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Investigating paradoxical hysteresis effects in the mouse neocortical slice model

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ABSTRACT

Clinically, anesthetic drugs show hysteresis in the plasma drug concentrations at induction versus emergence from anesthesia induced unconsciousness. This is assumed to be the result of pharmacokinetic lag between the plasma and brain effect-site and vice versa. However, recent mathematical and experimental studies demonstrate that anesthetic hysteresis might be due in part to lag in the brain physiology, independent of drug transport delay — so-called "neural inertia". The aim of this study was to investigate neural inertia in the reduced neocortical mouse slice model. Seizure-like event (SLE) activity was generated by exposing cortical slices to no-magnesium artificial cerebrospinal fluid (aCSF). Concentration–effect loops were generated by manipulating SLE frequency, using the general anesthetic drug etomidate and by altering the aCSF magnesium concentration. The etomidate (24 μM) concentration–effect relationship showed a clear hysteresis, consistent with the slow diffusion of etomidate into slice tissue. Manipulation of tissue excitability, using either carbachol (50 μM) or elevated potassium (5 mM vs 2.5 mM) did not significantly alter the size of etomidate hysteresis loops. Hysteresis in the magnesium concentration–effect relationship was evident, but only when the starting condition was magnesium-containing "normal" aCSF. The in vitro cortical slice manifests pathway-dependent "neural inertia" and may be a valuable model for future investigations into the mechanisms of neural inertia in the cerebral cortex.

1. Introduction

Identification of the mechanism by which the brain transitions between conscious and unconscious states remains a problematic question in the field of neuroscience. When applying an anesthetic, a hysteresis is observed between the concentrations needed to achieve anesthesia compared to the concentration observed when emerging from anesthesia. That is, patients wake at a lower anesthetic concentration than they go to sleep. It is generally assumed that anesthetic hysteresis is a result of the time taken for the drug to transit from the blood into the brain and vice versa, a phenomenon known as pharmacokinetic lag. Accordingly, pharmacokinetic–pharmacodynamic (PKPD) models collapse the anesthetic hysteresis loop into a single sigmoidal curve which is said to correspond to the effect–site concentration of the drug in the brain (Baars et al., 2006; Kuizenga et al., 2001).

However, recent research has shown that there might be more to hysteresis than just an effect of differences in measured drug concentrations in the blood and effective concentration in the brain. A mathematical study by Steyn-Ross et al. (2004) predicts the existence of hysteresis as an intrinsic property of interacting populations of neurons in the brain (Fig. 1). According to this work, so long as the level of cortical excitation is sufficiently low, the drug concentration within the cortex for anesthetic-induced unconsciousness is higher than that of emergence. This prediction has been supported recently by Friedman et al. (2010) in an experimental animal study. In this study mice and Drosophila were used to show a significant difference in anesthetic concentration needed to induce sleep compared to emergence from sleep, i.e. hysteresis, which was referred to by these authors as "neural inertia". These results were generated using very slow experiments and also measuring concentrations in the brain so as to avoid pharmacokinetic lag bias.

The aim of our study was to utilize the no-magnesium mouse cortical slice preparation to investigate whether neural inertial, as demonstrated by Friedman et al. (2010) and modeled by Steyn-Ross et al. (2004), is manifest in isolated neocortical slices. The advantage of the slice model is the high level of control over experimental conditions and ease of electrophysiological measurement. By removing magnesium ions from the artificial cerebrospinal fluid (aCSF) solution, seizure-like events (SLEs) are generated by the activation of N-methyl-D-aspartate (NMDA) channels. In this study we investigated hysteresis by manipulating the frequency of SLE activity by altering the aCSF magnesium concentration or by utilizing the general anesthetic etomidate.

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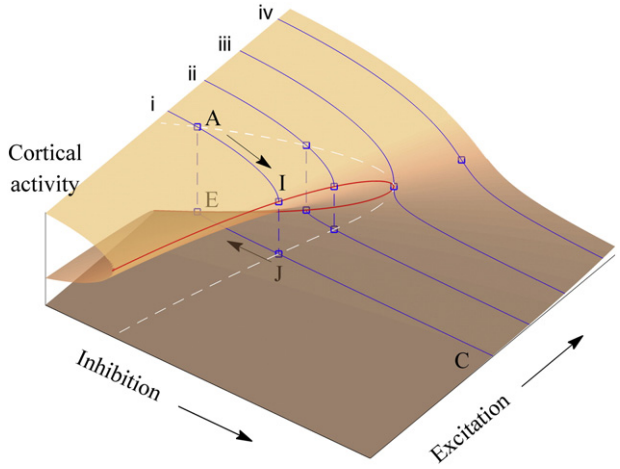

Fig. 1. Distribution of cortical steady states in the Steyn-Ross mathematical model (Steyn-Ross et al., 2011) showing cortical activity as a function of inhibition (anesthetic effect) and excitation (membrane depolarization). The upper surface represents conscious activity and lower surface shows anesthetic coma. Labels i to iv indicate four possible trajectories into anesthesia. For tour-i, the path runs from A (awake) to I (point of induction) to J (downwards jump) to C (coma), and returns via C to E (emergence) back to awake at A. The area AIJE defines the extent of the hysteresis loop. If the level of background excitation is raised, then the path into coma might proceed along tours-ii, -iii, or -iv. As excitation increases, the region of hysteresis diminishes, and vanishes completely for tours-iii and -iv.

