Stochastic and Spatial Influences on Drug-Induced Bifurcations in Cardiac Tissue Culture

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The addition of a drug that specifically blocks a potassium channel in spontaneously beating aggregates of chick heart cells leads to complex bifurcations over time. A stochastic partial differential equation model based on discrete ionic currents recorded in these cells demonstrates that drug diffusion and noise can induce the coupled beats and bursting rhythms observed. These results provide further evidence that stochastic events at a subcellular level are needed to understand complex cardiac arrhythmias and play an important role in the onset of these arrhythmias.

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Even a cursory examination of texts describing abnormal cardiac rhythms in people reveals a cornucopia of complex dynamics [1]. Viewed from a physical perspective, the onset of these arrhythmias is associated with a bifurcation in the dynamics of the heart as a consequence of a change in the structure of the heart, or by a change in the parameters controlling the cellular processes in the heart induced by circulating agents such as electrolytes, hormones, or drugs that affect cardiac tissue [2,3]. Since at a subcellular level, cardiac activity results from the stochastic opening and closing of ion channels and calcium release, stochastic influences should play an important role in both the normal dynamics of the heart [4–7] and also in the bifurcations associated with the generation of arrhythmias. Thus, models of cardiac arrhythmias share common features with other physical systems that involve noise in spatially extended systems [8,9]. In the case of the heart, the noise can have observable effects at a macroscopic level. Models of cardiac arrhythmias require inclusion of noise terms to obtain qualitative agreement with long term clinical recordings [10,11].

These theoretical considerations have practical significance in the analysis of drug-induced cardiac arrhythmias. In particular, many drugs unexpectedly block the human ether-à-go-go related gene (hERG) channel which underlies the I_{Kr} potassium current. The blocking of I_{Kr} leads to the generation of a second abnormal heart beat generated with a short delay following a normal heart beat, and this in turn may lead to the initiation of serious arrhythmia [12–14]. This scenario of drug-induced arrhythmia poses a significant barrier to the development of new drugs [15,16].

In this Letter, we study dynamics following administration of a drug that blocks the hERG channel in spontaneously beating aggregates of cells from embryonic chick hearts. By using optical methods to record the heart beat [17,18], we are able to simultaneously monitor the dynamics in multiple aggregates all of which have been treated in identical fashion. We simulate the dynamics in a single cell and a hemispheric aggregate of cells using deterministic ordinary and partial differential equations to model the effect of drug addition. We also consider the dynamics associated with noise generated by the stochastic opening and closing of ion channels in a single cell and during drug diffusion into the aggregates [4,5]. The model of the aggregate reproduces the complex time dependent rhythms observed experimentally and illustrates a novel mechanism for the dynamics involving noise in a system undergoing bifurcations due to a changing spatial structure.

The heart cell aggregates were prepared according to the method described by DeHaan [19]. Ventricular portions of hearts from 7-day-old White Leghorn chick embryos were dissected, dissociated into single cells by trypsinization, added to Erlenmeyer flasks containing a culture medium (818A) gassed with 5% CO_2, 10% O_2, 85% N_2 (pH = 7.4), and placed on a gyratory shaker for 24–48 hours at 37 °C. This procedure generates aggregates with a diameter of approximately 100–200 μm, and a regular beating pattern with a period of approximately 1–2 s and coefficient of variation of $\approx$2%. The intracellular voltages of different cells in the same aggregate have upstrokes that occur with a time difference of at most 50 μs [20], so the aggregate is essentially homogeneous electrically. Experiments were performed 2 to 6 hours after the aggregates were plated in plastic tissue culture dishes maintained at 37 °C. The I_{Kr} channel blocker E-4031 (Alomone Labs, Jerusalem) was added at various concentrations in the range of 1.0–2.0 μM.

Recent studies have used the motion resulting from contractions of cardiac cells to monitor dynamics in tissue culture [17,18]. This method has the advantage of enabling recordings for long times without the complicating effects that may be induced by the addition of voltage or calcium sensitive dyes, and enables us to track the drug effects in several aggregates simultaneously from an area of $\approx$1 cm^2 for up to two hours. We record the beating as reflected by the light intensity variation at the edge of the

aggregate using phase contrast imaging sampled at 40 Hz using a CCD camera (RedShirtImaging, LLC. NeuroCCD-SM) with an 80×80 spatial resolution. We report results on 91 aggregates in 7 different culture dishes.

