Paroxysmal Starting and Stopping of Circulating Waves in Excitable Media

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Levels of intracellular Ca^{2+} were monitored using fluorescence from Ca^{2+}-sensitive dyes in chick embryonic heart cells cultured in an annular geometry. There was spontaneous starting and stopping of reentrant waves of activity. The results are modeled using modified FitzHugh-Nagumo equations representing pacemakers embedded in a conducting medium. These results provide a potential mechanism for spontaneous abnormal cardiac rhythms in which there are rapid heart beats (tachycardias) that repetitively start and stop.

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In excitable media, such as the Belousov-Zhabotinsky reaction or the heart, constant amplitude waves propagate, but the collision of two waves leads to the annihilation of both. This observation leads one to expect that, in a ring or annulus composed of an excitable medium, a circulating wave, once started, will continue to propagate endlessly [1–6]. This reflects a principal that Winfree originally called "the conservation of winding number" [1]. If the local state of a system is described by a single phase variable defined by a phase on a circle that is coupled diffusively to its neighborhood, then the number of waves trapped on a ring must remain constant over time. Similar observations apply to some discrete rings of coupled nonlinear oscillators [7].

There are circumstances in which the conservation of winding number does not apply. Many serious heart conditions are associated with circulating waves of excitation on anatomically defined rings or annuli in the intact heart, leading to an abnormally rapid heartbeat (tachycardia) [8]. Cardiologists hypothesize that such abnormal reentrant waves are initiated by blockade of one of the two waves of excitation on a ring starting from a single source. Following this unidirectional block, the nonblocked wave can revisit the site of blockade from the opposite direction, leading to a reentrant rhythm. Moreover, such rhythms may suddenly (paroxysmally) start and stop, giving rise to serious and complex rhythms [9,10]. In this Letter we describe an experimental cardiac system in which there is a spontaneous starting and stopping of circulating waves on an annulus and offer a theoretical model for this phenomenon.

To study the dynamics of excitation waves trapped on rings, we have developed a tissue culture model in which chick ventricular cells were grown in a ring geometry [11] and studied optically by monitoring Ca^{2+} fluorescence using a specially constructed macroscope similar to one used previously [12–14]. Fifteen preparations were studied. In five of the preparations, there were one or more localized pacemakers which generated waves that collided and annihilated. In five of the preparations there was circulation of a single wave (3 clockwise, 2 counterclockwise), that was present initially and continued circulating throughout the recording (4/5) or terminated without restarting (1/5). However, in 5 preparations, there were complex repetitive rhythms in which there were repeated initiation and termination of circulating rhythms.

Figure 1A shows the fluorescent images and Fig. 1B shows a schematic representation of the dynamics from a preparation displaying complex dynamics. The top line shows two waves initiated by a pacemaker at 12 o'clock that collide and annihilate at 6 o'clock (row 1, panel 5). Following a quiescent period of 1.75 sec (not displayed), there is a second wave initiated by the pacemaker at 12 o'clock (rows 2 and 3). In addition, a second pacemaker at 4 o'clock spontaneously fires (row 2, panel 3). The counterclockwise circulating wave from the pacemaker at 4 o'clock collides with the clockwise wave from the pacemaker at 12 o'clock and both annihilate. However, the clockwise wave from the pacemaker at 4 o'clock continues circulating, presumably because the original counterclockwise wave from the pacemaker at 12 o'clock was unidirectionally blocked at 8 o'clock (row 2, panel 4). The newly initiated counterclockwise reentrant wave is itself blocked before a complete circulation at 2 o'clock (row 3, panel 5). Following another quiescent period of 1.75 sec (not shown), there is again an initiation of a reentrant wave by the pacemaker at 12 o'clock (row 4).

In the current experiment, regions in which excitation is blocked leading to reentry (e.g., 8 o'clock in Fig. 1B: row 2, column 4; row 4, column 3) can also successfully conduct excitation in the opposite direction (Fig. 1B: row 1, column 4). This indicates that the properties of the medium must be changing in response to the ongoing activity. Our theoretical model is based on the interactions of pacemakers embedded in an excitable medium in a ring geometry. In response to a stimulation more rapid than its intrinsic frequencies, a cardiac pacemaker displays overdrive suppression [15] in which its frequency transiently decreases and its refractory period transiently increases. Previous studies demonstrated overdrive suppression in

