Spontaneous activity resembling tone-evoked activity in the primary auditory cortex of guinea pigs

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ABSTRACT

In the primary auditory cortex (AI), a pure tone evokes propagating activity along a strip of the cortex. We have previously shown that focal activation of AI triggers autonomously propagating activity that resembles tone-evoked activity (Song et al., 2006). Because a focal spontaneous activity is expected to trigger similar activity propagation, spontaneous activity resembling tone-evoked activity may exist in AI. Here we tested this possibility by optical imaging of AI in guinea pigs. After obtaining tone-evoked activities, we made long-duration optical recordings (9–40 s) and isolated spontaneous activities from respiration and heartbeat noises using independent component analyses. Spontaneous activities were found all over AI, in all animals examined. Of all spontaneous events, 33.6% showed significant correlation in spatio-temporal pattern with tone-evoked activities. Simulation using a model that captures the temporal feature of spontaneous response in single channels but sets no constraint among channels, generated no spontaneous events that resembled tone-evoked activations. These results show the existence of spontaneous events similar in spatio-temporal pattern to tone-evoked activations in AI. Such spontaneous events are likely a manifestation of cortical structures that govern the pattern of distributed activation in AI.

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1. Introduction

In the primary auditory cortex (AI), neurons with the same characteristic frequency are aligned together to form a strip, often referred to as an isofrequency strip (IS) (Merzenich and Brugge, 1973; Redies et al., 1989; Ehret, 1997; Wallace et al., 2000; Buonomano and Merzenich, 1998). An IS manifests as a strip of activation (AS) to a suprathreshold pure tone stimulation, as revealed in optical imaging studies (Fukunishi et al., 1992; Taniguchi et al., 1992; Song et al., 2006). Neurons within an AS are not activated at the same time, but with progressively longer delays along the isofrequency axis, i.e., the long axis of AS. As a result, activities within an AS apparently propagate spatially (Fukunishi et al., 1992; Song et al., 2006). Thus a pure tone is represented in AI by a propagating activity in an AS. Different tone frequencies are represented by the spatially shifted AS within AI (tonotopy; Fukunishi et al., 1992; Taniguchi et al., 1992; Song et al., 2006).

We have previously found in guinea pigs that focal activation within an AS triggers autonomous activity propagation within the AS (Song et al., 2006). Because a focal spontaneous activity, activity occurring with no relation to external stimulation, is also expected to trigger similar activity propagation, spontaneous activities that resemble tone-evoked activities in spatio-temporal pattern may exist in AI. Neurons in AI have been repeatedly shown to exhibit spontaneous activities (Evans and Whitfield, 1964; Eggermont, 1992, 1994; Bitterman et al., 2008). In the visual cortex, Arieli and colleagues have made surprising and interesting observations that spontaneous activity is not random but exhibits patterns that resemble those evoked by certain visual stimuli (Arieli et al., 1996; Kenet et al., 2003). It is natural to ask whether the similarity between spontaneous and evoked activities is limited to the visual system.

Here we compared the spatio-temporal pattern of spontaneous activities and tone-evoked activities in guinea pig AI. Our results show that spontaneous activities with highly similar spatio-temporal pattern to tone-evoked activities can be found all over AI.

2. Methods

2.1. Animal

Six weeks old Hartley guinea pigs with normal Preyer's reflex were used ($n = 22$). Experiments were carried out according to the

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Guidelines for Use of Animals in Experiments of Kumamoto University. A mixture of ketamine (46 mg/kg) and xylazine (24 mg/kg) was used for the induction of anesthesia, and half dose of the mixture was injected once an hour to maintain anesthesia. A local anesthetic (4% lidocaine) was applied to the skin where an incision was made. During the recording period, the animal was paralyzed with pancuronium bromide (0.2 mg) and artificially ventilated. Adequacy of anesthesia was gauged by the absence of reflexes to toe pinches, prior to injection of the paralyzing agent. Rectal temperature was maintained at $35 \pm 1\,°C$, with the aid of a heating pad. Heartbeat rate and end-tidal CO_2 concentration were monitored throughout the experiments.

After induction of anesthesia, a piece of skull over the left auditory cortex was removed, and the dura matter was resected. The cortex was then stained with the voltage-sensitive dye RH-795 (0.7 mg/ml in saline) by surface application for 1 h, rinsed with saline, and covered with silicone oil. The AI was located as an area immediately posterior to the pseudosilvian fissure. The exact location of AI was identified by examining the tonotopy in AI with pure tones at frequencies of 1 kHz, 7 kHz, and 16 kHz (see below).

