Obstructive Sleep Apnea in a Rat Model: Effects of Anesthesia on Autonomic Evaluation from Heart Rate Variability Measures

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Abstract

Rat model of Obstructive Sleep Apnea (OSA) is a realistic approach for studying physiological mechanisms involved in sleep. Rats are usually anesthetized and autonomic nervous system (ANS) could be blocked. This study aimed to assess the effect of anesthesia on ANS activity during OSA episodes. Seven male Sprague-Dawley rats were anesthetized intraperitoneally with urethane (1g/kg). The experiments were conducted applying airway obstructions, simulating 15s-apnea episodes for 15 minutes. Five signals were acquired: respiratory pressure and flow, SaO_2, ECG and photoplethysmography (PPG). In total, 210 apnea episodes were studied. Normalized power spectrum of Pulse Rate Variability (PRV) was analyzed in the Low Frequency (LF) and High Frequency (HF) bands, for each episode in consecutive 15s intervals (before, during and after the apnea). All episodes showed changes in respiratory flow and SaO_2 signal. Conversely, decreases in the amplitude fluctuations of PPG (DAP) were not observed. Normalized LF presented extremely low values during breathing (median=7,67%), suggesting inhibition of sympathetic system due to anesthetic effect. Subtle increases of LF were observed during apnea. HRV and PPG analysis during apnea could be an indirect tool to assess the effect and deep of anesthesia.

1. Introduction

Rat model of Obstructive Sleep Apnea (OSA) is a realistic approach for understanding and studying physiological mechanisms involved in sleep apnea.

Rats are usually anesthetized for in vivo experiments. But, anesthetics can affect cardiovascular functions and even the autonomic nervous system (ANS) could be blocked. The type, dose and method of administrating anesthetics can disturb in different manner the sympathetic activity of instrumented rats [1].

Our group developed a rat model of chronic recurrent airway obstructions [2,3] to study the sleep apnea syndrome and its hypoxia effects. Recently, this rat model has been completed [4] with a control of duration and frequency of induced apneas. Different sensors have been also included to analyze cardiac and respiratory activity, such as flow and pressure transducers, electrodes and a specific pulse oximeter for rat monitoring.

This study aimed to assess the effect of anesthesia on ANS activity during OSA episodes in this rat model. Evaluation of anesthetic effect is analyzed by measures from pulse rate variability (PRV) analysis.

2. Materials and methods

2.1. Rat model

Seven male Sprague-Dawley rats (390 – 465 g) were intraperitoneally anesthetized with uretane (1g/1kg). One of them was excluded due to technical problems. They were connected to an electronically controlled nasal mask system that consists of two tubes, one open to atmosphere and the other connected to a positive pressure pump avoiding rebreathing on the animal.

Airway obstructions on the tubes by electrovalves, simulate OSA episodes. This nasal mask obstruction by electrovalves was controlled by the software AcqKnowledge v.4.1 from Biopac ® Systems (Figure 1).

Once the animal was anesthetized and shaved, the electrodes, nasal mask, and SaO_2 sensor, were placed on

the animal (Figure 1).

During spontaneous breathing and simulated OSA episodes, photoplesthysmography signal and SaO_2 were measured by a pulse oximeter (Mouse OxPlus ®) positioned in the rat leg. Respiratory pressure, respiratory flow signal, and electrocardiogram signal (lead I and lead II), were registered by Biopac ® Systems.

Table 1 describes the characteristics of rat population and anesthetic details.

Rat #	Weight (g)	Uretane (10%) (ml)	Ad. anest. (hour)	End protoc. (hour)
1.1	433	8.5	10:50h	15:45h
1.2	450	7.5	9:10h	13:45h
1.3	391	7.2	15:15h	20:55h
1.4	457	7	9:20h	14:40h
1.6	428	7	16h	20:30h
1.7	465	7.2	11:30h	17:20h

Table 1. Features of rats and measurements conditions.

