1. TITLE PAGE

Journal:

Measurement

Title of paper

Wireless sensor node for non-invasive high precision electrocardiographic signal acquisition based on a multi-ring electrode

Abbreviated title

Wireless ECG acquisition with CRE

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2. Abstract

Concentric ring electrodes (CRE) have been proposed for sensing bioelectrical activity with high spatial resolution. Computational studies have revealed that the CRE dimensions are closely related to the electric dipole depth they can sense, but further experimental confirmation is needed. We aimed to develop and test a wireless multichannel ECG recording system based on a new flexible multi-ring electrode and to check the influence of CRE dimensions on the acquired signals. The system provided high-precision ECG signals by a simple procedure. The bipolar concentric ECG signal amplitude and SNR increase with the CRE's outer ring dimension. Differences in the signal morphologies, associated with different sensitivities to the location of the active dipoles, were also obtained in the case of diameters smaller than 34mm. A system with several wireless sensor nodes developed could be easily used by clinical staff for non-invasive cardiac monitoring and diagnosis with high spatial and temporal resolution.

Keywords: flexible multi-ring electrode, wireless sensor node, concentric ring electrode.

TEXT

1. Introduction

Cardiovascular diseases are the main cause of mortality worldwide and are on the increase with the current trend towards an ageing population [1]. Early detection is a key factor in reducing the death rate from coronary diseases, as continuous and regular monitoring of the heart can increase timely diagnoses [2]. The diagnosis of cardiovascular disease is typically performed by using the standard 12-lead ECG with disc electrodes. Previous studies have shown that this provides limited spatial information on the ventricle and even less on the atria, i.e. it only provides general information on the direction and propagation of the heart's electrical activity [3,4]. Nowadays other diagnostic techniques such as invasive electrophysiology are used to detect and treat certain arrhythmias. New non-invasive monitoring systems with high spatial resolution are needed to reduce the time and risks associated with invasive electrophysiology exploration.

In this context, concentric ring electrodes (CRE) have been proposed to acquire more localized electrical activity than conventional disc electrodes do [3,5-8]. By obtaining the difference of potential between the rings and an inner concentric disc, these electrodes can directly estimate the Laplacian potential providing further detail to differentiate between multiple concurrent dipole sources [3,5-7,9]. In previous computational studies the size of the CRE has been proven to be closely related to the electric dipole depth sensed by these electrodes [10], but no experimental results have been reported to confirm this. In this respect, the use of multi-ring electrodes could permit an evaluation of the influence of electrode size on the bioelectrical signal sensed by a CRE. Despite the advantage of having high spatial selectivity, the bioelectric signal amplitude sensed by CRE is much lower than those picked up with disc electrodes (in the order of tens of microvolts) [11]. High precision instrumentation systems that reduce electronic noise and interference are needed for properly conditioning signals from CRE. Most research groups have used active sensors that consisted of a reusable preamplifier circuit located as close as possible to the CRE (usually on the back of the sensing electrode), and then transmit the captured signal by cable to an external bioamplifier for further analogue processing [3,5-7,11]. In this context, the trend in ECG monitoring is towards minimizing wiring and developing smart wearable or wireless systems that provide greater patient comfort and simplify the recording system and protocol [2,12-14]. Therefore, the development of a high-precision wireless recording system to pick up the ECG signals sensed by the CRE would allow to obtain high spatial information on cardiac activity and would enhance its clinical applicability and patient comfort by eliminating the wiring.

The present work aims to walk some steps towards the clinical application of CRE for the early detection of cardiac pathologies. Specifically, we developed a real-time high-precision wireless recording system of bipolar concentric electrocardiographic (BC-ECG) signals. So as to look for optimal CRE size, three simultaneous BC-ECG signals were picked up by a flexible multi-ring electrode and the influence of ring dimensions on the signal amplitude and morphology were studied. In the following sections it is described the electronic hardware and CRE that were developed; as well as the details of the signal recording protocol and analysis. Our results show that high quality BC-ECG signals can be obtained with the proposed system. Regarding the effect of electrode dimensions, experimental results show that the BC-ECG signal amplitude and SNR increase with the size of the CRE's outer ring. This is the main effect for ring dimensions of 34 mm and 46 mm in diameter, but in the case of smaller rings different signal morphologies can also be obtained. The differences in the spatial distribution of sensitivity to active electric dipoles of the ring electrodes of different sizes were only noticed for electrodes of 22 and 34 mm in diameter. A system composed of multiple sensor wireless nodes such as the one developed in this work could be easily used by clinical staff for non-invasive cardiac monitoring and diagnosis with high spatial and temporal resolution and without discomfort to patients.

