In the auditory cortex, primitive features of acoustic stimuli are represented for auditory scene analysis. A typical example of a feature representation is the tonotopic map, in which sound frequencies are spatially arranged in an orderly manner. Some neurons in the auditory cortex are sensitive to sound source location, which is another important feature for auditory scene analysis. In the present study, using the intrinsic optical imaging technique, we attempted to visualize the two-dimensional pattern of neuronal population responses in the primary auditory cortex of rats to pure tones presented at various frequencies and sound intensities. The observed arrangements of sound frequencies were consistent with those obtained by electrophysiological mapping, which indicates that our intrinsic optical recording can visualize population responses of neurons. We also found different temporal patterns of intrinsic signals elicited in response to contralateral, ipsilateral, and bilateral ear stimulations. Finally we try to explain the observed differential time courses of intrinsic signal responses from the theoretical point of view on the conduction of neural activities, based on the so-far anatomically identified neural pathways in the rodent auditory system.

**INTRODUCTION**

In the primary auditory cortex (AI), the characteristic frequencies and binaural response properties are represented tangentially [1–5]. The tonotopic representation is considered to be involved in the auditory scene analyses, such as pitch perception and sound source localization. Thus far, the orderly representations of information inherent in acoustic stimuli have been investigated mainly using electrophysiological mapping [6,7], Tonotopic arrangements in the auditory cortex have also been examined using PET and fMRI in humans [8,9] and intrinsic optical imaging in rodents [10,11]. In this study, to visualize spatio-temporal representations of acoustic features in AI, we recorded intrinsic optical signals from anesthetized rats in acute experiments. Our intrinsic optical recording could reveal tonotopic arrangements of sound frequencies that significantly evoke intrinsic signals. The obtained tonotopic maps were consistent with previously reported electrophysiological maps of characteristic frequencies in rat AI. We also investigated temporal patterns of intrinsic signals elicited in response to contralateral, ipsilateral, and bilateral ear stimulation with a pure tone. The resulting time courses indicated contralateral dominance in the maximum response to the stimulus. The bilateral ear stimulation evoked intrinsic signals whose time courses were quite similar to those evoked by contralateral ear stimulation, but the magnitude of the strength of the signal was weaker. The magnitude of signals elicited in response to ipsilateral ear stimulation rapidly increased following a latency of about 100 ms after the stimulus onset, and the signals where sustained long after the stimulus offset. These findings imply that ipsilateral stimuli modulate cortical activities in response to contralateral stimulation in a manner of gain control. To interpret our experimental results, we propose a hypothetical mechanism of the conduction of neural activities along the so-far anatomically identified neural pathways in the rodent auditory system.

**MATERIALS AND METHODS**

Imaging of intrinsic signals was performed in 26 adult rats (Sprague–Dawley: weight 250–500 g; age 2–3 months). For anesthetization, atropine sulfate (0.04 mg/kg body weight, i.m., Bayer Corp.) followed 5 min later by xylazine hydrochloride (2.0 mg/kg body weight, i.m., Bayer Corp.) and ketamine hydrochloride (20 mg/kg body weight, i.m.; Ketalar-50, Parke-Davis) was injected [7]. Supplementary ketamine hydrochloride (10 mg/kg body weight, i.m.) was injected about 1 h later to maintain a constant level of anesthesia during the experiment [7]. The anesthetized level was determined based on the heart rate, which was continuously monitored using an electrocardiometer. The rat’s temperature was monitored and maintained with an adjustable heating pad. An acrylic pedestal was affixed to
the surface of the skull and anchored by a steel stick on the stereotaxic frame without ear bars. At the end of the experiments, the animals were sacrificed by an overdose of nembutal (200 mg/kg body weight, i.p., Bayer Corp.). All procedures were carried out according to the standards of the animal care committee of RIKEN.

Surgical preparation: At the start of surgical preparation, the skin at the dorsal and lateral parts of the head was removed. The left lateral wall of the skull was exposed by removing the temporal muscle. The cranium above the auditory cortex was removed [12] using a dental drill.

