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Binaural interaction in the inferior colliculus of the big brown bat, *Eptesicus fuscus*

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Abstract

Binaural interaction plays an important role in shaping response properties of central auditory neurons. Using single unit recording and iontophoresis, we examined frequency tuning curves (FTCs), interaural intensity difference (IID) curves, and rate–intensity functions of inferior collicular (IC) neurons of the big brown bat, *Eptesicus fuscus*, under closed system or free field stimulation conditions. We isolated 46 EI (excitation–inhibition), 24 EO (monaural excitation) and 6 EE (excitation–excitation) neurons. Inhibitory FTCs of EI neurons plotted under ipsilateral sound stimulation fell within ($n = 10$, 22%), partly overlapped ($n = 26$, 56%), or almost entirely encompassed ($n = 10$, 22%) excitatory FTCs plotted by contralateral sound stimulation. The discharge rate of EI neurons was a sigmoid function of IID. The peak discharge rate occurred at IIDs at which contralateral sound stimulation was stronger than ipsilateral sound stimulation. Application of bicuculline, an antagonist for γ-aminobutyric acid A receptors, raised the IID curves and broadened the excitatory FTCs but partly or completely abolished the ipsilateral inhibitory FTCs. For EE neurons, excitatory FTCs and rate–intensity functions plotted by contralateral sound stimulation were always broader and higher than those plotted by ipsilateral sound stimulation. The sharpness of FTCs of EI neurons was significantly greater at ipsilateral 30° than at 30° contralateral. This direction-dependent frequency tuning was effectively abolished by occlusion of the ipsilateral ear. Possible mechanisms underlying these observations are discussed.

Key words: Aurality; Binaural inhibition; Frequency tuning curve; γ-Aminobutyric acid; Rate–intensity function

1. Introduction

In the ascending auditory pathway, the central nucleus of the inferior colliculus (IC) receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei (Adams, 1979; Casseday and Covey, 1995; Pollak and Casseday, 1989; Schneiderman and Oliver, 1989). The IC also receives descending inputs from the auditory cortex through the cortico-collicular pathways (Huffman and Henson, 1990; Saldana et al., 1996; Winer et al., 1998). In addition, intrinsic interactions within the IC and between the two ICs are profound (Oliver et al., 1994). Interaction between these inhibitory and excitatory inputs contributes importantly to temporal signal processing (Covey and Casseday, 1999; Casseday and Covey, 1995).

One of the major inhibitory transmitters in the IC is γ-aminobutyric acid (GABA) (Fubara et al., 1996; Roberts and Ribak, 1987). The $GABA_A$ receptor allows Cl^- to flow into neurons resulting in hyperpolarization in the neurons (Bormann, 1988; Perkins and Wong, 1997). Many studies have determined the contribution of GABAergic inhibition to auditory temporal processing in the IC by means of iontophoretic application of bicuculline, an antagonist for $GABA_A$ receptors. These

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Abbreviations: BF, best frequency; DNLL, dorsal nucleus of the lateral lemniscus; EI, excitation–inhibition; EE, excitation–excitation; EO, monaural excitation; FTC, frequency tuning curve; GABA, γ-aminobutyric acid; IC, inferior colliculus; MT, minimum threshold; PST, peristimulus time

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studies have shown that GABAergic inhibition shapes the discharge pattern, response latency, frequency tuning, duration selectivity, binaural signal processing, recovery cycle and selectivity for frequency modulation of IC neurons under free field stimulation conditions (Casseday et al., 1994; Faingold et al., 1991; Fuzessery and Hall, 1996; Jen and Feng, 1999; Klug et al., 1995; Koch and Grothe, 1998; Le Beau et al., 1996, 2001; Lu and Jen, 2001; Park and Pollak, 1993; Pollak et al., 2002; Yang et al., 1992). Bicuculline application also abolishes direction-dependent frequency tuning of IC neurons (Jen and Zhang, 2000; Zhang et al., 1999) and increases sensitivity to sound motion cues (McAlpine and Palmer, 2002).

Under closed system stimulation conditions, previous studies have shown that IC neurons have three types of aurality (Erulkar, 1972; Fuzessery and Pollak, 1985; Semple and Kitzes, 1985, 1987). The EI (excitation–inhibition) neurons are excited by sound stimulation to the contralateral ear and inhibited by sound stimulation to the ipsilateral ear. The EE (excitation–excitation) neurons are excited by sound stimulation presented to either ear. The EO neurons are only excited by sound stimulation to the contralateral ear. The role of these three types of IC neurons in sound localization has been studied by plotting their interaural intensity difference (IID) curves (see review by Erulkar, 1972). It has been shown that IID curves and binaural response properties of IC neurons are shaped by binaural GABAergic inhibition that is mainly from the dorsal nucleus of the lateral lemniscus (DNLL) (Klug et al., 1995; Li and Kelly, 1992; Park and Pollak, 1993; Pollak et al., 2002).

Previous free field studies have shown that excitatory frequency tuning curves (FTCs) of IC neurons were sharper when plotted with sounds delivered from ipsilateral than from contralateral angles (Gooler et al., 1993; Jen and Zhang, 2000; Zhang et al., 1999). However, whether this direction-dependent sharpening of FTCs might be related to aurality of IC neurons has not been determined. Because both EE and EO neurons typically receive little or no inhibition from the ipsilateral ear, it is conceivable that direction-dependent sharpening of FTCs of EE and EO neurons might not be as great as in EI neurons.

The main objective of this study was to determine how binaural interaction shapes response properties of IC neurons under combined closed system and free field stimulation conditions. Specifically, we determined the aurality of IC neurons under contralateral and ipsilateral sound stimulation. For EI neurons, we studied contralateral excitatory and ipsilateral inhibitory FTCs as well as IID curves before and during bicuculline application. For EE neurons, we plotted FTCs and rate–intensity functions with sound stimulation from each ear. We then studied how disruption of binaural input by monaural occlusion of the ipsilateral ear might affect direction-dependent sharpening of FTCs of IC neurons of different aural types under free field stimulation conditions. This approach of combined closed system and free field study of responses of IC neurons allowed us to determine the correlation between neuronal aurality and direction-dependent frequency tuning.

