Isoflurane increases cardiorespiratory coordination in rats

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ABSTRACT

Anesthetics such as isoflurane adversely affect heart rate. In this study we analysed the interaction between heart rhythm and respiration at different concentrations of isoflurane and ventilation rates. In two rats, the electrocardiogram (ECG) and respiratory signals were recorded under the influence of isoflurane. For the assessment of cardiorespiratory coordination, we analysed the phase locking between heart rate, computed from the R-R intervals of body surface ECG, and respiratory rate, computed from impedance changes, using Hilbert transform. The changes in heart rate, percentage of synchronization and duration of synchronized epochs at different isoflurane concentrations and ventilation rates were assessed using linear regression model. From this study it appears that the amount of phase locking between cardiac and respiratory rates increases with the increase in concentration of isoflurane. Heart rate and duration of synchronized epochs increased significantly with the increase in the level of isoflurane concentration while respiratory rate was not significantly affected. Cardiorespiratory coordination also showed a considerable increase at the ventilation rates of 50-55 cpm in both the rats, suggesting that the phase-locking between the cardiac and respiratory oscillators can be increased by breathing at a particular respiratory frequency.

Keywords: respiration, heart rate, ventilation rate, synchronization, heart rate variability, respiratory sinus arrhythmia

1. INTRODUCTION

Synchronization between two periodic oscillators can be defined as the appearance of some relationship in the form of locking of their phases or adjustment of rhythms. Cardiorespiratory coordination is an aspect of the interaction between heart rate and respiration rate which has been reported not only in subjects at rest[1,2,3]or during exercise[4] but also in subjects under the influence of anesthesia and drugs[5,6]. The oscillations of the cardiac and respiratory systems synchronize in a hierarchy of different phase-locked states[5]. Nonlinear coupling between the respiratory system and the heart results in cyclical modulation of heart rate by respiration, known as respiratory sinus arrhythmia (RSA). RSA, which is considered to be related with the intermittent phase coordination[7], is expressed as the high frequency power component of heart rate variability (HRV). However, little is known about the physiological basis and the underlying mechanism responsible for the cardiorespiratory coordination.

Although isoflurane, a volatile anesthetic, has been reported to cause a marked increase in heart rate and decrease in high frequency spectral power of HRV[6], its effect on the phase-locking between heart rate and respiration has not been fully investigated. In this study we aimed to investigate the effect of different stages of anesthesia and paced respiration on cardiorespiratory coordination in rats.

2. METHODOLOGY

2.1 Animal preparation

Two male Wistar Hooded rats, weighing 275 and 293 g (obtained from Flinders University Animal House, Bedford Park, South Australia), were used for this study. Experiments were conducted in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Flinders University Animal Welfare Committee.

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Genaral anesthesia was induced by placing rats in a container perfused with 3% isoflurane in O2. Following induction, the trachea was cannulated, and gas mixture was delivered to the lungs via the trachea tube. CO2 level in the expired air was measured using DATEK monitor, and respiratory frequency was derived from CO2 signal. ECG was recorded using two stainless-steel subcutaneous electrodes connected to BioAmp amplifier and PowerLab data acquisition system (ADInstruments, Australia). Rats were placed in a supine position on a heating pad, and their body temperature (measured with a rectal thermometer) was maintained at 37-38C. Respiratory signal was sampled at 100 Hz, and ECG signal – at 1 KHz.

During the experiment, ECG and respiratory rate, obtained from spontaneous breathing of the rats, were recorded continuously while changing the concentration of isoflurane at 15-min intervals. For the analysis of paced respiration, the isoflurane level was set to 1.5 mg/kg while the rats were artificially ventilated. The signals were acquired using MacLab interface and Chart software (ADInstruments, Sydney, Australia).

2.2 Data analysis

Pre-processing: Custom developed computer software developed under MATLAB® was used to extract times of the R-peaks from the recorded ECG signal using parabolic fitting. The RR intervals obtained from the time-points of the R-peaks were scanned for artifacts and, if necessary, manually edited. For the analysis of synchronization, we selected 10 minute intervals for each concentration of isoflurane, starting 2 minutes after each change of the dose of the anesthetic.