Optical recording: Using a headholder, the animal was rigidly fixed with dental cement onto the stereotaxic frame placed on a vibroisolated table. A chamber made of dental wax (4–6 mm i.d.; height 3–4 mm) was constructed above the hole on the skull. To suppress the cortical tissue pulsation originating from respiratory and cardiovascular movements, the chamber was filled with agar, which is important for the stable recording of intrinsic signals. Then the chamber was sealed with a cover glass to further suppress the cortical pulsation. The CCD camera (320 × 240 pixels, CS-8310, Tokyo Electronic Industry Corporation) was positioned above the chamber and directed such that its optical axis was perpendicular to the cortical surface as much as possible. The focusing plane was manipulated 300 μm below the cortical surface. Intrinsic signals were recorded using the Capos system (Laboratory for Integrative Neural System, RIKEN), on the Windows NT platform. The recording area of the cortex was illuminated with a light of 540 nm wavelength [10,13,14] delivered through dual optic-fiber lightguides.

At the start of the optical recording, the gray-scale image of the region of interest (ROI) was obtained. Each stimulus consists of a pure tone of 500–16 000 Hz generated by the Wave Factory 1941 system (NF Electronic Instruments), and the duration of stimuli was set at 200 ms. The sound stimulus was presented via small earphones. The linearly increasing onset and decreasing offset of stimulus envelops were set at 20 ms. All stimuli were calibrated before optical recording and the sound pressure was set at 45 dB. During recording, the stimuli were presented in a quasi-random sequence, and the interstimulus interval was set at 12 s. The sound stimulation began at the first frame and ended at the onset of the sixth frame. The intrinsic signals from the auditory cortex elicited in response to different stimuli during each recording session were collected in five frames of 330 ms each in the case of investigation of tonotopicity and 20 frames of 33 ms each in the case of investigation of time courses of the signals.

Data analysis: Analysis of the recorded image data was performed using IDL (Research Systems Inc., Boulder, Co.). Since optical signals in the first frame were taken prior to the presentation of sound stimuli, they contained only random fluctuations irrelevant to neural activities and no signals that provided information on the pattern of stimulus-driven activities. To extract the images reflecting neural activities in response to sound stimuli, we applied the so-called first-frame analysis [13,15,16], in which signals in the first frame were subtracted from signals in all the subsequent frames.

The images were the filtered with a band-pass filter composed of two Gaussian kernels passing signal components whose wavelengths were between 40 μm and 1000 μm [16]. The filtered images were combined to construct a frequency map. The temporal changes of signals after the onset of a stimulus (3000 Hz, 45 dB, duration of 200 ms) for contralateral, ipsilateral and bilateral stimulations were examined in the small rectangular domain of 40 × 60 pixels (1.0 × 1.5 mm²) inside the ROI. To define the position of this domain, we employed the following two criteria: (1) The significantly activated domain should be contained in this rectangle; and (2) thick blood vessels should not be located inside this rectangle. The time courses of the optical signals were averaged over the total number of rats examined. Generally, there is fluctuation of net signal strengths among individual rats. To eliminate such fluctuation, before the averaging procedure, we normalized signals using the maximum levels of signals in response to contralateral stimulation of individual rats. The temporal changes of light reflectance images are shown in Fig. 3 using the color scale. To construct tonotopic maps in color representation, images of optical signals were first converted in the range of 0–255 (8-bit grayscale image) [16]. Figure 1 shows 8-bit grayscale images in which dark areas represent stronger intrinsic signals produced by cortical regions activated by the sound stimulus. The intrinsic signals were then thresholded by 50% of the maximum response inside the ROI to determine the areas significantly activated by a particular frequency of sound. Thus, the obtained strongly activated areas were colored according to the stimulus frequencies and superimposed on the image of the cortical surface [14], as shown in Fig. 2.

