Comparative Characterization of Electrical and Panoramic Optical Mapping in Langendorff-Perfused Rabbit Hearts: From Sinus Rhythm to Fibrillation

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Abstract

Cardiovascular diseases (CVDs) are the leading causes of death globally. Comparative optical (gold standard) and electrical experimental setups have been developed to characterize the cardiac substrate and improve commercial medical equipment. We aim to characterize signals provided by a novel electrical and optical animal experimental setup across the entire heart, atrium, or ventricle and present differences between them using local activation time (LAT), cycle length (CL), frequency (F), heart rate (HR) and signal amplitude. New Zealand rabbit hearts were Langendorff-perfused, and electrical activity was acquired simultaneously with panoramic optical mapping and contact electrical mapping setup. Four rhythms were characterized: sinus with AVB (LA:162, V:42 bpm), atrial tachycardia with AVB (LA:463, V:73 bpm), atrial fibrillation with AVB (LA:428, V:0.4 bpm), and ventricular fibrillation (LA 223, V:490 bpm). The difference in electrical and optical LAT is smaller in SN-AVB (RA:12.2±4.3 ms) than in AF-AVB (RA:100.3±69.2 ms). The difference in CL, F, and HR was less than 0.1 unit for SN and increases in AF up to 27 ms, 0.6 Hz, and 43 bpm, respectively. Optical results showed lower deviation values compared to electrical. Differences between electrical and optical results were presented since sinus to fibrillation.

1. Introduction

The absolute global burden of all Cardiovascular diseases (CVDs) increased over time between 2000 and 2016 [1]. This modification of the characteristics of excitation and electrical propagation of the heart by CVDs leads to modifications in the conduction system [2]. Electrophysiological tests are essential for the identification of mechanisms for specific treatments. However, for complex arrhythmias such as atrial and ventricular fibrillation, the current commercial systems used for clinical evaluation of the substrate can distort the electrograms, resulting in an erroneous diagnosis and reducing the patient's quality of life [3]. Optical mapping, considered an advanced technique and often regarded as the gold standard, serves as a valuable tool for enhancing current commercial systems. Its implementation has facilitated a more precise identification of mechanisms involved in initiating and perpetuating arrhythmias. These systems utilize voltage-sensitive dyes, which change the emission spectrum proportional to the voltage fluctuations across transmembrane myocardial cells. This enables the mapping of the heart's electrical activity with high spatiotemporal resolution. Unlike traditional electrode-based electrograms, the fluorescent signal corresponds more closely to transmembrane potential and is less influenced by wavefront propagation direction. Moreover, employing multiple cameras allows for a panoramic view of the entire heart. This study presents the characterization of signals in sinus rhythm, atrial tachycardia, atrial fibrillation, and ventricular fibrillation obtained with an experimental model that simultaneously employs electrical and optical panoramic mapping. The aim is to identify differences between results of local activation time (LAT), cycle length (CL), and frequency (F)[4] calculated from both systems. This allows us to observe the progression from a normal and constant rhythm, such as sinus, to the irregularities seen in fibrillation.

2. Methods

2.1. Experimental process and setup

As is presented in Figure 1, four New Zealand rabbits (3.44 ± 0.36 kg) were used under protocol no. 3947230519 approved by the local Committee on Ethics in the Use of Animals (CEUA). As previously reported in [5], The animals were anesthetized and the euthanasia was performed by thoracotomy, under deep anesthesia. Then, was connected to the langendorff system, and a heated modified Krebs-Henseleit buffer [6] was perfused trow the aorta. Heart contraction motion was suppressed using

uncoupler (-)-Blebbistatin. The potentiometric dye Di-4-ANBDQPQ. The heart was located in the middle of the panoramic optical mapping system comprising six high-power deep-red LEDs (650 nm, Luminus Devices Inc., USA) and three high-speed cameras (Emergent Technologies) separated by 120°. Camera A captures a frontal view of the left atria (LA) and ventricle (LV), camera B captures the right atria (RA) and ventricle (RV), and finally, camera C captures the anterior face of the heart. The video recording software StreamPix 9 was used to acquire images at 500 frames/s for the three cameras simultaneously.

