The pinwheel experiment revisited: effects of cellular electrophysiological properties on vulnerability to cardiac reentry

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Yang MJ, Tran DX, Weiss JN, Garfinkel A, Qu Z. The pinwheel experiment revisited: effects of cellular electrophysiological properties on vulnerability to cardiac reentry. Am J Physiol Heart Circ Physiol 293: H1781–H1790, 2007. First published June 22, 2007; doi:10.1152/ajpheart.00014.2007.—In normal heart, ventricular fibrillation (VF) can be induced by a properly timed strong electrical stimulus delivered during the T wave, while a low-intensity stimulus rarely induces VF, even when delivered in succession (6, 16, 18, 20, 30). A mechanism for the initiation of reentry by strong electrical stimulation from a point electrode was first proposed by Winfree and coined the “pinwheel experiment” or “singular point hypothesis,” and later as the “critical point hypothesis” (50–52), coined the “pinwheel experiment” or “singular point hypothesis.” Experimental evidence of this mechanism was shown by Cheng et al. (8) in the rabbit heart. Moreover, the effects of cellular electrophysiological properties on vulnerability to reentry in the pinwheel scenario have not been investigated. In this study, we extend Winfree’s pinwheel experiment to show how the vulnerability to reentry is affected by the graded action potential responses induced by a strong premature stimulus, action potential duration (APD), and APD restitution in simulated monodomain homogeneous two-dimensional tissue. We find that a larger graded response, longer APD, or steeper APD restitution slope reduces the vulnerable window of reentry. Strong graded responses and long APD promote tip-tip interactions at long coupling intervals, causing the two initiated spiral wave tips to annihilate. Steep APD restitution promotes wave front-wave back interaction, causing conduction block in the central common pathway of figure-of-eight reentry. We derive an analytical treatment that shows good agreement with numerical simulation results.

vulnerable window; cardiac simulation; arrhythmias

IN A NORMAL HEART, ventricular fibrillation (VF) can be induced by a properly timed strong electrical stimulus delivered during the T wave, while a low-intensity stimulus rarely induces VF, even when delivered in succession (6, 16, 18, 20, 30). A mechanism for the initiation of reentry by strong electrical stimulation from a point electrode was first proposed by Winfree and coined the “pinwheel experiment” or “singular point hypothesis,” and later as the “critical point hypothesis” by Frazier et al. (15). The mechanism, illustrated in Fig. 1A, proposes that a premature electrical stimulus (S2) applied from a point electrode at a critical time Tc during the repolarization phase of a rectilinear (S1) wave, such as a sinus beat, depolarizes a spatial domain of critical size Ac (symbols T* and S* were used in Refs. 40 and 50). The S2 excitation cannot propagate upwardly in the same direction of the S1 beat, since the tissue in this region has not yet recovered excitability, but can propagate downward where the tissue has already recovered. This unidirectional conduction block of the S2 beat produces two singularities (wave breaks), which subsequently turn inward and attempt to form counterrotating spiral waves. “Figure-of-eight” reentry results if the two spiral wave tips have enough room to form a central common conduction pathway. However, if the S2 depolarizes a spatial domain smaller than the critical area, or if the S2 is given too early or too late, the two spiral wave tips will collide and annihilate (annihilation occurs if the 2 dashed circles in Fig. 1A intersect). For a given S2 stimulation strength, there is a range of coupling intervals for which reentry can be induced, which is called the “vulnerable period”; the range of both coupling intervals and stimulus intensities inducing reentry is called the “vulnerable domain.” In this article, we refer to both the vulnerable period and vulnerable domain interchangeably as the vulnerable window. Winfree (50) described the vulnerable domain as “a compact island surrounded by stimulus combinations that never evoke fibrillation.” He showed that the duration of the vulnerable period is approximately equal to the diameter of the depolarized domain divided by the conduction velocity of the S1 wave front (see Fig. 11a in Ref. 50). Winfree (50, 52) also suggested “if [the S2 stimulus] were so strong that the entire medium is stimulated beyond [Ac], there would be no intersections of [Ac and Tc], thus defining an upper limit to vulnerability.” Since its inception, the critical point hypothesis has been validated experimentally (6, 15, 44).

