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Role of frequency band integration in sharpening frequency tunings of the inferior colliculus neurons in the big brown bat, *Eptesicus fuscus*

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Abstract **By means of a particular two-tone stimulation paradigm in combination of using a pair of electrodes for simultaneously recording from two inferior colliculus (IC) neurons, the current *in vivo* study is undertaken to explore the role of frequency band integration (FBI) in sharpening of frequency tuning in the big brown bat, *Eptesicus fuscus*. Three major results are found: (1) The paired neurons correlated to FBI are located not only within the same frequency filter bandwidth (FFB), but also across different FFBs. The relations of their frequency tuning curves (FTCs) are mainly of two types: the flank-overlapped and overlaid patterns. (2) Although the sharpness of FTCs between paired neurons is mutual, the sharpening efficiency of neurons located within the same FFB is higher than that of neurons across FFBs, and the FTCs of neurons with the best frequencies (BF) of 20—30 kHz are most strongly sharpened. (3) The strength of FBI is weak near the BF but gradually increased with frequencies away from the BF of sound stimuli. This suggests that the dynamical FBI of the IC neurons located within and across the FFBs might be involved in the formation of functional FFB structures.**

Keywords: frequency band integration, frequency tuning, sharpen, inferior colliculus, bat.

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The inferior colliculus (IC) is one of the most important stations of information relay and processing in the auditory system. It is characterized with a typically tonotopic organization in which the functional frequency filter bandwidth (FFB) is arranged along tonotopic axis (from dorsal to ventral)[1]. Under two-tone stimulation conditions, the frequency tuning curves (FTCs) of IC neurons are sharpened. Blocking the inhibitory projections convergent upon IC neurons makes their FTCs broadened while the inhibitory areas at the limb of excitatory FTCs completely or partially disappeared[2, 3], implying that the mutual integration between the excitatory and inhibitory inputs plays a key role in shaping the frequency filter features of the IC neurons. Earlier neuro-anatomical studies have shown that the IC neurons mainly receive matched projections from multiple sources in the central auditory nuclei. Moreover, it has been also reported that axon collaterals of IC neurons often run in several directions to provide inputs to adjacent FFBs, and massive intrinsic neurons could be observed in the IC[4,5], which leads some investigators to the viewpoint that the local integrating networks within the IC might make a contribution to the formation of FFB.

Analogous to other sensory modality, it is conjectured that sound signals, on the one hand, could be decomposed into discrete frequency components by parallel FFBs along the central auditory pathways. On the other hand, the integration of acoustic information streams between different frequency filter channels would make an important contribution to eliminating the frequency ambiguity originated from periphery, and to performing cognitive function of natural sounds[6]. Recently, the latter feature has just begun to be explored in the auditory cortex (AC)[7, 8], nevertheless, it still remains an open question in the IC. For better elucidating the neural mechanism underlying the perception of complex sound in the auditory system, the two IC neurons were simultaneously recorded in the present study by using a pair of micropipette electrodes with a particular two-tone stimulation paradigm under free field stimulation conditions, which to our knowledge is different from the conventional recording mode of a single electrode. We reported here that the effects of the neuronal FBI within and across FFBs on sharpening frequency tunings were directly tested.

1 Materials and methods

(i) Animal preparation. The experiments were conducted on 12 big brown bats (4 males, 8 females, 18—25 g b. w.) tranquilized and anesthetized with Innoval-Vet (Fentanyl 0.08 mg/kg b. w., Droperidol 4 mg/kg b. w.) and Nembutal (50 mg / kg b. w.). The surgical procedures have been described in our previous paper[9]. In order to simultaneously record from two neurons, two small holes (200—500 μm in diameter) were made over the center of the IC to permit insertion of two micropipette electrodes with the use of skull and brain surface landmarks. The level distance between the two recording electrodes (about 100—200 μm in diameter apart) was measured by the ocular micrometer in an operative microscope and the recording depth of each neuron was read from each microdrive.

