Performance of Anesthetic Depth Indexes in Rabbits under Propofol Anesthesia

Prediction Probabilities and Concentration-effect Relations

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ABSTRACT

Background: The permutation entropy, the approximate entropy, and the index of consciousness are among the most recently studied electroencephalogram-derived indexes. In this work, a thorough comparison of these indexes was performed using propofol anesthesia in a rabbit model.

Methods: Six rabbits were anesthetized with three propofol infusion rates: 70, 100, and 130 mg·kg⁻¹·h⁻¹, each maintained for 30 min, in a random order for each animal. Data recording was performed in the awake animals 20, 25, and 30 min after each infusion rate was begun in the recovered animals and consisted of electroencephalogram recordings, evaluation of depth of anesthesia according to a clinical scale, and arterial blood samples for plasma propofol determination. Median and spectral edge frequencies were analyzed for single-scale permutation entropy and composite multiscale permutation entropy, approximate entropy, index of consciousness, and the spectral parameters. The spectral parameters and single-scale and multiscale permutation entropies were corrected for the presence of burst suppression. Performance of the indexes was compared by prediction probability and pharmacodynamic analysis.

Results: The single-scale and composite multiscale permutation entropies with a burst suppression correction showed better prediction probabilities than did the other electroencephalogram-derived parameters but not better than the electromyographic activity.

Conclusion: Single-scale and multiscale permutation entropies may be promising measures of propofol anesthetic depth when corrected for burst suppression. Additional studies should investigate the information measured by electromyography algorithms from commercial monitors of anesthetic depth. The rabbit may be a promising animal model for electroencephalographic studies because it provides a good-quality signal.

What We Already Know about This Topic
- A number of electroencephalogram-based measures have been studied to determine their usefulness for intraoperative monitoring of anesthetic depth
- In rats anesthetized with a potent volatile anesthetic, burst suppression-corrected permutation entropy had the best correlation with anesthetic dose

What This Article Tells Us That Is New
- In rabbits receiving propofol by infusion, burst suppression-corrected permutation entropy and burst suppression-corrected composite multiscale permutation entropy had the best propofol concentration-effect relationships and predictions of clinical signs

In recent years, the electroencephalogram has been actively studied for intraoperative monitoring of anesthetic depth, resulting in a multitude of measures extracted from this signal to translate it into a simple number.

Most recent parameters are based in nonlinear time series analysis, attempting to overcome limitations of older measures based on the power spectrum.¹,² However, there is no consensus on the choice of the best parameter for clinical use. Recently, permutation entropy (PE) was introduced and has shown great potential because of ability to predict anesthetic concentration, high resistance to artifacts, and fast computation.³ It has been compared with approximate entropy (AE) in patients receiving sevoflurane and propofol anesthesia and has shown better results.¹,² PE has been further explored in a multiscale approach in an attempt to better understand the dynamic characteristics of the electroencephalogram, resulting in composite multiscale permutation entropy

What Are the Clinical Implications of This Article?

Additional studies should investigate the information measured by electromyography algorithms from commercial monitors of anesthetic depth. The rabbit may be a promising animal model for electroencephalographic studies because it provides a good-quality signal.

What Do the Findings Mean for Clinical Practice?

In rabbits receiving propofol by infusion, the single-scale and multiscale permutation entropies with burst suppression correction showed better prediction probabilities than did the other electroencephalogram-derived parameters but not better than the electromyographic activity.

What Do the Findings Mean for Research?

Additional studies should investigate the information measured by electromyography algorithms from commercial monitors of anesthetic depth. The rabbit may be a promising animal model for electroencephalographic studies because it provides a good-quality signal.
New Zealand white rabbits, average weight 2.79 kg. Six healthy male rabbits were anesthetized for this study. Animals were group housed in floor pens with shelters, and behavior was assessed daily. In this study, we aimed to compare several electroencephalographic (EEG) indexes for the evaluation of anesthetic depth in propofol-anesthetized rabbits. We hypothesized that a rabbit model may allow the recording of an electroencephalographic signal with small electromyographic contamination, which is important to guarantee that brain effects, and not muscle tone, are being measured and reflected in the indexes. This is essential for future applications of the indexes in the electroencephalography-rich human frontal montages and may contribute a rapid clarification on the potential use of different electroencephalogram-derived indexes.

In the current study, the rabbit was used as a potential translational research model for the comparison of clinical indexes because of its anatomic characteristics. The performance of different electroencephalogram-derived parameters was compared: IoC, PE, CMSPE, AE, MEF and SEF and the burst suppression-corrected MEF, SEF, PE and CMSPE (BSMEF, BSSEF, BSPE, and BSCMSPE). The ability of these parameters to distinguish between different depths of propofol anesthesia was assessed using prediction probability analysis and pharmacodynamic modeling.

**Materials and Methods**

**Animals**

All procedures were carried out under personal and project licenses approved by the national regulatory office (Direcção Geral de Veterinária, Lisbon, Portugal). Six healthy male New Zealand white rabbits, average weight 2.79 ± 0.25 kg, were anesthetized for this study. Animals were group housed in floor pens with shelters, and behavior was assessed daily.

