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Increased electroencephalographic gamma activity reveals awakening from isoflurane anaesthesia in rats

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Editor's key points

- Measuring the precise point of a wakening from anaesthesia may be crucial in animal studies.
- The use of EEG to indicate awakening from isoflurane anaesthesia was studied in rats.
- Change in gamma power parameters of EEG activity was at the moment of awakening.
- Monitoring anaesthetic state of rats is possible using EEG.
- This may be useful for testing new anaesthetic agents.

Background. Animal studies often require reliable measures for anaesthetic drug effects. Lately, EEG-based depth of anaesthesia estimation has been widely applied to rat models. This study investigated the reliability of different EEG spectral properties in revealing awakening from isoflurane anaesthesia in rats.

Methods. Adult Wistar rats with previously implanted frontal epidural electrodes were anaesthetized using isoflurane. The anaesthesia was slowly lightened until awakening, as observed by the first spontaneous movement, after which anaesthesia was induced again by increasing the isoflurane concentration. EEG was recorded during the recovery and induction periods, and the spectrograms and 23 quantitative spectral parameters used in the depth of anaesthesia estimation were calculated from the signals.

Results. The awakening was accompanied by a decrease in EEG activity at frequencies below 25 Hz, while the activity at higher frequencies (25–150 Hz) was increased. Whereas the behaviour of parameters used to measure activity in the lower frequencies was subject to variability between animals, the increase in higher frequency activity was more consistent, resulting in a statistically significant change in the relative gamma power parameters at the moment of awakening.

Conclusions. The increase in frontal relative gamma activity, especially in the 50–150 Hz frequency band, seems to be the most reliable EEG indicator for the awakening of a rat from isoflurane anaesthesia. A number of other spectral measures can also be used to detect this event. However, the role of gamma frequencies in the performance of these parameters is crucial.

Keywords: anaesthesia, depth; anaesthetics volatile, isoflurane; monitoring, depth of anaesthesia; monitoring, electroencephalography; rat

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EEG is useful when estimating anaesthetic drug effects in humans. Increasing concentrations of anaesthetics in the blood produce a continuum of EEG changes. For example, during induction of propofol anaesthesia, a characteristic frequency progression pattern is seen. This includes a shift in EEG activity from high frequencies to low frequencies and a simultaneous increase in signal amplitude.[1 2] Under deep anaesthesia, the burst suppression pattern (BSP) begins. During emergence, the EEG changes proceed in approximately reverse order compared with induction.[3 4] Potent inhaled GABAergic anaesthetics such as isoflurane have been shown to produce similar EEG changes.[5] Several quantitative parameters utilizing these signal characteristics have been proposed for the depth of anaesthesia estimation.

Animal studies also often require reliable measures for anaesthetic drug effects. These are needed, for example, in the studies of pharmacological properties of anaesthetic drugs in the early phase of drug development. The anaesthetic state of the animals is also of interest during clinical experiments. Lately, EEG-based depth of anaesthesia estimation in rats has been reported.[6–9] The focus has been on determining anaesthetic drug effects by applying square quantitative depth of anaesthesia parameters originally developed for humans to rat experiments. Generally, these parameters rely on spectral changes in EEG.[10]

This study investigated the reliability of different EEG spectral properties in revealing awakening from isoflurane anaesthesia in rats. In addition to the analysis of the signal spectrum, the behaviour of a number of quantitative spectral parameters measuring depth of anaesthesia was examined and an attempt to determine the most reliable parameters for identifying awakening is presented.

Methods

Animals and electrode placement

The experimental protocol used in this study was approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions. Ten adult male Wistar rats weighting between 312 and 348 g were included in the study. Before the experiment, three epidural screw electrodes (Plastics One, Roanoke, VA, USA) for EEG recording were implanted in the skull of each animal under general anaesthesia with local anaesthetic infiltration. Two of the electrodes were located 1.5 mm anterior to the bregma symmetrically over the left and right frontal lobes at a distance of 3 mm from each other. One electrode was implanted in the sagittal midline 1.5 mm posterior to the bregma to serve as a reference. Dental cement was used to cover the electrodes, lead wires, and the exposed portion of the skull. With this approach, the electrodes were not close to any muscles and thus not affected by EMG artifact. During the electrode placement, the animals were anaesthetized using 2% isoflurane mixed with 50% oxygen in nitrogen and the adequacy of the anaesthesia was assessed by observing the clinical signs of the animal. Isoflurane was supplemented with subcutaneous injections of lidocaine before skin incision to guarantee appropriate local anaesthesia.

