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Frequency-Modulation Component of the Mimic Echolocation Sound can Increase the Sensitivity of Inferior Collicular Neurons to Sound Amplitude in the Leaf-Nosed Bat, *Hipposideros armiger*

Zi-Ying Fu, Jia Tang, Yang Li, Hong Zeng, and Qi-Cai Chen*

College of Life Sciences and Hubei Key Lab of Genetic Regulation and Integrative Biology, Central China Normal University, Wuhan 430079, China

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Zi-Ying Fu, Jia Tang, Yang Li, Hong Zeng, and Qi-Cai Chen (2011) Frequency-modulation component of the mimic echolocation sound can increase the sensitivity of inferior collicular neurons to sound amplitude in the leaf-nosed bat, *Hipposideros armiger*. *Zoological Studies* **50**(5): 537-545. A frequency modulation (FM) component widely exists in many bats' echolocation calls and human speech sounds. In order to investigate the role of the FM component in sound communication, 6 leaf-nosed bats, *Hipposideros armiger*, were used in this experiment. In total, 113 inferior collicular (IC) neurons were obtained, and the responses of 61 neurons among these neurons to constant frequency (CF) and mimic CF-FM sounds and their rate-amplitude functions (RAFs) were studied. According to the responses of the neurons to CF and CF-FM sounds at different amplitudes, RAFs of these neurons were classified into 3 types, monotonic, saturated, and non-monotonic. When CF sounds were changed into CF-FM sounds, the best amplitudes of the neurons decreased from 93.0 ± 9.8 to 84.4 ± 11.3 dB SPL ($p < 0.01$), and the dynamic ranges decreased from 27.5 ± 9.4 to 22.5 ± 8.5 dB ($p < 0.05$), but the slopes of the RAFs increased from 3.1 ± 1.4 to 4.1 ± 1.7%/dB ($p < 0.05$). By further analyzing the influence of the FM component on the firing rate of the neurons, it was shown that the FM component elevated the firing rate of the neurons at < 30 dB above the minimum threshold (MT), while reducing the firing rate at > 30 dB above the MT ($p < 0.001$). The results indicate that the FM component of CF-FM sound can increase the sensitivity of IC neurons to sound amplitude. http://zoolstud.sinica.edu.tw/Journals/50.5/537.pdf

Key words: FM component, Rate-amplitude function, Sound amplitude sensitivity, Inferior collicular neurons.

Echolocation signals of leaf-nosed bats (*Hipposideridae*), horseshoe bats (*Rhinolophidae*), and mustached bat *Pteronotus parnellii* (*Mormoopidae*) consist of a long segment of constant-frequency (CF) component and a short segment of frequency-modulation (FM) component (Gustafson and Schnitzler 1979, Jones et al. 1993, Hiryu et al. 2006, Gu et al. 2008). Therefore, they are called CF-FM bats, the recorded biosonar signals of which generally contain 4 harmonics (H1-H4); the 2nd harmonic (H2) is usually called the dominant one.

The CF and FM components of CF-FM bats' echolocation signals play different roles during echolocation. The CF component can generate a Doppler shift during bat flight and insect wing beating; therefore, the CF component is thought to be responsible for detecting the wing size, wing-beat velocity, and flying speed of insects (Kober and Schnitzler 1990, Schnitzler and Denzinger 2011), while the FM component is used to detect the target distance and texture (Simmons and Vernon 1971, Fitzpatrick et al. 1991). Because of the need for different functions corresponding to different components of bats' echolocation signals, there are some specialized areas in the bats'

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*To whom correspondence and reprint requests should be addressed. Zi-Ying Fu and Jia Tang contribute equally to this work. Tel: 86-27-67867229. Fax: 86-27-67861936. E-mail:qcchen2003@yahoo.com.cn auditory cortex (AC). For example, there are a Doppler-shifted CF processing (DSCF) area, that allows bats to detect small frequency differences, a CF-CF area that is involved in encoding the magnitude of Doppler shifts in echoes which is thought to identify the velocity of insects, and an FM-FM area that contains neurons tuned to particular time delays between the pulse FM_1 and the echo FM_2, FM_3 or FM_4 are believed to process the target range (Asanuma et al. 1983). These specialized areas are not only found in the AC, they are also found in subcortical auditory structures such as the medial geniculate body (MGB) and inferior colliculus (IC) (Olsen and Suga 1991, Portfors and Wenstrup 1999, Ulanovsky and Moss 2008). Although the 2 components of the CF-FM sound play independent roles during bat echolocation, they may also affect each other. An earlier study on the peripheral auditory system showed that the FM component of the CF-FM sound could inhibit the response, including the cochlear microphonic (CM) potential of hair cells and the compound action potential (AP, N1) of the auditory nerve, to CF components (Suga et al. 1975), but the possible role of this inhibition in bat echolocation remains little understood. Two recent studies in our lab showed that the FM component can enhance the selectivity of bat IC neurons to the CF duration (Luo et al. 2008), while the FM component also participates in shaping the discharge pattern and determining the response latency and firing rates to CF stimuli (Fu et al. 2010). Our studies also found that when a mimic CF-FM sound is used to stimulate IC neurons, 2 types of the responders, single-on responders which discharge only to the onset of CF-FM sounds and double-on responders that discharge to the onset of CF and FM components of CF-FM sounds, were observed, and partial single-on neurons could be changed into double-on neurons when stimulated with higher-intensity sounds (Fu et al. 2010).

