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Time-varying spectral analysis revealing differential effects of sevoflurane anaesthesia: non-rhythmic-to-rhythmic ratio

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Background: Heart rate variability (HRV) may reflect various physiological dynamics. In particular, variation of R-R peak interval (RRI) of electrocardiography appears regularly oscillatory in deeper levels of anaesthesia and less regular in lighter levels of anaesthesia. We proposed a new index, non-rhythmic-to-rhythmic ratio (NRR), to quantify this feature and investigated its potential to estimate depth of anaesthesia.

Methods: Thirty-one female patients were enrolled in this prospective study. The oscillatory pattern transition of RRI was visualised by the time-varying power spectrum and quantified by NRR. The prediction of anaesthetic events, including skin incision, first reaction of motor movement during emergence period, loss of consciousness (LOC) and return of consciousness (ROC) by NRR were evaluated by serial prediction probability (P_K) analysis; the ability to predict the decrease of effect-site sevoflurane concentration was also evaluated. The results were compared with Bispectral Index (BIS).

Results: NRR well-predicted first reaction ($P_K > 0.90$) 30 s ahead, earlier than BIS and significantly better than HRV indices. NRR well-correlated with sevoflurane concentration, although its correlation was inferior to BIS, while HRV indices had no such correlation. BIS indicated LOC and ROC best.

Conclusions: Our findings suggest that NRR provides complementary information to BIS regarding the differential effects of anaesthetics on the brain, especially the subcortical motor activity.

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Anaesthesia comprises several components. Different modalities reveal different aspects of the complicated, differential effects of anaesthetics, including hypnosis, amnesia, anti-nociception, immobility and autonomic nerve system control.[1–3] Bispectral Index (BIS), for example, allows us monitoring the level of hypnosis, and we rely on heart rate to assess analgesia, which is mainly mediated at the subcortical level.[4–8] To better understand anaesthesia, we study the clinical finding that deeper anaesthetic levels are associated with the appearance of more regular oscillatory patterns in the R-to-R peak interval (RRI) time series of electrocardiography (ECG).[9,10] We call this regular oscillatory pattern *rhythmicity*. To our knowledge, this peculiar rhythmicity feature has not been quantified or studied before.

The rhythmicity in the RRI emerges during anaesthesia and gradually disappears during recovery.[9,10] These suggest that the RRI during anaesthesia consists of two components: one more rhythmic, less affected by anaesthesia, and one more non-rhythmic, suppressed by deep anaesthesia, and the strength of these two components vary according to the anaesthetic depth.

We hypothesised that the quantification of rhythmic–non-rhythmic pattern can reflect the anaesthetic effects during routine clinical anaesthesia. A new index, referred to as non-rhythmic-to-rhythmic ratio (NRR), was introduced to quantify the relative strength of the rhythmic component and non-rhythmic component in the RRI. The aim of this study was to investigate how NRR changes during

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sevoflurane anaesthesia and whether it better indicates anaesthetic events, in particular the first reaction of motor movement.

Methods

To investigate the relationship between the RRI rhythmicity feature and anaesthetic effects, this study was designed as a prospective, clinical and observational study spanning the whole period of typical surgical anaesthesia. ECG (1000 Hz), BIS index and the end-tidal concentration of sevoflurane were collected for subsequent comparative analysis. Important anaesthetic events, including loss of consciousness (LOC), skin incision, first reaction of motor movement during emergence period and return of consciousness (ROC), were registered.

The protocol was approved on 10/15/2007 by the Institutional Review Board, Taipei Veterans General Hospital, Taipei, Taiwan, 201 Shih-pai Road, Sec.2, protocol number 96-08-12A. We enrolled 31 female patients with American Society of Anesthesiologists physical status (ASA) of I or II scheduled for laparoscopic gynecological surgery under general anaesthesia. Written informed consent was obtained from each patient. It is well known that many diseases affect heart rate variability (HRV), and we would like to investigate it under a normal physiological condition, so exclusion criteria were designed to exclude cardiovascular disease, arrhythmia, regular medication affecting the neurological or cardiovascular systems, presumed cancer, diabetes mellitus, anticipated difficult airway, aged younger than 20 or older than 55 years, and a body mass index of more than 30 or below 19. All surgeries were performed between 8:00 and 19:00 h.

