Concentration–Effect Relations, Prediction Probabilities (P_k), and Signal-to-noise Ratios of Different Electroencephalographic Parameters during Administration of Desflurane, Isoflurane, and Sevoflurane in Rats

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Background: The authors investigated the suitability of different electroencephalographic parameters to quantify the anesthetic effect of desflurane, isoflurane, and sevoflurane in rats.

Methods: Ten male Sprague-Dawley rats were anesthetized in a randomized crossover design with maximum values of 11% desflurane, 2.1% isoflurane, and 3.5% sevoflurane. The electroencephalogram was recorded with implanted electrodes and a wireless telemetry system. Concentration–effect relations and signal-to-noise ratios were determined for the approximate entropy and for the median frequency and the spectral edge frequency, which were modified to account for spikes and burst suppression. The prediction probability \( P_k \) with respect to the response to a painful stimulus was determined.

Results: All drugs produced deep anesthesia with burst suppression and no response at the highest concentrations. The occurrence of spikes and burst suppression made a modification of median frequency and spectral edge frequency necessary to obtain \( P_k \) values greater than 0.5 and monotonic sigmoid concentration–effect relations. The \( P_k \) values were between 0.89 and 0.98, with significantly higher values for modified median frequency and spectral edge frequency during desflurane and sevoflurane. The signal-to-noise ratios were between 3.0 and 6.4 dB, with significantly better values for modified spectral edge frequency and approximate entropy during sevoflurane.

Conclusions: If modified for spikes and burst suppression, median frequency and spectral edge frequency as well as the unmodified approximate entropy were able to assess the anesthetic effect of desflurane, isoflurane, and sevoflurane in rats. For sevoflurane, the modified spectral edge frequency was best with regard to signal-to-noise ratio and prediction probability.

ANIMAL studies may help to investigate the pharmacodynamics of anesthetic drugs for example in the early phase of drug development or in interaction studies where drugs and dose combinations beyond the clinical use can be studied. However, such studies require reliable measures of drug effect. Besides the classic approach of measuring the response to various stimuli, the analysis of the spontaneous electroencephalogram has become an important tool for this purpose. Hereby, the complex information of the electroencephalogram is usually condensed in one variable, and numerous variables derived from the electroencephalogram have been proposed, from spectral parameters such as median frequency (MEF) or spectral edge frequency (SEF) to parameters of higher order such as Bispectral Index or parameters from nonlinear system analysis such as entropy measures. A suitable electroencephalographic parameter should meet the following conditions: (1) There must be a correlation between the anesthetic effect measured by the electroencephalographic parameter and the anesthetic effect measured by an appropriate clinical parameter, e.g., response to stimuli. (2) There should be a clear relation between the concentration of the anesthetic drug and the effect measured by the electroencephalographic parameter. (3) Because the raw data of an electroencephalographic parameter must be smoothed to extract the “signal” out of the “noisy” data, and because every smoothing leads to an inevitable delay of the parameter with regard to sudden changes, the parameter should have a good signal-to-noise ratio (SNR) to keep the extent of necessary smoothing low.

In the current exploratory study, we investigated two parameters derived from the power spectrum of the electroencephalogram—MEF and SEF—together with approximate entropy (AE) with regard to their suitability to assess the anesthetic effect of desflurane, isoflurane, and sevoflurane in rats.

Materials and Methods

Animals

After approval by the appropriate animal investigation committee (Tierschutzkommission, Regierung von Mittelfranken, Ansbach, Germany), 10 adult male Sprague-Dawley rats, weighing 501 ± 61 g (mean ± SD), were included into the study. Animals were delivered by Charles River Wiga GmbH (Sulzfeld, Germany) at least 7 days before the instrumentation for quarantine and acclimatization. Animals were healthy with respect to serology, bacteriology, parasitology, and pathology. The rats were housed in pairs in polycarbonate cages type III (Uno Roestvaststaal b.v., Zevenaar, The Netherlands) on standard research bedding (soft wood fiber; Altromin GmbH, Lage, Germany) at 21.0°C, 60% humidity, 12-h light–dark cycle, with pelleted standard rodent diet (No. 1320; Altromin GmbH, Lage, Germany) and tap water ad libitum.
Instrumentation

At least 7 days before starting the experiments, the rats were anesthetized with 150 mg/kg ketamine (100 mg/ml Ketavet®; Pharmacia GmbH, Erlangen, Germany) and 3 mg/kg xylazine (20 mg/ml Rompun®; Bayer AG, Leverkusen, Germany) intraperitoneally. Using a stereotactic device, five stainless steel screw electrodes (1.3-mm diameter) with isolated copper wires were implanted into the skull 1.5 mm posterior and ±3 mm lateral to bregma (frontal electrodes F₁, F₂), and 7.5 mm posterior and ±3 mm lateral to bregma (occipital electrodes O₁, O₂), and on the sagittal midline 3 mm anterior bregma (F₃) as reference electrode. The electrodes were connected to a miniature socket that was fixed to the skull with dental cement.

