Greenwood frequency–position relationship in the primary auditory cortex in guinea pigs

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ABSTRACT

Although orderly representation of sound frequency over space is a hallmark feature of the primary auditory cortex (A1), the quantitative relationship between sound frequency and cortical position is unclear. We examined this relationship in the guinea pig A1 by presenting a series of stimulus tones with a wide frequency range, and recording the evoked cortical responses using an optical imaging technique with high spatial resolution. We identified the cortical positions of three best-frequency indices for each tone: the onset response position, the peak amplitude position, and the maximum rise rate position of the response. We found a nonlinear log frequency–position relationship for each of the three indices, and the frequency–position relationship was always well described by a Greenwood equation, with correlation coefficients greater than 0.98. The cortical magnification factor, measured in octave/mm, was found to be a function of frequency, i.e. not a constant. Our results are novel in that they demonstrate a quantitative relationship between sound frequency and cortical position in the guinea pig A1, as described by the Greenwood equation.

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Introduction

The orderly representation of sound frequency (tonotopy) has been established in many mammalian species as a hallmark feature of the primary auditory cortex (A1; Aitkin, 1990; Ehret, 1997). The cochlea transforms sound frequencies into a place code, such that low frequency tones evoke maximal oscillations of the basilar membrane near the apical region of the cochlea, and tones with higher frequencies evoke oscillations at successively more basal positions (Hudspeth, 2008; von Békésy, 1970). Basilar membrane oscillations are then transformed into neuronal activity by the inner hair cells, and this neuronal signal is transmitted through the auditory fibers to the brain. Thus, moving from the base to the apex of the cochlea, the frequencies to which emerging auditory fibers are most sensitive (i.e. characteristic frequencies, CFs) gradually decrease (Liberman, 1982). Psychological, electrophysiological, and anatomical studies have established that the frequency–position relationship in the cochlea can be described in a quantitative manner using the equation $f = a(10^{bx} - c)$, where f is frequency, x is the normalized distance of the basilar membrane from the apex, and a, b, and c are constants (Greenwood, 1961, 1990; Liberman, 1982). This equation is referred to as the Greenwood equation, and has been fundamentally important in cochlear modeling (Fleischer et al., 2010; Skrodzka, 2005; Yoon et al., 2007) and in the design of cochlear implant electrodes (Baumann and Nobbe, 2006; Dorman et al., 2007; Stakhovskaya et al., 2007).

Despite the number of animal (Barkat et al., 2011; Imaizumi and Schreiner, 2007; Kilgard and Merzenich, 1998; Merzenich and Brugge, 1973, 1975, 1976; Song et al., 2006; Wallace et al., 2000) and human (Striem-Amit et al., 2011) tonotopy studies in A1, to the best of our knowledge, the frequency–position relationship has not been described in the form of an equation. In electrophysiological mapping studies of A1, sites with the same CF are often found along an isofrequency line (Merzenich and Brugge, 1973, 1975, 1976). An iso-frequency line can also be defined in imaging studies as a contour line of the response evoked by a pure tone (Kalatsky et al., 2005; Song et al., 2006). A frequency–position relationship has been proposed in A1 by measuring the cortical distance in a direction perpendicular to isofrequency lines (Kalatsky et al., 2005; Merzenich and Brugge, 1973, 1975). However, isofrequency lines are seldom straight (Aitkin, 1990; Ehret, 1997). A recent study that tested the cortical linear magnification factor (CMF) index (using octaves/mm and its inverse) reported constant CMFs in rats (Kalatsky et al., 2005), implying a linear relationship between cortical distance and sound frequency on a log scale. Other imaging studies that assessed either the position of maximum response to a pure tone (optical best frequency; Storace et al., 2011) or the centroid of the response area (Mrsic-Flogel et al., 2006) reported similar results in the A1 of both rats and ferrets. Mrsic-Flogel et al. (2006), however, observed that the CMF value depended on the frequency band examined, indicating a nonlinear relationship between cortical distance and log frequency. This notion has been supported by several extensive electrophysiological studies in a number of species (Merzenich and

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Brugge, 1973, 1975, 1976; Robertson and Irvine, 1989). Additionally, Imaizumi and Schreiner (2007) presented direct evidence of a nonlinear relationship between cortical distance and log frequency in the cat A1. They found that CMF had a linear relationship with log frequency in two cats, although this correlation was weak (correlation coefficient < 0.5). Considering the discrepancies among previous studies, the frequency–position relationship in A1 requires further investigation.

Here, we examined the frequency–position relationship in the guinea pig A1. We used an optical imaging technique with a high spatial resolution to record responses in the A1 to tones that roughly spanned the audible frequency range of guinea pigs. We identified a unique position for each frequency using the response onset, response peak, or maximum rise rate of the response. Our results show that the frequency–position relationship in the guinea pig A1 is well represented by the Greenwood equation. Thus, the CMF appears not to be a constant, but instead, a function of frequency.

Materials and methods

Animal surgery and imaging

All experiments were performed according to the Kumamoto University Guidelines for the Experimental Use of Animals, and the protocol was approved by the Kumamoto University Committee for Animal Experiments. The experimental procedures were based on those previously described by our group (Nishimura and Song, 2012; Nishimura et al., 2007; Song et al., 2006). Briefly, adult albino guinea pigs weighing 400–600 g were anesthetized with a mixture of ketamine (16 mg/kg) and xylazine (50 mg/kg) for surgery and recording. Expiratory CO_2 levels and heart rate were monitored throughout the experiment. Rectal temperature was maintained at 38.0 ± 0.3 °C. We removed 8 mm of the skull over the left auditory cortex in both the rostrocaudal and dorsoventral directions to reveal A1 and surrounding regions, such as

the dorsocaudal field (Nishimura et al., 2007; Fig. 1A). Following resection of the dura mater, the cortex was stained twice with the voltage-sensitive dye RH-795 (0.5 mg/ml in saline; Invitrogen, Grand Island, NY, USA) for 60 min each time (Grinvald et al., 1994; Song et al., 2006).

