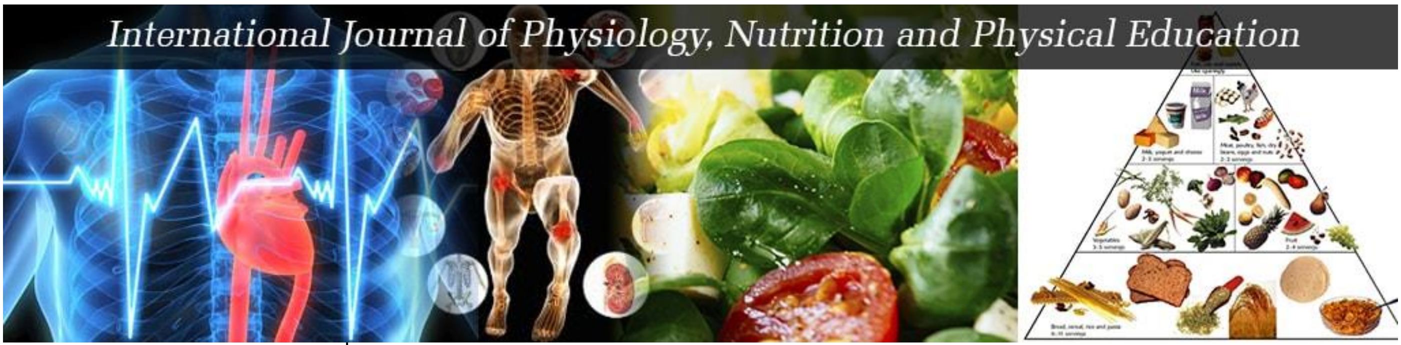

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Experimental monitoring of surface ECG on conscious rodents

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Abstract
Autonomic work of heart can be read through heart rate variability (HRV). Numerous studies are available on rodent electrocardiography. Heart is sensitive to various therapeutic agents. Small rodents like rats and mouse are used instead of large animals because of their low cost and easy handling. In recent years there has been shift in the ways of ECG monitoring in rats and has enabled researchers to receive ECG method that closely resembles human ECG monitoring. Use of anesthesia on rodent for HRV has proven to alter the autonomic activity of heart and also the implanted telemetric electrode will have its impact on rodent electrocardiography. Surface recording of ECG in a conscious rodent model will provide exact electrocardiographic assessment without the influence of external factors.

Keywords: Heart rate variability, ECG, conscious animal

Introduction
HRV is the beat to beat variation of the heart interval (RR) that is the fluctuation over the time course of successive heart beats. HRV is a definite tool for reflection of the many physiological agents modulating the normal rhythm of the heart (Pagani *et al.* 1986) [1]. The HRV is determined by different physiological factors includes reflex arc (respiration, carotid sinus, Bainbridge) pacemaker activity, and connection between central nervous system (CNS) to the autonomic nervous system (ANS), circadian oscillations in plasma hormones, genetics and thermoregulation, receptors for dopamine, angiotensin II, and adenosine located in sinusal node (Dreifus *et al.*, 1993) [2]. HRV is a reliable for signal accepting the status of ANS. The change in normal variability in HR is due to autonomic neural regulations of the heart and circulatory system (Berger *et al.*, 1986) [3]. HR is controlled by the balancing action of sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) which are the branches of ANS.

Heart rate (HR) increased when the sympathetic stimulation increases in conditions like stress, exercise and disease. Heart rate decreased during parasympathetic stimulations like trauma, resultant of internal organ functions, allergic reactions and inhalations of irritants. The degree of fluctuations in the HR suggests the details about the functions of the nervous control over the HR and hearts capability to respond. In humans HRV analysis can be applied to several areas. As stated above it is used to evaluate the functions of the autonomic nervous system, in addition it is used to analyze the blood pressure and myocardial infarction, (Pagani *et al.*, 1986) [1]. It is also aware that HRV is influenced by age, gender, exercise, with intake of drugs, alcohol and cigarette smoking (Mukilan *et al.*, 2016; Archana *et al*., 2016; Manibalan & Mukilan. 2019) [6, 6, 5].

