Anaesthesia 2017, 72, 570–579 doi:10.1111/anae.13843

Original Article

Sevoflurane-induced isoelectric EEG and burst suppression: differential and antagonistic effect of added nitrous oxide

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Summary

The objective of this study was to investigate whether nitrous oxide influenced the ED50 of sevoflurane for induction of isoelectric electroencephalogram ($ED50_{isoelectric}$) differently from its influence on the ED50 of sevoflurane for electroencephalogram burst suppression ($ED50_{burst}$). In a prospective, randomised, double-blind, parallel group, up–down sequential allocation study, 77 ASA physical status 1 and 2 patients received sevoflurane induction and, after tracheal intubation, were randomly allocated to receive sevoflurane with either 40% oxygen in air (control group) or 60% nitrous oxide in oxygen mixture (nitrous group). The $ED50_{isoelectric}$ in the two groups was determined using Dixon's up and down method, starting at 2.5% with 0.2% step size of end-tidal sevoflurane. The electroencephalogram was considered as isoelectric when a burst suppression ratio of 100% lasted > 1 min. The subsequent concentrations of sevoflurane administered were determined by the presence or absence of isoelectric electroencephalogram in the previous patient in the same group. The $ED50_{isoelectric}$ in the nitrous group 4.08 (95%CI, 3.95–4.38)% was significantly higher than that in the control group 3.68 (95%CI, 3.50–3.78)% ($p < 0.0001$). The values for $ED50_{burst}$ were 3.05 (95%CI, 2.66–3.90)% and 3.02 (95%CI, 3.00–3.05)% in nitrous group and control group, respectively ($p = 0.52$). The addition of 60% nitrous oxide increases $ED50_{isoelectric}$, but not the $ED50_{burst}$ of sevoflurane. Neither result indicates an additive effect of anaesthetic agents, as might be expected, and possible reasons for this are discussed.

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Accepted: 26 January 2017
Keywords: anaesthesia mechanisms; depth of anaesthesia; nitrous oxide; sevoflurane
This article is accompanied by an editorial by Pandit, Anaesthesia 2017; 72: 555–60.

Introduction

General anaesthesia produces distinct patterns on the electroencephalogram (EEG), the most common of which is a progressive increase in low-frequency, high-amplitude activity. As the administered concentration of general anaesthetic is increased, the brain reaches a burst suppression state, in which high-voltage brain electrical activities (bursts) intermittently alternate with very low voltage, suppressed EEG (burst suppression). The duration of these suppressed periods are prolonged as anaesthesia deepens further, finally progressing to an isoelectric EEG. Using burst suppression or isoelectric EEG as endpoints might be useful to compare anaesthetic potencies on cerebral function [1–3].

Volatile anaesthetics and nitrous oxide (N_2O) appear to act at different molecular targets and neural

circuits to produce distinct brain states that are visible in the EEG. For example, adding N_2O to halothane anaesthesia resulted in a reduction in higher frequencies and increase in lower frequencies of EEG power, whereas adding N_2O to isoflurane anaesthesia resulted in a general reduction of spectral power in all frequency bands [4]. Nitrous oxide activated the EEG during burst suppression in isoflurane anaesthesia [5]. Nitrous oxide is well reported to have an additive effect on volatile anaesthetics for preventing movement and haemodynamic response to surgical incision [6, 7], but this may not translate to an additive suppression of brain electrical activity [5].

In contrast to these reports of N_2O–isoflurane interaction, the effect of N_2O on sevoflurane-induced isoelectric and burst suppression EEG patterns has not been studied in humans and we wished to fill this apparent gap in the literature. We hypothesised that, in accordance with its additive effect on preventing movement, N_2O would also exhibit an additive effect on EEG parameters such as isoelectric EEG and burst suppression.

Methods

We obtained approval from the institutional review board of Huazhong University of Science and Technology.

Between October 2012 and October 2013, adult patients from 45 to 65 years, ASA 1 or 2, and scheduled for abdominal surgery, were evaluated for eligibility. Exclusion criteria included: any neurological disease; medication with neurological impact; cardiac ejection fraction < 40%; difficult airway; alcohol or drug addiction; obesity; and inability to consent.