(ii) Acoustic stimuli and recording procedures. Under free field stimulation conditions, two independently controlled sound generation systems were used. Adjustment of the two-tone stimuli was basically the same as

that in the previous experiment[10]. After the two recording electrodes (impedance: 5—10 MΩ; tip diameter < 1 μm) filled with 3 mol/L KCl were inserted into the IC by each microdrive (David-Kopf 640), respectively, the sound stimulation system was switched on and the frequency and amplitude of sound pulses were changed systematically for isolation of the acoustically evoked IC neurons. Action potentials of isolated neurons were amplified (HP 465A) and band-pass filtered (KH 3500 & KH 3362) independently. Then the neuronal activities were synchronously sent to an oscilloscope (Tek 5113) for visual monitoring, an audio monitor (Grass AM6), and a window discriminator (WPI 121), which was sconnected with a computer (Gateway 2000) for acquisition of post-stimulus-time histograms (PSTH). Sampling parameters of the computer were the same as in an earlier paper[11].

(iii) Determining the paired IC neurons and measuring the acoustic responses. A particular two-tone stimulation paradigm distinguished from the traditional mode of two-tone inhibition was employed in this study. The major difference between them was the selection of inhibitory tone/or modulating tone prior to probe tone. To investigate the FTC characteristics of one of the paired neurons, we chose the best frequency (BF) sound of the other paired neuron as a modulating sound (MS). Briefly, once upon determining the BFs and minimum thresholds (MT) of two IC neurons, called neurons A and B for convenience of description, respectively, the firing rate of neuron A at 10 dB above MT of at BF sound stimulus was chosen as control. When the control was affected by presentation of a 20 dB above MT of neuron B's BF sound (MS) prior to the MT + 10 dB BF sound (probe sound, PS), the inter-tone interval (ITI) between the MS and PS was varied to obtain the optimal effect at which the number of impulse from neuron A decreased or increased at least 20%. In this scenario we regarded neurons A and B as the paired IC neurons that were correlated to FBI. If the response of neuron A was not affected by the combined paired tones, neurons B was considered not to be related to neuron A and further isolation should be done until paired neurons were determined.

Then the following data collection was taken up: i) amplitude-FTC (aFTC) and rate-FTC (rFTC) at 20 dB above MT for neuron A; ii) repeated measures of them after addition of a MS (20 dB BF above MT sound for neuron B); iii) and iv) were the same as i) and ii) but the procedures were for neuron B, therefore, the MS presented was a 20 dB above MT BF sound for neuron A.

(iv) Data processing. The neuron's aFTC and rFTC were separately plotted in the light of each responsive frequency determined at different thresholds and PSTHs. For analyzing the sharpening features of FBI, the $Q_{10\,dB}$ and $Q_{30\,dB}$ values of aFTC, BW_{50} and BW_{75} values of rFTC, and their percent variations without and with the particular two-tone stimulation were calculated. In addition, percent inhibition in amplitude and in the firing rate at each selected frequency relative to the BF (re BF) of aFTC and rFTC was figured. Statistics and charting of the data were performed on the software of SigmaPlot (in PC computer) and InStat (in Macintosh computer). The values given in the text and in the figures were expressed as mean ± standard deviation (M ± SD). The statistical significance of experimental effects was assessed using the repeated measures one-way ANOVA (analysis of variance) and the unpaired *t*-test for independent samples.

2 Results

In this study, 55 pairs of neurons (n = 110) correlated to FBI were recorded. The optimal ITIs between MS and PS ranged from 1.5—15 ms. Under the particular two-tone stimulation conditions, 94 paired IC neurons (85.5%) displayed the suppression of firing rates and FTCs (inhibitory FBI), while the rest (n = 16, 14.5%) manifested facilitatory FBI, in which the firing rates were increased and the FTCs were broadened (this kind of FBI will be discussed in other paper specially). For a given neuron, we did not observe the case that there were not only inhibitory but also facilitatory FBI. However, it was worthy of note that the sharpening action of FBI between paired IC neurons in FTC was mutual. In other words, the BF sound of neuron B would sharpen the FTC of neurons A while the BF sound of neuron A sharpened the FTC of neuron B.

