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Impact of Movement on Cardiorespiratory Coordination in Conscious Rats

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Abstract—**In this study we assessed the impact of movement on the interaction between the heart rhythm and respiration in rats while they were conscious and freely moving. In eight male adult Sprague-Dawley (SD, n=4) and Hooded Wistar (HW, n=4) rats, we recorded respiratory rate using whole-body plethysmography with a piezoelectric sensor attached to simultaneously monitor body movement. Heart rate was recorded using a radio-telemetry transmitter. For the assessment of cardiorespiratory coordination, we analysed the phase-locking between heart rate and respiration, estimating the instantaneous phases using Hilbert transform. For statistical analysis, the piezoelectric signal was dichotomized into low-intensity (LIm) and high-intensity (HIm) movement. The R-R intervals, respiratory intervals and cardiorespiratory coordination between LIm and HIm of each rat were assessed with Student's t-test. A significant decrease in the mean values for respiratory interval (0.34±0.1 vs. 0.23±0.1 s, $p<0.01$ in HW rats) and R-R interval (0.19±0.01 vs. 0.17±0.01 s, $p<0.001$ in SD rats) was observed during HIm. The phase-locking between the cardiac and respiratory signals also decreased significantly during HIm (overall coordination during LIm vs. HIm: 89.3±3.3% vs. 8.7±1.7%, $p<0.001$). In conclusion the interaction between the cardiac and respiratory oscillators is affected by voluntary movements in rats.**

I. Introduction

THE appearance of some relationship between two periodic oscillators in the form of locking of their phases or adjustment of rhythms can be termed as synchronization. Cardiorespiratory coordination is an aspect of the interaction between heart and respiratory rhythm which has been reported not only at rest [1,2] or during exercise [3], but also in subjects under the influence of anesthesia [4,5] and drugs [6,7,8]. However, little is known about the physiological basis and the underlying mechanism responsible for the cardiorespiratory coordination.

Although previous studies on animals, investigating respiration and heart rhythms, have focused on the effect of anesthesia, drugs and induced movements on the cardiorespiratory interaction [4,5,7-10], the association between movement and cardiorespiratory coordination in freely moving animals has never been established. In this study, we investigated the impact of movement on the phase-locking between the cardiac and respiratory cycles in conscious rats. We hypothesized that movement disturbs the phase-locking.

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II. Methods

A. Animal Preparation

Male Hooded Wistar (n=4) and Sprague Dawley (n=4) rats, weighing 275-320 g 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 University of Newcastle Animal Care and Ethics Committee.

Preparatory surgery was conducted under isoflurane (1.5% in 100% oxygen) anesthesia, with carprofen (5 mg/kg) being used as a post-surgery analgesic. Telemetric ECG radio-transmitters (TA11CA-F40, Data Sciences International, St. Paul, MN) were used, with electrodes positioned in accordance with the method described by Sgoifo et al. [11], which permits the recovery of 95-99% of heart beats even during vigorous movements. Once the animals recovered from the anesthesia, they were returned to the animal house for one week before the start of the experiments. Respiratory signals were recorded using a custom-built whole-body plethysmograph. Under the plethysmograph, a piezoelectric pulse transducer (MLT1010/D, ADInstruments, Sydney, Australia) was placed to monitor animals' motor activity.

The protocol consisted of a 40-min recording period in each rat. ECG, respiratory and motion signals were sampled at 1 KHz and acquired using a PowerLab A/D converter and ChartPro 6.0 software (ADInstruments, Sydney, Australia).

B. Data Analysis

1) R-R Interval Analysis: Custom written computer software developed under MATLAB® was used to detect the R-peaks from the recorded ECG signal using parabolic fitting. The R-R time series obtained from the time-points of the R-peaks were visually scanned for artifacts.

2) Respiration Analysis: Respiratory signals consist of linear, nonlinear and non-stationary components, usually contaminated to some degree by noise. For our analysis, the respiratory signal was low-pass filtered at 10 Hz. The

inspiratory onsets, used to compute the breath-to-breath time series, were determined as the zero-crossings of the first derivative of the respiratory signal.

