Suppression of Alternans and Conduction Blocks Despite Steep APD Restitution: Electrotonic, Memory and Conduction Velocity Restitution Effects

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Abstract

In this paper, we examine the utility of the action potential duration (APD) restitution curve slope in predicting the onset of electrical alternans when electrotonic and memory effects are considered. We develop and use two ionic cell models without memory that have the same restitution curve with slope greater than one but different action potential shapes, and therefore different electrotonic effects. We also study a third cell model that incorporates short-term memory of previous cycle lengths, so that it has a family of S1-S2 restitution curves as well as a dynamic restitution curve whose slope is greater than one. Our results indicate that both electrotonic and memory effects can suppress alternans even when the APD restitution curve is steep. In the absence of memory, electrotonic currents related to the shape of the action potential as well as conduction velocity (CV) restitution can affect how alternans develops in tissue and in some cases can prevent its induction entirely even when isolated cells exhibit alternans. When short-term memory is included, alternans may not occur in isolated cells despite a steep APD restitution curve, and may or may not occur in tissue depending on CV restitution. We show for the first time that electrotonic and memory effects can prevent conduction blocks and stabilize reentrant waves in 2D and 3D. Thus, we find that the slope of the APD restitution curve alone does not always well predict the onset of alternans, and incorporating electrotonic and memory effects may provide a more useful alternans criterion.

**Key Words:** action potential morphology, arrhythmias, reentry
From its discovery in experimental (37) and clinical (47) settings in the early 1900s, and although considered rare until the 1940s (41), alternans has been recognized as a precursor to the induction of some ventricular arrhythmias. Some more recent clinical studies (60,62) have shown a correlation between the presence of alternans in the ECG and the likelihood of developing cardiac fibrillation, suggesting the possibility that interventions that eliminate alternans may inhibit conduction blocks and thereby prevent fibrillation. Building on the work of Nolasco and Dahlen (51), who showed by graphical arguments how steep APD restitution curves could produce alternans, much emphasis has been placed over the last decade on the slope of the APD restitution curve as one of the main factors in the induction of ventricular fibrillation both experimentally (31,46,57) and numerically (11,15,42). This theory-derived condition for the onset of alternans when the slope of the APD restitution curve as a function of the diastolic interval (DI) is greater than one (here called the APD restitution condition) has prompted what has been referred to as the restitution hypothesis, which postulates (43,52) that flattening the APD restitution curve inhibits alternans and subsequent conduction blocks, thereby preventing the occurrence of fibrillation. As an outgrowth of this hypothesis, altering the restitution curve has been proposed as a target for antifibrillatory drug design (33,43,48).

However, some questions and criticisms concerning the applicability of restitution curve slopes as a predictor of fibrillation have been raised recently (2,32,40), such as the existence of mechanisms for fibrillation besides alternans (3,6,7,21,23,44,56,58,61), the existence of mechanisms for alternans other than restitution (1,13,14,34,50,53,54,63), and difficulties and complexities in measuring and analyzing restitution curves (23,40). Furthermore, recent studies in animal models (2,36) have shown that preparations having dynamic APD restitution curves with slopes exceeding one do not necessarily exhibit alternans, as the original restitution criterion of Nolasco and Dahlen (51) would suggest, but in fact may exhibit stable behavior even at short cycle lengths where the restitution curve slopes are steepest. Recent theoretical studies in simulated isolated cardiac cells and 1D cables have confirmed these experimental findings and have demonstrated that two important physiological characteristics, electrotonic effects (17,18) and memory (26,64), should be included in the alternans criterion to explain both theoretical and experimental results.

In this paper, we use a mathematical model that allows direct variation of three features not easily altered experimentally, namely, the APD and CV restitution curves and action potential (AP) shape, to analyze their effects on the development of alternans. We explain under what conditions electrotonic effects and memory can suppress alternans and conduction block and show for the first time that sustained wave break may not develop in 2D and 3D tissue, despite APD restitution curve slope much greater than one.

Methods

Because our study is focused on restitution, and because the restitution hypothesis is concerned with the properties of the restitution curve rather than the specific currents that underlie it, we selected a model (25) whose parameters, such as the opening and
closing of the gate variables and the ionic conductances, can be varied to fit and reproduce specific mesoscopic characteristics like the APD and CV restitution curves as well as the AP shape. Versions of this phenomenological ionic model have been shown previously (8,9,23,25) to accurately reproduce the dynamics of other numerical models such as the Beeler-Reuter (5), Luo-Rudy-I (49), and Courtemanche et al. (16). It also has been used to reproduce experimentally obtained restitution curves for guinea pig (25) and rabbit (21) ventricular myocardium, as well as the AP shapes for canine epicardial, endocardial, and M cells (including appropriate spike-and-dome morphology for epicardial and M cells) (10).