Anaesthesia protocol

Standard anaesthetic monitoring (HP Agilent patient monitor system), including ECG, pulse oximeter (SpO_2) and non-invasive blood pressure, were applied to all patients. A BIS electrode (BIS-XP sensor) and the ECG recorder (MyECG E3–80; Micro-Star Int'l Co., New Taipei City, Taiwan) were applied to patients before anaesthetic induction. The BIS sensor was applied on the right side of the forehead after the skin was cleaned with alcohol. As our usual practice, all the anaesthetic managements, including the administration of medications, and airway management were under the discretion of the anaesthesiologist to whom the information of BIS monitoring is available also. After intravenous infusion of lactated Ringer's solution was started, we started anaesthetic induction with fentanyl 2.5–3 μg/kg; after pre-oxygenation, hypnosis was induced with propofol 2–2.5 mg/kg. LOC was assessed by no response to verbal command. Cisatracurium 0.15 mg/kg was used to facilitate tracheal intubation. Subsequently, mechanical ventilation was started from volume control mode with oxygen-gas-sevoflurane mixture as low flow anaesthesia. The respiratory rate and tidal volume of the ventilator was adjusted to maintain an end-tidal carbon oxide ($ETCO_2$) between 35 and 40 mmHg, and to keep the peak airway pressure lower than 25 mmHg. Laparoscopic skin incision was made by a blade. The adequacy of anaesthetic depth (during the whole period of surgery) was determined by BIS index (< 60) and the anaesthesiologist's judgement. Bolus dose of fentanyl was given if inadequate analgesia was determined. Near the end of the surgery, the peritoneal gas was deflated, and the body position of the patient was recovered from Trandelenburg position to level-supine position. As the wound closure began, the anaesthetic gas was gradually decreased. Muscle relaxation was reversed with a combination of neostigmine 0.05 mg/kg and glycopyrrolate 0.01 mg/kg during controlled ventilation. When the patient regained adequate spontaneous breathing (SB), controlled ventilation was stopped. Patients showing inadequate SB ($ETCO_2$ more than 50 mmHg or SpO_2 less than 95%) were assisted with manual positive ventilation; otherwise, SB was allowed until regaining consciousness without the interference of positive-pressure ventilation. Because ECG and BIS are the main electrophysiological signals we analyse, during the observation of the emergence period, physical contact to the patient was avoided and minimised if necessary to prevent the interference on the sensors of ECG and BIS monitor. The unnecessary interference to patient's own respiration from manual (bag) ventilation was avoided also. Based on our clinical judgement, the endotracheal tube was removed when the patient showed adequate SB. Corrective actions including mask ventilation or nasopharyngeal airway insertion were given in cases of inadequate ventilation or upper airway obstruction after extubation. During the emergence period, first reaction was carefully assessed by the anaesthesiologist (Y.T.) as any first visible motor reaction such as movement of the arms or legs, coughing, or grimace. ROC was assessed by opening of eyes and ability to follow simple commands.

Data acquisition

The BIS index was continuously recorded from an Aspect A-2000 BIS monitor (version XP, Host Rev:3.21, smoothing window 15 s; Aspect Medical Systems, Nattick, CA, USA) connected to a laptop computer (Asus Corp., Taipei, Taiwan). During surgery, corrective measures were taken to improve signal quality of the BIS sensor when the signal quality index was lower than 50; otherwise, the BIS data during this period were discarded. The raw limb lead ECG sampled at 1000 Hz and 12-bit resolution, was recorded for offline analysis. The clocks in the laptop and ECG recorder were synchronised with a time accuracy of ± 1 s. Time stamps of BIS record were provided by the laptop. The inhaled and end-tidal concentrations of anaesthetic gas, which was sampled from the connection piece close to the endotracheal tube and detected by the gas analyser on a Datex-Ohmeda S/5 anaesthesia machine (GE Health Care, Helsinki, Finland), were also recorded on the laptop. All data records were uninterrupted until the ROC was determined. We registered the precise timestamps of events, including LOC, skin incision, first reaction and ROC. All pieces of data collected for analysis of anaesthetic concentration and anaesthetic events, including skin incision, first reaction and ROC, are under single ventilation mode. In other words, there is no transition from the controlled ventilation to SB or vice versa in these data. For LOC, however, the transition from the SB to positive pressure ventilation is necessary.

Data analysis

The offline ECG data were analysed in several steps. The R peak detection was automatically determined by taking lead I, II and III ECG signals into account to have a better accuracy. The ectopic beats were removed and interpolated. Results were visually verified to eliminate incorrect R peaks and ectopic beats. Whenever electrocauterisation severely interfered the ECG, this data segment was discarded. RRI was derived from the R peaks by the cubic spline interpolation. RRI were resampled to be equally spaced at 4 Hz for subsequent analyses. Please see the Supporting information Appendix S1 for details. In order to compensate the hysteresis between the end-tidal sevoflurane concentration and the anaesthetic effect on the brain, the estimated effect-site sevoflurane concentration (C_{eff}) was derived from the end-tidal sevoflurane concentration (C_{et}) by the following first-order differential equation, which is based on the pharmacokinetic-pharmacodynamic modelling:[11]

$$\frac{dc_{eff}}{dt} = K_{e0}(C_{et} - C_{eff}) \tag{1}$$

The constant K_{e0} was assumed to be constant for all patients and defined as 0.20/min according to previous studies.[5]

NRR

We call the amount of variance inside the RRI signal HRV, which has been shown closely related to the autonomic activity.[12] The main tool to quantify HRV is the power spectrum (PS).[12] As useful as the PS is, however, it is well known that the PS cannot capture the momentary dynamics in the RRI time series. This issue limits its application to human under highly dynamic situation – in particular, the rhythmic-to-non-rhythmic pattern in surgery and anaesthesia. The main technical focus of this paper is to resolve this limitation.