Thirty minutes before anaesthesia, all patients received phenobarbital 0.1 g and atropine 0.5 mg by intramuscular injection as premedication, according to our local standard. EEG monitoring was established before induction. According to the manufacturer's instruction, before induction, frontal electrodes of Narcotrend Monitor (MonitorTechnik, Bad Bramstedt, Germany, Version 4.0) and Entropy Module (S/5 Compact Anaesthesia Monitor, Datex-Ohmeda, Helsinki, Finland) were placed on the forehead for EEG recording and isoelectric EEG recognition, respectively. Electrode impedances were set to ensure adequate contact defined as 6 kΩ or less for the Narcotrend and 7.5 kΩ or less for the Entropy Module as per manufacturer's instructions. Heart rate, invasive arterial pressure, S_pO_2, partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) and end-tidal fractions of oxygen, sevoflurane and N_2O (F_EO_2, FE_{Sevo}, F_EN_2O) were monitored and recorded every minute by a research assistant (YF), who was responsible for anaesthesia induction and intra-operative care but was not involved in EEG outcome assessment.

Inhalational anaesthesia was induced with 8% sevoflurane in 100% oxygen. Cisatracurium 0.15 $mg.kg^{-1}$ was administered after loss of movement to command and of the eyelash reflex, and tracheal intubation performed with loss of the train-of-four measured by nerve stimulator. Mechanical ventilation was used to maintain normocapnia (mean (SD) $PETCO_2$ 4.7 (0.7) kPa) with 2 $l.min^{-1}$ fresh gas flow in a circle system. A continuous invasive arterial pressure monitor was used; an audible alarm was set to indicate > 20% reduction in mean arterial pressure (MAP) from baseline values. Phenylephrine 0.05–0.1 mg was administered intravenously and repeated if necessary to maintain MAP to within this limit.

We used a sequential allocation design running two groups in parallel. Subjects were blinded to group allocation. To determine the isoelectric EEG ED50 ($ED50_{isoelectric}$) of sevoflurane, patients were randomly allocated to one of two groups receiving sevoflurane with either 40% oxygen (control group) or 60% N_2O in oxygen mixture (N_2O group) by a computer-generated sequence of numbers contained in sealed envelopes. The intra-operative caregivers (BZ and JL) were not blinded to the allocation and were also not involved in any other data collection and outcome assessment. Operating room noise was kept at a minimum to prevent auditory stimulation. The FE_{Sevo} of the first enrolled patient was 2.5% in each group. The presence or absence of isoelectric EEG of the previous subject determined the FE_{Sevo} of the following subject within the same group according to Dixon's up-and-down method. Briefly, the FE_{Sevo} was kept constant for 30 min before surgical incision to allow equilibration between arterial and brain tensions, the FE_{Sevo} for the next patient was decreased by 0.2% if isoelectric EEG was achieved with this concentration. Otherwise, the

FE_{Sevo} for the next patient was increased by 0.2% (Fig. 1). At the end of the data collection period, for the remainder of surgery, anaesthesia maintenance was left to the discretion of the anaesthetist in charge.

Decisions on how to proceed with protocol violations were made by an independent investigator (JX), who was blinded to the EEG outcome but not blinded to allocation. The study design required recruitment to continue until at least eight 'turning points' were achieved in both groups, judged by the study chairs (WM and YT). 'Turning points' were defined as the points of successive assigned doses at which the observed response changed direction based on Wetherill's methods, representing the peaks and troughs of the response series [8]. Data were analysed by a blinded investigator (FC).

Two independent investigators (BN and JX), blinded to the patients assignment, assessed the presence of burst suppression and isoelectric EEG. To calculate burst suppression ratio (BSR), suppression was defined as periods > 0.5 s during which EEG voltage was < 5 mV [9]. The time spent in a suppressed state was measured and burst suppression ratio was reported as the fraction (proportion) of the epoch during which the EEG was suppressed. The burst suppression ratio was averaged over at least 15 epochs (i.e. > 60 s) [10]. A burst suppression ratio of 100% indicates isoelectric EEG. If an isoelectric EEG was not attained, the maximal burst suppression ratio was recorded.

