Anesthesia Depth–dependent Features of Electroencephalographic Bicoherence Spectrum during Sevoflurane Anesthesia

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**Background:** Growth pattern in the electroencephalographic bicoherence spectrum has recently been found to relate to anesthetic depth, and bicoherence analysis can reflect behavior of the thalamocortical reverberating network. Because the thalamocortical network is known to represent a key factor in sleep by anesthesia, systematic and qualitative bicoherence studies of different anesthetic depths is necessary throughout all pairs of frequencies.

**Methods:** Sixteen patients were anesthetized using sevoflurane (1, 2, or 3%) combined with remifentanil (0.4 µg · kg⁻¹ · min⁻¹). Raw electroencephalographic signals were collected, and bicoherence was estimated in all pairs of frequencies, between 0.5 and 40 Hz at 0.5-Hz intervals.

**Results:** Sevoflurane (1%) caused two main peaks, spindle frequencies (11.0 ± 1.2 Hz, 44.7 ± 12.3% [bicoherence growth]) and δ-θ frequencies (5.4 ± 0.5 Hz, 33.0 ± 8.4%), in the diagonal line of biphase bicoherence plots. High concentrations of sevoflurane (2% and 3%) shifted these peaks to 9.8 ± 1.1 Hz, 46.2 ± 12.7%; 8.7 ± 1.3 Hz, 37.2 ± 13.7%; and 4.9 ± 0.5 Hz, 44.6 ± 7.0%; 4.3 ± 0.8 Hz, 45.2 ± 10.6%, respectively. Sevoflurane caused a third bicoherence peak to appear in another heterogeneous pair frequency (pair of α basal frequency and its double frequency), outside the diagonal line, which also inherited the behavior of α bicoherence peaks at different anesthetic depths.

**Conclusions:** Sevoflurane anesthesia caused bicoherence peaks in α and δ-θ areas and also formed secondary third peaks. Deeper sevoflurane anesthesia shifted all bicoherence peaks to lower frequencies and caused increased bicoherence growth in the δ-θ area. The obtained features are consistent with characteristics of the thalamocortical reverberating network and suggest the importance of bicoherence analysis for the thalamic system.

BICOHERENCE analysis, a power-independent bispectral analysis, has been developed to detect cross-frequency phase coupling, as a method to examine nonlinear regulation of brain electrical activities. Although a theoretical link between neural network physiology and phase coupling had not been fully established,¹ a certain reverberating system can contribute to high bicoherence values. Bicoherence is a signal-processing technique capable of tracking changes in any reentry system, investigating phase relations between two input signals (f₁, f₂) by introducing an output signal (f₁ + f₂), and by quantifying the quadratic phase coupling between these signals.² In nonlinear modulation such as seen in a certain reverberating system, the output signal from the reverberating circuit is expected to reenter into the system as the input signal and cause self-modulated characteristics, namely the components of intermodulation products (a signal component produced by multiplication of input signal components). Because this results in quadratic phase coupling between input signal components, bicoherence is expected to grow in these frequency components. A reverberating source such as seen in the thalamocortical network can therefore contribute to the growth of bicoherence.

In the synchronous neural oscillation in electroencephalographic rhythms, although the linear aspect, *i.e.*, the increase or decrease in amplitude as a result of the number of active neurons firing in synchrony, can be detected by synchronous peaks of the corresponding frequency area on the power spectral analysis,³ nonlinear thalamic contributions to synchronous rhythms are reflected only by phase analysis of the wave synchronization. Coherence, which is sometimes used to investigate the similarity of two signals, also examines wave synchronization, and seems to be important for binding certain brain areas among cortical areas, which is necessary for cognition and special functions.⁴ However, coherence alone cannot be used for the analysis of nonlinear relations. Moreover, thalamocortical reverberant features representing massive synchronizations cannot be examined by coherence between two certain cortical signals. Bicoherence analysis is thus a rare and powerful candidate to elucidate thalamocortical regulation linking to the cortical area, by determining the nonlinear reverberating configuration using one-channel cortical electroencephalogram.