Electroencephalographic Recording and Processing

The four-lead electroencephalogram was transmitted using a wireless telemetric system (TSE Technical & Scientific Equipment GmbH, Bad Homburg, Germany). This system consisted of a battery powered transmitter (25 × 15 × 5 mm, weight: 5 g) which was connected to the implanted socket, a receiver, and a computer interface. The raw electroencephalogram was filtered (high pass: 0.5 Hz, low-pass: 60 Hz, notch filter: 50 Hz), amplified and transmitted using pulse-width modulation at a frequency of 417 MHz and a maximum output of 1 mW. The antenna to receive the signal was placed approximately 1 m above the animal. The demodulated signal was digitized (sampling rate: 128 Hz, resolution: 16 bit) and stored for further analysis. From epochs of 8 s, the 50% quantile (MEF) and the 95% quantile (SEF) of the power spectrum (0.5–49 Hz) were estimated. In previous studies, we found that the electroencephalogram is suppressed; Nspike is the number of spikes in the epoch as determined by the spike analysis; and kspike is a coefficient that was determined in the current study by maximizing the prediction probability Pk between the electroencephalographic parameter and the reaction to a noxious stimulus (see Prediction Probability). With increasing number of spikes and/or increasing suppression, the term \((1 - BSR) \times (1 - k_{spike} \times N_{spike})\) decreases and the modified parameter becomes smaller than the original parameter. Whereas burst suppression ratio is a number between 0 (no suppression) and 1 (complete suppression), the term \((1 - k_{spike} \times N_{spike})\) can theoretically become negative if the number of spikes is very high. Therefore, mMEF and mSEF were set to zero if \((1 - k_{spike} \times N_{spike}) < 0.\)

As an additional electroencephalographic parameter that is not derived from the power spectrum, we also calculated the AE, which was introduced by Bruhn et al. as a measure for anesthetic drug effect.

Anesthesia

Experiments were performed at the animal laboratory of the Department of Anesthesiology and started always at approximately 14:00 (2:00 PM) to minimize the influence of circadian rhythms. For anesthesia, rats were placed in an acrylic glass cylinder of 20 cm diameter and 20 cm height with a gas inflow port at the bottom and an effluent port at the top of the wall. In the removable cap of the box, there was also a sealed slot for the test of response to stimuli (see Stimulus–Response Measure). After 20 min of baseline recording, desflurane (Suprane®; Baxter GmbH, München, Germany), isoflurane (Forene®; Abbott GmbH, Wiesbaden, Germany), or sevoflurane (Sevorane®; Abbott GmbH) was administered with a flow of 4 l/min and 30% oxygen (Servo Ventilator 900C; Siemens AG, Erlangen, Germany). Each animal received each inhalational agent in a randomized order with an interval of at least 5 days between two consecutive treatments. The inspired concentration of the agents was measured in the effluent route (Siemens Multi-gas and SC 9000XL; Siemens AG). The concentration of the inhalational agent was increased in steps of 20 min duration to allow equilibration of end-tidal and inspired concentration. The applied concentrations were 2.9, 5.0, 6.5, 7.9, 9.4, and 11 vol% for desflurane; 0.6, 1.0, 1.3, 1.5, 1.8, and 2.1 vol% for isoflurane; and 0.9, 1.6, 2.1, 2.6, 3.1 and 3.5 vol% for sevoflurane, respectively. Assuming a minimum alveolar concentration (MAC) of 7.6 vol% for desflurane, 1.3 vol% for isoflurane, and 2.4 vol% for sevoflurane, the applied concentrations equaled approximately 0.4, 0.7, 0.9, 1.1, 1.3, and 1.5 MAC. During the experiments, rats were allowed to breathe spontaneously, and the respiratory frequency was measured regularly. To determine the recovery time, we used a tape removal test that was originally proposed by Schallert et al. as a test for sensorimotor integration. After termination of the last concentration step, the animals were taken out of the cylinder and were placed in a large open box. The paws were fixed on the
ground with strips of adhesive tape, and the recovery time was defined as the time until complete removal of the strips.