Experimental protocol

The experimental protocol was performed approximately a week after electrode placement. Anaesthesia was induced in a chamber with 5% isoflurane mixed with 50% oxygen in nitrogen. The rat was then moved from the chamber to the table and the isoflurane concentration was decreased to 2% and delivery was continued using a nose cone. The wire for EEG recording was attached to the implanted electrodes and two needle electrodes were also attached to the rat's forepaws for ECG recording. Baseline recording was performed for 10 min with 2% isoflurane. After this, stepwise reduction in the isoflurane concentration was performed at 0.05% min^{-1} (recovery period). During the reduction, the beginning of withdrawal reflex was monitored by pinching the interdigital fold of the rat's right hind limb every 60 s, and spontaneous movements of the rat were noted. After the occurrence of the first spontaneous movement (moment of awakening), the isoflurane concentration was increased back to 2% for 20 min (induction period). The recording was then stopped, the nose cone and the wires were removed, and the animal was moved back to its cage.

During the experiment, EEG was recorded with the Tucker Davis Technologies (TDT, Alachua, FL, USA) System 3 data acquisition system using a sampling rate of 305.2 Hz and bipolar montages left frontal reference and right frontal reference. The signals were bandpass filtered between 1 and 150 Hz and the 60 Hz AC noise was removed using a second-order Butterworth filter.

Spectrograms

All the EEG signal processing presented from this point forward was performed with the Matlab® technical computing language (The MathWorks Inc., Natick, MA, USA).

Spectrograms were calculated from the EEG using a short-time Fourier transform with a moving 5 s Hamming window and 4 s overlap. The general spectral changes related to the moment of awakening were further examined by calculating the median of all spectrograms representing the signal segments around this event. The signal segments from 10 min before to 10 min after the first spontaneous movement were used. The median spectrogram was calculated by choosing the median value at every time–frequency point. To better visualize the relative activity in different frequency bands, the large difference between the amplitudes of high and low frequencies of the median spectrogram was reduced with amplitude normalization. In this procedure, the values in a specific frequency were divided by the mean value in that frequency calculated over the whole median spectrogram. The amplitude normalization was utilized only for visualization purposes, and it was not used when the quantitative spectral parameters were calculated.

Spectral parameters

A total of 23 quantitative spectral parameters generally used in depth of anaesthesia estimation were calculated from the spectrograms. These were: powers in different frequency bands (delta, theta, alpha, beta, gamma1, gamma2, and gamma3 powers), total spectral power, relative powers in different frequency bands (relative delta, theta, alpha, beta, gamma1, gamma2, and gamma3 powers), median power frequency (MPF), spectral edge frequency 90% (SEF90%) and 95% (SEF95%), peak power frequency, spectral entropy (SE) calculated using three different frequency bands (SE1, SE2, and SE3), and relative beta ratio (RBR).

Delta, theta, alpha, beta, gamma1, gamma2, gamma3, and total spectral powers were calculated using the frequency bands 1–4, 5–8, 9–12, 13–25, 26–50, 51–100, 101–150, and 1–150 Hz, respectively. Relative powers were determined by dividing the power in the corresponding frequency band by the total spectral power. The activities in different frequency bands represent classical parameters for depth of anaesthesia estimation and were therefore included in the study.[11 12] As the association between gamma activity and consciousness has been suggested recently,[13 14] the traditional bands of lower frequencies were supplemented with three different gamma bands in the analysis.

MPF, SEF90%, and SEF95% also represent classical parameters used in depth of anaesthesia estimation.[11 15] and have also been widely applied to animal experiments.[16 17] Spectral edge frequency *X*% is defined as the frequency below which *X*% of the power in the spectrum resides. MPF thus corresponds to spectral edge frequency 50%. The peak power frequency indicates the single frequency containing the highest power at that moment.