Hon & Lee, (1945) were the first to conduct the clinical study on Heart Rate Variability. In the last few decades there was an increase in number of studies that employed HRV analysis for different purposes: the analysis of physiological rhythm embeds in the beat to beat heart rate intervals, studies on risk of mortality due to infarction and studies on diabetes. The standardization of HRV measurement and analysis of signals regarding standardization of measurements and physiological understanding for clinical use, were said forth by European Society of Cardiology and the "North American Society of Cardiology and Electrophysiology" published their result on Task Force on HRV ("Task Force of The European Society of

Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

HRV in Animal Model

Small rodent ECG analysis may be an inexpensive and sensitive to study the cardio toxic potential and helps to find mechanisms of actions of common cardio toxicants. HRV analysis methodology could not be directly transferred to the animal studies. For small rodents short term recordings, HRV shows the frequency bands as three distinct bands: frequencies lower than 0.2 Hz is stated as very low frequency band (VLF), frequencies between 0.2 Hz and 0.8 Hz is stated as low frequency band (LF), and frequencies between 0.8 Hz and 2.5 Hz is stated as high frequency band (HF).

Electrophysiological difference is there among the lab animals. The most easily seen difference between human and rats are in the heart rate and Action Potential (AP), average heart rate in humans at rest in conscious state is around 70 beats / min (Sheu & Colecraft *et al.*, 1997) [9], whereas the heart rate in rats is much faster (300-500 beats / min) (Tontodonati *et al.*, 2010) [10]. In addition to that duration of ventricular AP in humans is five times more than rats (Karmer *et al.*, 1993) [11].

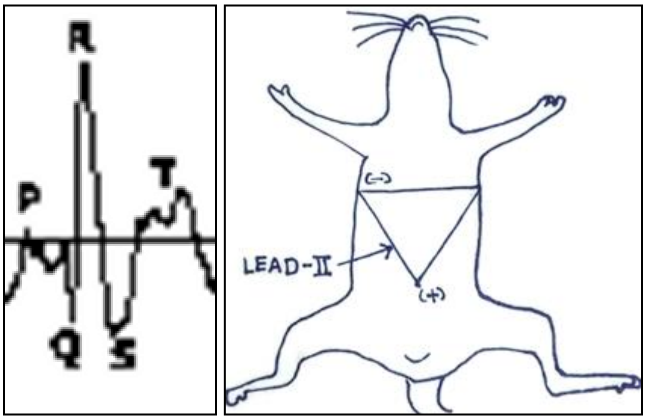


Fig 1: Lead II ECG waves of rat.

According to earlier studies the most commonly utilized setup in rats and mouse studies is a single lead II arrangement with one (+) in left leg and one (–) electrodes on right shoulder (Chen *et al.*, 2008; Meng *et al.*, 2009) [12, 13]. Lead II in the rodent is valuable since it shows powerful P, Q, R, S, and T waves.

Materials and Method

Animal preparation for ECG recording

After obtaining the institutional ethical committee approval 6 male Wister albino rats were obtained from the animal house and put in a cage for 1 week for accommodation. ECG was acquired from all the animals. The animals were conditioned inside a transparent restrainer for 20 minutes on everyday for a week prior to recording of ECG. The ventral surface of the animals was shaved. An electro conductive gel was applied over the self-adhesive platinum electrode with care was taken to avoid the formation of Gel Bridge among them. Two metal electrodes were used on the two limbs, the right limb and left leg which can be compared to Lead II electrode of human unipolar leads (Mongue-Din *et al.*, 2007) [14].

Recording of ECG for conscious rats

The electrodes were connected to the cable to reach the 2 channel data acquisition system INCO NAVIQURE. The animal was placed inside the clear transparent retainer which has ventilation holes. After 10 minutes inside the transparent retainer, the ECG was acquired for 5 minutes in all the groups. The diestrous stage was confirmed by the vaginal smear. The entire ECG recording was performed at the same time between 9am to 11am under neutral condition.

Recording of ECG for anaesthetized rats

To find the effect of anesthesia on cardiovascular performance, combination of ketamin hydrochloride and xyaline through injection was administered. Lead II ECG leads were connected to the animal and recording was done using polygraph for 5 minutes. QRS waves were detected and RR intervals identified. Through this time and frequency datas were obtained which gives sympathetic and parasympathetic action of heart.

The final result was obtained with KUBIOS HRV Analysis, which runs on MATLAB program was implemented for the HRV analysis.

HRV analysis

The same set up and algorithms are used for the data acquisition and HRV analysis. The ECG signals were recorded at the sampling rate of 1 kHz and the time constancy for the RR interval (the time duration between successive RR waves in ECG signals) was 1ms. ECG signals from each measurement were saved in a computer for time duration of 5 min.

The ECG signals were in reference to the repeated sequence of the five principal waves which were represented by P, Q, R, S and T. The QRS complex was composite of Q, R and S waves. For HRV parameters calculation of the QRS peaks were detected and a time series of the RR intervals were obtained.

