Equipotent Subanesthetic Concentrations of Sevoflurane and Xenon Preventing Cold-stimulated Vocalization of Neonatal Rats

Hannah Gill, F.R.C.A., Marianne Thoresen, Ph.D., Sarah Bishop, M.Sc., Elisa Smit, M.R.C.P.C.H., Xun Liu, Ph.D., Lars Walloe, Ph.D., John Dingley, M.D.

ABSTRACT

Background: The effects of inhaled anesthetics on the developing brain are studied using neonatal rodents exposed to fractions of minimum alveolar concentration (to avoid cardiorespiratory compromise). However, these fractions cannot be assumed to be equipotent. Xenon's anesthetic and neuroprotective properties warrant investigation in these models. Therefore, equipotent, subanesthetic concentrations of inhaled anesthetics are needed.

Methods: Forty-eight Wistar rats (Charles River Laboratories, Kent, United Kingdom) on postnatal day 9 were randomized to eight concentrations of inhaled anesthetics: isoflurane, sevoflurane, or xenon. Exposure was closely monitored in individual metal-based chambers resting on a 35°C mat to maintain normothermia. A 25°C mat was used to stimulate vocalization and a sound recording made (1 min, 1 to 100 kHz). Rectal temperature or partial pressure of carbon dioxide and pH of mixed arteriovenous blood were measured immediately after the exposure. Concentration–response models were constructed using logistic regression (dependent variable: vocalization and explanatory variable: concentration). The effects of all other explanatory variables were assessed by inserting them individually into the model.

Results: The effective inhaled concentrations preventing cold-stimulated vocalization in 50 and 95% of neonatal rats (EiC50 and EiC95) on postnatal day 9 were 0.46 and 0.89% sevoflurane and 20.15 and 34.81% xenon, respectively. The effect on the EiC50 of all other explanatory variables, including duration, was minimal. Stability of EiC50 isoflurane was not achieved over three durations (40, 80, and 120 min exposure). Partial pressure of carbon dioxide and pH in mixed arteriovenous blood appeared normal.

Conclusions: The authors report equipotent subanesthetic concentrations of sevoflurane and xenon in neonatal rats with preserved cardiopulmonary function. This may be useful in designing neonatal rodent models of anesthesia.
(ANESTHESIOLOGY 2014; 121:1194-202)

EVERY year millions of fetuses, babies, and young children are exposed to inhaled anesthetics.[1] Evidence supports a role for early exposure to anesthetics or sedative drugs in the development of neuropathological and neurobehavioral deficits later in childhood.[2–5] Retrospective clinical studies contain multiple confounding variables,[1,6–8] and although three prospective clinical studies are underway, they will take several years to report.[4] Therefore, experimental models continue to build on our understanding of anesthesia-associated neurotoxicity in the developing brain.[4]

Halogenated anesthetics induce both neuroapoptosis and neurocognitive deficit in laboratory models of neonatal anesthesia.[9–20] In neonatal rodent models of anesthesia, single, relatively prolonged exposure to inhaled anesthetic[3] results in cardiorespiratory compromise[11,19] and mortality.[10,15,18] There is evidence that isoflurane-induced brain cell death in neonatal rats may, at least in part, be caused by hypercarbia.[21]

What We Already Know about This Topic

- Comparative analysis of the toxicity of inhaled anesthetics in the developing brain requires the use of equipotent doses
- Minimum alveolar concentration fractions have been utilized when subanesthetic doses are evaluated; however, equal minimum alveolar concentration fractions may not reflect equipotency
- The authors utilized cold stimulation–induced vocalization as an endpoint for anesthetic action and determined equipotency of xenon and sevoflurane

What This Article Tells Us That Is New

- The effective concentrations of sevoflurane and xenon that prevented cold-induced vocalization were 0.46 and 20.15%, respectively; this indicates that sevoflurane is approximately 43 *times* more potent than xenon in neonatal rodents
- The use of cold-stimulated vocalization can be used as a measure of anesthetic potency
- As such, the research adds to our armamentarium, a method of measuring anesthetic potency, especially in the study of the effects of subanesthetic concentrations of inhaled agents

The methods and some of the primary analyses have previously been presented, by the first author Dr. Gill, at the Anaesthetic Research Society Spring Meeting, Merton College, Oxford, United Kingdom, April 11 and 12, 2013 (abstract published, *British Journal Anaesthesia*; August 2013, 315P-316P), and at the Joint Meeting of the Association of Paediatric Anaesthetists and European Society of Paediatric Anaesthetists, Cambridge, United Kingdom, June 19-21, 2013.

Submitted for publication November 27, 2013. Accepted for publication July 30, 2014. From Neonatal Neuroscience, School of Clinical Sciences, University of Bristol, St. Michael's Hospital, Bristol, United Kingdom (H.G., M.T., S.B., E.S., X.L.); Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway (M.T., L.W.); and Department of Anaesthesia, Swansea University College of Medicine, Wales, United Kingdom (J.D.).

 Anesthesiology 2014; 121:1194-202

Using repeat exposures to subanesthetic concentrations of inhaled anesthetics, maintaining cardiorespiratory function, may constitute a superior model of neurotoxicity in neonatal rodents.

To compare subanesthetic concentrations in neonatal rodents, equal fractions of the minimum alveolar concentration (MAC) preventing purposeful movement in 50% have been used[18,19]: However, *equal fractions of MAC cannot be assumed to be equipotent*[22,23] as the concentration–response curves may not be parallel. A large fraction of MAC in an anesthetic with a steep concentration–response curve results in an ineffective concentration, whereas an equal fraction is much more potent if the anesthetic has a shallow concentration–response curve (fig. 1).

Both pretreatment with and coadministration of xenon have been shown to reduce isoflurane-induced neuroapoptosis in neonatal rodent models[16,20] at concentrations as low as 30%.[12] In a perinatal rat model of hypoxia–ischemia, 35% xenon and 0.35% sevoflurane have been shown to provide labor analgesia and fetal neuroprotection.[24] Whether higher concentrations of xenon administered alone induce neuroapoptosis in neonates is contested.[16,17] Xenon is regarded as a cardiostable anesthetic with proven neuroprotective properties.[25]

As fractions of MAC cannot be guaranteed to provide equipotency, a subanesthetic concentration of inhaled anesthetics such as isoflurane, sevoflurane, and xenon in neonatal rats may improve comparison of their neurotoxic properties as well as facilitate investigation of any potential neuroprotection provided by coadministration of halogenated anesthetics and xenon in neonatal rodent models of anesthesia.

