Nonlinear Dynamics of Rate-Dependent Activation in Models of Single Cardiac Cells

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Recent studies in isolated cardiac tissue preparations have demonstrated the applicability of a one-dimensional difference equation model describing the global behavior of a driven nonpacemaker cell to the understanding of rate-dependent cardiac excitation. As a first approximation to providing an ionic basis to complex excitation patterns in cardiac cells, we have compared the predictions of the one-dimensional model with those of numerical simulations using a modified high-dimensional ionic model of the space-clamped myocyte. Stimulus–response ratios were recorded at various stimulus magnitudes, durations, and frequencies. Iteration of the difference equation model reproduced all important features of the ionic model results, including a wide spectrum of stimulus–response locking patterns, period doubling, and irregular (chaotic) dynamics. In addition, in the parameter plane, both models predict that the bifurcation structure of the cardiac cell must change as a function of stimulus duration, because stimulus duration modifies the type of supernormal excitability present at short diastolic interval. We conclude that, to a large extent, the bifurcation structure of the ionic model under repetitive stimulation can be understood by two functions: excitability and action potential duration. The characteristics of these functions depend on the stimulus duration. (Circulation Research 1990;67:1510–1524)

Since the original work of Fitzhugh1–2 leading to a bidimensional approximation to the Hodgkin-Huxley model (H-H),3 there has been extensive use of the Fitzhugh-Nagumo4 equations to provide analytical predictions and qualitative representations of the behavior of isolated nerve cells under a variety of conditions (see References 5, 6, and 7 for reviews). This was a major step forward, because in most instances (repetitive stimulation, extended structures), the mathematical complexity of the H-H model precludes any significant analysis beyond numerical simulation.

The situation is even more complicated for cardiac tissue. To model the electrical activity of ventricular myocytes, Purkinje fibers, or cells, an intermediate time scale, with associated gating variables and current, must be added to the original H-H two–time scale formulation.5,9 These changes are necessary if one wishes to reproduce the long plateau of the cardiac action potential and the resulting lengthening of the refractory period. In fact, some behaviors observed in these models based on ordinary differential equations may not be described in fewer than three dimensions,10–12 which makes any qualitative or analytical work far more difficult.

In the last several years, a large body of work has appeared that deals with repetitive stimulation of single cardiac cells and of extended cardiac tissue in terms of return maps, phase resetting, and nonlinear dynamics [e.g., Reference 13; also, see Reference 14 for review]. Recently, a difference equation (DE) model15 was developed on the basis of previous formulations15–20 to reproduce a wide variety of behaviors that have been observed in ventricular tissue and isolated Purkinje fiber preparations. Such behaviors include locking phenomena, period doubling, and chaos.15,21,22 The major feature of this model is that it uses three experimentally derived curves to explain observed dynamical behaviors, without any direct reference to underlying ionic mechanisms. As such, the model gives a global picture of the dynamics at a low computational cost and stresses the basic properties responsible for different classes of dynamics. Thus, if equivalence of the dynamical properties of the DE and full ionic models could be demonstrated, an integrated modeling approach then may become possible; that is, the DE model should provide global predictions whose ionic basis may be studied in more complex, highly dimensional models. To investigate this possibility, we have compared dynamical properties of the DE model with those of an ionic model of the space-clamped...
cardiac cell, particularly in the case in which super-
normality was present.

Methods

The Difference Equation Model

The DE model was devised to reproduce activity of nonpacemaking cardiac cells challenged by repetitive depolarizing current stimuli. The only independent variable of the model is the diastolic interval (DI); that is, the time from the end of a previous response, or action potential, to the beginning of the next stimulation. Three functions are required: the threshold (Thr[DI]), which corresponds to the minimal intensity of current needed to induce an action potential; the action potential duration (APD[DI]), which is the duration of an active response to a suprathreshold stimulus; and the latency (Lat[DI]), or time interval between the onset of the stimulation and the beginning of the action potential. Activity then is reconstructed in an iterative fashion. Consider DIᵢ, the diastolic interval preceding the i-th application of a stimulus of intensity Iᵢ. If the stimulus is subthreshold (Iᵢ<Thr[DIᵢ]), the response fails and the next diastolic interval will be

\[ DI_{i+1} = DI_i + T_{i+1,i} \]  \hspace{1cm} (1)

where \( T_{i+1,i} \) is the time between onset of the i-th and i-th +1 stimuli.

If the stimulus is suprathreshold (Iᵢ≥Thr[DIᵢ]), then the latency (Iᵢ=Lat[DIᵢ]) and the duration (APDᵢ=APD[DIᵢ]) of the resulting action potential are computed. The next diastolic interval then becomes

\[ DI_{i+1} = T_{i+1,i} - Lᵢ - APDᵢ \]  \hspace{1cm} (2)

The process then is repeated with DIᵢ₊₁. The original model also includes a memory process (M) whereby a slow time-dependent variable modulates the next APD (APDᵢ=APD[DIᵢ]×[1-Mᵢ]). Such a variable is a function of the previous APD and DI (Mᵢ=Mᵢ₋₁×APDᵢ₋₁×DIᵢ₋₁, Mᵢ∈[0,1]). However, it will be shown below that memory is negligible in the ionic model and need not be included in our calculations. During repetitive stimulation with a fixed period (BCL, basic cycle length) and current amplitude, the DE model thus is reduced to the iteration of a unidimensional map:

\[ DI_{i+1} = BCL - Lᵢ - APDᵢ = BCL - g(DIᵢ), \quad Iᵢ=Thr(DIᵢ) \]

\[ = BCL + DIᵢ, \quad Iᵢ<Thr(DIᵢ) \]  \hspace{1cm} (3)

with \( g(DIᵢ)=APD(DIᵢ)+Lat(DIᵢ) \).

