Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

Inherent Dispersion in Restitution Properties Over Space

Rupinder Singh[1], Harold Bien[1], Emilia Entcheva[1], and Flavio F. Fenton[2]

Abstract— **Cardiac tissue heterogeneities can result in spatially dependent restitution properties. We propose a method for quantifying the dispersed nature of these restitution curves (RCs) over a large number of imaged pixels/locations. Cardiac propagation in response to point stimulation was recorded in cardiomyocyte monolayers with voltage-sensitive dye over a large field of view using high resolution imaging. When examining restitution properties of cardiac tissue, the probabilistic nature of these relationships was observed even for macroscopically homogeneous tissue. The method outlined here allows for comprehensive quantification of restitution over space, and the degree of dispersion may provide information complementary to traditional parameters used to predict propensity to arrhythmias such as RC steepness and diastolic interval range.**

I. INTRODUCTION

The underlying mechanisms of lethal arrhythmias contributing to the primary cause of death in the industrialized world, cardiovascular disease, are poorly understood (Thom *et al.*, 2006). The onset of arrhythmias has been extensively studied by dynamically pacing cardiac myocytes, cardiac tissue or whole hearts (Bian & Tung, 2006). These experimental results have revealed a tight link between the time interval preceding an action potential – the diastolic interval (DI) - and the action potential duration (APD) following such an interval as well as the conduction velocity (CV). In most cases, these two relationships have been represented by a single curve, known as the APD or the CV restitution curve (RC), respectively (Gilmour & Chialvo, 1999). In general, a decrease in the DI leads to a shorter APD and a lower CV to the point of refractoriness. The cells that reach refractoriness exhibit an apparent functional block and if coupled with neighbors allowing propagation can facilitate the onset of reentrant activity, e.g. spirals.

Manuscript received April 24, 2006. This work was supported in part by the *Whitaker Foundation*.

[1]Rupinder Singh is with the Biomedical Engineering Department, Stony Brook University, Stony Brook, NY, 11794 USA (phone: 631-444-1498; fax: 631-444-6466; e-mail: rusingh@ic.sunysb.edu).

[1]Emilia Entcheva, PHD, is with the Biomedical Engineering Department, Stony Brook University, Stony Brook, NY, 11794 USA (e-mail: emilia.entcheva@sunysb.edu).

[1]Harold Bien, PHD, is with the Biomedical Engineering Department, Stony Brook University, Stony Brook, NY, 11794 USA (e-mail: harold.bien@hsc.stonybrook.edu).

[2]Flavio Fenton, PhD, is with the Biomedical Engineering Department, Cornell University, Ithaca, NY, 14853 USA

The spiral can further accelerate and become symptomatic of tachycardia, or can deteriorate due to wavebreaks and transition into less organized activity such as lethal fibrillation (Adamson *et al.*, 2005;Wu *et al.*, 2002).

The RC steepness has been proposed as a predictive tool for assessing the propensity to arrhythmic events (Fenton *et al.*, 2002). Therefore, the assumptions associated with estimation of RC curves and the accuracy of these curves is of great interest. It has been recognized that cardiac tissue heterogeneities can cause spatially different restitution properties (Poelzing *et al.*, 2004). However, even macroscopically homogeneous cardiac tissue may exhibit dispersed APD and CV restitution over space due to memory and coupling (electrotonic) effects under variable wave geometry and subtle microscopic structural features. In fact, by accounting for the diffusion current of a group of cardiac myocytes, computational models have shown that regions close the point of stimulation have longer APD for shorter DIs relative to regions elsewhere (Laurita *et al.*, 1997). This implies that when reporting RC curves multiple factors have to be taken into account, including the geometry of stimulation and readout, the particular local tissue structure.

In this study we use ultra-high resolution optical mapping over a large field of view to quantify spatial variations in restitution properties in macroscopically homogeneous cardiomyocyte monolayers. We present a method for probabilistic (histogram-based) approach of restitution curve assessment. Our analysis suggests that the APD and CV restitution in macroscopically homogenous cardiac tissue have an inherent amount of dispersion that can be quantified.