2.2. Optical recording

The principles of optical imaging using voltage-sensitive dyes have been described elsewhere (Cohen et al., 1978). A 12×12 photodiode array (effective channels = 128) was used to detect optical signals (Fukunishi et al., 1992; Tokioka et al., 2000; Song et al., 2006). With our optics, the array covered a cortical area of 3 mm × 3 mm, which is slightly smaller than the size of AI (Wallace et al., 2000; Nishimura et al., 2007). One edge of the photodiode array was aligned in parallel with the midline of the animal's head, so that leftward in the recorded image corresponds to rostral direction, and upward corresponds to dorsal direction (Fig. 1A). In guinea pigs, the isofrequency axis runs roughly in the dorsoventral direction (Redies et al., 1989; Wallace et al., 2000; Song et al., 2006). The position of the animal was adjusted so that the area responding to 1 kHz tone was recorded at the left end of the photodiode array, and the area responding to 16 kHz tone was recorded at the right end (see Fig. 3). In the dorsoventral direction, the upper edge of the photodiode array was adjusted to the dorsal end of the area responding to the tones.

The photodiode array was mounted on a custom-made microscope (NA: 0.4). Epi-illumination from a tungsten halogen lamp was used to activate the voltage-sensitive dye (excitation: $\lambda = 520 \pm 20$ nm; dichroic mirror: $\lambda = 580$ nm), and the fluorescence was collected and projected onto the photodiode array (absorption: $\lambda > 600$ nm). To minimize bleaching of the voltage-sensitive dye, the tungsten–halogen bulb was used at a low power (<80 W). The microscope was focused 300 μm beneath the cortical surface, to suppress interference from blood vessels. The microscope and the animal were set on a vibration isolation table to prevent unpredictable mechanical stimulation to the animal.

Signals from all photodiodes were amplified and sampled synchronously at 1 kHz via a sample and hold circuit. Recordings of 9–40 s long were obtained to facilitate the detection of spontaneous events. The long recordings also allow a better estimation of the amplitude distribution of low frequency components in independent component analysis (ICA; see below). Short recordings of 1.1 s long were obtained for tone-evoked responses. Optical recording in vivo is interfered by heartbeat and respiration noises (Fukunishi et al., 1992; Grinvald et al., 1994). These noises were removed by a subtraction method for short-duration recordings (Song et al., 2006) and by ICA for long-duration recordings (Maeda et al., 2001; Inagaki et al., 2003). We have previously shown that for short-duration recordings, ICA and our subtraction method yield virtually identical results (Maeda et al., 2001).
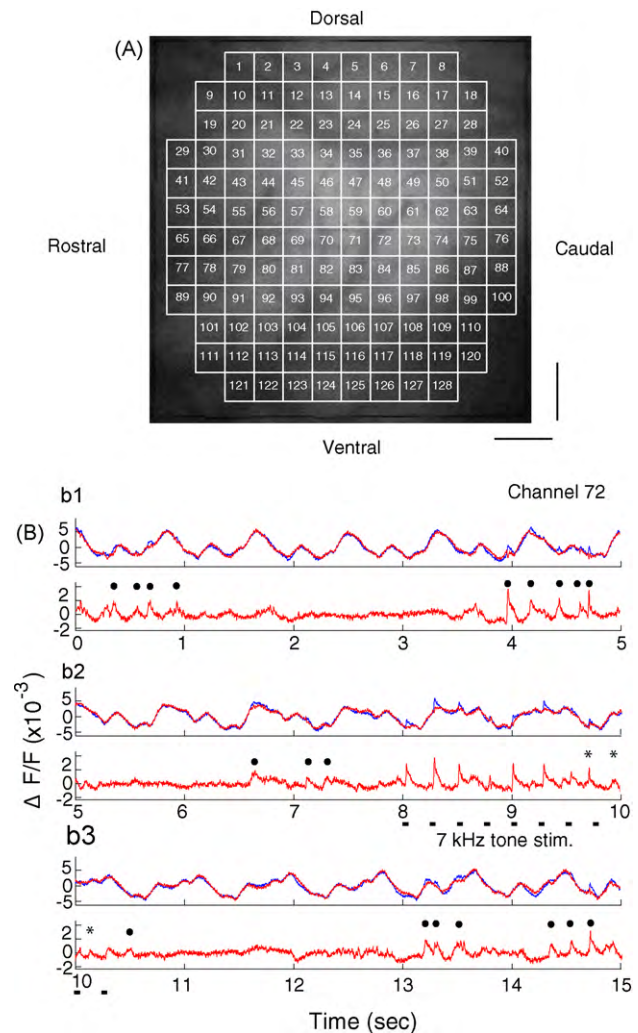