(i) Distribution of paired neurons in the IC and the mutual relations between neuronal aFTCs. In line with the BFs of paired neurons, Fig. 1 was drawn to show the distribution of these neurons in different FFBs. 22 pairs of neurons (46.8%, 22/47, indicated by solid circles) were located within the same FFB and, moreover, most of them (86.4%, 19/22) were gathered in the FFB of 20—30 kHz. The others (53.2%, 25/47, indicated by solid diamonds) were distributed over different FFBs and the biggest span between the two FFBs for a pair of IC neurons was extended to as far as four FFBs. The iso-frequency contours

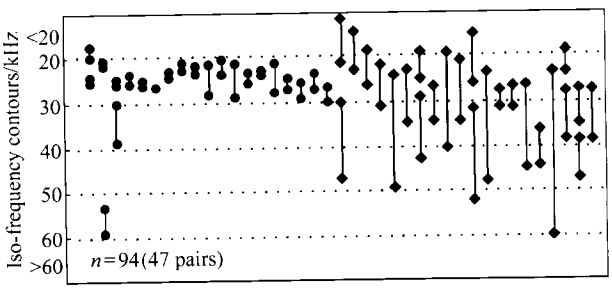

Fig. 1. Distribution of the paired IC neurons in different FFBs. The paired neurons located within the same FFB and across different FFBs were indicated by solid circles and solid diamonds, respectively. n, Number of paired neurons sampled.

in the figure were defined according to Casseday and Covey's report[12]. Note that the real thickness of each FFB in the IC is not equal.

There were three types of relations between the aFTCs of paired neurons (Fig. 2): i) The flank-overlapped pattern (Fig. 2(a), curves 1 vs. 2). The flanks of 55% (11/20) paired neurons' aFTCs were mutually overlapped. ii) The overlaid pattern (Fig. 2(b), curves 1 vs. 2). The aFTC of one of the two paired neurons was overlaid with the aFTC of the other neuron (30%, 6/20). iii) The nearly apart pattern (Fig. 2(c) curves 1 vs. 2). A few aFTCs of paired neurons (15%, 3/20) were either wholly separated or overlaid with a small area at the higher stimuli intensities.

(ii) Sharpening effects and features of the FBI on FTCs. The sharpening effects of FBI on FTCs were evaluated by using the values of $Q_{10\,dB}$ and $Q_{30\,dB}$ of FTC, BW_{50} and BW_{75} of rFTC. The $Q_{10\,dB}$ and $Q_{30\,dB}$ values were calculated by dividing the excitatory BF by the frequency bandwidths of aFTC at 10 and 30 dB above the MT. Each rFTC was normalized by defining the highest firing rate as 100% response and, afterwards, the BW_{50} and BW_{75} values were figured out by the frequency bandwidths at 50% and 75% of normalized responses. An increase in Q_n or a decrease in BW_n reflects more sharpening of FTC. As shown in Fig. 3(a) and (d), with two-tone stimulation the FTCs of two representative neurons were compressed (curves in (a), 1 vs. 2; in (d), 1 vs. 2). Compared with the FTCs obtained without and with addition of MS, their $Q_{10\,dB}$ and $Q_{30\,dB}$ values were increased by 58.6% and 46.2%, and the BW_{50} and BW_{75} were decreased by 30.9% and 52.8%, respectively.

In comparison with the 80 aFTCs and 96 rFTCs attained without and with the FBI (Fig. 4), it can be found that the Q_n values increased and the BW_n values decreased with presentation of a MS. Statistical analysis revealed that the $Q_{10\,dB}$ and $Q_{30\,dB}$ values have gone up from 3.5 ± 1.6 to 5.5 ± 2.1 ($P < 0.0001$, t-test), and from 1.6 ± 1.1 to 2.5 ± 1.5 ($P < 0.05$, t-test). Meanwhile, the BW_{50} and BW_{75} values have gone down from 7.2 ± 1.9 to 5.1 ± 1.7 kHz and from 4.1 ± 1.4 to 3.1±1.2 kHz ($P < 0.0001$, t-test).

