Noninvasive recording of electrocardiogram in conscious rat: A new device

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Abstract:
Aim: Electrocardiogram (ECG) is an important tool for the study of cardiac electrophysiology both in human beings and experimental animals. Existing methods of ECG recording in small animals like rat have several limitations and ECG recordings of the anesthetized rat lack validity for heart rate (HR) variability analysis. The aim of the present study was to validate the ECG data from new device with ECG of anesthetized rat.

Materials and Methods: The ECG was recorded on student's physiograph (BioDevice, Ambala) and suitable coupler and electrodes in six animals first by the newly developed device in conscious state and second in anesthetized state (stabilized technique).

Results: The data obtained were analyzed using unpaired t-test showed no significant difference (P > 0.05) in QTc, QRS, and HR recorded by new device and established device in rats.

Conclusion: No previous study describes a similar ECG recording in conscious state of rats. Thus, the present method may be a most physiological and inexpensive alternative to other methods. In this study, the animals were not restrained; they were just secured and represent a potential strength of the study.

Key words:
Conscious, electrocardiogram, noninvasive recording, rat

Methods

The electrocardiogram (ECG) is the record of algebraic sum of the action potentials of myocardial fibers during the cardiac cycle.⁰ Thus, the ECG is an important tool for the study of cardiac electrophysiology both in human beings and experimental animals. Cardiac electrophysiology is highly useful in clinical assessments of cardiovascular disorders and acutely sensitive to the various effects of chemical and drugs.¹,²

Existing methods of ECG recording in small animals like rat have several limitations, which can influence the electrophysiological parameters. For example, some methods require anesthesia and other require surgical procedures for ECG electrode implantation. Methods using anesthesia (drugs) and surgical procedures may affect the heart rate (HR), rhythm and various segment, and intervals of ECG.³,⁴ In addition, ECG recording of the anesthetized rat lacks validity for HR variability analysis.⁵,⁶

Various authors suggested that ECG can be recorded using specially designed boxes, stages, and jacket fitted with electrodes.⁷,⁸ These devices require complicated proceedings. Therefore, development of noninvasive devices for ECG and blood pressure recording in conscious animal rat is need of the day. These devices must be free from anesthesia, surgical invasions, or implant of electrodes/transmitter. Although it has been claimed that telemetry method is the gold standard⁹ for ECG recording in rats, this device is highly expensive and irrational for the developing countries like India. Hence, in the present study, we described a new device for ECG recording for conscious rat which is noninvasive and free from anesthesia. The aim of the present study was to validate the ECG data from conscious with an anesthetized rat.

Materials and Methods

The study was approved by institutional ethics committee (31/IAH/Pharma/14 dated 17/05/14) and conducted on six male Wistar rats (220–300 g), kept in wooden floored cages, in a room with temperature of about 27°C and 12 h day-night cycle. Animals were acclimatized in...
ambient conditions and protocol of our experimental laboratory for 1 week and procedures were performed in accordance with the CPCSEA guidelines.

**Experimental Protocol**
The ECG was recorded on student’s physiograph (BioDevice, Ambala) and suitable coupler and electrodes in six animals first by the newly developed device in conscious state and secondly in anesthetized state (stabilized technique). Thereafter, the recorded data were compared for accuracy of new device. ECG was recorded in each animal (both in conscious and anesthetized state) at 9:30 am daily for 5 days.

**Electrocardiogram Recording by New Device in Conscious Rat**
A diagram of the developed device for ECG recording of conscious rat is shown in Figure 1a and b. It comprised four separate strips of fabric hook and loop fasteners (Velcro) mounted on the block board of a small table. Animal (rat) was placed on the surface of the block board so that its feet were not in contact with each other. The rat was kept in stationary position by securing their body by Velcro as shown in Figure 2 and one fine sheet of polyethylene was placed between two limbs for insulation. Disk electrodes were tied on palmer surface of clean shaven limbs of rat. The front limbs and left hind limb were used for recording of ECG in standard leads while the right hind limb was attached with grounded electrode. ECG was recorded on a student’s physiograph machine moving at a speed of 50 mm/s at normal filtering. ECG parameters, i.e. RR interval, QRS duration, and QT interval were measured from the lead II and analyzed by one of the authors himself. ECG was recorded for 5 min in each group. The mean value of six consecutive waves was calculated.

**Electrocardiogram Recording in Anesthetized Rat: Anesthetization of Rat**
Animals were anesthetized with thiopental (50 mg/kg body weight) intraperitoneally[11] and secured on animal operation table for ECG recording. ECG was recorded on a student’s physiograph machine moving at a speed of 50 mm/s at normal filtering. ECG parameters, i.e., RR interval, QRS duration, and QT interval were measured from the lead II using calipers.

