Cortical Intrinsic Circuits Can Support Activity Propagation through an Isofrequency Strip of the Guinea Pig Primary Auditory Cortex

A pure tone evokes propagating activities in a strip of the primary auditory cortex (AI), an isofrequency strip (IS). A fundamental issue concerns the roles that thalamocortical input and intracortical connectivity play in generating the activities. Here we addressed this issue in guinea pigs using in vivo and in vitro real-time optical imaging techniques. As reported previously, tone-evoked activity propagated dorsoventrally along a strip (an IS) in AI. We found that an electrical pulse applied focally within the strip, triggered activity propagation with a spatiotemporal pattern highly similar to tone-evoked activation. The propagation velocity of electrically evoked activity was significantly slower than that of tone-evoked activity, but was comparable to the velocity of lateral activity propagation in cortical slices, suggesting that the electrically evoked activity propagation in vivo is mediated by intracortical circuits. To test this notion, we lesioned the auditory thalamus chemically; in such animals, electrically evoked activity in AI was not affected, although tone-evoked activity was abolished. Further, in slices of the AI, the extent of electrically evoked activity propagation in layer II/III was significantly larger in coronal slices than in horizontal slices. Together, our results suggest that intracortical connectivity in AI enables a focally evoked activity to propagate throughout an IS.

Keywords: cortical dynamics, electrical stimulation, intracortical circuits, optical recording, propagating activity

Introduction

A pure tone causes a travelling wave of displacement of the basilar membrane in the cochlea; the largest displacement occurs at a particular location that depends on the frequency of the tone (von Bekesy, 1960; Johnstone et al., 1986). Basilar membrane displacement is sensed and encoded into neural activity by inner hair cells and auditory nerve fibres. An auditory nerve fibre thus responds to a limited range of frequency, with highest sensitivity to a particular frequency, i.e. the fibre’s characteristic frequency. Sound frequency can thus be mapped into neural space in the cochlea in terms of characteristic frequency. This mapping, or tonotopy, is seen throughout the central auditory pathway (Brugge, 1992; Schreiner et al., 2000). In the primary auditory cortex (AI), neurons that have the same characteristic frequency are aligned in a band; thus a frequency is represented by a band of neurons, called an isofrequency strip (IS) (Merzenich and Brugge, 1973; Redies et al., 1989a; Ehret, 1997; Buonomano and Merzenich, 1998; Wallace, et al., 2000). ISs are aligned on AI in sequence, according to the frequency that they represent. A tone can thus be mapped to a strip in the cortex according to its frequency. Recent imaging studies on the auditory cortex have confirmed the presence of ISs, and have further demonstrated propagating activities within an IS (Fukunishi et al., 1992; Taniguchi et al., 1992; Uno et al., 1993). Bakin et al., 1996; Hess and Scheich, 1996; Tsytserc et al., 2004). The propagating nature of activity within an IS can also be inferred from single electrode studies that have demonstrated progressive changes in onset latency of responses in an IS along the isofrequency axis (the long axis of an IS; Mendelson et al., 1997; Schreiner et al., 2000).

How the propagating activity is generated and why it occurs along a strip (an IS) of the cortex is not fully understood. Because that there is tonotopy in the auditory thalamus (Aitkin and Webster, 1971; Morel et al., 1987) and that there is topography in thalamocortical projection along the frequency axis (Andersen et al., 1980; Redies et al., 1989b; Velenovsky et al., 2003) and the isofrequency axis (Redies et al., 1989b), thalamocortical inputs are expected to play an important role in defining cortical ISs. Cortical intrinsic circuits, however, also seem to contribute to anisotropic activation of the cortex. It has been shown in several species that neurons of cortical layer II/III tend to extend their axons along the isofrequency axis (Matsubara and Phillips, 1988; Ojima et al., 1991; Read et al., 2001; Wallace et al., 2002). It is not known, though, to what extent cortical intrinsic circuits contribute to the propagation of activity within an IS. Neither is it clear the role played by cortical intrinsic circuits and thalamocortical inputs in determining the dynamics of activity propagation within an IS. Therefore, the aims of the present study were twofold: to examine the role of cortical intrinsic circuits in supporting activity propagation in an IS and study the role of cortical intrinsic circuits and thalamocortical inputs in determining the dynamics of activity propagation within an IS. Using in vivo and in vitro real-time optical imaging techniques, we show that intracortical connectivity in AI enables a focally evoked activity to propagate throughout an IS. We also show that the propagation of activity in cortical intrinsic circuits is slower than the propagation of tone-evoked activity. The dynamics of tone-evoked activity propagation is thus likely to be determined by thalamocortical inputs.

Materials and Methods

Animal Preparation for In Vivo Recording

Experiments were carried out according to the Guidelines for Use of Animals in Experiments of Osaka University. Six weeks old Hartley guinea pigs with normal Preyer’s reflex were used. Anaesthesia was induced with a mixture of ketamine (46 mg/kg) and xylazine (24 mg/kg), and was maintained throughout the experiments by injecting half dose of the mixture once an hour. Adequacy of anaesthesia was gauged by the absence of reflexes to toe pinches. A local anaesthetic (lidocaine) was applied to all incision sites. During the recording period, the animal was paralysed with pancuronium bromide (0.2 mg) and artificially ventilated. Rectal temperature was maintained at 34 ± 1°C, with the aid of a heating pad. This temperature is slightly lower than normal, but it is our experience that a lower temperature often resulted in stable recordings.
Heart rate (180–200 beats/min) was monitored throughout the experiments. Tracheostomy was performed for artificial ventilation and for monitoring end-tidal CO2 concentration, which was kept at 4–6%.