**Stimulus–Response Measure**

As a clinical measure of anesthetic drug effect, we assessed the response to a painful stimulus 6 min before the end of a concentration step and again 1 min before the end of a concentration step. A thin stick with a rounded end of 1 mm diameter and an additional weight made of 200 g lead was inserted through the slot at the cap of the box, and a painful squeezing stimulus was applied at an interdigital fold of a paw, performing consecutive tests at different locations. A purposeful withdrawal reaction of the paw within 10 s after the stimulus was defined as positive response. All further analysis was performed with the second response measurement 1 min before the end of a concentration step.

**Prediction Probability**

The association between the electroencephalographic parameters mMEF, mSEF, and AE and the response to the painful stimulus was assessed by the prediction probability $P_k$. For positive correlation, this measure has a value of 1 when the indicator (i.e., the electroencephalographic parameter) predicts the observed effect (i.e., the response to stimulus) perfectly, and a value of 0.5 when the indicator predicts no better than a 50:50 chance. The mMEF, mSEF, and AE were averaged over the last minute of the corresponding concentration interval, after the second painful stimulus, to obtain one representative value for each concentration. The $P_k$ values were calculated for each drug and each parameter from the pooled data pairs of all rats, using the jackknife technique to obtain estimates of the SEs.

For the two modified spectral parameters, mMEF and mSEF, the $P_k$ value depends on the parameter $k_{\text{spike}}$, which defines the degree of modification. Therefore, we estimated the $P_k$ for the unmodified parameters MEF and SEF, and for the modified parameters mMEF and mSEF when only burst suppression modification was performed ($k_{\text{spike}} = 0$), and for the modified parameters with burst suppression and spike modification. $P_k$ was calculated as a function of $k_{\text{spike}}$ with values $0 \leq k_{\text{spike}} \leq 0.1$, and the optimum value of $k_{\text{spike}}$ was determined by searching the maximum of $P_k$.

**Pharmacodynamic Modeling**

The electroencephalographic effect and the response to the painful stimulus were modeled with a sigmoid $E_{\text{max}}$ model:

$$E = E_0 - E_{\text{max}} \frac{c^y}{c^y + EC_{50}}$$

where $E$ is the predicted effect at the steady state concentration $c$, $E_0$ is the effect at baseline, $E_{\text{max}}$ is the maximum effect, $EC_{50}$ is the concentration that produces half-maximum effect, and the Hill exponent $y$ is a measure of curve steepness. Because the parameters mMEF and mSEF approach a value of zero if the electroencephalogram is completely suppressed, $E_{\text{max}}$ was set equal to $E_0$ for mMEF and mSEF. For the pharmacodynamic modeling, we used the steady state values of mMEF, mSEF, and AE obtained by averaging over the last minute of the corresponding concentration interval. For modeling of the stimulus response, the effect $E$ was defined as the response probability, which was calculated from the individual dichotomous responses, dividing the number of positive responses by the number of animals. Accordingly, $E_0$ and $E_{\text{max}}$ were set to 100% for the response modeling. The pharmacodynamic parameters were estimated by population analysis using NONMEM® (GloboMax LLC, Hanover, MD) with a proportional error model for the interindividual variability of the pharmacodynamic parameters and a constant error model for the residual intrividual variability.

**Signal-to-noise Ratio**

In each animal, the SNR was estimated for the electroencephalographic parameters mMEF, mSEF, and AE. If the data are written as data $= \text{signal} + \text{noise}$, SNR is defined as $\text{SNR} = \text{Variance(signal)}/\text{Variance(noise)}$. The signal must be identified from the data. This can be done, for example, by smoothing the data with a moving average. However, it is difficult to find a rational choice for the length of the averaging interval, which strongly affects the SNR. We therefore decided to use a cubic spline interpolation for identification of the signal. With this approach, the degree of smoothing depends on the number of knots. If the number of knots equals the number of data points, there is obviously no smoothing, and with decreasing number of knots, the degree of smoothing increases. The optimum number of knots was estimated using a modified Akaike criterion penalizing the number of knots. Using the estimated spline interpolation as the “signal,” the SNR was defined as $\text{SNR} = \text{Variance(spline)}/\text{Variance(data − spline)}$, and was expressed in decibels by taking the logarithm: $\text{SNR(dB)} = 10 \times \log_{10}(\text{SNR})$.