To quantify the rhythmic-to-non-rhythmic pattern in the RRI time series, in this paper, we introduce a novel index referred to as NRR. NRR is motivated by the clinical finding that deeper anaesthetic levels are associated with the appearance of more regular oscillatory patterns in the RRI time series. Thus, under deeper anaesthesia, the RRI time series oscillates more regularly. We call such regular oscillatory RRI time series 'rhythmic', which appears as a sharp peak on the PS. On the other hand, when the subject is under lighter anaesthesia, the RRI time series varies irregularly, and we call such RRI time series 'non-rhythmic'. The non-rhythmic component exhibits an irregular, random-like behaviour that appears as a plateau on the PS. As a result, the RRI time series is composed of one 'non-rhythmic component' and one 'rhythmic component' with varying ratio. Based on the earlier facts, we hypothesise that the time-varying change of the NRR components may reflect the anaesthetic effect on the brain.

To quantify the NRR components, we need a new signal-processing tool to capture the momentary behaviour of the RRI time series. We replace the PS with the time-varying PS (tvPS) by applying the recently developed time-frequency analysis technique,[10,13] referred to as multitaper synchrosqueezing transform.[14–16] The tvPS is a non-parametric generalisation of the PS, which provides the PS information at each time instant. As a result, we can trace the momentary strength of the rhythmic component and non-rhythmic component by analysing the tvPS.

With the tvPS available, we extend the definition of the classical frequency domain HRV parameters.

Recall that traditionally, the high-frequency power (HF), the low-frequency power (LF) and the LF-to-HF ratio (LHR) are determined from the PS of the RRI time series. It is known that vagal activity mainly contributes to HF power, while sympathetic activity influences LF power and the LHR.[12,17] Using the tvPS of RRI, we can obtain time-varying HRV parameters, namely, the time-varying HF (tvHF), the time-varying LF (tvLF) and the time-varying LHR (tvLHR), which are more suitable for the dynamical situation of anaesthesia.

The main index NRR is defined as the ratio of the momentary non-rhythmic power to the momentary rhythmic power. Mathematically speaking, we compute the power of rhythmic component by identifying the location of peak on tvPS of the RRI time series. Then, the non-rhythmic power is defined as the tvHF subtracted by the momentary rhythmic power. Finally, NRR is defined as:

$$\mathrm{NRR} = \log_{10}\left(\frac{\text{Non-rhythmic power}}{\text{Rhythmic power}}\right) \tag{2}$$

where the value of NRR is high when RRI is non-rhythmic and low when RRI is rhythmic.

We refer readers to the Supporting information for the rigorous mathematical meaning of the rhythmicity, the tvPS and its relationship with the PS, and the computation of NRR, tvHF, tvLF and tvLHR.

Statistical analysis

We took BIS and ECG-derived continuous indices, including NRR, tvHF, tvLF, tvLHR and HR, as anaesthetic depth indices. The performances of the indices were evaluated in two aspects: their ability to predict anaesthetic events and their correlations with effect-site sevoflurane concentration during emergence period. Because the concentration of anaesthetic gas was decreasing and the influence from surgery was relative minor, we consider the emergence period for correlation analysis. A P value less than 0.05 was considered as statistically significant. Multiple significant tests were calculated with Bonferroni correction. Statistical results are expressed as mean (standard deviation).

Prediction probability (P_K) analysis is a versatile statistic method measuring the performance of an anaesthetic depth index.[18] A value of one means that the indicator always correctly predicts the observed depth of anaesthesia, a value of 0.5 means that the indicator predicts no better than 50/50 chance. The ability to predict anaesthetic events was investigated by serial P_K analysis.[18] The correlation with sevoflurane concentration employed P_K analysis and Spearman rank correlation. Estimation of P_K and its standard error was obtained by the Jackknife method. Before the null hypothesis, if the averaged P_K value is less than 0.5, all P_K values were converted into one minus the P_K value.[18]

Serial P_K analysis in predicting anaesthetic events

To evaluate the performance of the anaesthetic indices in predicting the anaesthetic events, serial P_K analysis was used as discussed later. We define the following timestamps as the base time: 1 min before LOC, 5 s before skin incision, 3 min before the first reaction and 3 min before ROC. The serial P_K analysis is performed as successive P_K analyses of data pairs: index on baseline timestamp vs. indices on subsequent successive timestamps spaced by 5 s. As the result, the output of serial P_K analysis is a sequence of time-varying P_K values revealing the temporal relation between the anaesthetic events and the indices. By plotting the serial P_K value and its standard error bar, we can do hypothesis test simply with the naked eye. The significant difference ($P < 0.05$) between indices can be established if 1.5 times of their standard error bars do not overlap.[18,19]

Correlations with sevoflurane concentration

Correlation between the previous indices and sevoflurane concentration was investigated in the emergence period. We chose the time interval when the sevoflurane concentration monotonically and continuously decreased. The period of SB is from the start of adequate spontaneous breath to ROC. We employed the P_K analysis and Spearman rank correlation to analyse performance of indices sampled every 4 s. The results were tabulated as weighted averages according to the data length of each patient. The bootstrap method was used to calculate the 95% confidence interval of Spearman correlation based on 10,000 samplings. A value of Spearman correlation closer to −1 is better because we expect that the indices will increase to correlate with the decrease of sevoflurane concentration.