Fig. 1. Spontaneous activities in the primary auditory cortex. (A) A schematic drawing of the photodiode array (128 channels), superimposed in a photograph of the surface of the primary auditory cortex of a guinea pig. The photograph appears out of focus because the microscope was focused 300 μm beneath the cortical surface, to suppress interference from blood vessels. The photodiode array covered a cortical area of 3 mm × 3 mm. Vertical and horizontal scale bar = 0.5 mm. (B) Tone-evoked, as well as spontaneous activities obtained from one of the 128 channels (channel 72). A recording is shown in 5-s-long segments in b1–b3 for 15 s. The blue trace in the upper panel of b1–b3 is the raw recording. The red trace is the sum of heartbeat components and respiratory components, isolated with independent component analysis. The difference between the two is therefore neuronal activity that is shown in the lower panel of b1–b3. Note the difference in vertical scale between upper and lower panels. In b2 and b3, pure tones of 7 kHz were applied for a total of 10 times, at the timings marked by the black bars. Black dots in b1–b3 mark spontaneous events. Part of the data has been used for the demonstration of the validity of the ICA method (Inagaki et al., 2003). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.3. Stimulation

Pure tones (50 ms; 10 ms rise and 10 ms fall, 30 ms plateau) were applied at the beginning of the experiments for the identification of AI, and were also applied in some experiments during recordings of long duration. Tone signals were generated digitally at 100,000 samples per second (Masscomp, Concurrent Computer Corporation) and were fed through a pair of active attenuators (HF-Eichleitung, Germany) after D/A conversion. Stimulation was applied through earphones to both ears in a double-walled, dark anechoic room (IAC, NY). The sound-delivery system was calibrated

with a sound level meter (Bruel and Kjaer, Denmark) at sound pressure level (SPL) of 30–70 dB (re. 20 μPa). All tone stimuli were applied at 60 dB SPL.

2.4. Data analyses

Data were processed with algorithms applied in the Matlab programming environment (The MathWorks, Natick, MA). Fractional fluorescence (the ratio of a fluorescence change caused by a membrane potential change to the basal fluorescence level; $\Delta F/F$) was calculated to compensate irregularities in staining.

To quantify the similarity between a tone-evoked response and a spontaneous event, the average of Pearson's correlation coefficient (r) between 10 pairs of frames from the two recordings was calculated, with the first frame starting from the response onset. The 10 frames, every other frame in a 20 ms duration sampled at 1 kHz, covered all rise phase and the early falling phase of tone-evoked responses. All spontaneous events were identified by eye and the onset time of each event was measured by eye with the aid of a hand-made software. To suppress errors in the measurement of onset times, we shifted the spontaneous event at the step of one frame forward and backward for five frames, and calculated r at each step of shift; the maximum r (referred to as r value) was taken to express the correlation between the spontaneous event and the tone-evoked response. Because of the propagating nature of activity over the cortex (see Section 3), it is evident for each recording that activities among neighboring pixels are spatially correlated and that activities within one pixel are serially correlated. These autocorrelations make the test of the statistical significance of r values difficult. Although some formulae have been proposed to account for temporal correlations (e.g., Ebisuzaki, 1997), none has been proposed to our knowledge for both spatial and temporal autocorrelations. Evoked responses randomly shuffled both in time and space for 1000 times each, had correlation coefficient with spontaneous events less than 0.2, but such shuffling underestimates the bias. To better estimate the bias caused by autocorrelations, we calculated r values after rotating the frames of spontaneous activities for 90° (r_o). Such a rotation changes the orientation of the frames but reserves completely autocorrelations of spontaneous events, allowing an estimation of the bias on r values caused by autocorrelations. Level of significance for r values was determined from the distribution of r_o.

2.5. Simulation

To test if spontaneous activity in AI is generated by a random process, we simulated spontaneous activity with temporal constraints obtained from experimental observations but without spatial constraint. Waveforms of experimentally observed spontaneous activity were approximated with an alpha function of the form $(t/\tau)\exp(t/\tau)$, where t is time and τ determines the time course of the function. Spontaneous events in each channel were simulated to occur at the highest frequency of experimentally observed spontaneous events. No constraint was set among channels. Gaussian noise with a SD of 3% of the peak value of the alpha function, approximately the lower limit of noise in actual recordings, was convolved to the activity in each simulated channels. All simulations were carried out in Matlab environment.

