Monitoring of the responsiveness to noxious stimuli during sevoflurane mono-anaesthesia by using RIII reflex threshold and bispectral index

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Background. We investigated the accuracy of the (normalized) RIII reflex threshold, the bispectral index (BIS), and the end-tidal sevoflurane concentration for predicting movement responses during mono-anaesthesia using sevoflurane.

Methods. Fourteen male subjects were included. Each received a sevoflurane mono-anaesthesia for which the end-tidal concentration was increased in steps of 0.2 vol% every 10 min. Every 5 min, the reactions to noxious stimuli (10 s trapezius squeeze and 30 s 80 mA tetanic stimulus) were tested. The administration of sevoflurane was halted after no movement reactions occurred for three concentration steps. RIII reflex threshold and BIS were recorded continually in all subjects.

Results. Thirteen subjects completed the study. The prediction probabilities for movement reactions to the noxious stimuli were 0.79 for the BIS, 0.91 for the RIII threshold, and 0.89 for the end-tidal sevoflurane concentration (PKDMACRO-Statistics: BIS vs RIII, P<0.05; BIS vs Csevo, P<0.05; RIII vs Csevo, P>0.05). All population prediction probability values differed significantly from 0.5 (P<0.01, PKDMACRO).

Conclusions. All three instruments can be used for a prediction of movement responses to a noxious stimulus under sevoflurane mono-anaesthesia with an accuracy exceeding prediction by chance. The accuracy of the BIS to predict these responses appears to be lower compared with the RIII reflex threshold or the end-tidal sevoflurane concentration.

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Movement responses to noxious stimuli under general anaesthesia are an important determinant of anaesthetic depth. However, this determinant can only be evaluated by singular testing and therefore further surrogate parameters are needed to allow for a continual monitoring. We have demonstrated in previous studies that the RIII reflex threshold can be used as such a surrogate.1,2

The RIII reflex as a component of the nociceptive flexion reflex is a polysynaptic spinal withdrawal reflex that is elicited by stimulation of nociceptive nerve afferents. To assess the RIII reflex, biceps femoris muscle activity is monitored using an EMG during the application of electrocutaneous stimuli to the ipsilateral sural nerve. On the basis of the observed EMG response, the stimulus intensity required to elicit the RIII reflex can be used as an objective measure of the individual nociceptive threshold.3,4

In former studies, we compared the accuracy of the RIII reflex threshold and the bispectral index (BIS) in their ability to predict movement after a noxious stimulus either during propofol mono-anaesthesia1 or during interactions between propofol and remifentanil.2 These studies reveal that the performance of the BIS is comparable with that of the RIII reflex threshold for detecting movement when only propofol is used for anaesthesia. In contrast, when high doses of remifentanil are given, the RIII reflex threshold outperforms the BIS. Therefore, it can be assumed that the RIII reflex threshold provides additional information on the probability of movement compared
with the BIS when movement is prevented by analgesic effects.

In this study, we compared the performance of the BIS and the RIII reflex threshold during sevoflurane monoaesthesia using a comparable protocol as published before. Movement suppression by sevoflurane is mediated predominately through mechanisms on the level of the spinal cord. Therefore, we hypothesized that the spinal RIII reflex threshold would have a higher accuracy in predicting movement responses during sevoflurane monoaesthesia, compared with the BIS, which mainly reflects the cortical drug effects.

Methods

Subjects and setting

After approval of the local ethics committee (Berlin, Germany) and written informed consent, the study was performed in 14 healthy (ASA class I) male volunteers, ranging in age from 23 to 30 yr. Only male volunteers were included to reduce the variability of the RIII reflex threshold. During the course of the study, the subjects were comfortably rested in therapy beds with a flexed leg-section to maintain angles of 120° in the hip and 130° in the knee.

Automated RIII threshold tracking

To elicit the RIII reflex of the left biceps femoris muscle, the left sural nerve was repeatedly stimulated at its retro-malleolar pathway via surface electrodes (inter-electrode distance: 30 mm). Stimuli were applied automatically at randomized intervals of 8–12 s to avoid habituation, with each stimulus consisting of a volley of five rectangular electrical pulses of 1 ms duration each, at 200 Hz (2× DS5, Digitimer Ltd, Hertfordshire, UK). To record the RIII reflex of the left biceps femoris muscle, surface electrodes were placed over its lateral tendon and over the muscle itself, 10 cm proximal of the popliteal fossa. The recorded signals were amplified (g.BSamp, g.tec, Schiedberg, Austria), digitized at a sampling rate of 5 kHz (Mikro 1401 mk II, CED Ltd, Cambridge, England), rectified, and analysed using Signal 3.10 (CED Ltd).