After induction of anaesthesia, external auditory canals were cleaned and the ear drums were inspected. To prevent visual interference from excitation light for voltage-sensitive dyes, both eyes were closed. A piece of the skull over the auditory cortex on the left hemisphere, 6 mm in rostrocaudal direction and 5 mm in dorsosentral direction, 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 direct application on the cortical surface. The cortex was stained for one h, rinsed with saline, and covered with a drop of silicone oil. The Al was first located as an area immediately posterior to the suprasylvian fissure. The exact location of Al was identified by examining responses to pure tones at frequencies of 1, 7 and 16 kHz (see below).

Optical Recording In Vivo

The principles of optical imaging using voltage-sensitive dyes have been described elsewhere (Cohen et al., 1978). In all in vivo experiments, a 12 × 12 photodiode array (effective channels = 128) was used to detect optical signals (Fukunishi et al., 1992; Maeda et al., 2001; Inagaki et al., 2003). With our optics, the array covered a cortical area of 3 × 3 mm2, which is slightly smaller than the size of Al in guinea pigs (Wallace et al., 2000). 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. 2a). In guinea pigs, the isofrequency axis runs roughly in the dorsosentral direction, and thus the frequency axis runs in the rostrocaudal direction (Hellweg et al., 1977; Redies et al., 1989a; Wallace et al., 2000). 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; the area responding to 16 kHz tone was thus recorded at the right end and the area responding to 7 kHz tone was usually at the center of the image (see Figs 2 and 3). In the dorsosentral direction, the upper edge of the photodiode array was adjusted to the dorsal end of the area responding to 1 kHz tones. Thus, visual markers and responses to pure tones of 1, 7 and 16 kHz provided a firm identification of Al.

The photodiode array was mounted on a custom-made bright microscope (NA: 0.4; Tokioka et al., 2000). Epi-illumination from a tungsten-halogen lamp was used to activate the voltage-sensitive dye (excitation filter: λ = 520 ± 20 nm; dichroic mirror: λ = 580 nm), and the fluorescence was collected and projected onto the photodiode array (absorption filter: λ = 600 nm). The tungsten-halogen bulb was used at a low power (<80 W) to minimize bleaching of the voltage-sensitive dye and to suppress photo-damage of cortical neurons. The microscope was first focused on the surface of the cortex, and the brain was then raised by 300 μm, to suppress interference from blood vessels and to focus on cortical layer II/III. Signals from all photodiodes were amplified and sampled synchronously via a sample and hold circuit. Sampling intervals were 0.5–1 ms. Because heartbeat interferes with optical recording in vivo (Fukunishi et al., 1992; Grinvald et al., 1994), we synchronized our recordings with the animal’s heartbeat, and subtracted recordings without a stimulus from recordings with a stimulus (Fukunishi et al., 1992). To suppress respiratory interference, respiration was stopped temporarily during recording for 1.2 s. Because spontaneous activity has been observed in the guinea pig auditory cortex (Tokioka et al., 2000) and spontaneous activity in the cortex has been shown to affect the response to sensory stimulus (Arieli et al., 1996), we considered the mean of 16 consecutive recordings, to suppress the effect of spontaneous activity and random noises.

Optical Recording In Vitro

Identification of Al in Slices

To eliminate the influence of subcortical structures, electrically evoked activity propagation in slices of Al was examined in 14 guinea pigs, weighing 200–330 g. For identification of Al in slices, Al was identified and visually marked in vivo in five animals (240–330 g) by recording responses with a CMOS camera system (MiCAM Ultima, BrainVision Inc., Tokyo, Japan) to pure tones of 1 kHz, and 16 kHz; the CMOS camera has a much better spatial resolution than the photodiode array (100 × 100 pixels, covering an area of 6.25 × 0.25 mm2 in the present study). The dorsal and ventral ends of the IS of 1 and 16 kHz were marked by a needle coated with Pontamine-Sky-Blue. The animals were then perfused with 0.3% formaldehyde in 50 mM phosphate buffer. The distances from the dorsal end of the suprasylvian fissure to the line connecting the dorsal ends of the ISs, and to the line connecting the ventral ends of the ISs, were measured (1.3 and 5.3 mm on average, respectively). These two distances were used to determine the dorsal and ventral end for blocking the Al region for slicing. The distances from the rostral end of the suprasylvian fissure to the long axis of the ISs of 1 and 16 kHz were measured (1.1 and 5.0 mm on average, respectively), and were used to determine the rostral and caudal end for blocking the Al region.