In his 1998 paper (40), Roth extended the pinwheel experiment to the more realistic case of bidomain tissue (in which both intracellular and extracellular resistivities are represented). He proposed that the shock creates virtual electrodes, consisting of cathodal and anodal regions in a “dog bone” pattern (Fig. 1B), which would affect the size of the vulnerable period and the number of rotors created. In numerical simulations using bidomain tissue, Lindblom et al. (27) investigated the effects of virtual electrodes on reentry initiation in two-dimensional (2D) tissue. Sambelashvili and Efimov (42, 43) extended this finding to three-dimensional (3D) tissue and showed that virtual electrodes induced three distinct types of filaments. The effects of a bidomain model on rotor initiation has also been shown in the real heart (26). In another interesting study in the bidomain model of cardiac tissue (41), Roth showed that “an S1 gradient of refractoriness is not essential for reentry induction by an S2 stimulus.” Experimental evidence of this mechanism was shown by Cheng et al. (8) in the rabbit heart.

Despite the extension of Winfree’s original theory to bidomain tissue, few studies have addressed how the cellular...
to investigate how general electrophysiological factors affect wave propagation, particularly APD restitution, rather than the effects of specific ionic currents, we chose a simple action potential model, phase I of the Luo and Rudy model (LR1) (28) to generate the ionic current $I_{\text{ion}}$ in Eq. 1. This model is much more computationally efficient than newer, more detailed action potential models. The maximum Na+ and slow inward Ca2+ channel conductances were fixed at $G_{\text{Na}} = 16 \text{ mS/cm}^2$ and $G_{\text{Ca}} = 0.06 \text{ mS/cm}^2$, respectively. Other parameters were modified from the original LR1 model to achieve the desired APD and restitution properties. We also simulated a one-dimensional (1D) cable model with the following equation to represent the diagonal line in the tissue, i.e.,

$$\frac{\partial V}{\partial t} = -(I_{\text{ion}} + I_{\text{f}})C_m + D \left( \frac{\partial^2 V}{\partial x^2} + \frac{\partial^2 V}{\partial y^2} \right)$$

(2)

The length of the cable is the equivalent to the diagonal line of the 2D tissue, which is $6\sqrt{2} \text{ cm}$. $I_{\text{ion}}$ in Eqs. 1 and 2 was the stimulation current density with intensity $30 \mu \text{A/cm}^2$ and duration 2 ms (the stimulation threshold for the LR1 is $\sim 18 \mu \text{A/cm}^2$ at the same 2-ms duration). $S_1$ was applied in a 1.5-mm $\times$ 1.5-mm area at the lower left corner of the 2D tissue or a 1.5-mm segment at the left end of the cable. It should be noted that the action potential wave elicited by $S_1$ is not a planar wave; however, the curvature becomes small when the wave reaches the tissue center, which should only have minor effects on reentry induction (compare to the scenario shown in Fig. 1A).

The S2 stimulation models the following three scenarios. The first scenario is a physiological activation occurring in an area during the T wave of the ECG, such as an early afterdepolarization in a region of the ventricles. The second is an external mechanical impact during the T wave, as in commotio cordis (25, 29), which activates mechanosensitive channels to depolarize the impacted region. The third scenario is a high-strength stimulus from a small electrode, as used in experiments for shock-induced reentrant arrhythmias (4, 6, 13, 15, 26, 44). In the third case, high-strength stimuli form virtual electrodes in a dog bone pattern dependent on the different diffusion rates and anisotropic ratios of the extracellular and intracellular domains (39, 49). The formation of these electrodes is an intrinsic property of bidomain tissue. However, in a monodomain model, such as the one used in this study, these bidomain properties cannot be correctly represented. In Winfree’s original paper (50), he assumes that as the stimulus from an electrode increases, the depolarized (circular or elliptic) area will also increase. Once it increases to a critical size, reentry can be induced. To model this effect in a monodomain model, we encounter the following problems. First, the ionic model cannot support a high stimulus current. Second, once the stimulus strength reaches a certain value, a target wave is induced because the passive diffusion speed is slower than the active wave propagation. In other words, in the monodomain model, we cannot depolarize a large enough area (>1 cm in radius) by increasing the stimulus strength alone. Therefore, to model the stimulus strength in monodomain, instead of increasing stimulus strength we fix the $S_2$ strength to be the same as that of the $S_1$ stimulus, but increase the size of the stimulation area. In 2D, the $S_2$ stimulus was applied in a circular area with radius $R$ in the center of the tissue, and in 1D cable, it was applied over a 2R segment in the center of the cable. We refer to the $S_2$ stimulation area as the “$S_2$ domain” in this article.

