Corticofugal Shaping of Frequency Tuning Curves in the Central Nucleus of the Inferior Colliculus of Mice

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Yan, Yun, Yunfeng Zhang, and Günter Ehret. Corticofugal shaping of frequency tuning curves in the central nucleus of the inferior colliculus of mice. J Neurophysiol 93: 71–83, 2005. First published August 25, 2004; doi:10.1152/jn.00348.2004. Plasticity of the auditory cortex can be induced by conditioning or focal cortical stimulation. The latter was used here to measure how stimulation in the tonotopy of the mouse primary auditory cortex influences frequency tuning in the midbrain central nucleus of the inferior colliculus (ICC). Shapes of collicular frequency tuning curves (FTCs) were quantified before and after cortical activation by measuring best frequencies, FTC bandwidths at various sound levels, level tolerance, Q-values, steepness of low- and high-frequency slopes, and asymmetries. We show here that all of these measures were significantly changed by focal cortical activation. The changes were dependent not only on the relationship of physiological properties between the stimulated cortical neurons and recorded collicular neurons but also on the tuning curve class of the collicular neuron. Cortical activation assimilated collicular FTC shapes; sharp and broad FTCs were changed to the shapes comparable to those of auditory nerve fibers. Plasticity in the ICC was organized in a center (excitatory)-surround (inhibitory) way with regard to the stimulated location (i.e., the frequency) of cortical tonotopy. This ensures, together with the spatial gradients of distribution of collicular FTC shapes, a sharp spectral filtering at the core of collicular frequency-band laminae and an increase in frequency selectivity at the periphery of the laminae. Mechanisms of FTC plasticity were suggested to comprise both corticofugal and local ICC components of excitatory and inhibitory modulation leading to a temporary change of the balance between excitation and inhibition in the ICC.

INTRODUCTION

Excitatory frequency tuning curves (FTCs) delimitate the excitatory frequency response areas of auditory neurons and thus are an important and basic measure of frequency selectivity and spectral resolution of the auditory system. FTC-shapes of auditory nerve fibers are rather uniform (e.g., Kiang et al. 1965; Liberman 1978) but show great variability in the central auditory system, including primary-like, closed, tilted, narrow, broad, symmetrical, unsymmetrical, and multi-peaked (complex) shapes. These various shapes of the FTCs are the result of the integration of excitatory and inhibitory inputs over a broad frequency range (e.g., Cresswell and Covey 1992; Egorova et al. 2001; Ehret and Merzenich 1988; Lu and Jen 2001; Suga 1969; Sutter et al. 1999; Yang et al. 1992; Young et al. 1988).

The auditory cortex (AC) can influence, via corticofugal projections, neural response properties to sounds in lower centers of the ascending auditory pathway. Thus cortical plasticity induced by learning or state of vigilance (e.g., Edeline 2003; Scheich et al. 1997; Suga et al. 2002; Weinberger 1998) may feed back to the ascending auditory system. For the central nucleus of the inferior colliculus (ICC), such feedback has been shown in electrophysiological studies on bats (Ma and Suga 2001; Sun et al. 1989; Yan and Suga 1996, 1998; Zhang and Suga 1997; Zhang et al. 1997; Zhou and Jen 2000a,b) and other mammals (Syka and Popelař; 1984; Syka et al. 1988; Torerolo et al. 1998; Yan and Ehret 2001, 2002). Focal stimulation or suppression in the mustached bat’s fovea region, the Doppler-shifted-CF processing area (Suga and Jen 1976), of the AC lead to a shift of best frequencies (BFs) of ICC neurons away from or toward the frequency of the cortical tonotopy where the cortical output is manipulated (Zhang and Suga 2000; Zhang et al. 1997). Electrical stimulation at a given frequency of the cortical tonotopy of the big brown bat shift the BFs of ICC neurons toward the stimulated cortical frequency without much influence on the tuning curve shape (Gao and Suga 2000; Yan and Suga 1998). Such an egocentric shift is also seen in the mouse ICC (Yan and Ehret 2001, 2002). Altogether corticofugal modulation greatly shifts ICC BFs. This should practically alter the FTC shapes. So far, it is not clear how corticofugal activity might change FTC shapes or selectivity of frequency tuning and thus the spectral analysis of sounds by neurons in the ICC.

Here, we quantified the influence of the AC on the FTCs of ICC neurons for the same neurons before and after cortical activation. The FTC classification according to Egorova et al. (2001) was introduced to analyze possible effects of corticofugal modulation on neurons of different FTC shape in a quantitative way. This classification (classes I–IV) contains all seven classes of FTCs from a previous study on the ICC of the mouse (Ehret and Moffat 1985) and is compatible in the relative numbers of neurons per class with measurements in the cat ICC (Ehret and Merzenich 1988) and primary auditory cortex (Sutter 2000). We show that corticofugal activation not only changes neuronal BFs and minimum thresholds (MTs) but also has differential effects on the sharpness of frequency tuning. The direction (sharpness increase or decrease) and magnitude of the effect depends on the FTC shape before cortical activation. Our data support the hypothesis that the auditory cortex significantly modulates local balances between...
excitation and inhibition in the auditory midbrain and thus specifies frequency tuning to preferentially process sounds of behavioral significance that have repeatedly elicited activity at a certain spot of the tonotopy of the primary AC before.

