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Real-Time Closed Loop Diastolic Interval Control Prevents Cardiac Alternans in Isolated Whole Rabbit Hearts

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Abstract

Cardiac alternans, a beat-to-beat alternation in action potential duration (APD), can lead to fatal arrhythmias. During periodic pacing, changes in diastolic interval (DI) depend on subsequent changes in APD, thus enhancing cardiac instabilities through a ‘feedback’ mechanism. Recently, an anti-arrhythmic *Constant DI* pacing protocol was proposed and shown to be effective in suppressing alternans in 0D and 1D *in-silico* studies. However, experimental validation of *Constant DI* in the heart has been unsuccessful due to the spatio-temporal complexity of 2D cardiac tissue and the technical challenges in its real-time implementation. Here, we developed a novel closed loop system to detect T-waves from real-time ECG data, enabling successful implementation of *Constant DI*, and performed high-resolution optical mapping experiments on isolated whole rabbit hearts to validate its anti-arrhythmic effects. The results were compared with: (1) *Periodic* pacing (feedback inherent) and (2) pacing with heart rate variability (*HRV*) (feedback modulation) introduced by using either *Gaussian* or *Physiological* patterns. We observed that *Constant DI* significantly suppressed alternans in the heart, while maintaining APD spatial dispersion and flattening the slope of the APD restitution curve, compared to traditional *Periodic* pacing. In addition, introduction of *HRV* in pacing failed to prevent cardiac alternans, and was arrhythmogenic.

Keywords

optical mapping; arrhythmias; pacing; beat-to-beat; HRV; periodic

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

INTRODUCTION

Fatal arrhythmias and ventricular fibrillation (VF) which lead to sudden cardiac death are often preceded by alternans, a beat-to-beat alternation in the cellular action potential duration (APD) [5, 7, 12, 23]. APD alternans often manifest as T-wave alternans, an alternation in the shape or duration of the T-wave, in an electrocardiogram (ECG). When the heart is paced at progressively increasing rates, electrical restitution, which is a fundamental property of cardiac myocytes, causes the heart to transition from a constant APD response at lower frequencies to an alternating long-short APD pattern (alternans) at higher frequencies [6, 18]. Although important for life at moderate heart rates, at higher rates, restitution may result in life-threatening cardiac rhythms such as alternans. Multiple mechanisms have been suggested to be responsible for the onset of cardiac alternans, from restitution dependent electrical instabilities to intracellular calcium cycling abnormalities [31, 32]. It is hypothesized that alternans is a precursor of cardiac electrical instability and previous studies have established a plausible link between alternans and ventricular arrhythmias, suggesting that elimination of alternans could lead to the prevention of VF and eventual arrhythmias in the heart [7, 12, 17, 21].

Over the past two decades, several groups have attempted the control of alternans through *in-silico*, *in-vitro* and *ex-vivo* experiments [2–4, 8–11, 15, 16, 25–30]. Although early studies reported positive results in single cell models and *in-vitro* preparations [8, 9], control of alternans in more spatiotemporally complex two-dimensional (2D) settings has proved unsuccessful [4, 24]. Many groups since have highlighted the significant challenges in the spatiotemporal control of cardiac alternans, revealing that control failed above a critical cable length [4, 27] and became attenuated spatially as alternans amplitude increased [3, 10, 24]. However, a major flaw in the control algorithms was the use of periodic stimulation, wherein the duration between consecutive stimuli (basic cycle length (BCL)) is constant from beat-to-beat, i.e.

$$APD_n + DI_n = BCL_n = BCL = \text{constant} \quad (1)$$

where n is the stimulus number and DI_n denotes the n^{th} diastolic interval (the cardiac relaxation phase). During periodic pacing (Eq.1), there is a partial dependence of the DI on the preceding APD ($DI_n = BCL - APD_n$), i.e. *feedback*, that can lead to subsequent destabilization of normal cardiac rhythms [15, 25]. We recently reported the drawbacks of periodic pacing using single cell and 1D cable numerical simulations [15, 25], in comparison to the beneficial anti-arrhythmic effects of feedback elimination by keeping the DI constant, i.e.

