Alternans by non-monotonic conduction velocity restitution, bistability and memory

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Abstract. Conduction velocity (CV) restitution is a key property that characterizes any medium supporting traveling waves. It reflects not only the dynamics of the individual constituents but also the coupling mechanism that mediates their interaction. Recent studies have suggested that cardiac tissues, which have a non-monotonic CV-restitution property, can support alternans, a period-2 oscillatory response of periodically paced cardiac tissue. This study finds that single-hump, non-monotonic, CV-restitution curves are a common feature of in vitro cultures of rat cardiac cells. We also find that the Fenton–Karma model, one of the well-established mathematical models of cardiac tissue, supports a very similar non-monotonic CV restitution in a physiologically relevant parameter regime. Surprisingly, the mathematical model as well as the cell cultures support bistability and show cardiac memory that tends to work against the generation of an alternans. Bistability was realized by adopting two different stimulation protocols, ‘S1S2’, which produces a period-1 wave train, and ‘alternans-pacing’, which favors a concordant alternans. Thus, we conclude that the single-hump non-monotonicity in the CV-restitution curve is not sufficient to guarantee a cardiac alternans, since cardiac memory interferes and the way the system is paced matters.

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1. Introduction

It is important to understand the instability of the heart, since it is related to various cardiac arrhythmogenesis. From the viewpoint of dynamic systems, cardiac instabilities can originate from either individual cardiac cells or the population dynamics of a coupled system of cells. The most well-known example of cardiac instability is the period-doubling bifurcation leading to alternans, which is often perceived as a precursor to cardiac fibrillation. At the level of a single cell, an alternans refers to a period-2 response of a periodically paced cardiac myocyte. For excitable cells, such as the cardiac myocytes, the characteristics of action potentials vary depending on the rate of the external pacing. When the pacing frequency is increased gradually, a period-doubling bifurcation can result in a sequential alternation in both action potential duration (APD) and diastolic interval (DI). The pacing-rate-dependent functional relation of APD to DI is termed as ‘APD-restitution’.

The period-doubling bifurcation based on APD restitution was discussed quite some time ago by Nolasco and Dahlen [19] and later recapitulated by Courtemanche et al [4]. Under the condition that the period of external pacing $\tau = \text{APD} + \text{DI}$, the induced beating sequence undergoes a period-doubling bifurcation as the slope of the APD restitution map $S = \frac{\text{dAPD}}{\text{dDI}}$ increases beyond 1. Subsequently, numerous experimental studies have examined the applicability of the APD-restitution hypothesis to various cardiac systems, but the results were very often not consistent with the hypothesis [8, 11, 13, 22]. For example, in some cases, alternans occurred even when $S < 1$ [19] or, conversely, it was absent even when $S > 1$. Furthermore, some alternans, now termed as discordant alternans (DA) [3], accompanied spatial defect structures named as ‘nodal lines’. These findings all together suggested that a more realistic explanation for the alternans must incorporate the spatial degree of freedom of the coupled system of myocytes.
The most essential property characterizing the medium supporting traveling waves is conduction velocity (CV) restitution—the dependence of the CV of a wave pulse on the time interval to its preceding pulse (i.e. inter-beat interval (IBI)). The shape of CV restitution reflects not only the intrinsic properties of the local elements (i.e. individual cells) but also the way they are coupled together. In this sense, CV restitution is more inclusive than APD restitution. Incidentally, several recent studies have examined the different effects of CV restitution on cardiac alternans [2, 11, 18, 26]. For example, Watanabe et al [26] examined the interaction between APD and CV and its effect on the generation of DA. Other recent studies discussed the importance of the steepness of CV restitution with respect to the creation and suppression of DA [2, 18].

More significantly, a series of recent studies indicated that cardiac tissues can support non-monotonic CV restitution, which is also known as ‘supernormal conduction’, and that it may be responsible for the generation of alternans [1, 15, 23, 24]. In figure 1, we recapitulate the proposed instability mechanism. It shows a simple non-monotonic CV-restitution function (figure 1(A))—a single-hump piecewise-linear function having three different regions with different slopes: zero, positive and negative. Then, for each region, we consider a small localized perturbation in an initially periodic wave train and follow its temporal evolution, as shown in figures 1(B)–(D). The periodic wave train can become unstable only when in a negative slope regime. For the case of figure 1(B) (slope = 0), the initial perturbation, displacing the position of the pulse in the middle, will remain the same, since the moving velocity of the pulse is constant, independent of the distance (or time) to the preceding pulse. For the case of figure 1(C), the perturbation gradually dies away, since the positive slope has a stabilizing effect. In the case
of figure 1(D), the negative slope confers a positive feedback on the initial perturbation, thus leading to an alternans.

