

Spatial Heterogeneity of Restitution Properties and the Onset of Alternans

Hana M. Dobrovolny, Carolyn M. Berger, Ninita H. Brown, Wanda Krassowska Neu, and Daniel J. Gauthier

Abstract—Traditionally, it was believed that cardiac rhythm stability was governed by the slope of the restitution curve (RC), which relates the duration of an action potential to the preceding diastolic interval. However, a single RC does not exist; rate-dependence leads to multiple distinct RCs. We measure spatial differences in the steady-state action potential duration (APD), as well as in three different RCs: the S1-S2 (SRC), constant-basic-cycle-length (BRC), and dynamic (DRC), and correlate these differences with the tissue's propensity to develop alternans. The results show that spatial differences in APD, SRC slope, and DRC slope are correlated with the tissue's propensity to exhibit alternans. These results may lead to a new diagnostic approach to identifying patients with vulnerability to arrhythmias, which will involve pacing at slow rates and analyzing spatial differences in restitution properties.

I. INTRODUCTION

Ventricular fibrillation is one of the leading causes of death in the United States. Thus, there is great interest in finding a way to identify patients who are susceptible to fibrillation. A significant body of research concentrates on action potential duration (APD) alternans, a long-short alternation in APD that was found to be a precursor to more complex arrhythmias [1]. Researchers are interested in understanding the mechanisms underlying the transition from a stable 1:1 rhythm to alternans.

It has long been thought that the stability of the 1:1 response is determined by the slope of the *restitution curve* (RC), a curve given by plotting APD as a function of the previous diastolic interval (DI). However, experimental studies have contradicted this hypothesis by showing that 2:2 responses occur with restitution slopes <1 or that 1:1 responses occur with restitution slope >1 [2], [3]. Part of the reason for these contradictory results is that there are several different restitution curves: dynamic RC (DRC), which consists of steady-state responses, the S1-S2 RC (SRC), which consists of responses to an extra stimulus, and the constant-basic-cycle-length RC (BRC), which consists of recovery beats after an extra stimulus. Further, existing stability criteria are derived from models of single-cell dynamics, but experimental measurements on which they are tested have been made in multicellular preparations, where restitution properties are known to exhibit spatial variation [1], [4]. Alternatively, some studies suggest that spatial variation in

restitution properties, rather than the value at any one spatial location, may be linked to the onset of arrhythmias [1], [4].

In this paper, we investigate spatial differences in steady-state APD, and slopes of the SRC (S_{SRC}), BRC (S_{BRC}), and DRC (S_{DRC}) in a bullfrog ventricle in vitro. The measurements are initially performed with simultaneous impalement of two microelectrodes and later extended to optical mapping. Our study intends to answer two questions: (a) Are there dynamical, pacing-induced spatial differences in any of the measured restitution properties? and (b) Are spatial differences in any of these properties correlated with the tissue's propensity to exhibit alternans?

II. METHODS

Microelectrode Study – This study was performed in accordance with a protocol that conforms to the Research Animal Use Guidelines of the American Heart Association and was approved by the Duke University Institutional Animal Care and Use Committee. Seven bullfrogs were anesthetized and double-pithed. The heart was excised and the anterior surface of the ventricle was removed and pinned in a dish. The tissue was superfused with a standard Ringer's solution, buffered by CO_2 , at room temperature. The tissue was paced at a constant basic cycle length (BCL) of 1,000 ms for 20 minutes before any pacing protocols were performed.

The tissue was paced using a perturbed downsweep protocol [3] (Fig. 1). Beginning at an initial BCL (e.g. 1000 ms), the tissue is paced for 60 s (small dots) until steady state is achieved. Five steady-state paces (diamonds) are applied at the initial BCL before an S2 pace at BCL+50 ms ('+') is applied. This is followed by five recovery paces (filled circles) at the initial BCL and another S2 pace at BCL-50 ms ('x'). The sequence ends with five recovery paces (filled circles) at the initial BCL. The BCL is then decremented by 50 or 100 ms and the sequence of Fig. 1 is repeated. Pacing at decreasing BCLs continues until either a 2:1 or 2:2 response is seen. Electrical signals were recorded simultaneously from two locations in the tissue using glass microelectrodes. Microelectrodes were placed 1-2 mm apart perpendicular to the line connecting the two terminals of the pacing electrode with the proximal microelectrode placed ~ 1 mm from the pacing electrodes.

