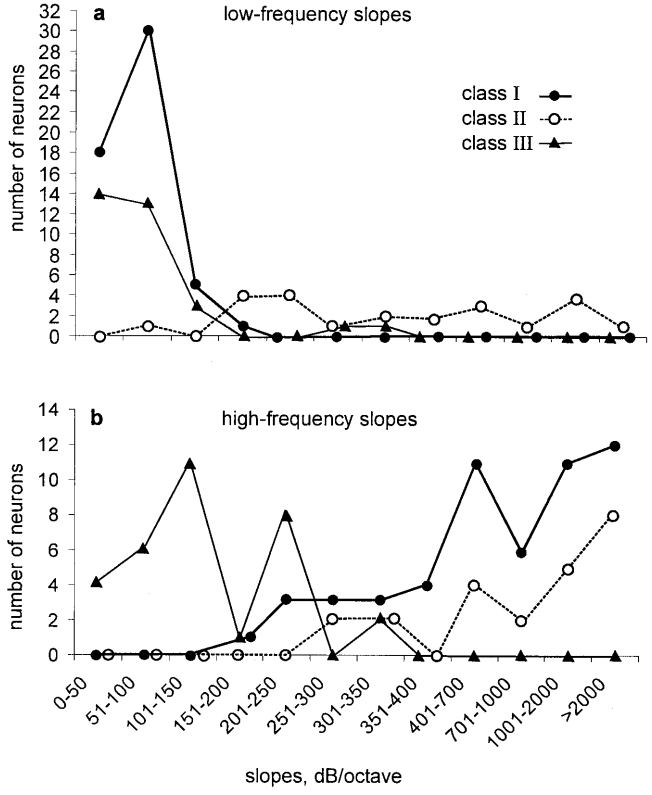

Fig. 5a, b Classification of neurons in classes I–III according to the slopes (dB/octave) of their excitatory tuning-curves (calculated between 10 dB above threshold at CF_E and the highest sound levels applied). Class I and III neurons separate well from class II neurons by low-frequency slopes of 150 dB/octave or less **(a)**. Class III neurons separate well from class I and II neurons by high-frequency slopes of 250 dB/octave or less **(b)** (cf. text)

Fig. 4). Figure 5a demonstrates that class II neurons were quantitatively different from class I and class III neurons in that they had significantly steeper low-frequency slopes (i.e., >150 dB/ocave) than class I and class III neurons (χ^2-test, $P<0.001$ in each case). Figure 5b shows that class III neurons had significantly shallower high-frequency slopes (<250 dB/octave) compared with class I and class II neurons (χ^2-test, $P<0.001$ in each case). Neurons from all four classes have been found over the whole CF_E range. Class II neurons rarely had CFs_E above 46 kHz (n=3) and class III neurons rarely below 10 kHz (n=3).

In the following, we will quantify and compare additional properties of excitatory and inhibitory frequency areas of the neurons in the four classes and show that there are further significant differences between the classes.

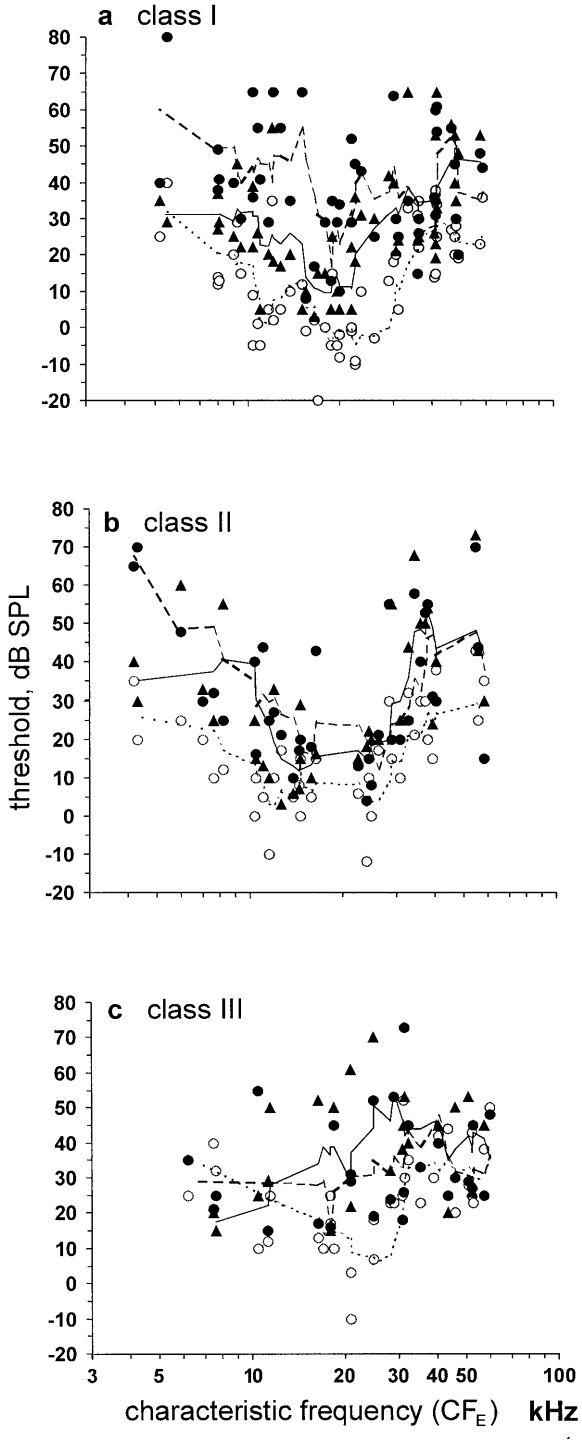

Fig. 6 Excitatory and inhibitory thresholds and average curves (polynomial fits) as a function of the neurons' CF_E in the three classes **(a–c)** indicated [*black dots-dashed lines* thresholds of low-frequency inhibition (at CF_{IL}), *open circles-dotted lines* excitatory thresholds (at CF_E), *black triangles-solid lines* thresholds of high-frequency inhibition (at CF_{IH})]

Thresholds of excitation and inhibition

Excitatory response thresholds at CF_E and the lowest thresholds of inhibition below and above the CF_E are plotted against the CF_E in Fig. 6 for the three main classes of neurons. The frequency dependence of the average excitatory thresholds of class I neurons (Fig. 6a) is very similar to that of all sampled units (Fig. 1). Average inhibitory thresholds in class I units up to about 30 kHz CF_E repeat the curve shape of average excitatory thresh-

olds. Thresholds of inhibition at the best inhibitory frequency below the CF_E (CF_{IL}) are about 20–50 dB higher than thresholds of excitation, and thresholds of inhibition at the best inhibitory frequencies above the CF_E (CF_{IH}) are typically 5–20 dB higher than thresholds of excitation (Fig. 6a). For units with CFs_E higher than about 30 kHz, average thresholds of high- and low-frequency inhibition are similar and about 10–30 dB higher than excitatory thresholds. Altogether, inhibitory thresholds below the CF_E are significantly higher than those above the CF_E (Mann-Whitney *U*-test with the differences between excitatory and inhibitory thresholds below and above CF_E respectively, $P<0.005$).

