Respiratory sinus arrhythmia as a surrogate measure of respiratory frequency: validity and robustness to activity in rats

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RESPIRATORY SINUS ARRHYTHMIA

Respiratory sinus arrhythmia (RSA) as a surrogate measure of respiratory frequency: validity and robustness to activity in rats. J Appl Physiol 118: 238–243, 2015. First published November 20, 2014; doi:10.1152/japplphysiol.00799.2014.—Recording of breathing frequency is a basic requirement for respiratory physiology. Usual techniques are invasive and constraining. Respiratory sinus arrhythmia (RSA) has recently been demonstrated to be a simple way to obtain respiration frequency at rest. In this study, we investigated whether this correlation is also observed during activity. We first compared RSA to the respiration frequency obtained in anesthetized rats using a pneumotachograph connected to the trachea (TRF). Data analyses using Passing and Bablok regression confirmed the absence of bias and proportional differences. Accordingly, the Bland-Altman plot did not show any significant differences in data sets. In a second experiment, we compared RSA to the respiration frequency obtained in freely moving rats using a subpleurally inserted telemetric catheter (PRF). Comparisons between RSA and PRF revealed no significant difference in determination of respiratory rate with the two methods, although the bias and confidence interval were greater when activity increased. This was, however, not the case during short episodes of sniffing-like tachypnea, during which no matching RSA peaks were observed. In conclusion, RSA frequency reflected regular respiration frequency independently of the level of activity and appears to be a good surrogate to usual techniques.

Heart rate variability; RSA; respiratory frequency; vigilance; method

Recognition of alterations in tidal volume and respiration pattern is an important early clue to disease detection. In particular, careful observation of the respiratory frequency is a crucial part of physical examination. Respiratory function in humans is usually evaluated by using a plethysmograph chamber (the whole body is placed in a sealed box) or a pneumotachograph device (measurement of chest wall displacement). Both techniques can also be performed in conscious (3) or anesthetized (16) animals, respectively. However, these techniques are either invasive or constraining, and they do not allow measurement of the respiratory rate for extended period of time or during periods of behavioral activity. The heart rate (HR) time series is characterized by a wide range of beat-to-beat variability. Parasympathetic (vagal) influences are present throughout all frequency ranges of the HR power spectrum, while sympathetic influences disappear at higher frequencies (7). It is therefore commonly accepted that the HR power spectrum contains two major components: high frequency (HF), which reflects cardiac vagal tone, and low frequency (LF), which reflects a mixture of parasympathetic and sympathetic influences. Respiratory sinus arrhythmia (RSA) reaches a peak in the HF domain (15). It reflects afferents to cardiac vagal motoneurones from lung stretch receptor and respiratory central pattern generators (located in the lower brainstem) (12). RSA is the heart pattern that occurs when HR increases during inhalation and decreases during exhalation and can be interpreted as influences of respiration on the sinoatrial node of the heart (9). It is therefore characterized by periodic increases and decreases in HR that occur at a frequency similar to that of respiration. RSA frequency is derived from electrocardiogram (ECG) recordings and can therefore be easily obtained without imposing any constraints on the subject. Therefore, RSA can be used as a surrogate technique to determine respiratory frequency. In 2007, Denver et al. (5) demonstrated in humans that RSA frequency is the same as respiratory frequency in healthy subjects by comparing the RSA frequency to the respiratory frequency obtained from a pneumotachograph. In animals, Boychuk and Hayward (3) also established a correlation between RSA frequency and respiratory frequency monitored by whole body plethysmography. However, in the abovementioned studies, comparisons were based on simple regression analysis and RSA was measured at rest, in the absence of activity. This raises the question of the real correlation between RSA and respiratory frequency when 1) analysis is based on Passing-Bablok regression, and 2) the level of activity increases. To answer this question, we compared RSA to respiratory frequency obtained by either tracheal recordings in anesthetized animals or subpleural recordings (13) in conscious animals during low, medium, or high levels of activity measured by telemetric recordings.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Centre d’Elevage R. Janvier, Le Genest-Saint-Ile, France), weighing 250–300 g, were used (n = 15). All animals were kept under controlled environmental conditions (22 ± 1°C; 60% relative humidity; 12-h light/dark cycle; food and water ad libitum). Procedures involving animals and their care were all performed in conformity with institutional guidelines, in compliance with national and international laws and policies (Council Directive 87–848, October 19, 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Sante et de la Protection Animale; Permission 75855 to C. Sévoz-Couche).