RR interval was recorded to calculate the HR using formula: number of RR interval (ECG small square) covered in 1 minute.

**Statistical Analysis**
All data are expressed as mean ± standard deviation; differences in data between two devices were calculated using GraphPad QuickCalcs (Graph Pad Software, Inc., La Jolla, CA 92037 USA).

**Results**
Typical ECG in lead II records obtained from the various rats are shown in Figure 1. Amplification was adjusted and paper speed was kept at 50 mm/s to obtain comprehensible waveforms in each animal. Electrocardiographic data obtained from the different animals are shown in Table 1. In lead II, the P waves, QRS complexes, and T waves were observed. We were not able to quantify the S-T segment in the ECG recordings. The reliable method for correcting the QT interval for rate is the Bazett’s formula[12] that is QTc = QT/√RR, where QTc is the QT interval corrected for rate and RR is the interval

**Table 1: Electrocardiogram parameters in conscious and unconscious rat**

| Paper-speed 50 mm/s | ECG parameter | Group-I (n=6) | Group-II (n=6) | P* |
|---------------------|---------------|--------------|--------------|----|
| Baseline (day-1)    | QTc (ms)      | 72.4±2.34    | 73.3±1.96    | 0.4962 |
| QT (ms)             | 60.8±11.1     | 58.0±10.0    | 0.7093 |
| HR (bpm)            | 368±27.6      | 352±23.8     | 0.3075 |
| Day-2               | QTc (ms)      | 72.1±1.85    | 73.4±3.13    | 0.4017 |
| QRS (ms)            | 55.0±10.0     | 53.3±8.9     | 0.7726 |
| HR (bpm)            | 374±27.1      | 359±27.7     | 0.3726 |
| Day-3               | QTc (ms)      | 71.2±3.64    | 72.7±1.37    | 0.3671 |
| QRS (ms)            | 55.0±9.17     | 48.3±6.05    | 0.1161 |
| HR (bpm)            | 352±15.1      | 378±24.8     | 0.084 |
| Day-4               | QTc (ms)      | 72.2±3.74    | 73.7±2.37    | 0.3877 |
| QRS (ms)            | 52.3±6.05     | 49.0±9.17    | 0.4788 |
| HR (bpm)            | 362±15.1      | 378±24.8     | 0.1961 |
| Day-5               | QTc (ms)      | 72.6±3.24    | 73.3±1.97    | 0.4001 |
| QRS (ms)            | 58.0±10.0     | 60.8±11.1    | 0.7141 |
| HR (bpm)            | 371±27.6      | 352±23.8     | 0.2188 |

*By unpaired t-test. ECG=Electrocardiogram, HR=Heart rate
between two consecutive R-waves measured in milliseconds. HR was calculated from the ECG records and it was >300 bpm. The mean values of ECG findings are shown in Table 1. The data showed that the mean HR (bpm) was 365.80 ± 7.95 and 363.80 ± 13.27 in conscious and anesthetized rats, respectively. QTc (ms) was 72.72 ± 0.36 in animals for recording new device, while it was 73.8 ± 0.56 (ms) in anesthetized animals. QRS was 56.220 ± 3.25 (ms) in ECG of conscious animal, and the value of QRS was 53.8 ± 5.48 (ms) in ECG of anesthetized animals. Our data demonstrated that there was no significant difference in QTc, QRS, and HR.

**Discussion**

Adverse effects of drugs and toxicants on the heart often manifest as a change in the ECG.[13] The ECG has been used since years to assess human cardiovascular health. Electrocardiographic recordings usually help to detect abnormal myocardial action potential, conduction of impulse, disturbances in cardiac rate and rhythm, and altered autonomic activities.[2] The method described, in this study, has many advantages over existing methods for recording ECG in conscious laboratory animals. Only a time of a week is needed to be spent on training/acclimatization of animals for this recording procedure, i.e., securing their body by Velcro and attachments of electrodes to limbs. In this way, the animals remain in a natural sitting position and there are no restrain or stress. Although the principle of the new device is same as existing one, the recording technique is unique and it will be able to record the ECG in conscious rats. ECG recording in conscious rats rely either on surgical placing of electrodes or implanted transmitter type electrodes.[10] It has shown that inflammatory responses may alter the physiological parameters of ECG. Although anesthesia may influence the ECG parameters, our data revealed that there was no statistically significant difference.

**Conclusion**

No previous study describes such type of ECG recording in conscious state of rats. Thus, the present method may be a most physiological and inexpensive alternative to other methods. In our study, the animals were not restrained; they were just secured and represent a potential strength of the study. The usefulness of this method will be in assessing the effect of drugs/toxicity on the ECG and HR.

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**Conflicts of Interest**

There are no conflicts of interest.

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