Resetting and Annihilating Reentrant Waves in a Ring of Cardiac Tissue: Theory and Experiment

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Theory predicts that a stimulus delivered to an excitation wave circulating on a ring of excitable media will either have no effect, or it will reset or annihilate the excitation depending on the phase and magnitude of the stimulus. We summarize the basis for these theoretical predictions and demonstrate these phenomena in an experimental system consisting of a tissue culture of embryonic chick heart cells cultured in the shape of a ring.

§1. Introduction

In the normal heart, a specialized region of the heart called the sinus node sets the rhythm of the heart beat leading to a wave of excitation and contraction that spreads throughout the heart. Cardiac arrhythmias are abnormal heart rhythms reflecting disturbances in the initiation and/or the conduction of the heart beat. An important class of serious arrhythmias are reentrant arrhythmias in which the cardiac excitation traverses a circuitous pathway. In this class of arrhythmias the time between successive heart beats is set by the time it takes the excitation to travel in the circuitous path. Since this time is usually shorter than the period of the sinus node, the heart beat is abnormally rapid and is called a tachycardia. Mines proposed a conceptual model for a reentrant tachycardia in which the excitation travels in a one-dimensional annulus consisting of various normal and abnormal anatomical features in the heart.[1] In the clinic, a single stimulus may be effective in either resetting or annihilating a reentrant tachycardia, and Mines' model is often invoked to help understand these phenomena.[2] Because of the relevance to cardiac arrhythmias, there have been many analyses of the effects of electrical stimuli delivered to theoretical models of cardiac waves traveling on a ring or an annulus.[3-7] These theoretical studies have demonstrated that a single stimulus can be effective in either resetting or annihilating a circulating excitation. In addition, experimental systems in which stimulation is delivered to an excitation traveling in a ring of cardiac tissue have shown similar results.[8,9]

Because of the importance of geometrical features of the substrate on cardiac arrhythmia formation, researchers have developed methods to grow cardiac cells in

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tissue culture in predetermined geometries.[10)-12)] However, these earlier studies in tissue culture did not investigate the effects of electrical stimulation delivered during the course of a model cardiac arrhythmia. In this article, we summarize earlier theoretical work that shows that a single stimulus should be capable of either resetting or annihilating a circulating excitation.[5)-7)] We then show experimental results demonstrating resetting and annihilation of reentrant excitation in embryonic chick heart cells cultured in the form of a one-dimensional ring.

§2. Theoretical predictions

Our theoretical analysis is based upon numerical simulation and conjectures that were initially presented in Refs. 5)-7). We briefly summarize the earlier results. The original papers should be consulted for details.

The conjectures deal with the effects of stimulation on a wave of excitation traveling in a one-dimensional ring of excitable medium. By excitable medium we refer to a physical or biological system, or a mathematical model of these systems that support traveling waves, called excitation waves. Following an excitation in a localized region, the tissue in that localized region is refractory and cannot support another excitation for a length of time called the refractory time. As a consequence, two excitations in a one-dimensional ring traveling towards each other in opposite directions will annihilate each other. The FitzHugh-Nagumo equations are often used to model excitable media such as the heart and nerve cells.[13)]

We assume that the excitable medium is in the form of a one dimensional ring that is sufficiently large that a traveling wave will propagate around the ring at a constant velocity (in smaller rings there can be complex quasiperiodic fluctuations of cycle time,[14)] but we do not consider these situations). Further we assume that a stimulus delivered to a ring far outside of the refractory time will lead to the initiation of two waves traveling around the ring in opposite directions. In the simplest case all waves travel around the ring with the same constant velocity, though more realistically the velocity of propagation is often slowed for waves following closely after another wave.[13)]

These assumptions have some interesting and experimentally testable consequences. To illustrate these we plot curves that show the perturbed cycle for a schematic model of excitable medium embodying only the above two assumptions and assuming that all propagating waves travel at constant velocities. The perturbed cycle length T_n is the time from the first beat before a stimulus to the nth beat after a stimulus. According to the above assumptions, if the stimulus falls during the refractory period, then $T_n = nT_0$, where T_0 is the intrinsic cycle length. The consequences of these two assumptions are illustrated by the solid lines in Fig. 1. The key feature of this figure from the current perspective is that at the end of the refractory period there is a discontinuity in the plot of the perturbed cycle length curve.