The production of cold-stimulated ultrasonic vocalizations by an isolated neonatal rat has been used as a model of anxiety[26]: The difference in number of vocalizations emitted while on a warm or cold metal plate was reduced by benzodiazepines.[26] It has been reported that ultrasonic vocalizations are emitted by neonatal rats from birth up to postnatal day 21 (probably associated with weaning).[27]

The primary aim of this study was to measure equipotent subanesthetic concentrations of isoflurane, sevoflurane, and xenon using traditional, fully randomized, dose–response study design. The effective inhaled concentration preventing cold-stimulated vocalization in 50 and 95% of neonatal Wistar rats (EiC50 and EiC95, respectively). Furthermore, we aimed to test the null hypotheses that the concentration–response curve slopes would be identical and cardiopulmonary function would be equally suppressed by the three inhaled anesthetics at equipotent inhaled concentrations.

Materials and Methods

These experiments were carried out under Home Office License in accordance with United Kingdom guidelines and approved by the Animal Ethical Review Panel at the University of Bristol, Bristol, United Kingdom. Litters of cross-fostered Wistar rats were supplied with lactating females (dams) by Charles River Laboratories, Kent, United Kingdom. MAC preventing movement of neonatal rats on postnatal day 9 (P9) has previously been reported,[28] and to allow direct comparison to this, all experiments were performed on P9.

Four litters of 12 neonatal rats were used for each anesthetic agent: isoflurane, sevoflurane, and xenon. After weighing, sexing, and marking with litter and individual identity, the rats were randomized to one of eight target concentrations: 0.1 to 0.8% in 0.1% steps for isoflurane, 0.2 to 1.6% in 0.2% steps for sevoflurane (Abbott Laboratories, Maidenhead, United Kingdom), and 8 to 56% in 8% steps for xenon (BOC, Manchester, United Kingdom). These concentrations were predicted from pilot data to produce a response rate (the percentage of animals failing to vocalize at a given concentration) ranging from 0 to 100% at the three highest concentrations: producing a concentration–response curve with a defined plateau and range of concentrations as narrow as was just required to achieve this. At each concentration, neonatal rats were randomized to one, two, or three durations of exposure: 40 min for isoflurane, 15 min for

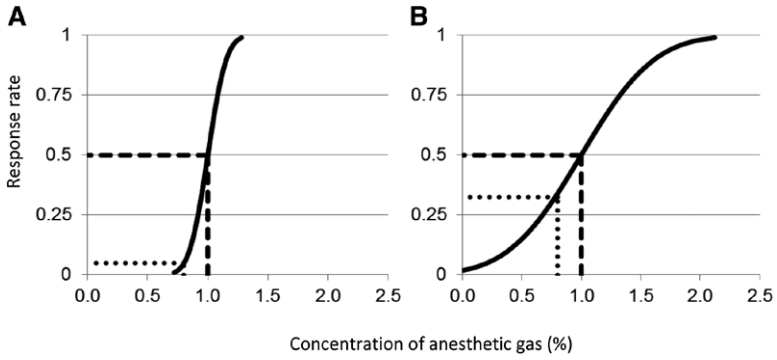

Fig. 1. Illustration to demonstrate that equal fractions of minimum alveolar concentration (MAC) cannot be assumed to be equipotent. Theoretical concentration–response curves for two inhaled anesthetics (*A* and *B*) with equal MAC of 1.0% (*dashed line*). (*A*) 0.8 MAC of an agent with a steep concentration–response curve (*dotted line*) results in an ineffective concentration (5% response rate). (*B*) 0.8 MAC of an agent with a shallow concentration–response curve (*dotted line*) results in a concentration which is seven times more effective (35% response rate).

sevoflurane, and 10 min for xenon. Therefore, pairs of neonatal rats were randomized to each concentration and duration. The durations were predicted both *a priori* and from pilot data, to allow adequate equilibration between inhaled and end-organ partial pressure before stimulation and assessment of response. Exposure of neonatal rats to anesthetic for one, two, and three durations allowed statistical assessment of this assumption in our multivariable regression analysis.

The Procedure for Exposing the Neonatal Rats to Isoflurane and Sevoflurane

Three pairs of neonatal rats, each randomized to the same concentration (chosen at random immediately before the exposure) and duration, were placed into six, individual, gas-tight chambers (500 ml volume), connected in parallel to an agent-specific vaporizer (sevoflurane: Penlon, Abingdon, United Kingdom; isoflurane: Medicalia, Liverpool, United Kingdom). All connecting tubing in this open gas delivery system was of equal length and internal diameter, and fresh gas flow (air) was maintained at 1 l/min to each chamber throughout. The concentration of anesthetic in the gas leaving the chambers was monitored using a gas analyzer (Andros 4800; LumoSense Technologies, Richmond, CA). The target concentration was equal in all chambers throughout the exposure.

The Procedure for Exposing the Neonatal Rats to Xenon

The first of a pair of neonatal rats randomized to the same target concentration (chosen at random immediately before the exposure) and duration was placed into a custom-built chamber (500 ml volume) with a xenon analyzer (GKM-03-INSOVT; ZAO "Insovt," St. Petersburg, Russia [delivered by Alfa-Impex, Oy, Finland]) and oxygen analyzer (MX300-I; Teledyne Analytical Instruments, City of Industry, CA) mounted in the top. This chamber was part of a closed gas delivery system containing a pump to circulate gas and carbon dioxide absorber (Intersurgical Ltd., Berkshire, United Kingdom). A 3 l gas calibration syringe (CareFusion, San Diego, CA) was filled one third with oxygen, then with a volume of xenon calculated to give the target concentration of xenon and the remaining volume filled with air. Half of this was used to flush the system, containing the neonatal rat, which was then sealed and the gas circulated at a flow rate of 1 l/min. After 3 min, to allow initial mixing within the system, the concentrations of xenon and oxygen were recorded every minute. Carbon dioxide concentration was monitored throughout to ensure complete removal from the circulating gas mixture. This procedure was repeated for the second neonatal rat in the pair.