2. Materials and methods

2.1. Surgery, stimulation and recording

As described in a previous study (Jen et al., 1987), a 1.8 cm nail was glued onto the exposed skull of each of six Nembutal-anesthetized (45–50 mg/kg b.w.) *Eptesicus fuscus* (four males and two females, 12–24 g b.w.) with acrylic glue and dental cement 1 or 2 days before the recording session. Exposed tissue was treated with an antibiotic (Neosporin) to prevent inflammation. During recording, the bat was administered the neuroleptanalgesic, Innovar-Vet (fentanyl 0.08 mg/kg b.w., droperidol 4 mg/kg b.w.) and was placed inside a bat holder (made of wire mesh) which was suspended in an elastic sling inside a sound-proof room (temperature 28–30°C). The ceiling and inside walls of the room were covered with 3 inch convoluted polyurethane foam to reduce echoes. A local anesthetic (lidocaine) was applied to the open wound area. After immobilizing the bat's head by fixing the nail with a set screw, a small hole was drilled in the skull above the IC for insertion of piggy-back multibarrel electrodes for recording auditory responses and for iontophoretic application of bicuculline. The recording depth was read from the scale of a microdrive (David-Kopf). An indifferent electrode (silver wire) was placed at the nearby temporal muscles.

For sound stimulation, continuous sine waves from an oscillator (KH model 1200) were formed into 4 ms tones (0.5 ms rise–decay times, at 2 pps) by an electronic switch driven by a stimulator (Grass S88). These tones were amplified after passing through a decade attenuator (HP 350D) before being fed to a small condenser loudspeaker (AKG model CK 50, 1.5 cm diameter, 1.2 g). The loudspeaker was placed 23 cm away from the bat and 30° contralateral to the recording site. The loudspeaker was calibrated with a Brüel and Kjaer 1/4 inch microphone (4135) placed at the position where the bat's head would be during recording. The output was expressed in dB SPL referred to 20 μPa root mean square.

Construction and calibration of earphones for closed system stimulation have been described in previous studies (Schlegel, 1977; Shen et al., 1997). Briefly, two 4 ms tones generated by two independent stimulation

systems were fed into two 1/4 inch Brüel and Kjaer (4135) microphones. Each microphone was snugly fitted into a custom-made plastic adapter with its tip inserted into the funnel of the external ear. These 'earphones' were calibrated with a 1/8 inch Brüel and Kjaer (4138) microphone placed at about 1 mm in front of the adapter tip. Its output was expressed in dB SPL referred to 20 μPa root mean square.

The construction of piggy-back multibarrel electrodes for recording and iontophoretic application of bicuculline has been described in a previous study (Lu et al., 1997). Briefly, a three barrel electrode (tip: 10–15 μm) was 'piggybacked'to a 3 M KCl single barrel electrode (tip: less than 1 μm; impedance: 5–10 MΩ) whose tip was extended about 10 μm beyond the tip of the three barrel electrode. The 3 M KCl single barrel recording electrode was connected by a silver wire to an amplifier (HP 465A) followed by an electronic filter (KH 3500). One of the barrels of the three barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 M NaCl, pH 3.0, Sigma). The bicuculline was prepared just prior to each experiment and the electrode filled immediately before use. This drug channel was connected via a silver–silver chloride wire to a microiontophoresis constant current generator (Medical Systems Neurophore BH-2) which was used to generate and monitor iontophoretic currents. During bicuculline application, a 1 s pulse of 40 nA was applied at a rate of 0.5/s for 1–2 min before data acquisition. The application current was changed to 10 nA during data acquisition. The other two barrels were filled with 1 M NaCl (pH 7.4), one of which was used as the ground and the other as the balanced barrel. The balance electrode was connected to a balance module. The retention current was negative 8–10 nA.

2.2. Data acquisition and analysis

Upon isolation of an IC neuron by contralateral sound stimulation, its best frequency (BF) was determined by changing the frequency and intensity of sound pulses. The minimum threshold (MT) at the BF was defined as the intensity at which the probability of responding to BF sounds was 50%. The neuron's FTC was plotted with the threshold of each responsive frequency. The rate–intensity function was plotted with the number of impulses obtained with a BF sound delivered at MT and at 10 dB increments above the MT.

The aurality of the neuron was determined by delivering BF sounds at different intensities to each ear as described in a previous study (Shen et al., 1997). When a neuron responded to sound stimulation at each ear, and the MT difference between stimulation at two ears was less than the acoustic isolation (50 dB), the neuron was referred to as an EE neuron. When a neuron did not respond to ipsilateral sound stimulation, a BF sound at 20 dB above the neuron's MT was delivered to the contralateral ear to elicit an excitatory response. The sound intensity at the ipsilateral ear was then systematically adjusted in 10 dB increments from 20 dB lower to 40 dB higher than contralateral sound intensity such that the neuron's binaural response was examined over a maximal range of 60 dB. When the neuron's response to contralateral sound stimulation was reduced by more than 20% by ipsilateral sound stimulation at any IID, the neuron was referred to as an EI neuron. When a neuron's response to contralateral sound stimulation was affected by less than 20% by ipsilateral sound stimulation at all IIDs, the neuron was referred to as an EO neuron. IID curves were plotted with the number of impulses obtained at different IIDs before and during bicuculline application.

Under closed system stimulation conditions, the contralateral excitatory FTC of an EI neuron was plotted by contralateral sound stimulation. An ipsilateral inhibitory FTC was then plotted with combinations of frequency and intensity of ipsilateral sound stimulation that decreased at least 20% of a neuron's excitatory responses elicited by contralateral sound stimulation (a BF sound at 10 dB above the MT). These excitatory and inhibitory FTCs were plotted before and during bicuculline application. For EE neurons, the excitatory FTCs and rate–intensity functions were obtained by both contralateral and ipsilateral sound stimulation. For EO neurons, only excitatory FTCs were plotted with contralateral sound stimulation.

