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Research Paper

Salicylate-induced frequency-map reorganization in four subfields of the mouse auditory cortex

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ABSTRACT

Salicylate is the active ingredient in aspirin, and in high-doses it is used as an experimental tool to induce transient hearing loss, tinnitus, and hyperacusis. These salicylate-induced perceptual disturbances are associated with tonotopic-map reorganization and neural activity modulation, and such neural correlates have been examined in the central auditory pathway, including the auditory cortex (AC). Although previous studies have reported that salicylate induces increases in noise-burst-evoked neural responses and reorganization of tonotopic maps in the primary AC, little is known about the effects of salicylate on other frequency-organized AC subfields such as the anterior auditory, secondary auditory, and dorsomedial fields. Therefore, to examine salicylate-induced spatiotemporal effects on AC subfields, we measured sound-evoked neural activity in mice before and after the administration of sodium salicylate (SS, 200 mg/kg), using flavoprotein auto-fluorescence imaging. SS-treatment gradually reduced responses driven by tone-bursts with lower (≤8 kHz) and higher (≥25 kHz) frequencies over 3 h, whereas evoked responses to tone-bursts within middle-range frequencies (e.g., 12 and 16 kHz) were sustained and unchanged in the four subfields. Additionally, in each of the four subfields, SS-treatment induced similar reorganization of tonotopic maps, and the response areas selectively driven by the middle-range frequencies were profoundly expanded. Our results indicate that the SS-induced tonotopic map reorganizations in each of the four AC subfields were similar, and only the extent of the activated areas responsive to tone-bursts with specific frequencies was subfield-dependent. Thus, we expect that examining cortical reorganization induced by SS may open the possibility of new treatments aimed at altering cortical reorganization into the normative functional organization.

1. Introduction

Aspirin is a well-known nonsteroidal anti-inflammatory drug that has long been used as an anti-pyretic and analgesic drug (Vane and Botting, 1998). Biotransformation of aspirin yields salicylate, which has similar anti-inflammatory potency as aspirin (Amann and Peskar, 2002). In addition, sodium salicylate (SS) is widely known to induce hearing loss and tinnitus, after acute and chronic doses to human and animals (Myers et al., 1965; McFadden et al., 1984; Brien, 1993). Therefore, SS is used to study behavioral, anatomical, physiological, and perceptual effects on the auditory system (Yang et al., 2007; Chen et al., 2013; for a review, Sheppard et al., 2014). These salicylate-induced perceptual disturbances are associated with a massive reduction in the neural output of the auditory periphery (Huang et al., 2005; Chen et al., 2010, 2013). However, this diminished neural output is accompanied by an increase in sound-evoked activity in the auditory cortex (AC) (Stolzberg et al., 2011; Jiang et al., 2017).

In particular, previous electrophysiological studies indicate that after a high dose of salicylate (300 mg/kg) to anaesthetized adult rodents, neurons in the central auditory system tuned to low characteristic frequencies (CF) in the primary AC (A1) shift upward. Conversely, neurons tuned to very high frequencies shift downward following salicylate administration resulting in an over representation of the mid frequencies (Stolzberg et al., 2011; Jiang et al., 2017), and altering tonotopy. Also, excessive SS is thought to induce tonal tinnitus with a pitch around 10–20 kHz (Brennan and Jastreboff, 1991; Kizawa et al., 2010; Lobarinas et al., 2004; Yang et al., 2007; Lowe and Walton, 2015).

However, as pointwise electrophysiological recordings provide

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Abbreviations

A1	primary auditory cortex
A2	secondary auditory field
AA	activated area
AAF	anterior auditory field
AC	auditory cortex
BF	best frequency
CF	characteristic frequency
D	duration
DM	dorsomedial field
GABA	γ-aminobutyric acid
L	peak latency
LFP	local filed potential
MPT	mitochondrial permeability transition
SPL	sound pressure level

little spatial information about neural activity in different AC areas simultaneously, how salicylate affects sound-driven activated areas (AAs) and tonotopic organization in the AC remains unknown. Additionally, recent studies have demonstrated that the mouse AC can be delineated into at least four frequency-organized subfields: i.e., A1, the anterior auditory field (AAF), the secondary auditory field (A2), and the dorsomedial field (DM) (Tsukano et al., 2015, 2016).

Therefore, in this study, we investigated the spatial and temporal properties of sound-driven responses, focusing on A1 and the surrounding three AC subfields. Using flavoprotein-autofluorescence imaging to evaluate larger areas than possible with electrodes, we evaluated the effects of SS on fluorescence changes as an indicator of elevated neural activity in four subfields of the mouse AC *in vivo*. To this end, we measured the characteristic responses of fluorescence changes, including peak intensity, latency, duration, and AAs, to sound stimuli to determine the differences between neural activity in the subfields of the AC. To examine salicylate-induced spatiotemporal effects on the AC subfields using flavoprotein auto-fluorescence imaging, we measured sound-evoked neural activity before and after administering SS (200 mg/kg). We particularly focused on the temporal neural changes and tonotopic map reorganization over three 1-h periods, following SS treatment. We hypothesized that examining cortical reorganization induced by SS could open the possibility of new treatments aimed at altering cortical reorganization to relieve tinnitus and reduce hyperactivity in the AC.

