BJA

British Journal of Anaesthesia, 125 (3): 308–320 (2020)

doi: 10.1016/j.bja.2020.05.031
Advance Access Publication Date: 11 July 2020
Neuroscience and Neuroanaesthesia

Resistance to state transitions in responsiveness is differentially modulated by different volatile anaesthetics in male mice

Andrzej Z. Wasilczuk[1,2], Benjamin A. Harrison[1], Paula Kwasniewska[1], Bo Ku[1], Max B. Kelz[1,2,3], Andrew R. McKinstry-Wu[1,*] and Alex Proekt[1,3]

[1]Department of Anaesthesiology and Critical Care, Perelman School of Medicine, Philadelphia, PA, USA, [2]Department of Bioengineering, School of Engineering and Applied Science, Philadelphia, PA, USA and [3]Chronobiology and Sleep Institute, University of Pennsylvania, Philadelphia, PA, USA

*Corresponding author. E-mail: andrew.mckinstry@pennmedicine.upenn.edu

This article is accompanied by an editorial: Advances in precision anaesthesia may be found by testing our resistance to change by Eagleman & MacIver *Br J Anaesth* 2020:125:235–237, doi: 10.1016/j.bja.2020.06.007

Abstract

Background: Recent studies point to a fundamental distinction between population-based and individual-based anaesthetic pharmacology. At the population level, anaesthetic potency is defined as the relationship between drug concentration and the likelihood of response to a stimulus. At the individual level, even when the anaesthetic concentration is held constant, fluctuations between the responsive and unresponsive states are observed. Notably, these spontaneous fluctuations exhibit resistance to state transitions R_{st}. Therefore, the response probability in each individual depends not just upon the drug concentration, but also upon responses to previous stimuli. Here, we hypothesise that R_{st} is distinct from drug potency and is differentially modulated by different anaesthetics.
Methods: Adult (14–24 weeks old) C57BL/6J male mice (n=60) were subjected to repeated righting reflex (RR) assays at equipotent steady-state concentrations of isoflurane (0.6 vol%), sevoflurane (1.0 vol%), and halothane (0.4 vol%).
Results: Fluctuations in RR were observed for all tested anaesthetics. Analysis of these fluctuations revealed that R_{st} was differentially modulated by different anaesthetics (F[2, 56.01]=49.59; P<0.0001). Fluctuations in RR were modelled using a stochastic dynamical system. This analysis confirmed that the amount of noise that drives behavioural state transitions depends on the anaesthetic agent (F[2, 42.86]=16.72; P<0.0001).
Conclusions: Whilst equipotent doses of distinct anaesthetics produce comparable population response probabilities, they engage dramatically different dynamics in each individual animal. This manifests as a differential aggregate propensity to exhibit state transitions. Thus, resistance to state transitions is a fundamentally distinct, novel measure of individualised anaesthetic pharmacology.

Keywords: general anaesthesia; individual-based pharmacology; inhalational anaesthetics; population-based pharmacology; responsiveness; state transitions

Received: January 31, 2020; **Accepted:** 3 May 2020

Editor's key points

- Fluctuations in the state of responsiveness at fixed anaesthetic concentrations exhibit resistance to state transitions, such that knowing just the drug concentration is insufficient to predict the probability of response.
- Resistance to state transitions could depend solely on the effective concentration of an anaesthetic, or be independent of drug potency and differentially modulated by different anaesthetics.
- To distinguish between these possibilities, the authors exposed male mice to equipotent concentrations of three volatile anaesthetics and quantified the degree of resistance to state transitions.
- At equipotent concentrations, resistance to state transitions depended on the anaesthetic agent, and is thus a dissociable feature of anaesthetic pharmacology that has been obscured by conventional population-based measures of drug efficacy.
- Even when the population-based measures, such as effective concentration, are the same, the state of anaesthesia induced with different volatile anaesthetics is associated with distinct dynamics at the level of individual mice, which has implications for defining depth of anaesthesia.

Anaesthesiologists aim to deliver an appropriate anaesthetic dose, such that each individual patient remains unconscious throughout the procedure and recovers consciousness swiftly and uneventfully once the procedure concludes. To accomplish this goal, clinicians currently rely on fundamental principles of pharmacology. The relationship between anaesthetic concentration and its effect in a target population is quantified by constructing a concentration–response curve.[1] A classic measure of inhaled anaesthetic pharmacology, the minimal alveolar concentration (MAC), is defined as the anaesthetic concentration at which the likelihood of a response to a painful stimulus is 50%.[2–4] Other anaesthetic endpoints, such as MAC-awake, have been defined as the concentration at which 50% of subjects respond to a verbal command.[2] In rodent research, the ability of an animal placed on its back to turn over onto its paws, the righting reflex (RR), has been used as a proxy for the state of wakefulness.[5,6] Across many mechanistically distinct anaesthetics, the effective concentrations for 50% of subjects (EC_{50}) for loss of RR in rodents and MAC-awake in humans are closely correlated.[7]

Current measures of anaesthetic efficacy, including MAC-awake, define the response at the level of the population rather than of the individual. To apply population-based measures to an individual, it is typically assumed that the individual's probability of wakefulness mirrors that of the population for every anaesthetic concentration. At the EC_{50}, for instance, each individual is expected to be responsive on 50% of trials. However, this line of reasoning reveals an essential shortcoming of population-based measures of anaesthetic potency. It is not clear which 50% of stimuli will trigger a response in each individual.

To illustrate this limitation, consider two hypothetical examples of individuals at EC_{50} (Fig. 1). With respect to the across-trial probability of wakefulness, these individuals are the same and each responds to 50% of stimuli. Yet, the overall righting probability does not directly inform the underlying dynamics of the responses in the two individuals. The first individual randomly fluctuates between responsive and unresponsive states (Fig. 1a). Thus, on average, the state switches approximately every other trial. In contrast, the fluctuations in the second individual are more predictable. The second individual tends to dwell in its previously observed state (Fig. 1b). We define this inertial tendency 'resistance to state transitions' R_{st}. One manifestation of this inertia is that the probability of unresponsiveness is significantly higher if the individual was unresponsive on the previous trial. The key point is that, with respect to the population-based measures of anaesthetic potency, the two individuals in Figure 1 are identical. Thus, population-based measures of anaesthetic pharmacology cannot account for the tendency to resist state transitions.

The scenario illustrated in Figure 1 is not just a hypothetical possibility. In a study of the fluctuations in responsiveness in mice and in zebrafish exposed to isoflurane or propofol,[8] fluctuations in the state of wakefulness under fixed anaesthetic concentrations exhibited resistance to state transitions. Thus, knowing just the drug concentration is insufficient to predict the probability of response. To more precisely determine the probability of response, an

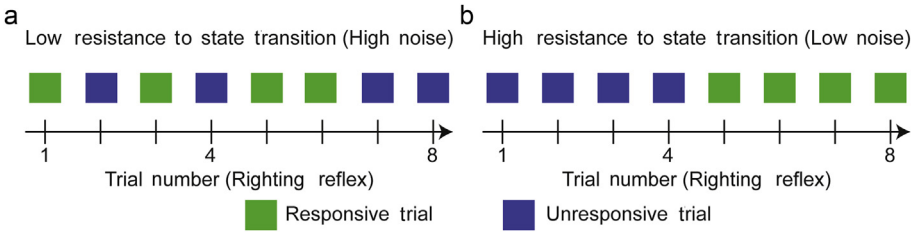

Fig 1. Identical anaesthetic potencies can be obtained with varying degrees of resistance to state transitions R_{st}. Schematic representations of performance of two individuals on eight consecutive righting reflex trials at a fixed anaesthetic concentration. (a) Low R_{st} results in a system that changes between states randomly. (b) High R_{st} results in a system that tends to persist in a given state. Although the two systems differ in their R_{st}, both produce an identical measure of anaesthetic potency, given by the proportion of responsive trials observed compared with the total amount of observed trials (50%).

individual's previous state of responsiveness must also be considered.

Here, we further characterise resistance to state transitions. One possibility is that resistance to state transitions depends solely on the effective concentration of an anaesthetic. If so, then different anaesthetic agents administered at equipotent concentrations will be associated with identical resistance to state transitions. Alternatively, it is possible that resistance to state transitions is independent of drug potency and can be differentially modulated by different anaesthetics. To distinguish between these possibilities, we exposed mice to equipotent concentrations of distinct volatile anaesthetics and quantified the degree of resistance to state transitions. We show that, at equipotent concentrations, the degree of resistance to state transitions depends strongly on the anaesthetic agent. Thus, resistance to state transitions is a dissociable feature of anaesthetic pharmacology that has been obscured by conventional population-based measures of drug efficacy. Our results illustrate that, even when the population-based measures, such as effective concentration, are the same, the state of anaesthesia induced with different volatile anaesthetics is associated with distinct dynamics at the level of individual mice.