When E-4031 is added to the culture medium, the beating activities of most aggregates change in a time dependent manner. In order to monitor the changes in the beat pattern, we measure the interbeat interval (IBI) as a function of time. Figure 1 displays typical transitions. The upper figure is the interbeat interval as a function of time, and the lower panels show the motion recording at selected times during the recording as indicated in the upper figure. Following addition of the drug, there is a time interval, typically between 10–40 min during which there are negligible changes in the interbeat interval. Following this initial interval, several different rhythms may arise. The times of the transitions between the rhythms and the rhythms themselves show a great deal of variability, even for aggregates in the same culture dish that were subjected to exactly the same preparation procedures. The predominant rhythm in these experiments, observed in 70/91 aggregates, was an alternation between long and short intervals. In 39/91 aggregates, there is a gradual development of the alternation, similar to dynamics occurring as a consequence of a period-doubling bifurcation, whereas in 21/91 aggregates, there are added beat rhythms

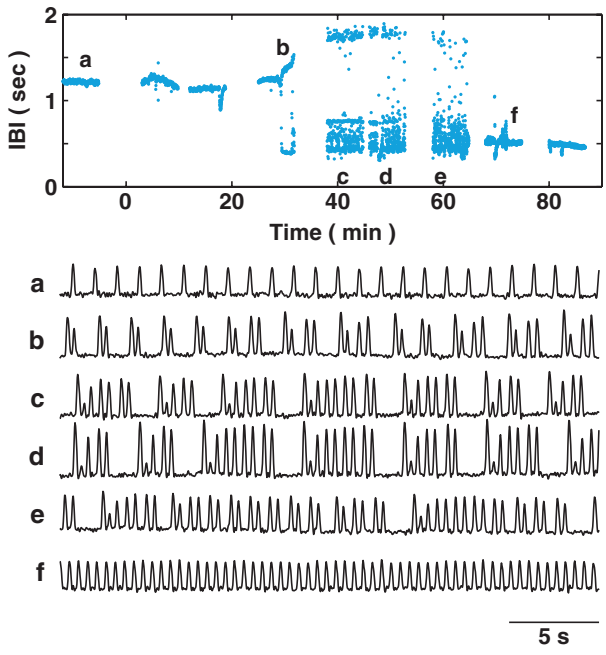

FIG. 1 (color online). Experimental observations of the motion in embryonic heart cell aggregates following addition of 2 μM of I_{Kr} channel blocker, E-4031 to the culture medium at $t = 0$. The interbeat intervals (IBIs) as a function of time are plotted in the upper panel with samples of corresponding motion recordings at selected times during the recording. The brief pauses in the data in the upper panels represent the times when the recording was interrupted to downloaded data to the hard disk.

in which there are occasional doublets in which there are two consecutive beats with an abnormally short interbeat interval. A chaotic rhythm, identified from a one dimensional map in which the IBI is plotted as a function of the preceding IBI, was observed 5/91 aggregates, all from the same dish, following an alternating rhythm. Finally, in 45/91 aggregates, there were bursting rhythms, eventually leading to a rapid rhythm.

As a first step in understanding the origin of these transitions, we develop a Hodgkin-Huxley type ionic model for drug effects on the beating aggregates. We assume that $\dot{V} = -I_{tot}/C_i$ where V is the membrane potential, I_{tot} is the total membrane current, and C_i is the input capacitance. Based on earlier studies of the ionic mechanisms underlying cardiac activity in embryonic chick heart cell cultures [4,6,21], we assume that $I_{tot} = I_{Na} + I_{Ca} + I_{Ks} + I_{Kr} + I_{K1} + I_b + I_f + I_{noise}$, where I_{Na} is the inward sodium ion current which controls the maximum rate of rise of the action potential upstroke, I_{Ca} is the calcium ion current primarily responsible for the inward current during the plateau phase, I_{Ks} is the slowly activating potassium current that underlies the primary repolarization, I_{Kr} is the rapidly activating delayed rectifier potassium current responsible for the repolarization phase of the action potential, I_{K1} is the inward rectifier potassium channel, I_b is a time independent background current, I_f is the hyperpolarization activated pacemaker current, and I_{noise} is a noise current that is used in the stochastic models. We further assume that the effect of E-4031 on potassium conductance g_{Kr} is described by

$$g_{Kr} = \frac{g_{Kr,0}}{(\frac{c}{c_{1/2}})^n + 1} \quad (1)$$

where $g_{Kr,0}$ is the maximum value of g_{Kr}, c is the concentration of E-4031, $c_{1/2}$ is the value of c at half maximum, and n is a parameter. We consider two different situations: dynamics in a single spatially homogeneous cell as a function of concentration, and dynamics in a theoretical model of the spatially heterogeneous aggregate as the drug diffuses into the aggregate. The integration of the spatially heterogeneous model uses a finite element method [22]. The time dependence of the drug concentration is obtained by integrating the diffusion equation in a hemispherical geometry [23]. For both the single cell and the aggregate, we consider both the deterministic equation and a stochastic equation that reflects random fluctuations in ion channel opening and closing [5–7]. Parameters and the detailed equations are included in the supplementary online material [24].

