RESEARCH ARTICLE

Short term Heart Rate Variability in Rats

Om Lata Bhagat[1*], Prasunpriya Nayak[1], Renu Gupta[2]

Abstract

Objective: A sustainable animal model is needed to detect Cardiovascular autonomic dysfunctions, which can be assessed by recording electrocardiogram and analysis of heart rate variability (HRV). Therefore, the present study tried to find out the duration for HRV analysis for reliable results.

Material and methods: Recording of electrocardiogram (ECG) is reported in many articles. However, there is no information regarding the duration of ECG to be considered for the HRV analysis. Adult Wistar rats were used for a recording of ECG and HRV analysis. A combination of Ketamine 50 mg/kg and Xylazine 10 mg/kg was used for anesthésia in all the recordings. We analyzed the HRV parameters for all the records with 10 different durations starting from 1 minute to 10 minutes and compared.

Results and Discussion: It was observed that, ECG parameters were within normal range while the data from HRV analysis from different duration showed wide discrepancies depending on the duration of ECG recording used for HRV analysis.

Conclusion: The minimum duration of eight minutes of recording is likely to produce acceptable data for HRV parameters.

Keywords: Electrocardiogram (ECG), Heart rate variability (HRV), Wistar rat.

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Introduction

Cardiovascular autonomic dysfunctions can be studied extensively if we could develop a sustainable animal model for it. For developing a standard cardiac autonomic assessment method, we need the recording of electrocardiogram and analysis of heart rate variability. Respiration is a known modulator of heart rate and heart rate variability therefore, simultaneous recording of respiration and ECG is important. Heart rate variability represents the cardiac oscillations; used to assess the autonomic tone and is a well-established parameter in humans. This is calculated as beat-to-beat variation in RR interval or/and heart rate in the time domain, frequency domain and nonlinear methods. In last ten years, with the improvements and innovation in recording instruments and equipment, the interest of researchers have risen in animal studies, especially rats, which are reflected in an increase in the number of paper published. For the estimation of short-term heart rate variability in rats, duration if recording is not standardized yet. HRV analysis is more a mathematical algorithmic method like Fast Fourier transform in frequency domain. These algorithms can only be applied on stationary signal or stable part of the signal. Usually, at least 256 data points are required for these analysis.[1] In rats as the heart rate is more than 300 BPM so powers in frequency bands in HRV can be calculated for an ECG signal of one minute. Researchers have used the durations from 1 min to 10 min for HRV analysis. Most frequently used duration for HRV in rat is 5 minutes that is similar to what is being used for analysis in humans.[2,3] Also, one minute,[4,5] 3 minutes[6,7] and 10 minutes[8] durations are used often.

The present study attempted to find out duration for HRV analysis for reliable results. Thus we analyzed the HRV parameters for all the records with 10 different durations starting from 1 minute to 10 minutes and compared them.

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Material and Methods

We attempted to develop a sustainable animal model for studying autonomic response to various maneuvers like Valsalva maneuver. The first step was to standardize the ECG recording and calculate short-term heart rate variability.

Animals

All the animals were procured from the JNVU, Jodhpur. All the procedures for animal handling and experimentations were performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Anesthetization of Rats

For identifying the suitable anesthetic agents, we searched the literature for last 10 years using PubMed database and selected 44 articles in which the recording for respiratory and cardiac activity were done in Wistar rats. Anesthetic agent used in each study were noted and it was observed that out of these studies eighteen has used either inhalational or intramuscular anesthetic agent, rest have used intraperitoneal injection for inducing anesthesia. The

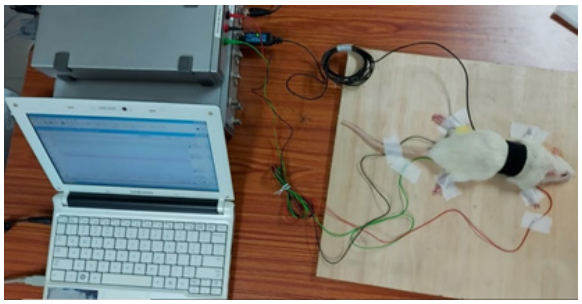

Figure 1: Recording setup for ECG and respiratory movements in Rats.

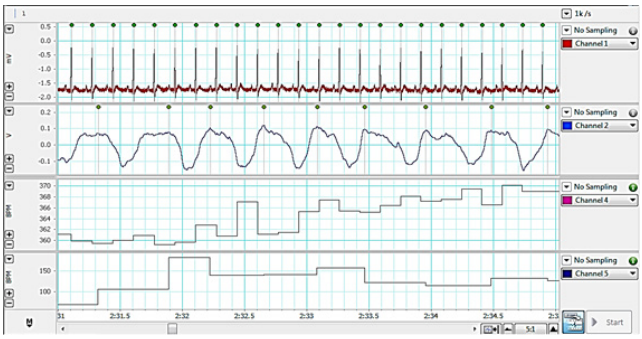

Figure 2: Sample recording of ECG and respiratory movements in Rats.