In this paper, we investigate whether a non-monotonic CV-restitution property described in figure 1 can alone really be sufficient for the emergence of alternans. This question is addressed by analyzing very long (>40 cm), quasi-one-dimensional tissue strands of cardiac cells grown on Petri dishes using a propagation-induced phase-contrast imaging system, which we developed earlier for studying cardiac reentries. In our cultured tissue of rat cardiac cells, non-monotonic CV restitution was commonly observed. Unexpectedly, however, even in the supposedly unstable negative-slope CV-restitution regime, a simple periodic sequence of stimulating pulses did not result in an alternans, and this result was confirmed with more than ten different preparations.

After a series of many different experiments, we finally realized that a finite-amplitude periodic modulation such as the alternans-pacing protocol in the report of Kucera’s [15] was necessary for generating a stable alternans. This finding was also consistent with our simulation results using the Fenton–Karma model. The initial small fluctuation in the pacing period disappeared convectively even in the negative-slope regime, whereas small, yet finite-amplitude alternating perturbations amplified to yield a stable alternans. In other words, an alternans was not globally stable. Another important observation was ‘cardiac memory’. We found that even after a long period of pre-conditioning S1 stimulation of our S1S2 stimulation protocol, the shape of the CV-restitution curve could drift rather significantly during the S2 phase, usually toward a monotonic CV-restitution regime.

2. Materials and method

2.1. Quasi-one-dimensional strands of a cardiac cell culture

As for obtaining a cardiac CV-restitution curve, a one-dimensional system of cardiac tissue would be ideal, since it would exclude any two-dimensional issues such as curvature or lateral wave instability. Moreover, long strands of tissue would be favorable for investigating steady-state wave trains. The pacing period relevant for the alternans that we found in confluent cultures of rat ventricular cells was about 200 ms, and the CV was about 20 cm s\(^{-1}\), thereby the wavelength was about 4 cm. Thus, a wave train of 11 pulses would easily cover a distance of 40 cm. With these figures in mind, we developed an experimental technique for making very long quasi-one-dimensional tissue strands of rat ventricular cells in the form of a spiraling track (see figure 2). The spiral construct was very useful since we could use a conventional charge-coupled device camera to visualize the wave activity along the strands. We cast a spiraling polydimethylsiloxane (PDMS) block out of a master polycarbonate mold, in which a narrow spiral groove was machined. Then, the spiral PDMS block was transferred to a culture dish (area 20.8 cm\(^2\)) and its top surface was sealed with a sheet of silicone rubber. Then, the narrow spiraling groove became a long, narrow, spiraling tunnel as shown in figure 2(A).

Dissociated rat ventricular cells were harvested by the same procedure reported earlier [10], and the cells suspended in culture medium were injected into the spiraling tunnel using a syringe. Then, the whole preparation was stored in an incubator (5% CO\(_2\) and 95% air, 37 \(^{\circ}\)C) approximately for 1 day to allow the cells to settle down onto the bottom surface. Then, the top silicone rubber sheet was removed carefully, and the dish was filled with 5 ml of culture medium. The whole procedure resulted in a well-defined quasi-one-dimensional track of cardiac tissue.
Figure 2. Quasi-one-dimensional strand of cardiac cell culture in spiral form. (A) A schematic diagram (the green dot in the middle indicates the position of a pair of stimulating electrodes). The cells are cultured only on the bottom substrate (red) along the spiral track guided by a spiral PDMS block. The spiraling groove (cyan) has physical dimensions of $0.5 \times 2.0 \times 415$ mm$^3$. (B) A processed snapshot image showing several excitation wave pulses (white area) spiraling out from the stimulation site. (C) Immunostaining image of a small part of the cardiac strand, showing myosins (green) and nuclei (red, PI staining) in the cell culture. The dashed lines delineate the boundaries of a cell culture.