2. Materials and methods

2.1. System Architecture

The conceptual diagram of the developed wireless ECG sensor node is shown in Figure 1. A disposable flexible multi-ring electrode is used for sensing non-invasively the bioelectrical signals. The differential biopotentials are then amplified and bandpass filtered within 0.3 Hz and 150 Hz. The conditioned signals are then digitalized by means of a precision 24-bit A/D converter (ADC), and then they are wirelessly transferred to the receiver node via a microcontroller (MCU). This latter also handles communications with the micro SD card for local data storage, so that the developed system can operate as a standalone module. In addition, analog circuitry is isolated from the digital circuitry so as to break the ground loop and thereby reduce digital noises coupled to the sensed bioelectrical signals, which is a critical issue for acquiring ultra-low amplitude biosignals of the order of ten microvolts. The receiver node consisted of a Bluetooth-USB adapter connected to a PC in which a custom-made software developed in LabView ® platform runs for managing the user interface and data storage. Further details on the implementation of the system are provided in the next subsections.

Insert Figure 1 here (2-column fitting image)

2.2. Multi-ring sensing electrode design & development

The sensing electrode consists of a bilayer multi-ring electrode. The sensing elements are implemented onto a first layer and consist of four hook-shaped electrodes and an inner disc made of silver (Gwent C2020522D1). The hook design avoids the use of vias for their connection and that of the inner disc to the electronic circuit. Open spaces of the hooks are very small (see Fig. 2), with opening angles that range from 4.26% for internal hook to 4.64%

for external hook. So that the hook electrodes present very similar spatial sensitivity to that of closed rings [15]. A second layer made of a dielectric material (DuPont LuxPrint 8153) was implemented on top of the connecting paths of the hooks and disc electrodes so as to avoid possible shortcuts when the electrodes are in contact with the body surface.

Regarding to the electrode dimension, it has been shown that the optimal diameter for the outer ring of the CRE is related to the depth of the target signal [7]. So as to check the influence of CRE dimension on the BC-ECG signals and taken into account that there is a 30-50mm distance between the heart wall and the sensor, in the present work the outer diameters of the sensing hooks ranged from 21.6 to 45.6 mm. In addition, equal inter-electrode distance (D) between hooks, and between the inner disc and internal hook was considered in the design, i.e. *D=R2a-R1=R3a-R2b= R4a-R3b=R5a-R4b=4.8mm*.

Insert Figure 2 here (1-column fitting image)

Flexible substrates were preferred to conventional rigid substrates to implement the multi-ring electrode, since the former has been proven to present lower skin-electrode contact impedance and also to provide more stable baseline drift for ECG recording [11]. They are more comfortable for the patient and less affected by motion artifacts and interference [11]. A flexible polyester film was used as the CRE substrate. Further details on the screen printing technique and equipment used can be found in a previous work [16].

2.3. Hardware Development

The biosignal analog processing stage for obtaining one of the three BC-ECG signals out of the potential differences sensed by the three middle hooks and the inner disc (*U2-U1*, *U3-U1* and *U4-U1*) is shown in figure 3. The outer hook was connected to the circuit analog ground to reduce common mode noises, using the three-electrode technique for biosignal acquisition. In this way no additional external reference electrode is required. The signal conditioning is made up of two stages with the minimum number of components without compromising the conditioned signal performance. The first stage consists of an ultra-high input impedance, differential input, and quasi high-pass instrumentation amplifier that provides unity gain for the DC component generated from the half-cell potentials between the skin and electrode while amplifying the AC component of the differential potential [6]. The preamplifier gain was set to 42.7, being the cut-off frequency of the quasi high pass filter 0.3 Hz. The second stage consists of a bandpass filter in the frequency range within 0.3 Hz and 150 Hz implemented with a single operational amplifier. The gain at medium frequencies of this stage was 98.07 V/V. Three identical biosignal analog conditioning circuits were implemented for simultaneously recording the three BC-ECG signals captured by the multi-ring electrode.

$$BC_1 = U_2 - U_1 \tag{1}$$

$$BC_2 = U_3 - U_1 \tag{2}$$

$$BC_3 = U_4 - U_1 \tag{3}$$

Where U_1, U_2, U_3 and U_4 are the biopotentials sensed by the inner disc and the three middle hooks (from the inside out) of the multi-ring electrode, respectively.