RESULTS
Intrinsic signals elicited in response to pure tone stimuli of frequencies from 500 Hz to 8000 Hz at the sound pressure of 45 dB were observed in a cortical area anatomically corresponding to AI of the left hemisphere for all the rats examined. Figure 1 shows the cortical domains differentially responding to the stimuli of different frequencies, which indicates a tonotopic representation of sound frequencies. Figure 2, we show three examples of tonotopic maps in which the stimulus frequencies are indicated by colors. Low frequencies were represented in the posterior part of AI and high frequencies in the anterior part, although there was considerable overlapping in the domains activated by different sound frequencies. The frequencies that evoked strong signal responses were in the range from 2000 Hz to 5000 Hz; the domains activated by frequencies < 2000 Hz or > 5000 Hz occupied smaller cortical territories. All these properties of frequency representation could be reproducibly observed in the rats examined. There was a tendency of relatively smaller overlapping among domains activated by different sound frequencies at lower sound pressures (data not shown). These observations are consistent with previous findings, obtained by electrophysiological mapping in terms of the location and direction of the representation of characteristic frequencies. This indicates that our intrinsic optical signals are relevant to populational activities of
cortical neurons, although the recording method only indirectly measures neural activities through metabolic changes.

Next, time courses of the optical signals were investigated using 11 rats stimulated by a pure tone of 3000 Hz at 45 dB, which was the frequency that activated larger territories in the ROI. There was no difference in location of activated domains among contralateral, ipsilateral, and bilateral stimulations, but the temporal patterns of evoked signals were different (Fig. 3). The time courses of the optical signals, which were averaged over the total number of rats examined after normalization of signal strengths, are shown in Fig. 4. Each division on the horizontal axis indicates one frame, in which the duration of a single frame was set at 33 ms. The vertical axis represents the normalized level of absorption of 540 nm light in a rectangular domain of 40 x 60 pixels which contained a strongly activated domain, as shown in Fig. 1.

For contralateral stimulation, the stimulus-driven optical signals appeared at the frame immediately after the stimulus onset, reached a maximum at the 10–12 frames (about 350 ms after the stimulus onset), and then decayed monotonically during the following few hundred milliseconds. For ipsilateral stimulation, the signals were weaker than those elicited following contralateral stimulation, but were sustained for as long as 400 ms. Interestingly, the magnitude of signals rapidly increased following a latency of about 100 ms after the stimulus onset. For bilateral stimulation, the signals were also weaker but the time course was quite similar to that for contralateral stimulation. It should be noted that the time courses for each type of stimulation were not markedly different for different rats.

**DISCUSSION**

Intrinsic optical signals reflect metabolic activities rather than neuronal electric activities. Since metabolic activities partially reflect neural activities evoked by particular stimuli, the visualization of functional maps has been possible based on these activities in the primary visual cortex of cats, ferrets and monkeys [13,16]. Therefore, we can reasonably assume that the intrinsic optical recording can also be applied to the visualization of acoustic feature representation in AI. Indeed, the tonotopic representation of sound frequencies in this study demonstrated that the location and direction of sound frequency arrangement closely agree with those of the arrangement of the best frequencies characterized electrophysiologically in rat AI [2,6,17]. The consistency in tonotopic maps with electrophysiological recording indicates that intrinsic optical recording is a powerful tool for measuring activities of
Fig. 3. Diagram of sound stimulus presentation and data acquisition of intrinsic optical signals, where image data in a single data acquisition are divided into 20 subsequent frames of 33 ms duration. The optical signal patterns inside the ROI at the 1st, 10th and 20th frames, which where elicited to contralateral, bilateral and ipsilateral ear stimulations are shown below the diagram. For the time course analysis, signal strengths are estimated as sums of signals at each pixel within the small rectangular domain placed inside the ROI.
neuronal population even in AI. Indeed, recent studies using the optical imaging of intrinsic signals in the auditory system of guinea pigs [10] and chinchillas [11] have shown tonotopic maps.

The notable feature in the present experimental findings is the difference in temporal patterns of intrinsic signals elicited in response to contralateral, ipsilateral, and bilateral stimulations. Among the three types of stimulations, the strongest intrinsic signals were elicited following contralateral stimulation, whereas the time courses were quite similar between contralateral and bilateral stimulations. These observations indicate dominance of contralateral inputs, as observed previously in electrophysiological recording studies [2,18,19]. On the other hand, the time course for ipsilateral stimulation was completely different. The magnitude of observed intrinsic signals rapidly increased about 100 ms after the stimulus onset to reach the maximum and the signals were then sustained long after the stimulus offset.

