Spatio-temporal Evolution and Prediction of Action Potential Duration and Calcium Alternans in the Heart

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1 Introduction

The beat-to-beat variation in cardiac action potential durations (APD) is a phenomenon known as electrical alternans (Figure 1A, B). In recent years, alternans has been considered a strong marker of electrical instability and a harbinger for ventricular fibrillation, which is a major cause of sudden cardiac death (Armoundas et al., 2002; Zipes et al., 1981). This beat-to-beat variation in the cardiac APD in the single cell has been shown to correspond to T-wave alternans at the whole heart level, seen in the electrocardiogram (Zipes et al., 1981; Pastore et al., 1999). Moreover, T-wave alternans is recognized as a precursor of ventricular arrhythmia since it was subsequently observed in a wide variety of clinical and experimental conditions associated with such arrhythmias (Salero et al., 1986; Hellerstein et al., 1950; Schwartz & Malliani, 1975; Rosenbaum et al., 1994).

**Figure 1:** The response of periodically paced cardiac tissue to stimulation (arrows) during (A) normal pacing (1:1 response) and (B) alternans.

In the heart, membrane voltage and \([\text{Ca}^{2+}]_i\) are bi-directionally coupled with each exerting an influence on the other during the course of an action potential. \([\text{Ca}^{2+}]_i\), transient is influenced by membrane voltage through its effect on the L-type calcium channel, termed calcium-induced-calcium-release (Armoundas et al., 2003). On the other hand, \([\text{Ca}^{2+}]_i\), amplitude controls APD via its effects on \([\text{Ca}^{2+}]_i\), sensitive membrane currents such as the sodium-calcium exchanger current (Mullins, 1979) and the L-type calcium current (Lee et al., 1985). Therefore, APD alternans can be accompanied by alternans in intracellular calcium ([\(\text{Ca}^{2+}]_i\)) transients. \([\text{Ca}^{2+}]_i\), transient amplitude alternans has been linked to mechanical alternans (Lee et al., 1988); and the simultaneous occurrence of \([\text{Ca}^{2+}]_i\), and APD alternans, termed electromechanical (EM) alternans, is believed to be a substrate for various cardiac arrhythmias (Weiss et al., 2006). It has been postulated that \([\text{Ca}^{2+}]_i\), alternans might be responsible for the fluctuations in APD that produce T-wave alternans in the whole heart (Lee et al., 1988; Wan et al., 2005). It is suggested that \([\text{Ca}^{2+}]_i\), alternans develops first due to dynamical instabilities in calcium cycling (Shiferaw et al., 2003; Chudin et al., 1998; Morita et al., 2008), which then drives APD alternans, leading to EM alternans in the heart (Lakireddy et al., 2005). Moreover, it was demonstrated that by suppressing \([\text{Ca}^{2+}]_i\), cycling and buffering \([\text{Ca}^{2+}]_i\), transients, APD alternans under rapid pacing was abolished even though APD was shortened with faster pacing (Goldhaber et al., 2005). These results point towards a primary role for
[Ca$^{2+}$], in the genesis of EM alternans. Despite the important role of [Ca$^{2+}$], in the development of EM alternans, no criteria has been proposed to predict the onset of [Ca$^{2+}$], and EM alternans.

Various theories have been put forward to explain how impaired calcium handling can induce calcium and APD alternans. Previous studies have suggested that diminished [Ca$^{2+}$] reuptake and instability in the beat-to-beat feedback control of sarcoplasmic reticulum (SR) content leads to [Ca$^{2+}$] alternans (Eisner et al., 2005). In areas with impaired calcium handling or larger calcium transients, complete reuptake of [Ca$^{2+}$], back into SR may not always be possible before the next activation, leaving the region vulnerable to [Ca$^{2+}$], transient amplitude (CaA) alternans. This is because the next [Ca$^{2+}$] transient is from a higher diastolic [Ca$^{2+}$], content and vice versa. These beat-to-beat changes in the [Ca$^{2+}$], transient amplitude has a huge effect on the APD due to the aforementioned coupling between membrane voltage and [Ca$^{2+}$]. Thus, impaired calcium handling can induce both calcium and APD alternans in the heart.

It has been proposed that the onset of APD alternans in cardiac myocytes can be determined by analysing their responses to periodic stimulation and constructing a restitution curve (Courtemanche, 1996; Guevara et al., 1984; Riccio et al., 1999; Nolasco & Dahlen, 1968), which represents the nonlinear relationship between the APD and the preceding diastolic interval (DI). Furthermore, APD restitution slope has been shown to be an important indicator of wave stability, and it has been proposed theoretically that a slope of the restitution curve equal to one predicts the onset of APD alternans in cardiac myocytes (Nolasco & Dahlen, 1968).