In the model, the dynamics of the transmembrane potential $V$ is governed by the cable equation \[ \partial_t V(x,t) = \nabla \cdot (D \nabla V) - (I_f(V,v) + I_{so}(V) + I_{si}(V,w)) / C_m \], where the ionic currents determine cell dynamics. The fast inward current $I_f$ represents the sum of the Na$^+$ currents, the slow outward current $I_{so}$ represents the sum of the K$^+$ currents, and the slow inward current $I_{si}$ represents the sum of the Ca$^{2+}$ currents. The membrane capacitance $C_m$ is set to 1 µF/cm$^2$. The diffusion tensor $D$ defines tissue structure and anisotropy (23). All simulations except those in 3D are isotropic, so that $D$ is a diagonal matrix whose off-diagonal elements are 0 and whose diagonal elements are 0.001 cm$^2$/ms. In 3D simulations, propagation is set to be three times faster along cardiac fibers than across.

The three phenomenological currents are given by the following equations:

$$I_f(V,v)= -v \ p \ (V-V_f) \ (I-V) / \tau_{d},$$
$$I_{so}(V)= V \ (1-r) \ (1-v k_2) / \tau_{o} + r / \tau_{r},$$
$$I_{si}(V,w)= -w \ (1+ \tanh(k_1 \ (V-V_{si}))) / (2 \ \tau_{si}),$$

and the two gate variables of the model, $v$ and $w$, follow first order equations in time:

$$\partial_t v(x,t) = (1-p) (I-V) / \tau_{v} - p \ v / \tau_{v}^{+},$$
$$\partial_t w(x,t) = (1-p) (I-w) / \tau_{w} - p \ w / \tau_{w}^{+},$$

where $\tau_{v}^{+} = (1-q) \ \tau_{v}^{i} + q \ \tau_{v}^{2}$ and $p$, $q$, and $r$ are defined by

$$p = \begin{cases} 0 & \text{if } V < V_c \\ 1 & \text{if } V \geq V_c \end{cases}, \quad q = \begin{cases} 0 & \text{if } V < V_v \\ 1 & \text{if } V \geq V_v \end{cases}, \quad r = \begin{cases} 0 & \text{if } V < V_r \\ 1 & \text{if } V \geq V_r \end{cases}$$

In the equations $V$ varies between 0 and about 1.4 and is rescaled here as $V_m=100 \ V - 85$ when comparing with other models or experiments. Initial conditions are $V=0$, $w=1$, and $v=1$. Although the step functions $p$, $q$, and $r$ are used for simplicity, we have verified that all results hold when using continuous functions, such as tanh functions.

We use three different sets of parameters to construct what for ease of discussion we refer to throughout this manuscript as Model 1, Model 2, and Model 3 (see Table 1). Models 1 and 2 are used to demonstrate electrotonic effects in the absence of memory
and are constructed to have the same APD restitution curve (for APD voltage thresholds of -60mV and lower), as shown in Figure 1A. However, the shape of the AP for \( V > -60 \) mV is different in the two models, thereby producing different electrotonic effects when coupled in tissue. When simulating in spatially extended systems, we further subdivide Model 2 into 2a, 2b, and 2c to incorporate different shapes of CV restitution curves. This is done by changing \( \tau_{v1} \) to 20 for Model 2a and to 150 for Model 2c, while leaving \( \tau_{i1} \) unchanged at 100 for Model 2b. Note that changing \( \tau_{v1} \) affects only the CV restitution shape in this model and does not alter the APD restitution or affect dynamics in isolated cells. The three different shapes of CV restitution for Model 2 are shown in Figure 2A.

Model 3 is used to demonstrate the effects of short-term memory of previous activations (19,30) on the order of seconds related to the adaptation to new cycle lengths (22,34,53). We increased the effect of memory beyond what has been shown previously for some parameter regimes of this model (64) by slightly altering the \( K^+ \) current in the manner of Fox et al. (26), who have shown that the rapid delayed-rectifier \( K^+ \) current \( I_{Kr} \) can play a role in increasing or decreasing memory effects. Therefore, we change \( I_{so} \) to the form \( I_{so}(V) = V (1-r) (1-v_{k2}) / \tau_o + r V y / \tau_y \), where an extra gate variable \( y \) is used that evolves in time according to the equation

\[
\partial_t y(x,t) = p (1-y) / \tau_y^+ - (1-p) (y-0.1) / \tau_y^-,
\]

where the rest value of \( y \) is 0.1. As with Model 2, Model 3 is subdivided into Model 3a, with \( \tau_{v1} = 20 \), and Model 3b, with \( \tau_{v1} \) unchanged, to produce two different CV restitution curves, as shown in Figure 7A. The effects of long-term memory associated with remodelling (59), which has a time scale of days, is not considered.

Two different types of restitution curves are discussed in this paper: S1-S2 restitution and dynamic restitution. The S1-S2 restitution curve is obtained by pacing at a given fixed cycle length (S1) until steady state is reached, then introducing a subsequent stimulus (S2) after a certain DI and recording that DI and the resulting APD. By varying the DI before the introduction of S2, a full restitution curve is obtained. The S1-S2 restitution curve depends on the S1 cycle length, and the protocol can be used to determine a family of S1-dependent restitution curves, which may be related to each other in a unique relationship (20). The dynamic restitution curve is obtained by pacing at a fixed cycle length until steady state is reached, at which time a single DI,APD pair is recorded. During alternans, the last two DI,APD pairs are recorded so that both the long and short APs are included (35,46,64). This process is repeated for decreasing cycle lengths until conduction block occurs. APD restitution curves for isolated cells are obtained in the same way as in Refs. 46 and 57, and the spatially extended APD and CV restitutions are obtained in strips and rings of simulated tissue following the method described in Ref. 23.