**APD restitution.** APD was defined as the duration of time in which transmembrane voltage ($V$) was greater than $-72 \text{ mV}$, and the diastolic interval (DI) as the duration during which $V$ was less than $-72 \text{ mV}$. APD restitution was measured with an $S_1$-$S_2$ protocol in the 1D cable (Eq. 2), i.e., a train of $S_1$ beats at a pacing cycle length of 500 ms was applied at one end of the cable, with an $S_2$ beat applied at the same end after a variable $S_1$-$S_2$ interval. APD of the $S_2$ beat and the DI preceding the $S_2$ beat were measured in the middle of the cable. APD restitution properties were adjusted by modifying conductance and/or the gating kinetics of the potassium or calcium currents. The

**Methods**

**Mathematical model.** We simulated a 6-cm $\times$ 6-cm isotropic 2D tissue using the following reaction-diffusion equation for the membrane voltage ($V$):

$$\frac{\partial V}{\partial t} = -(I_{\text{ion}} + I_{\text{f}})C_m + D \left( \frac{\partial^2 V}{\partial x^2} + \frac{\partial^2 V}{\partial y^2} \right)$$

(1)

where $D = 0.001 \text{ cm}^2/\text{ms}$ is the diffusion constant and $C_m = 1 \mu \text{F/cm}^2$ is the membrane capacitance. Since the goal of the study was
restoration curves used in this study and the corresponding parameter modifications are shown in Fig. 2.

**Numerical methods.** Simulations were performed by an explicit Euler’s method with an adaptive time step as previously described by Qu and Garfinkel (32).

**RESULTS**

The vulnerable window of reentry based on Winfree’s pinwheel experiment. Based on the pinwheel experiment (50), Winfree estimated that the duration of the vulnerable period is approximately equal to the diameter of the depolarized domain divided by the conduction velocity of the S1 wave front (see Fig. 11a in Ref. 50). In fact, one can derive the vulnerable window more precisely. Figure 3A illustrates graphically the calculation of the vulnerable period for a depolarized domain of radius \( R \). At time \( T_c \), a critical radius \( R_c \) is needed for figure-of-eight reentry to form. For a depolarized domain of radius \( R > R_c \), reentry can be induced between \( T_c - \Delta T \) and \( T_c + \Delta T \). The S2 depolarized domain intersects the S1 beat at these two critical time points, forming two singularities \( 2R_c \) apart. On the basis of this simple geometric argument, we obtain the relation between \( R \) and \( \Delta T \) as:

\[
R = \sqrt{R_c^2 + Z^2} = \sqrt{R_c^2 + (\Delta T \theta)^2}
\]

where \( Z = \Delta T \theta \) is the distance that S1 propagates from time \( T_c \) to \( T_c + \Delta T \), and \( \theta \) is the conduction velocity of S1. By definition, the size of the vulnerable period is \( 2\Delta T \) and \( \Delta T \) can be calculated for given \( R, R_c \), and \( \theta \) from Eq. 3. Since a critical distance is needed, unidirectional conduction block does not always result in reentry. The relation between \( R \) and \( \Delta T \) for unidirectional conduction block, as illustrated in Fig. 3B, is:

\[
R = \frac{\Delta T \theta}{\Delta T} \quad \text{or} \quad \Delta T = \frac{R}{\theta}
\]

which is Winfree’s estimate of the vulnerable period. When the vulnerable windows for unidirectional block and reentry, determined by Eqs. 3 and 4, are plotted together, we see that their difference lies mainly at small \( R \) (Fig. 3C).