**Statistics**

Data are presented as mean and SE unless otherwise stated. Because the $P_k$ values were obtained from pooled data pairs, we used the jackknife technique to obtain estimates of the paired differences between the $P_k$ values of the investigated electroencephalographic parameters. These differences were tested for statistic significance using a one-sample $t$ test with a null hypothesis of zero and the Bonferroni correction for multiple comparisons. The individual SNRs were compared using paired $t$ tests with Bonferroni correction for multiple comparisons. A value of $P < 0.05$ was considered significant.
For all investigated anesthetics, the response to the painful stimulus was suppressed in all animals at the highest concentration. Figure 1 shows the measured response data together with the concentration–response curve as predicted by the estimated pharmacodynamic models (table 1). Because there were no obvious differences between the different leads, the electroencephalographic analysis was performed with the data of the frontal lead F1-Fz. With increasing concentration of the inhalational agents, characteristic changes of the electroencephalogram were observed for all investigated anesthetics (fig. 2). Whereas the baseline electroencephalogram was dominated by frequencies in the theta band (4–7 Hz), there was initially a shift to lower frequencies with increasing concentration. At concentrations of 1.5 vol% isoflurane, 2.6 vol% sevoflurane, and 7.9 vol% desflurane (corresponding to approximately 1.1 MAC), spike patterns, but no burst suppression, were observed in the electroencephalogram. The number of spikes further increased with increasing concentration, and at the highest concentration, the electroencephalogram was partially suppressed with a burst suppression ratio between 0.5 and 0.8. The high-frequency components of the spikes and bursts caused a paradoxical increase of the

**Table 1. Pharmacodynamic Parameters for the Response to a Painful Stimulus**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>EC_{50}, vol%</th>
<th>( \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desflurane</td>
<td>7.80 ± 0.20</td>
<td>20.0 ± 2.0</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1.44 ± 0.08</td>
<td>21.4 ± 1.5</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>2.40 ± 0.10</td>
<td>18.8 ± 1.2</td>
</tr>
</tbody>
</table>

Data are estimate ± SE.

EC_{50} = concentration for 50% response probability; \( \gamma \) = Hill exponent.

Results

For all investigated anesthetics, the response to the painful stimulus was suppressed in all animals at the highest concentration. Figure 1 shows the measured response data together with the concentration–response curve as predicted by the estimated pharmacodynamic models (table 1). Because there were no obvious differences between the different leads, the electroencephalographic analysis was performed with the data of the frontal lead F1-Fz. With increasing concentration of the
spectral parameters MEF and SEF with deeper level of sedation. Figures 3 and 4 show the mean time courses of the unmodified parameters, the time courses if only burst suppression modification was performed ($k_{\text{spike}}/H11005\ 0$), and the time courses of mMEF and mSEF with the optimum value of $k_{\text{spike}}$ during sevoflurane anesthesia.

The AE continuously decreased from baseline values of approximately 1.3 to minimum values of approximately 0.7 (fig. 5). After termination of anesthesia, all electroencephalographic parameters rapidly regained baseline values.

For the parameters MEF and SEF, the prediction probability $P_k$ with regard to stimulus response was poor when the unmodified parameters were used. The mod-

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**Fig. 3.** Time courses of the unmodified median frequency (MEF; A), the median frequency modified for burst suppression only (MEFBS; B), and the median frequency modified for burst suppression and spikes (mMEF; C) in all rats during anesthesia with sevoflurane. Data are mean ± SEM. The raw data were smoothed by applying a moving average over eight epochs.

**Fig. 4.** Time courses of the unmodified spectral edge frequency (SEF; A), the spectral edge frequency modified for burst suppression only (SEFBS; B), and the spectral edge frequency modified for burst suppression and spikes (mSEF; C) in all rats during anesthesia with sevoflurane. Data are mean ± SEM. The raw data were smoothed by applying a moving average over eight epochs.
Correction for burst suppression yielded markedly better $P_k$ and with modification for burst suppression and spikes, $P_k$ achieved values between 0.92 and 0.98 (table 2). For isoflurane, figure 6 demonstrates the effect of the modification on the $P_k$ value of MEF, and figure 7 shows the plot of $P_k$ as a function of the modification parameter $k_{spike}$. For isoflurane, the AE revealed a $P_k$ that was as good as for mMEF and mSEF. The mSEF showed a significantly better $P_k$ than the AE both for desflurane and for sevoflurane, whereas the mMEF was superior to the AE only for sevoflurane (table 2). There were no differences between mMEF and mSEF with regard to the prediction probability.