**Electroencephalographic Recording**

The electroencephalographic recordings were performed using the IoC-View monitor (Aircraft Medical, Barcelona, Spain).

Before induction of anesthesia, all of the animals’ heads were shaved, cleaned, and surface layers removed with fine sandpaper and acetone. Gel-coated, silver-silver chloride electrodes (Swaromed, Innsbruck, Austria) were applied to record the electroencephalogram. Two electrodes (one for each eye) were placed 1 cm caudal to the lateral eye canthus; a central electrode was placed on the midline on the frontal bone 3 cm from each previously applied electrode. This localization was based on previous works for the BIS monitor (Aspect Medical Systems, Newton, MA) in rabbits, which has been determined to give the best quality electroencephalographic signal after testing of different positions in pilot studies with the IoC-View Monitor (Morpheus-Medical, Barcelona, Spain).

Impedance was automatically checked by the monitor and maintained at less than 15,000 ohms with a digitizing rate of 1.024 Hz. The electrodes were connected to the IoC-View monitor, which was connected by Bluetooth (Bluetooth SIG, Inc., Kirkland, WA) to a personal computer with the IoC-View Graph software version 1.4 (Morpheus-Medical), a storage software provided by the manufacturer.

**Anesthesia and Monitoring**

After electroencephalogram baseline recording in the fully awake animals for a period of 5 min, the fur on the ears was clipped, the skin cleaned with alcohol, and a local analgésic cream was applied to the ear skin (EMLA; Nycomed US Inc, Melville, NY). Thirty minutes later, two 22-gauge catheters were placed, one in the marginal ear vein and the other in the central ear artery for arterial pressure monitoring. Both auricular catheter systems were flushed with heparinized saline and fixed to the skin. The animals were then oxygenated with a facial mask at 5 l/min for 5 min.

Anesthesia was induced with intravenous propofol (20 mg/kg) using a syringe pump (Asena GH; Alaris Medical Systems, San Diego, CA) controlled by the Rugloop II software (developed by Tom DeSmet, DEMED Engineering, Gent, Belgium) at an infusion rate of 200 ml/h. After blind orotracheal intubation with an endotracheal tube with an internal diameter of 2.5 mm, propofol administration began according to an infusion scheme in which three infusion rates were performed in every animal: each infusion (70, 100, and 130 mg·kg⁻¹·h⁻¹) was maintained for 30 min. The order of the administration rates for each animal was randomized using the RAND function in Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA).

The rabbits were placed in ventral recumbence above a heating blanket, and rectal temperature was continuously monitored and maintained between 37° and 38°C. Anesthetic monitoring included cardiorespiratory monitoring provided by a Datex S/5 Anesthetic station (Datex Ohmeda, Inc., Herts, UK).
Table 1. Definition of the Anesthetic States and Attributed Numerical Scale

<table>
<thead>
<tr>
<th>Anesthetic State</th>
<th>Clinical Signs Observed</th>
<th>Numerical Scale of Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>Fully awake and alert</td>
<td>0</td>
</tr>
<tr>
<td>Sedated</td>
<td>Relaxed but still responsive to stimulation and with righting reflex present</td>
<td>1</td>
</tr>
<tr>
<td>Shallow anesthesia</td>
<td>Lost its righting reflex but still responds to any stimulation</td>
<td>2</td>
</tr>
<tr>
<td>Medium anesthesia</td>
<td>Responds only to painful stimulation (ear pinch and pedal withdrawal reflexes)</td>
<td>3</td>
</tr>
<tr>
<td>Surgical anesthesia</td>
<td>Does not respond to painful stimulation (ear pinch and pedal withdrawal reflexes) but still has corneal reflex</td>
<td>4</td>
</tr>
<tr>
<td>Close to death</td>
<td>Without corneal reflex, mean arterial blood pressure below 40 mmHg; apneic</td>
<td>5</td>
</tr>
</tbody>
</table>

Definition of six anesthetic states based on clinical signs. The main clinical signs observed in each state are described.

Helsinki, Finland), which included pulse oxymetry and pulse rate monitoring with the probe placed in the ear, invasive arterial pressure, and inspired and end-tidal concentrations of oxygen and carbon dioxide. These data were stored using the Rugloop II software. Animals were mechanically ventilated with 100% oxygen, with ventilation parameters set to maintain the $\text{EtCO}_2$ between 35 and 45 mmHg.

At the end of the infusion scheme, the fresh gas flow rate was increased to 5 l/min of 100% oxygen until the rabbits regained swallowing reflexes, and at this point extubation was performed. Animals were considered recovered from anesthesia when they exhibited an alert stance and had regained ambulation and limb coordination. Continuous infusion of physiologic saline at a rate of 10 ml $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$ was maintained during anesthesia.

A clinical evaluation of depth of anesthesia was performed according to a subjective numerical scale of anesthesia from 0 (awake) to 5 (excessive depth of anesthesia) (table 1) based on the evaluation of the postural reflex, muscular tone, palpebral reflex, corneal reflex, laryngeal reflex, ear pinch, and digital (pedal) reflexes. This scale was elaborated according to literature descriptions of clinical anesthetic depth evaluation in rabbits.$^{10-12}$ These evaluations were performed by the same investigator, who was blinded to the electroencephalogram, and recorded at specific time points of the study (fig. 1): in the awake animals, 20, 25, and 30 min after the beginning of each infusion rate, at extubation, and in the totally recovered animal. Animals were considered totally recovered when they exhibited an alert stance and had regained ambulation and limb coordination.

