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RESEARCH ARTICLE

# Masking effect of different durations of forward masker sound on acoustical responses of mouse inferior collicular neurons to probe sound

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**Abstract** To study the effects of different durations of forward masker sound on neuronal firing and rate-intensity function (RIF) of mouse inferior collicular (IC) neurons, a tone relative to 5 dB above the minimum threshold (re MT+5 dB) of the best frequency of recorded neurons was used as forward masker sound under free field stimulation condition. The masker durations used were 40, 60, 80, and 100 ms. Results showed that as masker duration was increased, inhibition in neuronal firing was enhanced ($P <$ 0.000 1, $n = 41$) and the latency of neurons was lengthened ($P<0.01$, $n = 41$). In addition, among 41 inhibited IC neurons, 90.2% (37/41) exhibited narrowed dynamic range (DR) when masker sound duration was increased ($P <$ 0.000 1), whereas the DR of 9.8%(4/41) became wider. These data suggest that masking effects of different durations of forward masker sound might be correlated with the amplitude and duration of inhibitory input to IC neurons elicited by the masker sound.

**Keywords** neurophysiology, masker duration, forward masking, dynamic range, mouse inferior colliculus

## 1 Introduction

Earlier auditory psychophysical tests have demonstrated that a subject would perceive two separate sound signals as one "fused" sound when these two signals are presented with short enough intervals from different positions, the apparent location of which is dominated by the leading sound. The lagging (probe) sound is masked by the leading (masker) sound (*i.e.* forward masking) and the masking degree was correlated with the inter-stimulus onset asynchrony (SOA) interval, inter-stimulus level difference (SLD), and frequency difference between the masker and probe sound (Litovsky and Colbum, 1999). Forward masking has been known to play an important role in sound location, frequency analysis, and sound intensity coding(Brosch and Scheich, 1999; Oxenham, 2001). Aside from its relation with suppression occurring in the cochlear, there is a growing body of literature in neurophysiological experiments which show that the central auditory system participates in the formation of forward masking (Shore, 1998; Frisina, 2001). With methods of recording electroencephalograph (EEG) recording and functional magnetic resonance imaging (fMRI), for instance, recent studies were able to show that children with speech impediment were accompanied by non-linguistic sensory disorder and neurophysiological impairment, such as a kind of impairment in auditory memory for complex, non-linguistic sounds. Meanwhile, under different masking conditions, their primary auditory cortex (AC) and other auditory-associated brain areas exhibited different activity patterns (Dijk and Backes, 2003; Griffith et al., 2003), suggesting that the temporal integration of sound signal in the central auditory system plays a key role in speech recognition (Ahissar et al., 2001; Geissler and Ehret, 2002).

Sound duration is one of the most important parameters of acoustic information. Previous behavioral tests conducted on humans have shown that different durations of forward masker have different masking effects on the discrimination of probe intensity (Schlauch et al., 1997; Oxenham and Plack, 2000). So far, however, there is little literature available on its neuronal mechanism. To further explore the characteristics of temporal integration on the inferior colliculus (IC) neurons, the current *in vivo* study was undertaken to investigate the effect of different forward masker durations on the response properties of mouse IC neurons under free field stimulation condition. The masker sound we employed was a tone burst of which the intensity

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was relative to 5 dB above the minimum threshold (re MT+5 dB) and the frequency was the same as the best frequency (BF) of recorded neurons.

## 2 Materials and methods

### 2.1 Animal and microelectrode preparation

Experiments were conducted on 8 mice weighing 20-25 g from Hubei Medical Science Center. All animals used in the experiments were with normal Pryer's pinna reflex (with a positive Preyer's pinna reflex and good health). Animals were anesthetized with nembutal (50~60 mg/kg, b. wt, ip) before surgery. Supplemental injection of Nembutal-maintained animals in light anesthetizing state was performed throughout the course of the experiment. No differences were noticed from the results of experiments conducted with this anesthetic. A midline incision was made in the scalp while the skin and muscles were retracted laterally, and a hole of 200~500 μm in diameter was made on the skull above the IC to permit insertion of the microelectrode for recording. The head of a 1.8-cm nail was mounted on the skull with superglue 502 and dental cement, and then the rod of the nail was screwed to a metal rod holding the head firmly during the experiment. The animal was then secured to a metal plate with a plastic band inside a sound proof room (temperature 28ºC~30ºC). All procedures used in these experiments were approved by Central China Normal University Animal Care Committee.