Methods

Animals

Studies were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania and were conducted in accordance with the National Institutes of Health guidelines. Inbred male wild-type C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) aged 16–24 weeks (n=60) were used in RR behavioural assays.

Righting reflex assay during volatile anaesthetic exposure

Before anaesthetic exposure, the mice were acclimatised to gas-tight temperature-controlled 200 ml experimental chambers, as described.[9] The RR protocol was performed, as described.[8] In brief, separate cohorts of 20 mice were exposed to either isoflurane 0.6 vol%, sevoflurane 1.0 vol%, or halothane 0.4 vol% in 100% oxygen on four separate occasions over the course of 1 month. These drug concentrations were determined in preliminary experiments and chosen such that they are close to the EC_{50} and such that the overall righting probability was statistically indistinguishable amongst the

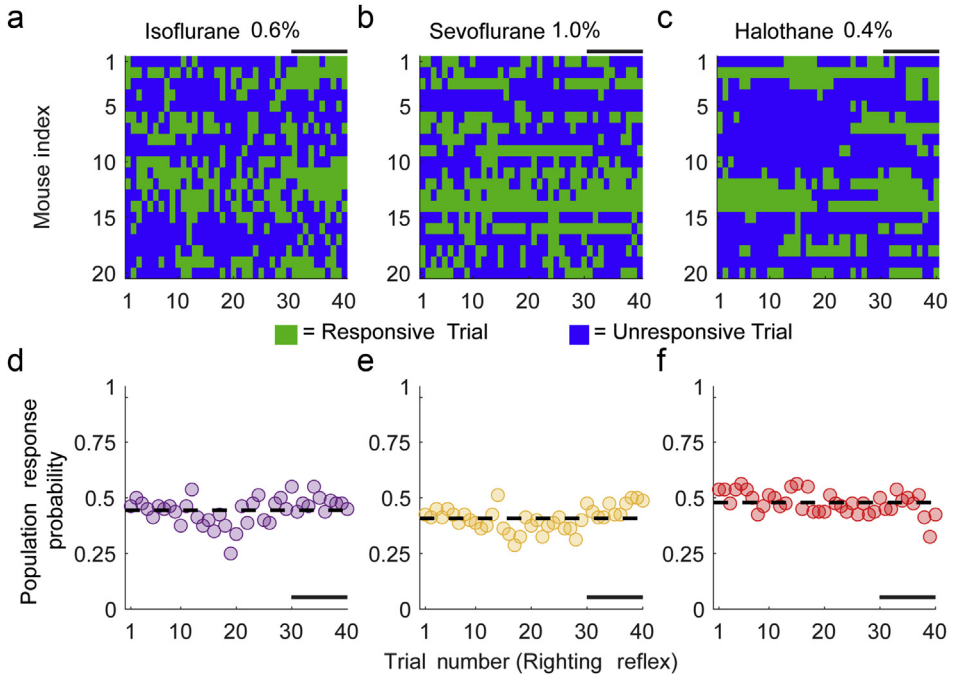

Fig 2. Steady-state response probability at the population level is associated with spontaneous behavioural fluctuations at the individual level. Groups of 20 mice were exposed to either isoflurane 0.6%, sevoflurane 1.0%, or halothane 0.4% on four separate occasions. The duration of each anaesthetic exposure was 4 h, with righting reflex assessments made every 3 min during the final 2 h of each exposure (40 trials per exposure). One experimental exposure from (a) isoflurane 0.6%, (b) sevoflurane 1.0%, and (c) halothane 0.4% is shown. Green squares depict trials, in which the animal successfully righted itself. Blue squares depict trials, in which the animal failed to right itself. Fluctuations between the responsive and unresponsive states were observed in each mouse and for every anaesthetic. At the population level, anaesthetic potency, defined as the proportion of unresponsive individuals on a given trial, remained constant across trials for all anaesthetics. (d) Isoflurane 0.6%: 0.44 (0.06) mean [standard deviation], dotted line. (e) Sevoflurane 1.0%: 0.41 (0.05). (f) Halothane 0.4%: 0.48 (0.05). Response probability was approximately constant across trials under isoflurane (R^2=4.4×10^{-16}; P=1), sevoflurane (R^2=4.2×10^{-16}; P=1), and halothane (R^2=1.4×10^{-15}; P=1), as measured by Pearson's R correlation between trial number and response probability. Scale bars represent 30 min.

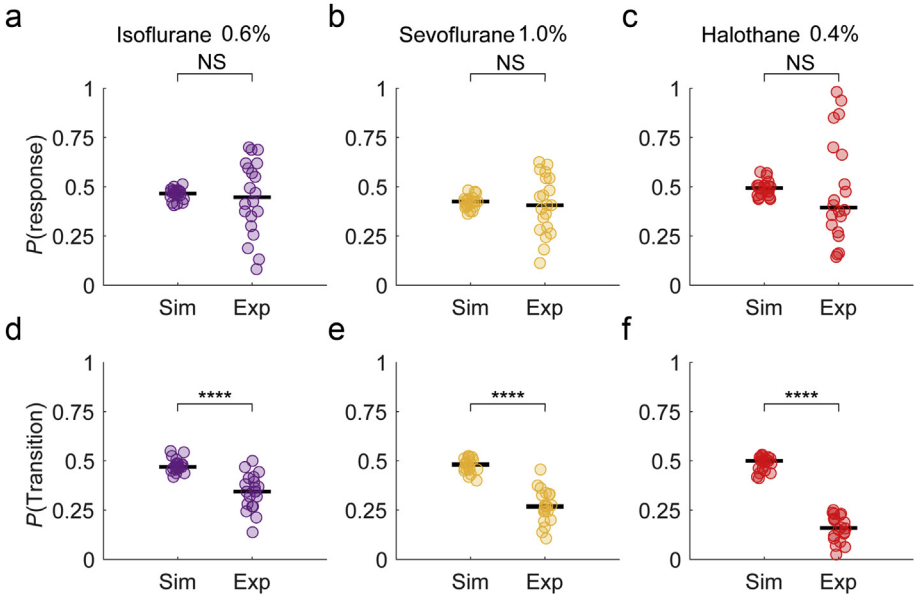

Fig 3. Righting probability depends on previous behavioural state. Bernoulli process simulations consisting of the same number of trials as experimental data sets were computed for each anaesthetic based on the experimental population response probabilities from Figure 2. Individual response probability, the proportion of responsive trials, was not significantly different between simulations and experimental data for (a) isoflurane (median experimental=0.45; simulated=0.47; U=194; P=0.88), (b) sevoflurane (median experimental=0.41; simulated=0.42; U=190; P=0.79), or (c) halothane (median experimental=0.4; simulated=0.49; U=144; P=0.13). However, mice exposed to (d) isoflurane (median experimental=0.34; simulated=0.47; U=29; P<0.0001), (e) sevoflurane (median experimental=0.27; simulated=0.48; U=5.5; P<0.0001), or (f) halothane (median experimental=0.16; simulated=0.5; U=0; P<0.0001) all had fewer state transitions than Bernoulli simulations predict. Statistical significance is shown by ****P<0.0001. All comparisons performed using Mann–Whitney U-test. Circles show individual points; black lines show medians. Exp, experimental; Sim, simulated.

different anaesthetics. A fresh gas flow of one-volume turnover-per-minute exchange rate was used to assure rapid drug equilibration, with 4 h anaesthetic exposures begun between ZT12-14. Righting reflex assessments were performed every 3 min during the final 2 h of each 4 h anaesthetic exposure. The first 2 h of drug exposure ensured anaesthetic equilibration. Each RR trial consisted of two rotations of the chamber performed in rapid succession. An animal was considered to be responsive if it was able to return to an upright posture on both chamber rotations. All other outcomes (righting on zero or on just one rotation) were defined as unresponsive. Chamber anaesthetic concentration was measured and confirmed to be