**Plasma Propofol Sampling**

Arterial blood samples were collected at exactly the same time points that clinical evaluation of depth of anesthesia was performed, except at the extubation moment: in the awake animals, 20, 25, and 30 min after the beginning of each infusion rate and in the totally recovered animal (fig. 1). This infusion scheme was designed to achieve a steady-state in the last 10 min of each infusion rate based on pharmacokinetic data of clearance from Cockshott et al.$^{15}$

After the blood was collected, the serum was separated through centrifugation at 3,000 rpm for 15 min and immediately placed at $-77^\circ$C and stored until analysis.

Propofol plasma concentrations were determined by gas chromatography mass spectrometry according Guitton et al.$^{14}$ with some modifications. Briefly, 50 $\mu$L internal standard thymol solution (0.01 mg/ml) and 1 ml water were added to 0.5-ml aliquot serum or propofol calibration standards. To this solution, 0.5 ml borate buffer (pH 9) was added and mixed by inversion. Then, 300 $\mu$L chloroform: ethylacetate (70:30, v:v) was added and mixed for 20 min at 50 rpm, after which 1 $\mu$L organic phase solution was injected into the gas chromatography-mass spectrometry injector in splitless mode at 250°C. The quantification of propofol was performed in a Varian CP-3800 gas chromatograph (Varian, Walnut Creek, CA) equipped with a ion trap mass detector (Varian Saturn 4000). The chromatographic column was a Varian Factor Four ms (30 m $\times$ 0.25 mm $\times$ 0.25 $\mu$m). The column temperature was programmed to 100°C (1 min), 15°C/min until 300°C (10 min). The detection of propofol and thymol was conducted in Full Scan mode, and the quantification performed by monitoring the characteristic mass-to-charge ratio.
charge ratio (m/z) fragments of each molecule: for propofol, the m/z used were 178 and 163, and for thymol, the m/z used were 150 and 135. The retention times for each compound were, respectively, 5.6 and 5.0 min.

**Indexes Derivation**

The IoC-View Graph 1.4 software stored the electroencephalographic data as binary files, which were further converted to the MATLAB format to be processed offline using the MATLAN® software (MathWorks, Natick, MA).

The IoC and the electroencephalogram suppression ratio, equivalent to the burst suppression ratio (BS), and the electromyographic activity were automatically derived by the IoC-View Monitor. The monitor includes an electromyographic activity filter that eliminates most of the potential interfering electromyographic activity before the derivation of the index of consciousness and calculates the electromyographic activity, which is given as a percentage and shows the energy of the electromyographic activity level in the frequency band of 30–45 Hz.

The monitor also displayed the signal quality index every second. All of the other parameters (MEF, SEF, AE, PE, and the traditional BS) were derived offline. The signal was first decreased eight times, resulting in a sampling frequency of 128 Hz. The indexes were derived in epochs of 8 s, and only epochs that showed a signal quality index value of 100 were considered. Visual inspection of every analyzed electroencephalogram fragment was also performed to ensure that no visible artifacts were included in the analysis. A Butterworth bandpass filter of eighth order with cutoff frequencies of 0.5 and 30 Hz was used, followed by removal of the mean value of the signal to get out any threshold.

The spectral parameters were corrected for the presence of burst suppression pattern, using the value of BS, according to the correction factor proposed by Rampil for human electroencephalogram studies and applied in rats. The same correction factor was applied to PE, resulting in BSPE, calculated as follows: BSPE = PE × (1 − BS/100), where BS is defined as the epoch length in which the electroencephalogram voltage did not exceed 5 μV. The analysis was also performed with a higher limit of 10 μV, and the results of each threshold applied were compared.

The AE and PE were computed according to published algorithms. Briefly, the calculation of the AE depends on three factors: the embedding dimension (m), the number of samples considered for each calculation (N), and the noise threshold (r). In the current study, N = 1,024, m = 2, and r = 0.2 were selected for AE calculation, based on previous studies. For the PE calculation, the length of subvectors (m) and the analyzed signal interval (length N) are main factors. In the current study, we used m = 3 and N = 1,024. A more detailed description of the AE and PE calculation can be found in published works.

The CMSPE was recently introduced. It is based in the multiscale entropy previously proposed for sample entropy applied to the PE. According to results by Li et al., we used scale values of 1, 2, and 3 for the calculation of the CMSPE. With the scale value of 1, the scalar PE is equal to the original PE, which we designate as single scale PE. The composite index consists of the average of the PE values calculated with scales 1, 2, and 3. More details on the calculation of the CMSPE can be found in the literature.