A single barrel glass electrode (tip diameter < 1 μm, resistance 5~10 MΩ) was prepared by microelectrode puller (Bioscience,GB) and filled immediately with 2 M NaCl before use.

### 2.2 Acoustic and recording setup

The electronic instruments used to generate acoustic stimuli consisted of two function generators (GFG-8016G，Good Will Inst Co.，Ltd, Malaysia), two tone burst generators (homemade), two attenuator (LAT-45，Leader，Japan), a power amplifier (homemade), and a small loudspeaker (AKG model CK 50，1.5 cm in diameter，1.2 g，frequency response 1-100 kHz ). The loudspeaker was placed at the mouse's ear and calibrated with a 1/4 inch microphone (4939, B & K, Denmark) connected with a measuring amplifier (2610, B & K, Denmark). This was fixed at 0° in elevation and 60° contralateral to the middle axis of recording site in azimuth. Its output was expressed in dB SPL referred to as 20μPa root mean square.

### 2.3 Sound stimuli and recording procedures

The distance between the loudspeaker and mouse's ear was 30 cm. Durations of first tone (masker) are 40, 60, 80, and 100 ms, while the second tone (probe) was fixed at 40 ms. The intensity of masker is 5 dB above the recorded minimum threshold (MT) of neurons. Rise-and-decay times and frequency of all sound stimuli are 2 ms and the recorded best frequency of neurons, respectively. These two sounds were successively delivered from the loudspeaker at 2 per second in free field.

A single glass electrode was driven orthogonally into the IC by a hydrolic micropositioner (KOPF 640, USA) with a resolution of 1 μm The depth, BF, and MT of isolated neurons were recorded. Thirty two samplings of neuron response were digitized and discriminated on the basis of amplitude using an A/D convector and a software (homemade) for the post-stimulus time histograms (PSTH) (bin width: 0.5 ms; sampling period: 100 ms) and rate intensity functions by 10-dB step increment. All data were fed into a computer for data processing after the end of the experiment.

### 2.4 Data analysis

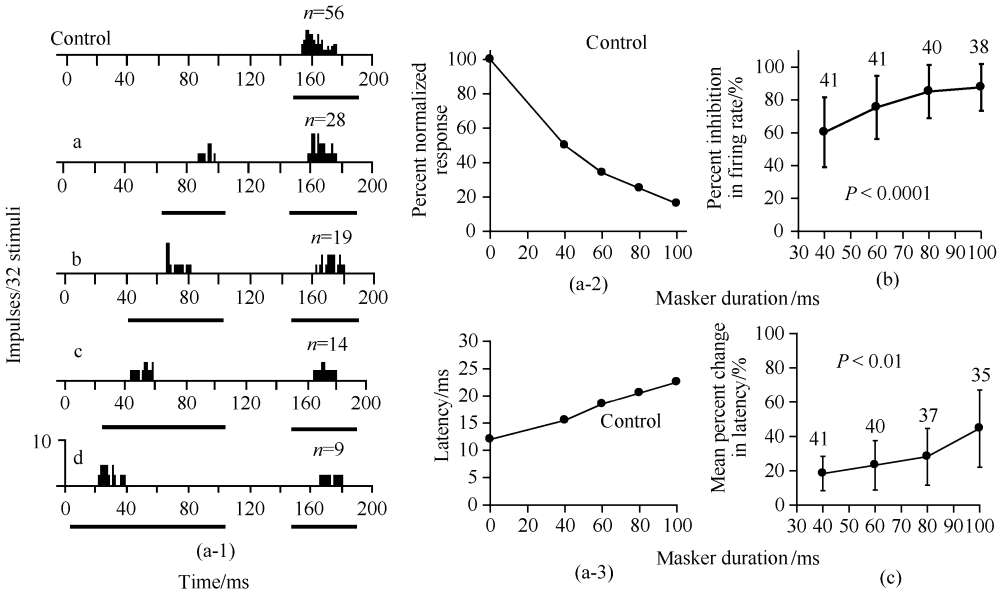
Statistical analysis and charting of the data were performed on the software Origin 6.0 and SigmaPlot 2000. The values given in the text and figures were expressed as mean ± standard deviation (M ± SD). Paired *t*-test for independent samples and one-way analysis of variance (one-way ANOVA) were used for statistical analysis in this paper.