Result

Patient demographics

Thirty-one women receiving laparoscopic surgery were enrolled in the study (age, 42 ± 7 years; weight, 53 ± 6 kg; height, 158 ± 4 cm; duration of anaesthesia, 169 ± 59 min). The surgeries included 12

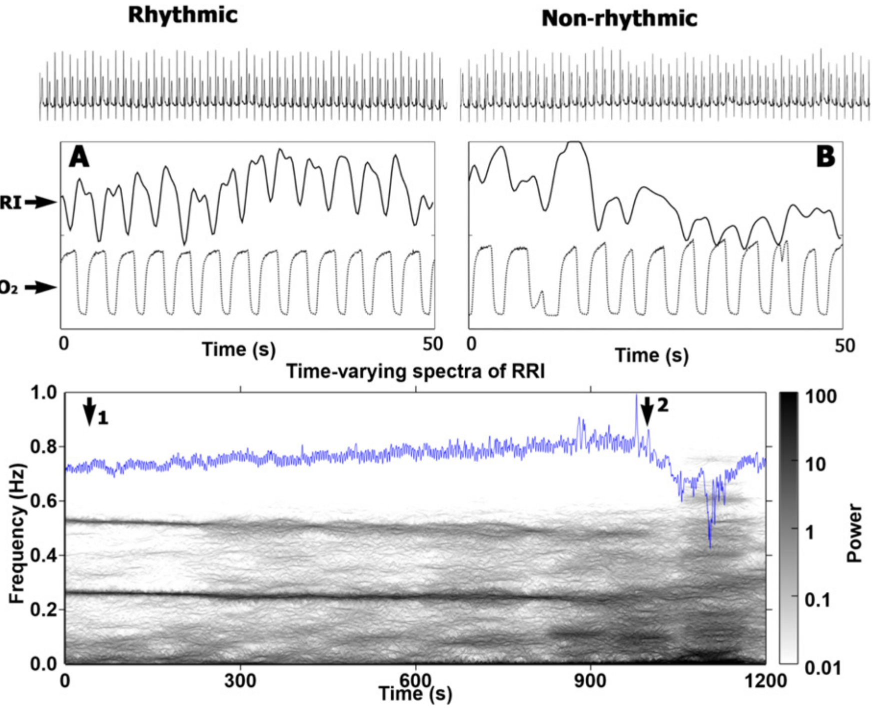

Fig. 1. Diagram demonstrating the typical rhythmic vs. non-rhythmic pattern in electrocardiography (ECG), respiratory waveform (CO_2 concentration), R-R interval (RRI) recorded during the spontaneous breathing period. (A,B) The RRI time series is shown in the solid curve, and the respiratory waveform is shown in the dotted curve. The coupling effect between the respiratory signal and the RRI time series can be seen visually in panel A, while the coupling effect is less apparent in panel B. In the bottom panel, the time-varying power spectra (tvPS) is shown with the RRI time series superimposed as the blue curve. Visually, the tvPS reveals the transition of the RRI time series from the rhythmic pattern to non-rhythmic pattern, which cannot be read directly from ECG. Arrow 1 indicates time of panel A; Arrow 2 indicates time of panel B.

laparoscopic cystectomy, 10 laparoscopic hysterectomy, 8 laparoscopic myomectomy and 1 for ectopic pregnancy. The myomectomy surgeries in general spent more time. Blood loss was less than 300 ml in all surgeries. BIS indices were lower than 60 in all cases during skin incision. No patient experienced inadequate ventilation or unsuccessful extubation after the mechanical ventilator was switched off; none of the patients needed supraglottic airway devices or positive pressure bag ventilation after extubation. Meanwhile, there was no temporal relationship between the first reaction and endotracheal tube extubation. (The period between the first reaction and extubation is -163 ± 275 s; max: 762 s; min: -590 s) None of the patients received bolus dose of fentanyl 30 min before the emergence period.

Observations on the time-varying power spectra

Abundant information can be read visually from the tvPS of RRI. It is demonstrated (Figs 1 and 2) that the tvPS is more concentrated on deeper anaesthetic levels and more scattered on lighter anaesthetic levels. In particular, during SB (Figs 1 and 2), there are dominant curves in the first part (left half part) of the tvPS, while no dominant curve can be identified in the second part (right half part). The dominant curve represents the typical rhythmicity feature. Also, observe that the tvPS becomes non-rhythmic before the appearance of first reaction (Fig. 2).

The earlier visual findings are quantified by NRR and HRV indices, such as tvHF, tvLF and tvLHR for statistical analysis.

Ability to predict anaesthetic events

In the serial P_K analysis for the first reaction (Fig. 3A), NRR is ahead of BIS ($P < 0.05$ for 30 s). NRR well-predicted the first reaction 30 s in advance ($P_K > 0.9$). At the instance of first reaction (0 s), both NRR and BIS were significantly better than other parameters. These time-varying HRV indices and HR ($P_K < 0.81$) were significantly worse than NRR.

In the serial P_K analysis for skin incision (Fig. 3B), tvLF reaches maximum ($P_K > 0.94$) 30 s after skin incision. It reflects skin incision best, followed by tvHF ($P_K < 0.85$). tvLF is significantly better than BIS and NRR ($P_K < 0.65$).