Ionic Model

As a model of the space-clamped ventricular myocyte, we have used the Beeler-Reuter model with modification of the sodium current as proposed by Drouhard and Roberge. This modified Beeler-Reuter (MBR) is a seven-dimensional H-H-type model including the potential (V), the reversal potential of the calcium current (E₉), and five gating variables (Yᵢ, i=1,5). The model equations are as follows:

\[ \frac{dV}{dt} = -I_{on}(V,E_{Ca},Yᵢ,i=1,5) + I_{st} \]

\[ I_{on}=I_{Na}(V,Y₁,Y₂)+I_{ad}(V,Y₃,Y₄,E₉)+Iₚ(V,Y₅) \]

\[ \frac{dYᵢ}{dt} = \frac{1}{τ(V)}(Yᵢ(V) - Yᵢ,i=1,5) \]

\[ \frac{dCₐ}{dt} = k₁Y₃Y₄(V-E₉) - k₂Ca \]

\[ E₉ = k₃\ln(Ca) \]  \hspace{1cm} (4)

All functions and parameters can be found in Reference 8, except for the sodium current (I_{Na}), which is taken from Reference 24.

Constructing the Difference Equation Model Functions

We have used in the MBR model a protocol similar to that used to construct Thr, APD, and Lat curves from experimental preparations. In experiments for which this protocol was described, the preparation is paced at a relatively low frequency (0.5–66 Hz) with suprathreshold stimuli (S_i) until a stable 1:1 response pattern is reached (~10 beats). Test stimuli (Sᵢ) then are applied at various time intervals (S_I–Sᵢ) after the last pacing stimulus. Threshold is obtained for each Sᵢ–Sᵢ and then APD is measured for barely suprathreshold stimuli. The time at which the potential time derivative reaches its maximum usually is taken as the end of the latency period and the beginning of the action potential. The end of the action potential is taken as the time at which the potential reaches a specified value after repolarization (Vₑ). DI is obtained from Sᵢ–Sᵢ by subtraction of latency and APD of the action potential induced by Sᵢ.

The Thr and APD functions were constructed from the MBR model for test pulses (S_i) of various durations. Stability of the response was diagnosed before the application of the Sᵢ stimulus by comparing the successive vectors \( X_i=[V,E_{Ca},Yᵢ,i=1,5] \) of the state of the system at the onset of each pulse. During a full-blown action potential, the variation of V and E₉ is on the order of 100 mV and that of the Yᵢ on the order of 1. The difference vector \( ΔX=[a_i||xᵢ^{t+1} - xᵢ^t|| j=1,7] \) with aᵢ=0.01, and aᵢ=1, j=3,7 was computed and the system was considered to be stable when all the components of \( ΔX \) were lower than 0.01 for five successive cycles. The same procedure of period diagnosis was used for the MBR model in all subsequent steps. Numerical simulations were performed using a hybrid integration technique, with time steps between 0.001 and 0.1 msec.
program was written in FORTRAN, using double precision, and run on a Sun-4 workstation using a floating point accelerator.

Simulations

Repetitive stimulation in the DE model was carried out using functions obtained as described above. Using BCL and stimulus current amplitude (I0) as parameters, a systematic exploration of the phase plane was performed. For each BCL and current, initial condition was reset at DI=∞, which corresponds to starting stimulation with the cell in its resting state. Unless otherwise indicated, runs containing up to 1,000 stimuli were carried out for each value of the parameter. Results were presented as activation ratio (AR), that is, the number of action potentials divided by the number of applied stimuli. Stimulation was stopped as soon as the same pattern of APD had repeated 11 times, with a maximum difference no greater than 0.5 msec between corresponding action potentials of the “locking” sequences (APD of failed responses was automatically set to zero). When no stable pattern was detected, mean AR for the entire run was used to describe the response pattern. Changes of AR greater than 0.001 were considered.

Equivalent simulations were carried out for the MBR model, but with larger parameter increments and a maximum of 100 beats because of the long time of simulation involved. As in the DE model, all variables were reset to their resting values after each run at a given set of current and BCL values. The procedure described in “Ionic Model” above was used to test for periodicity; criteria were established to compute the AR (see “Results”), and the bifurcation structures of the two models were compared.

In a few instances, bistability and hysteresis were studied in both models. BCL then was decreased and increased over specific intervals, the stable state for each BCL becoming the starting condition for the next BCL value.

Results

Thr(DI), APD(DI), and Lat(DI) Functions

There is no formal consensus among experimentalists regarding the precise definition of a suprathreshold response, except for the occurrence of an active phase of depolarization (I0<0, Equation 4). In addition, the current must remain activated long enough so that the response “looks like a real action potential.” Difficulties in classifying borderline cases inevitably occur. Mathematically, threshold refers to the separation of distinct dynamics converging toward different stable states. However, with nominal values of its parameters, the MBR model has only one single stable resting state. Thus, we have divided threshold evaluation into two steps. First, for each S1-S2, an approximation to threshold function (Thr) was obtained as the lowest value of the stimulus current resulting in activation of the ionic current for at least 0.5 msec (precision, 0.01 μA/cm²). Second, APD curves were constructed for sets of stimulation
threshold (bottom curves). Figures 1B–1D show, respectively, the APD, the absolute value of maximum ionic current (−Ion_max), and the maximum depolarization potential (V_max) resulting from the same stimulation parameters as in panel A. All curves were obtained using an S1 stimulus duration of 25 msec, after pacing with 1-msec S1 stimuli of 37.5 μA/cm² at a BCL of 1,500 msec. Two distinct families of curves are apparent in panels B, C, and D, each corresponding to data associated either with passive electrotonic responses (bottom curves) or with active potentials (top curves). These results fulfill the first prerequisite of the DE model that, as long as current intensity remains at suprathreshold levels, the characteristics of the action potential remain relatively insensitive to current amplitude, which also has been demonstrated in experimental preparations. The threshold condition is very critical, as demonstrated by the wandering of points associated with Thr between the two possible states in all variables (panels B–D). The fluctuations come from the 0.01 μA/cm² precision used to calculate Thr. It could be argued that a real threshold has not been detected, because the system always returns to the same resting point, and that smaller increments of the stimulation current will have given points filling the space between the two groups of curves. We thus have used an empirical definition of "threshold" as being a