Sample size determination using Dixon's up and down method is relatively speculative. According to a previous study, at least six crossovers are needed for

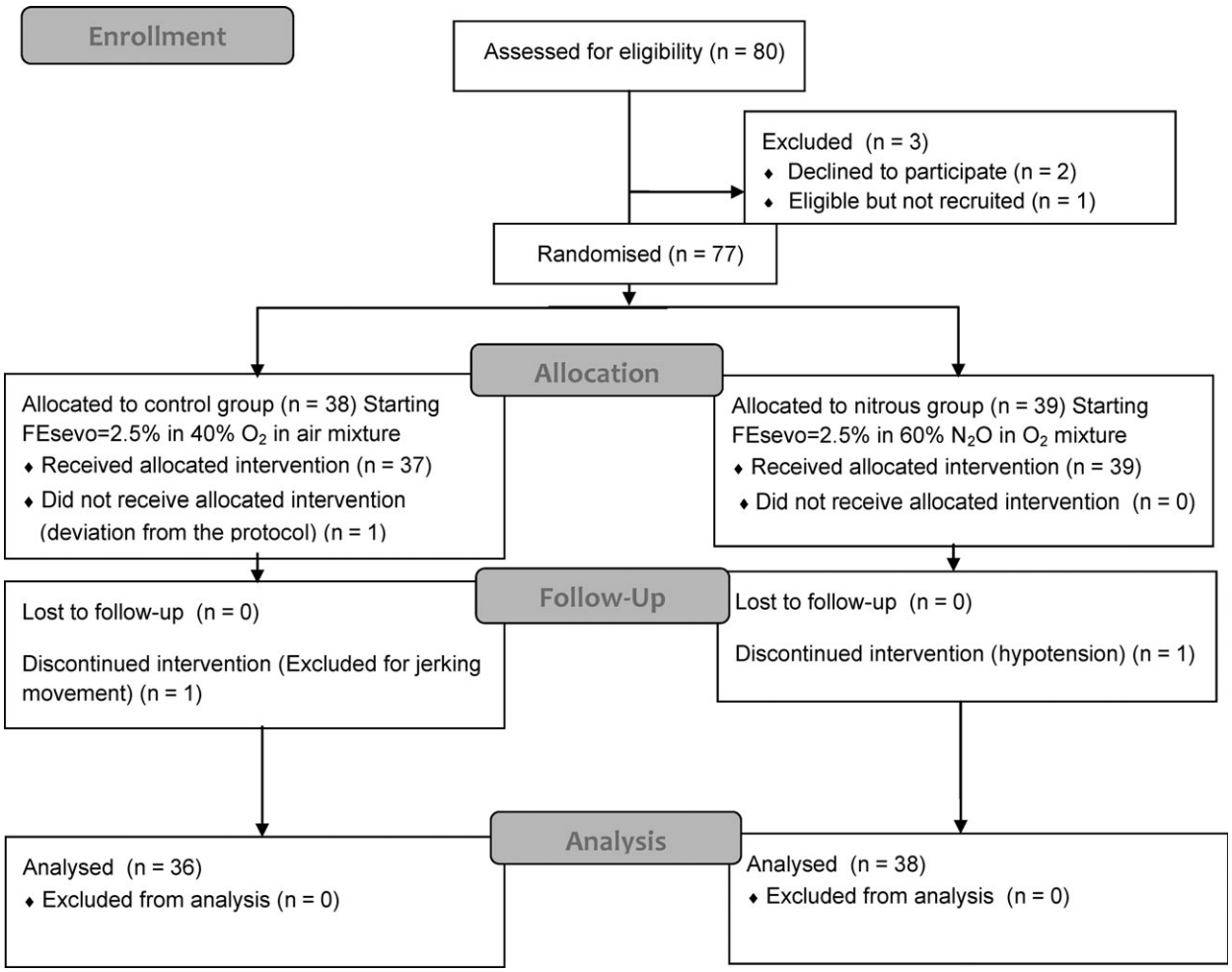

Figure 1 CONSORT flow diagram.

accurate MAC calculation [11]. Based on our previous report, the $ED50_{isoelectric}$ of sevoflurane in 100% oxygen is 3.5% (SD of < 0.2%) [2]. We planned to obtain at least eight turning points to get enough patients per group that would satisfy the needed sample size for both $ED50_{isoelectric}$ calculation and the comparison of $ED50_{isoelectric}$ between two groups. The study was therefore powered at > 80% at a 0.05 alpha level to detect a 10% difference in $ED50_{isoelectric}$ value between the two groups.

The $ED50_{isoelectric}$ and $ED50_{burst}$ values and 95% confidence interval (CI) were primarily calculated using Dixon and Mood's method. However, this does not yield CIs, so the $ED50_{isoelectric}$ and $ED50_{burst}$ values were also calculated using isotonic regression with μ3 estimator, the 95%CI was derived from 2000 bootstrap replicates [12, 13]. Isotonic regression was performed with R statistical package (version 3.3.2, R Foundation for Statistical Computing, Vienna, Austria).