In the serial P_K analysis for LOC (Fig. 3C), BIS reflects perfectly ($P_K = 1$) 50 s after LOC. LOC is also associated with decrease in tvLF, tvHF and HR. NRR does not reflects LOC well. In the serial P_K analysis for ROC (Fig. 3D), BIS is the best, and surpassed NRR, HR and the time-varying HRV indices significantly.

Representative values of the indices during anaesthetic events are tabulated in Table 1. Because of the inadequate data quality, we removed one patient from the serial P_K analysis of first reaction. When

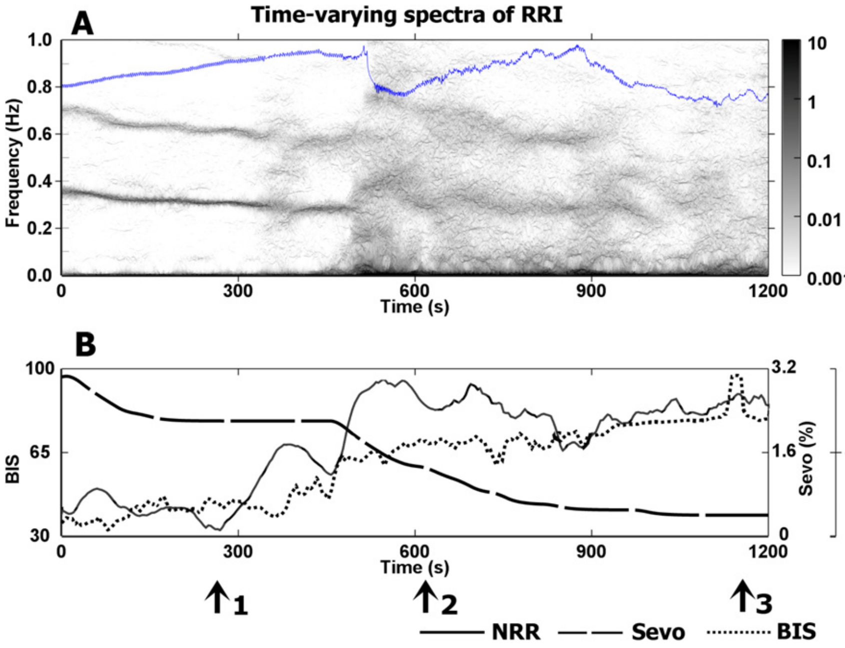

Fig. 2. A representative data obtained from a patient breathing spontaneously during emergence period. Panel A shows the 1200-s R-R interval (RRI) superimposed as a blue curve on its time-varying power spectra (tvPS). Panel B shows simultaneous recorded Bispectral Index (BIS), effect-site sevoflurane concentration (Sevo) and non-rhythmic-to-rhythmic ratio (NRR). Arrow 1: endotracheal suction and extubation; Arrow 2: first reaction; Arrow 3: regain of consciousness. The rhythmic component and its harmonics are time-varying in the tvPS of RRI. With the overall decrease of sevoflurane, the tvPS of the RRI time series exhibited a rhythmic-to-non-rhythmic transition. Note that the tvPS of the RRI time series became non-rhythmic before the first reaction.