The function of the central auditory system in sound amplitude analysis is very important for humans and many other mammals. For example, humans use the interaural amplitude difference to localize a sound source (Chadderton et al. 2009), while insectivorous bats extract information about the size, range, and texture of insects from the amplitude differences between the emitted pulse and the echo (Simmons and Vernon 1971). Because the FM component widely exists in bat echolocation calls and human speech sounds (Fuzessery et al. 2006), it may play an important role in shaping the amplitude sensitivity. To test this hypothesis, we studied the role of the FM component in an amplitude analysis of IC neurons using a CF-FM bat, the leaf-nosed bat (*Hipposideros armiger*). The leaf-nosed bat, a common species in China, is the largest insectivorous bat (Zhang 1997, Wang et al. 2010). Previous evolutional and ecological studies on this species found that the body size and echolocation frequency are negatively correlated (Zhang et al. 2000), the colony size and composition exhibit temporal changes due to the birth and natal dispersal of the young and seasonal migration of adults (Cheng and Lee 2004), and the growth rate is greater than that of many tropical bat species (Lin et al. 2010).

MATERIALS AND METHODS

Surgical procedures

This study used 6 *Hipposideros armiger* (all females, 42-63 g in body weight (BW)) caught from a cave of Tongshan County, Hubei Province, China. The surgical procedures were basically the same as our previous studies (Luo et al. 2007, Tang et al. 2007). Briefly, the flat head of a 2.0-cm nail was glued onto the exposed skull of each Nembutal-anesthetized bat (45-50 mg/kg BW) with acrylic glue and dental cement 1 or 2 d before the recording session. Exposed tissues were treated with an antibiotic (Neosporin) to prevent inflammation. The bat was administered the neuroleptanalgesic, Innovar-Vet (fentanyl 0.04 mg/kg BW and droperidol 2 mg/kg BW) and placed inside a bat holder (made of wire mesh) that was suspended in an elastic sling inside a custom-made double-wall sound-proof room (at a temperature of 28-30°C). The ceiling and inside walls of the room were covered with 8-cm convoluted polyurethane foam to reduce echoes.

After fixing the bat's head with a set screw, small holes (200-500 μm) were made in the skull above the IC for orthogonal insertion of 2 M NaCl glass pipette electrodes (with a tip diameter of < 1 μm, and an impedance of 5-10 MΩ) to record sound-activated responses. Additional doses of Innovar-Vet were administered during later phases of the recording when bats showed signs of discomfort. A local anesthetic (lidocaine) was applied to the open wound area to reduce any possible pain. The recording depth was read from the scale of a microdrive (David Kopf Instrument, model 640, Tujunga, California, USA). A common indifferent electrode (silver wire) was placed on the nearby temporal muscles. Each bat was used in 1 to 5 recording sessions on separate days, and each recording session typically lasted 2-6 h. The number of bats used was minimized to obtain the data required for this study. The experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Central China Normal Univ., Wuhan, Hubei, China.

Acoustic stimuli

For acoustic stimulation, continuous sine waves from a function generator (GFG-8016G, Good Will Instrument, Bayan Lepas, Penang, Malaysia) were formed into pure-tone pulses (5 ms with 0.5-ms rise-decay times, delivered at 2 pulses/s, referred to as the CF sound) by a custom-made tone burst generator (electronic switch) driven by a stimulator (model SEN-7203, Nihon Kohden, Shinjuku, Tokyo, Japan). The tone pulses were then amplified (by a custom-made amplifier) after passing a decade attenuator (LAT-45, Leader, Kohokuku, Yokohama, Japan) before they were fed into a small loudspeaker (AKG model CK 50, 1.5 cm in diameter, 1.2 g, with a frequency response of 1-100 kHz). The loudspeaker was placed 30 cm away from a bat's ear and 30° contralateral to the recording site. Calibration of the loudspeaker was conducted with a 1/4-in. microphone (4939, B&K, Narum, Denmark) placed at the bat's ear using a measuring amplifier (2610, B&K). The output of the loudspeaker was expressed in decibel sound pressure level (dB SPL) in reference to 20 μPa root mean square. A frequency-response curve of the loudspeaker was plotted to determine the maximal available sound amplitude at each frequency. The maximal stimulus amplitude ranged 110-125 dB SPL between 10 and 80 kHz but dropped off sharply to 80 dB SPL at 100 kHz and thereafter.