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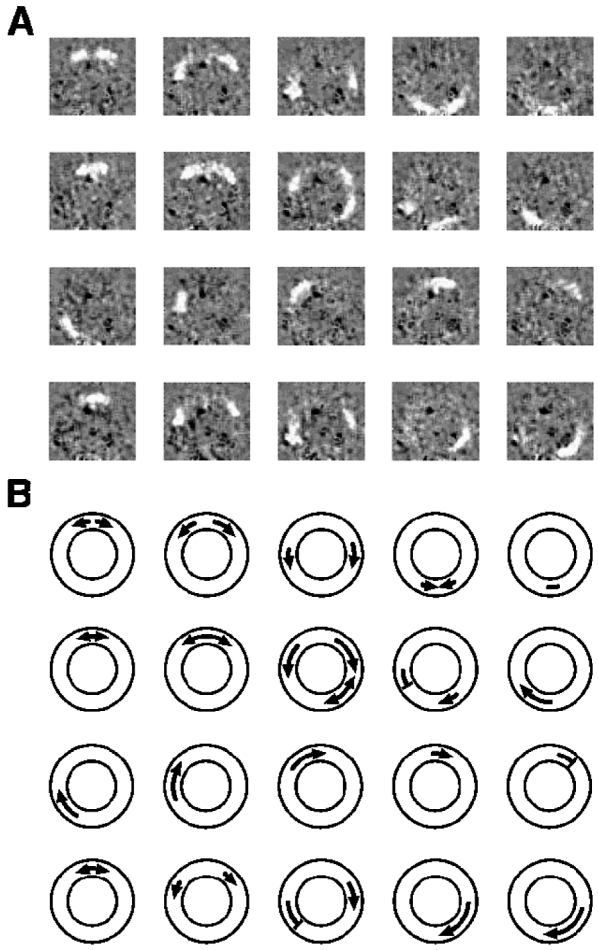

FIG. 1. Complex rhythms in a preparation with pacemakers and reentry. (A) Fluorescent images displayed with a 250 msec interval between frames. There are two quiescent intervals of 1.75 sec that are not recorded, separating the recordings in the first and second rows and the third and fourth rows. The outer diameter of the ring is 8 mm. (B) Schematic diagram of the dynamics in (A).

an experimental preparation consisting of spontaneously beating aggregates of chick heart cells, and developed a quantitative model of these phenomena [16]. However, this work did not couple the aggregates by an excitable medium.

In order to model the paroxysmal starting and stopping of a circulating excitation wave, we used modified FitzHugh-Nagumo (FHN) equations [6,17] with two localized pacemakers. Similar to real physical and biological excitable medium, the FHN equations allow propagation of waves in response to a stimulus, and also display a refractory period following the passage of a wave, during which stimuli are not effective in inducing a new propagating wave. In the current modification, v corresponds to the membrane voltage, w is a variable that is associated with refractoriness, and z is a variable that tracks the recent history of the activity of a pacemaker and reflects its excitability. We imagine z to represent a positive ion that enters a pacemaker cell during an action potential. In order to maintain appropriate ionic concentrations the positive ion must be pumped out of the cell, and this current counters the tendency of the cell to fire spontaneously. Thus, increased recent activity leads to increased refractoriness, and a longer cycle time. The equations are

$$\begin{aligned}\frac{\partial v}{\partial t} &= -(v + 0.1)(v - 0.9)(v - 0.039) \\ &\quad - w + D\frac{\partial^2 v}{\partial r^2} + I, \\ \frac{\partial w}{\partial t} &= (0.005v - 0.01w + 0.0005)R(\zeta, v), \\ \frac{dz}{dt} &= -\gamma_\alpha z + (\Delta z)\delta(t - t_{AP}),\end{aligned} \tag{1}$$

where D is the diffusion coefficient, I is the input current for the pacemaker activity, γ_α is a decay constant for pacemaker α, Δz is the increment of z resulting from an activation of the pacemaker, and $\delta(t - t_{AP})$ is a delta function that equals 1 at the initiation of an action potential at a pacemaker at time t_{AP}. $R(\zeta, v)$ is a function that controls the refractory period of the pacemaker cells [17] in terms of $\zeta(z)$,

$$\begin{aligned}\zeta(z) &= \frac{0.015}{z + 1.0}, \\ R(\zeta, v) &= \begin{cases} \frac{(1-\zeta)}{1+10e^{-10(v-0.1)}} + \zeta, & \text{pacemaker cells,} \\ 1, & \text{otherwise.}\end{cases}\end{aligned} \tag{2}$$

We assume that t_{AP} represents the time the v variable at a pacemaker passes through a threshold of $v = 0.5$ during the upstroke of the action potential. The net consequence of this equation is that, if a pacemaker is stimulated rapidly, there is a buildup of z and a corresponding decrease of ζ, leading to a longer cycle time and a longer refractory period (overdrive suppression) at the pacemaker. The form of this equation is similar to kinetic equations that we have used in related contexts to model the effects of rapid stimulation on cardiac tissue [16,18], but the parameter values and functions have been adapted to model the current experiments [19].