Data were analysed with a logistic regression model as a backup analysis (XLSTAT 2014.9.26, Microsoft™, Redmond, WA, USA). The logistic regression aims at modelling the probability of isoelectric EEG and burst suppression EEG depending on the values of independent explanatory variable 'FE_{Sevo}' using the logit model:

$$\log\left[\frac{\mathrm{p}}{(1-\mathrm{p})}\right] = A + (BX)$$

where A (intercept) and B (regression slope) are the model parameters [14]. The quality of the fit was based on maximisation of the likelihood function using an iterative algorithm (Newton–Raphson). The maximum number of iterations was fixed at 100 and the convergence threshold at 0.000,001. The logistic regression was also evaluated by the Hosmer–Lemeshow test. The significance of the regression coefficients was assessed by the likelihood ratio test and the Wald statistic.

Numerical data were analysed with Student's t-test or the Mann–Whitney U-test. Categorical data were compared between groups by two-tailed Fisher's exact test or chi-squared test. The alterations of MAP and heart rate after incision were evaluated by repeated measures analysis of variance. All analyses were performed with SPSS for Windows (Version 12.0; SPSS, Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

Results

In total, 80 patients were assessed for eligibility (Fig. 1). Two declined to participate and one was not recruited for technical problems leading to a lack of EEG recordings. Baseline characteristics are shown in Table 1.

There were three exclusions. In one patient, a momentary episode of jerking of limbs and trunk muscles occurred for 1 min 3 min after induction (with no postoperative consequences) with 4% FE_{Sevo} in one healthy 52-year-old patient in the control group. Sevoflurane was immediately stopped and propofol was given as 'rescue' medication. Another patient was unintentionally administrated remifentanil by an assistant. A third patient from the N_2O group was not studied because of severe hypotension during the equilibration period. No further complications occurred in the trial.

Examples of positive (presence of isoelectric EEG) and negative (absence of isoelectric EEG) electroencephalographic traces are shown in Fig. 2.

The up-and-down progression is shown in Fig. 3. Using Dixon and Mood's method, the mean (95%CI) $ED50_{isoelectric}$ values were 3.61 (3.47–3.75)% and 3.97

Table 1 Baseline characteristics and intra-operative data. Temperature and $P_{ET}CO_2$ were recorded 2 min before incision. Values are mean (SD), number or number (proportion).

	Control group* n = 36	N_2O group† n = 38
Age; years	54 (7)	54 (7)
Sex; women	13	7
Height; m	165 (7)	167 (6)
Weight; kg	62 (14)	63 (13)
Temperature; °C	36.1 (0.4)	36.1 (0.3)
$P_{ET}CO_2$‡; kPa	4.9 (0.1)	5.1 (0.3)
Episodes of burst suppression	31 (86.1%)	29 (76.3%)
Burst suppression rate	71 (35%)	49 (45%)

*Control group; patient received sevoflurane with 40% oxygen in air.
†N_2O group; patient received sevoflurane with 60% N2O in oxygen mixture.
‡$P_{ET}CO_2$; partial pressure of end-tidal carbon dioxide.

(3.53–4.41)% in the control and N_2O groups, respectively ($p < 0.0001$).

Using isotonic regression estimators, the mean $ED50_{isoelectric}$ values were 3.68 (3.50–3.78)% and 4.08 (3.95–4.38)% for control and N_2O groups, respectively ($p < 0.0001$). The $ED50_{burst}$ values were 3.02 (3.00–3.05)% and 3.05 (2.66–3.90)% for control and N_2O groups, respectively ($p = 0.52$). In both the control ($n = 36$) and N_2O groups ($n = 38$), the lowest FE_{Sevo} inducing isoelectric EEG was 3.3%, 15 patients showed isoelectric EEG.

The dose–response curves for $ED50_{isoelectric}$ and $ED50_{burst}$ modelled by logistic regression are shown in Fig. 4. It was striking that N_2O produced a right shift in the dose–response relationship for isoelectric EEG; that is, a greater, not lesser sevoflurane concentration was needed in the presence of N_2O to produce the same degree of isoelectric response (Fig. 4, panel a).

