Monitoring of the responsiveness to noxious stimuli during anaesthesia with propofol and remifentanil by using RIII reflex threshold and bispectral index

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Background. Movement responses are an important indicator of noxious perception in the unconscious state. To allow for a continual monitoring of the responsiveness to noxious stimuli during general anaesthesia, surrogate parameters are needed. Here we compare the performance of the bispectral index (BIS) and the RIII threshold in predicting reactions to noxious stimuli during anaesthesia with propofol and remifentanil.

Methods. Twenty male volunteers were included. The first 10 subjects received constant concentrations of propofol while remifentanil concentrations were increased stepwise. The other 10 subjects each received high propofol concentrations combined with different low remifentanil concentrations and also low propofol concentrations combined with different high remifentanil concentrations. In all subjects, the reactions to an 80 mA 30 s tetanic stimulus were tested every 5 min. BIS and RIII threshold were recorded continually in all subjects.

Results. Nineteen subjects completed the study. The population prediction probability for reactions to the noxious stimuli amounted to 0.86 for the BIS and to 0.84 for the RIII threshold in the first 10 subjects (P<0.05, PKDMACRO). In the other nine subjects, the prediction probabilities amounted to 0.64 for the BIS and to 0.77 for the RIII threshold (P<0.05, PKDMACRO). All population prediction probability values differed significantly from 0.5 (P<0.01, PKDMACRO).

Conclusions. RIII threshold and BIS are both influenced dose-dependently by remifentanil at those concentrations that suppress reactions to noxious stimuli. The susceptibility of the parameters to remifentanil concentration seems to be of a similar quality. Under different ratios of propofol and remifentanil concentrations, the RIII threshold correlates with non-responsiveness better than the BIS.

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Anaesthetic depth can be defined as the probability of a non-response to stimulation.1 This non-response has to be evaluated in the context of the strength of the stimulus, the definition of the response, and the drug concentration at the site of action that blunts responsiveness. In this model, two components are further defined as necessary to create the anaesthetic state: hypnosis created with drugs such as propofol or inhalation anaesthetics and analgesia created with opioids, nitrous oxide, or other means. Simplified these components can be regarded to act together in a hierarchical order where the net effect of analgesics is to attenuate the transmission of painful sensation to the cortex, reducing the amount of hypnotic required to obtain the state of non-responsiveness.

As a difficulty for the application of this model, the components hypnosis and analgesia cannot be measured directly and therefore clinical relevant endpoints have to be defined instead, such as the responsiveness to verbal commands or the responsiveness to painful stimuli such as laryngoscopy or surgical incision. Again, these endpoints...
have the drawback that they can only be evaluated by singular testing and therefore further parameters as surrogates for these endpoints are needed to allow for a continual monitoring. To act as a surrogate for the responsiveness to verbal commands, several rather precise parameters have already been developed, such as indices derived from the processed EEG like the bispectral index (BIS). For the responsiveness to painful stimuli on the contrary, no precise surrogate parameters exist.

Here we would like to propose the RIII reflex threshold as a possible surrogate for the responsiveness to painful stimuli. 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.

Recently, we have demonstrated that the prediction probability of this RIII reflex threshold for reactions to noxious stimuli during propofol mono-anæsthesia has a comparable precision to that of the BIS. Also the responsiveness of the RIII threshold to influences from analgesic substances has been demonstrated in several studies. Therefore, a susceptibility of the RIII reflex threshold to both hypnotic and analgesic influences seems to be evident, which can be regarded as the prerequisite for a good performance as a surrogate for non-responsiveness to painful stimuli since the non-responsiveness is usually a product of the combination of these influences.

However, for a good performance as a surrogate parameter, the parameter does not only have to be susceptible to hypnotic and analgesic substances. Also the relative susceptibility to each of the two substance classes has to be similar to the influence of the substances on the mechanisms producing the non-responsiveness to painful stimuli. As an example, a parameter could respond in a dose-dependent manner to both propofol and opioids. But if the relative effect of the different substances on the parameter would be in a different relative proportion as they would affect the non-responsiveness to painful stimuli itself, no conclusions can be drawn from the parameter to the state of non-responsiveness.