$$DI_n = DI = \text{constant} \quad (2)$$

Indeed, by controlling and maintaining a *Constant DI* on a beat-by-beat basis, we eliminated feedback and the inherent dependence of the DI on the preceding APD, which was successful in suppressing alternans [15, 25]. More recently though, contradictory findings

demonstrating the presence of alternans *in-silico* during *Constant DI* pacing was reported by some groups [29, 30], particularly under abnormal calcium cycling conditions. In addition, closed loop experimental validation of beat-to-beat control of alternans has exhibited limited success, especially in 2D cardiac tissue preparations [11, 24]. Experimental implementation of *Constant DI* pacing is challenging and previous attempts involving the control of DI were limited to point sensing using microelectrodes which failed to address the prevention of alternans in the heart [24]. In order to precisely control the DI in the whole heart, on a beat-to-beat basis, there is a need to develop a sophisticated control algorithm that can sense an action potential in real-time based on comprehensive cardiac electrical information, and apply a stimulus after a predefined time interval at the end of the APD.

The main goal of the current study was to develop a real-time beat-to-beat control system to successfully implement *Constant DI* pacing and demonstrate its efficacy in suppressing alternans in the whole heart. We successfully addressed the technical limitations mentioned previously and implemented a novel method to validate the anti-arrhythmic benefits of feedback elimination using *Constant DI*, in *ex-vivo* isolated whole rabbit hearts. Specifically, we implemented a closed loop algorithm to detect T-waves from real-time ECG data on a beat-by-beat basis, measure real-time APD and apply stimuli after a predefined *Constant DI*. To our knowledge, this is the first study to use whole heart 2D optical mapping for high resolution spatio-temporal assessment of feedback between DI and APD. In addition, we compared the electrophysiological and anti-arrhythmic effects of *Constant DI* pacing with different pacing protocols. Namely, the following protocols were used: (1) *Periodic* (feedback inherent), (2) *HRV* pacing (feedback modulation), introduced by using both (2.1) *Gaussian* and (2.2) *Physiological* variability patterns, and (3) *Constant DI* (feedback eliminated).

METHODS

1. Optical Mapping

All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of Minnesota. Optical mapping procedures were performed as previously described [13, 22]. Briefly, New Zealand White rabbits (Bakkom Rabbitry, 1.3 – 2.0 kg, $n=8$) were injected with heparin sulfate (550 U/100 g) and anesthetized with ketamine and xylazine (35 mg/kg and 5 mg/kg, resp.). The heart was quickly excised after a thoracotomy and immersed in cold cardioplegic solution (in mM: glucose 280, KCl 13.44, NaHCO₃ 12.6, and mannitol 34). The aorta was quickly cannulated and retrogradely perfused with warm ($37 \pm 1^\circ \text{C}$) oxygenated Tyrode's solution (in mM: NaCl 130, CaCl₂ 1.8, KCl 4, MgCl₂ 1.0, NaH₂PO₄ 1.2, NaHCO₃ 24, glucose 5.5, and pH 7.4) under constant pressure. The heart was immersed in a chamber and superfused with the same Tyrode's solution. Motion uncoupler Blebbistatin (10 μM) was added to Tyrode's solution when needed to reduce motion artifacts.

A bolus of 4 mL of the voltage-sensitive dye di-4-ANEPPS (10 μM) was injected and excited with a diode pumped, continuous-excitation green laser (532 nm, 1 W; Shanghai Dream Lasers Technology, Shanghai, China). Optical movies corresponding to the fluorescence signal were recorded from the epicardial surfaces of the left or right ventricle

(LV and RV) at 1000 frames per second, with 14-bit, 80×80 -pixel resolution cameras (Little Joe, RedShirt Imaging, SciMeasure) after a period of stabilization of approximately 30 minutes. Simultaneously, continuous 3-electrode volume conducted ECG was recorded throughout the experiments.