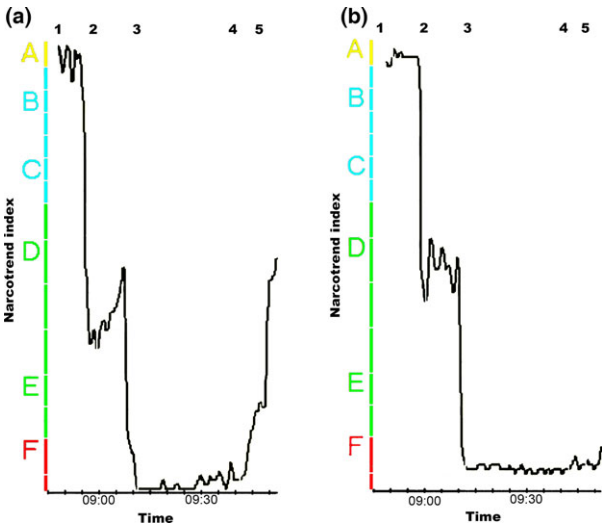

Figure 2 Time course of the Narcotrend EEG (index, y-axis) stages over time (x-axis; 24-h clock) of one example of a control group patient (panel a) and one example of a N_2O group patient (panel b). The marked events are: 1, induction; 2, intubation; 3, isoelectric or burst suppression (these appear with the same values on the raw Narcotrend index); 4, incision; 5, end of data collection. Symbols A to F: A awake (Narcotrend index 95 to 100); B sedated (Narcotrend index 80 to 94); C light anaesthesia (Narcotrend index 65 to 79); D general anaesthesia (Narcotrend index 37 to 64); E general anaesthesia with deep hypnosis (Narcotrend index 13 to 36); F general anaesthesia with increasing burst suppression. (Narcotrend index 1 to 12)

This was not the case with the burst suppression response, where there was no shift with N_2O (but some reduction in slope, Fig. 4, panel b).

Patients in the control group had higher maximal burst suppression ratio compared with patients in N_2O (Table 1).

Figure 5 shows the change in MAP and increase in heart rate following incision in two groups. Mean arterial pressure values were similar between the groups before incision ($p = 0.357$). Repeated measurement analysis of variance showed a statistically significant higher overall MAP in the control group than that in the N_2O group ($p = 0.031$). Heart rate increased more dramatically from baseline in the control group compared with the N_2O group after skin incision ($p = 0.008$).

Discussion

The main result of this study is that the $ED50_{isoelectric}$ of sevoflurane is significantly higher with N_2O than without. In other words, not only was N_2O not additive in combination with sevoflurane for this end-point of isoelectric EEG, but it was 'antagonistic'. This is a very surprising result, since the current paradigm is that all anaesthetic combinations should be additive. Some support to the robustness of our data is that the $ED50_{isoelectric}$ of sevoflurane in oxygen (in the control group) we report here is consistent with $ED50_{isoelectric}$ in oxygen reported in our previous study [2].

One potential concern with our study design is that inducing burst suppression or an isoelectric EEG is undesirable as it may lead to adverse patient outcomes. This argument is derived from observational studies that have demonstrated some association between, on the one hand, a state of intra-operative hypotension, low processed EEG values and low volatile anaesthetic concentrations (a so-called 'triple low') and, on the other hand, postoperative mortality [15]. However, this association has not been demonstrated in other studies [16], and it has only been reported for the bispectral index and not other measures of depth of anaesthesia. To us, this level of evidence does not make avoiding 'deep' anaesthesia (as judged by EEG criteria) inherently undesirable.

Rather, the occurrence of hypotension and deep hypnosis, despite a low volatile anaesthetic

