Supernormal Excitability as a Mechanism of Chaotic Dynamics of Activation in Cardiac Purkinje Fibers

Dante R. Chialvo, Donald C. Michaels, and José Jalife

Supernormality, which can be defined as greater than normal excitability during or immediately after action potential repolarization, has been observed in a variety of cardiac preparations. However, as yet, no description of the dynamics of tissue response to repetitive stimulation in the presence of supernormal or relatively supernormal excitability has appeared. Isolated sheep cardiac Purkinje fibers (2–5 mm in length) were superfused with Tyrode’s solution and stimulated with depolarizing current pulses through a suction pipette. Recovery of excitability, restitution of the action potential duration, and response patterns were measured in each fiber for a wide range of current amplitudes and stimulation frequencies. When the potassium chloride concentration of the Tyrode’s solution was decreased from 7 to 4 mM, the excitability recovery function consistently changed from monophasic (“normal”) to triphasic (“supernormal”). During repetitive stimulation at increasing rates, normal preparations responded only with gradual changes in the activation ratio, expressed as periodic phase-locked responses (i.e., Wenckebach-like patterns, etc.). Supernormal preparations showed a nonmonotonic change in the activation ratio, as well as complex aperiodic response patterns. Numerical results from an analytical model gave a quantitative basis for the relation between nonmonotonicity in the excitability function and the development of complex rhythms in cardiac Purkinje fibers. Both our experimental and theoretical results indicate that the presence of supernormality and the slope of the action potential duration restitution curve at short diastolic intervals are responsible for the development of chaotic dynamics. Moreover, our results give an accurate description of the supernormality phenomenon, predict the behavior expected under such conditions, and provide insight about the role of membrane recovery in determining regular and irregular frequency-dependent rhythm and conduction disturbances. (Circulation Research 1990;66:525–545)

Action potential propagation in the heart is characterized by an entire spectrum of rate-dependent activation patterns ranging from normal electrical discharges in which each impulse propagates from atrium to ventricle in a 1:1 fashion to the appearance of complete block in some portion of the conduction system. At the boundaries between 1:1 propagation and complete block, various intermediate processes can be demonstrated in all types of cardiac tissues, which may differ in either their manifestation or mechanism. As a result, a wide variety of arrhythmias can appear, many of which have rate dependency as their only common characteristic. A classical example of this type of behavior is the Wenckebach phenomenon, in which an increase in frequency leads to a predictable sequence of proximal to distal block patterns and ultimately 2:1 or higher degrees of block.

From a mechanistic point of view, the common denominator in all these rate-dependent phenomena may indeed be the fact that there exists a noninstantaneous (i.e., finite) recovery time for membrane excitability after each activation. As a result, with increasing stimulation frequency, there is usually a progressive decrease in the activation ratio (AR, defined as m:n ratio, where m is the number of action potentials or responses and n is the number of stimuli).

Recently, experimental evidence was provided suggesting that rate-dependent block processes in Purkinje fibers can be described according to universal rules previously developed for many biological, chemical, or electrical nonlinear systems. Moreover,
results in very different experimental preparations, ranging from clinical studies in humans to single guinea pig ventricular myocytes, also suggest that this novel dynamic approach can be useful for investigating the general behavior of such time-dependent dynamic processes.

In spite of their differences, many nonlinear systems with finite recovery time behave in much the same way. Providing that the relevant parameters (e.g., excitability) recover monotonically, the sequence of n:m patterns (stimulus/response locking, or locked patterns) that occur as the stimulation frequency is systematically altered can be theoretically predicted by the so-called Farey tree. Moreover, by assuming that the atrioventricular node has such a mode of recovery, Keener formulated a complete theory (partially based on previous models) to describe these dynamics.

However, under certain circumstances, cardiac Purkinje fibers do not have a monotonic recovery of excitability. In fact, in some cases, a relatively super-normal phase of excitability at early diastolic intervals may result in more complex types of phase-locking behavior in these tissues. For example, both experimental and clinical observations of 4:2 block have been related to the presence of supernormality. In addition, even more complex (i.e., chaotic) rhythms were found in experiments under similar conditions. No satisfactory theoretical formulation exists to explain the appearance of all of these patterns.

The present study was designed toward the following goals: first, to extend previous observations of periodic and aperiodic dynamics in cardiac Purkinje fibers during repetitive stimulation and to analyze which parameters are relevant to this behavior, and second, to use these relevant parameters as variables for the formulation of a simplified analytical model that would allow further investigation of the theoretically expected dynamics.

Materials and Methods

Experiments

Cardiac Purkinje fibers were obtained from seven young sheep (10–25 kg) of either sex anesthetized with sodium pentobarbital (35 mg/kg). Hearts were removed rapidly through a thoracotomy and immersed in warm, oxygenated Tyrode’s solution. Thin, externally unbranched free tendons were excised from the left ventricle and mounted in a 6-ml Plexiglas chamber superfused with Tyrode’s solution of the following millimolar composition: NaCl 130, NaHCO₃ 24, NaH₂PO₄ 1.2, MgCl₂ 1.0, CaCl₂ 1.8, KCl 4.0 or 7.0, and glucose 5.6. The solution was gassed with a mixture of 95% O₂-5% CO₂, which resulted in a pH of 7.4 at 36 ± 0.1°C. Temperature was monitored continuously on a pen recorder (model 2400S, Gould Instruments, Cleveland, Ohio).

Transmembrane potentials were recorded 1–2 mm from the stimulation electrode with a glass microelec-

trode filled with 3 M KCl (DC resistance, 10–30 MΩ) coupled to a high input impedance amplifier (model RS 700, World Precision Instruments, New Haven, Connecticut). The signals were displayed on a digital oscilloscope (model 5222, Tektronix, Beaverton, Oregon) and recorded on analog tape (model D, A.R. Vetter, Rebersburg, Pennsylvania) for later analysis.

Fibers were driven near one end of the preparation with rectangular constant depolarizing current pulses (duration, 10–20 msec) applied through a suction pipette (0.3 mm o.d.), which was made of heat-thinned polyethylene tubing (PE 50, Intramedics, Parsippany, New Jersey) filled with Tyrode’s solution. To reduce electrode impedance to a minimum and thus allow for true constant current operation, a long Ag/AgCl wire (0.02 mm diameter) was inserted into the Tyrode’s-filled tube and connected to the metal terminal in the Plexiglas microelectrode holder. Another Ag/AgCl wire, placed near the pipette, was used as a reference electrode. Pulses were delivered through a modified P6i Frederick Haer stimulator (Brunswick, Maine). Current strength was measured as the voltage drop across a 1-kΩ resistor in series with the suction pipette.

Strength-interval curves were determined using single test pulses (S₁) delivered after every tenth basic pulse (S₀) at a basic cycle length (BCL) of 2,000–3,000 msec (see Figure 1). The S₁S₂ interval was decreased progressively from the BCL value to the refractory period, while the threshold current was
measured at each step. After excitability curves were obtained, we tested for stability in the intensity of the current pulses applied by checking critical current values (e.g., “dip” and “peak” values of supernormal phase; see Figure 5). The response of the preparation to repetitive stimulation was then determined under similar experimental conditions. Values of current strength close to the measured strength-interval curve were selected and used for stimulation at several different values of BCL. Each test was always initiated after at least 10 seconds of quiescence, and the resultant pattern was evaluated after reaching a steady state (whenever possible). Patterns were considered to be stable stimulus/response locking when the same sequence of action potentials and dropped beats was repeated several times (typically, for short [e.g., 4:3, 5:3] sequences, 10 times, and for long [e.g., 9:2] sequences, four or five times). The same protocol was repeated at low and high potassium chloride concentrations (4 and 7 mM). At the end of the experiment, the recordings obtained during the strength-interval protocol were played back and action potential duration (APD) and latency-to-activation measurements were done to construct the APD restitution and latency curves. Steady-state APDs were measured from 1:1 stimulus/response locking at different frequencies during repetitive stimulation. The same protocols were carefully applied to seven preparations. All data were collected from preparations in which pacemaker activity was absent.