**METHODS**

**Subjects and surgery**

Twenty-five female house mice (Mus musculus, outbred strain, NMRI) aged 6–12 wk and weighing 23–31 g were used in the present experiments. All animals were housed with littermates of the same sex in plastic (Makrolon) type III cages with free access to food and water and with a 12-h day-night cycle. Animals were anesthetized with a mixture of ketamine (Ketavet, 120 mg/kg ip) and xylazine (Rompun, 5 mg/kg ip). Additional doses of ketamine (35 mg/kg) and xylazine (1 mg/kg) were applied when necessary to keep the animal in a quiet and motionless state during the experiment. The scalp overlaying the skull in a horizontal plane. Two holes of ~2 mm in diameter were drilled in the skull to expose the ICC and AC, both of the left side, drilled in the skull to expose the ICC and AC, both of the left side, which was contralateral to the position of the loudspeakers. The dura was driven into the cortex perpendicularly to the brain surface, and the electrode was advanced to a depth of ~700 μm below the surface of the AI for stimulation of the cortical layer V (Paxinos and Franklin 2001; Sidman et al. 1971). Layer V pyramidal cells of the AI send massive descending projections to the ICC (Doucet et al. 2003). An indifferent electrode was placed on the brain surface just beside the stimulating electrode. A 1-ms long monophasic electrical pulse with 500-nA constant negative current was created by a constant current isolator (WPI A360) that was triggered with the CED 1401-plus. Every electrical pulse was synchronized to the offset of a tone burst of which frequency and amplitude was set at the BF and 10 dB above MT of the measured cortical neurons, respectively (Yan and Ehret 2002). The combined acoustic and electric stimuli were delivered to the cortex through the stimulating electrode at a rate of 4/s for 7 min. With regard to recommended microstimulation of the human cortex (Dostrovski 1999), we used the same type of electrode and stimulus at about one-third of current density over time.

This stimulation paradigm ensured a constant relationship between acoustical and electrical stimulation in which the AI neurons were never electrically stimulated before the tone-evoked response had occurred (response latencies of AI neurons are ~10–30 ms for tone bursts with 5-ms rise/fall times) (Phillips 1998). Thus the contingency of the tonal and electrical stimulation is comparable with a conditioning paradigm using the electrical pulses as an unconditioned stimulus evoking a cortical response and the tone bursts as the stimuli to be conditioned (conditioned stimulus). The cortical response to every tone stimulus is reinforced by a presumably strong response to the electric pulse.

**Data acquisition**

Activities of neurons in the central nucleus of the ICC, located according to their coordinates relative to the lamba point of the skull (see Egorova et al. 2001; Stiebler and Ehret 1985), were recorded by using the same type of tungsten electrodes as mentioned above. Multiunit recordings were amplified 10,000 times and filtered with a band-pass of 0.3–10 kHz (WPI DAM 80 preamplifier and Rockland 852 filter). Once the recording was stable and the BF was estimated audiovisually, frequency-amplitude scanning was used to measure the frequency-tuning curves of collicular neurons. One frequency scan consisted of 21 blocks. In block 1–20, the frequency of the tone burst was systematically varied in steps of 1 or 0.5 kHz. The last block had no stimulus. Identical scans were repeated five times with 300-ms intervals at a given amplitude. After a 5-s interval, the frequency scans were repeated at another amplitude. Frequency scans were done at amplitude steps between 0 and 100 dB attenuation with 5 dB/step. Responses of collicular neurons were monitored by the oscilloscope and stored together with trigger signals on tapes by a RACAL 4-channel tape recorder. Without moving the recording electrode, the whole recording procedure was done immediately before and 3 h after cortical stimulation. We have shown already that with this stimulus paradigm, the maximum effects of cortical stimulation were reached at ~2–4 h after stimulation (Yan and Ehret 2001). In this way, we obtained one set of data (1 cortical stimulation site, 1 collicular recording site) from each animal.

Control measurements were done in two mice either with only tone stimulation not paired with electrical stimulation of the primary auditory cortex or with paired (tone + electrical) stimulation of non-AI cortex (dorsoposterior field) (see Stiebler et al. 1997).

**Data processing**

Stimulus, trigger, and data files recorded on tapes were played back and analyzed off-line with a DISCOVERY data-acquisition system (DATAWAVE Tech.). Single-unit action potentials, usually of two to five units, were isolated from the multiunit recording by using eight parameters of the action-potential waveform, i.e., peak, valley, height,
width, peak time, valley time, and two user-defined time-voltage values (see details in Yan et al. 2002, 2003). Single-unit data were automatically sorted and stored in an UFF file (DATAWAVE Tech) and eventually displayed in dot rasters, peristimulus time histograms (PSTs), and cumulative PSTs (PSTCs). Spike numbers of each frequency-amplitude block were counted based on five identical stimuli. The excitatory frequency-tuning curve was derived from threshold responses in each frequency-amplitude block. The criterion for threshold was the boundary between a block showing more than one spike and a block showing no response. In case of the influence of spontaneous activity, the threshold was set between blocks differing by at least one spike depending on the spontaneous rate (Suga et al. 1997).

