Acta Physiologica Sinica, December 25, 2007, 59 (6): 805-813
http://www.actaps.com.cn

Research Paper

GABAergic inhibition modulates intensity sensitivity of temporally patterned pulse trains in the inferior collicular neurons in big brown bats

LUAN Rui-Hong[1,*], WU Fei-Jian[2], Philip H. -S. Jen[3], SUN Xin-De[1]

[1]School of Life Sciences, East China Normal University, Shanghai 200062, China; [2]School of Life Sciences, Central China Normal University, Wuhan 430079, China; [3]Division of Biological Sciences, University of Missouri-Columbia, MO 65211, USA

Abstract: The echolocating big brown bats (*Eptesicus fuscus*) emit trains of frequency-modulated (FM) biosonar signals with duration, amplitude, repetition rate, and sweep structure changing systematically during interception of their prey. In the present study, the sound stimuli of temporally patterned pulse trains at three different pulse repetition rates (PRRs) were used to mimic the sounds received during search, approach, and terminal stages of echolocation. Electrophysiological method was adopted in recordings from the inferior colliculus (IC) of midbrain. By means of iontophoretic application of bicuculline, the effect of GABAergic inhibition on the intensity sensitivity of IC neurons responding to three different PRRs of 10, 30 and 90 pulses per second (pps) was examined. The rate-intensity functions (RIFs) were acquired. The dynamic range (DR) of RIFs was considered as a criterion of intensity sensitivity. Comparing the average DR of RIFs at different PRRs, we found that the intensity sensitivity of some neurons improved, but that of other neurons decayed when repetition rate of stimulus trains increased from 10 to 30 and 90 pps. During application of bicuculline, the number of impulses responding to the different pulse trains increased under all stimulating conditions, while the DR differences of RIFs at different PRRs were abolished. The results indicate that GABAergic inhibition was involved in modulating the intensity sensitivity of IC neurons responding to pulse trains at different PRRs. Before and during bicuculline application, the percentage of changes in responses was maximal in lower stimulus intensity near to the minimum threshold (MT), and decreased gradually with the increment of stimulus intensity. This observation suggests that GABAergic inhibition contributes more effectively to the intensity sensitivity of the IC neurons responding to pulse trains at lower sound level.

Key words: bat; intensity sensitivity; inferior colliculus; pulse repetition rate; echolocation

GABA 能抑制调制大棕蝠下丘神经元对串声刺激的强度敏感性

栾瑞红[1,*]，吴飞健[2]，Philip H. -S. Jen[3]，孙心德[1]

[1]华东师范大学生命科学学院，上海 200062；[2]华中师范大学生命科学学院，武汉 430079；[3]密苏里大学哥伦比亚分校生物学系，密苏里州 65211，美国

摘 要：本文采用不同重复率的串声刺激，模拟大棕蝠回声定位不同阶段听到的调频声纳信号，利用电生理方法和微电泳技术研究不同重复率串刺激条件下GABA能抑制对下丘神经元强度敏感性的影响。结果发现，随串刺激重复率的增加，有的神经元强度敏感性增强，有的神经元强度敏感性则降低。在不同串刺激条件下，微电泳荷包牡丹碱，神经元放电率均增加，随重复率增加强度敏感性增强或减弱的趋势消失，提示GABA能抑制调制下丘神经元对不同重复率串刺激反应的强度敏感性。串刺激强度在最低阈值附近时，微电泳荷包牡丹碱导致放电率增加的百分率最大，随串刺激强度增加，放电率增加的百分率逐渐减小。提示刺激强度较低时，GABA能抑制对下丘神经元强度敏感性的影响更有效。

关键词：蝙蝠；强度敏感性；下丘；重复率；回声定位
中图分类号：Q42

Received 2007-06-28 Accepted 2007-10-11
This work was supported by the research grants from East China Normal University (No. J912) and University of Missouri.
*Corresponding author. Tel: +86-21-62232765; Fax: +86-21-62233754; E-mail: rhluan@bio.ecnu.edu.cn

Microchiropteran bats, cetaceans and some rodents are known to produce and detect ultrasounds (frequencies greater than 20 kHz) for the purpose of communication and/or echolocation. The big brown bat emits relatively long, shallow frequency sweeps while searching for insect prey and obstacles. During pursuit it switches to shorter pulses of wider bandwidth at an increased repetition rate[1,2]. In the real world, natural sounds such as vocal communication sounds of many animal species typically occur as sequential sound pulses. Therefore, the responses of auditory neurons to a sound pulse would be affected when the sound pulse is preceded and succeeded by other sound pulses (i.e., forward and backward masking). In the mammalian auditory pathway, the central nucleus of the inferior colliculus (IC) receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei. For the same IC neuron, the response to a single sound pulse is often stronger than that to each pulse of a sound train at the same stimulus intensity[3,4].

Neurotransmitters that mediate the inhibitory inputs are γ-aminobutyric acid (GABA) or glycine in the central nervous system. As a major relay nucleus in the auditory pathway, the IC receives complex excitatory and inhibitory inputs from several lower brain stem nuclei, contralateral IC, and higher auditory as well as nonauditory structures[5]. During synaptic transmission, an inhibitory postsynaptic potential (IPSP) that follows the excitatory postsynaptic potential (EPSP) could limit the cell's ability to respond to the subsequent inputs[3]. As one of the two major inhibitory transmitters in the IC, GABA binds to $GABA_A$ receptor to increase Cl^- conductance allowing influx of Cl^- into neurons and this ionic influx produces hyperpolarization in most neurons. Conversely, bicuculline, an antagonist for $GABA_A$ receptors, blocks GABA-mediated inhibition[7]. For this reason, many studies examined the role of GABAergic inhibition in dynamic aspect of auditory temporal processing in the IC using iontophoretic application of bicuculline[8]. GABAergic inhibition contributes to shaping many auditory response properties of IC neurons, such as discharge rate, discharge pattern, response latency, duration selectivity, directional sensitivity, binaural signal processing, recovery cycle, sharpening of frequency tuning curves and so on[9-11].