with a final cell density of $2.0(\pm 0.5) \times 10^3$ cells mm$^{-2}$ (figure 2(B)). The distribution of nuclei was quite uniform, but that of myosins varied significantly across the narrow channel (i.e. in the direction perpendicular to the wave conduction) (see figure 2(C)). A pair of tungsten electrodes was carefully placed near the tip of the spiraling track before the whole culture assembly was transferred to a custom-made observation chamber (5% CO$_2$ and 95% air, 37 $^\circ$C). Stimulation experiments were performed with 4–6-day-old cultures, and the culture medium was replaced once every two days.

2.2. Optical recording and image analysis

The mechanical contractive activity of the cultured cardiac strands was optically mapped with a phase-contrast imaging system, which we introduced earlier [10] (see figure 2(B)). The imaging technique was non-invasive and very useful for long-term imaging, since it did not require any fluorescent dyes, which not only suffer from photo-bleaching but also introduce photo-toxicity. Images were acquired continuously at 60 Hz by a Dalsa (Falcon 1.4M100) complementary metal-oxide-semiconductor (CMOS) camera with a 25 mm macro lens (Apollo). The camera
was connected to a frame grabber card (Helios eCL/XCL, Metrox) in the host computer (dual AMD Opteron 2.6 GHz and 4 GB RAM). The field of view was set to 48 mm × 48 mm, and it covered the entire spiraling track of cardiac tissue. The raw images had a spatial resolution of 900×900 pixels, but a 10×10 real-time binning was applied before they were saved. The details of the image acquisition and processing can be found in [9].

2.3. Electrical stimulation protocol

A pair of stimulating electrodes was placed just above the cardiac tissue near the center of the view field, barely touching the culture surface. We used the following S1S2 stimulation protocol (see supplementary figure S1, available from stacks.iop.org/NJP/15/013046/mmedia). Initially, a sequence of 20 biphasic pulses (±6.5 V amplitude, 5 ms duration for each phase) was delivered every 720 ms (S1 phase) to ‘pre-condition’ the sample tissue, and this was immediately followed by another sequence of five or more successive biphasic pulses (S2 phase). In order to obtain a CV-restitution curve, the pacing interval τ2 of the S2 phase was scanned, typically with 10 ms resolution for the range of 500–700 ms, with 5 ms resolution for the range of 500–400 ms and with 2 ms resolution for the range of 400–180 ms. In the case of alternans pacing, the pre-conditioning S1 phase was identical to that of the S1S2 stimulation protocol but for the S2 phase a small Δt = 5–10 ms was either added or subtracted to τ2 alternatively.

3. Results

3.1. Non-monotonic conduction velocity (CV) restitution supported by cultured rat cardiac tissues

The aforementioned long spiraling strands of cardiac cells were subjected to a sequence of S1S2 stimulations to obtain their CV restitution. Electrical pulses were delivered near the central tip of the spiraling track, as marked by the green dot in figure 2(B). The amplitude and duration of each electrical pulse were chosen such that they guaranteed one-to-one excitatory wave generation for the period range of interest. Figures 3(A)–(C) illustrate three distinct responses for three different values of τ2, respectively.

In figure 3(A), the five wave pulses generated by S2 stimuli propagate more or less with the same constant velocity, which is essentially identical to that of the wave pulses created during the preceding S1 phase. Note that only a small part of the S1 phase, just before the S2 phase, is depicted in the figure. Clearly, the slopes of the white lines in the space–time plot of figure 3(A) are almost identical to each other and not varying in time. The same stimulation protocol was repeated with a smaller value of τ2 (see figure 3(B)). The space–time plot of figure 3(B) is not significantly, yet delicately, different from that of figure 3(A). The S2 lines, especially the first, behave differently from those of the S1 phase, and this difference in behavior is evident from figure 3(D) (green dots): the first S2 pulse is moving slightly faster than those of the S1 waves, thereby reducing the distance between itself and the preceding S1 wave with time. When the same stimulation experiment was repeated again with an even smaller τ2, the S2 pulses moved slower than the S1 waves, so the distance between them and the preceding S1 wave increased with time, as shown in figures 3(C) and (D) (blue dots).