Preparation of Slices and Recording

The method for preparation of slices followed those published previously (Tominaga et al., 2000; Otsuka et al., 2004). The animals were deeply anaesthetized with a mixture of ketamine (92 mg/kg) and xyloseine (48 mg/kg), and were perfused with a high sucrose solution containing (in mM): 200 sucrose, 2.5 KCl, 0.5 CaCl2, 10 MgSO4, 1.25 KH2PO4, 26 NaHCO3 and 10 glucose (300 ± 5 mOsmol/l, pH 7.4). After perfusion, the suprasylvian fissure was readily recognizable, and the Al-containing region was blocked using references to the fissure as described above. The block was cut either coronally or horizontally on a vibrating slide microtome (VT 1000 E, Leica) into 400-μm-thick slices, while being bathed in the high sucrose solution. Coronal slices from the caudal one-third of the tissue block were not collected to avoid possible contamination from the DC area; Horizontal slices from the ventral one-third of the tissue block were not collected to avoid possible contamination from the ventrorostral belt region (Wallace et al., 1999). Slices were then kept for 10 min in oxygenated Kreb’s solution (composition in mM: 126 NaCl, 2.5 KCl, 1.25 KH2PO4, 1 MgSO4, 2 CaCl2, 26 NaHCO3 and 10 glucose, 300 ± 5 mOsmol/l, pH 7.4, bubbled with 95% O2–5% CO2). Each slice was then transferred onto a membrane filter (JH, Millipore, MA) glued to one end of a glass ring (i.d. = 10 mm, o.d. = 12 mm, height = 2 mm), kept in a moisture chamber for 1 h at room temperature, and stained with the voltage-sensitive dye di-2-ANEPQ (0.25 mg/ml; Molecular Probes Inc.) for 25 min. The slice was stuck to the membrane filter, and could then be handled with the glass ring. After staining, slices were rinsed once with the Kreb’s solution and kept in a moisture chamber for >30 min before being used for recording. During recording, the slice was continuously perfused with the Kreb’s solution. The temperature was kept either at 28 or 35°C with a feedback controller (TC-324B, Warner, Hamden, CT) for estimation of temperature dependence of activity propagation and for comparison with in vivo recording. After recording, the slice was fixed and stained with cresyl violet for identification of cortical layers.

The CMOS camera was mounted on a microscope with epifluorescence optics (THT-AIV, SciMedia Ltd, Tokyo, Japan). A halogen light source was used with filters similar to those used for the photodiode-array imaging system described above (excitation: λ = 550 ± 3 nm; absorption: λ > 590 nm). Recordings were made with a sampling frequency of 2.5 kHz. The CMOS camera has a high spatial resolution (100 × 100 pixels), but has a shortcoming that an image frame is not sampled at the same time. The 100 horizontal lines are divided into 25 equal blocks. Within each block, images are sampled at the same time, but there is a fixed delay between the blocks. The total delay (D) in a frame depends on the sampling interval (T), being 320 μs for a sampling interval of 0.4 ms. This delay was corrected offline, according to the following equation:

\[
\text{Block}(m)\text{-in-frame}(n)_{\text{corrected}} = (1 - a) \times \text{Block}(m)\text{-in-frame}(n) + a \times \text{Block}(m)\text{-in-frame}(n-1)
\]

where \(m\) is the block number in a frame, \(n\) is the frame number and \(a = \left(\frac{1}{(m-1)}\right) \times \text{D} \times ((25 - 1) + T)
\)

Stimulation

Pure tones (50 ms; 10 ms rise and 10 ms fall, 30 ms plateau) were used for acoustic stimulation. 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-Eichleitnerg, Cerebral Cortex May 2006, V. 16 N 5, 719, Downloaded from http://cercor.oxfordjournals.org/ by guest on November 4, 2016)
was plotted against distance from the activity initiation point; propagation velocity was then calculated as the slope of the regression line of the plot. For recordings in vitro, the latency of activities detected at pixels along the maximum extent of propagation in layer II/III was plotted against distance; propagation velocity was calculated again as the slope of the regression line of the plot. Latency of a response was defined as the time interval from stimulus onset to when the signal exceeds twice the SD of the baseline noise. Data are presented as mean ± SD. Wilcoxon rank sum test was used for statistical comparison between groups.

Results

Optical Signals In Vivo Primarily Reflected Activity in Cortical Layer II/III

In response to stimulation of a pure tone, the optical signal recorded with the voltage-sensitive dye RH-795 from AI in a single channel exhibited a fast, transient reduction in fluorescence, followed by a slower, transient increase (Fig. 1a, polarity reversed). Because we focused our microscope 300 μm beneath the surface of the cortex (see Materials and Methods), it is expected that the optical signals would primarily reflect activities in cortical layer II/III. To actually test this point, we measured the cortical depth profile of fluorescence resulted from the in vivo staining of the cortex with RH-795. After a brief in vivo optical recording, the animal was perfused with formaldehyde and coronal sections of the AI were prepared in dim red light to minimize bleaching of the voltage-sensitive dye. Fluorescence of the sections was then immediately measured, and the sections were subsequently processed for staining with cresyl violet to identify cortical layers. Shown in Figure 1b are a photomicrograph of a Nissl-stained cortical section and its fluorescence depth profile. As is clear from the figure, maximum fluorescence was observed just beneath the border between layer I and layer II. The intensity of the fluorescence declined rapidly towards deeper layers. At layer IV, the fluorescence was <20% of the maximum value. Similar observations were obtained in 12 sections from two other animals. These results, together with the fact that we focused our microscope at 300 μm beneath the cortical surface (see Materials and Methods), suggest that our optical signal primarily reflected activity in cortical layer II/III.

