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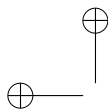
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Noninvasive method for electrocardiogram recording in conscious rats: feasibility for heart rate variability analysis

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ABSTRACT

Heart rate variability (HRV) analysis consists in a well-established tool for the assessment of cardiac autonomic control, both in humans and in animal models. Conventional methods for HRV analysis in rats rely on conscious surface electrocardiogram (ECG) recording based on prior invasive surgical procedures for electrodes/transmitters implantation. The aim of the present study was to test a noninvasive and inexpensive method for ECG recording in conscious rats, assessing its feasibility for HRV analysis. A custom-made elastic cotton jacket was developed to fit the rat's mean thoracic circumference, with two pieces of platinum electrodes attached on its inner surface, allowing ECG to be recorded noninvasively in conscious, restrained rats ($n=6$). Time- and frequency-domain HRV analyses were conducted, under basal and autonomic blockade conditions. High-quality ECG signals were obtained, being feasible for HRV analysis. As expected, mean RR interval was significantly decreased in the presence of atropine ($p<0.05$) and increased in the presence of propranolol ($p<0.001$). Also, reinforcing the reliability of the method, low- and high-frequency HRV spectral powers were significantly decreased in the presence of propranolol ($p<0.05$) and atropine ($p<0.001$), respectively. In summary, the present work describes a novel, inexpensive and noninvasive method for surface ECG recording in conscious rats.

Key words: electrocardiography, heart rate variability, methodology, noninvasive, rat.

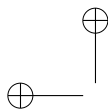
INTRODUCTION

The electrocardiogram (ECG) is a remarkably important tool for the study of cardiac electrophysiology, both in the clinical and in the experimental setting. It is known that the rat constitutes an important model for cardiovascular physiology research and, for long, ECG-based studies have been conducted in this animal model.

Heart rate variability (HRV) analysis, a technique that relies on the assessment of fluctuations on the intervals among successive ECG R waves, has been used as a powerful tool for the assessment of cardiac autonomic control (Alkhalaf et al. 1981, Task Force 1996).

Decreased HRV is associated with an increased risk of ventricular arrhythmia, and has been shown to constitute an independent prognostic factor for mortality in cardiac patients (Kleiger et al. 1987, Nolan et al. 1998). In rats, power spectral analysis of HRV has been shown to be an effective method of detecting disturbances in cardiac autonomic control in some experimental models and in pathologic conditions, such as myocardial infarction and diabetic neuropathy (Krüger et al. 2000, Sanyal et al. 2002).

Approaches based on ECG recording in the anesthetized state lack validity for HRV analysis, since



thetia may represent an important additional risk for animal mortality in some pathological conditions, such as in myocardial infarction and diabetes models (Tivesten et al. 2000, Flumignan et al. 2006, Cohen-Boulakia et al. 2000). Thus, the development of a new method that does not rely on surgical procedures for electrodes/transmitter implants may be of considerable importance.

Although telemetry represents the gold-standard for ECG recording in rats, the implementation of telemetric systems entails high costs. Additionally, some approaches based on electrodes implants may be limited by progressive decreases in electrodes viability across a couple of days. So, the development and application of a noninvasive method for conscious state ECG recording may represent a consistent alternative approach, allowing long-term HRV studies in the rat model.

Thus, the aim of the present study was to test a noninvasive and inexpensive method for ECG recording in conscious rats, assessing its feasibility for heart rate variability analysis.

MATERIALS AND METHODS

The study was in accordance with the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985), and was approved by the Institution’s Animal Care and Use Committee. Experiments were conducted on 6 male Wistar rats (330-370g), kept in cages floored with wood shavings, in a room with constant temperature (23°C) and 12h dark-light cycle. All animals had access to food and water *ad libitum*.

Prior to ECG recordings, animals were conditioned for 7 consecutive days, 20 minutes each day, inside a plexiglass restrainer. All posterior recordings were conducted on a constant environment, during the morning (0700-0900h).