The CF_E dependence of the excitatory and inhibitory thresholds in class II neurons (Fig. 6b) is similar to that of the class I units (Fig. 6a) and to that of the whole sample (Fig. 1). In these neurons, the average thresholds of low- and high-frequency inhibition do not differ systematically, and both are about 5–20 dB higher than the excitatory ones (Fig. 6b).

For class III neurons, average excitatory and inhibitory thresholds do not run in parallel (Fig. 6c) as in the other two classes of neurons (Fig. 6a, b). There are no statistically significant differences between average inhibitory thresholds above and below CF_E in this class of neurons. Class III neurons have higher excitatory thresholds than both class I ($P<0.005$, *U*-test) and class II neurons ($P<0.01$). Also, inhibitory thresholds above the CF_E are higher in class III neurons than in class I neurons ($P<0.05$).

Relationships between excitatory and inhibitory thresholds are shown for the three classes of neurons in Fig. 7. In class I and class II neurons (Fig. 7a, b), thresholds from low- and high-frequency inhibitory areas correlate significantly ($P<0.05$ for low-frequency inhibition in class I units; $P<0.001$ for the other cases) with excitatory thresholds. In class III neurons, there is no relationship between excitatory and inhibitory thresholds (Fig. 7c). A statistical comparison of the slopes of the regression lines (Sachs 1999) indicates the following significant differences: the slope (0.35) of low-frequency inhibition in class I units is shallower than the slope (0.73) of high-frequency inhibition in the same units ($P<0.05$) and shallower than the slope (0.95) of low-frequency inhibition in the class II neurons ($P<0.002$). Slopes (0.95, 0.81) for low- and high-frequency inhibition in class II units do not differ significantly.

Complex neurons (class IV) were defined as having a rather W-shaped excitatory area with a low-frequency CF_E (CF_{EL}) and a high-frequency CF_E (CF_{EH}). The response thresholds at the two CFs_E were either equal within 5 dB ($n=2$), or the threshold at the CF_{EH} was more than 5 dB lower than that at CF_{EL} ($n=6$; e.g., Fig. 3b). In six of the units, there was an inhibitory area between the two CFs_E so that these neurons had three inhibitory areas and, in addition to the CF_{IL} and CF_{IH}, a CF_{IC} (central characteristic inhibitory frequency). The threshold at CF_{IC} was always between those at CF_{EL} and CF_{EH}.

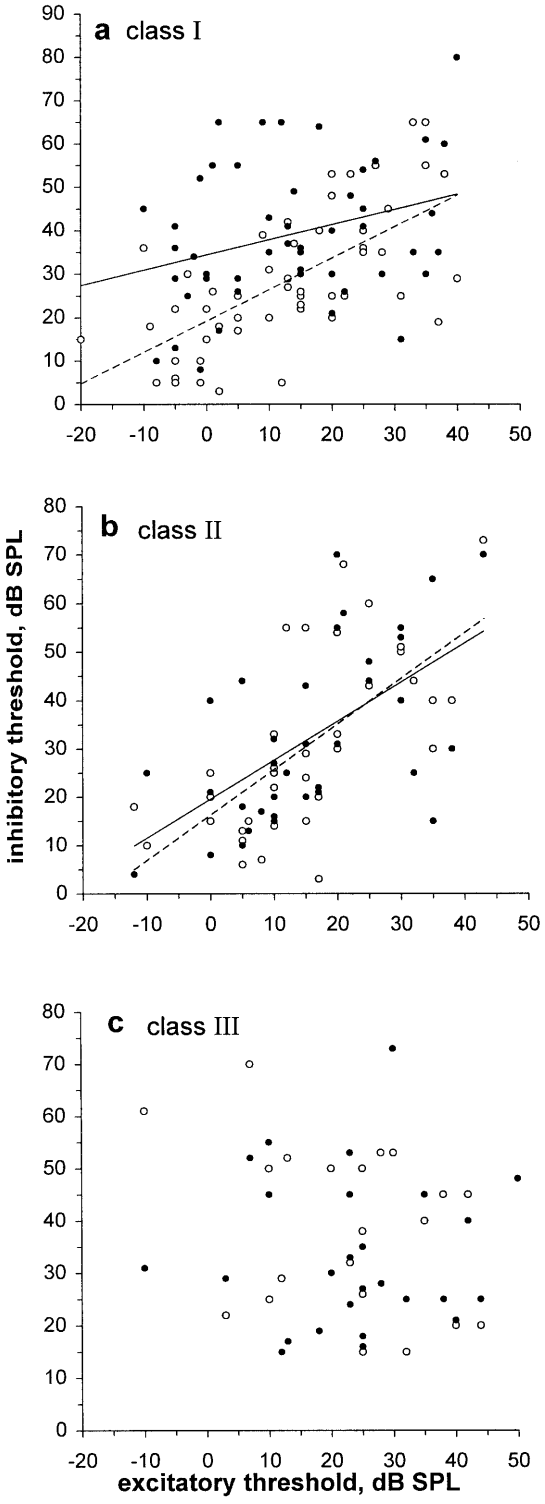

Fig. 7 Relationships between excitatory and inhibitory thresholds of neurons in the three classes **(a–c)** indicated. *Linear regression lines* show statistically significant relations (*black dots-heavy lines* thresholds of low-frequency inhibition, *open circles-dashed lines* thresholds of high-frequency inhibition)