During the stimulation with temporally patterned trains of sound pulses, the interplays between the excitatory and inhibitory inputs to the IC neurons contribute to auditory temporal processing and shape multi-parametric selectivity, such as duration, frequency, amplitude and direction[6]. The sound intensity is one of the most important parameters and the dynamic range (DR) of the rate-intensity functions (RIFs) represents the sound intensity sensitivity as a common criterion[12]. Many studies have focused on the acoustic intensity sensitivity of IC neurons and the enhancement of the intensity sensitivity is mainly attributed to the GABAergic inhibition[8]. But it has not been known how the repetition rate of sound pulse trains affects the intensity sensitivity of the IC neurons and the GABAergic inhibition function. In the present study, we investigated the effects of GABA-mediated inhibition on intensity sensitivity of the IC neurons in the big brown bat, with the string of the different repetition rate in sound pulse trains to mimic the pulses or echoes during search, approach, and terminal stages of pursuit in the real environments. Variation in the number of impulses and intensity sensitivity of IC neurons with sequentially presented sound pulses was then quantitatively determined and statistically compared.

1 MATERIALS AND METHODS

1.1 *Surgical procedures*

Thirteen brown bats *(Eptesicus fuscus)* weighing 12-28 g were used in the experiments. Each animal was anesthetized with Nembutal (45-50 mg/kg body weight) for surgery. Briefly, a 1.8-cm nail was glued on the exposed skull of each anesthetized bats with acrylic glue and dental cement 1 or 2 d before the recording session. Before recording, the bat was administered with the neuroleptanalgesic Innovar-Vet (Fentanyl, 0.08 mg/kg body weight; Droperidol, 4 mg/kg body weight) to let the bat calm during the recording session. Then the bat was put in a soundproof room (temperature 28-30 ºC). Its head was immobilized by fixing the shank of the nail into a metal rod with a set screw. Small holes were bored in the skull above the IC for insertion of electrodes to record auditory responses of IC neurons and for iontophoretic application of bicuculline. The inner surface of the ceiling and inside walls of the room were covered with 3-inch convoluted polyurethane foam to reduce echoes.

1.2 *Acoustic stimuli*

The wave form of the acoustic stimuli is sine wave produced by an oscillator (KH 1200). The frequency of the pure tone of the acoustic stimuli is in the range of 10 kHz to 100 kHz. The switch (HP 8015A), which is driven by the stimulator (Grass S88), is a tone burst generator shaping the continuous sine wave into tone bursts. These stimuli were then amplified after passing through a decade attenuator

(Leader Lat-45) before they were fed to a small condenser loudspeaker (AKG model CK 50) that was placed 29 cm away from the bat and 30° contralateral to the recording site. Calibration of the loudspeaker was performed with a 1/4-inch microphone (B&K 4135) placed at the bat's ear. The calibrated intensity-frequency curve of the loudspeaker was plotted to determine the maximal available stimulus intensity at each frequency. The sound was expressed in dB SPL with reference to 20 μPa root mean square. The acoustic stimuli were delivered at 2 pulses per second (pps). The signals were short tone bursts with 4-ms duration, 0.5-ms rise-decay times. Firstly the best frequency (BF), minimum threshold (MT) and response latency of the isolated IC neurons were determined by changing the frequency and level of single pure tone. At the MT, the neuron had a 50% probability of responses to presented BF pulses. Intensity sensitivity of an IC neuron was studied with 300-ms temporally patterned trains of 4-ms BF pulses. These pulse trains were delivered at the pulse repetition rates (PRRs) of 10, 30 and 90 pps by setting the inter-pulse interval within pulse trains at 100, 33.33 and 11.11 ms (i.e., the number of pulses was 3, 9 and 27 within each pulse train). The stimulus paradigm of the temporally patterned pulse trains at the three different PRRs is shown in Fig.1. These three PRRs are comparable to that occurring during the search, approach and terminal phases of hunting in the big brown bat. To avoid the potential effect of presentation order of pulse trains on intensity sensitivity of IC neurons, the three kinds of pulse trains were randomly presented. For example, while intensity sensitivity of one neuron was studied with pulse trains of 10, 30 and then 90 pps, another neuron was studied with trains of 90, 10 and then 30 pps. The intensity of the pulse trains increased from the MT to the highest level of the loudspeaker in 10 dB steps.

1.3 *Bicuculline application*

Iontophoretic application of bicuculline to recorded IC neurons was given with a three-barrel electrode (tip: 10-15 μm) 'piggybacked' to a 3 mol/L KCl single-barrel electrode (tip: less than 1 μm; impedance: 5-10 MΩ). The tip of the 3 mol/L KCl single-barrel electrode was extended about 10 μm from the tip of the other three-barrel electrode. The 3 mol/L KCl single-barrel electrode was used to record neural responses. One of the barrels of the three-barrel electrode was filled with bicuculline (10 mmol/L in 0.16 mol/L NaCl, pH 3.0, Sigma). The bicuculline was prepared just prior to each experiment and the electrode filled immediately before use. This barrel filled with bicuculline was connected via silver-silver chloride wire to a microiontophoresis constant current generator (Medical Systems Neurophore BH-2) that was used to generate and monitor iontophoretic currents. During bicuculline application, a 1-s pulse of positive 40 nA at 0.5 pps was applied for 1 min before data acquisition. The application current was changed to positive 10 nA during data acquisition. The other two barrels were filled with 1 mol/L 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 retaining current was negative 8-10 nA.

