INTRODUCTION

The presentation of a sinusoid with a small interaural frequency difference (IFD) in each ear elicits various perceptions resulting from neural interactions in the central auditory pathway, which receives input from both ears. When the IFD is zero, a single fused image is heard. Furthermore, the image is centered in the listener’s head when both the interaural differences in phase and level are zero. If the IFD is sufficiently large, two discrete images are heard (Perrott and Barry 1969). In the transitional range from a single stationary fused image to two nonfused images, a continuum of subjective effects is perceived (Perrott and Musicant 1977). These sensations are called “binaural beats” (BBs). Licklider et al. (1950) suggested that in a broad sense, BBs could be described as a triad of subjective effects. When the IFD is small, a single auditory image moves between the two ears, depending on the phase of the two signals (Kuwada et al. 1979). This movement perception has been called the “rotating tone” (Perrott and Musicant 1977). As the IFD increases, the localization effect is replaced by a beating tone with a rate equal to the IFD. This perception corresponds to “BBs in the narrow sense” and has been described as “a periodic fluctuation in loudness” (Perrott and Musicant 1977) or “a fluctuation in amplitude” (Yin and Kuwada 1983b). Further increases in the IFD produce a fast-beating tone (roughness), which gives way to a smooth bitoneal experience (Licklider et al. 1950).

In the cochlea, neural spikes tend to occur at a particular phase of the sinusoidal waveform (phase locking), and information on the phase of the acoustic stimuli is preserved in the auditory nerve fibers (Palmer and Russell 1986) and the spherical bushy cells of the antero-ventral cochlear nucleus (Goldberg and Brownell 1977), the axons of which project to the medial superior olivary (MSO) nucleus (Goldberg and Brown 1969; Smith et al. 1993). In the central auditory system, the interaural relative phase is detected by integrating information from each ear. BB reflects an orderly and continuously changing interaural phase difference (IPD) through one IFD cycle. Electrophysiological studies showed that when BBs are presented to mammals, the central auditory system responds to a continuously changing IPD. Kuwada et al. (1979) found that the responses of cat inferior colliculus neurons are phase-locked to the frequency of BBs. Moreover the discharges are highly periodic and synchronize to a particular phase of the BB cycle (Yin and Kuwada 1983a). Reale and Bruggé (1990) found that some neurons in the primary auditory cortex (PAC) of anesthetized cats are sensitive to the dynamically changing IPD created by BBs.

To detect noninvasively responses synchronized to BB in the human brain, we must record the corresponding neuronal responses in a steady state because BB is a periodical hearing phenomenon caused by continuously changing IPD. A steady-state response is an evoked potential the constituent discrete frequency components of which remain constant in amplitude and phase and is considered stable over a temporal window much longer than the duration of a single stimulus cycle. Steady-state responses are recorded when stimuli are presented periodically, and they demonstrate how the brain follows a stimulus or how the stimulus drives a response (Picton et al. 2003; Regan 1989). In previous studies using click trains (Forss et al. 1993; Galambos et al. 1981; Gutschalk et al. 1999; Mäkelä and Hari 1987) or amplitude-modulated (AM) tones (Engelien et al. 2000; Herdman et al. 2002; Pantev et al. 1996; Ross et al. 2002), conventional auditory steady-state responses (ASSR) were recorded as brain activity synchronized to peri-
odic acoustic stimulations. In human, ASSR to BB with IFD of 40 Hz was recorded by electroencephalography (EEG) (Schwar and Taylor 2005). The IFD of 40 Hz was adopted because ASSR at this rate was easily evoked in preceding studies using click train or AM tones. However, BB with this IFD does not correspond to BB in the narrow sense with subjective fluctuations.

Two points should be stressed about the importance of investigating ASSR to BB. First, a precise coding of tone frequency by phase locking in the peripheral auditory system on each side is indispensable for the detection of BB. The BB ASSR assesses this ability of temporal coding, which is also related to other auditory functions, such as speech and musical pitch recognition. Various diseases of the peripheral (Karino et al. 2005) and central auditory systems would be expected to perturb the temporal coding. Thus the binaural beat ASSR may enrich the diagnostic repertoire of the EEG and magnetoencephalography (MEG). Second, brain activities related to BB contain information induced by integrating information from both ears. Therefore even if neuronal responses to BB are analyzed in only one hemisphere, the results reflect the information from one ear to which a higher frequency tone is presented and the other ear to which a lower tone is presented. Furthermore, whether the higher tone is presented to the ear ipsilaterally or contralaterally from the viewpoint of the observed hemisphere may influence the findings in one hemisphere.

The periodic loudness fluctuations of BBs are based not only on the properties of acoustic stimulation itself but also on a product of binaural integration in the brain. The goal of this study was to verify whether the fluctuations of BBs are represented in ASSR by using MEG with high temporal resolution.

**Methods**

**Subjects**

Nine normal-hearing subjects (6 males, 3 females; age, 23–57 yr; 36.1 ± 11.2 (SD) yr) participated in this study. The subjects had no history of otological or neurotological disorders and had normal audiological status. Handedness was established with the Edinburgh handedness questionnaire (Oldfield 1971). Laterality quotients ranged from 89.5 to 100, except for one subject whose value was 0. Informed consents were obtained from all subjects after a full explanation of the aim and methods. The procedure used in this study was approved by the Ethics Committee of the University of Tokyo and conducted in accordance with the principles of the Declaration of Helsinki.