**Difference-Differential Model**

During repetitive stimulation, several different rhythms, either periodic or aperiodic, may arise that depend on the presence or absence of supernormal recovery of excitability. Whether these rhythms are the result of different specific tissue properties may be determined if the same basic theoretical argument can be applied to all the observations.

We hypothesized that these rhythms arise as a direct consequence of the peculiar mode of restitution of three parameters—excitability, latency, and APD—modulated by an additional slow (“memory”) process.

Figure 1 presents the most important assumptions used in the model. Panel A shows the action potential parameters used in our formulation. As is generally accepted for experiments using the “S1S2 protocol,”9,11 the test threshold (Th), APD (APD), and latency (L) are functions of the test coupling interval (S1S2) or, more specifically, of the diastolic interval (DI) after the tenth basic beat. DI can be approximated as the coupling interval minus the last basic APD (APD) minus latency (L). Thus,

\[ DI = (S_1S_2) - APD - L \]

\[ L = F(DI) \]

\[ APD = R(DI) \]

\[ Th = Z(DI) \quad \text{For } St > Th \]  

(1)

where St is the strength of the stimulation and Th is the threshold current requirement at the moment at which the pulse is applied. Obviously, the L value is a function of both the degree of recovery of excitability (F) and the stimulus strength. The APD restitution function R is well known for these experimental situations;12 it decreases monotonically as DI decreases. The Z function (threshold) is the strength-interval curve obtained experimentally.

Figure 1B illustrates the condition during periodic stimulation. Here, S1 represents the first stimulus following a sudden change in frequency (i.e., equivalent to an S1 in panel A), after which constant BCL (i.e., equivalent to the S1S2 interval in panel A) is maintained. Using the same formulation as before, we can rewrite Equation 1 as

\[ DL_n = BCL - APD_n - L_n \]

\[ L_{n+1} = F(DL_n) \]

\[ APD_{n+1} = R(DL_n) \]

\[ Th_{n+1} = Z(DL_n) \quad \text{For } St > Th \]  

(2)

Then, during repetitive stimulation, the recovery of the membrane during the nth cycle (i.e., at the S1S2 interval) will determine the value of APD, Th, and L in the next cycle, and so on.

Basically, Equation 2 is an algebraic representation of the conceptual steps usually followed in the literature for the analysis of rate-dependent block processes. However, the characteristics of the function governing APD under these circumstances are known only for two very particular cases: one, during the steady state (i.e., after several beats at the same BCL), described as a hyperbolic process,12 and the other representing the situation described by the restitution curve,12 which is responsible for the short term APD adjustment as a function of the previous diastolic interval. The latter is well fitted by two exponentials and is described by the following equation:

\[ APD = APD_{max} \times \left[ 1 - A_1 \times \exp \left( -DI/T_1 \right) \right] \]

\[ -A_2 \times \exp \left( -DI/T_2 \right) \]  

(3)

where DI is the diastolic interval, APD_{max} is the maximum action potential duration, and A and T are the amplitude and time constant of the fast (A_1, T_1) and slow (A_2, T_2) exponential components of the curve. Because in our experiments the (dynamic) process was more complex than in the previously mentioned two cases, we needed to provide for a continuous estimation of time-dependent changes on R. We propose here that the function R in Equation 2 shifts each beat gradually from one case (restitution) to the other (steady state) as a function of the memory dissipation (M). Based on previously reported models13 we considered M as growing exponentially toward 1 during each action potential and decreasing in reverse fashion toward 0 during the diastolic interval. M is thus described by the following equation:
\[ M = \beta AP - \Delta M/dt \quad (4a) \]

where \( \Delta \) is the memory time constant and \( \beta AP \) equals 1 during the action potential and 0 otherwise. Then, during the action potential, memory grows from the previous value \([M_{t0}] \) equal to 0 at the first action potential as described by

\[ M_{t0} = 1 + [(M_{t0}) - 1] \times \exp(-t/\Delta) \quad (4b) \]

where \( t = APD \). From \( M_{t0} \) (i.e., memory value at the end of the action potential) during the diastolic interval memory decays following the equation:

\[ M_{t+1} = M_{t0} \times \exp(-x/\Delta) \quad (4c) \]

where \( x = DI \).

By using Equation 4, it becomes possible to rewrite Equation 2 to incorporate the memory effect. Thus,

\[ DI_t = BCL + APD_n - L_n \quad a) \]

\[ L_{n+1} = F(DI_n) \quad b) \]

\[ APD_{n+1} = R(DI_n) \times (1 - M) \quad c) \]

\[ Th_{n+1} = Z(DI_n) \quad d) \]

For \( St > Th \) (5)

The iterative procedure used to solve Equation 5 is as follows: first, assuming that initial conditions are such that \( APD_0 = 0 \) and \( L_0 = 0 \) (i.e., there was no previous beat), for a given BCL calculate the recovery (\( DI_n \)) which will determine in (b) the next latency (\( L_{n+1} \)), and in (c) \( APD_{n+1} \) as well as in (d) \( Th_{n+1} \). Furthermore, since each beat modifies the value of \( M \), the next APD is affected (solving Equation 4) proportionally. The values obtained are now used to establish the next recovery time (\( DI_n \)). The condition of suprathreshold stimulus (\( S > Th \)) is also tested for each beat. If that comparison results in \( S > Th \) then \( DI_{n+1} \) is calculated as \( BCL + DI_n \). The overall procedure is repeated iteratively until a steady-state response is achieved. For the present study we used an iterative program, which was written in BASIC and run on a personal computer.

**Parameter values used in the simulations.** The parameter values incorporated in the model are representative of our experimental data and consistent with previously reported measurements. The APD restitution curve (e.g., Equation 3) to parameters was as follows: \( A_0 = 0.40 \), \( T_1 = 150 \) msec, \( A_2 = 0.13 \), and \( T_2 = 1,400 \) msec. Memory was calculated solving Equation 4 in each iteration for \( \beta AP = 0 \) during a time equal to the previous diastolic interval, and for \( \beta AP = 1 \) for an interval equal to the APD, using the Equations 4b and 4c, respectively, with a time constant (\( \Delta \)) of 100 seconds. For the examples discussed in Figures 12 and 13 the strength-interval curve was oversimplified using a five-step linear piecewise approximation. Threshold current in this curve was normalized, in arbitrary units (AU), taking the late diastolic threshold as the minimum (e.g., 2.5 AU in Figures 12A and 12B) and the threshold corresponding to the absolute refractory period as 10 AU. For the simulations presented in Figure 14 we used the experimental curves (strength-interval and APD restitution) illustrated in Figure 2. Linear interpolation between experimental data points was used to obtain an adequate description of such curves. It has been shown that the latency to activation (\( L \)), for a given stimulus amplitude, is directly related to the threshold function. Thus, for the sake of simplicity, \( L \) was considered to be proportional to the calculated threshold (e.g., \( Th = 2 \) AU \( \rightarrow L = 2 \) msec).

To our knowledge, a general analysis of Equation 5 (hereafter called “difference-differential model”) has not been given previously. Two special cases (in which memory is not included) have been described by Glass and Mackey (where an overview of these types of equations and references to previous work can be found). These equations may admit several solutions (different dynamics) whose detailed description exceeds the scope of this paper and will be given elsewhere.

