Neuroscience Letters 548 (2013) 149–154

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Sevoflurane-induced loss of consciousness is paralleled by a prominent modification of neural activity during cortical down-states

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HIGHLIGHTS

- Cortical down-states contain information that are altered by the volatile anaesthetic sevoflurane.
- Non-linear analysis methods are suitable tools to reveal the actions of sevoflurane on cortical down-states.
- Different sevoflurane-concentrations change cortical down-state activity in distinct ways.

ARTICLE INFO

Article history:
Received 6 February 2013
Received in revised form 12 April 2013
Accepted 15 May 2013

Keywords:
Sevoflurane
Cortex
Up-states
Down-states
Non-linear parameter analysis

ABSTRACT

Networks of neocortical neurons display a bistable activity pattern characterised by phases of high frequency action potential firing, so called up-states, and episodes of low discharge activity (down-states). We hypothesised that during down-states neocortical neurons are vulnerable to anaesthetic agents. To tackle this issue, it is necessary to identify analytical methods, which are sufficiently sensitive for resolving anaesthetic effects during phases of scarce neuronal activity. The local field potential was recorded in organotypic cultures (OTC) from rat neocortex under control conditions and in the presence of increasing concentrations of sevoflurane by extracellular electrodes. Epochs from down-states were cut from the local field potential and analysed using power spectrum density as well as non-linear parameters approximate entropy (ApEn) and order recurrence rate (ORR). ApEn and ORR proved to be suitable tools for analysing the actions of volatile anaesthetics on cortical down-states. During these phases of low neuronal activity, sevoflurane caused prominent changes in the local field potential. Time series analysis using ApEn showed a reduction of signal predictability in the presence of sevoflurane. Furthermore, the ORR displayed an abrupt decrease at sevoflurane concentrations corresponding to loss of consciousness *in vivo*, indicating a drug-induced decrease in the signal to noise ratio. The actions of volatile anaesthetics on cortical down-states have been neglected so far, perhaps due to the lack of suitable analysis tools. In the current *in vitro* study the non-linear parameters ApEn and ORR are introduced to characterise volatile anaesthetics actions. Sevoflurane alters cortical down-states as indicated by non-linear parameter analysis of local field potential recording from cultured neuronal networks. ORR even displays an abrupt change, i.e., a step-like behaviour indicating an increased signal complexity at concentrations of sevoflurane corresponding to loss of consciousness in humans.

1. Background

Volatile anaesthetics depress neuronal activity in the brain, thereby inducing sedation and hypnosis [31]. However, neuronal activity in the brain is by no means uniform, but regionally and temporally alternating between phases of high and low activity, depending on the current need of information processing. Responsiveness of most receptor systems like $GABA_A$ receptors or potassium channels – being molecular targets of general anaesthetics – depends on the current activity of the neuronal network. Hence, efficacy of anaesthetics critically depends on the activity state of the brain.

A simplified model of neuronal activity within a network of neurons consists of two alternating states: phases of high neuronal activity characterised by frequent action potential firing of principal cells and thereby, also high activity of inhibitory interneurons (up-states), and phases of sparse or near absent neuronal activity,

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http://dx.doi.org/10.1016/j.neulet.2013.05.040

termed down-states [19,27,32]. In the past years research efforts largely focused on the actions of anaesthetics in the presence of high neuronal activity, i.e., during neuronal up-states [4,28]. There are good tools to analyse and quantify the actions of anaesthetics during up-states, e.g., on action potential activity or on neuronal oscillations in the γ-band [21]. Yet, phases of sparse neuronal activity, down-states, have been neglected so far, presumably due to two reasons: first, these phases have spuriously been considered as not containing any useful information at all; and second, suitable tools to analyse down-states have been missing until now. On the other hand, evidence was provided that neuronal down-states could well be of interest for analysing the actions of general anaesthetics, as during cortical down-states information processing may be performed within sub-networks of highly active neurons [9,10,35].

In the current study we therefore addressed the question whether the volatile anaesthetic sevoflurane alters neuronal down-states *in vitro*. As neuronal activity of single cortical neurons is scarce during down-states by definition, the local field potential (LFP) of small neuronal networks derived from organotypic cultures (OTC) from the neocortex of rats was recorded by means of extracellular electrodes. Since a small number of neurons are unlikely to generate large changes in the LFP, a major challenge of the current study was to clearly separate the bioelectric voltage signal from noise. To account for this problem recordings were evaluated by means of classical power density analysis (PSD) and, in addition, by deploying non-linear analytical methods.

It is demonstrated that (i) in addition to classical power spectrum density analysis, non-linear parameters like order recurrence rate (ORR) and approximate entropy (ApEn) are useful tools to analyse sparse neuronal activity found during cortical down-states; and that (ii) the use of the non-linear ORR and ApEn provided clear evidence that down-state neuronal activity was non-random as well as that (iii) the volatile anaesthetic sevoflurane markedly alters cortical down-states *in vitro*, especially at concentrations corresponding to loss of consciousness (LOC) in animals and humans.

