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The role of medial olivocochlear activity in contralateral suppression of auditory steady-state responses

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ABSTRACT

Objective: The auditory steady-state response (ASSR) amplitudes fall in the presence of contralateral noise. However, whether and to what extent medial olivocochlear (MOC) activity involves in contralateral suppression of ASSR remain unclear. Therefore, we assess the role of MOC activity in contralateral suppression of ASSR.

Methods: Mice were treated with strychnine to completely eliminate MOC activity and then measured ASSR amplitudes in the presence of contralateral noise.

Results: The contralateral noise reduces ASSR amplitudes at some stimulus intensity. After treating with the strychnine to eliminate MOC activity, ASSR amplitudes recovered again.

Conclusions: MOC activity participated in contralateral suppression of ASSR.

1. Introduction

Medial olivocochlear (MOC) fibers running through the cochlea stems from both sides of the medial portion of the superior olivary complex. These fibers create synapses with the outer hair cells (OHCs) [1]. MOC efferent fibers block the electromotility of OHCs by inducing hyperpolarization of OHCs, which reduces the gain of cochlear amplifier leading to a reduction in otoacoustic emissions (OAEs) [1]. Thus, OAEs have been extensively used to evaluate MOC activity [2]. The MOC activity includes both contralateral suppression and ipsilateral suppression. Contralateral suppression of OAEs is typically used to evaluate MOC activity, wherein OAEs are measured without and with contralateral noise that activates the contralateral MOC pathway. Contralateral suppression has been described for all types of OAEs, including spontaneous otoacoustic emissions (SOAEs) [3], stimulus frequency otoacoustic emissions (SFOAEs) [4], distortion-product otoacoustic emissions (DPOAEs) [5], and transient-evoked otoacoustic emissions (TEOAEs) [2,6]. The MOC activity may function in the optimization of hearing and selective attention in the incidence of interfering noise and may also have a role in slowing the aging process of the cochlea [1].

Studies showed that contralateral suppression of OAEs can be an effective approach for exploring MOC activity [2–6]. However, during hearing loss, OAEs are mostly absent or

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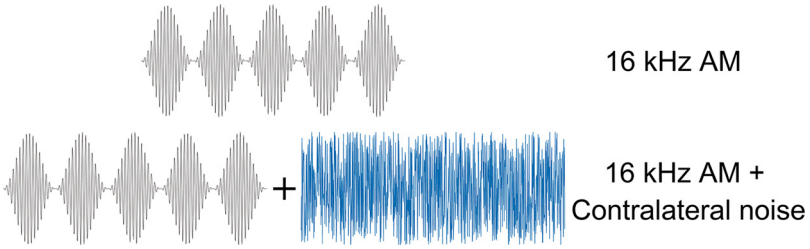

Fig. 1. Schematic of stimuli. The SAM tone modulated at 100% depth and the 1 kHz and its carrier frequency was the 16 kHz. The contralateral noise was white noise and given in synchronization with SAM tone. SAM, sinusoidally amplitude-modulated.

weak, which increases the difficulty of studying MOC activity in individuals with hearing loss [7]. Mertes and Leek [2] believe that there are several reasons why contralateral suppression of ASSR may be a promising tool for studying MOC reflex: (a) Contralateral suppression of auditory neural responses is often larger than contralateral suppression of OAEs [8], (b) ASSR amplitudes fall in the presence of contralateral noise [9], and (c) ASSR measured at suprathreshold stimulus levels in ears with significant hearing loss demonstrates similar amplitudes as in individuals with normal hearing [10]. However, whether and to what extent MOC activity involves in contralateral suppression of ASSR remain unclear. To assess the role of MOC activity in contralateral suppression of ASSR, we treated mice with strychnine to completely eliminate MOC activity and then measured ASSR amplitudes in the presence of contralateral noise.

2. Methods

All the procedures were approved by the Institutional Animal Care and Use Committee of our hospital. Six-week-old male CBA/Ca mice were used in all experiments. Two study groups included the control group ($n = 8$) and the MOC-null group ($n = 8$).

The MOC-null group: Additional mice were connected to a respirator (RWD Life Science) via a tracheal cannula after anesthesia. MOC activity was completely eliminated by intraperitoneally (i.p) treating mice with α-D-tubocurarine and strychnine (1.25 and 10 mg/kg, respectively) [11].

Xylazine (i.p, 10 mg/kg) and ketamine (i.p, 100 mg/kg) were used to anesthetize the mice. Rectal thermometer was used for monitoring the body temperature which was kept at 37 °C in the course of testing through heating the experimental chambers. An additional one-third of the original anesthetic dose was administered depending on the need.

