Hyperventilation-induced hypocapnia changes the pattern of electroencephalographic bicoherence growth during sevoflurane anaesthesia

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Background. Hyperventilation, with the resulting hypocapnia, reduces cerebral blood flow and causes slowing of the EEG activity. However, neuronal oscillating properties including the thalamocortical network during hyperventilation have not been elucidated. To assess these features provoked by hyperventilation, the present study examined quadratic phase coupling features by means of bicoherence analysis.

Methods. Twenty-two patients were anaesthetized using sevoflurane 1.5% combined with remifentanil or epidural anaesthesia. After a stable normocapnic period, hypocapnia was induced by hyperventilation, and the raw EEG signals were collected. Bispectral analysis (bicoherence) and power spectrum analysis were performed before and after hypocapnia.

Results. Mean (SD) peak bicoherence in the δ–θ area increased from 35.6 (10.9)% during normocapnia to 43.8 (10.9)% during hypocapnia (P<0.05), whereas mean (SD) peak bicoherence in the α area decreased from 42.8 (14.4)% during normocapnia to 37.5 (12.3)% during hypocapnia (P<0.05). Normalized power in the δ–θ frequencies on the power spectrum increased from 60.2 (13.1)% to 72.5 (12.7)% (P<0.05). Bispectral index and spectral edge frequency changed from 45.9 (7.0) to 40.1 (5.6) (P<0.05) and from 15.0 (2.3) to 14.0 (2.5) Hz (P<0.05), respectively. No significant differences in these values were observed between the two types of anaesthesia.

Conclusions. Hypocapnia enlarged bicoherence growth in the δ–θ frequency range, suggesting the contribution of subcortical oscillating mechanisms in regulating EEG during hyperventilation.

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Hyperventilation is an often-used strategy during anaesthesia to reduce brain volume by decreasing cerebral blood flow through cerebral vasoconstriction. During hyperventilation, hypocapnia is known to cause slowing of the EEG, which is related to an alteration of consciousness in awake persons.1–3 Reductions in the thalamic metabolism and blood flow have recently been shown to be closely related to unconsciousness, suggesting the possibility that the thalamus may serve as a consciousness switch,4 although the thalamus receives massive innervation from the cortex and the switch in thalamic firing activity is known to be driven primarily through a reduction in afferent corticothalamic feedback, more than by any direct effect of anaesthesia on thalamic neurones.5 Assessment of the combined network properties including thalamocortical oscillating system is thus important when considering the regulation of consciousness, anaesthesia, and EEG. However, the traditional methods of linear analysis, such as power spectrum, cannot directly assess these themes.

Bicoherence analysis, a normalized form of bispectral analysis, can elucidate the non-linear oscillating features of the corticothalamo-cortical network under anaesthesia, representing some features of the regulatory properties of thalamoreticular (RE) and thalamocortical relay (TC)
neurons.6–8 That is, an oscillatory source, such as that seen in the thalamocortical network, can contribute to the growth of bicoherence. This is because, in certain oscillating systems, the output signal from the oscillating circuit is expected to re-enter into the system as the input signal and cause self-modulated characteristics resulting in quadratic phase coupling between input signal components. As bicoherence plots during EEG slowing in hyperventilation have not yet been examined, we are interested in the growth pattern of bicoherence under hyperventilation–hypocapnia. Examination of bicoherence in EEG regulation thus seems warranted to examine systematic neuronal activity during hyperventilation.

