Effects of backward masking on the responses of the inferior collicular neurons in the big brown bat, *Eptesicus fuscus*

LUAN Rui-Hong\(^1\), WU Fei-Jian\(^2\), Philip H.S. Jen\(^3\), SUN Xin-De\(^1\,\ast\)

\(^1\)College of Life Sciences, East China Normal University, Shanghai 200062, China; \(^2\)College of Life Sciences, Central China Normal University, Wuhan 430079, China; \(^3\)Division of Biological Sciences, University of Missouri-Columbia, MO 65211, USA

Abstract: Temporal features of sound convey information vital for behaviors as diverse as speech recognition by human and echolocation by bats. However, auditory stimuli presented in temporal proximity might interfere with each other. Although much progress has been made in the description of this phenomenon from psychophysical studies, the neural mechanism responsible for its formation at central auditory structures especially at the inferior colliculus (IC), a midbrain auditory nucleus which practically receives massive bilateral projections from all the major auditory structures in the brainstem, remains unclear. This study was designed to investigate it in vivo by using electrophysiological recording from the inferior collicular neurons of the big brown bat, *Eptesicus fuscus*. In our results, the responses of 12 (38\%, \(n = 31\)) neurons to the test sound (leading sound) were obviously inhibited by the masker (lagging sound). The inhibitory effects in these neurons were correlated with the inter-stimulus level difference (SLD) and the inter-stimulus onset asynchrony (SOA) interval. The strength of backward masking increased with the masker intensity increasing, the test sound intensity decreasing and the SOA interval shortening. There were no obvious effects of backward masking on the responses of many other neurons (52\%, 16/31), and yet in a part of these neurons, the neural inhibition of responses to the test sound was observed at the special SLD and the special SOA intervals. Moreover, few of the 31 sampled IC neurons (10\%, 3/31) displayed facilitating responses to the test sound at the special SLD and the special SOA intervals. These data demonstrate that a lot of IC neurons are involved in the generation of the backward masking of acoustical perception. It is conjectured that the temporal dynamic integration between the leading inhibitory inputs evoked by the masker sound and the excitatory inputs evoked by the test sound might play a key role in shaping the acoustical response characteristics of the IC neurons.

Key words: backward masking; masker; test sound; bat; inferior colliculus; echolocation
The ability to detect a signal (or test sound) is reduced if the signal is followed, after a short delay, by another stimulus called masker. This phenomenon is known as backward masking. The detectability of a signal may also be reduced if it is preceded by another stimulus (forward masking). Auditory psychophysical studies show that forward and backward masking play an important role in many auditory phenomena, including sound localization. The effective strength of this temporal masking is correlated with the inter-stimulus onset asynchrony (SOA) interval, inter-stimulus level difference (SLD) and other factors [1, 2]. For most intervals between the time of presentation of the masker and test sound, forward masking exerts a greater effect than backward masking, and also extends over a larger range of intervals than backward masking does. Although a generally accepted view is that the forward maskings results from, at least partly, the cochlear suppression, a growing body of literature in neurophysiological studies has demonstrated that the central auditory system is largely attributable to the generation of not only backward but also forward masking [3,4]. Recently, a pioneering experiment of exploring the acoustical response characteristics of the duration-tuned IC neurons in bat revealed that the responses to the excitatory tone were integrated by the non-excitatory tone, which might interpret the phenomena of temporal masking [5].

It is noteworthy that backward masking is a widespread, if not universal, feature of sensory system. For example, in a backward visual masking paradigm when the SOA intervals were shortened to less than 30 ms, the neural responses to the test stimulus were obviously affected in the visual cortex of awake, behaving macaques [6]. The auditory tests to the children with language impairment indicated that they had significantly higher (poorer) signal thresholds than their nonimpaired controls in backward masking, whereas their auditory cognitive processes were improved after suitable trainings [7], suggesting that the plasticity of functional connections between the central auditory neurons are correlated with the auditory cognitive processes. In a recent behavioral study conducted on insectivorous bats, which can detect the target size and distance by the echo amplitude and delay, Jen and Gold have shown that the reflected echoes from the obstacles behind the targets can affect the bat to discriminate the targets [8]. This means that echolocating bat has to encounter the effect of backward masking on detecting the targets [9]. We noticed in our previous studies that the responses of IC neurons to the first pulse of a tone pulses train in a high repetition rate were fewer than the responses to the same single sound. In the experiment of forward masking, it was obvious that the responses of the IC neurons to the first sound were depressed by the second sound [10–12]. Thus, it is necessary to explore the mechanism for these physiological phenomena. The present study is an extension of our preliminary work and was designed to investigate the neural mechanism underlying the backward masking in vivo by using electrophysiological recording from the inferior collicular neurons of the big brown bat, Eptesicus fuscus. We report here that a lot of IC neurons are involved in the generation of the backward masking of acoustical perception.

