Effects of Volatile Anesthetic Agents on Cerebral Cortical Synchronization in Sheep

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ABSTRACT

Background: The exact neurophysiological mechanisms of anesthetic-induced unconsciousness are not yet fully elucidated. The cortical information integration theory hypothesizes that loss of consciousness during general anesthesia is associated with breakdown of long-distance cortical connectivity across multiple brain regions. However, what is the effect of anesthetics on neural activities at a smaller spatial scale?

Methods: The authors analyzed a set of previously published eight-channel electrocorticogram data, obtained from a 14-mm-long linear array of electrodes in eight adult merino sheep during general anesthesia induced by sevoflurane, desflurane, isoflurane, and enfurane. The S-estimator was applied to the bi-channel coherence matrix to construct an overall index called the SI, which is the entropy of the eigenvalues of the cortical coherence for each pair of channels within the multichannel electrocorticographic dataset.

Results: The SI values increased ~30–50% from the waking to the burst-suppression states, and returned to baseline during recovery. The anesthetic-induced increase in synchrony was most marked in the α (8–13 Hz) and β (13–30 Hz) frequency bands (P < 0.05). Using prediction probability (P_K) analysis, we found a significant correlation between the increase in spatial synchrony (as estimated by the SI at various frequency bands) and anesthetic-induced cortical depression (as estimated by the approximate entropy).

Conclusions: The results suggest that it is feasible to use the SI to measure cortical synchrony, and over a local spatial scale of 2–14 mm, synchrony increased during general anesthesia.

What We Already Know about This Topic

- The neurophysiological mechanisms of general anesthesia-induced loss of consciousness are unclear
- Loss of cortical connectivity among widely distributed brain regions has been proposed as an important anesthetic effect, but effects on local connectivity are not well characterized

What This Article Tells Us That Is New

- The effects of volatile anesthetics on synchronization between electrocorticogram recordings in sheep were analyzed
- In contrast to global synchrony, local cortical synchrony increased during anesthesia, which might contribute to the loss of long-range synchrony and information integration critical to consciousness

UNDERSTANDING how general anesthetic agents induce unconsciousness in the central nervous system may provide a direction for future rational anesthetic drug design and improved intraoperative monitoring. Despite the cellular and molecular pharmacology of anesthesia having been studied extensively,1,2 the neurophysiological mechanisms of general anesthesia-induced loss of consciousness have not been fully clarified.2 Over the last decade, many theories have been proposed to explain the mechanisms of consciousness, such as the cognitive binding paradigm3 and the cortical information integration and information capacity theory.2,4–7 Common among these theories is the idea that consciousness is supported by an optimal balance between segregation of functionally diverse regions, and global coupling. The implication is that loss of consciousness during general anesthesia is caused by a breakdown of cortical connectivity, resulting in a reduction in coupling between widely distributed regions, but, paradoxically, with increased local synchronization.2,8

During the transition from waking to unconsciousness in humans, electroencephalographic coherence in the γ band (20–80 Hz) decreases between homologous regions within the two hemispheres, and between the anterior and posterior regions on each hemisphere.9 Isoflurane impairs...
anteroposterior phase synchronization in 5–25 and 25–50 Hz frequency bands, and disrupts frontal-to-posterior information transfer at high γ band frequencies in rats stimulated with light flashes. The mean information integration capacity is reduced in several frequency bands after induction with propofol; specifically, frontal-to-parietal transfer is inhibited, while communication in the other direction—from the parietal forward to the frontal region—persists. These studies focused on long-range neural communication associated with anesthesia-induced unconsciousness across multiple brain regions.

On the contrary, what is the effect of general anesthesia on neural activities on a millimeter–centimeter spatial scale? There is evidence that local synchrony is increased with commonly used anesthetic drugs. Because of the local cytoarchitectonic packing, cortical activity is inclined to synchronize into a common resonance mode with anesthetics. Recently, Kreuzer recorded local field potentials from the somatosensory cortex of three rats and found that the cortical signals were more synchronous during anesthesia. Increase in local synchrony also accounts for the increase in amplitude of the electroencephalogram signal during anesthesia. In the aforementioned study, the neural synchrony between signals from each channel pair was measured in terms of cross-approximate entropy (AE), which is a non-linear statistical parameter that quantifies the dissimilarity of patterns in a series of pairs.