A mimic echolocation sound, a CF-FM sound, was generated by means of a linear voltage ramp from a function generator (33220A, Agilent, Santa Clara, CA, USA) driven by the same electronic switch. A 7-ms CF sound was first generated. Then, the onset of the linear voltage ramp was adjusted and synchronized at 5 ms of the CF sound such that the amplitude did not change at the boundary between the CF and FM sounds when the frequency of the CF sound (referred as the CF frequency) swept downward 20% within the FM portion. That is, the end frequency of the 2-ms FM component was 80% of the CF frequency.

Recording of neuronal responses

Upon isolation of a neuron with 5-ms CF sounds (0.5-ms rise-decay times), its response was amplified (ISO-DAM, WPI, Sarasota, FL, USA), band-pass filtered (Krohn-Hite 3500, Oceanside, CA, USA), and then fed through a window discriminator (WPI 121) before being sent to an oscilloscope (TDS210, Tek, Beaverton, OR, USA) and an audio monitor (Grass AM9, Warwick, RI, USA). The neuron's threshold at each responsive frequency was audio-visually determined by changing the sound amplitude, which on average elicited a 50% response probability from the neuron. The sound frequency that elicited a neuronal response at the lowest amplitude was defined as the best frequency (BF). The threshold at the BF was defined as the minimal threshold (MT). Rate-amplitude functions (RAFs) were plotted with firing rates obtained at the MT and 10-dB increments above the MT with 5-ms CF and 7-ms CF-FM sounds (i.e., added FM components). The best amplitude (BA) was defined as the specific amplitude which elicited maximal firing rates from a neuron. The dynamic range (DR) was defined as the amplitude range from 10% below the maximal to 10% above the minimal firing rates. The slope of an RAF was obtained by dividing the percent change in firing rates within the dynamic range by the dynamic range and was expressed in %/dB. We used the influence rate to evaluate the effect of the FM component on the firing rate elicited by the CF component of CF-FM sounds. The influence rate was defined as $(n_2 - n_1)/n_1$, i.e., the difference value between the firing rate (n_2) evoked by the CF-FM stimulus and the firing rate (n_1) evoked by the CF stimulus divided by n_1. After the CF stimulus was changed into a CF-FM one, i.e., the FM component was added to the CF component, the obtained positive, negative, and zero influence rates respectively indicated increasing, decreasing, and no change in the firing rates caused by the FM component.

The neuron's responses were sent to a computer (Kaitian 4600, Lenovo, Beijing, China) for acquisition of post-stimulus-time (PST) histograms (with a bin width of 250 μs and a sampling period of 145 ms) to 32 sound presentations. The PST histogram showed the neuron's temporal discharge pattern in relation to a sound stimulus. Total firing rates in each histogram were used to quantify the neuron's response under each stimulation condition.

Data analysis

All data obtained under different stimulation conditions were processed and plotted using Sigmaplot 2000 (Systat Software, San Jose, CA, USA). They were then quantitatively examined and statistically compared using SPSS 13.0 (Student's *t*-test and one-way analysis of variance (ANOVA) at $p < 0.05$) (SPSS, Chicago, IL, USA).

RESULTS

In this study, 113 neurons were isolated from the IC of leaf-nosed bats. The ranges of recording depth, BF, and MT of these neurons were 703-4012 (2761.8 ± 796.5) μm, 6.5-75.6 (48.4 ± 20.4) kHz, and 5-107 (58.6 ± 23.5) dB SPL, respectively. Because of restrictions of recording conditions and time, the RAFs of 61 neurons among the 113 neurons were examined, and effects of the FM component on the BA, DR, and RAF's slope (%/dB) of the neurons were studied using CF and mimic CF-FM sound stimuli. The response patterns of these 61 IC neurons stimulated with CF sounds at 10 dB above the MT (MT+10 dB) were phasic ($n = 37$, 60.6%), phasic bursts ($n = 12$, 19.7%) and tonic ($n = 12$, 19.7%), respectively. The response patterns of the 25 phasic responders, 10 phasic bursters and 9 tonic responders did not change with an increasing amplitude of the sound, but 12 phasic responders changed into 10 phasic bursts and 2 tonic responders, 2 phasic bursters changed into 1 phasic responder and 1 tonic responder, and 3 tonic responders changed into 2 phasic bursters and 1 phasic responder. When using the CF-FM sound stimulus at MT+10 dB, the response patterns of 30 phasic responders, 9 phasic bursters and 9 tonic responders were consistent with those using CF sound, whereas 3 phasic responders, 1 phasic burster and 2 tonic responders were changed into double-on responders, 4 phasic responders were changed into 2 phasic busters and 2 tonic responders, 2 phasic bursters were changed into 1 phasic responder and 1 tonic responder, and 1 tonic responder was changed into a phasic burster.