Methods

Protocol
The institutional ethics committee approved the present study, and informed consent was obtained from all patients. Subjects comprised 22 patients [mean (range): age, 48.9 (15–71) yr; mean (sd): weight, 54.8 (7.9) kg; height, 158.9 (5.9) cm; American Society of Anesthesiologists physical status, I] who were undergoing non-cranial surgery. Patients were premedicated with 0.5 mg atropine approximately 30 min before induction of anaesthesia. Patients were facilitated with vecuronium 0.15 mg kg⁻¹, and maintained using sevoflurane 1.5% and oxygen 35%, with epidural anaesthesia using ropivacaine 0.375% at a rate of 7–12 ml min⁻¹, and overlapping by 75%. After applying Blackman’s window, the Fourier transform of each epoch was computed and averaged power spectra were made from these 120 epoch data. The averaged power spectrum recorded from 0.5 to 40.0 Hz at 0.5 Hz intervals was converted to a normalized form, that is, the ratio of individual power to total power, as along with the resulting EEG data. Mean arterial pressure and heart rate were continuously collected. The raw EEG signal was also stored on a personal computer for off-line analysis. Another anaesthetist, who was blinded to our protocol, determined whether epidural anaesthesia should be performed. In patients who were to receive epidural anaesthesia (Group R, n=11), an epidural catheter was inserted into the interspace of thoracic vertebrae 9 and 10 and positioned 4 cm within the epidural space, then a bolus dose of lidocaine 1% (initial dose, 70–100 mg) was injected to confirm correct positioning of the epidural catheter. Anaesthesia was induced using propofol (2.5 mg kg⁻¹) facilitated with vecuronium 0.15 mg kg⁻¹, then maintained with sevoflurane 1.5% and oxygen 35%, with epidural anaesthesia using continuous infusion of ropivacaine 0.375% at a rate of 7–10 ml h⁻¹ after an initial bolus shot of lidocaine. In patients who did not require epidural anaesthesia (Group NR, n=11), anaesthesia was induced using propofol (2.5 mg kg⁻¹) facilitated by vecuronium 0.15 mg kg⁻¹, combined with continuous infusion of remifentanil 0.5 μg kg⁻¹ min⁻¹, and maintained using sevoflurane 1.5% and oxygen 35%, combined with remifentanil 0.4 μg kg⁻¹ min⁻¹ after intubation. Concentration of expired sevoflurane and end-expired partial pressure of CO₂ were continuously monitored using a Smart Anesthesia Multigas module (GE Medical Systems, Milwaukee, WI, USA). Vecuronium was administered at 0.08 mg kg⁻¹ h⁻¹ to obtain muscle relaxation during surgery. Mechanical ventilation was adjusted to keep the end-expired partial pressure of CO₂ at 35–40 mm Hg. After 1 h of normocapnic conditions, stored raw EEG signals were submitted for off-line analysis. Hyperventilation was then induced by doubling the ventilatory frequency, and was maintained until the end-expired concentration of CO₂ reached 20 mm Hg. We adjusted ventilatory frequency to maintain end-expired concentration of CO₂ at 20 mm Hg, and confirmed stable sevoflurane and CO₂ concentrations in end-expired gas monitoring. After 5 min of stable condition, stored raw EEG signals were once again submitted for off-line analysis as hyperventilation–hypocapnia data. Mean arterial pressure and heart rate were maintained throughout the study at >60 mm Hg and >50 beats min⁻¹, respectively, using phenylephrine and atropine as required. Arterial blood was sampled and analysed; every time, raw EEG signals were checked for detailed off-line analysis.

Data acquisition and analysis
A BIS sensor® (Aspect Medical Systems) consisting of four EEG electrodes was applied to the forehead. Raw EEG wave signals (converted from analogue to digital at 128 Hz frequency) as along with the resulting EEG indices were collected using BSA version 3.22B2 software (Bispectrum Analyzer for A-2000 BIS monitor) developed by Hagihira and colleagues, via an RS232 interface on a personal computer (CF-W2; Panasonic, Osaka, Japan). Signals <0.5 or >50 Hz in frequency were excluded from analysis. BIS values were calculated by the A2000® from the preceding 1 min period of EEG recording and were extracted to a personal computer directly from the A2000®. EEG signals in the preceding 1 min period were divided into 120 epochs, with each epoch 2 s in length and overlapping by 75%. After applying Blackman’s window, the Fourier transform of each epoch was computed and averaged power spectra were made from these 120 epoch data. Spectral edge frequency (SEF) below which 95% of the power in the spectrum resides (SEF95) was calculated from the averaged power spectrum. EEG amplitude (AMP), defined as the voltage halfway between the positive and negative peaks, was calculated from waves with AMPs >5 μV, and averaged over 1 min.

The averaged power spectrum recorded from 0.5 to 40.0 Hz at 0.5 Hz intervals was converted to a normalized form, that is, the ratio of individual power to total power, within the frequency range from 0.5 to 40.0 Hz at 0.5 Hz intervals. The sum of normalized power in each frequency range of the δ area (0.5–3.5 Hz), θ area (4–7.5 Hz), and α area (8–12.5 Hz) was calculated.

