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Population activity in auditory cortex of the awake rat revealed by recording with dense microelectrode array

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Abstract— **Cortical mechanisms of auditory perception include temporal interaction between neuronal ensembles in a functional cortical structure such as a place code of frequency, or tonotopic map. To investigate the mechanism, a recording method is needed to densely map spatio-temporal activity pattern within the predefined tonotopic organization specifically in the awake condition. The present study has proposed and developed an experimental system that is capable of simultaneous neural recording with a grid array of 100 sites with 400-μm inter-electrode distance in the 4th layer of auditory cortex of awake rat. Both multiunit activities (MUA) and local field potentials (LFPs) confirmed the tonotopic map in the auditory cortex. In addition, spectral powers in higher frequency components (4-120 Hz) were enhanced and a lower frequency component (1-4 Hz) was reduced during waking. Phase synchronization between recording sites in the gamma-band oscillatory activity was generally smaller in the awake cortex than in the anesthetized cortex. These results have proven the feasibility of our recording and will open a new avenue to investigate neural activities in the functional map of awake cortex.**

I. Introduction

The perceptual organization is owing not only to extracting stimulus features but also to binding distinct features. This underlying mechanism may include cooperative activities of neuronal ensembles, or cell assemblies [1], within the place code in the cortex, e.g., the tonotopic map in the auditory cortex (AC). The developments of microelectrode array in a number of previous studies have contributed to gaining further insights in this field. In rodents, despite the importance as animal models, cortical mapping with microelectrode array has been established only in the anesthetized preparation, but not fully in the awake conditions. Specifically, AC is difficult to access because a large part of temporal muscle and cranium has to be surgically removed. Furthermore, the number of recording sites has to increase up to 100 in order to visualize the tonotopic map.

In the present study, we report an experimental system that is able to densely map spatio-temporal activities in AC of awake rats. We first describe the design of recording chamber that enables a quick access to AC for microelectrode recording, and recording procedure to maintain awake rats in a head-fixed state. Second, we demonstrate simultaneous extracellular recording at 10 × 10 sites within 4 × 4 mm area in AC, which is able to visualize the tonotopic map. Furthermore, we investigate the difference between anesthetized and awake states in terms of band-specific synchronizations across channels during tone presentation and silence.

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II. Materials and Methods

A. Animal preparation & Surgery

All procedures were approved by our institutional committee and performed in accordance with "Guiding Principles for the Care and Use of Animals in the Field of Physiological Science" by the Japanese Physiological Society. A Schematic for the recording system of AC of rat is shown in Figure 1 (a). Chamber was designed for head fixation of the animal in order to realize cortical recording under awake resting state. The chamber composed of polyacetal with U-form shape (Fig. 1 (b)) covers around temporal cortex, where almost whole area of rat AC was included. This chamber also protected these areas from scratching behaviors of rats. After handling of the animal for a few days, 4 adult male Wistar rats (270-340 g) were anesthetized with isoflurane (2.5–3.5%), and the chamber was surgically attached to the parietal bone with dental cement. The reference electrode was implanted above the dura of parietal lobe. After a few days of recovery, the rats were trained to get used to head-fixed state. During recording, sugar water was periodically provided to rats to maintain their resting state.

B. Recording

All electrophysiological experiments were performed in a sound attenuating chamber. A Microelectrode array used in this study constituted a grid of 10 × 10 silicon probes with

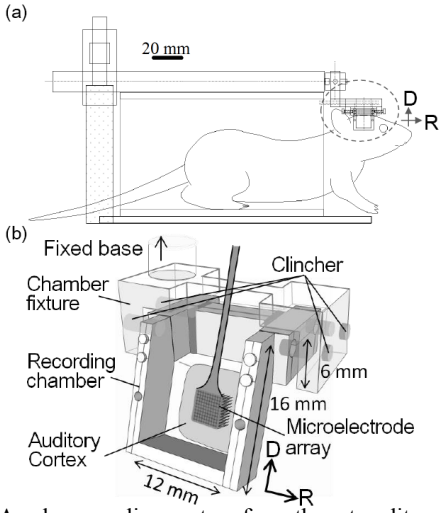

Figure 1. Awake recording system from the rat auditory cortex. (a) Overview of the head-fixed condition during awake recording. (b) Schematic of the recording chamber, corresponding to the part bounded by dashed line in Fig 1 (a). D, dorsal; R, rostral.

