Comparison of Anesthetic Depth Indexes Based on Thalamocortical Local Field Potentials in Rats

Aura Silva, D.V.M., M.Sc.,* Hélder Cardoso-Cruz, M.Sc.,† Francisco Silva, M.Sc.,‡ Vasco Galhardo, Ph.D.,§ Luis Antunes, Ph.D.,∥

ABSTRACT

Background: Local field potentials may allow a more precise analysis of the brain electrical activity than the electroencephalogram. In this study, local field potentials were recorded in the thalamocortical axis of rats to (i) compare the performance of several indexes of anesthetic depth and (ii) investigate the existence of thalamocortical correlated or disrupted activity during isoflurane steady-state anesthesia.

Methods: Five rats chronically implanted with microelectrodes were used to record local field potentials in the primary somatosensory cortex and ventroposterolateral thalamic nuclei at six periods: before induction of anesthesia; in the last 5 min of randomized 20-min steady-state end-tidal 0.8, 1.1, 1.4, and 1.7% isoflurane concentrations; and after recovery. The approximate entropy, the index of consciousness, the spectral edge frequency, and the permutation entropy were estimated using epochs of 8 s. A correction factor for burst suppression was applied to the spectral edge frequency and to the permutation entropy. The correlation between the derived indexes and the end-tidal isoflurane was calculated and compared for the two studied brain regions indexes. Coherence analysis was also performed.

Results: The burst suppression–corrected permutation entropy showed the highest correlation with the end-tidal isoflurane concentration, and a high coherence was obtained when comparing the two studied areas.

Conclusions: The permutation entropy corrected with the classic burst suppression ratio is a promising alternative to other indexes of anesthetic depth. Furthermore, high coherence level of activity exists between the somatosensory cortical and thalamic regions, even at deep isoflurane stages.

© Ph.D. Student, ‡ Associate Professor of Veterinary Anesthesiology and Researcher, Centro de Ciência Animal e Veterinária (CECAV), Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, and Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto. † Ph.D. Student, Instituto de Histologia e Embriologia, Faculdade de Medicina and IBMC, ‡ Research Student, Departamento de Física, Faculdade de Ciências, § Associate Professor, Instituto de Histologia e Embriologia, Faculdade de Medicina and Principal Investigator at IBMC, Universidade do Porto.


Address correspondence to Dr. Silva: Rua do Campo Alegre, 824, 4150-180 Porto, Portugal. asilva@ibmc.up.pt. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY’s articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

What We Already Know about This Topic
- Surface electroencephalographic analysis of anesthetic depth reflects overlapping activity
- Analysis of intracranial local field potentials may improve understanding and advancement of electroencephalographic analysis

What This Article Tells Us That Is New
- In rats, there was high coherence between cortical and thalamic activity during steady-state anesthesia
- Permutation entropy corrected with classic burst suppression was noted as a promising new index of anesthetic state

There are several indexes of anesthetic depth developed to translate the information of the complex electroencephalogram signal into a number. However, the electroencephalogram reflects noisy overlapping of activity from different brain regions and only its lower frequency components (below 30 Hz) are normally analyzed because of potential electromyographic activity interferences. Intracranial techniques, such as the local field potentials (LFPs), are not contaminated by electromyographic activity and allow the analysis of higher frequencies, such as the γ-band. Thus, the effect of anesthetics on the brain could be more accurately reflected on the LFPs than on the electroencephalogram, allowing a more precise analysis of the performance of anesthetic depth indexes.

LFPs represent the extracellular low-frequency summed postsynaptic potentials from local cell groups and have been applied in neurophysiology studies to record the electric signal from specific brain areas. By recording thalamocortical LFPs, it is possible to compare the activity in the cortical and thalamic neurons and answer the controversy regarding which region is responsible for anesthetic-induced unconsciousness, the cortex or the thalamus. These structures are densely and reciprocally interconnected, even during quiet physiologic sleep, but it is not known whether this connection is interrupted by anesthetics. The use of electroencephalogram-derived indexes to analyze LFPs is a strong tool to address these questions. The most recently investigated indexes of anesthetic depth are the approximate entropy (AE), the permutation entropy (PE), and other commercial monitors such as the most recently introduced

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index of consciousness (IoC).\textsuperscript{18} On the other hand, classic spectral edge frequency (SEF) is mathematically simpler and has been suggested to adequately reflect depth of anesthesia from the electroencephalogram of rats, when corrected for the burst suppression ratio.\textsuperscript{19}

In this study, LFPs were recorded in isoflurane-anesthetized rats with two main objectives: (i) to compare the performance of the more recently introduced indexes of anesthetic depth and (ii) to investigate the existence of thalamocortical correlated or disrupted activity during isoflurane steady-state anesthesia.