1.4 *Recordings of neural activities*

Microelectrodes were pushed gently into the IC region with a microdrive (David-Kopf). The recording depth was read from the scale. The recordings were made with micropipette electrodes. The action potentials were amplified (HP 465A), filtered (KH3362), and fed through a window discriminator (WPI 121) before being sent to an oscilloscope

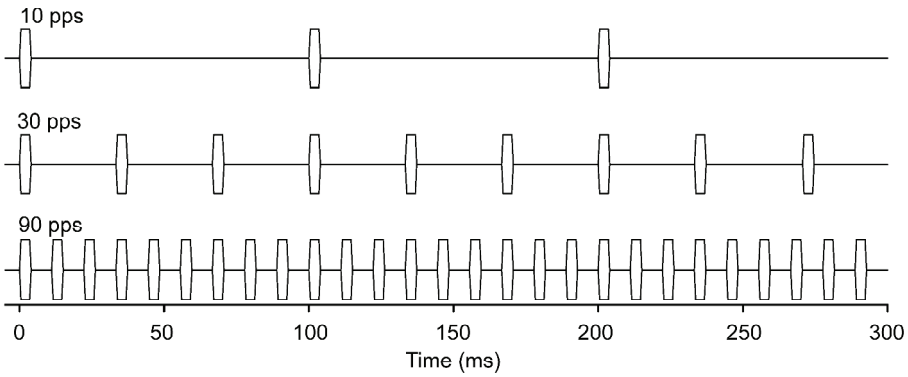

Fig. 1. The stimulus paradigm of the temporally patterned pulse trains at the three different PRRs. The duration of temporally patterned trains was 300-ms. Duration of each short tone burst was 4-ms with 0.5-ms rise-decay times. The PRRs of the temporally patterned pulse trains were 10, 30 and 90 pulses per second (pps). The number of pulses was 3, 9 and 27 within each pulse train.

(Tektronix 5113) and audio monitor. The neural activities detected were input to a computer with an A/D converter. To generate the post-stimulus-time histograms (PSTHs) and record the rate of acoustic responses of the neurons, the neural signals were collected for 32 trials using a bin width of 500 μs. The number of impulses and intensity sensitivity of the IC neuron were studied with 32 presentations of a 300-ms pulse train and at two trains per second. Thus there was always 200-ms silent period between pulse trains. The number of impulses of each neuron discharged to each train was obtained before and during bicuculline application.

1.5 *Data analysis*

The data recorded were input to the software Sigmaplot 2000. The RIFs of the responses to the stimulation trains were acquired when the intensity of the pulse trains increased from the MT to the highest level of the loudspeaker in 10 dB steps. DR as an intensity range was defined in each RIF when the responses were between 90% of the maximal response and 10% of the minimal response above the MT. For the statistical analysis of the differences of DR at 10, 30 and 90 pps, the DRs of the RIFs were produced from the normalized responses (Fig.2). DR was considered as a criterion of sensitivity of the neuron to the intensity of pulse train. It means that the decrease of DR reflects a genuine increment of intensity sensitivity to the pulse train for the IC neurons. One-way analysis of variance (ANOVA) was used in statistical analysis by SPSS.

2 RESULTS

In this study, 68 IC neurons were isolated at depths between 387 and 1 660 μm. Their BFs and MTs ranged at 17.86-80.37 kHz and 18-56 dB SPL, respectively. The mean value of their latencies was (10.83±2.08) ms. Among the 68 IC neurons, 40 were received bicuculline application.

2.1 Properties of the IC neurons responding to the pulse trains at different PRRs

When stimulated with the pulse trains, 52 neurons responded to each sound pulse of the trains at different PRRs. The other 16 neurons discharged impulses to each sound pulse of the trains at 10 and 30 pps, but these neurons only discharged impulses to the initial few pulses or the first pulse of the trains at 90 pps. According to the response patterns of IC neurons to the pulse trains, we divided 68 IC neurons into two groups. The 52 neurons with the former response pattern belonged to group I, and the other 16 neurons with the latter response pattern belonged to group II[13].