3. Results

3.1. Spontaneous activity in AI

At the beginning of each experiment, responses to pure tones of frequencies ranging from 1 kHz to 16 kHz were examined in short-duration recordings (1.1-s-long). As we have reported previously
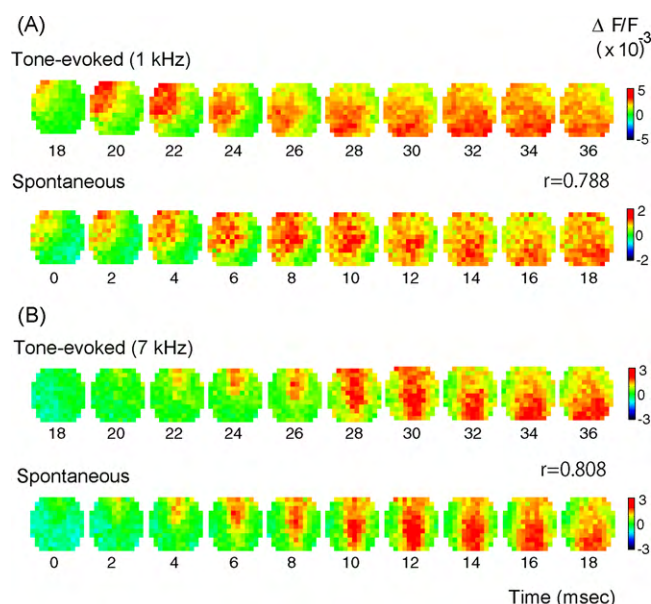

Fig. 2. Spontaneous events resembling tone-evoked activities in AI. Data shown in this figure and those shown in Fig. 1B are from the same animal. (A) Upper panel: The response of AI to stimulation of a 1 kHz tone. The response amplitude was encoded in color, as shown on the right. Each image shows the spatial pattern of response in a 3 mm × 3 mm cortical area, at the time in msec after onset of stimulus, specified under the image. The orientation of the image is the same as that shown in Fig. 1A. Lower panel: The spontaneous event marked by the first black dot in Fig. 1Bb1. The number below each image denotes the time in msec from the onset of the spontaneous activity. Note the similarity in the pattern of activity to the evoked activity shown in the upper panel ($r = 0.788$). (B) Upper panel: The first evoked activity in Fig. 1Bb2. Lower panel: A spontaneous event occurring at about 27 s after the evoked activity (at 15 s of the following recording to that shown in Fig. 1B). Again the similarity between the evoked and the spontaneous activity is remarkable ($r = 0.808$). Conventions are as in A. Tones were applied at 60 dB SPL.

(Maeda et al., 2001; Song et al., 2006), each tone-evoked propagating activity along a strip of cortical area (an AS), with its long axis, the isofrequency axis, running roughly in the dorsoventral direction (Fig. 2A and B, upper row; Fig. 3A–C, upper row). A tone of lower frequency activated an AS in rostral AI (Fig. 2A and Fig. 3A, upper row), with the AS shifting towards caudal direction with increasing tone frequency (Fig. 3B and C, upper row).

After having obtained responses to pure tones, we made successive recordings of 9–40 s long, to study spontaneous activities in AI. Neuronal activities were isolated from heartbeat and respiration interferences using ICA, as we have reported previously (Maeda et al., 2001; Inagaki et al., 2003). Occasionally tone stimulations were also applied during long-duration recordings, to verify the method of activity isolation with ICA. Fig. 1B shows an example of the first 15 s of a 20-s-long raw recording (blue traces in the upper panel in Fig. 1B, b1–b3) from 1 of the 128 channels, together with the isolated noise component (red traces in the upper panel in Fig. 1B, b1–b3) and neuronal activities (the lower panel in Fig. 1B, b1–b3). From the eighth second, a pure tone of 7 kHz was applied 10 times, at an interval of 250 ms (Fig. 1B, b2 and b3, bars below the lower panels indicate the timing of tone stimuli). Tone-evoked activities with a rapid rise phase and a slower decay, appearing with a delay of ~20 ms, were clearly isolated and are readily recognizable in the figure (Fig. 1B, b2 and b3, lower panels; responses to the last three tones were weak and may had been suppressed by the preceding activities marked by the asterisks). Meanwhile, neuronal activities that had a rapid rise phase followed by a slower decay, were also observed before or long-after tone stimuli (Fig. 1B, b1–b3, lower panels, events marked by the black dots). Here we defined an event whose occurrence was not temporally related with tone stimuli as a spontaneous event. A total of 4858 spontaneous events were iden-
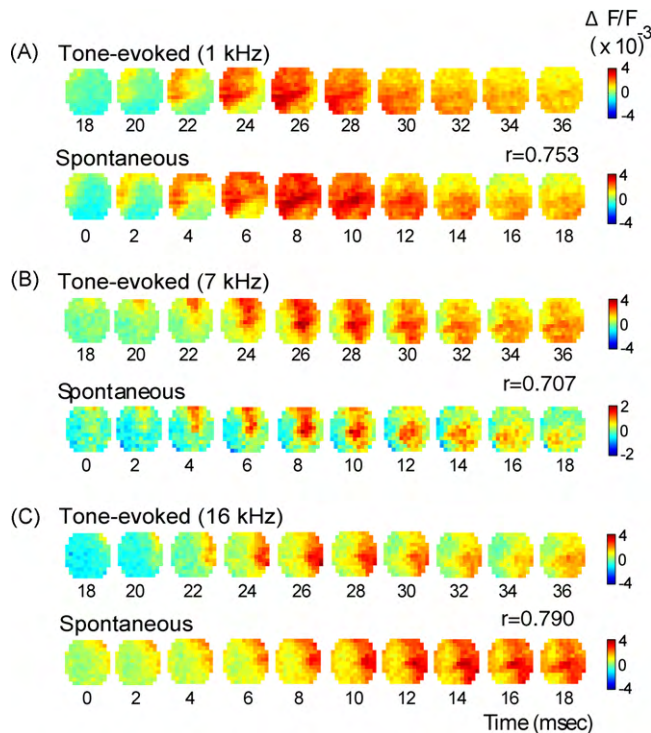
Fig. 3. Spontaneous activity resembling activities evoked in AI by low-, middle-, and high-frequency tones. (A) Upper panel: The response of AI to stimulation of a 1 kHz tone. Lower panel: Spontaneous activity found in the same animal. Note the similarity in the pattern of activity to the evoked activity shown in the upper panel. (B) An example demonstrating the similarity between a 7-kHz-tone-evoked activity to a spontaneous event in the same animal. (C) An example showing the similarity between a 16-kHz-tone-evoked activity to a spontaneous event in the same animal. Sound pressure level was 60 dB in A–C. Conventions are as in Fig. 2.