II. METHODS

A. Cell Culture and Imaging

Neonatal rat cardiomyocytes were cultured as described previously (Entcheva, 2001). Samples were stained with the calcium indicator dye Fluo-4 AM (Molecular Probes, Oregon) before imaging. High resolution optical mapping was carried out using an intensified CMOS camera with 1280x1024 pixels at 200fps covering a field of view approximately 24mm. All experiments were conducted at room temperature to enable better imaging of the wavefront.

B. Capturing Macroscopic Propagation

We mapped propagation in five macroscopically confluent and homogeneous cell monolayers. Particularly, we recorded cardiac cell propagation using a standard dynamic pacing protocol, reaching steady state for each

frequency in incremental manner (stepping up by 0.2 or 0.5Hz until failure to capture). Stimuli were delivered via a point electrode with closely positioned Platinum leads. Each recording consisted of on average four beats at steady state and the field of view did not include the stimulating electrode. Half of the samples were kept at room temperature, while the other three samples were heated to 30°C. Furthermore, for five out of the five samples the measurements were done with a voltage-sensitive dye (di-8-ANEPPS).

C. Filtering Signals

In the analysis of the recordings, each movie was spatially averaged (binned) either 5x5 or 3x3 pixels. The varying gray-scale intensity, which varied from 0 to 255 (8 bit signals converted from the 10-bit camera recordings), were centered to a bias of 127 and then stretched for wider dynamic range by setting the maximum and minimum of each pixel to 0 and 255, respectively. The signals were further filtered using a Savitzky-Golay polynomial filter with a width of 7 frames and order of 2. The preceding steps vastly improved the quality of the signal for our analysis.

D. Obtaining Restitution Curves and Spatial Maps

The analysis consisted of three main parts: the analysis of the histogram based distribution and averaged spatial distribution of the APDs, DIs and CVs; and finally, the analysis of five APD and CV RCs for the voltage recordings. For each part of our analysis, the APDs, DIs, and CVs were quantified over time for every pixel in space. This was done by first determining 50% threshold intensity for each pixel using the median amplitude and median baseline of the transients that varied in time due to noise and cardiac excitation. Then, using the 50% threshold, the APDs, DIs and activation times for each transient were determined from the time corresponding to the intersection of the transient and the 50% threshold. Particularly, the activation times for each depolarization-repolarization transient was determined by finding the time at which the threshold intersected with the rising (depolarizing) portion of the transient. Next, APDs were determined by subtracting activation times from the subsequent time that corresponded to the repolarization of the cardiac cells or the falling portion of each transient, and the corresponding DIs were determined by subtracting the APDs from the appropriate intervals between the subsequent depolarizations, known as the basic cycle length (BCL). Finally, by computing the spatial gradient in the activation times for each frequency of propagation, we computed our CVs.

Histograms of the APD, DI, and CV for a particular frequency and sample were used to exemplify the mean distribution of the values. For the distribution of these values in space, the APDs, DIs, and CVs were averaged over time and then presented spatially. The isochrones of our propagation in space were also depicted to show the smooth propagation of our cells, and indirectly, the confluence of our cells.

For restitution curves, each preceding DI was paired with a subsequent APD and CV for a particular frequency. By plotting the two pairs for all of the measured frequencies, we were able to present the characteristic APD RCs and CV RCs. However, since the pairs in our RCs superimposed on a 2-D linear plot due to our large samples of APD, CV, and DI in space and time, we presented our RCs as 2D histograms (density plots) for greater clarity.

IV. Results

The histogram of our APDs, DIs, and CVs for a particular sample and frequency demonstrated that the distribution in these values were Gaussian in nature (Fig 1.A). By observing the random spatial distribution of these values in space, the basis of the Gaussian distribution became evident (Fig 1.C&E). That is, we noticed that the APDs, which varied reciprocally to the DIs, were not constant over space. The differential distribution of the values in space was also noticed for the CVs. In general, the spatial distribution was probabilistic in accordance to the range of our histograms.