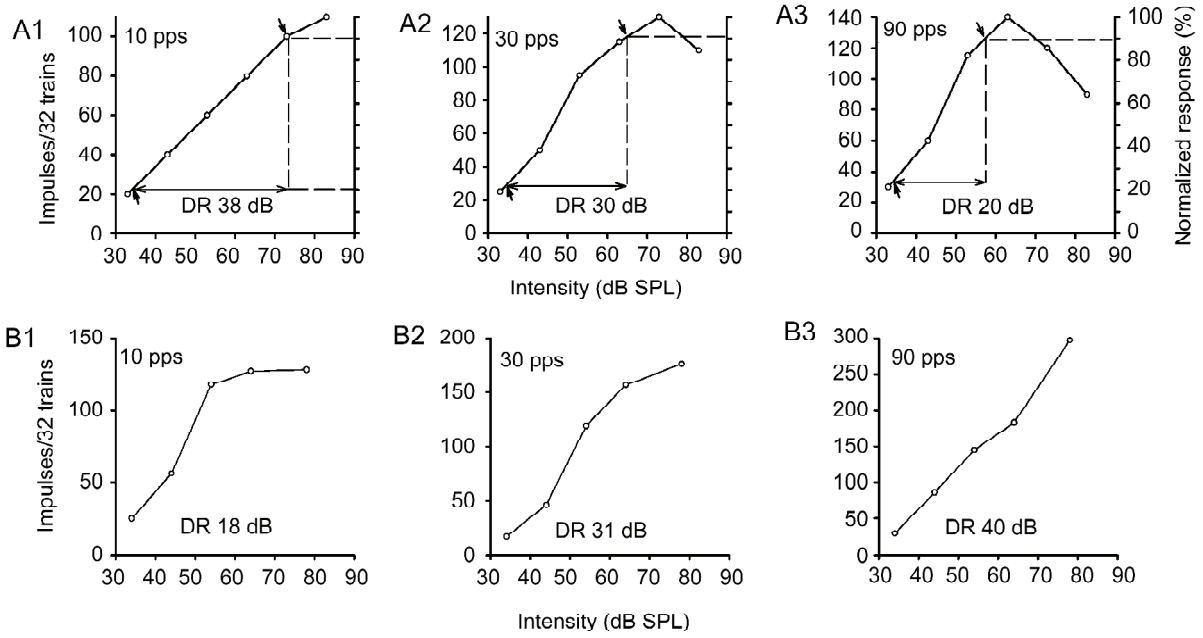

Fig. 2. Rate-intensity functions (RIFs) of two representative neurons (*A*, *B*) in group I responding to pulse trains at different pulse repetition rates (PRRs) (10, 30, 90 pps). The left ordinate represents the number of impulses in response to 32 stimuli and the abscissa represents stimulus intensity. The right ordinate represents the normalized response according to the maximal response. Dynamic range (DR) is the range of intensity between 90% of the maximal response and 10% of the minimal response above the minimum threshold (MT). The best frequency (BF, kHz), MT (dB SPL), latency (ms) and recording depth (μm) of the two neurons were 22.36, 33, 10 and 795 (*A*); 24.22, 34, 12 and 710 (*B*), respectively.

2.2 RIFs of the IC neurons responding to the pulse trains at different PRRs

The DR in each RIF, as a criterion of intensity sensitivity of the neuron responding to the pulse train, was acquired when the responses were normalized. The decrease of DR indicated an increment of intensity sensitivity to the pulse train for the IC neurons.

The RIFs of two representative neurons in group I responding to the pulse trains at 10, 30 and 90 pps were shown in Fig.2. In Fig.2*A*, DR was minimal at 90 pps and progressively decreased with the increment of PRRs from 10 to 30 and 90 pps. It means that this neuron exhibited the maximal intensity sensitivity to pulse train at 90 pps, and the intensity sensitivity was gradually improved with the increment of PRRs from 10 to 30 and 90 pps. In Fig.2*B*, the DR was minimal at 10 pps. However, DR increased progressively with the increment of PRRs from 10 to 30 and 90 pps. This neuron exhibited the maximal intensity sensitivity at 10 pps and the minimal intensity sensitivity at 90 pps.

The RIFs of an IC neuron in group II were shown in Fig.3. The DR was minimal at 30 pps. Variation of DRs with the different PRRs was stochastic.

2.3 Intensity sensitivity of the IC neurons responding to the pulse trains at different PRRs

In IC neurons ingroup I, there were two different trends of variation in DRs at different PRRs. In some neurons, DRs gradually decreased with the increment of PRRs from 10 to 30 and 90 pps, and the average DRs of the RIFs decreased significantly with increasing PRRs of pulse trains (one-way ANOVA, $P<0.05$, Fig.4*A*). In the other neurons, DRs gradually increased with the increment of PRRs from

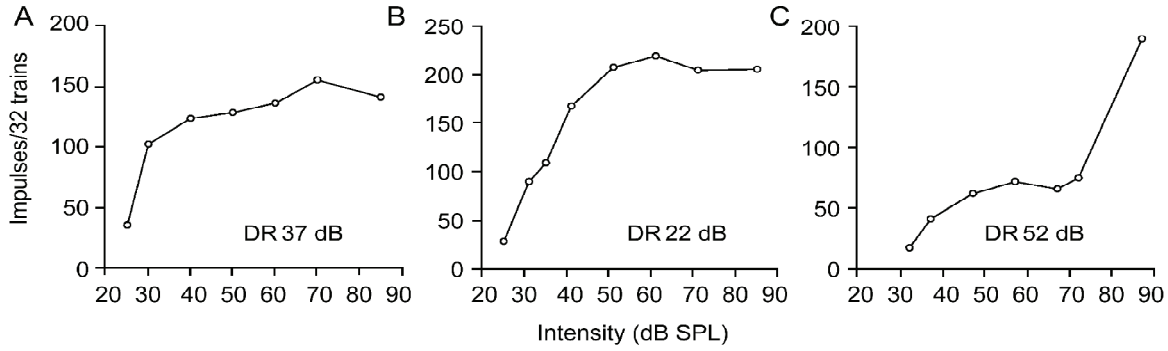

Fig. 3. Rate-intensity functions (RIFs) of a representative neuron in group II responding to pulse train at different pulse repetition rates (PRRs). The best frequency (BF, kHz), minimum threshold (MT, dB SPL), latency (ms) and recording depth (μm) were 34.57, 25, 9 and 789, respectively. *A*: 10 pps. *B*: 30 pps. *C*: 90 pps.