SE is one of the more recently proposed measures for the depth of anaesthesia assessment and has been utilized in the M-Entropy® module (GE Healthcare Finland Oy, Helsinki, Finland).[18] It can be considered to quantify the flatness of the power spectrum and is calculated as

$$\mathrm{SE} = \frac{-\sum_{i=f_l}^{f_h} P(i)\log P(i)}{\log N} \quad (1)$$

where f_l and f_h indicate the lower and higher limits of the used frequency band, respectively, P is the normalized power spectrum ($\sum_{i=f_l}^{f_h} P(i) = 1$) of the signal, and N is the number of frequency components in the range [f_l, f_h]. In this study, f_l and f_h were 1 and 32 Hz for SE1, 1 and 47 Hz for SE2, and 1 and 150 Hz for SE3. The frequency band limits of SE1 and SE2 were chosen according to the state and response entropy band limits used in the M-Entropy® module, respectively.

RBR is one of the subparameters used in the calculation of bispectral index.[11] It is defined as the logarithm of the ratio of the spectral powers in two different frequency bands:

$$\mathrm{RBR} = \log_{10}\frac{P_{30-47\mathrm{Hz}}}{P_{11-20\mathrm{Hz}}} \quad (2)$$

To extract the underlying trends of the spectral parameters during the experiment, the parameter values of each rat were smoothed in time with curve fitting. The values were not assumed to follow any specific pattern and thus a parametric fitting could not be used. Instead, the trends were extracted with non-parametric cubic spline smoothing.[19] The general behaviour of spectral parameters related to the moment of awakening were further investigated by calculating a median curve for each parameter.

Statistical analysis

The values of the 23 spectral parameters at three different points, that is, 2 min after the end of BSP (recovery period), 3 min before the first spontaneous movement (recovery period), and 2 min after the first spontaneous movement (induction period), were statistically compared with those at the moment of awakening, that is, first spontaneous movement. These three points were considered to represent characteristic signal properties related to recovery and induction periods and were also not affected by BSP. The parameter values at different points were not assumed to follow normal distribution and thus the comparison was performed with a non-parametric Mann–Whitney U-test. P-values of <0.05 were considered statistically significant. Owing to the multiple comparisons, the Bonferroni correction was included in the statistical analysis to reduce the probability of type I errors. Statistical analysis was carried out with The Statistics Toolbox for Matlab®.

Results

In two rats, poor quality EEGs necessitated their exclusion from analysis. In addition, since spectral changes in the two bipolar montages were almost identical, only the results of left frontal reference are reported here.

In Figure 1 an example of EEG is given. The figure presents the whole signal recorded from one rat during the entire experiment, four short signal segments picked from different phases of the recording, and the spectrogram of the whole signal. The corresponding isoflurane concentration administered is also shown. Figure 1 also shows the occurrence of different events during the experiment: the ending of BSP during the recovery period, the beginning of the pedal withdrawal reflex, the first spontaneous movement, and the beginning of BSP during the induction period. The times of these events for all 10 rats are given in Table 1. In the beginning of the recovery period, BSP was observed in all but one rat. As in Figure 1, a clear change in the EEG, that is, a sudden decrease in amplitude and increase in frequency, preceded the first spontaneous movement. Again, in one rat, such a switch-like change was not observable.