Types of RAFs obtained by CF and CF-FM stimuli

According to variations of the firing rates with the sound-stimulus amplitude, all RAFs could be described as 3 types under CF sound stimulation condition. These 3 types were (1) monotonic ($n = 13$), in which the firing rates increased monotonically with the stimulus amplitude (Fig. 1A-1); (2) saturated ($n = 13$), in which the firing rates increased with the stimulus amplitude to a plateau or with a subsequent change of < 25% (Fig. 1B-1); and (3) non-monotonic ($n = 35$), in which the firing rates increased with the stimulus amplitude to a peak and then decreased > 25% (Fig. 1C-1).

Also, the RAFs obtained by the CF-FM sound stimulus exhibited the same 3 types similar to the CF sound stimulus. Compared to the CF sound stimulus, although most RAF types ($n = 46$, 75.4%) of neurons exhibited no change under CF-FM sound stimulation condition (Figs. 1B-2 VS B-1, C-2 VS C-1), a minority of RAF types ($n = 15$, 24.6%) of neurons changed (Fig. 1A-2 VS A-1). Table 1 shows the RAF types of all 61 neurons obtained by the CF and CF-FM sound stimuli. Numbers of monotonic, saturated, and non-monotonic RAF types obtained by the CF-FM stimulus were 5 (8.1%), 16 (26.3%), and 40 (65.6%), respectively.

Changes in the BA, DR, RAF slope, and firing rate of neurons induced by CF and CF-FM stimuli

The BA, DR, and slope were calculated according to the RAFs obtained from the neurons (Fig. 2A). Figure 2Ba-1, b-1, and c-1 respectively show the distribution of BAs, DRs, and slopes of neurons under CF and CF-FM stimulation conditions. Ranges (mean ± S.D.) of BAs, DRs, and slopes of the neurons were 41-124 (93.0 ± 9.8) dB SPL, 3.1-76.8 (27.5 ± 9.4) dB, and 0.5-12.3 (3.1 ± 1.4)%/dB under CF stimulation conditions, respectively, while the ranges (mean ± S.D.) of BAs, DRs, and slopes of these neurons became 17-121 (84.4 ± 11.3) dB SPL, 1.4-75.0 (22.5 ± 8.5) dB, and 0.5-13.5 (4.1 ± 1.7)%/dB under CF-FM stimulation conditions, respectively. In a comparison of the CF stimulus, most of the neuronal BAs and DRs decreased under CF-FM stimulation, and most of the RAF slopes of neurons increased. The statistical analysis also showed that there were significant difference between changes in BA (Fig. 2Ba-2, paired *t*-test, $p < 0.01$), DR (Fig. 2Bb-2, paired *t*-test, $p < 0.05$), and slope (Fig. 2Bc-2, paired *t*-test, $p < 0.05$) of neurons induced by the CF and CF-FM stimuli.

The effect of the FM component on the firing rate elicited by the CF component of the CF-FM sound was also studied by calculating the influence rate. When the amplitude of the CF-FM stimulus was < 30 dB above the MT, firing rates evoked by the CF-FM stimulus were higher than those by the CF stimulus in 59.0% (36/61) of neurons. Therefore, the influence rates of these neurons were positive (Fig. 3A). When the amplitude of the CF-FM stimulus increased to > 30 dB above the MT, firing rates of neurons induced by the CF component of the CF-FM gradually decreased. As such, the average influence rates changed from a positive value to a negative one (Fig. 3B). By further analysis with a linear regression, statistical results showed that the influence rate of the FM component of the CF-FM sound on firing rates evoked by the CF stimulus decreased with an increasing amplitude of the CF-FM stimulus (Fig. 3A, $n = 61$, $r = 0.358$, $p < 0.001$). Also, the average influence rate appeared to significantly decrease with an increasing amplitude of the CF-FM stimulus (Fig. 3B, one-way ANOVA, $p < 0.001$).