The three cases depicted in figures 3(A)–(C) represent, respectively, three different regimes in the CV-restitution curve of the cardiac culture system under study, as marked in figure 3(E).
Figure 3. Non-monotonic CV restitution supported by a strand of cultured cardiac cells. (A)–(C) The space–time plots of cardiac wave trains produced by S1–S2 stimulation protocols at the position marked by a red dot: (A) $\tau_2 = 520$ ms, (B) $\tau_2 = 374$ ms and (C) $\tau_2 = 224$ ms. (D) The IBI traces of the first S2 pulse as a function of distance for the three different cases. The IBI is barely changing for (A). On the other hand, it decreases (increases) when the slope of CV restitution is negative (B) (positive (C)). (E) A single-hump non-monotonic CV restitution measured by sweeping the value of $\tau_2$ in S1–S2 stimulation experiments. (F) Space–time plot showing many S2 pulses propagating along the spiral track. The stimulation interval $\tau_2$ is the same as the one used for the case of (B). The return maps of IBI sequence acquired near and far away from the stimulation site (red dot) are shown in (G) and (H), respectively. The two positions are marked by arrows in (F). Note that only the first few IBIs in (G) show different values from those of the imposed $\tau_2$. 

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To obtain figure 3(E), we defined CV as the mean velocity over the distance range of 29.5–113.7 mm of the first S2 wave pulse and the period $\tau$ to be the mean temporal distance to the last S1 wave pulse over the same distance range. Although there were some variations from one sample to another, the single-hump non-monotonic CV restitution was commonly observed (21 out of 59 cases). The visual similarity between the CV-restitution curve of figure 3(E) and that of figure 1(A) is striking: both have three distinctive regimes that have different slopes, either positive, negative or zero. This result was somewhat unexpected, since non-monotonic CV-restitution curves were found only when $[K^+]_o$ was abnormally low in previous experimental investigations by Kucera et al, who used similarly prepared cardiac cell cultures [15, 16].

According to the schematic illustration of figure 1(D), a periodic pacing of an excitable medium, which supports non-monotonic CV restitution, in the negative slope regime may generate an unstable period-1 oscillatory wave train. In other words, a small local perturbation in the period-1 wave train can be amplified to result in a period-2 oscillatory wave train (i.e. alternans). But the pedagogical instability argument did not work for the case shown in figure 3(B). The instability set in only briefly for the first few wave pulses, and the disturbance disappeared rather quickly, yielding a simple period-1 wave train. We repeated the same S1S2 stimulation experiment with a much longer S2 phase duration only to find the same result (see figure 3(F)). The return map of the IBI sequence (figure 3(G)) taken far away from the pacing site well illustrates how quickly the initial disturbance goes away. In the long run, the return map cannot be distinguished from that acquired near the pacing site (figure 3(H)).

3.2. ‘Period-1 pacing’ versus ‘alternans pacing’

The single-hump CV restitution depicted in figure 3(E) was a common feature of our quasi-one-dimensional culture, yet we were not able to generate a stable alternans. In other words, the negative CV-restitution hypothesis for alternans did not work. Therefore, as a next step, we started to add a small but finite $\Delta t$ modulation in the $\tau_2$ of the S2 phase. This was modeled by following the alternans pacing introduced by de Lange and Kucera [15]. In the alternans-pacing experiment of figure 4, the small periodic modulation given at the pacing site grew with time as the waves moved outward: see the space–time plots figures 4(B) and (D) and their corresponding return maps, figures 4(C) and (E). The wave train gradually stabilized to a stable concordant alternans. Without the finite $\Delta t$ modulation, the culture sample used produced only a period-1 wave train (not shown) as in the case of figure 3. Thus, the nature of pacing was an important factor in determining the final steady state of the system. We repeated the whole set of stimulation experiments several times and reached the same conclusion. The very existence of two different responses implied that the cardiac culture systems could support multi-stability.