However, the actual dynamics of periodically paced cardiac myocytes are more complex, and the APD usually depends on the entire pacing history (Gilmour, 2002; Goldhaber et al., 2005; de Diego et al., 2008; Koller et al., 1998; Lou et al., 2009; Hall et al., 1999, Tolkacheva et al., 2003), and not only on the preceding DI. One of the main consequences of this phenomenon is a dependence of the restitution curve on the pacing protocol used to obtain it. Several pacing protocols have been used experimentally to construct different restitution curves, where the dynamic and S1-S2 restitution curves are the most common. In a dynamic protocol, steady state APD and DI are measured as the basic cycle length (BCL) decreases. In an S1-S2 protocol, a premature stimulus (S2) is applied at various times relative to the end of a series of paced (S1) beats. Thus, the dynamic and S1-S2 restitution curves describe different aspects of cardiac dynamics: steady state responses and responses to perturbations, respectively. In the presence of memory, these different restitution curves have different slopes, and none of them have been clearly linked to the onset of alternans (Goldhaber et al., 2005; de Diego et al., 2008; Koller et al., 1998). Indeed, several experimental studies demonstrated the existence of alternans for a shallow restitution and no alternans for a steep restitution (Guevara et al., 1984; Riccio et al., 1999; Hall et al., 1999). In addition, the restitution hypothesis has been shown to have little clinical relevance, with studies showing poor correlation between APD alternans, APD restitution, and clinical outcome (Narayan et al., 2007).

One of the possible reasons why the restitution hypothesis does not always hold experimentally is the fact that it does not take into account [Ca$^{2+}$], cycling. Although different mechanisms exist to explain the formation of APD alternans without [Ca$^{2+}$], cycling (Nolasco & Dahlen, 1968), it has been postulated in several numerical and single cell experimental studies that [Ca$^{2+}$], alternans might be responsible for the alternations in APD (Wan et al., 2005; Shiferaw et al., 2003). Even though these results point towards a primary role for [Ca$^{2+}$], in the genesis of EM alternans, no studies have looked into predicting the onset of [Ca$^{2+}$], alternans.

Another reason for the failure of the restitution hypothesis is the complex spatio-temporal evolution of alternans in the heart. Indeed, it has been demonstrated that APD alternans has a local onset in the
heart, i.e. alternans develops in a small region of the heart, and then occupies the entire surface as the pacing rate increases (Cram et al., 2011). However, not much is known about spatio-temporal evolution of [Ca$^{2+}$]$\text{}_{i}$ alternans in the heart (Visweswaran et al., 2013).

Recently, a perturbed downsweep pacing protocol was developed theoretically (Tolkacheva et al., 2003) and implemented experimentally (Tolkacheva et al., 2006) leading to the concept of the restitution portrait, which consists of several restitution curves measured simultaneously at various pacing frequencies. It has been shown that the local onset of APD alternans in a small region of an isolated rabbit heart can be predicted by calculating one of the slopes measured in the restitution portrait (Cram et al., 2011). This suggests that the restitution portrait may be a better approach to predict APD alternans compared to individual restitution curves since it captures several aspects of cardiac dynamics simultaneously. Even though the restitution portrait has proved useful in predicting APD alternans, it is still unclear whether it can be applied to predicting the onset of [Ca$^{2+}$]$\text{}_{i}$ alternans, which might be the primary driving force of EM alternans.

The main objective of this chapter is to discuss the local development of [Ca$^{2+}$]$\text{}_{i}$ and APD alternans in the isolated rabbit heart using simultaneous recording of both transmembrane voltage and [Ca$^{2+}$]$\text{}_{i}$. Specifically, we aimed to study the spatio-temporal evolution of both [Ca$^{2+}$]$\text{}_{i}$, transient amplitude (CaA) and duration (CaD) alternans in relation to APD alternans. In addition, we aim to demonstrate whether the slopes measured in the restitution portrait can predict the onset of APD and [Ca$^{2+}$]$\text{}_{i}$ alternans in the heart.

## 2 Methods

### 2.1 Downsweep Pacing Protocol

All investigations conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, Revised 1996), and the protocol was approved by the Institutional Animal Care and Use Committee at the University of Minnesota. To determine the development of APD alternans, we used high resolution optical mapping to visualize the electrical activity in N=6 Langendorff-perfused isolated rabbit hearts using the voltage-sensitive dye di-4-ANEPPS, as described previously (Mironov et al., 2008; Cram et al., 2011). Two synchronized CCD cameras were used to record membrane voltage from the left (LV) and right ventricular (RV) epicardial surfaces of the heart. In order to study the spatial and temporal development of [Ca$^{2+}$]$\text{}_{i}$ and APD alternans, we used simultaneous voltage-calcium optical mapping using voltage-sensitive (RH-237) and calcium-sensitive (Rhod-2AM) dyes in N=8 rabbits, as described previously (Choi & Salama, 2000; Pruvot et al., 2004; Visweswaran et al., 2013). Briefly, the fluorescence signal emitted from the heart upon excitation (532 nm) was split at 630 nm using a dichroic mirror. The split signals were further filtered using a 720 long pass and 585 ± 20 nm to obtain the voltage and calcium signals. The choice of dyes was made such that the emission spectra do not overlap. Two cameras were used to measure the calcium and voltage signal simultaneously from the RV surface. A mechanical uncoupler blebbistatin was added to the Tyrode’s solution to reduce motion artifacts in both sets of experiments.