The FTC shapes before and after cortical stimulation were characterized and compared on the basis of the following measures: 1) MT: the lowest dB SPL producing an excitatory response; 2) BF: the frequency at the MT; 3) bandwidth of frequency tuning: the width in kHz of the FTC at 10, 30, 50, and 70 dB above MT; 4) quality factor (Q value): Q10, Q30, Q50, and Q70 were calculated by dividing the BF by the bandwidth at 10, 30, 50, or 70 dB above threshold (BW10, BW30, BW50, or BW70), respectively; 5) slope_{low} and slope_{high}: slopes at the low-frequency (slope_{low}) and high-frequency (slope_{high}) sides of FTCs were calculated in dB/octave by using the values at MT/BF and the frequency and SPL values at 60 dB above MT; 6) level tolerance: as a measure to express the change of FTC bandwidth with increasing SPL, the FTC bandwidth at 60 dB above MT was divided by the bandwidth at 10 dB above MT; 7) asymmetry index (ASI): The ASI was calculated as ASI = (a - b)/(a + b) with a representing the bandwidth between BF and the low-frequency (left) side of the FTC and b the bandwidth between BF and the high-frequency (right) side of the FTC, both at 60 dB above MT. This asymmetry index leads to values between −1 and +1. When the ASI is zero, the FTC is symmetric with reference to the BF. The corticofugally evoked changes in all of these parameters were expressed by the differences in the values before and after cortical activation (poststimulus minus prestimulus).

**Statistics**

The t-test or nonparametric U test, paired t-test or Wilcoxon matched-pairs signed-rank test, χ² test, and one-way ANOVA (all 2-tailed) were employed to compare differences among groups of data. A P value <0.05 was considered to be of significant difference. Except stated otherwise, average data, and their variability are presented in the text and the figures as means ± SD.

**RESULTS**

Excitatory frequency-tuning curves of 77 single units of the ICC were sorted into four classes according to the criteria shown in Fig. 1A (Egorova et al. 2001). The validity of the classification criterion using systematic differences in the slopes of the tuning curves and thus in the general shapes of the FTCs between neurons in the classes I–III is shown in Fig. 1C. FTCs were measured before and after focal stimulation of the primary auditory cortex, and their individual shapes were quantified according to the seven measures indicated in the preceding text. Their BFs and MTs ranged from 5.4 to 35.1 kHz and from 0 to 27.3 dB SPL, respectively. About one-third of the neurons were each of the classes I–III, whereas class IV neurons were rather rare (Fig. 1B).

Figure 2 (left) shows an example of collicular FTCs that were derived from the matrix of displayed PSTCs. Before cortical stimulation (A, control), this unit was sharply tuned to 10 kHz. At 3 h after cortical stimulation (B), the shape of the FTC was largely changed; the BF shifted to 12 kHz, the MT increased by 20 dB, and the bandwidth of the FTC increased significantly. These changes in FTC properties had partly recovered 8 h after cortical stimulation (C). Different from the changes in frequency tuning, the waveform of the isolated action potential was constant over the whole recording period (Fig. 2, right), suggesting that the recording was very stable through our experiment.

More examples of collicular FTCs in each tuning-curve class before and after cortical stimulation are shown in Fig. 3. Regardless of the classes, the BFs, MTs, and shapes of the FTCs were systematically modulated depending on the relationship of BFs and MTs between the activated cortical neurons and the recorded collicular neurons. In general, cortical activation shifted collicular BFs to lower values when collicular BFs were higher than cortical BFs (Fig. 3, A1, B1, and C1) and to higher values when collicular BFs were lower than cortical BFs (Fig. 3, A3, B3, and C3). Cortical activation increased MTs of collicular neurons with BFs different from the cortical BF of stimulation. When collicular and cortical BFs were similar (within 2 kHz), cortical activation did not
three class IV neurons (Fig. 3) were recorded here, they were evoked responses. were drawn along the lowest frequency/amplitude blocks that showed tone- and collicular neurons (Fig. 4) were plotted as a function of the BF difference between cortical nal class as shown in Fig. 3, BF shifts of class I–III neurons were independent of the neuro- change collicular BFs (Fig. 3, A2, B2, and C2). Because only three class IV neurons (Fig. 3D) were recorded here, they were not considered in the following quantitative evaluations.

Correlation of shift in BF and MT with difference in BF

Because BF shifts as a function of the BF difference between collicular and cortical neurons were independent of the neuronal class as shown in Fig. 3, BF shifts of class I–III neurons were plotted as a function of the BF difference between cortical and collicular neurons (Fig. 4A). Positive (negative) BF shifts were seen in collicular neurons with BFs lower (higher) than stimulated cortical BFs. The relationship between BF shift and BF difference was linear (n = 66) for BF differences between about –10 to +14 kHz (Fig. 4A) as has been shown by Yan and Ehret (2002). The larger the BF difference between cortical and collicular neurons, the larger was the shift in collicular BFs (Fig. 4A). Hence, the average absolute difference between collicular and cortical BFs was significantly larger before than after cortical activation, 6.0 ± 4.6 versus 3.4 ± 3.9 kHz (P < 0.001). The shift in collicular BFs was independent from the classes of collicular neurons.