Characteristic frequency of excitation and inhibition

The relationships between CF_E (CF_{EH} in class IV) and the inhibitory CFs (CF_{IL}, CF_{IH}, CF_{IC}) are shown for all four classes of neurons in Fig. 8. All correlations are statistically highly significant ($P<0.001$ in each case). The regression lines follow the equations (frequencies in kHz):

class I,	low-frequency inhibition:	$CF_{IL}=0.83\ CF_E-1.2$;	$r=0.944$,	$n=48$	(1)
	high-frequency inhibition:	$CF_{IH}=1.12\ CF_E+2.4$;	$r=0.986$,	$n=51$	(2)
class II,	low-frequency inhibition:	$CF_{IL}=0.92\ CF_E-1.3$;	$r=0.987$,	$n=36$	(3)
	high-frequency inhibition:	$CF_{IH}=1.03\ CF_E+2.6$;	$r=0.990$,	$n=36$	(4)
class III,	low-frequency inhibition:	$CF_{IL}=0.95\ CF_E-3.7$;	$r=0.960$,	$n=27$	(5)
	high-frequency inhibition:	$CF_{IH}=0.98\ CF_E+8.6$;	$r=0.962$,	$n=22$	(6)
class IV,	low-frequency excitation:	$CF_{EL}=0.70\ CF_{EH}+2.7$;	$r=0.929$,	$n=8$	(7)
	low-frequency inhibition:	$CF_{IL}=0.72\ CF_{EH}-0.16$;	$r=0.973$,	$n=8$	(8)
	central-frequency inhibition:	$CF_{IC}=0.97\ CF_{EH}-1.6$;	$r=0.996$,	$n=8$	(9)
	high-frequency inhibition:	$CF_{IH}=1.02\ CF_{EH}+3.2$;	$r=0.943$,	$n=8$	(10)

The slopes of the regression lines for CF_{IL} and CF_{IH} differ significantly in class I ($P<0.001$) and class II ($P<0.005$) neurons. Further, the slope of CF_{IH} for class I units is significantly steeper than that for class II ($P<0.01$) and class III ($P<0.05$) neurons. Despite these differences, all slopes of class I, II, and III neurons are close to 1.0. Thus, one can take the *y*-intercepts at $CF_E=0$ as average distances between CF_E and CF_{IL}, and CF_E and CF_{IH}. For these three classes of neurons, the CFs_{IH} have about twice the frequency distance from CFs_E compared with CFs_{IL} (2.4 vs 1.2; 2.6 vs 1.3; 8.6 vs 3.7 kHz). The absolute distances, however, are much larger (about threefold) in class III neurons than those of neurons in the other two classes.

Frequency bandwidth of excitatory and inhibitory response areas

As the examples of frequency response areas shown in Figs. 2 and 3 demonstrate, for most units the low-frequency borders of inhibition below the CFs_E and the high-frequency borders of inhibition above the CFs_E could not be determined over the whole intensity range because of the frequency limits of our tone stimuli (two octaves below CF_E and one octave above the CF_E). Hence, in many units the borders of inhibitory areas could be obtained only up to 40 dB or 50 dB above the threshold at CF_E. To be able to compare the bandwidths of excitatory and inhibitory response areas of neurons with various CFs_E, thresholds, and shapes of response areas among class I–III neurons, we did the following: For each neuron, a one-octave and a two-octave bandwidth was placed symmetrically around its CF_E (i.e.,

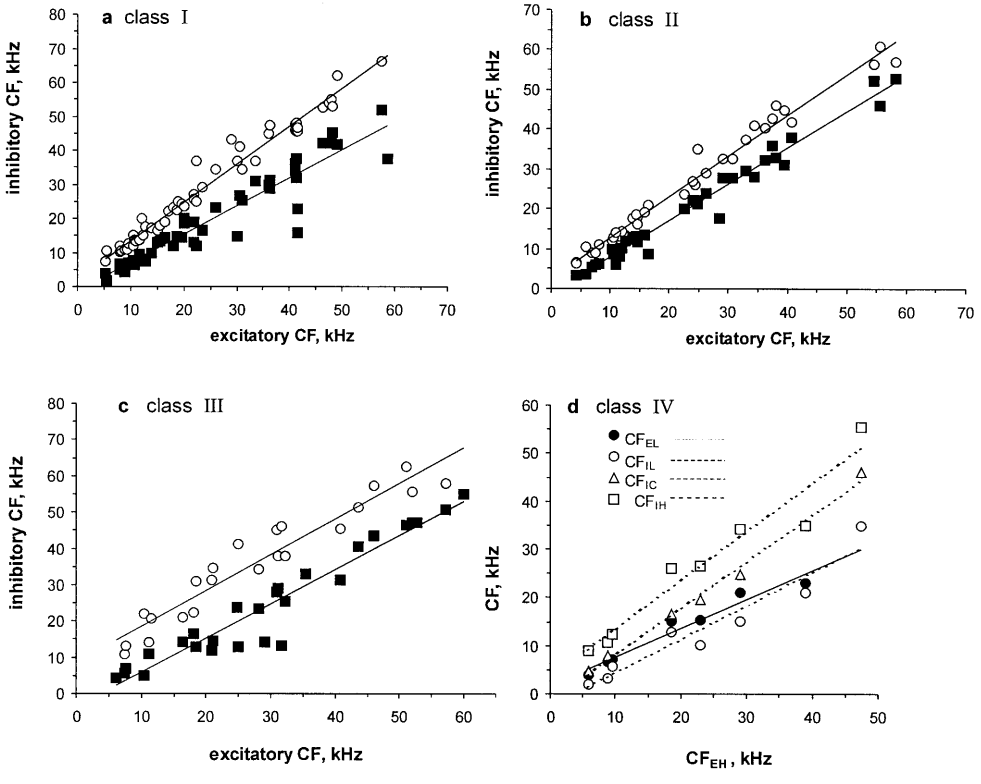

Fig. 8 Relationships between excitatory and inhibitory characteristic frequencies of neurons in the four classes **(a–d)** indicated. *Linear regression lines* show statistically significant relations (Eqs. 1–10) [**a–c** *black squares* low-frequency inhibitory CFs (CF_{IL}), *open circles* high-frequency inhibitory CFs (CF_{IH})]