In the current study, the effect of volatile anesthetic agents (sevoflurane, desflurane, isoflurane, and enfurane) on neural synchrony—measured over a scale of 2–14 mm—was investigated through a reanalysis of previously published electrocorticogram data recorded from an eight-channel electrode array in sheep. We quantified the synchrony of signals from each channel pair by the coherence at different frequency bands, then an S-estimator was used to the bi-channel coherence matrix, which is a novel method to estimate the synchronization in multichannel electroencephalographic series, and finally, the index derived from the S-estimator (SI) was used to track the overall synchronization changes during anesthesia.

Materials and Methods

Data Recordings

In this study, we reanalyzed the electrocorticogram data from a previously reported study. After approval by the Animal Ethics Committee (University of Adelaide, Adelaide, South Australia), the effects of sevoflurane, desflurane, isoflurane, and enfurane were studied in eight adult merino sheep, each weighing approximately 50 kg. Under halothane anesthesia, a midline craniotomy was performed through the frontal bone, 1 cm anterior to the base of the cornual process. A standard, premanufactured linear array of eight stainless-steel electrodes (2 mm spacing–total length 14 mm) was placed over the para-sagittal frontoparietal cortex, penetrating 1–2 mm into the outer layers of the cortical gray matter. The electrocorticogram was obtained using two electroencephalogram monitors (A1000, Aspect Medical Systems, Natick, MA), and the signal was digitally sampled at 256 Hz. After at least 24 h for the animal to recover, the sheep were given a series of short inhalational anesthetics in oxygen. Sevoflurane, desflurane, isoflurane, and enfurane were administered in random order and increasing concentration until burst suppression was noted on the electrocorticogram, and the sheep was then allowed to recover fully (at least 2 h) before the administration of the next agent. More details can be found in the references.

In order to assess the effects of these volatile anesthetic agents at different depths of anesthesia, the cortical anesthetic effect was monitored using AE, calculated in the standard manner as described by Bruhn (sample rate of 128 Hz, embedding of m = 2, and noise threshold r = 0.2 x SD). Due to the incorporation of a lower threshold for noise, the AE could correctly classify the occurrence of burst suppression with increasing anesthetic drug effect. In the following, the electrocorticogram data with more than four channels failed were rejected, thus the sample sizes in subsequent synchronization analysis were N = 8 for sevoflurane, and N = 7 for desflurane, isoflurane, and enfurane anesthesia. The average of all the available electrocorticographic recordings was subtracted from each channel to remove the interference of baseline drift. Raw data segments of 1 min at different anesthetic states were extracted from each sheep to calculate the coherence-based SI indices. Each segment was visually inspected, and any segment with significant artifacts was excluded from the analysis.

The synchronization analysis of a single epoch is difficult to determine the accurate relations in the neural signal. Thus, in the current study, each segment was subdivided into 10-s epochs, with 75% overlap, then the SI values were computed for each epoch, and finally, a reliable estimate was obtained by averaging the SI values of all epochs.

Coherence Analysis and S-estimator

To explore the synchronization between two signals at a prescribed frequency band, the coherence function is a simple and widely used method, which quantifies linear correlations in the frequency domain. Given two electrocorticographic series \( x_i \) and \( x_j \), \( i, j = 1, 2, \ldots, M \), and \( M \) is the number of available channels, the average synchronization at a frequency band \( [f_i, f_j] \) can be calculated and denoted as \( d_{ij} \). Detailed information is shown in the appendix. Then for the \( M \)-channel electrocorticographic series, the synchrony at frequency band \( [f_i, f_j] \) can be described by the coherence matrix, \( D \), with each element of it, \( d_{ij} \), denoting the correlation between the electrocorticographic signals from the \( i \)-th channel and \( j \)-th channel.