Time domain analysis

In time domain analysis the calculations were made from the mean RR interval, mean heart rate (HR), and SDNN is the mean of 5 minute standard deviation of NN (normal to normal) interval, which means variability of cycles shorter than 5 minutes. The other commonly used measures include RMSSD, (the square root of meaned square differences of successive NN interval, NN50 the number of interval differences of successive NN interval greater than 50 ms. All the measurements are short term variations to estimate high frequency variations of heart rate.

SDNN indicates the sympathetic activity of heart, whereas RMSSD and PNN50 represent para sympathetic activity of heart (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology., 1996; Akselrod *et al.*, 1985) [16].

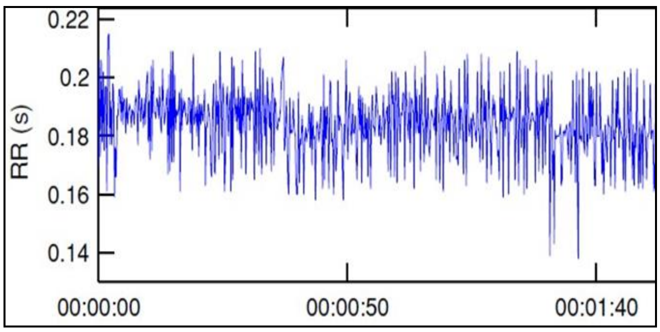


Fig 2: Tachogram.

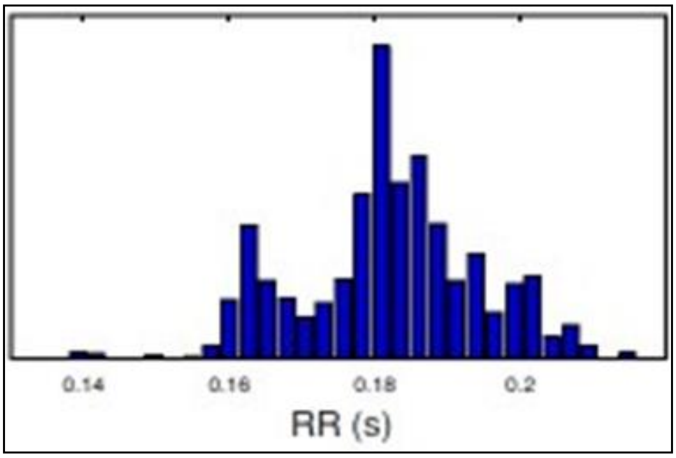


Fig 3: Histogram RR interval.

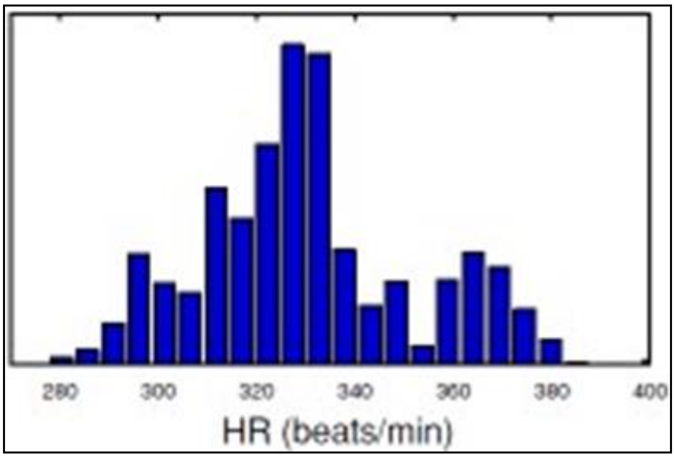


Fig 4: Histogram Heart rate. (Avg 340 bpm)

Frequency domain analysis

In the short term recording of HRV three main components are distinguished in a spectrum, VLF, LF and HF, very low frequency (VLF) are frequencies lower than 0.04Hz, Low frequency (LF 0.2 – 0.8 Hz) and High frequency (HF 0.8 -2.5 Hz). The distribution of HF and LF may vary in relation to changes in autonomic modulations of heart (Pagani *et al.*, 1986; Malliani *et al.*, 1991) [1, 17]. The physiological cause for VLF is not well defined.

The peak of HF is believed to reflect the parasympathetic activity of heart, while the peak of LF is more complex and is often found to be associated with sympathetic activity of heart (Furlan *et al.*, 1990) [18]. The changing relationship between the sympathetic and parasympathetic activity over heart can be obtained from ratio of LF to HF (LF/HF), (sympatho vagal imbalance) (Billman, 2011) [19]. The LF/HF ratio is a widely accepted tool to access cardiovascular autonomic regulations (Houle and Billman, 1999) [20], whereas the increase in LF/HF reflects 'sympathetic dominance' and decrease in this index denotes 'parasympathetic dominance'.