The 18 parameters used in the various models are given in Table 1. Further description of model parameters and their functions can be found in Refs. 23 and 25.
Parameter $\tau_v^+$ $\tau_v^-$ $\tau_{v1}$ $\tau_{v2}$ $\tau_w^+$ $\tau_w^-$ $\tau_d$ $\tau_0$ $\tau_r$ $\tau_{si}$

| Model 1 | 10 | 350 | 80 | 562 | 48.5 | 0.15 | 1.5 | 12.5 | 10 |
| Model 2 | 10 | 100 | 20 | 800 | 45  | 0.15 | 1.5 | 31  | 26.5 |
| Model 3 | 3.33 | 45  | 300 | 600 | 40  | 0.075 | 8.3 | 38  | 127 |

| Parameter $\tau_y^+$ $\tau_y^-$ $k_1$ $K_2$ $V_{c1}^+$ $V_c$ $V_r$ $V_v$ $V_{fi}$
| Model 1 | —  | —  | 15  | 0  | 0.2 | 0.25 | 0.16 | 0.001 | 0.15 |
| Model 2 | —  | —  | 10  | 1  | 0.7 | 0.25 | 0.6  | 0.05  | 0.11 |
| Model 3 | 1000 | 230 | 60  | 0  | 0.7 | 0.25 | 0.25 | 0.05  | 0.25 |

Table 1. Parameter values used in the ionic model to produce the simulations included in this study.

Results

Even when the slope of the APD restitution curve is greater than one, alternans can be suppressed. Here we present results showing how electrotonic and memory effects can prevent the induction of alternans and how CV restitution in each case can further affect the development or suppression of alternans.

Suppression of Alternans by Electrotonic Effects

To illustrate how electrotonic currents can affect alternans development, we use Models 1 and 2. As described in the Methods section, these models have different AP morphologies, with Model 2 having a longer plateau and faster late repolarization than Model 1. However, both models exhibit the same APD restitution curves when measured at repolarization thresholds less than −60mV, as shown in Figure 1. In addition, both models are constructed so that they have no memory, and the APD restitution curve therefore is unique and independent of pacing protocol. Thus, the APD depends only on the previous DI, and a map can be constructed as described by Guevara et al. (35), where alternans appears exactly when the slope of the restitution curve is greater than one (51). In this case, since both models have the same APD restitution (see Figure 1B), alternans occurs in isolated cells for both models when the cycle length decreases below 318ms (APD ≈ 222ms, DI ≈ 96ms, slope = 1), and the two models share the same DI range and voltage amplitudes of alternans and the same transition to 2:1 rhythm for cycle lengths below 260 ms, as shown in Figure 1A.

The dynamics of alternans in tissue can vary from what is observed in isolated cells depending on CV restitution (18,23,29,67). Figure 2A shows three different CV restitution curves, corresponding to Models 2a, 2b, and 2c, which share the same APD restitution but exhibit different dynamics when paced in a 1D cable. Figure 2B shows the spatial distribution of APDs obtained using the three models for two successive beats on a cable 10cm long that is paced from the left edge at a cycle length of 285 ms. While in an isolated cell the APD alternates between 107ms and 257ms for Models 2a, 2b, and 2c, in tissue the models exhibit concordant alternans, discordant alternans, and discordant alternans that progresses to conduction block (29) and 2:1 propagation, respectively.
Figure 1. (A) APD as a function of cycle length in an isolated cell for Model 1 (green dotted line) and Model 2 (red solid line). Alternans develops at shorter cycle lengths, where the slope of the APD restitution curve is greater than one. Insets show action potentials for Models 1 and 2 during 1:1 and 2:2 rhythms, where Model 1 is more triangular than Model 2. (B) APD restitution curves for Models 1 (green dotted line) and 2 (red solid line) in an isolated cell, with slope greater than one in the gray region (Di<96ms). Since Models 1 and 2 do not include memory, the dynamic and S1-S2 restitution curves for all S1 cycle lengths are identical.