Tone-evoked Activity Propagation

The spatiotemporal patterns of responses in AI to stimulation with pure tones of 1, 7 and 16 kHz are exemplified in Figure 2, with the amplitude of the responses encoded in color. As expected from the tonotopy of the guinea pig AI (Hellweg et al., 1977; Redies et al., 1989a; Taniguchi et al., 1992; Uno et al., 1993; Wallace et al., 2000), tones of lower frequency activated rostral AI (Fig. 2b), whereas tones of higher frequency activated caudal AI (Fig. 2d). In agreement with previous reports (Fukunishi et al., 1992; Taniguchi et al., 1992; Uno et al., 1993; Hess and Scheich, 1996), the activity evoked by the pure tones did not appear simultaneously in space, but was in most cases initiated at a dorsal site (24–31) and then propagated mainly ventrally (in other cases activities were either initiated at a middle site or at multiple sites) (Fig. 2b–d). For the first a few milliseconds after the response onset, the activity evoked by all tones propagated in a narrow strip (an IS) of the cortex, with its long axis, the isofrequency axis, running approximately in the dorsoventral direction (Fig. 2b–d; see the next section for an analysis of long axis orientation). At later times, activity spread in
the rostrocaudal direction also became evident. The spread was always limited in the dorsal part of AI, although considerable spread was often observed in the ventral part (Fig. 2b–d). This is consistent with the observation by Redies et al. (1989a) that IIs slightly fan out towards ventral AI (also see Wallace et al., 2000).

Because there is topography in thalamocortical projection along the isofrequency axis of guinea pigs (Redies et al., 1989b), the sequential activation along the long axis of an IS by tones suggests that the arrival of thalamic inputs at the cortex is dispersed over time. The contribution of cortical local circuits to the activity propagation in an IS is, however, unknown.

**In Vivo Activity Propagation Evoked with a Focally Applied Electrical Pulse**

To address the role of cortical intrinsic circuits in the propagation of activity in an IS, we first identified an IS with a pure tone, and then stimulated the cortex at a site within the strip, using a single electrical pulse (duration = 100 μs; amplitude = 0.1-0.5 mA). An example of such an experiment is shown in Figure 3A. Figure 3Aa shows the response to the stimulation of a 1 kHz tone. A stimulating electrode was then placed at a position where activity was initiated by the tone (Fig. 3Ab, arrow). For the first 2 ms, activities evoked by an electrical pulse applied through the electrode were observed only within 250 μm of the electrode tip (Fig. 3Ab). This was consistent for all 31 experiments, suggesting that the spread of stimulus current is spatially limited. Over time, the activity propagated, primarily along the dorsoventral direction. Ten milliseconds after stimulation, the activity had propagated throughout AI along the dorsoventral axis, forming a strip of activity (Fig. 3Ab). Further, the pattern of activity evoked with the electrical pulse (Fig. 3Ab) was remarkably similar to that evoked by the 1 kHz tone (Fig. 3Aa), although the latencies of the electrically evoked activities were much shorter. To quantify the similarities in the spatiotemporal patterns, we calculated the maximum mean spatial correlation coefficient \( r \); see Materials and Methods for definition) between tone-evoked and electrically evoked activities. A significant correlation was found for the case of Figure 3Aa,b \( (r_{ab} = 0.814; P < 0.01) \) and for all 20 other cases studied \( (r = 0.655-0.907; P < 0.01) \). Electrical stimulation of AI regions that responded to higher frequency tones (7–16 kHz) also evoked activity similar to the tone-evoked activity in all 17 animals tested \( (r = 0.688-0.828, P < 0.01) \), as exemplified in Figure 3B, where a comparison of activity evoked with a 7 kHz tone to that elicited with an electrical pulse is shown \( (r = 0.692, P < 0.01) \). Comparison between activities evoked electrically at the 1 kHz site (Fig. 3Ab) to activities evoked by a 16 kHz tone in the same animal revealed no correlation \( (r = -0.100) \). The same was true when activities evoked at the 7 kHz site (Fig. 3Bb) were compared to the activities evoked by a 1 kHz tone in the same animal \( (r = 0.108) \).

To further compare the acoustically- and electrically evoked activities, activity contour defined by pixels having a response more than half the maximum response, was fitted with an ellipse with least mean squared error; and the ratio of the major axis to the minor axis of the ellipse, as well as the orientation of the major axis, was compared between the responses evoked by the two kinds of stimuli. Taking the difference in response latency into account, the response evoked by tone stimulation at 30 ms was compared to that evoked by electrical stimulation at 10 ms in the same animals \( (n = 15) \); the electrical stimulation was applied to where activity was initiated by the tone stimulation. As shown in Figure 4a, the ratio of axis was always more than one (>1.5 in most cases) for both kinds of stimuli. The ratio of electrically evoked activity was linearly related with the ratio of tone-evoked activity (Fig. 4a), Pearson’s correlation coefficient = 0.729; \( P < 0.005 \), suggesting again the similarity in the pattern of activities evoked by the two kinds of stimuli. The major axis of activity contour evoked by both kinds of stimuli had an angle between 69 and 116°, reflecting the dorsoventral propagation of the activities (Fig. 4b). This axis is likely to parallel the isofrequency contour demonstrated in guinea pig AI in single electrode studies (Redies et al., 1989a; Wallace et al., 2000). The relationship between the angle of the major axis of electrically evoked activity contour and that of tone-evoked activity contour could be well described by a straight line with a slope of one (Fig. 4b), Pearson’s correlation coefficient = 0.638; \( P < 0.01 \), suggesting that the activity