tified by eye in 150 successful single recordings obtained from 22 animals. Spontaneous event occurred occasionally in isolation, but appeared to have a trend to occur in a train of several events (Fig. 1B, b1, right end). Events within a train occurred mainly at 4–6 Hz.

3.2. Spatio-temporal pattern of spontaneous activity in AI

To test if there are spontaneous events in AI that resemble tone-evoked activities, we calculated the correlation coefficient (r value; see Section 2) between a spontaneous event and the response to a pure tone. A high r value suggests the resemblance of spontaneous event to tone-evoked activity. Such analysis did reveal spontaneous events that resembled tone-evoked activity in the same animal. The third spontaneous event marked by a black dot in Fig. 1Bb1, for example, was found similar to the activity evoked by a 1 kHz tone in the same animal ($r = 0.788$; Fig. 2A). The upper row in Fig. 2A shows the spatio-temporal pattern of the response evoked by a 1 kHz tone, with the amplitude of the responses encoded in color; and the lower row shows the spontaneous event. As is clear from the figure, the similarity between the evoked and the spontaneous activity in spatio-temporal pattern is remarkable. The first response evoked by the 7 kHz tone shown in Fig. 1Bb2, was found similar to a spontaneous event occurred about 27 s after the evoked response (at 15 s of the following recording; not shown in Fig. 1B) ($r = 0.808$; Fig. 2B). The r values for repeated trials of tone-evoked activities were found to be 0.798–0.894 ($n = 18$). The values of 0.788 and 0.808 found here between spontaneous events and tone-evoked activity are thus striking.

Fig. 3 shows more examples of spontaneous events that resembled responses evoked by low- (1 kHz), middle- (7 kHz), and high-frequency (16 kHz) tones, with the upper row in Fig. 3A–C
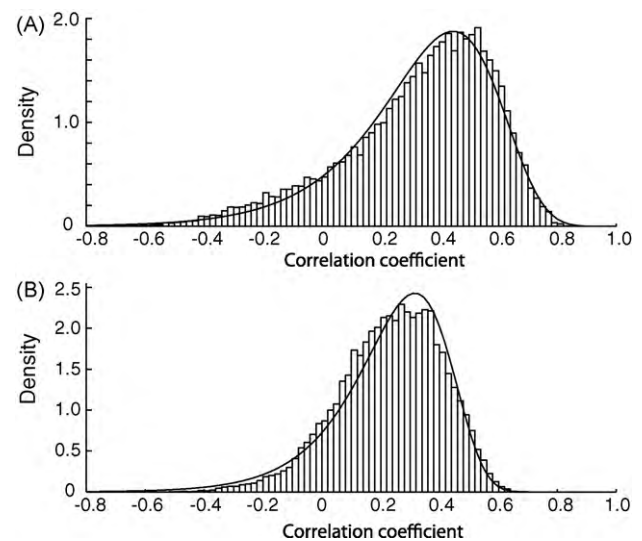