For AE and for mMEF and mSEF with the optimum values of $k_{spike}$, the concentration–effect relation could be described by a sigmoid $E_{max}$ model (table 3). For isoflurane, the concentration–effect relations of the three electroencephalographic parameters are shown in figure 8.

The SNRs showed a trend to better values for mSEF and AE, which was statistically significant only for sevoflurane (table 4). The raw electroencephalographic parameters and the corresponding spline interpolations in one animal during anesthesia with sevoflurane are shown in figure 9.

All rats continued to breathe spontaneously during anesthesia, while the respiratory frequency decreased from a baseline value of $82 \pm 5$ min$^{-1}$ to minimum values of $42 \pm 1$, $42 \pm 2$, and $39 \pm 2$ min$^{-1}$ at the maximum concentration of desflurane, isoflurane, and sevoflurane, respectively. The recovery times as defined by the tape removal test were $113 \pm 8$, $199 \pm 17$, and $272 \pm 30$ s after anesthesia with desflurane, sevoflurane, and isoflurane, respectively, with all differences being statistically significant ($P < 0.05$, paired $t$ test with Bonferroni correction).

**Discussion**

It was the aim of this study to compare different electroencephalographic parameters with regard to their ability to assess the anesthetic effect of desflurane, isoflurane, and sevoflurane in rats. As with higher concentrations specific patterns with high-frequency components, i.e., spikes and burst suppression, occurred in the electroencephalogram, commonly used parameters of the power spectrum, such as MEF and SEF, increased or remained almost constant with increasing concentration and increasing level of sedation. Therefore, the association between the clinical effect (response to stimulus) and the spectral parameters MEF and SEF was poor, and these parameters would not be suitable to assess the anesthetic effect of the studied drugs. A modification of the SEF to account for burst suppression was proposed in early studies, and we therefore introduced an extension of this modification by taking into account the occurrence of spikes. Whereas this approach was initially introduced for the electroencephalogram of rats during propofol anesthesia, it could be successfully applied also in the current study with inhalational agents.

![Fig. 5. Time course of the approximate entropy (AE) in all rats during anesthesia with sevoflurane. Data are mean ± SEM. The raw data were smoothed by applying a moving average over eight epochs.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931051/)
However, this modification introduces also an additional parameter, $k_{\text{spike}}$, which controls the extent of the modification, and this raises the problem of the best choice for this parameter. One approach to estimate the optimum value of $k_{\text{spike}}$ would be to search that value that maximizes the prediction probability between the concentration of the drug and the modified electroencephalographic parameter. However, because a suitable electroencephalographic parameter should mainly be able to reflect the anesthetic state and should be sensitive to alterations caused by external stimuli, we decided to use the prediction probability of the parameter with regard to the response to a noxious stimulus, and we therefore estimated $k_{\text{spike}}$ by maximizing this $P_k$ value. For the spectral parameters modified in this way, a monotonic concentration–effect relation could be established which is favorable particularly for automated drug control in anesthesia.

Interestingly, there was no modification of the AE necessary to achieve an adequate association between this parameter and the response. This parameter seems to correctly classify special patterns such as spikes and burst suppression. This was also found in a study of Bruhn et al. with isoflurane in patients. In their study, AE reached values close to zero if the burst suppression ratio was near 100%, whereas in our study, the AE did not fall below 0.4. This may be caused by residual electroencephalographic activity during suppression and depends on the choice of the “noise” filter in the entropy calculation.

The findings of the current study are contradictory to those of Rampil et al., who found no correlation be-

![Fig. 6. Association between the response to a painful stimulus and the unmodified median frequency (MEF; A), the median frequency modified for burst suppression only (MEFBS; B), and the median frequency modified for burst suppression and spikes (mMEF; C) for all rats during anesthesia with isoflurane. The rhombic dots show the individual values, the square dots show the mean, and the bars show the 95% confidence intervals of the means. The prediction probability $P_k$ is a measure for the degree of association.](image)

![Fig. 7. Plot of the prediction probability $P_k$ for the modified median frequency (mMEF) during anesthesia with isoflurane as a function of the spike modification parameter $k_{\text{spike}}$. The optimum value of $k_{\text{spike}}$ that led to a maximum prediction probability was 0.033.](image)