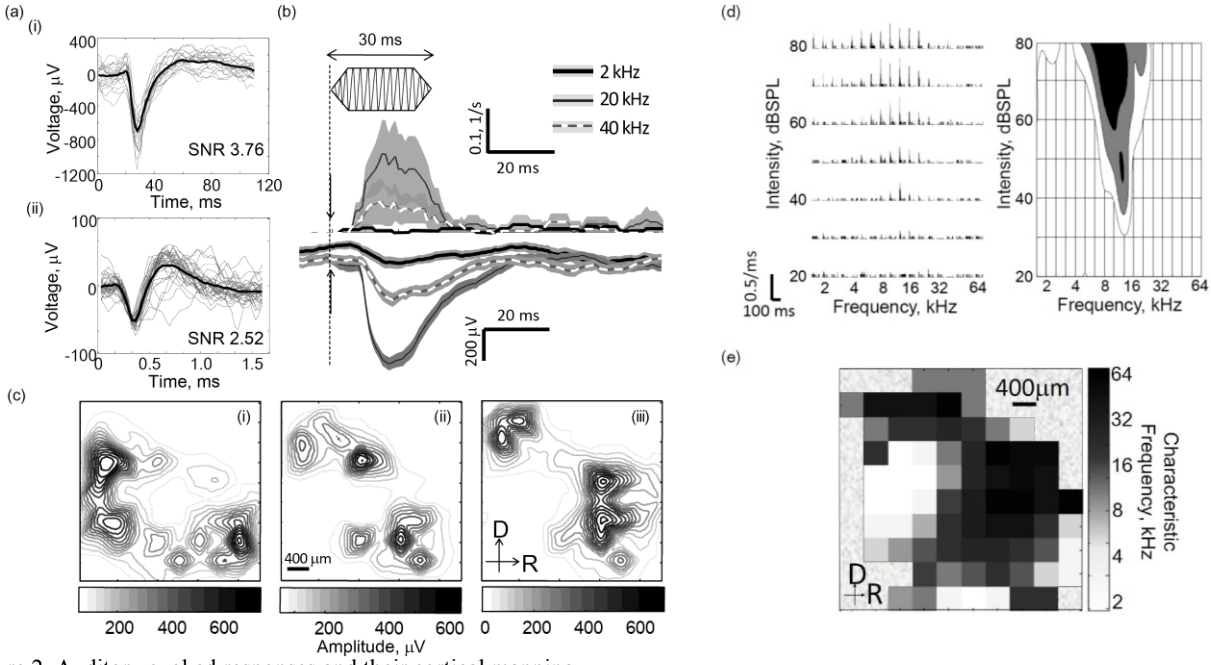

Figure 2. Auditory evoked responses and their cortical mapping.
(a) Representative traces of neural signals. (i) Tone-evoked LFPs and (ii) spike waveforms. Black and gray line indicates averaged waveform and waveform in a given trial respectively. (b) Representative LFP waveforms (lower inset) and PSTHs of MUA (upper inset) in response to 5-, 20-, and 40-kHz tone bursts The average (bold line) and s.e.m (shade) are shown. (c) Representative spatial distributions of LFP maxima to tone bursts of 2 kHz (i), 20 kHz (ii), and 40 kHz (iii). (d) Frequency response area based on the response to the 18 test frequencies at 7 sound intensities. Left inset, PSTHs; Right inset, Bicubic interpolated tuning curve at the same recording site. (e) Representative example of tonotopic map in the auditory cortex of awake rat. D, dorsal; R, rostral.

inter-electrode distances of 400 μm in 4 × 4 mm substrates (Blackrock Microsystems Inc., ICS-96 Array). This array can cover the rat AC of approximately 3 × 4 mm [2,3]. Diuretics were administered intravenously to prevent cerebral edema under isoflurane anesthesia (1.4-2.2%). Temporal skull was first removed and temporal cortex was exposed just before recording to prevent brain inflammation. The electrode arrays were inserted into the depth of the cortical layer IV with a custom-made inserter. After turning off anesthesia, some mild voluntary movements of four limbs confirmed that the rats were awake. We first characterize frequency response area (FRA) on the basis of MUA at each recording site. The test stimuli were tone bursts with 30-ms duration and 5-ms linear rise / fall ramps. The test frequencies ranged between 1.6–64 kHz with 1/3-octave increments and intensities between 20–80 dB SPL with 10-dB increments. Each tone was presented 20 times in a pseudorandom order with inter-tone interval of 600 ms through free-field speaker. Alternating tone sequence with 8-s duration was also presented 20 times. The sequence consisted of 20-kHz and 40-kHz tones with an inter-tone interval of 100 ms. After presentation of those sound stimuli, anesthesia was administered again. In the anesthetized state, hind-paw reflex was absent and typical up-and-down neuronal states were observed. The test stimuli used in the awake condition was presented again under anesthesia.