Fig. 4. The distribution of correlation coefficient between spontaneous events and responses evoked by pure tones. (A) The distribution of correlation coefficient (r values; see Section 2) between all 4858 spontaneous events and responses evoked by tones of 1 kHz, 4 kHz, 7 kHz, 10 kHz, and 16 kHz. The distribution was fitted with an extreme value distribution with a mean of 0.332 and a variance of 0.063. (B) The distribution of correlation coefficient after rotating the frames of spontaneous activities (r_o values; see Section 2). The distribution is also fitted with an extreme value distribution, with a mean of 0.229 and a variance of 0.038. From the fitted curve, probability reached 99.99% at $r_o = 0.608$.

showing the evoked responses and the lower row showing spontaneous events. Again, the similarity between the evoked and the spontaneous activities in spatio-temporal pattern is evident ($r = 0.707$–0.790). In Fig. 3C, the response to 16 kHz tone stimulation spread towards the center of AI at latencies >26 ms; such a spread was also found in the pattern of spontaneous activity (Fig. 3C, lower row, >10 ms after onset).

The distribution of r values of all 4858 spontaneous events found here with the responses evoked by tones of 1 kHz, 4 kHz, 7 kHz, 10 kHz, and 16 kHz, is shown in Fig. 4A. Shown in Fig. 4B is the distribution of correlation coefficients calculated after rotating the frames of spontaneous activities for 90° (r_o; see Section 2), which provides an estimation of bias on r caused by autocorrelation in the activities (see Section 2). Both distributions were skewed to the right and could be best fitted with an extreme value distribution. The r_o value for $p < 0.001$ in Fig. 4B was 0.608, and was taken as the criterion to judge if an r value is significant. With this criterion, 1633 spontaneous events had significant r values ($r = 0.609$–0.826), out of the 4858 spontaneous events found here (33.6%). Spontaneous events resembling activities evoked by low frequency tones (1 kHz) and high-frequency tones (16 kHz) were found more frequently (1 kHz, 35% of total; 16 kHz, 27%) than those resembling activities evoked by middle frequency tones (4 kHz, 13%; 7 kHz, 18%; 10 kHz, 7%).

3.3. Simulation results

The observation of spontaneous events resembling tone-evoked activities suggests the role of circuits in AI in determining the distributed pattern of activation in the cortex. The possibility that all spontaneous events are manifestations of a random process, however, cannot be excluded (Mokeichev et al., 2007). To test this possibility, we did a simulation in which simulated spontaneous events were generated using waveforms resembling recorded spontaneous activity, but without posing any constraint among pixels (channels). The waveform in one channel of the first spontaneous event recorded from 10 animals is superimposed in Fig. 5Aa1,
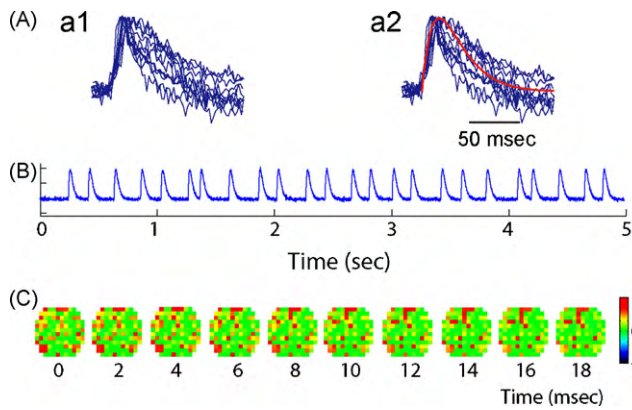
Fig. 5. Spontaneous activity simulated without spatial constraint did not show similarity to tone-evoked activity. (A) a1: Single channel waveforms of spontaneous events normalized and superimposed, to illustrate the time course of the activities. a2: The time course could be roughly captured by an alpha function ($\tau = 15$ ms; the red trace). (B) An example of simulated spontaneous activity in one channel. (C) Spatio-temporal pattern of a 20-ms segment of simulated spontaneous activity that had a correlation coefficient of 0.337 to a tone-evoked activity template (1 kHz). Amplitudes in A–C are arbitrary. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

with the amplitude normalized to illustrate the time course of the activities. The time course could be roughly approximated with an alpha function ($\tau = 15$ ms; Fig. 5Aa2, red trace). The activity in each of the 128 channels was generated by letting simulated events occur at the frequency of 4–6 Hz that matches the frequency of observed spontaneous events in a train (see Section 3.1). Fig. 5B illustrates an example of simulated activity in one channel. Without posing any constraint among channels, we simulated for a total duration of 10 h. The correlation coefficient between simulated spontaneous activity and tone-evoked activity templates from five animals never exceeded 0.34. Similar results were obtained when the parameter τ of the alpha function was reduced to 10 ms or increased to 25 ms. Fig. 5C shows a segment of simulated activity that had a correlation coefficient of 0.337 to a 1 kHz tone-evoked activity.