The heart was paced at the base and optical mapping movies were recorded at different BCLs $B$. External stimuli (5 ms durations, twice the threshold) were applied to the base of the heart using a perturbed downsweep pacing protocol (Kalb et al., 2004), in which the following steps were applied at each BCL $B$, starting with $B = 300$ ms:

I. 100 stimuli were applied at BCL $B$ to achieve a steady state (SS).
II. One additional stimulus (long perturbation, LP) was applied at a longer BCL $B_{LP} = B + 10\text{ms}$.

III. 10 stimuli were applied at BCL $B$ to return to SS.

IV. One additional stimulus (short perturbation, SP) was applied at a shorter BCL $B_{SP} = B - 10\text{ms}$.

V. 10 stimuli were applied at BCL $B$ to return to SS.

After completing steps (I) - (V), the BCL $B$ was progressively reduced in 20 ms decrements until $B = 100\text{ ms}$ or until ventricular fibrillation occurred.

At each BCL, APD was measured at 80% repolarization and CaA was calculated as the difference between the local maxima and minima of the same response, representing the systolic and diastolic values of $[\text{Ca}^{2+}]_i$, after subtraction of the background fluorescence, as described previously (Qian et al., 2001). The CaD was determined from the maximum first derivative of the $[\text{Ca}^{2+}]_i$ upstroke to the time point of 80% recovery of $[\text{Ca}^{2+}]_i$ to its original baseline, as was described previously (Choi & Salama, 2000; Lakireddy et al., 2005).

### 2.2 Alternans Measurements

The amplitudes of APD and CaD alternans were calculated at each pixel as a difference in corresponding values of the SS responses between odd and even beats: $\Delta \text{APD} = \text{APD}_{\text{even}} - \text{APD}_{\text{odd}}$, and $\Delta \text{CaD} = \text{CaD}_{\text{even}} - \text{CaD}_{\text{odd}}$. The degree of CaA alternans, $\Delta \text{CaA}$, was calculated at each pixel as the alternans ratio, $1-X/Y$, where X is the net amplitude of the smaller $[\text{Ca}^{2+}]_i$ transient and Y is the net amplitude of the larger $[\text{Ca}^{2+}]_i$ transient, as was described previously (Wu & Clusin, 1997). The temporal thresholds for both APD and CaD alternans were set at 5 ms; and for CaA alternans at 0.15. 2D $\Delta \text{APD}$, $\Delta \text{CaD}$, and $\Delta \text{CaA}$ maps were constructed to reveal the spatial distribution and amplitude of each type of alternans for the RV surface of the heart. To eliminate a possibility of inclusion of more complex rhythms or ectopic beats, each movie that was used for alternans analysis was visually inspected to ensure the absence of alternans phase change (long-short to short-long). If such a phase change occurred, the corresponding movie was excluded from the analysis.

The local spatial onset of alternans was defined as the BCL $B_{\text{Onset}}$, at which at least 10% of the RV surface exhibited alternans separately for APD ($B_{\text{Onset}}^{\text{APD}}$), CaA ($B_{\text{Onset}}^{\text{CaA}}$), or CaD ($B_{\text{Onset}}^{\text{CaD}}$). Two spatial regions of the heart were defined at each $B_{\text{Onset}}$: the 1:1 alt region, which exhibited alternans, and the 1:1 region, which exhibited 1:1 behaviour and no alternans. These two regions were back-projected to all BCLs $B$ preceding $B_{\text{Onset}}$, and the mean values and standard errors for all parameters were calculated and averaged separately for these two regions. The notations 1:1_{alt} and 1:1 reflect the fact that both regions exhibit 1:1 behaviour prior to $B_{\text{Onset}}$, and only at $B_{\text{Onset}}$ the 1:1_{alt} region exhibits alternans. Since alternans occurred at different BCLs during different experiments, a normalized value of BCL according to formula $B_N = B - B_{\text{Onset}}$ was introduced.

### 2.2 Slopes Calculations

The responses at each pixel were used to construct restitution portraits of the heart separately for voltage and $[\text{Ca}^{2+}]_i$. Only data from different BCLs prior to $B_{\text{Onset}}$ were considered for restitution portrait construction. Specifically, at each pixel, the restitution portrait consists of a dynamic restitution curve measuring SS responses at each BCL $B$, and several local S1-S2 restitution curves for each BCL $B$. The following slopes were calculated separately for APD, CaA, and CaD responses at each pixel: 1) slope of dynamic restitution curve, $S_{\text{dyn}}^{BP}$, by fitting SS responses from all $B$ values with a second degree polynomial curve;
and 2) slopes of S1-S2 restitution curve $S_{12}$ (measured at SS) and $S_{12}^{Max}$ (measured at SP) by fitting LP and SP responses from all $B$ values respectively with a second degree polynomial function.