To keep the animals warm during exposure to the anesthetic, the chambers, with nonferrous metal bases, were placed inside a custom-built plexiglass box on a fluid-filled mat maintained at 35°C (Tecotherm TSmed 200N; Inspiration Healthcare Ltd., Earl Shilton, United Kingdom). This maintained rectal temperature of the isolated neonatal rats in the chamber approximately equal to that measured when taken immediately from dam and littermates in a previous experiment (33.2°C).

The Procedure for Determining the Presence or Absence of Vocalizations in Response to a Cold Stimulus

To stimulate vocalization of the neonatal rat, the chamber was placed onto a cooler mat maintained at 25°C and immediately a 1-min sound recording was made using a digital microphone (Ultramic200K; Dodotronic, Castel Gondalto, Italy) inserted *via* a latex port in the top of the chamber and using Seawave computer software (Gianni Pavan, Centro Interdisciplinare di Bioacustica e Ricerche Ambientali, Pavia, Italy). The recordings were made with a range from 0 to 100 kHz and a sampling frequency of 200 kHz. A vocalization was defined as a sonogram seen in the ultrasonic range or a sonogram visible in the audible range and accompanied by audible vocalization (fig. 2). Recordings were observed in real time in silence to ensure quality of the recording and reviewed with sound by an assessor blinded to concentration after completion of experiments.

Temperature and Blood Gas Analysis

Immediately after recording, the neonatal rat was quickly removed from the chamber and temperature was measured using a probe inserted 5 mm rectally (calibration accuracy 0.1°C; Physitemp Instruments, Clifton, NJ). After the two longer durations of exposure, the second rat in each pair at the concentration and duration was quickly removed from the chamber, decapitated, and mixed arteriovenous blood was collected using a heparinized capillary tube. Blood gas analysis was performed (iSTAT, G3+ cartridge; Abbott Laboratories) and corrected to the rectal temperature of the first neonatal rat in the pair. It was not technically feasible to measure both rectal temperature and immediate blood collection in a single animal. Four additional neonatal rats were exposed to 0.0% anesthetic using both the open and closed gas delivery system.

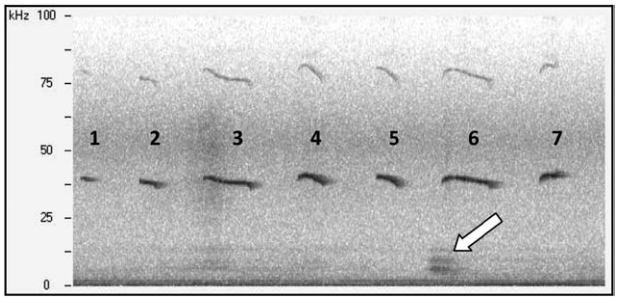

Fig. 2. Examples of vocalizations. A 2-s snapshot taken from a sound recording made using Seawave software (Gianni Pavan, Centro Interdisciplinare di Bioacustica e Ricerche Ambientali, Pavia, Italy). Time is on the x-axis and frequency on the y-axis (0 to 100 kHz). The sonograms of seven ultrasonic vocalizations, labelled one to seven, can be seen at approximately 40 kHz. Harmonics can also be seen at approximately 80 kHz. Vocalization number 6 has a component audible to humans (below 20 kHz) and is marked with the *arrow*.

Statistical Analysis

Data collected included litter identity, weight, sex, duration and concentration of exposure, presence or absence of vocalization, rectal temperature, pH, and partial pressure carbon dioxide (P_{CO_2}) in mixed arteriovenous blood. Where rectal temperature was not measured, animals were assigned the median measured value for the anesthetic. Mann–Whitney U test was used for comparison between the two groups. Bivariate correlation was assessed using Spearman rank correlation (two tailed). Significance was set at a P value of 0.05.

Concentration–response models were built for each anesthetic using univariate binary logistic regression in SPSS 19.0 (IBM, Armonk, NY), where prevention of vocalization (coded: 1) was the dependent variable and concentration was the explanatory variable. Sample size was chosen from pilot data to achieve a P value less than 0.05 for the coefficient of concentration. Using this model, we calculated the effective inhaled concentration preventing vocalization in 50% (EiC50) using the coefficients reported for the constant and concentration to solve the equation: ln (probability (y_1)/probability (y_2)) = $\beta_O + \beta_1 X_1$, where X_1 is EiC50 and hence y_1 and y_2 are equal (50% probability).[28] The concentration–response model was accepted if the change in the calculated EiC50 was less than 10% when duration was inserted as a second explanatory variable. If the change in EiC50 was greater than 10%, we attempted construction of a new model using the data from the two longer durations of exposure only and repeated the sensitivity analysis by inserting duration of exposure. If the change in EiC50 was again greater than 10%, then the model was rejected.

All other explanatory variables were then individually inserted into the accepted concentration–response model to assess the percentage change in EiC50.

The concentration–response curves and 1.96 SDs or 95% confidence intervals were plotted using R package (R Core Team [2012]; R Foundation for Statistical Computing, Vienna, Austria), and the effective inhaled concentration preventing cold-stimulated vocalization in 95% (EiC95) was read from this. To allow comparison of the steep section of the concentration–response curves for sevoflurane and xenon, $\log_{10}$concentration–response curves were plotted up to EiC95.

Results

Experiments Performed to Measure Concentration–Response

The calculated effective inhaled concentrations preventing cold-stimulated vocalization in 50% (EiC50) for sevoflurane and xenon in neonatal rats on P9 were 0.46 and 20.15%, respectively (table 1). Concentration–response curves were estimated using responses at 15, 30, and 45 min exposure to sevoflurane and 20 and 30 min exposure to xenon as this resulted in less than 10% change in the EiC50 (table 2), suggesting the concentration at the effect site had not materially changed. However, EiC50 for isoflurane changed more than 10% using responses at three durations and responses at the two longer durations (80 and 120 min). Therefore, the model for isoflurane was rejected and we do not report any further analysis. Individually inserting all other explanatory variables into the concentration–response models for sevoflurane and xenon changed the calculated EiC50 less than 6.7% (table 2).