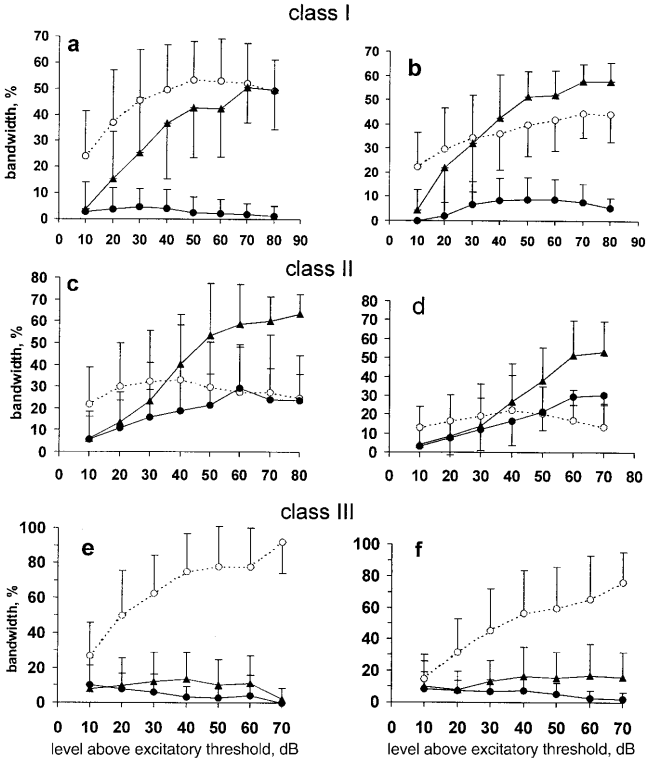

Fig. 9 Average percentages and standard deviations (shown only unilaterally for clarity) of the bandwidths of the excitatory area (*open circles, dotted lines*), the low-frequency inhibitory area (*black dots*) and the high-frequency inhibitory area (*black triangles*) within one octave **(a, c, e)** and two octaves **(b, d, f)** around CF_E as a function of the level above excitatory threshold

CF_E±1/2 octave and CF_E±1 octave), and within these frequency bandwidths the percentages were calculated occupied by the excitatory and the inhibitory response areas (inhibitory areas below and above CF_E separately) for tone levels of 10 dB, 20 dB,..., 80 dB above CF_E threshold. The percentages of the excitatory area and of the inhibitory areas within these one-octave or two-octave bandwidths were averaged for all neurons within each class and plotted in Fig. 9. Because of the possible overlap of excitatory and inhibitory areas (cf. Figs. 2, 3), the percentages may add up to more than 100%.

In the class I neurons, the proportion of the one-octave (±1/2 octave; Fig. 9a) or the two-octave (±1 octave; Fig. 9b) bandwidths occupied by the low-frequency inhibitory area is always below average 10%, and rather independent of the tone level. The proportion of bandwidths covered by the high-frequency inhibitory area and by the excitatory area both increase with increasing tone level and reach a plateau at about 50 dB superthreshold level, when all of the one-octave (Fig. 9a) and two-octave (Fig. 9b) bandwidths are filled with excitatory and inhibitory response areas. Figure 9b indicates that, on average, the high-frequency inhibitory area is wider than the excitatory area for tone levels of more than 40 dB above threshold.

Class II neurons (Fig. 9c, d) are characterized by rather constant, intensity-independent excitatory bandwidths covering a proportion of about 30% of the CF_E±1/2-octave bandwidths (Fig. 9c) and about 15% of the CF_E±1-octave bandwidths (Fig. 9d). The inhibitory areas of these units increase with increasing tone level. Finally, areas for low- and high-frequency inhibition become wider than the excitatory area within the CF_E±1-octave bandwidths (Fig. 9d).

Class III neurons are dominated by the excitatory response area (Fig. 9e, f). The inhibitory areas below and above the CF_E do not change much with increasing tone level and are very narrow, compared to the excitatory one. Together, they cover, on average, less than 20% of the one- or two-octave bandwidths around the CF_E (Fig. 9e, f).

Sharpness of excitatory and inhibitory tuning

Sharpness of tuning is often expressed by the Q_{10dB} value (CF_E divided by the bandwidth of the excitatory tuning curve 10 dB above threshold at CF_E). In analogy, we calculated Q_{10dB} values also for the inhibitory areas below and above the CFs_E using the CF_{IL} or the CF_{IH} as reference. In Fig. 10, the Q_{10dB} values of excitatory and inhibitory tuning are shown as a function of the CFs_E together with significant regression lines. Q_{10dB} values of excitatory areas (open circles, dotted lines) increase with increasing CF_E in all three classes of neurons. Q_{10dB} values of the low-frequency inhibitory areas (Q_{10IL}, black dots, dashed lines) increase significantly with increasing CF_E only in class II neurons. Q_{10dB} values of high-frequency inhibitory areas (Q_{10IH}, black triangles, solid lines) increase significantly only in class I and class III neurons. A statistical comparison (*U*-test) of Q_{10E}, Q_{10IL}, and Q_{10IH} values between the three classes of neurons (values from neurons with CFs_E <20 kHz and CFs_E >20 kHz were compared separately) did not reveal significant differences (P>0.1). However, statistically significant correlations between Q_{10dB} values from excitatory and inhibitory areas occurred (P<0.05) except for Q_{10IL} vs Q_{10E} in class I neurons. On average, the sharpness of excitatory and inhibitory tuning near absolute threshold correlated positively with the neuron's CF_E.

Further, we calculated Q values of the total excitatory areas obtained in the single-tone stimulation paradigm (the conventional tuning curves) and separately for their parts below and above the CFs_E and averaged them for neurons in the three main classes. Means and standard deviations are shown in Fig. 11. Linear regressions for values at 20 dB sound level and higher show that in class I neurons (Fig. 11a) the Q values from the whole excitatory bandwidths and from the part below the CF_E decrease significantly with increasing tone level (r=0.426, P<0.001, n=275; r=0.472, P<0.001, n=299). Q values from above the CFs_E do not show such a decrease. That is, excitatory tuning in these neurons becomes broader with increasing sound level, although only towards frequencies lower than the CFs_E. In class II neurons (Fig. 11b), the excitatory tuning is intensity independent.