![Diagram](image-url)
value of current around which a 1% variation induces a jump from the lower curves to the upper family of curves of $V_{max}$ -- $V_{ion}$, the maximum ionic current after the S2 stimulation, and APD. The S1-S2 value at which the respective lower and upper curves of $V_{max}$, $V_{ion}$, and APD come together at a brief S1-S2 interval (270 msec in panels B--D) was used to mark the end of the absolute refractory period (ARP), that is, the earliest interval at which an action potential can be induced (DI). A threshold no longer exists to the left of this point. For S1-S2 intervals briefer than the ARP, increasing the stimulus current intensity results in slight monotonic increases in $V_{max}$ -- $V_{ion}$ and APD.

It should be noted that the threshold at certain short S1-S2 intervals is lower than the saturation value (3.22 $\mu$A/cm$^2$) at very long S1-S2 intervals and that a maximum (3.65 $\mu$A/cm$^2$) is reached at a relatively short S1-S2 interval, before the beginning of the sharp decline to a minimum of 3.07 $\mu$A/cm$^2$ (see arrow in Figures 1A and 3B). This behavior has been termed "type-2 supernormality" and was shown to be associated with the occurrence of a chaotic regime during repetitive stimulation in isolated cardiac Purkinje fiber preparations. Some consequences of supernormal excitability at early intervals in the MBR model are illustrated in Figure 2 for a constant current intensity of 3.5 $\mu$A/cm$^2$, corresponding to the horizontal line in Figure 3B. In Figure 2, the 12 superimposed tracings of membrane potential show that at this level of current there is a "silent" S1-S2 interval bounded by two regions in which action potentials can be initiated.

Selection of Parameters

To study dynamics of excitation in the DE model, we have selected the APD curve obtained with Thr+0.01xThr to the right of the point at which the ARP begins (Figure 3B). The shape of the APD curve is relatively insensitive to the intensity of suprathreshold stimuli, although this does not hold true for the function Lat(DI) describing latency to activation. Indeed, once threshold has been achieved, latency diminishes as the stimulus intensity is increased. Nevertheless, for a fixed intensity of stimulation, the shape of Lat(DI) is similar to that of Thr(DI), which reflects at each point the excess of current above threshold (i.e., low threshold--large [I threshold]--brief latency). The function Lat(DI) (not shown) thus was constructed by multiplying Thr(DI) by a scaling factor (k) such that the resulting data points were within the range of the latencies observed for the interval of current amplitudes under study. This is a satisfactory approximation if the range of amplitude is not exceedingly large. The final one-dimensional map g(DI) = APD(DI)+Lat(DI) and its derivative (dg/dDI) obtained with Lat(DI) fixed to 7.0xThr(DI) are presented in panel C of Figure 3.

The superimposed APD(DI) curves shown in panel D of Figure 3 illustrate simulation results obtained using four different sets of pacing parameters: three with pulse duration of 1 msec, amplitude of 37.5 $\mu$A/cm$^2$, and BCLs of 300 (1), 1,500 (2), and 4,000 (3) msec; and one with pulses 25 msec in duration, 3.75 $\mu$A/cm$^2$ in strength, and BCL of 1,500 msec (4). All curves are practically identical, which demonstrates that "memory effects" on the APD are negligible in the MBR model.

Threshold, latency, and APD functions also were constructed for S2 stimuli of various durations. The normalized threshold curves (Thr S1/S1 x Thr S2) are presented in panel A of Figure 4. The S1 parameters (37.5 $\mu$A/cm$^2$ amplitude, 1 msec duration, 1,500 msec BCL) were the same in all cases. It can be seen that the shape of the Thr function changes from a monotonic to a nonmonotonically increasing curve with the prolongation in S2 duration. Such a transition was experimentally observed in cardiac Purkinje fibers where it was associated with the modification of the external potassium concentration. Short S2 durations give rise to monotonically increasing threshold curves. As the S2 stimulus duration increases, the curve changes in shape and gives rise to conditions of...
absolute (\(\text{Thr}_{\text{max}} > \text{Thr}[a] > \text{Thr}_{\text{min}}\)) ("type-2") and then relative (\(\text{Thr}_{\text{max}} > \text{Thr}_{\text{min}} > \text{Thr}[a]\)) ("type-1") supernormality.

Panel B of Figure 4 presents the final maps (\(g = \text{APD} + k \\text{Thr}\)) characterizing the DE model for stimulus durations of 1, 25, and 100 msec. Except for the detailed aspects of their respective derivatives (not shown), the curves associated with 1 and 25 msec are very similar to each other. On the other hand, when \(S_2\) stimuli of 100 msec are used, \(g\) values increase appreciably as a result of the fact that the latency to activation is always on the order of 100 msec for current amplitudes that are just above threshold. Under these conditions, the curve remains relatively flat, except for a slight dip at small \(S_1-S_2\) intervals.

In this article, we discuss only the 25-msec case, in which the presence of type-2 absolute supernormality allows the demonstration of a rich and complex family of behaviors, as was shown previously in biological experiments.\(^{15,21}\) The 25-msec case is also discussed in detail because, as explained later, it was found to be "generic": low-amplitude and high-amplitude stimulations lead to dynamics respectively similar to the 100-msec and 1-msec duration cases, whereas intermediate amplitudes induce new classes of dynamics that are irreducible to the two other cases. Details for the 1-msec and 100-msec cases can be found in Reference 27.