3) Movement Analysis: The intensity of movement was characterized by initially calculating the power of the piezoelectric signal (PowPz) and subsequently determining the slope of the cumulative sum of PowPz. Using a threshold value of 10^{-2} V^2, we dichotomized movement as: low-intensity movement (LIm) (slope $\leq 10^{-2}$) and high-intensity movement (HIm) (slope $> 10^{-2}$).

4) Synchronization Analysis: We used Hilbert transform to calculate the phases of the respiratory signal, and determined relationship between the respiratory phases at different R-peak instants. Theoretically, if we denote the phase of heartbeat as Φ_c and respiratory signal as Φ_r and considering that the heart completes m heartbeats in n respiratory cycles, then the condition for phase locking can be given as

$$\left| m\Phi_c - n\Phi_r \right| \leq const$$

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. If t_k is the time of the appearance of a k^{th} R-peak, then by observing the phase of the respiration at the instants t_k , denoted by $\Phi_r(t_k)$ and wrapping the respiratory phase into a $[0, 2\pi n]$ interval, we can generate cardiorespiratory synchrogram. This provides a visual tool to detect cardiorespiratory coordination (Figure 1), by plotting Ψ_n against t_k which, in case of m:n synchronization, results in m horizontal lines. Here Ψ_n is given by the equation

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

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,…,7 and n = 2: m = 5,7,9,11,13. We used a threshold value of 0.025 for the phase difference as it was suggested by Cysarz et al. [12].

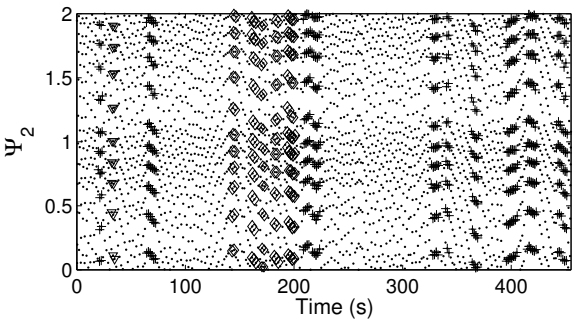

Fig. 1. Cardiorespiratory synchrogram plot showing time in seconds and the corresponding relative phases of heart beat normalized to 2 respiratory cycles. Dots indicate the normalized phases while delta, plus and diamond indicates 9:2, 10:2 and 11:2 phase-locked ratios respectively.

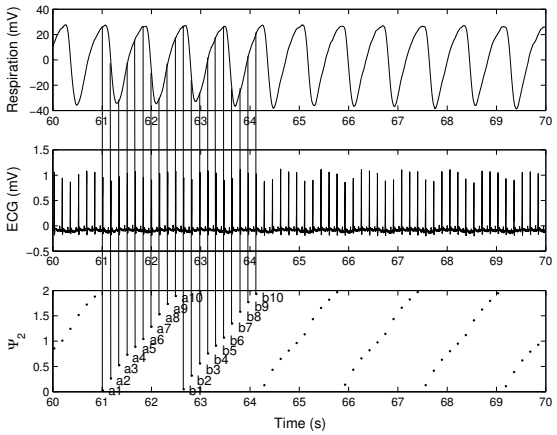

Fig. 2. Illustration of the generation of synchrogram from the respiratory and ECG signals. a1, a2,…,a10 and b1, b2,…,b10 in the synchrogram plot represent the respiratory phases, based on the time points of R-peaks, for the first two and the following two respiratory cycles.

We presented an illustration of the synchrogram plot in figure 2. The phases of the respiratory signal, corresponding to the time points of the R-peaks, were plotted as normalized phases between 0 and 2. Subsequently, the phases for every two respiratory cycles formed a relatively vertical line: a1,a2,…,a10; b1,b2,…,b10; and so on. We then determined the differences between each point of one line to each corresponding point of the next line: a1-b1, a2-b2,…,a10-b10. If the differences between all the corresponding points were below the threshold value of 0.025, the respective R-peaks were considered as coordinated.