Insert Figure 3 here (2-column fitting image)

The conditioned signals were then routed for their digitalization. In this work the analog front-end ADS1294 (Texas Instruments, Texas, USA) with programmable gain from 1 to 12 that incorporates four 24-bits sigma delta analog digital converters was chosen for data digitalization. The digitalized ECG data were then transmitted to the MCU through a digital isolator ADum7642 (Analog Devices Inc., Massachusetts, USA) by the standard serial peripheral interface. Taken into account the data rate needed (72 kbits/s being sampling frequency at 1 kHz), the nBlue Br-le-4.0-D2A module (Blue Radios, Colorado, USA) that incorporates: an ultra-low power MSP430F5438A MCU (Texas Instruments Texas, USA), a CC2564 Bluetooth and Dual-Mode Controller (Texas Instruments) and an antenna, was used to transfer in real-time the ECG signals to a host device. A micro SD card (2 GB) was connected to the MCU via serial peripheral interface to provide additional memory for data storage. A rechargeable 1000 mAh Lithium Polymer battery (measuring 53x33 mm and weighing 20 g), was used for providing power supply of the wireless ECG sensor node. The recharge circuitry for the battery was also integrated into the system. The power supply of the analog circuitry was isolated from the digital circuit to break the ground loop.

2.4. Firmware Development

In order to achieve maximum power saving the MCU is configured in a low-power mode and the analog circuitry is powered off until the user triggers data acquisition and transmission or local storage. The sensor node starts real-time wireless transmission when it receives the data request command from the host device via Bluetooth. The MCU is then programed to read the three channels of digitalized ECG data and to send them to a host device using the standard Bluetooth 2.0+EDRcommunication protocol. The receiver node consists of a standard Bluetooth dual mode USB Micro Adapter (IOGEAR GBU521W6, USA) connected via a serial port to a PC. The PC runs a custom-made software developed in LabView ® platform for receiving, displaying and storing data. Local data storage is available by inserting a micro SD in the slot of the sensor node, which triggers the reading of the digitalized ECG data from the ADS1294 and then writing it on the micro SD card. This enables the system to operate without the need for any external unit.

2.5. ECG signal acquisition

Fifteen records were conducted on 15 healthy volunteers (5 females and 10 males) with an age (mean±SD) 33.7±11.9 years (ranging from 23-64 years) and body mass index 23.6±3.8 kg/m^2 (ranging from 19.1 to 32.7 kg/m^2). The study was approved by the ethics committee of the University Polytechnic of Valencia. The volunteers were informed about the study and protocols, and they provided their written consent. The subjects were in a relaxed resting period lying on a stretcher during the whole recording session.

So as to minimize skin electrode impedance, the skin area under the electrodes was previously minimally exfoliated (Nuprep, Weaver and Company, USA) and was also shaved if needed. Since the body surface ECG is highly dependent on electrode position and the activity of the atria is considerably weaker than that of the ventricle, the dry multi-ring electrode was placed on the left infraclavicular fossa medial to the deltoid insertions to facilitate picking up electrical signals from both atria and ventricle. Five minutes of 3-channel BC-ECG signals were simultaneously recorded using the wireless sensor node described in the previous section.

2.6. Data processing

ECG can be corrupted by background noise and different types of interference, such as baseline drift, power line and abdominal muscle interference. The acquired signals were high pass filtered (0.3 Hz, a fifth-order Butterworth) for reducing remaining baseline drifts. ECG fiducial points were obtained by detecting the R-wave of filtered signals [17]. Then, it was computed the average beat ($\overline{ECG}$) in a 60s window, extending from 250 ms prior to, and 375 ms after the R-wave. The average values of the following parameters were then obtained for each recording session in order to value BC-ECG amplitude and signal quality:

- Amplitude of the different waves of the average beat ($\overline{ECG}$): P wave, QRS complex and T wave.
- Signal-to-noise ratio (SNR): ratio of the root mean square (rms) value of the average beat ($\overline{ECG}$) and that of the noise during the isolectric period between beats. Being this latter calculated as the rms value of the total isolectric periods over the 60 s window.

$$SNR\ (dB) = 20 \cdot \log_{10}\left[\frac{V_{rms}\left(\overline{ECG}\right)}{V_{rms}\left(noise\right)}\right] \qquad (4)$$

Given that a normal distribution of the data cannot be considered and being the data from different channels matched for the same recording, all these parameters obtained from the three BC-EHG signals were statistically compared using Wilcoxon rank sum test (α=0.05).