**Results**

**Isolated Tissue Experiments**

**Monotonic versus nonmonotonic recovery of excitability.** Consistently, in all the experiments, quiescent sheep Purkinje fibers showed two major types of behavior in response to repetitive stimulation, depending on the time course of recovery of excitability after an action potential. These behaviors could be identified, in the same fiber, by changing the potassium chloride concentration in the Tyrode’s solution used as superfuse. Results from one preparation are presented in Figures 2–4. In Figure 2, panels on the left (\( A_0 \) and \( A_2 \)) show data obtained at 7 mM KCl; panels on the right (\( B_0 \) and \( B_2 \)) illustrate results gathered with similar protocols at 4 mM KCl in the Tyrode’s solution. The strength-interval curves obtained by using the \( S_0 S_2 \) protocol (Figure 1A) are plotted on panels \( A_0 \) and \( A_2 \), together with an action potential, to illustrate the voltage responses obtained under each condition; the respective APD restitution functions are shown in panels \( B_0 \) and \( B_2 \). In Figure 2, under the conditions of 7 mM KCl/Tyrode’s solution superfusion, a monotonic decrease in threshold current (panel \( A_0 \)) and an increase in APD (panel \( A_2 \)) were observed as the coupling interval was increased. During superfusion with 4 mM KCl/Tyrode’s solution, the APD curve was shifted to the right and upward, and the rate of APD change greatly increased (panel \( B_2 \)), but the overall restitution function remained monotonic. However, as shown by the graph in panel \( B_0 \), the strength-interval curve became nonmonotonic and featured a phase of relative supernormality at \( S_0 S_2 \) intervals between 390 and 440 msec. As a result of these differences in the time course of recovery in excitability and APD, completely different dynamics were observed under each condition during repetitive stimulation at various BCLs.

**Monotonic recovery and staircase.** In the case of 7 mM KCl superfusion, application of repetitive depo-
Figure 2. Strength-interval curves (panels A1 and B1) and action potential duration (APD) restitution curves (panels A2 and B2) obtained from a sheep Purkinje fiber preparation at a basic cycle length (S1-S2 of 3,000 msec). In both A1 and B1, an action potential is presented (lower left) to indicate the phase of membrane potential at which the test stimulus was applied. Calibration bars in A1 and B1 are for the action potentials and represent 30 mV. Potassium chloride concentration was 7 mM for panels A1, A2, and 4 mM for panels B1, B2. While no supernormality is present at the higher concentration (A1), it becomes evident at the lower concentration (B1). Arrows indicate the stimulus amplitude used during the repetitive stimulation shown in Figures 3 and 4.

Polarizing pulses of constant duration (20 msec) and of amplitude (32 µA) equivalent to threshold at the interval of 600 msec in the strength-interval curve (arrow in Figure 2A1) led to predictable activation patterns. This is also illustrated by the analog recordings in Figure 3A1, which were selected from sections with steady-state patterns at the various cycle lengths. The overall sequence of patterns is presented in panel B as an AR versus BCL plot. Clearly, as the stimulus BCL was decreased in relatively small steps (see "Materials and Methods"), there was a progressive and predictable decrease in the activation ratio. The ratio was 1 for BCLs longer than 580 msec and became 0.66 (i.e., 3:2 locking) at BCLs between 580 and 570 msec. Further decrease of BCL to a range between 560 and 320 msec led to ratios of 0.60 (5:3 locking) followed by 0.50 (2:1 locking). Additional abbreviations of stimulus BCL yielded predictable locking patterns including 5:2 (AR = 0.4) between 320 and 310 msec, and 3:1 stimulus/response locking (AR = 0.33) for BCLs between 310 and 240 msec. With still further decrease of BCL, the activation ratio continued to diminish until a 7:1 pattern (AR = 0.14) was recorded at a BCL of 150 msec. Clearly, in this representative example, there is a monotonic relation between the activation ratio and the stimulus cycle length.

In all the experiments in which the recovery of excitability was monotonic, the AR versus BCL plot showed the characteristic staircase of predictable patterns exemplified in Figure 3B. An important theoretical feature of this type of staircase is that, if one explores carefully and with very small BCL changes between any two adjacent steps of n:m and N:M patterns, it should be possible to find additional smaller steps whose activation ratio is equivalent to (n+N):(m+M). For example, between 2:1 and 3:1 one should detect 5:2 (i.e., [2+3]:[1+1]) and between 5:2 and 2:1 one would expect to find 7:3 (i.e., [2+5]:[1+2]). The sequence of n:m numbers obtained by repeating such an operation is known as Farey’s sequence or series. Obviously, an infinite construction could be made by adding smaller and smaller adjacent steps. Yet, experimentally this would be impossible because the presence of noise would impede the observation of the predicted patterns.
Figure 3. Stimulus/response patterns obtained during repetitive stimulation of a sheep Purkinje fiber at a potassium chloride concentration of 7 mM; i.e., the strength-interval curve is monotonic (same conditions as in Figure 2, panels A1 and A3). Panel A shows action potential recordings. For each BCL, the upper trace is of transmembrane potential and the lower trace is the stimulus monitor. Numbers to the right indicate the ratio (N:M) of stimuli (N) to responses (M). Calibration bars for transmembrane potentials indicate 40 mV. Panel B shows a so-called “devil’s staircase” in which the activation ratio (AR) is plotted vs. the BCL. AR is the reciprocal of the stimulus/response ratio (SRR). For clarity, the locations of selected SRRs (1:1, 3:2, etc.) shown in panel A are indicated on the plot in panel B.

Supernormality and the discontinuous staircase. When similar measurements were repeated during exposure to 4 mM KCl, a completely different picture emerged (Figure 4A) with many complex cycle length-dependent changes in the AR. Indeed, under these conditions (pulse amplitude, 27 μA; duration, 20 msec; see arrow in Figure 2B1) there are three clearly apparent differences with respect to the 7 mM case. First, decreasing the stimulation cycle length led to abrupt transitions between 1:1 and 2:1 and between 2:1 and 3:1 without any intermediate patterns. Second, at brief cycle lengths the AR increased and decreased over narrow ranges of BCL. Third, there are discontinuities in the plot corresponding to regions of complex irregular dynamics. For example, at BCLs between 450 and 400 msec, the 3:1 pattern was replaced by 4:2 locking and that was followed...
by 5:2. The 4:2 pattern consisted of two successive action potentials followed by two failures (Figure 4A, left column), whereas 5:2 was characterized by two successive action potentials followed by three dropped beats (not shown). Finally, at the very brief BCL of 200 msec, unstable sequences of 7:2→10:3 (not shown)→9:3→5:2 appeared which finally stabilized in a 2:1 locking pattern. Clearly, under these experimental conditions, there is a complex relation between BCL and AR. In fact, unlike the monotonic recovery case, the presence of relative supernormality at 4 mM KCl allowed for the recurrence of some of the ARs at various BCL ranges. For example, as shown in Figure 4B, the AR of 0.5 was obtained at three different ranges: two of these were manifest as intermittent 2:1 locking and the third as a 4:2 locking pattern; 0.33 was detected twice, first as 3:1 at BCLs between 900 and 800 msec, and then again as an unstable 9:3 locking pattern at a BCL of 200 msec.