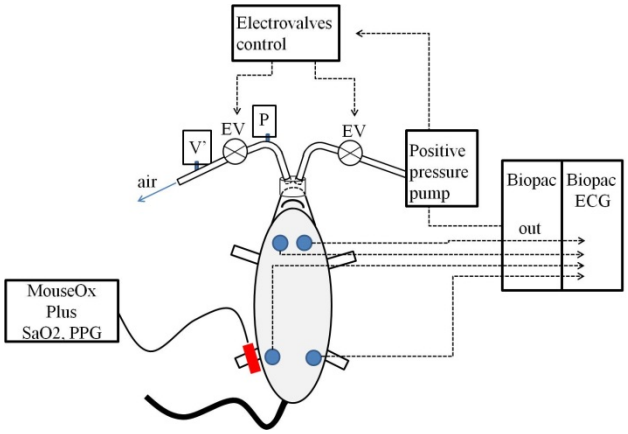

Figure 1. Experimental setup for the OSA rat model, using two electrovalves (EV) and a positive pressure pump. Signal acquired: respiratory flow (V') and pressure (P), SaO2 and PPG (with MouseOx Plus ®) and ECG (with Biopac ® ECG).

2.2. Protocol

In a first step, recurrent 15s-apnea episodes for 15 minutes intervals, with a subsequent resting period of 15 minutes were used (figure 2). Apnea index of 20, 40 and 60 apnea/hour were applied. In a second step (figure 3), apnea index of 60 apnea/hour was applied with different durations of the apnea episodes (5, 10 and 15 s). In both cases the order of the 15-minutes apnea intervals were randomly selected.

In this study, only the longer apneas (15-s duration) were considered since, individually, they are the most aggressive and so they should lead to the strongest autonomic response.

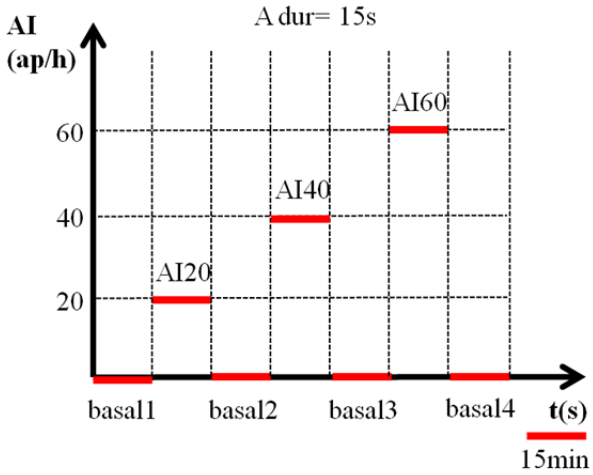

Figure 2. A sequence of 15s-apnea episodes for 15-minutes apnea intervals, with a subsequent resting period of 15 minutes (basal #).

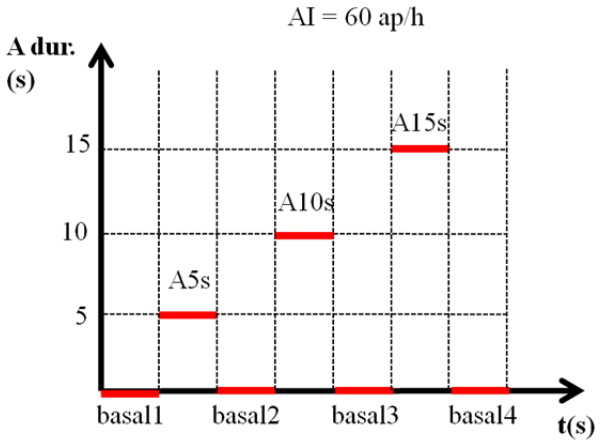

Figure 3. A sequence of AI=60 apnea episodes for 15-minutes apnea intervals, with a subsequent resting period of 15 minutes (basal #).

2.3. Signals

Five signals were acquired: respiratory pressure and flow, SaO_2, ECG and photoplethysmography (PPG). In total, 210 apnea episodes of 15-s duration were studied. All the signals were recorded simultaneously using software AcqKnowledge v.4.1 from Biopac ®System.

Figure 4 illustrates an excerpt of these signals during a 15-s episode of apnea.

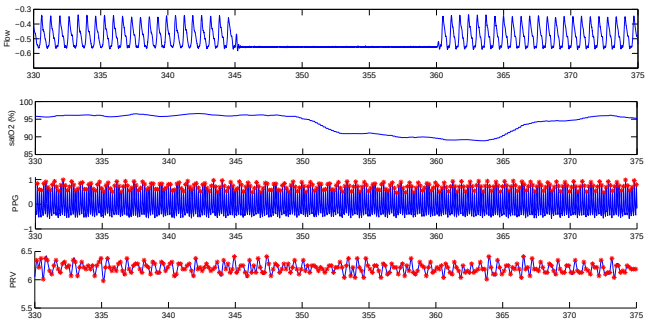

Figure 4. Respiratory Flow, SaO_2, PPG and PRV signals during a 15s-apnea episode.