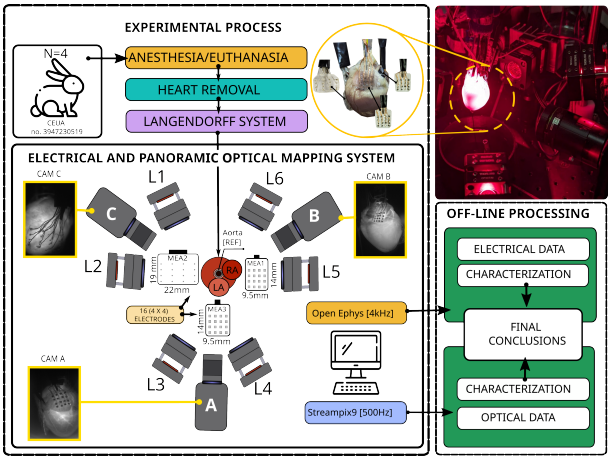

Figure 1. Diagram of the experimental process, the electrical and panoramic optical mapping system, and the off-line processing

For electrical mapping, the epicardial electrical activity was acquired using three handmade multi-electrode arrays (MEAs) with 16 silver electrodes positioned in a 4x4 arrangement on a polyethylene terephthalate (PET) surface (Fig. 1). Two of these arrays labeled as MEA1 and MEA3, were positioned over the right and left ventricle, respectively. The third array, designated MEA2, was placed over the anterior view of the heart. The MEAs were connected to a recording headstage (Intan Technologies) and the Open Ephys acquisition board (Open-source Electrophysiology), allowing the acquisition at 4 kHz. To prevent chamber collapse, latex balloons were developed, allowing the atrium to remain expanded and better contact with MEAs. A bipolar electrode was placed over the epicardium, and cardiac arrhythmias were induced by S1-S1S or S1-S2 protocols. For 3D heart surface generation, a three-pointed adapter was positioned at a fixed distance, and one optical camera recorded a sequence of 2D images every 5 degrees of rotation until a full rotation. To reduce ventricular influence in atrial signals AV node was ablated with 15 W of RF power to induce an atria ventricular block (AVB).

2.2. Off-line analyses

2.3. Pre-processing

For electrical signals, 60 Hz was filtered using a 6th-order harmonic filter with a notch filter designed using the "filtfilt" MatLab function. Afterward, a high-pass Butterworth filter with a cutoff of 1.2 Hz and a low-pass filter of 250 Hz were applied. For optical potentials, the baseline was restored using a 4th-order Butterworth high-pass filter with a cutoff frequency of 0.5 Hz. Subsequently, optical signals were filtered with a spatiotemporal Gaussian smoothing filter of 5x5 spatial filter kernel size and 1x7 temporal filter kernel size and fluorescence intensity (ΔF/F) was calculated by (F - F0) / F0, where F0 represents the fluorescence intensity of the polarized cell's membrane, and F of a depolarized cell's membrane)[7].

2.3.1. Signal characterization

For signal characterization, the first step involved resampling the original optical sample frequency from 500 to 4000. Subsequently, the following parameters were calculated. Local activation time was detected for all beats presented within a selected time frame using the first derivative dF/dt(max) for optical signals, and the first derivative -dV/dt for electrical signals. Once both parameters were calculated, optical and electrical local activation times were subtracted to determine the difference between activations and calculate the mean of all results as ΔLAT. CL represents the difference between two consecutive beats in ms, and ΔCl is the mean of all CLs obtained at each point during the time selected. F was calculated as the inverse of CL [8], and ΔF is the mean of all frequencies obtained at each point during the time selected. HR was calculated using the frequency results and presented in beats per minute (bpm) and the ΔHR represents the mean of all results. Finally, the amplitude for optical signals is the difference between the baseline and the highest peak, and for electrical signals, the peak-to-peak amplitude.