**Conditions for Stimulus/Response Locking**

**Monotonic recovery.** Demonstration of the various stimulus/response patterns illustrated in Figures 2–4 required specific sets of conditions. In the case of 7 mM KCl superfusion, it was necessary to keep the stimulus intensity at relatively low values to demonstrate not only the monotonic type of recovery (Figure 2A) but also the presence of postpolarization refractoriness, which permitted the establishment of the Wenckebach-like and intermittent block patterns observed as the BCL was changed over a wide range. Postpolarization refractoriness has been shown to occur under several experimental conditions as a result of a delayed recovery of excitability in response to critically timed stimuli of low amplitude. In Figure 3, when current pulses of low but constant amplitude were used, the Wenckebach-like stimulus/response patterns (e.g., 3:2 and 5:3) during high potassium chloride superfusion were characterized by progressive increases in the latency between the onset of the stimulus and the action potential upstroke, in the absence of APD change. Moreover, in all cases in which patterns with ARs between 0.5 and 1 were observed, failure usually occurred after repolarization was completed.

A second requirement for the establishment of these Wenckebach periodicities is that, at the relevant BCLs, the beat-to-beat APD changes must be negligible and the prolongation in the stimulus/response latency must be greater than the APD change. Although this is not very apparent in Figure 3 because of the slow recording speed, it was indeed the case in all experiments surveyed. Obviously this condition can only be achieved at the long BCLs, which correspond to S3s intervals at which APD restitution has reached a maximum level (i.e., between 1,000 and 3,000 msec in Figure 2A).

**Presence of supernormality.** In the presence of supernormality, the dynamic behavior of the Purkinje fibers in response to repetitive stimulation depended critically on the strength of the current pulse. Figure 5 shows a schematic representation of a strength-interval curve that has been divided into three different ranges of current intensity. Range I corresponds to stimulus amplitudes that are below the minimum current requirement at the relatively supernormal phase (dip); range III corresponds to stimulus amplitudes higher than the peak threshold current (peak) at intermediate intervals near the supernormal phase; and range II, also termed the "critical range," lies between I and III. When the stimulation strength was selected to be within the range of either I or III, changes in BCL led to monotonic decreases in the activation ratio. In fact, the usual patterns observed were those of "period adding" sequences (N+1 for N=1 to n) which began at the 1:1 level at long BCLs and changed to 2:1, 3:1, 4:1... N+1 and, finally 1:0 at very brief BCLs. However, unlike the monotonic case (e.g., see Figure 3B), no intermediate locking patterns were observed under the condition of supernormality (i.e., low potassium chloride superfusion) when the current pulse strength was within range I. In addition, in range III, stable APD alternans (2:2 rhythm?) was observed at BCL values that were intermediate to those resulting in 1:1 and 2:1 rhythms.

On the other hand, stimulus strengths within the critical range (range II in Figure 5) brought about a rich variety of regular and irregular BCL-dependent activation patterns, including those already presented in Figure 4. In addition, within this range of current amplitude, and for some BCL values, we commonly observed at least three forms of irregular (aperiodic) dynamics.

One type of irregular activity occurring at range II in the supernormal strength-interval curve (see Figure 5) was characterized by "recurrent fluctuations" between regular and irregular activation patterns. In the example presented in Figure 6, the preparation was superfused with 4 mM KCl; the stimulus parameters were the same (BCL, 370 msec; pulse ampli-
a single 2:1 event which was followed by four successive 1:1 responses. In a long run of hundreds of action potentials, the number of intervening 1:1 responses (e.g., 12 and 4 in Figure 6C) never repeated. Finally, although not very apparent in Figure 6, during 1:1 activation in any sequence of random fluctuation, there were small alternations in APD that decreased progressively and were eventually interrupted by the dropped beat. The significance of these decreasing alternations in the mechanisms of irregular activity at the critical range of current magnitude will become apparent below.

Another type of irregular activity, which we have termed "multirhythmicity," consisted of apparently random pattern fluctuations occurring at fixed stimulus magnitude and BCL. An example of multirhythmicity obtained from a Purkinje fiber superfused with 4 mM KCl/Tyrode's solution is presented in Figure 7. In all panels, the top trace is the transmembrane potential and the bottom trace is the current monitor. Depolarizing current pulses were applied repetitively. At constant pulse amplitude (64 μA), duration (20 msec), and BCL (190 msec) the response pattern fluctuated between three different stimulus/response patterns. An apparently stable 2:1 pattern (panel A) suddenly changed to a 6:3 (panel B), which eventually gave way to 16:8. Clearly, in this case, the presence of supernormal excitability led this Purkinje fiber to random transitions between multiple patterns whose common activation ratio was 0.5.

A third type of irregular phenomenon (termed "chaotic activity") observed within the critical range was characterized by highly aperiodic activity, with APD alternans. As shown by the recordings in Figure 8, the most important feature of this chaotic activity is that, during the successful 1:1 runs, the magnitude of APD alternation increases in a beat-to-beat manner and eventually leads to a failure. In this case also, the number of 1:1 responses within a given run never repeated even when the recording periods encompassed 100 or more stimuli.

**Influence of Noise**

It is conceivable that random noise (electrical, thermal, etc.) acting upon the stimulus parameters may have led to irregular activity in these experiments. It is conceivable that small variations in the bath temperature may have led to some of the instabilities in the recorded patterns. Indeed, although we did maintain strict control of this parameter, it is possible that variations of a few tenths of a degree may have been responsible for the coexistence of different patterns at certain BCLs. As discussed for the example in Figure 4, different patterns could be seen at a BCL of 200 msec even though the experimental conditions remained unchanged. The possibility that some kind of noise may have affected this pattern is suggested by the fact that the rhythm eventually stabilized at 2:1. However, in several experiments, we recorded apparently random back and forth fluctuations from one to another of those
disparate patterns, and the fluctuations were independent of temperature change. When these “multi-stable” states occurred, the activation ratio increased and decreased randomly, unlike those cases of multi-rhythmicity (Figure 7) in which the activation ratio remained constant in spite of large changes in the stimulus/response pattern. Finally, “noisy rhythms” were detected also in the absence of supernormality. However, these rhythms were characterized by random alternation between neighboring predictable patterns in the monotonic staircase (Figure 3); for example, between 2:1 and 1:1 or between 2:1 and 3:1.

In the following sections, theoretical arguments are used to analyze the experimental data presented thus far. Our intention is to provide a unified explanation for the different rate-dependent behaviors occurring at high and low potassium chloride concentrations. In analyzing irregular patterns (traces from Figures 6–8) we have used specific criteria which have enabled us to differentiate between random (noisy) rhythms and deterministic chaos.

Numerical Simulations and Theoretical Predictions: An Introductory Example

The tools derived from the theory of dynamical systems can be useful in providing insight into the complex behavior demonstrated by our preparations. In all our experiments, changing the BCL to a new value after a long period of quiescence was usually accompanied by transient changes in the relevant variables, which eventually settled into a new pattern. This pattern could be periodic or irregular, depending on the particular experimental conditions and on the BCL. Our hypothesis is that all of these behaviors are the direct result of the interval dependence in the recovery of three variables: excitability, latency, and APD. We thus formulated an analytical model that enabled us to predict both graphically and numerically how these rate-dependent activation patterns are established. Consider the dynamics associated with a sudden BCL change in a purely hypothetical example. We will assume for the time being that the overall behavior of this system is determined solely by the BCL dependence of APD (Figure 9). We will assume also that the APD in a given beat (\( \text{APD}_{n+1} \)) during a run of repetitive stimulation is determined exclusively by the preceding diastolic interval (\( \text{DI}_n \)). This assumption requires that we ignore for the moment the contributions of latency (\( \text{L}_n \)) and memory (M) in determining beat-to-beat APD changes (see “Materials and Methods”). Then, the set of Equation 5 can be simplified to

\[
\text{DI}_{n+1} = \text{BCL} - \text{APD}_n \\
\text{APD}_{n+1} = R(\text{DI}_n)
\]