<table>
<thead>
<tr>
<th>Term</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B$</td>
<td>Basic cycle length (BCL)</td>
</tr>
<tr>
<td>$B^{onset}$</td>
<td>B at which 10% of the heart exhibited alternans</td>
</tr>
<tr>
<td>$B^{Prior}$</td>
<td>B immediately prior to $B^{onset}$</td>
</tr>
<tr>
<td>$B_N$</td>
<td>Normalized BCL based on the formula $B_N = B - B^{onset}$</td>
</tr>
</tbody>
</table>

**Table 1**: Different terms and what each term represents.

<table>
<thead>
<tr>
<th>Pacing protocol (step)</th>
<th>Responses</th>
<th>Slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>APD and DI after LP</td>
<td>$S_{12}$ - Measured at SS at each B</td>
</tr>
<tr>
<td>I</td>
<td>APD and DI after SS</td>
<td>$S_{12}^{Max}$ - Measured at SP at each B</td>
</tr>
<tr>
<td>IV</td>
<td>APD and DI after SP</td>
<td>$S_{12}^{dp}^2$ - Measured at SP from all B</td>
</tr>
<tr>
<td>I</td>
<td>APD and DI after SS</td>
<td>$S_{12}^{dp}$ - Measured at SS from all B</td>
</tr>
</tbody>
</table>

**Table 2**: Different responses and corresponding slopes measured from the pacing protocols.

## 3 APD Alternans

### 3.1 Spatio-temporal Evolution of APD Alternans in the Heart

We observed that alternans first appeared locally at $B = B^{onset}$ in ventricles and evolved spatially throughout the heart as the BCL decreased. Figure 2 shows a representative example of the spatiotemporal evolution of alternans in the left ventricle of the rabbit heart at different values of $B$. The color bar represents the amplitude of alternans APD. Note the presence of 1:1 behavior (white) at large $B$, and appearance of alternans (red) as $B$ decreases. For instance, in this particular example, the onset of alternans occurred at $B^{onset} = 170$ ms. At that specific BCL, the two regions, 1:1$_{alt}$ and 1:1, were identified at the surface of the heart and projected back into all prior BCLs (see Figure 2). Note that alternans spread throughout the heart as the BCLs became smaller than $B^{onset}$, and spatially concordant alternans became spatially discordant, as has been described previously (Weiss et al., 2006; Mironov et al., 2008), and as indicated by the presence of white color (nodal line) at $B=150$ ms in Figure 2.

**Figure 2**: A representative example of the 2D ΔAPD maps in the rabbit left ventricle at different $B$. The color bar represents amplitude of alternans (red), and 1:1 responses (white). The local spatial onset of alternans occurs at $B^{onset}$ ($B^{onset}_{LP}$). At $B^{onset}$, the two regions, 1:1$_{alt}$ and 1:1 are introduced and projected to all preceding $B$ (black outlines).
3.2 2D Restitution Properties of the Heart

From Figures 1 and 2, we notice that alternans develop in a small region of the heart before spreading throughout the epicardial surface as the pacing rate increases. In order to determine whether there are any changes in the properties of the myocardium before this local onset of alternans appears, we constructed 2D APD maps for SS, LP, and SP responses at various BCLs. Representative examples of these APD maps are shown in Fig. 3A for large BCL $B=200$ ms, and for the BCL prior to $B^{onset}$, $B^{Prior}$. The color bar represents the duration of the action potentials, and the projected boundaries of the $1:1_{alt}$ and $1:1$ regions are shown as black lines on each APD map. Note, that for large $B$ (top panel in Figure 3A), the spatial distributions of APDs measured during LP and SP were similar to the one measured at SS, both for the $1:1_{alt}$ and $1:1$ regions. However, at $B^{Prior}$, the spatial distribution of APD measured at LP was similar to the one from SS, while the spatial distribution of APD measured at SP was different, both for the $1:1_{alt}$ and $1:1$ regions (see bottom panel in Figure 3A). This shows that, prior to the onset of alternans, all regions of the heart respond similarly both at steady state and after perturbations. However, just prior to the onset of alternans, the region of the heart which eventually develops alternans has different restitution properties compared to the region which does not develop alternans.

![Figure 3](image)