2.4. PRV analysis

In this work, heart rate variability measures were estimated by the Pulse Rate Variability (PRV) analysis.

For the pulse rate signal generation, the maximum of each pulse wave (n_{A_i}) was detected by applying a technique presented in [5], which is based on a linear filtering transformation and an adaptive thresholding rule. Then, these positions are used to generate the inverse interval function (IIF), which is inversely proportional to the pulse-to-pulse interval:

$$d_{\mathrm{IIF}}^{u}(n) = \sum_{i} \frac{1}{n_{\mathrm{A}_i} - n_{\mathrm{A}_{i-1}}} \delta(n - n_{\mathrm{A}_i}) \qquad (1)$$

where the superscript "u" denotes that the signal is unevenly sampled as the pulses occur non-uniformly in time. Subsequently, a median absolute deviation outlier-rejection rule was applied in order to reduce the effect of ectopic/misdetected pulses. Then, a 15 Hz evenly sampled version was generated by cubic-spline interpolation. This evenly sampled version is denoted $d_{\mathrm{IIF}}(n)$ in this paper.

In order to analyze the spectral parameters of the PRV in a time-frequency map, the smooth pseudo Wigner-Ville distribution (SPWVD) [6] was applied, using a 3-minutes-length time window centered at the middle of each apnea. Powers in the appropriate bands for a rat model [7] were computed: low frequency band (LF) (0.04-1 Hz) ($P_{\mathrm{LF}}(n)$), high frequency band (HF) (1-3 Hz) ($P_{\mathrm{HF}}(n)$), and low to high frequency ratio ($R_{\mathrm{LF/HF}}(n)$). Then, their normalized versions $P_{\mathrm{LF_n}}(n)$ and $P_{\mathrm{HF_n}}(n)$ with respect to the total power (($P_{\mathrm{LF}}(n) + P_{\mathrm{HF}}(n)$) were computed.

To quantify the evolution of autonomic variations, three time windows were defined in specific time intervals related to the j^{th} apnea: the 15 s before the apnea (w_{b_j}), the 15 s during the apnea (w_{d_j}), and the 15 s after the apnea (w_{a_j}). Figure 5 shows an example of PPG and air flow signals before, during, and after an apnea, with its associated $d_{\mathrm{IIF}}(n)$ and its time-frequency map obtained by SPWVD.

Means of $P_{\mathrm{LF_n}}(n)$, $P_{\mathrm{HF_n}}(n)$, and $R_{\mathrm{LF/HF}}(n)$ within each one of the three windows were calculated. In addition, the differences between w_{d_j} and w_{b_j}, and between w_{a_j} and w_{d_j}, were also computed for each one of these means.

In order to study if these three indexes (means of $P_{\mathrm{LF_n}}(n)$, $P_{\mathrm{HF_n}}(n)$, and $R_{\mathrm{LF/HF}}(n)$) show significant statistical differences between the three windows w_{b_j}, w_{d_j}, and w_{a_j}, the Friedman statistical test was applied.