**Stimulation**

Continuous pure tones were played on an Apple personal computer via MOTU 828 (Mark of the Unicorn, MA) audio interface and led to ER-3A (Etymotic Research, Elk Grove Village, IL) foam insert earphones with extended plastic tubes. The transmission delay of ~6 ms was compensated by an appropriate shift of the trigger signal. The earphones had almost a flat frequency response in the range from 100 to 1,000 Hz. First, hearing thresholds for continuous pure tones of 240 and 480 Hz were determined in both ears of all subjects seated in the same conditions as with a MEG recording in a shielded room. The intensity of pure tone was set at 40 dB above each subject’s sensation threshold to prevent cross-hearing. In healthy subjects with intact external and middle ears, bone conduction plays a negligible role at this level because the conduction between ears is attenuated at 50–60 dB (Zwislocki 1951, 1953). Therefore the addition of a contralateral tone 40 dB above the ipsilateral threshold would not perturb neuronal phase locking sufficiently to affect the sound source localization (Blauert 1996) or BB patterns. In our experiments, 4.00 and 6.66 Hz were employed as IFD. We realized 4-Hz BB by the combination of 240.00 and 244.00 Hz and that of 480.00 and 484.00 Hz. Moreover, 6.66-Hz BB was realized by the combination of 240.00 and 246.66 Hz and that of 480.00 and 486.66 Hz. Measurement under control conditions with a binaural presentation of the same pure tones of 240.00 or 480.00 Hz with the same starting phase (no-BB condition) was also executed to elucidate the characteristics of responses evoked by BB. One run of BB or no-BB presentation lasted for 5–10 min. In total, averaged steady-state responses in eight types of BB conditions and two kinds of no-BB conditions were recorded for each subject (Table 1). The subjects were instructed to watch a silent video movie on a screen to maintain vigilance level during recording.

**Recording**

Neuromagnetic cortical signals were recorded with a whole scalp neuromagnetometer (Vectorview; Neuromag, Helsinki, Finland), which has 204 first-order planar gradiometers. However, we found a channel that was noisy in the left antero-temporal zone, which was then inactivated. Thus a total of 203 channels were used for actual measurements. During the recordings, the subjects were seated under the helmet-shaped dewar in a magnetically shielded room. The position of the head under the helmet was determined by attaching four coils to the head surface and measuring the coil positions with respect to the helmet-shaped dewar in a magnetically shielded room. The position of the head under the helmet was determined by attaching four coils to the head surface and measuring the coil positions with respect to the helmet-shaped dewar in a magnetically shielded room.

**Table 1. Combination of frequency to the left ear (FL) and to the right ear (FR) and intensity of steady-state responses in each condition**

<table>
<thead>
<tr>
<th>FL, Hz</th>
<th>FR, Hz</th>
<th>IFD (FL − FR)</th>
<th>Left 39 Channels</th>
<th>Center 124 Channels</th>
<th>Right 40 Channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Hz BB</td>
<td>240.00</td>
<td>244.00</td>
<td>−4</td>
<td>2.44 ± 0.23</td>
<td>1.95 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>244.00</td>
<td>240.00</td>
<td>+4</td>
<td>2.52 ± 0.27</td>
<td>2.12 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>480.00</td>
<td>484.00</td>
<td>−4</td>
<td>2.62 ± 0.40</td>
<td>2.23 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>484.00</td>
<td>480.00</td>
<td>+4</td>
<td>3.05 ± 0.68</td>
<td>2.61 ± 0.52</td>
</tr>
<tr>
<td></td>
<td>240.00</td>
<td>246.66</td>
<td>−6.66</td>
<td>2.70 ± 0.36</td>
<td>2.23 ± 0.22</td>
</tr>
<tr>
<td>6.66-Hz BB</td>
<td>246.66</td>
<td>240.00</td>
<td>+6.66</td>
<td>2.72 ± 0.34</td>
<td>2.33 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>480.00</td>
<td>486.66</td>
<td>−6.66</td>
<td>2.78 ± 0.42</td>
<td>2.40 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>486.66</td>
<td>480.00</td>
<td>+6.66</td>
<td>2.58 ± 0.35</td>
<td>2.29 ± 0.29</td>
</tr>
<tr>
<td>No-BB</td>
<td>240.00</td>
<td>240.00</td>
<td>0</td>
<td>2.16 ± 0.27</td>
<td>1.82 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>480.00</td>
<td>480.00</td>
<td>0</td>
<td>2.08 ± 0.26</td>
<td>1.83 ± 0.25</td>
</tr>
</tbody>
</table>

In each of the eight types of binaural beat (BB) conditions [interaural frequency difference (IFD) = FL − FR = −6.66, −4, +4, or +6.66] and the two types of no-BB conditions (IFD = 0), the mean ± SE values (n = 9) of root mean square (RMS) intensity of the magnetic field (fT/cm) are shown separately for the three areas.
to landmarks on the skull with a three-dimensional (3-D) digitizer; the coil locations in the magnetometer coordinate system were determined by leading current through the coils and measuring the corresponding magnetic fields. The recording passband was 1.0–200 Hz, and the data were digitized at 600 Hz. A vertical electrooculogram (EOG) was recorded simultaneously, and all traces of EOG activity >150 μV or signals in planar gradiometers >3,000 fT/cm were excluded from the on-line averages. The averaged signals were low-pass filtered at 40 Hz.

In single measurements using each combination for BB, the magnetic fields were recorded while 1,000–2,000 cycles of BB were presented to obtain steady-state responses. The analysis time of four BB cycles was used, and consequently 250–500 responses were averaged on-line. Figure 1 demonstrates the arrangement of triggers for averaging. Each trigger was transmitted from the tone generating PC to the neuromagnetometer at the moment the two sinusoids crossed the zero line and IPD was zero (Fig. 1, A and B). However, fewer triggers with an interval of four BB cycles were employed for actual averaging to display a periodic fluctuation of responses evoked by four cycles of BBs (Fig. 1, C and D). The mean amplitude of the four BB cycles was used as the baseline.

To explore the effect of probable noise, measurements without a subject were performed. The earphones were attached to the portions corresponding to ears in the helmet-shaped dewar, and the same measuring procedure was conducted with each of the eight kinds of BB and two types of no-BB stimulations.

**Spectral analysis on each channel**

Fast Fourier transform (FFT) spectra were calculated on each channel across 8,192 samples of the continuously recorded magnetic field signals, and the FFT window was moved in steps of 4,096 samples; this procedure resulted in a frequency resolution of 0.074 Hz. Approximately 70 spectra were averaged to improve the signal-to-noise ratio. On each channel of each subject, spectra in BB conditions were compared with those in a corresponding no-BB condition within a spectral range of 1–20 Hz.