The IoC is a proprietary index that was developed to match the anesthetic depths observed in patients anesthetized with a variety of anesthetics. For its calculation, a fast Fourier transform is carried out after applying a hamming function to a 3-s window of the electroencephalogram. The calculation of the IoC is based on the energies in different frequency bands (1–6, 6–12, 10–20, and 30–45 Hz) combined with fuzzy logic rules. It also integrates the β ratio (frequency range between 11 and 42 Hz) during superficial anesthesia and the amount of suppression of the electroencephalogram (equivalent to the BS). In humans, decreasing values of IoC correspond to a gradual loss of consciousness and a deepening of the depth of anesthesia.

**Data Analysis**

**Prediction Probability Analysis.** The capacity of the studied indexes (MEF, SEF, AE, PE, CMSPE, IoC, electromyographic activity, BSMEF, BSSEF, BSPE, and CMSPE) to detect different anesthetic states, as reflected in the numerical scale of anesthesia (table 1), was evaluated using prediction probability (Pk) statistics by correlating the parameter values during 64 s (8 measurements) at each of the defined study periods (fig. 1) with the numerical scale. Pk was calculated using a custom spreadsheet macro, PKMACRO. A Pk of 1 means that the parameter always decreases as the subject reaches deeper anesthetic states. Alternatively, a Pk value of 0.5 or less would mean that the indicator is useless for predicting the depth of anesthesia. Pk was calculated using pooled data from all animals. The Pk value and the Jackknife SE are shown in Results.

**Pharmacodynamic Modeling.** The relation between the propofol plasma concentrations and the electroencephalographic effect reflected in each studied index was modeled with an inhibitory sigmoid model:

\[ E = E_0 - \frac{E_{\text{max}}}{1 + \frac{c}{EC_{50}} + c'^{\gamma}} \]

where E is the pharmacodynamic effect (i.e., the studied indexes of anesthetic depth), EC_{50} is the drug concentration that produces half-maximum effect, E_0 is the effect at zero concentration, E_{max} is the maximum effect, C is the drug plasma concentration effect, and the Hill exponent γ is a measure of curve steepness.

The pharmacodynamic parameters were estimated by population analysis using WinNonlin (WinNonlin Professional 5.0.1; Pharsight Corporation, Mountain View, CA). The goodness-of-fit for each index was compared using the values obtained for the coefficient of determination (R^2).
The indexes that showed the best goodness-of-fit according to these parameters were analyzed regarding the estimated pharmacodynamic parameters: $E_{max}, E_0, EC_{50}, \gamma$, and $E_{max} - E_0$.

**Statistical Analysis.** All of the indexes derived from the data resultant from the electroencephalographic 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 data sets were normally distributed. All tests were detailed with a statistical significance defined as $P < 0.05$.

The Spearman rank correlation coefficient was calculated between the propofol plasma concentration and the other studied parameters: clinical scale of anesthesia, MEF, SEF, AE, PE, CMSPE, BSMEF, BSSEF, BSPE, BSCMSPE, IoC, electromyographic activity, mean arterial pressure, and heart rate.

The Wilcoxon matched pairs signed-rank test was used to compare the parameter values corrected with a BS limit calculated with 5 or 10 $\mu$V thresholds.

Data are presented as mean $\pm$ SD in Results.

**Results**

In the current study, six rabbits weighing $2.79 \pm 0.25$ Kg were anesthetized with propofol in an infusion scheme that included three consecutive infusion rates (70, 100, and 130 mg $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$). Every animal could be successfully anesthetized with the three rates, each maintained for 30 min. The total duration of anesthesia (from induction to the stopping of the last infusion) was $109.5 \pm 6.8$ min. All of the animals recovered well from anesthesia, taking $21 \pm 6.8$ min from the end of infusion to extubation and $28 \pm 11.2$ min to recover limb ambulation.

Because of the randomization of the infusion schemes, the same infusions and duration did not produce the same propofol plasma concentrations. Different anesthetic depths were also seen with the same infusions when performed in a different order.

The duration of the infusions was stipulated, based on pharmacokinetic data of propofol in rabbits from Cockshott *et al.*, to produce a 10-min steady state in the end of each infusion rate. However, this steady state was achieved only in the lower infusion rate (70 mg $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$). Thus, the analysis of clinical and electroencephalographic data were performed based on the plasma concentrations determined and not on the infusion rates administered.

With regard to the clinical depth of anesthesia, it was necessary to attribute an intermediate grade (4.5) between depths 4 and 5 for animals that did not have a perceptible corneal reflex but responded to the pedal withdrawal reflex (deep noniceptive reflex).

With regard to the raw electroencephalographic findings, the electroencephalogram was gradually suppressed with increasing plasma concentrations of propofol (fig. 2). From the awake state to superficial depths of anesthesia, the electroencephalogram shifted from a low-amplitude, high-frequency pattern to higher amplitudes and lower frequencies. However, during medium and profound depths with plasma concentrations of propofol higher than 18 $\mu$g/ml, the electroencephalogram amplitude decreased progressively to values between −5 and 5 $\mu$V. In every animal, at the higher propofol infusion rate (130 mg $\cdot$ kg$^{-1}$ $\cdot$ h$^{-1}$) the electroencephalogram was below these amplitude limits.