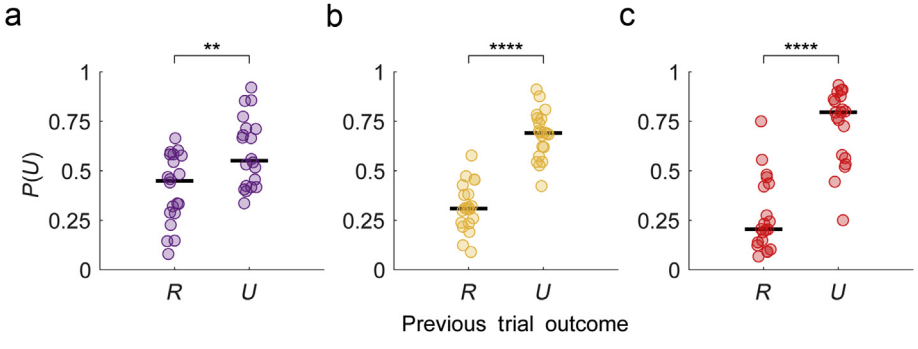

Fig 4. Probability of future response depends on the previous state of the animal. Probability of unresponsiveness $P(U)$ was higher if the animal was unresponsive on the previous trial for all three anaesthetics. (a) Isoflurane median $P(U)$ previously responsive=0.45; median $P(U)$ previously unresponsive=0.55; U=103; P=0.008. (b) Sevoflurane median $P(U)$ previously responsive=0.31; median $P(U)$ previously unresponsive=0.69; U=9; P<0.0001. (c) Halothane median $P(U)$ previously responsive=0.20; median $P(U)$ previously unresponsive=0.80; U=19; P<0.0001. Circles show individual points; black lines show medians. Statistical significance shown by **P<0.01; ****P<0.0001. All pairwise comparisons performed using Mann–Whitney U-test.

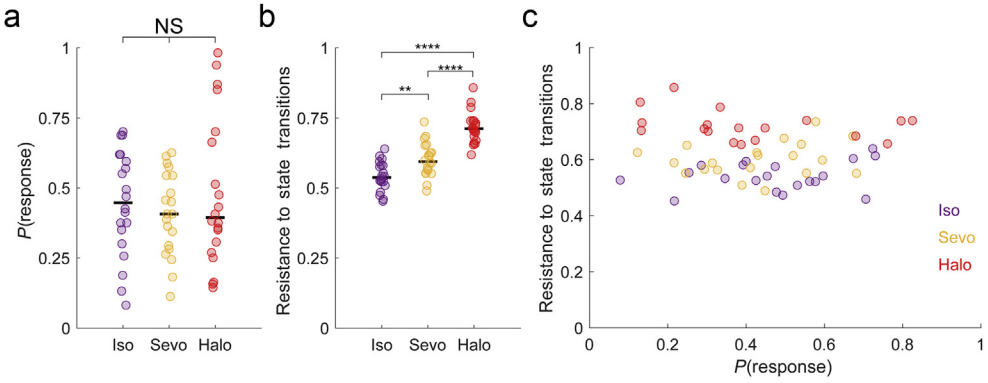

Fig 5. Resistance to state transitions is distinct from anaesthetic potency and altered by different anaesthetics. (a) The mean response probability was not significantly different across mice exposed to isoflurane, sevoflurane, or halothane (F[2, 46.30]=0.5915; P=0.5576). (b) The mean resistance to state transitions R_{st} was significantly different across mice exposed to isoflurane, sevoflurane, or halothane (F[2, 56.01]=49.59; P<0.0001) *post hoc* comparisons isoflurane *vs* sevoflurane (0.54 [0.05] *vs* 0.60 [0.06]; mean [standard deviation {SD}]; P=0.0062), sevoflurane *vs* halothane (0.60 [0.06] *vs* 0.72 [0.06]; mean [SD]; P<0.0001), and isoflurane *vs* halothane (0.54 [0.05] *vs* 0.72 [0.06]; mean [SD]; P<0.0001). *Post hoc* comparisons performed using Dunnett's multiple comparisons test. (c) Pearson's correlation coefficients between individual response probability and R_{st} did not reach statistical significance for any anaesthetic (isoflurane R^2=0.23, P=0.36; sevoflurane R^2=0.25, P=0.27; halothane R^2=−0.38, P=0.1). Statistical significance shown by **P<0.01; ****P<0.0001. Statistical comparisons performed using parametric analysis of variance (ANOVA) without assuming equal variance (Brown–Forsythe ANOVA). Halo, halothane; Iso, isoflurane; Sevo, sevoflurane.

constant throughout the experiment using a Riken FI-21 refractometer (A.M. Bickford, Wales Center, NY, USA). Isoflurane experiments have been described[8] and are shown here for the purposes of comparison with the other anaesthetics. Analysis of isoflurane data shown in Figures 5 and 6 has not been reported before.

Transition probability matrix estimation

Markov matrix modelling is a simple and convenient method for describing fluctuations in RR. Markov processes relate the current state of a system to the previous state of a system using a transition probability matrix (TPM). In the case of the binary responses (e.g. the RR assay), the probability of the responsive R or unresponsive U state can be modelled using the following Markov matrix M:

$$M = \begin{bmatrix} P(R_t|R_{t-1}) & P(U_t|R_{t-1}) \\ P(R_t|U_{t-1}) & P(U_t|U_{t-1}) \end{bmatrix}$$

M consists of four conditional probabilities in the form of $P(I_t|J_{t-1})$. This notation is expressed henceforth as $P(I|J)$ for simplicity, and reflects the probability that an animal is in state I (responsive or unresponsive) given that it was in state J (responsive or unresponsive) on the previous trial. Thus, $P(R|R)$ is the probability of being responsive on two consecutive trials, $P(U|U)$ is the probability of staying unresponsive on two consecutive trials, $P(U|R)$ is the probability of failing to right given a successful RR on the previous trial, and $P(R|U)$ is the probability of a successful RR given a failed response on the previous trial. Note that the sum of conditional probabilities in each row is exactly 1. Thus, M is completely specified by computing the two diagonal elements $P(U|U)$ and $P(R|R)$. Similar to previous work,[8] $P(U|U)$ and $P(R|R)$ were estimated empirically for each individual on the basis of four exposures each consisting of 40 RR trials (160 RR trials total).

Comparison of experimental data with simulations

To examine whether there is a state dependence on the observed behavioural fluctuations, simulations of a Bernoulli process were computed. Bernoulli process is a special case of the TPM M from the Transition probability matrix estimation section, but with a special condition of state independence. In other words, a Bernoulli process assumes that the probability of righting is independent of the previous state of the animal, $P(R|R)=P(R|U)$. Consequently, the TPM for a Bernoulli process, M_B, can be fully defined by knowing the fraction of responsive trials:

$$M_B = \begin{bmatrix} P(R) & P(U) \\ P(R) & P(U) \end{bmatrix}$$

M_B was constructed separately from the mean population response probability for isoflurane, sevoflurane, and halothane. Note that M_B mathematically expresses the assumption that the probability of response is solely dependent on anaesthetic concentration. For each anaesthetic, 20 simulations consisting of 160 trials were computed using M_B. State transition probabilities were then compared between experimental and simulated data sets. State transition probability was computed as

$$P(\text{Transition}) = \frac{P(U|R) + P(R|U)}{P(R|R) + P(U|R) + P(R|U) + P(U|U)}$$