Optical Mapping Study – Twenty-two bullfrogs were anesthetized and double-pithed. The heart was excised and a cannula was inserted into the ventricle through a small incision in the left auricle. The heart was perfused with standard Ringer's solution and 5 μM di-4-ANEPPs, a voltage-sensitive dye. The staining solution was re-circulated for

H.M. Dobrovolny, C.M. Berger and D.J. Gauthier are with Department of Physics, Center for Complex and Nonlinear Systems, Duke University, Durham, NC, 27708

N.H. Brown, W. Krassowska Neu and D.J. Gauthier are with Department of Biomedical Engineering, Center for Complex and Nonlinear Systems, Duke University, Durham, NC, 27708

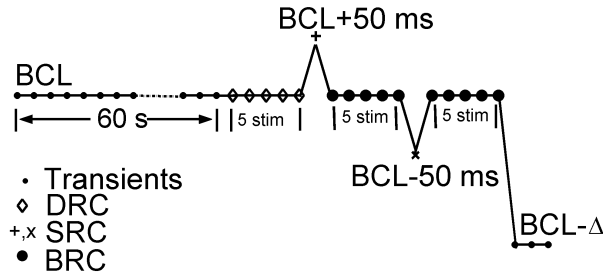


Fig. 1. Perturbed downsweep pacing protocol.

a minimum of 10 minutes (longer if the tissue was not adequately stained). Once the tissue was stained, the anterior surface of the ventricle was cut from the heart and pinned in a dish. The tissue was superfused with Ringer’s solution and paced at a constant BCL of 1,000 ms for 20 minutes, to allow it to recover, before any pacing protocols were performed. While the tissue was recovering, between 10 mM and 20 mM diacetyl monoxime (DAM) was added to the Ringer’s solution to eliminate muscle contraction. The tissue was illuminated with ultra-high power cyan LEDs (Lumileds Star/O). The light was absorbed by the voltage-sensitive dye and was emitted at higher wavelengths. The emitted light was passed through a high-pass cutoff filter and was collected with a high-speed 14-bit CCD camera (iXon, Andor Technologies).

Twelve animals were used to determine spatial variation of steady-state APD. The tissue was paced for 2 minutes at a constant BCL of 1000 ms and the final 10 action potentials were recorded for analysis. The stimulus location was changed and the pacing protocol is repeated. Three stimulus locations were used before the BCL was decreased and the process was repeated. In a separate set of experiments, ten animals were used to determine spatial variation of S_{DRC} . Beginning at BCL=1000 ms, the tissue was paced for one minute at a constant BCL and the final 10 action potentials were recorded. The BCL was decreased, without changing the stimulus location, and the protocol was repeated. Once 2:2 or 2:1 behaviour was seen, the stimulus location was changed and the entire process was repeated.

Analysis – APDs and DIs were found using a threshold of 70% of the action potential amplitude. Trials were categorized as ‘NoALT’, those that transition to 2:1 at rapid pacing, or ‘ALT’, those that exhibit alternans or another complex rhythm at rapid pacing. A response pattern was classified as displaying steady-state alternans if $\delta A = APD_{n+1} - APD_n$, where $n = 1...4$, alternated in sign from beat to beat and $|\delta A| > 2$ ms for microelectrode experiments and $|\delta A| > 10$ ms for optical experiments (the limits are determined by error in APD measurement). Steady-state APD was determined from the mean of the final 5 (10 for optical mapping) responses after 60 or 120 s of pacing at a constant BCL. The DRC was determined from an exponential fit to the steady-state (DI,APD) responses. Segments of SRCs were

determined from the responses to S2 paces and the steady-state APD at each BCL. Segments of BRCs were determined from the response to recovery paces and the steady-state APD at each BCL. For microelectrode measurements, we found spatial differences in restitution properties by subtracting the measured value at the distal electrode from the one at the proximal electrode. For optical mapping experiments, we determined the mean 3-point spatial gradient over the surface of the tissue to quantify the amount of spatial variation. All differences and gradients were analyzed as a function of normalized BCL, $BCL_N = BCL - BCL_t$, where BCL_t is the BCL at which 2:2 or 2:1 behaviour is observed. A t-test was used to determine if differences between ALT and NoALT trials were significant ($p < 0.05$).