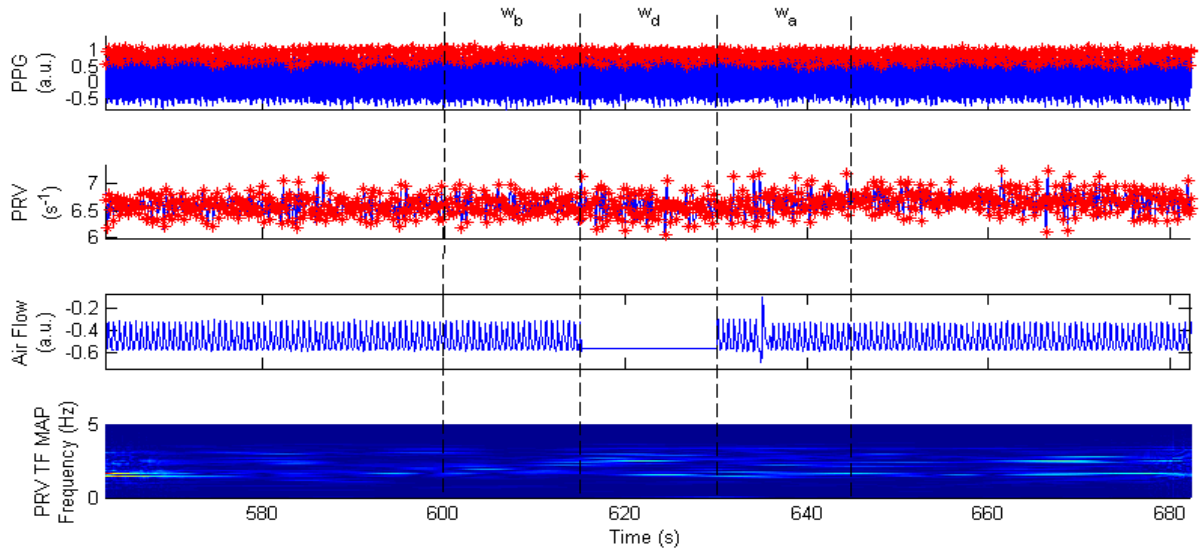

Figure 5. Example of PPG and air flow signals before, during, and after an apnea, with its associated $\boldsymbol{d_{\mathrm{IIF}}(n)}$ and its time-frequency map obtained by SPWVD.

Table 2. Median and interquartile range for means of $P_{\mathrm{LF_n}}(n)$, $P_{\mathrm{HF_n}}(n)$, and $R_{\mathrm{LF/HF}}(n)$, within the windows w_{b_j}, w_{d_j}, and w_{a_j}, and their differences between w_{d_j} and w_{b_j}, and between w_{a_j} and w_{d_j}, of the 210 studied apneas.

PRV	w_{b_j}		w_{d_j}		w_{a_j}		$w_{d_j} - w_{b_j}$		$w_{a_j} - w_{d_j}$	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
$\boldsymbol{P_{\mathrm{LF_n}}(n)}$	7.67%	28.71%	8.01%	31.11%	8.45%	26.63%	0.21%	4.50%	0.25%	4.44%
$\boldsymbol{P_{\mathrm{HF_n}}(n)}$	92.33%	28.71%	91.99%	31.11%	91.55%	26.63%	-0.21%	4.50%	-0.25%	4.44%
$\boldsymbol{R_{\mathrm{LF/HF}}(n)}$	0.08	0.46	0.09	0.52	0.09	0.42	0.00	0.07	0.00	0.06

This same test was also applied in order to study if significant statistical differences are shown in the differences between w_{d_j} and w_{b_j}, and between w_{a_j} and w_{d_j}. The Friedman test was chosen because it is a non-parametric statistical test (it makes no assumptions about the statistical distribution of the variables) for multiple group measures.

3. Results

Table 2 shows the median and interquartile range (IQR) for means of $P_{\mathrm{LF_n}}(n)$, $P_{\mathrm{HF_n}}(n)$, and $R_{\mathrm{LF/HF}}(n)$, within the windows w_{b_j}, w_{d_j}, and w_{a_j}, and their differences between w_{d_j} and w_{b_j}, and w_{a_j} and w_{d_j}.

According to the Friedman statistical test, there were no significant differences in mean of $P_{\mathrm{LF_n}}(n)$, $P_{\mathrm{HF_n}}(n)$, and $R_{\mathrm{LF/HF}}(n)$ during the three windows w_{b_j}, w_{d_j}, and w_{a_j} (p-value=0.2107, for three cases). The differences between w_{d_j} and w_{b_j}, and w_{a_j} and w_{d_j} also showed no statistical differences (p-value=0.5809, for three cases).

4. Discussion and conclusions

All episodes showed changes in respiratory flow and SaO_2 signal (desaturation <90%). Conversely, decreases in the amplitude fluctuations of PPG (DAP), which are associated with sympathetic activations caused by apnea, were not observed during apnea episodes. Mean of $P_{\mathrm{LF_n}}(n)$ presented extremely low values during breathing (median=7,67%), suggesting inhibition of sympathetic system probably due to anesthetic effect. Although subtle increases of mean of $P_{\mathrm{LF_n}}(n)$ were observed during apnea (median=8,01%) and after (median=8,45%), no significant differences were found in means of $P_{\mathrm{LF_n}}(n)$, $P_{\mathrm{HF_n}}(n)$ or $R_{\mathrm{LF/HF}}(n)$ during the three windows, according to the Friedman statistical test (p-value=0.2107).

This rat model is suitable to study the consequences of OSA in respiratory pattern, but anesthetic effects can limit assessment of ANS response. Consequently, HRV and PPG analysis during apnea could be an indirect tool to assess the effect and deep of anesthesia.

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