An additional assumption for the present analysis is that the stimulus amplitude is suprathreshold at any DI. If the function \( R \) is known, then the set in Equation 6 can be solved either numerically or graphically. This specific case was already analyzed by Guevara et al.\textsuperscript{17}

The solution of this system for an initial condition of BCL = 700 msec and APD = 365 msec is presented in Figure 9. APD is plotted on the ordinate scale as a function of the DI on the abscissa (zero DI corresponds to the time of full repolarization after an action potential). The diagonals (termed “BCL lines”) represent the solution of BCL minus APD\( _n \) (see Equation 6) at the indicated BCLs. For illustrative purposes in this particular example, the function \( R \) was approximated in a piecewise manner (three pieces), as represented by the thick solid line. For DI
values higher than 600 msec, APD is constant at 370 msec. For DI values lower than 600 msec, APD decreases linearly with two different slopes, one from 600 msec to 200 msec, and the other from 200 msec to zero DI. The sequence of steps used to obtain the numerical solution of APD after a change in BCL are shown by the inset. Given the initial conditions of BCL=700 msec and APD₀=365 msec, the solution was carried out as follows: 1) calculate DI₀ after the last action potential before the BCL change, that is, BCL−APD₀=400−365=35 msec; 2) find on the R function the first APD value (APD₁) that corresponds to DI₀=35 msec after the BCL change. In this case, APD₁=270 msec; 3) repeat step 1 using APD₁ to find DI₁ (130 msec); 4) repeat step 2 for APD₂ using DI₁; 5) use APD₂ (320 msec) to find DI₂; and so on. These iterations are continued in search of a steady-state value of APD.

The graphical solution of the set in Equation 6 is also straightforward, as indicated by the sequence of arrows in Figure 9. Each time BCL−APDₙ is calculated, the resultant DIₙ value falls on the straight line with slope=−1, whose origin on the abscissa corresponds to the BCL. Hence, to solve Equation 6 for APD at any given BCL the following steps are followed: 1) from the starting point on the R function before the BCL change (i.e., at APD₀=365 msec) a horizontal arrow of magnitude BCL−APD₀ will hit the BCL line exactly at the DI₀ value of 35 msec (dotted vertical line); 2) from DI₀, one must now draw a vertical toward the R function to find APD₁; then 3) move again horizontally toward the BCL line.

FIGURE 9. Graphic prediction, in a hypothetical case, of the dynamics induced by a sudden basic cycle length (BCL) change from 700 msec (BCL₁) to 400 msec (BCL₂). Parameters used for the graphic solution are defined in the panel to the right. Numbered trajectories indicate the following steps of the solution: From 1 to 2, BCL−APD₁=DI₀; 2 to 3, APD₁=f(DI₀); 3 to 4, BCL−APD₂=DI₁; 4 to 5, APD₃=f(DI₁). Successive steps (from point 5 on) follow the same rules. Dotted vertical lines indicate the predicted diastolic intervals (DIs). Horizontal dotted lines indicate predicted action potential durations (APDs).
to find DI, and so on. Eventually, this iteration of R will encounter the steady-state value of APD. In this particular case, such a value falls at the intersection of the R function with the BCL line. This simple procedure allows one to investigate the asymptotic behavior of any given function relating APD to the diastolic interval.

In the example of Figure 9, a change of BCL from 700 to 400 msec leads to a series of APD alternations of decreasing amplitude, but the system eventually settles into a steady-state APD. Intuitively, it is obvious that changing the slope of the R function at the intersection with the BCL line will change the magnitude of the APD alternation, as well as the number of iterations needed to reach the steady state. In fact, changing the slope means not only changing the quantity of the result, but also its quality for, at certain critical slope values, a steady-state APD may never be reached (see below).

Supernormality and Expected Stimulus/Response Dynamics

In the hypothetical example of Figure 9, it was assumed that the stimulus amplitude was large enough to induce a response regardless of the diastolic interval. We now use Equation 2 to analyze the experimental data obtained during repetitive stimulation when the diastolic recovery of excitability was nonmonotonic (see Figure 2B) using a stimulus strength such that excitation is achieved only for certain ranges of diastolic interval. In Figure 10, graphic solutions of the dynamics upon changing the BCL under these conditions are presented. Three graphs are shown in each panel; the top graph is the reconstructed R function (as in Figure 9), dots correspond to experimental data (APD and diastolic interval) values from the steady-state patterns (1:1, 2:1, 3:1, and 4:2) illustrated in Figure 4. The middle graph is the strength-interval curve (already presented in panel B of Figure 2). The shaded region indicates current strength (27 μA). Since strength is constant, it is subthreshold for an intermediate range of diastolic intervals and yields a discontinuity in the R function (dotted vertical lines). The diagonals in each panel represent the stimulation cycle length (see Figure 9) or, in the case of failure, a multiple integer of BCL (i.e., 2×BCL or 3×BCL). The bottom graph in each panel of Figure 10 illustrates the predicted APD changes after each particular BCL change. Panels A through D show the various dynamics predicted by this method for different cycle lengths. As in the case of Figure 9, the graphical iteration begins by drawing a horizontal arrow from the asymptotic value of APD at the starting cycle length to the new BCL line. In panel A (BCL=2,000 msec), the system rapidly settles into a steady-state 1:1 pattern with constant APD (425 msec, point 1). In panel B, the second iteration occurs within the discontinuity in the R function and failure occurs (point 2). Diastolic interval in the next beat is equal to DI+BCL (see "Materials and Methods"). Graphically, this operation can be solved using a second diagonal (indicated as 2×BCL), whose intersection with the R function predicts the next APD value (point 1). In this condition, a stable 2:1 pattern is established in which APD alternates between 0 and 425 msec, and recovery time changes between 575 and 1,575 msec. By the same procedure, when BCL is shortened to 500 msec, a 3:1 pattern is predicted in panel C, in which one action potential (APD=420 msec, DI=1,090 msec; point 1) is followed by two consecutive failures at diastolic intervals of 90 (point 2) and 590 msec (point 3). Finally, the development of a 4:2 pattern is predicted by the solution presented in panel D when the BCL is shortened to 400 msec. In this case the second iteration (point 2) occurs at the supernormal phase (DI=−10 msec) predicting a relatively short action potential (APD=265 msec). Two successive dropped beats occur (at DIs of 135 and 535 msec, points 3 and 4, respectively) before the iteration returns to the allowed portion of the R function (point 1: APD=410 msec, DI=935 msec).

A careful analysis at cycle lengths intermediate to those illustrated in Figure 10 (not shown) indicates that the unique sequence expected at this particular stimulation amplitude is 1:1→2:1→3:1→4:2 which is in complete agreement with the experimental results (see Figure 4). A second type of 2:1 response at an even briefer BCL was also reproducible in the graphical solution (not illustrated). In this case, the BCL line will intersect the R function on the left side of the discontinuity. Moreover, the solution suggests the possibility that this 2:1 pattern can be preceded by unstable patterns like those illustrated in Figure 4. Therefore, the model results confirm our experimental finding that the presence of supernormality can allow the demonstration of a given pattern at two widely different BCLs (e.g., 2:1 at BCLs 1,200 msec and 200 msec in Figure 4).

From the analysis of Figure 10 it is clear that the different behaviors observed experimentally in the three ranges of stimulus intensity (see Figure 5) can be associated with presence or absence of discontinuity in the R function. In range I, this function is continuous and linear, and graphical analysis shows that the expected behavior for any stimulation amplitude within this range is a period-adding sequence (1:1, 2:1, 3:1, etc.). On increasing the stimulation strength to range II, the function becomes discontinuous and is composed of a linear portion at long diastolic intervals and a nonlinear portion at short diastolic intervals (see Figure 10). Finally, in range III, the R function is again continuous and nonlinear as in the hypothetical example of Figure 9.