**Parameter Plane of the Difference Equation Model**

Using the \(\text{Thr(DI)}\) and \(g(DI)\) functions associated with a stimulus duration of 25 msec (panels C and D of Figure 3), we have studied the bifurcation sequences of the periodically driven DE model as a function of BCL and amplitude \((I_a)\) of the stimulus.
Figure 6. Iterations and stable patterns of the difference equation model for decreasing basic cycle lengths (BCLs). In panels A–C, the initial response has the saturation value of the total action potential duration (g(DI)). Panel A: 1:1 at BCL of 600 and 325 msec. Panel B: BCL of 288 msec. The first iterations are shown. Iterations finally converge to the stable 2:2 orbit appearing as a small square. Panel C: BCL of 270 msec. The beat after the saturation value fails (F), but iterations still converge to 2:2. Panel D: Final stable 2:1 at BCL of 200 msec. DI, diastolic interval.

For all DIs falling in the ARP (DI < DI_{min}), Thr(DI) was considered infinite such that no action potential could be generated. As stated in "Methods," DI initially was reset to infinity for each change in the value of the stimulation parameters, which corresponded to a system at rest at the beginning of stimulation. The parameter space in panels A and B of Figure 5 summarizes the results. The data points in the plots mark the stimulus BCLs and amplitudes at which changes occur in the AR or in the number of stimulations in the periodic sequence. Borders have been drawn to separate zones of constant period and AR.

Description of the DE parameter space. The most striking feature of the parameter space is the global modification of the bifurcation structure with the amplitude of the stimulus. As shown in Figure 5A, the parameter space could be divided into three regions of current amplitude that are briefly described as follows.

Region I, corresponding to low values of I_{in}, has the simplest bifurcation structure with successive n:1 ratios. In addition, short self-similar transitional regions (TI) exist in the 3:1, 5:1, and 6:1 zones (see details in the 3:1 zone of panel B). Their main transition sequence is everywhere the same, namely

\[ 1 \rightarrow 1+2 \rightarrow 2 \rightarrow 1 \]

where n = 3, 5, 6

Region II extends from the top of region I to the maximum level of threshold current (\( \sim 3.65 \) \( \mu A/cm^2 \), see Figure 3B). It has the most complex organization, with two additional types of transitions: TII, in the 2:1 and 4:1 regions (BCL \( \sim 270 \) and 135 msec), and TII' in the 3:1 region. Details of TII in the 2:1 region are given in Figure 5B. In the upper portion, successive 2n:2n-1 tongues are detected before the occurrence of the 2:2 phase locking (only 4:3 and 6:5 are labeled in Figure 5B). At low current values, the TII zone ends in an area of 7:4 and 5:3 activity, just at the border of the 3:1 tongue. These ratios, as well as all intermediate ratios also present at the bottom tip of the descending 2n:2n-1 tongues, could be deduced from continued fraction expansion of (4:3, 3:1) and (2:2, 3:1) (for example, 4:3 \( \rightarrow 7:4 \leq 3:1 \)).

The TII' zone is given in some detail in the inset of Figure 5A. The specific patterns detected change...
with $I_w$. At high amplitude, a region of irregular dynamics (hatched area in Figure 5A inset) becomes manifest between the 4:2 and 6:3 zones. As $I_w$ goes up, increasing numbers of points with aperiodic sequences are detected, intermingled with short (<0.5 msec) intervals of stable 0.5 AR.

Finally, region III corresponds to current amplitudes higher than the maximum threshold current. As $I_w$ decreased, the sequence of transitions 1:1→2:2→2:1→4:2 is observed within this range of current amplitude. In the thick bands before the 2:2 and 4:2 regions, the pattern of activity bounces back and forth between 1:1 and 2:2, or 2:1 and 4:2. This comes from the fluctuation in the derivative of g(DI) (see Figure 3C, left ordinate), which is an artifact resulting from the method used to construct the function from the MBR model. For BCLs between 119 and 137 msec (hatched area between 4:2 and 3:1 in Figure 5A), irregular dynamics are mixed with short bursts of stable activity. Using BCL steps of 0.1 msec, eight stable intervals were detected, each lasting less than 0.3 msec. Just at the border between the 4:2 and aperiodic zones, a cascade of decreasing 2n·n+1 ratios was detected within a BCL interval of ~1 msec. When this short interval was scanned with a BCL step of 0.1 msec, the AR was seen to increase continuously from 0.5 (4:2) up to 0.5625 (16:9), with occasional period doubling. Within the aperiodic zone, the AR remains between 0.5 and 0.75, which ends abruptly with the appearance of the 3:1 area.

**Explanation of the bifurcation structure.** The bifurcation structure predicted by the DE model undoubt- edly is very complicated. The main interest for the DE model, beyond its low computational cost, is that it allows a complete understanding of such a structure, as will now be illustrated.

In region III, the bifurcation sequence is no longer a function of $I_w$ because $I_w$ is everywhere greater than the maximum threshold, such that the complete g(DI) function is available for iterations. As illustrated in Figure 6, for each BCL, the fixed point of the iteration (DI=BCL−g(DI)) can be found graphically as the intersection of the line BCL−DI with the function g(DI). In panel A, for long BCL (e.g., 600 msec), the fixed point falls in the region where g(DI) is relatively flat. Iterations from any initial condition will converge to the fixed point, ending in a 1:1 stable pattern. As BCL decreases, the fixed point moves toward lower values of g(DI), where the slope of the function is steeper (see, for example, BCL=325
When the slope of $g(DI)$ at the fixed point becomes greater than 1, the fixed point is no longer stable.\textsuperscript{28} As shown in panel B, iterations now converge to a stable $2:2$ orbit, with alternation of short and long action potentials. In our case, a stable $2:2$ orbit appears at a BCL where the fixed point is still stable. There is a short range of BCL values at which iterations will converge to either $1:1$ or $2:2$, depending on initial conditions. Once in the $2:2$ region, as BCL is further decreased, alternation of APD increases until the shortest DI falls in the ARP (panel C). As illustrated in panel D, when a beat is missed, the next DI is shifted by an amount equal to one BCL, and a stable $2:1$ pattern appears. The stable successful beat now is given by the intersection of $g(DI)$ with the line $2 \times BCL - DI$. As BCL is further decreased, the line $2 \times BCL - DI$ repeats the movement done previously by the line $BCL - DI$ (panels A to D), leading to $4:2$ phase locking.