Statistics

The obtained results were compared between the conscious and anesthetized rats using student't' test.

Result

NN50%, rMSSD, SDNN in anaesthetized rats were found to be decreased, which indicates the decreased parasympathetic activity. Ketamin / xyaline combination has shown increased HR and decreased RR interval which shows the sympathetic dominance.

Overall increase in TP indicates sympathetic dominance in anesthesia rat. LF which indicates both sympathetic and parasympathetic activity. In anaesthetized rats LF was found to be increased than conscious rats shows sympathetic dominance.

Time domain analysis

Table 1: comparison with conscious rats anaesthetized rats shows increased HR and decrease RR interval, with significant decrease in SDNN and NN50%. * P< 0.01 is statistically significant.

	Conscious rats	Anesthetized rats
Mean RR (ms)	180 ± 3.8	168 ± 0.5*
Mean HR (beats/min)	306 ± 2.16	340 ± 1.81*
SDNN (ms)	14.01 ± 1.03	11.3 ± 0.9P
rMSSD (ms)	4.36 ± 0.13	3.86 ± 0.04
NN50%	18.8 ± 0.01	14.36 ± 0.7 *

Frequency domain analysis

Table 2: In comparison with conscious rats, anaesthetized rats shows significant increase in LF/HF ratio. * P< 0.01 is statistically significant.

	Conscious rats	Anesthetized rats
TP (ms^2)	84.6 ± 1.03	71.9 ± 6.3
LF (ms^2)	12.07 ± 0.6	14.3 ± 2.1
HF (ms^2)	14.1 ± 4.01	12.11 ± 0.05
LF/HF (ms^2)	0.86 ± 0.13	1.94 ± 0.16 *

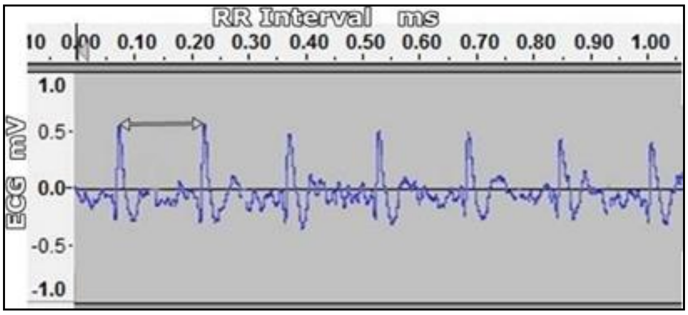
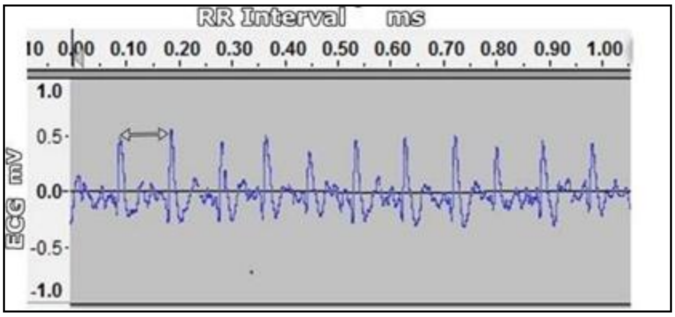


Fig 3: RR interval of conscious rat.



Decreased RR interval in anesthetized rat.

Discussion

Usually the injectable or inhalation type of anesthesia used for rodent studies have shown to affect the cardiac activity which may increase or decrease the heart rate by its action on cardiac myocyte and conducting system of heart. ketamine anesthesia has its effect on cardiac voltage sensitive calcium channels, which may significantly affects the cardiac myocytes electrical activity (Hirota and Lambert 1996) [22], ketamine anesthesia affects sympathetic activity of heart, thus heart rate is increased and RR interval is decreased when compare to the conscious rats (Suleiman *et al.*, 2012) [21].

Conclusion

Electrodes placed externally and introduced in a restrainer for ECG recording without anesthesia has provided the time and frequency parameters for HRV analysis, Influence of anesthesia which has shown to alter the shypathovagal activities of heart. Iatrogenic implantation of electrodes for telemetric recording of ECG will have its impact on cardiac activity. This surface monitoring conscious recording of HRV will provide exact action of heart without the influence of external agents.

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