## 3 Results

A total of 102 IC neurons were obtained in this experiment and their depth, BF, and MT ranged from 553~1954 μm, 6.03~16.58 kHz, 13~57 dB SPL, respectively. Using a variation of≥20% in firing rate at 10 dB above the MT before and during presentation of the masker sound as the critical standard, 41 among the 44 or approximately 93.2% of neurons examined for complete rate-intensity functions (RIF) exhibited response suppression. The remaining neurons were either facilitated (1/44, 2.3%) or unaffected (2/44, 4.5%) by the forward masker.However, the result for the latter have no statistical significance.

### 3.1 Effects of masker duration on the firing rate and response latency of neurons

The relationship among masker duration,firing rate and latency of neurons responding to the probe of 10 dB above the MT before and during presentation of the masker is shown in Fig. 1. When the intensity of the masker and probe was constant, the suppressing effect of masker on the responses of neurons to probe was related with masker duration (Fig. 1 a-1). Elongating the masker duration caused a decrease in firing rate of neurons responding to the probe (Fig. 1 a-2). To evaluate the masking efficiency, percent inhibition in firing rate at 10 dB above the MT was calculated by dividing the difference between the number of




(a-1): PSTHs showing the acoustical responses of a representative IC neuron before and during presentation of the masker. The horizontal black bars under abscissa indicate the probe sound, and the different durations of masker sound (a-d: 40, 60, 80, and 100 ms). N represents the firing rate of neuron;
(a-2): The normalized response (%) - masker duration function before and during presentation of the masker;
(a-3): The latency - masker duration function before and during presentation of the masker;
(b): Mean percent inhibition of all sampled neurons induced by the masker with different durations in firing rate;
(c): Mean percent variations of all sampled neurons in response latency after addition of the masker. The vertical line and number at each data point in A & B represent the SD and the number of neurons sampled. The depth, BF, and MT of this neuron were 990 μm , 13.41 kHz, 31 dB SPL, respectively

**Fig. 1** Effects of different masker durations on the responses of IC neurons to the probe sound

impulses before (control) and during the addition of masker by the number of impulses of control. As shown in Fig. 1 b, percent inhibition was increased with masker duration elongation ($P$<0.0 001, one-way ANOVA). However, this inhibition was not enhanced linearly. As masker duration lengthened, the increment of percent inhibition on firing rate was slowed down.

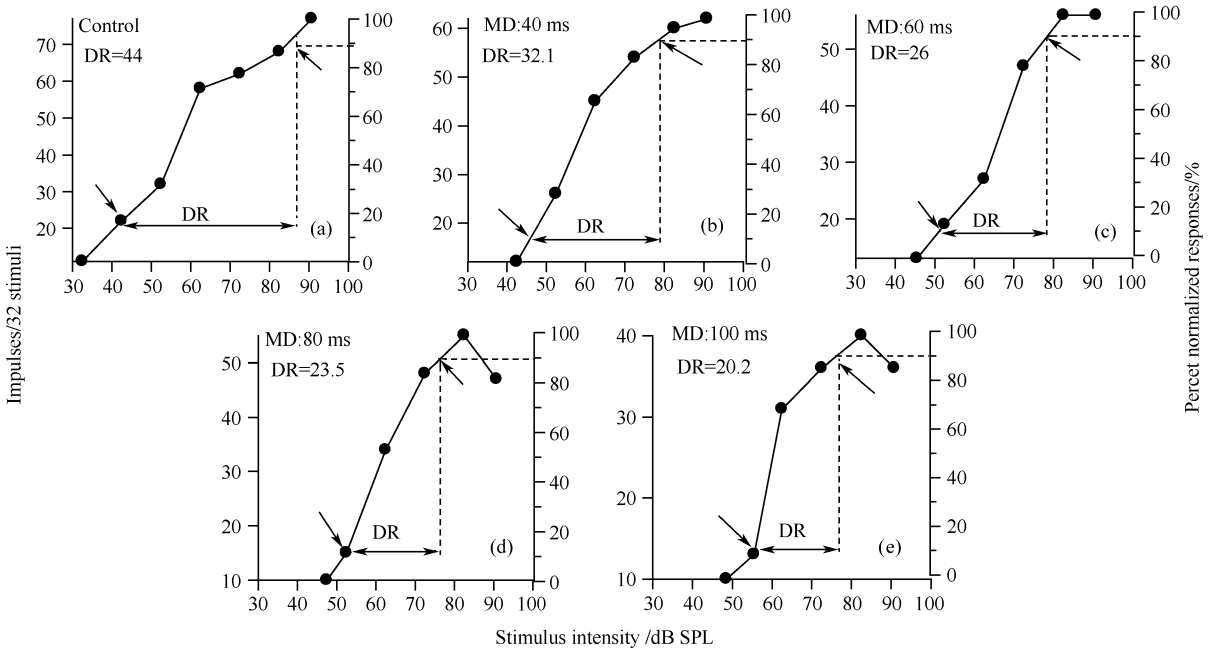
Likewise, by dividing the difference in response latency of neurons to the probe before and during addition of the masker by the latency of control, we found that mean percent variations of sampled neurons in response latency increased with masker duration elongation (Fig. 1 c, $P$ < 0.0 001, one-way ANOVA).