The complete evolution of the system can be seen in Figure 7A, which shows the stable $g(DI)$ values as a function of BCL. It is seen that the alternating successful beats of the $4:2$ region are identical to the $2:2$ beats occurring at twice the BCL. Alternation grows until the beginning of the irregular dynamics regions. Some of the iterations associated with the irregular dynamics are illustrated in Figure 8A. Irregular dynamics appear when the shortest action potential of the $4:2$ sequence becomes brief enough to be followed directly by a successful beat. When this occurs, the BCL already is brief enough for the first points of both the first ($DI = BCL - g(DI)$) and second iterate ($DI = 2 \times BCL - g(DI)$) to be unstable (slope of $g(DI) > 1$). Alternans grows around the first iterate, until the duration of the response becomes so large that the following stimulation fails. Iteration then moves back to the second iterate ($2 \times BCL - DI$), and alternation starts again. Figure 8B shows the return map of $g(DI)$ for successful responses from 1,500 successive stimulations at BCLs of 121 and 133 msec (labeled respectively 1 and 2). These BCLs are near each end of the zone of irregular dynamics. In each case, the lower branch corresponds to successive $1:1$ beats, and the upper branch to $2:1$ responses. Iteration is seen to jump back and forth between the two branches. This type of noninvertible
map has sensitive dependence on initial conditions as well as disconnected branches and typically is associated with chaotic dynamics.\textsuperscript{29–31} Chaotic dynamics appears as a series of successive alternating 2:1 responses, followed by a sequence of alternating 1:1, the number of beats in each sequence varying in a seemingly unpredictable fashion. Presence of chaos also was confirmed by calculation of the Lyapunov exponent.\textsuperscript{27}

All differences between regions I, II, and III of the parameter space are the result of the appearance of an “inexcitability” gap in the g(DI) function when current amplitude is less than the maximum threshold level. Indeed, because the system is operating under the conditions of absolute supernormality (\(\text{Thr}_{\text{min}} < \text{Thr}(\text{DI}[\ast]) < \text{Thr}_{\text{max}}\); see Figure 3), two other outcomes are possible in addition to that related to the globally subthreshold case discussed above: \(\text{Thr}_{\text{min}} < \text{I}_{\text{st}} < \text{Thr}(\text{DI}[\ast])\) and \(\text{Thr}(\text{DI}[\ast]) < \text{I}_{\text{st}} < \text{Thr}_{\text{max}}\). In the first case, only a short branch of g(DI) subsists near \(\text{DI}_{\text{min}}\). This branch is so short that 1:0 patterns are the only stable solutions. In the second case, as \(\text{I}_{\text{st}}\) increases above \(\text{Thr}(\text{DI}[\ast])\), a second branch is added that starts at longer DIs.

As an example, panel A of Figure 9 shows a BCL value at which a 2:2 pattern is the stable solution for \(\text{I}_{\text{st}} > \text{Thr}_{\text{max}}\). In panel B, the short beat of the 2:2 pattern now falls in the “inexcitability” gap introduced by the reduction of \(\text{I}_{\text{st}}\). It is now a failed response, and a 4:3 pattern (Short, Long, Failed, Long) now becomes the stable solution. Further reduction of \(\text{I}_{\text{st}}\) in panels C and D still enlarges the gap and changes the nature of the stable response to a 2:1 and finally a 6:3 phase-locking pattern. The entire bifurcation structure of regions I and II of current intensity can be explained similarly by the presence of the “inexcitability gap.” The succession of stable states for a current of 3.3 \(\mu\text{A/cm}^2\), in region I, is illustrated in Figure 7B.

On the basis of this analysis, it also can be understood why the case with 25-msec stimulus duration is “generic.” For a stimulus of 1 msec, the threshold function is almost flat, except for a sharp variation near the ARP (see Figure 4B). For \(\text{I}_{\text{st}}\) over the maximum threshold, the full g(DI) function is used for iteration. Because the g(DI) function for 1 msec is almost identical to the one for 25 msec (Figure 4B), the sequence of bifurcation will be the same for both cases. For 1-msec duration, if \(\text{I}_{\text{st}}\) is decreased below the maximum threshold, only a small portion of g(DI) is left for iteration, near the ARP. This segment has a high slope and cannot sustain stable solutions. It also is short and cannot sustain simultaneously beats from the first and second iterate (BCL, 2xBCL). Hence, phase locking decays abruptly to 1:0 for currents less than the maximum threshold. For 100-msec stimulus duration, the g(DI) function always has a slope less than 1 (see Figure 4B). The 1:1 patterns are stable, as long as the fixed point falls on the g(DI) function. As BCL is reduced, successive n:1 patterns appear, as is the case for the main sequence observed for a 25-msec stimulus with low current intensities (region I, Figures 5A and 5B). The results for the full parameter space for durations of 1 and 100 msec presented elsewhere\textsuperscript{27} confirm this analysis.
Modified Beeler-Reuter Model Parameter Space and Comparison with Difference Equation Model

The parameter space of the MBR model was obtained for a stimulus duration of 25 msec. The system was reset to rest at the beginning of each train of stimulation. To compute AR from the MBR model, an empirical definition of successful and failed responses must be provided. Those responses with ionic current active (Ion<0) for at least 0.5 msec were considered successful, which is the same criterion as that adopted for the first approximation of the threshold (see "Methods"). The parameter space is shown in Figures 5C and 5D. It can be seen readily that its structure is globally similar to that of the parameter space of the DE model (Figures 5A and 5B), particularly in reference to the existence of three separate regions of current intensity. Analysis of the DE model has shown that this was a consequence of supernormality in the threshold function. Comparison of the two models was made in each region regarding the similarity of bifurcation sequences and of the BCL location of transitions between various phase-locking patterns. Fine structure of the chaotic zone also was studied.

