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Flavoprotein fluorescence imaging-based electrode implantation for subfield-targeted chronic recording in the mouse auditory cortex

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HIGHLIGHTS

- A subfield-targeted electrophysiology using flavoprotein imaging is described.
- The proposed method improved the success rate of recording auditory responses.
- Flavoprotein imaging revealed large individual variation in the auditory cortex.

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ABSTRACT

Background: Chronic neural recording in freely moving animals is important for understanding neural activities of cortical neurons associated with various behavioral contexts. In small animals such as mice, it has been difficult to implant recording electrodes into exact locations according to stereotactic coordinates, skull geometry, or the shape of blood vessels. The main reason for this difficulty is large individual differences in the exact location of the targeted brain area.
New methods: We propose a new electrode implantation procedure that is combined with transcranial flavoprotein fluorescence imaging. We demonstrate the effectiveness of this method in the auditory cortex (AC) of mice.
Results: Prior to electrode implantation, we executed transcranial flavoprotein fluorescence imaging in anesthetized mice and identified the exact location of AC subfields through the skull in each animal. Next, we surgically implanted a microdrive with a tungsten electrode into exactly the identified location. Finally, we recorded neural activity in freely moving conditions and evaluated the success rate of recording auditory responses.
Comparison with existing method(s): These procedures dramatically improved the success rate of recording auditory responses from 21.1% without imaging to 100.0% with imaging. We also identified large individual differences in positional relationships between sound-driven response areas and the squamosal suture or blood vessels.
Conclusions: Combining chronic electrophysiology with transcranial flavoprotein fluorescence imaging before implantation enables the realization of reliable subfield-targeted neural recording from freely moving small animals.

1. Introduction

The auditory cortex (AC) is composed of multiple subfields to realize hierarchical processing for auditory perception. These subfields have been identified in a variety of species such as mice (Stiebler and Neulist, 1997; Tsukano et al., 2015), rats (Doron et al., 2002; Polley et al., 2007), songbirds (Amin et al., 2004), cats (Merzenich et al., 1975), monkeys (Merzenich and Brugge, 1973), and humans (Leaver and Rauschecker, 2010, 2016). In the mouse AC, Stiebler et al. used electrophysiological mapping to define the

Abbreviations: A1, primary auditory cortex; A2, secondary auditory field; AAF, anterior auditory field; AC, auditory cortex; DM, dorsomedial field; DP, dorsoposterior field; IAF, insular auditory field; PSTH, peri-stimulus time histogram; STRF, spectrotemporal receptive field; SPL, sound pressure level; UF, ultrasonic field.

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auditory subfields; A1 (primary auditory field), AAF (anterior auditory field), A2 (secondary auditory field), DP (dorsoposterior field), and UF (ultrasonic field) (Stiebler and Neulist, 1997). Some other studies used optical imaging to re-define DM (dorsomedial field) which largely corresponds to UF (Tsukano et al., 2015), and to find IAF (insular auditory field) which is an additional auditory subfield in mice (Sawatari et al., 2011). In addition, several studies demonstrated that each auditory subfield has specific functional roles in hierarchical sound processing (Romanski et al., 1999; Linden et al., 2003; Guo et al., 2012).

Most electrophysiological studies of the AC have been conducted under anesthetized acute conditions to analyze response properties of auditory neurons in each subfield. It is relatively easier to control the stimulus settings and recording quality under acute anesthetized conditions compared with chronic conditions of freely moving animals. However, a series of studies have reported that a variety of response properties change in a manner that depends on the type of anesthetic agent used (Osanai and Tateno, 2016; Yanagawa et al., 2016) and on the state (awake or anesthetized) of the animal (Gaese and Ostwald, 2001; Guo et al., 2012; Noda and Takahashi, 2015). Moreover, recent studies indicate that behavioral states of the animal influence neural activities in the AC (Rao et al., 2014; Schneider et al., 2014). Therefore, to examine functional roles of the AC in sound processing, stable long-term recordings from awake animals with identified AC subfields are profoundly important. However, no techniques for chronic recording that precisely implant electrodes into targeted AC subfields have been reported.

In most chronic recording experiments, implanted electrode positions are determined according to stereotactic coordinates, skull geometry, or the shape of blood vessels (Guo et al., 2012; Rao et al., 2014). However, the geometrical relationships between the activated areas and these structural landmarks are thought to have large individual differences (Willott et al., 1993; Stiebler and Neulist, 1997). Therefore, the success rate of implantation into the targeted areas has usually been lower than expected. Because electrode tips of most microdrives are not able to be relocated along the brain surface after surgery, it is important to identify the exact location of the activated area through the skull before surgery.

