Published in final edited form as:

Conf Proc IEEE Eng Med Biol Soc. 2008 ; 2008: 153–156. doi:10.1109/IEMBS.2008.4649113.

A sensitive algorithm for automatic detection of space-time alternating signals in cardiac tissue

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Abstract

Alternans, a beat-to-beat alternation in cardiac signals, may serve as a precursor to lethal cardiac arrhythmias, including ventricular tachycardia and ventricular fibrillation. Therefore, alternans is a desirable target of early arrhythmia prediction/detection. For long-term records and in the presence of noise, the definition of alternans is qualitative and ambiguous. This makes their automatic detection in large spatiotemporal data sets almost impossible. We present here a quantitative combinatorics-derived definition of alternans in the presence of random noise and a novel algorithm for automatic alternans detection using criteria like temporal persistence (TP), representative phase (RP) and alternans ratio (AR). This technique is validated by comparison to theoretically-derived probabilities and by test data sets with white noise. Finally, the algorithm is applied to ultra-high resolution optical mapping data from cultured cell monolayers, exhibiting calcium alternans. Early fine-scale alternans, close to the noise level, were revealed and linked to the later formation of larger regions and evolution of spatially discordant alternans (SDA). This robust new technique can be useful in quantification and better understanding of the onset of arrhythmias and in general analysis of space-time alternating signals.

I. Introduction

The onset of certain types of arrhythmias, such as ventricular tachycardia (VT) and the more malignant ventricular fibrillation (VF), has been linked to instabilities in transmembrane potential and intracellular calcium, known as alternans [1–3].

The beat-to-beat calcium alternans reflect instabilities due to interactions between the three main calcium cycling processes: calcium release, reuptake, and recovery. In theory [4], a large calcium release from the sarcoplasmic reticulum (SR) corresponds to a longer calcium transient duration (CTD) and a higher intracellular calcium concentration. At high frequency, the SR requires longer time to reload after a large release (longer transient). A premature stimulus then results in a partially refilled SR and a smaller release (shorter transient). Furthermore, if certain areas experience long transients and other areas experience shorter transients within the same beat, spatially discordant alternans (SDA)

arise. SDAs are more closely associated with the development of reentrant ventricular arrhythmias, irregularities in the ECG signals and sudden cardiac death [1, 4, 5].

Traditionally, confocal microscopy has been used to investigate alternans in individual myocytes or tissues at high spatial resolution [7–9]. A recent study [9] revealed SDA regions at a surprisingly fine scale, including at the sub-cellular level while imaging intact heart tissue. In such high-magnification experiments, the field of view (FOV) is limited to less than 100 cells, which precludes the examination of macroscopic wave propagation. Furthermore, confocal microscopy is currently limited in temporal resolution (usually <100fps) [10]. Nevertheless, with the rapid development of optical mapping technologies, macroscopic measurements can be performed with higher spatiotemporal resolution, approaching cell level while maintaining large FOV [11]. For example, we record simultaneously from more than 200,000 cells over 20×20mm area with 44μm spatial resolution after binning, and 200fps temporal resolution. The acquired large data sets prompted the development of a new noise-resistant algorithm for automatic identification of alternans, including SDAs.

Technically, two consecutive transients are sufficient to determine presence or absence of alternans. However, in the presence of noise and/or subtle alternans magnitude, certain reassurance (repeated measure) is needed. As a result, a conservative requirement has been adopted previously for strictly uninterrupted alternating sequence over a number of beats, chosen by experience [6]. This is only applicable for large enough alternans ratio (AR) or very high signal-to-noise ratio (SNR), while fine-scale alternans can easily be neglected. In this paper, we present a quantitative mathematical definition of alternans, which uses less-conservative temporal persistence (TP) criteria and is especially valuable for detection and quantification of subtle alternans and/or noisy records.

Here we validate the proposed alternans detection algorithm by comparison to combinatorics-derived theoretical probabilities and by test data sets with white noise. Furthermore, we assess the effects of SNR on the sensitivity of the algorithm. Finally, we apply the algorithm to uncover fine-scale intracellular calcium alternans and their evolution in neonatal rat cardiomyocyte monolayers subjected to progressively faster pacing rates. The algorithm should have general applicability to time-space alternating signals other than calcium as well as applications to other experimental models and systems.

II. Experimental methods

A. Primary myocyte culture and optical mapping

The protocol for primary cardiomyocyte culture has been published previously [12]. Briefly, neonatal rat cardiomyocytes were cultured on thin long silicone polymer (PDMS) surfaces (18×5mm) to form confluent monolayers. Optical mapping of intracellular calcium (using fluorescent dye Fluo-4) was performed on day 6 after culture using an intensified CMOS camera at room temperature in standard Tyrode's solution. Pacing was applied via a platinum line electrode on one of the short sides of the each sample. Spatial imaging resolution after 2×4 binning was (44×88μm in x-y); temporal resolution was 200 fps.