1 MATERIALS AND METHODS

1.1 Surgical procedures
Six male brown bats (Eptesicus fuscus) weighing 12–28 g were used in the experiments. Each animal was anesthetized with Nembutal (45–50 mg/kg) for surgery. The methods for surgical preparation, recording and stimulation techniques were similar to those described in one of our previous papers [12]. Briefly, a 1.8-cm nail was glued onto 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, droperidol 4 mg/kg) so as to let the bat become calm during the recording session. The bat was then put inside a sound-proof room (temperature 28–30 °C). Its head was immobilized by fixing the shank of the nail into a metal rod with a set screw. 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
Acoustic stimuli were generated by the oscillators (KH 1200 and KH 1600), shaped with switches (HP 8015A) and then through decade attenuators (Leader Lat-45), a signal mixer,
a power amplifier and a loudspeaker (AKG model CK 50). The loudspeaker was placed 29 cm from the bat and 30º contralateral to the middle line and calibrated with a Bruel & kjaer microphone (4135) that was placed at the bat ear. Frequency characteristics curve was plotted for the loudspeaker 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. The signals were short tone bursts with duration 2.5 ms and rise-fall time 0.5 ms. Firstly the characteristic frequency (CF), minimum threshold (MT) and response latency of the isolated IC neurons were determined by single pure tone. The neurons having the lowest threshold to sound stimulus at CF and responding to each of two consecutive presentations of CF pulses at the MT were selected. The stimulus paradigm of the inter-stimulus onset asynchrony (SOA) interval was shown in Fig. 1. The frequency of the masker and test sound was fixed at the CF of the IC neurons. The SOA interval was varied in 1, 3, 6 and 12 ms, respectively. The intensity of the test sound ($I_t$) was fixed at 10, 20 and 30 dB above the MT. The intensity of the masker ($I_m$) increased from 10 dB above the MT to the highest level of the loudspeaker in 10 dB steps. In some cases for the stimulation, the intensity parameters of the masker and test sound were separately presented at 10, 20 and 30 dB above the MT of the neuron.

1.3 Recordings from neural activities
Small holes were bored in the skull. Microelectrodes were pushed gently into the IC region with a micro-drive (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), sent to an oscilloscope (Tektronix 5113) and audio monitor and synchronously to a window discriminator (WPI 121). 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 16 trials using a binwidth of 500 µs.

1.4 Data analysis
The data recorded were input to the software SigmaPlot 2000. The rate-masker-intensity functions (RMIFs) and the inter-stimulus onset asynchrony functions (SOAFs) of the responses to the test sound were recorded. The RMIFs were acquired when the test sound intensity was at 10, 20 and 30 dB above the MT, the masker intensity at 10 dB increments above the MT and the SOA interval at 1, 3, 6 and 12 ms. The SOAFs were acquired when the intensity of the test sound and masker was shifted at 10, 20 and 30 dB above the MT and the SOA interval at 1, 3, 6 and 12 ms. The RMIFs and the SOAFs were normalized and input to the statistics software SPSS. One-way analysis of variance (one-way ANOVA) was used in statistical analysis.

2 RESULTS
Fifty-six IC neurons were measured while RMIFs and the SOAFs were successfully recorded in 31 neurons. The CF, MT, response latency and recording depth of the neurons were ranged in the region of 11.56~37.33 kHz, 20~72 dB SPL, 5~9 ms and 115~1214 µm, respectively. Backward masking was clearly observed in 12 IC neurons responding to the test sound. There was no obvious change in the rest 16 neurons responding if special SLD and the special SOA interval were used. But the responses of some neurons displayed backward masking in the cases of the special SLD and the special SOA interval. We also noticed that the effects on the responses to the test sound were facilitory at the special SLD and the special SOA in three IC neurons.

It was shown in Fig.2 that the responses of an IC neuron to the test sound were affected by backward masking. The strength of backward masking decreased with the SOA intervals increasing (Fig.2A). On the other hand, the masking effects increased with the masker intensity elevating when the SOA interval was at 3 ms (Fig.2B).