For exploring the sharpening characteristics of FBI in FTC, the percent inhibition in amplitude of aFTC (Fig. 3(b), (c)) and in firing rate of rFTC (Fig. 3(e), (f)) at nine selected frequencies, including 25%, 50%, 75%, and 100% responsive frequencies at low flank and at high flank as well as the BF of each FTC, were determined. By using of the BF as a reference, both ending responsive frequencies at low and at high flank of FTC were defined as −100% and +100%, respectively. The results demonstrated that the efficiency of inhibition either in amplitude or in firing rate was weak near the BF but became increasingly strong with frequencies away from the BF (Fig. 3(c), (f), $P < 0.0001$, One-way ANOVA).

(iii) Efficiency of the FBI within the same FFB and across FFBs. As illustrated in Fig. 1, there was hardly any difference of distribution percentage between the paired neurons located within the same FFB (46.8%) and across different FFBs (53.2%), and yet most of them were located in the FFB of 20—30 kHz (62.8%, 52/94). Statistical analysis of the Q_n and BW_n values reflecting sharpening degrees showed two findings: i) The sharpening degrees resulted from the FBI of paired neurons within the same FFB were significantly higher than that of those across different FFBs (Fig. 5(a), (b), $P < 0.05$—0.01, t-test); ii) comparing the sharpening degrees of neurons located within the FFB of 20—30 kHz with those located in the other FFBs revealed that the sharpening degrees of the former were far higher than that of the latter (Fig. 5(c), (d), $P < 0.05$—0.01, t-test), indicating that the FBI of paired neurons with different distributions in the IC might produce different sharpening effects on the FTCs.

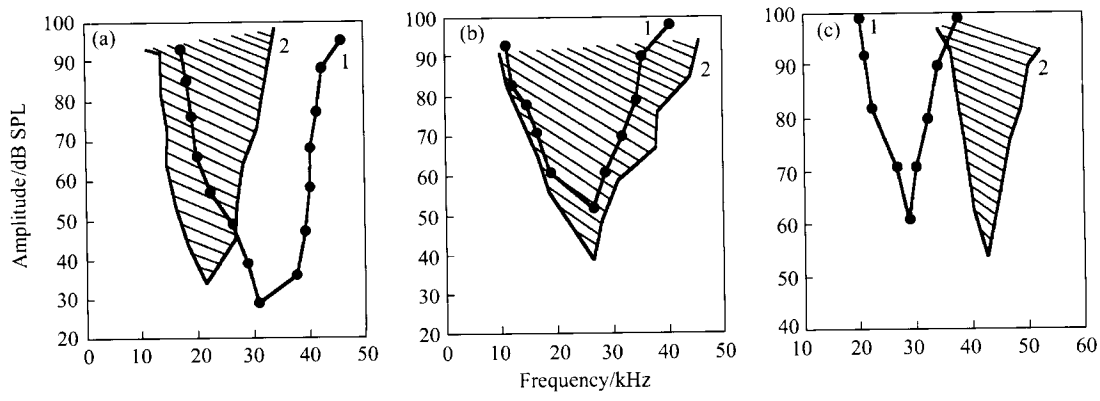

Fig. 2. aFTCs of 3 pairs of representative IC neurons. Three types of relations between aFTCs, including the flank-overlapped, the overlaid, and the nearly apart patterns were shown by (a), (b) and (c), respectively. The recording depth (μm) and latency (ms) of these representative neurons were 788, 6 (a1); 304, 10 (a2); 456, 7 (b1); 360, 7 (b2); 668, 8 (c1); 1109, 5 (c2).