**Materials and Methods**

**Subjects**

Five adult male Sprague-Dawley rats weighing between 350 and 400 g were used in this study. The rats were maintained on a 12-h light and dark cycle, with ad libitum access to food and water. All procedures and experiments were conducted in compliance with the Ethical Guidelines for Animal Experimentation and Animal Care Committee imposed by the European Community Directive Number 86/609/ECC, November 24, 1986, and approved by the national regulatory office (Direcção Geral de Veterinária—DGV, Lisboa, Portugal) under the title “Melhorias das técnicas anestésicas e analgésicas em animais de laboratório de 21 de Outubro de 2003,” and the project was approved by the Foundation for Science and Technology with the number POCI/CTU/59056/2004 and the same name.

**Surgery**

At least 7 days before the experiment, the rats were anesthetized with an intramuscular injection of a mixture of xylazine and ketamine (10 and 60 mg/kg, respectively). Anesthesia was maintained with additional injections of ketamine (one-third of the initial doses). A fresh gas supply of oxygen was delivered during the procedure by a facemask. Depth of anesthesia was assessed by regularly testing the corneal blink, hind paw withdrawal, and tail-pinch reflexes. Core body temperature was measured with a rectal thermometer and maintained at 37°C by a homeothermic blanket system. Core body temperature was measured with a rectal thermometer and maintained at 37°C by a homeothermic blanket system. An animal was transferred to a recovery cage. The analgesic carprofene (5–10 mg/kg) (Rimadyl; Pfizer Animal Health, Lisboa, Portugal) and the antibiotic amoxicillin (6 mg/kg) (Clamoxyl; Pfizer Animal Health) were administered subcutaneously every 24 h during 2 or 3 days. Rats were allowed to recover for 1 week before the recording sessions began.

**Histology**

After the end of all experiments, the rats were deeply anesthetized with ketamine and xylazine mixture, and the recording site was marked by injecting direct current (10–20 \( \mu \)A for 10–20 s) through one microwire per matrix group, marking the area below the electrode tips. After this step, the animals were perfused through the heart with 0.01 M phosphate buffer (pH = 7.2) in 0.9% saline solution followed by 4% parafomaldehyde. The brains were removed and post-fixed in 4% parafomaldehyde during 4 h and then stored in 30% sucrose before they were frozen and sectioned into 60-µm slices. The sections were stained to identify the recording site under the microscope. This technique, in conjunction with careful notation of electrode movements during implantation surgery, allowed localization of all recording sites (fig. 1B).

**Isoflurane Anesthesia Studies**

To reduce stress during induction, animals were placed in the induction chamber, connected to the data recording cables, and oxygen was administered several days before the experiment. This allowed animals’ acclimatization to the induction chamber and instrumentation. On the testing day, neuronal recording started 5 min before induction of anesthesia. Anesthetic induction was performed with 4% isoflurane (isoflu-
rane; Abbott, Amadora, Portugal) in 5 l/min of 100% oxygen. After loss of the righting reflex and evaluation of changes in respiratory rate, the animal was transferred to a smaller chamber placed above an homeothermic blanket (Harvard Apparatus, Edenbridge, Kent, United Kingdom), which also recorded the animal’s temperature by a rectal probe. A purpose-built hole in the chamber allowed the passage of the LFP signals recording cable. A gas sensor, connected to an anesthetic agent monitor (Capnomac II; Datex Ohmeda, Helsinki, Finland) was placed inside the chamber at the level of the rat’s nose. The animal was positioned with the head turned to the inflow port in ventral recumbence. The end-tidial isoflurane (etISO) concentration was stabilized at 0.8, 1.1, 1.4, and 1.7% concentrations for 20 min to allow equilibration between inspired and end-tidal concentration, according to a predetermined randomized sequence for each animal. These concentration stages were named as 0.8, 1.1, 1.4, and 1.7.