A day before ECG was firstly recorded, the ventral thoracic region of each animal was carefully shaved. A custom-made elastic cotton jacket was developed to fit the rat’s mean thoracic circumference, and two pieces of rectangular platinum electrodes (each one measuring 7.0×3.0 mm) were attached on the jacket’s inner surface, with each electrode being connected to a cable long

be acquired in a lead close to DII, with prominent R wave peaks. Details on the jacket can be observed in Figure 1B.

A conductive ECG gel was applied over each electrode, with care being taken to avoid the establishment of a gel bridge between them. After that, the elastic cotton jacket was dressed (Fig. 1A), the animal was placed inside the plexiglass restrainer (Fig. 1C), which had holes on its front end and on its other surfaces, allowing adequate ventilation, and electrodes were connected to a differential A/C amplifier (A-M Systems, USA), with signals being digitized by a 16 bit A/D interface converter (Axon 1322-A, USA), and sampled at 10 KHz by the software Axoscope 9.0 (Axon Instruments, USA). Data were stored in a PC for off-line processing. The ECG recording started 10 minutes after the animal was placed inside the plexiglass restrainer, and was conducted for 5 minutes. For each record, the most stable 180s continuous segment was chosen for HRV analysis. This segment duration was described in previous studies (Mangin et al. 1998, Krüger et al. 2000).

Additional records were conducted on different days (48h interval among them), with animals under vagal and sympathetic blockade conditions. For vagal blockade assessment, animals were treated with the muscarinic antagonist atropine sulfate (2 mg kg^{-1} i.p., Sigma), while for sympathetic blockade, the beta blocker DL-propranolol hydrochloride was used (4 mg kg^{-1} i.p., Sigma). Electrocardiogram records were carried out 10 minutes after drug injection.

All HRV signal processing in the present study was done using Matlab-based algorithms. ECGs were bandpass filtered (2-300 Hz) and, after R wave peak detection, 180-s tachograms were generated, containing all heart period fluctuations within this time segment. In the time-domain, the following indexes were obtained: RR (mean RR interval), SDNN (standard deviation of RR intervals), RMSSD (square root of the mean squared differences of successive RR intervals), and pNN5 [percentage of successive RR interval differences greater than 5 ms, as described by Aubert and colleagues (1999)]. For spectral (frequency-domain) anal-



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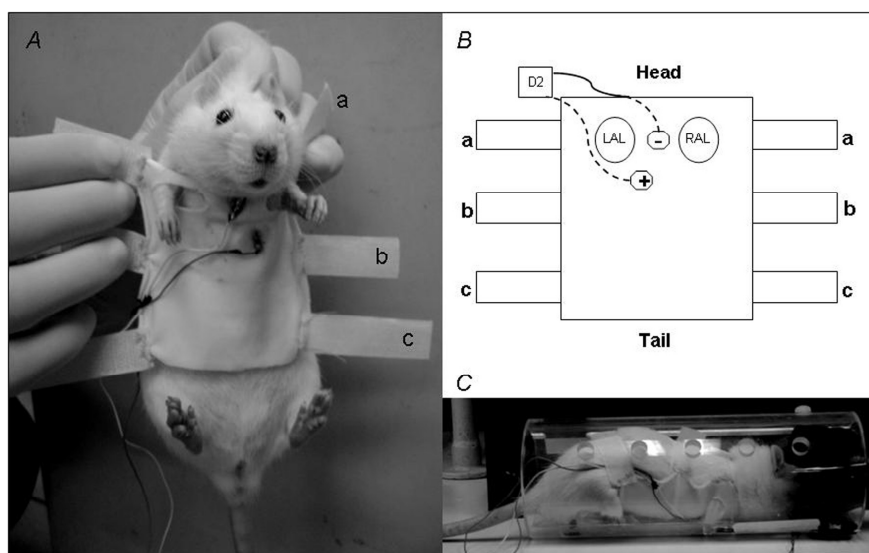


Fig. 1 – ECG acquisition method: A – jacket placement on the rat. B – schematic diagram of the inner face of the jacket, illustrating negative and positive electrodes positions. LAL – left anterior limb; RAL – right anterior limb. C – restrained animal in rest during signal acquisition.

obtained with a fast Fourier transform based method (Welch’s periodogram: 256 points, 50% overlap, and Hamming window). Two frequency bands were determined: low-frequency (LF: 0.2–0.8 Hz), and high-frequency (HF: 0.8–2.5 Hz). Power (in ms^2) was estimated as the area under the spectrum within these frequency ranges.