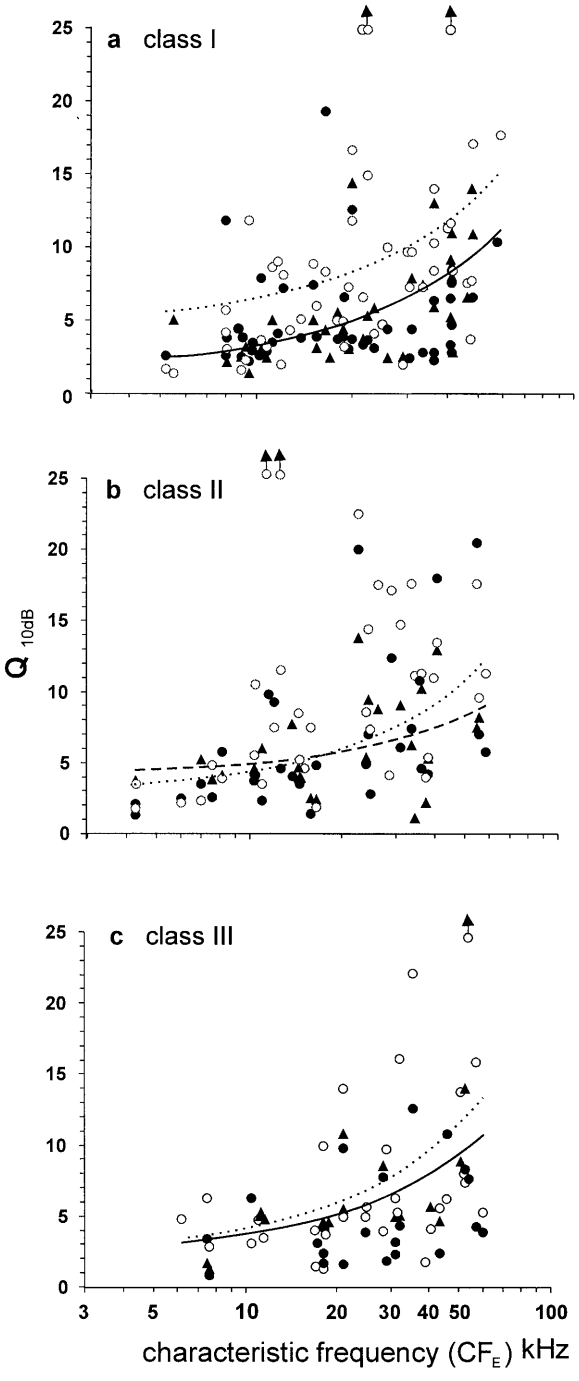

Fig. 10a–c Q_{10dB} values of the low-frequency inhibitory response areas (*black dots, dashed lines*), the excitatory response areas (*open circles, dotted lines*), and the high-frequency inhibitory response areas (*black triangles, solid lines*) as a function of the characteristic frequency of excitation of neurons in the classes as indicated. Linear regression lines of statistically significant relationships appear as curved functions because of the logarithmic scale on the *x*-axis

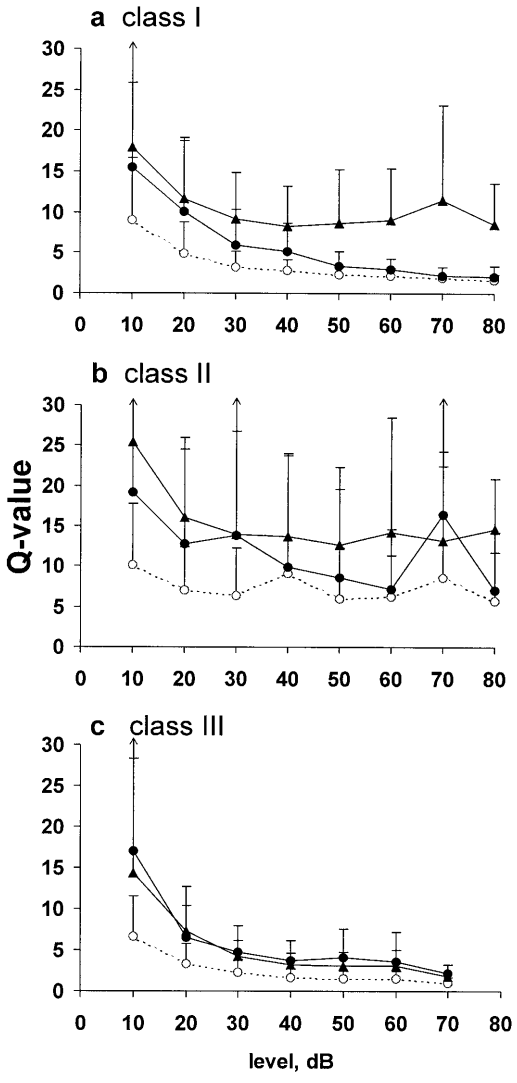

Fig. 11 Relationship between tone level above threshold and average *Q* values with standard deviations (shown only unilaterally for clarity) of the excitatory areas obtained at 10 dB, 20 dB,..., 80 dB above threshold at CF_E for neurons in the three classes **(a–c)** indicated (*open circles-dotted lines Q* values from the whole excitatory areas, *black dots Q* values from the excitatory areas below CF_E, *black triangles Q* values from the excitatory areas above CF_E)

In class III neurons (Fig. 11c), *Q* values from both the high- and low-frequency parts and, therefore, also those from the whole excitatory bandwidths decrease with increasing sound intensity (r=0.458, P<0.001, n=147; r=0,296, P<0.001, n=139; r=0.401, P<0.001, n=137) such that a symmetrical widening of the excitatory area with increasing sound level becomes apparent.

There are no significant differences of Q_{10dB} values between the classes of neurons. *Q* values measured at 20 dB, 30 dB..., up to 80 dB sound level above threshold are always larger (the tuning is sharper) in class II neurons than in class III neurons (P<0.05 for Q_{20}, P<0.005 for other *Q* values). Class II neurons also have larger *Q* values than class I units with regard to the whole excitatory area (P<0.05 for Q_{20}, P<0.005 for other *Q* values), the low-frequency part (all sound levels above 40 dB, P<0.01 except at 80 dB, P<0.05), and the high-frequency part (sound level of 20, 40, 50, and 80 dB with P<0.05). Class I neurons have larger *Q* values (whole excitatory area and high-frequency part of it) than class

III neurons at all sound levels above 10 dB ($P<0.01$ for whole excitatory area except $P<0.05$ at 20 dB; $P<0.005$ for the high-frequency part of the excitatory area).

These data show that, for most sound levels of more than 10 dB above threshold, class II neurons are more sharply tuned than class I and class III neurons. Class I units are, except for their low-frequency excitatory area, more sharply tuned than class III neurons.