C. Analysis

FRA was derived on the basis of MUA from 5- to 55-ms post-stimulus latency in response to test tones. Characteristic frequency (CF) at each recording site was determined at which test tones evoked a response at the lowest intensity. Signal-to-noise ratio (SNR) of MUA, which is based on the deviation from the average signal [4], was evaluated to pure tones in order to verify the recorded signals. Next, oscillatory power and synchrony was evaluated in each frequency band and compared them between during waking and during anesthesia. Power spectrum density was calculated during both the presentation of alternating tone sequence (8 s) and silence (3 s) and its median across trials was obtained. Finally, gamma-band synchrony was investigated because previous studies showed the enhancement of gamma-band activity during waking. Instantaneous phase was extracted from band-pass filtered LFPs through Hilbert transform. Phase synchrony was quantified by Phase Locking Value (PLV) across trials [5], and was applied to both tone-evoked and spontaneous LFPs.

III. RESULTS

Figure 2 (a) shows tone-evoked LFP waveform and spike waveform, respectively. Signal-to-noise ratio was 2.38±0.15 in LFP and 1.44±0.07 in MUA across trials, demonstrating that both LFPs and spike activities show significant large signals to noise. Figure 2 (b) shows tone-evoked LFPs (lower inset) and PSTHs of MUA (upper inset) at a representative recording site. Both the signals depended on the test frequency, and the test site corresponded to a local activation focus to the 20-kHz tone. The spatial distribution of LFP peak amplitudes also depended on a test frequency as shown in Figure 2 (c). This spatial localization in cortical activation to tones could confirm the recording area as AC. Figure 2 (d) shows a representative example of FRA, which has CF of 13 kHz-tone frequency. A total of 278 tone responsive sites were recorded from the arrays in 4 animals. Figure 2 (e) shows a representative cortical map of the CF, i.e., tonotopic map. Consistent with previous reports, a high CF was obtained at

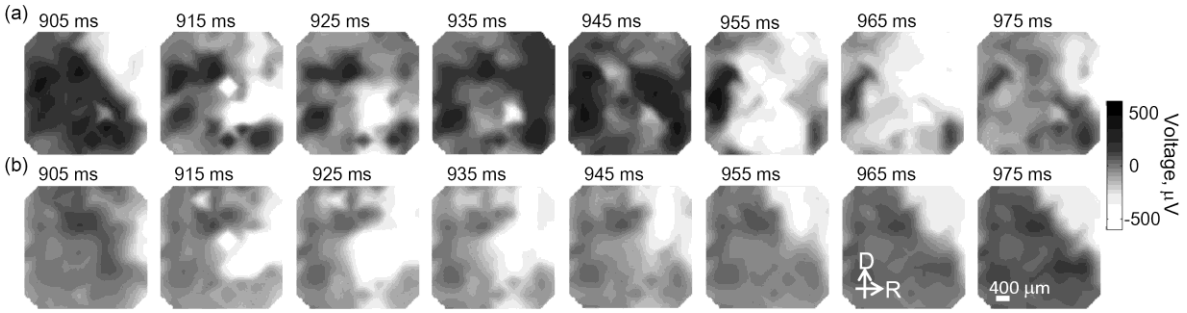

Figure 3. Temporal evolution of cortical mapping of tone-evoked LFPs.
(a) Spatio-temporal patterns of tone-evoked LFPs at cortical deeper layer during awake state when 40-kHz tone burst was presented at 900 ms. (b) Spatio-temporal tone-evoked LFPs during anesthetized state. D, dorsal; R, rostral.

the center of AC, while a low CF at the fringe [3]. Each adjacent recording site generally has different frequency selectivity one another, and secures frequency resolution of 1/3-octave, which is comparative to previous studies [6,7]. Thus, simultaneous recording in our experimental system showed sufficient spatial resolution to visualize the tonotopic map with a typical frequency resolution.