2. Real-time Closed Loop Control Algorithm

Figure 1A shows a schematic of the setup interfacing the real-time ECG-based *Constant DI* control algorithm with high-resolution optical mapping. We utilized RT intervals on the ECG to approximate an APD, while the TR interval was maintained constant to implement *Constant DI*. However, as the correspondence between RT, TR intervals and APD, DIs respectively has been well established [15], fundamentally our method implements feedback elimination by controlling the repolarization interval (for details, see Supplementary materials). Arrows in black indicate the original optical mapping set up that was used for the *Periodic* and *HRV* pacing implementation. Arrows in red indicate the closed loop system that enables real-time T-wave detection from ECG and implementation of *Constant DI* pacing. *Constant DI* pacing was implemented using a proprietary algorithm developed in LabVIEW that detected the ECG T-wave peak and applied a pacing stimulus after a predefined fixed duration equivalent to the required DI.

3. Pacing Protocols

The following pacing protocols were implemented:

(1) *Periodic* pacing protocol—Since *Periodic* pacing (Eq.1) forces the BCL to be constant during each beat, it creates an inherent dependency of the DI on the preceding APD, i.e. feedback. Hence, in this study, we refer to *Periodic* pacing protocol as the ‘*feedback inherent*’ protocol.

(2) *HRV* pacing protocol—Under normal physiological conditions, there exists intrinsic HRV, which ensures deviation of the heart from periodic pacing [14, 20]. To simulate known healthy human HRV and to in turn modulate the feedback between DI and APD, we incorporated stochasticity in pacing by varying the BCLs. Two different *HRV* pacing protocols were used to generate ‘*feedback modulation*’:

(2.1) *Gaussian* pacing: Here, we incorporated HRV in pacing by using a strictly Gaussian distribution of beat-to-beat intervals. *Gaussian* pacing was implemented as follows:

$$BCL_n = BCL_0 \pm \delta(HRV) \quad (3)$$

where $\delta(HRV)$ is a random number with a mean of zero and standard deviation of $BCL_0 * HRV$, and,

$$HRV = \frac{STD_{RR}}{AVG_{RR}} * 100\% \quad (4)$$

where STD_{RR} and AVG_{RR} denote the standard deviation and average of the RR intervals i.e. the distance between the RR peaks in the ECG. To mimic known human HRV values [15, 19] we used a Gaussian distribution with HRV set at 5%.

(2.2) Physiological pacing: Here, to mimic a physiological HRV pattern, we utilized the variation in RR intervals from a previously acquired anesthetized *in-vivo* rat ECG recording. The original HRV of 1.12% was scaled up to 5% to simulate a quasi-physiologically relevant human HRV value, and the scaled RR ‘pattern’ was superimposed onto BCL values to implement *Physiological* pacing for each BCL from 200 ms down to 120 ms in steps of 10 ms.

(3) Constant DI pacing protocol—To evaluate the proposed anti-arrhythmic effects of ‘*feedback elimination*’, we implemented *Constant DI* pacing as shown in Eq.2. The details of the algorithm implementation are presented in Supplementary Materials.

External stimuli at twice the activation threshold, were applied at the base of the LV for each rabbit, during each pacing protocol. One hundred stimuli were applied at each BCL for *Periodic* and *HRV* pacing, and the BCL was progressively decreased from 200 ms down to 120 ms in steps of 10 ms. Similarly, during *Constant DI*, 100 stimuli were applied at each DI and the DI was progressively decreased from 100 ms down to 30 ms in steps of 10 ms. It should be noted that since we aimed to investigate the propensity of the pacing techniques to the onset of alternans rather than VF, we did not perform pacing below $BCL = 120$ ms to preclude the heart to go to VF.

A total of $n=40$ pacing runs were analyzed for both LV and RV for the *Periodic*, *Gaussian*, *Physiological* and *Constant DI* pacing protocols respectively (10 runs of each protocol). Runs of each pacing protocol were acquired in each rabbit in a random manner, to eliminate any effect of pacing order on the results. Optical movies corresponding to the last ten stimuli were recorded for each BCL (DI) for all pacing protocols.