Discussion

Tone-response thresholds

The ranges of tone-response thresholds and CFs of ICC neurons in the present study compare favorably with those obtained in the ICC of the mouse previously (Ehret and Moffat 1985). The average neural excitatory thresholds are virtually identical with the behavioral auditory threshold curve of the mouse (Fig. 1) determined with conditioning techniques (Ehret 1974, 1983).

Excitatory response areas and classification of neurons

Willott and Urban (1978) sampled frequency response areas of mouse IC neurons and found shapes of excitatory areas compatible with the present ones. Excitatory frequency response areas of mouse ICC neurons (n = 159) have been classified previously into seven classes (Ehret and Moffat 1985), all of which are contained in the four present ones. Class I neurons were identified in almost the same proportion previously (43% of neurons in the former classes A + E vs 41.5% in the present study). Class II neurons were observed more frequently previously (42% of neurons in former classes C + D + F compared to almost 28% in the present study). The reverse is true for broadly tuned class III neurons (13% vs 24.6%). Class IV neurons were rare in both studies (2% vs 6%). Thus, the main difference between the two studies concerns class II and class III neurons. The increased percentage of class II and decreased percentage of class III neurons in the former study may have been caused by the barbiturate anesthetics known to enhance central nervous inhibition (e.g., Nelson and Erulkar 1963; Kuwada et al. 1989; Clarey et al. 1992; Zurita et al. 1994). In addition, tuning curves in the former study have been measured only up to 40–50 dB above threshold at CF. Thus, low- and high-frequency tails of units would have been detected less frequently in the former study leading to a lower percentage of class III neurons.

Excitatory response areas of the same types as found here have been described for ICC neurons of cats (Gersuni et al. 1971; Aitkin et al. 1975; Ehret and Merzenich 1988a; Ramachandran et al. 1999), bats (Grinnell 1963; Suga 1969, 1971; Vater et al. 1979; Yang et al. 1992; Casseday and Covey 1992; Fuzessery and Hall 1996), and chinchillas (Wang et al. 1996). However, only Ehret and Merzenich (1988a) found a proportion similar to our present work (27% class II neurons vs 28% here; 23% class III neurons vs 25% here; 50% class I and class IV neurons vs 48% here). In the other studies, excitatory response areas have not been classified or the proportions differed considerably. For example, Ramachandran et al. (1999) reported 53% of neurons with closed (upper threshold) frequency-response areas in the decerebrated cat compared to only 5% in the anesthetized cat (Ehret and Merzenich 1988a) and only 8% in the mouse (present study).

Taking only the excitatory properties, neurons in the classes differ significantly by: (1) average absolute thresholds (class III different from the others), (2) the steepness of slopes of excitatory tuning curves (Figs. 4, 5), (3) the relative bandwidths of their excitatory areas as a function of tone intensity (cf. Figs. 9, 11), and (4) the number of threshold minima (class IV neurons different from the others).

Inhibitory response areas

Inhibitory response areas of ICC neurons have been measured in few studies. In five of these (Suga 1969; Möller 1978; Ehret and Merzenich 1988a; Fuzessery and Hall 1996), two-tone stimuli were used as in the present work. However, parameters characterizing the inhibitory response areas have not been quantified and their relationships to excitatory response areas have not been evaluated. In the other four studies (Suga 1964; Willott and Urban 1978; Wang et al. 1996; Ramachandran et al. 1999), inhibitory response areas were detected by inhibition of spontaneous activity with tone bursts of frequencies and intensities outside the excitatory frequency response area. The application of this "one-tone" inhibition paradigm to characterize inhibitory response areas is problematic, however, for two reasons. First, inhibitory effects can be quantified with precision only in neurons of sufficiently high spontaneous rates. Since a considerable number of units in the ICC (17–31%) have no or little (<1 spike/s) spontaneous activity (e.g., Ehret and Moffat 1985; Ehret and Merzenich 1988a) even in awake animals (Ryan and Miller 1978), inhibition in these neurons is not measurable. Second, and more important, inhibition of spontaneous activity may be an unequivocal indicator of inhibitory input to ICC neurons only if the spontaneous activity is an intrinsic property of ICC neurons. Intracellular recordings from ICC neurons in brain slices of the mouse devoid of ascending and descending input from other auditory centers except the ipsilateral dorsal nucleus of the lateral lemniscus have shown that multipolar and disk-shaped cells are not spontaneously active at all (Reetz and Ehret 1999), or that less than 10% of multipolar cells have spontaneous activity (Wagner 1994, 1996). In view of these data, spontaneous activity of ICC neurons measured in vivo seems to reflect input from other spontaneously active auditory centers projecting to the ICC, such as the ventral and dorsal cochlear nucleus (data from in vitro brain slice studies:

Hirsch and Oertel 1988; Manis 1990; Zhang and Oertel 1993a, 1993b, 1994; Golding and Oertel 1996, 1997; Ferragamo et al. 1998) rather than intrinsic, i.e., true spontaneous, activity of ICC neurons. Thus, inhibitory areas obtained from ICC neurons by Ramachandran et al. (1999) may be due to inhibition occurring presynaptic to ICC neurons.

In the present experiments we chose a low suprathreshold tone level for the excitatory tone (10 dB above threshold at CF) in order to establish inhibition by a second tone. Doing this, we avoided, at least reduced, habituation and the possibility that the excitatory tone itself activated inhibitory input from frequency areas close to the CF of the neuron. The presence of such inhibition is visible in non-monotonic rate-intensity functions peaking near the threshold at CF (Ehret and Merzenich 1988b). In addition, the potentially inhibitory tone started 5 ms earlier than the CF tone to inhibit fast excitatory input to the ICC found in intracellular in vivo (Covey et al. 1996; Pedemonte et al. 1997) and in vitro recordings (Reetz and Ehret 1999). Almost all first postsynaptic potentials recorded in these studies were excitatory ones. Inhibitory postsynaptic potentials were seen with a delay of some milliseconds. Hence, a simultaneous onset of the CF tone and the potentially inhibitory tone could have produced less efficient inhibition because the inhibitory input would have been too late to suppress a phasic response to the onset of the CF tone. With these settings of stimulus parameters in our two-tone inhibition paradigm, we have detected inhibitory influences on ICC neurons starting with two-tone suppression in the auditory nerve and ending with actions of intrinsic inhibition in the ICC. Differentiation between effects of two-tone suppression and inhibition will be discussed below separately for the classes of neurons.