Bicoherence values were computed in all pairs of frequencies between 0.5 and 40 Hz at 0.5 Hz intervals from 3 consecutive minutes of artifact-free signals. The calculating method is the same as the method described in our previous manuscripts.6–8 Next, two-dimensional moving
averages of nine points of bicoherence were calculated every 0.5 Hz from 1.5 to 23.0 Hz. These computations were performed using Borland C++® (version 5.02J; Borland International, Tokyo, Japan) and MATLAB® (version 6.5.1; The MathWorks, Natick, MA, USA; Signal processing toolbox, Control system toolbox, and Data acquisition toolbox are included).

**Statistical analysis**

Before and after hypocapnia, changes in EEG parameters (SEF95, BIS, and AMP), bicoherence values, and normalized power spectrum in the δ−θ and α areas were analysed by two-factor repeated measure analysis of variance (ANOVA). Values of P<0.05 were considered statistically significant. Data are expressed as mean (SD).

**Results**

Figure 1 shows a raw EEG wave and the corresponding power spectra under normocapnic or hypocapnic conditions in a patient anaesthetized with sevoflurane combined with remifentanil. In comparison with normocapnia, hyperventilation–hypocapnia caused an increase in the lower frequency area (δ and θ range) of the power spectrum (Fig. 1c).

The corresponding mean biphasic bicoherence in the same patients are shown as two-dimensional frequency plots in Figure 2. During normocapnia, bicoherence peaks around the α and δ−θ areas appeared (Fig. 2a). Under hypocapnic conditions, bicoherence growths in the δ−θ area were more prominent, whereas bicoherence growth in the α area decreased (Fig. 2b).

Figure 3a summarizes the mean normalized power spectra of 22 superimposed cases, and Figure 3b shows the corresponding mean bicoherence spectra around the diagonal lines (\(f_1=f_2\)) on the bicoherence plane of the same 22 superimposed cases. As the figure indicates, hypocapnia increased normalized power in the δ frequencies and decreased normalized power in the α frequencies. Hypocapnia also increased the bicoherence values in the δ and θ areas, while decreasing bicoherence values in the α area.

Table 1 shows changes in BIS, SEF95, and AMP under normocapnic and hypocapnic conditions. Changes in bicoherence growth, bicoherence peak frequency, and normalized power spectrum in each frequency band are also summarized. The results are separately described for Groups R and NR. In comparison with normocapnia, hypocapnia as a whole decreased both BIS and SEF95, but increased AMP (P<0.05), irrespective of the anaesthetic agent used. With hyperventilation, normalized power in the δ−θ frequencies on the power spectrum increased from 60.2 (13.1)% to 72.5 (12.7)% (P<0.05), and normalized power in the α frequencies on the power spectrum decreased from 27.8 (10.6)% to 20.3 (9.6)% (P<0.05). Bicoherence in the α area was decreased from 42.8 (14.4)% to 37.5 (12.3)% (P<0.05), but bicoherence in the δ−θ area was significantly increased from 35.6 (10.9)% to 43.8 (10.9)% (P<0.05). When Groups R and NR were compared, no significant differences were observed in changes of EEG values (P>0.05).

**Discussion**

Hyperventilation was found to result in slowing of EEG, accompanied by increased power of the lower frequency
area on the power spectrum. BIS and SEF95 decreased and AMP increased with hyperventilation, reflecting the same slowed and synchronized EEG features. Previous research has shown that hypocapnia induced by hyperventilation is associated with a consistent slowing of EEG.1–3 Our results are consistent with these reports. However, the method used in previous studies was a simple power spectral analysis, which can only assess linear processes, thereby ignoring potential non-linear interactions of the EEG. The oscillatory wave in EEG includes some oscillatory features of the neural network.9 Among these oscillatory characteristics, broad synchronization, appearing in the low frequency-band oscillations, has a role in the neural mechanisms of sensory awareness and consciousness, reflecting the general features of integration of the extensive brain level. Cross-frequency phase synchrony is thus a salient characteristic of ongoing activity in the human cortex, related to consciousness and cognitive task demands.10–12 Bicoherence, an analysis for cross-frequency phase synchrony, is therefore a candidate for assessing the integration of spectrally distributed processing.8

Our study is the first of its kind to examine the effects of hyperventilation–hypocapnia on the EEG, by bicoherence analysis. We found that hyperventilation–hypocapnia resulted in augmentation of bicoherence growth in the δ–θ area, whereas reducing bicoherence in the α area. These EEG changes in bicoherence and in the power spectrum