The contribution of ipsilateral inhibition to direction-dependent FTCs of IC neurons was studied by plotting the excitatory FTCs with free field sounds delivered from two sound directions (30° contralateral and 30° ipsilateral relative to the recording sites) before and after occlusion of the ipsilateral ear. Occlusion was achieved by inserting a wet cotton ball into the external ear canal before covering with a plastic cap. Such an occlusion attenuated the sound intensity at the ipsilateral ear by an average of 20 ± 12 dB in the frequency range of 20–80 kHz.

Recorded action potentials were amplified, bandpass-filtered (Krohn-Hite 3500), and fed through a window discriminator (WPI 121) before being sent to an oscilloscope (Tektronix 5111) and an audio monitor (Grass AM6). They were then sent to a computer (Gateway 2000, 486) for acquisition of peristimulus time (PST) histograms (bin width: 500 μs, sampling period 300 ms) to 32 stimuli. The PST histograms quantitatively describe a neuron's discharge pattern under different stimulation conditions. The total number of impulses in each PST histogram was used to quantify a neuron's response under each specific stimulation condition.

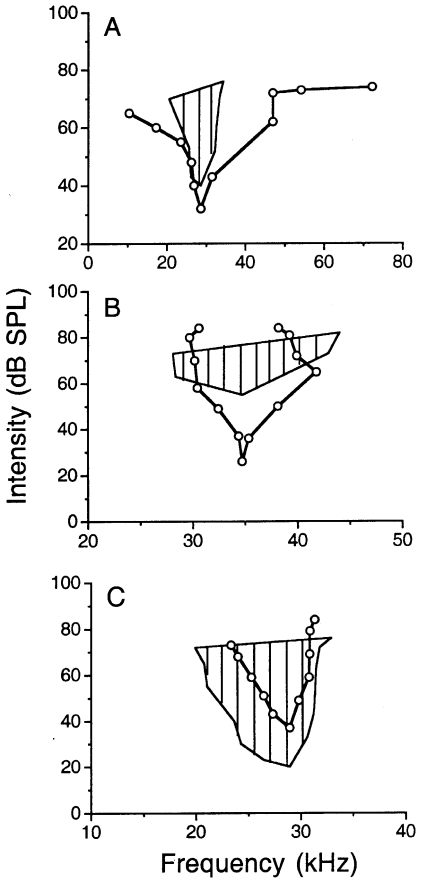

Fig. 1. Contralateral excitatory FTCs (unfilled circles) and ipsilateral inhibitory FTCs (shaded areas) of three EI neurons. The ipsilateral inhibitory FTCs either fell within (A), partially overlapped (B), or nearly encompassed (C) the contralateral excitatory FTCs. Note that the excitatory and inhibitory FTCs had similar BFs.

3. Results

Under closed system stimulation conditions, 76 IC neurons at depths of 115–1920 μm were isolated by contralateral sound stimulation. Most neurons discharged either fewer than three impulses (phasic responders, $n = 49$, 65%) or three to seven impulses (phasic bursters, $n = 17$, 22%) to 4 ms BF sounds. They did not further increase the number of impulses when the BF sounds were lengthened to 20 ms (for methods, see Lu et al., 1997, fig. 1). The remaining neurons (tonic responders, $n = 10$, 13%) discharged impulses throughout the entire BF sounds of 4 or 20 ms. The BF ranged between 20.8 and 75.3 kHz with MT between 21 and 77 dB SPL. The first spike latency in response to 4 ms BF sounds at 10 dB above the MT was between 4.5 and 22.0 ms. These response properties were similar to those reported previously (Casseday and Covey, 1995; Jen and Schlegel, 1982; Jen and Feng, 1999; Lu et al., 1997; Lu and Jen, 2001). The aurality of these neurons was 46 (60.5%) EI neurons, 24 (31.5%) EO neurons and six (8%) EE neurons.

3.1. Contralateral excitatory and ipsilateral inhibitory FTCs of EI neurons

Representative contralateral excitatory and ipsilateral inhibitory FTCs of three types of EI neurons are shown in Fig. 1. Ipsilateral inhibitory FTCs of the first type fell within corresponding excitatory FTCs ($n = 10$, 22%; Fig. 1A). These inhibitory FTCs were typically narrower with higher MTs than corresponding excitatory FTCs. Ipsilateral inhibitory FTCs of the second type also had higher MTs but had a wider frequency response spectrum than corresponding excitatory FTCs ($n = 26$, 56%; Fig. 1B). Ipsilateral inhibitory FTCs of the third type were broadly tuned with low MT to encompass corresponding excitatory FTCs ($n = 10$, 22%; Fig. 1C).

A comparison of BFs and MTs of contralateral excitatory and ipsilateral inhibitory FTCs of all 46 EI neurons is shown in Fig. 2. Excluding five neurons that had BF differences of 1.5, 1.7, 2.3, 2.3, and 2.5 kHz, excitatory and inhibitory BFs of remaining neurons matched each other such that their data points fell on the equal value dashed line (Fig. 2A). However, excluding two (4%) neurons that had identical excitatory and inhibitory MTs, inhibitory MTs of remaining

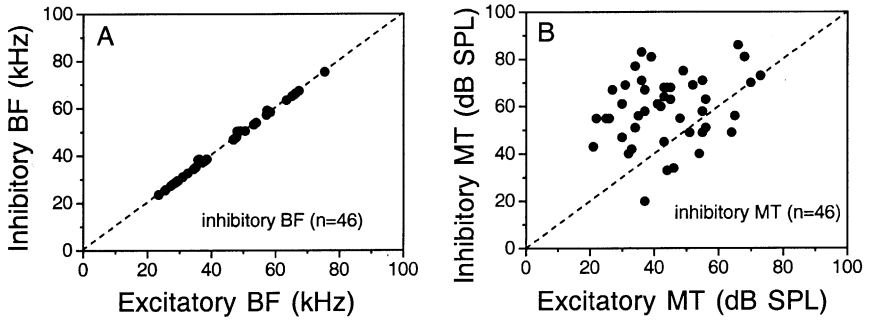

Fig. 2. Comparisons of BFs (A) and MTs (B) between excitatory and ipsilateral inhibitory FTCs. The dashed line indicates equal value. Note that most excitatory and inhibitory FTCs had similar BFs but different MTs (see text for details).