2. Materials and method

2.1. Ethics statement

All experimental procedures were approved by the Waikato Ethics Committee at Waikato University, Hamilton, Waikato, New Zealand.

2.2. Tissue preparation

Mouse-neocortical slices were obtained from adult male and female wild-type (C57/Bl6/129SV) animals. The mice were anesthetized with carbon dioxide prior to decapitation and brain dissection. Once removed the brain was placed in ice-cold artificial cerebrospinal fluid (aCSF), made according to Nowak and Bullier (1996) for cerebral protection. A vibratome (Campden Instruments, UK) was then used to section the brain in 400 μm coronal slices between Bregma −2 mm to −5 mm. The slices were then transferred to carbogenated no-magnesium aCSF. Recovery time was a minimum of one hour at room temperature (approximately 21 °C) before commencing recording. Post recovery the slices were moved to a recording bath and unless otherwise stated, perfused with carbogenated no-magnesium aCSF at a flow rate of 2.5 ml/min. Perfusion was performed using either a syringe (50 ml) infusion pump (Terumo, Medtel, Australia) or custom passive gravity feed system.

2.3. Solutions

The following solutions were made with double distilled water and were stored at 1–4 °C for no more than seven days. All solutions were saturated with carbogen (95% O_2; 5% CO_2) prior to use.

- *Protective aCSF* containing 92.7 mM NaCl, 24 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 3 mM KCl, 19 mM $MgCl_2$, 0 mM $CaCl_2$, and 25 mM D-glucose.
- *No-magnesium aCSF* composed of 124 mM NaCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 5 mM KCl, 0 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM D-glucose.
- *Magnesium-containing ASCF solutions* composed of 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 5 mM KCl, 2 mM $CaCl_2$ and 10 mM D-glucose, with $MgCl_2$ added to the desired concentration and NaCl reduced accordingly to balance osmolarity.

Table 1
Experimental protocols.

a) Etomidate loops	
With/without carbachol (n = 8)	Potassium 2.5 mM/5 mM (n = 10)
b) Magnesium loops	
Stepwise magnesium perfusion	Rapid magnesium perfusion
No-magnesium aCSF starting condition (n = 10) Normal aCSF starting condition (n = 7)	No-magnesium aCSF starting condition (n = 9) Normal aCSF starting condition (n = 6)

2.4. Extracellular field potential recording

Extracellular field potentials were recorded from a single 50 μm Teflon-coated tungsten electrode, referenced to a silver/silver-chloride electrode in the perfusion bath (Tissue Recording System, Kerr Scientific Instruments, New Zealand). The data were recorded with a gain of 250, low- and high-pass filtered at 100 Hz and 2 Hz (Model 1800 AC amplifier, A-M Systems, USA), respectively and sampled at 1 kHz (Power 1401, Cambridge Electronic Designs, UK). A 50 Hz notch-filter was applied to remove mains noise. The signal was recorded using LabChart software and saved for later analysis using Matlab software.

2.5. Experimental procedures

Spontaneous seizure-like events (SLEs) were generated utilizing no-magnesium aCSF. For investigation of hysteresis effects, the frequency of SLE activity was manipulated in two ways: by adding the anesthetic etomidate or through altering the magnesium concentration. A summary of the experimental protocols described below is given in Table 1.

2.5.1. Etomidate-mediated neural inertia

For this protocol, no-magnesium aCSF was perfused continuously. Etomidate was added directly to the no-magnesium aCSF and perfused over a 20 min period giving a gradual increase in drug concentration in the recording bath; followed by a return to drug-free no-magnesium aCSF. Because etomidate diffuses relatively slowly into cortical slices (Benkwitz et al., 2007), this procedure produced the anticipated diffusion-related concentration–effect hysteresis loops. We attempted to identify the presence of etomidate-mediated neural inertia indirectly, by seeing if these hysteresis loops could be altered while the pharmacokinetic parameters were held constant. This was done by repeating the etomidate concentration–effect cycles at different levels of tissue excitability; using either 50 μM carbachol (effecting potassium channel blockade) (n = 8) or altering the aCSF potassium concentration from 2.5 mM to 5 mM (n = 10). According to the Steyn-Ross model, neural inertia should be eliminated or reduced at higher levels of tissue excitability (Steyn-Ross et al., 2004). The experimental protocols for this part of the

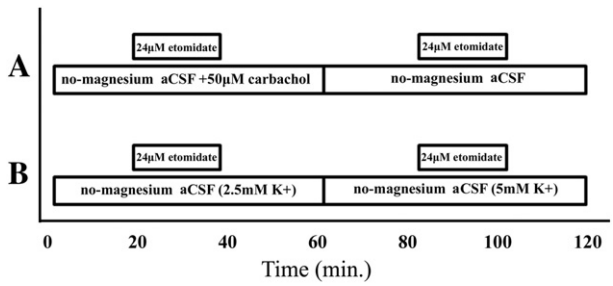

Fig. 2. Graph showing the experimental procedures for investigating the effect of etomidate in the presence or absence of carbachol (n = 8) and in the presence of low or high concentration of potassium (n = 10). Each 60 min cycle was run in randomized order, either (A) with or without carbachol (50 μM) or (B) with 2.5 mM or 5 mM potassium. For all cycles, etomidate was perfused for 20 min as shown. aCSF; artificial cerebrospinal fluid.