DISCUSSION

Effect of the FM component of the CF-FM sound on RAF types of neurons

RAF types of neurons obtained by the CF stimulus were basically similar to those in previous studies (Jen et al. 2001, Zhou and Jen 2002), i.e., monotonic, saturated, and non-monotonic

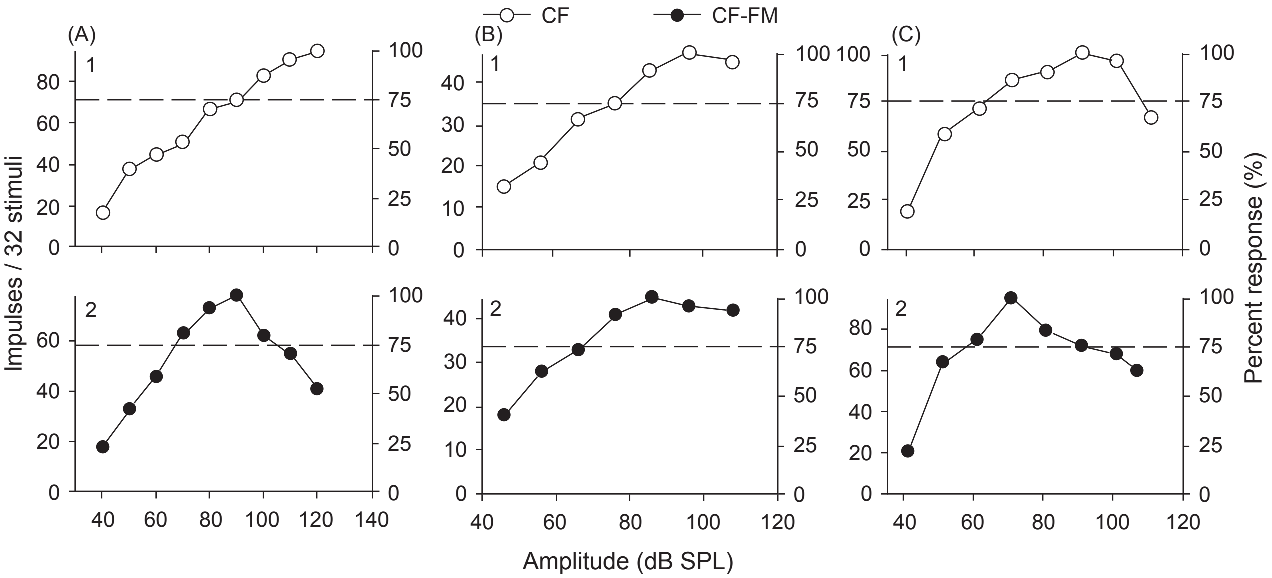

Fig. 1. Rate-amplitude functions (RAFs) of 3 representative inferior collicular (IC) neurons under constant frequency (CF) and CF-frequency modulation (FM) sound stimulation conditions. A-1, monotonic; B-1 and B-2, saturated; C-1, A-2, and C-2, non-monotonic. The best frequency (BF), minimal threshold (MT), and recording depth of these 3 representative neurons were 59.4 kHz, 40 dB sound pressure level (SPL), and 3524 μm (A); 68.3 kHz, 46 dB SPL, and 3097 μm (B); and 64.9 kHz, 41 dB SPL, and 2892 μm (C), respectively.

Table 1. The rate-amplitude functions (RAFs) of inferior collicular neurons of the constant frequency (CF)-frequency modulation (FM) bat, *Hipposideros armiger*, obtained with CF and CF-FM sounds

	CF	CF-FM		
	n = (%)	Monotonic	Saturated	Non-monotonic
Monotonic	13 (21.3) →	5 (8.1)	4 (6.6)	4 (6.6)
Saturated	13 (21.3) →	0 (0)	9 (14.7)	4 (6.6)
Non-monotonic	35 (57.4) →	0 (0)	3 (4.9)	32 (52.5)
Total	61	5 (8.1)	16 (26.3)	40 (65.6)

(Fig. 1A-1, B-1, C-1). After adding a segment of a down-sweep FM component to the end of the CF component (i.e., a CF-FM stimulus), the RAF types in 24.6% of neurons changed (Fig. 1A-1 VS A-2, Table 1), while the RAF types in 75.4% of neurons remained the same (Fig. 1B-1 VS B-2; C-1 VS C-2, Table 1). Previous studies showed that the amplitude which affected the RAF type of neurons was usually at a higher amplitude, and neural inhibition took part in the formation of different RAF types (Wu and Jen 2007 2009). Therefore, a possible reason that a segment of the down-sweep FM component connecting to the end of the CF component caused a change in the RAF type was due to the activation of some projection of the lateral inhibition to the recorded neuron at a higher amplitude (Suga and Tsuzuki 1985), which is similar to backward masking.