**Minimum-norm current estimate**

Source localization was performed using L1 minimum-norm estimation. The L1 estimation results in a current distribution with the smallest integral of the absolute value of the current density that could generate the measured magnetic field and provides a minimum-norm current estimate (MCE) (Uutela et al. 1999) with location and strength information for the current sources at each time point. The origin of the spherical model was determined for each subject based on his/her anatomical magnetic resonance image (MRI) by fitting a sphere to the curvature of the outer surface of the brain. For the source localization of BB ASSR, MCEs were calculated separately in each condition for each subject in the period of one BB cycle displayed in Fig. 1.

**RESULTS**

**Wave configurations of steady-state responses**

All the participants in our study reported that both 4 and 6.66 Hz BBs containing 240- or 480-Hz tones allowed them to perceive BB in the narrow sense. Steady-state responses with dominant amplitudes in the temporal channels were recorded from both hemispheres in all subjects. Figure 2A shows a typical example of averaged responses in a subject, evoked in 4-Hz BB and corresponding no-BB condition as control. In this example, four peaks were clearly recognized under BB stimulation, mainly in the temporal channels in the time window that corresponds to four BB cycles, but no such peaks were identified under control conditions. Figure 2, B and G, displays enlarged wave configurations on demonstrative channels selected from the left and right temporal channels, respectively. In most subjects and in most BB conditions, isofield contour maps showed a clear dipolar pattern over the temporal area in both hemispheres. Figure 2, B and C, displays examples of isofield contour maps in the left and right hemisphere, respectively. These field patterns imply a dominant current source in each temporal area. However, the time point at which such dipolar patterns were observed varied across subjects, conditions, and hemispheres, even in a single subject. The time points of magnetic fields shown in Fig. 2, B and C, are not the same because they were selected independently within one BB cycle to demonstrate a typical dipolar pattern in each hemisphere.

**Intensities of steady-state responses**

First, to present the time course of magnetic fields, the root mean square (RMS) of the magnetic field amplitude was calculated separately in the left (Fig. 2D) and right (E) temporal channels and also in the remaining 124 channels (center). The selected temporal channels covered the perisylvian areas, including the auditory cortices. Next, to present the mean intensity of the magnetic fields, we calculated the temporal

**FIG. 1.** A: 1 cycle of binaural beat (BB). In this example, the frequency of the sinusoid delivered to the left ear (FL: thin line) was lower than that delivered to the right ear (FR: thick line). For a schematic illustration, the amplitudes of the 2 sine waves are different, and the frequencies of the tones are much lower than those actually used. This illustration shows the procedure in which the interaural phase (IPD = right phase – left phase) slowly shifted from the left ear leading (−π < IPD < 0) to the right ear leading (0 < IPD < π) through 1 complete cycle of the beat frequency. B: each trigger (arrow) was transmitted to the neuromagnetometer at a moment when the 2 sinusoids crossed the 0 line and IPD was 0. C: fewer triggers with analysis time equivalent to 4 BB cycles were employed for actual averaging to display the periodic fluctuation of responses evoked by 4 cycles of BB. D: example of an averaged wave of magnetic field on a single channel.
mean of the RMS in one BB cycle at the center of the analysis period. Figure 3 shows the grand mean (±SE) values (n = 9) of the temporal mean of RMS intensity. The RMS measured without a subject (8 types of BB and 2 types of no-BB conditions) were much smaller in each of the three areas (left, center, and right). The intensity of magnetic fields under BB condition was ~2.5 times as large as the noise intensity demonstrated under no-body condition, and the intensity of magnetic fields under no-BB condition was about twice as large as the control. To examine the statistical significance of this finding, we first applied two-way ANOVA adopting three conditions (BB vs. no-BB vs. no-body) and three areas (left vs. center vs. right) as factors, although the correspondence of subjects was neglected. There were 72 data (8 types of BB stimulations * 9 subjects), 18 data (2 types of no-BB stimulations * 9 subjects), and 10 data (8 types of BB and 2 types of no-BB stimulations) under BB, no-BB, and no-body conditions, respectively. No significant main effect of areas was found; however, the main effect of conditions [F(2,4) = 26.38, P < 0.001] was significant. A post hoc test using Bonferroni’s multiple comparison procedure revealed that RMS intensity under BB condition was significantly greater than those under both no-BB (P = 0.013) and no-body (P < 0.001) conditions. The RMS intensity under the no-BB condition was significantly greater than that under the no-body condition (P < 0.001). There was no significant condition-area interaction. These results indicate that magnetic fields under BB and no-BB were large enough to be distinguishable from the noise level. Table 1 shows the details of RMS intensity under BB and no-BB conditions. To evaluate precisely the RMS intensity under BB and no-BB conditions using the correspondent data across subjects, we applied two-way repeated-measures ANOVA adopting 10 types of stimuli (including 8 types of BB and 2 types of no-BB stimulations) and three areas (left, center,
or right) as within-participant factors. No significant main effect of stimuli was found, though the RMS intensities under the two types of no-BB conditions were the least and second least in the 10 types of stimuli. The main effect of areas (left or right) as within-participant factors. No significant main effect of tone frequency nor a tone frequency-IFD interaction was found. In each of the three areas, neither significant main effect of tone frequency nor a tone frequency-IFD interaction was found. In each of the three areas, there was no significant difference between the left and right areas, and no significant stimulus-area interaction was found.

Furthermore, the RMS intensities in BB and no-BB conditions were compared separately in each of the three areas by two-way repeated-measure ANOVA adopting tone frequency (240 or 480 Hz) and interaural frequency difference (IFD = FL – FR = −6.66, −4, 0, +4, or +6.66) as within-subject factors. In each of the three areas, neither significant main effect of tone frequency nor a tone frequency-IFD interaction was found. In each of the three areas, the RMS intensity in IFD of 0 (namely, under no-BB condition) was least in the five types of IFD; however, there was no significant main effect for IFD. These findings indicate a lack of systematic effect of tone frequency or IFD on RMS intensity in the three areas.