Figures 3 show a representative example of spatio-temporal patterns of LFPs to alternating tone sequence during waking and during anesthesia, respectively. Pure tone bursts with 40-kHz frequency and 30-ms duration was presented at 900 ms. In both conditions, the onset response began as a negative deflection at about 915 ms around a center and anterior-sided location of the recording area. The onset response tended to terminate earlier during waking (at 935 ms in this example) than that during anesthesia (at 945 ms). In the awake state, another cortical activity was generated at 955 ms around the area including both 40-kHz-tone-evoked area and other auditory cortical areas. On the other hand, in the anesthetized state, cortical activity was quiet after the end of tone-evoked onset response (Fig. 3 (b)). Thus, the awake cortical activity tended to fluctuate spatio-temporally as compared to the anesthetized activity. Similar fluctuation was reported in a spontaneous neuronal activity [8].

In order to investigate properties of these fluctuating signals in the awake state, we investigated band-specific oscillatory activities of LFPs. Figure 4 (a) (i) demonstrates a representative example of power spectrum density of LFP during presentation of the alternating tone sequence. Steep harmonic components observed in both the awake (AW) and the anesthetized (AN) conditions corresponded to a rhythm of repetitive tones, by which responses were locked. Aside from these, AW condition had a smaller value at lower frequency component (< 6 Hz) than that in AN condition. On the other hand, AW condition tended to have larger values at higher frequency components (> 10 Hz), suggesting that these awake fluctuating activities are mainly composed of higher frequency components. Figure 4 (a) (ii) shows normalized power spectrums across all recording sites with CF, across all trials and subjects, with box plots showing medians of normalized power at a given frequency band. As is the case with Fig. 4 (a) (i), while normalized power spectrum in AN condition has significantly larger values (Two-tailed *t*-test, $p <$ 1E*-100) in delta band (1-4 Hz) than in AW condition, awake normalized power spectrum had significantly larger values (Two-tailed *t*-test, $p <$ 1E*-100) in all higher frequency bands above theta band (4-8 Hz). These tendencies were consistent with the previous studies reporting the enhancement of higher frequency oscillatory power [9,10].

Figure 4 (b) shows an example of time trace of phase synchrony between two recording sites during presentation of alternating tone sequence. Phase synchrony changed in time in both conditions, but was smaller in AW condition across time than AN condition. Figure 4 (c) represents the median of PLV histogram obtained from all pairs of recording sites with CF. Phase synchrony in neuronal activities both during tone presentation and during spontaneous activities was significantly small in awake state (Mann-Whitney's *U*-test, One-sided *p*-value, $p <$ 1.4E-2 during tone presentation; $p <$ 2.9E-2 during silence). Thus, oscillatory activities between frequency selective columns in auditory cortex tend to be desynchronized in awake state.

IV. Discussion

A. Summary

The present study proposed and developed the method of acute awake recording from auditory cortex of rat by using microelectrode array with 100 channels and 400-μm inter-electrode distance. The microelectrode array can cover several auditory cortical fields. Tone-evoked LFPs and MUAs were detected and tonotopic map was successfully estimated with MUA. Awake-specific neuronal activities were observed in auditory cortex with this recording method. First, both tone-evoked responses and spontaneous activities fluctuated spatio-temporally compared to the anesthetized state. Second, higher frequency oscillatory power was enhanced and lower frequency component was reduced. Third, oscillation in gamma band between recording sites became more desynchronized than that in the anesthetized state.

B. Validity of the recording method

Unlike the chronic recording with implantation of microelectrode array into cerebral cortex, recording in the present study is acute, and additional recording session with the same animal is impractical due to significant brain hemorrhage after removal of the electrode. Therefore, the cortex is always exposed just before recording. This means that cortical tissue is neither damaged by the electrode nor at higher risk of infection until beginning of recording. Thus, this acute setup may make the recoding possible at more recording sites than chronic recording methods, where the number of detectable neuronal activities often differs by the experimental day. Furthermore, the electrode array can be empirically usable for ten or more times of this acute recording. Unless intended neuronal activities are their

