Delayed inhibition creates amplitude tuning of mouse inferior collicular neurons

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Nonmonotonic intensity response neurons referred as amplitude-tuned neurons are considered to be created by high-threshold inhibition in auditory system. Very limited information, however, is available on how the inhibition works for amplitude-tuned neurons. We studied the temporal response properties of these neurons with or without iontophoresis of bicuculline (γ-aminobutyric acid A antagonist). In most neurons, the firing durations gradually reduced with the increasing amplitudes beyond the best amplitudes. Bicuculline application selectively blocked the inhibition of the sustained responses to high amplitudes and abolished the nonmonotonic intensity response properties. Our results suggest that a temporally delayed inhibition, whose latency reduced related to excitation with the increasing amplitude, is responsible for the creation of about 71% amplitude-tuned neurons in mouse inferior colliculus. *NeuroReport* 19:1445–1449 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The auditory neurons with nonmonotonic intensity responses (NIR), that is, the spike rates or counts initially increase, then decrease as sound amplitude increases, are referred as amplitude or intensity-tuned neurons for sound amplitude processing. These neurons have been found in every level of the auditory central pathway [1–10]. Peripheral auditory nerve fibers, however, have monotonic intensity responses, that is, the spike rates or counts increase monotonically with increasing amplitude of acoustic stimuli [11]. Thus, an inhibition from central neurons to high-intensity stimuli is thought to contribute to the creation of a neuron's amplitude tuning [1–10].

This notion has been favored by studies with two-tone masking paradigms [10,12]. Further studies have revealed that γ-aminobutyric acid-mediated inhibition is involved in the creation of amplitude tuning of inferior colliculus (IC) neurons [13–15]. Recently, studies using whole-cell recording indicate that the reduction of the relative time interval between the excitation and inhibition creates or sharpens amplitude tuning in auditory cortical neurons [16,17]. It remains unclear whether the NIR neurons in subcortical nuclei, however, are the case or not. Therefore, in this study we examined the temporal response properties of NIR neurons in mouse IC. Our data indicate that a temporally delayed inhibition creates about 71% amplitude-tuned neurons by reducing the time interval relative to excitation to high-amplitude sounds, and about 29% NIR neurons receive the NIR property from the below neurons in the auditory pathway.

Methods

Animal surgery, acoustic stimulation, data acquirement, and analysis were the same as described in our earlier paper [18]. The procedures for this study were approved by the Animal Care and Use Committee of the Institute of Biophysics, Chinese Academy of Sciences.

Extracellular recording

Twenty-five female BALB/c mice (4–6 weeks, 12–15 g) contributed data to this study. Under anesthetization (pentobarbital, 60 mg/kg intraperitoneally), each animal was glued with a 1.5-cm-long nail on the dorsal skull surface. After 3–4 day recovery from the surgery, the animal was reanesthetized and placed in a polyethylene-foam body mold, which was hung with an elastic band at the center of a 24–26°C soundproof room. The animal's head was immobilized and adjusted directly toward the loudspeaker located 30 cm away by fixing the glued nail into a metal bar with set screws. Then an about 2×2-mm^2 IC surface was exposed after the skull and dura were removed.

Acoustic signal generation and data acquirement and online processing are performed with Tucker-Davis Technologies System 3 controlled by BrainWare (Tucker-Davis Technologies, Alachua, Florida, USA). Of acoustic stimuli

(pure tone bursts) synthesized and generated with Tucker-Davis Technologies System, the parameters, such as amplitude (varied in 5 dB steps or 1–2 dB steps when it was close to minimum threshold), frequency (set as the neuronal characteristic frequency, CF, the most sensitive frequency), duration (50 ms), rise and fall times (5 ms), rate of delivery (1/s) were controlled with BrainWare. The loudspeaker was calibrated and sound amplitude was expressed as sound pressure level (SPL, 0 dB referred as 20 μPa). The neuronal activities recorded with a glass micropipette electrode (about 1 μm in tip diameter, filled with 2 M sodium acetate) at 100–1000 μm below the pial surface, were filtered (0.3–3 kHz), amplified (2000–10 000 ×), and isolated as single-unit responses. To obtain a mean response, an identical acoustic stimulus was repeated 20 times. The data were viewed with respect to raster plot of spikes in poststimulus time histogram, spike counts–amplitude plot, spike shape and feature space window by using BrainWare. The numbers and timings of spikes (with signal-to-noise ratio $>4:1$) were collected and stored as datasets.