![Figure 1. Optical signals reflected activity in cortical layer II/III.](http://cercor.oxfordjournals.org/)

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**Figure 1.** Optical signals reflected activity in cortical layer II/III. A, An example of an optical response in AI to stimulation with a 16 kHz tone (60 dB SPL), detected at one channel of our photodiode array, using the voltage-sensitive dye RH-795. The trace is the mean of 16 trials. Upward is reduction in fluorescence. Onset of stimulus is defined as time 0. b, Depth profile of the fluorescence of RH-795. The left panel is a photomicrograph of a Nissl-stained slice of AI, prepared after in vivo optical recording. The right panel shows the fluorescence detected at pixels corresponding to the vertical midline of the left panel, plotted against depth (distance from the pia). The vertical axis of the plot was adjusted to represent the vertical distance in the left panel. It is clear from the figure that the fluorescence was primarily restricted to layer II/III. Scale bar in b = 0.2 mm.
evoked by the two kinds of stimuli propagated along axes of similar orientations. Together, the above results suggest that a focally-applied electrical pulse evoked activity propagation throughout an IS in AI that was similar to tone-evoked activation.

To test whether the activity propagation within an IS depends on the site of activation along the isofrequency axis, we moved the stimulating electrode within the strip. As exemplified in Figure 3 Aa–d, the electrically evoked activities propagated primarily along the dorsoventral axis regardless of the placement of the stimulating electrode, all resulting in activity patterns highly similar to the tone-evoked activity ($r_{ro} = 0.814$, $r_{oc} = 0.804$, $r_{ad} = 0.658$). Similar results were obtained in all 11 tested animals for all AI regions tested (1–16 kHz; $r = 0.690–0.907$; $P < 0.01$).

The effect of moving the stimulating electrode along the frequency axis is exemplified in Figure 5. Shown in Figure 5a–c are responses evoked at three successive pixels along the frequency axis. It is evident that the response area shifted towards right along with the electrode. In Figure 5d, the response amplitudes are plotted against pixel number along a rostrocaudal line at a dorsal (line 2), a middle (line 6) and a ventral (line 10) position. It can be seen from the figure that early during the response, the peak amplitude shifted exactly with the shift of the electrode in a pixel-wise manner at all positions (4 and 8 ms for line 2; 8 ms for line 6; 12 and 16 ms for line 10; arrows in Fig. 5d). At later times, the shift of peak positions no longer matched the electrode shift, but there was nevertheless a trend of shift of the peaks towards right, along with the electrode shift. Similar observations were obtained in all five animals studied. To quantify the overall shift of response with electrode shift, we calculated the mean spatial correlation coefficient between responses; the coefficient dropped from 1 to 0.49–0.67 for shift of one pixel, to 0.25–0.33 for two pixels and to 0.09–0.14 for four pixels ($n = 5$). These results suggest that early during the electrically evoked response, the location of the activated area is strictly determined by the position of the electrode along the frequency axis. The loss of strict match between electrode shift and shift of response peak during later times of the response, suggests an active role of cortical and probably extracortical circuits in determining the response amplitude at later times.

Changing the intensity of the electrical stimulus increased the size of activated area in the rostrocaudal direction (frequency axis), but did not significantly affect the overall response pattern (Fig. 6a). For intensities from 0.2 to 0.5 mA, all responses were significantly correlated ($r = 0.64–0.83$; $P < 0.01$), although the response evoked at 0.1 mA did not have a significant...
correlation with other responses (Fig. 6a; P > 0.05). Similar observation was obtained in six animals tested. The non-significant correlation between the response evoked at 0.1 mA to other responses is probably attributable to the weakness of the response. Changing the intensity of tone stimulation had similar effects, as exemplified in Figure 6b. The responses evoked by tones from 30 to 60 dB were all significantly correlated (r = 0.69–0.80, P < 0.01; Fig. 6b). Similar observations were obtained in all five tested animals. This result is consistent with previous findings in guinea pigs that most AI neurons are tuned to a narrow band of frequency at low stimulus intensities, but to a broadened band at higher intensities (Hellweg et al., 1977; Redies et al., 1989a). Increasing the stimulus intensity also enhanced the maximum response (Fig. 6), and shortened the response latency in the case of tone stimulation (Fig. 6b).

The above results suggest that neurons in an IS in AI are activated sequentially in an autonomous manner, once part of the strip is activated.

Circuits Responsible for the Electrically Evoked Activity Propagation

In Vivo Experiments

What circuits then mediate the propagation of activity evoked by the electrical pulse? Although the stimulation electrode was placed in AI, cortical as well as extracortical circuits may be involved in the propagation, because it is not known what neuronal element is activated by the electrical stimulation.