The concentration–response curves are shown in figure 3. The effective inhaled concentration preventing cold-stimulated vocalization in 95% (EiC95) for sevoflurane and xenon were 0.89 and 34.75%, respectively. The slopes of the $\log_{10}$-concentration curves of sevoflurane and xenon for concentrations up to the EiC95 did not differ (fig. 4).

The characteristics of the neonatal rats used are shown in table 3. There were different proportions of females in the groups, but there was no difference in the time of day the recordings were made or the median weight. Statistical analysis was restricted to the sevoflurane and xenon groups as the concentration–response model for isoflurane was not successful. The rats exposed to xenon were significantly warmer than those in sevoflurane (by 0.6°C). The temperature of the neonatal rats exposed to sevoflurane was significantly correlated with both weight (+0.371, $P = 0.003$) and concentration (–0.660, $P = 0.0001$). There was a significant correlation between rectal temperature and weight of neonatal rats exposed to xenon (0.442, $P = 0.03$), but no correlation with concentration ($P = 0.9$).

Mixed arteriovenous blood sampling was successful in 12 neonatal rats exposed to sevoflurane and 9 neonatal rats exposed to xenon and an additional 3 rats exposed to 0.0% sevoflurane and xenon in the open and closed gas delivery systems (fig. 5). Median interquartile range P_{CO_2} and pH were: 46.15 mmHg (38.72 to 51.43) and 7.44 (7.39 to 7.51) in sevoflurane and 45.95 mmHg (38.63 to 51.38) and 7.45 (7.41 to 7.51) for xenon. There were significant correlations between sevoflurane concentration and both P_{CO_2} (0.937, $P = 0.2 \times 10^{-4}$) and pH (–0.878, $P = 0.2 \times 10^{-6}$), but this was not so for xenon: P_{CO_2}, $P = 0.08$ and pH, $P = 0.168$. In 12 neonatal rats exposed to isoflurane, the median (interquartile range) P_{CO_2} and pH were 46.80 mmHg (43.73 to 50.75) and 7.44 (7.41 to 7.47).

Discussion

We have measured equipotent inhaled concentrations of sevoflurane and xenon preventing vocalization of neonatal rats stimulated with a standardized cold stimulus. The effective inhaled concentration preventing cold-stimulated vocalization in 50% (95%) of neonatal rats (EiC50 [EiC95]) of sevoflurane and xenon were 0.46% (0.89%) and 20.15% (34.81%), respectively, in normothermic neonatal Wistar rats on P9, with no overlap of the 95% CIs. Therefore, sevoflurane was approximately 43 times more potent than xenon in this study. It has been reported to be 38 times more potent in newborn pigs at 33.5°C.[29] The similar relative potencies in two newborn anesthetic models would appear to support their respective potencies and the mechanism of action being similar across the species.

Table 1. SPSS 19.0 (IBM, Armonk, NY) Output from Univariate Binary Logistic Regression and Calculated EiC50

	Sevoflurane (n = 48)	Xenon (n = 32)
	Beta Coefficient (*P* Value)	Beta Coefficient (*P* Value)
Concentration	7.029 (0.002)	0.195 (0.007)
Constant	–3.207 (0.01)	–3.930 (0.16)
EiC50	0.46%	20.15%

EiC50 is the effective inhaled concentration preventing cold-stimulated vocalization in 50% of neonatal rats. Dependent variable is the absence (1) or presence (0) of vocalization. Explanatory variable is the concentration. EiC50 is calculated using the beta coefficients reported to solve the equation: ln (probability (y_1)/probability (y_2)) = $\beta_O + \beta_1 X_1$, where X_1 is EiC50 and hence y_1 and y_2 are equal.

Table 2. Sensitivity Analysis for Sevoflurane and Xenon: The Effect on EiC50 When a Second Explanatory Variable Is Individually Inserted into the Concentration–Response Model

	Sevoflurane (n = 48)	Xenon (n = 32)
Second Explanatory Variables	Effect on Calculated EiC50, %	Effect on Calculated EiC50, %
Duration	–1.7	–6.2
Litter identifier	–2.1	–0.5
Weight	–6.6	0.0
Sex	–5.9	–6.7
Time of day	+4.5	+2.1
Rectal temperature	+5.2	–0.5

EiC50 is the effective inhaled concentration preventing cold-stimulated vocalization in 50% of neonatal rats. The first explanatory variable is "Concentration" (table 1): The effect on the calculated EiC50 is equal to the percentage change in the beta coefficient reported for the explanatory variable "Concentration" before and after inserting the second explanatory variable into the logistic regression model.

We sampled mixed arteriovenous blood immediately after exposure to anesthetic as arterial blood sampling requires surgical anesthesia under United Kingdom ethical guidelines. In an animal model, the mean difference between venous and arterial pH and P_{CO_2} was shown to be small: 0.02 and 5.1 mmHg, respectively, widening to 0.13 and 20.3 mmHg only when severe circulatory failure was induced.[30] It has previously been shown that pH and P_{CO_2} in neonatal rats breathing 1.0% sevoflurane for 30 min on P7 were not statistically different to animals breathing air: pH 7.41 and P_{CO_2} 39.1 mmHg, but was significantly altered in 2.0% sevoflurane.[31] In another, median arterial P_{CO_2} of approximately 90 mmHg in neonatal rats exposed to 2% sevoflurane on P7 was associated with 25% mortality.[15] In our study, no neonatal rats appeared to have clinically severe cardiorespiratory compromise.

Assessment of Sensitivity of the Concentration–Response Model to Individual Explanatory Variables

We found consistency in the response to stimulation after different durations of sevoflurane and xenon exposure, suggesting that the concentration at the effect site had not materially changed between 15, 30, and 45 min exposure to sevoflurane and between 20 and 30 min exposure to xenon. We did not achieve consistency of response with isoflurane exposures of 40, 80, and 120 min and therefore have not reported EiC50 or EiC95 for isoflurane.