Mouse hearing functions, including auditory brainstem responses (ABRs) threshold, cubic ($2f_1 - f_2$) DPOAEs threshold, and MOC activity, were evaluated in two groups. The DPOAEs and ABRs were conducted by TDT system (BioSig software). A total of 3 platinum subdermal electrodes inserted subcutaneously at the vertex (+), over the mastoid process (−) and near the base of the tail (ground) were used to record the ABRs. An MF1 Multi-Field Magnetic speaker (TDT) provided tonepip (8, 12, 16, 20 and 24 kHz) stimuli. Thresholds at the various frequencies were defined as the lowest intensity at which ABRs waves could be detected.

Equal intensity primary tones were generated by the two MF1 speakers in the DPOAEs test, under the control of the TDT system (f_1 and f_2; f_2/f_1 was 1.2). Measurements of the recording were conducted at 8, 12, 16, 20 and 24 kHz. Thresholds at the various frequencies were evaluated reference when the cubic ($2f_1–f_2$) distortion product more than 3 dB above the noise floor, with increasing tone intensity.

The MOC activity was determined using contralateral suppression of DPOAEs while maintaining the DPOAEs-measuring probe on the ear. The contralateral suppression: the contralateral ear was subjected to 70 dB SPL, broadband suppressor noise (10 s continuously, closed field), and the DPOAEs were stimulated in the ipsilateral ear with 60 dB SPL, 16 kHz primary tones. The ABRs, DPOAEs and MOC activity recording protocols and procedures were similar to previous studies and more details can be found in them [12].

The ASSR amplitudes to sinusoidally amplitude-modulated (SAM) tone were measured with/without contralateral noise in the control and the MOC-null group. The carrier frequency of SAM tone was the 16 kHz and the duration was 200 ms (5ms rise and fall). The 16 kHz was chosen as carrier frequency because it falls within the most sensitive hearing regions in mice. Stimuli were given at a rate of 3.1/s. A total of 200 repetitions were done in a 300 ms acquisition window and the average amplitude was obtained. The SAM tone modulated at 100% depth and the 1 kHz were given at 40–80 dB SPL and raised in 5 dB increments (Fig. 1). For channel 1, a positive electrode was placed along the midline of the forehead, in the Cz to Fz position. A total of three platinum subdermal electrodes were subcutaneously inserted at the vertex (+), over the mastoid process (−), and near the base of the tail (ground). Data was exported as text files for analysis in the inhouse MATLAB programs. To omit transient auditory brainstem responses at the start and conclusion of the experiment, Fast Fourier transforms (FFT) were done on the time-domain waveform from 10 to 190 ms relative to stimulus onset [13,14]. Maximum magnitude of the evoked response at one of the six frequency bins (6 Hz/bin), around 1 kHz, was considered the peak FFT amplitude. Noise floor was calculated as the average magnitude of five frequency bins above and below the central six bins. Peak response at ≥5 dB above noise floor was considered to be markedly above noise level. A 200-ms duration white noise (60 dB SPL) was regarded as contralateral noise and was provided in synchronization with SAM tone.

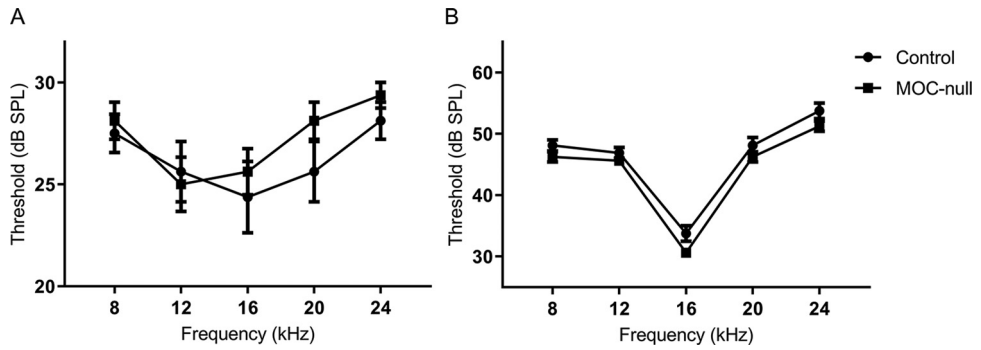

Fig. 2. Strychnine did not change ABRs and DPOAEs thresholds in mice. $N = 8$. (A) ABRs thresholds. (B) Cubic ($2f_1–f_2$) DPOAEs thresholds. Data are shown as mean ± SEM.