Respiration analysis: Respiratory signals consist of linear, nonlinear and non-stationary components, usually contaminated to some degree by noise. To extract the essential respiratory rhythm related components from the signal, we used empirical mode decomposition (EMD). EMD separates data into non-overlapping time-scale components. The multi-component signal is decomposed into a series of intrinsic mode functions (IMF) where each IMF represents a simple oscillatory mode embedded in the data[8]. The IMF that best matched the respiratory rhythm was selected for further analysis.

In order to determine the maximum and minimum points of each respiratory cycle, we calculated the derivative of the respiratory signal and considered those points that were equal to or very close to zero (window length: 0 - 0.2 mV). The average of the time series obtained from minima-to-minima intervals was used to calculate the average respiratory period.

Synchronization analysis: We used Hilbert transform to calculate the phases of the cardiac and respiratory signals. If we denote the phase of heartbeat as Φ_c and respiratory signal as Φ_r and considering that the cardiovascular system completes *m* heartbeats in *n* respiratory cycles, then the condition for phase locking can be given as

$$\left| m\Phi_c - n\Phi_r \right| \le const \tag{1}$$

In other words, if the phase difference between the two oscillators was within a certain threshold value and remained stable for *n* respiratory cycles, the oscillators were considered synchronized. The cardiorespiratory synchrogram, CRS, provide a visual tool to detect synchronization. If t_k is the time of the kth R-peak, then the normalized relative phase of heart beat is

$$\Psi_n = \frac{1}{2\pi}\left(\phi_r(t_k) \bmod 2\pi n\right) \tag{2}$$

CRS is generated by plotting Ψ_n against t_k which, in case of *m*:*n* synchronization, results in *m* horizontal lines (Fig.1). In order to determine the values of *m* and *n*, we selected one value of *n* at a time and looked for coordinations at different values of *m*. The study was carried for the following *m*:*n* coordinations: $n = 1$: $m = 2,\ldots,7$ and $n = 2$: $m = 5,7,9,11,13$. We used a threshold value of 0.035 for the phase difference as it was suggested by Cysarz et al.[9].