3. Results

Figure 2 presents four cardiac rhythm episodes: sinus with AVB (SN-AVB), atrial tachycardia with AVB (AT-AVB), atrial fibrillation with AVB (AF-AVB), and ventricular fibrillation (VF). Optical and electrical signals are compared from one electrode and the pixels related to it, over the RA, LA, and V (anterior view with parts of the right and left ventricles). On the right of each plot, the measured parameters are presented: two samples of cycle length (CL_1 and CL_2), the mean cycle length calculated during the time in seconds of each figure (CL_m), the mean frequency calculated (F), the heart rate in bpm (HR),

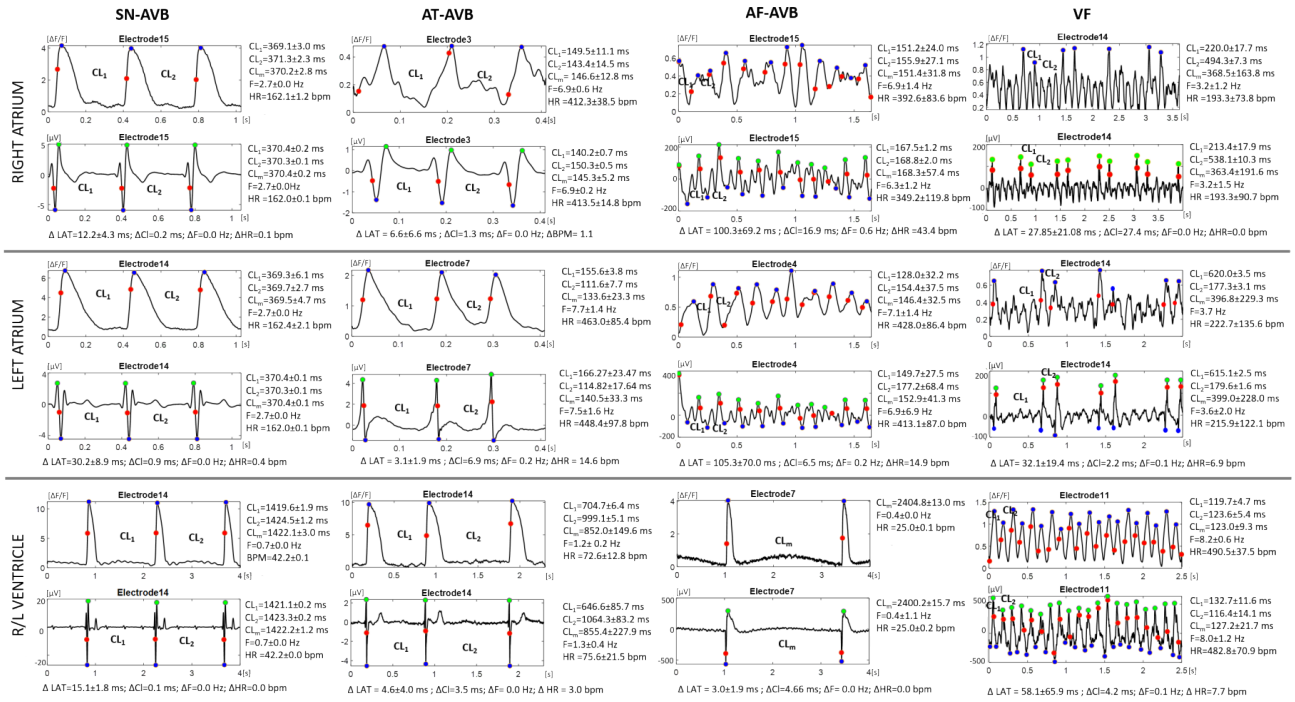

Figure 2. An optical (up) and electrical signal(down) from one electrode and the pixels around it located on the RA (top), LA (middle); and ventricle (V) (bottom) during, from left to right, sinus with AVB (SN-AVB), atrial tachycardia with AVB (AT-AVB), atrial fibrillation with AVB (AF-AVB), and ventricular fibrillation (VF). Next to each plot is presented the mean and standard deviation of cycle length (CL_1 and CL_2), the mean cycle length (CL_m), the mean frequency calculated in Hz (F), the mean HR calculated in beats per minute (bpm), and the mean total amplitude (Amp) in percentage of fluorescence and voltage for optical and electrical mapping, respectively. at the bottom of each plot the difference between the electrical and optical local activation of the time mean (Δ LAT), the mean cycle length (ΔCl), the mean frequency (ΔF), and the mean HR (ΔHR)