Noisy Rhythms or Deterministic Chaos Depending on the Slope of the APD Restitution Curve

As noted above, one of the dynamic consequences of R function discontinuity is the production of a complex sequence of stable stimulus/response locking patterns as the BCL is shortened. Moreover, when the R function is both discontinuous
FIGURE 10. Graphs of solutions for different dynamics (panel A, 1:1; panel B, 2:1; panel C, 3:1; panel D, 4:2) associated with supernormality using the reconstructed function from the experimental traces presented in Figure 4A (basic cycle lengths [BCLs] of 2,000; 1,000; 500; and 400 msec for panels A, B, C, and D, respectively). In each panel, top graphs are recovery (i.e., "R function") maps; middle graphs are excitability curves; bottom graphs show predicted beat-to-beat action potential duration (APD) changes. For the sake of simplicity, latency changes (less than 20 msec) are neglected in the analysis of these data. Solid diagonal lines indicate BCL or, in case of failure, multiples of BCL (2×BCL, 3×BCL). In each panel, dotted vertical lines mark the boundaries in the discontinuity in the R function resulting from the relatively low current pulse strength (shaded region).
experimentally, this unstable pattern was observed during the transition to a more irregular recurrent fluctuation, a very simple stability analysis was carried out to determine the origin of this irregular activity. For this analysis a different initial condition from that indicated as point 1 (Figure 11A) was selected and iterated in the usual manner. The new initial point (asterisk) had an APD 30 msec longer and a DI 300 msec longer than those of point 1. The shaded area indicates the possible trajectories for all points between point 1 and the asterisk. As the iteration proceeds, the initial difference becomes small, as indicated by the thinning of the shaded areas. By point 4, any differences have disappeared. This dissipation of the initial differences demonstrates that this pattern does not have sensitivity to initial condition and must be (at least theoretically) stable.

What, then, gives rise to the irregular activity? If one assumes that in the experimental situation there is a small amount of random noise, then irregular fluctuations between apparently stable patterns can be accurately reproduced. Such noise imposed on the stimulation strength would induce fluctuations in the width of the discontinuity in the R function. Under these conditions, the predicted stable sequence of Figure 11A could change abruptly to more irregular activity while still preserving the general structure observed experimentally; namely, a sequence of apparently stable 1:1 (with damped APD alternation) interrupted by a 2:1 pattern. Thus, the patterns of irregular fluctuation can be adequately explained by the addition of small amounts of random noise.

However, very irregular dynamics can arise in this preparation in the absence of noise. The conditions necessary to produce such deterministic chaos are illustrated in Figure 11B. For this example, experimental APD-DI data pairs from Figure 7 (middle trace, “multirhythmicity”) were used to reconstruct the R function. Similar results can be obtained from the data presented in Figure 8. The most important difference between this case and the preceding one (Figure 11A) is that the slope of the R function here is slightly greater than 1. Points 1–6 show the predicted graphical solution in complete agreement with the evolution of the experimental data presented in Figure 7. Application of the stability analysis described above highlights the essential difference between this case and the case of random noise. When a different initial condition (asterisk) from point 1 (APD 5 msec longer and DI 100 msec longer) is chosen, it is possible to determine the way in which any initial “error” is handled by the system. For illustrative purposes, dark shaded areas can be regarded as the predicted trajectories for the range of initial conditions between point 1 and the asterisk. From beats 1 to 4, the initial 5-msec difference in APD is amplified up to 30 msec, and the predicted trajectories are split into two groups: one group (stippled region) predicts two successive action potentials (close to points 3 and 4), while the other
Figure 12. Effect of the slope of the action potential duration (APD) recovery curve (panels A and B) on the dynamics of the behavior during repetitive stimulation. Results represent numerical solutions of the model (Equation 5) for two different values of the fast time constant (panel A, 150 msec; panel B, 100 msec) of the APD recovery curve. For descriptive purposes, the memory effect is neglected (i.e., \( M \) in Equation 5 was equal to zero). As described in the text, all other parameters were the same. Panels C and D show beat-to-beat APD changes during 300 beats when the initial diastolic interval was set to 10 seconds. Increasing the initial diastolic interval by only 1 msec produces the results shown in panels E and F. The difference between the two solutions (APD_C minus APD_E and APD_D minus APD_E) are shown in panels G and H, respectively. For the slope shown in panel A, results of the two simulations converged after an initial transient shown by the trace in panel G. However, at the higher slope of panel B, the results with the two initial conditions were markedly different (panel H), indicating sensitive dependence on the initial conditions.

Group (grid region) leads to two dropped beats (close to points 5 and 6) before reaching the vicinity of point 1 with a difference smaller than the initial one. In successive iterations, this remaining error increases and decreases but never dissipates. This results in an extremely aperiodic activity in which the main characteristic is a cycle of a variable number of action potentials with increasing alternans (i.e., amplification) followed by one or two opposite beats that “reset” (although not completely) the system. In any nonlinear system, the demonstration of this property of “amplification and reset” (which has also been termed “stretching and folding”18,19) is essential for demonstrating deterministic chaotic motion. Thus, we have concluded that, as in other nonlinear systems,14,18,19 multirhythmnicity is an expression of deterministic chaos in our preparations. In our case, the “amplification” or “stretching” is provided by a critical value (higher than 1) of the slope of the R function at very early diastolic intervals (see below), while the presence of supernormality at these same intervals induces discontinuity in the recovery function leading to “reset” or “folding.” It is this characteristic sensitivity to initial conditions which distinguishes deterministic chaotic activity from the recurrent fluctuations resulting from the addition of random noise.

Numerical Experiment Showing the Critical Role of the APD Restitution Curve Slope

The critical role of the slope in the recovery function is demonstrated in the example of Figure 12 in which the model equations (Equation 5) were used to determine the predicted dynamics for a specific BCL, under two slightly different conditions. Panels A and B show the recovery maps to illustrate the temporal relation between the excitability and APD restitution functions. The only difference between the two panels is that the fast time constant (\( T_f \), see “Materials and Methods”) of the APD restitution curve was decreased from 150 msec in panel A to 100 msec in panel B, thus increasing the average slope to values higher than 1. Panels C and D present the beat-to-beat APD changes predicted, for A and B, respectively, during the first 300 beats. Stimulation amplitude was in both cases 5 AU; that is, within the “critical zone.” For description purposes, the effects of memory were neglected in these simulations (see below). In the first case (panel C), after a transient period equivalent to 100 beats, a stable 3:1 pattern is
established and maintained throughout the entire run. On the other hand, in the second case (panel D), chaotic activity persisted for 300 beats and lasted as long as the simulation was prolonged.

An important test for the deterministic nature of an apparently irregular pattern is to search for the presence of sensitive dependence on initial conditions. As was graphically demonstrated in Figure 11, such a property implies a beat-to-beat amplification of any difference. This is clearly demonstrated in panels E–H of Figure 12 in which the influence of just a 1-msec change in the initial diastolic interval (see “Materials and Methods”) was determined for the two different cases. Panels E and F show the respective beat-to-beat APD values calculated with such different initial conditions. To better illustrate the dynamic effects of those initial changes, in panels G and H are presented the beat-to-beat APD differences (APD_0 – APD_1; APD_0 – APD_2) generated for each case. A comparison of the APD differences in the periodic (panel G) and irregular (panel H) results reveals that, in the former case, a 1-msec difference in the initial DI changes the outcome for just a few initial beats, but eventually, the original pattern is established and maintained. However, in the latter case involving chaotic dynamics, a lack of correlation is apparent for the entire period of the simulation. In fact, this exquisite sensitivity to changes on the initial conditions produces an amplification of the initial “error” introduced between panels D and F and explains the deterministic nature of such a chaotic fluctuation in the APD pattern.