2.1 Effects of the masker intensity on backward masking
The responses of some IC neurons to the test sound decreased with the masker intensity increasing when the SOA interval was unchanged. The RMIFs of a neuron responding to the test sound with the masker intensity changing systematically were shown in Fig.3. According to its ten-
dency of the RMIFs, the RMIFs were distinguished into two types. In one type (Fig. 3A), the responses to the test sound reduced with the increment of the masker intensity. The rate of the responses to the test sound was reduced to 75% of the rate of the control responses or below at higher intensity of masker. In the other type (Fig. 3B), the responses to the test sound did not relate to the changes of the masker intensity and the rate of the responses was always steady above 75% of the control responses.

For statistical analysis, each RMIF was normalized, that is, the percentages of the responses to the test sound were calculated based on the control responses as 100% in different stimulus conditions (Fig. 4). The results indicated that for the first type of the RMIFs the normalized responses to the test sound significantly decreased with the increment of the masker level (one-way ANOVA, P < 0.001) when the test sound was at 20 dB above MT and the SOA interval at 3 ms or 6 ms. On the other hand, for the

---

**Fig. 2.** PSTHs of an IC neuron determined under different stimulation conditions. Filled rectangle, test sound; unfilled rectangle, masker sound. $N_{xx}$: Firing rate of the response of an IC neuron to the single stimulus at xx dB SPL. $N_{txx}$: Firing rate of the responses to a test sound at xx dB SPL. $N_{mxx}$: Firing rate of the responses to a masker sound at xx dB SPL. A: Responses of an IC neuron in control and variety of the SOA intervals. B: Responses of an IC neuron when different maskers were used.

---

**Fig. 3.** Rate-masker-intensity functions (RMIFs) of two representative IC neurons responding to the test sound without and with presentation of a masker at different sound intensity MT and SOA intervals. The ordinate represents the firing rate of the responses to the test sound and the abscissa represents the masker intensity relative to the MT. A: Three groups of RMIFs of a neuron. B: Three groups of RMIFs of another neuron.
2.2 Effects of the test sound intensity on backward masking

As shown in Fig. 3A, the strength of backward masking was also related to the test sound intensity. The responses to the test sound were weaker than the control responses when the test sound intensity at lower level, such as 10 dB above MT, indicating that backward masking was strong. When the test sound intensity increased to 30 dB above MT, backward masking became weak. Only when the masker intensity was at high levels (40 or 50 dB above MT) did backward masking occur visibly.

But for some other neurons shown in Fig. 3B, there were no obvious effects of test sound on backward masking in the most stimulus conditions. Only when the intensity of the test sound and masker at 10 and 50 dB above MT respectively with 3 ms of the SOA interval, were the responses to the test sound fewer than 75% of the control responses.

2.3 Effects of the SOA interval on backward masking

The inter-stimulus onset asynchrony functions (SOAFs) of the responses to the test sound were classified into two types. The first type was that the responses to the test sound were mainly steady when the SOA interval was shortened. In Fig. 5A, the rate of the responses diminished to the level below 75% of the control responses with the SOA interval shortened. In Fig. 5A, backward masking occurred only in some special conditions, such as the SOA interval at 1 ms, the test sound at 10 dB and the masker at 40 dB and 50 dB above MT, or the SOA interval at 1 and 3 ms, the test sound at 20 dB and the masker at 40 dB above MT. In these cases, the responses to the test sound diminished to the level below 75% of the control responses.

For statistical analysis, the SOAFs of the two types were normalized (the percentages of the responses to the test sound were calculated based on the control responses as 100% in different stimulus conditions). The mean values and standard deviation (SD) were acquired for the normalized SOAFs of the two types respectively. The results shown in Fig. 6 indicated that the decrement of the normalized responses to the test sound was significant with the increment of the SOA interval for the first type of the SOAFs in Fig. 6A (one-way ANOVA, \( P < 0.001 \)). On the other hand, for the second type of the SOAFs, the variety of the normalized responses to the test sound with the increment of the SOA interval was not significant (\( P > 0.05 \)).
Psychoacoustic and electro-neurophysiological studies of the brain mechanisms responsible for children with primary language impairment have demonstrated that these children are accompanied with unlinguistic sensory disorder and neurophysiological impairment, such as impediment in auditory memory of complex, nonlinguistic sounds. Among these results, results of backward masking experimental indicated that children in the language impairment group had significantly higher (poorer) signal threshold than...
their nonimpaired controls. The latency of the responses prolonged and the amplitude of the responses diminished. It is assumed that, when the children with language impairment listen to and discriminate the recent acoustic signals, their auditory pathway in the central auditory system is impeded, thus resulting in weakening the acoustical information processing and integration for sound perception. This assumption is substantiated by findings from neural imaging and electrophysiological studies achieved in auditory cortex [7, 13-17]. As one of the main integrative centers for auditory information in the auditory pathways, whether or not the IC neurons participated in creating backward masking during auditory cognitive processes is not well understood [5, 8-12].