Spectral analysis on each channel

In each subject, we explored demonstrative channels on which a distinct peak was recognized at the corresponding BB frequency under BB condition and verified whether the peak represented specific synchronization to BB frequency. Figure 2, H and I, demonstrates the strategy adopted in our study to systematically explore the channels that show a spectral peak potentially relevant to a specific BB frequency.

FIG. 2. Steady-state responses by BBs and spectral analysis of each channel of a representative subject. A: averaged waveforms on 203 planar gradiometers, excluding 1 noisy channel. Responses evoked by BBs with 240-Hz tone in the left ear and 244-Hz tone in the right ear (4-Hz BB; black lines) and those evoked by a binaural presentation of 240-Hz tone (no-BB; gray lines) are arranged in parallel. Note the 4 peaks under 4-Hz BB condition in some channels, mainly in bilateral temporal areas. Demonstrative channels surrounded by rectangles are magnified in F and G. In the corresponding no-BB condition, there was no periodic fluctuation. The analysis time is equivalent to four cycles of 4-Hz BB = 1,000 ms. Temporal channels selected for evaluating amplitude in magnetic fields are surrounded by polygons (40 channels in the right hemisphere; 39 channels in the left hemisphere because of the exclusion of 1 noisy channel). The selected temporal channels covered the perisylvian areas, including the auditory cortices. B: isofield contour map on the sensor array in the left hemisphere. Outflux (red lines) and influx (blue lines) are stepped by 2 T. An arrow in F indicates the time point of this magnetic field, which was selected within 1 BB cycle at the center of the analysis period to demonstrate a typical dipolar pattern over the temporal area in the left hemisphere. C: isofield contour map on the sensor array in the right hemisphere. An arrow in G indicates the time point of this magnetic field, which was selected within 1 BB cycle at the center of the analysis period to demonstrate a typical dipolar pattern over the temporal area in the right hemisphere. Note that the time points of B and C are not the same because they were selected independently in each hemisphere. D: root mean square (RMS) of amplitude in magnetic fields was calculated to represent the time course of recorded fields in the left temporal channels. We adopted the temporal mean of the RMS in 1 BB cycle at the center of the analysis period to represent the mean intensity of the fields. E: RMS of amplitude in magnetic fields represented the time course of recorded fields in the right temporal channels. We adopted the temporal mean of the RMS in 1 BB cycle at the center of the analysis period to represent the mean intensity of the fields. F: magnification of the demonstrative channels in the left temporal area surrounded by the rectangles in A. Top: magnetic field under 4-Hz BB condition (L240R244 in our nomenclature). Four peaks are clearly recognized in the analysis time of 4 BB cycles. Bottom: magnetic field under the corresponding no-BB condition (L240R240 as control). Note the lack of periodic fluctuation. G: magnification of the demonstrative channels in the right temporal area surrounded by the rectangles in A. Top: magnetic field under 4-Hz BB condition (L240R244). Four peaks are clearly recognized in the analysis time of 4 BB cycles. Bottom: magnetic field under the corresponding no-BB condition (L240R240 as control). Note the lack of periodic fluctuation. H: fast Fourier transform (FFT) spectra on the same channel as that of F. Top: FFT spectra of response to L240R244 (4-Hz BB). Right: enlargement of the left panel around the BB frequency of 4 Hz. Thin horizontal line: the mean amplitude in the width of 1 Hz (from 3.5 to 4.5 Hz); dashed horizontal line: 95% confidence limit (1.64 times the SD) for response detection. The peak amplitude at just 4 Hz (arrows) is beyond the limit. Middle: FFT spectra of the response to L240R244 on the same channel are added (6.66-Hz BB as control). The amplitude at just 4 Hz is not beyond the 95% confidence limit. Furthermore, it was confirmed that the 4-Hz peak value in the top panels is also beyond the 95% confidence limit in the middle panels. Bottom: FFT spectra of response to L240R240 (no-BB as control). The amplitude at just 4 Hz is not beyond the 95% confidence limit. Furthermore, it was confirmed that the 4-Hz peak value in the upper panels is beyond the 95% confidence limit in the lower panels. Because the double requirements described in the text were fulfilled, we considered that the 4-Hz peak in this channel was specific to 4-Hz BB. I: FFT spectra on the same channel as that of G. Top: FFT spectra of response to L240R244 (4-Hz BB). The peak amplitude at just 4 Hz (arrows) is beyond the 95% confidence limit in the width of 1 Hz (from 3.5 to 4.5 Hz). Middle: FFT spectra of response to L240R244.66 on the same channel are added (6.66-Hz BB as control). The amplitude at just 4 Hz is not beyond the 95% confidence limit. Furthermore, we confirmed that the 4-Hz peak value in the upper panels was beyond the 95% confidence limit in the middle panels. Bottom: FFT spectra of response to L240R240 (no-BB as control). The amplitude at just 4 Hz is not beyond the 95% confidence limit. Furthermore, we confirmed that the 4-Hz peak value in the upper panels is beyond the 95% confidence limit in the lower panels. Because the double requirements described in the text were fulfilled, we considered that the 4-Hz peak in this channel was specific to 4-Hz BB.

FIG. 3. Grand means ± SE values (n = 9) of the temporal mean of root square (RMS) intensity determined separately in the left and right temporal channels and the other remaining 124 channels (center). The RMS measured without a subject (8 types of BB and 2 types of no-BB conditions) was smaller in each of the 3 areas. The intensity of magnetic fields in BB condition was 2.5 times as great in the left hemisphere than in the no-body condition, and the intensity of magnetic fields in the no-BB condition was about twice as great as the control.
Hz was beyond the 95% CI of amplitudes in the width of 1 Hz (from 3.5 to 4.5 Hz). To evaluate the spectral peak on each channel, we used two control conditions, namely, another BB (6.66-Hz BB) and no-BB stimulations, and we used these controls in two ways. The first application was to calculate the 95% CI of amplitudes in the width of 1 Hz (from 3.5 to 4.5 Hz) similarly in both controls (L240R246.66 and L240R240) and to recognize that the 4-Hz peak in L240R244 was adequately high only when the peak was also beyond both of the control 95% CIs on the same channel of the same subject. The results confirmed that the 4-Hz peak in L240R244 was beyond all of the three kinds of the 95% CIs. The second application of the two controls was to verify the specificity of the peak at BB frequency by confirming that a similar peak at 4 Hz was found on the same channel of the same subject in neither of the two types of control conditions. In other words, when the tone in the right ear was changed from 244 to 246.66 or 240 Hz, the amplitude at just 4 Hz did not exceed 95% CI in the width of 1 Hz. When the double requirements (i.e., a peak at BB frequency beyond each of the 3 kinds of 95% CI, no such peaks in any of the 2 control conditions) were fulfilled, we considered that the 4-Hz peak on this channel was specific to 4-Hz BB.