2. Methods

2.1. Organotypic slice cultures and electrophysiology

All procedures were approved by the animal care committee (Eberhard-Karls-University, Tuebingen, Germany) and were in accordance with the German Animal Welfare Act (TierSchG). Neocortical slice cultures were prepared from 2 to 5-days old rat pups as described by Gähwiler [12] and used after two weeks *in vitro*. Extracellular network recordings were performed in artificial cerebrospinal fluid (ACSF) consisting of (in mM) NaCl 120, KCl 3.3, NaH_2PO_4 1.13, $NaHCO_3$ 26, $CaCl_2$ 1.8 and glucose 11, bubbled with 95% oxygen and 5% carbon dioxide. All recordings were done using ACSF-filled glass electrodes (3–5 M at 34 °C. The signal was composed of action potentials and LFP, separated by digital band-pass filtering. Data were acquired at f_s = 10 kHz with the digidata 1200 AD/DA interface and Axoscope 9 software (Axon Instruments, Foster City, USA).

The test solutions containing sevoflurane (Abbott, Wiesbaden, Germany) were prepared by diluting in ACSF, filled into gas-tight glass syringes and applied *via* syringe pumps (ZAK, Marktheidenfeld, Germany) connected with Teflon tubing (Lee, Frankfurt, Germany) at a flow rate of 1 ml/min. To ensure steady state conditions, recordings during anaesthetic treatment were carried out 10 min after commencing the change of the perfusate. This time interval has been proven to be sufficient for steady state conditions [3], as diffusion times in slice cultures are considerably shorter compared to acute slice preparations [5,15].

Episodes of 2 s length were cut from the recorded LFP corresponding to cortical down-states as displayed in Fig. 1 after down-sampling to 500 Hz and applying a 0.5–100 Hz Butterworth band-pass filtering routine. Power spectrum density (PSD) from 0 Hz to 100 Hz in LFP down-states was determined using the LabView6i (National Instruments, Austin, TX, USA) PSD module.

2.2. Computation of non-linear parameters

ApEn quantifies predictability of a signal. When the analysed signal is predictable, ApEn is small. Complex, not predictable signals lead to high ApEn values. ApEn was first introduced by Pincus [29] and found its way into biosignal analysis for the evaluation of heart rate variability [18] until it was introduced to EEG analysis. The algorithm routine was realised with C++ according to Ho and Bruhn [6,18]. The variables window length m and tolerance r of the ApEn were set to $m = 2$ and $r = 0.2\,SD$ (standard deviation) as default value [6]. The parameter r defines the error tolerance that two data points are considered similar, similar to a low-pass filter. The idea of applying the order recurrence rate (ORR) on EEG data can be found in Groth [16]. ORR is a measure of statistical similarities in the signal. ORR computes the number of pairs of similar sequences of length n in the signal. Two sequences are considered similar when the order of ranks in both sequences is equal. For ORR calculation the time series is split into single vectors of dimension d. This method has the advantage that the data of the time series is reduced to order patterns overcoming the problem of changing amplitudes and offsets with the time.

2.3. Statistical analysis

For statistical analysis, a Wilcoxon two-sample test was performed with R 2.8.0 (The R Foundation for Statistical Computing, Vienna, Austria). Significance level was set to $p < 0.05$. Figures were plotted with SigmaPlot 8.0 (SPSS Inc., Chicago, IL, USA). Mean and median calculations were performed with Excel 2000 (Microsoft Corp., Redmond, WA, USA). All data are given as mean ± *SD* if not stated otherwise.

3. Results

3.1. Sevoflurane alters cortical down-states

The effects of sevoflurane on cortical down-states were investigated using OTC from the neocortex. The firing pattern of neocortical OTC was characterised by episodes of high neuronal activity (up-states), separated by phases of low activity named down-states (Fig. 1). This firing mode is also found in isolated cortical slabs and therefore, can be regarded as an intrinsic network property of the cerebral cortex [34].

Two hundred and forty-three LFP down-state episodes of were analysed. At first, PSD from LFP down-states was calculated for control recordings and in the presence of sevoflurane. Fig. 2 displays the power spectrum for 0.175 mM and 0.35 mM sevoflurane.

Under control conditions a peak at low frequencies (around 2 Hz) and relative low power at frequencies above 10 Hz was observed. However, in the presence of 0.35 mM sevoflurane the peak at 2 Hz was considerably reduced. On the basis of this observation it can be concluded that sevoflurane has effects on cortical down-states.

In a next step we sought to quantify these sevoflurane-induced LFP changes during cortical down-states using non-linear parameters ApEn and ORR.

3.2. ApEn of cortical down-states is increased in the presence of the volatile anaesthetic sevoflurane

ApEn describes the complexity of a signal with higher values indicating higher complexity, i.e., the signal becomes less predictable. For comparison of sevoflurane actions on cortical