In this study, we compared the performance of the RIII reflex threshold and the BIS as surrogate parameters of the non-responsiveness to painful stimuli by comparing their prediction probabilities for the responsiveness to painful electrical stimulation. The BIS was used in this study as a comparison since even though it is not designed as a surrogate of non-responsiveness to noxious stimuli, it can still be regarded as the standard monitoring device of anaesthetic depth and which has to be outperformed by any potential technique for monitoring immobility.

Methods

Subjects and setting

After approval of the local ethics committee (Berlin, Germany) and obtaining written informed consent, the study was performed in 20 healthy (ASA class I) male volunteers, ranging in age from 23 to 35 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 (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, Schiedlberg, Austria), digitized at a sampling rate of 5 kHz (Mikro 1401 mk II, CED Ltd, Cambridge, UK), rectified, and analysed using Signal 3.10 (CED Ltd).

In this study, 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. RI reflex occurrence was defined as an interval peak z score higher than 10.32 in the post-stimulation interval of 90–150 ms.

Testing procedure for reactions on verbal and noxious stimuli

During the whole course of the study, the reactions on 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, electrical tetanic stimulation in the area of the right ulnar nerve with 80 mA for 30 s. The different stimuli were applied immediately one after another, with a maximum of 5 s in between. Any verbal or movement reaction, regardless of purposeful or not, was considered as a positive response 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 face-mask for measuring end-tidal CO₂, surface electrodes for the BIS, and an i.v. access via a forearm vein were 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 through the face mask, or both.

Propofol and remifentanil were infused i.v. via computer-controlled infusion pumps, which were programmed with the weight- and age-corrected pharmacokinetic parameter set of Schnider and colleagues for propofol and with the body mass- and age-corrected pharmacokinetic parameter set of Minto for remifentanil. The dosing of the drugs followed two different drug administration protocols, which were each applied to 10 of the subjects.

In the first administration protocol (Fig. 1), the effect compartment concentration (Ce) of propofol was increased in steps of 1 µg ml⁻¹ every 15 min until the loss of consciousness, defined as the loss of reaction to verbal stimuli. Then propofol Ce was decreased by 1 µg ml⁻¹ and after 15 min, to allow for equilibration processes, the

Fig 1 Drug administration protocol 1: fixed propofol concentrations plus variable remifentanil concentrations. Shown are the effect compartment concentrations of propofol and remifentanil in an exemplary subject. The loss of consciousness is marked with LOC. Noxious stimuli were applied to the subject every 5 min starting from the loss of consciousness: plus sign represents a reaction and open circle the absence of a reaction to the noxious stimuli. For this drug administration protocol, the Ce of propofol was increased in steps of 1 µg ml⁻¹ every 15 min until the loss of consciousness, defined as the loss of reaction to verbal stimuli. Then propofol Ce was decreased by 1 µg ml⁻¹ and after 15 min to allow for equilibration processes, the additional administration of remifentanil was started. Remifentanil Ce was increased in steps of 0.5 ng ml⁻¹ every 10 min up to three steps above the loss of reactions to the noxious stimuli. After the maximum remifentanil concentration was maintained for 10 min, the administration of remifentanil was discontinued and after another 30 min, the administration of propofol was discontinued.

In the second drug administration protocol (Fig. 2), the Ce of propofol was increased in steps of 1 µg ml⁻¹ every 15 min until the loss of consciousness, defined as the loss of reaction to verbal stimuli. Then remifentanil Ce was increased in steps of 0.5 ng ml⁻¹ every 10 min until the loss of reaction to the noxious stimuli. After 10 min time to allow for equilibration processes, propofol Ce was decreased in steps of 1 µg ml⁻¹ every 15 min until reactions to the noxious stimuli reoccurred. After another 15 min for equilibration processes, remifentanil Ce was increased further in steps of 1 ng ml⁻¹ every 10 min until reactions to the noxious stimuli ceased again. Then the administration of propofol was discontinued first. After reoccurrence of the reactions to the noxious stimuli, the administration of remifentanil was discontinued as well.