**Figure 3:** (A) Representative examples of 2D APD maps for APDs calculated at LP, SP and SS responses $B = 200$ ms and $B^{Prior}$. (B) Representative examples of the restitution portraits constructed for two pixels taken from the $1:1_{alt}$ (solid circles) and $1:1$ (open circles) regions. The steady state restitution curve is shown in red, and the local S1-S2 restitution curves for different $B$ are shown in blue. The gray dotted lines with the slope of -1 represent the equal-BCL lines. The solid line shows the slope of equal one. Three different slopes are present in the restitution portrait for each $B$: $S_{dyn}^{RP}$, $S_{12}$, and $S_{12}^{Max}$. Representative examples of the restitution portraits for two pixels taken from the $1:1_{alt}$ and $1:1$ regions are compared in Figure 3B. Here, a unique steady state restitution curve (red) and several local S1-S2 restitution curves for different $B$ (blue) are shown along with the corresponding slopes $S_{dyn}^{RP}$, $S_{12}$, and $S_{12}^{Max}$. The gray dotted lines are the equal BCL lines with the slope = -1, and solid black line show the slope=1. Note that the dynamic restitution curves are almost parallel in both $1:1_{alt}$ and $1:1$ regions, indicating the absence of differences in $S_{dyn}^{RP}$ at all $B$. Similarly, local S1-S2 restitution curves are nearly par-
allel at large $B$, indicating that $S_{12}$ and $S_{12}^{\text{Max}}$ were similar between $1:1_{\text{alt}}$ and $1:1$ regions as well. However, at $B_{\text{Prior}}$, the difference between local S1-S2 restitution curves is pronounced, and both the $S_{12}$ and $S_{12}^{\text{Max}}$ slopes were visually steeper in the $1:1_{\text{alt}}$ region in comparison to the $1:1$ region. Therefore, Fig. 3B indicates that the slopes $S_{12}$ and $S_{12}^{\text{Max}}$ correlate with the local onset of alternans in the heart.

### 3.3 Prediction of the Local Onset of APD Alternans in the Heart

To determine whether the restitution portrait can predict the local onset of APD alternans, we calculated mean values of three slopes measured in the restitution portrait, $S_{\text{dyn}}^{\text{RP}}$, $S_{12}$, and $S_{12}^{\text{Max}}$, over the epicardial surface of the hearts in all experiments separately for the $1:1_{\text{alt}}$ and $1:1$ regions. The data are shown in Fig. 4A – C as a function of $B_N$.

Note that there is no significant difference between values of $S_{\text{dyn}}^{\text{RP}}$ calculated both for the $1:1_{\text{alt}}$ and $1:1$ regions at any $B_N$ (Figure 4A). However, the behavior of $S_{12}$ and $S_{12}^{\text{Max}}$ slopes are different (Figure 4 B and 4C). At large $B_N$, both $S_{12}$ and $S_{12}^{\text{Max}}$ are similar between the $1:1_{\text{alt}}$ and $1:1$ regions, but as $B_N$ decreases, the values of both slopes becomes significantly larger in the $1:1_{\text{alt}}$ region compared to the $1:1$ region ($p < 0.05$). Note that this significant difference appears first for the $S_{12}$ slope (at $B_N = 20$ ms, Figure 6B) and later for the $S_{12}^{\text{Max}}$ slope (at $B_N = 10$ ms, Figure 4C). However, the difference in $S_{12}^{\text{Max}}$ values is significantly larger than the difference in $S_{12}$ values between the $1:1_{\text{alt}}$ and $1:1$ regions immediately prior to the onset of alternans, at $B_{\text{Prior}}$. Therefore, both $S_{12}$ and $S_{12}^{\text{Max}}$ can serve as an indicator of the local onset of alternans in the heart.

**Figure 4**: Mean values of A, $S_{\text{dyn}}^{\text{RP}}$; B, $S_{12}$, C, and $S_{12}^{\text{Max}}$ as a function of $B_N = B - B_{\text{Onset}}$. All slopes were calculated separately for the $1:1_{\text{alt}}$ (solid circles) and $1:1$ (open circles) regions. D, Mean values of these slopes measured at $B_{\text{Prior}}$ in comparison to the theoretical predicted value of 1. * denotes statistically significant data ($p < 0.05$).
To determine whether any of the slopes measured in our experiments were comparable with the theoretically predicted value of one, we calculated the mean values of $S_{b_{\text{on}}}$, $S_{12}$, and $S_{12}^{\text{max}}$ immediately prior to the onset of alternans, at $B_{\text{Prior}}^{\text{onset}}$. Figure 4D compares the values of all slopes for both the $1:1_{\text{alt}}$ and $1:1_{\text{alt}}$ regions with the value of one. Note that $S_{b_{\text{on}}}$, and $S_{12}$ were less than one in both the $1:1_{\text{alt}}$ and $1:1_{\text{alt}}$ regions, while $S_{12}^{\text{max}}$ was larger than one in $1:1_{\text{alt}}$ and smaller than one in $1:1_{\text{alt}}$ region. Nevertheless, the values of all slopes were significantly different than the theoretically predicted value of one.