III. RESULTS

Figure 2 shows the results of the measurements made with simultaneous microelectrode impalement. The mean (taken over all ‘ALT’ or ‘NoALT’ trials) spatial difference of APD is positive for all BCL, indicating that APD decreases as the wave propagates away from the stimulus. The mean spatial difference in ALT trials are larger than in NoALT trials with the difference becoming significant below $BCL_N = 200$ ms (indicated by ‘*’). The mean SRC slope difference is positive in ALT trials and negative in noALT trials, with both differences moving toward zero as the BCL decreases. The mean spatial difference in S_{SRC} in ALT and NoALT trials differs significantly at most BCL_N s, the exceptions being $BCL_N = 600, 350, 50$ ms. The mean BRC slope difference is generally positive in ALT trials and negative in NoALT trials, though the trend is not as clear as for S_{SRC} . The mean spatial difference in S_{BRC} in ALT and noALT trials differ significantly only at $BCL_N = 550, 500, 250$ ms. The mean DRC slope difference is mostly positive in both ALT trials and noALT trials and increases as BCL decreases, particularly in ALT trials. The mean spatial difference in S_{DRC} in ALT and noALT trials differ significantly when $BCL_N \leq 200$ ms.

Figure 3 shows the results of the optical mapping experiments. The steady-state APD (top row) is longest near the stimulus site and decreases as the wave propagates away. The mean spatial gradient of APD is larger in ALT trials than in NoALT trials, consistent with the microelectrode results. However, mean spatial gradient does not differentiate between ALT and NoALT trials as well as spatial difference in APD. S_{DRC} (bottom row) is also higher near the stimulus site and decreases as the wave propagates away. The mean spatial gradient of S_{DRC} shows trends consistent with the spatial differences seen in the microelectrode experiment, with significant differences between ALT and NoALT trials at most BCLs.

IV. DISCUSSION

Our study found spatial differences in several characteristics of restitution. Spatial differences in restitution properties have previously been seen in experiments using mammalian cardiac tissue, although they seem to be linked to intrinsic

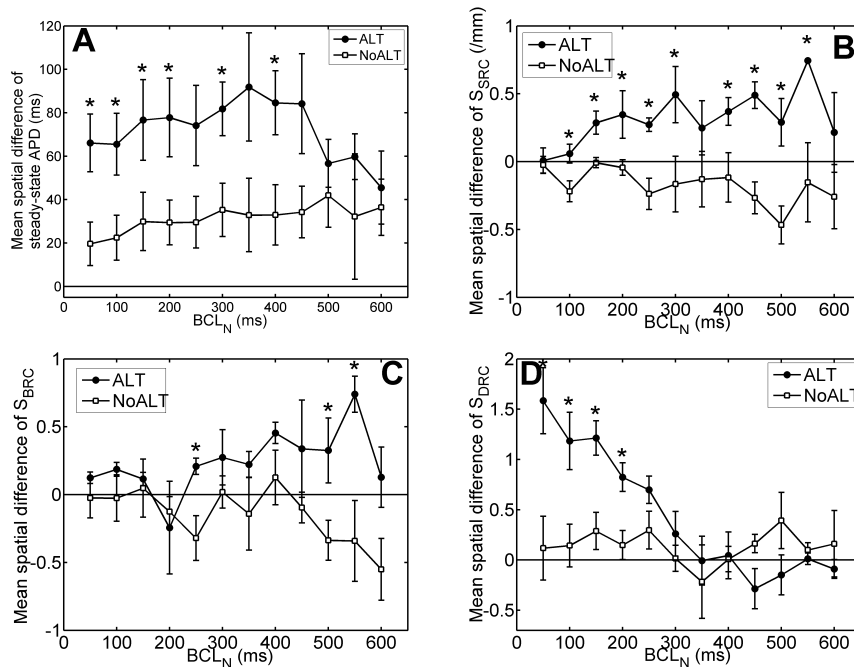


Fig. 2. Spatial differences in restitution properties. Mean spatial difference is shown for (A) steady-state APD, (B) slope of SRC, (C) slope of BRC, and (D) slope of DRC. A '*' indicates that the difference in the measured value of the restitution property in ALT and NoALT trials is significantly different.