As a result of cortical activation, we observed a significant increase of average collicular MTs from 10.8 ± 7.7 to 23.2 ± 15.2 dB SPL (P < 0.001). In general, the MT increase was positively correlated with the absolute BF difference between collicular and cortical neurons. The correlation was significant only when cortical BFs were lower than collicular BFs (Fig. 4B). The three FTC classes behaved differently with regard to MT increases after cortical activation. Class III neurons showed only a small (1.1 ± 14.3 dB) and nonsignificant MT increase (P > 0.05). The increases in MTs of class I and II neurons were significant (P < 0.001 in each case). The MT increase of class II neurons (21.3 ± 17.7 dB) was significantly larger than that of class I (10.1 ± 7.5 dB, P < 0.01) and class III neurons (P < 0.001). There was also a significant difference in the MT increase between class I and III neurons (P < 0.05). Changes in sharpness of collicular frequency tuning

The bandwidths of collicular FTCs systematically changed after cortical activation according to the shifts in collicular BFs and MTs. These changes are shown for four amplitude levels above MT in Fig. 5 (A, a–d, and B, a–d). When the BF showed no or little (between −4 and +2 kHz) shift, bandwidths became either wider (positive values) or narrower (negative values; Fig. 5A). Larger BF shifts to either higher or lower BFs caused a widening of the FTC bandwidths at all amplitude levels. When the BF shifted to lower values, the increase in bandwidths was systematically related to the shift in BFs for measurements taken at 10 and 30 dB above threshold (r = 0.53, P < 0.05 and r = 0.58, P < 0.01, respectively). This is indicated by the regression lines in Fig. 5A. The bandwidths of collicular FTCs systematically changed after cortical activation according to the shifts in collicular BFs and MTs. These changes are shown for four amplitude levels above MT in Fig. 5 (A, a–d, and B, a–d). When the BF showed no or little (between −4 and +2 kHz) shift, bandwidths became either wider (positive values) or narrower (negative values; Fig. 5A). Larger BF shifts to either higher or lower BFs caused a widening of the FTC bandwidths at all amplitude levels. When the BF shifted to lower values, the increase in bandwidths was systematically related to the shift in BFs for measurements taken at 10 and 30 dB above threshold (r = 0.53, P < 0.05 and r = 0.58, P < 0.01, respectively). This is indicated by the regression lines in Fig. 5A.

At all levels above threshold, we found that, after cortical activation, bandwidths of collicular FTCs became generally smaller if MTs shifted to lower values, and bandwidths became larger if MTs shifted to larger values (Fig. 5B). Thus changes in bandwidths showed a linear correlation with shifts in MTs (see regression lines in Fig. 5B). The slopes of the regression lines increased systematically with increasing sound level above threshold. The slopes were 0.07 at 10 dB above the MT (r = 0.47, P < 0.001), 0.12 at 30 dB above the MT (r = 0.58, P < 0.001), 0.21 at 50 dB above the MT (r = 0.63, P < 0.001), and 0.24 at 70 dB above the MT (r = 0.54, P < 0.01).

The preceding changes in sharpness of collicular tuning were explicitly different among the neurons of the three classes of tuning curves. This is quantified in Fig. 6. Class I neurons significantly increased the bandwidths of their FTCs measured at 10, 30, and 50 dB above MT (Fig. 6A, left) after compared with before cortical stimulation. At the same time, the Q values (BF/FTC-bandwidth at a given level above MT) did not show a significant change or even increased at 70 dB above MT (Fig. 6A, right). Class II neurons showed large increases of bandwidths and corresponding decreases of Q values (Fig. 6B), i.e., FTCs of class II neurons broadened after cortical stimulation. On the other hand, FTC bandwidths of class III neurons generally decreased and Q values increased (Fig. 6C), leading to a sharper tuning in many of these neurons. By definition, a bandwidth increase must lead to a Q-value decrease, if the BF of a neuron remains constant. Because BFs of most collicular neurons from all classes of tuning curves changed after cortical stimulation (Figs. 3, 4A, and 5A), changes in FTC bandwidths and in Q values are not equivalent measures here.

Changes in slopes of tuning curves

Classes I–III of collicular neurons are defined and discriminated by the slopes of their low-frequency (slope low, B, right) and high-frequency (slope high) FTCs. This is shown in Fig. 6. Class I neurons increased their slopes significantly after cortical activation (Fig. 6A, left) and remained constant (P > 0.05) after 480 min. of stimulation. The slopes increased significantly after cortical activation (Fig. 6A, right) and remained constant (P > 0.05) after 480 min. of stimulation. Furthermore, the slopes of class II neurons increased significantly after cortical activation (Fig. 6B, left) and remained constant (P > 0.05) after 480 min. of stimulation. On the other hand, the slopes of class III neurons decreased significantly after cortical activation (Fig. 6C, left) and remained constant (P > 0.05) after 480 min. of stimulation.
and high-frequency (slope$_{\text{high}}$) sides (Fig. 1, A and C). Figure 7 shows the slope$_{\text{low}}$ and slope$_{\text{high}}$ of class I–III neurons before (●) and after (○) cortical activation. In class I neurons, slope$_{\text{low}}$ and slope$_{\text{high}}$ were either increased or decreased resulting in a larger variation of slopes after cortical activation (Fig. 7A). The average slope$_{\text{low}}$ and slope$_{\text{high}}$ before cortical activation were 74.75 ± 28.8 and 377.63 ± 131.0 dB/octave, respectively. They changed to 71.25 ± 71.0 and 574.55 ± 502.4 dB/octave, respectively, after cortical activation. Because of the large increase of variability of slopes, these changes were not statistically significant.