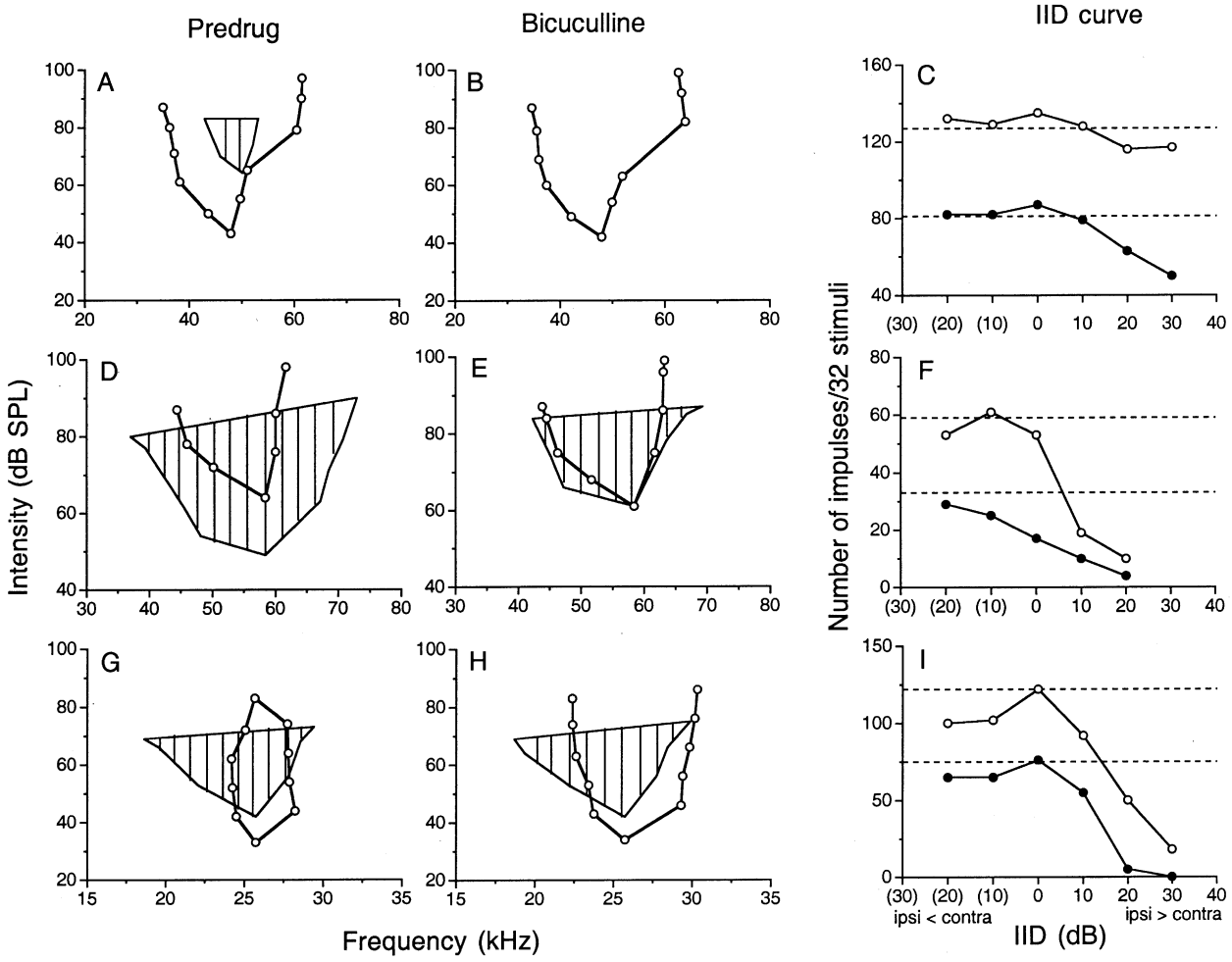

Fig. 3. Excitatory and inhibitory FTCs and IID curves of three EI neurons obtained before and during bicuculline application. Bicuculline application broadened all three excitatory FTCs (unfilled circles in A,B,D,E,G,H) but either completely abolished, partly abolished or did not affect the ipsilateral inhibitory FTCs (A vs. B, D vs. E, G vs. H, shaded areas). The IID curves of all three neurons were shifted upward to varying degrees during bicuculline application (C,F,I, unfilled circles vs. filled circles). The dashed lines indicate the discharge rate to contralateral sound stimulation at the BF and 20 dB above the excitatory MT obtained before (lower dashed line) and during (upper dashed line) bicuculline application.

neurons were either higher ($n = 35$, 76%) or lower ($n = 9$, 20%) than corresponding excitatory MTs. As such, their data were distributed either above or below the equal value dashed line (Fig. 2B).

3.2. *Effects of bicuculline application on excitatory and inhibitory FTCs and IID curves of EI neurons*

The effect of bicuculline application on excitatory and ipsilateral inhibitory FTCs as well as IID curves was examined in 10 EI neurons. As shown in Fig. 3, ipsilateral inhibitory FTCs of three representative EI neurons either fell within or largely covered corresponding excitatory FTCs (Fig. 3, unfilled circles vs. shaded areas). Contralateral excitatory FTCs of these three neurons plotted before bicuculline application were either V-shaped or closed (Fig. 3A, D vs. G, unfilled circles). Bicuculline application broadened all three excitatory FTCs and opened the closed FTC into V-shaped (Fig. 3B,E,H, unfilled circles). Bicuculline application completely abolished, partly decreased or did not affect ipsilateral inhibitory FTCs of these three EI neurons (Fig. 3A vs. B, D vs. E, G vs. H, shaded area). In total, bicuculline application broadened eight excitatory FTCs and completely ($n = 2$), partly ($n = 7$) or did not ($n = 1$) abolish corresponding ipsilateral inhibitory FTCs.