4. Data Analysis

4.1 APD Alternans—Optical movies were recorded from the epicardial surfaces of both the LV and RV. Mean APD at 80% repolarization ($\langle APD_{80} \rangle$) was calculated for RV and LV for the even (APD_{EVEN}) and odd (APD_{ODD}) beats separately. 2D mean APD_{EVEN} and APD_{ODD} maps were generated for each BCL and DI. Temporal threshold for alternans was set at $|APD_{EVEN} - APD_{ODD}| = 4$ ms and 2D alternans maps were generated as a difference of mean APD_{EVEN} and APD_{ODD} maps. For spatial onset of alternans, the BCL (or DI) at which at least 20% of the area of the RV or LV was occupied by alternans was defined as the BCL_{onset} (or DI_{onset}). The presence of spatial alternans was then quantified for all the pacing runs.

4.2 APD Heterogeneity—To assess the dispersion of APD_{80} repolarization across the LV and RV surfaces, we calculated APD_{80} heterogeneity index μ as follows:

$$\mu = (APD^{95} - APD^5) / APD^{50} \quad (5)$$

wherein, APD^{95} and APD^5 represent the 95th and 5th percentiles of the APD_{80} distribution, respectively, and APD^{50} is the median of the distribution. For each BCL and DI, heterogeneity μ was calculated based on 2D APD maps generated for an average APD_{80} of 3 – 5 beats.

4.3 Comparison between BCL and DI—In order to compare data between the different pacing protocols, the corresponding DI for each BCL was calculated for each *Periodic* and *HRV* pacing run as follows:

$$DI = BCL - \langle APD_{80} \rangle \quad (6)$$

wherein, $\langle APD_{80} \rangle = \langle (APD_{EVEN}, APD_{ODD}) \rangle$. Finally, mean DI values corresponding to each BCL were calculated for the *Periodic* and *HRV* pacing protocols by combining DI values for individual runs.

4.4 Slopes of APD Restitution Curve (S_{max})—To evaluate the effect of the different pacing protocols on the slope of the APD restitution curve, and in turn the propensity to arrhythmias, we calculated the minimum DI (DI_{min}) for every pacing run (DI corresponding to the last BCL for each run). APD restitution curves were generated as a function of change in $\langle APD_{80} \rangle$ with DI and fit with a 2nd degree polynomial. The maximum slope of restitution, S_{max} , was calculated for each run by evaluating the derivative of the polynomial fit at DI_{min} . Finally the individual mean S_{max} values, corresponding to change in 2D $\langle APD_{80} \rangle$ with DI, for each pacing run were combined to generate average S_{max} values for each pacing protocol.

5. Statistics

All data are presented as mean $\pm$ standard error. Statistical comparisons between the pacing protocols were performed using 1-way ANOVA (Origin Software, Northampton, MA, USA). Fisher's exact test was used to perform statistical comparison of the number of runs that exhibited arrhythmias across the different pacing protocols. Values of < 0.05 were considered statistically significant.

RESULTS

1. Real-time T-wave measurements

We first validated accurate T-wave detection by the real-time algorithm and execution of *Constant DI* pacing. The time instances of each T-wave peak detected and pacing stimulus applied was generated by the algorithm for each run. Figure 1B shows representative real-time ECG data collected during a *Constant DI* run. Two checks were performed to demonstrate the successful implementation of *Constant DI* pacing. First, the difference between expected and actual DI, i.e. algorithm error, was calculated and was less than 8 ms across all experimental runs. Figure 1C displays the algorithm error quantified for all beats at each DI, for one representative *Constant DI* run. Second, for each DI, the ECG data acquired was also analyzed post experiment and the T-wave peak detected by the algorithm was compared to the visible T-wave peak on the ECG to ensure accurate detection (Fig. 1B).

Any run that incorrectly detected a T-wave or missed the expected DI by more than 8 ms due to technical limitations of the current study, was excluded from further analysis. Details of the runs excluded are provided in the Supplementary Materials.