B. Data pre-processing

A series of advanced methods were applied for data pre-processing using custom-designed software in Matlab (MathWorks, Natick, MA). Signal filtering included temporal Savitzky-Golay filter (2nd order, 7 frames window) and spatial Bartlett filter (3 pix kernel). After baseline subtraction and enhancement of contrast, each beat was automatically detected by time of activation. Further automated detection of peak height of calcium transients was

done for all spatial locations (pixels) over all beats to form 3D data sets. Some of these steps are outlined in Figure 1 as part of the algorithm described below.

III. Alternans detection algorithm

A. Alternans Quantification

For a perfect period-2 rhythm, i.e. perfectly alternating calcium transients, the amplitude of all transients during even beats should be consistently smaller (or larger) than transients during odd beats. However, in the presence of noise, due to local instability dynamics and due to spatial interactions, the alternans pattern may break in time – that is the small-large-small sequence may be interrupted at different time points for different spatial locations. We use an index of temporal persistence (TP) of calcium alternating transients as the basis of our algorithm for automatic detection. TP is defined as the ratio between the longest length of a segment of uninterrupted alternans and the total temporal length (number of beats) recorded. The classification of a temporal sequence as “alternans” depends on the TP of that signal, i.e. TP must be above a pre-set threshold value, as described below. With natural heterogeneity between cells, there is no guarantee that all cells will be alternating in the same phase. Therefore, the data are analyzed individually for each pixel (44 μ m*88 μ m).

Figure 1 shows the steps in calculating TP. After pre-processing, the 3D spatiotemporal intensity data are further used to extract a representative parameter at every beat and for each pixel - peak intracellular calcium concentration is extracted, in this case. This reduced data set is further converted to an essentially binary temporal sequence as follows: A derivative and sign are taken along the third dimension (over sequential beats) to exaggerate ‘large-small-large’ or ‘small-large-small’ alternating pattern. Then another derivative is taken to detect discontinuities (interruptions) in the alternans pattern, i.e. where the derivative value equals zero. The longest length of uninterrupted alternans is chosen between discontinuity points. At last, TP is calculated as the longest length of alternans (in beats), divided by the total length of the recorded signal (in beats).

It is important to understand that measurement noise can also display alternation (‘large-small-large’ patterns). Therefore, to distinguish with sufficient confidence alternans signal from noise, it is essential to determine a threshold level (minimum TP). For practical purposes, the regions with TP smaller than the selected threshold are considered noise, while the signals with TP larger than the threshold can be regarded as alternans.

In addition to TP, prior to conversion of the signal to binary, the magnitude of the alternans was quantified using the alternans ratio (AR), defined as $AR = |A_e - A_o| / \max(A_e, A_o) * 100\%$, where A_o and A_e are the amplitudes of odd and even calcium transients, respectively. For every pixel, the AR is the average AR in the maximum continuous segment (used to calculate TP).

Dealing with spatially-resolved measurements of alternans prompted quantification of relative phase over space, i.e. spatial concordance/discordance was quantified. SDA regions can be easily recognized as two alternating regions with opposite phase. However, the determination of phase is not trivial since it can be easily altered by noise (disruption in the sequence). Therefore, it becomes critical to determine a phase which can be representative and consistent between different spatial locations. Based on the TP concept, we use representative phase (RP) derived in time-overlapping segments for different spatial locations to help identify SDA regions; RP assumes values of -1 or +1.

As the TP in our algorithm is always set to be bigger than 50% (usually is 60%), even in the worst situation of TPs exactly equal to 60%, for any two points qualified the TP criteria,

there will be always at least 20% temporal overlap in the middle. As a result, the phase determined in this overlapping time interval of is the RP and can be used for characterization of SDAs.

B. Theoretical and empirical validation

In order to examine the possibility for false identification due to random noise-related and/or short-lived alternations, we applied the detection algorithm to simulated data and compared the results with theoretical prediction.

From combinatorics, one can derive a simple formula for the probability of a given binary data set with length n to exhibit uninterrupted alternating pattern with length m (minimum length 2). Intuitively, the TP will be equal to the ratio of m and n .

$$P(m, n) = \frac{(n - m + 3) * 2^{(n-m-1)}}{2^n} \quad (n > m \geq 2)$$

Empirical testing of the algorithm was performed using uniformly distributed pseudo-random numbers, generated in Matlab. To be comparable to the experiments (considering total number of observed pixels), 40 000 random data sets were created as noise samples, with a length ranging from 4 to 40 beats. After processing the noise sample data with our alternans identification algorithm, we obtained results perfectly matching those produced by the theoretical approach. The empirical and theoretical curves are plotted in the following figure.