The vulnerable window of reentry is affected by APD and APD restitution. Using the mathematical and numerical approaches described in METHODS, we investigated the effects of APD and APD restitution on the size of the vulnerable window. The four APD restitution curves shown in Fig. 2 were investigated. Figure 4A shows the vulnerable window for the case of short APD and shallow APD restitution slope. The vulnerable window of reentry is close to that of unidirectional conduction block, forming a triangular shape. This case is similar to the analytical results shown in Fig. 3C. The upper border of the vulnerable domain is Winfree’s “upper limit of
Fig. 4. Vulnerable domains from computer simulations for the 4 APD restitution curves shown in Fig. 2. Gray areas represent unidirectional conduction block with reentry, and hatched areas represent unidirectional conduction block in which reentry fails because of tip-tip interactions (vertical stripes) or wave front-wave back interactions (cross-hatched). A: shallow APD restitution slope with short APD. B: control APD restitution. C: steep APD restitution. D: shallow APD restitution slope with long APD.

vulnerability” corresponding to the S2 stimulus exceeding the size of the tissue. It should be noted that alternative mechanisms for the upper limit of vulnerability have been proposed based on tissue experiments (16) and computer simulations in bidomain tissue (38), for which tissue size does not play an important role. Figure 4B shows the vulnerable window for the case in which APD restitution is normal (i.e., long APD at long DIs decreasing gradually to short APD as DI decreases). In this case, the vulnerable window of reentry is reduced, shrinking inward from the long S1-S2 interval side as well as from the apex of the triangle, forming a notch. If the APD restitution curve becomes even steeper (Fig. 4C), the vulnerable window decreases further, with the notch becoming more prominent. In the final case (Fig. 4D), APD is long but the APD restitution curve is shallow. The vulnerable window of reentry becomes substantially smaller as longer S1-S2 intervals fail to induce reentry (Fig. 4D).

There are several distinct behaviors represented in the phase diagrams in Fig. 4, corresponding to different mechanisms that prevent reentry. If the S1-S2 coupling interval is too short, the S2 stimulation fails to propagate because of the refractoriness from the S1 wave. If the S1-S2 coupling interval is too long, the S2 beat propagates as a target wave without unidirectional conduction block. If the S2 depolarizes a region less than the critical area, unidirectional conduction block occurs, but reentry does not result (vertically striped regions in Fig. 4) because the broken wave tips do not have enough spatial separation to form a central common pathway. Reentry fails because of tip-tip interactions. Voltage snapshots of this scenario are shown in Fig. 5A. Figure 5B illustrates the successful initiation of reentry where the S2 depolarizes a spatial domain larger than the critical domain during the vulnerable period (gray areas in Fig. 4). In the notch area (cross-hatched areas in Fig. 4), reentry fails because of a different mechanism. In this case (Fig. 5C), the two spiral wave tips have enough separation to successfully form a central common pathway, but the wave front in the central common pathway subsequently collides with the repolarizing wave back of the same S2 beat and blocks. Failure to induce reentry in this case is a consequence of wave front-wave back interactions.

Mechanisms by which cellular electrophysiological properties affect the vulnerable window. According to the classic pinwheel experiment, the vulnerable window of reentry is similar to that of unidirectional conduction block. Using numerical simulations, we have shown above that this is true when APD is short and the APD restitution curve is shallow (see Fig. 4A). However, as APD lengthens and/or APD restitution becomes steeper, the vulnerable window for reentry no longer matches the vulnerable window for unidirectional conduction block as a result of increased tip-tip interaction and wave front-wave back interaction. Additionally, in the classic pinwheel experiment, the vulnerable window for reentry is symmetrical with respect to \( T_c \), but this symmetry is also lost, as shown in Fig. 4, B–D. In the following sections, we develop analytical explanations for how graded action potential responses, APD, and APD restitution affect the properties of the vulnerable window of reentry.

Effects of APD and graded action potential responses. When restitution is flat, only two behaviors occur given that unidirectional conduction block occurs. The first is reentry, and the second is spiral tip annihilation due to a tip-tip interaction. As Winfree described in the original pinwheel hypothesis, the tip-tip interaction occurs when an area with a radius less than
a critical radius $R_c$ is depolarized. This critical radius for reentry $R_c$ is an unknown quantity governed by the dynamic properties of the tissue. Here we attempt to derive $R_c$. As illustrated in Fig. 1A for the critical case, the time, $T_{sc}$, taken for the newly formed spiral wave tips (dashed fronts) to rotate (along the thin dashed circle) from their formation sites to where the S2 was applied is:

$$T_{sc} = \frac{\pi R_c}{20_c}$$ or $$R_c = \frac{20_c T_{sc}}{\pi}$$

(5)

where $v_c$ is the tip velocity of the spiral wave and is determined by the conduction velocity restitution and the wave front curvature (11, 36). However, for the spiral wave tips to successfully pass this point without being blocked or annihilated because of tip-tip interactions, $T_{sc}$ must be equal or longer than the refractory time, $T_R$, at the stimulation site after the S2 is given. Thus, if $T_R > T_{sc}$, then $R_c$ is determined by $T_R$. Therefore,

$$R_c = \begin{cases} \frac{20_c T_{sc}}{\pi} & \text{if } T_{sc} \geq T_R, \\ \frac{20_c T_R}{\pi} & \text{if } T_{sc} < T_R \end{cases}$$