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Table 3. Results of the Pharmacodynamic Modeling

<table>
<thead>
<tr>
<th></th>
<th>$E_0$</th>
<th>$E_{\text{max}}$</th>
<th>$EC_{50}$, vol%</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desflurane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mMEF</td>
<td>$4.3 \pm 0.3$</td>
<td>$4.3 \pm 0.3$</td>
<td>$7.4 \pm 0.5$</td>
<td>$8.8 \pm 3.7$</td>
</tr>
<tr>
<td>mSEF</td>
<td>$24 \pm 2$</td>
<td>$24 \pm 2$</td>
<td>$7.8 \pm 0.3$</td>
<td>$7.1 \pm 1.2$</td>
</tr>
<tr>
<td>AE</td>
<td>$1.21 \pm 0.05$</td>
<td>$0.46 \pm 0.08$</td>
<td>$7.0 \pm 0.3$</td>
<td>$8.0 \pm 2.0$</td>
</tr>
<tr>
<td>Isoflurane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mMEF</td>
<td>$4.5 \pm 0.7$</td>
<td>$4.5 \pm 0.7$</td>
<td>$1.3 \pm 0.2$</td>
<td>$5.2 \pm 2.0$</td>
</tr>
<tr>
<td>mSEF</td>
<td>$24 \pm 2$</td>
<td>$24 \pm 2$</td>
<td>$1.5 \pm 0.1$</td>
<td>$6.5 \pm 1.4$</td>
</tr>
<tr>
<td>AE</td>
<td>$1.27 \pm 0.04$</td>
<td>$0.50 \pm 0.11$</td>
<td>$1.4 \pm 0.1$</td>
<td>$5.8 \pm 1.7$</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mMEF</td>
<td>$4.6 \pm 0.3$</td>
<td>$4.6 \pm 0.3$</td>
<td>$2.0 \pm 0.2$</td>
<td>$4.6 \pm 1.2$</td>
</tr>
<tr>
<td>mSEF</td>
<td>$23 \pm 1$</td>
<td>$23 \pm 1$</td>
<td>$2.1 \pm 0.4$</td>
<td>$6.9 \pm 1.3$</td>
</tr>
<tr>
<td>AE</td>
<td>$1.22 \pm 0.02$</td>
<td>$0.38 \pm 0.27$</td>
<td>$2.3 \pm 0.7$</td>
<td>$5.4 \pm 0.6$</td>
</tr>
</tbody>
</table>

Data are estimate ± SE. For modified median frequency (mMEF) and modified spectral edge frequency (mSEF), maximum effect ($E_{\text{max}}$) was set equal to baseline effect ($E_0$). AE = approximate entropy; $EC_{50}$ = concentration at 50% maximum effect; $\gamma$ = Hill exponent.
between electroencephalogram and movement to response in rats during isoflurane anesthesia. However, these authors investigated the “naive” SEF and the SEF with a modification only for burst suppression but not for spikes. For these parameters without spike modification we found also a poor correlation with the response (table 3) and apparently no concentration–effect relation (fig. 4). Another difference between their study and the current investigation concerns the electroencephalo-

graphic analysis. Whereas Rampil et al. correlated the electroencephalographic measurements immediately before the stimulus, we used the electroencephalogram after the stimulus. Particularly at lower and medium concentrations, there may be an interference of drug-induced sleep by natural sleep, and the prestimulus electroencephalographic data may suggest a deeper level of sedation than really given. In our study, there were indeed small peaks at the end of the low- and medium-concentrations steps, after the stimulus had been applied (figs. 3–5). When using prestimulus electroencephalographic data, one gets some information about how well the electroencephalogram can predict a future response, whereas with poststimulus data, one gets some information about how well the electroencephalogram reflects the current anesthetic state in presence of stimuli. Using poststimulus data, there may be a problem with motion artifacts when needle electrodes are used, as by Rampil et al., whereas the implanted electrodes and the wireless transmission in our setup allowed us to record an artifact-free electroencephalogram even if the animal moved.