Table 2, the number and spatial distribution of such “BB-synchronized” channels varied across subjects. For example, in the left temporal area in L480R484, few subjects presented a channel that met the strict requirements, and the median number of positive channels was zero. However, there was no difference in the number of synchronized channels between 4- and 6.66-Hz BBs.

Figure 4 displays the spatial distribution of BB-synchronized channels in the arrangement of 204 sensors. On each channel in Fig. 4A, the sums of numbers of BB-synchronized channels in nine subjects and in four kinds of 4-Hz BB conditions are indicated by the diameter of the circle. Besides the temporal areas defined in Fig. 2, the remaining central area was divided into three zones: frontal, parietal, and occipital. Although the anatomical variations of individual brains were not taken into account in this division, the results confirmed the presence in both temporal zones bilaterally of comparatively many channels showing frequent synchronization to 4-Hz BB. However, large circles were also found in the frontal, parietal, and occipital zones, and small circles were scattered throughout the five zones. As indicated in Table 2, these findings emphasize that synchronization to 4-Hz BB was detected not only in the temporal zones but also in the other zones.

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**MCE**

The MCEs suggested several areas as possible generators of BB ASSR. Figure 5 shows typical examples of MCE in one BB cycle in L244R240. In most subjects, dominant current sources >0.5 nAm were found in the superior temporal areas bilaterally, which were considered to contain bilateral auditory cortices (Fig. 5, A and B). In some subjects, current sources were also found in the posterior parietal cortex including the superior or inferior parietal lobule (Fig. 5, C and D). However, we found no specific IPD at which these current sources were activated within one BB cycle across conditions or subjects. In consideration of the great variability in IPD of the activations, we investigated within one cycle in each subject the presence of activated sources over 0.5 nAm in the temporal and poste-
explore the correlation between the IPD cycle and wave channels.

Phase analysis of magnetic fields on BB-synchronized and 6.66-Hz BBs in the tendency of the activation.

Table 3 also shows that there was no difference between 4-spatial distribution of BB-synchronized channels shown in Fig. 4. These findings were consistent with the frontal lobe, although no specific tendency was noted in the weakly activated regions were found scattered mainly in the prior parietal areas. Table 3 shows the number of subjects who showed a current source in each brain area. Furthermore, weakly activated regions were found scattered mainly in the frontal lobe, although no specific tendency was noted in the spatial distribution. These findings were consistent with the spatial distribution of BB-synchronized channels shown in Fig. 4. Table 3 also shows that there was no difference between 4- and 6.66-Hz BBs in the tendency of the activation.

**Phase analysis of magnetic fields on BB-synchronized channels**

BB stimulation consists of regularly repeated IPD cycles. To explore the correlation between the IPD cycle and wave con-figuration of the steady-state response to BB, we investigated the IPD at which waves formed peaks and troughs in BB-synchronized channels. In particular, we analyzed the phase of extracted FFT components of BB frequency to identify a peak and a trough within one BB cycle.

The top panels in Fig. 6, A and B, display the same waveforms on the demonstrative channels in L240R244 as shown in Fig. 2, F and G, respectively (the middle panels are their magnifications in 1 BB cycle). The sine waves shown in gray lines are FFT components of BB frequency of 4 Hz. The bottom panels show dichotic sine waves to make possible a comparison of the changing IPD with responses in one BB cycle. From the standpoint of the channel in the left hemisphere (Fig. 6A), the IPD (= right phase – left phase) shifted continuously from the ipsilateral (left) ear leading (−π < IPD < 0) to the contralateral (right) ear leading (0 < IPD < π). In contrast, with regard to the channel in the right hemisphere (Fig. 6B), the IPD shifted continuously from the contralateral (left) ear leading (−π < IPD < 0) to the ipsilateral (right) ear leading (0 < IPD < π). Arrows in the middle panels indicate the IPD at which the sine component of BB frequency reached a peak value within one BB cycle.

Each panel of Fig. 6C is a summed plotting of peak IPDs on BB-synchronized channels of all nine subjects in each BB stimulation. The ordinate represents the amplitude of the FFT component of BB frequency. BB-synchronized channels are represented by five symbols according to the five zones used in Fig. 4. The two arrows in the panel of L240R244 correspond to the channels the IPDs with peak values of which are indicated in Fig. 6, A and B. In this subject and with L240R244 stimulation, the IPD with peak value was located approximately at 0 on each hemisphere. However, when the data of all the nine subjects were plotted together, each panel of Fig. 6C shows that the peak IPDs in the left temporal area (blue triangles) and in the right temporal area (red triangles) did not correlate with laterality (positive vs. negative IPD). If the arrangement and angles of 203 employed sensors are considered, a delay of 180° should be considered an identical phase. However, in each of the eight types of BB condition and in each of the five brain areas shown in Fig. 6, the changing IPD with responses in one BB cycle was represented by 5 symbols according to the 5 zones used in Fig. 4.