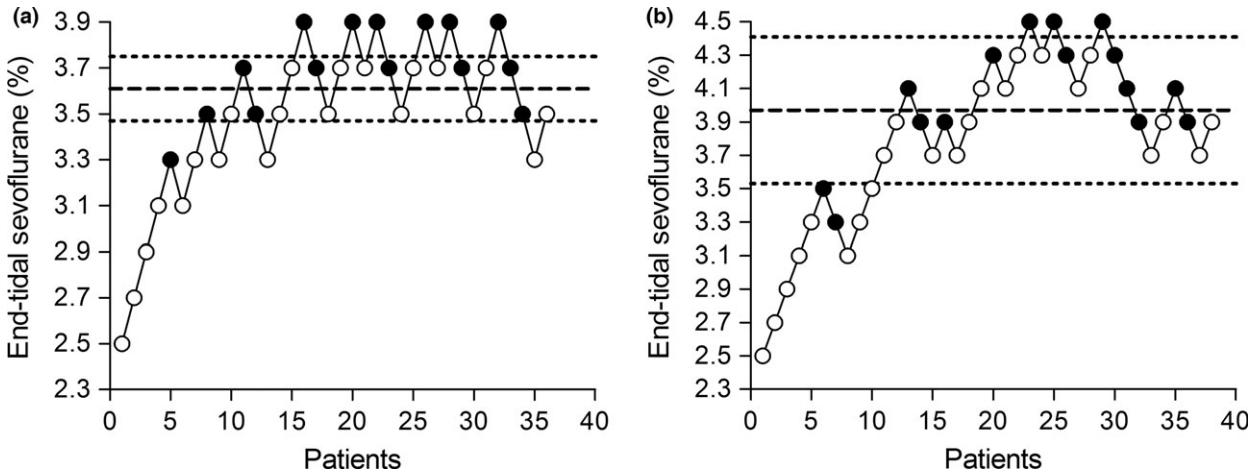

Figure 3 Response of each subject to pre-determined end-tidal sevoflurane concentrations in control group (panel a) and N_2O group (panel b). The solid black points (●) indicate attainment of isoelectric EEG, and open white points (○) indicate the absence of isoelectric EEG. The horizontal dashed line (– – –) represents the calculated $ED50_{isoelectric}$ value and the horizontal doted line (···) represents 95% confidence limits, both were determined by Dixon and Mood's method.

concentration might simply represent a naturally increased sensitivity to volatile anaesthetics in some patients. Routine practice consists in the main of administering possibly very high concentrations of volatile anaesthetics without EEG monitoring. For example, the NAP5 study in the UK showed that < 3% of anaesthetics employ any form of depth of anaesthesia monitoring [17, 18], so no comments can be made of whether burst suppression or isoelectric EEG commonly occur (which they probably do) and at similar agent concentrations as we employed in our study.

Moreover, it can be beneficial to evoke profound brain inactivation with burst suppression or isoelectric EEG, as in to treat status epilepticus or to facilitate traumatic brain protection or in neurosurgery.

Our finding of lack of additivity for N_2O and sevoflurane for isoelectric EEG appears similar to the reported interactions between ketamine and intravenous hypnotics [19]. Most previous studies have reported that N_2O and volatile anaesthetics were additive for immobility [7, 20, 21], but some studies have reported infra-additive interactions for hypnosis and learning [22, 23]. Although (using a response surface modelling approach) N_2O exhibits an additive effect on the MAC of sevoflurane [6], addition of N_2O to a propofol or sevoflurane does not result in clinically significant changes in EEG-derived indices [24]. Yli-Hankala et al. reported a significant decrease in the burst suppression ratio when the air was replaced by 65% N_2O during isoflurane anaesthesia (69.5% vs. 43.7%) [5]. Another study showed that isoflurane-elicited BSR decreased from 53.5% to 34% when the air was replaced by 60% N_2O [25]. Similarly, N_2O has a moderate effect on raising the threshold of sevoflurane for induction of major epileptiform signs in children [26]. In accordance with previous studies [5, 25, 26], addition of 60% N_2O resulted in about 10% increase of $ED50_{isoelectric}$ in our study, and the patients in the N_2O group had lower maximal BSR when compared with patients in the control group.

With regard to burst suppression, we found that N_2O was neither additive nor antagonistic to the effect of sevoflurane (Fig. 4, panel b). However, the slope of the relationship lessened, suggesting N_2O reduced the sensitivity of the response to sevoflurane.

It is now clear that the anatomical and molecular targets of anaesthetics are different for different anaesthetic end-points including mobility, sedation and amnesia [27]. Most volatile anaesthetics, acting perhaps principally through enhancement of gamma-amino-butyric acid (GABA) action, induce a typical dose-dependent increase in frontal slow wave activity of the EEG [28, 29]. As a non-competitive N-methyl-