Drug application

To evaluate the role of GABAnergic inhibition in shaping the amplitude tuning, we iontophoretically applied bicuculline methiodide (BMI, a $GABA_A$ antagonist) to some NIR neurons with microiontophoresis (Neurophore BH-2, Harvard, USA) and 'piggy-backed' electrodes, of which a single recording electrode with the tip (1–2 μm) sticking out about 10 μm was 'piggy-backed' to a three-barrel electrode (tip diameter: 10–15 μm). One of the three barrels was filled with BMI (10 mM, pH 3.0) and connected to one iontophoresis channel of the BH-2, whereas the other two barrels were filled with NaCl (1 M, pH 7.4) and used as the ground and balance, respectively. BMI was ejected by applying a positive current, usually less than 80 nA, and held with −20 nA to prevent drug leakage. To determine the effect of BMI, an ejection current was increased in 10 nA steps until an obvious and reliable response increasing was observed. The responses of a neuron were evaluated before, during, and after the drug application.

Data analysis

On the basis of acquired datasets, the best amplitude (BA, the amplitude of acoustic stimulus which evoked most spikes), the monotonicity ratio defined as the ratio of the spike counts at the BA to that at the highest amplitude (HA), the first spike latency (FSL), the firing duration (FD, the mean time interval between the first spike and the last spike in 20 trials) and the differences of FSL and FD between those at BA and HA were calculated for each neuron and expressed with mean ± SD. So were those for the BMI-ejected neuron before, during, and after BMI application. Repeated measures analysis of variance (SPSS 11, SPSS Inc., Chicago, Illinois, USA) was applied to test the difference of BMI application for each and all administrated neuron(s). Reported P values were corrected.

Results

Data of 88 isolated single neurons were collected from the central nuclei of 42 (23 right, 19 left) ICs. The CFs of these neurons ranged from 4 to 37 kHz (17 ± 6.3 kHz), minimum thresholds ranged from 5 to 75 dB SPL (38 ± 18.2 dB SPL).

Figure 1 shows the responses of a NIR neuron to different amplitudes of CF (33 kHz) tone bursts before, during, and after BMI application. In control, its spike counts increased from 59 to 64 dB SPL, peaked at 64 dB SPL and then decreased from 64 to 86 dB SPL [Fig. 1(a1 and b), the open circles]. The BA and monotonicity ratio (spike counts at the HA/that at the BA) of this neuron were 64 dB SPL and 0.24 (17/70). The FSL, however, decreased monotonically with increasing stimulus amplitude and asymptotically to a minimum latency at high amplitudes [Fig. 1(a1 and c), the open circles]. The FD was a function of stimulus amplitude and also peaked at the BA [Fig. 1(a1 and d), the open circles] as the spike counts amplitude function. The temporal properties of this NIR neuron demonstrated that the inhibition delayed to the excitation was evoked by high amplitude and increased with the increasing sound amplitude.

Out of the 88 neurons, 46 (52%, Fig. 2a, the filled circles) with monotonicity ratios (0.5 ± 0.2) less than 0.8 were NIR neurons with the BAs ranging from 13 to 78 dB SPL, whereas the remaining 42 (the open circles) were monotonic intensity response neurons, according to the criteria (the line in Fig. 2a) of earlier papers [15,19]. In a majority of these NIR neurons, the FSLs (43 out of 46), and FDs (42 out of 46) at BAs were longer than those at HAs (85–91 dB SPL) (Fig. 2b and c, the filled circles above the lines). These temporal response properties suggested that a delayed inhibition for high amplitude (beyond BA) created non-monotonic spike counts amplitude function of a given neuron. In contrast, the remaining FSLs (3 out of 46) and FDs (4 out of 46) at the BAs were shorter than those at the HAs (within 0.5 and 3.4 ms for FSLs and FDs, respectively). The neurons with 'paradoxical' FSL changes were not those with 'paradoxical' FD changes. The 'paradoxical' FSL changes might attribute to variation or selection of non-CF frequency [18] or an early inhibition [20], which might contribute to formation of nonmonotonic intensity response.