Figure 2. (A) CV restitution curves corresponding to Models 2a (long dashes, blue), 2b (short dashes, red), and 2c (solid line, black). The values of the ratio of the slope of the curve to the square of the CV, which is inversely proportional to the minimum length required to form discordant alternans (18), are shown in the inset. (B) Alternans in a 10 cm long 1D cable using the three different CV restitution curves shown in (A). APD is shown as a function of length on two successive beats after reaching steady state for a pacing cycle length of 285ms, with Model 2a (long dashes, blue) producing concordant alternans, Model 2b (short dashes, red) discordant alternans with one node, and Model 2c (solid line, black) conduction block leading to a 2:1 rhythm with a fixed APD of 270ms. (C) Growth in alternans amplitude with distance from the pacing site for Model 1 when paced at a cycle length of 295ms. Over time, this transient state evolves into a steady state with uniformly spaced nodes and equal amplitudes. (D) In contrast, when Model 2c is paced at the same cycle length of 295ms in a cable of the same length, alternans decreases in amplitude as the distance from the pacing site increases. Note that steady state has been reached and that alternans close to the pacing site persists at this period (295ms) and does not develop conduction block, as occurred for the shorter cycle length (285ms) in (B). In all cases, the pacing site is at the left boundary.
Like CV restitution, electrotonic effects in tissue can alter the dynamics observed in isolated cells, since a cell whose membrane potential is more negative (positive) than a neighboring cell will slightly repolarize (depolarize) that neighbor by diffusive currents. These effects can have important consequences during fast pacing and at boundaries. For example, Models 1 and 2 have the same APD restitution, but Model 2 repolarizes more quickly than Model 1 and produces stronger electrotonic effects along the wave back. Therefore, even though Models 1 and 2 exhibit the same pacing responses in isolated cells (see Figure 1A), differences can be observed in tissue. While Model 1 always exhibits alternans, regardless of changes in CV restitution, Model 2 can suppress alternans. Figure 2C shows discordant alternans for Model 1 when paced at 295 ms on a cable, while Figure 2D shows alternans suppression far from the pacing site for Model 2c when paced at the same period.

Alternans can be reduced or suppressed completely at all points when no external pacing is present, such as during reentry on a ring of tissue, depending on a combination of the CV restitution curve and its slope \( c'/c^2 \) and the electrotonic currents. Figure 3A shows the maximum and minimum APDs obtained on a ring as a function of the period (where the various periods of the pulse are obtained by changing the ring size) for Models 2a, 2b, and 2c, which have different CV restitutions (see Figure 2A). For Model 2a, alternans occurs over nearly the full range of periods for which alternans occurs in an isolated cell (small filled circles). The more sloping CV restitution of Model 2b also gives rise to alternans in a ring, but both the range of periods and the range of APDs over which it occurs is reduced. For Model 2c, which has more pronounced CV restitution over a broader range of DIs (larger \( c'/c^2 \)), alternans is completely suppressed over the full range of periods in a ring due to increased electrotonic currents that stabilize the APD and prevent alternans induction. This effect of alternans suppression as a function of \( c'/c^2 \) is described further in the Discussion. Figure 3A also shows in the inset the spatial profile of the pulse along the ring at one instant in time for four different periods using Model 2c (first column, solid line) and Model 2a (second column, long dashes).

![Figure 3](image-url)
range of cycle lengths, but when the CV decreases gradually over a wide range of DI’s, the alternans can be decreased in amplitude and in DI range (Model 2b, short red dashes) or even suppressed entirely (Model 2c, solid black line). In the inset, spatial profiles along the ring using Model 2c (solid black line) and Model 2a (long blue dashes) are shown for four different ring sizes corresponding to periods of (a) 400, (b) 270, (c) 150, and (d) 76 ms. Note that while no alternans occurs for Model 2c (solid black line), alternans gives rise to different spatial profiles for Model 2a for periods of 270 and 150ms. (B) APD restitution curves using Model 2c obtained for an isolated cell (solid line), for a 1D cable (long dashes), and for a 1D ring (short dashes). Differences between the cable and ring restitutions become pronounced at short DI’s due to electrotonic effects. In the inset, the slopes of the three restitution curves are shown, with a maximum slope of 4.6 for the ring, which displayed no alternans at any period.

Figure 3B shows the APD restitution curves obtained using Model 2c in an isolated cell, a 1D cable, and a 1D ring. Although there is no memory, several differences in the restitution curves arise due to coupling. First, the restitution curve obtained in an isolated cell can have a DI as small as 0, since the cell can be excited at any time, provided a strong external stimulus is applied, while in 1D there is a minimum DI below which propagation is not possible for any stimulus strength. Second, differences between the 1D cable (long dashes) and ring (short dashes) appear at short DI’s due to electrotonic effects, which are weaker in the cable near the pacing site (note that the alternans amplitude at the pacing site in Figure 2D is similar to that of an isolated cell, shown in Figure 1A). In the ring, the lack of external pacing allows CV restitution and electrotonic effects to eliminate alternans (17) (similar to the suppression of alternans far from the pacing site in a cable, as in Figure 2D), thereby allowing adaptation to shorter cycle lengths without reaching alternans and conduction block compared to a cable. Therefore, a steeper restitution is obtained in the ring. It is important to note that although the maximum APD restitution slope obtained in the ring is 4.6, no alternans occurred for any period.

As in the 1D case, the difference in AP shapes between Model 1 and Model 2 is enough to produce substantial differences in dynamics in 2D. A spiral wave initiated using Model 1, which has a shorter plateau and more gradual repolarization, develops discordant alternans and sustained breakup, as shown in Figure 4A. Model 2a, however, with its longer plateau and faster repolarization, is capable of winding into an extremely tight spiral with a period below 50 ms without developing any alternans. Note that as the spiral wave forms in Figure 4B, it adjusts directly from a long AP to a series of short APs without alternans or conduction block. Similar stable behavior can be observed using Models 2b and 2c, since the spiral period is in the region with no alternans in the ring in all three cases.