2. Presence and onset of alternans

We quantified the number of pacing runs that exhibited alternans in the heart for each pacing protocol and the corresponding BCL_{onset} , for both LV and RV. As seen in Fig.2A, *Constant DI* pacing significantly reduced the instances of cardiac alternans in the heart (only 1 run demonstrated the presence of alternans for $DI = 40ms$ in the RV), compared to the other three pacing protocols. *Periodic* and *HRV* pacing always induced alternans and also had instances of VF. Furthermore, comparing BCL_{onset} between the *Periodic* and *HRV* pacing techniques (Fig.2B), we observed that the onset of alternans in both the LV and RV is promoted with an increase in stochasticity in *Physiological* pacing showed the largest BCL_{onset} . In case of the RV, *Periodic* pacing had significantly smaller BCL_{onset} in comparison to both *Gaussian* and *Physiological* pacing.

3. Spatio-temporal evolution of alternans

Next, we quantified the spatial evolution of alternans for the different pacing protocols as a percentage of RV or LV, for decreasing values of DI. Since *Physiological* pacing significantly preponed BCL_{onset} compared to both *Gaussian* and *Periodic* pacing, we only showed data comparing *Periodic*, *Gaussian* and *Constant DI* from here on. Figure 3 shows representative 2D APD_{EVEN} and APD_{ODD} maps along with 2D alternans maps for decreasing BCLs and DIs, for *Periodic*, *Gaussian* and *Constant DI* pacing. For both *Periodic* and *Gaussian* pacing, we observed an increase in the area of the heart occupied by alternans as we decreased the BCL (shown in terms of calculated corresponding DIs), as demonstrated in Fig.4. Note that *Constant DI* pacing prevented the spatio-temporal evolution of alternans in both LV and RV.

4. APD Heterogeneity μ

Figure 5 demonstrates the change in APD heterogeneity μ with a decrease in DIs for the three pacing protocols, along with representative 2D APD maps. There were no significant differences in μ between the three pacing protocols, although we observed a slightly higher μ in the RV during *Gaussian* pacing, in comparison to *Periodic* and *Constant DI* pacing. Representative 2D APD maps for all three pacing protocols are shown in Fig.5 for a DI of $\sim 50ms$.

5. APD Restitution Curve

Figure 6 shows APD_{80} as a function of different DIs for *Periodic*, *Gaussian* and *Constant DI* protocols. *Constant DI* pacing significantly lowered S_{max} in both the LV and RV in comparison to other pacing protocols. It is important to note that both *Periodic* and *Gaussian* pacing had $S_{max} > 1$ for both LV and RV, while $S_{max} < 1$ during *Constant DI* pacing, demonstrating its anti-arrhythmic effect. In addition, the calculated DI_{min} for the *Gaussian* pacing was significantly larger compared to both *Periodic* and *Constant DI* pacing for the LV (Fig.6 bottom panel).

DISCUSSION

Here, we extended our previous *in-silico* findings of the anti-arrhythmic benefits of *Constant DI* pacing [15, 25] to the whole heart level and implemented a novel real-time closed loop system for beat-by-beat cardiac control. Using our system, we investigated the effect of feedback on the propensity to cardiac alternans, using three different pacing protocols: *Periodic* (feedback inherent), *HRV* (feedback modulation) and *Constant DI* (feedback elimination). Our major findings are as follows: First, we successfully implemented an algorithm for real-time detection of T-waves and validated its efficacy in controlling the DI on a beat-to-beat basis in the heart. Second, *Constant DI* pacing prevented the occurrence of alternans in the heart, thus providing a clear anti-arrhythmic effect. *Constant DI* pacing was associated with an increase in APD and decrease in S_{max} , while not affecting spatial heterogeneity of APD, μ , in comparison to *Periodic* pacing. On the other hand, feedback modulation using *HRV* pacing, which was modeled with either *Gaussian* or *in-vivo Physiological* patterns, promoted the formation of alternans and VF in the heart, thus enhancing arrhythmogenicity. *HRV* pacing was associated with both increased S_{max} and μ , and earlier occurrence of alternans (larger BCL_{onset}), in comparison to *Periodic* pacing, thus providing clear pro-arrhythmic effects.