The durations of sevoflurane and xenon exposure in our study are relatively short when compared with the original MAC studies of dogs ventilated with halothane where 20 min was allowed after equilibration of inhaled and end-tidal partial pressure before assessing response to stimulation.[32] In studies of small neonatal rodents, only the inhaled partial pressure of anesthetic can be measured and time to

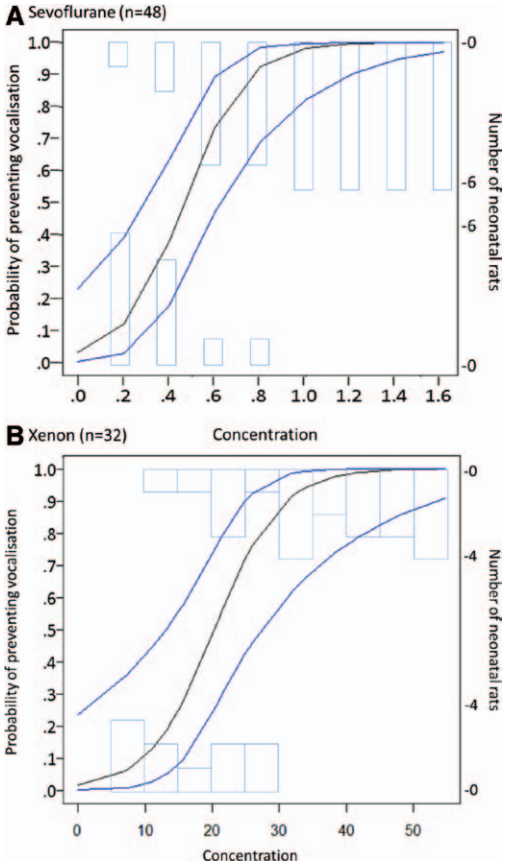

Fig. 3. Concentration–response curves and individual responses for sevoflurane and xenon. Concentration–response curves (*black line*) and 95% CI (*blue lines*) plotted from binary logistic regression in R package where the dependent variable is prevention of vocalization and explanatory variable is concentration. (*A*) The *bars* display the number of neonatal rats vocalizing (*lower*) and prevented from vocalizing (*upper*) at each concentration of sevoflurane (0.2 to 1.6%). (*B*) The histogram displays the number of neonatal rats vocalizing (*lower*) and prevented from vocalizing (*upper*) in 5% ranges of measured concentrations of xenon. Effective inhaled concentration preventing cold-stimulated vocalization in 50% of neonatal rats (95% CI): 0.46% (0.29 to 0.62%) for sevoflurane and 20.15% (13.24 to 27.75%) for xenon. Effective inhaled concentration preventing cold-stimulated vocalization in 95% of neonatal rats (95% CI): 0.89% (0.73 to 1.42) for sevoflurane and 34.81% (27.75 to approximately 60%) for xenon.

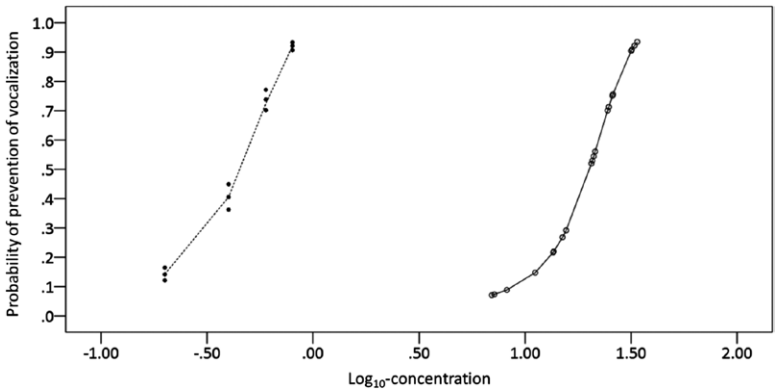

Fig. 4. Log10-concentration response curves for sevoflurane (*dotted line*) and xenon (*solid line*) up to the effective concentration preventing cold-stimulated vocalization in 95% of neonatal rats. The slopes of the curves at this, the steepest section, are indistinguishable.

equilibration with the effect site has only been measured in one study: Partial pressure of isoflurane in the brains of neonatal rats was shown to be lower than the inhaled partial pressure after 1 h exposure and was equal at 4 h.[33] However, in most MAC studies, 15-min duration to allow for equilibration has been used.[34] In humans, sevoflurane has a faster onset of action than isoflurane and this is attributed to its lower solubility in blood,[35] whereas that of xenon is extremely low, explaining its very rapid onset and offset of anesthesia.[36] The solubility of inhaled anesthetics in the blood differs between rats and humans[37] and neonates and adults[35] and with temperature, but solubility in neonatal rat blood has not been reported. Factors that may enhance the wash-in of sevoflurane and xenon to the effect site in healthy neonatal rats could include small lung volumes with relatively high alveolar minute volume, low cardiac output, high brain solubility, and high cerebral blood flow.[38] These physiological parameters may be differentially affected by inhaled anesthetics, complicating assumptions regarding wash-in times. The blood gas analysis, revealing similar ranges of P_{CO_2} and pH in our study, do not suggest that isoflurane equilibration times were slowed by cardiorespiratory compromise.

Reduction in core temperature lowers MAC of the halogenated anesthetics.[34] MAC of sevoflurane and isoflurane in newborn pigs was reduced from 4.1 to 3.05% with 5°C reduction[29] and 2.47 to 1.83% with 4°C reduction,[39] respectively, whereas MAC of xenon was not affected (120 and 116% with 5°C reduction).[29] MAC of sevoflurane and isoflurane has been measured in neonatal Wistar rats with rectal temperatures between 33.7° and 35°C on P9, but the effect of individual's temperature was not studied.[28] The rectal temperatures in that study are reported, as in ours, to be consistent with that measured when neonatal rats on P9 are removed immediately from the nest (dam and littermates). In our study, despite a narrow range of temperatures and weights, both these explanatory variables had a greater effect on the calculated EiC50 for sevoflurane than xenon. However, this may be caused by the correlation of rectal temperature with weight for both anesthetics and the correlation with concentration of sevoflurane, but not xenon. This correlation suggests that sevoflurane caused a concentration-dependent loss of body temperature, whereas xenon did not. Xenon has good thermal insulating properties and, along with the fact that it was delivered in a closed delivery system, probably prevented temperature loss from the isolated neonatal rats in the xenon-filled chamber. Therefore, we do not feel we can comment on whether the difference in temperature represents a pharmacological effect.