The electroencephalogram was recorded along with the IoC, electromyographic activity, and the electroencephalogram suppression ratio all derived automatically by the IoC-View Monitor. These parameters were included in the overall analysis of the indexes of anesthetic depth. The other indexes were derived offline from the raw electroencephalogram and consisted of the MEF, SEF, AE, PE, CMSPE, BS, BSMEF, BSSEF, BSPE and BSCMSPE. Visual inspection of the raw electroencephalogram and analysis of the signal quality index given by the IoC-View Monitor were performed before derivation of the indexes of anesthetic depth to discard poor quality electroencephalogram epochs. Four epochs were discarded in the extubation measurements of one rabbit and four epochs on the recovery measurements of another.

The values of the studied indexes at increasing propofol plasma concentrations are shown in figure 3. To simplify this representation, the propofol plasma concentrations were or-
organized in theoretical ranges: 0, 1–10, 10–20, 20–30, 30–40, and 40–50 μg/ml. The real concentrations for each of these ranges were, respectively, 0, 4.87 (1.98–7.22), 14.7 (10.70–18.99), 25.9 (20.15–29.00), 34.8 (30.48–38.42) and 45.6 (41.70–48.85) μg/ml.

The capacity of all these indexes to adequately assess the clinical anesthetic depth was evaluated by Pk analysis, which revealed higher Pk values for BSPE, BSCMSPE, electromyographic activity, BSSEF, and IoC than did the other parameters, as shown in table 2.

No significant differences between the Pk values of the corrected indexes BSMEF, BSSEF, BSPE, and BSCMSPE with a BS correction calculated with 5 or 10 μV thresholds.

The correlation of the parameters with the plasma concentration of propofol is shown in table 3.
creasing propofol plasma concentrations, but the response correlation with propofol plasma concentrations (table 3). Heart rate also decreased with increasing propofol concentrations until 0.001; table 3). However, at higher concentrations, the depth of anesthesia stabilized. Mean arterial pressure achieved low values at propofol plasma concentrations, the depth of anesthesia stabilized. Mean arterial pressure; MEF (Hz) = median edge frequency; PE = permutation entropy; SEF (Hz) = spectral edge frequency 95%.

The clinical scale of anesthesia increased monotonically with increasing propofol plasma concentrations until the range of 20–30 μg/ml (fig. 3A), revealing an increasing deepening of anesthesia. However, at higher concentrations, the depth of anesthesia stabilized. Mean arterial pressure achieved low values at propofol plasma concentrations above 10 μg/ml (fig. 4) and showed a negative correlation with propofol plasma concentrations (−0.73; P > 0.001; table 3). Heart rate also decreased with increasing propofol plasma concentrations, but the response was less consistent than that of mean arterial pressure (fig. 4), with a lower correlation coefficient (−0.53; P > 0.001; table 3).

The IoC-View Monitor was incapable of detecting the low voltage of the electroencephalogram during the higher concentrations in two rabbits. With the electroencephalogram completely suppressed (voltage between −5 and 5 μV) and propofol plasma concentrations of 35.3 ± 4.8 μg/ml, the IoC showed values of 70.6 ± 8.5 and an electroencephalogram suppression ratio (provided automatically by the monitor) of 0. In these animals, offline analysis with the BS algorithm revealed high values of BS (95.1 ± 3.4%). The representation of two animal index values at the different study periods are shown in figure 5.

With regard to pharmacodynamic modeling, the highest coefficient of determination was shown by the electromyographic activity with regard to the estimated pharmacodynamic parameters EC50 and E0 and, with regard to all of the pharmacodynamic parameters, than did BSSEF (table 5). Figure 6 shows the pharmacodynamic fitting curves obtained for the studied parameters that showed the higher coefficients of determination.

Animal exhibited normal behavior at daily monitoring for 2 weeks after the procedure.

**Discussion**

In the current study, propofol anesthesia in a rabbit model was used to compare the performance of different electro-

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**Table 2.** Prediction Probabilities Calculated Between the Subjective Anesthetic Depth Scale and the Studied Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pk</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>0.59</td>
<td>0.05</td>
</tr>
<tr>
<td>BSCMSPE</td>
<td>0.83</td>
<td>0.02</td>
</tr>
<tr>
<td>BSMEF (Hz)</td>
<td>0.73</td>
<td>0.04</td>
</tr>
<tr>
<td>BSPE</td>
<td>0.82</td>
<td>0.02</td>
</tr>
<tr>
<td>BSSEF (Hz)</td>
<td>0.77</td>
<td>0.04</td>
</tr>
<tr>
<td>CMSPE</td>
<td>0.53</td>
<td>0.06</td>
</tr>
<tr>
<td>EMG (%)</td>
<td>0.84</td>
<td>0.03</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>0.71</td>
<td>0.05</td>
</tr>
<tr>
<td>IoC</td>
<td>0.75</td>
<td>0.04</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>0.74</td>
<td>0.03</td>
</tr>
<tr>
<td>MEF (Hz)</td>
<td>0.48</td>
<td>0.05</td>
</tr>
<tr>
<td>PE</td>
<td>0.52</td>
<td>0.06</td>
</tr>
<tr>
<td>SEF (Hz)</td>
<td>0.54</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Prediction probability (Pk) values calculated with pooled data from all animals (n = 6). The standard error (SE) is also shown. AE = approximate entropy; BS = burst suppression; BSCMSPE = BS-corrected multiscale permutation entropy; BSMEF (Hz) = BS-corrected median edge frequency; BSPE = BS-corrected permutation entropy; BSSEF (Hz) = BS-corrected spectral edge frequency; CMSPE = composite multiscale PE; EMG (%) = electromyographic activity; HR (beats/min) = heart rate; IoC = index of consciousness; MAP (mmHg) = mean arterial pressure; MEF (Hz) = median edge frequency; PE = permutation entropy; SEF (Hz) = spectral edge frequency 95%.