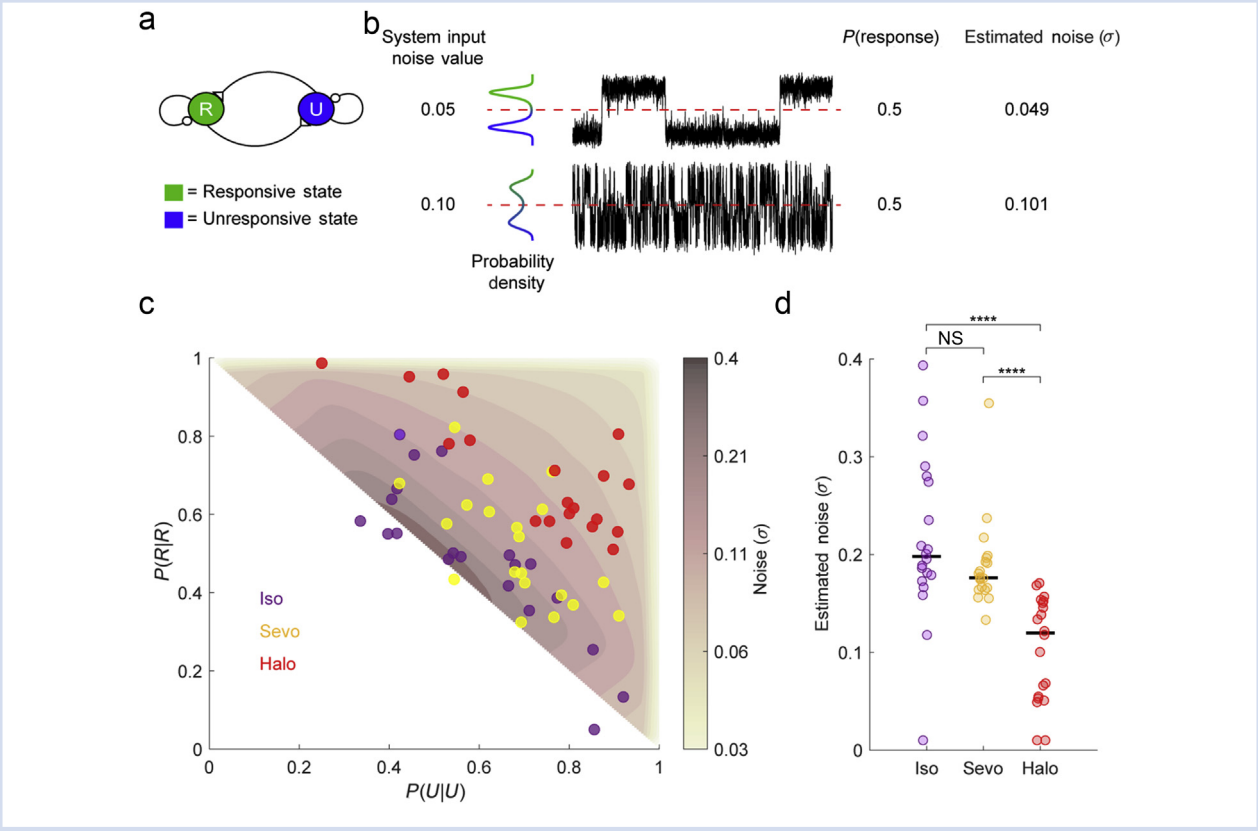

Fig 6. Noise driving state transitions is dissociable from anaesthetic potency and differs amongst equipotent anaesthetics. (a) A network composed of mutually inhibitory (and self-excitatory) neuronal populations. (b) Simulation of neuronal dynamics under lower-noise (top trace) and higher-noise (bottom trace) conditions. Red dashed line shows the threshold used for binarisation of dynamics into the responsive and unresponsive states. Distribution of states in each simulation is shown on the margin. Noise does not strongly affect the probability of being responsive or unresponsive, but affects the frequency of state switching. Noise recovered from the simulations using our fitting procedure gives a precise and unbiased estimate of the noise driving the fluctuations. (c) Neuronal dynamics model simulations performed by parametrically varying noise (shading) are plotted in the parameter plane of the two-state transition probability matrix (TPM). Because the neuronal dynamics model gives rise to state stabilisation, no simulations are found in the bottom triangle of the plane. Each point corresponds to the TPM fitted independently to each mouse (colour shows anaesthetic agent). (d) Noise estimated from behavioural fluctuations for isoflurane, sevoflurane, and halothane. (H=30.09; P<0.0001); non-parametric Kruskal–Wallis analysis of variance; *post hoc* comparisons isoflurane *vs* sevoflurane (P=0.84), isoflurane *vs* halothane (P<0.0001), sevoflurane *vs* halothane (P=0.0001); median σ isoflurane 0.20; sevoflurane 0.18; halothane 0.12. *Post hoc* comparisons performed using Dunnett's multiple comparisons test. Statistical significance shown by $^{****}P \leq 0.0001$. Halo, halothane; Iso, isoflurane; Sevo, sevoflurane.

As an additional test to assess state dependence, the probabilities of staying unresponsive, $P(U|U)$, were compared with the probabilities of becoming unresponsive, $P(R|U)$, across individuals for each anaesthetic tested. Note that these conditional probabilities in the Bernoulli process case are identical ($P(U)$).

Determination of resistance to state transitions

The 2×2 TPM is fully determined by specifying $P(R|R)$ and $P(U|U)$, the probabilities that an animal remains in the responsive or unresponsive state on two consecutive trials. Adding these two values together results in a metric of the propensity to stay in the same state, or the resistance to state transitions R_{st}. We quantify resistance to state transitions as

$$R_{st} = \frac{P(R|R) + P(U|U)}{2}$$

The normalisation is used to assure that R_{st} varies between 0 and 1. Zero indicates a system that never stays in the same state, and 1 indicates a system that never transitions from its initial state. Thus, R_{st} value of 1 is only attainable when fixed anaesthetic concentration reliably leads to either loss or preservation of the RR in each animal.

Fitting behavioural fluctuations to a stochastic dynamical system

Markov modelling of fluctuations in responsiveness can be seen as a discrete approximation to a continuous dynamical system. To fit the behavioural fluctuations to a continuous system, we adapt the mathematical formalism described previously.[8,10] The purpose of this procedure is to estimate the amount of noise that drives fluctuations between the responsive and unresponsive states. Briefly, behavioural

fluctuations in RR can be approximated by diffusion on an energy landscape that contains two wells corresponding to the responsive and unresponsive states. The overall dynamics of the system are given by

$$\frac{dx}{dt} = -D\frac{\delta E(x)}{\delta x}\bigg|_{ec} + \varepsilon \quad (1)$$

where x is the state of the system, D is the diffusion constant (assumed to be 1 for mathematical convenience), E is the energy function, ec is the effective anaesthetic concentration at which the partial derivative is evaluated, and ε is noise modelled as a Gaussian process with mean zero and standard deviation σ; σ controls the amount of noise. Following previous work,[8] we define the energy function as

$$E(x, ec) = x^2\left(\frac{x^2}{2} - 2\right) + ec(x-1)^2 + (1-ec)(x+1)^2 \quad (2)$$

This energy function forms two local energy minima at $(x \approx -1; x \approx 1)$ corresponding to the unresponsive and responsive states, respectively, and a local energy maximum at $x \approx 0$. With increasing effective concentration ec, the energy minimum corresponding to the unresponsive state is deepened and the well corresponding to the responsive state becomes shallower. To simulate the continuous system, equation (1) is integrated using the standard Euler method. The simulated dynamics are then binarised into 'responsive' and 'unresponsive' states using $x = 0$ (local energy maximum) as threshold. The resultant binary time series is then fit to a Markov process using the same method as for the RR assays. This predicted Markov model is specified by two parameters: $\{P(U|U)_{pred}$ and $P(R|R)_{pred}\}$. The effective concentration ec for the simulations was fixed to the overall probability of successful righting computed across trials in each individual. This leaves only one free parameter: the noise level σ. To approximate the amount of noise present in the behavioural observations, we find σ such that the Euclidean distance between the experimentally observed Markov model given by a point $\{P(U|U)_{exp}, P(R|R)_{exp}\}$ and the predicted Markov model given by $\{P(U|U)_{pred}, P(R|R)_{pred}\}$ is minimised. This constrained minimisation was implemented in MATLAB (MathWorks, Natick, MA, USA) using particle swarm optimisation algorithm. Using this strategy, we estimated the amount of noise present in each individual exposed to a given anaesthetic. The accuracy of the fitting procedure was demonstrated by constructing synthetic time series using equation (1) with pre-specified σ and recovering the correct σ from the fitting procedure.

Statistical analyses

Analyses were performed using custom code written in MATLAB implementing the statistics, machine learning, and global optimisation toolboxes. Statistical comparisons were performed using Prism 8.3 (GraphPad Software, San Diego, CA, USA). Pearson's correlation coefficient was used to confirm steady-state population response probabilities. R^2 values approaching zero indicate constant population response probabilities across trials. Statistical comparisons between experimental and simulated data sets were performed using the Mann–Whitney U-test. Testing for equal variances across experimental *vs* simulated response probabilities and experimental response probabilities *vs* resistances to state transitions was performed using the Brown–Forsythe test for equal variances. After verifying that the experimental observations do not deviate significantly from normal distribution (Anderson–Darling test), parametric analysis of variance (ANOVA) without assuming equal variances (Brown–Forsythe ANOVA) test was used for comparing results across different anaesthetics. If experimental observations deviated significantly from a normal distribution, the non-parametric Kruskal–Wallis test was used to compare results across different anaesthetics. $P<0.05$ was considered statistically significant for all comparisons.