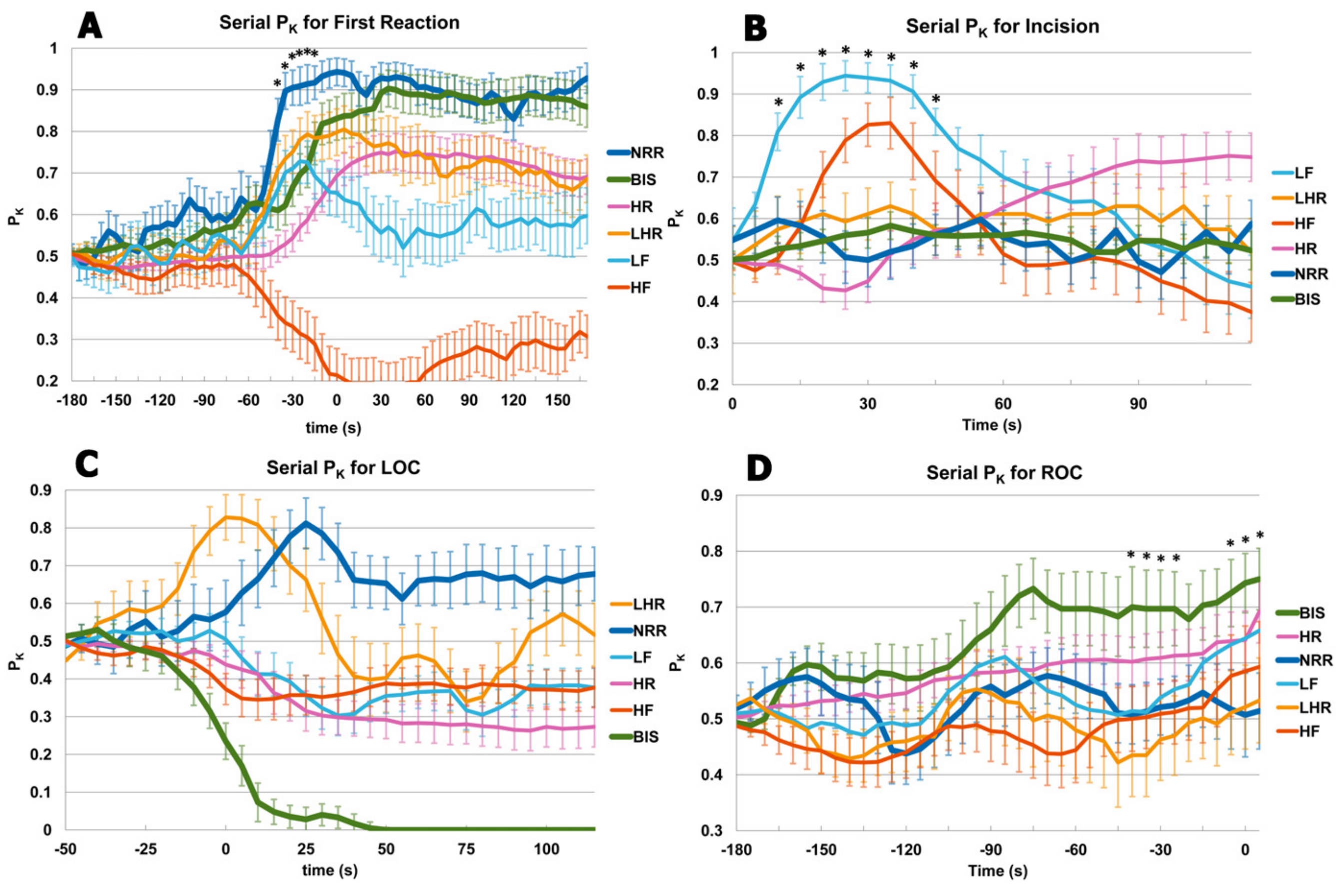

Fig. 3. Tracings and their faded error bars demonstrate the prediction probability (P_K) values of the indices and their standard errors in serial P_K analysis for first reaction (A), incision (B), loss of consciousness (LOC) (C) and return of consciousness (ROC) (D). The baseline is 3 min before first reaction, 5 s before skin incision, 1 min before LOC and 3 min before ROC. NRR, non-rhythmic-to-rhythmic ratio; HF, high-frequency power; LF, low-frequency power; LHR, LF-to-HF ratio; BIS, Bispectral Index; HR, heart rate. *P < 0.05, NRR vs. BIS (A); LF vs. BIS (B); BIS vs. NRR (D).*

Table 1

Representative values of the indices during important anaesthetic events.

	LOC		Skin incision		First reaction		ROC	
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
BIS	97 (93, 98)	54 (42, 68)	38 (34, 42)	41 (36, 46)	60 (57,65)	74 (69,81)	79 (77, 83)	86 (81, 94)
NRR	0.49 (0.27, 0.74)	0.70 (1.04, 0.54)	0.72 (0.50, 0.96)	0.76 (0.57, 0.87)	0.12 (−0.11, 0.46)	1.14 (0.88, 1.47)	0.71 (0.49, 1.05)	0.70 (0.49, 1.06)
HF	2.34 (1.76, 2.70)	1.44 (1.26, 1.65)	1.03 (0.70, 1.40)	1.21 (0.94, 1.56)	1.48 (1.11, 1.92)	1.24 (0.83, 1.42)	1.22 (0.95, 1.44)	1.19 (0.91, 1.63)
LF	1.43 (1.09, 1.93)	0.53 (0.15, 1.05)	0.45 (0.06, 0.74)	1.58 (1.09, 1.81)	−0.24 (−0.93, 0.08)	0.53 (−0.22, 0.93)	0.63 (−0.32, 1.19)	0.56 (0.33, 1.17)
LHR	−0.82 (−1.07, −0.47)	−0.77 (−1.03, −0.56)	−0.46 (−0.93, −0.27)	0.17 (−0.30, 0.53)	−1.80 (−2.27, −1.08)	−0.47 (−1.05, −0.14)	−0.40 (−1.36, −0.14)	−0.47 (−0.69, 0.13)
HR	74 (64, 91)	64 (57, 72)	64 (58, 72)	69 (59, 76)	70 (61, 80)	81 (67, 87)	75 (67, 81)	84 (77, 94)

Values are presented as median values (lower and upper quartiles). tvHF, tvLF and tvLHR are expressed in common logarithm (10 base) of millisecond square. Heart rate (HR) is expressed in beat per minute.

BIS, Bispectral Index; NRR, non-rhythmic-to-rhythmic ratio; HF, high-frequency power; LF, low-frequency power; tvHF, time-varying HF; tvLF, time-varying LF; tvLHR, tvLF-to-tvHF ratio; LOC, loss of consciousness; ROC, return of consciousness; T_0, 60 s before LOC; T_1, 60 s after LOC; T_2, 5 s before incision; T_3, 30 s after incision; T_4, 180 s before first reaction; T_5, 5 s before first reaction; T_6, 180 s before ROC; T_7, 5 s after ROC.

Table 2

Prediction probability (P_K) and Spearman rank correlation for indices vs. estimated sevoflurane effect-site concentration in spontaneous breathing.