The same behavior also results when Models 1 and 2 are simulated in a three-dimensional model of rabbit ventricles (65) that includes anisotropy and fiber directions. As in the 2D case, Model 1 gives rise to alternans, which initiates self-sustaining breakup throughout the ventricles, as shown in Figure 5A. When Models 2a, 2b (shown in Figure 5B), and 2c are used, alternans does not occur, and an initiated spiral remains stable despite steep APD restitution, even with the complex anatomy and fiber orientation of the ventricles.
Figure 4. (A) Simulation of spiral wave dynamics using Model 1. Discordant alternans develops away from the core as the wave rotates, as seen in frames 3 and 4, where the nodes separating long action potentials from short ones move as the wave rotates. Eventually, the alternans leads to conduction block and sustained breakup. Times are 1.8, 2.8, 9.5, 9.6, 13.7, and 14.3 s. (B) Simulation of spiral wave dynamics using Model 2, which has a longer plateau and faster repolarization than Model 1. Alternans is suppressed due to the increased coupling currents from the sharp wave back, and the induced spiral wave curls tightly and remains stable. The slight irregularities along the waves visible in the two bottom panels are due to the meandering of the tip trajectory and not to alternans. Times are 34, 74, 118, 188, 254, and 396 ms. Excited tissue is shown in white and light gray and quiescent tissue in black. Tissue size is 12.5x12.5 cm for (A) and 17.5x17.5 cm for (B) (bars represent 5 cm), with Δx=0.025 cm and Δt=0.05 ms for both (A) and (B).

Figure 5. Electrotonic effects in rabbit ventricles (65) using Model 1 (A) and Model 2b (B). Spiral waves using Model 1 break into multiple waves, while the electrotonic effects arising from the faster-repolarizing
Suppression of Alternans by Memory Effects

Because it includes memory, Model 3, unlike Models 1 and 2, has a family of restitution curves resulting from different S1-S2 protocols as well as a distinct dynamic restitution curve. Figure 6 shows five different APD restitution curves obtained in an isolated cell using Model 3, where the solid line represents the dynamic restitution curve and the four dashed lines S1-S2 curves obtained for S1 cycle lengths of 600, 300, 200 and 150 ms. While the slope of the dynamic APD restitution curve (shown in the inset), which has been suggested as the most relevant to alternans onset (35,46,57), is greater than one for DIs less than 130 ms (with a maximum slope of 2.2), no alternans occurs when the cells are paced at any cycle length. An improved criterion for determining the onset of alternans is the memory-corrected single-cell stability criterion of Tolkacheva et al.(64), which uses both the slope of the dynamic restitution curve and the slopes of S1-S2 curves and predicts alternans when the criterion has a value greater than one. This criterion, shown over the same range of DIs in the inset of Figure 6, never exceeds one and therefore correctly predicts that alternans does not occur.

Figure 6. Dynamic and S1-S2 restitution curves obtained using Model 3. Significant memory effects cause the curves to differ and prevent the induction of alternans despite dynamic restitution slope greater than one. Adding a memory correction to the APD restitution condition correctly predicts the absence of alternans for this case despite dynamic restitution slope larger than 2.
In tissue, changes to the CV restitution curve can influence whether alternans develops even when no alternans is present in an isolated cell. As an example, Figure 7A shows two different CV restitution curves used with Model 3, one flat over a large range of DI’s but becoming steep at short DI’s (Model 3a, $\tau_{v1} = 20$), and the other more gently sloped over a larger range of DI’s (Model 3b, $\tau_{v1} = 45$). The dynamics obtained in a 1D cable when each model is paced at a cycle length of 154 ms is illustrated in Figure 7B. While Model 3a remains stable with no alternans, Model 3b exhibits alternans due to its more sloping CV restitution, which facilitates the development of alternans in long cables (23,67). When the basic cycle length (BCL) is decreased further to a BCL of 153 ms, Model 3b exhibits conduction block and 2:1 dynamics, while Model 3a remains stable, as shown in Figure 7C. Furthermore, Model 3a does not produce alternans in a cable at any period of stimulation up to the minimum BCL of 140 ms obtained at the minimum DI ($\approx 23$ ms) of the model (see Figure 6).

![Figure 7](image_url)

**Figure 7.** (A) CV restitution curves corresponding to Model 3a (solid line) and Model 3b (dashed line). (B) On a 1D cable paced from the left at a cycle length of 154 ms, Model 3a (solid line) remains stable, while Model 3b (dashed lines) exhibits alternans whose amplitude increases with distance from the pacing site. The APDs for two successive beats after reaching steady state is shown along the length of the cable. (C) When the cycle length is reduced to 153 ms, Model 3a continues 1:1 conduction, while the alternans of Model 3b develops conduction block along the cable and transitions to 2:1 behavior far from the pacing site. Alternate beats 20 and 21 are shown. Over time, the site of the transition to 2:1 rhythm moves toward the pacing site at the left, resulting in 2:1 rhythm throughout the cable. Results were achieved by decreasing the BCL slowly to allow time for the cable to adjust to the new APD. In all cases, $\Delta x = 0.025$ cm and $\Delta t = 0.1$ ms.
In 2D, reentrant waves using either Model 3a or Model 3b do not produce alternans and are stable even though the slope of the dynamic restitution curve is greater than one, as shown in Figure 8. Two snapshots during one rotation are shown using Model 3b, where the spiral’s period of rotation is about 170ms, well within the region of slope greater than one.