Results

Individuals fluctuate between responsive and unresponsive states at steady-state concentrations

Separate cohorts of 20 mice each were exposed to isoflurane 0.6%, sevoflurane 1.0%, or halothane 0.4%. All data for isoflurane in Figures 2–4 are from experiments previously analysed,[8] and are plotted here for the sake of comparison with other anaesthetics, which have not been previously reported. Figure 2 shows the results of 40 consecutive trials of RR assay performed every 3 min after a 2 h equilibration period (Fig. 2a–c). The population-level response probability for animals remained constant for all three anaesthetics (Fig. 2d–f). Whilst the population-level righting probability did not change over time, the ability of each mouse to successfully right itself did fluctuate from trial to trial. Fluctuations are observed for each mouse and each anaesthetic agent at constant anaesthetic concentration. Animals exposed to halothane appeared to switch states less frequently than those exposed to isoflurane.

The simplest model of behavioural fluctuations in Figure 2 is that the effective concentration of an anaesthetic sets the probability of successful righting as assumed in population-based anaesthetic pharmacology.[11] A clear prediction of this model is that the probability of successful righting should be the same for all trials of the RR, be independent of the previous state of the animal and of the anaesthetic agent, so long as the effective concentration is the same. For instance, at EC_{50}, we expect the probability of righting to be 50% on each trial. Mathematically, this corresponds to modelling trial-to-trial fluctuations in Figure 2 as a Bernoulli process. Thus, we compared the trial-to-trial fluctuations observed for isoflurane, sevoflurane, and halothane anaesthesia with those observed in a Bernoulli process constrained to have the same population-level righting probability. The overall righting probability in experiments and simulations was not statistically different for each anaesthetic (Fig. 3a–c). In contrast, switches between the responsive and unresponsive states happened significantly less frequently for all three anaesthetics than in those generated by a Bernoulli process (Fig. 3d–f). Thus, a Bernoulli process does not adequately capture the observed behavioural fluctuations.

To further expose the limitation of a Bernoulli process, we directly compared the probability of failure on the RR trials preceded by either success or failure. The probability of failure on the RR assay was significantly larger if the animal failed to right itself on the previous trial (Fig. 4a–c). This clearly illustrates that, whilst the effective concentration is an indispensable measure of population-based anaesthetic pharmacology, it is not sufficient to explain the trial-to-trial fluctuations observed in individuals. This is because the

effective concentration fails to account for the fact that righting probability depends not just on drug concentration, but also on the ability of the animal to successfully right itself on the preceding trial.

Degree of resistance to state transitions differs between equipotent doses of distinct anaesthetics

It is generally assumed that the probability of successful response to the stimulus ought to be independent of the identity of the anaesthetic agent, so long as the effective concentration is the same. This assumption underlies the concept of equipotency. In contrast to this prediction of population-based pharmacology, results in Figure 2 suggest that animals exposed to halothane switched between the responsive and unresponsive states less frequently than those exposed to other anaesthetics. Indeed, the probability of the state switch was different amongst the anaesthetic agents (F [2, 57]=25.89; P<0.0001). *Post hoc* pairwise comparisons revealed statistically significant differences between all pairs of anaesthetics (isoflurane *vs* sevoflurane, P=0.018; isoflurane *vs* halothane, P<0.0001; sevoflurane *vs* halothane, P<0.0001).

To further quantify the propensity to switch between the responsive and unresponsive states, we quantitatively defined resistance to state transitions R_{st}. This is accomplished by quantifying the propensity of each animal to stay in its previously observed state. Complementary analysis that quantifies the propensity of the system to switch from the responsive to the unresponsive state and *vice versa* is shown in Supplementary Table S1. We then estimated R_{st} for different anaesthetics. Figure 5 shows the distribution of R_{st} observed for isoflurane, sevoflurane, and halothane. Whilst there were no statistically significant differences in the mean righting probability (Fig. 5a), R_{st} depended strongly on the anaesthetic agent (Fig. 5b). This finding is consistent with the observation that animals exposed to halothane exhibited fewer switches between the responsive and unresponsive states (Fig. 2). We next determined whether, at the level of an individual, probability of righting and R_{st} are correlated. Results in Figure 5c fail to reveal significant correlation between these two measures of anaesthetic responses. Thus, resistance to state transitions is distinct from anaesthetic sensitivity and is differentially affected by different anaesthetics at equipotent concentrations. This further illustrates the limitation of the concept of equipotency. Even at equipotent concentrations, different anaesthetics engage different dynamics at the level of an individual.

In contrast to highly variable sensitivity to anaesthetics, even amongst genetically identical age-matched mice,[12] the tendency to resist state transitions was tightly controlled amongst individuals (isoflurane P [response] *vs* R_{st}, F[1, 38]=23.72, P<0.0001; sevoflurane, F[1, 38]=13.03, P<0.001; halothane, F[1, 38]=15.03, P<0.0001; Brown–Forsythe test for equal variance). Thus, resistance to state transitions is tightly biologically controlled.

Noise driving state transitions is modified by different anaesthetics

Until now, we have inferred resistance to state transitions by empirically estimating transition probabilities between states of responsiveness and unresponsiveness from behavioural data. In what follows, we more rigorously define the tendency of the system to maintain its previous state using a mathematical model of neuronal dynamics. As proposed,[8,10] the simplest model of spontaneous state switching at a constant drug concentration is that of a diffusion on an energy landscape with two energy wells. These diffusion models can be used to model the activity of neuronal networks composed of mutually inhibitory neuronal populations[13] (Fig. 6a). Such neuronal architecture is thought to underlie switches between states of sleep and wakefulness.[14] Addition of noise to the system results in stochastic switches between network states, where one or the other neuronal population temporarily dominates network activity (Fig. 6b). Increasing the amount of noise does not fundamentally alter the distribution of states (i.e. response probability). Rather, increasing noise results in increase in the frequency of state switching[8,10,13] (Fig. 6b). Thus, resistance to state transitions at its most fundamental level is related to the amount of noise required to recapitulate the observed behavioural dynamics.

Using this simple intuition, we explicitly fitted the observed behavioural fluctuations at a fixed drug concentration to a neuronal dynamics model. We first computed the transition probability matrices that correspond to different combinations of drug sensitivity and noise. The results of these simulations are plotted in the parameter space of the TPM spanned by $P(U|U)$ and $P(R|R)$, the probability of staying in the unresponsive and responsive states, respectively. The amount of noise needed to give rise to a TPM is shown by shading in Figure 6c. Because of stability afforded by the energy wells, the behavioural fluctuations given by the model are predicted to reside exclusively in the upper triangle of the parameter plane (Fig. 6c, shaded area). Consistent with this prediction, behavioural observations in most animals are confined to the upper triangle of the plane (circles). Low-noise systems tend to the perimeter of the plane (tan colours). This is because under low-noise conditions, the system tends to stay in its previous state and state transitions are rare. In contrast, systems with high noise tend towards the centre of the plane (brown colours). This behaviour arises because, under high-noise conditions, the system becomes independent of the energy landscape and switches state every other trial on average. Note that behavioural fluctuations observed under halothane are located closer to the perimeter of the plane, whilst those observed under isoflurane are found close to the main diagonal.

By fitting the neuronal dynamics model to the observed fluctuations in RR, we estimated the amount of noise driving behavioural fluctuations under different anaesthetic conditions. This analysis revealed statistically significant differences in the amount of noise driving behavioural fluctuations for different anaesthetics. This provides further independent confirmation that resistance to state transitions is fundamentally distinct from drug potency and is differentially modulated by different anaesthetics.

Discussion

Fluctuations in the state of responsiveness in individual animals were observed even when volatile anaesthetic concentration was held constant. These fluctuations were reliably observed for three different volatile anaesthetics. These fluctuations in responsiveness were not random. Rather, for all three volatile anaesthetics, fluctuations in responsiveness exhibited resistance to state transitions. Resistance to state transitions was fundamentally distinct from anaesthetic potency. Several lines of reasoning support this last claim.

Anaesthetic potency is traditionally defined at the level of a population. In contrast, resistance to state transitions is an individual-based measure of anaesthetic pharmacology, and is obscured by averaging response probabilities across trials and across individuals. No significant correlations were observed between individual sensitivity to an anaesthetic and resistance to state transitions. Most importantly, however, we show that, even when anaesthetic agents were administered in equipotent doses, resistance to state transitions varies depending on the anaesthetic agent.