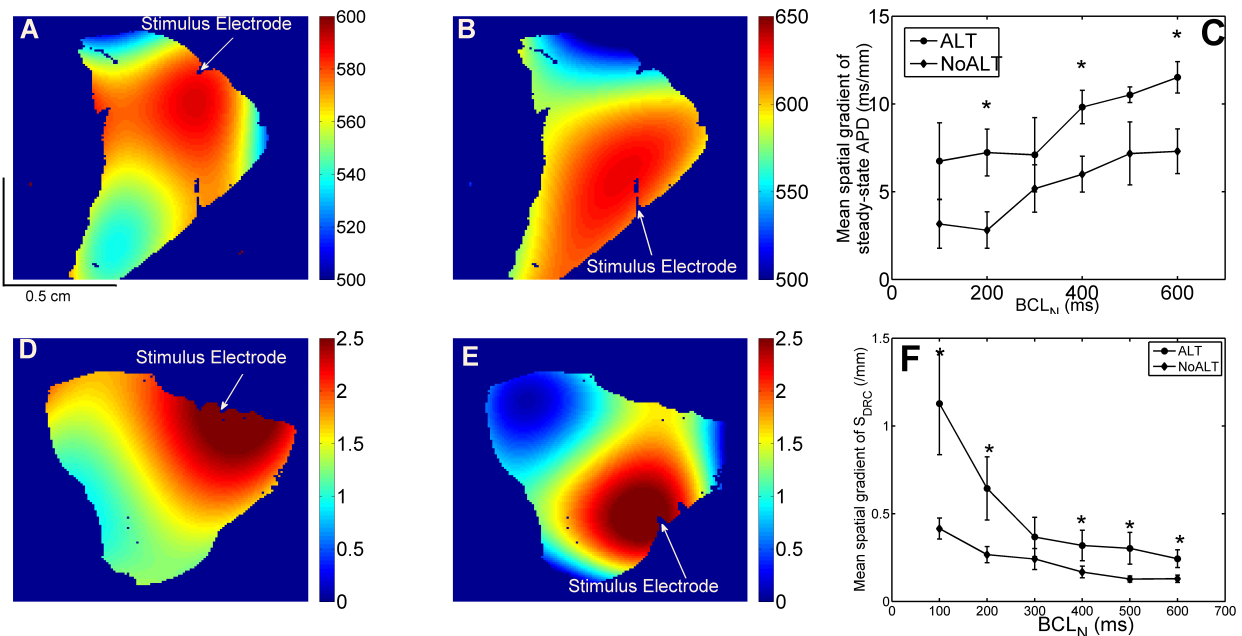


Fig. 3. Spatial variation in restitution properties. The steady-state APD (top) or S_{DRC} (bottom) over the entire surface of the tissue is shown for a stimulus applied at the top right (A) and (D) and bottom right (B) and (E). Colorbars indicate the APD in ms (A and B) and S_{DRC} (D and E). The mean spatial gradient for ALT and NoALT trials is shown for APD (C) and S_{DRC} (F). Spatial maps (A, B, D, and E) are produced by fitting experimental data to a cubic surface.

spatial heterogeneity of the tissue [5]. While we also observed spatial differences in restitution properties that seemed to be linked to intrinsic spatial heterogeneity of the tissue in some of our experiments (data not shown), the results presented here indicate that tissue heterogeneity may not be entirely responsible for spatial heterogeneity of restitution properties since the heterogeneity we observe seems to be tied to the stimulus location. The results presented here also indicate that spatial interactions may play a critical role in the stability of the 1:1 response. We found that spatial differences in APD and SRC slope are correlated with alternans at many BCLs, while spatial differences and spatial gradients in S_{DRC} are correlated with alternans at fast pacing, where they become positive and grow in magnitude in ALT trials.

Our results may have important implications for the diagnosis and treatment of patients with arrhythmias. Current diagnostic procedures analyze spatially-averaged temporal response patterns or temporal response patterns at a single location. Our study suggests an entirely new approach, based on spatial differences in restitution properties. The proposed spatial approach may have significant advantages. Our results show that spatial differences may be able to detect tissue's propensity to alternans at cycle lengths much longer than the cycle length of the transition to alternans, which would be safer and better tolerated by patients. These experimental observations are compelling enough to stimulate future work on developing the theory linking spatial differences in restitution properties and tissue's propensity to alternans.

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