4 \[\text{Ca}^{2+}\] Alternans

4.1 Spatio-temporal Evolution of \[\text{Ca}^{2+}\] Alternans in the Heart

With our simultaneous voltage and calcium optical mapping experiments, we observed that \[\text{Ca}^{2+}\] alternans also occurred locally in a small region of the heart and spread to occupy the entire heart as the BCL $B$ decreased, similar to APD alternans. Figure 5 shows the evolution of \[\text{Ca}^{2+}\] alternans in relation to APD alternans. Fig. 5 A shows representative examples of the spatiotemporal evolution CaA (top panel), CaD (middle panel), and APD (bottom panel) at different values of $B$. The color bar represents the amplitude of alternans (red) or the presence of 1:1 responses (white). Note that at large $B$, there is no alternans in APD, CaD or CaA; but as $B$ decreases, alternans gradually develops in the heart. Note that in this particular example, alternans in CaA develops first at $B_{\text{CaA}}^{\text{onset}} = 170$ ms, followed by APD alternans at $B_{\text{APD}}^{\text{onset}} = 160$ ms, and CaD alternans occurs last at $B_{\text{CaD}}^{\text{onset}} = 150$ ms. Fig. 5 A clearly demonstrates that APD and CaD alternans develop in the same area that was already occupied by CaA alternans. Fig. 5 B shows single pixel traces of \[\text{Ca}^{2+}\] transients and action potentials taken from the regions marked as ‘*’, ‘+’, and ‘#’ in Fig. 5 A. At large BCL $B = 190$ ms, 1:1 behaviour is seen in both \[\text{Ca}^{2+}\] and APD (see ‘*’); and as the BCL $B$ is decreased to 170 ms (see ‘+’), note that beat-to-beat changes occur first only in CaA, while 1:1 behaviour is still seen in APD. As the BCL $B$ is further decreased to 150 ms, the alternans in CaA, APD and CaD are seen (see ‘#’). It is also worth mentioning that CaD alternans, in contrast to CaA and APD alternans, was rarely present and was generally observed only during pacing at a very high frequency.

The spatio-temporal dynamics of alternans illustrated in Fig. 5 A was present across all experiments. Fig. 5 C shows a box plot representation of minimum, median, and maximum values of $B^{\text{onset}}_{\text{CaA}}$, $B^{\text{onset}}_{\text{CaD}}$, and $B^{\text{onset}}_{\text{APD}}$. Note that the onset of CaA alternans, $B^{\text{onset}}_{\text{CaA}}$, always preceded the onset of APD alternans, $B^{\text{onset}}_{\text{APD}}$, while the onset of CaD alternans, $B^{\text{onset}}_{\text{CaD}}$ always developed last. Fig. 5D shows the spatial development of CaA, APD, and CaD alternans in all experiments quantified by the percentage of the total RV surface occupied by each type of alternans as a function of $B$. Note the gradual development of each type of alternans in the heart as the $B$ decreases. Therefore, our results show that CaA and CaD alternans, similar to APD alternans (Cram et al., 2011), has a local onset in the heart, and that the local onset of CaA precedes the local onset of APD and CaD alternans.

Here, we demonstrated that the local onset of \[\text{Ca}^{2+}\] alternans always occurs first, which then causes alternations in APD as has been previously demonstrated in numerical investigations and single cell experiments (Shiferaw et al., 2003; Chudin et al. 1998). In order for \[\text{Ca}^{2+}\] alternans to be considered the cause of accompanying APD alternans, it is necessary to show that, for a given cell within the intact heart, the two phenomena are inexorably linked. Initially, this link has been demonstrated using monophasic action potential electrodes recordings (Lee et al., 1998), and later using more rigorous optical mapping studies (Pruvot et al., 2004). The fact that \[\text{Ca}^{2+}\] transient and APD alternans occur together...
is consistent with the hypothesis that the $[\text{Ca}^{2+}]_i$ transient "controls" APD, but this never has been sufficiently proven, because it is possible that purely voltage-dependent currents could produce APD alternans (Clusin, 2008). Our results show that APD alternans occur in the same region that was already occupied by $[\text{Ca}^{2+}]_i$ alternans, which seem to indicate that calcium alternans drives APD alternans in the whole heart even with complex spatial factors. This might be due to the steep dependence of the SR $\text{Ca}^{2+}$ release on SR $\text{Ca}^{2+}$ load (Diaz et al., 2004) and SR $\text{Ca}^{2+}$ uptake (Shiferaw et al., 2003), which has been implicated as the primary cause of $[\text{Ca}^{2+}]_i$ alternans in single cells. APD alternans can occur passively due to the close dependence between $[\text{Ca}^{2+}]_i$ and membrane voltage mediated by the $[\text{Ca}^{2+}]_i$-dependent currents.

**Figure 5:** (A) Representative example of the 2D $\Delta \text{CaA}, \Delta \text{CaD}$ and $\Delta \text{APD}$ maps showing spatio-temporal evolution of alternans at different $B$ values. The color bar represents the amplitude of alternans (red) and 1:1 responses (white). The local spatial onsets of alternans occur at $B^{\text{Onset}}$. At $B^{\text{Onset}}$, two regions (1:1$_{alt}$ and 1:1) are introduced and back-projected to all prior $B$ values (black outlines). (B) Representative traces of $[\text{Ca}^{2+}]_i$ (red) and action potential (black) taken from pixels marked as ‘*’, ‘+’, and ‘#’ in A. (C) Local onset, $B^{\text{Onset}}$ of CaA, CaD, and APD alternans and (D), spatial evolution of alternans as a function of $B$. Dashed horizontal line indicates spatial threshold for alternans (10% of RV surface).
4.2 Prediction of the Local Onset of CaD and CaA alternans