Thalamic reticular (RE) and thalamocortical relay (TC) neurons in the thalamus are known to be fundamental to the genesis of oscillatory activity during slow-wave sleep as α spindle waves and δ waves.⁵ These electrophysiologic oscillations driven from thalamocortical neurons are modulated by sleep depth.⁷ That is, RE and TC neurons participate in the 7- to 14-Hz spindling α rhythm of early sleep and the slower rhythms of deeper sleep, with different firing patterns. Because sleep regulation is thus closely re-
lateral to the thalamocortical network, examination of anesthesia-dependent changes in the bicoherence spectrum is important. Recently, the pattern of the bicoherence spectrum has been reported as dependent on anesthetic depth. The quantity of bicoherence growth in α and δ-θ areas is reportedly related to the concentration of isoflurane and sevoflurane. However, in previous studies, anesthetic-related peak frequency shifting among each α and δ-θ area in bicoherence plots has not been sufficiently examined. Our previous work found that ketamine increases bicoherence peak frequency in the α spindle area. Because basal frequency changes in spindle and slower rhythms by anesthetic depth are another important characteristic of the thalamocortical system, examination of shifts in all bicoherence peak frequencies during anesthesia may also be important, as a reflection of electrophysiologic activity of RE and TC neurons.

Furthermore, bicoherence growth appearing in heterogeneous pairs of biphasic frequencies outside the diagonal line has not been investigated in previous studies. Generally, in the electroencephalographic rhythm generated in the system including the reverberating network, because a certain single frequency component contains both original and reverberating modulated subcomponents, electroencephalographic bicoherence mainly appears around the diagonal lines \( f_1 = f_2 \). However, if the thalamocortical reverberating circuit contributes to the growth of bicoherence, then bicoherence might increase in other heterogeneous pairs of biphasic frequencies besides the diagonal line, as any output signals from the thalamocortical system might reverberate again, and might be coupled with other reverberating signals from the thalamocortical system. Bicoherence growth in the diagonal line is thus insufficient to extract anesthesia-related changes in bicoherence growth. A systematic bicoherence study throughout all pair of frequencies seems warranted.

The current study systematically examined how different anesthetic depths using sevoflurane affected the entire bicoherence spectrum, from the different perspectives of quantity of bicoherence growth and shifts in bicoherence peaks in all pairs of frequencies. We were interested in how the anesthesia-related bicoherence spectrum reflects already known thalamocortical reverberating features.

Materials and Methods

Protocol

The Institutional Review Board on Human Experiments, Kyoto Prefectural University of Medicine (Kyoto, Japan), approved the current study, and informed consent was obtained from all patients. Subjects comprised 16 patients (mean age, 50.2 yr; range, 21–69 yr; American Society of Anesthesiologists physical status I or II) who were scheduled to undergo noncranial surgery. None of the patients had any neurologic or psychiatric disease. Patients were premedicated with 0.5 mg atropine at approximately 30 min before induction of anesthesia, as the routine prescription at our institution. In the operating room, electroencephalography was started using an Aspect A-2000 BIS® monitor (version 4.0; Aspect Medical Systems, Natick, MA), and electroencephalographic data were continuously collected. Anesthesia was induced with propofol (2 mg/kg) combined with continuous infusion of remifentanil (0.5 \( \mu g \cdot kg^{-1} \cdot min^{-1} \)), facilitated by 0.15 mg/kg vecuronium. After tracheal intubation, the continuous infusion rate of remifentanil was changed to 0.4 \( \mu g \cdot kg^{-1} \cdot min^{-1} \). Anesthesia was maintained with sevoflurane (1, 2, or 3%) with 30% oxygen, combined with 0.4 \( \mu g \cdot kg^{-1} \cdot min^{-1} \) remifentanil. A dose of 0.08 mg/1 kg · h · min remained in the end-expired concentrations of carbon dioxide at 35–40 mm Hg. Mean arterial blood pressure and heart rate were maintained at greater than 60 mm Hg and greater than 50 beats/min, respectively, using phenylephrine and atropine as required. Concentration of expired sevoflurane was continuously monitored using a Smart Anesthesia Multigas module® (GE Medical Systems, Milwaukee, WI). From 1 h after induction of anesthesia, end-tidal concentration of sevoflurane was deliberately kept at the goal concentration (1, 2, or 3%) for 30 min to achieve a steady state for data collection periods, and then changed to another concentration. Electroencephalographic analyses (bicoherence analysis and other electroencephalographic indices) were then performed in each setting.