It is important to note that transient breakup can still occur in some cases, even when alternans is suppressed. This breakup results from abrupt changes in cycle length, which can lead to conduction block as the cell tries to adjust to the new pacing but fails to capture the next beat. Therefore, when a reentrant wave is created using the memory model (particularly in Model 3a), depending on initial conditions, conduction block can still occur in some cases following initiation as the reentrant wave turns, leading to breakup. Nevertheless, this breakup is not due to alternans and, more importantly, it is not sustained and therefore does not lead to a continuous generation and annihilation of multiple reentrant waves as in Figure 4A. Transient breakup of this nature generally does not occur when electrotonic effects in the absence of memory suppress alternans, as the faster repolarization makes it easier for the wave front and back to adjust to sudden large changes in pacing cycle length without developing conduction blocks (17).

![Figure 8](image.png)

**Figure 8.** Stable spiral wave obtained using Model 3b, despite dynamic APD restitution curve slope greater than one. Two snapshots during one rotation are shown. Memory effects suppress the alternans predicted by the APD restitution condition. Tissue size is 13x13cm (bar represents 5 cm), with $\Delta x=0.025$cm and $\Delta t=0.1$ms.

**Discussion**

In this study we show that diffusion currents due to the specific shape of the AP, memory of previous activations, and CV restitution can all alter the criterion for the development of alternans when the slope of the restitution curve is greater than one. Variations of a phenomenological ionic cell model that separate electrotonic, memory, and CV restitution effects are used to illustrate these effects. However, the theory underlying the main points of the paper, described below, can be interpreted independently of any particular model.
Alternans in Isolated Cells Does Not Guarantee Alternans in Tissue

Even when alternans occurs in isolated cells, it is possible for electrotonic effects due to the AP shape, combined with CV restitution, to suppress alternans in tissue. Previous analytical studies using delay equations have shown that incorporating the role of CV restitution and electrotonic effects leads to a new criterion for the development of alternans (17,18); specifically, alternans develops when the slope of the APD restitution curve exceeds unity by an amount that is a function of $\xi c'/c^2$, where $\xi$ accounts for diffusion effects related to the AP morphology, especially its repolarization, and $c'/c^2$ relates to the CV $c$ and the slope of the CV restitution curve $c'$ (inset of Figure 2A). Thus, both large values of $\xi$, corresponding to strong electrotonic effects, and large values of $c'/c^2$, corresponding to steeply sloped CV restitution or slow CV, increase beyond one the value that the slope of the APD restitution curve must achieve before alternans develops. Model 1 and Model 2, with different AP morphologies, have correspondingly different values of $\xi$ that change wave front-back interactions (17) causing differences in the formation of alternans in tissue, even though alternans is present in isolated cells in both cases. Therefore Model 1, which has small electrotonic effects along the wave back, exhibits discordant alternans throughout the cable, as shown in Figure 2C, similar to previous numerical simulations (18,23,27,28,55,67). However, Model 2, with stronger electrotonic effects due to faster phase 3 repolarization, shows suppression of alternans far from the pacing site (Figure 2D) when paced at the same cycle length, an effect which has not been demonstrated previously.

Unlike in a 1D cable, where alternans is always present at the pacing site, in a 1D ring where there is no pacing, alternans can be suppressed at all points during reentry. The faster the repolarization of the AP, the easier it is for alternans to be suppressed, as the value of $\xi$ becomes larger (17). However, CV restitution can diminish the stabilizing effect of $\xi$, which accounts for the different dynamics obtained using Models 2a, 2b, and 2c in a ring (Figure 3A). The ratio $c'/c^2$ is relatively large over a wide range of DI’s (as shown in the inset of Figure 2A) for Model 2c, and thus electrotonic currents completely suppress alternans in a 1D ring (single-valued solid curve in Figure 3A) for any ring size (period) since $\xi c'/c^2$ remains large. On the other hand, when $c'/c^2$ is small close to the onset of alternans in an isolated cell but increases at smaller DIs, alternans may be suppressed for sufficiently small periods (ring sizes), as for Models 2a and 2b, in which alternans is suppressed for periods below 130ms and 196ms, respectively.