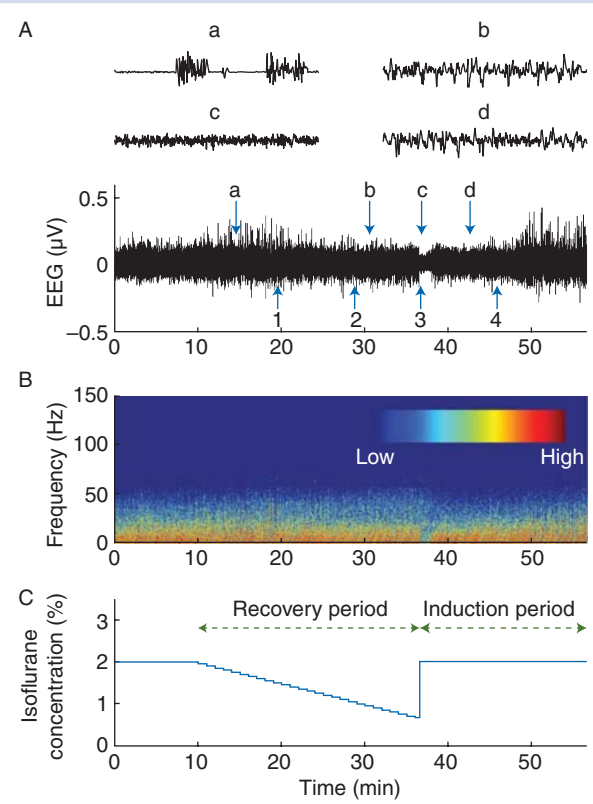

Fig 1 (A) An example of an EEG (Rat 3) recorded during the experiment. The numbers 1, 2, 3, and 4 below the recording indicate the beginning of the withdrawal reflex, ending of BSP, first spontaneous movement, and beginning of BSP, respectively. In addition to the whole signal, four 6 s signal segments picked from different phases of the recording are given. (B) The spectrogram of the signal. (C) Isoflurane concentration administered during the experiment. Time 0 indicates the beginning of baseline recording.

Table 1 Times of different events during the experiment. *Owing to the poor quality of the signal, the events detected from the EEG were not observable for Rats 1 and 5. **No clear switch-like change in the EEG preceded the first spontaneous movement of Rat 6. ***For Rat 7, BSP did not occur during the recovery period

Event	Time (min)										
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8	Rat 9	Rat 10	Mean
Burst suppression pattern ends	*	25.9	28.7	20.1	*	17.9	***	18.6	21.1	17.7	21.4
Withdrawal reflex begins	32.5	26.5	19.5	20.5	26.5	21.5	31.5	29.5	25.5	26.5	26.0
Change in EEG	*	39.6	36.5	27.4	*	**	40.2	35.9	40.5	33.5	36.2
First spontaneous movement	42.3	39.9	36.6	28.2	30.7	35.6	40.6	36.2	41.2	33.6	36.5
Burst suppression pattern begins	*	43.4	45.8	33.2	*	38.1	44.3	39.4	53.8	45.4	42.9

Spectrograms

Figure 2 illustrates the median spectrogram and its amplitude normalized version. A clear decrease in the activity below 25 Hz can be related to the moment of awakening. Simultaneously, activity in higher frequencies (>25 Hz) substantially increases. These changes are both seen slightly before awakening. In addition, some increase in the 40–60 Hz activity preceding this was seen ~4 min before the first spontaneous movement.

Spectral parameters

In Figure 3, the smoothing procedure and calculation of the median curve is illustrated for gamma2 spectral parameter. The median curves of all 23 different spectral parameters are shown in Figure 4. In Figure 5, the parameter values at four different points, i.e. 2 min after the end of BSP, 3 min before the moment of awakening, the moment of awakening, and 2 min after the moment of awakening, are given. Figure 5 and Table 2 present the results of the statistical comparison between the parameter values at the moment of awakening and the other three points.

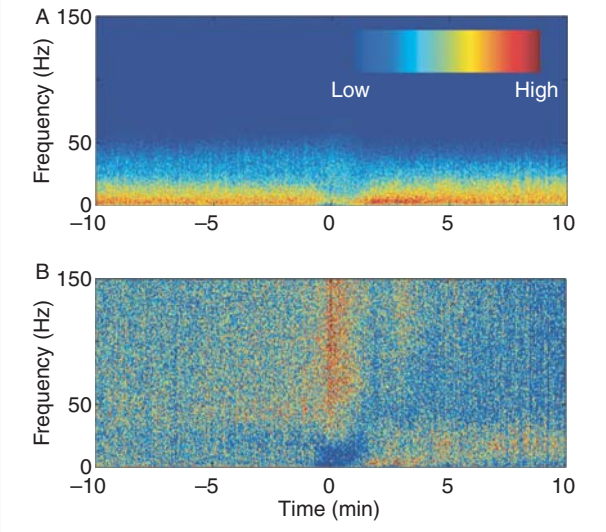

Fig 2 (A) The median spectrogram. (B) The amplitude normalized median spectrogram. Time 0 indicates the moment of awakening, that is, the first spontaneous movement.