We then performed a similar analysis to investigate if any of the slopes of the restitution portrait constructed from CaD responses could predict the local onset of CaD alternans. Figure 6A shows representative examples of 2D ΔCaD maps at $B^\text{Onset}_{\text{CaD}}$ and the BCL prior to the onset of CaD alternans, $B^\text{Prior}$. The dotted lines indicate the border between 1:1 and 1:1$_{alt}$ regions that was determined at $B^\text{Onset}_{\text{CaD}}$. Figure 6B shows a representative example of a restitution portrait from the pixel marked as “#” along with the definitions of measured slopes (top). The spatial distribution of $S^\text{RP}_{dyn}$, $S_{12}$, and $S^\text{Max}_{12}$ slopes calculated at $B^\text{Prior}$ is shown in Figure 6B (bottom). In this case, both $S^\text{Max}_{12}$ and $S^\text{RP}_{dyn}$ have larger values in the 1:1$_{alt}$ compared to the 1:1 region. The mean $S^\text{RP}_{dyn}$, $S_{12}$, and $S^\text{Max}_{12}$ slopes calculated at $B^\text{Prior}$ from all our experiments are shown in Figure 6C separately for 1:1 and 1:1$_{alt}$ regions. Note that in the case of CaD, both $S^\text{Max}_{12}$ and $S^\text{RP}_{dyn}$ are significantly larger in 1:1$_{alt}$ region compared to 1:1 region immediately prior to onset of CaD alternans; while the $S_{12}$ slopes remain the same. This indicates that both $S^\text{Max}_{12}$ and $S^\text{RP}_{dyn}$ slopes calculated prior to the onset of CaD alternans, at $B^\text{Prior}$, can indicate which region of the heart is susceptible to alternans.

![Figure 6](image.png)

Figure 6: (A) Representative examples of 2D ΔCaD maps at $B^\text{Onset}_{\text{CaD}}$ and $B^\text{Prior}$. (B) Representative example of CaD restitution portrait for pixel marked as “#” in A showing the slopes measured (top); 2D distribution of $S^\text{RP}_{dyn}$, $S_{12}$, and $S^\text{Max}_{12}$ slopes at $B^\text{Prior}$ (bottom). Dashed lines outline the boundary between the 1:1 and 1:1$_{alt}$ regions and C, Average $S^\text{RP}_{dyn}$, $S_{12}$, and $S^\text{Max}_{12}$ slope values at $B^\text{Prior}$. * indicates statistical significance (p<0.05).
Our experiments demonstrate that the rate dependent shortening/reduction of CaA with BCLs, i.e. steady state dynamic restitution of CaA, is not present in the isolated hearts. This is in contrast to APD and CaD restitution, as indicated by the red dynamic restitution curves in Figure 3B and 4B, top, respectively. However, CaA after SP and LP responses was different from steady state responses, especially at lower BCLs. Therefore, the restitution portraits obtained for CaA appear different from the one obtained for APD and CaD. Figure 7A shows representative examples of 2D ΔCaA maps at BCaA and the BCL prior to the onset of CaA alternans, BPrior. The dotted lines indicate the border between 1:1 and 1:1alt regions that was determined at BCaA. Figure 7B shows a representative example of a restitution portrait from a pixel marked in Figure 7A as ‘#’. Note the presence of the flat dynamic restitution curve indicating that the steady state responses are rate-independent, and thus steady state CaAs has similar values across all BCLs leading to a very flat dynamic restitution curve. However, the presence of local S1-S2 curves at different BCLs indicates that there are differences between the steady state and perturbation (SP and LP) responses. Note that this difference is more pronounced at lower BCLs, closer to the onset of CaA alternans. The spatial distribution of S12, S12, and S12Max slopes calculated at BPrior is shown in Figure 7B. In this example, both S12Max and S12 have larger values in the 1:1alt compared to the 1:1 region, while S12 is close to zero in all regions. The mean S12, S12, and S12Max slopes calculated at BPrior from all our experiments are shown in Figure 7C separately for 1:1 and 1:1alt Regions. Both S12 and S12Max are significantly larger in 1:1alt region compared to 1:1 region immediately prior to the onset of CaA alternans at BPrior, while the S12Max slopes remain close to zero in both regions. This indicates that both S12Max and S12 slopes calculated prior to the onset of CaA alternans, at BPrior, can indicate which region of the heart is susceptible to alternans.