The discharge rate of these EI neurons was a sigmoid function of IID (Fig. 3C,F,I). The large discharge rate occurred at IIDs when contralateral sound stimulation was stronger than ipsilateral sound stimulation. Discharge progressively dropped to a minimum rate when ipsilateral sound stimulation became stronger than contralateral sound stimulation. Bicuculline application in-

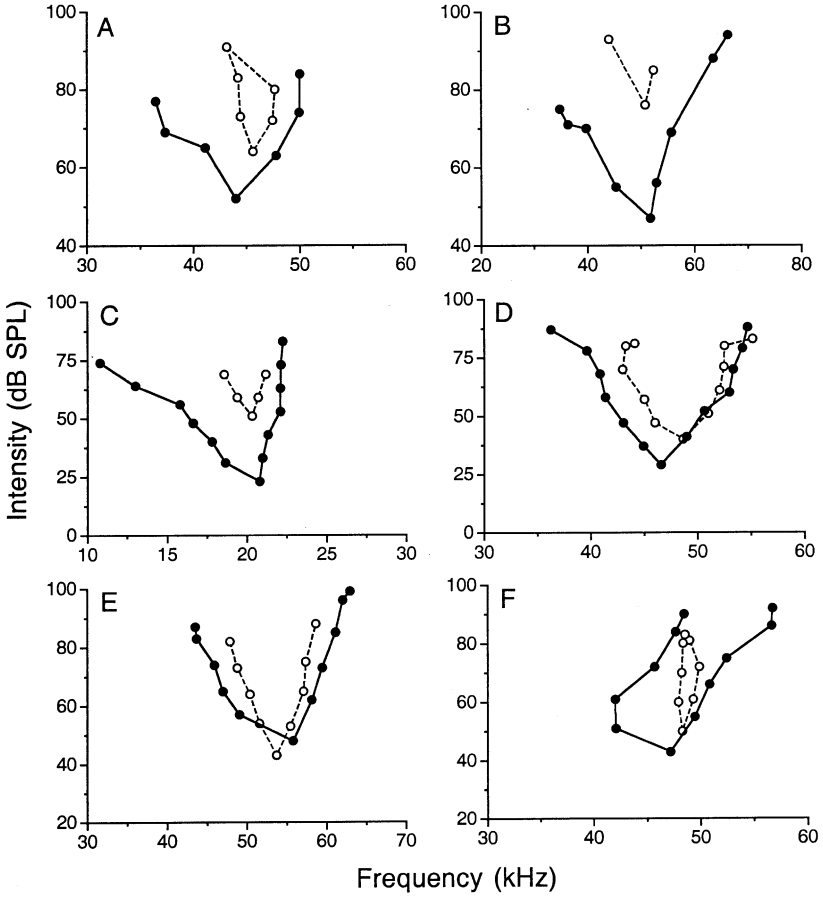

Fig. 4. Excitatory FTCs of six EE neurons determined with contralateral (filled circles) and ipsilateral (unfilled circles) sound stimulation. Contralateral FTCs were typically broader and lower than ipsilateral FTCs. Two neurons had closed FTCs when determined with ipsilateral sound stimulation (A,F) (see text for details).

creased the discharge rate and raised the sigmoid IID curves to varying degrees among individual EI neurons. For example, bicuculline application increased the discharge rate of one neuron almost with equal amounts at each IID (Fig. 3I). Conversely, bicuculline application differentially increased the discharge rate of another two EI neurons at different IIDs (Fig. 3C,F). The aurality of eight EI neurons was not affected during bicuculline application. However, two neurons changed from EI into EO neurons because of a large increase in the discharge rate when ipsilateral sound stimulation was stronger than contralateral sound stimulation.

3.3. *Excitatory FTCs and rate–intensity functions of EE neurons*

Excitatory FTCs of the six EE neurons were plotted by both contralateral and ipsilateral sound stimulation. The FTCs obtained by contralateral sound stimulation (referred to as contralateral FTCs) were broader than those obtained by ipsilateral sound stimulation (referred to as ipsilateral FTCs) (Fig. 4, filled vs. unfilled circles). Contralateral and ipsilateral FTCs of four EE neurons were both V-shaped (Fig. 4B–E, filled vs. unfilled circles). Another neuron had a closed ipsilateral FTC but had an irregular contralateral FTC with low frequency branch bending toward the high frequency side at high sound intensities (Fig. 4F, unfilled vs. filled circles). The remaining neuron had a V-shaped contralateral FTC but had a closed ipsilateral FTC (Fig. 4A, filled vs. unfilled circles).

The BFs of contralateral FTCs were either lower (Fig. 4A,D,F) or higher (Fig. 4B,C,E) than BFs of ipsilateral FTCs. However, BF differences were never larger than 3 kHz. The MTs of all but one contralateral FTC were lower than the MTs of corresponding ipsilateral FTCs (Fig. 4A–D, F vs. E).

Fig. 5A shows PST histograms of a representative EE neuron obtained under different stimulation conditions. This neuron responded to a wide range of sound intensity during contralateral sound stimulation but only responded to a narrow range of sound intensity during ipsilateral sound stimulation. At a given sound inten-