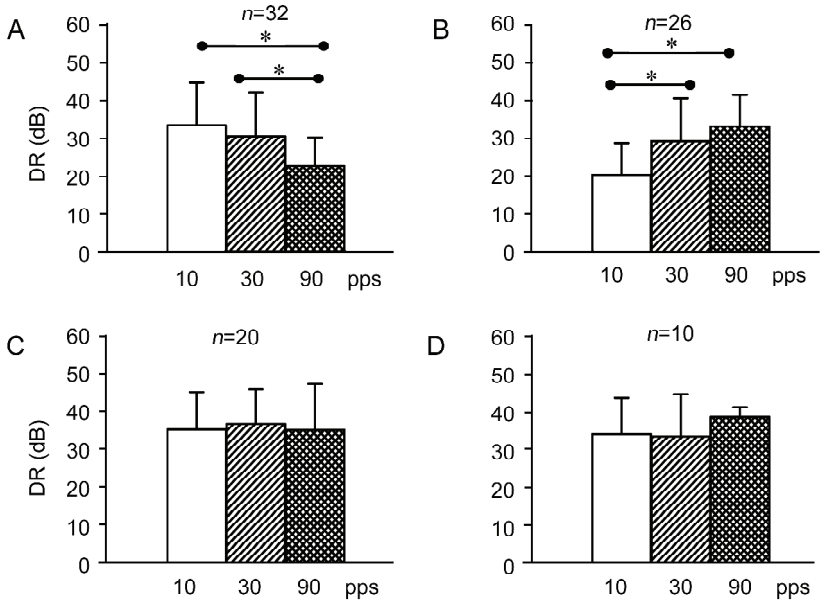

Fig. 4. Dynamic range (DR) values of the rate-intensity functions (RIFs) in neurons in group I. *A*, *B*: Two trends of variation in DRs before bicuculline application. $^{*}P<0.05$. *C*, *D*: Changes of DRs during bicuculline application.

10 to 30 and 90 pps, and the average DRs of the RIFs increased significantly with increasing PRRs of pulse trains (one-way ANOVA, $P<0.05$, Fig.4*B*). The results indicated that some neurons in group I exhibited higher intensity sensitivity at higher PRR such as 90 pps and the other neurons in group I exhibited the reversed characteristics in intensity sensitivity at different PRRs.

2.4 GABAergic inhibition affects the intensity sensitivity at different PRRs

In order to investigate the effects of GABAergic inhibition on the intensity sensitivity at different PRRs, we compared all the DRs of the RIFs in IC neurons in group I before and during bicuculline application. Since the DRs of the RIFs in IC neurons in group II varied randomly before and during bicuculline application, we did not take further investigation by statistics. In IC neurons in group I, the two different trends of variation in DRs with different PRRs vanished during bicuculline application (one-way ANOVA, $P>0.05$) (Fig.4*C*, *D*). This indicates that the effects of the GABAergic inhibition on the intensity sensitivity are not consistent at different PRRs for each IC neuron.

2.5 Changes in responses and RIFs of IC neurons before and during bicuculline application

Changes of RIFs of a representative neuron in group I responding to the pulse trains before and during bicuculline application were shown in Fig.5. Bicuculline application resulted in considerable increments in the number of impulses under all stimulating conditions. Compared to that before bicuculline application, the DR increased significantly during bicuculline application (*t*-test, $P<0.05$) (Fig.6). This result indicated that GABAergic inhibition modulated the intensity sensitivity at different PRRs in IC neurons.

The percentage changes in response before and during bicuculline application was maximal when the stimulus intensity was near the MT and progressively decreased. In some cases, the high percentage of changes in response before and during bicuculline application also presented when the stimulus intensity was near the maximal sound intensity. The percentage of changes in response to pulse

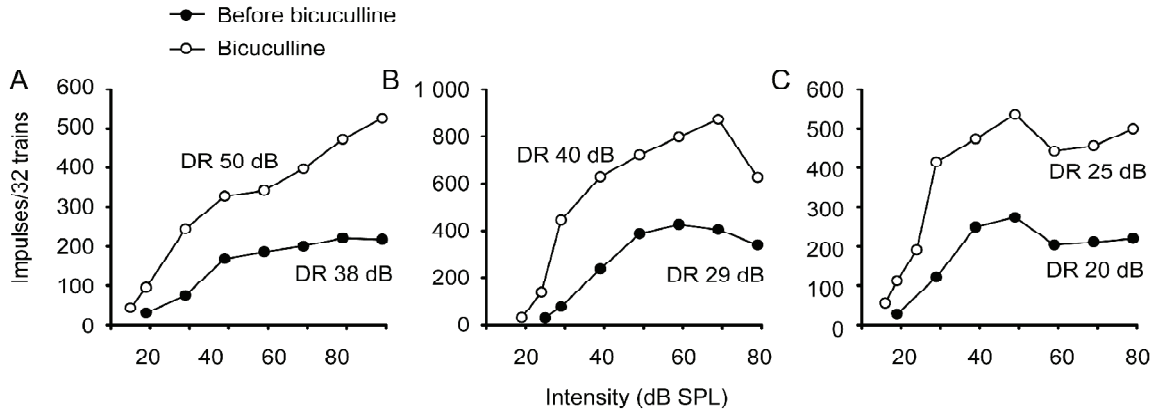

Fig. 5. A family of rate-intensity functions (RIFs) of a neuron in group I responding to pulse train at different pulse repetition rates (PRRs) before and during bicuculline application. The best frequency (BF, kHz), minimum threshold (MT, dB SPL), latency (ms) and recording depth (μm) were 25.3, 29, 10 and 850, respectively. *A*: 10 pps. *B*: 30 pps. *C*: 90 pps.