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**Table 3.** Correlation Coefficients (Spearman R) between the Studied Parameters and the Plasma Concentration of Propofol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spearman R with Cp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pk</td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>0.59</td>
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<td>0.77</td>
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<tr>
<td>CMSPE</td>
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<td>0.48</td>
</tr>
<tr>
<td>PE</td>
<td>0.52</td>
</tr>
<tr>
<td>SEF (Hz)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Spearman rank correlation coefficient from pooled data of all animals (n = 6) is shown.

**Fig. 4.** Mean and SD of mean arterial pressure [MAP (mmHg)] (A) and heart rate [HR (bpm)] (B) at different ranges of propofol concentration (at 0, 1–10, 10–20, 20–30, 30–40, and 40–50 μg/ml plasma propofol) in the six rabbits.
encephalogram-derived parameters: MEF, SEF, AE, PE, CMSPE, and IoC. The ability of these parameters to distinguish different depths of propofol anesthesia was assessed using prediction probability analysis and pharmacodynamic modeling.

The classic BS correction was applied to the MEF, SEF, PE, and CMSPE, and the performance of the corrected parameters (BSMEF, BSSEF, BSPE, and BSCMSPE) was also compared.

Both BSPE and BSCMSPE showed better correlation coefficients with propofol plasma concentrations than did the other studied indexes. They also showed better Pk than did the other electroencephalogram-derived indexes. However, the electromyographic activity, as detected by the IoC-View Monitor, showed a higher Pk value than did BSPE and BSCMSPE. The Pk value and the correlation with propofol plasma concentrations were slightly higher for BSCMSPE than for BSPE, which also showed less variability with regard to the estimated pharmacodynamic parameters.

Previous results with volatile anesthesia in rats showed a better correlation of BSPE than AE, IoC, and spectral parameters with the anesthetic dose. However, the signal analyzed in that study was recorded intracranially, and thus is less likely to be contaminated with movement artifacts and noise than are extracranial signals. The current results also show a superior capacity of BSPE and BSCMSPE to be used in extracranial signals during propofol anesthesia, with better concentration-effect relations and greater capacity to predict clinical signs than the other electroencephalogram-derived indexes.

Despite the thin muscle layer between the skull and the skin on the rabbit head, electromyographic activity was captured by the IoC-View Monitor, showing better ability to...
detect clinical signs of anesthesia than the electroencephalogram-derived indexes. The cortical depression reflected in the electroencephalogram would be expected to have better prediction probability with propofol clinical effects.\textsuperscript{21} We point out some possible explanations for the ability of the electromyographic activity to predict clinical signs: First, there are some important limitations related to the use of clinical scales of anesthetic depth.\textsuperscript{22} Although applied in the daily clinical practice, their correlation with real cerebral hypnotic drug effect is not well established because surrogate observations, such as reflex responses and eyeball position, result from independent neurologic pathways and are not directly involved in the neurologic process of consciousness.\textsuperscript{23} In addition, propofol myorelaxant properties have been demonstrated,\textsuperscript{24,25} and a plausible explanation for the good correlation of electromyographic activity with the clinical scale of anesthesia could be that the mechanism by which propofol induces myorelaxation could be closer to the mechanism by which it produces the loss of autonomic reflexes evaluated by the clinical scale of anesthesia, rather than the cortical depression mirrored by the electroencephalogram.\textsuperscript{26}

In addition, the band frequencies considered by the IoC-View Monitor as electromyographic activity are between 30 and 45 Hz.\textsuperscript{4} The electroencephalogram also shows activity in these frequencies, called the \( \gamma \) band. One possibility is that the electromyographic activity recorded by the monitor in such a thin skull as the rabbit’s was incorporating information on the \( \gamma \) band, which decreases from the awake to the anesthetized state.\textsuperscript{27} This possibility was not assessed in the current study and should be ruled out in future investigations using neuromuscular blocking agents to abolish muscle ac-

### Table 4. Goodness-of-fit for the Pharmacodynamic Modeling of the Studied Indexes

<table>
<thead>
<tr>
<th>Index</th>
<th>( R^2 )</th>
<th>MIN–MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>0.59</td>
<td>(0.29–0.79)</td>
</tr>
<tr>
<td>BSCMSPE</td>
<td>0.79</td>
<td>(0.52–0.91)</td>
</tr>
<tr>
<td>BSMEF</td>
<td>0.75</td>
<td>(0.65–0.95)</td>
</tr>
<tr>
<td>BSPE</td>
<td>0.79</td>
<td>(0.42–0.91)</td>
</tr>
<tr>
<td>BSSEF</td>
<td>0.87</td>
<td>(0.76–0.94)</td>
</tr>
<tr>
<td>CMSPE</td>
<td>0.57</td>
<td>(0.49–0.73)</td>
</tr>
<tr>
<td>EMG</td>
<td>0.92</td>
<td>(0.79–0.99)</td>
</tr>
<tr>
<td>IoC</td>
<td>0.68</td>
<td>(0.33–0.98)</td>
</tr>
<tr>
<td>MEF</td>
<td>0.50</td>
<td>(0.10–0.96)</td>
</tr>
<tr>
<td>PE</td>
<td>0.60</td>
<td>(0.42–0.96)</td>
</tr>
<tr>
<td>SEF</td>
<td>0.61</td>
<td>(0.55–0.90)</td>
</tr>
</tbody>
</table>