Spontaneous breathing		
	P_K (SE)	Spearman rank correlation (95% CI)
BIS index	0.839 (0.014)*	−0.836 (−0.881, −0.778)*
NRR	0.736 (0.019)	−0.619 (−0.688, −0.536)
tvHF	0.489 (0.025)*	0.001 (−0.117, 0.113)*
tvLF	0.669 (0.022)*	−0.416 (−0.506, −0.317)*
tvLHR	0.666 (0.022)*	−0.420 (−0.514, −0.319)*
HR	0.532 (0.022)*	0.027 (−0.075, 0.133)*

*$P < 0.05$ between non-rhythmic-to-rhythmic ratio (NRR) and other indices.

BIS, Bispectral Index; tvHF, time-varying high-frequency power; tvLF, time-varying low-frequency power; tvLHR, time-varying LF-to-HF ratio; HR, heart rate; SE, standard error; CI, confidence interval.

analysing the serial P_K of skin incision, LOC and ROC, we removed three, five and four patients, respectively.

Correlation with sevoflurane concentration

The correlation with the estimated effect-site sevoflurane concentration is evaluated for the SB period (Table 2). The data number of SB is 7155 (28,620 s), and average duration per patient was 923 ± 384 s. The rankings of indices are in general consistent between P_K analysis and Spearman rank correlation (R). While BIS correlates with effect-site sevoflurane concentration best (SB: $P_K = 0.839$, $R = -0.836$, $P < 0.0001$), NRR is second ($P_K = 0.736$, $R = -0.619$, $P < 0.0001$). tvLF is the best HRV indices ($P_K = 0.669$, $R = -0.416$, $P < 0.0001$). Compared with tvLF, NRR is significantly better ($P < 0.01$). BIS surpasses NRR significantly in SB ($P < 0.001$). NRR is significantly better than HR ($P < 0.0001$).

Discussion

The main findings of this prospective study are twofold. First, NRR well-predicts first reaction during the emergence period. Second, NRR correlates with sevoflurane concentration during SB. However, NRR is not a good predictor for LOC and ROC. These results jointly suggest that momentary information hidden inside RRI and revealed by the tvPS should possess a distinct physiological interpretation of the anaesthetic depth compared with the hypnosis measured by surface electroencephalography (EEG). There is an additional finding that tvLF correlates well with skin incision.

We start from understanding the genuineness of NRR. First, NRR generally increased before the onset of first reaction, so it is unlikely that the motion artefacts interfere NRR. Second, there is no transition from the mechanical ventilation to SB or vice versa in these pieces of data we analysed, except for LOC. In particular, there is no positive pressure ventilation for all the first reaction data. Thus, the observed NRR performance in not confounded by the ventilation mode transition. Furthermore, although not quantitatively analysed in the existing literature, the association between non-rhythmic-to-rhythmic transition of the oscillatory pattern in RRI and level of anaesthesia has been qualitatively described in the past. In fact, by using the classical power spectral analysis, Kato et al. described that the high-frequency component of RRI spreads during the awake period, whereas it concentrates during anaesthesia and controlled ventilation.[9] Based on the earlier facts, the genuineness of different, novel information provided by NRR is convincing.

The existing findings

Our results of BIS (Fig. 3C, Table 2) are in agreement with previous studies showing that BIS can monitor awake status vs. anaesthesia, the first reaction and the decreasing concentration of anaesthetic gas.[2,5,7] Our BIS results also agree with the reported studies that BIS or other EEG-derived indices cannot well-indicate the response to noxious stimulation.[3,4,6]

Our analysis results are also consistent with previous reported association between anaesthetic depth and the traditional HRV parameters: HRV is decreased during general anaesthesia and is increased during recovery.[9,13,20,21] Our results are also consistent with the reported finding that combining the classical HRV indices with BIS does not outperform BIS in discriminating awake from asleep during anaesthetic induction.[22]

It was reported that the classical HRV parameters do not outperform HR in predicting noxious stimulation.[1,23] Contrarily, our results demonstrate that tvLF predicts skin incision better than HR. It seems that the noxious stimuli of the skin incision elicited a momentary increase of sympathetic activity, which leads to this finding. Although the study is not designed for noxious stimulation, the finding that tvLF correlates with skin incision shows the technical advantage of our approach and its potential in noxious stimulation study. We mention that Huang et al. was the first group employing time-frequency analysis to study HRV during anaesthetic induction.[13]

Physiological interpretation of NRR

The association between NRR and anaesthetic depth may be partially explained by the cardiopulmonary coupling[24] as well as the respiration. When the coupling effect is strong, the respiration pattern is faithfully reflected in the RRI. Thus, the respiratory mechanism also contributes to the differential influence of anaesthetic agents. It is known that neural respiratory control comprises the involuntary automatic control system mediated by the respiratory centre in the pontomedullary areas and the voluntary control system in the forebrain.[25] The anatomies of these two systems are distinct. Respiratory signals from these two systems compete with each other and are integrated at the spinal level to control the respiratory motor neuron. During spontaneous respiration, it is known that the involuntary automatic respiratory pattern generated in the pontomedullary centre,[25] or more specifically the pre-Bötzinger complex,[26–28] is rhythmic. On the contrary, the breathing pattern of the voluntary respiratory motor control is non-rhythmic, which involves cortical processing and thalamic integration in response to peripheral and descending inputs.[25,29–31] Specifically, the thalamus actively participates in many motor functions, including respiration,[30,31] speech[32] and cough.[33] Hence, our NRR findings suggest the following possibility – the non-rhythmic respiratory activity involving widespread brain regions is more susceptible to sevoflurane than the rhythmic respiration generated in the medulla. This suggestion is supported by the following studies. In addition to the classical observation on respiratory pattern by Guedel,[34] Bimer et al. showed that the respiratory irregularities accompany electroencephalographic arousal reaction in human breathing spontaneously.[35] Also, it is shown that the respiratory activity of pre-Bötzinger complex is less depressed by sevoflurane,[36,37] and the literature indicating that the effect of anaesthetic gas is less prominent in the brainstem than in areas with more synaptic transmission, such as the cortex.[38]