Table 3. Characteristics of Neonatal Rats

	Xenon (n = 32)	Sevoflurane (n = 48)	Mann–Whitney U Test *P* Value	Isoflurane (n = 48)
Durations of exposure (min)	20, 30	15, 30, 45		40, 80, 120
Number of litters	4	4		4
% female	25	46		31
Time of recording	10:00–19:30	10:30–19:00	0.466	10:30–19:00
Rectal temperature (°C)	35.0* (34.4–35.3) n = 16	34.4* (33.8–34.8) n = 32	<0.001	34.3 (33.8–34.7) n = 32
Weight (g)	17.95 (15.08–19.28)	18.05 (16.93–19.00)	0.507	16.90 (15.40–18.10)

Concentration–response models were accepted if duration of exposure resulted in <10% change in the calculated EiC50. This was not achieved for isoflurane and data are presented for information only (not subjected to statistical analysis). EiC50 is the effective inhaled concentration preventing cold-stimulated vocalization in 50% of neonatal rats.

* Significant correlation with weight: Spearman rank correlation, $P < 0.05$.

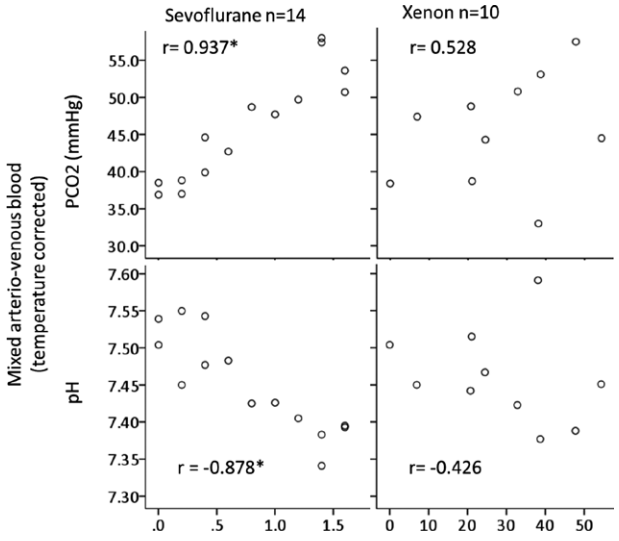

Fig. 5. Mixed arteriovenous blood gas analysis after exposure to 30 or 45 min of sevoflurane or 20 or 30 min of xenon. Values are corrected to the rectal temperature of a paired neonatal rat exposed to the same concentration and duration. Sevoflurane concentration, but not xenon concentration, is significantly correlated with partial pressure carbon dioxide (P_{CO_2}) and pH (*$P < 0.0001$).

Although sex is generally accepted to have no effect on MAC, sex of the neonatal rats in this study changed the EiC50 in our study by the greatest degree of all the explanatory variables studied: both sevoflurane and xenon appearing to prevent vocalization of female neonatal rats at a lower concentration than in males. Sex differences in anesthetic requirement have been reported. MAC of xenon was lower in elderly Japanese females[40] and less anesthetic was required by female rats undergoing intensive care[41] but has not been a consideration in neonatal rodent models of anesthesia.

Difference between the slopes of the $\log_{10}$concentration–response curves for sevoflurane and xenon preventing cold-stimulated vocalization in neonatal rats on P9 is not distinguishable, suggesting that equal fractions or multiples of the EiC50 (up to the EiC95) may be equipotent. Although the ratio of EiC95 to EiC50 is not exactly equal, the wide 95% CIs seen at the upper end of the concentration–response curves, despite randomization of subjects to all concentrations, indicate that the reported EiC95 are less reliable than the EiC50. We did not measure anesthetic MAC and cannot comment whether EiC50 of sevoflurane and xenon are equal fractions of it, and due to failure to establish a concentration–response model for isoflurane, we cannot directly compare our EiC50 to MAC measured in the study reported by Orliaguet *et al.*[28]

Our study uses randomization of all neonatal rats to all concentrations as in a true dose–response study. The extreme steepness of the concentration–response curves serves to illustrate the narrow *therapeutic window* of inhaled anesthetics: the range of concentration of an anesthetic that can be used to produce prevention of vocalization while staying within the safe range. Unlike anesthetic MAC studies, there is no evidence of toxicity or risk of death at the higher concentrations in our study and this justifies the use of randomization to all concentrations. MAC preventing purposeful movement in 50% of subjects in response to a standardized surgical stimulus is determined using the up and down method[34] which brackets the concentrations used close to the EC50 for this reason. Hence, this method is not designed to measure the entire concentration–response curve, as our method is.

Another drawback to the modified up and down method used in neonatal rodent MAC studies is the repeated stimulation of the subjects. Repeated stimulation was avoided in the original (unmodified) description of the technique, as one stimulation is likely to alter the response to the next.[42] MAC for isoflurane and sevoflurane in neonatal Wistar rats on P9[28] has been measured using repeated stimulation in a modified up and down method and reported narrow CIs around the MAC when univariate logistic regression was used to construct the concentration–response curves.[34,43] However, the assumption of this analysis is broken; repeated measures are not independent variables and therefore an unverifiable assumption of symmetry is made. The wider CIs evident in our study do not necessarily suggest greater variability of our model.

One published concentration–response curve plots the potency of isoflurane causing loss of righting reflex in neonatal, adolescent, and adult rats (8 to 10 per group). Repeated assessments were performed every 35 min as isoflurane concentration was increased from 0.1 to 0.5% in 0.1% steps.[44] The published concentration–response curve has extremely narrow CIs. This method is not either an up and down design or randomized design and it is therefore difficult to draw conclusions from the CI reported.