Cortical activation decreased slope$_{\text{low}}$ values of class II neurons (Fig. 7B) from 386.97 ± 292.8 to 148.44 ± 79.1 dB/octave ($P < 0.001$) and slope$_{\text{high}}$ values from 1081.21 ± 640.9 to 855.36 ± 918.4 dB/octave (0.05 < $P < 0.06$). In class III neurons, both slope$_{\text{low}}$ and slope$_{\text{high}}$ were increased from 81.49 ± 18.9 to 117.98 ± 77.8 dB/octave (nonsignificantly) and from 154.07 ± 58.3 to 302.01 ± 165.9 dB/octave ($P < 0.001$), respectively.

Because cortical activation changed the slope values of FTCs that were used as the criterion for classification of the neurons, collicular neurons were re-sorted into the four classes after cortical activation according to the criteria explained before (see Fig. 1). Of 24 class I neurons, 20 (83.3%) neurons remained in class I, 3 (12.5%) became class II, and 1 (4.2%) became class IV. Of 27 class II neurons, 12 (44.4%) neurons became class I, 9 (33.3%) remained class II, and 6 (22.2%) became class III. Of 23 class III neurons, 8 (34.8%) became class I, 7 (30.4%) became class II, and 8 (34.8%) remained class III. Thus significantly more class II and III neurons compared with class I neurons changed their class after cortical activation ($\chi^2$-test, $P < 0.001$). Qualitatively, about two-thirds
both slopehigh and slopelow evoked by cortical activation were
declined. In contrast, the percentage of class I neurons with
tuned after cortical activation. Table 1 summarizes the propor-
tions of neurons in the four FTC classes before and after
cortical activation.

Changes in FTC symmetry

Asymmetry indexes differed significantly between the
groups of neurons (ANOVA, \( P < 0.001; t \)-test for each of the
comparisons \( P < 0.01 \)). On average, neurons of class I had an
asymmetry index (ASI) of 0.66 ± 0.12, class II neurons of
0.45 ± 0.21, and class III neurons of 0.27 ± 0.24. This
indicates that more class III neurons than neurons of the other
classes had rather symmetric FTCs (ASI closer to 0) and even
negative ASIs (high-frequency branch of the FTC wider than
the low-frequency branch); this was never the case in the other
neurons (Fig. 10). Class I neurons had the most asymmetric
FTCs with the low-frequency branch always being much wider
than the high-frequency branch (ASI has positive, large val-
ues). Figure 10 shows how collicular ASIs change as a function
of the original ASI. A positive change of the ASI indicates that
the low-frequency branch widened and/or the high-frequency
branch narrowed more than the respective other branch after
the cortical activation. A negative change of the ASI occurred
when the low-frequency branch of the FTC narrowed and/or
the high-frequency branch widened more than the respective
other branch. There was a significant correlation between ASI
change and original ASI for class II neurons only (Fig. 10B).
This regression line shows that class II neurons of high or low
asymmetry before cortical activation tended to become more or
less symmetric, respectively, after cortical activation.

Figure 11 shows how ASI changes relate to BF shifts and
MT shifts of neurons in the three classes (Fig. 11, class I: A and
B; class II: C and D; class III: E and F). For the class I neurons,
the change in ASI was positively related to the shift in
collicular BF (\( r = 0.74, P < 0.001, \) Fig. 11A) but unrelated to the shift in collicular MT (\( P > 0.05, \) Fig.
11B). For the class II neurons, the change in ASI was posi-
actively related to the shift in collicular BF (\( r = 0.49, P < 0.05, \) Fig.
11C) but negatively to the shift in collicular MT (\( r = 0.65, P < 0.001, \) Fig.
11D). The change in the ASI of class III
neurons showed no correlation with the shift in both collicular
BF and MT (\( P > 0.05, \) Fig. 11, E and F).

Controls

Neither acoustical stimulation alone (2 neurons examined)
nor the pairing of acoustical stimulation with electrical stimu-
lation in the dorsoposterior field of the AC (3 neurons exam-
ined) changed the BFs, MTs, or shapes and classes of FTCs of
ICC neurons. Two neurons were of class I, one of class II, and
two of class III. The shapes of the FTCs of the neurons were
compared by superimposing the FTC plots.

Discussion

Effectiveness of the conditioning paradigm

Studies on bats have shown only some minor plasticity of
frequency tuning of ICC neurons in response to repetitive tone
stimulation without electrical reinforcer (Ma and Suga 2003;
Suga et al. 2000; Yan and Suga 1998). The bats were stimu-
lated by tone bursts at a rate of 10/\( s \) for 30 min. In our control
experiment with only acoustical stimulation, tone bursts were
presented at a much lower rate (4/\( s \) for a much shorter time (7
min) than in the bats, so that we did not expect and actually did
not observe any changes in frequency tuning to acoustic stimulation alone.

The here-applied pairing of tone bursts with electrical stimulation for the activation of the primary auditory cortex at a given location of its tonotopy had profound effects on BFs (Yan and Ehret 2001), MTs, tuning and dynamic ranges (Yan and Ehret 2002), and frequency tuning curves in the ICC (present study) taken 3 h after the conditioning of the AC. Shifts of BFs in the ICC had not fully recovered 8 h after cortical conditioning (Yan and Ehret 2001). Such a long phase of plasticity in the auditory midbrain induced by auditory cortical conditioning seems not to be reported before.