Our experimental results and numerical simulations thus far suggest that a necessary condition for the demonstration of chaotic behavior is that the average slope of the restitution curves at DIs corresponding to the supernormal phase of excitability must be greater than one. Provided this prerequisite does exist, then sensitivity to initial conditions and chaotic dynamics will occur within the “critical zone” at certain ranges of BCL and stimulation strength. On the other hand, if the average slope of the restitution curve is less than 1, initial “errors” will be dissipated, sensitivity to initial conditions will not be demonstrable, and the resulting patterns will be mostly regular.

Effects of Latency

Although latency was included in our original description of the analytical model (see “Materials and Methods”), it has not yet been mentioned in regard to the graphical analysis of the results. Introducing latency only requires that we modify the R function according to the set in Equation 5. Using those equations, it is apparent that we can define a new function, R’, in which we add the value of latency to the value of APD. R’ can be regarded as the curve describing the time to full repolarization as a function of the previous diastolic interval. Under conditions of repetitive stimulation with pulses whose duration is not longer than 20 msec, the values for latency will range from zero (at long DI) to a maximum of 20 msec (at the shortest DI). Therefore, under these conditions, R’ will not differ significantly from R, and there will be minimal alterations in the dynamics. However, under conditions where the pulse duration is a substantial fraction of APD, the addition of latency can significantly decrease the slope of R’ at short diastolic intervals with respect to R. Indeed, the addition of substantial latencies might linearize the original function, or even make its slope positive at short diastolic intervals, thereby inducing Wenckebach-like periodicities.

Effects of Memory

When stimulation is initiated after a relatively long period of quiescence, the so-called memory effect can act by gradually reducing APD until a steady-state value is eventually achieved. In our simulations, the time required for that steady state is a function of the memory time constant (Δ). In addition, since memory grows toward 1 throughout the duration of the action potential and decreases toward zero during the diastolic interval, the time to steady state also is a function of the ratio DI/APD at the pacing cycle length (see “Materials and Methods”). Accordingly, the time to steady state and the final value of APD are both functions of the BCL. The effects of memory on the APD versus BCL relationship are illustrated in Figure 13A. The three curves were obtained by computing with the model (Equation 5) the action potential duration of the first, fifteenth, and five hundredth beat at the respective BCL. It is clear from these data that the memory process leads to a progressive decrease in the nonlinearity of the restitution curve. In fact, as the number of beats increases, the relation becomes almost linear. These simulations accurately reproduce previous experimentally derived restitution and steady-state curves during 1:1 excitation. Moreover, with our formulation, it becomes possible to study the predicted effects of memory on activation patterns other than 1:1.

Memory versus Chaos

Our model, and the analysis of the experimental results, predicts the demonstration of sensitivity to initial conditions and chaotic dynamics of excitation when there is supernormal excitability, as well as a critical slope in the APD restitution function. On the other hand, we have demonstrated that by modifying the original restitution curve the memory process would lead to a gradual decrease in the steepness of the APD versus DI function. Thus, it would be expected that the possibility of demonstrating chaotic dynamics should decrease as the memory process gradually develops. The results of one simulation in which we studied the effects of memory on the irregular APD dynamics are shown in Figure 13B. The parameters used were the same as those in panels D, F, and H of Figure 12, but with the addition of a time-dependent change in the restitution of APD (see Equation 5). In Figure 13B, the
shown in Figure 13B can be regarded as transient chaos. Yet, for practical purposes and because of its potential electrophysiological implications, such a transient event is long enough to be considered more than a mathematical curiosity (see "Discussion").

**Predicted Parameter Planes**

Several simulations have been carried out by numerical solution of the model for monotonic as well as nonmonotonic forms of recovery of excitability. Wide ranges of BCLs (1-msec steps) and current strengths (0.5-μA steps) were explored searching for the presence or absence of responses characterized by constant n:m ratios. In each case, at any selected combination of BCL and strengths, the activation n:m ratio was calculated (after transients) for every single iteration, until a constant pattern, or lack thereof, was detected. Typically, for periodic stimulus/response locking (i.e., 3:2, 3:1, etc.) the ratio was constant at 100 beats. If the responses were aperiodic, the number of iterations was doubled or tripled depending on the circumstances.

The results of these simulations, in which we used the experimental curves (strength-interval and APD restitution) illustrated in Figure 2 are presented in the form of parameter planes. In Figure 14, panel A shows data obtained for the 7 mM KC1 case (monotonic recovery), and panel B shows the results gathered in the presence of supernormal excitability (4 mM KC1). The lines are the boundaries between the most important activation ratios. In the absence of supernormality (panel A), gradual changes in BCL or strength yields gradual and predictable changes in the activation ratio. In fact, it is clear that a horizontal section through this plane results in a monotonic staircase plot similar to those found experimentally during high potassium chloride superfusion (Figure 3). On the other hand, when the supernormal recovery of excitability is incorporated into the model equation, the structure of the parameter plane (Figure 14B) is similar to that inferred from the experimental results during low potassium chloride superfusion (see Figure 4). In Figure 14B, the parameter plane has been divided into three major regions, depending on the patterns recorded. Within region I, the organization of the various stimulus/response sequences is similar to that described for the entire parameter plane of panel A; any change in BCL or strength led to a smooth change in conduction ratio. The same holds true for region III above the upper boundary of the parameter plane. However, within relatively narrow BCL ranges at these current levels, changing the BCL leads the system to a new pattern, but the activation ratio remains the same. For example, 1:1 rhythm (AR = 1) is followed by 2:2; 2:1 (AR = 0.5) by 4:2, and so on. Between these two boundaries there lies a "critical zone" (region II) in which parameter changes may lead to complex transitions in the activation ratio (encircled region) and to chaotic dynamics. For instance, as the BCL was decreased from 1,000 msec to 200 msec through the

**FIGURE 13.** Influence of memory on the results of model simulations (Equation 5). Panel A: Action potential duration (APD) as a function of basic cycle length (BCL) showing the progressive shift from the restitution curve obtained in the absence of memory effects (1 beat), to an intermediate condition (50 beats), to the steady state (500 beats). Panel B: Effects of memory on the chaotic patterns. Two slightly different initial conditions (same parameters as those used in Figure 12B) generate very different beat-to-beat APD sequences (panels B1 and B2). The initial lack of correlation between these two results is represented in panel B3 as the difference between the two solutions (B1 minus B2) and persists for up to 200 beats, after which the solutions converge.

beat-to-beat APD changes for condition 1 (equivalent to Figure 12D) are presented in the top trace; those for condition 2 (i.e., after the initial DI has been changed by 1 msec; see Figure 12F) are shown in the middle trace, and the difference between the two (APD2 - APD1) is shown in the bottom trace. In this simulation, the addition of memory suppressed the chaotic dynamics and eventually led to a 2:1 pattern in both cases. However, as shown by the bottom trace, the establishment of regular periodicity required more than 200 consecutive events. From a rigorous mathematical point of view, the behavior
region indicated by the horizontal dashed line (i.e., at constant strength of 30 μA), changes in the stimulus/response ratios occurred as follows: 1→0.5→0.75→0.66→0.5→0.33→chaotic activity.

Predicted regions for irregular dynamics agree with experimental observations. For example, in the black region (AR=0.66), conditions for recurrent fluctuations occur which, in the presence of noise, could lead to patterns similar to those presented in Figure 6. Chaotic activity in the model (occurring inside the encircled region) exhibits the dynamic properties ("amplification and reset") that were suggested by the analysis of the experimental traces (see Figure 11B).