We first consider the bifurcations in the theoretical model as a function of E-4031 concentration assuming a spatially homogeneous deterministic equation, Fig. 2(a). At low drug concentrations, there is stable rhythm. As the concentration increases, there is a period-doubling bifurcation at ≈ 0.9 μM leading to an alternation of IBI inter-

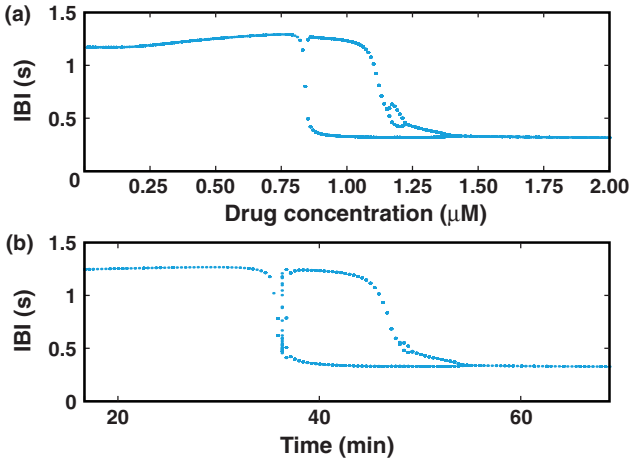

FIG. 2 (color online). (a) Bifurcation diagram of interbeat intervals (IBIs) in a deterministic single cell model as a function of concentration of the drug E-4031. (b) Bifurcation diagram of IBIs in the deterministic spatially extended model as a function of time assuming an external concentration of E-4031 of 2 μM.

vals in the range $\approx$0.9–1.2 μM. In the model, E-4031 blocks the I_{Kr} leading to a prolonged action potential and a slowing of repolarization. This provides the opportunity for sufficient recovery of the sodium channel from inactivation to initiate another beat with a shorter IBI. A second bifurcation leading to four different IBI intervals (2 of which are almost equal $\approx$0.3 s) occurs at drug concentrations between $\approx$1.2–1.25 μM. As the drug concentration further increases, there is a rapid rhythm with alternating IBIs and finally a rapid stable rhythm.

We also computed the dynamics in a 150 μm radius hemispheric aggregate as a function of time, Fig. 2(b). We solved the diffusion-reaction equation using the finite element method with a model comprising 1954 nodes. Drug concentrations within the aggregate were dependent on time and radial position to account for diffusion from the bath into the aggregate [24]. Comparison of the bifurcation diagrams in Fig. 2 shows a striking similarity between the bifurcations in the spatially homogeneous model as a function of drug concentration, and the hemispheric aggregate as a function of time. However, neither of these simulations display the more complex rhythms observed in the experiments.

In order to model such dynamics, we consider models in which a stochastic term is added to the total current. We carried out computations on both the single cell model, Fig. 3, and the spatially extended model, Fig. 4. The simulated time series in Figs. 3 and 4 appear very similar to each other, and also to the experimental recordings. In particular, added beat rhythms and bursting rhythms occur in the stochastic models, but not in the deterministic models. Although we had expected that there should be differences between the bifurcations induced by drug in a single homogeneous cell as a function of concentration, and the bifurcations occurring over time induced by diffusion of