To address this issue, we first studied the velocity of activity propagation. The response latencies measured at pixels along a dorsoventral line, were plotted against distance from the activity initiation point; the latencies were found to be significantly correlated with distance (Pearson’s correlation coefficient was 0.830–0.990 for electrically evoked responses and 0.742–0.979 for tone-evoked responses; analysis of tone-evoked activity was limited to cases where the activity was initiated at one site). Propagation velocity was thus calculated as the slope of the regression line of the plot (Fig. 7a). For both acoustic and electrical stimulation, the propagation velocity changed little with the change of stimulus intensity (Fig. 7b; 0.0008 m/s/db for acoustic stimulation; 0.0229 m/s/mA for electrical stimulation). It is clear from Figure 7a–c that tone-evoked activities propagated faster than electrically evoked activities. This is better illustrated in Figure 7d, where the propagation velocity of tone-evoked activities was plotted against that of electrically evoked activities measured in the same animals (n = 11; tone pressure: 50–60 dB; electrical pulse amplitude: 0.3–0.4 mA; tone-evoked activity propagated at an average speed of 0.38 ± 0.10 m/s (n = 11), whereas electrically evoked activity propagated at a significantly lower speed.

![Figure 3](http://cercor.oxfordjournals.org/)

Figure 3. A single electrical pulse applied focally to AI evoked activity similar to that evoked with a tone. (A) A comparison of responses to a 1 kHz tone to those evoked by an electrical pulse. (a) The response to stimulation of a 1 kHz tone at 70 dB SPL. (b) The response to electrical stimulation of the cortex at a dorsal site, marked by the arrow in the first graph. (c) The response to electrical stimulation of the cortex at a middle site, marked by the arrow. (d) The response to electrical stimulation of the cortex at a ventral site, marked by the arrow. All recordings are from the same animal. Note the similarity in responses evoked by the tone and by the electrical stimulus at different locations. (B) A comparison of responses to a 7 kHz tone to those evoked by an electrical pulse in a different animal. (a) The response to a 7 kHz tone at 70 dB SPL. (b) The response to electrical stimulation of the cortex at a site where activity was initiated by the 7 kHz tone, marked by the arrow in the first graph. Again the patterns of activities evoked by the two kinds of stimuli are remarkably similar. All electrical pulses had a duration of 100 µs and a strength of 0.3 mA.
Figure 4. Comparison of the shape and orientation of electrically- and acoustically evoked activity patterns. Activity contour, defined by pixels having a response more than half the maximum response, was fitted with an ellipse with least mean squared error. (a) The ratio of the major axis to the minor axis of the ellipse fitted to the electrically evoked activity contour was plotted against that of acoustically evoked activity contour. The response evoked by tone stimulation at 30 ms was compared to that evoked by electrical stimulation at 10 ms in the same animals, because of the difference in latency; the electrical stimulation was applied to where activity was initiated by the tone stimulation. The regression line had a correlation coefficient of 0.729 ($P < 0.005$). (b) The angle of the major axis of the ellipse fitted to the electrically evoked activity contour was plotted against that of the acoustically evoked activity contour. The fitted straight line with a slope of one had a correlation coefficient of 0.638 ($P < 0.01$).

Figure 5. Effect of moving the stimulating electrode pixel-wise in rostrocaudal direction. (a–c) Responses to an electrical stimulation at the same strength (0.3 mA), applied at sites indicated by the arrow in each graph. The stimulation site was shifted to the right by one pixel from (a) to (b) and from (b) to (c). In (c) the electrode was also shifted downwards by one pixel to avoid a blood vessel. In (d) the response amplitude in pixels along a rostrocaudal line at a dorsal (line 2), a middle (line 6) and a ventral (line 10) position was plotted against the pixel number. Note early during the response, the peak of the amplitude profile shifted exactly in a pixel-wise manner as the electrode was shifted pixel-wise, at all positions (peaks marked by the arrows). At later times, the shift of peaks no longer matched the shift of electrode, but there was a trend for the peaks to shift with the electrode.
(0.26 m/s ± 0.08 m/s, \( n = 11; \ P < 0.05 \)). Test of significance for the difference in velocity between tone-evoked and electrically evoked activities in the same animal (e.g. Fig. 7a) also revealed that 10 out of 11 animals had significantly different velocities (\( P < 0.01; \ t\)-test). As shown below, the average velocity of activity propagation in a slice preparation of AI was 0.23 m/s at 34°C. Thus the velocity of electrically evoked activity propagation is close to the velocity in slices, suggesting the possibility that electrically evoked activity propagation is intrinsic to the cortex.

To produce the electrically evoked, patterned activity, i.e. activity propagation along an IS, cortico-thalamo-cortical interaction is likely to be involved, in addition to cortical intrinsic circuits. To test the involvement of cortico-thalamo-cortical connections, we chemically lesioned neurons of the auditory thalamus, and studied the effect of electrical stimulation in the deafferented AI. Selective destruction of thalamic neurons was achieved by targeted injection of ibotenic acid (see Materials and Methods; also see Song and Murakami, 1998). As exemplified in Figure 8, after the thalamic lesion, the response to a 4 kHz tone was diminished (Fig. 8a), but electrical stimulation of the tonotopic area evoked activity propagation similar to that in a normal animal (Fig. 8b). Similar observations with test tones of varying frequencies were obtained in all five lesioned animals. The propagation velocity of electrically evoked activity in lesioned animals was 0.25 ± 0.02 m/s (\( n = 8 \)), which is not significantly different from electrically evoked propagation in normal animals (0.28 ± 0.08; \( P > 0.05 \)) but is significantly different from the tone-evoked propagation in normal animals (0.38 ± 0.10; \( P < 0.01 \)). These observations argue against a major role of thalamo-cortical connections in the electrically evoked activity propagation in AI.