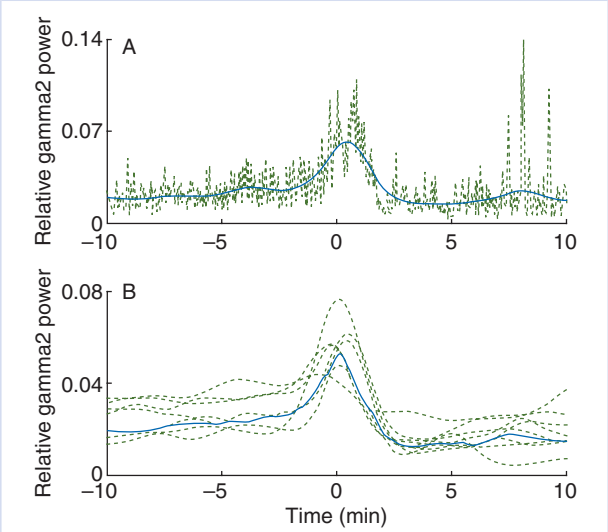

Fig 3 (A) An example of the smoothing procedure. The underlying trend (solid line) of the spectral parameter (dashed line) is extracted with a non-parametric cubic spline smoothing. In this case, the procedure is illustrated with the relative gamma2 power parameter. (B) The median curve (solid line) determined from the smoothed spectral parameters of all subjects (dashed lines). Time 0 indicates the moment of awakening, that is, the first spontaneous movement.

The spectral changes seen in the median spectrogram also affect the behaviour of spectral parameters. The suppression of activity in frequencies below 25 Hz result in a decrease in the curves of delta, theta, alpha, beta, and total spectral powers related to the moment of awakening. However, due to the large variation, the values of these parameters do not differ statistically significantly at the moment of awakening compared with those at the three other points. Furthermore, the relative versions of the first four parameters are unable to reliably reveal the first spontaneous movement. The increase in high frequency activity preceding the moment of awakening yields a peak in the gamma power curves. This is emphasized in the relative versions of the parameters resulting in statistical significance in all three