So as to study the influence of ring dimensions on the captured signals, the ratio of amplitudes for each cardiac wave between each pair of BC-ECG signals was computed for each subject. To quantify the morphological similarity between the signals it was calculated the correlation coefficient of the waveform of average beat identified in the three BC-ECGs signals.

3. Results

The developed wireless ECG sensor node is shown on the left of photograph of Fig. 4. It can be seen the disposable flexible multi-ring electrode that is directly connected to the reusable circuitry without any additional wiring. Table 1 shows the main features of the wireless sensor node circuitry which were experimentally obtained. The module presents high CMRR and low input-referred noise which suggests its feasibility for picking up very low amplitude biosignals. On the other hand, the mean consumption is 10.4 mA in standby mode, 59.1 mA when performing real-time wireless transmission, with the effective data rate of 72 kbit/s, and 34.7 mA when storing local data.

Insert Figure 4 here (2-column fitting image)

Insert table 1 here

The whole BC-ECG recording system running can be seen on the right of Fig. 4. As observed no additional wiring is necessary to pick up and record the ECG signals. Figure 5 shows 10s of simultaneous recordings of raw BC-ECG signals acquired by the flexible multi-ring electrode and the proposed wireless recording system. The quality of the three BC-ECG signals is good being the ECG fiducial points clearly identifiable and the background noise is negligible. The raw ECG signals were free of power-line interference without having included a notch filter in the analog circuitry. It can also be observed that the larger the size of the outer hook, the higher the signal amplitude of the BC-ECG, ranging from tens of microvolts in BC_1-ECG to hundreds of microvolts in BC_3-ECG.

Insert Figure 5 here (1-column fitting image)

Mean and standard deviation of the ECG parameters obtained from all the subjects are shown in table 2. As expected, the BC-ECG signal amplitude showed large variations between subjects, which caused high SD values. The smallest signal amplitude was obtained in the BC_1-ECG channel and the highest in the BC_3-ECG channel. These differences in amplitude among signals from the rings of different dimensions proved to be statistically significant (α=0.05), except for the amplitude of P wave and T wave in which no statistically significant differences were found between BC2 and BC3. Figure 6 shows the distribution of the amplitude ratio of the ECG waves obtained from the different pairs of BC-ECG signals. In general, the ratio of amplitude for *BC3*/*BC1* ranges from 2 to 8, with small variations for the different ECG waves and larger variations between individuals. It can also be seen that in the case of *BC3*/*BC2* this ratio is not only practically the same for the different ECG waves of any given subject but is also similar between the different subjects. This would indicate that increasing the distance from the second to the third ring, would only result in an almost constant scaling factor on the cardiac signals acquired. Concerning signal quality, the SNR mean value was 14.8±5.6 dB, 20.1±4.4 dB and 22.0±4.2 dB for BC_1-ECG, BC_2-ECG and BC_3-ECG respectively, again increasing with the ring size, being statistically significantly difference found between BC1-ECG and BC2-ECG, and between BC1-ECG and BC3-ECG (α=0.05).

Insert Table 2 here

Insert Figure 6 here (1-column fitting image)

In general, the signal morphology of the three BC-ECGs was very similar. This is reflected in the values of the correlation coefficients shown in Figure 7, which were close to 1 in most cases. Nonetheless, there are some other cases (3 out of 15) in which signal morphology in the three signals from the multi-ring electrode was clearly different, presenting lower correlation coefficient values. It is noticeable that signal morphology showed greater variations between *BC1-BC2* and *BC1-BC3*, whereas BC2 and BC3 signals remained morphologically more similar for almost all the subjects. Figure 8 shows an example of both these situations. Figure 8a depicts the normalized average beats obtained from a 1-min recording from one patient in which signal morphology was very similar between the channels. In contrast, different signal morphology can be seen in the three BC-ECGs signals recorded by the sensor node in the case shown in Figure 8b.