Fig 2 Drug administration protocol 2: high propofol concentrations plus low remifentanil concentrations vs low propofol concentrations plus high remifentanil concentrations. Shown are the effect compartment concentrations of propofol and remifentanil in an exemplary subject. The loss of consciousness is marked with LOC. Noxious stimuli were applied to the subject every 5 min starting from the loss of consciousness: plus sign represents a reaction and open circle the absence of a reaction to the noxious stimuli. For this drug administration protocol, the Ce of propofol was increased in steps of 1 µg ml⁻¹ every 15 min until the loss of consciousness, defined as the loss of reaction to verbal stimuli. Then remifentanil Ce was increased in steps of 0.5 ng ml⁻¹ every 10 min until the loss of reactions to the noxious stimuli. After 10 min time to allow for equilibration processes, propofol Ce was decreased in steps of 1 µg ml⁻¹ every 15 min until reactions to the noxious stimuli reoccurred. After another 15 min for equilibration processes, remifentanil Ce was increased further in steps of 1 ng ml⁻¹ every 10 min until reactions to the noxious stimuli ceased again. Then the administration of propofol was discontinued first and after the reactions to the noxious stimuli reoccurred again, the administration of remifentanil was discontinued as well.
Data analysis and statistical analysis

Analysis was performed with RIII reflex threshold and BIS data points obtained after the loss of consciousness. For each sequence of reaction testing as described above, the last RIII reflex threshold value and the last BIS value 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 RIII reflex threshold with that of the BIS in distinguishing between reactions and the absence of reactions on the stimuli in each individual, the prediction probability, was calculated for each method for every individual subject. A value of 1 stands for a 100% correct differentiation between reactions and the absence of reactions, whereas a value of 0.5 represents only a 50:50 chance of a correct differentiation. The estimation of individual 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 K-values for the RIII threshold and the BIS were compared for each drug administration protocol using a Wilcoxon signed-rank 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 which 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 wake subjects and therefore to reduce the inconvenience of the procedure when the method would be used on patients.

To compare the overall performance of the RIII reflex threshold and the BIS in distinguishing between reactions and the absence of reactions on the noxious stimuli in all subjects, the population prediction probability P was calculated for the BIS, the normalized RIII reflex threshold, and remifentanil effect compartment concentrations. However, the statistic used here is based on the assumption of independent data, since no comparable statistical method has been developed that permits non-independent data. Therefore, we used the Pstatistic 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. As a result of this, standard errors may have been underestimated in our analysis. Statistical testing of the prediction probabilities was performed using the spreadsheet macro PKDMACRO as described by Smith and colleagues.

Results

No relevant changes in arterial pressure, heart rate, arterial oxygen saturation, or end-tidal CO₂ were observed throughout the study. The loss of reaction to repeated verbal commands for all 20 subjects occurred at a median effect compartment concentration of 4 μg ml⁻¹ (range: 2–5 μg ml⁻¹) propofol.

The median stimulus intensity that was applied through the automated threshold tracking system after the loss of consciousness of the subjects was 23.75 mA (min: 7.75 mA, max: 50 mA). One subject was excluded from further analysis because the RIII reflex threshold under influence of propofol alone already reached the maximum output of the stimulator (50 mA), after which the experimental session was discontinued.

The individual mean RIII reflex threshold values, BIS values, and probabilities of responses to the noxious stimuli at the different remifentanil effect compartment concentrations are shown in Figure 3.

The individual prediction probabilities for reactions and the absence of reactions to the noxious stimuli are shown in Table 1. While for the first drug administration protocol (fixed propofol concentrations plus variable remifentanil concentrations), no significant difference between the prediction probabilities of the RIII reflex threshold and the BIS could be observed (P>0.05, n=10, Wilcoxon signed-rank test), the prediction probabilities of the RIII reflex threshold and the BIS differed significantly (P<0.01, n=9, Wilcoxon signed-rank test) for the second drug administration protocol (high propofol concentrations plus low remifentanil concentrations vs low propofol concentrations plus high remifentanil concentrations).

For the comparison of the population prediction performance, the RIII reflex threshold values were normalized by subtraction of the first threshold that was estimated after the subject’s loss of consciousness. All recorded BIS- and normalized RIII reflex values of all subjects after the individual losses of consciousness for the first drug administration protocol are shown in Figure 4. For this drug administration protocol, the population prediction probability P for the combined data from all subjects amounted for the BIS to 0.84 (0.02) [estimate (se)], for the normalized RIII reflex threshold to 0.86 (0.02) [estimate (se)], and for remifentanil effect compartment concentration to 0.88 (0.02) [estimate (se)]. All P-values differed significantly from 0.5 (P<0.01, PKDMACRO), but the differences between the P-values were not statistically significant (P>0.05, PKDMACRO).

Normalization of the BIS did not improve the prediction probability P which amount to 0.81 (0.03). The prediction probability for non-normalized RIII reflex threshold values amounted to 0.70 (0.04).