The eigenvalue decomposition of coherence matrix is

\[
D_{ij} = \lambda^\text{raw}_i v_i
\]
where $\lambda_i^{\text{raw}}, i = 1, 2, \cdots, M$ are eigenvalues, and $v_i$ are the corresponding eigenvectors. As $D$ is a real symmetric matrix, all eigenvalues are real numbers, and they can provide information about the synchronization among individual elements of the matrix. If the multichannel electrocorticographic signals $x_i, i = 1, 2, \cdots, M$ are statistically independent, all the eigenvalues tend to be equal to 1. In contrast, if the signals are well-synchronized, only a few numbers of eigenvalues will remain prominent, and the others will be close to 0.28

However, for real data, the distribution of eigenvalues exhibits some bias due to the length of data, noise, and so on. Therefore, a surrogate technique was proposed to reduce the spurious coupling information.21 In the current study, the estimate of coherence mainly depends on the phase relation between neural activities, thus we randomize the phase relationship using a typical nonlinear resampling method—the iterative amplitude-adjusted Fourier transform algorithm,29 which can retain both the amplitude distribution and the power spectrum to a high degree of precision.29 Details can be found in the references.29,30 For the two signals $x_i$ and $x_j$, first resampling one signal through the iterative amplitude-adjusted Fourier transform method, a surrogate coherence matrix is obtained, and surrogate eigenvalues can be derived by eigenvalue decomposition. This procedure is run $N^{\text{run}}$ ($N^{\text{run}} > 19$) times to obtain $N^{\text{run}}$ groups of eigenvalues. The averaged surrogate eigenvalues are calculated and denoted as $\lambda_{i}^{\text{surro}}, i = 1, 2, \cdots, M$. Thus, the improved eigenvalues of statistical significance can be derived by the raw eigenvalues divided by the averaged surrogate eigenvalues, i.e., $\lambda_i^{\text{sig}} = \lambda_i^{\text{raw}} / \lambda_i^{\text{surro}}$.

In intuitive terms, the significant eigenvalues might be considered to be a measure of the complexity within the matrix. In our case, to reliably describe the synchronization patterns of coherence matrix, the S-estimator that quantifies the flatness of the distribution is applied, which is defined as20,21

$$SI = 1 + \frac{\sum_{i=1}^{M} \tilde{\lambda}_{i}^{\text{sig}} \log(\tilde{\lambda}_{i}^{\text{sig}})}{\log(M)}$$

where $\tilde{\lambda}_{i}^{\text{sig}} = \lambda_{i}^{\text{sig}} / \sum_{i=1}^{M} \lambda_{i}^{\text{sig}}$ are the normalized improved eigenvalues. The SI index takes values between 0 and 1, thus behaving as a unitary and normalized measure for the synchronization of multichannel electrocorticographic series. If there is no synchrony among multiple channels, the normalized eigenvalues $\lambda_{i}^{\text{sig}}, i = 1, 2, \cdots, M$ are all equal to $1/M$, so $SI = 0$. On the contrary, if all the series are perfectly correlated, the variation in the coherence can be described by a single normalized eigenvalue $\tilde{\lambda}_{1}^{\text{sig}} = 1$, and the others are zeros, so $SI = 1$.28

Statistical Analysis

The electrocorticogram data were exported from Matlab to SPSS (Version 13.0, SPSS Inc., Chicago, IL) for statistical analysis. For each anesthetic agent, one-way repeated measures ANOVA with Bonferroni post hoc tests were used to explore the existence of significant differences in the synchronization at different study periods. If the assumption of homogeneity of covariance was violated by Mauchly’s test of sphericity, a Greenhouse–Geisser correction method was used. For each sheep, the association between the SI measure and the underlying anesthetic depth was assessed by the prediction probability ($P_K$).31 $P_K$ was calculated for each sheep, with the averaged AE index (as an indicator of the depth of anesthesia) as an independent variable, and the SI indices at the studied frequency bands as dependent variables. A $P_K$ value of 1 means that the SI index is perfectly concordant with the underlying anesthetic depth. A value of 0.5 means that the SI is not superior to that obtained by chance. The resultant $P_K$ value is replaced by $1-P_K$ when there is a negative correlation between the AE and the SI indices. A further comparison of these $P_K$ values at different frequency bands was performed by using one-way repeated measures ANOVA with Bonferroni post hoc tests. For all tests, $P < 0.05$ were considered significant. Data are presented as mean ± SD, unless specially stated.