Tracheal cannulation and ECG recordings in anesthetized rats. A group of rats (n = 9) were anesthetized with pentobarbital sodium (60
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mg/kg ip). The trachea was cannulated to monitor respiration (16). The cannula was connected to a pneumotachograph (Fleisch 0000), linked to a volume transducer (Digitimer Neurolog, NL 905). ECG was recorded using stainless steel pins placed subcutaneously into fore- and hindpaws. ECG signals were amplified and filtered (Universal Amplifier; Gould, Courtaboeuf, France). ECG and respiratory signals were relayed to a 1401 interface (1401 Plus; CED) connected to running Spike 2 Software (6.14; CED). Recordings lasted 1 h in each animal. Rectal temperature was maintained at 37°C with a thermostatically controlled heating blanket.

Radiotelemetric probe implantation. Another group of rats (n = 6) was implanted with radiotelemetric probes (CXT P50; Data Sciences International, St. Paul, MN) for subpleural pressure and ECG recording, according to the technique previously used in monkeys (13). Briefly, rats were anesthetized with 2% inhaled isoflurane. The transmitter fluid-filled catheter was threaded through the serosal layer of the oesophagus and advanced cranially past the junction with the diaphragm and into the thoracic cavity. The ECG sensor leads were tunneled from the abdominal cavity. One lead (wire loop) was optimally fixed to the dorsal surface of the xyphoid process, while the other lead was subcutaneously tunneled on the thorax to the superior surface of the diaphragm and into the thoracic cavity. The ECG sensor leads were inserted into the oesophagus and advanced cranially past the junction with the diaphragm. Dataquest A.R.T 4.1 software was used to record telemetry signals associated to respiration. These analyses were done with SigmaPlot 12.0 (Systat Software). ECG signals were relayed to a 1401 interface (1401 Plus; CED) connected to offline respiratory and HR variability (HRV) analyses.

Data analysis. Tracheal respiratory frequency (TRF) was determined as the mean of four 90-s time intervals. Pleural respiratory frequency (PRF) was determined for each level of activity: low (activity <2 per 10 s), medium (activity between 2 and 5 per 10 s), and high (activity greater than 5 per 10 s). HRV analyses were performed on the same time intervals as those used for PRF or TRF measurement. Power spectra (0–2.5 Hz) were obtained by Fourier transformation (size 256, Hanning window, sample interval: 0.2 s). When tachypnoeic episodes were observed (usual maximal duration of ~5 s), corresponding power spectra (0–12.5 Hz) were obtained using a Fourier transformation of 0.04 Hz sample interval. Very LF, LF, and HF powers were situated in the ranges of 0–0.2, 0.2–0.7, and 0.7–2.5 Hz, respectively. HF power is exclusively under parasympathetic (vagal) control and includes the RSA peak (15), i.e., the change in heart period corresponding to the inspiratory and expiratory phases of the respiratory cycle.

Statistical analysis. Data are presented as means ± SE. One-way ANOVA followed by Dunnett’s pairwise comparison for respiratory rate during activity (activity between 2 and 5 or >5 per 10 s) vs. control (activity <2 per 10 s) was used. Similarly, comparison for respiratory rate during mixed pattern of respiration (regular breathing associated to tachypnoeic episode) vs. control (regular breathing only) was used. These analyses were done with SigmaPlot 12.0 (Systat Software), P = 0.05 was defined as the limit of statistical significance. The interchangeability of RSA determined by TRF and PRF was checked using Passing-Bablok regression. In addition, the Bland-Altman plot was applied to determine intermethod bias and limits of agreement. These analyses were performed with XLSTATS software (v. 2014.2.07; Addinsoft).

RESULTS

In anesthetized animals (Fig. 1A), mean TRF was 93.1 ± 3.7 cycles/min (range: 81 to 112 counts/min), corresponding to a respiratory frequency of 1.551 ± 0.063 Hz (range: 1.35 to 1.9 Hz).

![Image](http://jappl.physiology.org/Download/1.152/japplphysiol00799.2014/www.jappl.org)

Fig. 1. Representative R-R interval (RRi), tracheal tidal volume (TVt) obtained by pneumotachograph measurement connected to the trachea, and ECG time series recorded in 1 anesthetized rat. Power spectra of RRi in the same animal as in A showing low-frequency (LF; 0.2 to 0.7 Hz) and high-frequency (HF; 0.7 to 2.5 Hz) domains. Note the peak HF (large dot) corresponding to respiratory sinus arrhythmia (RSA) at 1.56 Hz in this case. Passing-Bablok regression scatter diagram with the regression line (red line), the 95% confidence interval for the regression line (dashed lines) and identity line (x=y, dotted line), for respiratory frequency obtained by determination of respiratory cycles from tracheal pressure (TRF) or RSA in all anesthetized rats. Corresponding Altmann-Blund plots are shown. The dashed line indicates the bias, large dotted lines indicate the 95% limits of agreement, and small dotted lines indicate the 95% limits of confidence.
Innovative Methodology