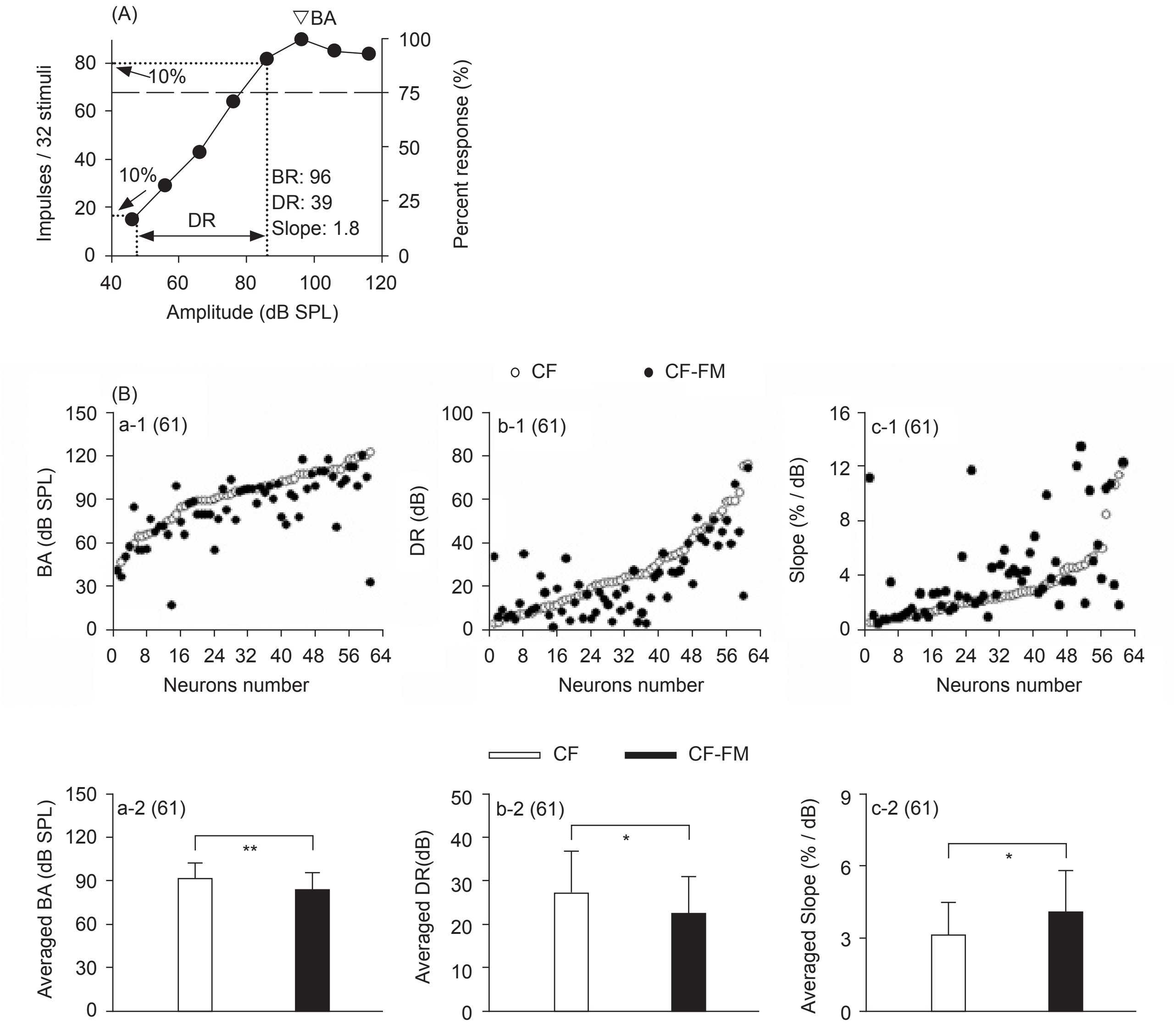

Fig. 2. Best amplitudes (BAs), dynamic ranges (DRs), and slopes of the inferior collicular (IC) neuronal rate-amplitude functions (RAFs). (A) The method of calculating the BA, DR, and slope. Ba-1, b-1, and c-1, show the distributions of BAs, DRs, and slopes of IC neuronal RAFs determined with constant frequency (CF) and CF-frequency modulation (FM) stimuli, respectively. Ba-2, b-2, and c-2 are the average BAs, DRs, and slopes of IC neuronal RAFs under CF and CF-FM sound stimulation conditions, respectively. The upward bars of each mean data point are the half standard deviation, and the number inside the parentheses is the number of neurons evaluated. $^*p < 0.05$; $^{**}p < 0.01$. The best frequency (BF), minimum threshold (MT), and recording depth of the representative neuron in panel A were 29.9 kHz, 46 dB sound pressure level (SPL), and 1034 μm, respectively.