Optical signals were sampled at 500 Hz using a CMOS imaging system (MiCAM Ultima, Brainvision, Tokyo, Japan), with a resolution of 100×100 pixels. The dimensions of the recording field were 6.25×6.25 mm^2; one edge of the recording field was made parallel to the midline (Fig. 1A), and the rostrocaudal position of the recording field was determined by referencing the skull opening and the orientation of the blood vessels running along the pseudosylvian sulcus. These efforts were made to ensure that the position and orientation of the recording field were similar across animals.

Heartbeat and respiration have been shown to significantly interfere with optical in vivo recording (Inagaki et al., 2003; Maeda et al., 2001). Thus, we removed ventilation interference by high-pass filtering the signal at 2.5 Hz. To suppress heartbeat noise, we synchronized the recordings with the first R wave of the electrocardiogram after the end-inspiratory period of ventilation. Evoked responses were obtained by subtracting recordings without stimulus presentation from those with stimulus presentation (Nishimura et al., 2007; Song et al., 2006). The inter-recording interval was greater than 7 s.

Acoustic stimuli

All acoustic stimuli were digitally generated with a sampling rate of 195 kHz using custom-made software, and loaded into a TDT real-time processor (RX6, Tucker-Davis Technologies, Alachua, FL, USA). Tone pulses (30-ms plateau, 10-ms on/off cosine ramp) were presented at 60 dB SPL to both ears simultaneously via two earphones (ATH-C602, Audio-Technica, Tokyo, Japan), which were driven separately by a two-channel headphone buffer (HB7, Tucker-Davis Technologies, Alachua, FL, USA). An ear speculum was attached to each of the
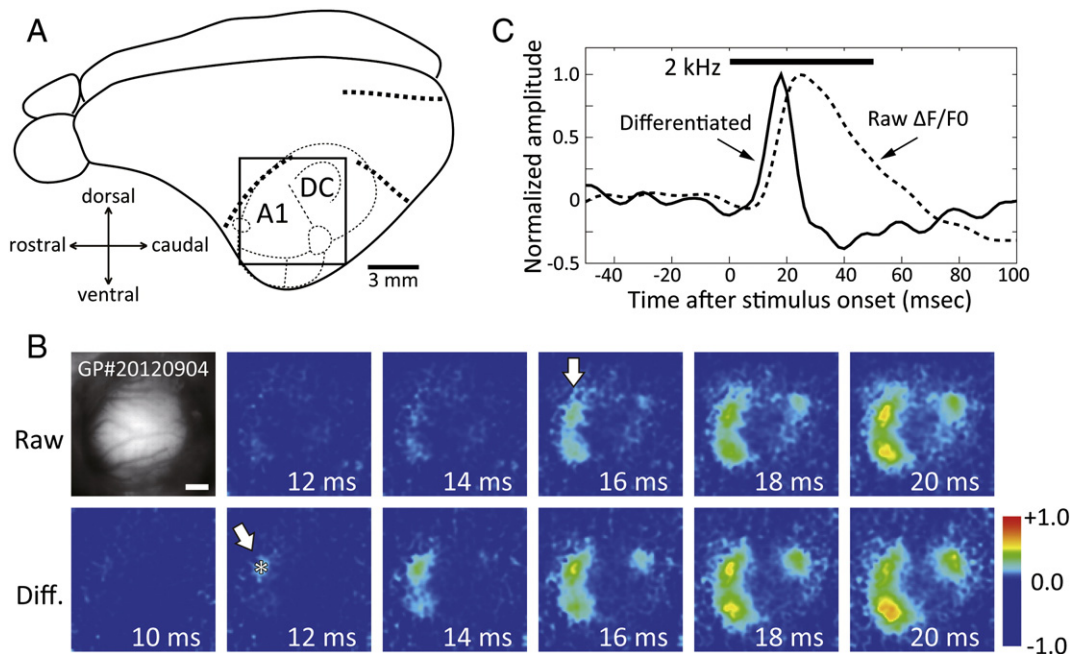
Fig. 1. Best-frequency positions in A1 as determined by differentiated fractional fluorescence responses to pure tones. (A) A schematic illustration of the location of the auditory cortex in guinea pigs. The square marks the approximate position for imaging. A1: primary auditory cortex. DC: dorsocaudal field. The thick dotted lines represent the cortical sulci. The depicted orientation applies to all imaging figures and distance–distance plots. The upper panel in (B) shows the color-encoded fractional fluorescence response ($\Delta F/F_0$) to a tone of 2 kHz, with the leftmost graph showing a basal fluorescence image of the cortex. Each of the remaining five panels is a frame taken at a specific time after stimulus onset (specified at the bottom of the image). The arrow points to the response in A1. The dorsocaudal field response, which occurred after the initial response in A1, appears in the upper right corner. The lower panel in (B) shows the corresponding differential signal $\Delta(\Delta F/F_0)$. The response-onset position, marked with an asterisk, was determined using the differential signal, and is emphasized with an arrow. The scale bar in (B) is 1 mm. (C) An example of $\Delta F/F_0$ signal at 1 pixel, and the differential signal. The peaks of all signals were normalized to one. The black bar shows the timing of the tone stimulation.