Numerical studies show that discontinuities in this type of resetting curve persist even in mathematical models that are formulated as nonlinear partial differential equations supporting waves circulating on rings.[5)] However, there is an important difference between the dynamics in the nonlinear partial differential equations and

the model described in the above paragraphs. Numerical studies carried out on the FitzHugh-Nagumo equation show that stimuli delivered just after the end of the refractory period lead to a single wave traveling in a direction opposite to the original wave.[19),5)] Although these numerical results were confined to particular equations, we have conjectured they are a much more general consequence of discontinuities in the resetting curves. *Conjecture: Given an excitable medium in a one-dimensional ring supporting reentrant excitation, stimuli can be found which would lead to annihilation of the reentrant wave.*[5)] Gedeon and Glass[7)] developed a strategy for a proof based on the following steps.

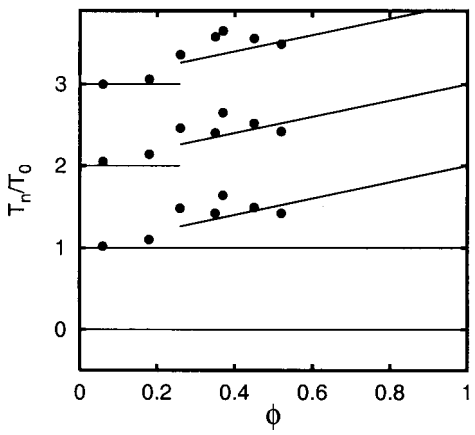

Fig. 1. The normalized perturbed cycle as a function of the phase of a stimulus delivered to a ring supporting a circulating excitable wave with a refractory period that is about one quarter the period of the cycle. The solid lines represent theoretical predictions for the model described in the text. The symbols represent an experiment carried out as described in the following section.

1. Excitable media in the geometry of a one-dimensional ring, support a stable circulating pulse, i.e., an asymptotically stable limit cycle in the associated partial differential equation.
2. Continuity theorem: If a perturbation delivered at any phase of a limit cycle oscillation leaves the state point in the basin of attraction of the asymptotically stable limit cycle, then the resetting curves characterizing the effects of the stimuli, will be continuous.
3. Resetting curves for stably circulating pulses on a one dimensional ring of an excitable medium are discontinuous.
4. It follows from 2 and 3 that there is a critical stimulus (or stimuli) that will lead to a point in the phase space outside of the basin of attraction of stable periodic orbit.

The above argument is based on continuity considerations for dynamics in idealized mathematical models of excitable media. A key element, the Continuity Theorem was proved in Ref. 7), but points 1 and 3 still remain unproved. Moreover, the conjecture does not specifically assert that a stimulus can be found that will annihilate the rhythm (though this is what was found in mathematical models of excitable media,[5),6)] but rather that a stimulus can be found that will lead to a qualitatively different dynamics from the original circulating wave. Consequently, it is important to test these predictions in experimental settings.

§3. Experimental studies

In order to test the theoretical predictions experimentally, we have studied dynamics in tissue cultures consisting of embryonic chick heart cells grown in a ring geometry and studied the propagation of traveling waves in these cells using Ca^{2+} sensitive dyes. Complex spontaneous rhythms that are observed in these preparations can be modeled using modified FitzHugh-Nagumo equations.[12] We now describe initial results describing the dynamics observed when these preparations are subjected to electrical stimulation with single stimuli.