Optical imaging techniques have been a useful tool for fine-grained mapping in the AC (Kalatsky et al., 2005). In particular, flavoprotein fluorescence imaging (Shibuki et al., 2003), which uses intrinsic signals coupled with aerobic metabolism, is useful for delineating small AC subfields with a scale of around several hundred micrometers because it avoids the non-homogeneous staining associated with exogenous fluorescent dyes. More importantly, intensity changes of flavoprotein fluorescence can be detected above the thin skull of mice (Tsukano et al., 2015).

In this study, we developed a new method for targeting electrode implantation by taking advantage of the transcranial flavoprotein fluorescence imaging technique. We demonstrate that the procedure dramatically improves the success rate of recording auditory responses in chronic neural recording from freely moving mice. We also found large individual differences in positional relationships between responsive AC areas and the squamosal suture or blood vessels, which is thought to be a main reason for the low success rate by conventional procedures without imaging.

2. Materials and methods

2.1. Animals

Two male and thirty female C57BL/6J mice (Japan SLC, Hamamatsu, Japan; 7–25 weeks old) and 16 male Wistar/ST rats (Japan SLC, Hamamatsu, Japan; 6–15 weeks old) were used in this study. All experiments were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and with approval of the Institutional Animal Care and Use Committee of Hokkaido University.

2.2. Surgical procedures before optical imaging

Nine mice were used for optical imaging before electrode implantation to identify the AC in each animal. The mice were anesthetized with a mixture of midazolam (10 mg/kg, i.p.; Astells Pharma, Japan) and xylazine (10 mg/kg, i.p.; Bayer AG, Germany) in saline as the initial dose (Inaoka et al., 2011; Tateno et al., 2013; Noto et al., 2016). The adequacy of anesthesia was confirmed by the absence of toe-pinch reflexes. Supplemental doses were administered every 1 h with half the initial dose (midazolam, 5 mg/kg, i.m. and xylazine, 5 mg/kg i.m.) to maintain anesthesia during the surgical and optical imaging procedures. The skin and muscle over the parietal and the left AC were carefully removed, and the parietal portions of the skull were attached to a custom-made metal post using dental cement to avoid vibration during optical imaging. A local anesthetic (xylocaine gel; AstraZeneca K.K., Japan) was applied to all incision sites. 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 $35 \pm 1\,^{\circ}C$ using a pocket heater or a heating pad.

2.3. Sound stimuli

For flavoprotein fluorescence imaging in mice, 5, 10, and 20 kHz tone-burst sounds (70 dB SPL, 100 ms duration with 5 ms linear rise and fall ramps) were presented pseudo-randomly 15 times at 5–10 s intervals to identify activated areas by optical imaging. In rat experiments, we used 1 kHz, 4 kHz, and 16 kHz tone-bursts as sound stimuli because the hearing frequency range of rats shifted downward compared to that of mice. These sound signals were generated digitally at a rate of 100 kHz, and processed by digital-to-analog conversion, attenuation, and amplification using TDT-system-3 hardware (RP2.1, PA5, and SA1; Tucker-Davis Technologies, USA) and software (RPvdsEX, Tucker-Davis Technologies). The sound parameters of each output signal in RPvdsEX were controlled through Active X with a custom-made Matlab program (MathWorks, MA, USA).

For chronic neural recording in mice and acute neural recording in rats, tone-bursts of 30 ms duration and 5 ms linear rise and fall ramps were presented pseudo-randomly 30 times at 200–250 ms intervals. Stimulus frequencies ranged from 0.625 to 20 kHz with 1/16 octave increments at 60 dB sound pressure level (SPL). A total of 2430 sound stimuli were presented in an electrophysiological recording session. Sound signals were generated with a sound card (USB Sound Blaster Digital Music Premium HD; SB1240; Creative Technology, Singapore) at a sampling rate of 96 kHz, and amplified with a stereo amplifier (SA1; Tucker-Davis Technologies, Alachua, FL, USA).

The sound stimuli were delivered in an open-field through a speaker (MF1, Tucker-Davis Technologies) located 20 cm in front of the animal in a double-walled sound-proof room (Comany, Japan). The sound delivery system was calibrated using a sound level meter (Type 2636, Brüel and Kjaer, Denmark) with a 1/4-in. microphone (Type 4939-L-002, Brüel and Kjaer) close to an animal's right ear.

2.4. Transcranial flavoprotein fluorescence imaging

Transcranial flavoprotein fluorescence imaging was performed on nine mice to identify the position of the AC according to previously reported imaging procedures (Shibuki et al., 2003; Tsukano et al., 2015; Osanai and Tateno, 2016; Yanagawa et al., 2016;