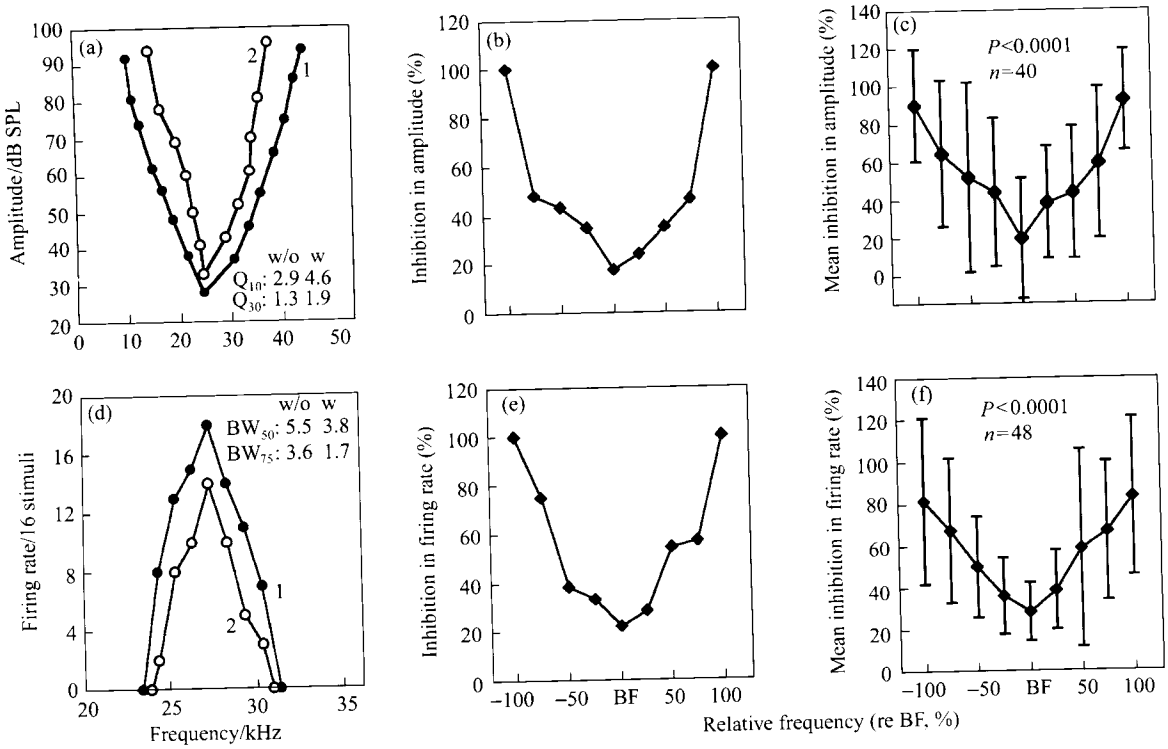

Fig. 3. Suppression of FTCs by the FBI. (a) and (d) aFTC and rFTC of two representative neurons plotted without (solid circles; a1, d1) and with (unfilled circles; a2, d2) the FBI. (b) and (e) Percent inhibition in amplitude and firing rate in relation to selected responsive frequencies relative to the BF (re BF). Percent inhibition was calculated by dividing the difference in threshold and in firing rate at each selected frequency by the threshold and firing rate obtained without the FBI. (c) and (f) Average percent inhibition curves obtained from all IC neurons studied. The vertical bar represents a standard deviation. w/o, without presentation of a MS; w, with presentation of a MS; *n*, number of neurons studied. The recording depth (μm) and latency (ms) of these two representative neurons were 512, 11 (a); 586, 9 (d).