Paired Student’s t-tests were conducted in order to compare power spectral indexes (LF and HF powers) before and after autonomic blockades, while mean RR comparisons were carried out by one-way analysis of variance (ANOVA) with Bonferroni post-test. All data are expressed as mean \pm standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 4 (GraphPad Software, Inc.). Statistical significance was established at the $p < 0.05$ level.

RESULTS

Electrocardiography in conscious state animals is a widely used tool for cardiac autonomic control, allowing the extraction of HRV parameters. With the present noninvasive method, we have shown the possibility of

surgical implants of electrodes/transmitters, we show that the present approach is feasible for HRV analysis in the time- and frequency-domain, as illustrated in Figure 2B and Figure 2C, which show, respectively, a representative tachogram and RR power spectrum. Additionally, in Table I we present the individual HRV time-frequency-indexes from each of the 6 animals used in the present work.

In addition, to assess the HRV responses to autonomic blockades, atropine and propranolol were used. As expected, RR was significantly decreased in the presence of the muscarinic antagonist atropine sulfate ($p < 0.05$), and increased in the presence of the beta-adrenergic antagonist propranolol ($n=6$; $p < 0.001$), as shown in Figure 3A. Also, reinforcing the reliability of the present method, as expected, low-frequency HRV was significantly decreased in the presence of atropine (Fig. 3B; $p < 0.05$), and high-frequency HRV was significantly decreased in the presence of atropine sulfate (Fig. 3C; $p < 0.001$).

DISCUSSION

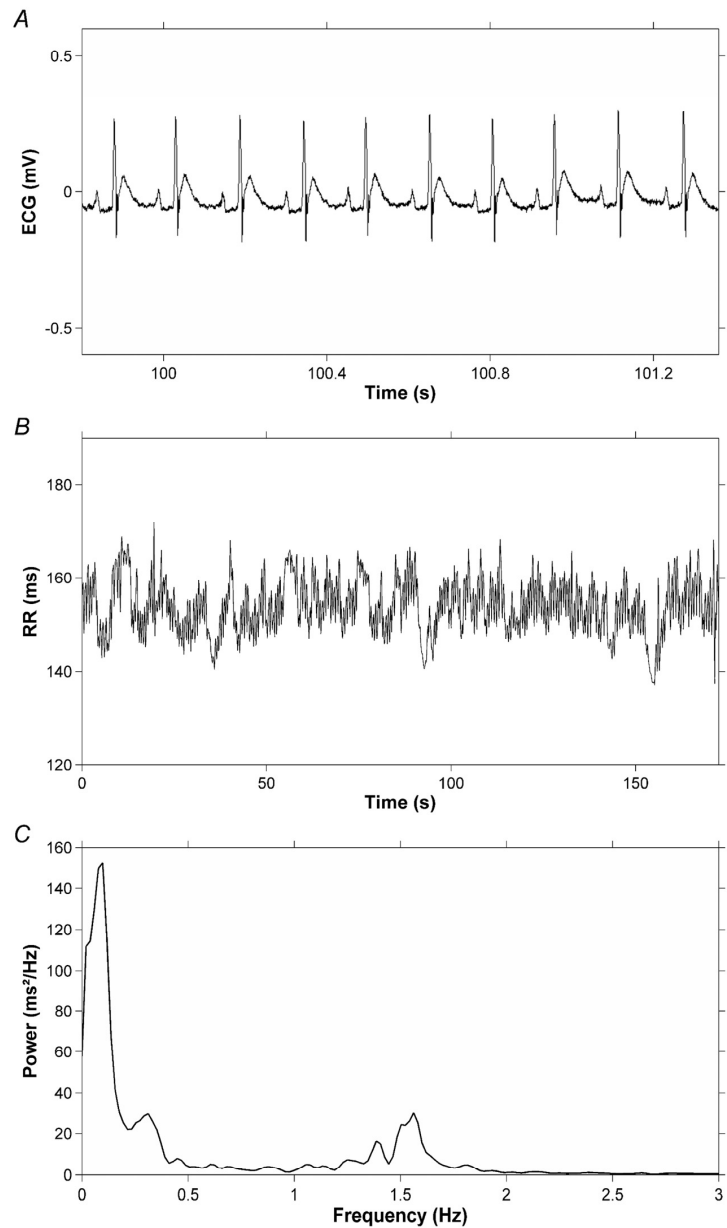
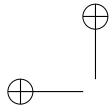