3.3. Non-monotonic CV-restitution and alternans pacing revisited with a Fenton–Karma model

Subsequently, we conducted a similar set of studies with a Fenton–Karma cardiac model system [20], a well-known model commonly used for exploring issues regarding cardiac reentries and alternans. The model includes a fast inward sodium current ($I_{fi}$), a slow inward calcium current ($I_{si}$), a slow outward potassium current ($I_{so}$) and a stimulation current ($I_{stim}$). The membrane potential is governed by the following equation:

$$\frac{\partial V(x, t)}{\partial t} = D \nabla^2 V - (I_{fi}(V, v) + I_{si}(V, w) + I_{so}(V) - I_{stim})/C_m,$$

(1)
**Figure 4.** The enhancement of $\Delta t$ modulation by a non-monotonic CV restitution in the negative slope regime. (A) Space–time plot showing a long sequence of S2 pulses driven by alternans pacing. Panels (B) and (D) are blown-up images of the yellow boxed areas in (A), and (C) and (E) are IBI return maps that are built based on the time series acquired at the positions indicated by arrows in (B) and (D), respectively. The negative slope range of the CV-restitution curve of this sample is approximately from $\tau = 0.2$ to $0.4$ s.

where $C_m$ is the membrane capacitance and $D$ is the diffusion constant. The three ionic currents have the following forms:

\begin{align}
I_{fi} &= -vp(V - V_c)(1 - V)/\tau_d, \\
I_{so} &= -V(1 - p)/\tau_o - p/\tau_r, \\
I_{si} &= -w(1 + \tanh[k(V - V_{si}^c)]/(2\tau_{si}).
\end{align}

Two gating variables $v$ and $w$ are governed by

\begin{align}
\frac{\partial v(x, t)}{\partial t} &= (1 - p)(1 - v)/\tau_v (V) - pv/\tau_v^+(V), \\
\frac{\partial w(x, t)}{\partial t} &= (1 - p)(1 - w)/\tau_w (V) - pw/\tau_w^+(V),
\end{align}
where
\[ \tau_v^- = (1 - q)\tau_v(\bar{V}) + q\tau_v^-(\bar{V}) \] (7)
and
\[ p = \begin{cases} 1 & \text{if } V \geq V_c, \\ 0 & \text{otherwise}, \end{cases} \quad q = \begin{cases} 1 & \text{if } V \geq V_v, \\ 0 & \text{otherwise}. \end{cases} \] (8)

The simulation result of figure 5 in a physiologically relevant parameter regime [20] almost exactly matched the experimental result shown in figure 3. The model supported a single-hump CV restitution (see figure 5(B)) as in the experiment of figure 3(B). However, the system developed only a period-1 wave train even when it was paced in the supposedly unstable (negative slope) regime. On the other hand, with the addition of small modulation \( \Delta t = 10 \text{ ms} \), the model system developed a concordant alternans as depicted in figure 6(A). The space–time plot of \( G(x, t) \), a measure revealing the local periodicity in color (black-yellow), well illustrated how quickly the system established a stable alternans with time as well as in space (figure 6(B)).

A fully stable alternans was established approximately beyond the distance of 35 cm and the initial transient time period of 12 s. The pacing period \( \tau_2 \) was chosen from the unstable negative regime, as shown in figure 6(C) (purple dot). Figures 6(D) and (E) show a nearly period-1 time series obtained near the pacing site and the corresponding return map of IBIs, respectively. On the other hand, the time series obtained at the far end (figure 6(F)) exhibited a well-developed alternans. The enhanced period-2 modulation is rather clear in the return map of figure 6(G), and the two stabilizing period-2 modulations and their corresponding CV profiles are given in figures 6(H) and (I), respectively.