The way in which this theoretical model leads to cycling between reentrant excitation and pacemaker activation can be appreciated by following the levels of z at the slow pacemaker (Fig. 2A). At first, excitation from the fast pacemaker entrains the slow pacemaker, leading to an increase of z. During this time, the two waves generated by the fast pacemaker collide at a position directly opposite from the location of the fast pacemaker (Fig. 2B). This process continues until the refractory period of the slow pacemaker increases to a level that leads to block one of the waves from the fast pacemaker. However, the other wave from the fast pacemaker travels by a longer route, and activates

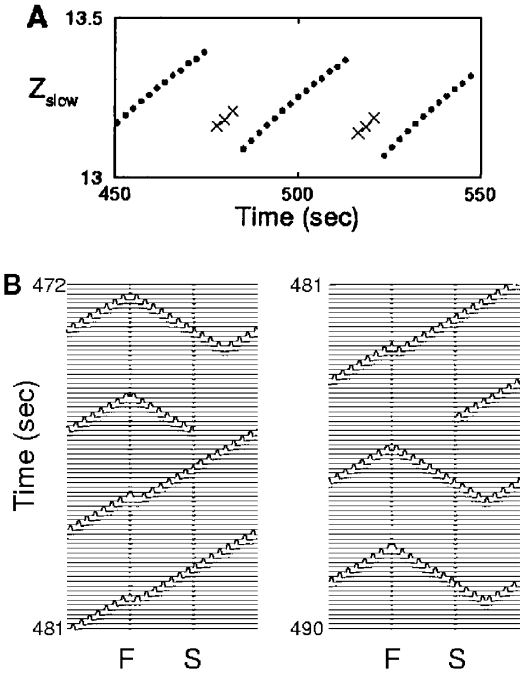

FIG. 2. (A) z measured at the time of activation at the slow pacemaker as a function of time during the paroxysmal starting and stopping of the excitation for the FHN model. The data is plotted after transients have dissipated. Dots represent the pacemaker cycles and $\times$'s represent the reentrant cycles. (B) Space-time plot of the v variable for the FHN model. Successive traces show the value of v around the ring at time intervals of about 121 msec, with cyclic boundary conditions. The left column shows blockade of the wave originating from the pacemaker leading to reentry. The right column shows blockade of reentry leading to a dominant pacemaker. F and S represent the locations of the fast and slow pacemakers, respectively. The plotted data in (B) include the first three reentrant cycles in (A).

the slow pacemaker. This leads to a reentrant rhythm that will continue to circulate until the refractory period builds up to a point that again leads to blockade of the excitation. Following this block, the fast pacemaker will once again take over and then the whole cycle repeats. The essence of this mechanism is that, following the blockade of excitation, there is a prolonged interval during which z can decay to a level that once again permits circulating excitation through the region of the slow pacemaker.

The dynamics in Eq. (1) can be approximated by using a difference equation. There are two pacemakers embedded in a ring of circumference L with a distance l between them. The velocity of propagation of an excitation in the ring is c. The pacemaker at 12 o'clock is a fast pacemaker with period T_f and the second pacemaker has a longer period T_s, $T_f < L/c < T_s$. Rapid activation of the slow pacemaker leads to a buildup of substance z according to the relationship, $z_{n+1} = (\Delta z + z_n)e^{-\gamma_s t}$, where n is the index that counts the successful activations of the slow pacemaker. We assume that the refractory period of the slow pacemaker increases as a function of z_n. From an analysis of Eq. (1) (not shown), we find that the refractory period, θ is approximately $\theta = az + b$, where $a = 150.7$ and $b = -8.3$.