**In Vitro Experiments**

To further test the role of cortical intrinsic circuits in supporting activity propagation over an IS, we studied propagation of activity evoked by an electrical pulse in slice preparations that were deprived of subcortical circuits. Coronal slices of AI were prepared (see Materials and Methods) to study activity propagation along the isofrequency axis, and activity propagation along the frequency axis was examined in horizontal slices of AI. For comparison with our *in vivo* experiments, stimulation electrode was placed approximately 200 \( \mu \)m below the pia in most experiments. This depth corresponds to the upper part of the AI cortex.
Figure 7. The propagation velocity of electrically evoked activity was slower than that of tone-evoked activity. (a) The latency of tone-evoked activities and the latency of electrically evoked activities in pixels along a line in the dorsoventral direction in the AI of the same animal, plotted against distance from the activity initiation point. Propagation velocity was calculated as the slope of the regression line of the data. (b) The relationship between propagation velocity and tone pressure level. The propagation velocity changed little with stimulus intensity. Data from five animals. (c) The relationship between propagation velocity and the intensity of electrical stimulation. The propagation velocity changed only modestly with stimulus strength. Data from nine animals. (d) The propagation velocity of tone-evoked activity was plotted against that of electrically evoked activity measured in the same animals. The dotted line is the equal-velocity line. Electrically evoked activity propagated at a significantly lower speed ($P < 0.05$).

Figure 8. Lesion of the auditory thalamus abolished tone-evoked activity but spared electrically evoked activity. (a) Recordings showing the cortical response to a 4 kHz tone at 60 dB after chemical lesion of the ventral medial geniculate body (MGBv). Only weak responses of long latencies were detectable. (b) Electrical stimulation (0.3 mA) of the rostral part of AI, corresponding to the 4 kHz tonotopic area, evoked activity propagation as in a normal animal. (c) A photomicrograph showing the lesioned side of the MGB in a Nissl-stained section. Note the absence of cells in the ventral nucleus. Broken lines outline the area of total loss of neurons. (d) A photomicrograph showing the control side of the MGB in the same coronal section as in (c). The broken line marks the medial edge of MGBv. The scale bar in (d) is 0.5 mm and also applies to (c).
Cortical Intrinsic Circuits Can Support Activity Propagation through an IS

The initial piece of evidence for our conclusion is based on electrical stimulation in vivo. Electrical stimulation is often problematic when used to define functional circuits, because it is usually difficult to know which neuronal elements at the tip of the stimulating electrode are actually activated (Ranck, 1975). Therefore, all possibilities need to be carefully pursued. In the present case, electrical stimulation of AI may activate neurons in AI, but it may also activateafferent fibers to AI and efferent fibers from AI, as well as fibers of passage. Thus the electrically evoked activity in AI can be attributed either to circuits intrinsic to AI, thalamocortical interactions or a combination of these possibilities. Lines of evidence provided in the Results section strongly support the hypothesis that the propagation of electrically evoked activity through the span of an IS in AI, which occurred in the first 10 ms, is attributable to intrinsic circuits of AI.

The similarity between tone-evoked activity and electrically evoked activity demonstrated here is surprising, because a tone is expected to activate contrary to the finding that electrically evoked activity propagated a longer distance in cortical slices and propagated over a larger extent along the isofrequency axis; and (iv) electrically evoked activity in vivo propagated at a velocity comparable to that of lateral propagation in cortical slices, albeit it had a shorter latency and slower time course after initiation compared to tone-evoked activity. These observations strongly suggest that intracortical connectivity in guinea pig AI can support a focally evoked activity to propagate throughout an IS with a slower speed than tone-evoked activities.

Discussion

Using in-vivo and in-vitro real-time optical imaging, we showed in guinea pig AI that (i) an electrical pulse applied focally within an IS triggered autonomous activity propagation throughout the IS, with a pattern highly similar to the tone-evoked activation; (ii) the electrically evoked activity was not significantly affected by the chemical lesion of the auditory thalamus; (iii) electrically evoked activity propagated a longer distance in cortical slices and propagated over a larger extent along the isofrequency axis; and (iv) electrically evoked activity in vivo propagated at a velocity comparable to that of lateral propagation in cortical slices, albeit it had a shorter latency and slower time course after initiation compared to tone-evoked activity. These observations strongly suggest that intracortical connectivity in guinea pig AI can support a focally evoked activity to propagate throughout an IS with a slower speed than tone-evoked activities.
Activity Propagation in Primary Auditory Cortex

The velocity we measured in vivo is also close to that reported in the visual cortex of the macaque monkey (Grinvald et al., 1994). In contrast to our finding that electrically evoked activity propagated a longer distance along the isofrequency axis, a previous study on lateral activity propagation in rat auditory cortex has reported no difference with cortical orientation (Kubota et al., 1999). The reason for this discrepancy is not clear at this time, but it should be pointed out that neuronal connections at longer distances are more likely to be subjected to destruction by slicing. Previous anatomical findings that neuronal processes labelled in AI tend to elongate preferentially in the isofrequency direction (Matsubara and Phillips, 1988; Ojima et al., 1991; Read et al., 2001; Wallace et al., 2002) should be a major structural basis for our findings. The contribution of other mechanisms, such as selective synaptic connection in the isofrequency direction, awaits future studies.