Similarly, electrotonic effects can be very important in determining the stability of reentrant waves in two and three dimensions, as shown in Figures 4 and 5, where Model 1 produces breakup and Model 2 produces a stable spiral or scroll wave. (Breakup in Model 1 no longer occurs when the tissue size is decreased below 7.5x7.5 cm, which indicates alternans rather than meander (3) is responsible for the breakup in this case since discordant alternans can be prevented in smaller tissues (18,23,67).) Model 2 can even produce an exceptionally tightly wound spiral, as shown for an extreme case in Figure 4B, which while not typical of reentrant waves observed in cardiac tissue has a frequency within values reported in the literature (61,66). More realistic reentrant waves
can be produced that similarly exhibit no alternans despite a steep APD restitution by varying some of the model parameters, such as the sodium conductance (23). Although in 3D thickness and fiber rotation (25,56) as well as structure (21,58) can destabilize reentrant waves with some specific dynamics, reentrant waves can remain stable when electrotonic effects are very strong, even when initiated in a realistic model of rabbit ventricular anatomy with fiber anisotropy, as shown in Figure 5B.

AP shape may be one explanation of the results of Hall et al. (36) and Banville and Gray (2), where APD restitution slope greater than one was measured but no alternans was observed. The repolarization of the AP in both rabbit and frog ventricular myocardium in those experiments is relatively fast and is similar to Model 2. Furthermore, differences in AP shape may be important in determining the stability of reentrant waves, as illustrated in two recent studies. Samie et al. (61) showed stable reentry in a simulation of LV tissue using a variation of the LR1 model (49) with a large $I_{K1}$ current and unstable reentry in RV tissue with small $I_{K1}$, which produced an AP with slower repolarization than in the LV. Likewise, Hondeghem et al. (39) found that triangular APs with slower repolarization were more likely to be proarrhythmic.

**Suppression of Alternans in Isolated Cells Does Not Guarantee Suppression of Alternans in Tissue**

Cardiac tissue’s memory of previous activations can prevent alternans in isolated cells, even when the APD restitution curve has slope greater than one. Analytical studies (26,64) have shown how incorporating memory can change the stability of the 1:1 rhythm and prevent alternans. In particular, Tolkacheva et al. (64) generalized the original analysis of Guevara et al. (35), which assumed that APD was a function only of the preceding DI, for a case that included memory of the previous APD as well (as in Refs. 12 and 53). Because of this short-term memory, the stability criterion of the 1:1 rhythm was found to depend not only on the slope of the dynamic restitution curve, but also on the entire family of S1-S2 restitution curves obtained at different S1 cycle lengths. The criterion for 1:1 stability under these conditions becomes $|1-S_{S1,S2}(1+(1/S_{dyn}))|<1$ and not simply $|S_{dyn}|<1$ as the 1D map without memory predicts, where $S_{dyn}$ is the slope of the dynamic restitution curve for a given cycle length and $S_{S1,S2}$ is the slope of the S1-S2 restitution curve of the same cycle length that crosses the dynamic restitution at the given DI. Although it requires further testing in other models and different experimental conditions, in this case the memory-corrected stability criterion for Model 3, shown in the inset of Figure 6, never exceeds one and correctly predicts that alternans will not occur in an isolated cell although the slope of the dynamic APD restitution curve is greater than one.

Nevertheless, even when memory suppresses alternans in an isolated cell, alternans still may develop in tissue due to electrotonic effects related to the CV restitution. We have found this to be the case for the Fox et al. ionic model, which in an isolated cell with the maximum conductance of $I_{Kr}$ increased by a factor of two does not alternate despite APD restitution slope greater than one (26), but which does exhibit alternans in 1D cables when paced at high frequencies. In the same way, Model 3b exhibits alternans in a 1D cable, but not in an isolated cell. As described above, a CV
restitution curve that is sufficiently steep over a wide range of DIs (as in Model 3b, compared to Model 3a) promotes the induction of discordant alternans because the value of \( \frac{c'}{c^2} \), which is inversely proportional to the size of tissue needed for discordant alternans to develop, is large (18,23,67). Thus pacing at a certain cycle length may induce discordant alternans and conduction block in a cable of a given length for Model 3b, but not for Model 3a, as shown in Figure 7.

**CV Restitution Can Promote or Suppress Alternans in Tissue**

Based on the results shown here, steepening the CV restitution over a broad range of DIs may appear to be a useful strategy to prevent the induction of alternans. However, it is important to consider that the combination of CV restitution and tissue size determine whether APD alternans in isotropic tissue will be concordant or discordant (18,23,67). For tissue paced at a constant period, discordant alternans requires a minimum tissue size that is inversely proportional to \( \frac{c'}{c^2} \) (18), so that when \( \frac{c'}{c^2} \) is small (see Model 2a in Figure 2A-B) only concordant alternans is formed. When \( \frac{c'}{c^2} \) is larger (see Model 2b in Figure 2A-B), discordant alternans can develop, and conduction block can occur as \( \frac{c'}{c^2} \) grows (see Model 2c is Figure 2A-B). Even when no alternans occurs in an isolated cell due to memory, as in Model 3, CV restitution in tissue can still induce discordant alternans and conduction block far from the pacing site when \( \frac{c'}{c^2} \) is large, as shown for Model 3b in Figure 7, where \( \frac{c'}{c^2} \) is larger for Model 3b than for Model 3a.