The solid and dashed lines are the theoretical probabilities calculated from the combinatory formula above, which fluctuate because of the discrete parameters and round-off error. The symbols represent empirical results, computed from white noise data sets generated in Matlab. With two TP values of 60% and 80%, the data from the combinatorics calculations and Matlab noise test match perfectly. Additionally, from both the theoretical and empirical data, is seen that the length threshold for $p < 0.05$ is around $n = 13$ (if TP threshold is set at 60%). This means that, if we discover a sequence with $TP \geq 60\%$ in a signal with $n \geq 13$ beats, this sequence must be true alternans, not noise, with 95% confidence.

C. Signal-to-noise ratio

In order to investigate the sensitivity and general performance of the detection algorithm in different conditions, white noise was added to 3 perfect alternans sequences ($TP = 100\%$, $n = 30$) of different alternans ratio $AR = 10\%$, 20% and 50% . As noise level increases and SNR decreases (right-to-left on the x-axis), the detected TP starts dropping following a sigmoid curve. If the alternans detection threshold is set at $TP \geq 60\%$, all signals with SNR below the dark horizontal line will be misclassified (false negative). As expected, signals with very subtle alternans (low AR) are more sensitive to noise and will produce more false negatives.

In our data set, the average SNR was approximately 50, which guarantees the sensitivity of our algorithm for fine scale alternans detection, including such with AR of only 10%.

IV. Results

Figure 4 shows the application of the algorithm described above to actual optical mapping data, obtained in thin long strips of cultured myocytes, paced on one side at progressively higher frequency (steps of 0.3Hz, 30sec per frequency, starting from 1Hz). Panel A depicts

the experimental setup. Cardiomyocytes were cultured on PDMS strips (18×5mm) and during experiments samples were paced with a Pt line electrode on one side. A pre-processed Ca^{2+} fluorescence intensity signal from a single pixel is shown on the right.

Figure 4 (panel B) tracks the evolution of alternans patterns over different pacing frequencies. Color shows the magnitude of alternans at different spatial locations, quantified by $\text{RP} \cdot \text{AR} \%$. Light green areas, whose value is zero, are regions with no alternans ($\text{AR} \approx 0$) or noise regions with $\text{TP} < 60\%$. Red and blue identify opposite RP. Images on the left display all points with confirmed alternans. The images from the right are computed from an intuitive method, which is the sum of ARs from 14 continuous beats (located in the middle of temporal length). As predicted, the results from intuitive method match well with those from our algorithm, when the AR is large enough to override noise at higher pacing frequency. But, there are a lot mismatches at lower pacing frequency, as the AR is relatively small.

Figure 4 (panel C) shows the original intensity traces and derivatives of peak height in calcium for two selected spatial points A and B over different pacing frequencies. Blue and red in the left column represent the original data for points A and B. Blue and red in the middle and right column refer to derivative values during odd and even beats. For a perfectly alternating segment, all odd and even beats should be evenly distributed above and under the zero line, just like the derivatives for points A and B at 3.12Hz ($\text{TP} = 100\%$ for both locations).

Some limitations of the intuitive method can be clearly uncovered from Figure 4. For instance, point A would have been classified as alternans at 2.45Hz if only 14 beats are considered. However, our method reveals that only the central 14 beats show continuous alternating pattern, which couldn't satisfy 95% confidence when $\text{TP} = 60\%$ according to Fig. 2.

V. Conclusion

A novel algorithm for automatic alternans detection is proposed here, which can efficiently handle macroscopic data with ultra-high spatiotemporal resolution. For validation, this algorithm was applied to simulated white noise data and the results matched perfectly with the combinatorics-derived theoretical outcome, confirming the specificity of the algorithm. Also the performance of this algorithm was examined under different signal quality (SNR), and its sensitivity was confirmed for our optical mapping system. Finally, macroscopic spatiotemporal data from paced neonatal rat cardiomyocyte monolayers were analyzed by the algorithm to uncover the evolution of early stage fine-scale alternans under progressively faster pacing.

Acknowledgments

The work presented in this paper was supported by a grant from the National Science Foundation (BES-0503336). The authors would like to acknowledge Chiung-Yin Chung and Sunita Chugh for cell culture preparation.

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Fig. 1.
Sequence of processing steps for identification and characterization of alternans over time.

Fig. 2.

Algorithm validation based on TP – calculation of false positive results as function of the total number of observed beats at two thresholds of detection (TP of 60% and 80%). The horizontal black line crosses the probability curves at the required temporal signal length for 95% confidence.

Fig. 3.
Sensitivity of alternans detection to SNR

Fig. 4.
Alternans development with increasing pacing frequencies