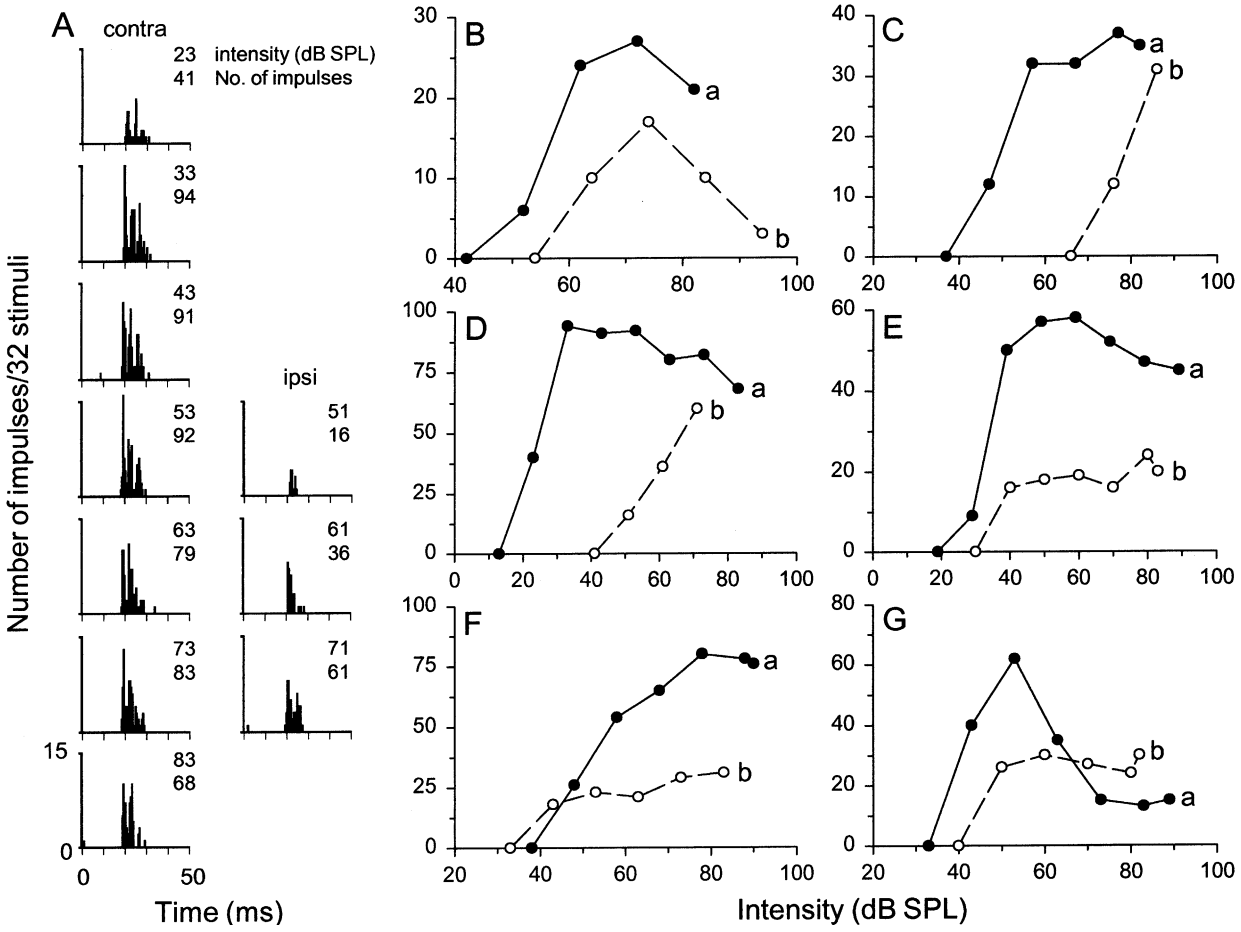

Fig. 5. (A) PST histograms showing discharge patterns and number of impulses of a representative EE neuron obtained with BF sounds delivered at different stimulus intensities under contralateral and ipsilateral stimulation. Note that this neuron discharged fewer impulses with longer first spike latency to ipsilateral stimulation than to contralateral stimulation. (B–G) Rate–intensity functions of six EE neurons determined by contralateral (filled circles) and ipsilateral (unfilled circles) sound stimulation. Note that contralateral sound stimulation produced higher rate–intensity functions than ipsilateral sound stimulation.

sity, the neuron discharged more impulses with shorter latency under contralateral than ipsilateral sound stimulation.

EE neurons did not always display the same discharge pattern under contralateral and ipsilateral sound stimulation. While all six EE neurons discharged phasically during ipsilateral sound stimulation, three were phasic bursters and the other three were phasic responders during contralateral sound stimulation.

The rate–intensity functions of these six EE neurons obtained under contralateral and ipsilateral sound stimulation are shown in Fig. 5B–G. Contralateral sound stimulation produced higher rate–intensity functions than ipsilateral sound stimulation (Fig. 5, solid vs. unfilled circles). Rate–intensity functions of these EE neurons plotted under contralateral sound stimulation were either non-monotonic or saturated in which the number of impulses either decreased more than 25% from the maximum or reached a plateau at strong stimulus intensity (Fig. 5Ba–Ga). Monotonic rate–intensity functions were only obtained under ipsilateral stimulation (Fig. 5Cb,Db).

3.4. *Effect of monaural occlusion on excitatory FTCs plotted at two sound directions*

To determine the role of ipsilateral inhibition on direction-dependent FTC, we plotted FTCs of 30 IC neurons (21 EI neurons, six EO neurons, and three EE neurons) with sounds delivered at 30° ipsilateral and 30° contralateral before and after occlusion of the ipsilateral ear. Fig. 6 shows excitatory FTCs of three representative IC neurons of each aural type plotted at two sound directions before and after occlusion of the ipsilateral ear. Excitatory FTCs of all three IC neurons were sharper at 30° ipsilateral than at 30° contralateral (Fig. 6A vs. B, C vs. D, E vs. F, unfilled circles). However, occlusion of the ipsilateral ear produced different effects on these FTCs. For example, occlusion of the ipsilateral ear broadened the excitatory FTC of an EI