The RIII reflex threshold was traced continually by an automated RIII threshold tracking system. This system varies the stimulus intensity according to an up–down staircase algorithm with a variable step length to estimate the stimulus intensity associated with a 50% probability of RIII reflex occurrence, which is defined as the reflex threshold. RIII reflex occurrence was defined as an interval peak z-score \( >10.32 \) in the post-stimulation interval of 90–150 ms. RIII reflex recording was started 10 min before administration of sevoflurane to accustom the subjects to the stimulation. The applied stimuli are perceived in the range of not painful to slightly above the pain threshold.

Testing procedure for reactions on vocal and noxious stimuli

During the whole course of the study, the reactions to verbal and noxious stimuli were tested every 5 min. The testing sequence was performed in the following order: one single verbal command, loudly repeated verbal commands, trapezius squeeze of 10 s duration, and electrical tetanic stimulation in the area of the right ulnar nerve with 80 mA for 30 s. The testing was performed by the same investigator for all subjects. Any verbal or movement reaction, regardless of purposeful or not, was considered as a positive response to the innocuous stimuli and the sequence of reaction testing was aborted. Any movement reaction, regardless of purposeful or not, was considered as a positive response to the noxious stimuli and the sequence of reaction testing was aborted.

Drug administration and monitoring

The subjects fasted at least 6 h before the administration of the drugs. Before the study period, standard monitoring including non-invasive arterial pressure, electrocardiography, pulse oximetry, a tight-fitting facemask for measuring end-tidal CO₂, surface electrodes for the bispectral EEG index (BIS), and an i.v. access via a forearm vein was established. To avoid hypoventilation and to maintain a stable level of end-tidal CO₂ under higher drug concentrations, some subjects received Guedel tubes, assisted ventilation via the facemask, or both.

Sevoflurane was administered via the facemask using an anaesthetic workstation (Primus, Dräger Medical, Lübeck, Germany). End-tidal concentrations were measured continuously using the infrared spectrophotometer of the anaesthesia monitor (iMM Anesthesia Monitor, Datex Ohmeda S/5 FM, Helsinki, Finland). According to the experimental protocol, sevoflurane was increased every 10 min in steps of 0.2 vol% end-tidal concentration. To accelerate the distribution of sevoflurane when increasing the concentration, higher concentrations than the target level were administered to reach the next concentration level within 2–3 min. After the loss of responses to the noxious stimuli as described above, three more steps to increase the concentration were conducted, before the administration of sevoflurane was ended.

Data analysis and statistical analysis

Analyses were performed with RIII reflex threshold data, BIS data, and sevoflurane end-tidal concentrations obtained after the loss of consciousness. For each sequence of reaction testing as described above, the last RIII reflex threshold value, the last BIS value, and the sevoflurane end-tidal concentration that were obtained immediately before starting the sequence of testing for a reaction and which therefore were not influenced by the testing sequence were used for analysis.
To compare the performance of the three parameters in predicting reactions and the absence of reactions to the stimuli in each individual, the prediction probability $P_K$ was calculated for each parameter for every individual subject. A $P_K$-value of 1 stands for a 100% correct differentiation between reaction and absence of reaction, whereas a value of 0.5 represents only a 50:50 chance of a correct differentiation. The estimation of individual $P_K$-values was performed using the spreadsheet macro PKMACRO as described by Smith and colleagues. Standard errors of the estimates were computed by the jackknife method. Individual $P_K$-values of the three parameters were compared using a Friedman’s test with a Dunn’s post-test.

To adjust for the large inter-individual variance of the RIII reflex threshold, the individual reflex threshold values were normalized to the first threshold that was estimated after the subject’s loss of consciousness. This mode of normalization has been chosen to avoid the necessity of recording the RIII reflex in awake subjects and therefore to reduce the inconvenience of the procedure when the method would be used on patients. To adjust for the influence of age on the responsiveness to sevoflurane, we also analysed age-adjusted minimum alveolar concentration (MAC) values.