**Fig 3** (A) Superimposition of 22 averaged power spectra. Means with standard errors of the mean (SEMs) are shown. SEMs are shown as perpendicular lines (n=22). (B) Superimposition of 22 averaged bicoherence spectra around the diagonal lines ($f_1=f_2$). Means with SEMs. SEMs are shown as perpendicular lines (n=22). We described SEMs instead of SDs, because the larger SDs would make the figure unintelligible. Grey line: averaged normalized power (A) or bicoherence spectra (B) under normocapnic conditions. Bold line: averaged normalized power (A) or bicoherence spectra (B) under hyperventilation–hypocapnia conditions.
Hypocapnia and alkalosis induced by anaesthesia are known to be closely reflecting the oscillating features of neuronal network. Oscillating characteristics seen in deepening anaesthesia, causing similar effects to the thalamocortical oscillatory thalamocortical neural activity and thus to the hyperpolarization of thalamic neurones by hyperventilation. However, hyperventilation cannot be elucidated by the present study, directly responsible for provoking the response during anaesthesia. Conversely, hypoxia is known to cause depolarization in cerebral neurones, after an initial temporary hyperpolarization lasting a few minutes induced by an increase in K⁺ conductance. Although the area directly responsible for provoking the response during hyperventilation cannot be elucidated by the present study, hyperpolarization of thalamic neurones by hyperventilation is one of the conceivable candidates to contribute to the oscillatory thalamocortical neural activity and thus to the anaesthesia, causing similar effects to the thalamocortical oscillating characteristics seen in deepening anaesthesia.

One of the well-known mechanisms explaining this EEG slowing during hyperventilation—hypocapnia is the hypoaxia theory that EEG slowing is secondary to hypoxia arising from sequential cerebral vasoconstriction resulting from hypocapnia. However, Hoshi and colleagues suggested that mechanisms other than hypoaxia theory could also be responsible for this slowing. A study using indomethacin also suggested that EEG slowing is not directly related to reductions in cerebral flow, as cerebral vasoconstriction without concomitant alkalosis does not induce EEG slowing. Patel and Maulsby reported that hypocapnia decreased activity in the mesencephalic reticular formation and that lesions of the thalamus abolish the hyperventilation response. Thus, although hyperventilation is well known to slow down EEG rhythms, the mechanism has not been completely elucidated. The result of increased quadratic phase coupling in the δ area in the present study indicates the contribution of subcortical oscillating mechanisms including the corticothalamocortical network to the regulation of slowing EEG rhythms under hyperventilation, even if EEG slowing is first provoked by direct cortical suppression such as by cortical ischaemia, and the regulation is noteworthy, representing a key factor in the regulation of anaesthesia and consciousness. Thus, although we cannot offer any further discussion of mechanisms from the present study, the results suggest the contribution of corticothalamo-cortical oscillation in slowing EEG by hyperventilation.

Some authors have claimed that the spectral information in the frequency domain is largely contained in the power spectrum of the signal, and that bicoherence and bispectrum could explain only a small percentage of variance in the investigated data. Certainly, the quality of the recursive circuit of cerebral neurone activity is known to appear in the synchronous characteristics of EEG analysis, and both linear and non-linear processes can cause synchronization. In the synchronous neural oscillation in EEG rhythms, the linear aspect, that is, the increase or decrease in AMP as a result of the number of active neurones firing in synchrony, can be detected by synchronous peaks in the corresponding frequency area on the power spectral analysis. When most EEG synchronizes to the rhythm formed in thalamocortical oscillating circuit, as seen in many cases of clinical anaesthesia, the peaks in power spectrum and bicoherence coincide. However, in the specific condition in which the regulation of thalamocortical network was disrupted, the power spectrum dissociates from the bicoherence plot, as activity of the cortical neurones was