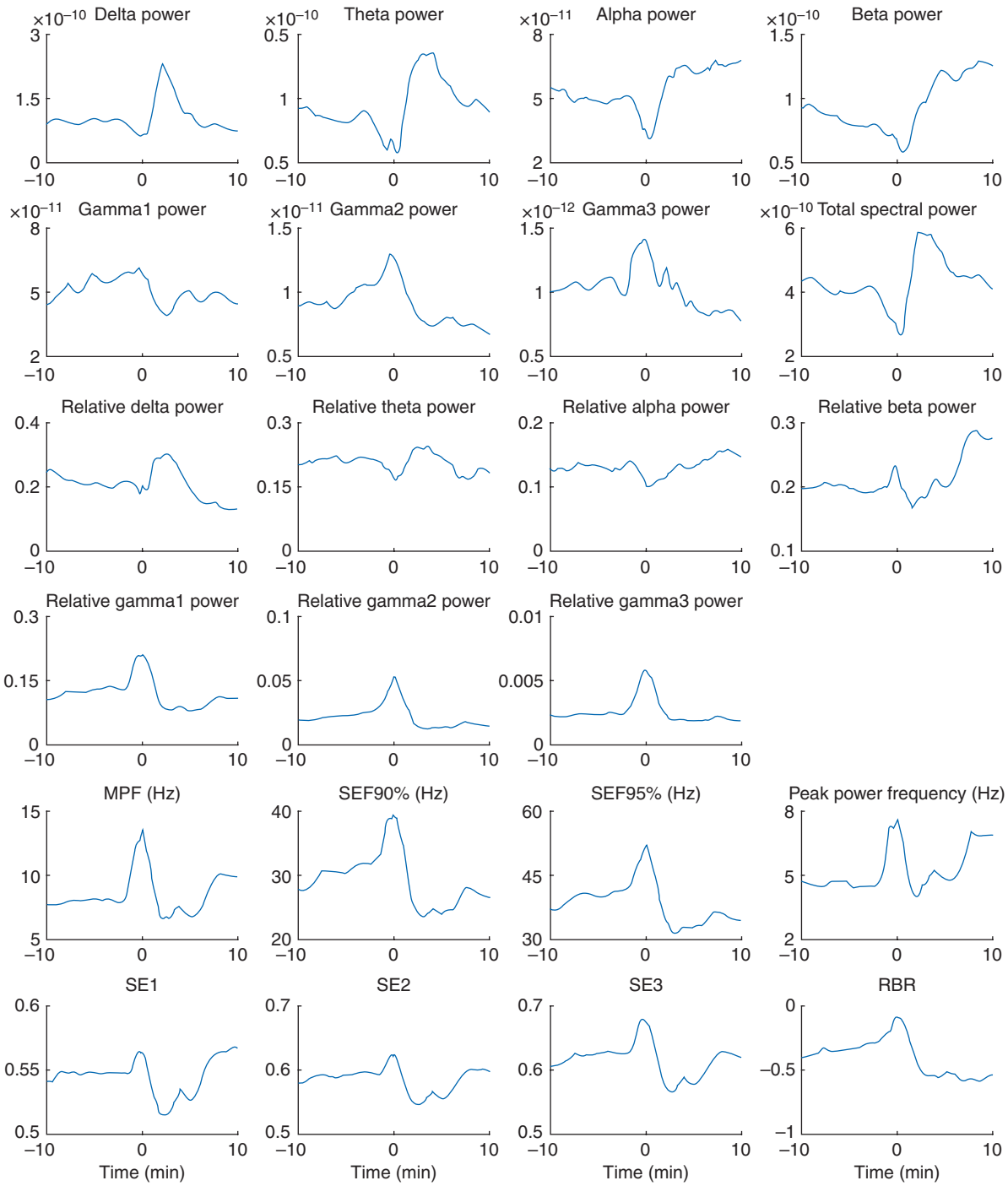

Fig 4 The median curves of all 23 different spectral parameters. Time 0 indicates the moment of awakening, that is, the first spontaneous movement.

comparisons of both gamma2 and gamma3 values. In two comparisons, the statistical significance is also reached with gamma1. However, due to the increase of 40–60 Hz activity preceding the awakening, the values were not considered to differ statistically significantly in one of the comparisons. The spectral changes related to the moment of awakening influence also MPF, SEF90%, and SEF95% parameters leading to statistical significance in all comparisons with these parameters. The peak power frequency, on the other hand, is not able to discriminate between different levels of anaesthesia reliably. SE is also unable to detect the changes in the anaesthetic state reliably when the narrow-frequency bands are used in the calculation of the parameter (SE1 and SE2). However, including the higher frequencies

Fig 5 The parameter values at four different points. A, B, C, and D correspond to 2 min after the end of BSP, 3 min before the moment of awakening, the moment of awakening, and 2 min after the moment of awakening, respectively. The statistically significant difference between the values and the ones at the moment of awakening (C) is indicated (+) above the box plots. The five horizontal lines in each box plot show the medial, intra-quartile, and full range.

significantly improves the performance of this measure (SE3). Similarly, RBR is able to discriminate different levels of anaesthesia. Like gamma1, SE3 and RBR both seem to be affected by the 40–60 Hz activity preceding the awakening and statistical significance is thus not reached in one of the comparisons.

Discussion

In this study, the reliability of different EEG spectral properties in revealing awakening from isoflurane anaesthesia was investigated in rats. In addition to the analysis of signal spectra, the behaviour of 23 different quantitative

Table 2 Statistical analysis. Data are *P*-values resulting from the statistical comparison between the values of the 23 spectral parameters at three different points and those at the moment of awakening. A, B, and D correspond to 2 min after the end of BSP, 3 min before the moment of awakening, and 2 min after the moment of awakening, respectively. *Statistically significant after the Bonferroni correction

Parameter	A	B	D
Delta power	0.9591	0.8785	0.2786
Theta power	0.2786	0.2786	0.1049
Alpha power	0.1949	0.1949	0.1605
Beta power	0.3823	0.3282	0.3823
Gamma1 power	0.0499	0.8785	0.1949
Gamma2 power	0.0047	0.0207	0.0070
Gamma3 power	0.0650	0.0148	0.1304
Total spectral power	0.6454	0.4418	0.1605
Relative delta power	0.8785	0.2345	0.0104
Relative theta power	0.0011	0.0104	0.0011
Relative alpha power	0.0104	0.0104	0.5737
Relative beta power	0.4418	0.8785	0.0499
Relative gamma1 power	0.0002*	0.0011	0.0003*
Relative gamma2 power	0.0002*	0.0002*	0.0002*
Relative gamma3 power	0.0003*	0.0006*	0.0002*
MPF	0.0002*	0.0003*	0.0006*
SEF90%	0.0002*	0.0006*	0.0002*
SEF95%	0.0002*	0.0003*	0.0002*
Peak power frequency	0.0148	0.0207	0.0104
SE1	0.1049	0.3282	0.0207
SE2	0.0070	0.2345	0.0070
SE3	0.0003*	0.0070	0.0003*
RBR	0.0002*	0.0030	0.0002*

spectral parameters used in the depth of anaesthesia estimation was illustrated. The increase in the relative gamma activity, especially in the 50–100 and 100–150 Hz frequency bands, was found to be the best indicator for the moment of awakening. This event was also detected by the MPF, SEF90%, SEF95%, RBR, and SE parameters. However, the role of the gamma frequencies in the performance of these parameters is crucial.