3.4. Generating alternans with a simple toy map

To further investigate the significance of the alternans pacing, we calculated the sequence of IBIs assuming a piecewise linear, single-hump, CV restitution as shown in figure 7(A). The IBI between two successive pulses is merely the difference between two integrals that integrate the inverse of the CV over a traveling distance. Thus, we can write \( \text{IBI}_n(x) \), the time difference between the \( n \)th and \((n - 1)\)th pulses, as
\[ \text{IBI}_n(x) = \tau + \int_0^x \frac{1}{V(\text{IBI}_n(x'))} - \frac{1}{V(\text{IBI}_{n-1}(x'))} \, dx', \] (9)
where \( \tau \) is the pacing period and \( V(\text{IBI}_n) \) is the CV of the \( n \)th pulse [5, 26]. We assumed that \( V(\text{IBI}_0(x)) = V_s = 16.4 \text{ cm s}^{-1} \) is an initial condition. Then, at one end of the one-dimensional chain of model cardiac cells, a series of pacing pulses was introduced at a time interval \( \text{IBI}_n(0) = \tau = 310 \text{ ms} \), which lies in the negative slope CV-restitution regime. The time evolution of eight leading wave pulses is shown in figure 7(B). As expected, the first two leading wave pulses developed an increasing modulation as they propagated outward. The subsequent wave pulses also tended to develop an alternating modulation. However, the higher the pulse number, the smaller the modulation became and the longer the time for developing an alternans. In other words, the system became period-1 oscillatory in the long run almost for the entire range (\( \sim 40 \text{ cm} \)).

Interestingly, however, with the addition of small but finite \( \Delta t \) to \( \tau \), such that \( \text{IBI}_n(0) = \tau + \Delta t \cos(n\pi) \), the system did support a very stable alternans as shown in figure 7(C). The initially small \( \Delta t \) was quickly amplified and stabilized to yield two alternating periods.
Figure 5. Non-monotonic CV-restitution property of a one-dimensional strand Fenton–Karma model. The following set of parameter values is used for the simulation: $\tau^+ = 5.75$, $\tau^- = 40.0$, $\tau_2 = 82.5$, $\tau_1 = 300.0$, $\tau_0 = 0.249$, $\tau_e = 64.7$, $\tau_r = 222.9$, $\tau_v = 226.9$, $k = 10$, $V_s = 0.85$, $V_c = 0.160$, $V_a = 0.040$, $C_m = 1.99$ and $D = 0.0016$. The pacing periods $\tau_1$ and $\tau_2$ are chosen to be 1.20 and 0.80 s, respectively. (A) Space–time plot of S2 pulses stabilizing into a period-1 wave train. Only the first few pulses show some modulations in their IBIs ($\tau_2 = 0.80$ s). (B) A single-hump non-monotonic CV restitution supported by a Fenton–Karma model. CV and $\tau$ are measured for the first S2 pulse. (C) Time series acquired near the pacing site (green dot located near 0.0 in (A)) and its corresponding return map of IBIs (D). (E) Time series acquired away from the pacing site (green dot located near 50.0 in (A)) and its corresponding return map of IBIs (F).

$\tau_1 = 272$ ms and $\tau_2 = 348$ ms for the steady state. $\tau_1$ and $\tau_2$ fell into the positive slope regime and the zero slope regime, respectively, and their mean $(\tau_1 + \tau_2)/2$ was, of course, the same as $\tau$. Also, the larger the value of $\Delta t$, the quicker the system stabilized into a stable alternans. Thus, considering all three cases (cell cultures, a Fenton–Karma model and a toy map) together, we...
Figure 6. Concordant alternans produced by alternans pacing in a strand of Fenton–Karma model cells. (A) Space–time plot of S2 pulses. (B) Space–time plot revealing the local periodicity measure $G(x, t) = \int_0^t |V(x, t') - V(x, t' - \tau)| dt'$ ($G = 0$ (black) represents period-1 oscillation, while $G \neq 0$ (yellow) represents period-2 oscillation (i.e. alternans)). (C) A single-hump CV restitution acquired by an S1S2 protocol and the time course of period modulation showing the development of alternans (blue and green lines). (D) Time series acquired near the pacing site (distance $= 0.0$) and its corresponding return map of IBIs (E). (F) Time series acquired at the end of model tissue (distance $= 50.0$) and its corresponding return map of IBIs (G). (H) Time course of two successive IBIs as a function of distance from the pacing site. The red line represents the average IBI. (I) Time course of the conduction velocities of two neighboring pulses. The pulse with a smaller IBI (green line) is moving faster than the one with a larger IBI (blue line) until the neighboring pairs stabilize into a concordant alternans. Parameter values are the same as those given in the figure caption of figure 5 except for $\tau_v = 61.875$ and $\tau_o = 97.05$. The basic pacing period $\tau_2$ is 685 ms and its modulation $\Delta t$ is $\pm 5$ ms.
Figure 7. Generating alternans using a simple, non-monotonic, CV-restitution map. (A) A single-hump CV restitution ($T_{\text{max}} = 290 \text{ ms}$, $V_{\text{max}} = 16.8 \text{ cm s}^{-1}$, $V_s = 16.4 \text{ cm s}^{-1}$, $T_{\text{pacing}} = 310 \text{ ms}$). (B) Transient IBI(x) in the non-monotonic CV restitution shown in (A). (C) At steady state, the fully grown $\Delta t$ modulation yields a stable alternans with $\tau_1 = 272 \text{ ms}$ and $\tau_2 = 348 \text{ ms}$.