In region III, for high $I_{st}$, the main sequence of bifurcation from 1:1 to 4:2 is present in both models, and there is a zone of irregular dynamics between 4:2 and 3:1 phase-locking regions. The transitional zone between 4:2 and irregular dynamics was scanned using $\Delta$BCL of 0.1 msec with a maximum of 400 beats. A sequence of period doubling was found (4→8→16 while decreasing BCL) in an interval shorter than 2 msec. Because the AR in this interval was highly dependent on the criterion used to define an active response, there were one or two borderline responses. At even shorter BCLs, aperiodic responses were found, mixed with short bursts of periodic patterns as seen in the DE model. Progression of the bifurcation structure for the DE and MBR models is presented in more detail in Figure 7, panels A and C, respectively, for $I_{st}$=4 μA/cm$^2$. For MBR, maximum ionic current after each stimulation ($I_{on}$) is presented because APD is not available when the next stimulation comes before the end of repolarization (V≤V$\text{mem}$, see "Methods"). Finally, bistability and hysteresis also were found in the MBR model at the junctions of the 1:1, 2:2, 2:1, and 4:2 zones.

In addition to the difference in the cascade leading from 4:2 to irregular dynamics, other differences between the two models exist in region III. In the DE model, the BCLs at which there are transitions between successive phase-locking zones do not depend on $I_{st}$ (i.e., transition lines are vertical in region III), whereas transition points shift toward lower values of BCL when $I_{st}$ is increased in the MBR model (i.e., transition lines are no longer vertical in region III, see Figure 5C). Two factors can explain this effect. In the MBR model, latency diminishes as $I_{st}$ is increased above the threshold. The resulting shortening of the total APD allows any regime to be maintained at shorter BCL values as $I_{st}$ is increased. In a regime in which missed beats are intercalated between successful responses, the DE model ignores the effect of subthreshold responses that tend to prolong the previous action potential. In the MBR model in a 2:1 regime, for example, because of the effect of subthreshold responses, the DI of successful action potentials will be shorter than the 2×BCL→g (DI) predicted by the DE model. These shorter action potentials will result in an enlargement of the BCL range with 2:1 phase locking. Furthermore, in a 4:2 regime, this also should decrease the amount of alternation, the subthreshold response increasing the interaction between the successful action potentials. In the context of the DE model, this is equivalent to making the slope of the lower portion of the g(DI) function smoother at short DIs and may explain some differences in the bifurcation structure of the two models near the onset of chaos. Indeed, we have demonstrated previously that in the DE model this portion of g(DI) controls the exact scenario of bifurcation before the onset of chaos. The 2n→n+1 sequence was specifically a result of the change in slope of g(DI) at short DIs. Finally, as seen in Figure 7C, a small region of 4:2 interrupts the 2:1 zone in the MBR model. This is not found in the DE model but has been observed in different systems.

In region I, corresponding to low current intensity, successive n:1 ratios are encountered in both models. From TI transitions described for the DE model in the 3:1, 5:1, and 6:1 zones, only 3:2 and 5:2 ratios were detected in the MBR model (see Figure 7D). As shown in Figure 8, TI transitions occur in the DE model when beats fall in the gap introduced in g(DI) by using $I_{st}$ less than the maximum threshold. For complicated ratios (e.g., 6:3), at least one successful beat falls near the gap, in a region where $I_{st}$ comes very near the threshold. In the MBR model, the latency of each of these beats will be maximum, and this may prevent the stabilization of the pattern.

Within region II of the MBR model, TII transitions occur as in the DE model at BCLs between 350 and 250 msec (2→1→2→2) and between 150 and 130 msec (4→1→4→2) (compare Figures 5B and 5D). Also, as in the DE model, a third type of transition (TII') occurs within region II at BCL=150 msec, but at higher current intensity. However, and this is the most striking difference between the two models, the family of ratios and periods for the MBR model within this range is far less complex than that in the corresponding transition of the DE model (inset, Figure 5A). Activation ratios of 6:3 occur in both models as a mix of 3:2 (Short, Failed, Long) and 3:1. Zones with higher periods and large numbers of missed beats in the DE model become a homogeneous 3:2 region in the MBR case. The effect of subthreshold responses certainly can explain part of this difference. However, with stimulations of relatively high amplitude applied at high frequency, the limit may have been reached where the MBR model simply no longer behaves as a one-dimensional system. One main
hypothesis of the DE model in our simulations is that the system does not have "memory" (the original version includes a memory effect\textsuperscript{15,21} and that the action potential returns always following the same path of repolarization, regardless of the frequency or amplitude of the stimulus. Furthermore, if differences between the two models in the TI zone of region II actually reflect high-dimensional behavior of the MBR model, then such a behavior also should appear in the zone of irregular dynamics that spans the same BCL interval at still higher $I_n$.

\textit{Modified Beeler-Reuter Nonperiodic Region}

To explore the region of irregular dynamics of the MBR model, return maps of $V_{\text{max}}$ were constructed from 1,500 successive stimulations at a constant pulse amplitude of 3.7 $\mu$A/cm$^2$ and duration of 25 msec. Results are presented in Figure 10 for four BCL values (177.5, panel A; 176.5, panel B; 175, panel C; and 173.5 msec, panel D) in the range of irregular dynamics at this amplitude of stimulation (see Figure 5C). $V_{\text{max}}$ was selected because it eventually should allow for direct comparison with experimental results.

Consider first the map of Figure 10A, which has four branches. Iteration proceeds from branch 1 to branch 4 and gives rise to successive sets of four responses. Branches 1 and 3 correspond to full-blown action potentials with sodium current-mediated upstrokes; branch 2 is related to passive electrotonic responses in which $I_{\text{on}} \geq 0$. $V_{\text{max}}$ is high because the stimulus falls in the ARF, when the membrane is still depolarized. The structure of branch 4 is more complex. Potentials associated with the small segment after the minimum (dotted box) are purely electrotonic responses with no inward ionic current.