We presented the sound free-field and contralateral to the recorded ICC from an angle (45°) which focused the sound directly in the ear canal of the mouse, because the natural pinna position of the mouse is at 45° from head midline (Chen et al. 1995). In this sound presentation, the contralateral input dominated by 5–30 dB for most frequencies between 10 and 60 kHz (Saunders and Garfinkle 1983; Chen et al. 1995). Since the overall shapes of excitatory tuning curves of mouse ICC neurons have been found to be independent of the azimuthal angle of the loudspeaker (Cain and Jen 1999), our classification of ICC neurons according to tuning curve shapes is comparable with previous data from monaural contralateral stimulation in the cat (Ehret and Merzenich 1988a).

We detected inhibitory response areas in all 130 neurons analyzed. Möller (1978) and Fuzessery and Hall (1996) also found inhibitory response areas in all of their ICC neurons recorded from awake horseshoe bats or anesthetized pallid bats. In the cat, 9% (Ehret and Merzenich 1988a) or 12% (Ramachandran et al. 1999) of the ICC neurons had no inhibitory areas. In the latter study, all class III neurons (their type V units, Ramachandran et al. 1999) had no inhibitory areas. This can be explained by the very low spontaneous activity of these neurons preventing a detection of inhibition in their study. The two-tone experiments (present study), however, indicate that most if not all ICC neurons show inhibitory areas. This conclusion is emphasized by studies in which application of antagonists of inhibitory receptors (binding γ-aminobutyric acid, GABA) directly demonstrated effects of inhibition on the shapes of frequency-response areas (Yang et al. 1992; Palombi and Caspary 1996; Fuzessery and Hall 1996), on the response magnitude within these areas (Faingold et al. 1989; Yang et al. 1992; Le Beau et al. 1996; Palombi and Caspary 1996; Fuzessery and Hall 1996) also as a function of the type of binaural input (Pollak and Park 1993; Park and Pollak 1993, 1994; Klug et al. 1995) and input timing (Casseday et al. 1994; Saitoh and Suga 1995; Lu et al. 1997, 1998; Burger and Pollak 1998).

Relations between and possible origins of excitatory and inhibitory response areas

Class I neurons

Class I neurons differ significantly from neurons in all other classes by asymmetries of inhibitory areas below and above CF_E. Inhibition below CF_E has higher average thresholds than inhibition above CF_E, especially for CF_E below about 30 kHz (Fig. 6a) and for neurons with low excitatory thresholds (Fig. 7a). Also, the inhibitory area in one- or two-octave bandwidths below the CF_E is smaller at all sound levels compared to that above CF_E (Fig. 9a, b), and Q_{10I} from inhibitory areas below CF_E does not correlate with Q_{10E}. In addition, the widths of excitatory areas below CF_E increase (Q values decrease) with increasing sound levels while those above CF_E remain rather constant (Figs. 4a, 11a). These properties together with the partial overlap of excitatory and inhibitory areas (Fig. 2a, b) reflect characteristics of excitatory and two-tone suppression areas of auditory nerve fibers. Response areas of mouse auditory nerve fibers have not been determined, so we will base our comparison on data from cats (Sachs and Kiang 1968; Arthur et al. 1971; Delgutte 1990) and Mongolian gerbils (Schmiedt 1982). Thresholds of low-frequency suppression in auditory nerve fibers are largely independent of (Schmiedt 1982) and always considerably (20–50 dB) higher than the threshold at CF_E (Sachs and Kiang 1968; Arthur et al. 1971; Delgutte 1990). This is what we see here for two-thirds of the individual neurons (Figs. 6a, 7a). Thresholds for high-frequency suppression are lower and closer to the CF_E threshold (Sachs and Kiang 1968; Schmiedt 1982; Delgutte 1990). This is true also for high-frequency inhibition (Fig. 7a). Further, we compared the relationship between CF_E and CF_{IH} or CF_E and CF_{IL} (Eqs. 1, 2; Fig. 8a) with equations calculated from the auditory nerve data. Taking CF_E, CF_{IL}, and CF_{IH} of the six neurons shown in Sachs and Kiang (1968) and the two neurons (only CF_E and CF_{IH}) in Delgutte (1990) to-

gether, we get for the cat: CF_{IL}=0.898 CF_E–0.591 (r=0.995, P<0.001) and CF_{IH}=1.192 CF_E+0.22 (r=0.993, P<0.001). From the nine auditory nerve fibers shown in Schmiedt (1982) for the gerbil, we extracted the CFs_{IH} (the CFs_E were given in the paper) and found the equation: CF_{IH}=1.09 CF_E+1.08 (r=0.981, P<0.001). These equations have slopes very similar to our Eqs. 1 and 2, and they have the slope differences we found (Fig. 8a). That is, properties of excitatory and suppression areas of auditory nerve fibers such as relationships between excitatory and suppression thresholds and between characteristic frequencies of excitation and suppression generally occur in the excitatory and inhibitory areas of class I neurons in the ICC.

We arrive at the following conclusion: About two-thirds of the neurons in the ICC that we termed class I have primary-like field characteristics. The areas of lateral inhibition observed by two-tone stimulation in these neurons seem to arise from areas of suppression in auditory nerve fibers created by non-linear interactions in the cochlea (Ruggero 1992). This conservation of shapes of peripheral excitatory and suppressive receptive fields up to the midbrain requires neurons in the lower brainstem with primary-like field properties such as type I neurons in the ventral cochlear nucleus (Young et al. 1988) projecting to the ICC (Romand and Avan 1997). In harmony with that, many neurons have been found in the ICC responding to a loss or reduction of GABAergic inhibition in the ICC with increases of spike rates to tones within their excitatory response areas but not with increases of the sizes of these areas (Yang et al. 1992; Palombi and Caspary 1996; Fuzessery and Hall 1992). The majority of these neurons have class I (primary-like) or class III (V-shaped) excitatory response areas (Yang et al. 1992). The other one-third of class I units deviate from primary-like characteristics, mainly by low threshold levels of inhibition compared to the threshold at CF_E (e.g., Figs. 2b, 7a) or by only unilateral inhibitory fields. This shows that the excitatory receptive field of auditory nerve fibers can be preserved in class I neurons of the ICC while the properties of lateral suppression are modified by more central mechanisms.