Our results support our hypothesis that the presence of feedback, or dependence of the DI on the preceding APD, can lead to electrical instability and cardiac arrhythmias, especially at higher pacing rates. This was demonstrated by the onset of spatio-temporal cardiac alternans during all *Periodic* pacing runs. Feedback modulation or *HRV* further perturbed the stability of the system and promoted arrhythmogenesis. To incorporate *HRV* in pacing, we used two methods. *Gaussian* distribution with 5% HRV was used to introduce perturbations in BCL, based on previously shown human HRV values and distributions [15, 19]. Furthermore, to investigate a truly 'random' yet physiological perturbation, we also implemented *Physiological* pacing by utilizing RR interval patterns from previously recorded *in-vivo* rat ECG traces. However, our results demonstrated an increase in arrhythmogenicity during *HRV* pacing, with *Physiological* pacing showing earlier onset of alternans than *Gaussian* pacing. This can be attributed to the fact that incorporating HRV in pacing only modulates feedback and does not eliminate the dependence of the DI on prior perturbations. *Constant DI* pacing however, ensures that the DI during each beat is independent of any prior instabilities or perturbations in the APD and hence, is more electrically stable.

HRV pacing also showed an increase in APD heterogeneity compared to both *Periodic* and *Constant DI* pacing. Since the beat-to-beat intervals are varied randomly when incorporating HRV in pacing, it is possible that APD repolarization is heterogeneously affected. While certain regions might fully repolarize, other regions might be forced to reactivate before complete repolarization, which eventually could lead to heterogeneity in APD distribution across the surface of the heart. This could lead to abnormal activations such as early after depolarizations and delayed after depolarizations, which are known to be arrhythmogenic. This is supported by our data wherein we observed earlier onset of alternans, instances of VF and an earlier loss of pacing response (conduction block) during *HRV* pacing, demonstrated by a significantly larger DI_{min} in case of the LV.

Constant DI pacing also demonstrated a flattening in the slope of restitution. Numerous studies have suggested the anti-arrhythmic effects of flattening the restitution slope [1, 8, 21]. Expectedly, our data showed significantly steeper restitution curves during *Periodic* and *HRV* pacing, wherein we see an onset of alternans. Since *Constant DI* pacing aims to maintain a fixed DI during each heartbeat, we saw an almost linear decrease in APD as we shortened our target DI, again demonstrating the beneficial effects of feedback elimination. Overall, our data shows promising anti-arrhythmic effects of feedback elimination and beat-to-beat DI control. Furthermore, all of our findings were consistent between the LV and RV and we did not observe any significant differences between ventricles in terms of the anti-arrhythmic benefits of *Constant DI* pacing.

LIMITATIONS

There are some limitations of the current study. First, we utilized volume conducted 3-electrode ECG that was easy to integrate with an existing 2D *ex-vivo* optical mapping system, to implement *Constant DI* pacing. Hence, our study is limited by the temporal resolution achieved by volume conducted ECG and observing a distinct T-wave. Given the rabbit model and *ex-vivo* setup, at very high pacing rates, the T-wave cannot be discerned, making it difficult to precisely detect the DI. However, feedback elimination can still be achieved by detecting a T-wave and incorporating a time offset in the target DI. Hence, the minimum DI successfully implemented was 30 ms for our study. Second, we used healthy rabbit hearts for our study and hence, the size of the hearts limits the spatial validation of the pacing techniques. Further pre-clinical validation would be required to explore the benefits of *Constant DI* pacing in a large animal model, as well as under diseased conditions. While similar ECG based approaches can be used for sensing T-waves and applying constant TR intervals in larger animals, the size of the heart could limit the spatial control achievable from single point pacing. Hence, there may be a need to utilize multi point intracardiac recordings for sensing instead of average ECG (which could mask local activity) for better spatio-temporal control in larger hearts. However, since rabbits are known to have electrical and ionic characteristics similar to humans, we do not expect a change in model to significantly alter the concepts established in this study.