Each one of those in the box is followed by an active response on branch 1. However, branch 1 is folded (more apparent in panel B of Figure 10), and the action potentials after the passive response of branch 4 all fall on its lower segment. Responses on the other upward-going segment of branch 4 are active responses, with relatively low ionic current. Bottom points of this segment of branch 4 are calcium-mediated upstrokes, with an increasing sodium current contribution as $V_{\text{max}}$ moves toward the top of the branch. Branch 4 thus is the result of supernormality, and its minimum corresponds to the end of the ARP. Tightly packed $V_{\text{max}}$ values on branch 3 are widely dispersed when they fall in branch 4, meaning sensitive dependence on initial conditions, a sine qua non condition for chaotic regime.\textsuperscript{28}

Iteration sends the point labeled X on branch 1 to the minimum of branch 4. After iteration from branch 1, corresponding to successful upstrokes (U), all points originally on the XB segment return to the small folded segment of branch 1 after the 4:2 firing sequence (UFUF) (F, failed beat). On the other hand, all points of the remaining AX segment end up covering the complete original AB segment (point A now falls at B, and X at A), after the 4:3 firing sequence (UFUU). Among those points, some go through another round of 4:3 firing, whereas others are followed immediately by a sequence of 4:2. The resulting dynamics thus appear as a varying number of 4:3, interrupted by a single 4:2. Chaotic dynamics would mean that the number of 4:3 intercalated between the 4:2 becomes unpredictable. As BCL is decreased in panels A–D of Figure 10, the length of the folded segment of branch 1 increases such that successive 4:2 sequences also become possible. It
strated in single myocytes in which it was induced with current pulses of low amplitude and long duration.\textsuperscript{33} Hence, the results may be relevant for cells receiving stimulation emerging from regions of depressed conduction velocity or excitability.\textsuperscript{21,22}

APD(DI) is the second determinant of the dynamics in the DE model. In the MBR model, the Thr and APD functions were shown to be almost independent of the previous pacing history of the system (S\textsubscript{2}-S\textsubscript{3} period was varied from 4,000 to 300 msec). APD also is relatively insensitive to duration or intensity of stimulation. This fulfills the main hypothesis of the DE model\textsuperscript{15} and discards any possibility that long-term memory processes are involved in the dynamics of the MBR model. This also means that, irrespective of the precise nature of the upstroke, the system always returns to rest by following more or less the same trajectory controlled by the slow variables. DI then becomes a measure of the position on this invariant trajectory. The huge variation of APD(DI) near the end of the ARP mainly is controlled by the state of the slow variables at the beginning of stimulation. For high current stimulus (I\textsubscript{M} > Thr\textsubscript{MAP}), the DE model shows that the bifurcation structure depends precisely on the details and extension of this high slope region\textsuperscript{32} and that even slight modifications may profoundly change the exact sequence of bifurcations (e.g., cascade of 2n·n+1 at onset of chaos, compared with more usual period doubling observed in Reference 34).

Analysis of the DE model is relevant because it reproduces many important features of the MBR dynamics. The DE model predicts that the 25-msec case is “generic,” embedding short and long stimulus duration bifurcation structures at respectively high and low amplitudes. This was verified in the MBR model.\textsuperscript{33} For type-2 supernormality, the bifurcation structure is highly dependent on stimulation amplitude, and the parameter phase could be divided into three regions of I\textsubscript{M} whose borders closely coincided in both models.

Moreover, for high I\textsubscript{M}, the sequence of bifurcations from 1:1 to 4:2 is identical in both models, except for a short region of 4:2 intercalated in the 2:1 region of MBR. Hysteresis and bistability are also found in both cases. There are differences in BCL loci of transitions because they vary with I\textsubscript{M} in the MBR model. This comes from the fact that the latency is assumed to be constant in the DE model. For any specific I\textsubscript{M}, concordance of BCL values of transition can be very much improved by using latency curves constructed from an S\textsubscript{1}-S\textsubscript{2} protocol with this specific I\textsubscript{M} for S\textsubscript{2} stimulation.

At lower BCL, chaotic dynamics are found for both models, between 4:2 and 3:1 phase locking. The two models show different cascade roads to chaos (2n·n+1 in DE, incomplete period doubling in MBR), as well as differences in the fine structure of the chaotic zone (mixture of alternating 2:1 and 1:1.

<table>
<thead>
<tr>
<th>Table 1. Modified Beeler-Reuter Model Maximum Lyapunov Exponent</th>
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<tr>
<td>Basic cycle length (msec)</td>
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<td>Lyapunov exponent (10\textsuperscript{-3})</td>
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should be noted that similar maps have been observed in Purkinje muscle preparations.\textsuperscript{22} Table 1 shows the maximum Lyapunov exponent calculated for each map of Figure 10 (see "Appendix" for details of calculation). Their values greater than zero show that MBR is indeed a chaotic regime for all these BCLs. However, the structure of the chaotic regime, a mixture of 4:2 and 4:3, is slightly different from that predicted by the DE model (mixture of 2:1 and 1:1, see Figure 8 and related discussion). Furthermore, the increasing level of folding observed in the MBR maps (see Figure 10, panel D) may indicate loss of unidimensionality,\textsuperscript{31} particularly in branch 4, associated to stimulation falling in or near the ARP.