The use of a stimulus, which is not supramaximal (where an increase in the intensity of a stimulus does not increase the response to it), has previously been shown to increase variation in models of anesthesia.[45,46] However, when multiple different stimuli were used to define anesthesia depth in 26 adult patients ventilated with isoflurane, laryngoscopy and intubation required the highest concentration to prevent movement, suggesting it was most likely to be a supramaximal stimulus, but had a wider error of the mean compared with other stimuli.[47] Classifying different stimuli as sub- or supramaximal is evidently not simple.

In conclusion, we have reported clinically and experimentally relevant equipotent subanesthetic concentrations of sevoflurane and xenon in neonatal rats on P9 and shown evidence of preserved cardiopulmonary function at these concentrations. Equipotent subanesthetic concentrations of anesthetic in neonatal rats measured using prevention of vocalization, rather than using equal fractions of MAC, may allow researchers to better optimize neonatal rodent models of anesthesia from birth to weaning.

Acknowledgments

The authors thank the kind assistance of Hiroko K. Solvang, Ph.D., Institute of Marine Research, Bergen, Norway, for help with R package code.

Supported by The Moulton Charitable Foundation and Sparks charity, London, United Kingdom.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Thoresen: Neonatal Neuroscience, School of Clinical Sciences, University of Bristol, Level D, St. Michael's Hospital, Southwell Street, Bristol, BS2 8EG, United Kingdom. marianne.thoresen@bristol.ac.uk.

References

1. Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, Sprung J, Weaver AL, Schroeder DR, Warner DO: Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics 2011; 128:e1053–61
2. Davidson AJ: Neurotoxicity and the need for anesthesia in the newborn: Does the emperor have no clothes? ANESTHESIOLOGY 2012; 116:507–9
3. Sanders RD, Hassell J, Davidson AJ, Robertson NJ, Ma D: Impact of anaesthetics and surgery on neurodevelopment: An update. Br J Anaesth 2013; 110(suppl 1):i53–72
4. Olsen EA, Brambrink AM: Anesthetic neurotoxicity in the newborn and infant. Curr Opin Anaesthesiol 2013; 26:535–42
5. Istaphanous GK, Loepke AW: General anesthetics and the developing brain. Curr Opin Anaesthesiol 2009; 22:368–73
6. DiMaggio C, Sun LS, Kakavouli A, Byrne MW, Li G: A retrospective cohort study of the association of anesthesia and hernia repair surgery with behavioral and developmental disorders in young children. J Neurosurg Anesthesiol 2009; 21:286–91
7. DiMaggio C, Sun LS, Li G: Early childhood exposure to anesthesia and risk of developmental and behavioral disorders in a sibling birth cohort. Anesth Analg 2011; 113:1143–51
8. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, Gleich SJ, Schroeder DR, Weaver AL, Warner DO: Early exposure to anesthesia and learning disabilities in a population-based birth cohort. ANESTHESIOLOGY 2009; 110:796–804
9. Ma D, Williamson P, Januszewski A, Nogaro MC, Hossain M, Ong LP, Shu Y, Franks NP, Maze M: Xenon mitigates isoflurane-induced neuronal apoptosis in the developing rodent brain. ANESTHESIOLOGY 2007; 106:746–53
10. Ramage TM, Chang FL, Shih J, Alvi RS, Quitoriano GR, Rau V, Barbour KC, Elphick SA, Kong CL, Tantoco NK, Ben-Tzur D, Kang H, McCreery MS, Huang P, Park A, Uy J, Rossi MJ, Zhao C, Di Geronimo RT, Stratmann G, Sall JW: Distinct long-term neurocognitive outcomes after equipotent sevoflurane or isoflurane anaesthesia in immature rats. Br J Anaesth 2013; 110(suppl 1):i39–46
11. Zhou X, Song FH, He W, Yang XY, Zhou ZB, Feng X, Zhou LH: Neonatal exposure to sevoflurane causes apoptosis and reduces nNOS protein expression in rat hippocampus. Mol Med Rep 2012; 6:543–6
12. Sanders RD, Xu J, Shu Y, Januszewski A, Halder S, Fidalgo A, Sun P, Hossain M, Ma D, Maze M: Dexmedetomidine attenuates isoflurane-induced neurocognitive impairment in neonatal rats. ANESTHESIOLOGY 2009; 110:1077–85
13. Liang G, Ward C, Peng J, Zhao Y, Huang B, Wei H: Isoflurane causes greater neurodegeneration than an equivalent exposure of sevoflurane in the developing brain of neonatal mice. ANESTHESIOLOGY 2010; 112:1325–34
14. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF: Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 2003; 23:876–82
15. Shih J, May LD, Gonzalez HE, Lee EW, Alvi RS, Sall JW, Rau V, Bickler PE, Lalchandani GR, Yusupova M, Woodward E, Kang H, Wilk AJ, Carlston CM, Mendoza MV, Guggenheim JN, Schaefer M, Rowe AM, Stratmann G: Delayed environmental enrichment reverses sevoflurane-induced memory impairment in rats. ANESTHESIOLOGY 2012; 116:586–602
16. Cattano D, Williamson P, Fukui K, Avidan M, Evers AS, Olney JW, Young C: Potential of xenon to induce or to protect against neuroapoptosis in the developing mouse brain. Can J Anaesth 2008; 55:429–36
17. Sabir H, Bishop S, Cohen N, Maes E, Liu X, Dingley J, Thoresen M: Neither xenon nor fentanyl induces neuroapoptosis in the newborn pig brain. ANESTHESIOLOGY 2013; 119:345–57
18. Istaphanous GK, Howard J, Nan X, Hughes EA, McCann JC, McAuliffe JJ, Danzer SC, Loepke AW: Comparison of the neuroapoptotic properties of equipotent anesthetic concentrations of desflurane, isoflurane, or sevoflurane in neonatal mice. ANESTHESIOLOGY 2011; 114:578–87
19. Kodama M, Satoh Y, Otsubo Y, Araki Y, Yonamine R, Masui K, Kazama T: Neonatal desflurane exposure induces more robust neuroapoptosis than do isoflurane and sevoflurane and impairs working memory. ANESTHESIOLOGY 2011; 115:979–91
20. Shu Y, Patel SM, Pac-Soo C, Fidalgo AR, Wan Y, Maze M, Ma D: Xenon pretreatment attenuates anesthetic-induced apoptosis in the developing brain in comparison with nitrous oxide and hypoxia. ANESTHESIOLOGY 2010; 113:360–8
21. Stratmann G, May LD, Sall JW, Alvi RS, Bell JS, Ormerod BK, Rau V, Hilton JF, Dai R, Lee MT, Visrodia KH, Ku B, Zusmer EJ, Guggenheim J, Firouzian A: Effect of hypercarbia and isoflurane on brain cell death and neurocognitive dysfunction in 7-day-old rats. ANESTHESIOLOGY 2009; 110:849–61
22. Cross DA: Multiples of minimal alveolar concentration of volatile agents are not necessarily equipotent. ANESTHESIOLOGY 2011; 114:221–2
23. de Jong RH, Eger EI II: MAC expanded: AD50 and AD95 values of common inhalation anesthetics in man. ANESTHESIOLOGY 1975; 42:384–9
24. Yang T, Zhuang L, Rei Fidalgo AM, Petrides E, Terrando N, Wu X, Sanders RD, Robertson NJ, Johnson MR, Maze M, Ma D: Xenon and sevoflurane provide analgesia during labor and fetal brain protection in a perinatal rat model of hypoxia-ischemia. PLoS One 2012; 7:e37020
25. Sanders RD, Ma D, Maze M: Xenon: Elemental anaesthesia in clinical practice. Br Med Bull 2004; 71:115–35
26. Groenink L, Verdouw PM, van Oorschot R, Olivier B: Models of anxiety: Ultrasonic vocalizations of isolated rat pups. Curr Protoc Pharmacol 2008; Chapter 5:Unit 5.18
27. Hashimoto H, Moritani N, Katou M, Nishiya T, Kromkhun P, Yokosuka M, Tanaka M, Saito TR: Ontogenetic changes of ultrasonic vocalizations emitted from infant rats. Exp Anim 2007; 56:315–8
28. Orliaguet G, Vivien B, Langeron O, Bouhemad B, Coriat P, Riou B: Minimum alveolar concentration of volatile anesthetics in rats during postnatal maturation. ANESTHESIOLOGY 2001; 95:734–9
29. Liu X, Dingley J, Elstad M, Scull-Brown E, Steen PA, Thoresen M: Minimum alveolar concentration (MAC) for sevoflurane and xenon at normothermia and hypothermia in newborn pigs. Acta Anaesthesiol Scand 2013; 57:646–53