Results

A seven-channel electrocorticographic recording from one sheep during sevoflurane anesthesia is shown in figure 1A. The electrocorticogram shows the transition from the awake to the anesthetic state, to burst suppression, and then back to the waking state. At different states, 1-min segments were extracted, and labeled as I (awake), II (anesthesia), III (burst suppression), IV (anesthesia), and V (recovery). Each segment was then subdivided into a series of 10-s epochs, and five example epochs at different states are enlarged and shown in figure 1B. It is obvious that both the waveform and the frequency distribution are distinct at different states. For each channel pair, the coherence in the $\alpha$ band (8–13 Hz) was computed, and the coherence matrix was obtained, as shown in figure 1Bc. Figure 1Bd shows the raw eigenvalues from the eigenvalue decomposition of the coherence matrix (in circles), the averaged surrogate eigenvalues derived from the coherence analysis of the resampled electrocorticographic series (in stars), the resulting improved eigenvalues (in diamonds), and the output of the S-estimator. From the figure, it is evident that the S-estimator of the electrocorticographic signals indicates the changes in anesthetic state, with the larger SI values occurring during burst suppression (III), followed by those during anesthesia (II and IV). The awake and recovery states (I and V) have the lowest S-estimator values. Note that an S-estimator with a low value corresponds to having most randomness in the coherence matrices—as seen by the multiple shades of blue in figure 1Bc. In contrast, the higher SI in the burst-suppression state reflects the fact that the coherence matrix is not flat, but is dominated by few red and yellow peaks in the coherence matrix (fig. 1Bc).
Cortical Synchrony in Sheep during Anesthesia

Fig. 1. Example electrocorticogram, power spectra, correlation matrix, and the distribution of their eigenvalues at different study periods. (A) Preprocessed seven-channel electrocorticographic data recorded from a sheep during sevoflurane anesthesia (one of the eight channels failed). ECoG is the abbreviation for electrocorticogram. The sampling frequency is 256 Hz. Five 60-s segments are extracted, labeled as I (awake), II (anesthesia), III (burst suppression), IV (anesthesia), and V (recovery), respectively. To obtain a reliable estimate of synchrony, each segment is subdivided into 10-s epochs, with 75% overlap. (Ba) The enlarged representative 10-s electrocorticographic epochs at different states, from the dark-colored shaded region in A. (Bb) The power spectra of each channel (in thin blue) and the averaged power spectra (in thick red). (Bc) The correlation matrix based on the coherence analysis at the α band (8–13 Hz) for each channel pair. The different colors represent different degrees of coherence between two channels at α band. (Bd) The distribution of eigenvalues (circles: raw eigenvalues from the eigenvalue decomposition of the correlation matrix in Bc; stars: averaged surrogate eigenvalues; diamonds: improved eigenvalues) and the output of the S-estimator (SI).
Figure 2 shows the SI values derived from the coherence analysis across the whole frequency spectrum (fig. 2A), and also the five traditionally defined frequency bands (fig. 2, B–F): δ (0.5–4 Hz), θ (4–8 Hz), α (8–13 Hz), β (13–30 Hz), and γ (30–45 Hz), for all eight sheep under sevoflurane anesthesia. As can be seen from the figure, the SI values generally increase with deepening anesthesia (states I–III: from awake to burst suppression), and decrease during the recovery process (states III–V: from burst suppression to recovery). This result indicates that the cortical electrocorticographic signals are more synchronous during sevoflurane anesthesia.

To further evaluate these changes in synchrony, multiple comparisons (and corresponding post hoc tests) were conducted for SI values from awake to burst suppression (states I–III), and from burst suppression to recovery (states III–V), respectively. As shown in table 1, all the comparisons revealed significant differences ($P < 0.05$). Further, post hoc tests were carried out and are shown in figure 2. In almost all the cases, the burst suppression (III) can always be distinguished from the anesthesia (II and IV) and awake/recovery states (I and V; $P < 0.05$). The SI values at the α and β bands could also differentiate the anesthesia and awake/recovery states ($P < 0.05$).