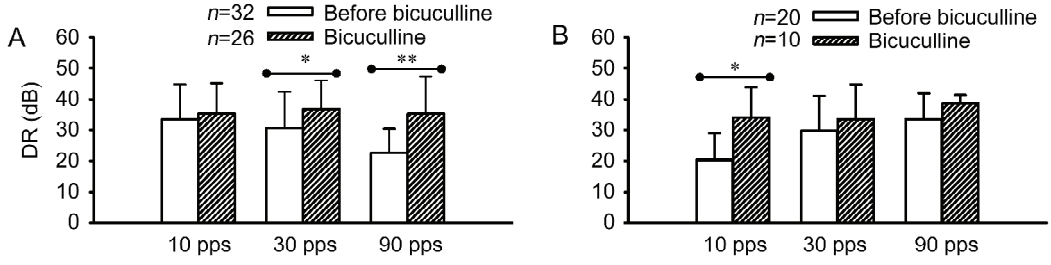

Fig. 6. The mean dynamic range (DR) values of the rate-intensity functions (RIFs) in IC neurons in group I before and during bicuculline application. *A*: One trend of the average DR values responding to pulse trains before ($n=32$) and during bicuculline application ($n=26$). $^{*}P<0.05$, $^{**}P<0.01$. *B*: The other trend of the average DR values responding to pulse trains before ($n=20$) and during bicuculline application ($n=10$). $^{*}P<0.05$.

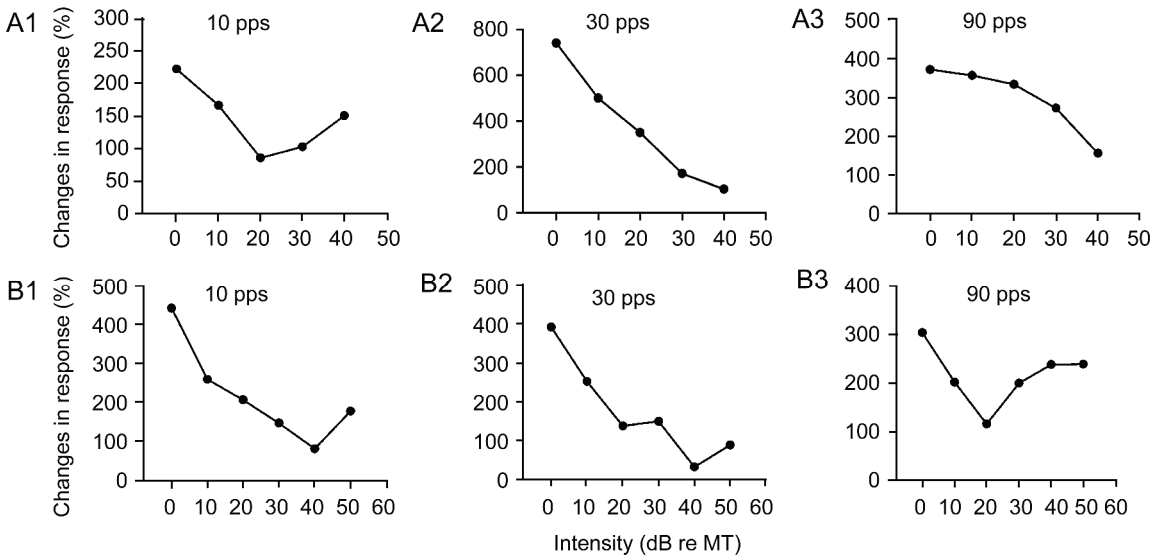

Fig. 7. Percentage changes in impulses responding to pulse trains at differen pulse repetition rates (PRRs) before and during bicuculline application from two representative neurons in group I. The best frequency (BF, kHz), minimum threshold (MT, dB SPL), latency (ms) and recording depth (μm) of the two neurons were 24.20, 34, 12 and 910 (*A*); 46.2, 30, 11 and 960 (*B*), respectively.

trains at different PRRs before and during bicuculline application from two representative neurons in group I was shown in Fig.7. The results indicated that the modulation of GABAergic inhibition was stronger when the neuron responded to the sounds at lower intensity level. In some cases, strong GABAergic inhibition presented possibly at higher sound intensity level.

3 DISCUSSION

The big brown bat emits sonar pulses at a low emission rate during searching for prey. While tracking and closing in on the prey, the bat reduces its sonar pulse duration to prevent pulse-echo overlap, increases pulse rate to increase the sampling rate, and reduces the pulse intensity to keep the perceived amplitude of the echoes essentially constant.

The IC is an obligatory relay station that receives excitatory and inhibitory inputs from all lower auditory nuclei and is very important for auditory information processing. The previous studies demonstrate that the changes in stimulus repetition rate can affect frequency sensitivity and duration sensitivity of IC neurons and increasing sound level either increases or decreases the recovery cycle of central auditory neurons[1,3,5,9,14-17]. The intensity is one of the important parameters of sound, as well as frequency or duration. The changes in stimulus repetition rate might affect the intensity sensitivity of IC neurons. GABAergic inhibition might play an important role in intensity sensitivity in IC.

3.1 Intensity sensitivity of IC neurons responding to the pulse trains at different PRRs

The big brown bat is an insectivorous species that uses sonar to locate and track flying prey. During pursuit, the bat shortens its sonar signals and increases their rate of emission as it closes in to seize the target. However, during a pursuit, when the emission rate of sonar signals is changed, bats also simultaneously adjust their emission intensities to stabilize the perceived echo intensity. Specially, in terminal phase of echolocation, while closing to the prey, the bat increases its emitting pulse rate and reduces the pulse intensity to keep the amplitude of echoes essentially constant and in high sensitivity, which is called automatic gain control[18]. Echo intensity is correlated with the target size and distance. As a result, bats can use the echo intensity as reliable information for estimating the target size and distance[1,11,14]. The studies in the bat's IC show that each emission and each echo evoke its own responses during the approach and tracking stages of pursuit. But echoes do not explicitly evoke neural responses in the IC distinct from responses evoked by the broadcast during the terminal stage because the delay of echoes is too short for responsiveness to recover from the emissions. Local multiunit responses recorded from the IC of *Eptesicus* reveal a format for encoding the phase of echoes at all stages of interception[19]. The changes of IC neurons responding to the pulse trains at different PRRs depend on the regulation of neuronal membrane potential by excitatory and inhibitory

inputs[3,5,20,21]. The rate of sound pulses influenced over the intensity sensitivity of the IC neurons and it could be correlative with temporal masking phenomenon. Temporal masking exhibited in the responses of some IC neurons and affected the intensity sensitivity in our previous study[4,11,15,22,23].