### 3.2 Effects of masker duration on the DR of neuron response

The dynamic range (DR, dB) is a measure of the intensity range within which a neuron's response monotonically increased with sound intensity. It is thus an indication of a neuron's intensity sensitivity. Based onthe RIF of neuron responding to the probe, we defined the intensity range over which the normalized response (refer to ordinate in Fig. 2) was elevated from 10% to 90% of the DR. Fig. 2 shows the variations in DR of a representative neuron under different forward masking conditions. It can be observed that all durations of masker stimuli caused the DRs of this neuron to narrow. Among 42 inhibited IC neurons, variations in DR can be classed into two types (Table 1). In most neurons (37/41, 90.2%), the DR narrowed during presentation of different durationsof masker and decreased when the masker duration was increased ($P$<0.001, One-way ANOVA). The DR of the rest (4/41, 10.5%) became wider during presentation of different durations of masker , but the values did not typically risewhen the masker duration was increased ($P$>0.05, One-way ANOVA).

**Table 1** Variations of neuronal dynamic range induced by different durations of masker

| | Masker duration /ms | | | | |
|---|---|---|---|---|---|
| | control | 40 | 60 | 80 | 100 |
| | DR decreased （$n$=37） | | | | |
| M±SD | 30.7±11.2 | 24.4±7.8 | 20.8±8.7 | 20.3±9.3 | 19.5±10.3 |
| Range | 16.5~54 | 8~39.2 | 6~38.4 | 5.4~48 | 2.7~43 |
| $P$ | | <0.0 001 | <0.0 001 | <0.0 001 | <0.0 001 |
| | DR increased （$n$=4） | | | | |
| M±SD | 16±5.1 | 25.5±6.5 | 26.1±8.2 | 16.1±6.6 | 22.8±6.3 |
| Range | 11~23 | 20.5~35 | 19.5~38 | 19.5~34 | 16~30.8 |
| $P$ | | 0.0 023 | 0.0 085 | 0.0 032 | 0.0 064 |




(a): The RIF and DR obtained without presentation of the masker.
(b—e): Variations in DR induced by differentdurations of masker . MD represents the masker duration. The depth (μm) , BF, and MT of neuron were 742 μm, 14.8 kHz, and 33 dB SPL, respectively.

**Fig. 2** Effect of different masker durations on the DR of a representative neuron

## 4 Discussion

In the present study, we found out that the firing rate, latency, and DR of most neurons responding to probe sound were negatively masked by different durations of weak masker with intensity of 5 dB above the MT and the same frequency as the recorded BF of neurons. The forward masking strength increased with the lengthening of masker duration.