During the experiments, the animals breathed spontaneously, and the respiratory frequency was monitored every 5 min. In the end of the last isoflurane concentration stage, the animal was transferred to the induction chamber, where oxygen was delivered, and the recording continued for 5 min after the animal recovered its righting reflex.

LFP Recording and Processing
LFPs represent the extracellular low-frequency current flow that reflects the linearly summed postsynaptic potentials from local cell groups around the microelectrode. This type of neural signal has a temporal structure mainly in the frequency of 0–100 Hz. Extracellular LFP activity was recorded from the implanted microwires and processed by using a 16-channel Multineuron Acquisition Processor (Plexon Inc., Dallas, TX). LFP signals recorded from electrodes were pre-amplified (500×), filtered (0.5–400 Hz), and digitized at 1,000 Hz using a Digital Acquisition card (NIDAQ; National Instruments, Austin, TX) and sent to the 16-channel Multineuron Acquisition Processor system. Only two LFP channels per array per area were recorded and considered for posterior analysis (channel 3 [medial] and channel 6 [lateral]). The LFP frequencies analyzed ranged from 1 to 100 Hz.

Data were validated by offline analysis using NeuroExplorer 4 (Plexon Inc.) and exported to MatLab R14 (Version 2008a) for complementary analysis (MathWorks, Natick, MA).

Calculation of AE, PE, SEF, and BS Ratio. The LFP sampling frequency was first decreased to 250 Hz. All four parameters were derived from epochs of 8 s from the mean of the two cortical channels and the mean of the two thalamic channels.

The AE and the PE were computed according to the published algorithms. The AE measures the predictability of a time series. There are three essential parameters on its calculation: the embedding dimension (m) that refers to the number of points used for prediction, the number of samples considered for each calculation (N), and a noise threshold (r). These parameters and the AE algorithm are explained in more detail in the literature. Studies carried out by Bruhn et al. showed that the AE yielded the highest pre-
diction probability with desflurane effect site concentration using $m = 2$, $r = 0.2$, and an epoch length equal or higher than 1.024 points. $N = 2,000$, $m = 2$, and $r = 0.2$ were selected in our study for AE calculation.

The PE is a method for ordinal time series analysis. It analyzes consecutive subvectors of constant length ($m$) in the analyzed signal interval (length, $N$). Then, it orders the samples in every subvector according to their amplitude and defines permutations of order $m$ ($m!$). The parameter value is given by the resultant normalized probability distribution of the obtained permutations, using the Shannon entropy formula. In this study, we used $m = 3$ and $N = 2,000$. A more detailed description of the PE calculation can be found in the published works.15–17

The SEF that consists of the power spectrum in the 95% quantile was also calculated and was corrected for the presence of BS patterns according to the correction factor proposed by Rampil23 for human electroencephalogram studies. The same correction factor was applied to PE, calculated as follows: BS-corrected PE = PE × (1 − BS/100), where the BS is defined as the epoch length in which the LFP voltage did not exceed 50 µV.

**Calculation of the IoC.** The IoC was derived from the signal using an executable file provided by the manufacturer (Aircraft Medical, Barcelona, Spain). Its calculation is based on the symbolic dynamics method that transforms a time series into a symbol sequence that can reveal the nonlinear characteristics of the electroencephalogram. It also integrates the $\beta$-ratio (frequency range between 11 and 42 Hz) during superficial anesthesia and the amount of suppression of the electroencephalogram (equivalent to the BS). Similar to entropy methods, this method also expresses the complexity of the signal that makes it a correlate to the depth of anesthesia. In humans, decreasing values of IoC correspond to a gradual loss of consciousness and a deepening of the level of anesthesia. In a unitless scale from 99 to 0, an index of 99 indicates an awake patient and an index of 0 indicates a flat electroencephalogram. More details on its calculation were recently published.18