concluded that a finite-amplitude alternans pacing might be an additional requirement, besides the existence of a negative slope regime in CV restitution, for generating a stable alternans. Without a modulation in the pacing period, it was impractical, even if possible, to realize a stable alternans in a physically relevant domain size.

3.5. Cardiac memory effect on non-monotonic CV restitution

So far, we have discussed the significance of a finite-amplitude modulation in the pacing cycle, as for creating a stable alternans. But we found another property of a medium, which was in a way self-suppressed by the formation of alternans: it was ‘cardiac memory’, whose significance is illustrated in figure 8. The CV-restitution functions, which were discussed in previous figures, all were based on the first S2 pulses that immediately followed the S1 pulses. Figure 8(A) shows the non-monotonic restitution curve of figure 3(E) (red dots) based on the first S2 pulse. We re-evaluated the CV-restitution curve based on the subsequent S2 pulses, and the results were plotted on the same frame. As the figure clearly shows, there is a very notable, gradual, downward drift of the initial CV-restitution curve. In other words, the CV-restitution property was not stationary but evolving with increasing the number of stimuli—a phenomenon that can be coined as ‘fatigue’ or ‘cardiac memory’. Meanwhile, the system moved into a more typical monotonic CV-restitution regime. Note that the system tended to stabilize only after many pulses had passed, as shown in figure 8(B). The time needed for the stabilization increased as the pacing interval got longer.

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Figure 8. The adaptation of CV and its CV restitution due to cardiac memory. (A) CV-restitution curves measured for different pulses. (B) The adaptation of CV as a function of the pulse number for several different values of pacing interval. The data set of figure 3 is used for obtaining the CV-restitution curves in (A).

3.6. A new toy map with a memory

Subsequently, we devised a modified toy model to capture the experimentally observed memory effect discussed in figure 8. The idea was borrowed from the adaptive map having a memory variable M, which is assumed to accumulate exponentially during APDs and dissipate during DIs, thereby changing the shape of the APD restitution curve [7]. In our new toy model, the memory variable M is assumed to be a function of conduction velocity V and IBI, instead of APD and DI, and V in turn is assumed to depend on the value of M according to the following two-dimensional return map:

\[ M_{n+1}(x) = e^{-IBI_n(x)/\bar{\tau}} \left[ 1 + (M_n(x) - 1)e^{-\beta V_n(x)/\bar{\tau}} \right], \]

\[ V_{n+1}(x) = [1 - \alpha M_{n+1}(x)] h[IBI_n(x)], \]

where IBI_n(x) is the nth inter-beat interval for the pulse at position x, determined by equation (9). \( \bar{\tau} \) is a time constant for the accumulation and dissipation of memory, \( \beta \) is a parameter for scaling purpose and \( \alpha \) adjusts the influence of \( M(x) \) on \( V(x) \). The function \( h[IBI_n(x)] \) is the CV restitution depicted in figure 7(A). Basically, we have replaced the variable APD in the return map memory model of Fox et al [7] with \( V \), since we were dealing with CV restitution instead of APD restitution. The replacement seems reasonable if we assume that locally \( V \) depends on the preceding IBI (i.e. APD+DI) linearly and so do APD and DI. However, its physiological foundation is yet to be justified. As such, the new model should be considered only as a descriptive toy model.