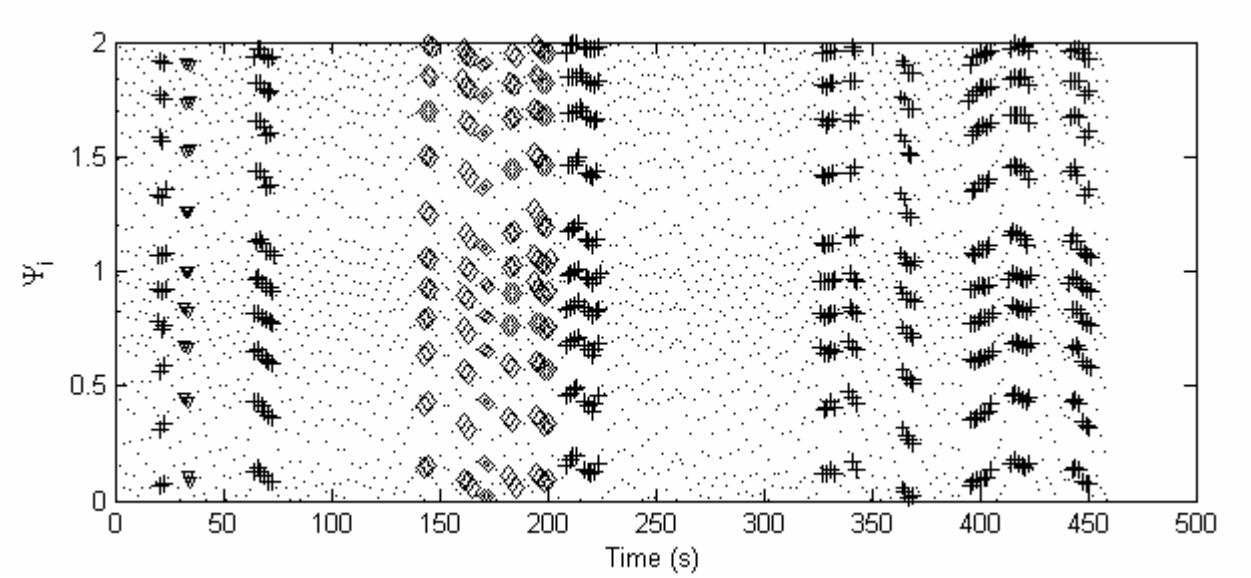

Fig. 1. Cardiorespiratory synchrogram plot showing time in seconds and the corresponding relative phases of heart beat normalized to 2 respiratory cycles. Dots indicate the normalized phases while delta, plus and diamond indicates 9:2, 10:2 and 11:2 phase-locked ratios respectively.

Paced respiration: To analyze the effect of paced respiration on cardiorespiratory coordination, the level of isoflurane concentration was set to 1.5 mg/kg and respiratory frequency was increased by 5 cycles per minute (cpm) every 15 minutes starting from 35 cpm, while recording the ECG and respiration continuously.

Measurements: We selected only artifact-free recording segments to generate the results. Since the duration of each segment may vary, we calculated percentage of synchronization by adding up the time for each synchronized epoch and then divided it by the total duration of the segments. We have also recorded the average duration of each synchronized epoch.

Statistical analysis: Statistical analysis was performed with GraphPad Prism® version 5.0. Linear regression model was used to test the effect of isoflurane on heart rate, respiratory rate, percentage of synchronization, duration of synchronized epochs. Data are expressed as the mean±SD. For the analysis, $p < 0.05$ was considered statistically significant.

3. RESULTS

The data for sections 3.1-3.3 were acquired from rats during spontaneous breathing. Since both the rats showed the same pattern in their results, regression model analysis was performed by taking the mean values of the corresponding results from both rats for different isoflurane concentrations. For section 3.4, the isoflurane level was set to 1.5 mg/kg and data were recorded at different ventilation rates. Since only two rats were used for this study and the percentage of synchronization and duration of synchronized epochs showed a high increase at a particular ventilation rate, it was not possible to use linear regression model for section 3.4, as linear regression model determines the relationship between two variables depending on the slope.

3.1 Effect of isoflurane concentration on heart rate

Heart rate increased with the increase in the concentration of isoflurane (Fig.2). Using linear regression it was observed that the heart rate was significantly associated with isoflurane ($r^2 = 0.98$, $F(1,3) = 160.3$, $p < 0.001$).

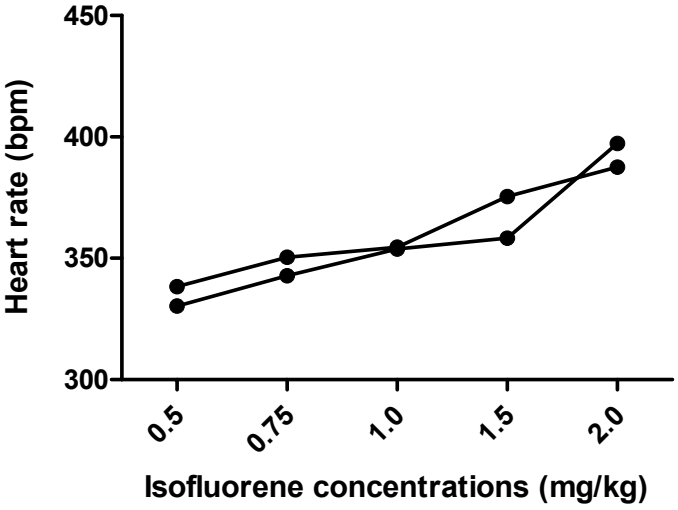

Fig. 2. Changes in heart rate (beats per minute) obtained from two rats at different levels of isoflurane concentrations. Heart rate showed a marked increase with the increase in the concentration of isoflurane.