**Spectral Analysis.** Spectrogram analysis was used to visualize LFP power at different frequency bands as a function of time for each etISO concentration (fig. 2). LFP spectrograms were computed in NeuroExplorer (Version 4, Plexon Inc.), with a 0.5-Hz spectral resolution and a 100-ms temporal resolution. Fig. 2. Effects of isoflurane on intracranial local field potentials (LFPs) recorded simultaneously in the primary somatosensory cortex (SI, black trace) and in the ventroposterolateral thalamic nuclei (VPL, blue trace). (A) Raw LFP recordings representing a sample of 10 s of ongoing LFP activity recorded during the last 5 min before anesthesia induction (unaesthetized period) and during the last 5 min of each isoflurane concentration (0.8, 1.1, 1.4, and 1.7%). Visual inspection of the LFP traces revealed similar patterns of signal amplitude oscillations developed by the two recorded areas across the anesthesia stages. (B) Correspondent power spectrogram of each SI trace from A. Spectrograms showed the different patterns of signal power across the frequency range analyzed, with the appearance of alternating periods of quiescence and high-frequency activity, at higher anesthetic depths. This is compatible with the burst suppression pattern observed on the LFP correspondent traces (A).
resolution, using a 512-point fast Fourier transform and a
50-ms Hanning window. The power was normalized by the
logarithm of the power spectral density (in decibel) and a
smoothing was applied (Gaussian filter, width = 3).

The power spectra of cortical ($P_{xx}$) and thalamic ($P_{yy}$)
LFP signals were calculated between 1 and 100 Hz by fast
Fourier transform (512 point) of nonoverlapping 1-s ep-
ochs (Hanning window). Data are shown as the percent-
age of total power spectral density within each considered
frequency band: $\delta$ (1–4 Hz), $\theta$ (4–9 Hz), $\alpha$ (9–15 Hz), $\beta$
(15–30 Hz), $\gamma$-low (30–50 Hz), and $\gamma$-high (50–100 Hz). Values are means ± SE. Comparisons based on two-way analysis of variance test, followed by Bonferroni post hoc test. *$P < 0.05$; **$P < 0.01$, and ***$P < 0.001$. Color version of this figure is available at www.anesthesiology.org.

Coherence $K_{xy} = (P_{xy}^2/(P_{xx} P_{yy}))$ for two signals, $x$ and $y$, is equal to the average cross power spectrum ($P_{xy}$) normalized by the averaged power spectra of the signals. This technique was used to measure the strength of the linear relationship at every frequency band between the cortical and thalamic LFP signals across each etISO concentration (fig. 3C). Its value lies between 0 and 1, where $K_{xy} = 0$ means phases are eventually dispersed, and high coherence ($K_{xy} = 1$) means phases of signals $x$ and $y$ are identical.

Intervals of 5 min were used to calculate signal power spectral density and coherence ($K_{xy}$). Isoflurane stages were calculated during the last 5 min, 5 min before anesthesia induction, and 5 min after anesthesia recovery.

Statistical Analysis

Data resultant from the LFP processing were exported from Matlab to Graphpad Prism (Version 5; GraphPad Software Inc., San Diego, CA) for statistical analysis. The Kolmogorov-Smirnov test was used to determine whether datasets were normally distributed. The correlation between the studied indexes AE, IoC, SEF, PE, BS-corrected SEF, BS-corrected PE, and the etISO was calculated using the Spearman rank correlation coefficient ($r$), with data from all animals. Statistical comparisons for the derived indexes, between animals and between SI and VPL channels (including comparisons of the $r$ values) were made using the Mann–Whitney and the Wilcoxon matched-pairs tests, respectively. One-way repeated-measures ANOVA with Bonferroni post hoc was used to address the existence of significant differences in the spectral frequency bands of the LFP signal at the different study periods.

Results

Spontaneous intracranial LFP activity was recorded from the SI and VPL of awake and anesthetized rats ($n = 5$).

Six indexes of anesthetic depth were derived from the SI and VPL recordings: the AE, the IoC, the SEF, the PE, the BS-corrected SEF, and the BS-corrected PE. The values of the indexes, at each study period, for the five rats are pre-
sented in figure 4. The Spearman rank correlation coefficient ($r$) between the indexes and the etISO concentration was calculated for the indexes derived from the SI and the VPL and compared (table 1).

The indexes tended to decrease similarly with increasing concentrations of etISO, except the SEF and PE. Both SEF and PE decreased from the period before induction to the period 0.8 but increased paradoxically with higher etISO concentrations, as shown in figures 4C and D. When the correction factor for BS was applied, both parameters decreased monotonically with increasing etISO concentrations (figs. 4E and F).