The coefficient of determination (\( R^2 \)) values are shown. The minimum and maximum values for the \( R^2 \) obtained after analysis of each animal’s data (\( n = 6 \)) are shown as MIN–MAX. The best models are in italic.

AE = approximate entropy; BS = burst suppression; BSCMSPE = BS-corrected composite multiscale permutation entropy; BSMEF (Hz) = BS-corrected median edge frequency; BSPE = BS-corrected permutation entropy; BSSEF (Hz) = BS-corrected spectral edge frequency; CMSPE = composite multiscale PE; EMG (%) = electromyographic activity; IoC = index of consciousness; MEF (Hz) = median edge frequency; PE = permutation entropy; SEF (Hz) = spectral edge frequency 95%.

### Table 5. Results from the Pharmacodynamic Modeling for the Indexes that Showed the Best Goodness-of-fit

<table>
<thead>
<tr>
<th>Index</th>
<th>( E_{\text{max}} )</th>
<th>( EC_{50} )</th>
<th>( E_{\text{d}} )</th>
<th>( \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSCMSPE</td>
<td>0.94 ± 0.04</td>
<td>23.06 ± 3.86</td>
<td>0.21 ± 0.14</td>
<td>3.97 ± 1.71</td>
</tr>
<tr>
<td>BSPE</td>
<td>0.87 ± 0.04</td>
<td>22.76 ± 3.91</td>
<td>0.19 ± 0.13</td>
<td>3.85 ± 1.66</td>
</tr>
<tr>
<td>BSSEF</td>
<td>30.98 ± 1.94</td>
<td>10.10 ± 9.42</td>
<td>0.25 ± 10.8</td>
<td>0.82 ± 0.41</td>
</tr>
<tr>
<td>EMG</td>
<td>99.02 ± 5.30</td>
<td>6.58 ± 0.74</td>
<td>−0.25 ± 5.15</td>
<td>1.95 ± 0.38</td>
</tr>
</tbody>
</table>

Data are estimate ± standard deviation.

BSCMSPE = burst suppression-corrected composite multiscale permutation entropy; BSPE = burst-suppression corrected permutation entropy; BSSEF = burst suppression-corrected spectral edge frequency; EMG = electromyographic activity; \( E_{\text{max}} \) = maximum effect; \( EC_{50} \) = concentration at 50% maximum effect; \( E_{\text{d}} \) = baseline effect; \( \gamma \) = Hill exponent.
tivity, and the γ band input in indexes of anesthetic depth should be investigated.

Another interesting finding of the current study was the smaller correlation between propofol plasma concentration and the clinical scale of anesthesia than between propofol plasma concentration and the BSCMSPE and BSPE. This may be related to the limitations of clinical assessment referred to above, which is mainly based on movement responses that are merely spinal cord reflexes, and thus may not be related to the cortical effects of anesthetics. Studies highlight the need for the development of objective alternatives to the evaluation of clinical signs for assessment of depth of anesthesia. However, these are the standard methods for evaluating depth of anesthesia in rabbits. Our results also confirm that knowledge of the drug concentration may be an important complement for a more complete anesthetic depth monitoring. There are several factors influencing the anesthetic depth in addition to anesthetic concentration, such as patient and surgery characteristics, existence and magnitude of the noxious stimulation, and concomitant use of other drugs, that limit the use of anesthetic concentration as a unique means to assess anesthetic depth in clinical practice. Nevertheless, the use of propofol plasma concentrations has the advantage of being an objective measure and providing a continuous range of propofol plasma concentrations has the advantage of being an objective measure and providing a continuous range of propofol plasma concentrations has the advantage of being an objective measure and providing a continuous range of propofol plasma concentrations has the advantage of being an objective measure and providing a continuous range of propofol plasma concentrations. This may be related to the appearance of BS, which has been recognized as a difficulty with PE and CMSPE, which increases paradoxically during this pattern and has been attributed to the presence of noise during the suppression periods. The spectral parameters (MEF and SEF) also lacked correlation with propofol plasma concentration and showed very low prediction probabilities with clinical scale of anesthesia. This is in accordance with the literature and was expected, particularly with the current experimental protocol, in which high levels of hypnosis were achieved with increased BS values. When a BS correction was applied to MEF, SEF, PE, and CMSPE, the parameters became able to detect clinical changes and correlate with propofol plasma concentrations, albeit with inferior performances of BSMEF and BSSEF. Consequently, a BS component seems indispensable when high anesthetic depths are expected. The PE and CMSPE capacities for detecting nonlinear properties of the electroencephalogram and their high resistance to artifacts may be in the origin of their better performance when corrected for BS.