3.4. Spontaneous events that had low correlation with tone-evoked activity

It is curious to know what patterns spontaneous events show when they have low correlation with tone-evoked templates, although it is difficult to characterize such events. We noticed four response patterns: (1) spontaneous activity initiated from a ventral site, (2) spontaneous activity initiated at a middle site, (3) spatially separated but temporally overlapping multiple spontaneous events, and (4) apparent shift of strip-like activity along the rostro-caudal axis. Shown in Fig. 6A is a spontaneous event in which the activity was initiated from a rostroventral site, in contrast to the rostrodorsal site of tone-evoked activity (see Figs. 2 and 3). The late phase of the event appeared to be similar to a low frequency tone-evoked activity, but overall the event had a correlation coefficient of 0.569 to a template evoked by a 1 kHz tone. Shown in Fig. 6B is an example of spontaneous events in which activity was initiated at the center of AI. Despite the spread of activity along the dorsoventral axis, the event had a correlation coefficient as low as 0.263 to a template evoked by a 7 kHz tone. This low correlation is likely to be attributable to the slow spread of activity. Fig. 6C shows spatially separated but temporally overlapping spontaneous events in AI. In Fig. 6D, when a spontaneous event at the caudal end of AI was diminishing, another event was initiated at a neighboring site (arrowhead in the frame at 26 ms); As a result, strip-like activity appeared to shift in a caudorostral direction.
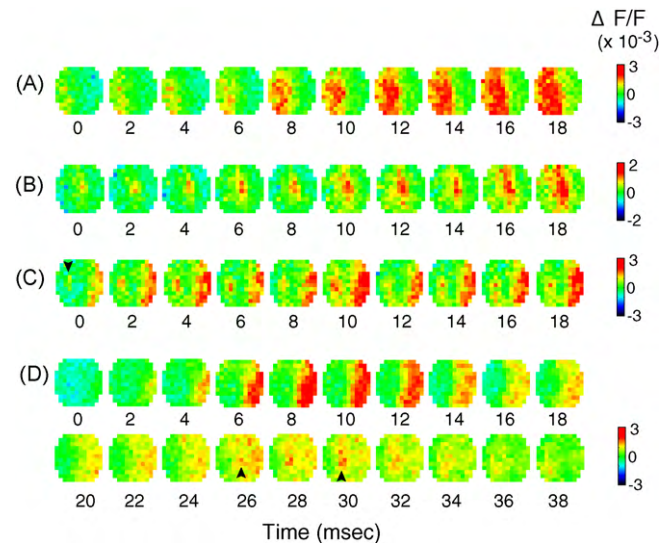

Fig. 6. Some spontaneous events that had low correlation with tone-evoked activity. (A) A spontaneous event initiated from a rostroventral site in AI. (B) A spontaneous event initiated from the center of AI. (C) Two spontaneous events in AI, with the rostral one being initiated from a middle site (arrowhead in the first image). (D) Apparent shift of activation. A spontaneous event was initiated from a middle site at the caudal end at 2 ms; when this event was diminishing, another event was initiated at a neighboring site (arrowhead in the 26 ms image), inducing a strip-like activation a few msec later (arrowhead in the 30 ms image). Overall, the strip-like activation at 10 ms apparently shifted rostrally at 30 ms. Conventions are as in Fig. 2.

The response patterns described above certainly do not exclude other patterns, but we found no spontaneous activity that was initiated all over AI at the same time, nor did we find spontaneous activity that spread in a spatially isotropic manner.

4. Discussion

By long-duration optical recording, we showed in this study the existence of spontaneous events in guinea pig AI that had remarkably similar spatio-temporal pattern to that of tone-evoked activities. Such spontaneous events were found at all locations examined in AI.

Both evoked and spontaneous activities often showed rather broad spread within AI, especially after the response had reached the peak (see Fig. 3). One may argue that the significant correlation between spontaneous and evoked activities described here is a result of such activity spread. The drop of correlation coefficient after rotating the frames of spontaneous events (see Fig. 4), however, argues against this possibility. In fact, by visual inspection, the similarity between evoked and spontaneous activities remains in Fig. 3 even when one focuses on dark red colors (large response amplitudes).