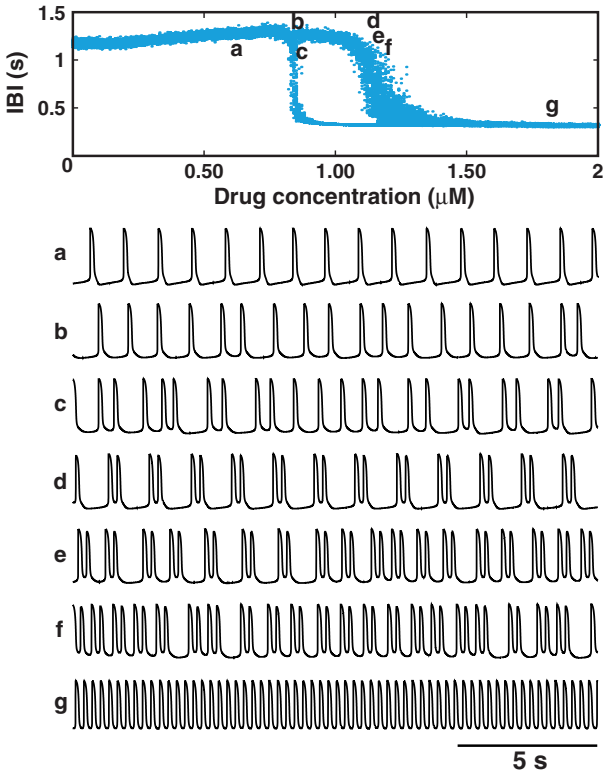

FIG. 3 (color online). Uppermost panel: bifurcation diagram of the single cell stochastic model. Panels (a)–(g): Representative time series at increasing drug concentration of the single cell stochastic model.

the drug into the spatially extended aggregate, we were surprised to find that the qualitative features of the bifurcations appear to be quite similar. If the cells in the aggregate were uncoupled, then in general, there would be different dynamics in the outer and inner regions of the aggregate reflecting the local drug concentration, but the coupling leads to a spatial uniformity in the model, as it does in the aggregate. However, the spatially homogeneous model does not account for the time dependence of the bifurcations. The simulations support the notion that the observed time dependence of the dynamics are due to the changing concentration profile of the drug as a consequence of diffusion of drug into the aggregate.

The current work has implications in both physical and biological realms. The dynamics observed in the cardiac tissue in this work bears qualitative similarities to rhythms observed in nerve cells under a variety of different experimental manipulations and in mathematical models of those systems [25,26]. This earlier work analyzes the bifurcations leading to added beat, bursting, and chaotic rhythms in nonlinear ordinary differential equations modeling neural dynamics as a function of parameter values. The current observations enlarge the class of physiological systems and associated mathematical models in which these phenomena are found. Although deterministic models of both the single cell and aggregate did not display added

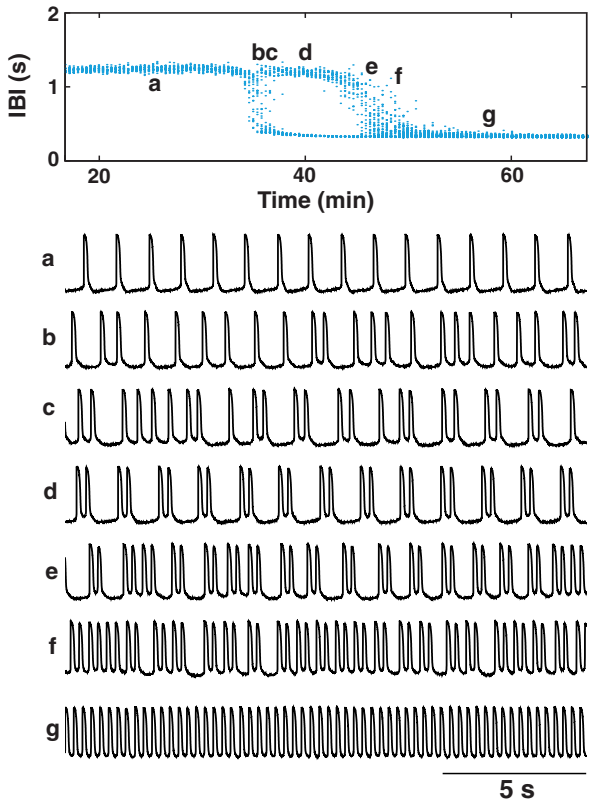

FIG. 4 (color online). Uppermost panel: bifurcation diagram of the spatially extended stochastic model. Panels (a)–(g): Representative time series at increasing drug concentration of the spatially extended stochastic model.

beat or bursting rhythms, Fig. 2, addition of the stochastic terms did lead to a generation of these complex dynamics, Figs. 3 and 4. This further supports the important role of stochastic factors in partial differential equations in the neighborhood of bifurcations and phase transitions [8,9].

Some patients display coupled beats and short bursts of activity, preceding sudden cardiac death [11,27]. A recent study has pointed to a particular electrophysiological abnormality called early afterdepolarizations in inducing similar rhythms in individual rabbit heart cells and whole rabbit heart [3]. We propose that the doublets and short bursts of activity observed here may offer a biological model for these arrhythmias and insight into their genesis [11]. Although the current experimental model is different from the one in [3], we believe that the underlying mechanism generating couplets in these experiments may be similar to mechanisms generating early afterdepolarizations in other contexts [7,12,14]. Consequently, the irregular couplets and bursting rhythms under compromise of potassium channel blockade may help provide insight into the genesis of complex rhythms including those that precede sudden cardiac death.

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