(6)

When $R_c$ is constant, Eq. 3 is parabolic and thus the vulnerable window is symmetrical. Generally, $T_R$ is not constant but is affected by cellular electrophysiological properties, S1-S2 coupling interval, and the S2 domain, all of which break the symmetry of the vulnerable window.

We first show how $T_R$ is affected by the above factors. Figure 6A shows voltage traces from the center of the tissue for two different S1-S2 intervals in the case of a shallow APD restitution curve with longer APD (triangles in Fig. 2). A full action potential is elicited if the S2 is applied after the S1 has fully recovered (gray trace in Fig. 6A), whereas a slightly prolonged APD (what we refer to as “graded response”) is elicited if the S2 is given too early (black trace in Fig. 6A). The APD or the duration of the graded response of the S2 beat depends on the cellular electrophysiological properties. In Fig. 6B, we show spatial $T_R$ distributions (calculated in the 1D cable with Eq. 2) for the four APD restitution curves shown in Fig. 2. $T_R$ of a spatial location is defined as the time difference between the onset of S2 and the time at which this location has repolarized to $-72$ mV. When a full action potential is elicited for a cell inside the S2 domain $T_R = \text{APD}$, but when a graded response results $T_R$ is the duration of the graded response. If the cell is outside the S2 domain, then $T_R$ is APD plus the time that it takes S2 to propagate to that location, i.e., $T_R = \text{APD} + \Delta l/v$, where $\Delta l$ is the distance from the spatial location to the S2 domain and $v$ is the conduction velocity of the S2 beat. Therefore, $T_R$ generally increases from right to left as shown in Fig. 5.
Fig. 6. Effects of graded action potential responses on the vulnerable domain. **A**: sample voltage traces within the area of S2 stimulation exhibiting graded action potential responses. Traces were taken from the shallow APD restitution with long APD case. **B**: Spatial distribution of refractory times $T_R$ after S2 along the diagonal of the tissue for the 4 different APD restitution cases. The center of the diagonal is denoted as 0 cm. $R = 1.2$ cm, $T = 320$ ms. 
- Shallow APD restitution with short APD; 
- Shallow APD restitution with long APD; 
- Control APD restitution; 
- Steep APD restitution. 
**C**: refractory time $T_R$ at the center of the cable (0 cm) fit by Eq. 7 and plotted for various $R$ (cm) and S1-S2 intervals (175, 190, 205, and 210 ms) for the shallow APD restitution with short APD case. $D$: same as in C for the shallow APD restitution with long APD case (for S1-S2 intervals of 280, 295, 310, and 325 ms). $E$ and $F$: analytically derived vulnerable domains, using Eqs. 3, 6, and 7. $T_R$ was derived from the data in $C$ and $D$. For the shallow APD restitution with long APD case, the following fitting parameters were used: $\alpha = -6.279$, $\beta = 65.61$, $\delta = 1.63$, and $\tau = 0.24$. For the shallow APD restitution with short APD case, the following fitting parameters were used: $\alpha = 47.8$, $\beta = 157.37$, $\delta = -0.23$, and $\tau = 0.124$. For the steep APD restitution allows the reentrant spiral tips to propagate into a central common pathway. However, the fused wave front subsequently collides with the refractory tail of S2 and disappears (Fig. 5C). This phenomenon only occurs when APD restitution is steep and only at small $R$. The following questions are now raised: Why does wave front-wave back interaction occur only with steep restitution? How does it occur? Why only at small $R$?