Fig. 2 – HRV graphs extracted from one animal: A – Non-filtered ECG segment tracing. B – Tachogram. C – Power spectrum of RR fluctuations.

sible prognostic value of HRV in rodents (Rowan et al. 2007). The rat has been shown to constitute an important model for the study of HRV, being suitable for investigations involving diseases, toxicological, and environ-

methods in the study of HRV in rats has been recently described (Beckers et al. 2006).

Conventional methods for conscious state ECG recording in rats rely on surgical procedures for electrodes/



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TABLE I
Individual HRV parameters.

Animal #	RR (ms)	SDNN (ms)	RMSSD (ms)	pNN5 (%)	LF Power (ms ²)	HF Power (ms ²)	LF/HF
1	153.54	9.89	5.41	24.40	14.74	11.30	1.30
2	149.48	6.23	3.47	13.72	3.37	4.33	0.78
3	147.78	8.40	4.47	20.79	9.93	6.69	1.48
4	153.65	5.85	4.64	27.81	5.83	8.71	0.67
5	147.88	7.78	2.95	2.71	5.65	3.30	1.71
6	146.97	4.94	3.23	12.83	4.72	3.19	1.48
Mean	149.88	7.18	4.03	17.04	7.37	6.26	1.24
S.E.M	1.21	0.75	0.39	3.74	1.72	1.34	0.17

RR (mean RR interval), SDNN (standard deviation of RR intervals), RMSSD (square root of the mean squared differences of successive RR intervals), pNN5 (percentage of successive RR interval differences greater than 5 ms), LF/HF ratio.

to implanted custom-made electrodes generally occur about a week after surgery, leading to progressive worsening in the signal to noise ratio, even resulting in the complete absence of detectable ECG tracings after some days or weeks (data not shown). Long-term ECG recording has already been described with telemetric systems (Howarth et al. 2008). Nevertheless, to our knowledge, no previous report describes long-term ECG recordings using setups based on custom-made implanted electrodes and direct cable connection. Thus, the present method may represent an interesting and inexpensive alternative to telemetry, allowing ECG repeated measures to be taken across a given time period, which may benefit study designs such as the ones used to assess the time course of complications in cardiovascular disease models, drug therapies, or cardiovascular adaptations to exercise training. Besides that, if adequate safety precautions are taken in animal handling, using a noninvasive method may possibly represent a way of reducing the risk of accidental infection, compared to surgical methods, in cases where conscious state ECG recording is necessary in rat models of infectious diseases (eg, Chagas' Disease).

Animal restraining may represent a potential limitation of our method, as no free activity or grooming was possible. Thus, we can not discard the hypothesis that restraining related stress could have some interference on the HRV indexes obtained. Nevertheless, some

ure 2C, it is possible to notice the presence of a low-frequency spectral peak occurring at about 0.3 Hz and a high-frequency peak close to 1.5 Hz. This spectral profile being comparable to those presented in previous HRV characterization and validation studies, which were conducted in freely moving rats, and relied on standard procedures for cable-based data acquisition or telemetric data transmission (Kuwahara et al. 1994, Aizawa et al. 1999). Moreover, the normalized LF and HF values obtained in this study are in agreement with previous findings in unrestrained conscious rats with implanted telemeters (Mangin et al. 1998, Ning et al. 2006). It seems important to remark that previous habituation to the procedure (see Methods) may be a very important requirement for optimizing this noninvasive approach.

Previous works have described blood pressure measurement in conscious rats, allowing the detection of blood pressure variability and baroreflex sensitivity (Ramaekers et al. 2002). We should remark that the present method is only suited for the measurement of ECG, and is not valid if blood pressure recording is needed.