In addition, to implement *Physiological* pacing, we utilized *in-vivo* rat ECG rather than pre-recorded rabbit data. While species related differences in the RR interval patterns may be present, these differences may not be very relevant as the main focus of the current study was to investigate the effects of feedback modulation vs feedback elimination. Our data demonstrates that variability, irrespective of the model used to implement it (Gaussian or Physiological) seems to be pro-arrhythmic. Finally, while our study demonstrates the efficacy of *Constant DI* in preventing restitution dependent alternans by eliminating feedback, its effect on alternans driven by calcium cycling abnormalities remains unclear, and needs to be experimentally investigated in future studies.

CONCLUSIONS

We developed a novel real-time closed loop system to successfully validate the efficacy of *Constant DI* pacing in suppressing alternans using isolated whole hearts, hence

demonstrating its potential in the prevention and control of cardiac arrhythmias. While the presence of feedback between the DI and APD, as demonstrated by use of *Periodic* pacing, induced cardiac alternans at higher pacing rates, incorporating *HRV* in pacing further enhanced the electrical instabilities and promoted the onset of alternans. In addition, irrespective of the model used to incorporate *HRV* in pacing i.e. *Gaussian* or *Physiological* pattern, incorporating stochasticity in pacing showed pro-arrhythmic effects. Hence, our data suggests that feedback elimination using *Constant DI* might be essential in the prevention of restitution dependent cardiac alternans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Schematic of our optical mapping system for real-time closed loop control of alternans. Red arrows highlight the real-time ECG T-wave detection and *Constant DI* algorithm implementation. Black arrows indicate 2D optical imaging and implementation of *Periodic* and *HRV* pacing. (B) Validation of *Constant DI* pacing using real-time ECG recording for representative target DI=40 ms. The interval between dashed vertical lines represent achieved DI values. Green marks represent implemented *Constant DI* pacing. (C) Error, calculated as difference between achieved and target DI, is shown for the entire DI range, for one representative pacing run.

Figure 2.

(A) Quantification of the occurrence of alternans in the heart for different pacing protocols.

(B) Comparison of BCL_{onset} between *Periodic*, *Gaussian* and *Physiological* pacing. *

denotes statistical significance of $p < 0.05$

Figure 3.

Representative 2D APD maps for APD_{EVEN} (B1), APD_{ODD} (B2) and Δ APD (alternans), for (A) *Periodic*, (B) *Gaussian* and (C) *Constant DI* pacing, from the LV. Action potential traces depicting alternans ($\star$) at lower BCL and no alternans ($\blacklozenge$) at higher BCL are shown for *Periodic* and *Gaussian* pacing. Note that no alternans were observed for *Constant DI* pacing and representative action potential traces at two distinct DIs are denoted by $\star$ and $\blacklozenge$.

Figure 4. Spatial evolution of alternans across the (A) LV and (B) RV surfaces of the heart as DI decreases, for *Periodic* (blue), *Gaussian* (red) and *Constant DI* (green) pacing protocols. Dotted black line indicates the threshold used for spatial onset of alternans i.e. BCL_{onset}

Figure 5.

Change in APD₈₀ heterogeneity μ with decreasing DIs for *Periodic* (blue), *Gaussian* (red) and *Constant DI* (green) pacing, for both (A) LV and (B) RV. Representative 2D APD maps are shown alongside for each pacing protocol, demonstrating the distribution of APD₈₀ across the ventricles for a calculated DI of ~ 50ms.

Figure 6.

Top: Restitution curves demonstrating change in mean APD₈₀ with decreasing DIs for *Periodic* (blue), *Gaussian* (red) and *Constant DI* (green) pacing. *Mid:* Maximum restitution slopes S_{max} calculated at DI_{min} . *Bottom:* Mean DI_{min} across all runs. Data shown for (A) LV (left panel) and (B) RV (right panel). * denotes statistical significance of $p < 0.05$