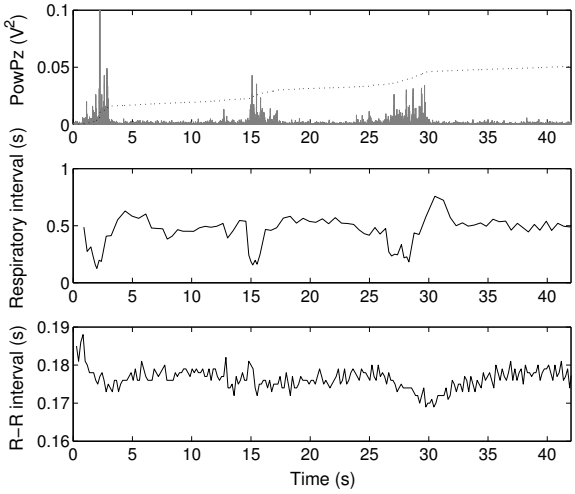

Fig. 3. Power of piezoelectric signal (PowPz) (top panel) and the corresponding changes in respiratory and R-R interval (middle and bottom panel, respectively). The dotted line in the top panel indicates the cumulative sum of PowPz (divided by 10^{-3} to fit in the graph). A shortening in respiratory and R-R interval can be observed with the increase in the movement intensity.

5) Statistical Analysis: We selected only artifact-free recording segments to generate the results. Since the duration of each segment may vary, we calculated percentage of cardiorespiratory coordination by adding up the time for each coordinated epoch and then divided it by the total duration of the segments. Subsequently, we determined the percentage of the coordination during LIm and HIm. We also recorded the phase-locking ratio and average duration of each coordinated epoch.

Statistical analysis was performed with GraphPad Prism® version 5.0 software (GraphPad, San Diego, CA, USA). Student's t-test was used to compare R-R intervals, respiratory intervals and cardiorespiratory coordination between LIm and HIm epochs. Data were expressed as mean±SD. A value of $p < 0.05$ was considered statistically significant.

III. Results

During the recording period, we observed a variation in respiratory rate and heart rate associated with movement. This is illustrated by plotting the respiratory and R-R intervals against PowPz (Figure 3).

A. Effect of Movement on Respiratory Interval

The respiratory interval was shortened in all rats during HIm as compared to LIm (0.23±0.1 vs. 0.34±0.1 s, p<0.01 respectively in HW and 0.27±0.1 vs. 0.39±0.1 s, p<0.05 respectively in SD) (Figure 4).

B. Effect of Movement on R-R Interval

All rats showed a shortening in R-R interval during HIm as compared to LIm (0.15±0.01 vs. 0.16±0.01 s, p<0.01 respectively in HW and 0.17±0.01 vs. 0.19±0.01 s, p<0.001 respectively in SD) (Figure 5).

C. Effect of Movement on Cardiorespiratory Coordination

The total percentage of synchronization between cardiac and respiratory signals was significantly higher during LIm as compared to HIm (for example, 88.4±3.7 vs. 10.3±1.7%, p<0.001 respectively for HW1) (Table 1). High-intensity movement also caused a slight but significant decrease in the duration of cardiorespiratory coordination (for example, LIm vs. HIm: 2.12±0.3 vs. 0.88±0.2 s, p<0.001 respectively for SD1) (Table 1).

TABLE I
MEAN VALUES (± STANDARD DEVIATION) OF THE PERCENTAGE OF COORDINATION AND DURATION OF COORDINATED EPOCHS DURING LOW-INTENSITY AND HIGH-INTENSITY MOVEMENT

Rats	Percentage of coordination		Duration of coordination (s)	
	LIm	HIm	LIm	HIm
HW1	88.4±3.7	10.3±1.7***	1.66±0.3	0.74±0.2***
HW2	90.1±2.9	7.8±1.8***	2.02±0.3	0.67±0.2***
HW3	88.6±3.8	9.7±2.2***	1.85±0.2	0.81±0.2***
HW4	81.9±4.6	15.1±1.9***	1.97±0.3	0.65±0.2***
SD1	96.5±1.8	2.4±0.9***	2.12±0.3	0.88±0.2***
SD2	91.7±3.3	5.7±1.2***	1.78±0.2	0.72±0.2***
SD3	88.2±3.5	9.6±2.2***	1.91±0.3	0.77±0.2***
SD4	89.1±3.0	8.8±1.4***	1.88±0.3	0.59±0.2***

Asterisks indicate differences with low-intensity movement.
*** represents p<0.001.