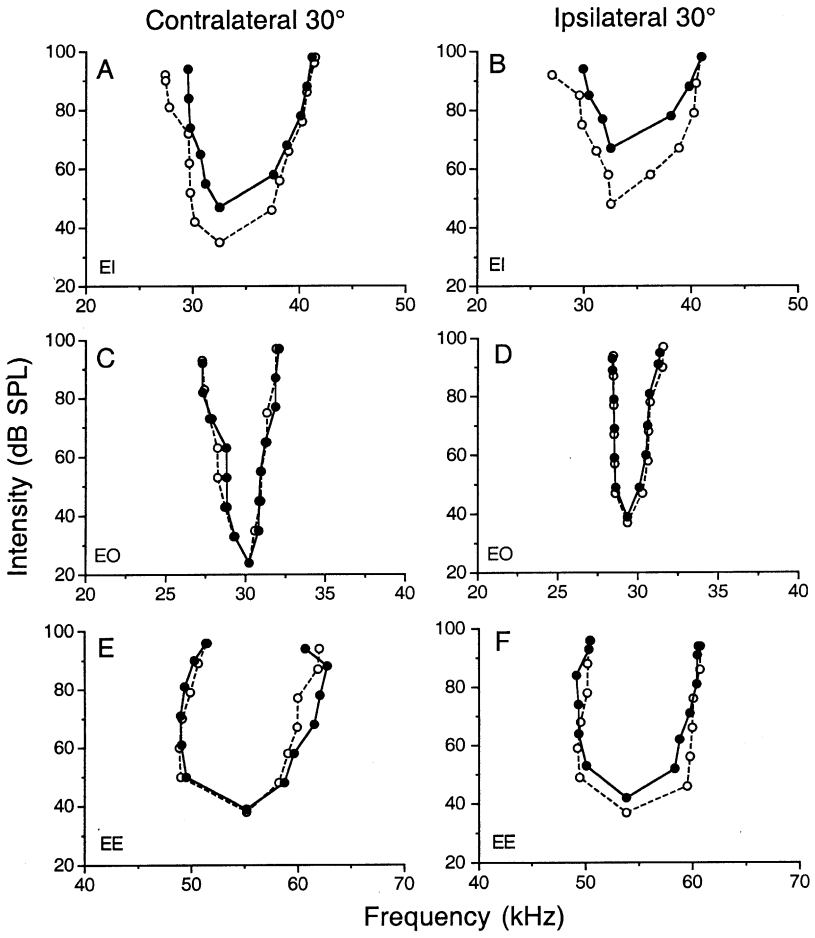

Fig. 6. Free field excitatory FTCs of EI, EO, and EE neurons plotted at two sound directions (30° contralateral and 30° ipsilateral relative to the recording site) before (filled circles) and after (unfilled circles) occlusion of the ipsilateral ear. Note that broadening of FTC of the EI neuron was more pronounced at 30° ipsilateral than at 30° contralateral (A vs. B). However, monaural occlusion hardly broadened the FTCs of EO (C,D) or EE (E,F) neurons (see text for details).

neuron and lowered its MT to a greater degree at 30° ipsilateral than at 30° contralateral (Fig. 6A,B, unfilled circles). However, occlusion of the ipsilateral ear hardly affected excitatory FTCs of an EO (Fig. 6C,D, unfilled vs. filled circles) neuron and decreased the MT of an EE neuron by only 2 dB at ipsilateral 30° (Fig. 6E vs. F, unfilled vs. filled circles).

We quantitatively compared the sharpness of FTCs of these neurons at two sound directions before and after monaural occlusion by calculating the Q_{10} and

Table 1
Average Q_{10} and Q_{30} values of excitatory FTCs of EI, EO and EE neurons determined at two sound directions before and after occlusion of the ipsilateral ear

		Sound direction		t-test, P
		Contralateral 30°	Ipsilateral 30°	
EI neurons				
Q_{10}	open	9.1 ± 5.2 (21)	15.9 ± 13.2 (18)	0.036*
	occlusion	7.6 ± 4.4 (21)	10.6 ± 7.4 (18)	0.8873
Q_{30}	open	4.0 ± 1.9 (16)	6.3 ± 2.3 (5)	0.0361*
	occlusion	4.1 ± 2.0 (17)	5.2 ± 2.3 (10)	0.2033
EO/EE neurons				
Q_{10}	open	7.5 ± 6.5 (9)	12.1 ± 9.4 (7)	0.2658
	occlusion	7.4 ± 6.8 (9)	11.0 ± 8.1 (7)	0.3498
Q_{30}	open	5.7 ± 4.6 (7)	6.8 ± 4.9 (6)	0.6845
	occlusion	5.4 ± 3.9 (7)	6.8 ± 5.9 (6)	0.6185

Number of neurons is shown in parentheses. *: significant at $P < 0.05$.

Q_{30} values. The Q_{10} and Q_{30} values were obtained by dividing the BF by the bandwidth of a FTC at 10 and 30 dB above the MT. Large Q_{10} and Q_{30} values indicate sharper frequency tuning. As shown in Table 1, Q_{10} and Q_{30} values of EI neurons were significantly larger when obtained at 30° ipsilateral than at 30° contralateral before occlusion of the ipsilateral ear ($P < 0.05$). However, this direction-dependent variation in Q_{10} and Q_{30} values was abolished after occlusion of the ipsilateral ear ($P > 0.05$).

4. Discussion

Consistent with previous studies (Erulkar, 1972; Fuzessery and Pollak, 1985; Semple and Kitzes, 1985, 1987), we observed that most IC neurons were EI neurons that were excited by contralateral stimulation but inhibited by ipsilateral stimulation. Possible sources for inhibition of EI neurons include glycinergic inputs from the ipsilateral lateral superior olivary nucleus and GABAergic inputs from the contralateral DNLL (Adams and Mugnaini, 1984; Pollak et al., 2002; Pollak and Casseday, 1989; Roberts and Ribak, 1987; Schneiderman and Oliver, 1989). These two inhibitory inputs are excited by ipsilateral stimulation and exert their inhibitory influence on EI neurons.

While contralateral excitatory FTCs and ipsilateral inhibitory FTCs typically had similar BFs, most excitatory MTs were lower than corresponding inhibitory MTs (Fig. 2). However, Yang and Pollak (1998) showed that contralateral excitatory FTCs and ipsilateral inhibitory FTCs of neurons in the bat DNLL match in both BF and MT. They plotted ipsilateral inhibitory FTCs of DNLL neurons by combinations of frequency and intensity of ipsilateral sound stimulation that suppressed background activity elicited by glutamate application. We do not know if these different observations between the two studies are due to different inhibition in the DNLL and IC or due to different experimental paradigms.