Data Acquisition

A BIS® sensor (Aspect Medical Systems) consisting of four electroencephalographic electrodes was applied to the forehead. Electrode impedance was checked every 10 min and was maintained at 5 kΩ or less throughout the study by the A-2000®. Raw electroencephalographic wave signals (converted from analog to digital at 128 Hz) in addition to the resulting electroencephalographic indices were collected using BSA version 3.22B2 software (Bispectrum Analyzer for A-2000 BIS® monitor) via an RS232 interface on a personal computer (CF-W2; Panasonic, Osaka, Japan). Signals less than 0.5 Hz and greater than 50 Hz were excluded. The Bispectral Index (BIS) values were calculated by the A-2000® from the preceding 1-min period of electroencephalogram and were extracted to a personal computer directly from the A-2000®, Spectral edge frequency (SEF95; the frequency below which 95% of the power in the spectrum resides) was calculated using BSA software as the average over 1 min. Bicoherence values were computed in all pairs of frequencies be-
between 0.5 and 40 Hz at 0.5-Hz intervals from 3 consecutive minutes of artifact-free signals. Signals were divided into a series of 2-s epochs, with each epoch overlapping by 75%. After applying the Blackman window function, the Fourier transform of each epoch was computed. Raw bicoherence values were calculated, using the following equations:

- **Triple product**: \[ TP_j (f_1, f_2) = X_j (f_1) X_j (f_2) X_j^* (f_1 + f_2) \]
- **Bispectrum**: \[ B (f_1, f_2) = \sum_j |TP_j (f_1, f_2)| \]
- **Bicoherence**: \[ BIC (f_1, f_2) = \frac{B (f_1, f_2)}{\sum |TP_j (f_1, f_2)|} \cdot 100 \]

with \( j \) referring to epoch number, \( X_j (f_1) \) representing a complex value calculated with Fourier transformation of \( j \)th epoch, and \( X_j^* (f_1) \) as the conjugate of \( X_j (f_1) \). Next, two-dimensional moving averages of nine points of bicoherence were calculated every 0.5 Hz from 1.5 to 40.0 Hz as averaged bicoherence. These computations were performed using Borland C++® (version 5.02J; Borland International, Tokyo, Japan) and MATLAB® (version 6.5.1; The MathWorks, Natick, MA; Signal Processing Toolbox, Control System Toolbox, and Data Acquisition Toolbox are included).

To determine the bicoherence peak in each frequency area (such as the \( \alpha \) area), we detected the corresponding peak as the highest bicoherence value under the corresponding area on the bicoherence plane in every analysis process. First, averaged bicoherence spectra were plotted around the diagonal lines \( f_1 = f_2 \). We labeled the maximum value of the bicoherence in the diagonal lines between 2.5 and 6.5 Hz as the bicoherence \( \delta \)-\( \theta \) peak. Similarly, we labeled the maximum value of the bicoherence in the diagonal lines between 7 and 13 Hz as the bicoherence \( \alpha \) peak. The bicoherence ridge along \( \alpha \) frequency lines, i.e., the pair of \( \alpha \) frequencies and other frequency signals, were analyzed by the frequency of the detected \( \alpha \) peak, every 1 Hz (8–11 Hz). We then labeled the maximum value on the bicoherence line of the detected \( \alpha \) peaks as the third peak.

**Fig. 1.** Representative raw results of electroencephalogram (EEG) and corresponding power spectra along with changes in sevoflurane concentrations (A: 1%; B: 2%; C: 3%). Arrows indicate \( \alpha \) spindle peaks. (A) At 1% concentration, a conspicuous peak of around 10 Hz was predominant in the power spectrum. (B and C) Shifts of the \( \alpha \) peak to a lower frequency were noted in the power spectrum (shown by arrows), along with increased sevoflurane concentrations to 2% and 3%. Power of the lower frequency area (\( \delta \) range) also increased.
Statistical Analysis

Changes in bicoherence peaks, bicoherence values, and electroencephalographic parameters (SEF95, BIS) were analyzed by one-way analysis of variance followed by a Tukey multiple-comparison test. These data were statistically analyzed using KyPlot 5.0® software (KyensLab, Tokyo, Japan). Values of $P < 0.05$ were considered statistically significant. Data are expressed as mean $\pm$ SD.