3.2 Effect of isoflurane concentration on respiration rate

No significant change in respiratory frequency was observed with the increase in the level of isoflurane (Fig.3) (r^2 = 0.53, F(1,3) = 3.52, $p > 0.1$).

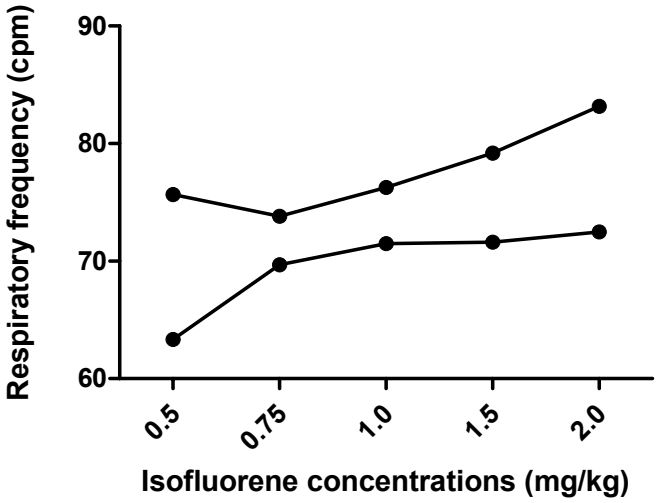

Fig. 3. Changes in respiratory frequency (cycles per minute) obtained from two rats at different levels of isoflurane concentrations. Respiratory rate showed a slight increase with the increase in the concentration of isoflurane.

3.3 Effect of isoflurane concentration on percentage and duration of synchronized epochs

Isoflurane caused a dose-dependent increase in the percentage of synchronization (r^2 = 0.99, F(1,3) = 350.30, $p < 0.001$) and duration of synchronized epochs (r^2 = 0.84, F(1,3) = 15.9, $p < 0.05$) (Fig.4).

The increase in percentage of synchronization with the increase in the level of isoflurane concentration was considered highly significant.

