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# Age-related deterioration of cortical responses to slow FM sounds in the auditory belt region of adult C57BL/6 mice

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## HIGHLIGHTS

- The UF area in mice auditory cortex was activated by slow FM sound stimulation.
- The UF activities to slow FMs were deteriorated in C57BL/6 mice of 18–25 weeks old.
- The MGd–UF pathway disappeared in 22 weeks old C57BL/6 mice.
- The MGv–UF pathway was not affected by aging.
- C57BL/6 mice are a good model of age-related hearing diseases arising from the brain.

## ARTICLE INFO

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## ABSTRACT

To compare age-related deterioration of neural responses in each subfield of the auditory cortex in C57BL/6 mice, we evaluated amplitudes of tonal responses in young (5–11 weeks old) and adult (16–23 weeks old) groups using transcranial flavoprotein fluorescence imaging. Cortical responses to 20-kHz amplitude-modulated (AM) sounds, which were mainly found in the anterior auditory field (AAF) and the primary auditory cortex (AI) of the core region, were not markedly different between the two groups. In contrast, cortical responses to direction reversal of slow frequency-modulated (FM) sounds, which were mainly found in the ultrasonic field (UF), were significantly disrupted in the adult group compared with those in the young group. To investigate the mechanisms underlying such age-related deterioration, biotinylated dextran amine (BDA) was injected into UF. The number of retrograde labeled neurons in the dorsal division of the medial geniculate body (MGd) was markedly reduced in the adult group compared with that in the young group. These results strongly suggest that cortical responses to FM direction reversal in UF of adult C57BL/6 mice are mainly deteriorated by loss of non-lemniscal thalamic inputs from MGd to UF due to aging.



Age-related hearing loss (presbycusis) is caused by hair cell impairment in the cochlea [1,24]. However, some hearing problems are caused by pathological changes in the central nervous system (CNS) [4,5,18]. Elderly people without hearing loss experience marked difficulty in detecting temporal information which is thought to be processed in the CNS [22]. Information of frequency modulation (FM) is an important temporal factor required for understanding speech and detecting surroundings, and is processed in the CNS [6,12,29,31]. Age-related effects on cortical responses to FM sounds have previously been studied in rodents; auditory information received by the cortex is mediated by the lemniscal and non-lemniscal pathways [16], and the lemniscal pathways are known to be resistant to aging [15,19,20]. However, no reports are available on effects of aging in the non-lemniscal pathways. We have recently demonstrated that slow FM information around 24 kHz/s sweep is mediated in the belt region of the auditory cortex i.e., the ultrasonic field (UF) and the dorsoposterior field (DP) via the non-lemniscal pathways [9], suggesting a possibility that these pathways may be susceptible to aging. The present study aims to test this hypothesis

*Abbreviations:* AAF, anterior auditory field; AI, primary auditory cortex; AII, secondary auditory cortex; AM, amplitude-modulation or amplitude-modulated; BDA, biotinylated dextran amine; CNS, central nervous system; DP, dorsoposterior field; FM, frequency-modulation or frequency modulated; MG, medial geniculate body; MGd, dorsal division of MG; MGm, medial division of MG; MGv, ventral division of MG; SPL, sound pressure level; UF, ultrasonic field.

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in C57BL/6 mice, in which the auditory functions are susceptible to aging [10,17,21].

This study was approved by the Animal Care and Use Committee of Niigata University. C57BL/6 mice were obtained from Charles River Japan. The mice were divided into young (5–11 weeks old) and adult (16–23 weeks old) groups. Both the groups were housed in cages with ad libitum access to food pellets and water under a 12 h light/dark cycle.

Flavoprotein fluorescence imaging experiments were performed as described previously [9–11,23]. In brief, images of the auditory cortex (128 × 168 pixels) were taken at 9.7 frames/s in mice anesthetized with urethane (1.65 g/kg, i.p.) using a CCD camera system (AQUACOSMOS/Ratio with ORCA-R2 camera; Hamamatsu Photonics, Hamamatsu, Japan). Fluorescence changes ($\Delta F$) elicited by tonal stimuli were averaged over 24–40 trials and normalized by the baseline fluorescence intensity ($F_0$), which was obtained by averaging five images immediately before the onset of tonal stimuli or FM direction reversal. The response amplitude was evaluated as $\Delta F/F_0$ in a circular window of 19 × 19 pixels. The position of the window was adjusted so that the $\Delta F/F_0$ was the largest. The center of the window with the largest $\Delta F/F_0$ was defined as the response peak position.

Sound waves were synthesized using LabVIEW programs (National Instruments, Austin, TX, USA) at 500 kHz, low-pass filtered at 50 kHz, and transmitted through a speaker (SRS-3050A; Stax, Saitama, Japan) placed in front of the mice. The overall intensity of sounds used in this study was adjusted to approximately 60 dB sound pressure level (SPL) with the microphone (Type 4135 and Type 2669, Brüel & Kjær, Nærum, Denmark) and the sound level meter (Type 2610, Brüel & Kjær, Nærum, Denmark). All the types of auditory stimuli emitted from the speaker were recorded and analyzed using spectrogram calculated by MATLAB software (Mathworks, Natick, MA, USA) to verify that apparent physical distorted sounds were not emitted. To stimulate cortical responses to slow FM sweeps, direction reversal of superimposed FM sweeps was used (Fig. 1C) [9]. The overall duration of superimposed FM sweeps was 8 s. Three FM sweeps ranging between 5 and 11 kHz with linearly changing frequencies at a speed of 24 kHz/s were repeated at 83 ms intervals. The FM stimuli used in this study included three FMs in any point of time; however, their relation about frequencies was not necessarily harmonic. At 6 s after the onset of FM sweeps, the direction was reversed from downward to upward or upward to downward. The neural activities for the directional change were recorded (Fig. 1D), whose details were written in our previous paper [9]. As a control stimulus, a 20 kHz sine wave was amplitude-modulated (AM) to the depth of 100% with a 20 Hz sine wave. The 20 kHz AM sound was presented to mice for 0.5 s.