To date, contralateral dominance has been suggested based on the strength of neuronal response in electrophysiological recording [6,7]. Our finding implies that aside from the contralateral dominance in neuronal response, the neural pathway [20] that sends information on stimuli to the contralateral ear is the main stream for the auditory information processing in AI, because the time course of intrinsic signals elicited in response to bilateral stimulation was quite similar to that for contralateral stimulation but different from that for ipsilateral stimulation. In addition, the observation that intrinsic signals in response to bilateral stimulation were weaker than those in response to contralateral stimulation indicates that a neural pathway originating in the ipsilateral ear for suppressing neural response in AI is evoked by contralateral stimulation. The observation that the time course of intrinsic signals in response to ipsilateral stimulation was sustained long after the stimulation offset suggests the involvement of reverberating circuits in the neural pathway for conduction of activities evoked in response to ipsilateral ear stimulation. Interestingly, we can observe the residual response after the stimulus offset for bilateral stimulation as well which was statistically significant. Such a residual response could not be observed in the time course of intrinsic signals for contralateral stimulation. This long-tailed but weak residual response is, in turn, suggested to be the effect of contralateral suppression of responses to ipsilateral stimulation. Therefore, if there are reverberating circuits, they should be observed following the contralateral inhibition.

To interpret our findings of differential cortical responses to different types of stimulation to the ears, we postulate the following hypothetical mechanism of the conduction of neural activities based on the well identified neural pathways in the rodent auditory system, as shown in Fig. 5.
Neurons in the lateral olivary nucleus, which receives direct inputs from the ipsilateral cochlear nucleus, are inhibited by inputs from the contralateral cochlear nucleus [21]. It has been reported that the medial geniculate body sends feedback inputs to the ipsilateral inferior colliculus [3,22] and the inferior colliculus also sends axons back to the lateral lemniscus [3] and the lateral olivary nucleus [7,22]. Therefore, the presence of these feedback connections may support the involvement of reverberating circuits in the ipsilateral pathway. In AI, some neurons receive inputs originating from the contralateral ear, whereas some neurons receive inputs from the ipsilateral pathway with the reverberating circuits. The observed intrinsic optical signals should be the sum of signals elicited from both types of neurons. Consequently, for contralateral stimulation, signals show a phasic response evoked only by the contralateral pathway; for ipsilateral stimulation, signals show a sustained response due to the reverberating circuits in the ipsilateral pathway in the absence of contralateral inhibition; and for bilateral stimulation, a weak sustained response due to the reverberating circuits suppressed by the contralateral inhibition and phasic response driven by the contralateral stimulation.

In the present study, we observed that intrinsic optical signals were elicited unexpectedly fast after the stimulus onset. Fast components of intrinsic optical signals have been recorded in some brain regions including the auditory cortex [5,23]. These data support the claim that the fast components are related to neural activity. Probably, such signal components are localized within an area ≤ 5–10 mm in diameter [24]; therefore, fast optical imaging can be used to obtain the time course of brain activity in localized cortical areas [5]. Although the physiological mechanism of the fast change of optical properties of the brain tissue has not been fully clarified yet, it is currently presumed that movement of ions across the neuronal membrane might play a central role, probably because ions carry with them a large amount of water molecules due to both osmotic and ionic forces [23,24]. We think that recording of the fast optical signals can be applied to the observation of neuronal population activities.

**CONCLUSION**

Tonotopic maps were obtained, which can in turn be regarded as proof of the validity of our recording method. Different temporal patterns of intrinsic signals elicited in response to contralateral, ipsilateral, and bilateral ear stimulations are novel findings of our present study. We suggest that neural pathways composed of both afferent and efferent projections may be involved in the differential transfer of information from the contralateral and/or ipsilateral ears to AI of the cortical hemisphere.

**REFERENCES**