Table 1. Passing–Bablok regression analysis comparing respiratory frequency measurement obtained by either tracheal (regression analysis 1) or subpleural (regression analyses 2 to 4) approaches and RSA determination

<table>
<thead>
<tr>
<th>Variable X Measurement</th>
<th>Variable Y: RSA Measurement/Value 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tracheal measurement</strong></td>
<td></td>
</tr>
<tr>
<td>Intercept A</td>
<td>$-0.36$ From $-0.29$ to $0.027$</td>
</tr>
<tr>
<td>Slope B</td>
<td>$1.022$ From $0.982$ to $1.200$</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$0.819$</td>
</tr>
<tr>
<td><strong>Regression analysis 1</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Subpleural measurement (activity &lt;2)</strong></td>
<td></td>
</tr>
<tr>
<td>Intercept A</td>
<td>$0.034$ From $-0.122$ to $0.165$</td>
</tr>
<tr>
<td>Slope B</td>
<td>$0.980$ From $0.900$ to $1.080$</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$0.441$</td>
</tr>
<tr>
<td><strong>Regression analysis 2</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Subpleural measurement (activity &lt;2–5&gt;)</strong></td>
<td></td>
</tr>
<tr>
<td>Intercept A</td>
<td>$-0.193$ From $-0.755$ to $0.273$</td>
</tr>
<tr>
<td>Slope B</td>
<td>$1.136$ From $0.833$ to $1.500$</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$0.900$</td>
</tr>
<tr>
<td><strong>Regression analysis 3</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Subpleural measurement (activity &gt;5)</strong></td>
<td></td>
</tr>
<tr>
<td>Intercept A</td>
<td>$0.125$ From $-0.568$ to $0.600$</td>
</tr>
<tr>
<td>Slope B</td>
<td>$0.927$ From $0.600$ to $1.394$</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$0.699$</td>
</tr>
<tr>
<td><strong>Regression analysis 4</strong></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; RSA, respiratory sinus arrhythmia.

Hz. Mean RSA peak frequency (Fig. 1B) was $1.557 \pm 0.064$ Hz. Passing-Bablok regression analysis (Fig. 1C) showed that intercept A and slope B values were contained in their relative confidence intervals (CIs; Table 1, regression analysis 1), confirming signal interchangeability. An Altman-Bland plot of the same data set (Fig. 1D) showed a bias of 0.005 with limits of agreement of $-0.018$ to $0.007$. In conscious animals, pleural PRF (Fig. 2) varied with the level of activity (Fig. 3). Mean PRF was $1.59 \pm 0.03$ Hz for rat activity >5 per 10 s (high), $1.53 \pm 0.02$ Hz for rat activity between 2 and 5 per 10 s (medium) and $1.49 \pm 0.02$ Hz for rat activity <2 per 10 s (low). PRF at high activity was significantly higher than at low activity ($P = 0.008$) but not compared with medium activity ($P = 0.280$). Passing-Bablok regression with RSA frequency (Fig. 3, A.1–C.1) showed that intercept A and slope B values were contained in their relative CIs (Table 1, regression analyses 2–4), confirming signal interchangeability, regardless of the animal’s level of activity. An Altman-Bland plot of the same data sets (Fig. 2, A.2–C.2) showed a bias of $-0.001$ with limits of agreement of $-0.007$ to $0.007$ for low activity, $-0.016$ with limits of agreement of $-0.030$ to $-0.002$ for medium activity, and $-0.006$ with limits of agreement of $-0.113$ to $0.100$ for high activity. RSA was also looked for during sniffing-like tachyphoeic episodes, be them, or not, associated to increased activity. As these episodes were of very short duration (<10 s), we analyzed tachyphoeic (regular breathing only) or mixed (tachyphoea intermingled with regular breathing) periods (Fig. 4). Regular breathing frequency was equal to $1.59 \pm 0.05$ Hz during every activity (Fig. 4A.I) and $1.88 \pm 0.04$ Hz (Fig. 4B.I), with corresponding RSA observed in power spectra (Fig 4, A.2 and B.2). During mixed (regular and tachyphoeic) episodes, maximum respiratory rate was significantly increased to $6.5 \pm 1.2$ and $7.5 \pm 1.0$ Hz ($P < 0.001$, respectively). No RSA peaks above 4 Hz were observed (Fig. 4, A.3 and B.3).