All recorded BIS- and normalized RIII reflex values of all subjects after the individual losses of consciousness for the second drug administration protocol are shown in Figure 5. For this drug administration protocol, the population prediction probability P for the combined data from all subjects amounted for the BIS to 0.64 (0.05) [estimate (se)], for the normalized RIII reflex threshold to 0.77 (0.04) [estimate (se)], and for the remifentanil effect compartment concentration to 0.67 (0.04) [estimate (se)].
These $P_K$-values differed significantly from 0.5 ($P<0.01$, PKDMACRO). The $P_K$-value of propofol effect compartment concentration amounted to 0.52 (0.03) and did not differ significantly from 0.5 ($P>0.01$, PKDMACRO). The difference between the $P_K$-value of the normalized RIII reflex threshold and those of the BIS and the remifentanil effect compartment concentration was statistically significant ($P<0.05$, PKDMACRO). The difference between the $P_K$-values of the BIS and the remifentanil effect compartment concentration was not statistically significant ($P>0.05$, PKDMACRO).

Normalization of the BIS did not improve the prediction probability $P_K$ which amounted to 0.63 (0.05). The prediction probability for non-normalized RIII reflex threshold values amounted to 0.69 (0.04).

**Discussion**

As stated in the Introduction section, several prerequisites have to be fulfilled for a parameter to qualify as a surrogate parameter for the responsiveness and non-responsiveness to noxious stimuli. In general anaesthesia without the use of neuromuscular blocking agents, the non-responsiveness is usually a product of the combined use of hypnotic drugs such as propofol and analgesic drugs such as remifentanil. For both, propofol and remifentanil, a dose-dependent effect in suppressing the reactions to noxious stimuli can be assumed. Therefore, the first prerequisite for a possible surrogate would be a pronounced dose-dependent susceptibility to both drugs.

However, only the dose-dependency at that narrow concentration range at which the response to the noxious stimuli is actually lost is of importance for a good performance of a surrogate. A parameter could show a steep consistent relationship between concentration and effect, but this would not qualify the parameter as a surrogate as good as another parameter which showed no significant concentration–response except for an extremely steep raise at exactly that concentration at which the reaction to the stimulus is lost. Therefore, the concentration–response plots should not be overrated for judging the quality of a surrogate, since they could never focus that exactly on the specific concentration range which is of importance.

Another possibly misleading factor in the comparison of concentration–response plots is that no information is given on whether the surrogate actually responds correctly in the individual cases. For example, at the specific

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Protocol 2</th>
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<tbody>
<tr>
<td><strong>Subject</strong></td>
<td><strong>RIII reflex threshold</strong></td>
</tr>
<tr>
<td>A</td>
<td>0.98 (0.02)</td>
</tr>
<tr>
<td>B</td>
<td>0.92 (0.07)</td>
</tr>
<tr>
<td>C</td>
<td>0.88 (0.08)</td>
</tr>
<tr>
<td>D</td>
<td>1.00 (0.00)</td>
</tr>
<tr>
<td>E</td>
<td>0.92 (0.06)</td>
</tr>
<tr>
<td>F</td>
<td>0.86 (0.08)</td>
</tr>
<tr>
<td>G</td>
<td>0.74 (0.03)</td>
</tr>
<tr>
<td>H</td>
<td>0.97 (0.03)</td>
</tr>
<tr>
<td>I</td>
<td>0.72 (0.12)</td>
</tr>
<tr>
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<tr>
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<tr>
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<td>0.03</td>
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concentration at which a subject would respond to noxious stimuli at a probability of 50%, one can imagine a surrogate which also predicts a probability of response of 50%. Nevertheless, no information is yielded whether the surrogate predicts the particular responses correctly or not. A surrogate which predicts only half of the responses correctly and also gives a false positive prediction for half of the non-responses could still have a concentration–response function which is identical to one of a surrogate which predicts all responses and all non-responses correctly. Therefore, we prefer the $P_k$ statistic as a way of directly linking surrogate prediction and response without the detour over the concentration.