An IS is originally defined in single electrode studies by the strip-like alignment of neurons having the same characteristic frequency (Merzenich and Brugge, 1973; Redies et al., 1989a; Ehret, 1997). In imaging studies, a pure tone, as demonstrated here, activates a strip-like area of the cortex, also referred to as an IS here. Although the IS demonstrated in single electrode studies and in imaging studies are obviously correlated in the sense that they have similar isofrequency axis orientation and that they show similar tonotopy, they might not be identical. The major cause for this difference is that ISs in single electrode studies are determined by near-threshold stimulation while in imagining studies suprathreshold stimulations are usually used. To suprathreshold stimulations, AI neurons still respond only to a limited range of frequency including the characteristic frequency (Merzenich and Brugge, 1973; Redies et al., 1989a; Ehret, 1997; Stecker et al., 2005). This explains why the ISs defined in single electrode studies and in imaging studies are closely correlated. Phillips et al. (1994) have actually mapped the response in cat AI to suprathreshold stimulations using single electrodes, and have demonstrated that at low stimulus intensities, the distribution of activated neurons roughly agrees with the threshold IS contour of the test frequency, although the distribution appears patchy. At high stimulus intensities (80 dB), the distribution of activated neurons no longer respects the threshold IS contour. This change in distribution pattern of activated neurons according to stimulus intensity is thought to be attributable to spatial segregation of monotonic and non-monotonic cells in AI (Phillips et al., 1994). Our results cannot be directly compared to those of Phillips et al. (1994) at high stimulus intensities, because the intensity was limited to 60 dB in most of our experiments. Up to this intensity, we did not find a dramatic change in response pattern (see Fig. 6b). One must be cautious, however, in comparing optical signals with unit recordings, because optical signals are thought to stress more on synaptic potentials (Grinvald et al., 1994; Arieli et al., 1996). How the monotonicity of cortical neurons would affect the spatial distribution of acoustically evoked activity is nevertheless an interesting subject to explore in future imaging studies.

Functional Significance

Our results suggest that cortical intrinsic circuits can support activity propagation throughout an IS. The response of AI neurons to tone stimulations would thus be expected to be the result of interaction between thalamic inputs and propagating activity in the IS. It is a task in future studies to elucidate how exactly this interaction occurs.

Tone-evoked propagating activity in AI has been reported before in imaging studies using voltage-sensitive dyes (Fukunishi et al., 1992; Taniguchi et al., 1992; Uno et al., 1993). The propagating nature of activity can also be inferred from progressive changes in response latency along the isofrequency axis, demonstrated in single unit recordings (Mendelson et al., 1997; Schreiner et al., 2000). Here we have characterized the propagation velocity of tone-evoked activity and have found that it was significantly faster than the propagation velocity of activity in cortical intrinsic circuits (see Fig. 7). Because activity propagation within cortical intrinsic circuits is slower, we interpret the velocity of tone-evoked activity propagation as the velocity of tangential dispersion of thalamic inputs over the cortex. This interpretation requires that the projection from the auditory thalamus covers the entire region of an IS demonstrated here, which needs to be further elucidated in future works (but see Redies et al., 1989b). The currently proposed temporal dispersion of thalamic inputs may originate from the thalamo-cortical neurons, but it may also be created at an earlier stage of the auditory pathway. The propagation of activity along an IS creates temporal delays in firing between neurons within the IS. The functional role of the delays is not clear at this time, but an obvious possibility is the use of the delay for temporal differentiation. Recent simulation studies have shown that a ‘delay layer’, a layer of modelled neurons responding to the same frequency but with progressively longer delays, may serve for detection of amplitude transients of sound (Fishbach et al., 2001, 2003). Neurons in an IS of guinea pig AI share the properties of ‘delay layer’ neurons, but it remains to be elucidated whether they work for detection of amplitude transients. Many response properties of AI neurons, such as excitatory bandwidth and intensity-dependent coding, have been shown to change along the isofrequency axis (Ehret, 1997; Schreiner et al., 2000). It would be interesting in future studies to examine the role of cortical intrinsic circuits and thalamocortical inputs in creating such spatial coding.

In summary, our results suggest that cortical intrinsic circuits primarily determine the distributed activation pattern in AI by a pure tone. The dynamic property of tone-evoked activity along the isofrequency axis is likely to be a reflection of temporally dispersed arrival of thalamic inputs, because cortical intrinsic circuits had a slower dynamics. Our finding that tone-evoked activity can be well reproduced with an electrical pulse applied through a single electrode should also have implications on restoring hearing for the deaf by electrical stimulation of the cortex.

Notes

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