Evidently, the Gaussian distribution could have been a clear sign of false positives in our detection of APDs, DIs, and CVs; however, if this was the case, the APD-DI pairs would not sum to a constant frequency. That is, by observing a set of APD-DI pairs for a particular sample and frequency, we noticed that most of the values fell on a 45 degree line. This suggested that we had obtained a realistic dispersion in the APD-DI pairs rather than a false one. The CV dispersion was thus also real since it was obtained using a subset of the values used to obtain our APDs and DIs. In spite of the dispersion, the isochrones and thus the propagation appeared to be regular (Fig 1.B).

Though there seemed to be dispersion in our APD RCs, this did not prevent us from seeing the typical exponential trends (Fig 1.D). That is, we noticed that for small DIs, the APDs decreased sharply, while for larger DIs, the APDs increased slowly. The dispersion in the RCs, however, suggested that our cardiac myocytes did not have a single APD-DI pair for a particular frequency. Rather, the values were probabilistic over space, with ranges defined by our APD histograms.

The dispersion in the CV RCs also did not prevent us from seeing the typical exponential characteristics (Fig 1.F). That is, we noticed that for small DIs the CV decreased sharply to the point of refractoriness, and for larger DIs the CV increased slowly. As we noticed for the APD-DI pairs, the dispersion in the CV suggested a probabilistic distribution in the CVs over space.

V. DISCUSSION & CONCLUSION

Five samples of neonatal cardiac cells stained with di-8 ANEPPS were studied on a 35mm dish with ultra-high

spatiotemporal resolution. After determining the APDs, DIs, and CVs for different frequencies of propagation, which were set using a dynamic pacing protocol, the histograms, spatial maps, isochrones and RCs for five different movies were analyzed. The dispersion in the APD and CV RCs suggested that there were multiple single-curve RCs over space in spite of the normal propagation evident by the isochrones. Furthermore, the dispersion and spatially scattering of the APDs, DIs, and CVs, suggested that these properties had a Gaussian distribution over space.

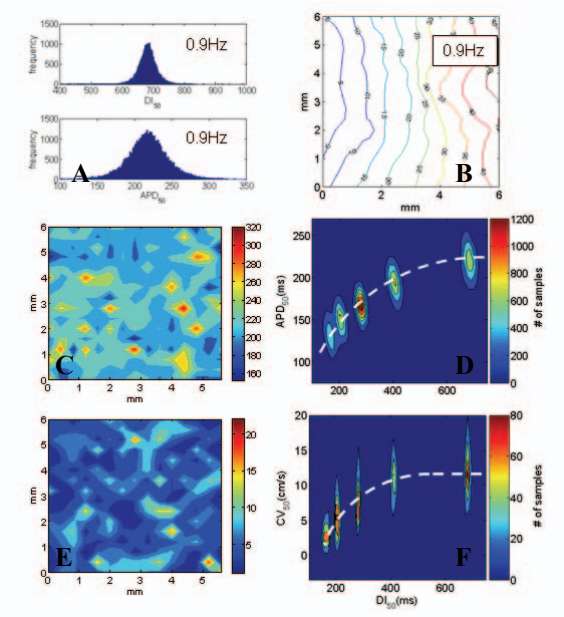

Figure 1: The APD and DI histograms depict Gaussian dispersion (A). The dispersion is present in the presence of uniform propagation (B) and it does not completely mask the exponential APD and CV restitution curves (D & F). The random distribution of these electrical properties in space contributes to the probabilistic dispersion (C & E).

Past studies have only exemplified the spatial dispersion due to electrotonic effects and spatial heterogeneities(Laurita *et al.*, 1997;Poelzing & Rosenbaum, 2004). Our study suggests that there is a Gaussian dependent change in single curve APD RCs and CV RCs in space due to the inherent biological variability of cardiac myocytes, which lead to spatially different electrical properties and or cell-to-cell coupling. However, since APD and CV vary randomly in space, there is a probability that certain regions in space are more prone to experience refractoriness. This makes the dynamics of the heart more complex. By quantifying the spread in the spatial Gaussian distributions for APD and CV and by understanding the trend in the spread for a particular sample in space may make it feasible to identify which regions are more probable to experience an arrhythmic event and to what extent are we confident this might be the case.

In conclusion, RCs and thus the dynamical behavior of cardiac cells need to be reconsidered since the dynamics of cardiac myocytes seem to be more complex due to our finding of the random nature of electrical properties over space.

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