In this study, we have shown that in the first drug administration protocol, the prediction probability for reactions to noxious stimuli is for both parameters significantly better than a 50:50 chance. Since in this protocol, only the concentration of remifentanil was altered, a dose-dependent effect on both the RIII reflex threshold and the BIS can be assumed at those concentrations that divide between reactions and the absence of reactions. This fulfils the first prerequisite of remifentanil concentration-dependence for both parameters. Since there is no significant difference between the prediction probabilities of the two parameters, one can assume a similar quality of the susceptibility of the two parameters to remifentanil.
For propofol, we have shown a similar result in a previous study; therefore, the first prerequisite of propofol concentration-dependence is fulfilled for both RIII reflex threshold and the BIS as well.4

A second prerequisite for a possible surrogate for the responsiveness to noxious stimuli would be that the relative effect of different drugs on the surrogate parameter has to be similar as on the mechanisms producing the non-responsiveness to painful stimuli. Only by fulfilling this second prerequisite, it is ensured that different concentrations of drugs that create the same state of non-responsiveness to painful stimuli are reflected in the same value for the surrogate.

To test this second prerequisite, the second drug administration protocol has been set up, during which different combinations of propofol and remifentanil concentrations were administered to the subjects. Here both parameters, RIII reflex threshold and BIS, show a significant better prediction probability for reactions to noxious stimuli compared with a 50:50 chance. However, both the individual prediction probabilities and the population prediction probability of the RIII reflex threshold are significantly higher than that of the BIS. Therefore, one can assume that the relative influences of propofol and remifentanil on the RIII reflex threshold seem to be more similar to their relative influences on the physiological mechanisms producing the non-responsiveness to painful stimuli compared with those on the BIS.

The rather weaker performance of the BIS when propofol and remifentanil are used in different concentrations compared with its performance when only the concentration of one of the drugs is altered could be related to the negligence of EEG-derived indices towards the subcortical drug effects. The suppression of movement on noxious stimuli during general anaesthesia is mediated predominantly through mechanisms on the level of the spinal cord.22 Therefore, a different relative effect of the drugs on cortical and spinal mechanisms would corrupt the performance of a monitor based on their cortical effects. It has been demonstrated that the effect of propofol on the BIS is much stronger than that of opioids on the BIS.23 This implies that for propofol in comparison with remifentanil, the effects on cortical structures are relatively stronger than those on subcortical structures. The better performance of the RIII reflex threshold in the second drug administration protocol could therefore be related to its functional location in the spinal cord.

As a conclusion, the RIII reflex threshold and the BIS could be used as surrogates for the non-responsiveness to noxious stimuli under propofol and remifentanil. However, the RIII reflex threshold shows a considerably better performance in terms of prediction probability when different concentrations of propofol and remifentanil are used. Therefore, the RIII reflex threshold should be chosen over the BIS as a surrogate parameter for the responsiveness to noxious stimuli, when the setting allows for the normalization of the reflex.

To put the absolute prediction probability values calculated in this study into perspective when comparing with other studies, one has to keep in mind that here only those data points were included, that occurred after the loss of consciousness. The concentrations of the drugs were increased only slightly above the concentrations when the reactions to the noxious stimuli were lost. Therefore, the given prediction probabilities are the values for a more demanding task compared with investigations in which data from awake states and states of deeper anaesthesia have been compared.

However, the RIII reflex threshold also has several drawbacks compared with EEG-derived parameters as the BIS. First of all, the procedure of setting up the electrodes and apparatus to record the reflex is much more complex than simply applying frontal EEG electrodes and therefore takes more time (10–20 min) and experienced personnel. Also the necessity of normalizing the RIII reflex threshold for inter-individual comparability is a certain disadvantage, since the method therefore cannot be applied when anaesthesia has already been started. Owing to inter-individual anatomical and physiological differences influencing the electrical current necessary to elicit the RIII reflex, the use of RIII reflex threshold values without normalization does not seem practical and results in a far worse performance compared with normalized values.

A limitation of the present study is especially 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 should be expected in female subjects.

In summary, we have shown in this study that the RIII reflex threshold and the BIS show a comparable prediction probability for reactions to noxious stimuli under a constant concentration of propofol and varying concentrations of remifentanil. Under different combinations of high propofol concentrations with low remifentanil concentrations and vice versa in the same individuals, however, the prediction probability of the RIII reflex threshold for reactions to noxious stimuli is significantly higher than that of the BIS. Therefore, the RIII reflex threshold rather than the BIS could be used as a surrogate parameter to monitor the responsiveness to noxious stimuli during anaesthesia with propofol and remifentanil.

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