Comparisons between the SI and VPL revealed significant differences only in the AE ($1.4$ and $1.7$; $P < 0.05$), PE ($0.8$, $1.1$, $1.4$, and $1.7$; $P < 0.05$), and BS-corrected SEF ($0.8$, $1.1$, and $1.4$; $P = 0.0025$). No significant differences were found between the $r$ values of the SI- and VPL-derived indexes ($P = 0.089$), although the indexes derived from the SI tended to have higher $r$ values with the etISO (table 1). A correlation was found between AE and BS ($r = -0.73$; $P < 0.001$).

Data obtained from a representative recording are given in figure 2. The visual inspection of the raw LFPs confirmed that large signal amplitude oscillations were developed simultaneously in both recording areas during all anesthesia concentrations (fig. 2A). In addition, anesthetic induced changes in the signal power in the frequency range considered (1–100 Hz) (fig. 2B).

The LFP signal power spectral density over all experimental conditions is shown in figures 3A and B. The statistical comparison of the signal power of each considered frequency band between the two recorded areas revealed that no differences were present for $\delta$ ($F(5,40) = 0.0136$, $P = 0.99$),
Table 1. Correlation Coefficients (Spearman Rank $r$) with End-tidal Isoflurane Concentration for the Studied Parameters

<table>
<thead>
<tr>
<th></th>
<th>SI</th>
<th>VPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>-0.89</td>
<td>-0.87</td>
</tr>
<tr>
<td>IoC</td>
<td>-0.85</td>
<td>-0.83</td>
</tr>
<tr>
<td>PE</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BSPE</td>
<td>-0.97</td>
<td>-0.95</td>
</tr>
<tr>
<td>SEF</td>
<td>-0.42</td>
<td>-0.41</td>
</tr>
<tr>
<td>BSSEF</td>
<td>-0.94</td>
<td>-0.93</td>
</tr>
</tbody>
</table>

Correlation coefficients (Spearman rank $r$) for the studied parameters: approximate entropy (AE), index of consciousness (IoC), permutation entropy (PE), burst suppression–corrected permutation entropy (BSPE), spectral edge frequency 95% (SEF), and burst suppression corrected spectral edge frequency (BSSEF). The $r$ values calculated from pooled data of all rats indexes derived from the primary somatosensory cortex (SI) and ventro-posterolateral thalamic nuclei (VPL) are presented. Correlations were considered statistically significant with $P < 0.05$; correlation was not statistically significant (NS) at the 0.05 level.

Discussion

LFPs may allow a more precise analysis of the brain electrical activity than the electroencephalogram. In this study, LFPs were recorded in the thalamocortical axis of rats to (i) compare the performance of several indexes of anesthetic depth and (ii) investigate the existence of thalamocortical correlated or disrupted activity during isoflurane steady-state anesthesia. The use of electroencephalogram-derived indexes to analyze LFPs was a strong help for the interpretation of the results obtained for the second objective.

Two main results were obtained with this study: (i) the BS-corrected PE had the best correlation with steady-state etISO concentration and (ii) high coherence activity exists between the cortical and thalamic regions during steady-state anesthesia, even at deeper stages.

Both the SEF and the PE values decreased from the awake state to 0.8% isoflurane etISO concentration but paradoxically increased when higher concentrations were used. This phenomenon is well described for the SEF in electroencephalographic signals and is assumed to be caused by the appearance of the BS pattern. This paradoxical increase was also seen in the spectral analysis, with increasing spectral power on the $\alpha$, $\beta$, and $\delta$ bands with increasing etISO concentration. A similar paradoxical increase occurred in PE in humans anesthetized with sevoflurane. The inclusion of a BS component in the PE would, therefore, contribute to the improvement of the parameter, and the correction applied in this study seems appropriate to adapt the PE for deeper anesthetic stages.

There was no correction necessary to achieve an adequate association between AE and etISO concentration. This parameter seems to correctly classify the BS pattern, which is in accordance to previous findings in humans and rats. However, AE has the drawback of being very sensitive to artifacts. In the study by Ihmsen et al., higher etISO concentrations were achieved (a maximum of 2.1%) and the AE did not decrease to values lower than 0.4, whereas in this study lower values were achieved with etISO concentration of 1.7%. This may be due to the presence of residual noise during BS pattern in the study by Ihmsen et al., which could have been classified as brain activity by AE. In our study, with a cleaner signal from intracranial recordings this situation could be avoided. The IoC had an acceptable performance in detecting the effects of different isoflurane concentrations in the LFPs. It is a proprietary monitor and its exact calculation algorithm is not published. However, it is known that, as other commercial monitors, it has a BS component. The lower correlation value obtained with the IoC, when compared with BS-corrected PE, BS-corrected SEF, and AE, could be related to the fact that its algorithm is adjusted for the human electroencephalogram and the BS limit might be.