Effect of the FM component of the CF-FM sound on the BA, DR, RAF slope, and firing rate of neurons

In this study, the BA, DR, and slope were calculated according to the RAF of each recorded neuron (Fig. 2A), and the sensitivity of the neuronal responses to the stimulus amplitude was evaluated. Compared to the sole CF sound stimulus, the BAs (Fig. 2Ba-1, a-2) and DRs (Fig. 2Bb-1, b-2) of neurons significantly decreased under a CF-FM stimulation condition. The reason that the neuronal BAs and DRs decreased was also likely due to the lateral inhibition activated by a segment of the down-sweep FM component connected to the end of the CF component (Suga and Tsuzuki 1985). The DR represents the range of neuronal responses to the stimulus amplitude, within which a neuron's firing rate or the number of impulses can increase monotonically with an increase in the stimulus amplitude. Therefore, a large DR represents a wide range of amplitudes that a neuron can respond to. In the real world, the range of biologically relevant sound amplitudes is generally from -10 to 100 dB SPL (Gulick et al. 1989). However, the average DR of the recorded IC neurons in the present and previous studies (Jen et al. 2001, Zhou and Jen 2002) was just 20-30 dB (Fig. 2Bb-2). If the auditory system wants to encode such a wide range of sound amplitudes, cooperation is necessary among neuronal populations having different MTs and DRs as proposed by previous studies (Aitkin 1991, Zhou and Jen 2002). This basic principle found in bats is also suitable for human hearing. When a neuron's MT remains invariant, the decrement in the BA results in a reduction in the DR which plays an important role in echolocation of CF-FM bats. When a CF-FM bat approaches a target, the proportion of the FM component gradually increases accompanied by a shortening of the CF duration (Simmons 1979), and the difference in the amplitude between the emitted pulse and returning echo also gradually decreases (Wang et al. 2008, Hiryu et al. 2010), so a smaller DR can readily satisfy the bats' need to analyze the echo amplitude. In essence, decrements in the BA and DR of neurons induced by the FM component of the CF-FM sound well matched the echolocation behavior of CF-FM bats. Because the slope of the