GABAnergic inputs are the extensive inhibition sources. To clarify the above suggestions, BMI was iontophoretically applied to 16 NIR neurons (including the three 'paradoxical' FSL change neurons), of which all the FDs at BAs were longer than those at HAs. With the administration of BMI, the dynamic range of response was extended and the responses for the high amplitudes (beyond BA) were selectively enhanced with less effect on those at the amplitudes lower than the BA [Fig. 1(a2), illustrated by an example neuron] so that the spike counts and FD amplitude functions of this neuron changed from nonmonotonic to monotonic (Fig. 1b and d, filled circles). In contrast, disinhibition changed the temporal discharge pattern of the neuron to high amplitudes. Nevertheless, BMI application did not affect the FSLs for the high amplitudes but shortened them for the low amplitudes (Fig. 1c, filled circles). The responses of this neuron recovered completely 25 min after BMI application [1Fig. 1(a3 and b–d), the open triangles].

Fourteen neurons' data were selected as datasets by complete recovery, that is, without difference of spike counts, FSL, and FD between pre-BMI and post-BMI ($P>0.087$), the other two with differences were discarded. In 13 BMI administrated neurons, the spike counts were significantly ($F>12.765$, P for BMI vs. control <0.000) enhanced by BMI application, further, the FSLs were shortened ($F>10.422$, $P<0.006$), and the FDs at the HAs