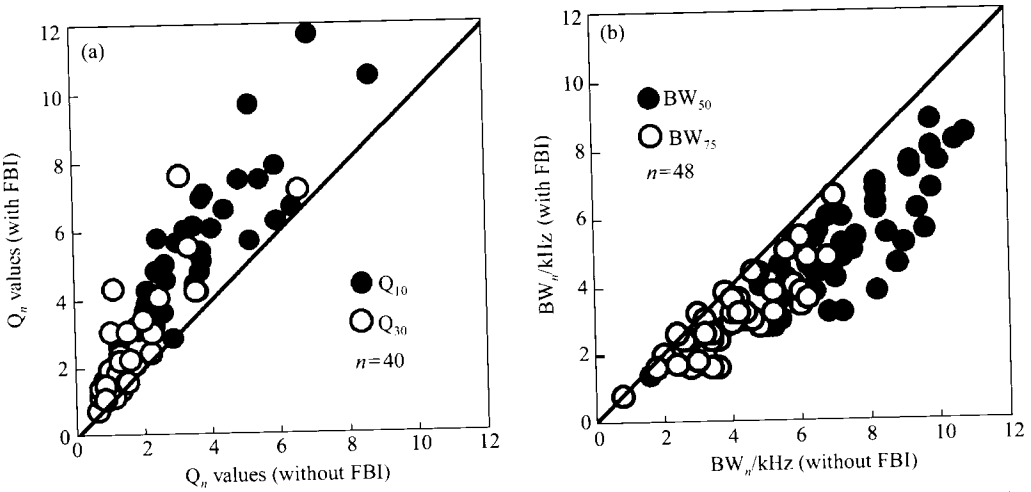

Fig. 4. Comparison of $Q_{10\,dB}$, $Q_{30\,dB}$, BW_{50}, and BW_{75} values obtained without and with the FBI. Isometric lines were indicated by the solid diagonals. *n*, number of neurons studied.

3 Discussion

As a result of methodological limitation, there has long been no immediate evidence for addressing the question whether the IC neurons located within the same FFB and across different FFBs could produce the FBI. Many previous studies have observed the sharpening effects on FTC of IC neurons by using a single recording electrode under two-tone stimulation conditions[2, 3]. Lately, Wenstrup and Leroy[13] reported that the acoustic responses of neurons with high BF in the IC of mustache bat (*Pteronotus parnellii*) were modulated when additional low frequency tones were applied. On the contrary, Biebel and

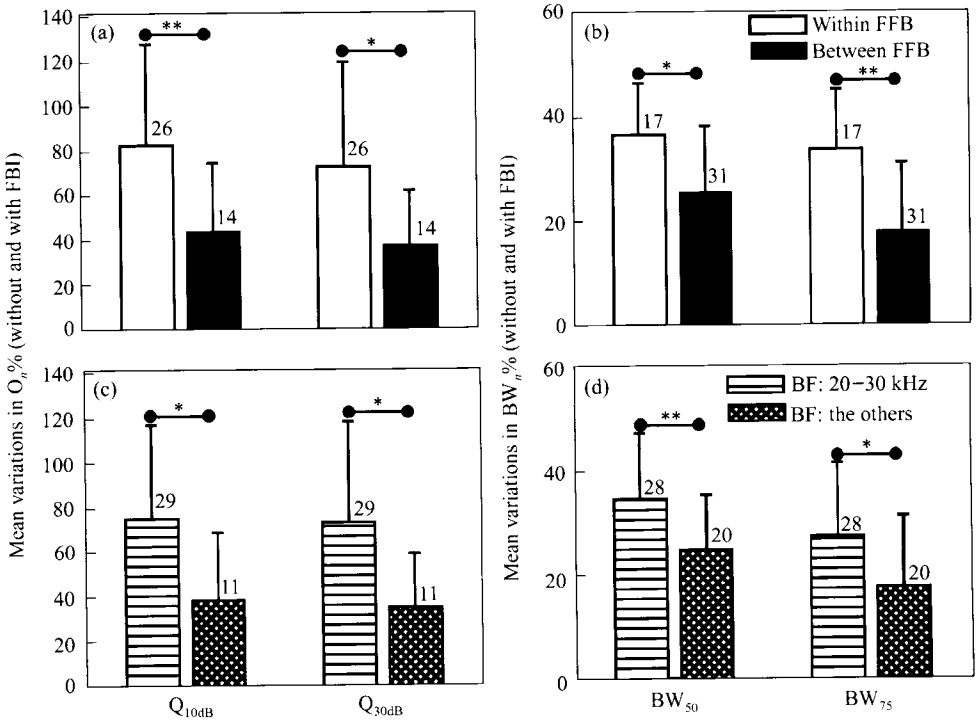

Fig. 5. Statistical comparison of $Q_{10\,dB}$, $Q_{30\,dB}$, BW_{50}, and BW_{75} of paired neurons with different distributions in the IC. The vertical bar and number at each column represent a standard deviation and number of neurons studied. * $P < 0.05$, ** $P < 0.01$.