We observed that ipsilateral inhibitory FTCs plotted under the binaural stimulation paradigm either fell within, partly overlapped, or entirely encompassed contralateral excitatory FTCs (Fig. 1). However, previous studies have shown that inhibitory FTCs were at one or both flanks of excitatory FTCs of IC neurons using a two tone (i.e. a probe and a masker) stimulation paradigm under free field stimulation conditions (see review in Suga, 1995). Under this paradigm, the inhibitory FTC of a neuron was plotted with frequency–intensity combinations of a masker that decreased the neuron's probe-elicited excitatory response by at least 20%. When studied under closed system stimulation conditions, these inhibitory FTCs can also be plotted at one or both flanks of excitatory FTCs of IC neurons despite their aurality (Ehret and Merzenich, 1988; Lu and Jen, 1999). Inhibition derived from these inhibitory FTCs has been called lateral or sideband inhibition which contributes to sharpening of excitatory FTCs. For example, we have previously shown that excitatory FTCs of IC neurons with lateral inhibitory FTCs were sharper than those without inhibitory FTCs and excitatory FTCs with two lateral inhibitory FTCs were sharper than those with one inhibitory FTC (Lu and Jen, 2001). The different spectral distribution of binaurally derived ipsilateral inhibitory FTCs and monaurally derived lateral inhibitory FTCs relative to excitatory FTCs might simply be due to different experimental paradigms used to plot these inhibitory FTCs. It may also be an indication that these two types of inhibitory FTCs are formed through different inhibitory pathways and they play different roles in signal processing. As described earlier, binaurally derived ipsilateral inhibition shapes various binaural response properties of IC neurons including IID curves, aural type and directional sensitivity (Erulkar, 1972; Klug et al., 1995; Li and Kelly, 1992; Park and Pollak, 1993; Pollak et al., 2002).

In agreement with previous studies (Gooler et al., 1996; Jen and Zhang, 2000; Zhang et al., 1999), we observed that excitatory FTCs of IC neurons were sharper at 30° ipsilateral than at 30° contralateral and sharpening of FTCs occurred mostly at the tip of FTCs (Fig. 6, Table 1). We have previously suggested that decreased excitation due to ipsilateral inhibition and/or head shadowing with sound direction contributes to direction-dependent frequency tuning of IC neurons (Jen and Zhang, 2000). For example, when a sound is delivered from 30° ipsilateral, it produces a strong intensity at the ipsilateral ear and thus a strong neural inhibition to an EI neuron. Conversely, head shadowing reduces the sound intensity and thus produces a weak neural excitation to the EI neuron at the contralateral ear. This increase in the strength of ipsilateral inhibition and decrease in the strength of contralateral excitation differentially elevate the MTs across a wide range of frequencies especially at off-BFs resulting in a sharpening of the FTC at 30° ipsilateral (e.g. Figs. 1 and 2 in Jen and Zhang, 2000). When the same sound is delivered at 30° contralateral, reducing head shadowing increases the strength of excitation at the contralateral ear while increasing head shadowing decreases the strength of inhibition at the ipsilateral ear. This variation in the strength of excitation at the two ears differently lowers the MTs across a wide range of frequencies resulting in a broadening of the FTC at 30° contralateral. For EO neurons, contralateral sound stimulation should produce maximal excitation while ipsilateral sound stimulation should produce weak excitation due

to head shadowing. Because EE neurons typically have higher MTs for ipsilateral than for contralateral sound stimulation (Fig. 5), a sound delivered from 30° ipsilateral would be less effective in exciting these neurons than from 30° contralateral resulting a sharper FTC at 30° ipsilateral than at 30° contralateral.

However, neural inhibition appears to contribute more significantly to direction-dependent frequency of IC neurons than head shadowing. This is evidenced by the fact that sharpness of FTCs of EI neurons but not EO or EE neurons significantly increased when sound direction changed from 30° contralateral to 30° ipsilateral (Table 1). Furthermore, occlusion of the ipsilateral ear produced more pronounced broadening of excitatory FTCs of EI neurons at 30° contralateral (Fig. 6A vs. B). As a result, significant direction-dependent difference in Q_{10} and Q_{30} values of EI neurons was abolished (Table 1). Previous studies have shown that direction-dependent sharpening of FTCs of EI neurons was primarily due to differential GABAergic inhibition with sound direction (Jen and Zhang, 2000; Zhang et al., 1999). These studies showed that bicuculline application significantly broadened FTCs of IC neurons at both sound directions and abolished direction-dependent frequency tuning of EI neurons.

Blocking $GABA_A$ receptors by bicuculline application produced an increase in discharge rate that did not differ substantially across IIDs of EI neurons even when the ipsilateral sound stimulation was weaker than contralateral sound stimulation (i.e. left half of IID curves of Fig. 3C,F,I). Hence, the IID curves were shifted upwards almost in parallel to predrug ones and the aurality of most neurons was not changed (Fig. 3C,F,I). These data suggest that bicuculline application did not effectively alter binaural interactions that shape the IID curves of these neurons. Presumably, bicuculline application had removed all GABAergic inhibition regardless of its origin as such ipsilateral GABAergic inhibition from DNLL that shapes the IID curves was masked by the large increase of discharge rate across IIDs (Li and Kelly, 1992; Pollak et al., 2002).

Bicuculline application broadened the excitatory FTCs but did not completely abolish corresponding inhibitory FTCs of some EI neurons (Fig. 3D vs. E, G vs. H). These data indicate that excitatory FTCs of these EI neurons are not solely shaped by GABAergic inhibition. For example, we have previously shown that glycinergic inhibition contributes exclusively to sharpening of excitatory FTCs of 12.5% of IC neurons (Lu and Jen, 2001). It is also conceivable that some EI neurons simply inherited the inhibitory FTC from the lower order neurons.

We observed that EE neurons typically discharged fewer impulses with longer latency and had lower rate–intensity functions and sharper FTCs with higher MTs by ipsilateral than by contralateral stimulation (Figs. 4 and 5). Similar findings have been reported for IC neurons in cats (Aitkin and Reynolds, 1975), frogs (Gooler et al., 1996), bats (Fuzessery et al., 1990) and gerbils (Semple and Kitzes, 1985). It has been suggested that this difference in contralateral and ipsilateral excitatory FTCs is due to stronger ipsilateral than contralateral inhibitory inputs (Gooler et al., 1996). Conversely, Semple and Kitzes (1985) suggested that this observation is due to weaker ipsilateral than contralateral excitatory inputs.

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