Up to now, most quantitative information on negative masking effects comes from auditory psychophysical investigations. Its neuronal mechanism has remained elusive. Many early studies argued that the suppression occurring in the periphery auditory system may be one of the main reasons of its cause (Litovsky and Colbum, 1999; Oxenham, 2001). However, the results of ionophoresis experiments obtained in recent years suggested that central neural inhibition might play a more important role in creating negative masking effect (Frisina, 2001). Further immediate evidence emerged from the electrophysiological studies of intracellular recording *in vivo*. For example, by using the technique of whole-cell clamp and neuroph-armacological recordings, Covey (1996) showed that sound-evoked excitatory postsynaptic currents (EPSCs) were frequently accompanied by an inhibitory postsynaptic currents (IPSCs) in the ICs of live bats and the latter could modulate the response characteristic of neuron to the stimuli (Covey, 1996). In addition, there are some investigators holding a viewpoint that all sound stimuli could induce inhibitory input. It is strongest at its onset and sustained throughout the full duration of a sound for at least more than 100 ms, then gradually decays (Park and Pollak, 1993; Brosch and Schreiner, 1997; Fatur et al., 2003). Therefore, it would be logical to believe that the negative masking effects we observed in the current investigation are related to the inhibitory input activated by forward masker. It was notable that acoustical response duration to the forward masker of most IC neurons was not lengthened with increasing forward masker duration, but the latency of neurons responding to the probe was gradually increased (Fig. 1). This demonstrated that the inhibitory input induced by the forward masker not only shaped the response properties of IC neurons to the forward masker, but also might affect the IC neurons' response to the probe sound by means of dampening the recovery cycle of neural circuits (Lu et al., 1997; Luan et al., 2003). Thus we would like to speculate that longer durations of forward masker might activate more inhibitory inputs and its inhibitory influences are sustained with longer time. i.e. the time decay of inhibitory input was delayed with forward masker duration lengthening.

On the other hand, lengthening the masker duration did not always follow the linear increment of masking strength (Fig. 1b, c). This observation closely mirrors a previous report in which the authors found out that after the masker duration went beyond 100 ms, the inhibitory strength was increased by no more than ten percent in the IC neurons of anesthetized mice (Finlayson, 1999). Of course, our data imply that forward masking effects caused by weak masker, having the same frequency as the probe under the condition of fixing gap, couldn't be fully accounted for by the temporal integration between excitatory and inhibitory

inputs upon the recorded neurons. Rammsayer and Leutner (1996) suggested that there might be two kinds of mechanism underlying the temporal information processing of masker duration. Nonlinear mechanism of peripheral basilar membrane motion is most likely involved in this temporal processing. Hence, it would be reasonable to believe that the nonlinear effects of forward masking on the recorded acoustical responses of IC neurons we observed, at least to some extent, are attributable to the nonlinear suppression occurring in the cochlea.

Although most auditory psychophysical tests showed that lengthening the duration of forward masker within a certain extent would cause decrease in the subject's discrimination ability for signal intensity, contrary results were also reported. Moreover, previous study performed by Zhou and Jen (2002) confirmed that alteration of sound duration will change the RIF patterns and the values of DR. Likewise, our recent investigation conducted on the IC of big brown bat (*Eptescus fuscus*) had shown that sensitivities to signal intensity and frequency are dynamically modulated under the condition of two-tone stimulation (Wu et al., 2004a,b). Our present data, together with these findings, make it most likely that there might be complex temporal integration of the IC neurons under the forward masking stimulation condition. Another interesting cue in present experiments to focus on is that, although the MTs and DRs of majority of the neurons were elevated and narrowed, DRs of a smallfraction of neurons were broadened (Fig. 2, Table 1), implying that the intensity coding feature of recorded neurons is altered by forward masking. A widespread view is that narrowing / broadening DR means a decrease / increase in intensity scope necessary for an auditory neuron to reach the maximal acoustical responses. Namely, in such two cases the auditory neuron would either be more sensitive to the intensity variations or have a much broader scope for detecting signal. Thus, to clarify whether this two-way variation in DR of IC neurons under the forward masking condition we described here are interrelated with the central auditory strategy for intensity perception in animals and humans, and whether it is somewhat interrelated with the function of speech recognition in humans under the environment with background noise, masking should be an important consideration for further verification.

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