Processing of ECG parameters

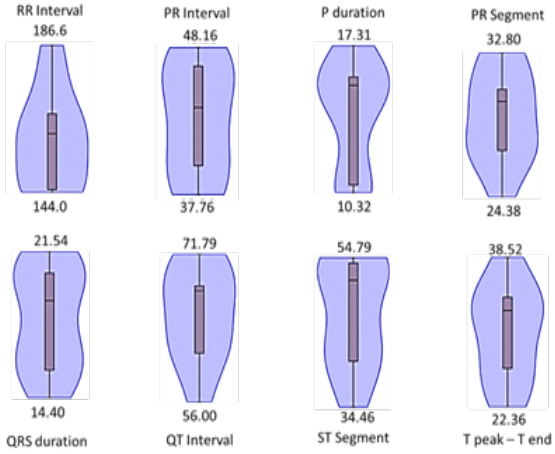

Figure 3: Distribution of ECG parameters of 10 recordings. All values are expressed in miliseconds.

most used anesthetic agents are, ketamine and xylazine combination, Phenobarbital, thiopental and urethane. The doses for these anesthetic agents were reported noticably varied and there is no consensus over that. The ketamine used in the range of 30-100 mg/kg body weight in combination with xylazine 3-15 mg/kg body weight (Table: 1). We used the combination of Ketamine 50 mg/kg and xylazine 10 mg/kg for anesthésia in all the recording.

Table 1: Studies with use of Ketamine and Xylazine for anesthesia for recording ECG

Authors; Year	*Ketamine (doses mg/Kg)*	*Xylazine (doses mg/Kg)*
Nascimento *et al.*, 2019 [12]	80	10
Nwokocha *et al.*, 2019 [13]	42	5
Silva *et al.*, 2017 [14]	50	10
Shiogai *et al.*, 2012 [15]	45	7
Baeeri *et al.*, 2013 [16]	30	3
Grandič *et al.*, 2011 [17]	100	15
Yenişehirli *et al.*, 2008 [18]	75	10
Musizza *et al.*, 2007 [19]	45	7
Sumitra *et al.*, 2004 [20]	50	5
Veliks *et al.*, 2004 [21]	30	3.5

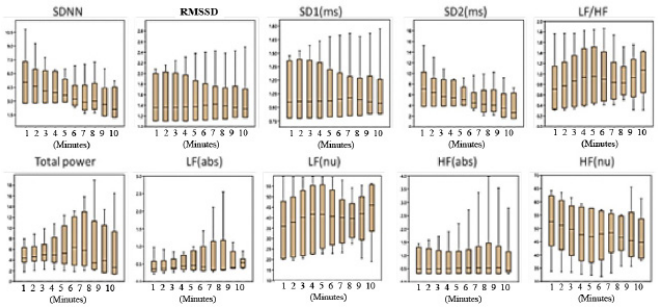

Figure 4: Distribution of HRV parameters of 1 minute to 10 minutes duration .

Comparison of the Breathing Rate and Heart Rate measurements

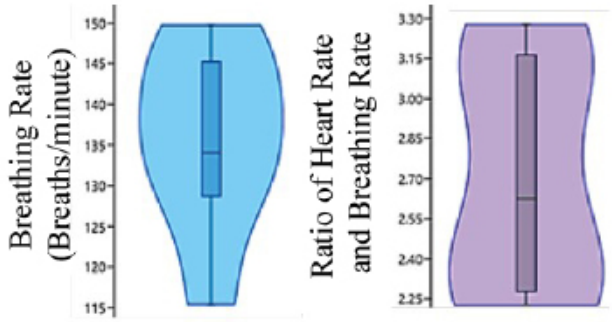

Figure 5: Distribution of recorded breathing rate and ratio between heart rate and breathing rate.

Recording of Ecg and Respiratory Movements from Anaesthetized Rats

The overnight food-restricted male Wistar rats (BW 140-180g) were anesthetized with ketamine (*i.p.*). After 5 minutes, limb electrodes and reference electrode were connected and the pulse transducer with piezo-electric element was fixed in the midline using a velcro strap. The animal was stabilized in prone position and electrodes for ECG recording were connected to a channel of 4-channel PowerLab

(AD Instruments) with the help of an Animal Biopotential module (Figure 1). Another channel was used for recording of respiratory movements through pulse transducer. The recording was continued for 15 minutes A sample screen-shot has been presented in Figure 2.

Standardization of the HRV Parameters

Parameters of heart rate variability were analyzed from the recorded ECGs using LabChart 8. The standard criteria for human HRV analysis is more than 256 data points (which generally come with 5 minutes of ECG recording). However, there is no available standard protocol for HRV analysis in rats. As the normal heart rate of rats is >300 BPM, the required 256 data points could have been collected by 1 minute of ECG recording. However, the preliminary data processing showed high level of discrepancies between individual values. Thus, to standardize the minimum recording duration and inclusion in processing, we analyzed the HRV parameters for all the records with 10 different durations starting from 1 minute to 10 minutes. During the processing, the RR and PR intervals, durations of P wave, PR segment, QRS complex, ST segment, QT interval and time required for T-peak to T-end are calculated for all the recordings with different time epochs (1-10 seconds). The distribution of these parameters indicates wide variations in their central tendencies (Figure 3). Similar trend related to non-uniformity in terms of epoch-related variations was also observed in case of the time domain parameters of HRV analysis like standard deviation of normal to normal intervals (SDNN), root mean square of standard deviation (RMSSD), frequency domains like total power, low frequency power – absolute (LFabs) and normalized (LFnu), high frequency power – absolute (HFabs) and normalized (HFnu), as well as LF/HF ratio (Figure 4). Interestingly, normal distribution was observed in case of breathing rate; however, the normalcy was disturbed when the ratio of heart rate and breathing rate was computed (Figure 5).

DISCUSSION

From the data presented here, it appears that the ECG parameters are well within the normal range while the data from HRV analysis from different duration showed wide discrepancies depending on the duration of ECG recording used for HRV analysis. Therefore, the preliminary data presented here suggest that the minimum duration of eight minutes of recording is likely to produce acceptable data for HRV parameters. However, a larger data size is required to confirm these observations.

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