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need an adaptation for this type of signal to achieve a better performance. Its major drawback is being proprietary, which makes it impossible to adapt the algorithm to the type of signal and species. On the other hand, the other three parameters (AE, SEF, and PE) have a greater advantage of having published algorithms thereby allowing its continuous improvement. The calculation of the SEF is very simple relying only on frequency changes. Its major problems are the sensitivity to artifacts and the need for a correction for BS. With this correction, it seems to be able to classify different anesthetic depths, with a correlation with the anesthetic concentration even better than the AE in this study.

The type of signal analyzed here represents a relevant difference to previous studies comparing anesthetic depth indexes. No artifact-filtering systems were applied before deriving the indexes and only a 50-Hz notch filter was applied because, contrary to the electroencephalogram, the LFPs do not suffer interference from the electromyographic activity. This way, the γ-band oscillations could be preserved, which are known to be important markers of the conscious state.2 In this study, the γ-band power decreased from the awake to the anesthetized state, but this decrease was only significant in the SI. This may be due to the association of the γ oscillations to cortical conscious activity2 and may be the reason for the higher correlation coefficients with the etISO concentration obtained with the SI indexes. Significant differences between the two areas were found for the θ, α, and β bands. This could also be related to the different origins of these rhythms: while the spindle activity (14 Hz) is generated on the thalamus10 and may increase the power in the α band (9–15 Hz), the β rhythms (15–30 Hz) generated by cortico-cortical interactions caused higher power on the SI.14

The BS pattern simultaneously appeared in the SI and VPL and was highly synchronized, as previously suggested.27

Although there was a tendency for the correlation coefficients from the SI indexes to be higher than those from the VPL indexes, the difference was not statistically significant. It could be expected that the SI indexes had a better correlation with the anesthetic concentration, because they were developed to analyze the electroencephalogram that reflects mainly the cortical activity, contrary to previous findings with neuroimaging techniques in humans.28 But this result together with the high coherence obtained between the two areas may indicate that the cortical and thalamic neurons remain connected to some extent during steady-state anesthesia. Our results support the existence of a "permanent corticothalamic dialogue" during steady-state anesthesia, as has been suggested for quiet physiologic sleep.10 However, the existence of a separate role of the cortex27 or the thalamus,9 as targets of anesthetic effects, cannot be clarified with the present results because the dynamic phases of anesthesia were not studied but only steady-state effects. These results are in accordance with the ideas that loss of consciousness may not necessarily require that thalamocortical neurons are inactivated10,11 and that an anesthesia causes a drop in γ-frequency power.2,11 According to the information or integration theory proposed by Alkire et al.,11 anesthetic-induced unconsciousness presupposes two mechanisms: (1) a loss of information capacity reflected by reduction in the number of electrophysiological patterns with increasing anesthetic depth and (2) a loss of integration capacity reflected by an interruption in the interactions between brain structures. Although this study does not reveal a disruption in the thalamocortical interactions, it may support the loss of information idea, because a decrease in the electrophysiological patterns was observed, shrinking to the stereotypic BS pattern at deeper stages.

Meanwhile, these results do not guarantee the existence of equal activity in both areas, because output neuronal activity is not contemplated on the LFPs, but only slower postsympathetic activity. Nevertheless, this animal preparation is a promising method for future investigations on the mechanisms of anesthetic action, especially by analyzing the dynamic phases of anesthesia, at induction and recovery and including other recording techniques such as multi-unit recordings. Also, by knowing how the SI and VPL neurons interact during steady-state anesthesia, it will be easier to draw conclusions on the effects of intraoperative noxious stimuli and analgesic administration on pain processing by the somatosensory system, with the same animal preparation.29

LFP recordings performed in this study resulted in two main conclusions: (i) the PE corrected with the classic BS ratio is a promising alternative to other indexes of anesthetic depth and (ii) data showed high coherence level of activity between cortical and thalamic regions, even at deep isoflurane stages.

References


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