These findings expose a fundamental distinction between population-based and individual-based anaesthetic pharmacology. At the level of the population, anaesthetic effects are described using a smooth concentration–response curve.[1,4,7,11,15,16] Whilst many different anaesthetic endpoints, such as MAC,[2,3] MAC-awake,[2,17] MAC-amnestic,[18,19] and others, have been defined, all share one thing in common: an individual responses collapses to a binary outcome: a given individual either does or does not respond to a given stimulus.[20] The distinction between binary responses of individuals, on the one hand, and the graded population-level responses, on the other, has been reconciled by invoking individual differences in anaesthetic sensitivity.[11,20] One interpretation of this model is that, at the EC_{50}, 50% of the individuals will be consistently unresponsive, whilst the other, more resistant 50% of individuals, will consistently respond to stimuli. Alternatively, it is possible that each individual would respond to 50% of stimuli.[11] The first interpretation is categorically ruled out by our data presented herein. Whilst the second interpretation of the population-level dose–response relationship is *prima facie* consistent with our results, it is not complete. Specifically, failures to respond to the stimulus are not randomly distributed amongst trials of the RR assay. If an individual failed to respond on a previous trial, its probability of response on the next trial is significantly decreased. Thus, the response to a stimulus is not solely a function of the effect-site anaesthetic concentration, as is assumed in pharmacokinetic–pharmacodynamic models of anesthetics,[21,22] but also of the state of the individual when the stimulus is applied.

Our observation of spontaneous behavioural state switching at a fixed anaesthetic concentration is consistent with neurophysiological experiments. It has been shown using invasive recordings of brain activity in rodents that, under anaesthesia, the state of the brain fluctuates spontaneously between several discrete activity patterns.[23,24] Such fluctuations are also observed in the EEG of patients undergoing anaesthesia.[25–27] State transitions are a feature of mathematical models of anaesthetic effects on the cortex.[28–30] Resistance to state transitions is consistent with the stabilisation of neuronal dynamics by mechanistically distinct anaesthetics.[31–33]

Pharmacokinetic–pharmacodynamic models would predict that, all things held equal, a person who starts out more deeply anaesthetised should take longer to recover consciousness after anaesthetic administration ceases. Yet, human subjects who exhibit burst suppression, a universally accepted marker of deep anaesthesia, recover consciousness and cognitive function at the same rate as those who were less deeply anaesthetised.[34] One potential explanation for this puzzling observation is that resistance to state transitions exerts a strong influence on the transitions between the states of arousal. This resistance to state transition is a hidden variable obscured by population-level pharmacology.

It has recently been proposed that spontaneous switching between discrete states is sufficient to give rise to anaesthetic hysteresis.[10] Anaesthetic hysteresis, or neural inertia, refers to the observation that recovery of consciousness after anaesthesia reliably happens at a lower anaesthetic concentration than loss of consciousness.[35] Anaesthetic hysteresis is evolutionarily conserved from *Drosophila*[36] to mice[37] and humans.[38] Whilst evidence for anaesthetic hysteresis in humans has not been found in all studies,[39] the inability to measure anaesthetic concentration in the brain experimentally complicates the analysis of human data.[35] Definitive evidence concerning anaesthetic hysteresis in humans will ultimately have to be obtained using novel experimental approaches.[40] The evidence for anaesthetic hysteresis in animal models, however, is unequivocal.

According to a previous model,[10] the key determinant of anaesthetic hysteresis is 'noise' that drives switching between different states under constant anaesthetic conditions. The model predicts that, all things held equal, increasing noise results in faster collapse of the hysteresis. Thus, less noisy systems are expected to have greater anaesthetic hysteresis. The meaning of the term 'noise', however, was left unclear. Traditionally, the term 'noise' is reserved for describing microscopic processes that are not directly observed experimentally. This does not necessarily imply that the processes that give rise to the observed phenomena are ultimately stochastic. For instance, fluctuations between conductive and non-conductive states of an ion channel are described using stochastic processes.[41] Yet, molecular dynamic simulations of conformational switches are deterministic.[42] Similarly, firing of many cortical neurones can be well approximated by a stochastic Poisson process,[43] but Hodgkin and Huxley[44] models of neuronal activity are deterministic. We invoke the term 'noise' here in a similar spirit of statistical mechanics.[45] As we are not observing all of the microscopic parameters that ultimately result in a transition between the responsive and unresponsive states, we can approximate the sum total of these unobserved processes as noise.

It is thus interesting to ask whether 'noise' is entirely determined by the effective concentration of anaesthetic. This is equivalent to asking whether resistance to state transitions is the same for different anaesthetic agents. Using two complementary analytical approaches, we demonstrate that this is not the case. Whilst the overall probability of righting under different anaesthetics failed to exhibit statistically significant differences, the probability of switching between the states of arousal depended strongly on the anaesthetic agent. Specifically, halothane anaesthesia was characterised by the largest resistance to state transitions or, alternatively, the smallest noise. We were able to detect statistically significant differences in resistance to state transitions for isoflurane and sevoflurane using empirically estimated transition probability matrices. In the continuous model, whilst there was a trend towards decreased noise in response fluctuations for sevoflurane anaesthesia, these differences failed to reach statistical significance when compared with isoflurane. One interpretation of this difference between the two approaches is that more trials of the RR may be required to unequivocally determine whether anaesthesia produced by isoflurane or sevoflurane is characterised by different noise levels.

Whilst both noise and R_{st} analysis attempt to quantify the tendency of the system to stay in its previously observed state, they are distinct parameters. R_{st} is proportional to the sum of

$P(U|U) + P(R|R)$, and therefore remains constant along diagonal lines parallel to $y = -x$ in the plane spanned by $P(U|U)$ and $P(R|R)$ shown in Figure 6c. The amount of noise driving state transitions, in contrast, remains constant for points spanning this plane in elliptic-like curves centred around the point (0.5, 0.5), as shown in Figure 6c. Both R_{st} and noise change concomitantly when the point moves along the line $y = x$. Away from this line, however, R_{st} and noise behave in more complex ways: R_{st} can stay constant whilst estimate of noise will change or *vice versa*. Thus, R_{st} and noise are distinct but related measures of the propensity of the system to exhibit resistance to state change.

Regardless of the differences between R_{st} and noise analysis, both show that halothane anaesthesia is associated with greatest resistance to state transitions and the least amount of noise. Consistent with this observation, anaesthetic hysteresis observed under halothane anaesthesia is larger than that observed for isoflurane.[36,37] Increased resistance to state transitions for halothane is unrelated to its pharmacokinetics. All experiments were performed at pharmacokinetic steady state, assured by a 2 h equilibration period. Furthermore, the across-subject response probability remained constant throughout the experiment.

How can different volatile anaesthetics differentially affect noise? Whilst volatile anaesthetics are known to act on a large number of receptors,[7,16,46] this molecular-level promiscuity[47] does not imply that all volatile anaesthetics act on the same molecular targets. For instance, whilst halothane binds broadly throughout the nervous system, this binding cannot be readily displaced by isoflurane.[48] Unfortunately, very few studies have directly compared the effects of different volatile anaesthetics on their molecular targets. Yet, some differential activity of volatile anaesthetics on muscarinic signalling,[49] gamma-aminobutyric acid inhibition,[50,51] and potassium channels[52] have been documented. Different volatile anaesthetics also exert differential effects on cortical activity. EEG oscillations under halothane anaesthesia are more 'wake-like' than for comparable doses of isoflurane.[53] For instance, whilst isoflurane reliably elicits burst suppression at 1.2% and above in rats, even when administered at 2%, halothane fails to elicit burst suppression.[54]

The differential effects of volatile anaesthetics on subcortical structures are of particular significance for the study of noise-driven fluctuations in arousal. Whilst isoflurane suppressed activity of orexinergic neurones in the hypothalamus[55] and noradrenergic neurones in the pontine *locus coeruleus*, equipotent halothane fails to do so *in vivo*.[56] Loss of orexinergic neurones or suppression of their signalling results in narcolepsy/cataplexy.[57] Narcolepsy is characterised by a decrease in stability, or alternatively, increase in the noise, in the networks that mediate transitions between sleep and wake states.[58–60] In a large part, orexinergic neurones affect sleep–wake transitions by acting upon *locus coeruleus* neurones.[61] Thus, it is possible that increased resistance to state transitions observed for halothane is because of the continued activity of orexinergic neurones. Whilst little is known about the specific effects of sevoflurane on orexinergic neurones,[55] sevoflurane activates locus neurones in brain slices.[62] This observation is consistent with modest increase in resistance to state transitions observed for sevoflurane relative to isoflurane.