2. Materials and methods

All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and with approval of the Institutional Animal Care and Use Committee of Hokkaido University.

2.1. Surgical procedures

In this study, a total of twelve male C57BL/6J mice (Japan SLC, Japan) (5–7 weeks old) were used. Intraperitoneal (IP) doses of urethane (1.5 g/kg; Wako, Osaka, Japan) initiated anesthesia, and anesthesia levels were monitored using pedal withdrawal reflexes. Supplemental doses of 1/4 of the initial dose were administered as needed. To reduce salivary and bronchial secretions, atropine (0.04 mg/kg, Mitsubishi Tanabe Pharma, Japan) was injected before anesthesia.

Our surgical procedure has been described previously (Yanagawa et al., 2016). Briefly, to prevent sensory interference, the whiskers were trimmed and the eyes were kept closed using cyanoacrylate adhesives. The skin and muscle over the parietal and left AC were removed, and the parietal skull fragments were attached to a piece of metal using dental cement. The skull was covered with liquid paraffin (Wako, Osaka, Japan) for transparency. During all experiments, body temperature was monitored with a thermo-recorder (RT-30S; ESPEC, Japan) and maintained at 36.5 ± 0.5 °C using a heating plate.

2.2. Sound stimulation

In the main experiments, tone-burst sound stimuli (60 dB sound pressure level (SPL), 500 ms duration with 5 ms linear rise and fall ramps) at 4, 8, 12, 16, 25, 32, and 40 kHz were randomly generated using a digital-to-analog converter (NI USB-6341, National Instruments, USA) at a 500-kHz sampling rate, band-pass filtered at 2–100 kHz (Multifunctional Filter 3611, NF Electronic Instruments, Japan) and amplified with a stereo amplifier (SA1, Tucker-Davis Technologies, USA). Sound stimuli were presented via magnetic speakers (T250D, Fostex, USA; MF1, Tucker-Davis Technologies, USA). Prior to starting each experiment, the stimuli were calibrated with a sound-level meter (Type 2636, Brüel and Kjaer, Denmark) and a 1/4" microphone (Type 4939-L-002, Brüel and Kjaer).

2.3. Optical imaging

Flavoprotein optical imaging was performed according to a previously reported method (Yanagawa et al., 2016). Briefly, a complementary metal-oxide semiconductor (CMOS) camera system (MiCAM02; Brainvision, Japan) was mounted on a tandem-lens upright microscope (THT, Brainvision). Excitation light was provided with a 465-nm blue light emitting diode (LED) (LEX2-B; Brainvision) through a blue band-pass filter (466 ± 20 nm). Endogenous flavoprotein green fluorescence (500–550 nm) was detected by the CMOS camera system through a dichroic mirror and a green band-pass filter (525 ± 22.5 nm). Cortical images (188 × 160 pixels) of fluorescent signals were recorded at 20 frames/s before and after tone-burst sound stimulation. Trials were repeated 10 times at 5–10 s random intervals unless otherwise noted, and the averaged images were displayed using acquisition and analysis software (BV_Ana; Brainvision). Spatial averaging of 5 × 5 pixels and temporal averaging of five consecutive frames were applied for further analysis. Fluorescent intensities in the obtained image were normalized pixel-by-pixel with respect to the reference image (F_0), which was obtained 200 ms before acoustic stimuli.

IP doses of saline alone (50 ml, normal saline), as the control condition, or SS (Kanto Chemical, Tokyo, Japan; 200 mg/kg) was administered. Although the dose concentration applied to mice was smaller relative to that typically administered to rats, e.g., 250–350 mg/kg (Norena et al., 2010; Lu et al., 2011; Stolzberg et al., 2011), the concentration was the upper bound for the stable imaging of mice over 4 h. Without this precaution, we would be unable to obtain stable recordings due to death of the animal. Following the imaging protocol described above, SS ($n = 6$) or saline ($n = 6$) was slowly injected via IP catheter, and the imaging protocol was repeated each 10–12 min beginning 1.5 h before injection (i.e., the control or pre-dose condition) to 4 h following the salicylate or saline treatment. Additionally, in the pre-dose and post-dose conditions, optical imaging was repeated with each tone-burst stimulation. Tested frequencies were categorized into four frequency groups: lower- (4 and 8 kHz), lower middle- (12 and 16 kHz), upper middle- (25 and 32 kHz), and higher-frequency (40 kHz) ranges,