The ability of NRR to predict first reaction suggests a connection between the rhythmicity feature of the RRI and motor reaction. Antognini et al. demonstrated that immobility to surgical stimulation by anaesthetic gas in goats is mainly modulated by the spinal cord, which also indirectly affects the thalamic response to noxious stimulation.[39] Velly et al. recorded the human subcortical electrophysiological activity and demonstrated that subcortical activity predicts suppression of movement to noxious stimuli, but not changes of consciousness, whereas cortical EEG predicts LOC but not motor suppression.[4] Based on the earlier discussion, NRR might reflect the subcortical activity as it better reveals information about motor reaction, but not consciousness, compared with BIS. Because immobility to noxious stimulation is similar to, although not the same, the first reaction under surgical wound pain in the present study, this relationship suggests the role of the thalamus in NRR. Besides NRR, in our serial P_K analysis, BIS also responded to first reaction, which is in agreement with other studies.[7] Therefore, it is possible that a cortical mechanism is also related to the first reaction.

Accordingly, sevoflurane might affect non-rhythmic respiration at the level of the spinal cord, subcortical supraspinal regions or cortex. Although we cannot pin down the exact anatomic location, the subcortical supraspinal areas, probably the thalamus, is most plausible because of the NRR's differential results in the serial P_K analysis and its close connection with respiration. Thus, we hypothesise that the rhythmicity of the RRI signal exhibits the central respiratory activity via central or peripheral mechanisms,[24] and it reflects the integration of rhythmic and non-rhythmic respiratory activities. Although more evidence is needed to clarify this mechanism, we propose that the methodology of NRR quantification is a potential tool to evaluate the depth of anaesthesia from a different aspect compared with EEG-based monitoring.

Strength, limitation and future

The first strength of this study is a new quantitative approach, referred to NRR, to analyse a particular RRI pattern – the rhythmicity feature. We showed the potential of NRR to reflect levels of anaesthesia and anaesthetic events, and the underlying physiological mechanism is partially supported by the literature. Second, the signal processing technique, synchrosqueezing transform, has been well studied in the literature, so we have adequate theoretical support for our index. In particular, the limitation of PS is resolved, and the momentary behaviour of the RRI can be captured by the tvPS, which leads to the NRR index.

The limitations of this study need to be mentioned. First, the patients enrolled were mainly ASA class I or II females, and only sevoflurane-based anaesthesia was studied. Further studies taking different patient groups and different regimen of anaesthesia into account are necessary to better understand the relationship between NRR and anaesthesia. Second, although the findings of the current study suggest some directions as being discussed earlier, more evidences, for example experiments based on animal model, are needed to draw the conclusion. Third, normal sinus rhythm is necessary for the derivation of NRR as ectopic beats might lower the signal quality of NRR. Fourth, the influences of other physiological factors,[40] for example the respiration and the cardiopulmonary coupling, on NRR deserve further investigation. Fifth, how the anaesthetics and extubation process influence the respiration and cardiopulmonary coupling need to be addressed, and the study is undergoing. Furthermore, although we did not design the study for noxious stimulation, from the data analysis result, we found that the indices we apply are potential in detecting pain reaction under anaesthesia. In particular, tvLF index correlates with the noxious stimulation better than heart rate as an additional finding. A further study is needed to survey this finding, as well as comparing the performance of tvLF with the well-known indices Surgical Stress Index® (GE Healthcare) and Analgesia Nociception Index® (Metrodoloris, Lille, France) that are useful in monitoring surgical stress.[8]

In conclusion, this prospective study showed that we may extract hidden information regarding the anaesthetic level from the routine ECG monitor. In particular, our quantitative study of the NRR index supports our initial hypothesis, that is, the rhythmic-to-non-rhythmic transition correlates with motor reaction during emergence in advance and earlier than BIS, and correlates with sevoflurane concentration. In addition, the potential of the time-varying HRV indices can be expected from the observed relationship between tvLF index and the noxious stimulation. The notion of dynamics rooted in the recently developed signal processing technique; without tvPS, the earlier quantification cannot be easily achieved with enough theoretical rigor. Overall, our study suggests that the ECG signal could contain complementary information to the EEG-based depth-of-anaesthesia index BIS, and further study is warranted.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. R-peak to R-peak interval (RRI) signal.

Appendix S2. From power spectrum to time-varying power spectrum.

Appendix S3. Frequency domain parameters of HRV.

Appendix S4. Quantification of nonrhythmic to rhythmic ratio.