30. Adrogue HJ, Rashad MN, Gorin AB, Yacoub J, Madias NE: Arteriovenous acid-base disparity in circulatory failure—Studies on mechanism. Am Journal Physiol 1989; 257:F1087–93
31. Kato R, Tachibana K, Nishimoto N, Hashimoto T, Uchida Y, Ito R, Tsuruga K, Takita K, Morimoto Y: Neonatal exposure to sevoflurane causes significant suppression of hippocampal long-term potentiation in postgrowth rats. Anesth Analg 2013; 117:1429–35
32. Eger EI II, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of anesthetic potency. Anesthesiology 1965; 26:756–63
33. Stratmann G, Sall JW, Eger EI II, Laster MJ, Bell JS, May LD, Eilers H, Krause M, Heusen Fv, Gonzalez HE: Increasing the duration of isoflurane anesthesia decreases the minimum alveolar anesthetic concentration in 7-day-old but not in 60-day-old rats. Anesth Analg 2009; 109:801–6
34. Quasha AL, Eger EI II, Tinker JH: Determination and applications of MAC. Anesthesiology 1980; 53:315–34
35. Malviya S, Lerman J: The blood/gas solubilities of sevoflurane, isoflurane, halothane, and serum constituent concentrations in neonates and adults. Anesthesiology 1990; 72:793–6
36. Goto T, Suwa K, Uezono S, Ichinose F, Uchiyama M, Morita S: The blood-gas partition coefficient of xenon may be lower than generally accepted. Br J Anaesth 1998; 80:255–6
37. Soares JH, Brosnan RJ, Fukushima FB, Hodges J, Liu H: Solubility of haloether anesthetics in human and animal blood. Anesthesiology 2012; 117:48–55
38. Kety SS: The physiological and physical factors governing the uptake of anesthetic gases by the body. Anesthesiology 1950; 11:517–26
39. Satas S, Haaland K, Thoresen M, Steen PA: MAC for halothane and isoflurane during normothermia and hypothermia in the newborn piglet. Acta Anaesthesiol Scand 1996; 40:452–6
40. Goto T, Nakata Y, Morita S: The minimum alveolar concentration of xenon in the elderly is sex-dependent. Anesthesiology 2002; 97:1129–32
41. Torbati D, Ramirez J, Hon E, Camacho MT, Sussmane JB, Raszynski A, Wolfsdorf J: Experimental critical care in rats: Gender differences in anesthesia, ventilation, and gas exchange. Crit Care Med 1999; 27:1878–8V4
42. Dixon W, Mood AM: A method for obtaining and analyzing sensitivity data. J Am Statist Assoc 1948; 43:109–26
43. Pace NL, Stylianou MP: Advances in and limitations of up-and-down methodology: A précis of clinical use, study design, and dose estimation in anesthesia research. Anesthesiology 2007; 107:144–52
44. Sanders RD, Patel N, Hossain M, Ma D, Maze M: Isoflurane exerts antinociceptive and hypnotic properties at all ages in Fischer rats. Br J Anaesth 2005; 95:393–9
45. Eger EI II, Johnson BH, Weiskopf RB, Holmes MA, Yasuda N, Targ A, Rampil IJ: Minimum alveolar concentration of I-653 and isoflurane in pigs: Definition of a supramaximal stimulus. Anesth Analg 1988; 67:1174–6
46. Davidson AJ, Wong A, Knottenbelt G, Sheppard S, Donath S, Frawley G: MAC-awake of sevoflurane in children. Paediatr Anaesth 2008; 18:702–7
47. Zbinden AM, Maggiorini M, Petersen-Felix S, Lauber R, Thomson DA, Minder CE: Anesthetic depth defined using multiple noxious stimuli during isoflurane/oxygen anesthesia. I. Motor reactions. Anesthesiology 1994; 80:253–60