An alternans pacing with a basic cycle length \( \tau = 300 \) ms and \( \Delta t = 5 \) ms was delivered at one end of the model strand. But the modified toy model produced only a period-1 wave train as shown in the space–time plot of figure 9(A) and the IBI return map of figure 9(B). Figure 9(B) well illustrates how the initially growing period-2 modulation around the pacing site recedes for the subsequent pulses far away from the pacing site. The results shown in figures 9(A) and (B) matched those of figures 3(F) and (G) surprisingly well. Furthermore, the memory effects rendered visible in figures 9(C) and (D) match well with those based on experimental data shown in figure 8. In the absence of cardiac memory (i.e. \( \alpha = 0 \)), a stable alternans of figure 7(C) was simply recovered. In other words, the cardiac memory that we added to the toy
model had an alternans-suppressing effect. Indeed, we could systematically tune the degree of alternans modulation by varying the parameter $\alpha$ (see supplementary figure S2, available from stacks.iop.org/NJP/15/013046/mmedia).

4. Discussion

The purpose of this study was twofold. Firstly, we wanted to find out how well the non-monotonic CV-restitution hypothesis would apply to physiologically relevant cardiac model systems, namely long strands of cultured populations of rat cardiac cells and a Fenton–Karma ionic model. Secondly, we wanted to know whether the non-monotonicity in CV restitution alone would be sufficient to induce an instability to yield an alternans. We found that cultured networks of rat myocytes commonly supported single-hump, non-monotonic CV-restitution curves. With that, they were likely to exhibit an alternans. However, there were two other important factors in alternans generation, namely multi-stability and memory.

The multi-stability issue was not limited to the cultures of rat myocytes but also found relevance to the Fenton–Karma model, a general cardiac ionic model. We found that the same system could support either a period-1 oscillatory traveling wave or a period-2 oscillatory traveling wave (i.e. alternans), depending on the way it was paced. For creating an alternans, a finite-amplitude modulation seemed necessary in the pacing period. In addition, there was also a significant memory effect in cultures of rat myocytes, which suppressed alternans, converting...
the initially non-monotonic CV restitution into a monotonic one as in the case of figure 3(F) or figure 8. This memory effect is analogous to the one discussed in connection with APD restitution in [2, 27], in which a cardiac memory curtailed the steepness of the APD restitution map. Similarly, our toy restitution map, which was based on the memory model of [7], showed that increasing the memory effect tended to change a non-monotonic CV-restitution curve to a monotonic one. In some cases, however, the cardiac memory effect did not remove the non-monotonicity completely. Thus, with a proper alternans pacing, a stable alternans could be established, as shown in figure 4.

A few years ago, de Lange and Kucera reported simulation as well as experimental results that were very similar to what was reported here. By using in vitro cultures of rat myocytes grown on a multi-electrode array and by simulating the Luo–Rudy model, they addressed the condition in which a non-monotonic CV restitution could be realized in their systems. For obtaining CV-restitution curves, they employed an S1S2 protocol that was essentially identical to what we used here except that only a single pulse was given for the S2 phase. They reported that low potassium concentration in the culture medium was a critical factor, conferring non-monotonicity on the CV-restitution curve [15]. For that matter, it was unclear how our cardiac cell cultures supported a non-monotonic CV restitution even under a normal (physiological) concentration of potassium. The difference perhaps lied in the conditions under which the culture samples were prepared: for example, cell density, days in culture and the existence of fibroblasts. They explored neither the cardiac memory effect on CV restitution nor the significant effect of modulation in the pacing.

The significance of non-monotonic CV restitution for excitable media in general was first discussed by Winfree [28]. Winfree indicated that such a property may create a situation in which two different wave trains having different wavelengths can coexist in a separate territory. A non-monotonic CV restitution was also accounted for in the phenomenon of ‘wave bunching’ in the work of Steinbock et al [17, 25]. They found various complex traveling wave states when the driving frequency was varied. More recently, it was also reported that the non-monotonicity of CV restitution can be a basis for the formation of period-2 oscillatory reentries [6, 12, 14].

Taken together, we conclude that non-monotonicity in CV restitution can lead to a host of interesting complex oscillatory wave states. However, as far as cardiac tissues are concerned, we also need to consider cardiac memory and multi-stability. Indeed, as to obtain a stable alternans, the degree of CV non-monotonicity, the strength of the memory effect and the degree of modulation in the pacing all are important. Finally, the alternans-pacing protocol that we employed in this study can also be important in in vivo and in situ settings, since the larger the modulation in pacing, the more easily a stable alternans can be established.

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