Langner[14] noticed that neurons with low BF displayed across frequency integration when stimulated with high frequencies in the IC of awake chinchilla. With a particular two-tone stimulation paradigm in combination with two electrodes for simultaneously recording from two IC neurons, the current investigation not only offers the new evidence of across FFB integration, but also reveals the mutual interaction features of different FFBs. It is quite clear that our results cannot be accounted for by the integration model of cross frequency channels advanced by Biebel and Langner[14]. These data strongly imply that there might be a kind of directly mutual interactions between the IC neurons. Even though we have no way to exclude the possibility totally that the phenomena we observed are caused by the non-linear suppression occurring in the cochlea, there are two clues obtained in this study giving firm backing to the above inference: i) the ITIs of a few paired neurons were as long as 10—15 ms, and ii) the MS and PS of some paired neurons were at a distance of multiple FFBs. Morphological studies[4,5] and tests of disinhibition by micro-iontophoresis of drugs that selectively block $GABA_A$ or glycine receptors in the IC under two-tone stimulation conditions[2, 13] have also supported the notion that the FBI may be taken place within the IC. We assume that the generation of functional FFB structure might be achieved not only by use of the interactions of IC neurons within the same FFB, but also by virtue of the integration of across frequency channels, thus, FTCs of the IC neurons are sharpened. It may be really necessary for eliminating the information vagueness of peripheral frequency.

It was first noticed in the present research that the inhibition degrees of IC neurons located in the FFB of 20—30 kHz were significantly higher than that of those distributed over the other FFBs, suggesting that it seems to have a striking resemblance between ICs of the big brown bat and the chinchilla[14]. Namely, when the IC neurons located on frequency band laminas more ventrally project their terminals onto neurons in the medial geniculate body (MGB), their axons may form collaterals onto the IC neurons of dorsal laminas, which leads to creating the FFB of 20—30 kHz characterized with a great extent of FBI. This result functionally annotated the two facts early found: i) The experiments conducted on the big brown bat's behavior have demonstrated that its most sensitive frequencies of sound signal are 20—30 kHz[15], and ii) the neurophysiological studies have shown that there is a vast amount of neurons with level-tolerant FTC in that FFB[12].

To explain the neural mechanism responsive to sharpening frequency tunings of the central auditory neurons, near-BF (matched) and off-BF (unmatched) hypotheses have been put forward for many years[16], but they have been being a disputed subject under discussion

ever since. Both assumptions have received supports from experimental data. In this paper, the observation of the FBI within the same FFB and across FFBs hints broadly that either matched or unmatched projections has a hand in the sharpness processing of frequency tuning in the IC. Of course, we noticed that the flank-overlapped and overlaid patterns were predominant over all relations of paired neurons' aFTC, and the integration efficiency of paired neurons located within the same FFB was higher than that of those across FFBs. It is inviting to combine these two findings and arrive at a conjecture that the matched projections onto IC neurons are still the major sources for sharpening FTC. Hence, it would be reasonable to believe that the shaping of different types of FTCs should be resolved on the inhibitory projection patterns and their modulating fashions received by the IC neurons. Another interesting cue in this study worthy to note is that the inhibitory degrees either in amplitude of aFTC or in firing rate of rFTC are gradually enhanced with stimuli frequencies away from the BF, indicating that there are dynamic interactions between excitatory and inhibitory inputs to the IC neurons during the frequency band integrating. This is highly consonant to the earlier observations in awake bats[17] and the cats[18], in which the integration of postsynaptic currents was interrelated with the stimuli frequencies. Our data taken together with these findings from other authors make it most likely that the dynamic FBI between IC neurons participates in the formation of FFB. For animals and humans, it would be of a vital significance in perception of more complex and natural acoustic information.

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