There are immediate effects of local electrical stimulation of the AC on tone responses in the ICC. These corticofugal effects are excitatory or inhibitory, occur at a short latency, and disappear within milliseconds or seconds (Bledsoe et al. 2003; Jen et al. 1998) or ∼30 min (Zhou and Jen 2000b) after the end of AC stimulation. Corticofugal effects with regard to inducing BF shifts in the ICC last for a maximum of about 3 h after conditioning the AC with electrical stimulation or combined acoustical and electrical stimulation (Ma and Suga 2001; Yan and Suga 1998; Zhang and Suga 2000). In the cases of pairing acoustical with electrical stimuli (Yan and Suga 1998; Zhang and Suga 2000), four equally spaced electrical pulses were presented over the first 6 ms of the 20-ms-long tone bursts. Considering tone-response latencies of AI neurons of >10 ms (e.g., Goldstein and Abeles 1975; Phillips 1998) the electrical stimulation of the AC had ended before the AC could respond to the tone. Hence, this is a case of backward conditioning of AC neurons, which, in general, is a very ineffective conditioning strategy (e.g., Tarpy and Mayer 1978). Thus it can be understood that the combined stimulation (electric and acoustical) of the AC did not add in effect to the changes produced by electrical stimulation alone (Yan and Suga 1998).

If an effective forward conditioning is used by, for example, presenting tone bursts followed by an aversive stimulus (electrical leg stimulation) (Gao and Suga 1998, 2000; Ji et al. 2001) or tone bursts followed by electrical stimulation of the basal forebrain (Bakin and Weinberger 1996; Bjordahl et al. 1998; Kilgard and Merzenich 1998; Ma and Suga 2003), changes in BFs and response strength of neurons in the AC are lasting longer than 3 h and, depending on the stimulation parameters, even longer than 24 h. However, conditioning effects on neurons in the ICC still last for less than ∼3 h (Gao and Suga 1998, 2000; Ji et al. 2001) or less than ∼5 h (Ma and Suga 2003). In our stimulus paradigm, the plastic changes in ICC neurons last >8 h. The presumably decisive differences between the above-mentioned and our forward conditioning par-

FIG. 5. Changes in bandwidth of collicular frequency tuning curves as a function of the shift in collicular BFs and MTs due to cortical stimulation. Cortical stimulation changed collicular bandwidths at all amplitude levels above minimum threshold (BW10–BW70). The increase in bandwidths was significantly related to the downward shift of the BF at 10 and 30 dB above the MT (regression lines in A, a and b). The corticofugally evoked changes in bandwidth were also significantly related to the shift in collicular MTs (B). The higher the amplitude level was above threshold (B, a–d), the larger was the slope of the regression lines.
adigm concern the presentation of the reinforcing stimulus to the AC. Different from the reinforcing electrical leg stimulation or basal forebrain stimulation, the stimulus in our study is a direct stimulation of particular loci of the AC where the neuronal BF represented the frequency of the conditioned tone. We propose that this cortical congruence of acoustical with electrical stimulation in a forward conditioning paradigm is responsible for the long-lasting plasticity in the ICC observed here. This hypothesis has to be tested in further experiments.

**Contribution of cortical and local factors to ICC plasticity**

The primary auditory cortex (AI) is apparently responsible for the plastic changes in the ICC frequency tuning because activation of other auditory cortical areas does not affect the frequency tuning of ICC neurons as shown in the present (control with stimulation in the dorsoposterior field) and other reports (Yan and Suga 1996; Zhang and Suga 1997). Further, inactivation of the AI by application of muscimol before the cortical conditioning abolishes changes in frequency tuning in the ICC (Gao and Suga 1998). This shows that corticofugal influence is necessary for the induction of collicular plasticity in the frequency domain. If the AI is inactivated, however, after changes of frequency tuning in the ICC have been established according to cortical conditioning, these changes persist without further corticofugal control for the same time as in the case of the intact AC (Gao and Suga 1998). This suggests that the ICC has its own mechanisms for maintaining plasticity of frequency tuning independent of permanent corticofugal feedback. Provided that the corticofugal system is working, it seems even possible that neurons of the ICC can change their frequency tuning independently of whether BF changes in the AC in response to conditioning occur or not because suppression of cholinergic effects by application of atropine to the AC prevents BF shifts in response to conditioning in the AC but not in the ICC (Ji et al. 2001). Taken together, these data suggest that plasticity of frequency tuning in the ICC is both under corticofugal and local control.

Corticofugal and local factors of plasticity of frequency tuning in the ICC are also evident from our present data. The cortical factors are demonstrated by the systematical relationships of the changes in ICC BFs and MTs to the differences between cortical and collicular BFs or MTs (Figs. 2–4). If BFs and MTs of collicular neurons are very similar to those of the activation center of the cortex, cortical activity does not change the average identity of collicular neurons, not BFs or MTs or properties of frequency tuning (Fig. 4, A and B, values at 0 BF difference; Fig. 5, A and B, values at 0 BF or MT shift). If BFs and/or MTs are different, collicular neurons mostly lose their identity with regard to BF, MT, and shape of FTC. As a general effect, changes in FTC bandwidths (Fig. 5) appear to be linked to MT shifts because FTCs tend to sharpen (decrease of

**FIG. 6.** Comparison of the bandwidths and Q values of the frequency tuning curves before and after cortical stimulation, separately for class I (A), class II (B), and class III (C) neurons. Bandwidths and Q values were measured 10, 30, 50, and 70 dB above minimum threshold (BW10–BW70; Q10–Q70). Significant differences are indicated, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. 