$T_R = (T - \beta) + \frac{\alpha - (T - \beta)}{1 + e^{(x + \frac{\delta}{\tau})}}$ (7)

where $\alpha$, $\beta$, $\delta$, and $\tau$ are fitting parameters. By combining Eqs. 3, 6, and 7, we can analytically determine the vulnerable window of reentry. Figure 6, $E$ and $F$, show the vulnerable windows calculated with these equations for the two shallow APD restitution cases, which agree well with the simulation results shown in Fig. 4, $A$ and $D$, although they are not quantitatively to scale.

**Effects of APD restitution steepness.** In the case of reentrant failure due to wave back-wave front interactions, the tips of the two spiral waves do not annihilate but successfully propagate into a central common pathway. However, the fused wave front subsequently collides with the refractory tail of S2 and disappears (Fig. 5C). This phenomenon only occurs when APD restitution is steep and only at small $R$. The following questions are now raised: Why does wave front-wave back interaction occur only with steep restitution? How does it occur? Why only at small $R$?

Figure 6B shows that $\partial T_R/\partial x$ with steep restitution is greater than $\partial T_R/\partial x$ with shallow restitution. Specifically, $T_R(x)$ near the S2 domain for steep restitution is shorter than $T_R(x)$ for shallow restitution at long APDs. This difference in $T_R(x)$ for steep APD restitution allows the reentrant spiral tips to prop-
agate into the S2 domain and form a central common pathway, whereas for shallow APD restitution, especially at long APDs, the S2 domain remains refractory and the spiral tips cannot propagate inward.

To see why wave front-wave back interactions only occur at small R, we examine the diagonal of the tissue to see how the reentrant wave front interacts with the S2 wave back. This case is analogous to a previously studied case of unidirectional conduction block in a 1D homogeneous cable (34), where S1 begins from one end of the cable and S2 and S3 are applied in the other end of the cable. In the present case, the reentrant wave after S2 is equivalent to the S3, as illustrated by the arrow in Fig. 7A. A major difference is that S2 has a finite size in the present study, which affects APD or $T_R$. An analytical explanation for why wave front-wave back interactions only occur at small R is as follows. In cardiac cells, APD can be expressed as a function of its preceding DI, i.e.,

$$a = f(d)$$

where a represents APD and d the previous DI. In general, f is a function that increases monotonically with d. The DI $d(x)$ preceding S2 at location x is governed by

$$\frac{d[d(x)]}{dx} = \frac{1}{\theta_1(x)} - \frac{1}{\theta_2(x)}$$

where $\theta_1(x)$ is the wave front velocity of S1 at location x and $\theta_2(x)$ is the wave front velocity of S2. If S2 depolarizes the whole cable simultaneously, then $\theta_2(x) \to \infty$. By setting $\theta_1(x) = \theta = \text{constant}$, then $d(x) = d(0) - x/\theta$. If S2 only depolarizes a small region and propagates with a constant speed $\theta$, i.e., $\theta_2(x) = -\theta$ (negative sign is due to S2 propagating in the opposite direction to S1), then $d(x) = d(0) - 2x/\theta$.

Therefore, the DI gradient is $-2/\theta$, which is twice the former case, and will give rise to a larger APD gradient in space as indicated by $Eq. 8$. To demonstrate this effect, we show the profile of $T_R$ in space in Fig. 7B for an S2 depolarizing either the whole space (dashed line) or a small space in the center (solid line). At small R, $T_R$ is shown to be steeper. When S2 is larger, it takes more time for the reentrant wave to reach the wave back of S2, leaving more room for the tips to propagate. Therefore, wave front-wave back interaction preferentially occurs with steep APD restitution and a weaker S2 stimulation, in agreement with the numerical results.

**DISCUSSION**

In this study, we have extended Winfree’s pinwheel experiment to characterize how cellular electrophysiological properties affect vulnerability to reentry by a premature stimulus in homogeneous monodomain 2D tissue. We found that graded action potential responses, APD, and APD restitution all affect the shape and size of the vulnerable window. Larger graded responses and long APD reduce the vulnerable window by promoting tip-tip interactions at long S1-S2 coupling intervals, causing the two initiated spiral wave tips to collide and annihilate. Steep APD restitution promotes wave front-wave back interaction, allowing the tips to form a central common pathway but preventing their exit because of collision with the S2 wave back. Our analytical treatment provides mechanistic explanations for the phenomena observed in the numerical simulations.