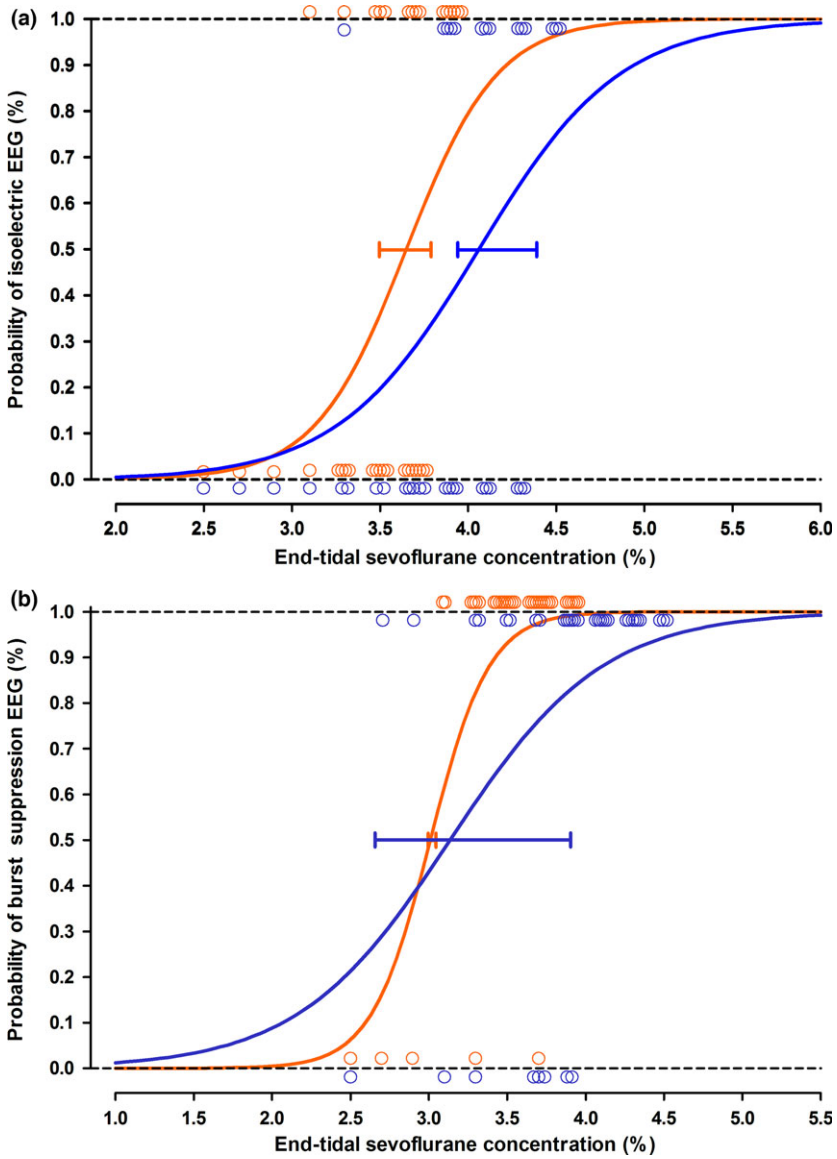

Figure 4 Dose–response curves plotted from logistic analysis of individual end-tidal sevoflurane concentrations and the respective EEG responses for control, sevoflurane-only (orange line) or sevoflurane with N_2O (blue line), for isoelectric (panel a) and burst suppression ration (panel b) data. Orange circles and blue circles represent individual patients in control and N_2O group, respectively, with the circles at probability 0 being 'no response' and those at 1 being 'response'. Error bars represent 95%CIs, determined by isotonic regression estimators, respectively.

D-aspartate (NMDA) receptor antagonist, N_2O has quite different EEG effects compared with many other general anaesthetics [30]. In accordance with analyses offered by Dilger [31] (and based on this, by Pandit [32]), the steeper the slope of a dose–response relationship, the more likely a drug (or combination) is working on a single receptor; whereas the less steep, the more likely the drug(s) work on several different receptors [31, 32]. If this analysis holds for our results, sevoflurane and N_2O might act competitively on a single-receptor system to influence the isoelectric EEG (wherein the slope of the dose–response relationship is the same in control and combination groups). However, they may act by different molecular mechanisms to influence burst suppression (where the slope is influenced by the combination).