Cell cultures were prepared from embryonic chick ventricles according to previously established procedures[16)-18),12)] which should be consulted for details. The cells were maintained in the medium at 5% CO_2 at 36°C for 3-4 days before experiments were undertaken. To construct the ring, dishes were first coated with cell adhesive (Cell-Tak, Collaborative Biomedical Products) diluted in sodium bicarbonate 0.1 mol/L and pH 8.0. After drying, the culture dishes were thoroughly rinsed with doubly distilled water. To create the ring geometry, we constructed a template from a cylindrical piece of Plexiglas. On one end of the template, an annular depression with dimensions of 8 mm outer diameter and 6 mm inner diameter was machined into the Plexiglas. The raised portions of the template were first coated with silicon polymer (3140 Dow-Corning) and then applied to the culture dishes. The regions of culture dishes that contacted the siliconized regions of template did not favor cell adhesion, leaving an annular region as the substrate for tissue culture growth.

Cell cultures were maintained at 36 ± 1°C during the recordings. Electrical stimulation was delivered by means of a bipolar teflon coated platinum electrodes (diameter 120 μm) separated by 1 mm. Cells were stimulated with rectangular pulses of 20 or 30 ms at twice diastolic threshold current delivered from a Frederick-Haer stimulator. Resetting experiments were carried out in two preparations that showed stable reentrant excitation in the absence of stimulation. In each experiment, stimuli were delivered at several different phases of the cycle from a single location.

To allow low light level measurements at low magnification scales, we constructed a tandem lens macroscope[15)] as previously described.[18)] Images were collected using a cooled CCD camera (Princeton Instruments, Model TE CCD 576). Adjacent pixels in the CCD were binned (3×3) and up to 30000 consecutive images at a sampling speed of 50 msec/frame were transferred directly to a Pentium 90 computer for storage and analysis. The recorded data was filtered using a rotationally symmetric 2-by-2 Gaussian low pass filter with mainlobe width $\sigma = 1.5$ pixels. We used a Ca^{2+} sensitive dye (Calcium Green-1, Molecular Probes, Eugene, Oregon) to image the propagation of the wave. To measure the timing of the activity waves in a region, the local fluorescence is plotted as a function of time (see panels C in Figs. 2 and 3), and the crossing time of a threshold lying at about half maximum amplitude is determined by interpolation. This gives an upper estimate of the error of timing of events ± 50 msec.

Figure 2 shows the effect of an electrical stimulus applied at 11 o'clock at a sufficiently long time interval after a wave of excitation that the tissue was no longer

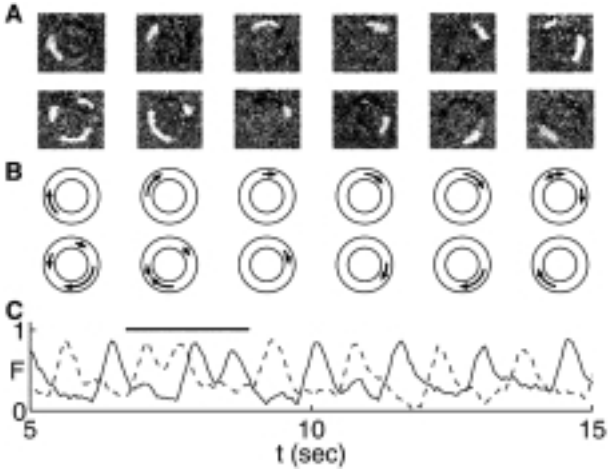

Fig. 2. Resetting of a reentrant rhythm. In this and the following figure, the normalized intensity of fluorescence F recorded from 11 o'clock is indicated by a dashed curve and from 5 o'clock is indicated by a solid curve. The bar over the trace in panel (C) indicates the time interval for which fluorescence is displayed in panels (A) and (B). The stimulus delivered at 11 o'clock after the fifth panel in the first row leads to a resetting of the rhythm. (A) Fluorescent images displayed at 200 msec intervals. (B) Schematic diagram. (C) Normalized intensity of fluorescence. The excitation wave recorded at 5 o'clock was advanced by approximately 0.7 sec due to the stimulus.