Histological experiments were performed as described previously [9,10]. In brief, biotinylated dextran amine (BDA; molecular weight 3000; Molecular Probes, Eugene, OR, USA) was injected into UF after this area was functionally identified using flavoprotein fluorescence imaging. Seven days after the injection, the mice were deeply anesthetized with an overdose of pentobarbital (1.0 g/kg, i.p.), and cardiac perfusion was performed with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Coronal brain sections (40 μm thick) were prepared using a cryotome. The sections were counterstained with 0.1% cresyl violet (Chroma Gesellschaft, Kongen, Germany). The medial geniculate body (MG) is placed 2.8–3.4 mm posterior to the bregma according to the mouse brain atlas [25,26]. Consecutive coronal sections ranging 2.8–3.4 mm posterior to the bregma were prepared every other section (at 80 μm interval) to observe distribution of BDA-positive neurons in the whole MG. Three major subdivisions of the ventral division of MG (MGv), the dorsal division of MG (MGd), and the medial division of MG (MGm), were identified according to the atlas [25,26] and by immunostaining the adjacent sections using

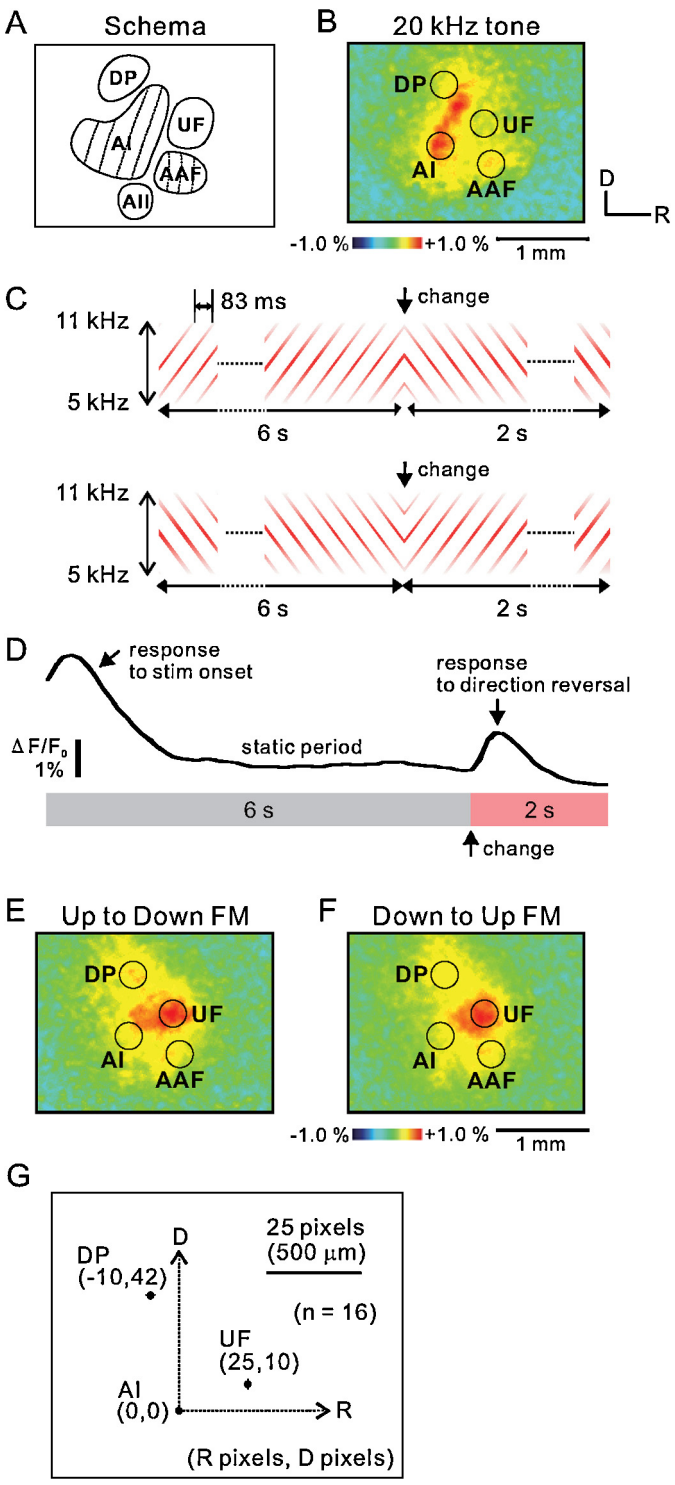


**Fig. 1.** Flavoprotein fluorescence responses to tonal stimuli in young mice. (A) Schematic illustration of subareas of the right auditory cortex. (B) Fluorescence responses to 20 kHz AM sound. (C) Schematic drawings of FM direction reverse stimulation. At 6 s after the onset, direction was changed from upward to downward FM (*top*), and from downward to upward FM (*bottom*). (D) Sample trace of fluorescence signal on the auditory cortex during 8 s stimulation. The neural response specific to the direction change was observed. (E, F) FM direction reversal from upward to downward (E), or downward to upward (F). These responses were recorded in the same of 7-week-old mouse. (G) Relative position of UF and DP with respect to the response peak to 20 kHz AM sound in AI. Mean and S.E.M. obtained in 16 young mice are shown. The numbers in parentheses representing the coordinates are rounded off to the nearest integer.