Relationships between pair frequencies composing the third peaks were analyzed by simple regression analysis and the Pearson correlation coefficient. The regression line was shown with 95% confidence limits.

To examine reproducibility of the bicoherence calculation, variances of bicoherence peaks about 10 series of bicoherence calculations in every steady state of different sevoflurane concentrations were tested, and bicoherence peak frequencies and their bicoherence values were summarized as means, SDs, and skewness of $\alpha$, $\delta-\theta$, and third peaks.

Results

Background data of the 16 patients (4 men, 12 women) were as follows: age, 50.2 $\pm$ 15.4 yr; weight, 56.4 $\pm$ 13.9 kg; and height, 158.2 $\pm$ 12.4 cm. Hemodynamic parameters of mean arterial blood pressure and heart rate did not differ significantly between points of data measure-
ments along with changes of sevoflurane concentration (heart rate: 61.6 vs. 62.9 vs. 62.8 beats/min; mean arterial blood pressure: 71.3 vs. 69.3 vs. 68.5 mm Hg for 1, 2, or 3% sevoflurane, respectively; \( P < 0.05 \)).

Raw electroencephalographic data and the corresponding power spectrum along with changes in sevoflurane concentrations are shown in figure 1. With 1% sevoflurane, conspicuous peaks of around 11 Hz predominated in the power spectrum (fig. 1A). The \( \alpha \) peak on the power spectrum shifted markedly to a lower frequency with increased sevoflurane concentrations. Power of the lower frequency area (\( \delta \) range) also increased. The corresponding averaged bicoherence in the same patients is shown in figure 2. During 1% sevoflurane anesthesia, bicoherence peaks around \( \alpha \) and \( \delta - \theta \) area appeared (fig. 2A). The frequency of the \( \alpha \) bicoherence peak was almost consistent with the \( \alpha \) peak frequency of the power spectrum. In contrast, the frequency of the \( \delta - \theta \) bicoherence peak did not completely coincide with the \( \delta - \theta \) peak frequency of the power spectrum. A third peak also appeared in another heterogeneous pair frequency, namely the pair with \( \alpha \) basal frequency and the double frequency (11 and 22 Hz), outside the diagonal line. We again found that all bicoherence peaks shifted toward lower frequencies, along with increases in sevoflurane concentration to 2% and 3% (figs. 2B and C).

Changes in BIS and SEF \(_{95}\) during changes in sevoflurane concentration are shown in figure 3. Both BIS and SEF \(_{95}\) decreased with deepening of sevoflurane anesthesia, as is well known in anesthesia.

Figure 4 shows mean bicoherence values of 16 superimposed cases around the diagonal lines \((f_1 = f_2)\), whereas figure 5 summarizes bicoherence peak frequencies and bicoherence values regarding \( \alpha \) and \( \delta - \theta \) ranges during changes in sevoflurane concentration. The bicoherence growth in diagonal line of slower frequency area was recognized to form a combined broad large peak in \( \delta - \theta \) band. Higher sevoflurane concentration (3%) significantly shifted both \( \alpha \) and \( \delta - \theta \) peaks to lower frequencies \(( P < 0.05 \)) resulting in peaks at about 8.6 and 4.3 Hz instead of 11 and 5.4 Hz, respectively. Bicoherence values in \( \alpha \) peaks tended to decrease with increases in sevoflurane concentration from 1% to 3%, but no significant difference was seen. On the contrary, bicoherence values in \( \delta - \theta \) peaks increased \(( P < 0.05 \))