Another potential limitation of the present method may be due to its potential interference over the respiratory pattern, so we took care to ensure that the custom-made jacket was complacent, in order to minimize this potential issue. In this way, it is important to remark that the intra-group sample dispersion of HF

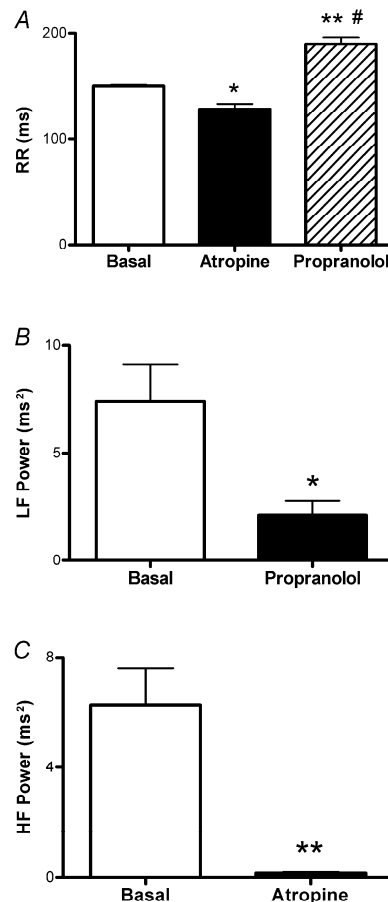
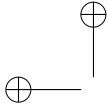


Fig. 3 – Autonomic blockade data: A – Mean RR values under basal conditions and after atropine (2 mg kg^{-1}) or propranolol (4 mg kg^{-1}) blockade. B – Low-frequency spectral power under basal conditions and after propranolol blockade. C – High-frequency spectral power under basal conditions and after atropine blockade. Data expressed as mean \pm SEM. * $p < 0.05$ vs. basal; ** $p < 0.001$ vs. basal; # $p < 0.001$ vs. atropine. For all conditions, $n = 6$.

indexes to sympathetic and parasympathetic autonomic blockades have shown a similar physiological pattern comparable with previous works in the literature that used telemetric systems (Kuwahara et al. 1994). We should remark that this approach may be specially suited for repeated ECG measures, so that paired comparisons would not be affected by such methodological concerns.

In summary, the present work describes a novel

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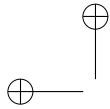
RESUMO

A análise da variabilidade da frequência cardíaca (VFC) consiste em uma metodologia bem estabelecida para o estudo do controle autônomo cardíaco, tanto em humanos como em modelos animais. As metodologias convencionais para o estudo da VFC em ratos utilizam-se de procedimentos cirúrgicos para o implante de eletrodos ou transmissores, o que possibilita a posterior aquisição do eletrocardiograma (ECG) no estado consciente. O objetivo do presente trabalho foi o de desenvolver e aplicar um método não-invasivo para o registro do ECG em ratos conscientes, verificando sua validade para a análise da VFC. Uma vestimenta de tecido elástico em algodão foi desenvolvida de acordo com as dimensões médias da circunferência torácica dos animais, e dois pequenos eletrodos retangulares de platina foram aderidos à superfície interna da vestimenta, permitindo o registro do ECG de forma não-invasiva em ratos conscientes ($n=6$), sob contenção. Foram conduzidas análises de VFC nos domínios do tempo e da frequência, tanto para a condição basal, como para as condições de bloqueio autônomo. Foram obtidos sinais de ECG de alta qualidade, viáveis para a análise de VFC. Conforme esperado, o intervalo RR médio foi significativamente reduzido na presença de atropina ($p < 0.05$), e aumentado na presença de propranolol ($p < 0.001$). Reforçando a validade do método, as potências espectrais de baixa e alta frequência da VFC sofreram reduções significativas, respectivamente, na presença de propranolol ($p < 0.05$) e atropina ($p < 0.05$). Em resumo, o presente trabalho descreve um novo método, de baixo custo e natureza não-invasiva, para a realização do ECG de superfície em ratos conscientes.

Palavras-chave: eletrocardiografia, variabilidade da frequência cardíaca, metodologia, não-invasivo, rato.

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