**FIG. 4.** Spatial distribution of the BB-synchronized channels in the arrangement of 204 channels. A: sums of numbers of BB-synchronized channels in 9 subjects and in 4 kinds of 4-Hz BB conditions are indicated by a diameter of a circle. Besides the temporal zones explained in Fig. 2, the remaining central area was divided into 3 zones: frontal, parietal, and occipital (gray lines). Although the anatomical variations of individual brains are not taken into account in this schema, it is confirmed that in both temporal zones, bilaterally there are many channels where synchronization to 4-Hz BB was frequently observed. However, large circles are found also in frontal, parietal, and occipital zones, and small circles are scattered over the 5 zones. B: sums of numbers of BB-synchronized channels in 9 subjects and in 4 kinds of 6.66-Hz BB conditions. A synchronization to 6.66-Hz BB was frequently observed, especially in the frontal and parietal zones, besides the temporal zones.

**FIG. 5.** Examples of source distribution (color coded) by minimum-norm current estimates (MCEs) in the same subject shown in Fig. 2. In this subject in L244R240, dominant current sources > 0.5 nAm were recognized in the left temporal (A: at 0.81°π in 1 IPD cycle), right temporal (B: at −0.68°π in 1 IPD cycle), left posterior parietal (C: at −0.80°π in 1 IPD cycle), and right posterior parietal (D: at 0.64°π in 1 IPD cycle) areas.
areas, there was no specific phase for channels synchronized to 4- or 6.66-Hz BB. Furthermore, within a single subject there was no consistent correlation between the left and right temporal areas, unlike the example shown in Fig. 6, A and B. It is also noticeable that most BB-synchronized channels with a high amplitude of BB-frequency component belonged to the left or right temporal areas. This finding suggests that the BB-synchronized channels in the temporal areas tend to have a comparatively high spectral peak of BB frequency, although Table 2 and Fig. 4 show that the number of BB-synchronized channels was not exceedingly large in the temporal areas compared with other areas.

**DISCUSSION**

**Synchronization to BB and possible sources of BB ASSR**

In an analysis of magnetic fields on each channel, we used two methods to search for channels that represented ASSR to BB in each subject. One method examined the configuration of averaged magnetic field in each channel (Fig. 2, A, F, and G), and the other involved searching the channels for FFT with a specific peak at BB frequency (Fig. 2, H and I). The comparison of another BB and no-BB condition confirmed that the magnetic field itself under BB condition was ASSR to BB. Such spectral analysis of a magnetic field on a single channel has been performed and validated in the study of ASSR to AM tones (Fujiki et al. 2002; Patel and Balaban 2000; Ross et al. 2000). However, to identify more strictly the channels that were synchronized to a specific BB frequency, we adopted two requirements, namely, a peak at BB frequency beyond the 95% confidence limits and no such peaks in any of the two control conditions (another-BB and no-BB conditions). Especially, a spectral peak at BB frequency was considered significantly evident only when it exceeded all of the three 95% CIs (BB, another-BB, and no-BB conditions). These strict requirements using two types of controls reduced the number of channels that were considered to represent synchronization to BB. It is possible that some channels had false negative synchronization to BB; however, the selected channels showed with adequate reliability that periodic responses with the same frequency as IFD are evoked in the cerebral cortex.

Consequently, the small numbers of selected channels might cause a large variance in the spatial distribution of BB-synchronized channels. This might be partly because anatomical variations of individual brains were not taken into account in this calculation. Gender difference in the location of auditory cortex (Elberling et al. 1982; Nakasato et al. 1995; Ohtomo et al. 1998; Reite et al. 1995) might be another cause of the varied distribution of BB-synchronized channels because we pooled the data of six male and three female subjects. Furthermore, the Edinburgh handedness questionnaire confirmed that one of our nine subjects was ambidextrous. Because the only single neurophysiological study on BB perception in humans (Schwarz and Taylor 2005) did not discriminate the handedness of subjects and because auditory processing using IFD was considered to be less influenced by handedness than by other auditory mechanisms, such as the processing of language sounds (Griffiths and Warren 2002; Jancke et al. 2002; Josse et al. 2003; Liegeois-Chauvel et al. 1999; Mazoyer et al. 1993; Zatorre et al. 2002), we did not exclude the data of the ambidextrous subject. Actually, the values of this subject were not different from those of others and did not affect the results of any analysis.

MCE requires no a priori information concerning the possible source configuration or restriction of the MEG channels included in the model. Therefore the MCE was considered suitable for making an approximate estimation of brain areas activated by BB stimulation. As shown in Table 3, most subjects showed dominant activation in the left and right temporal areas, which contained the auditory cortices. This finding supports the notion that the main source for BB ASSR might be within the temporal area similar to conventional ASSR, although MCE cannot demonstrate whether these activities contain BB specific spectral components.

In humans, the primary auditory cortex (PAC) is generally identified with Brodmann (1909) area 41, which is in the depth of the Sylvian fissure where it occupies a sizable part of Heschl’s gyrus (HG). Studies using implanted electrodes in surgical patients confirm the localization of the PAC in humans to HG, particularly its middle part (Howard et al. 1996; Liegeois-Chauvel et al. 1991). Homologies of the belt and parabelt subdivisions defined in the macaque (Kaas and Hackett 2000) have never been precisely determined in the human brain, and thus areas surrounding the PAC are simply referred to as nonprimary auditory cortex (Hall et al. 2003). Extensive studies using MEG have been made of conventional ASSR evoked by stimulus rates near 40 Hz. In previous studies, the source of ASSR was located on the PAC (Engelien et al. 2000; Forss et al. 1993; Pantev et al. 1993, 1996). Animal studies in which intracortical (Karmos et al. 1993) and subdural field potentials (Franowicz and Barth 1995) were recorded suggested PAC as the source of the 40-Hz ASSR.

Previous neurophysiological studies provided evidence that the auditory cortex can represent BB frequency component. BBs demonstrate that the discharges of the auditory nerve fibers preserve information on the phase of acoustic stimuli. Neural spikes tend to occur at a particular phase of the sinusoidal waveform (phase locking), and the central auditory system has the capacity to preserve temporal information (frequency coding). Animal studies confirmed that the central auditory system preserves and utilizes information of continuously changing IPD on a presentation of BBs. Reale and Brugge (1990) examined the sensitivity to IPD in single neurons of PAC in anesthetized cats. They demonstrated the sensitivity of neurons to fixed IPD produced by a dichotic

<table>
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<th>Temporal Region</th>
<th>Posterior Parietal Region</th>
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presentation of two tones that differed from each other only in the starting phase. Approximately 26% of cells that were sensitive to static IPD were also sensitive to dynamically changing IPD created by BBs. They used tone combinations of 600 and 600–635 Hz to elicit BB, and BB-synchronized neuronal spikes were recorded in the range of IFD of 5–29 Hz.