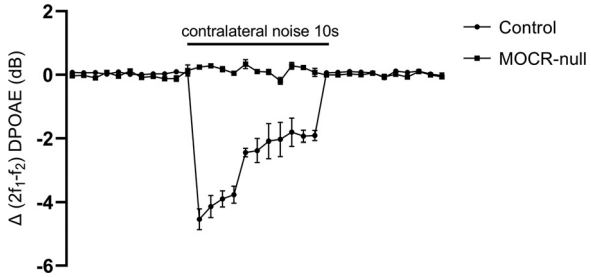

Fig. 3. Strychnine completely eliminates MOC activity in mice. $N = 8$. Data are shown as mean ± SEM.

2.1. Statistical analysis

Continuous variables were represented as mean ± SEM. The Student's t test and one-way analysis of variance were used for analysis. The analysis was performed using SPSS software (IBM, Armonk, NY), and statistical significance was set at $p < 0.05$.

3. Results

At all frequencies, based on the threshold for cubic ($2f_1–f_2$) DPOAEs and threshold for ABRs, baseline hearing functions in the MOC-null group were comparable to those in the control group ($p > 0.05$, Fig. 2). MOC activity was completely eliminated by strychnine ($p < 0.05$, Fig. 3).

ASSR amplitudes without contralateral suppression were higher than those recorded with contralateral suppression for SAM tone at 55, 60, and 65 dB SPL (Control vs Control+contralateral noise, 65 dB SPL: 0.42 ± 0.04 μV vs 0.24 ± 0.02 μV; 60 dB SPL: 0.28 ± 0.02 μV vs 0.20 ± 0.01 μV; 55 dB SPL: 0.24 ± 0.02 μV vs 0.17 ± 0.003 μV; $p < 0.05$, Fig. 4). Upon treatment with strychnine, ASSR amplitudes recovered in contralateral suppression for SAM tone at 55, 60, and 65 dB SPL and did not differ from pre-strychnine treatment values (Control vs MOC-null+contralateral noise, 65 dB SPL: 0.42 ± 0.04 μV vs 0.38 ± 0.05 μV; 60 dB SPL: 0.28 ± 0.02 μV vs 0.28 ± 0.04 μV; 55 dB SPL: 0.24 ± 0.02 μV vs 0.23 ± 0.03 μV; $p>0.05$, Fig. 4).

4. Discussion

This investigation revealed that contralateral noise reduced ASSR amplitudes at some stimulus intensities. After treating with strychnine to eliminate MOC activity, ASSR amplitudes recovered again. These findings showed that MOC activity participated in contralateral suppression of ASSR amplitudes.

The auditory efferent system improves sound detection and protects the peripheral auditory system from acoustic trauma by modifying peripheral hearing function. The MOC bundle consists of fibers that predominately project from the medial superior olive to synapse on the OHCs. These fibers release acetylcholine (ACh) into the synaptic cleft, leading to hyperpolarization of OHCs. Thereafter, hyperpolarization of OHCs decreases the motility and gain of the cochlear amplifier. Background noise reduces the effective dynamic range of auditory nerve responses to the tone bursts; that is, the noise partially masks the tone-burst response. With MOC activity, the response to the background noise is inhibited, which reduces the adaptation, partially restores the fiber dynamic range for responses to short tone bursts. Thus, in a continuously noisy background MOC activity can increase the response to transient sounds by reducing the response to the noisy background [1]. In individuals with normal-hearing, MOC activity correlates with capability of understanding speech in the context of background noise [15], which confirms to the hypothesis that the efferent system aids hearing in the presence of background noise. Individuals with impaired hearing experience significant difficulty when communicating in the presence of background noise. In some cases, issues associated with hearing-in-noise may be due to impaired MOC function [16]. However, due to methodological barriers, the function of MOC system in patients with hearing loss problems is not known.