Therefore a dilemma arises when considering the effects of altering CV restitution. On the one hand, for a fixed tissue size discordant alternans is more likely to appear as \( \frac{c'}{c^2} \) increases (18,23,67), but on the other hand, in the presence of strong electrotonic effects alternans suppression is also more likely to occur with large \( \frac{c'}{c^2} \) (17) (Model 2c in Figures 2A, 2D, and 3A). Therefore, steeper CV restitution curves may promote the alternans suppression effect of a strongly repolarizing wave back, but at the same time steep CV restitution may facilitate the progression of any alternans that does occur from concordant to discordant.

**Other Mechanisms May Cause Fibrillation and Alternans**

It is important to note that although alternans is a mechanism that can lead to conduction block and initiation of VF, it is not the only one, and other possible mechanisms may need to be considered as well, especially since the induction of complex electrical activity resembling fibrillation has been shown to occur using mathematical cell models without steep APD restitution or alternans. For example, the Luo-Rudy I model with calcium speedup of a factor of two has flat APD restitution but still produces complex VF-like dynamics (23). Certain types of trajectories of reentrant waves have been shown to lead to conduction blocks and continuous generation of multiple wavelets despite flat APD restitution curves (3,23). Tissue thickness (6,23), rotational anisotropy (23,56), and ventricular anatomy (21,58) have also been shown to cause breakup for some types of reentrant wave trajectories, independent of APD restitution and cell model. Regional differences in ion channel density (61) as well as decreased conductivity and
coupling (7,44) have also been shown in some cases to lead to complex dynamics characteristic of VF. Even the results of experimental studies asserting that drugs that flatten APD restitution have antifibrillatory effects can be interpreted in other ways, so that other conditions may be involved in causing fibrillation. For instance, some of these studies (31,52) also show an increase in the minimum DI, which itself has been suggested as an antifibrillatory target (23,61) since a larger minimum DI can increase the core size and period of reentrant waves.

Similarly, steep APD restitution may not be the only mechanism that can produce alternans in cardiac tissue. A number of other possible mechanisms for alternans have been postulated, including tissue heterogeneities (1,13,54), memory effects even with APD restitution slope less than one (34,53), alternans in intracellular calcium concentration (13,14) and ischemia (1,14,50), even though the APD restitution curve becomes flatter in ischemic tissue (63).

**Limitations**

Several limitations are associated with this study. Although for ease of discussion we have used a model that is able to separate electrotonic and memory effects, both are present in cardiac tissue and should be considered together. It was assumed here that memory did not extend to CV, although the maximum CV has been shown to become a function of cycle length during ischemia (38), and how CV memory would affect alternans development and suppression is unknown. Furthermore, we have not studied the effect of varying the minimum DI, which also may alter the conditions necessary for alternans to develop. Finally, the effects of tissue complexities such as localized heterogeneities, transmural differences in ventricular cell types, and electromechanical coupling were not considered.

Another more general limitation arising in theoretical or experimental studies involving restitution is how to measure APD restitution curves. First, different choices of thresholds for defining the APD and DI may produce APD restitution curves with different shapes, and it is not known how to determine which threshold is most relevant for predicting alternans. Furthermore, because of electrotonic effects due to the cores of VF wavelets, small double potentials (19,23,45) and short APDs (4) can be recorded each time a wave tip passes by when measuring dynamic APD restitution curves during VF. These double potentials can be identified as true activations, although they are not (68), and can add points to the restitution curve with very small APD and DI. Including these spurious points in the restitution curve often produces a steeply sloped region at very short DIs and therefore can result in an erroneously higher value for the slope (40). For example, in Ref. 46, the restitution obtained by feeding the signal from VF into tissue does not produce many of the extremely small-valued APDs obtained during VF, even after increasing the stimulus intensity and temperature, most likely due to short APDs resulting from electrotonic double potentials by wave tips in VF that cannot be present during pacing. In addition, data points from experiments are often scattered widely during VF probably due to double potential recordings. To avoid both the problem of data from wave tips and data scattering, Ref. 23 suggests the use of a density plot, a method that
works well for numerical simulations but remains to be verified experimentally, especially if strong memory is present.

**Conclusions**

In this study we have shown that even when the slope of the APD restitution curve is greater than one over a large range of pacing cycle lengths, the generation of electrical alternans and conduction blocks are a function of the AP shape, cardiac memory, and CV restitution. While memory effects can prevent alternans in single cells, electrotonic effects due to AP shape and CV restitution can suppress alternans in tissue even when alternans is present in single cells, but they can also induce alternans in tissue even when no alternans is present in single cells. The results presented here and recently by others (17,26,64) give a possible explanation for the absence of alternans in some experiments even when the slope of the restitution was observed to be greater than one (2,36) and also support the finding of Hondeghem et al. (39) that prolonging APD while making the AP more triangular tends to be proarrhythmic, while predominantly prolonging the plateau may not be. Because the fundamental mechanisms underlying VF remain largely unknown, and because the onset of alternans and the dynamics of reentrant waves in cardiac tissue cannot always be well predicted by only the APD and CV restitution curves, our results support the idea (2,32,40) that designing antiarrhythmic drugs targeted at altering only the slope of the APD restitution curve may not be appropriate at this time.

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