![Graphs](image-url)
bandwidths) when MTs in the ICC are decreased (negative shifts in Fig. 5B) or to broaden when MTs are increased (positive MT shifts in Fig. 5B).

The local factors are demonstrated by the fact that many details of the plasticity of frequency tuning in the ICC depend on the tuning-curve class of particular neurons (Figs. 6–10). Neurons in the four tuning-curve classes largely reflect local properties of excitatory and inhibitory inputs to the neurons (Egorova et al. 2001) and neurons of classes II and III are distributed along spatial gradients on frequency-band laminae of the ICC (Ehret et al. 2003; Hage and Ehret 2003). Neurons of just these two classes respond differently to cortical conditioning. FTCs of class II neurons tend to broaden, whereas those of class III neurons to sharpen (Fig. 6, B and C, Q values) in both classes more or less asymmetrically with regard to the BF. The steeper the slopes of the FTCs are before cortical activation (Fig. 8), the larger are these asymmetric changes.

The general result of all these changes is an assimilation of the tuning-curve shapes of class II and III neurons to the shapes of class I neurons (increase in the number of class I neurons, Table 1) and of the level tolerances among the neurons of the different classes (Fig. 9) after cortical stimulation.

The classification of FTC shapes must be understood as a simplified method to analyze the continuous variability of tuning-curve shapes and their alterations by corticofugal modulation. The criteria for the assessment of the FTC shapes into four classes and the percentages of neurons in classes found here (Fig. 1) are consistent with earlier studies on the ICC of the anesthetized mouse (Egorova et al. 2001; Ehret et al. 2003; Hage and Ehret 2003), cat (Ehret and Merzenich 1988), and guinea pig (Le Beau et al. 2001). The classification of ICC tuning-curve shapes of the decerebrated cat into three classes

<table>
<thead>
<tr>
<th>Before Stimulation</th>
<th>After Stimulation</th>
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<tr>
<td>Class I</td>
<td>24 (31)</td>
</tr>
<tr>
<td>Class II</td>
<td>27 (35)</td>
</tr>
<tr>
<td>Class III</td>
<td>23 (30)</td>
</tr>
<tr>
<td>Class IV</td>
<td>3 (4)</td>
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</tbody>
</table>

Percentages are in parentheses.
by Ramachandran et al. (1999) is based on excitatory FTCs and inhibition of spontaneous activity by tone bursts. The proportions of neurons in FTC classes comparable with ours are very different. For example, \( \frac{70\%}{100\%} \) of ICC neurons in the decerebrated cat have closed FTCs compared with only 5% in the anesthetized cat (Ehret and Merzenich 1988) and 8% in the anesthetized mouse (Egorova et al. 2001). Whether anesthesia in our present experiment or the lack of corticofugal influences in the decerebrated cat has a significant influence on the assessment of tuning-curve shapes (and their plasticity) is subject of further study.

**Suggested mechanisms of corticofugal control**

Direct glutamatergic projections from the primary auditory cortex to the ICC (Feliciano and Potashner 1995) appear to be weaker than projections to the pericentral collicular nuclei (mainly data from cats and rats) (e.g., Herbert et al. 1991; Huffman and Henson 1990; Winer et al. 1998) but still they are substantial and topographically arranged (Saldaña et al. 1996). Corticofugal fibers end in the ICC with excitatory synapses predominantly at the distal dendrites of collicular neurons of very similar BF, which may themselves be excitatory or inhibitory (GABAergic) (Saldaña et al. 1996; see also discussion there). Thus corticofugal innervation has a direct modulatory influence on neurons in the ICC that may facilitate the responses of neurons of matched BF and MT without altering their FTC shapes (Fig. 3, A2, B2, and C2). If the corticofugal activity is acting on a neuron of matched BF but nonmatched, i.e., higher, MT, the corticofugal excitation could lower the neuron’s tone threshold as it is seen in many class III neurons (Fig. 4C and 5B). In the case of neurons of nonmatched BF, there is an inhibitory net-effect causing an increase of tone response threshold (Fig. 4C) and a resulting increase in the tuning-curve bandwidth (Fig. 5B). The reason for this inhibitory net effect may then be the missing excitatory corticofugal influence because of BF mismatch and an inhibitory influence on the examined neuron by corticofugal excitation of GABAergic neurons of a frequency-band lamina in the neighborhoods of the lamina containing the examined neuron. Previous studies have shown GABAergic inhibition intrinsic to the ICC influencing excitatory and inhibitory receptive fields of ICC neurons (e.g., Faingold et al. 1989; Fuzessery and Hall 1996; Le Beau et al. 1996; Lu and Jen 2001; Palombi and Caspary 1996; Pollak and Park 1993; Yang et al. 1992). That is, inhibition between frequency-band laminae has been shown as well as GABAergic neurons that could mediate this inhibition (e.g., Oliver et al. 1994). Altogether, the plastic changes in ICC neurons evoked by local cortical conditioning follow a center-surround pattern (center is excitatory, surround is inhibitory) as described before (Yan and Ehret 2002).