The ASSR detection thresholds of 40 Hz, but not 80 Hz, were reported to be increased in the presence of contralateral noise [17]. Due to this observation, it was argued that the involvement of MOC activity may have influenced both 40- and 80-Hz ASSR, because they originate from subcortical structures which become suppressed when MOC activity is activated. Probably, the effect occurred only at 40-Hz ASSR because signal-to-noise ratios and response amplitudes

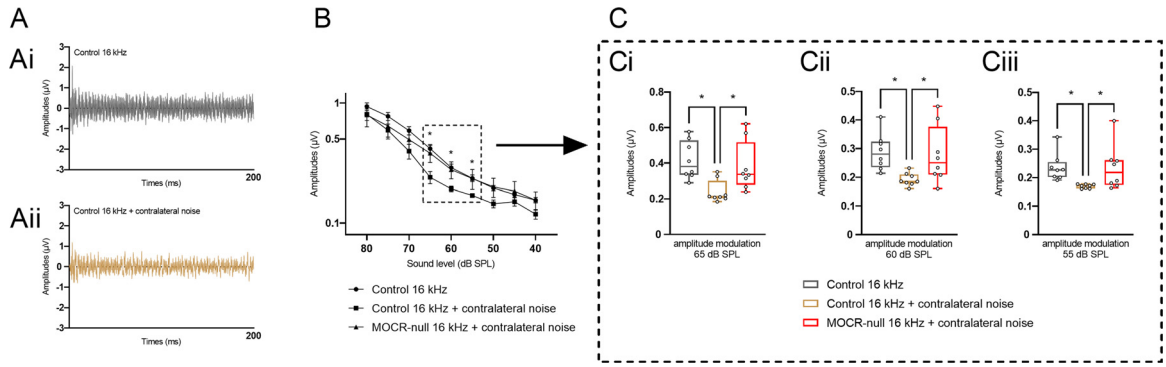

Fig. 4. The time waveforms of ASSR without (Ai) and with (Aii) contralateral noise for SAM tone at 65 dB SPL. ASSR amplitudes without contralateral suppression were larger than those recorded with contralateral suppression for SAM tone at 55, 60, and 65 dB SPL ($p < 0.05$) and were found recovered ($p > 0.05$) in contralateral suppression for SAM tone at 55, 60, and 65 dB SPL after strychnine treatment (B) at 65 (Ci), 60 (Cii), and 55 (Ciii) dB SPL.$N = 8$. (A) Data are shown as mean ± SEM. (C) Box plots have boundaries indicating 25th and 75th percentile, dashed line was median, with individual data overlaid, error bars indicated the minimum and maximum of all of the data.

were larger than at 80 Hz [2], which are important considerations when detecting small MOC-induced changes in response amplitudes [18]. A similar phenomenon was reported for ASSR masking level difference measures [19]. These observations indicate a discrepancy between ASSR threshold and psychophysical auditory threshold, even though it is believed that the ASSR threshold reflects hearing threshold. In some cases, visual detection tasks can decrease auditory nerve neural response and alter OAEs in humans [6]. Because we suspected that advanced functions of the brain, including vision and selective attention, might affect the experiment, we used anesthetized mice and chose the 16 kHz carrier frequency because it falls within the most sensitive mouse hearing regions, and it is commonly used to detect MOC activity [5,20].

MOC activity can also be mimicked by ACh perfusion [21] but its pharmacological profile differs from that of typical muscarinic or nicotinic receptors in that strychnine is among the most potent antagonist [22]. The α-D-tubocurarine is a nicotinic antagonist that has been shown to block the ACh response as well as the mechano-electrical transducer (MET) channels. It did not affect the compound action potential, DPAOEs, OHCs and MOC activity [23–25]. OHCs have been shown to harbor two subunits of ACh receptor in the nicotinic family, 9 and 10, which exhibit similar sensitivity as strychnine and Ca^{2+} flux during MOC activity [26]. Our findings indicated that strychnine completely eliminated MOC activity. The largest MOC-induced decreases in OAEs are at 45 to 75 dB SPL and not at the lowest sound levels [27]. ASSR amplitudes decreased with contralateral suppression when the intensity of SAM tone (55, 60, and 65 dB SPL) was similar to the contralateral noise intensity (60 dB SPL). When the stimulus sound intensity was greater than 70 dB SPL, the cochlear amplification was small [28], so contralateral noise has little effect on ASSR amplitudes through MOC reflection. Additionally, when the stimulus intensity was low, MOC activity induced by the contralateral noise was small [29,30]. Therefore we speculated that when the intensity of SAM tone was less than 55 dB SPL, MOC activity induced by the contralateral noise was small enough to have a significant effect on the ASSR amplitudes. If the intensity of the contralateral sound was increased or decreased, the intensity of MOC activity also changes, and the differences in ASSR amplitudes between the control and control+contralateral noise group changed accordingly.

In conclusion, MOC activity participated in contralateral suppression of ASSR amplitudes. Our findings highlight a new method that can be used to study MOC activity using mouse models.

Declaration of Competing Interest

None.

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