refractory. The last panel of the last row in Fig. 2(A) illustrates the generation of two new traveling waves. The counterclockwise traveling wave collides with the original clockwise wave. The newly initiated clockwise wave continues circulating. The net effect is that there is once again a single clockwise wave. However, the timing of this wave is reset, so that subsequent activations at a given location on the ring occur at different times than would have been observed if there had not been a stimulus. The normalized perturbed cycle length that was found in this preparation is displayed as the data points in Fig. 1. The discrepancies between the experiments and the solid lines, representing the theoretical predictions, arise as a consequence of errors made in estimating the timings of the waves as well as the slowing of the propagation velocity that occurs when a wave follows closely after an earlier one.

A different effect is observed from electrical stimulation delivered with a short latency (approximately in the range of 80 and 120 msec) following an activation, Fig. 3. Electrical stimulation at 12 o'clock applied with a short latency following the passage of a wave circulating in a counterclockwise direction, Fig. 3(A) second panel, second row, initiated only a single wave, traveling clockwise. This wave collides with the original counterclockwise traveling wave to lead to annihilation of the reentrant rhythm. The initiation of a single wave verifies theoretical predictions of a "vulnerable phase" of cardiac tissue.[19),5)] The annihilation of the reentrant wave following collision of the induced wave with the original reentrant wave also follows theoretical predictions.[5)] Following a pause of about 5 sec, a localized pacemaker leads to a single non-reentrant excitation.

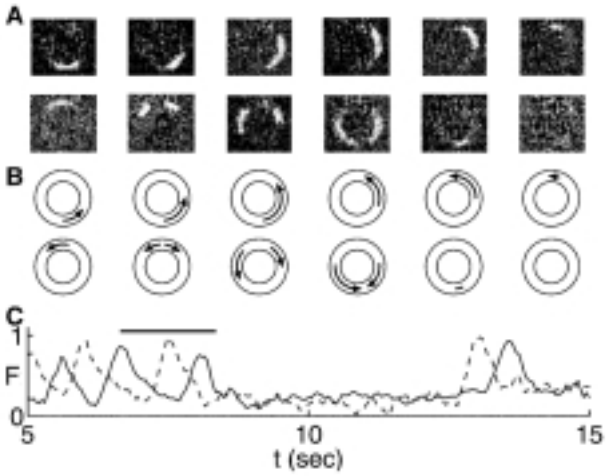

Fig. 3. Annihilation of a reentrant rhythm. The stimulus at 12 o'clock, delivered after the first panel in the second row, leads to the initiation of a single wave propagating in a clockwise orientation. This collides with the original wave traveling in a counterclockwise direction leading to annihilation of the reentrant rhythm. Following a pause, pacemaker oscillations reappear. (A) Fluorescent images recorded at 150 msec intervals. (B) Schematic diagram. (C) Normalized intensity of fluorescence.

§4. Conclusions

Although the real heart is an extraordinarily complex three dimensional structure, cardiologists have developed simplified conceptual models that have been useful for understanding mechanisms of arrhythmia and to help guide therapy. In this paper we have considered an extraordinarily simple model, in which an excitation is imagined to traverse a one-dimensional circular track. Earlier theoretical studies, based on modeling the excitation by nonlinear partial differential equations, had shown that a single stimulus can have different effects including resetting of the circulating rhythm and annihilation of the circulating pulse. In the current article we have shown initial experimental studies that indicate that resetting and annihilation can also be obtained by applying electrical stimuli to cardiac tissue cultured in the shape of a ring. From a medical perspective, the annihilation of the circulating rhythms here corresponds to the annihilation of serious tachycardias in a clinical setting by using single stimuli delivered directly to the heart either through a catheter or an implanted anti-tachycardia pacing device. These results show the feasibility of studying the interaction between electrical stimulation and complex arrhythmia in model systems constructed in controlled geometries.

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