We also compared the effects on cortical synchrony of three other volatile anesthetic agents: desflurane, isoflurane, and enflurane. The SI values all demonstrate results similar to those in figure 2 (figs. not shown). Multiple comparison of SI values for the θ, α, and β bands all revealed significant differences under desflurane ($P < 0.01$), isoflurane ($P < 0.05$), and enflurane anesthesia ($P < 0.01$); the SI values across the whole frequency spectrum and γ band also revealed significant differences under isoflurane ($P < 0.05$) and enflurane anesthesia ($P < 0.01$), and those at the δ band revealed significant differences under isoflurane anesthesia ($P < 0.01$). These results demonstrate that the cortical electrocorticographic signals are more synchronous in the presence of volatile anesthetic agents.

To quantify the relationship between the anesthetic effects on cortical activity, we calculated the prediction probability ($P_k$) between the SI and averaged AE values. As shown in figure 3, overall there was a modest, but significant, correspondence between AE and SI for all the frequencies. With the Bonferroni correction for multiple comparisons, there was no significant difference ($P > 0.05$) in the $P_k$ values between the different frequency bands. However, the strongest correlations with the AE seemed to be found for the SI applied to the α and β bands—with mean $P_k$ values between 0.85 and 0.9. These results probably reflect the well-described appearance of strong synchronous α activity during volatile-based anesthesia—the so-called anesthesia spindles.

![Fig. 2](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/930992/) Synchronization analysis of the electrocorticographic data at different study periods (I: awake, II: anesthesia, III: burst suppression, IV: anesthesia, and V: recovery) under sevoflurane anesthesia. The SI values derived from the coherence analysis at the whole frequency spectrum (A), and the five traditionally defined frequency bands: δ (0.5–4 Hz; B), θ (4–8 Hz; C), α (8–13 Hz; D), β (13–30 Hz; E), and γ (30–45 Hz; F), for all the six sheep. The values are given in the form of mean ± SD. The notation ***, **, and * indicate significant difference in the SI values at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively, through Bonferroni post hoc test.
Discussion

In this study, we investigated the effect of volatile anesthetic agents on the cortical synchrony of multichannel electrocorticographic recordings from sheep. The overall synchrony in different frequency bands was assessed using the SI measure, which quantifies the flatness of the distribution of the eigenvalues of the coherences among all the pairs of channels. With increasing anesthetic depth, the coherences among the cortical signals increase and become less complex; and hence can be described by a few dominant eigenvalues, as shown by an increase in the SI. If we look at subsets of different frequency bands, it seems that most of the increase in coherence occurs in the higher frequencies (the α and β bands), and less so in the δ and θ bands.

The increase in SI values in the presence of volatile anesthetics suggests that the electrocorticographic signals are more synchronous on a local spatial scale. This finding is consistent with previously reported results in the references. In the awake state, synaptic inputs into closely spaced neuronal populations represent diverse patterns, and

Table 1. Significance Test of One-way Repeated measures ANOVA for the SI Values at Studied States under Sevoflurane Anesthesia

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>States I, II, III</th>
<th>States III, IV, V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(2,14) = 12.231</td>
<td>F(1.062,14) = 13.178</td>
</tr>
<tr>
<td>0.5–4 Hz</td>
<td><strong>P &lt; 0.01</strong></td>
<td><strong>P &lt; 0.01(GC)</strong></td>
</tr>
<tr>
<td>4–8 Hz</td>
<td>F(2,14) = 25.749</td>
<td>F(2,14) = 16.224</td>
</tr>
<tr>
<td>8–13 Hz</td>
<td>*<strong>P &lt; 0.001</strong></td>
<td>*<strong>P &lt; 0.001</strong></td>
</tr>
<tr>
<td>13–30 Hz</td>
<td>*<strong>P &lt; 0.001</strong></td>
<td>F(1.075,14) = 22.914</td>
</tr>
<tr>
<td>30–45 Hz</td>
<td>F(2,14) = 9.386</td>
<td>F(1.066,14) = 10.196</td>
</tr>
</tbody>
</table>

GC means the Greenhouse–Geisser correction method was used when the assumption of homogeneity of covariance was violated. States I, II, and III mean from awake to burst suppression (I: awake; II: anesthesia; III: burst suppression). States III, IV, and V mean from burst suppression to recovery (III: burst suppression; IV: anesthesia; V: recovery).