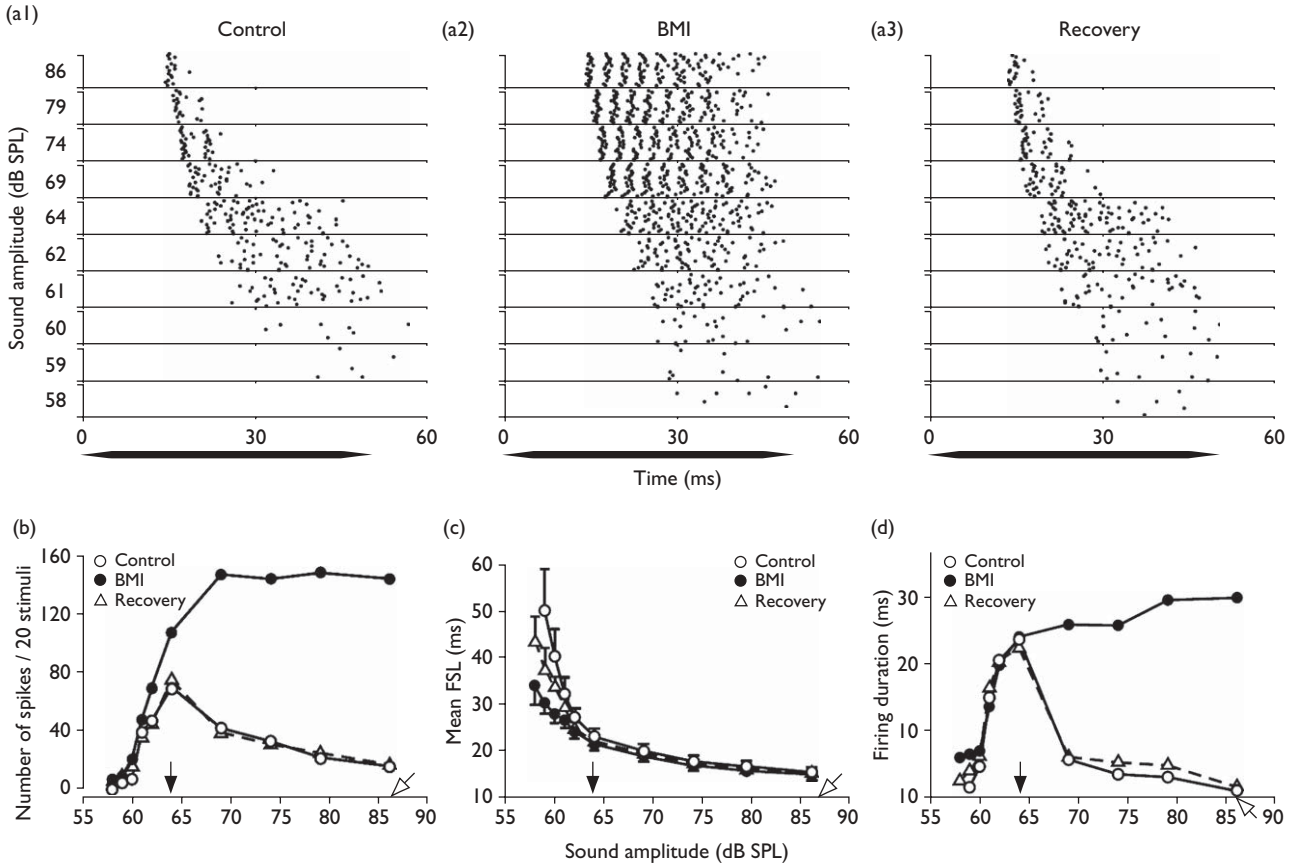

Fig. 1 Response properties of an inferior colliculus nonmonotonic intensity response neuron before, during, and after bicuculline methiodide (BMI) application. (a1–3) Spike timing rasterplot displaying the responses of the neuron to different amplitudes of acoustic stimuli before (control), during (BMI), and 25 min after (recovery) BMI application. Responses to repeated presentations of the identical amplitude (20 times) are shown in one block. Each dot represents a spike. The darkened line at the bottom shows the acoustic stimulus envelope. (b–d) The spike counts – first spike latency – and firing duration – amplitude functions, before, during, and after BMI application, respectively. The filled and open arrows show the best and highest amplitudes. SPL, sound pressure level.

were significantly ($F > 36.181$, P for BMI vs. control < 0.000) elongated. Only one neuron's spike counts and FSL were not affected by BMI ($F = 1.381$, P for BMI vs. control $= 0.243$).

For 10 out of the 13 BMI enhanced neurons, however, the ratios of spike counts at the HA to those at the BA (Fig. 2d) and the differences of FDs between BAs and HAs (Fig. 2f) were reversed, whereas the ratios and FD differences for the other three neurons were little changed (Fig. 2d and f). The differences of FSLs between BAs and HAs were significantly reduced by BMI application ($F = 5.928$, $P = 0.049$) (Fig. 2e). The three 'paradoxical' FSL changes, however, were not reversed by BMI application (Fig. 2e, the points blow the line). This suggested that no early inhibition was involved in the 'paradoxical' FSL changes and creation of IC NIR neurons. Anyhow, it seemed that the properties of 71% IC NIR neurons were created by delayed (not early) GABAnergic inhibition in the IC level and could be abolished by iontophoretical application of BMI, and of 29% IC NIR neurons might be created in the level below IC and relayed to IC and could not be abolished.

Discussions and conclusion

Nonmonotonic intensity response property of central auditory neurons was proposed to be formed by the interaction of excitation and high-threshold inhibition [10,12]. Further studies using whole-cell recording in auditory cortical neurons have revealed that the reduction of the relative time interval between the excitation and inhibition creates or sharpens amplitude tuning [16,17]. To check whether the NIR property of subcortical nucleus neuron is created in the same way as that of cortical neuron is, we investigated the temporal response properties of nonmonotonic neurons in mouse IC with or without BMI application.

In this study, nonmonotonic intensity responses were demonstrated in about 52% mouse IC neurons, which are similar to other studies on the IC of rats [13] and mustached bats [14] (60%). The FSL of a NIR neuron generally decreases with increasing amplitude (Fig. 1a and c, and Fig. 2b), and the FD also decreases with increasing amplitude beyond the BA although it increases from its minimum threshold to the BA [Fig. 1(a1 and d), and Fig. 2c]. Thus, an inhibition could be evoked by high amplitude of acoustic stimulation, for its latency shortens faster than FSL as sound amplitude increases. In contrast, the results of BMI (an antagonist of $GABA_A$ receptor) microiontophoresis favor this notion with reviving the inhibited spikes and extending FD [Fig. 1(a2 and d), and Fig. 2f] by disinhibition. Therefore, NIR property of IC neurons