In raw electroencephalographic changes, propofol caused an almost complete suppression on the electroencephalogram. The effects of low doses of propofol (from 4 to 15 μg/ml) were more characteristic of low-frequency, high-amplitude patterns present in shallow anesthetic depths or deep sedation. From this pattern, with increasing plasma concentrations of propofol, the electroencephalogram amplitude decreased until burst suppression and complete isoelectricity. The burst suppression pattern observed in this study was not the typical pattern seen in rabbits anesthetized with volatiles. As described previously, the bursts produced by propofol in rabbits were less distinct, consisting of spikes on slow waves with a smoother wave form than the abrupt change in direct current level seen in isoflurane burst suppression, without the appearance of the typical spindles seen with propofol anesthesia in humans. In general, high concentrations of the drug produced a complete suppression, rather than a burst suppression pattern. In patients, burst suppression may appear with propofol plasma concentrations of approximately 6 μg/ml. In this study, the higher concentrations achieved were much higher (almost 10 times). Therefore, although the metabolism of propofol seems to be faster in rabbits than humans, these are high plasma concentrations, which make the appearance of an almost isoelectric signal not surprising. As observed in previous studies in rabbits, the achievement of electroencephalographic silence was accompanied by considerable cardiovascular depression, with the occurrence of hypotension at propofol plasma concentrations higher than 10 μg/ml. Apart from the propofol effects on the brain electrical activity, the prolonged systemic hypotension also could contribute to the electroencephalographic silence, as observed previously in humans. Although no signs of brain ischemic damage were found after anesthesia (in that all animals recovered well and showed a normal behavior 2 weeks after recovery).
after the procedure), the presence of hypotension during the recordings is an important limitation of the current study because the extent to which cardiovascular depression influenced electroencephalographic patterns and consequently its effects on concentration-effect relations cannot be established.

The commercial index IoC showed high variability in the prediction probability results, which may be attributable to its difficulty in detecting BS in two animals, resulting in a paradoxical increase of the index in very high depths. This situation has been reported in patients with other commercial monitors of anesthetic depth. The monitors seem to confound the characteristic low voltage of the completely suppressed electroencephalogram with the awake state. Because in the current study this did not happen in every animal that achieved electroencephalogram isoelectricity, we may speculate that it is a situation dependent on the presence of residual noise on the almost isoelectric signal. The IoC is a proprietary monitor, and its exact calculation algorithm is not published, but it is known that it has a BS component.

In human patients, the depths of anesthesia achieved are not as deep as those achieved in the current study. Thus, the paradoxical increase of the indexes in case of isoelectricity may not be seen commonly in practice. However, the need for an accurate method for burst suppression detection should be emphasized because paradoxical increases in this situation could result in dangerous overdosage. From another point of view, this IoC difficulty could be explained by the fact that it is calibrated for human electroencephalographic characteristics. Although there are differences in electroencephalogram amplitude between species, the burst suppression limit capable of detecting the rabbits’ electroencephalographic silence was similar to that used in human patients.

With regard to translational research between animals and humans, it was expected that the rabbit would provide a high quality electroencephalographic signal because of the small muscle layer between the skull and the head skin and little electromyographic activity. However, the electromyographic activity as recorded by the IoC-View Monitor showed a very good dose-response relation, which may indicate that electromyographic activity recorded in the rabbit skull during the awake state and in lighter anesthetic depth. Nevertheless, this rabbit model provided good quality recordings with little artifact and low electromyographic activity during deeper anesthesia and may be an interesting model for future research in this area.

In the current study, plasma concentrations were used for the pharmacodynamic analysis instead of effect-site concentrations because the plasma effect-site equilibration constant ($k_{eq}$) was not determined. A first-order, effect-site model could be used to find the $k_{eq}$. However, the fact that the expected (according to previous pharmacokinetic data) steady state was not observed at the higher infusion rates may suggest nonlinear pharmacokinetics in this species, and applying the first-order, effect-site model could lead to inaccurate results. Another limitation of the current study was the use of different infusion rate orders in different animals. Although the order effects on pharmacokinetics are not an important interference because real plasma concentrations of propofol were used for statistical analysis, the possibility that the order interfered with the observed effects cannot be excluded.

In conclusion, using the most recent indexes of anesthetic depth, promising results were found with single-scale permutation entropy and composite multiscale permutation entropy during propofol anesthesia when a burst suppression correction was included in their calculation. Future research should investigate the information present in electromyographic activity seems to be a good correlate with anesthetic depth. Burst suppression algorithms incorporated into monitors for intraoperative use should be accurately calibrated, to avoid dangerous paradoxical increases during deep anesthesia.

The authors gratefully acknowledge the contributions of Pedro Oliveira, Ph.D. (Professor, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal), Nadja Bressan, M.Sc. (Ph.D. Student, Faculdade de Engenharia da Universidade do Porto, Porto), and Ana Castro, B.Sc. (Ph.D. Student, Faculdade de Engenharia da Universidade do Porto), who helped in the data analysis.

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