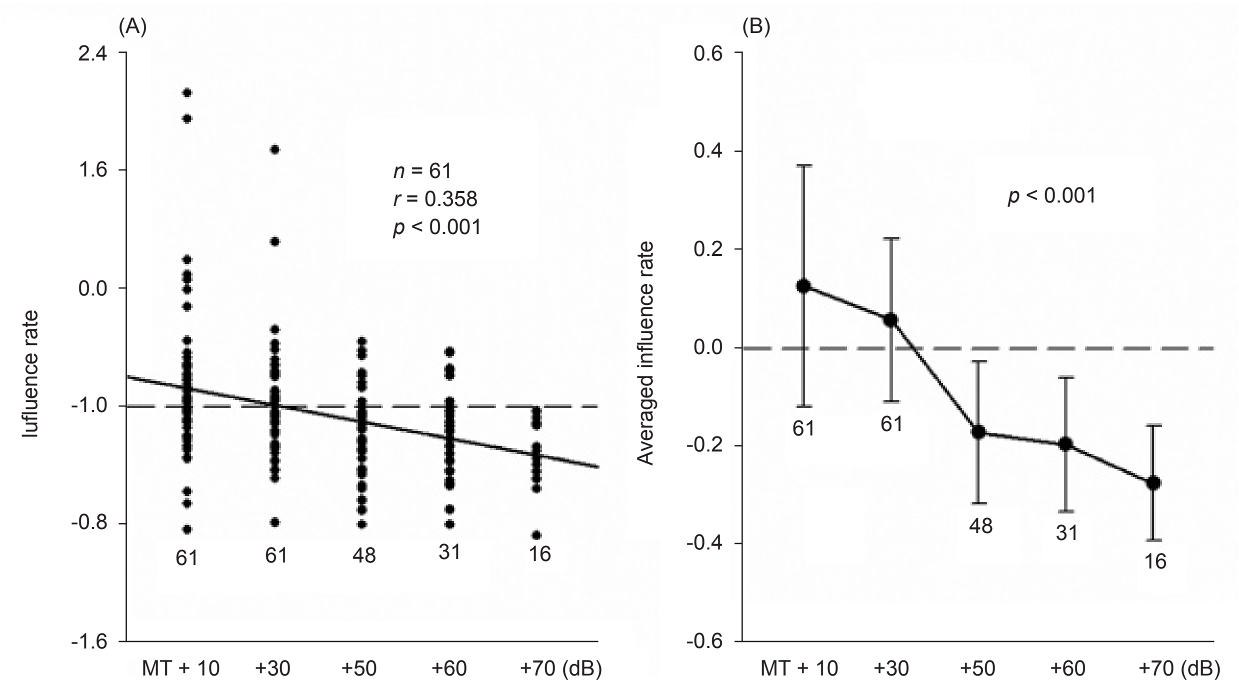

Fig. 3. Influence rate of the frequency modulation (FM) component of constant frequency (CF)-FM stimuli on firing rates elicited by the CF stimulus at various amplitudes above the minimum threshold (MT). (A) Scatterplot showing the distribution of the influence rate of the FM component on the firing rates. The figures under the sampling points show the number of neurons. The solid and dashed lines are the linear regression and zero level lines, respectively. (B) Scatterplot showing the average influence rate of the FM component on firing rates. The upward and downward bars of each mean data point are the half standard deviation, while the figure under each bar is the number of neurons evaluated. *n*, number of neurons; *r*, correlation coefficient; *p*, significance level.

RAF represents a neuron's sensitivity to change in the stimulus amplitude, the larger the slope of a neuron's RAF is, the higher is the neuron's sensitivity to change in the stimulus amplitude (Heil et al. 1992). In this study, we found that slopes of the RAFs of neurons significantly increased after adding a segment of the down-sweep FM component to the end of the CF component (Fig. 2Bc-1, c-2). These results indicated that the increment in the slopes accompanying the decrement in the BAs and DRs improved the sensitivity of the neurons to change in the amplitude and ensured successful capture by the bat during echolocation.

A previous study on the peripheral auditory system showed that the responses of the cochlear microphonic (CM) and compound action potentials (N1) elicited by the CF stimulus could be inhibited by the FM stimulus at a given amplitude (Suga et al. 1975). However, our results demonstrated that the FM component of the CF-FM sound had dual effects on central auditory neurons. When the neurons were stimulated by lower-amplitude CF-FM stimuli (< MT + 30 dB), firing rates elicited by the CF component were increased by the FM component (Fig. 3). When the neurons were stimulated by higher-amplitude CF-FM stimuli (> MT + 30 dB), firing rates elicited by the CF component were decreased by the FM component. By analyzing the extent to which the FM component influenced firing rates elicited by the CF component at various amplitudes, it was found that the extent of the influence decreased monotonically with an increasing amplitude of CF-FM stimuli (Fig. 3A, B). From the current observations, we propose that the initial part of the FM component close to the CF at lower amplitude plays a continual and accumulative role which is similar to the CF component and results in an increment in firing rates, while the latter part of the FM component cannot activate a response by neurons for frequencies far away from the neuron's BF. Therefore, the FM component of the CF-FM has an amplification role in the responses of neurons to lower-amplitude sound. This functional role possibly occurs when searching and in the early approach phases during CF-FM bat hunting, and the IC neurons are beneficial in accepting weaker echoes from a long-distance target (Griffin et al. 1960, Simmons et al. 1979). However, when IC neurons are stimulated by higher CF-FM amplitudes, the latter part of the FM component possibly sweeps the inhibition area of the recorded neurons, thereby eliminating the accumulated role of the initial part of the FM component and even reduces the responses elicited by the CF component. Therefore, the central auditory system is protected from generating too strong of a reaction through neural inhibition activated by the FM component at high amplitudes. This functional role of the FM component possibly occurs in the late approach and terminal phases during CF-FM bat hunting, and the IC neurons are beneficial for accepting stronger echoes from short-distance targets. This issue is consistent with the result that the MTs of the inhibition areas of most neurons are usually higher than those of excitatory areas (Jen and Zhang 1999). Just because the inhibitory effects of the FM component at higher amplitudes, the BAs and DRs of IC neurons decreased and RAF slopes of these neurons increased when a segment of the down-sweep FM component was added to the end of the CF component (Jen et al. 2001 2002, Suga 1995).

Also, human speech and many animal sounds used for communication, consisting of the frequency, amplitude, and duration, contain multiple FM components (Fuzessery et al. 2006). Thereby, our data could provide a new view that those FM components possibly improve the efficiency of communication using animal sounds and human speech.

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