Insert Figure 7 here (1-column fitting image)

Insert Figure 8 here (1-column fitting image)

4. Discussion

A wireless sensor node with a flexible multi-ring electrode has been developed for the simultaneous recording of three BC-ECG signals. This system combines ease-of-use for extensive clinical application (since it does not need any wires) with the enhanced performance of concentric ring electrodes for locating active dipoles. Even though the size and power consumption of the wireless sensor node circuitry are slightly larger than those described in other studies [13,14], the performance of the wireless system is superior in many respects. Firstly, 3 independent high-precision 24-bit resolution ADCs were used for data digitalization, i.e. the ADCs were not multiplexed for different input channels, thus allowing the simultaneous recording of various bioelectrical signals. In addition, our wireless system can store and transmit the signals at a sampling frequency of 1000 Hz. These two factors are of special importance when high temporal resolution is required, e.g. when detecting the activation time of atrial or ventricle depolarization in a body surface potential mapping study. Unlike previous studies focused on acquiring ECG signals sensed by standard wet Ag/AgCl electrodes in which the signal amplitude is of the order of millivolts [13,14], the system proposed here is one of the few wireless transmission systems that acquires bioelectric signals in microvolts and could therefore be easily adapted for surface monitoring of other weak bioelectrical signals, such as the electrohysterogram, electromyogram, electrogastrogram, electroenterogram, fetal ECG or the electroencephalogram. The proposed sensor node can also be easily adapted for acquiring BC-ECG signals from an array of bipolar concentric ring electrodes. Together with the ability to create a wireless sensor network, this would allow simultaneous recording of BC-ECG from different positions for body surface potential mapping without any wire. We therefore consider that our wireless sensor node has adequate potential for clinical monitoring and diagnostic applications due to its ease-of-use in the recording protocol. Finally, it can also store local data, which means that the system can work as a standalone module for a wider range of applications.

We also quantitatively evaluated the BC-ECG signal amplitude and quality sensed by the flexible multi-ring electrode to check the influence of the ring dimensions. Firstly, the acquired BC-ECG signal amplitude ranged from a few microvolts to a hundred microvolts, which is within the range of values reported in the literature [7,11]. The experimental results showed that as the CRE's outer hook size increases, the BC-ECG signal amplitude rises, which agrees with the findings of other groups, who reported that the signal amplitude depends on the radius of the central ring and its gap with the inner disc [10]. Furthermore, the size of the electrode seems to affect only the signal amplitude of the BC2 and BC3 channels, but no noticeable differences were found in the signal morphology of these two channels. This was responsible for the high correlation coefficient of the average beats, and the small variation of the amplitude ratio for different ECG waves. Nevertheless, changes in signal morphologies and greater variability of amplitude ratios for the different cardiac waves were found with the smaller rings of the multi-ring electrode (BC1 and BC2). This could mean that the effect of increasing the distance between the second (≈34 mm diameter) and third ring (≈46 mm diameter) could give an almost constant scaling factor on the acquired cardiac signals. Rings of these dimensions pick up more general information and other dimensions in this range do not affect the way the cardiac vectors are sensed, so that the external ring may be redundant. In contrast, the smaller ring (≈22 mm diameter) senses the electrical activity of different cardiac regions with a different distribution of the sensitivity to electrical dipoles, resulting in signals with possible morphological differences to those sensed by the outer rings. However, this latter phenomenon was not observed in all the subjects. This could be related to differences in the physiological constitution of individual subjects and the relative position and orientation of the heart with respect to the multi-ring electrode. Due to the specific characteristics of each patient, the different rings of the electrode may not have been in exactly the same plane with all test subjects, which can cause differences in signal morphology between BC1, BC2 and BC3 records. In this respect, further analysis should be carried out to consider the specific conditions of individual patients, by making slight changes to the position of the electrode or by using an array of multi-ring electrodes. This would allow to perform a body surface potential map and to check that the activity of specific areas of the heart could give different results according to different electrode rings and positions.

Finally, although the proposed wireless recording system has provided promising results, this study is not exempt from certain limitations. First, the number of subjects involved was limited and all were in good health. Further studies should be carried out on patients with cardiovascular diseases to determine the performance of BC-ECG recording in clinical diagnosis. Secondly, skin preparation was necessary for obtaining high-quality BC-ECG recording since dry CRE was used, this could be an important drawback for clinical application. In this sense, pre-gelled CRE could be a solution for obtaining high-quality BC-ECG recording without any special skin preparation. Although the CRE has been proven to provide more detailed information on localized electrical activity, so far no standard position has been proposed for extracting significant clinical data for diagnosis with this type of electrode. This could be due to the fact that the signal morphology is highly dependent on electrode position and also on the physiological constitution of individual subjects. A comparison of body surface potential mapping by multiple sensor nodes like the one developed in this work and simultaneously recorded 12-lead ECG would be helpful in determining the best CRE locations for registering the activity of different areas of the heart and would improve the clinical performance of this CRE.