We found another augmentation of bicoherence, as a ridge along \( \alpha \) lines. This was formed with a series of
bicoherence growth, in the pair of α frequencies and other frequency signals. We therefore examined bicoherence growth along the α frequency line, by the frequency at which α peaks appeared every 1 Hz (8–11 Hz; fig. 6). These peaks were found to be particularly augmented at pair frequencies of basal α frequencies and approximately doubled frequencies (10 vs. 20 Hz), and constituted the third peaks. The other side of the pair frequency in the third peak decreased with decreasing α peak frequency. We then examined the details of third peaks. Figure 7A shows bicoherence peak frequencies and bicoherence value regarding the third peak, in relation to basal α frequency every 1 Hz. Bicoherence peak frequency (the other side of the pair frequency in the third peak) decreased along with decreasing basal α frequency, but bicoherence value was not significantly changed. Figure 7B shows simple regression analysis between basal α frequency and another pair frequency in the third peak. We found that the two biphasic pair frequencies making the third peak display a linear relation, and that frequency of the other side is twice as high as α harmonics ($r = 0.87, P < 0.01$; fig. 7B). Biphasic pair frequency where the third peak appeared seemed to be dependent on basal α frequency. We therefore also analyzed bicoherence changes of these third peaks, in relation to changes in sevoflurane concentrations.

Figure 8 summarizes bicoherence peak frequencies and bicoherence values regarding third peaks, during changes in sevoflurane concentration. Higher sevoflurane concentrations significantly shifted third peak frequencies to lower in the bicoherence spectrum ($P < 0.05$), resulting in peaks at approximately 18 instead of 21 Hz, representing almost proportional shifts to changes seen in α and δ peaks. Bicoherence values in third peaks tended to increase with sevoflurane increments from 1% to 2% ($P < 0.01$), but no significant change was identified.

Although our calculation method for bicoherence has already been well established by the previous study,12 we lastly clarified the reproducibility of bicoherence calculated in the current study, by demonstrating the same case presented in figures 1 and 2. In every steady state under 1, 2, and 3% sevoflurane concentration, we calculated 10 series of bicoherence spectra every 10 s. By way of example, in figure 9, averaged bicoherence plots of 10 series in 2% sevoflurane are superimposed around the diagonal lines (B1) and around the α frequency lines (B2). Ten-series-averaged bicoherence spectra almost coincided and were piled up on each other. In 10 series-averaged bicoherence spectra, bicoherence peak frequencies and their bicoherence values for α, δ, and third peaks were summarized as means, SDs and skewness in 1, 2, and 3% sevoflurane, respectively (fig. 10). Observed small SDs and skewness indicated the stability and reproducibility of the bicoherence calculation in the current study.

**Discussion**

Bicoherence growth in the δ-θ area increased along with increasing sevoflurane concentration from 1% to 3% in this study. Conversely, bicoherence growth in the α area tended to decrease. Deeper anesthesia thus caused increased bicoherence in the δ-θ area but reduced bicoherence in the α area. This result supports the findings of previous studies.8,5 The physiologic background for bicoherence growth changes between the α and δ-θ range has not been sufficiently clarified. However, because bicoherence growth in the α and δ-θ area can reflect reverberating activity of the RE and TC system through α spindle and slower δ-θ rhythms, respectively, because those rhythms are driven by TC and RE neurons in an intrinsic and synaptic manner within the reverberating network process,13,14 this result suggests the superiority of TC neurons...
The oscillatory rhythm regressed with increasing sevoflurane concentration, with the spindle band seeming to decrease as isoflurane concentration increased. The observed decreases in bicoherence peak frequencies in sevoflurane anesthesia were compatible with these reports. Conversely, the authors did not find significant decreases in the frequency of the maximum bicoherence value between 2 and 6 Hz (pBIC-low) along with increasing isoflurane concentration, which is inconsistent with our result that bicoherence peak frequency in the δ-θ area was decreased. However, they did not show what pBIC-low indicates and whether pBIC-low coincides with the real bicoherence peaks in the δ-θ area. Furthermore, our averaging method for bicoherence was different from theirs, and we think their method assuming a diagonal distribution could cause bias. In addition, we used sevoflurane, not isoflurane. Because the bicoherence growth in the δ-θ area has a broad distribution pattern, we think the factors above may have contributed to the different result.