and the mean total amplitude (Amp) in percentage of fluorescence and voltage for optical and electrical mapping, respectively. Finally, for each rhythm, the difference between the electrical and optical local activation of the time mean (Δ LAT), the mean cycle length (ΔCl), the mean frequency (ΔF), and the mean HR (ΔHR) are presented at the bottom of each image. The heart rate for New Zealand rabbit hearts in normal conditions ranges from 187 to 250 beats per minute [9]; in ex vivo isolated rabbit hearts, it was found to be 162.1 bpm [10]. As presented in Figure 2, the SN-AVB exhibited a HR of 162 bpm for both optical and electrical signals in the RA and LA. The ventricle showed a constant CL of 1422 ms and 42 bpm of HR. Comparing amplitudes, electrical and optical recordings showed higher values in the ventricles, almost double those recorded from the atrium. ΔLAT during SN-AVB was 12.2±4.3 ms, 30.2±8.9 ms, and 15.1±1.8 ms for the LA, RA, and V, respectively, while ΔF, ΔCL, and ΔHR were less than 1 unit for all. In the case of AT-AVB (more than 400 bpm), CL_1 and CL_2 are different, resulting in higher values of standard deviation of both systems (≥5ms). ΔCl presented values from 1 to almost 7 ms between the electrical and optical systems. At this point, it is possible to detect that optical results presented lower deviation values. For AF-AVB, CL values for the RA and LA are under 150 ms, presenting higher HR, between 349 and 428 bpm. It is detected that RA and LA presented a difference in frequency. Comparing electrical and optical recordings, deviation increased, especially for electrical results. The difference in frequency was 0.6 and 0.2 Hz for RA and LA, respectively, resulting in a difference in HR between 14.9 and 43.4 bpm. Figure 3 presents an electrical (left) and optical (right) phase map of the RA (middle), with a central rotor observed in the middle of the optical map and the electrical map cached the left inferior part of this mechanism.

Finally, the last column presented in figure 2 shows a VF episode, in this case, the AVB procedure was not done. but was possible to detect two atrial beats contaminated with ventricular activity. Those two consecutive beats are separated for 220.0±17.6 ms (optical) and 213.4±17.9 ms (electrical) for the RA, and 177.3±3.1 ms (optical) and

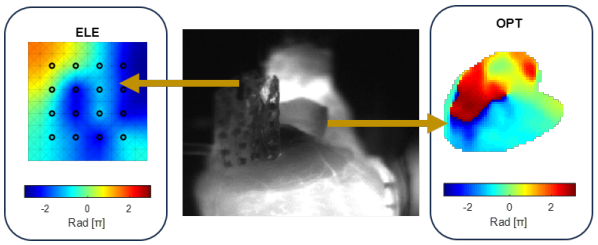

Figure 3. Electrical (left) and optical (right) phase map of the Right atrium (middle) expressed in radians

179.6±1.6 ms (electrical) for the LA. These two beats appear after 494.3±7.3 ms (optical) and 538.1±10.3 ms (electrical) for the RA and 620.0±3.5 ms (optical) and 615.1±2.5 ms (electrical) for the LA. At this same time, the ventricle presents a cycle mean of 123.0±9.3 ms (optical) and 127.2±21.7 ms (electrical), giving as a result 7.7 bpm difference between systems.

4. Discussion

The presented system can provide results of the complete heart, and study the atria or ventricles separately. However, some considerations must be taken into account for both electrical and optical measurements. Even though optical signals exhibit greater immunity to contamination from far-field electrical activity compared to bipolar or numerous unipolar electrodes [8], they also present an influence, especially when conducting studies in the atrial area. This is due to the delicacy of the atrial tissue compared to the ventricular tissue, which is thicker and more abundant. For this reason, other options such as AV node ablation should be implemented to preserve the atrial structure without removing the ventricle, thus contributing to better measurement by the electrodes.

5. Conclusion

According to the results obtained, it can be seen that the deviation of the different measured parameters increased from sinus rhythm to cases of fibrillation. The optical mapping system showed less variation than the electrical.

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