5. Conclusions

It was developed and tested a wireless recording system for acquiring in real-time high precision electrocardiographic signal using a flexible multi-ring electrode. The experimental results show that the BC-ECG signal amplitude and SNR increase with the size of the CRE's outer ring. It was also shown that the electrode dimensions do not only affect the signal amplitude, but in certain cases can also affect morphology, putting different emphasis on the electrical activity of different cardiac vectors in the signals picked up by the multi-ring electrode. A system composed of multiple sensor wireless nodes such as the one developed in this work could be used in a simple procedure by clinical staff for non-invasive cardiac monitoring and diagnosis with high spatial and temporal resolution and without discomfort to patients.

Acknowledgments

Research supported in part by a grant from the Conselleria d'Educació, Cultura I Esport, Generalitat Valenciana Conselleria (GV/2014/029); by a grant from the Universitat

Politècnica de Valencia (SP20120490); and by the Ministry of Economy and Competitiveness and the European Regional Development Fund (DPI2015-68397-R (MINECO/FEDER)).

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Figure Caption:

Fig. 1. Conceptual diagram of the wireless ECG sensor node.

Fig. 2. Bilayer design of the multi-ring concentric electrode; electrodes and connecting paths are shown in black (first layer), and the dielectric paste layer is shaded in gray (second layer). *U1*, *U2*, *U3*, *U4* and *U5* are the biopotentials sensed by the inner disc and the subsequent four hooks from the inside out, respectively.

Fig.3. Analog signal conditioning circuit for one of the three BC-ECG channels (*U2-U1* in this case), where *U1*, *U2*, *U3*, *U4*, *U5* are the biopotentials picked up by the flexible multi-ring electrode, corresponding to the inner disc and the four hooks from the inside out, respectively.

Fig. 4. Left: Wireless sensor node for the recording of three BC-ECG signals; Right: whole recording system running.

Fig. 5. Example of 3 BC-ECG signals from subject #3 using the flexible multi-ring electrode. (a) BC_1-ECG (b) BC_2-ECG (c) BC_3-ECG.

Fig. 6. ECG wave amplitude ratio identified in the three BC-ECG signals (*BC3*/*BC1*: black; *BC3*/*BC2*: dark red).

Fig. 7. Correlation coefficient of the average number of beats from a 1 min record of chest surface signals.

Fig. 8. Normalized average beats from a 1 min record of the BC-ECG signals. (a) subject #8. (b) subject #4.

Tables and table caption:

Table 1.Wireless sensor node circuitry main features.

Feature	
Nº Channels	3
Bandwidth	0.35-155 Hz
Average differential gain at mid-band frequencies	4084 V/V
Average CMRR at mid-band frequencies	129 dB
Input referred noise	0.28 µVrms
Mean current consumption (real-time wireless transmission)	59.1 mA@Rx; 10.4 mA@inquiry mode
Mean current consumption (local data storage)	34.7 mA
Sampling rate	1000 Hz/channel
Physical size	80x42x10mm^3
Weight (g)	17.9g

Table 2. BC-ECG parameters obtained from 15 subjects (mean±SD). $^{*o□}$ Indicates a statistically significant difference in this parameter between BC1 and BC2, BC1 and BC3 , BC2 and BC3 respectively (α =0.05).

Channel	Amp-Pwave ($\mu V_{p\text{-}p}$)	Amp-QRS ($\mu V_{p\text{-}p}$)	Amp-Twave ($\mu V_{p\text{-}p}$)	SNR (dB)
BC_1-ECG	4.2±2.0^{*o}	41.6±22.2^{*o}	15.5±10.0^{*o}	14.8±5.6^{*o}
BC_2-ECG	10.3±5.2*	105.8±56.0$^{*□}$	38.5±19.7*	20.1±4.4*
BC_3-ECG	14.1±7.3^{o}	157.8±79.2$^{o□}$	52.6±31.1^{o}	22.0±4.2^{o}