Regarding changes in bicoherence peak frequency, sevoflurane-induced hyperpolarization in thalamocortical neurons shifts voltage-dependence membrane conductance toward the less sensitive, through the modulation of activation variables about hyperpolarization-activated inward current, which leads to lower activation kinetics and activation dynamics in TC–RE

**Fig. 6.** The bicoherence ridge along the α frequency lines, i.e., the series of bicoherence growths in the pair of α frequencies and other frequency signals, were analyzed by the frequency of α peaks every 1 Hz (8–11 Hz). SDs are shown as perpendicular lines. In particular, bicoherence augmented at the pairs between basal α frequencies and doubled frequencies (e.g., 10 vs. 20 Hz), and caused third peaks. Arrow indicates the third peak appearing in the bicoherence ridge along each α frequency line. A bicoherence ridge of 12-Hz α frequency lines (series of bicoherence growth in the pair of 12-Hz and other frequency signals) was not described, because the large SDs would make the figure unintelligible.
neurons. This can contribute to changes in /H9251 spindle and /H9254 frequency and cause lower shifts in bicoherence peaks in each band, because those changes of activation dynamics in TC–RE neurons are included in thalamocortical reverberant loops. Although the suggested mechanism cannot necessarily explain the proportional decrease in both bicoherence peak frequencies in /H9251 and /H9254 areas by deepening anesthesia, we think the finding is a notable feature in bicoherence analysis reflecting anesthetic depth.

We found that the frequency of the /H9251 peak in the power spectrum is consistent with the bicoherence /H9251 peak (fig. 1). We consider that when a certain rhythm is dominantly formed in a thalamocortical reverberating network, a peak will often appear in the corresponding frequency of the power spectrum, because the number of active neurons firing in synchrony will increase by the reverberating signals in reentrant loops. Simultaneously, a peak in bicoherence will augment a similar frequency by forming phase coupling induced by reverberating waves. Therefore, the feature of synchronized activity is often reflected in both linear (power spectrum) and nonlinear (bicoherence) processes. In contrast, the peak of the power spectrum in the /H9251 area was low; however, the bicoherence peak was high. This suggests that the waves contributing to these rhythms are coupled with each other and synchronized, although the activity is low. Therefore, even if the power is small, bicoherence may grow when the component without phase coupling decreases. The discrepancy between power spectrum and bicoherence plot is considered to indicate the importance of bicoherence analysis for elucidating the features that cannot be analyzed by simple power spectrum.

A ridge seems to exist along /H9251 spindle lines (10 Hz), indicating a series of interactions between 10-Hz signals and other frequency signals, particularly the third peaks of bicoherence growth between /H9251 basal frequency and the double harmonics (10 vs. 20 Hz). These features in bicoherence growth are first reported in the current study, and the mechanisms remain unclear. We hypothesized that the characteristics of the thalamocortical reverberant network may be involved. For example, when bicoherence growth is seen in 10-Hz biphasic frequency, both input signals used in bicoherence analysis are 10 Hz, and the output signal is 20 Hz. The high bicoherence at the pair of 10-Hz frequencies thus shows that the output signal (20 Hz) from the thalamocortical system includes the components of intermodulation products produced by multiplication of input signal components at about 10 Hz. The output 20-Hz signal thus produced (including a 10-Hz intermodulation product) is expected to reenter into the thalamocortical reverberant circuit again as the input signal. Quadratic phase coupling, for example, may then occur between the new reverberating 20-Hz signals and the original 10-Hz signals, and may enlarge bicoherence at the new pair of input signals (10 Hz, 20 Hz). As bicoherence broadly grows.

Fig. 7. (A) Bicoherence peak frequencies (○) and bicoherence value (□) regarding the third peak, in relation to one side of paired biphasic frequency matching the basal /H9251 frequency (7 ≤ /H9251 frequency ≤12). Significant difference in these bicoherence data compared with the value at: * 7 Hz; # 8 Hz; ** 9 Hz. (B) Simple regression analysis among pair frequencies, describing third peaks in relation to basal /H9251 frequencies. The regression line is shown with 95% confidence limits. Two biphasic pair frequencies make a third peak, displaying a linear relation, and frequency of the other side was twice as high as in /H9251 harmonics.