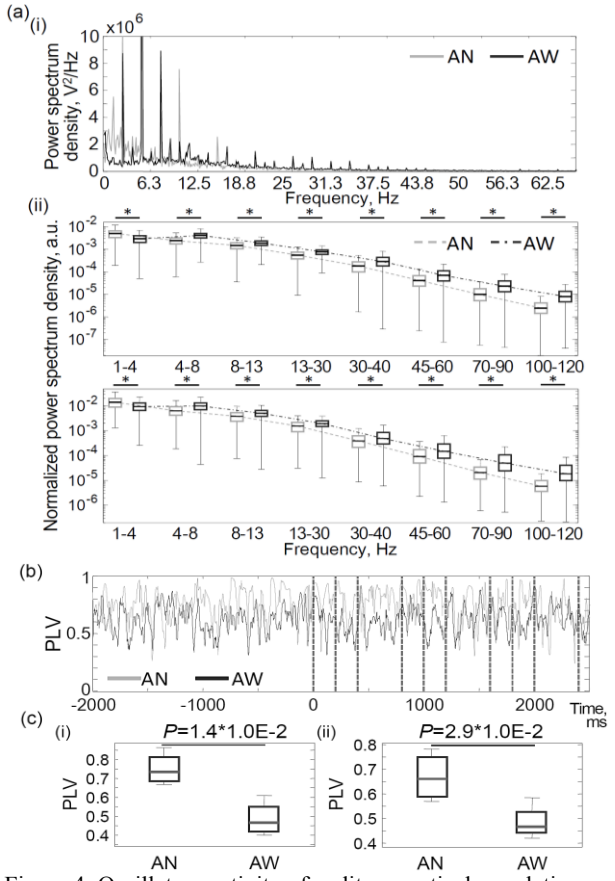

Figure 4. Oscillatory activity of auditory cortical population. (a) (i) Representative example of power spectrum density of LFP during awake (black) and during anesthesia (gray). (ii) Normalized power spectrum across channels and subjects. Upper inset, during alternating tone presentation; Lower inset, during silent. (b) Temporal change of PLV between a site with CF of 20 kHz (presented tone frequency) and a site with CF close to 20 kHz. Vertical broken lines indicate time of tone onsets. (c) Median of PLV histogram (i) during tone presentation, (ii) during silent.

day-by-day variations or plasticity, this acute setup should be more feasible for robust recording than the chronic recording methods.

C. Neuronal oscillation across recording sites

Lower frequency component in the present study (1-4 Hz) decreased during waking. Slow waves are frequently observed during anesthesia and NREM sleep. The origin of slow waves is referred to as the periodic neuronal fluctuation between a hyperpolarized downstate and a depolarized upstate [8]. In general, the variation between downstate and upstate disappears during waking, which was also the case with this study. This results in decrement of lower frequency component. On the other hand, higher frequency components (4-120 Hz) increased in this study. Awake cortical state generally reduces hyperpolarized state and disinhibits NMDA-mediated excitatory response, leading to increase of spontaneous firing rate, or shortening of inter-spike interval. Increase of firing rate and depolarization of membrane potential are highly correlated with the power of gamma-band oscillation [11,12]. These could result in the enhancement of higher frequency oscillatory power.

Desynchronization in higher frequency (including gamma band) oscillation or decorrelation during waking is reported in LFP [13] and spike activity [14]. However, auditory cortical desynchronization during waking has been poorly understood. Gamma-band activity is profoundly related to GABAergic inhibitory circuit [15], and this gamma desynchronization could be caused as follows: First, dominant inhibitory input to cortical neurons during anesthesia is replaced by enhanced excitatory and weakened inhibitory input in the awake state. Second, such relative decrease of inhibition possibly lead to reduction of gamma-band synchronized activity. Cortical desynchronization is also considered to be related to modulatory input to cortex like enhancement of tonic firing in thalamo-cortical relay neuron [16]. Furthermore, recurrent intra-cortical and cortico- cortical inputs could become stronger and elicit spatially localized oscillatory activity in each frequency selective column, also possibly contributing to desynchronization. Thus, increased higher frequency oscillatory components in the awake state, which can originate from thalamo-cortical and horizontal modulatory input, lead to auditory cortical desynchronization.

In conclusion, the proposed method in this study can investigate awake auditory cortices with spatial resolution of several hundreds of μm, opening a new avenue for assessing global functional network on cortical functional structure.

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