Effects of graded responses, APD, and APD restitution on vulnerability to reentry by an electrical shock. The pinwheel experiment or critical point hypothesis, first proposed by Winfree (50–52), was based on general features of an excitable medium (Fig. 1) yet has greatly improved our understanding of initiation of reentrant arrhythmias by extrastimuli such as electrical and mechanical shocks. This hypothesis has been further extended to bimodomain tissue (27, 40, 43). In this study, we extended Winfree’s hypothesis to include the effects of cellular electrophysiological properties, namely, graded action potential responses, APD, and APD restitution.

Spiral wave annihilation due to tip-tip interaction is one of the two major determinants affecting the vulnerable window and is influenced by the size and timing of the S2 stimulus, the APD, and the size of the graded response. The effects of size and timing of the S2 stimulus are well explained by Winfree’s pinwheel experiment theory (50–52). The effects of APD can be intuitively understood as related to its effects on spiral period ($2T_{sc}$) and core diameter, which are critical determinants of tip-tip interaction. How spiral wave period relates to APD and refractory period has been described in our previous study (36). Basically, a longer APD at the minimum DI before conduction failure has little effect on the critical conduction velocity but increases the diameter of the spiral wave core. Thus the critical radius $R_c$ required to avoid tip-tip interaction is greater when APD is long, thus reducing the vulnerable window.

![A](http://ajpheart.physiology.org/)
![B](http://ajpheart.physiology.org/)

**Fig. 7.** Mechanism of wave front-wave back interactions at small R. A: voltage snapshot illustrating the state of excitation along the diagonal 600 ms after S2. B: spatial distribution of refractory time $T_R$ under steepened restitution parameters along the first half of the diagonal (as indicated by dashed line in A) showing a steeper gradient of refractoriness with a small area of depolarization (solid line, $R = 0.6$ cm) compared with a large area of depolarization (dashed line, $R = 3\sqrt{2}$ cm).
The effects of graded responses were studied by Gotth et al. (16), who found that giving an S2 extrastimulus during the refractory period (wave back) of a propagating wave lengthened its refractory period in proportion to the intensity of the stimulus. Because the graded response forms an island of prolonged refractoriness in which the spiral tips cannot reenter, they travel around the island to eventually collide and annihilate, the tip-tip interaction as shown in our present study. Gotth et al. concluded that a stronger stimulus gave rise to a stronger graded response, which prevented figure-of-eight reentry from forming, thus providing an alternative explanation to the upper limit of vulnerability to Winfree’s pinwheel theory. As we explained in METHODS, we cannot directly simulate the effects of stimulus strength in monodomain tissue and thus cannot simulate the observations by Gotth et al. However, the upper limit of vulnerability can be explained with our analytical method as follows. It is known that under external stimulation the electrical potential decays exponentially in cardiac tissue (22). We assume that the stimulation strength follows the same decaying manner, i.e.,

\[ I(r) = I_0 e^{-rc} \]  
(10)

where \( I(r) \) is the effective stimulus strength at location \( r \), \( I_0 \) is the stimulus strength at the location of the electrode, and \( c \) is the space constant. At location \( R \), the effective stimulus strength decays to the threshold \( I_n \) needed for depolarization:

\[ R = c \ln \frac{I_n}{I_0} \]  
(11)

As shown by Gotth et al. (16) and Karaguezian and Chen (21), the graded response increases as stimulus strength increases; we modify Eq. 7 into the following form:

\[ T_h = T - \beta + G(I_0) \]  
(12)

where \( \beta \) is a parameter and \( G(I_0) \) is the duration of graded response as a function of \( I_0 \). On the basis of data from Karaguezian and Chen (21), \( G(I_0) \) is close to a sigmoidal function and can be roughly approximated by \( G(I_0) = 50 - 50[1 + \exp(-I_0 - 40)/7] \) (see Fig. 1 of Ref. 21). Combining Eqs. 3, 6, 11, and 12, we obtained an upper limit of vulnerability as \( I_0 \) increases, as shown in Fig. 8. Another mechanism for the upper limit of vulnerability based on virtual electrodes was proposed by Rodriguez and Trayanov (38).