Class II neurons

They differ significantly from neurons in all other classes with steep slopes on both sides of excitatory tuning curves (Figs. 4b, 5) and often symmetrical inhibitory areas reaching far into or even through the narrow excitatory area. Symmetry occurs in average thresholds and relationships between excitatory and both inhibitory thresholds (Figs. 2c, d, 7b), in increasing proportions within a one- or two-octave bandwidth around CF_E of inhibitory areas below and above CF_E with increasing sound level (Fig. 9c, d), in similar relations between Q_{10IL} and Q_{10IH} with Q_{10E}, and rather constant excitatory bandwidths for sound levels 20 dB and more above threshold at CF_E (Fig. 11b). These properties are not present in auditory nerve fibers and could be the result of excitatory and inhibitory interactions visible, for example, in response areas of type IV neurons of the dorsal cochlear nucleus. These neurons project to the ICC (Romand and Avan 1997), and several of them have response areas (Young and Brownell 1976; Young et al. 1988; Spirou and Young 1991) resembling those of our class II neurons. Studies in which GABAergic inhibition within the ICC was reduced or blocked have shown (Yang et al. 1992; Palombi and Caspary 1996; Fuzessery and Hall 1996) that the shapes of excitatory and inhibitory areas, especially of class II neurons according to our nomenclature (Yang et al. 1992), emerge as the result of excitatory and dominating inhibitory inputs. This means that class II units are inhibition-dominated neurons. Since there are several types of inhibitory neurons in the ICC (e.g., Oliver et al. 1994), the shaping of response areas of class II neurons could be an intrinsic property of the ICC itself, not only of convergence of ascending excitatory and inhibitory input (Helfert and Aschoff 1997).

Class III neurons

Class III neurons differ significantly from neurons in all other classes by lacking a relationship between excitatory and inhibitory thresholds (Fig. 7c), by showing no differences of slopes of the relations between excitatory and inhibitory CFs and larger distances between excitatory and inhibitory CFs than in the other classes (Eqs. 5, 6; Fig. 8), by constantly low proportions of inhibitory bandwidths within one or two octaves around CF_E (Fig. 9e, f), by a constant and symmetrical widening of excitatory response areas below and above CF_E with increasing sound level (Figs. 4c, 11c), by unilateral inhibitory areas in a large proportion (47%) of the neurons, and by no or little overlap between excitatory and inhibitory response areas (Fig. 3a). In addition, they have, on average, higher excitatory thresholds than class I and class II neurons (Figs. 6, 7). Together, these properties are not present in excitatory and suppression areas of auditory nerve fibers. Although class III neurons have variable tone response patterns, with their broad tuning and small inhibitory fields they resemble onset neurons of the ventral cochlear nucleus (Rhode and Smith 1986a). Such neurons are not frequent in the dorsal cochlear nucleus (Rhode and Smith 1986b; Rhode 1991; Romand and Avan 1997), the superior olivary complex (Goldberg and Brown 1969; Guinan et al. 1972; Harnischfeger et al. 1985), and the nuclei of the lateral lemniscus (Aitkin et al. 1970; Huffman et al. 1998; Kelly et al. 1998; Bajo et al. 1998). Thus, their excitatory and inhibitory properties may arise from convergence of excitatory and inhibitory input to the ICC. The rules or contributing neural populations for this convergence must be different from those leading to class I and class II neurons because the frequency distances between CF_E and both CF_{IL} and CF_{IH} are about 3 times the distances found in the other two classes of neurons (Eqs. 1–6, Fig. 8). Further, the

overlap of excitatory and inhibitory areas in class III neurons is negligible compared with that in the other two classes (Figs. 2, 3).

Class IV neurons

Class IV neurons with two branches of excitatory response areas were rarely encountered. Besides low- and high-frequency inhibitory areas, often a central inhibitory area between CF_{EL} and CF_{EH} occurred. The comparison of the CF relationships of class IV (Eqs. 7–10, Fig. 8d) with those of neurons of the other classes (Eqs. 1–6, Fig. 8a–c) suggests that receptive field characteristics of class IV neurons arise from the convergence of two inputs having class I and/or class II characteristics. W-shaped excitatory fields have been observed in small numbers of neurons of the dorsal cochlear nucleus (Rhode and Smith 1986b; Spirou and Young 1991) and the superior olivary complex (Guinan et al. 1972).

General properties of excitation and inhibition in the ICC

Some neurons in all four classes (altogether 11.5%) had areas of facilitation (in addition to inhibitory areas) below or above but always within ±1 octave of the CF_E. The facilitatory areas ranged between 2.8 and 52 kHz and the CFs_E of the respective neurons between 3.8 and 41 kHz. Hence, facilitation occurs virtually all through the tonotopy of the mouse ICC (Stiebler and Ehret 1985; Romand and Ehret 1990). Besides combination-sensitive neurons in the mustached bat ICC showing facilitation to certain frequency and delay combinations of the biosonar pulse and its echo (Mittmann and Wenstrup 1995; Yan and Suga 1996; Wenstrup et al. 1999), there seem to be only one study each of the auditory midbrain of frogs (Fuzessery and Feng 1983) and cats (Ehret and Merzenich 1988a) in which spectral facilitation has been observed. Since spectral facilitation has already been found in the ventral cochlear nucleus (Winter and Palmer 1995; Palmer et al. 1995), it can be expected to occur in the ICC.

All our 130 neurons had inhibitory receptive fields. In 106 (81.5%) of the neurons, inhibition was present on both sides of the excitatory field. In most neurons, the inhibitory areas most likely extended beyond the bandwidth limits within which we sampled. That is, the great majority if not all neurons of the mouse ICC had inhibitory receptive fields broader than their own excitatory fields. It seems that sound processing in the ICC is dominated by inhibition. Inhibitory mechanisms in the ICC can, for example, ensure the separation of spectral peaks by establishing critical bands (Ehret and Merzenich 1985, 1988a), a necessary condition for the perception and recognition of complex sounds.

We show that inhibitory CFs are linearly related to excitatory CFs in all classes of neurons, suggesting that they are based on the same frequency scale. This scale is based on the cochlear tonotopy which is reproduced in the regularity of frequency maps in most of the auditory brainstem centers including the ICC (e.g., Rouiller 1997). Hence, we propose that inhibitory projections and interactions in the ICC are tonotopically organized comparable to the excitatory ones (e.g., Ehret 1997). It is important to note that 93% of our neurons could be classified into classes I–IV by using the shapes of the excitatory response areas as a qualitative and the steepness of slopes of the tuning curves as a quantitative measure for the class separation. This indicates that the shape of the excitatory response area of the great majority of ICC neurons is an excellent predictor for the shapes of their inhibitory response areas.

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