In the present study, the auditory response properties for a large number of IC neurons are dependent on the temporal dynamics of acoustic stimuli. Specifically, rate of stimulation can markedly change the RIFs of many IC neurons. We found that for many IC neurons, an increase in PRR of stimulation changed the DR and produced a systematic increase in intensity sensitivity (Fig.4). The analysis reveals that, for about one-half of IC neurons, the DR decreased and the intensity sensitivity increased with the increment of PRRs of the pulse trains. On the other side, for some IC neurons, the DR increased and the intensity sensitivity decreased with the increment of PRRs of the pulse trains. These results demonstrated that for different IC neurons, the properties of the intensity sensitivity presented different tendency with the increment of PRRs of the pulse trains. The IC neurons with higher intensity sensitivity responding to the pulse train at 90 pps might dedicate to the terminal phase of echolocation and the other neurons with higher intensity sensitivity responding to the pulse trains at 10 or 30 pps might be involved in the search or pursue phase. These different kinds of IC neurons possibly contribute to processing acoustic information during different phases of bat echolocation.

3.2 GABAergic inhibition modulates the intensity sensitivity of IC neurons at different PRRs

GABA is the main inhibitory neurotransmitter in the central nervous system acting through three different receptors identified as: $GABA_A$, $GABA_B$ and $GABA_C$ receptors. The ionotropic $GABA_A$ receptor is $GABA_A$-gated chloride channels and bicuculline-sensitive, and causes a membrane chloride conductance increase for inhibitory postsynaptic potential in numerous areas in the mammalian brain. The metabotropic $GABA_B$ receptor is a G protein-coupled receptor and mediates pre- and postsynaptic inhibition by decreasing membrane Ca^{2+} conductance and increasing K^+ conductance, respectively[24]. Although the ionotropic $GABA_C$ receptors are expressed in several tissues such as the rodent retina, hippocampus, superior colliculus and spinal cord, the $GABA_C$ receptors are rarely investigated in the IC[25]. GABAergic inhibition shapes the recovery cycle of IC neurons, because the blockade of $GABA_A$ receptors by bicuculline application decreases the recovery cycle and increases the ability of IC neurons in following the PRR[16,20,21]. In our study, GABAergic inputs to IC neurons were activated at all intensities and stronger at lower level near the MT (Fig.7). In some cases, the higher activation occurs at higher intensities around the maximal level. During bicuculline application, the differences of intensity sensitivity among the three PRRs vanished with increasing PRRs of pulse trains in all neurons (Fig.4). The results indicated that for some IC neurons GABAergic inhibition contributed to the improvement of intensity sensitivity with increasing PRRs and inhibitory inputs introduce different effects on different IC neurons when the sound PRR changes. The IC receives extensive inhibitory input and the excitation-inhibition balance between different neurons is important to regulate the intensity sensitivity of IC neurons responding to pulse trains at different PRRs[5,6,9,17,22,26].

3.3 Possible mechanisms of GABAergic inhibition on sound intensity sensitivity of PRRs

Behavioral studies have shown that during insect pursuit, bats produce the next call immediately after hearing the echo from the prey. The bat has to wait for sound to reach and return from a target. All echoes arriving from the preceding call will be received before the emission of the next call. The call intensity has played an important role in calculating potential detection ranges for insect targets. Bats typically reduce call duration as they approach targets so that the signal overlap zone is equal to or less than the distance to the target and overlap between pulse and echo (forward masking) is avoided. If echoes from background clutter arrive soon after prey echoes, and interfere with neural activity evoked by the prey echoes, backward masking may occur. It results in echoes returning in an overlap-free window, in which both forward and backward maskings are avoided[27]. Previous studies have shown that the auditory system of bats is fundamentally similar to that of other mammals and the interplay of inhibition and excitation that shapes many response properties of IC neuron is also similar to that of other mammals[2,14,23]. The changes of stimulation intensity can influence the response of a neuron and the changes of intensity sensitivity with PRRs may be attributed to either intrinsic mechanism (e.g., biophysical properties of ion channels), or extrinsic mechanism (e.g., synaptic interactions)[3-5,17,20,21]. In our study, a local application of bicuculline in the IC enhanced the responses and reduced the DR of RIFs. Therefore it is possible that GABA-based inhibition may play a role in the regulating of intensity sensitivity of units responding to pulse trains at different PRRs[12,16,23]. Increasing strength of GABAergic inhibition at different PRRs in the present study might be the neural mechanism underlying the psychophysical phenomena

of temporal masking. At the level of the IC, a processing task was partly determined by the spatio-temoral interplay of excitatory and inhibitory inputs to IC neurons[26].

* * *

ACKNOWLEDGEMENTS: The authors would like to thank Prof. ZHANG Ji-Ping for valuable advice on this paper.

REFERENCES

1 Sanderson MI, Simmons JA. Target representation of naturalistic echolocation sequences in single unit responses from the inferior colliculus of big brown bats. J Acoust Soc Am 2005; 118(5): 3352-3361.