No remarkable difference was found in spike numbers between 5 and 9 Hz BB. The IFD sensitivity of some PAC neurons was markedly similar to that of IPD-sensitive neurons in the medial superior olive and central nucleus of the inferior colliculus. Based on these results, Reale and Brugge (1990) concluded that the sensitivity to IPD is transmitted from the lower brain stem to PAC, where it is preserved essentially undistorted. In their experiments with primates, Malone et al. (2002) reported the responses to changing IPD of BB in the auditory cortex of awake macaques. Their results were recorded in the core region of the auditory cortex (Hackett et al. 1998).

In human EEG recordings of ASSR to BB (Schwarz and Taylor 2005), a systematic phase shift raised the possibility of more than one current source along the rostrocaudal axis. In this EEG study, a BB-specific spectral component was detected in the frontal and parietal electrodes as well as in the temporal areas. In the current study, BB-specific spectral peaks were found also on channels in the parietal, frontal, and occipital areas. MCE demonstrated current sources in the posterior parietal area and scattered weak activation in the frontal lobe, in addition to the temporal areas. These results supported the possibility of multiple sources in the human EEG study.

A phase analysis of positive channels showed that BB-specific components with high amplitude tended to be frequently found in the temporal areas. The RMS intensity of averaged magnetic fields was larger in the temporal areas bilaterally than in other areas. These findings suggest that the main source for BB ASSR might be located in the auditory cortex, such as for conventional ASSR, but they do not confirm that the auditory cortex is the exclusive source.

**IPD for BB**

Rose et al. (1966) and later Yin and Kuwada (1983b) found that for certain cells of the cat central nucleus of the inferior colliculus there existed an ITD that evoked the same relative discharge rate regardless of stimulus frequency. Rose et al. (1966) referred to this ITD as the characteristic delay (CD) and

FIG. 6. A and B: phase of extracted FFT components of BB frequency was analyzed within 1 BB cycle. Top: waveforms on the demonstrative channels in L240R244 as shown in Fig. 2, F and G, respectively. The sine waves of the FFT component of BB frequency of 4 Hz are added (gray lines). Middle: magnifications of waves in the top. The abscissa is set in 1 BB cycle within which the IPD changes continuously. Arrows indicate the IPD with a peak value of the BB frequency component. Bottom: schematic dichotic sine waves to cause BB of 1 cycle. In this example, the frequency of the sinusoid delivered to the left ear (FL: thin line) was lower than that delivered to the right ear (FR: thick line). For a schematic illustration, the amplitudes of the 2 sine waves are different and the frequencies of the tones are much lower than those actually used under L240R244 condition. From the standpoint of the left hemisphere, the IPD (= right phase − left phase) shifted continuously from the ipsilateral (left) ear leading (−π < IPD < 0) to the contralateral (right) ear leading (0 < IPD < π). In contrast, from the standpoint of the right hemisphere, the IPD shifted continuously from the contralateral (left) ear leading (−π < IPD < 0) to the ipsilateral (right) ear leading (0 < IPD < π). C: each panel is a summed plot of peak IPDs on BB-synchronized channels of all the 9 subjects in each BB stimulation. The ordinate expresses the amplitude of the FFT component of BB frequency. BB-synchronized channels are represented by 5 symbols according to the 5 zones applied in Fig. 4 (left temporal, right temporal, frontal, parietal, and occipital). Two arrows in the panel of L240R244 correspond to the channels the IPDs with peak value of which are indicated in A and B. Note that in each of the 8 types of BB conditions and in each of the 5 brain areas, there is no specific phase for channels synchronized to 4- or 6.66-Hz BB. Furthermore, within a single subject, there is no consistent correlation between the left and right temporal areas, unlike the examples shown in A and B.
argued that it may be a property of some binaural neurons that allows them to detect the location of a sound source in space. Benson and Teas (1976) were unable to find in their results from chinchilla auditory cortex a registration of peaks or troughs in spike count versus IPD functions examined at three different frequencies. However, in the PAC of anesthetized cats, the discharges of neurons sensitive to dynamically changing IPD were highly periodic and tightly synchronized to a particular phase of the BB cycle (Reale and Brugge 1990). In the latter study, the ITD sensitivity of a single neuron was analyzed at a relatively large number of frequencies within the response area, and further consideration was given to the cortical presence of a CD. Their findings revealed that the IPD sensitivity in the cortex differs little from that recorded in the inferior colliculus or medial superior olive.

Because BB stimulation consists of regularly repeated IPD cycles, we had expected before our experiment to find a correlation between the IPD cycle and the wave configuration of steady-state response to BB. Reale and Brugge (1990) found a marked tendency for each neuron to fire maximally when the phase of the tone to the ipsilateral ear lagged that to the contralateral and for cell firing to reach its minimum when the reverse was true. Based on this finding, as illustrated in Fig. 1, we employed BB stimulation in which IPD shifted from one ear leading to another ear leading through one complete cycle of BB. For example, when the right ear lagged to the left ear \((-\pi < \text{right phase} - \text{left phase} < 0\), the response in the right auditory cortex had been expected to show maximal amplitude. In contrast, when the left ear lagged to the right ear \((0 < \text{right phase} - \text{left phase} < \pi\), the response in the left auditory cortex had been expected to show maximal amplitude. However, as shown in Fig. 6, our results showed that the distribution in the phase of peak amplitude did not exhibit such laterality. The IPD for peak showed wide interindividual variability within each type of BB stimulation. Furthermore, also within each subject, the peaks of BB-synchronized waves in the left and right temporal areas did not always show symmetry or mirror image. These findings might mean that there is no uniform phase of neuronal activity for BB detection in synchronized areas. Alternatively, the great variability in phase might imply that BB ASSR does not represent changing IPD per se but reflects a higher order of cognitive process corresponding to subjective fluctuations of BB. Our results did not show a systemic phase shift that was reported in the EEG study (Schwarz and Taylor 2005). This discrepancy in phase might reflect the essential difference between 40-Hz BB and slow BB, or the cognitive process for subjective fluctuations of slow BB. In summary, our results in source and phase analyses suggest the contribution of several cortical regions and imply the sequential recruitment of these regions for the high-order cognitive process of BB.