The percentage of spontaneous events that resembled tone-evoked activity appeared low (33.6%). This may have been caused by several factors. First, there are strict conditions for a spontaneous event to resemble tone-evoked activity. These conditions are not explicit from the value of correlation coefficient, but comparison between events that resembled tone-evoked activity (Figs. 2 and 3) to those that did not (Fig. 6), appears to suggest at least the following conditions for a spontaneous event to resemble tone-evoked activity: (1) the event was initiated at a dorsal location, (2) activity spread largely along a dorsoventral axis, and (3) the dynamics of activity spread was close to that of the tone-evoked activity. Second, we used correlation coefficient after rotating the frames of spontaneous activity (r_o) to estimate the bias caused by autocorrelation. When r values are large, r_o serves as a proper estimation; when r values are small, however, r_o may overestimate the bias. Lastly, we

used activities evoked by tones at a single SPL of 60 dB. This may have lowered the percentage of spontaneous events resembling tone-evoked activities.

Although the percentage of spontaneous events that resembled tone-evoked activity was low, our simulation study suggests that it is significant. Despite the temporal constraint imposed on spontaneous activity in our simulation, no simulated activity was found to resemble tone-evoked activity. Our simulation results suggest the importance of circuits that set spatial constraint on activity spread, in the generation of spontaneous events that resemble tone-evoked activity. Such circuits may be of cortical origin, but may also involve the thalamus.

Spontaneous events resembling activities evoked by low- and high-frequency tones were found more frequently than those in the middle frequency. The exact reason for this observation is not clear at this time, but it might be in part caused by the fact that responses evoked by low- and high-frequency tones were not completely covered by our camera. This may have imposed a moderate bias on the comparison with spontaneous events.

Spontaneous activities in AI have been reported in the earliest animal studies (Evans and Whitfield, 1964) and have been extensively studied by electrode-based recording techniques (Eggermont, 1992, 1994). Neurons in human AI also exhibit spontaneous activities (Bitterman et al., 2008). Here we have compared the spatio-temporal pattern of spontaneous activities to that of tone-evoked activities in guinea pig AI, and have found spontaneous activities that show a remarkable similarity to tone-evoked activities. Spontaneous events judged as not similar to tone-evoked activity also showed strip-like activation in AI (see Fig. 6). Consistent with our findings, Yoshida et al. (2008) have recently reported that spatial coherence (correlation between spatially separated channels) of spontaneous activities is stronger along the isofrequency axis in AI. Hosokawa et al. (1998), however, have reported spatially isotropic coherence under spontaneous condition in AI. The reason for this discrepancy is not clear at this time.

We have previously shown that cortical intrinsic circuit in AI can support autonomous activity propagation in an AS (Song et al., 2006). This finding predicts the presence of spontaneous activities in AI that resemble tone-evoked activities, because a focal spontaneous activity is expected to trigger activity propagation along an AS. Results obtained here are consistent with this prediction. The low percentage of spontaneous events resembling tone-evoked activities, however, suggests the complexity of mechanisms determining the pattern of spontaneous activities. Nevertheless, some spontaneous activities having low correlation with tone-evoked activities also appeared to have a tendency to spread along the dorsoventral axis (see Fig. 6). Thus cortical circuits alone or together with the thalamus appear to favor activity propagation along the isofrequency axis. Preferential elongation of cellular processes along the isofrequency axis may be one anatomical basis for such propagation (Matsubara and Phillips, 1988). Although cortical activities evoked by tones in the present study were all initiated at a dorsal site of AI, we have previously shown that cortical activity can be occasionally initiated at other sites by tone stimulation (Song et al., 2006). Cortical activities initiated from a rostroventral site (see Fig. 6A) is thus not limited to spontaneous activity. It seems that cortical circuits can support activity propagation along the isofrequency axis wherever an activity is initiated within an AS (Song et al., 2006). The apparent slow propagation of some of the spontaneous events (see Fig. 6B) is interesting, yet the mechanism for the variation of propagating velocity of spontaneous activity is not known. Brain-state-dependent modulation of synaptic transmission and/or cell excitability in the cortex might in part account for such variation. This issue also depends on the anatomical basis of spontaneous activity. At this time, it is not known how spontaneous events in AI are generated. In principle, spontaneous activity in AI can be initiated in AI or at any stage of the subcortical pathway.

In recent years, spontaneous activities have been extensively studied in the visual cortex (Arieli et al., 1995, 1996; Tsodyks et al., 1999; Kenet et al., 2003; Petersen et al., 2003). A surprising finding is the remarkable resemblance of spontaneous activity to orientation map in area 18 (Kenet et al., 2003). Another interesting finding in the visual cortex is the interaction between spontaneous and stimulus-evoked activities; the variability of stimulus-evoked response amplitude is not a "noise", but can be accounted for by the variability of ongoing activities (Arieli et al., 1996; Petersen et al., 2003). How spontaneous activity affects the spatio-temporal pattern of sound-evoked activity in AI would be an important subject for future studies.

Acknowledgements

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