| Table 1 EEG parameters and bicoherence values under normocapnic and hypocapnic conditions. The results are separately described for Group R (remifentanil+) and Group NR (epidural anaesthesia, remifentanil–), according to the presence or absence of remifentanil administration. Changes in bicoherence values, power spectra, and EEG parameters before and after hypocapnia were analysed by two-factor repeated-measures ANOVA. *Values of P<0.05 are considered statistically significant about the category of respiratory conditions (normocapnia or hypocapnia). **Values of P<0.05 are considered statistically significant about the category of anaesthetic conditions (with remifentanil or without remifentanil). However, there was not any statistical significance. Data are expressed as mean (sd). |
|-----------------|------------------|------------------|------------------|------------------|------------------|
|                | Normocapnia      | R               | NR               | Hypocapnia       | R               | NR               |
| n               | 22               | 11              | 11               | 22               | 11              | 11               |
| Pa CO₂ (mm Hg)  | 39.2 (3.1)       | 40.1 (3.4)      | 38.9 (3.0)       | 39.2 (3.1)       | 40.1 (3.4)      | 38.9 (3.0)       |
| pH              | 7.41 (0.04)      | 7.40 (0.05)     | 7.41 (0.03)      | 7.41 (0.04)      | 7.40 (0.05)     | 7.41 (0.03)      |
| BIS             | 45.9 (7.0)       | 45.1 (8.1)      | 46.7 (5.9)       | 45.9 (7.0)       | 45.1 (8.1)      | 46.7 (5.9)       |
| SEF 95 (Hz)     | 15.0 (2.3)       | 14.7 (2.8)      | 15.2 (1.7)       | 15.0 (2.3)       | 14.7 (2.8)      | 15.2 (1.7)       |
| AMP (µV)        | 14.6 (3.6)       | 14.3 (3.2)      | 15.0 (4.2)       | 14.6 (3.6)       | 14.3 (3.2)      | 15.0 (4.2)       |
| Bicoherence     |                  |                 |                  |                  |                 |                  |
| α peak (%)      | 42.8 (14.4)      | 44.4 (15.5)     | 41.1 (13.8)      | 37.5 (12.3)*     | 39.9 (12.7)     | 35.0 (12.0)      |
| α peak frequency (Hz) | 10.5 (1.0) | 10.2 (0.9) | 10.7 (1.1) | 10.2 (1.0) | 10.2 (1.0) | 10.3 (1.0) |
| δ–θ peak (%)    | 35.6 (10.9)      | 35.7 (11.2)     | 35.5 (11.2)      | 43.8 (10.9)*     | 46.5 (12.0)     | 41.0 (9.5)       |
| δ–θ peak frequency (Hz) | 5.2 (0.6) | 5.2 (0.3) | 5.3 (0.8) | 4.9 (0.7)* | 4.8 (0.8) | 5.0 (0.6) |
| Normalized power spectrum |                  |                 |                  |                  |                 |                  |
| α (%)           | 27.8 (10.6)      | 27.9 (11.4)     | 27.7 (10.3)      | 20.3 (9.6)*      | 20.9 (10.3)     | 19.7 (9.3)       |
| δ–θ (%)         | 60.2 (13.1)      | 59.0 (13.1)     | 61.5 (13.7)      | 72.5 (12.7)*     | 72.7 (13.4)     | 72.3 (12.6)      |
no longer regulated by the thalamocortical network. Non-linear thalamic contributions to the synchronous rhythms are reflected only by phase analysis of the wave synchronization. When the component without phase couplings increases, bicoherence becomes low even if the peak of the power spectrum is high. Clinically, the discrepancy between power spectrum and bicoherence spectrum has been found in the θ frequency area. Moreover, we have previously found such cases in EEG during hypothermic-cardiopulmonary bypass, in which EEG was slowed and δ power in the power spectrum was increased, whereas bicoherence in the δ wave was decreased at the same time (unpublished data). Bicoherence analysis, directly assessing reverberating network features, is thus necessary, along with the power spectrum.

We used the hyperventilation technique to induce hypocapnia, as hyperventilation is clinically used with this aim in the operating theatre. However, hyperventilation may change haemodynamic conditions such as intrathoracic pressure and cardiac output, and this may affect the results. Finally, in the present cases, we ensured adequate depth of anaesthesia and pain relief in all patients, with the use of either epidural anaesthesia or adequate doses of remifentanil. However, we cannot completely exclude the influences of surgical noceptive stimuli on the EEG. These are the limitations of our study.

In conclusion, we found that hyperventilation with hypocapnia to an $P_{a\text{CO}_2}$ of about 24 mm Hg is associated with bicoherence growth and an increase in the relative power spectrum in the δ–θ frequency area, along with a decrease in BIS and SEF. These changes in EEG during hyperventilation—hypocapnia resemble those observed during deeper anaesthesia, and indicate the modulation of oscillating features in the thalamocortical network.

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