**Discussion**

The DE model postulates that Thr(DI) and g(DI) are the only determinants of the dynamics. The MBR model was shown to yield different types of threshold curves, depending on the duration of the S\textsubscript{1} stimulus used in the S\textsubscript{1}-S\textsubscript{2} protocol. For a short stimulus duration (~1 msec), only the sodium current activates fast enough (τ<0.25 msec) for an upstroke to result from the short transient increase of potential associated with the stimulation. Except for a short interval near the end of the ARP,\textsuperscript{12} the system has an almost fixed voltage threshold for fast sodium upstroke. Thr(DI) becomes equivalent to a measure of the voltage distance from threshold. When stimulus duration is increased in the range of calcium current activation (τ<40 msec), the latter also may contribute to the upstroke and change the form of Thr(DI) to type-2 supernormality. This effect depends on the duration of the stimulus relative to the time course of the inward currents of the model and on the relative position of the activation window of these currents on the voltage axis. Because Thr(DI) is a major determinant of the dynamics, it gives a powerful test for estimating the dynamical implications of any reformulation of the inward currents. Possibly, a set of experimental Thr(DI) curves will become available. Such curves would provide a good way to evaluate the adequacy of the actual ionic models, as well as an indication of the time constant and potential range at which improvement is most needed. However, we have learned by experience that it is not an easy task to obtain such a set of curves with a satisfying precision from an isolated single myocyte. (Anomonymow and Vinet, unpublished observations, 1990). Experimentally, changes in supernormality have been induced by manipulation of the extracellular potassium concentration.\textsuperscript{15,22} MBR results suggest that this also could happen through a purely dynamical process involving only stimulation parameters. Indeed, Wenckebach phenomena have been demonstrated in single myocytes in which it was induced with current pulses of low amplitude and long duration.\textsuperscript{33} Hence, the results may be relevant for cells receiving stimulation emerging from regions of depressed conduction velocity or excitability.\textsuperscript{21,22}
for DE; 4:3 and 4:2 for MBR). These, as well as other differences in the two other ranges of I_n are partly explained by the fact that the DE model neglects the effect subthreshold events. This could be corrected by extending g(DI) into the ARP.

However, there is undoubtedly a region near the end of the irregular dynamics zone where MBR dynamics could no longer be seen as unidimensional. This occurs at short BCLs when multiple beats fall on both sides near the end of the ARP. The system does not have time to return to an invariant trajectory between stimulations, and there is a buildup in values of the slow variables (closure of calcium current and activation of potassium current) that depends on the one or two preceding stimulations. The same type of limit occurs also when phase locking of automatic systems is represented by iteration of a phase–transit curve (PTC).35

The present study shows that the DE model gives a good representation of the dynamic properties of the MBR model. The DE model already has been shown to apply in different experimental contexts.15,21,22 However, a comparison between measured and predicted results obviously could not be performed with the detail used for this article (ABCL ≤ 0.5 msec, ΔL ≤ 0.1 μA/cm²). When comparing with experimental data, one must remain aware of possible shifts of BCL location of phase-locking transition, because the latency may be either overestimated or underestimated. Underestimation is very likely to happen for stimulation very near threshold, and certain fine transitions predicted by the DE model may not be present (e.g., TF transition in region 1 of current, not found in the MBR model; see Figure 5). Similarly, high stimulus intensity may modify the steep slope region of the APD function, usually making it smoother and less abrupt,15,21 and may lead to some predictable alteration of the bifurcation sequence (e.g., incomplete period doubling sequence). Besides, biological noise certainly interferes with observable patterns. Study of noise effects on the DE model has not been performed because data are lacking on the distribution characteristics of the noise in excitable cells. This has been studied in different types of models (e.g., References 36 and 37). The effects of noise in models were shown to be nontrivial, especially in or near the chaotic regime, where periodicity could be alternatively restored or destroyed depending on the fine structure of the bifurcation sequence and of the noise.38

Finally, it has been shown that a memory factor, with a long time constant (1.4 sec), has to be included in the DE model to reproduce experimental results obtained with pieces of tissue.15 The effect of this memory is to smooth the APD function at short DIs. It remains to be seen if such a memory factor also is important when dealing with dynamics of isolated cells, where effects of ion accumulation in the interstitial medium obviously are absent.

**Conclusions**

In our opinion, the DE model is not an alternative, but rather is a complement to the ionic model. The DE model provides a global understanding of the dynamical properties of the ionic model and stresses the central structural properties responsible for its behavior in response to repetitive stimulation. All of this is done at a relatively low computational cost. In addition, the results of the DE model can be compared directly with those of the MBR model, which also allows the detailed analysis of the ionic bases of the phenomena in question. In turn, the combined results of both models should provide testable predictions in terms of the ionic mechanisms of complex dynamics of excitation of cardiac cells in response to repetitive stimulation. As new knowledge about the molecular processes involved in cardiac cell excitation arises, ionic models are constantly being revised (e.g., new currents, pumps, more accurate kinetics), and it is difficult to appreciate at the outset how each new addition could change the dynamical properties under repetitive stimulation. Using the DE model may help to provide such an insight, as well as serve as a guide for determining which additions or deletions are necessary for an accurate representation of experimental results.

**Appendix**

Maximum Lyapunov exponent was obtained for the MBR model using the method developed by Shimada and Nagashima.39 For each specific BCL, the system is reset to rest, and the trajectory is reconstructed with a fixed time step (Δt) of 0.005 m.sec. For each time step, evolution of the initial vector V(i) = (1, 0, 0, 0, 0, 0, 0) in the tangent space is calculated as V(i+Δt) = V(i) + ΔtJ(i)V(i), with J(i) the Jacobian of the system at time t. After each 100 time steps, the quantity

S_i = ln(||V(i+Δt)||/||V(i)||)

is calculated, V(i+Δt) is normalized, and calculation continues. At the beginning of each period of stimulation, evaluation of the Lyapunov exponent is obtained as

λ = (1/T) Σ S_i

where T is the total time elapsed and summation runs over all previously calculated values of S_i. Calculation is stopped when variation between two successive values of λ was less than 10⁻⁵. In all cases, convergence was detected after not less than 200 stimuli.

**Acknowledgment**

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**References**


**KEY WORDS** • cardiac action potential • entrainment • mathematical model • phase-locking • deterministic chaos