3.1 Effects of inhibition on backward masking
It is generally believed that inhibition plays an important role in processing auditory signals in the central auditory system. For instance, a lot of studies showed that the tip of the frequency tuning curves were progressively sharpened along periphery to primary auditory cortex. It was presumed that this enhancement of frequency selectivity of central auditory neurons was as a result of mutual integration of excitatory and inhibitory inputs [18]. The whole-cell patch-clamp and neuropharmacological recordings in the IC of awake bats (Eptesicus fuscus) showed that sound-evoked EPSCs were frequently preceded by an IPSC and the sustained leading inhibition was strongest at the stimulus onset and then gradually decayed. These results indicated that the response properties of the IC neurons were determined by the interaction of the inhibitory and excitatory inputs [19-20]. A conceptual model proposed by Covey et al. showed that the neurons fired only when the EPSP coincided with the excitatory rebound from the leading IPSP [21]. The present studies indicated that backward masking occurred in some of the IC neurons and enhanced with the SOA intervals shortening, suggesting that it is most likely that the masker sound could create a leading inhibition with time decay. The suppressed neural responses evoked by the test sound may account for this inhibition. Taken together with other neurophysiological studies, it is presumed that backward masking may be mainly generated by neural inhibition in the central auditory stations including the IC [2, 3, 5].

3.2 Relationship between the temporal integration and backward masking
In the present study, we noticed that the strength of backward masking in the IC neurons was related to the SLD and the SOA interval. The changes of the stimulus conditions, such as the test sound intensity decreasing, the masker intensity increasing or the SOA interval shortening, might cause the responses to the test sound interval shortening. Especially in some IC neurons, only on the conditions of some special SLDs and special SOA intervals could backward masking occur. We conjecture that the occurrence of backward masking is determined by two factors. One is the dynamic balance of the potential amplitude between the excitatory inputs evoked by test sound and the inhibitory inputs evoked by masker. The excitability of the neuron was enhanced by the increment of the test sound intensity and the backward masking induced by the masker was relatively diminished. The increment of masker intensity might activate more inhibitory interneurons in the auditory pathways, thus lead to an increment of inhibitory inputs upon the IC neurons. Therefore, the backward masking we observed would diminish or elevate with test sound intensity increasing or mask sound intensity decreasing (Fig.3 and 4). The other is that there is a temporally dynamic balance between the excitatory inputs evoked by the test sound and the inhibitory inputs evoked by the masker. For the most IC neurons with backward masking, the decrement of the SOA interval means the enhancement of the leading inhibition evoked by the masker. It was consistent with Covey’s recordings by the whole-cell patch-clamp method in the bats [19]. Our observations that for some IC neurons backward masking occurred only in the special SLD and the special SOA interval indicated that it was important for the generation of backward masking that a temporal coincidence between the EPSP evoked by test sound and the IPSP evoked by masker. Furthermore, few of IC neurons in our experiments displayed facilitating responses to the test sound at the special SLD and the special SOA intervals. Although the recent study revealed that there were two-tone facilitating neurons in the IC [22]. This phenomenon we showed here has not been reported under backward masking stimulations. As there is no direct functional and physiological information about it, further investigation should be done.

3.3 IC contributing to the generation of backward masking in auditory cognitive processes
Backward masking is a complex cognitive phenomenon. The recent studies using functional magnetic resonance imaging (fMRI) have shown that backward masking induced the enhancement of the actions in the primary auditory cortex and a number of other associated brain areas, exhibiting the complex dynamic interactions between different acoustical informative streams. The generation of backward masking was relative to the response properties of the auditory neurons and the dynamic integrations of
the information from different brain areas \[23\]. In the study of the acoustic response of AI cortex neurons, the effects from lateral amygdaloid nucleus (LA) were observed \[24\]. Our present studies showed that the responses to the test sound in a part of the IC neurons displayed backward masking, indicating that backward masking previously observed in the cortex might include a succession of the auditory responding properties from the IC neurons. Thus it would be reasonable to believe that, as an important subcortical structure, the IC contributes to the formation of the backward masking in the acoustic cognitive processes. We also noticed that a number of the IC neurons did not display the decrease in response to test sound as if they showed some sort of tolerance to backward masking. This might be related to the amplitude and duration of the leading inhibitory postsynaptic potential evoked by the masker\[5,19-21\]. Nevertheless, our present work only provides a first-step towards understanding the role of IC neurons in backward masking. Whether there might be different mechanisms underlying the backward masking between the IC and the auditory cortex is important for further investigation in the future.

REFERENCES