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A Method for Electrocardiographic Investigations in Experiments Creating Artificial Ischemia in Experimental Animals

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This report presents approaches to the development of methods for experiments creating artificial ischemia in experimental animals and specific methods for detecting, recording and storing electrocardiosignals (ECS) obtained by ultra-high-resolution electrocardiography (UHR ECG). Examples of ECG data recorded in experiments using experimental rats are also presented.

Introduction

Ischemic heart disease (IHD) is a serious problem in cardiology and medicine in general, confronting doctors and technical specialists working in the field of ECG technology. The relevance of this theme comes from the fact that circulatory system diseases in the 21st century constitute the most commonly encountered problem in current healthcare induced by factors including increases in ecological, technogenic, and psychological risks provoking negative changes in the people's state of health. With the aim of observing and detecting the early signs of cardiac pathology for the prevention, early diagnosis, and effective treatment of heart disease, recent years have seen the active development of new ECG methods based on improvements in apparatus, algorithms, and software for detecting and recording ECS [1].

The aims of the present work were to detect, record, and store ECS in standard and extended amplitude and frequency ranges to detect novel informative signs of cardiovascular system pathology in experimental animals and to assess the potential to diagnose the signs of ischemic heart disease at earlier stages of its development. The work contributes to advances in this area and addresses solution of a series of specific tasks: technical aspects of the development of methods for studies creating artificial cardiac ischemia in experimental animals [2] and development of specific methods for detecting, recording, and storing ECS using a new and original method for ultra-high-resolution electrocardiography (UHR ECG) [1, 3]. This method was developed in recent years by a group at one of the leading scientific schools in the Russian Federation, Radioelectronic and Informational Tools for Evaluating the Physiological Parameters of Living Systems (REIT LS) led by Doctor of Technical Sciences, Professor K. V. Zaichenko. The Grants Council of the President of the Russian Federation recognized this school with a Russian Federation Leading Scientific Schools award (Certificate No. NSh-3455.2012.8). This same decision awarded the group a grant for State Support for Leading Scientific Schools of the Russian Federation.

Methods for Procedures with Experimental Animals

These specific tasks were addressed by the authors of the present article – members of the REIT LS scientific school – in a series of experiments creating artificial ischemia in experimental animals at the Institute of Experimental Medicine, V. A. Almazov Federal Center for the Heart, Blood, and Endocrinology, with detection and recording of ECS using an SVR-4 cardiography system. This is the fourth modification of the experimental prototype (EP) for detecting and recording ultra-high-resolution electrocardiosignals throughout the heart

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cycle over wider amplitude and frequency ranges than in all other electrocardiography methods [4]. The minimum boundary of the amplitude range was of the order of 10 nV and the maximal upper limit of the frequency range could reach 2000 Hz or more [3]. The use of this approach provides for recording of low-amplitude and high-frequency useful components of ECS, which are termed micropotentials and carry diagnostically significant information. Previous studies in the UK [5, 6] and USA [7, 8] on the recording and analysis of the high-frequency components of ECS for investigation of the potential for diagnosis of IHD used an ECS recording range of up to 2000 Hz and showed that patients with severe ischemia have have considerably high-frequency components in the ECG than healthy people. It should be emphasized that the UHR ECG method combines all potentials of other existing methods for electrocardiography, i.e., standard, high-frequency, and others as described in [3-8].

There are several models for studying myocardial ischemia; for example, investigation of pathology in living organisms, isolated hearts, etc. The present method used a model in which the experiment is run on the whole body, as we needed to preserve the physiological regulatory mechanisms – the operation of the nervous, endocrine, and other systems. The main studies were performed on laboratory rats – male Wistar rats weighing 240-260 g, which were kept on a standard diet [9]. Animals were prepared for experiments as follows: they were anesthetized via the i.v. route, connected to the artificial pulmonary ventilation apparatus via tracheostomy, and sensor electrodes were connected to the authors' original SVR-4 cardiograph unit and the Kardiotekhnika-EKG-8 control cardiograph. Throughout the experiment, body temperature was maintained at a constant 37.0 ± 0.5°C by placing the animal on a special thermostatted operating table. Animals then underwent thoracotomy (opening of the chest cavity) for further surgical manipulations to produce myocardial ischemia and to position the clamps required for controlling circulation during surgery [9].