Because of the simplicity of the above model, once the parameters are set, the dynamics can be analyzed. Starting from an initial value z_0, the value of z after n activations will be

$$z_n = g(z_0, n) = z_0 e^{-n\gamma_s T_f} + \Delta z e^{-\gamma_s T_f}\left(\frac{1 - e^{-n\gamma_s T_f}}{1 - e^{-\gamma_s T_f}}\right). \tag{3}$$

The slow pacemaker will be entrained for n_1 cycles until $T_f < az_{n_1} + b$, which in turn leads to a block of the excitation. However, calling $z_0' = z_{n_1} e^{-\gamma_s \xi}$, where $\xi = (L - l)/c - l/c$, provided $T_f + \xi > az_0' + b$, then the reentrant wave will propagate. The reentrant waves will continue until $z_{n_2} = g(z_0', n_2)$ increases so that $T_f < az_{n_2} + b$, which will lead to a block of the reentrant wave. Finally, we have $z_0'' = z_{n_2} e^{-\gamma_s (T_f - \xi)}$. Assuming n_1 and n_2 are fixed, since $g(z_0, n)$ is linear in z_0, by equating $z_0'' = z_0$, we can compute the value of the fixed point and show that it is stable.

There are oscillatory instabilities that arise prior to the blocking in the FHN equation, but do not occur in the schematic model [20].

Although the current model generates excitation paroxysmally starting and stopping similar to the experiments, we cannot be certain that this is the actual mechanism underlying the experimental observations. In the current experiments, although we frequently observe isolated pacemakers in the rings, we cannot directly study their response to stimulation, so we cannot verify the assumptions of the model. However, in earlier work on the same cells grown as aggregates rather than sheets, detailed studies of kinetics of the pacemakers showed behavior similar to what we have assumed here [16].

Previous studies of excitable chemical media in ring or annular geometries showed persistence of circulating waves once initiated. Although in many idealized settings the conservation of circulating waves is expected, in more realistic situations in which there are localized heterogeneities with time dependent properties there is no *a priori* reason to expect conservation of the numbers of

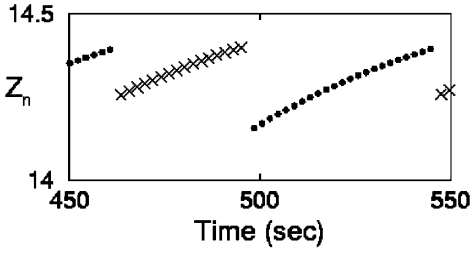

FIG. 3. z as a function of time for the schematic model. There are 23 pacemaker cycles and 16 reentrant wave cycles in the repeating sequence. Dots represent the pacemaker cycles and $\times$'s represent the reentrant cycles.

circulating waves. In particular, in clinical and experimental systems in cardiac electrophysiology, there are often circumstances in which there is paroxysmal starting and stopping of tachycardias [9,10,12,18,21].

This work shows that, with even two localized pacemaker sites in an excitable media in a ring geometry, a rich range of complex dynamics is expected. The observation that circulating waves can suddenly start and stop provides a new hypothetical model for complex repetitive rhythms that are sometimes associated with serious heart problems.

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[14] Since optical imaging using high intensity light and voltage sensitive dyes restricts the recording time to tens of seconds before serious damage to the cells occurs [S. Rohr, J. Cardiovasc. Electrophysiol. **6**, 551 (1995)], we used Ca^{2+} sensitive dyes which allow a longer recording time [12].

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[19] The number of cells is 1459. The fast pacemaker is located at 12 o'clock and the slow pacemaker is located at 4 o'clock. The number of cells between two pacemakers is 486. Each pacemaker has nine cells with $I = 0.202$. Equations (1) and (2) are solved using a Runge-Kutta algorithm with periodic boundary conditions, $\Delta t = 10^{-4}$ sec, $\Delta r = 1.51 \times 10^{-3}$ cm, and $D = 4.54 \times 10^{-3}$ cm^2/sec. The decay rates of the fast and slow pacemaker are $\gamma_f = 1.33$ sec^{-1} and $\gamma_s = 1.35 \times 10^{-2}$ sec^{-1} with intrinsic periods of $T_f = 2.13$ sec and $T_s = 5.13$ sec. The period reentrant wave is $T_r = 2.30$ sec, and the conduction velocity is 1.01 cm/sec. All parameters are set so that the FHN model produces periods of the reentrant wave and the fast pacemaker similar to the experiments.

[20] In Eq. (1), there is an alternation of action potential duration and amplitude immediately prior to the block at the slow pacemaker site. This alternation is similar to what is observed in experimental systems prior to block (Refs. [16,18]) but it is not included in the iterative model [Eq. (3)]. This alternation leads to a conduction block at different values of z_{slow} in the reentrant and pacemaker segments in Fig. 2A, and leads to different numbers of reentrant and pacemaker cycles in Figs. 2 and 3.

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