To compare the overall performance of the RIII reflex threshold, the BIS, and the end-tidal sevoflurane

![Graph A](image1.png)

![Graph B](image2.png)

**Fig 1** Concentration dependencies of RIII reflex threshold and BIS. Shown are the individual arithmetic means of the RIII reflex threshold (A) and the BIS (B) at different end-tidal sevoflurane concentrations.
concentration in predicting reactions and absence of reactions to the noxious stimuli in all subjects, the population prediction probability \( P_K \) was calculated for each of the three parameters. However, the \( P_K \) statistic used here is based on the assumption of independent data, since no comparable statistic method has been developed that permits non-independent data. Therefore, we used the \( P_K \) statistic still while the assumption of independent data was violated for our data by the inclusion of multiple stimuli for each subject, as it has been done in other investigations.\(^1\) \(^2\) \(^{10–13}\) As a result of the inclusion of dependent data, standard errors are underestimated if intra-individual variability is lower than inter-individual variability and overestimated in the reverse case. Statistical testing of the prediction probabilities was performed using the spreadsheet macro PKDMACRO as described by Smith and colleagues.\(^8\)

### Results

No relevant changes in arterial pressure, heart rate, arterial oxygen saturation, or end-tidal CO\(_2\) were observed throughout the study. Reactions to the repeated verbal commands were lost for all 14 subjects at a median end-tidal sevoflurane concentration of 1.6 vol\% (range: 0.8–2.0 vol\%). Reactions to the noxious stimuli were lost for all 14 subjects at a median end-tidal sevoflurane concentration of 2.6 vol\% (range: 2.2–3.0 vol\%). Overall, 178 positive responses occurred to the noxious stimuli and 110 negative responses. The effect of sevoflurane at different concentrations on the RIII reflex threshold and the BIS is shown in Figure 1.

One subject had to be excluded from further data analysis due to technical problems with the RIII reflex stimulation unit under the influence of sevoflurane, after which the experimental session was discontinued.

Individual prediction probabilities for RIII reflex threshold, BIS, and end-tidal sevoflurane concentration for reactions and absence of reactions to the noxious stimuli are shown in Table 1. The comparison between the three parameters showed that the prediction probabilities for the RIII reflex threshold were significantly higher compared with those of the BIS \((P<0.05)\), whereas no significant difference could be detected between RIII reflex threshold and end-tidal sevoflurane concentration \((P>0.05)\) or

### Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>RIII reflex threshold</th>
<th>BIS</th>
<th>Sevoflurane end-tidal (vol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.97 (0.03)</td>
<td>0.70 (0.11)</td>
<td>0.93 (0.07)</td>
</tr>
<tr>
<td>B</td>
<td>1.00 (0.00)</td>
<td>0.93 (0.06)</td>
<td>0.91 (0.10)</td>
</tr>
<tr>
<td>C</td>
<td>0.85 (0.10)</td>
<td>0.91 (0.06)</td>
<td>0.89 (0.07)</td>
</tr>
<tr>
<td>D</td>
<td>0.92 (0.08)</td>
<td>0.86 (0.09)</td>
<td>0.92 (0.08)</td>
</tr>
<tr>
<td>E</td>
<td>0.93 (0.05)</td>
<td>0.92 (0.07)</td>
<td>0.86 (0.09)</td>
</tr>
<tr>
<td>F</td>
<td>0.95 (0.04)</td>
<td>1.00 (0.00)</td>
<td>1.00 (0.00)</td>
</tr>
<tr>
<td>G</td>
<td>0.98 (0.02)</td>
<td>0.93 (0.05)</td>
<td>0.93 (0.05)</td>
</tr>
<tr>
<td>H</td>
<td>1.00 (0.00)</td>
<td>0.68 (0.14)</td>
<td>1.00 (0.00)</td>
</tr>
<tr>
<td>I</td>
<td>1.00 (0.00)</td>
<td>0.95 (0.05)</td>
<td>0.93 (0.07)</td>
</tr>
<tr>
<td>K</td>
<td>0.96 (0.04)</td>
<td>0.56 (0.13)</td>
<td>0.94 (0.04)</td>
</tr>
<tr>
<td>L</td>
<td>1.00 (0.00)</td>
<td>0.79 (0.12)</td>
<td>0.82 (0.20)</td>
</tr>
<tr>
<td>M</td>
<td>0.98 (0.02)</td>
<td>0.96 (0.03)</td>
<td>0.98 (0.02)</td>
</tr>
<tr>
<td>N</td>
<td>0.94 (0.04)</td>
<td>0.79 (0.09)</td>
<td>0.92 (0.08)</td>
</tr>
<tr>
<td>Mean</td>
<td>0.96</td>
<td>0.85</td>
<td>0.93</td>
</tr>
<tr>
<td>SE</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### Fig 2

All recorded data points for RIII reflex threshold, BIS, and end-tidal sevoflurane concentration. Shown are all recorded data points of all subjects for (a) the RIII reflex threshold, (b) the BIS, and (c) the end-tidal sevoflurane concentration, divided by whether movement followed the noxious stimulation sequence or not. Each data point shows the respective values of the three monitoring parameters at the moment immediately before the testing sequence for the movement reactions was started.
end-tidal sevoflurane concentration and BIS ($P > 0.05$, Friedman’s test with Dunn’s post-test).