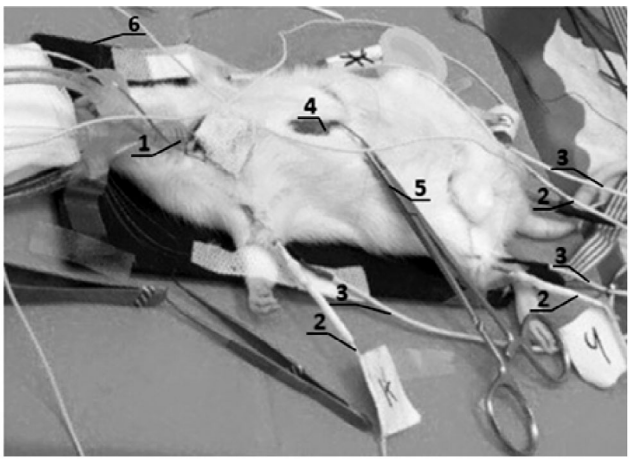

Fig. 1. An experimental animal connected to the experimental apparatus: 1) tubes connected to the artificial pulmonary ventilation apparatus; 2) sensor electrodes detecting ECS for the UHR-4 EP; 3) sensor electrodes detecting ECS for the Kardiotekhnika-EKG-8 control cardiograph; 4) incision; 5) clamp; 6) thermostatted operating table.

Figure 1 shows a photograph of an experimental animal during one of the experiments.

The experiment was divided into three stages. The first stage – the stabilization stage – was to obtain constant hemodynamic values for the experimental animal in the normal state (without pathological changes). This stage took about 30 min. Some minutes before completion of the stabilization stage, surgical intervention by left-sided thoracotomy was performed to apply a ligature, i.e., a propylene surgical filament. This provided for ligation (closing) of the left coronary artery to form an occluder, i.e., a controllable gap to regulate blood flow by altering the patency of the left coronary artery. This was used to model pathological transformation of the functional activity of the heart [9]. This manipulation began the second stage of the experiment – the ischemia stage. As all processes in the development of ischemia in these experiments in rats occur extremely rapidly [2, 9], the second stage also lasted about 30 min. The third stage was the reperfusion stage, during which the occluder was used to renew the flow of blood into the heart. This stage started at 60 min into the experiment. The experiment was complete at 180 min.

The whole of the experiment used a study protocol [9] in which the following parameters were recorded: the times at which the experiment and its stages started and finished, the condition of the experimental animal at different stages (arterial blood pressure and heart rate), the time of surgical intervention during the operation, and random events which might influence the experimental animal during the experiment.

From the medical point of view, as confirmed by medical experimenters, particularly important results on physiological indicators are obtained at the following time points: 1, 10, 15, 20, and 29 min of myocardial ischemia [2, 9]. Studies of this pathology using the UHR ECG method are directed towards detecting other significant time points to provide for faster, more accurate, and more reliable determinations of the onset and different phases of the course of ischemia.