*, **, *** Significant difference in the SI values at P < 0.05, P < 0.01, and P < 0.001, respectively, through Bonferroni post hoc test.

Fig. 3. Prediction probability (P_k) analysis between the SI values and averaged approximate entropy indices, derived from the electrocorticographic data under sevoflurane (A), desflurane (B), isoflurane (C), and enflurane (D) anesthesia, respectively. The values are given in the form of mean ± SD. The approximate entropy is computed with the embedding dimension m = 2 and noise threshold r = 0.2, and averaged across multiple channels.
these populations operate quite independently of each other. However, with volatile anesthesia, the diversity of these synaptic activities is transformed into a more uniform, synchronous pattern, reflecting a reduction in the independence of operation. Our results are in close agreement with those in a recent paper by Lewis et al., who found that—during propofol anesthesia—the slow (<1 Hz) oscillations strongly entrained all the high-frequency activity (see fig. 7 in Lewis’ article), thus increasing small-scale coherence (<4 mm). Conversely, they found that increased propofol caused asynchronous activity at large scales in the low-frequency band. Diverse anesthetic drugs have been shown to disrupt long-range cortical coupling. Thus it would appear that anesthetic drugs have the dual effect of reducing global cortical integration, while enhancing synchronization on a local scale. Indeed, we would speculate that the anesthetic-induced disruption of long-range cortical integration might actually be achieved by the anesthetic-induced increase in small-scale synchrony. By making small groups of neurons hyper-synchronous, they become insensitive to long-range input—the so-called cortical block phenomenon—that has been proposed as the final common pathway for unconsciousness. This effect may arise from the enhancement of inhibitory GABAergic (GABA: γ-aminobutyric acid) synaptic transmission. Augmented GABAergic interneuron action is likely to influence the cortical activity by coercing independent neuronal populations into more synchronous, uniform, activity patterns. If this shift in cortical dynamics reflects a final common pathway for unconsciousness, it would suggest that the anesthetic-induced disruption of long-range cortical information transfer can be measured and monitored by electrophysiological methods.

In conclusion, the SI measure based on the S-estimator and coherence analysis could be used to measure cortical synchrony; and the local cortical synchrony over a spatial scale of 2–14 mm is enhanced during general anesthesia induced by volatile anesthetic agents. We hypothesize that the anesthetic-induced increase in local synchrony might actually be the mechanism by which long-range integration of information in the cerebral cortex is disrupted by anesthetic drugs.

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Appendix: Coherence Analysis
Given two time series \( x_i \) and \( x_j \), the magnitude square coherence function, denoted as \( H_y(f) \), is defined as

\[
H_y(f) = \frac{\left| C_y(f) \right|^2}{C_x(f) \cdot C_y(f)} \tag{A}
\]

where \( C_y(f) = P_y(f)P_y^*(f) \) is the cross-spectrum, \( P_i(f) \) and \( P_j(f) \) are frequency spectra of \( x_i \) and \( x_j \), \( * \) stands for the complex conjugation, and \( C_x(f) \) and \( C_y(f) \) are auto-spectra created by \( x_i \) and \( x_j \). The value of \( H_y(f) \) ranges from 1 to 0, indicating the maximum and no synchrony, respectively. In this study, the estimate of coherence, \( \hat{H}_y(f) \), was calculated by running the Matlab (Version R2011a, MathWorks Inc., Natick, MA) function \texttt{mscohere.m}, using Welch’s averaged method.

The normalized average synchronization of signals \( x_i \) and \( x_j \) at a frequency band \( [f_L, f_H] \), can be described by the expression

\[
d_y = \frac{1}{N_w} \sum_{w=f_L}^{f_H} \hat{H}_y(f) \tag{B}
\]

where \( N_w \) is the number of summands in the summation, guaranteeing that the synchronization value of \( d_y \) is bounded between 0 and 1.