For the comparison of the population prediction performance, the RIII reflex threshold values were normalized to the first threshold that was estimated after the subject’s loss of consciousness. Normalization of BIS data or the end-tidal sevoflurane concentrations did not improve their population prediction probabilities and was therefore neglected. Also, normalization of the end-tidal sevoflurane concentration to age-adjusted MAC values did not improve the prediction probability. All recorded normalized RIII reflex values, BIS values, and end-tidal sevoflurane concentrations of all subjects after the individual loss of consciousness are shown in Figure 2.

The prediction probability $P_K$ for the combined data from all subjects amounted to 0.91 (0.02) [estimate (se)] for the normalized RIII reflex threshold, to 0.79 (0.02) [estimate (se)] for the BIS, and to 0.89 (0.02) [estimate (se)] for the end-tidal sevoflurane concentration. All $P_K$-values differed significantly from the $P_K$-value of 0.5 which corresponds to prediction by chance ($P < 0.01$, PKDMACRO). The difference between the $P_K$-values of the RIII reflex threshold and also the difference between the BIS and the end-tidal sevoflurane concentration were statistically significant ($P < 0.05$, PKDMACRO with Bonferroni’s correction). The difference between the $P_K$-values of the RIII reflex threshold and the end-tidal sevoflurane concentration was statistically not significant ($P > 0.05$, PKDMACRO with Bonferroni’s correction).

Non-normalized RIII reflex threshold values show a significantly lower accuracy of prediction, which is reflected in a $P_K$-value of 0.70 (0.03) [estimate (se)].

**Discussion**

The present study demonstrates that the normalized RIII reflex threshold can be used under sevoflurane mono-anaesthesia to predict reactions and absence of reactions after noxious stimulation. The accuracy of the prediction by the normalized RIII reflex threshold was comparable with that of the end-tidal sevoflurane concentration, whereas the BIS showed a significantly lower accuracy.

This is in line with previous findings that the RIII reflex threshold outperforms the BIS when high doses of remifentanil are given, whereas both instruments show a comparable performance when only propofol is used for anaesthesia. Movement suppression by sevoflurane is mediated predominately through mechanisms on the level of the spinal cord. So, it can be assumed that the RIII reflex threshold provides additional information on the probability of movement compared with the BIS especially when immobility is achieved through analgesic or spinal motor suppressing mechanisms.

For the RIII reflex threshold, a responsiveness to analgesic substances has been demonstrated in several studies. It remains to compare the performance of the RIII reflex threshold in predicting movement responses to noxious stimuli in the setting of general anaesthesia using analgesic substances in addition to sevoflurane with the performance of an interaction surface of sevoflurane and the applied analgesic substance.

A major drawback of the RIII reflex threshold as a monitoring instrument on the other hand is its inter-individual variability. To adjust for this variability in the present study, we normalized the RIII reflex threshold to the threshold value that is estimated immediately after the loss of consciousness, as it has been done in previous studies. Another possible approach would be to normalize to RIII reflex threshold values estimated in the awake state, but this would cause more inconvenience for the subject and therefore we decided to use the threshold value directly after the loss of consciousness as the reference point. Possibly in the future, it would be possible to assign absolute cut-off values for the RIII reflex threshold to different noxious stimuli, which could replace the necessity of normalization. Another disadvantage of the RIII reflex threshold compared with simpler monitoring instruments such as the end-tidal sevoflurane concentration or the BIS is the complexity of the setup to record the RIII reflex. The procedure of setting up the electrodes and apparatus to record the reflex takes time (10–20 min) and experienced personnel.

One limitation of the present study is that only male volunteers have been studied to reduce the inter-individual variability of the RIII reflex threshold a priori. However, since RIII reflex threshold values were normalized in the present study to individual values at the moment of the loss of consciousness, no different results would be expected in female subjects.

In summary, we demonstrated in the present study that the RIII reflex threshold shows a comparable prediction probability for reactions to noxious stimuli as the end-tidal sevoflurane concentration. The BIS shows a lower prediction probability.

**Conflict of interest**

None declared.

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