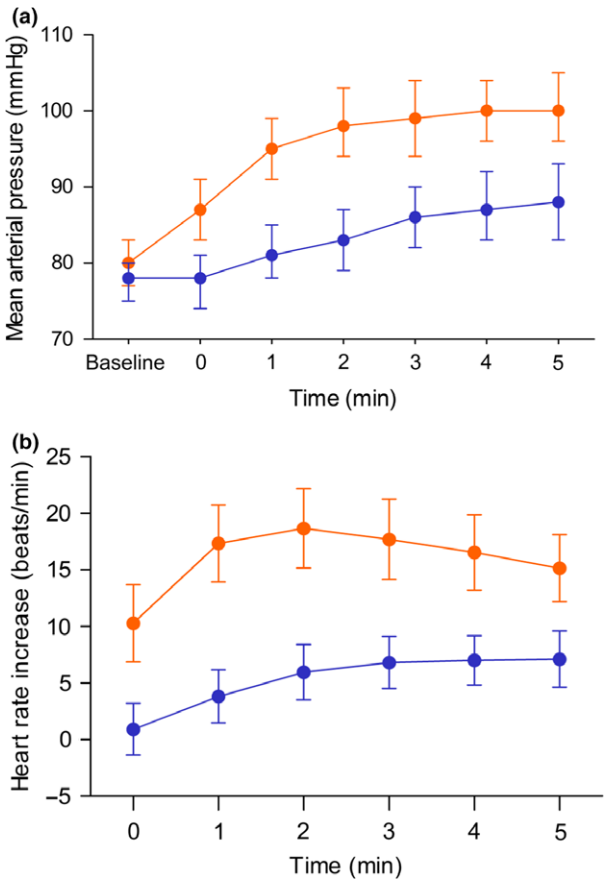

Figure 5 Mean arterial pressure and heart rate increase with 95% confidence intervals of 5 min following skin incision in control group (orange line) and N_2O group (blue line).

The neurophysiological mechanisms involved in the generation of burst suppression and isoelectric EEGs are not fully understood. Both glutamate and GABA synapses contribute to burst suppression EEG activity seen during anaesthesia [33]. Electrophysiological characteristics of burst suppression vary among different general anaesthetics [34]. Burst suppression has traditionally been considered as a homogeneous, deeply inactivated brain state. Substantial heterogeneity in bursting dynamics across the cortex has been demonstrated in a recent study [35]. More in-depth studies are necessitated to explore mechanisms involved in the interaction of N_2O and volatile anaesthetics on EEG activity.

A limitation of this study is that data were all collected from an Asian population. Although the MAC of sevoflurane has limited variability within a population [36], ethnic differences may still exist and, therefore, our results might not be extrapolated to other populations. Our random sampling led to unequal sex ratios in the two groups, but we think this was unlikely to have influenced our results, as MAC does not seem to be influenced by sex in most published studies [37]. Another important limitation of our study was that only one concentration of N_2O was tested. All patients were premedicated with phenobarbital, which may have an indeterminate effect on baseline EEG, and itself has an isoelectric effect when used at high doses [38]. However, both control and test groups received this medication. Although we attempted to control for bias, strictly our studied interactions were of three agents and not just two.

Some studies have claimed that the position of the EEG electrodes may be important [35, 39, 40]. The EEG data in our study were obtained from the forehead sensors and restricted to the frontal cortex; further studies are necessary to ascertain whether our results are applicable to other brain cortical regions. Although the use of ephedrine has been shown to increase brain norepinephrine release and minimum alveolar concentration (MAC) of halothane in dogs, phenylephrine, as used in our study, did not change MAC [41].

Use of Dixon's up-and-down method may yield inaccurate MAC estimates, but inaccuracy is minimised with the help of improved design by careful choice of starting concentration, increment size of concentration and number of turning points [11]. We used the Narcotrend monitor and Entropy module but we do not know if our results are reproducible using other monitors to estimate our end-points of burst suppression and isoelectric EEG. Regardless of the monitor, however, we think the latter end-point is robust and likely to be independent of the monitor.

In summary, our results suggest that there are end-points for anaesthetic effect (burst suppression ratio) where some anaesthetic combinations (sevoflurane and N_2O) produce neither additive nor antagonistic effects, but a reduced slope of the 'dose–response' relationship. It is possible for us to explain this using molecular terminology [31, 32]. Yet, for some other end-points (isoelectric EEG being one), these same anaesthetic combinations appear antagonistic or infra-additive, rather than additive. We cannot explain this

interaction within current paradigms for anaesthetic action.

Acknowledgments

The authors are grateful for the assistance of Prof Yuanshan Wu and Prof Zhijian Yang, School of Mathematics and Statistics, Wuhan University, China, for their statistical analysis. We thank Professor N. L. Pace, University of Utah, USA, and Dr M. P. Stylianou, Mathematical Statistician, Office of Biostatistics Research, National Heart, Lung, and Blood Institute, National Institutes of Health, USA, for providing us software packages for isotonic regression using the R statistical package and help with the statistical analysis. Trial registered with ClinicalTrials.gov (NCT01705743).

Competing interests

Funded by the National Natural Science Foundation of P.R. China, grant no. 81,571,357 to WM and no. 81,571,053 to YT. This work was supported partly by 2010 Clinical Key Discipline Construction Grants from the Ministry of Health of the People's Republic of China. No other competing interests declared.

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