Differential reshaping of FTCs may also be approached by local differences of corticofugal effects on the three-dimensional ICC. Class III neurons are found preferentially in the periphery of a physiologically defined frequency-band lamina of the ICC with a gradient of decreasing numbers toward its core (Ehret et al. 2003; Hage and Ehret 2003). Thus class III neurons are most abundant in the lateral, caudal, and medial ICC. These locations are bordered by the pericentral nuclei of the ICC that receive the most intense innervation by corticofugal fibers (e.g., Huffman and Henson 1990; Herbert et al. 1991; Hofstetter and Ehret 1992; Winer et al. 1998). When the corticofugal projections excite the GABAergic neurons in the pericentral nuclei projecting to the ICC (Jen et al. 1998, 2001), we can expect the largest inhibitory effects of cortical activation to occur in the peripheral regions of the ICC, just where class III neurons are most abundant (Ehret et al. 2003; Hage et al. 2004).

FIG. 10. Change in the asymmetry index (ASI) of frequency tuning curves due to cortical stimulation (ASI after minus before stimulation) as a function of the ASI before cortical stimulation. The changes of the ASIs of class I and class III neurons were independent of the original ASIs (A and C), the change of the ASI of class II neurons was significantly related to the original ASI (regression line in B).

FIG. 11. Changes in BF and MT of frequency tuning curves due to cortical activation are plotted as a function of the shift in collicular BF and MT. Statistically significant relationships are indicated by regression lines. Class I neurons: A and B; class II neurons: C and D; class III neurons: E and F.
and Ehret 2003), leading to the observed increase of FTC sharpness (Figs. 6 and 7).

In our previous study (Yan and Ehret 2002), we discussed the possibility that part of the corticofugally induced changes in the ICC could be mediated via corticofugal influences on the cochlea, cochlear nucleus and/or superior olivary complex. Results from electrical stimulation (100 nA) at a rate of 5/s for 7 min in the Doppler-shifted CF-processing area of the mustached bat’s AC have shown corticofugally induced BF shifts in the ICC (Zhang and Suga 2000) but no influence on the cochlear microphonic potential (Xiao and Suga 2002). Changes in the cochlear microphonic potentials were seen only after electrical pulses were delivered at a rate of 33/s (Xiao and Suga 2002). From these data, one could assume no corticofugal effect of our stimulus paradigm on cochlear hair cells if looking only at the rate of 4/s and a duration of 7 min of our electrical stimulation (500 nA). By considering, however, that we stimulated the AC effectively in a forward-conditioning paradigm with pairs of tones and electrical pulses, corticofugally mediated changes even down to the cochlear level cannot be ruled out and are investigated now.

Functional considerations

Although the presently applied cortical conditioning paradigm with pairs of tones and electrical pulses is artificial and may have produced stronger corticofugal effects than expected from the use of biological stimuli for conditioning, our data certainly show that the AI can change frequency filtering in the auditory midbrain, which is specified differently for neurons of different tuning-curve shapes. Considering the center-surround organization of ICC plasticity in the frequency domain, the processing of a frequency of interest in a sound representing the BF of activated cortical neurons, is enhanced in the ICC (BF-matched neurons). The inhibition of BF-unmatched neurons in the surround is maximally about one critical band wide toward both lower and higher frequencies with reference to a given BF (Yan and Ehret 2001). This width of inhibition resembles the BF distance between neighboring frequency-band laminae of the ICC (Schreiner and Langner 1997). This close relationship between the bandwidths of frequency resolution of the auditory system (expressed by the psychophysical measure of critical bands) and the frequency bandwidths of corticofugal adjustment of FTCs in the ICC suggests that a main function of auditory cortical efferents is to support spectral resolution of sounds. Thus corticofugal control in the center of frequency-band laminae of the ICC, working mainly via influences on neurons of class I and II tuning curves, seems to be an important factor to improve the perception of frequency components of biological significance, especially in background noise.

In the periphery of BF-matched frequency-band laminae, corticofugal influences decrease the tone-response thresholds and increase the frequency selectivity of class III neurons. These neurons seem to be specialized for time-domain processing because their spatial distribution on frequency-band laminae coincides with distributions of onset-spiking neurons that are able to respond to fast changes of frequency sweeps (Hage and Ehret 2003). Thus the auditory cortex sensitizes these class III neurons by decreasing their response threshold and specifying their frequency tuning to respond to fast changes in frequencies similar to the BF of the cortical activation. Cortical activation by disturbing noise off the frequency of a tone of interest would increase lateral inhibition in class III neurons with the effect of narrowing their FTCs to improve again time-critical processing of frequencies of interest.

On the first view, the assimilation of the class II and III FTC shapes to the class I shapes (Table 1) may be regarded as a transient change of specialized frequency filters to auditory-nerve like (general purpose) neurons in the ICC mediated by corticofugal activity. By considering the spatial distribution of FTC types in the ICC, however, it seems that the auditory cortex can adjust the auditory midbrain function in the spectral domain to improve both spectral and time-critical processing via its differential influence on specialized groups of neurons.

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