DISCUSSION

This study shows that the ECG HF peak attributed to respiratory sinus arrhythmia can be used as a surrogate for respiratory frequency measurements across a range of direct activity states in rats. Interchangeability decreased slightly at high activity levels.

Plural pressure can be measured to directly assess the mechanical properties of the lung. Pneumotachograph analyses of respiration are commonly used in humans (2), but this method induces constraint for the subject and may affect respiration itself. Pneumotachograph or pleural pressure methods can be useful in anesthetized animals and have been validated by the perfect fit of respiratory frequency obtained in a plethysmograph chamber (13). However, these methods appear to be excessively invasive when only this parameter needs to be recorded. In contrast, ECG recordings are easy to obtain and noninvasive and can be used to analyze HRV. RSA, observed in the HF domain of spectral analysis (15), is characterized by periodic increases and decreases in HR that occur at a frequency similar to that of respiration. Various authors have assumed that RSA and respiratory frequency are parallel outputs of a “common cardiopulmonary oscillator” (6). We first compared RSA to respiration frequency obtained with a pneumotachograph in anesthetized animals by Passing-Bablok analyses (1). This method has the advantage of being independent of the hypothesis of error distribution (8), unlike the regression analysis that is currently used to compare RSA and respiratory frequency (5). The Passing-Bablok procedure is used on variables that have a linear relationship and that are highly correlated. It is a nonparametric approach and the requirements for Passing-Bablok regression are continuously distributed measurements (covering a broad concentration range) and linear relationship between two methods. We found no systematic or proportional difference between the two methods, as 95% CI for the intercept included zero and 95% CI for the slope included one. This was confirmed by Bland-Altman analyses (most points were included within the CI).

Respiratory frequency was also obtained by subpleural recordings in conscious rats using telemetric implants for ECG recording, which can be used to determine different levels of activity. Respiratory frequency was found to increase when activity and respiratory frequency increased. The CI for the
intercept on Passing-Bablok graphs, although it increased with the level of activity, included zero. Similarly, the limits of agreement on the Bland-Altman plot were larger when activity increased but remained very close to and included zero. No significant difference was therefore observed between the two techniques to determine respiratory frequency, regardless of the level of activity.

Sniffing is an active behavior. There are two types of sniffing episodes: early studies showed that some (9–12 Hz) could be associated to a reward anticipation (4), but others are related to obvious exploratory behavior (6–9 Hz) (11, 14).

Olfaction is one of the principal sources of sensory information in rats, and respiratory pattern during exploration (associated with an increased activity level) is a dramatic and rapid change in the respiratory rate associated with rapid protraction/retraction of the nose. Of note, due to the metabolic need to coordinate breathing and swallowing, small animals (rats and mice) often lick at frequencies similar to those of sniffing (4–8 Hz) (11). These tachypnoeic episodes occur without increased activity. We did observe tachypnoeic episodes, occurring during the usual slow and regular breathing; some of these episodes were associated with increased activity and others were not of note; breathing frequency during these episodes was under 9 Hz and therefore did not correspond to anticipatory reward. We analyzed RSA with power spectra that was superior to 9 Hz (i.e., 12.5 Hz) to ensure the visualization of fast respiratory frequency. We found that during increased activity or not, RSA was mostly the reflect of the usual regular breathing only (inferior to 2 Hz). Analyses of mixed periods showed that faster breathing between 2 and 3 Hz was present, but the respiratory rate superior to 4 Hz was not observed, confirming previous findings on exploratory sniffing (10). It appears that this frequency corresponds, or is very close, to HR. Moreover, sniffing consist of a series of fast and forced nasal inhalations with small passive exhalations (19). As RSA is the reflect of HR modifications between inspiration and expiration.
expiration, it seems probable that RSA would not reflect a fast and incomplete respiratory cycle. It was shown that vagal feedback from pulmonary stretch receptors is obligatory for the generation of a neurally mediated RSA (18). During sniffing and fast only inspirations, it is possible that there is no activation of lung stretch receptors, explaining that RSA could not occur during these specific periods. Myographic recordings may be useful to answer this question. It is worthwhile to note that these periods are of very short duration (<10 s), scarcely distributed, and predominantly observed at the beginning or the end of the recording.

In conclusion, RSA frequency appears as an adequate reflection of breathing frequency, in anesthetized as well as in conscious animals. These observations provide an easy to obtain, noninvasive, unrestraining method to determine respiratory frequency that stays accurate independently of the level of activity, even if rare and very short tachypnoeic episodes cannot be detected.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