Although in our monodomain model the upper limit of vulnerability is the same as Winfree’s original hypothesis (50), i.e., the size of the tissue, our conclusions about the effects of graded response and their effect on the vulnerable window are still valid in the presence of the above new mechanisms for upper limit of vulnerability. A novel observation in the present study relates to the effects of steep APD restitution slope, which causes wave front-wave back interactions to reduce vulnerability to reentry by large-amplitude shocks. The effects of APD restitution on vulnerability to reentry by near-threshold extrastimuli have been studied previously in homogeneous and heterogeneous tissue models (9, 10, 33, 34). Steeper APD restitution promotes reentry initiation through the induction of discordant APD alternans by rapid pacing (5, 12, 31, 33) as well as by increasing dispersion of refractoriness by a premature near-threshold stimulation (10, 33, 34). In the case of high-strength (or large spatial domain) stimulation as shown in the present study, steep APD restitution induces dispersion of refractoriness, which tends to block the propagation in the central common pathway by wave front-wave back interaction.

As shown by our results, agents that shorten APD and reduce APD restitution slope will increase the vulnerability by electrical shocks. A very interesting experimental study by Cheng et al. (7) showed that the excitation-contraction uncouplers 2,3-butanedione monoxime (BDM) and cytochalasin D (Cyto D) affect vulnerability differently during electric shocks. BDM shortens APD and makes APD restitution shallower compared with Cyto D, which has also been shown by others (3, 24). These authors demonstrated that BDM resulted in a significant increase of vulnerability to shock-induced arrhythmias compared with Cyto D, in agreement with our simulation and theoretical results.

Arrhythmogenesis by physiological extrastimuli and mechanical shocks. For physiological premature extrasystoles, a critical gradient in refractoriness is required for reentry initiation (1, 23, 35). However, early or delayed afterdepolarizations can develop over a large area of tissue, which could induce figure-of-eight reentry and fibrillation as in the pinwheel experiment scenario without the need of a critical gradient of refractoriness. Another scenario relevant to our present study is mechanically induced depolarization of large spatial domains, as in commotio cordis (25, 29). In commotio cordis, the mechanical stimulus inducing ventricular fibrillation is likely to represent stretch-induced depolarization of a large region in the wake of a sinus beat. We believe that the effects of APD and APD restitution on vulnerability to reentry shown in the present study are also applicable to these clinically relevant scenarios.

Limitations. The findings of the present study were obtained in simulated monodomain homogeneous 2D tissue, in order to complement and facilitate the theoretical analysis of the underlying mechanisms. Accordingly, caution must be exercised in extrapolating the findings to real cardiac tissue, which is bidomain, 3D, and heterogeneous. For instance, in bidomain tissue, large electrical shocks create virtual electrodes with adjacent anodal and cathodal regions in a dog bone pattern, so that graded responses can be either positive or negative (48),
which may have different effects on vulnerability (14, 27). The waveform of the electrical stimulus, which modulates the dog bone pattern, also has an important impact on vulnerability (2, 14, 26, 27, 40). Another related limitation in our present study is that the monodomain tissue, unlike the bidomain tissue, cannot simulate the effects of increased stimulus strength directly; instead, we used a constant stimulation strength over a certain area of tissue. In real tissue, the stimulation strength over the depolarized area is nonuniform, which may produce additional effects. 3D tissue micro- and macroanatomy may also play important roles (37, 45–47) not accounted for in the present study. In addition, since our initial focus was to delineate the effects of APD and APD restitution properties on the present study. In addition, since our initial focus was to delineate the effects of APD and APD restitution properties on vulnerability, we chose a simple ionic action potential model that does not contain detailed intracellular calcium cycling as in more recent physiologically detailed models (17, 19). Intracellular calcium cycling is likely to have its own important effects, which will be important to characterize in future studies. Nevertheless, the pinwheel experiment and critical point hypothesis originally formulated by Winfree have played a valuable role in advancing our understanding of electrical fibrillation and defibrillation. We hope that the extension of Winfree’s pinwheel experiment in the present study to consider the role of cellular electrophysiological properties will play a similar role, serving as a useful platform from which to launch more advanced simulations such as bidomain studies or studies involving more detailed calcium cycling, as well as experimental tests of its predictions.

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REFERENCES


37. Rodriguez B, Li L, Eason JC, Efimov IR, Trayanova NA. Differences between left and right ventricular chamber geometry affect cardiac vulnerability to electric shocks. Circ Res 97: 168–175, 2005.