Whether the orexinergic system is causally involved in mediating the 'noise' will have to be directly tested in the future. Regardless of the specific mechanisms, however, the fact that the amount of 'noise' depends on the anaesthetic agent implies that this noise is not merely an automatic consequence of neuronal activity. The possibility that noise is just a function of effective concentration can also be ruled out. Even when different anaesthetics are administered at equipotent doses, the amount of noise still strongly depended on the anaesthetic agent. Thus, the apparent noise that drives fluctuations in the behavioural responsiveness under constant anaesthetic conditions is a distinct phenomenon likely controlled by specific elements within the networks that mediate transitions between unresponsiveness and arousal.

What are the clinical implications of these findings? This is a study on male rodents, and stochastic fluctuations in the state of responsiveness in female rodents and humans must be demonstrated experimentally. The fact that stochastic fluctuations and resistance to state transitions were observed in rodents and zebrafish,[8] species separated by 400 million yr of evolution, strongly suggests that similar fluctuations will be present in humans as well. If so, then this work suggests that a core concept in clinical anaesthesiology, depth of anaesthesia, must be fundamentally redefined.

The current conception of depth of anaesthesia is very closely tied to the effect-site anaesthetic concentration.[63] Because the concentration of anaesthetic is assumed to vary gradually along a single dimension, anaesthetic depth is usually thought to be a graded one-dimensional quantity. This assumption is reflected in the widely used clinical monitors of anaesthetic state that assign depth of anaesthesia a single value that varies gradually between 0 and 100.[64] In contrast, the constellation of neurophysiological recordings,[24–27] mathematical modelling,[10,28–33] and the results presented here suggest that the state of anaesthesia is a collection of discrete brain states more akin to sleep stages than a graded function of anaesthetic concentration. Even at constant anaesthetic concentration, the state of the brain,[24] and indeed the behavioural state, can spontaneously switch from unresponsive to responsive.[65] A novel monitor of anaesthetic depth that takes these observations into account might be better suited to defining the anaesthetic state in each individual patient rather than of a population. Having a better quantification of the anaesthetic state in each individual may in turn allow clinicians to avoid the complication of awareness under anesthesia,[66–71] whilst at the same time avoiding delays in restoration of consciousness and cognition common in older patients upon recovery from surgery.[71]

Based on findings in this paper, we hypothesise that resistance to state transitions is dissociable from anaesthetic potency and is controlled by specific neuronal mechanisms. If this hypothesis proves correct, we speculate that novel adjunct therapies that specifically affect resistance to state transitions may be developed in the future. Once the unconscious state is attained, a theoretical 'state stabiliser' drug could be used to lock the subject in the state of unconsciousness and prevent spontaneous awakening during surgery without requiring an increased anaesthetic dose. At the conclusion of surgery, the unconscious state could then be destabilised, allowing uneventful restoration of consciousness.

Authors' contributions

Experiment design: AZW, MBK, ARM, AP

Data collection: AZW, BAH, PK, BK
Data analysis: AZW, AP
Writing paper: AZW, AP
Editing paper: AZW, MBK, ARM, AP
All authors are responsible for the validity of the data and have approved the final paper.

Declarations of interest

The authors declare that they have no conflicts of interest.

Funding

National Institutes of Health grants K08 GM123317 to ARM, R01 GM124023 to AP, R01 GM088156 to MBK, and R01 GM107117 to MBK.

Acknowledgements

The authors are grateful to A Hudson, C Brennan, S Reitz, A Aggarwal, B Shortal, R Eckenhoff, and M Eckenhoff for their helpful discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2020.05.031.

References

1. Goodman LS. *Goodman and Gilman's the pharmacological basis of therapeutics*, Vol. 1549. New York: McGraw-Hill; 1996
2. Eger EI. Age, minimum alveolar anesthetic concentration, and minimum alveolar anesthetic concentration-awake. *Anesth Analg* 2001; **93**: 947–53
3. Steffey EP, Gillespie JR, Berry JD, Eger 2nd EI, Munson ES. Anesthetic potency (MAC) of nitrous oxide in the dog, cat, and stump-tail monkey. *J Appl Physiol* 1974; **36**: 530–2
4. Sonner JM, Gong D, Eger EI. Naturally occurring variability in anesthetic potency among inbred mouse strains. *Anesth Analg* 2000; **91**: 720–6
5. Franks NP. Molecular targets underlying general anaesthesia. *Br J Pharmacol* 2006; **147**: S72–81
6. Wasilczuk AZ, Maier KL, Kelz MB. The mouse as a model organism for assessing anesthetic sensitivity. *Methods Enzymol* 2018; **602**: 211–28
7. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci* 2008; **9**: 370–86
8. McKinstry-Wu AR, Wasilczuk AZ, Harrison BA, et al. Analysis of stochastic fluctuations in responsiveness is a critical step toward personalized anesthesia. *Elife* 2019; **8**, e50143
9. Sun Y, Chen J, Pruckmayr G, et al. High throughput modular chambers for rapid evaluation of anesthetic sensitivity. *BMC Anesthesiol* 2006; **6**: 13
10. Proekt A, Hudson AE. A stochastic basis for neural inertia in emergence from general anaesthesia. *Br J Anaesth* 2018; **121**: 86–94
11. Sonner JM. Issues in the design and interpretation of minimum alveolar anesthetic concentration (MAC) studies. *Anesth Analg* 2002; **95**: 609–14
12. Uchimura A, Higuchi M, Minakuchi Y, et al. Germline mutation rates and the long-term phenotypic effects of mutation accumulation in wild-type laboratory mice and mutator mice. *Genome Res* 2015; **25**: 1125–34
13. Moreno-Bote R, Rinzel J, Rubin N. Noise-induced alternations in an attractor network model of perceptual bistability. *J Neurophysiol* 2007; **98**: 1125–39
14. Lu J, Sherman D, Devor M, Saper CB. A putative flip-flop switch for control of REM sleep. *Nature* 2006; **441**: 589–94
15. Miller RD, Eriksson LI, Fleisher LA, Wiener-Kronish JP, Cohen NH, Young WL. *Miller's anesthesia E-book*. Elsevier Health Sciences; 2014. https://www.eu.elsevierhealth.com/about-us#:~:text=For%20over%20125%20years%2C%20the,Louis%20and%20New%20York
16. Campagna JA, Miller KW, Forman SA. Mechanisms of actions of inhaled anesthetics. *N Engl J Med* 2003; **348**: 2110–24
17. Stoelting R, Longnecker D, Eger E. Minimum alveolar concentrations in man on awakening from methoxyflurane, halothane, ether and fluroxene anesthesia. *Anesthesiology* 1970; **33**: 5–9
18. Alkire MT, Gorski LA. Relative amnesic potency of five inhalational anesthetics follows the Meyer–Overton rule. *Anesthesiology* 2004; **101**: 417–29
19. Aranake A, Mashour GA, Avidan MS. Minimum alveolar concentration: ongoing relevance and clinical utility. *Anaesthesia* 2013; **68**: 512–22
20. Dilger JP. From individual to population: the minimum alveolar concentration curve. *Curr Opin Anesthesiol* 2006; **19**: 390–6
21. Minto CF, Schnider TW. Contributions of PK/PD modeling to intravenous anesthesia. *Clin Pharmacol Ther* 2008; **84**: 27–38
22. van den Berg JP, Vereecke HEM, Proost JH, et al. Pharmacokinetic and pharmacodynamic interactions in anaesthesia. A review of current knowledge and how it can be used to optimize anaesthetic drug administration. *Br J Anaesth* 2017; **118**: 44–57
23. Clement EA, Richard A, Thwaites M, Ailon J, Peters S, Dickson CT. Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. *PLoS One* 2008; **3**, e2004
24. Hudson AE, Calderon DP, Pfaff DW, Proekt A. Recovery of consciousness is mediated by a network of discrete metastable activity states. *Proc Natl Acad Sci* 2014; **111**: 9283–8
25. Chander D, García PS, MacColl JN, Illing S, Sleigh JW. Electroencephalographic variation during end maintenance and emergence from surgical anesthesia. *PLoS One* 2014; **9**, e106291
26. Vlisides PE, Li D, Zierau M, et al. Dynamic cortical connectivity during general anesthesia in surgical patients. *Anesthesiology* 2019; **130**: 885–97
27. Li D, Vlisides PE, Kelz MB, Avidan MS, Mashour GA. Dynamic cortical connectivity during general anesthesia in healthy volunteers. *Anesthesiology* 2019; **130**: 870–84
28. Steyn-Ross M, Steyn-Ross D, Sleigh J, Wilcocks L. Toward a theory of the general-anesthetic-induced phase transition of the cerebral cortex. I. A thermodynamics analogy. *Phys Rev E* 2001; **64**, 011917
29. Steyn-Ross D, Steyn-Ross M, Wilcocks L, Sleigh J. Toward a theory of the general-anesthetic-induced phase transition of the cerebral cortex. II. Numerical simulations,