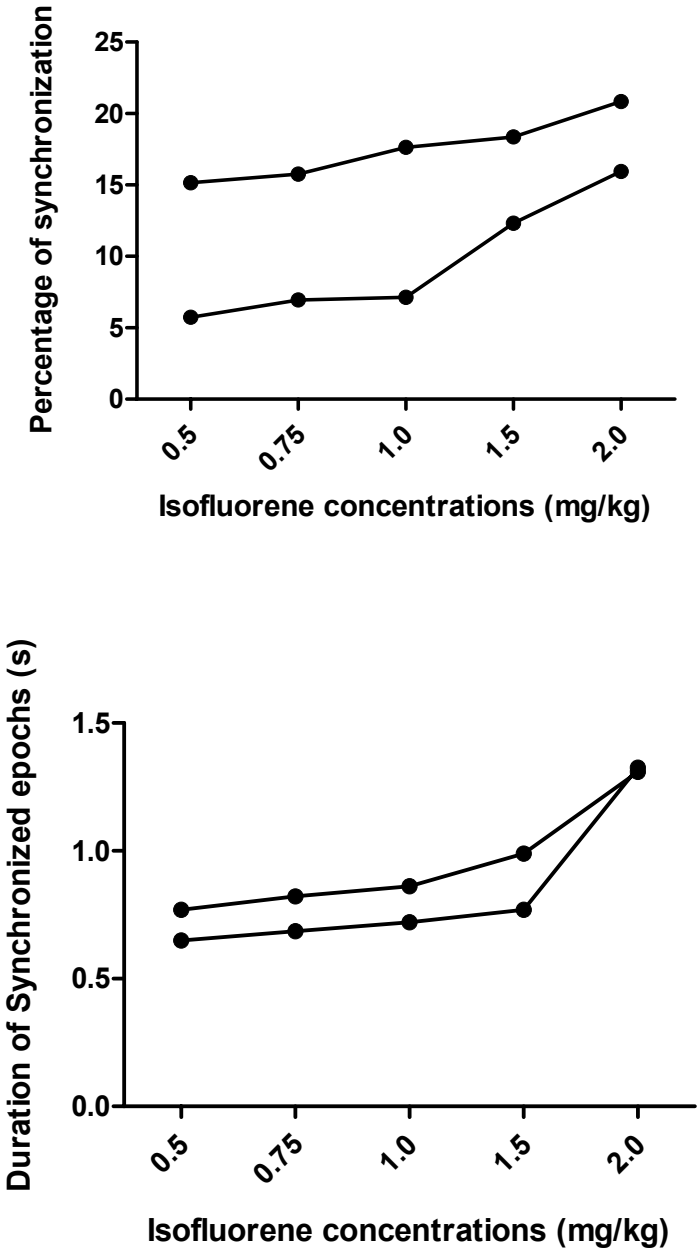

Fig. 4. Changes in percentage of synchronization and duration of synchronized epochs (seconds) obtained from two rats at different levels of isoflurane concentrations. Both showed an increase with the increase in the concentration of isoflurane.

3.4 Effect of ventilation rate on heart rate, percentage of synchronization and duration of synchronized epochs

The change in ventilation rate caused a change in heart rate, percentage of synchronization and duration of synchronized epochs. Both rats showed a decrease in heart rate and a considerable increase in percentage and duration of synchronized epochs at the ventilation rates of 50 and 55 cycles per minute (Table 1).

Table 1. Heart rate, percentage of synchronization and duration of synchronized epochs for different rates of ventilation.

	Ventilation rate (cpm)					
	35	40	45	50	55	60
Heart rate (bpm)	346.6±16.1	369.8±14.4	358.6±21.1	349.3±13.1	350.1±11.8	362.8±11.4
Percentage of synchronization	7.62±4.07	1.93±6.89	7.91±6.89	22.04±6.39	15.02±10.07	9.51±2.41
Duration of synchronized epochs (s)	1.16±0.27	0.85±0.32	1.51±0.79	1.62±0.56	1.47±0.49	1.22±0.19

4. DISCUSSIONS

This is the first study to deeply investigate the effect of isoflurane at several concentrations and ventilation rates on cardiorespiratory coordination in two rats. Our results show that isoflurane causes a dose-dependent change in heart rate, which is in accordance with the results reported by Marano et al.[6]. The increase in heart rate suggests that although isoflurane causes an overall reduction in autonomic tone[6], the sympathetic tone becomes the predominant factor under isoflurane. The cardiorespiratory coordination also showed an increase with the increase in isoflurane concentration while respiratory rate was not affected. This suggests that the heart rate rather than respiratory rate is the predominant factor causing the phenomenon of phase-locking between heart rate and respiration.

Cardiorespiratory coordination and RSA are caused by cardiorespiratory coupling[7]. The increase of cardiorespiratory coupling after the reduction in vagal tone is contradictory with the observation that vagal outflow to the sinus node of the heart is the predominant driver of RSA. Also, RSA results from modulation of heart rate by respiration which is uni-directional. On the other hand, synchronization can occur from a bi-directional interaction between heart rate and respiration. This suggests that RSA might not be an important factor responsible for cardiorespiratory coordination.

The increase in the percentage of synchronization and duration of synchronized epochs at the ventilation rates of 50-55 cycles per minute in both the rats indicates that the phase-locking between heart rate and respiration is maximized at a particular respiratory frequency similar to what was observed in human subjects[10].

5. CONCLUSIONS

Isoflurane causes a dose-dependent change in heart rate, percentage of synchronization and the duration of synchronized epochs in rats. The phase-locking between heart rate and respiration in rats increased at a certain ventilation rate, suggesting that the cardiorespiratory coordination can be achieved at a suitable respiratory frequency.

6. LIMITATIONS

For the purpose of this study we have only investigated the effects of isoflurane and paced respiration in two rats. A larger study will be required in order to draw a global conclusion.

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