Although previous literature defines [Ca^{2+}]i alternans as the beat-to-beat variation in the amplitude of the [Ca^{2+}]i transients, i.e. CaA alternans (Qian et al., 2001; Wu & Clusin, 1997), we wanted to investigate CaA alternans as well, since it has received limited attention. Here, we demonstrated that the local onset of CaA and CaD alternans occurred at statistically different values of BCLs. CaD is thought to have similar general features as action potential repolarization and duration maps (Choi & Salama, 2000). The importance of CaD lies in the fact that [Ca^{2+}]i overload and increases in CaD, due to spontaneous [Ca^{2+}]i releases from the sarcoplasmic reticulum (SR), can be arrhythmogenic. Spontaneous SR [Ca^{2+}]i releases are thought to be potential sources of early after depolarizations which can result in torsades de pointes, tachycardia, and other arrhythmias (Choi et al., 2002). However, physiological relevance of CaD alternans needs to be further investigated, and it remains unclear whether a combination of CaA and CaD alternans is more arrhythmogenic compared to beat-to-beat variation in CaA only.

It is important to predict the onset of CaA alternans since our results shows that CaA alternans consistently develops ahead of either APD or CaD alternans. Our experiments demonstrate that the rate dependent shortening/reduction of CaA with BCLs, i.e steady state dynamic restitution of CaA, is not present in the isolated hearts. However, CaA after SP and LP responses were different from steady state responses, especially at lower BCLs. Our analysis of the S1-S2 restitution curves reveals that both S12Max and S12 slopes were significantly higher in the 1:1alt region compared to the 1:1 region. Therefore, these slopes are reliable indicators of CaA alternans. Our results are consistent with previous findings in isolated rabbit ventricular myocytes. Specifically, (Tolkaicheva et. al, 2006) measured the peak L-type calcium current (I_{Ca-L}) during SS, SP and LP, and demonstrated SP does not affect peak I_{Ca-L} for large values of BCL (≥ 350 ms), but significantly reduces it at lower BCLs (200 ms). This is probably due to the complex interplay between reactivation and inactivation kinetics of I_{Ca-L}. I_{Ca-L}, which plays an important role in the upstroke of the cyclic calcium transient, controls the amount of Ca^{2+} present in the intracellular
Figure 7: (A) Representative examples of 2D ΔCaA maps at $B^{\text{Onset}}_{\text{CaA}}$ and $B^{\text{Prior}}_{\text{CaA}}$. (B) Representative example of CaA restitution portrait for pixel marked as ‘#’ in A (top); 2D distribution of $S^{\text{Rep}}_{\text{dyn}}$, $S_{12}$ and $S^{\text{Max}}_{12}$ slopes at $B^{\text{Prior}}$ (bottom). Dashed lines outline the boundary between the 1:1 and 1:1$_{alt}$ regions and C, Average $S^{\text{Rep}}_{\text{dyn}}$, $S_{12}$, and $S^{\text{Max}}_{12}$ slope values at $B^{\text{Prior}}$. ** indicates statistical significance ($p < 0.05$).

Mechanisms other than impaired calcium handling and electrical restitution have also been implicated as the cause of alternans, specifically spatially discordant APD alternans, in the heart. It has been demonstrated that pre-existing tissue heterogeneity can facilitate alternans formation – changes in pacing rate or an appropriately timed stimulus can induce APD alternans around the locations of heterogeneity (Pastore et al., 1999; Chinushi et al., 2003; Pastore et al., 2006). However, several numerical studies have shown that this is not a primary mechanism and that alternans can be formed in homogeneous tissue (Hayashi et al., 2007). Other mechanisms of alternans formation in the heart include steep conduction velocity restitution, i.e., when CV of a propagating wave has a steep dependence on the preceding diastolic space during each transient by mediating the entry of the Ca$^{2+}$ into the cell triggering the release of the Ca$^{2+}$ from the SR. The decrease in $I_{\text{Ca-L}}$ due to a perturbation at low BCLs would thus lead to smaller amplitude of the calcium transient similar to what is seen during our experiments.
interval (Watanabe et al., 2001; Fox et al., 2002; Fenton et al., 2002; Franz, 2003) or short term memory (Mironov et al., 2008).

5 Summary

In this Chapter, we discussed a spatio-temporal evolution of both $[\text{Ca}^{2+}]_i$ and APD alternans in the isolated rabbit heart, and determined if the local onset of $[\text{Ca}^{2+}]_i$ and APD alternans could be predicted using different slopes measured in the restitution portrait. The main results can be summarized as follows. First, $[\text{Ca}^{2+}]_i$ alternans has a local onset in the heart similar to APD alternans. It initially develops in a small area of the heart, and then evolves to occupy the entire heart. Second, the local onset of $[\text{Ca}^{2+}]_i$ alternans, specifically CaA alternans, always precedes the local onset of APD alternans, while CaD alternans always follows APD alternans. Third, the restitution portrait, can be used to predict the local onset of APD alternans, as well as the local onset of $[\text{Ca}^{2+}]_i$ (both CaA and CaD) alternans. Specifically, $S_{12}$ and $S_{12}^{Max}$ measured in the restitution portrait can predict the local onset of APD alternans; $S_{01}^{RP}$ and $S_{12}^{Max}$ slopes can be considered as indicators of CaD alternans; while $S_{12}$ and $S_{12}^{Max}$ are indicators of CaA alternans.

References


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