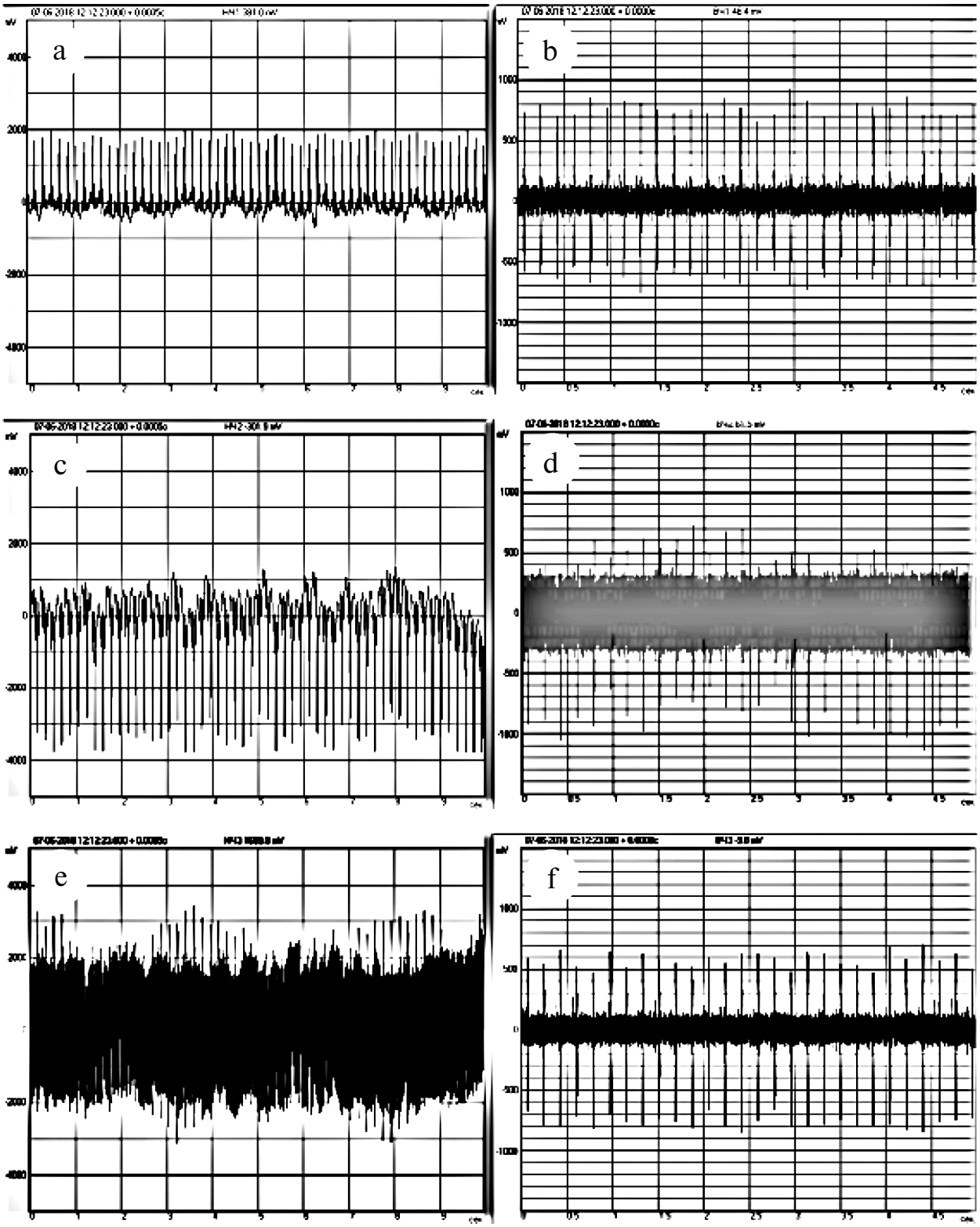

Fig. 2. ECG traces: a, c, e) LF channels of leads I, II, and III, respectively; b, d, f) HF channels of leads I, II, and III, respectively.

Method for Detecting ECS during Experiments

Apparatus was prepared as follows. The metal table on which the experiment was performed was grounded to prevent interference from the metal instruments used by the surgeon. The experimental animal was connected to the SVR-4 EP, which was mounted on a separate table. Electrodes were gold-plated to make electrical contact in the oxidation-reduction system (the rat's body), to increase conductivity, and to decrease the drop in potential of the useful signal on the sensors. Detected ECS were separated in the SVR-4 EP into low-frequency (LF) and high-frequency (HF) channels. Recording and display of data were with a portable PC (notebook) connected to the SVR-4 EP using two cables, each of which could switch the LF or HF channels. The SVR-4 EP and PC operated independently of the 220 V supply. These had their own separate power supplies based on 9-V and 5-V batteries, respectively. This prevents a number of problems during the experiments such as electric shocks to the experimental animal due to device malfunction or power surges in the power supply network, as well as direct superimposition of interference from these devices on the ECS recorded.

Method for Recording ECS during Experiments

ECS was recorded throughout the experiment from three leads, I, II, and III, each in the LF and HF channels in parallel. This recording of six independent channels yields ECS traces in both the standard amplitude (0.1-10 mV) and frequency (0.05-100 Hz) ranges (LF channels) and expanded amplitude (10 nV to 100 μV) and frequency (100-2000 Hz) ranges (HF channels) [1, 3]. *ZETLAB* software, which includes *SignalWriter* [10], was used to display signals on the PC monitor and record them for further processing and storage. The need to display signals from sensors on the monitor during the experiment in real-time mode was due to the need to follow changes in ECS amplitude on transferring to a new study object, to control the image scale, to record interference due to surgical intervention and the experimental animal's responses (changes in respiratory parameters, pressure drops, occurrence of arrhythmias), and other factors.

Method for Information Storage and Selection

At the end of the experiment, results were obtained from the experiment animal using our electrocardiographic studies in the form of ECS traces (Fig. 2) needed for subsequent detection of informative signs at the initial, early, and later stages of development of ischemic heart disease. Then, using specially developed algorithms, a database (DB) was formed from these ECS traces. Rapid access to ECS traces was ensured by using a compact embedded local *SQLite* database management system, which provides for parallel reading of data from the DB for simultaneous performance of multiple processes for streams. For example, the *SQLite* system allows simultaneous requests of signals recorded in the DB from all channels and leads, which increases the productivity and speed of secondary ECS processing procedures. In addition, the *SQLite* system allows a convenient programming language to be used for creating the interface for interaction with the database to allow convenient selection and presentation of information of interest to the user at any time.

The DB interaction interface is written in C++ and allows addition, editing, deletion, and presentation of all information recorded in the DB. All these data have their ID numbers corresponding to the experiment in the series, providing thereby for convenient search for desired information.

Conclusions

This article presents approaches to developing the first stage in a fundamentally expanded methodology for functional electrocardiography in ischemia, i.e., a method for electrocardiographic investigations in experiments creating artificial ischemia in experimental animals, as well as its constituent parts – specific methods for detecting, recording, and storing experimental ECS. All studies were based on using the original UHR ECG method.

The specific methods developed here for detecting, recording, and storing ECS are needed for further investigations in the field of finding informative features at the initial, early, and later stages in the development of myocardial ischemia. These methods are universal and can be applied to studies not only of ischemic heart disease, but also other organs and systems in animals and humans.

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