Our results illustrate the significant role of EEG gamma activity related to anaesthesia in rats. The issue has been actively addressed in previous research, but results have been conflicting. Some studies suggest that gamma activity is preserved or even enhanced during anaesthesia,[13 20] while others report that activity is suppressed.[14 21] Recently, Hudetz and colleagues[14] showed that the suppression of high-frequency gamma activity (70–140 Hz) in the frontal and visual cortex and also in the hippocampus during isoflurane anaesthesia correlates with the loss of righting reflex in rats. In contrast, activity in the 30–50 Hz frequency band was not decreased during anaesthesia.[14] The current results support their findings. We also found the gamma activity above 50 Hz to be the most reliable indicator for detecting if the rat is awake or not. However, in our study, the 25–50 Hz gamma activity was also decreased during isoflurane administration. The difference can be explained by the fact that the maximum isoflurane concentration used by Hudetz and colleagues[14] was 1.2%, while in our study, it was up to 2%. We showed activity in the 40–60 Hz frequency range during light anaesthesia which was suppressed during deeper anaesthesia. This lower gamma band can therefore potentially be used to discriminate between different levels of anaesthesia while activity in the higher band correlates with awakening.

We have previously reported EEG spectral changes and their relation to clinical signs during anaesthesia in humans.[2 22 23] We have also shown the behaviour of different quantitative spectral parameters.[24] Fundamental changes in the signal characteristics, that is, the change from low-amplitude high-frequency signal to high-amplitude low-frequency signal, are similar in humans and rats. However, the smooth frequency progression pattern is not apparent in rats. Instead, the transition between different levels of anaesthesia, especially the awakening in the end of the recovery period, was rapid, representing a switch-like behaviour. Furthermore, the increase in beta activity during light anaesthesia (also known as paradoxical excitation)[25] was not observed in the present study.

Even though not included in our previous studies, EEG gamma activity has also been investigated in humans during anaesthesia and its suppression has been connected to the loss of consciousness.[26 27] The measurement of gamma activity from the scalp has, however, several problems. The gamma waves have small amplitudes and are further attenuated by the skull and other tissues between the electrode and cortex. In addition, the signal is often contaminated by the EMG artifact. These measurement challenges have made it difficult to draw definite conclusions about the relationship between gamma activity and the depth of anaesthesia.

Our study had some limitations, which indicate directions for future research. First, the number of rats studied was rather small. Although ethical issues dictate sample size, confirmation of the results would require a larger study. Furthermore, the study does not define which of the spectral parameters is the most reliable for detecting the moment of awakening. Secondly, in order to assess the EEG phenomenon as a function of continuously changing anaesthetic concentration during recovery, the isoflurane concentration every minute and thus equilibrium could not be guaranteed. As such, it was not possible to analyse isoflurane. In addition, the induction had to be fast to prevent the rat struggling which would have disturbed the EEG recording. Ideally, to be able to compare the recovery and induction periods, the anaesthesia protocols should be identical but reversed. To achieve this, recording in an anaesthesia chamber would be required. The electrode locations may also have affected the results. For example, the proximity of the reference electrode in the sagittal midline may have had a greater impact on low-frequency activity than on high-frequency activity.

In conclusion, this study illustrates the significant role of frontal gamma activity related to anaesthetic drug effects in rats. By detecting the increase in relative power in frequencies above 50 Hz, the awakening of the rat can reliably be determined. Furthermore, the suppression of lower frequency gamma activity refers to the transition from light anaesthesia to deep anaesthesia. The findings could potentially be used to monitor the anaesthetic state of rats for experimental purposes and may offer an objective measure for comparing drug effects of new anaesthetic agents.

Declaration of interest

None declared.

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