spectral entropy, and correlation times. *Phys Rev E* 2001; **64**, 011918
30. Kim H, Moon J-Y, Mashour GA, Lee U. Mechanisms of hysteresis in human brain networks during transitions of consciousness and unconsciousness: theoretical principles and empirical evidence. *PLoS Comput Biol* 2018; **14**, e1006424
31. Alonso LM, Proekt A, Schwartz TH, Pryor KO, Cecchi GA, Magnasco MO. Dynamical criticality during induction of anesthesia in human ECoG recordings. *Front Neural Circuits* 2014; **8**: 20
32. Solovey G, Alonso LM, Yanagawa T, et al. Loss of consciousness is associated with stabilization of cortical activity. *J Neurosci* 2015; **35**: 10866–77
33. Alonso LM, Solovey G, Yanagawa T, Proekt A, Cecchi GA, Magnasco MO. Single-trial classification of awareness state during anesthesia by measuring critical dynamics of global brain activity. *Sci Rep* 2019; **9**: 4927
34. Shortal BP, Hickman LB, Mak-McCully RA, et al. Duration of EEG suppression does not predict recovery time or degree of cognitive impairment after general anaesthesia in human volunteers. *Br J Anaesth* 2019; **123**: 206–18
35. Proekt A, Kelz M. Schrödinger's cat: anaesthetised and not! *Br J Anaesth* 2018; **120**: 424–8
36. Joiner WJ, Friedman EB, Hung H-T, et al. Genetic and anatomical basis of the barrier separating wakefulness and anesthetic-induced unresponsiveness. *PLoS Genet* 2013; **9**, e1003605
37. Friedman EB, Sun Y, Moore JT, et al. A conserved behavioral state barrier impedes transitions between anesthetic-induced unconsciousness and wakefulness: evidence for neural inertia. *PLoS One* 2010; **5**, e11903
38. Warnaby CE, Sleigh JW, Hight D, Jbabdi S, Tracey I. Investigation of slow-wave activity saturation during surgical anesthesia reveals a signature of neural inertia in humans. *Anesthesiology* 2017; **127**: 645–57
39. Kuizenga MH, Colin PJ, Reyntjens KMEM, et al. Test of neural inertia in humans during general anaesthesia. *Br J Anaesth* 2018; **120**: 525–36
40. McKinstry-Wu A, Carspecken CW, Proekt A, Kelz MB. Xenon anesthesia and CT: noninvasive measures of brain anesthetic concentration. *Methods Enzymol* 2018; **602**: 289–98
41. Colquhoun D, Hawkes AG. The principles of the stochastic interpretation of ion-channel mechanisms. In: Sakmann B, Neher E, editors. *Single-channel recording*. Boston, MA: Springer; 1995. p. 397–482
42. McKinstry-Wu AR, Woll KA, Joseph TT, et al. Azi-medetomidine: synthesis and characterization of a novel α2 adrenergic photoaffinity ligand. *ACS Chem Neurosci* 2019; **10**: 4716–28
43. Destexhe A, Contreras D. Neuronal computations with stochastic network states. *Science* 2006; **314**: 85–90
44. Hodgkin AL, Huxley AF. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J Physiol* 1952; **116**: 449–72
45. Jaynes ET. Information theory and statistical mechanics. *Phys Rev* 1957; **106**: 620
46. Eckenhoff RG, Johansson JS. Molecular interactions between inhaled anesthetics and proteins. *Pharmacol Rev* 1997; **49**: 343–68
47. Eckenhoff RG. Promiscuous ligands and attractive cavities. *Mol Interv* 2001; **1**: 258–68
48. Eckenhoff MF, Eckenhoff RG. Quantitative autoradiography of halothane binding in rat brain. *J Pharmacol Exp Ther* 1998; **285**: 371–6
49. Nakayama T, Penheiter AR, Penheiter SG, et al. Differential effects of volatile anesthetics on M3 muscarinic receptor coupling to the Galphaq heterotrimeric G protein. *Anesthesiology* 2006; **105**: 313–24
50. Nishikawa K, MacIver MB. Agent-selective effects of volatile anesthetics on GABAA receptor-mediated synaptic inhibition in hippocampal interneurons. *Anesthesiology* 2001; **94**: 340–7
51. MacIver MB. Anesthetic agent-specific effects on synaptic inhibition. *Anesth Analg* 2014; **119**: 558–69
52. Barber AF, Liang Q, Covarrubias M. Novel activation of voltage-gated K+ channels by sevoflurane. *J Biol Chem* 2012; **287**: 40425–32
53. Lloyd-Thomas AR, Cole PV, Prior PF. Quantitative EEG and brainstem auditory evoked potentials: comparison of isoflurane with halothane using the cerebral function analysing monitor. *Br J Anaesth* 1990; **65**: 306–12
54. Brown PL, Zanos P, Wang L, Elmer GI, Gould TD, Shepard PD. Isoflurane but not halothane prevents and reverses helpless behavior: a role for EEG burst suppression? *Int J Neuropsychopharmacol* 2018; **21**: 777–85
55. Kelz MB, Sun Y, Chen J, et al. An essential role for orexins in emergence from general anesthesia. *Proc Natl Acad Sci U S A* 2008; **105**: 1309–14
56. Gompf H, Chen J, Sun Y, Yanagisawa M, Aston-Jones G, Kelz MB. Halothane-induced hypnosis is not accompanied by inactivation of orexinergic output in rodents. *Anesthesiology* 2009; **111**: 1001–9
57. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999; **98**: 437–51
58. Diniz Behn CG, Kopell N, Brown EN, Mochizuki T, Scammell TE. Delayed orexin signaling consolidates wakefulness and sleep: physiology and modeling. *J Neurophysiol* 2008; **99**: 3090–103
59. Diniz Behn CG, Klerman EB, Mochizuki T, Lin S-C, Scammell TE. Abnormal sleep/wake dynamics in orexin knockout mice. *Sleep* 2010; **33**: 297–306
60. Saper CB, Fuller PM, Pedersen NP, Lu J, Scammell TE. Sleep state switching. *Neuron* 2010; **68**: 1023–42
61. Carter ME, Brill J, Bonnavion P, Huguenard JR, Huerta R, de Lecea L. Mechanism for hypocretin-mediated sleep-to-wake transitions. *Proc Natl Acad Sci* 2012; **109**: E2635–44
62. Yasui Y, Masaki E, Kato F. Sevoflurane directly excites locus coeruleus neurons of rats. *Anesthesiology* 2007; **107**: 992–1002
63. Whitlock EL, Villafranca AJ, Lin N, et al. Relationship between bispectral index values and volatile anesthetic concentrations during the maintenance phase of anesthesia in the B-Unaware trial. *Anesthesiology* 2011; **115**: 1209–18
64. Palanca BJA, Mashour GA, Avidan MS. Processed electroencephalogram in depth of anesthesia monitoring. *Curr Opin Anaesthesiol* 2009; **22**: 553–9
65. MacIver MB, Bland BH. Chaos analysis of EEG during isoflurane-induced loss of righting in rats. *Front Syst Neurosci* 2014; **8**: 203
66. Avidan MS, Zhang L, Burnside BA, et al. Anesthesia awareness and the bispectral index. *N Engl J Med* 2008; **358**: 1097–108

67. Osterman JE, Hopper J, Heran WJ, Keane TM, van der Kolk BA. Awareness under anesthesia and the development of posttraumatic stress disorder. *Gen Hosp Psychiatry* 2001; **23**: 198–204
68. Sanders RD, Gaskell A, Raz A, et al. Incidence of connected consciousness after tracheal intubation: a prospective, international, multicenter cohort study of the isolated forearm technique. *Anesthesiology* 2017; **126**: 214–22
69. Gaskell AL, Hight DF, Winders J, et al. Frontal alpha-delta EEG does not preclude volitional response during anaesthesia: prospective cohort study of the isolated forearm technique. *Br J Anaesth* 2017; **119**: 664–73
70. Avidan MS, Mashour GA. Prevention of intraoperative awareness with explicit recall. *Anesthesiology* 2013; **118**: 449–56
71. Evered L, Silbert B, Knopman DS, et al. Recommendations for the nomenclature of cognitive change associated with anaesthesia and surgery—2018. *Anesthesiology* 2018; **129**: 872–9

Handling editor: Hugh C Hemmings Jr