2 Feng AS, Narins PM, Xu CH, Lin WY, Yu ZL, Qiu Q, Xu ZM, Shen JX. Ultrasonic communication in frogs. Nature 2006; 440: 333-336.

3 Casseday JH, Ehrlich D, Covey E. Neural measurement of sound duration: control by excitatory-inhibitory interaction in the inferior colliculus. J Neurophysiol 2000; 84(3): 1475-1487.

4. Faure PA, Fremouw T, Casseday JH, Covey E. Temporal masking reveals properties of sound-evoked inhibition in duration-tuned neurons of the inferior colliculus. J Neurosci 2003; 23(7): 3052-3065.

5 Pollak GD, Burger RM, Klug A. Dissecting the circuitry of the auditory system. Trends Neurosci 2003; 26(1): 33-39.

6 Wu CH, Jen PHS. The role of GABAergic inhibition in shaping duration selectivity of bat inferior collicular neurons determined with temporally patterned sound trains. Hearing Res 2006; 215: 56-66.

7 Fubara BM, Casseday JH, Covey E, Schwartz-Bloom RD. Distribution of $GABA_A$, $GABA_B$ and glycine receptors in the central auditory system of the big brown bat, *Eptesicus fuscus*. J Comp Neurol 1996; 369(1): 83-92.

8 D'Angelo WR, Sterbing SJ, Ostapoff EW, Kuwada S. Role of GABAergic inhibition in the coding of interaural time differences of low-frequency sounds in the inferior colliculus. J Neurophysiol 2005; 93(6): 3396-3400.

9 Jen PHS, Wu CH, Luan RH, Zhou XM. GABAergic inhibition contributes to pulse repetition rate-dependent frequency selectivity in inferior colliculus of the big brown bat, *Eptesicus fuscus*. Brain Res 2002; 948: 159-164.

10 Jen PHS, Zhang JP. The role of GABAergic inhibition on direction-dependent sharpening of frequency tuning in bat inferior collicular neurons. Brain Res 2000; 862: 127-137.

11 Luan RH, Wu FJ, Jen PHS, Sun XD. Effects of forward masking on the responses of the inferior collicular neurons in the big brown bats, *Eptesicus fuscus*. Chin Sci Bull 2003; 48(16): 1748-1752.

12 Wang D, Pi JH, Tang J, Wu FJ, Chen QC. Dynamic modulations on intensity sensitivity evoked by weak noise in the inferior collicular neurons. Acta Physiol Sin (生理学报) 2005; 57 (1): 59-65.

13 Luan RH (栾瑞红), Jen PHS, Sun XD. The effects of GABA on temporal discharge pattern of IC neurons to acoustic pulse train in the big brown bat, *Eptesicus fuscus*. Acta Biophy Sin (生物物理学报) 2002; 18(4): 399-403 (Chinese, English abstract).

14 Feng AS, Ratnam R. Neural basis of hearing in real-world situations. Annu Rev Psychol 2000; 51: 699-725.

15 Galazyuk AV, Llano D, Feng AS. Temporal dynamics of acoustic stimuli enhance amplitude tuning of inferior colliculus neurons. J Neurophysiol 2000; 83(1): 128-138.

16 Zhou XM, Jen PHS. Azimuth-dependent recovery cycle affects directional selectivity of bat inferior collicular neurons determined with sound pulses within a pulse train. Brain Res 2004; 1019: 281-288.

17 Sivaramakrishnan S, Sterbing-D'Angelo SJ, Filipovic B, D'Angelo WR, Oliver DL, Kuwada S. $GABA_A$ synapses shape neuronal responses to sound intensity in the inferior colliculus. J Neurosci 2004; 24(21): 5031-5043.

18 Hartley DJ. Stabilization of perceived echo amplitudes in echolocating bats. II. The acoustic behavior of the big brown bat, *Eptesicus fuscus*, when tracking moving prey. J Acoust Soc Am 1992; 91(2): 1133-1149.

19 Simmons JA, Dear SP, Ferragamo MJ, Haresign T, Fritz J. Representation of perceptual dimensions of insect prey during terminal pursuit by echolocating bats. Biol Bull 1996; 191(1): 109-121.

20 LeBeau FE, Malmierca MS, Rees A. Iontophoresis *in vivo* demonstrates a key role for $GABA_A$ and glycinergic inhibition in shaping frequency response areas in the inferior colliculus of guinea pig. J Neurosci 2001; 21: 7303-7312.

21 Ingham NJ, McAlpine D. GABAergic inhibition controls neural gain in inferior colliculus neurons sensitive to interaural time differences. J Neurosci 2005; 25(26): 6187-6198.

22 Luan RH, Wu FJ, Jen PHS, Sun XD. Effects of backward masking on the responses of the inferior collicular neurons in the big brown bats, *Eptesicus fuscus*. Acta Physiol Sin (生理学报) 2005; 57(2): 225-232.

23 Frisina RD. Subcortical neural coding mechanisms for auditory temporal processing. Hear Res 2001; 158: 1-27.

24 Bettler B, Kaupmann K, Mosbacher J, Gassmann M. Molecular structure and physiological functions of $GABA_B$ receptors. Physiol Rev 2004; 84(3): 835-867.

25 Kirischuk S, Akyeli J, Iosub R, Grantyn R. Pre- and postsynaptic contribution of $GABA_C$ receptors to GABAergic synaptic transmission in rat collicular slices and cultures. Eur J Neurosci 2003; 18(4): 752-758.

26 Covey E, Casseday JH. Timing in the auditory system of the bat. Annu Rev Physiol 1999; 61: 457-476.

27 Jones G, Holderied MW. Bat echolocation calls: adaptation and convergent evolution. Proc R Soc B 2007; 274: 905-912.