Previous studies indicated that the great variability in phase could be assumed within the auditory cortex. Reale and Brugge (1990) indicated that IPD-sensitive cells in the auditory cortex exhibited peaks and troughs in spike count at certain IPD within one BB cycle; however, the specific IPD of the peaks and troughs were different for each cell. This result implied that the auditory cortex contained a mixture of neurons the suitable IPDs of which differed from one another, and this variance may contribute to realizing auditory function such as sound localization. In fact, in experiments involving cats, Furukawa et al. (2000) suggested a model in which sound-source location is coded by a population of cortical neurons that are distributed widely throughout the auditory cortex, each of which can carry information of sound-source location by its own spike pattern (Middlebrooks et al. 1994, 1998). Our results were not contradictory to the model of functional ensembles of cortical neurons.

**Intensities of ASSR**

The intensity of magnetic field under BB condition was significantly greater than those of no-BB and no-body conditions. The recorded fields showed relatively weak responses to BB; however, our findings proved that the fields were strong enough to be distinguished from noise, which might be caused by acoustic stimulations or the circumstances of our recordings.

Although BB ASSR was greater than noise level and evidently contained a spectral component of BB frequency, its weakness may be due to the small number of IPD-sensitive neurons. Reale and Brugge (1990) demonstrated that approximately a fourth of the IPD-sensitive neurons isolated in of the cat’s PAC responded to continuously changing IPD. On the other hand, >90% of IPD-sensitive neurons in the inferior colliculus responded to both static and dynamic phase shifts (Yin and Kuwada 1983a).

In the study of ASSR to BB (Schwarz and Taylor 2005), BB ASSR was more evident when the subjects were instructed to attend to one warbling tone than when they were not. Our subjects watched video movies instead of attending to BB to maintain a vigilance level based on precedent finding that drowsiness and sleep reduce the amplitude of response in conventional ASSR to AM tone with rates around 40 Hz (Galambos et al. 1981; Jerger et al. 1986; Linden et al. 1985). It is possible, however, that the neglect of BB might attenuate ASSR. Although it was reported that selective attention to AM tone does not affect ASSR with rates around 40 Hz when arousal is controlled (Linden et al. 1987), a recent MEG study using an AM discrimination task, which required focused attention to the modulation rhythm, showed an enhancement in amplitude during auditory attention (Ross et al. 2004). Such significant effects of attention on amplitude have been reported also in steady-state visual- (Morgen et al. 1996; M{"u}ller et al. 1998) and somatosensory- (Giabbiconi et al. 2004) evoked potentials. Alternatively, BB ASSR could reflect both a cognitive process, which is influenced by attention, and an unconscious transporting process of IPD information, which is not affected by attention.

**Effects of tone frequency and IFD**

Previous behavioral studies (Licklider et al. 1950; Perrott and Musicant 1977; Perrott and Nelson 1969) reported that both 4- and 6.66-Hz BBs elicited similar sensations of a beating tone, which was also described as a “periodic fluctuation in loudness.” All the participants in our study also reported that both 4- and 6.66-Hz BBs containing 240- or 480-Hz tones allowed them to perceive BB in the narrow sense. This equality in subjective evaluations by subjects was reflected also in the properties of BB ASSR. The intensity of magnetic fields, spatial distribution of BB-synchronized chan-
channels, or MCEs: these properties of BB ASSR were not influenced by either tone frequency or IFD. Especially, an exchange of frequencies between ears causes an inversion of IFD, and the direction of rotating tone should be inverted if slow BB was perceived as a movement of auditory image. However, the properties were not influenced systematically by such exchanges of stimuli. Furthermore, whether a higher tone was presented to the ipsilateral or the contralateral ear made no specific effect on responses in a temporal area. These findings are consistent with results of previous animal studies. Although a sensitivity to the direction of BBs was reported in a small number of neurons in the inferior colliculus (Yin and Kuwada 1983a), Reale and Brugge (1990) could find no such sensitivity in the PAC. Most cells sensitive to BB in the PAC responded equally to BB, regardless of the direction of IFD change.

Originally, conventional ASSR are more difficult to record at low modulation frequencies because the EEG noise levels are higher. However, reliable responses can be recorded at lower frequencies, especially at 2–5 Hz for amplitude-modulated tones and at 3–7 Hz for frequency-modulated tones (Picton et al. 1987). These results confirm that ASSR could be synchronized even to fluctuation with a frequency <10 Hz. To our knowledge, there are no studies in which synchronized responses to BB were recorded using lower frequency tones in humans. In this regard, two neuroimaging studies employed 1-Hz BB; however, neither the PET studies (Griffiths et al. 1994) nor the functional MRI studies (Bremmer et al. 2001) detected a BB-specific component because of their limitations. Our results showed that BB ASSR can be elicited by such lower IFD. However, one limitation of our study is the use of only two types of BB frequencies. Further studies using BBs <4 Hz and beyond 6.66 Hz are required because any technical or physiological factors, such as intensity of BB stimulation (Karno et al. 2005), can influence the responses.

Conclusions

In our study, periodical steady-state responses with small amplitudes were evoked by slow BB. A spectral analysis of magnetic field on each single channel revealed that responses evoked by BB contained a specific spectral component of BB frequency, and the evoked responses were the ASSR to BB. The spatial distribution of BB-synchronized channels and MCEs suggested multiple BB ASSR sources in the parietal and frontal cortices in addition to the temporal areas, including auditory cortices.

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