Fig. 8. The bicoherence peak frequencies (○) and bicoherence values (□) regarding third peaks are summarized during sevoflurane concentration. Significant difference between: * 1% and 2% sevoflurane; ** 1% and 3% sevoflurane; # 2% and 3% sevoflurane.
in \(\alpha\) and \(\delta\)-\(\theta\) areas, other associated signals including other intermodulation products about \(\alpha\) and \(\delta\)-\(\theta\) waves may similarly reverberate into the thalamocortical reverberant circuit again as the input signal, which may result in quadratic phase coupling between \(\alpha\) signals. This may cause the 10-Hz line ridge. Equally, a ridge along the \(\delta\)-\(\theta\) line seemed to grow, although we have not studied the \(\delta\)-\(\theta\) line because of the broader pattern of growth in the \(\delta\)-\(\theta\) area than in the \(\alpha\) area. Although other possibilities may exist, the observed bicoherence pattern and hypothetical analysis fit these features of the thalamocortical reverberating system.

We found that higher sevoflurane significantly shifted third peaks to lower frequency in the bicoherence spectrum, in the same manner as seen in changes in \(\alpha\) peaks. Bicoherence frequencies in the third peaks decreased from 20.7 ± 2.6 Hz to 18.7 ± 2.4 Hz (90.3%) and 17.9 ± 2.3 Hz (86.4.1%) with increased sevoflurane from 1% to 2% and 3%, respectively (\(P < 0.01\)), coinciding with the proportional changes seen in \(\alpha\) peaks. In addition, one-sided frequency of the third peak was always twice as high as other-side \(\alpha\) basal frequency, even if \(\alpha\) basal frequency was changed by the deepened anesthesia (fig. 7B). Constitution of the third peak thus seemed to depend on appearance of the \(\alpha\) peak, and supports the idea that bicoherence spectra reveal a certain systematic reverberating mechanism. The most likely candidate for this mechanism is the thalamocortical reverberating system.

We used remifentanil combined with sevoflurane (1, 2, or 3%), with an intravenous infusion rate of 0.4 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\), to maintain anesthesia. The simulated blood concentration of remifentanil was 10.4 ng/ml, which is probably sufficient to regulate nociceptive stimulation in an adult during surgery.\(^{22,23}\) However, the effects of surgical stimulation might be not completely removed. These are the limitations to the current study. Furthermore, theoretical analysis about the result was based on our speculations.

### Figure 9
Averaged bicoherence spectra of 10 series superimposed around the diagonal lines (B_1) and around the \(\alpha\) frequency lines (B_2) in 2% sevoflurane. Means and SDs are shown as black bold lines and perpendicular lines, respectively (\(n = 10\)). Arrows indicate \(\alpha\) and \(\delta\)-\(\theta\) peaks appearing around the diagonal lines and also indicate third peaks appearing in the bicoherence ridge along each \(\alpha\) frequency line. \(\alpha =\) bicoherence peak around \(\alpha\) area; \(\delta\)-\(\theta =\) bicoherence peak around \(\delta\)-\(\theta\) area; third peak = third peak appeared in another heterogeneous pair, between \(\alpha\) basal frequency and the double harmonic.

### Figure 10
Variances of bicoherence peaks about 10 series of bicoherence calculations in every steady state condition of 1, 2, and 3% sevoflurane concentration are shown as representative data in the same demographic case described in figures 1 and 2. Regarding \(\alpha\), \(\delta\)-\(\theta\), and third peaks, bicoherence peak frequencies and bicoherence values are summarized as means and SDs for the different sevoflurane concentrations (1, 2, and 3%). Skewness about bicoherence peak frequencies and skewness about bicoherence values are also provided, respectively, in parentheses. Gray circles = 1% sevoflurane concentration; triangles = 2% sevoflurane concentration; black diamonds = 3% sevoflurane concentration.

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\(\epsilon\) = 0.05; \(\alpha\) = 0.05

\(N = 10\)
derived from already elucidated electrophysiologic knowledge. The pathways and networks must be directly assessed to clearly determine these points in the future.

In conclusion, we systematically examined how different sevoflurane concentration affects the entire bicoherence spectrum in all pairs of frequencies. Sevoflurane concentration increment from 1% to 3% results in a proportional decrease of $\alpha$, $\delta$, and third peak frequencies. Bicoherence in the $\delta$ area increases with deepening anesthesia. The observed features in bicoherence growth patterns seem to show the natures of anesthesia-related changes in electroencephalographic synchronization driven by modulating TC and RE networks. It suggests the possibility that bicoherence analysis is useful for unified estimation of anesthetic depth common to various kinds of anesthetic agents.

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