D. Effect on Phase-Locking Ratio

The phase-locking ratio of 3:1 was the most frequently ratio observed during LIm. Other phase-locking ratios for cardiorespiratory coordination were 2:1, 4:1, 5:2 and 7:2. However, in addition to a significant decrease in phase-locking between heart and respiratory cycles, 2:1 was the most frequently observed locking ratio during HIm.

IV. Discussion

We have previously shown that voluntary movements in rats are associated with increases in the respiratory rate [13]. This is the first study to investigate the interaction between cardiac and respiratory signals during movement in conscious freely moving rats. Our results show that the amount of interaction between the cardiac and respiratory oscillators is decreased during high-intensity movement. It is also evident that movement causes a shortening in the respiratory and R-R intervals.

Previous studies in humans have established the relation between respiration and body movement during certain

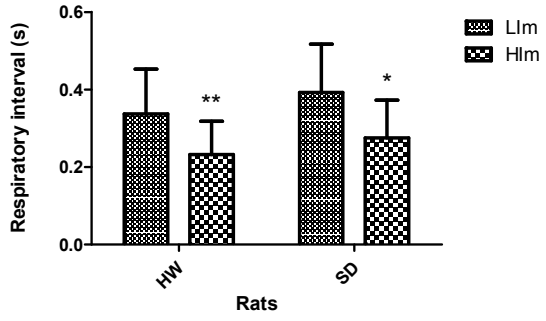

Fig. 4. Overall average respiratory intervals in HW and SD rats during LIm and HIm presented as mean±SD. * and ** represents p<0.05 and p<0.01 respectively.

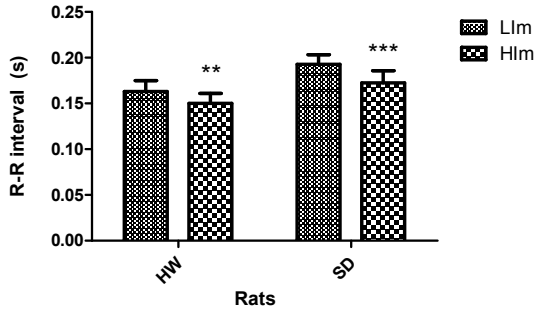

Fig. 5. Overall average R-R intervals in HW and SD rats during LIm and HIm presented as mean±SD. ** and *** represents p<0.01 and p<0.001 respectively.

activities such as exercise [3]. Our finding of a shortening in the respiratory interval in freely moving rat can be well explained from the exercise physiology in larger mammals including humans [14, 15]. The dominant cause of the change in respiratory interval would be to facilitate high demand of oxygen in the lungs during movement as compared to non-movement.

Respiratory sinus arrhythmia (RSA), the cyclical modulation of heart rate by respiration, is a well-known phenomenon that has been observed in humans as well as in mammals. RSA, which is significantly influenced by respiratory rate [16,17] and mostly reflects the changes in cardiac vagal tone [18], might be the cause of shortening in R-R intervals. Furthermore, the shortening in R-R interval during movement could also be due to the contraction of body muscles, which activates the muscle receptors causing a release from vagal tone, as reported in human subjects during exercise [19].

The phase-coupling between cardiac and respiratory rhythms has long been recognized [1,2]. Although the physiological significance of cardiorespiratory coordination is yet to be established, the relationship between heart rate and respiration has been suggested as a useful tool for assessing the autonomic nervous system functioning [20], diagnosis of sepsis [21] and obstructive sleep apnea [22]. However, for the accurate determination of the coordination process, the respiratory period and the ratio of heart rate to respiratory rate should also be considered [23]. In this study, a significant decrease in cardiorespiratory coordination was observed during high-intensity movement along with the shortening in the respiratory and R-R intervals. Movement also caused a decrease in the most frequent phase-locking ratio from 3:1 to 2:1. One of our previous studies on rats suggested that the phase-locking between the heart and respiratory rhythms is maximized at a particular respiratory frequency [5]; a similar result being reported in human subjects [16]. This would suggest that a change in respiratory interval during movement is the dominant cause of the decrease in cardiorespiratory coordination.

V. Conclusion

Movement in rats is associated with a shortening of respiratory and R-R intervals, which appear to confound the phase-locking between cardiac and respiratory cycles.

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