Another more general issue that has been discussed in the literature is the question whether movement as a response to a noxious stimulus is really a measure of depth of anesthesia. Various experiments with a cranial bypass model or decerebrated animals suggested that anesthetic action in the spinal cord plays an important role in producing immobility after noxious stimuli, whereas the electroencephalogram measures the cerebral activity. However, for an integral animal, there will be an action of the anesthetic drug both on the spinal and on the cerebral level, and these two actions are likely to be correlated. Accordingly, the response to noxious stimulus and the electroencephalogram may reflect different aspects of anesthesia, but these aspects should also be correlated when studying an integral organism. Therefore, the response to stimuli is still indispensable in pharmacodynamic research. Compared with other reflexes such as whisker reflex, startle reflex to noise, or righting reflex, the response to noxious stimulus has the advantage that it is usually lost at higher concentrations and may therefore be more suitable to assess the effect over the complete anesthetic concentration range.

**Table 4. Signal-to-noise Ratios of the Investigated Electroencephalographic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>mMEF</th>
<th>mSEF</th>
<th>AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desflurane</td>
<td>3.3</td>
<td>3.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>4.2</td>
<td>5.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>3.0</td>
<td>6.6</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Data are mean ± SEM, in decibels. *P < 0.05 compared with modified median frequency (mMEF).

AE = approximate entropy; mSEF = modified spectral edge frequency.
Regarding the SNR, significant differences were only observed for sevoflurane, but the mMEF showed a general trend to lower SNR compared with mSEF and AE. Similar results were also found for propofol in man.\(^{19}\) From figure 9, it is obvious that the AE shows lower noise than mSEF, but because the range of mSEF (0–25 Hz) is larger than that of AE (0.6–1.3), the variance of the signal is larger for mSEF and therefore the SNRs of mSEF and AE are similar. Because the necessary degree of smoothing depends on SNR, mSEF and AE need less smoothing than mMEF and should therefore react faster on sudden changes of the anesthetic effect, which make them more suitable for intraoperative monitoring.

There are some limitations of the current study that need to be stated. The inhalational agents were applied in increasing concentration steps, which may introduce some bias because time effects cannot be excluded. With randomized concentrations, on the other hand, the time to reach a new steady state would be longer, when there is a large difference in concentrations between two consecutive measurements. Therefore, with randomized concentrations, it would generally be useful to increase the duration of the steps, so that the complete experiment would be prolonged, which may introduce some new problems with respect to alterations of the general state of the animals. Another issue is the fact that the examiner of the response to the painful stimulus was not blinded with regard to the drug and the concentration. The missing blinding to agent is of less impact because we did not intend to compare the different agents, and because the response was assessed on a dichotomous scale there is also little impact of the missing blinding to the concentration.

The applied modification of the spectral parameters is obviously an empiric or phenomenologic approach to take into account the appearance of specific electroencephalographic patterns, with the intention to construct an electroencephalographic parameter that correctly reflects that the clinically defined level of sedation is deeper when spikes or burst suppressions are observed. We cannot give any physiologic explanation for these effects; however, most electroencephalographic analyses in anesthesia must be seen more as a phenomenologic rather than a physiologic approach. And, as already mentioned, the modification for burst suppression, which was introduced some time ago, has become an accepted technique.\(^{20}\) However, it would be worthwhile to investigate whether the spike patterns that we observed for isoflurane, desflurane, sevoflurane, and propofol also occur with other anesthetic agents, e.g., benzodiazepines or ketamine.

In conclusion, the current study showed that specific electroencephalographic patterns, namely spikes and burst suppression, made a modification of the parameters MEF and SEF necessary to obtain an adequate correlation between the electroencephalographic parameters and the response to noxious stimulus as a clinical sign of anesthetic effect. For these modified parameters, there was also a clear monotonic concentration–effect relation. For the AE, there was no modification necessary. The SNR tended to be better for mSEF and AE. All three parameters, mMEF, mSEF, and AE, were suitable to assess the anesthetic effect of desflurane, isoflurane, and

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**Fig. 9.** Time course of the modified median frequency (mMEF; A), the modified spectral edge frequency (mSEF; B), and the approximate entropy (AE; C) in one rat during anesthesia with sevoflurane. The dots depict the raw data, and the lines show the spline interpolations, which were used to estimate the signal-to-noise ratios (SNR).
sevoflurane in rats, with mSEF being the best parameter for sevoflurane.

References

15. Antognini JF, Carstens E, Atherley R: Does the immobilizing effect of thiopental in brain exceed that of halothane? Anesthesiology 2002; 96:980–6
17. Rampj J: Anesthetic potency is not altered after hypothermic spinal cord transection in rats. Anesthesiology 1994; 80:606–10