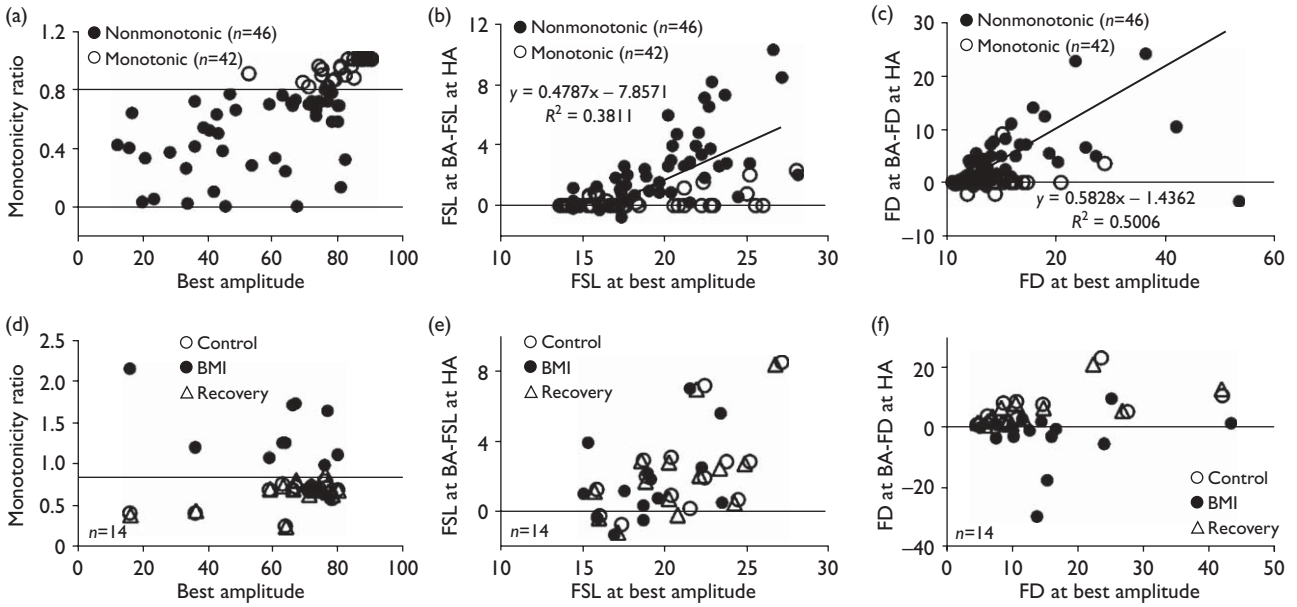

Fig. 2 Pooled data for recorded neurons without (a–c) and with (d–f) bicuculline methiodide (BMI) application. (a and d) Monotonicity ratios distributed over best amplitudes (BD) of neurons. The horizontal line is the criteria to classify the monotonic and nonmonotonic intensity responses. (b and e) The distribution of first spike latency (FSL) differences between the BA and highest amplitude (HA) over the FSLs at the BA. (c and f) The distribution of firing duration (FD) differences between the BA and (HA) over the FD at the BAs. The tilted lines indicate the distributed tendencies.

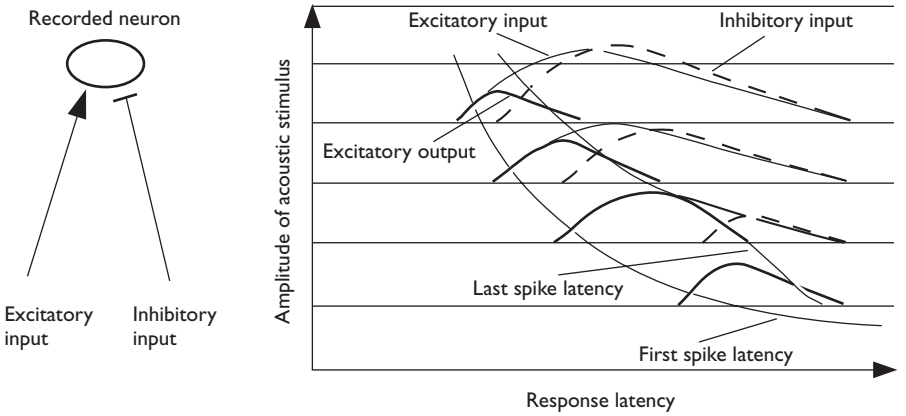

Fig. 3 A model for the creation of a nonmonotonic intensity response neuron. A recorded amplitude-tuned inferior colliculus neuron receive both inhibitory and excitatory inputs. The excitatory output (bold solid lines) is a summation of excitatory (gray solid lines) and inhibitory (dashed lines) inputs. Firing duration is the interval between first and last spike latency.

referred as amplitude-tuned neurons is created by the excitatory input and the delayed inhibitory input, whose latency shortens faster than the excitatory input with increasing amplitude (Fig. 3), as the way in cortex [16,17].

Not all amplitude-tuned neurons in IC, however, are created by delayed inhibition in the same level. Our present results demonstrate that the amplitude tuning of about 29% NIR IC neurons is not abolished by disinhibition of BMI application. The amplitude tuning of these neurons may be created in the level below IC and relayed to IC. Published data obtained from the ICs of rat and mustached bat have suggested that the NIR property of about 12% amplitude-tuned IC neurons in the rat [13] and 69% of those in mustached bat [14] may be relayed to IC from the neurons below IC. The proportion of this relayed property may differ among species.

In this paper, we conclude that not all nonmonotonic intensity response neurons of mouse IC are created in IC, about 29% are relayed from the below neurons and about 71% are created in IC by a delayed inhibition to high-sound amplitude.

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