**Discussion**

The strategy used in our investigation derives from the theory of dynamic systems. Such a strategy consisted of concentrating our attention on the analysis of beat-to-beat changes in those few action potential parameters that are known to have a nonlinear relation to time. In general, in excitable tissues, the prototypical functions with nonlinear time courses of recovery with respect to a previous response are APD, activation threshold, and stimulus/response latency. During repetitive electrical stimulation, a feedback between such nonlinearities endows these tissues with certain properties that allow them to behave either periodically or chaotically, depending on the stimulus magnitude and cycle length. The results of this study confirm and expand previous experimental data from our laboratory, in which we demonstrated that the general behavior of isolated Purkinje fiber during repetitive stimulation follows universal rules described for a wide variety of nonlinear systems.

Our in vitro experiments as well as numerical simulations suggest that normal recovery of excitability leads to a monotonic change in the activation ratio during repetitive stimulation at increasing pacing rates. Moreover, these results support our contention that in the presence of supernormal excitability there are three different stimulation amplitude ranges that give rise to different types of behavior: Pulses of relatively low or high current strength at increasing pacing rates induce monotonic changes in the activation pattern, and intermediate current intensity stimuli induce very complex nonmonotonic changes in the activation ratio and can also lead to chaotic dynamics.

**Relevance of the Analytical Model**

The model results reproduce very accurately our experimental observations. Both experimental and numerical analyses predict that as the BCL is decreased, the expected dynamics will depend very strongly on the time course of recovery of the tissue excitability. In both cases, a monotonic recovery of excitability is associated with activation patterns that follow the Farey rule (for a description of the Farey's arithmetic see References 3, 5, 7, 11, and 16). The same holds true for the nonmonotonic recovery at very low stimulus amplitudes. However, in the presence of supernormality, there exists a critical level of stimulus amplitude in which the rule is not applicable, but rather, periodic or chaotic rhythms may be demonstrable, depending on the BCL. Such a critical level is in fact equivalent to about twice the diastolic threshold strength; a value commonly used as the "standard" stimulus amplitude in many experimental reports.

Our model provides a quantitative basis for the experimentally observed stimulus/response pattern in the presence or absence of supernormality. Moreover, the demonstration that sensitivity to initial conditions is involved in the postulated mechanism of chaotic
responses in our Purkinje fibers is a novel explanation for complex rhythms that, in the past, were rejected as noisy and of nondeterministic nature. The fact is that the overall behavior in our experiments can be reproduced by this simple formulation. Additional efforts involving refinement of the fitting process may allow a better and more accurate representation of each of the functions used in our analyses, but probably such refinements will not modify the important qualitative predictions of the model. Indeed, mathematical arguments derived from the theory of dynamic systems can be used to prove that the important parameters in those functions are not violated by the proposed approximations.

Ordered Behavior and Rate-Dependent Block Processes

Ordered n:m patterns were observed during high potassium chloride superfusion, as well as outside of the critical range of stimulus strength (see Figure 5) during low potassium chloride superfusion. In either of these two cases, abbreviations of the BCL followed the Farey rule for stimulus/response locking patterns that has been demonstrated in other systems. However, since pulses of relatively brief duration (20 msec) were used in all experiments, the most common behavioral patterns observed upon abbreviating the BCL were sequences of period adding. Thus, beginning at 1:1, a BCL change usually led to 2:1 then 3:1 and so on. Although intermediate “Wenckebach-like” patterns (i.e., 3:2; 5:3) were indeed observed in some cases (e.g., Figure 3), the rule under these experimental conditions was that of period adding, which is in perfect agreement with previous results from this laboratory. In those experiments, complete Farey sequences, including Wenckebach rhythms, were obtained at relatively long pulse durations (>50 msec) but not when pulses of 50 msec or shorter were used.

Many combinations of stimulus/response patterns were observed in our Purkinje fiber preparations during repetitive stimulation at wide ranges of BCL. When present, the intermediate n:m patterns recorded were similar to those usually reported in the clinical situation as examples of rate-dependent activation sequences of the Mobitz type II second-degree heart block. This is not surprising since, by experimental design, the duration of the repetitive stimulus was always constant at 20 msec, which yielded patterns whose beat-to-beat changes in latency between onset of the stimulus and response never exceeded 20 msec. Nevertheless, careful measurements revealed that in some examples (e.g., Figure 3) the activation pattern resulted from gradual increases in latency culminating in a single failure, mimicking the so-called “millisecond Wenckebach” characteristic of intermittent block phenomena in the His-Purkinje system of patients with heart disease. Moreover, in some cases in which supernormality was demonstrable, Mobitz type I and II block phenomena were detected in the same preparation at widely separate BCL ranges. For example, in one experiment (not shown) a 3:2 pattern with typical Wenckebach structure (i.e., gradual increase in latency at decreasing increments) was recorded at a relatively long cycle length (650 msec). Acceleration of the BCL to 340 msec yielded a second type of 3:2 pattern with no measurable differences in the activation latencies of the first and second successful responses. The results obtained during low potassium chloride superfusion may provide the experimental counterpart to the clinical observation relating Mobitz type II block processes with supernormality. Through deductive electrocardiographic analysis in patients with intermittent heart block, Halpern et al. have suggested that the occurrence of some cases of sudden heart rate–dependent intermittent block in the setting of apparently normal ventricular activation time may be explained by the presence of a supernormal phase of excitability in the His-Purkinje system. It would not be valid at this point to extrapolate our analysis to the clinical situation. However, we could speculate that the activation patterns observed in some patients with second-degree heart block would not differ in essence from those predicted in the parameter spaces presented in Figure 14. Thus, analysis of some of these cases in light of this new information may provide further insight into their dynamics and cellular mechanisms.

The Purkinje Fiber as a Nonlinear System

Theoretically, it may be expected that any linear system should behave in a simple and totally predictable manner when one of its parameters is changed. For example, if the volume in a closed space is progressively reduced, the resulting pressure within the space will increase proportionately in a given range. Yet, in nature, it is very difficult (almost impossible) to find a completely linear system, whereas nonlinearity is probably the rule. Nonlinear dynamic systems, including those that are described by few variables (e.g., APD, latency, excitability, and memory) can behave in very complex ways. When the degree of nonlinearity is relatively small, the dynamics may be regular and predictable regardless of the initial conditions. Such is the case in our experimental and numerical results when the system is operating within region I in the parameter plane (see Figures 5 and 14B). However, for higher degrees of nonlinearity (e.g., within region II in Figure 14B), chaotic behavior can arise. This behavior may still be completely determined by the few variables of the system, but is highly dependent on small differences in the initial conditions (see Figures 11B and 12). Such “deterministic chaos” is very similar to that demonstrated recently in large numbers of nonlinear systems. At first glance, this behavior seems paradoxical and counterintuitive, since, by definition, any system that is determined by only a few variables should be orderly and predictable. Thus, for centuries investigators have accepted the notion that chaotic behavior must be the result of large numbers of variables interacting randomly and complexity. This
idea supported the erroneous concept that thousands of measurements must be carried out in the study of irregular behaviors to understand their mechanisms. Perhaps the most exciting contribution of chaos theory is that, through the direct analysis of the effects of nonlinearities, it is possible to explain very complex evolutions in very simple nonlinear systems.

**Supernormality and Vulnerability to Arrhythmias**

On the basis of the arguments outlined above, we decided to carry out a study in isolated Purkinje fibers of the respective conditions under which ordered and disordered behavior appear during repetitive electrical stimulation. We also attempted to determine whether the irregular activation patterns observed under our experimental conditions may be attributed to random (noisy) mechanisms and whether some of that behavior may be explained in terms of deterministic chaos theory. The possible pathophysiological implications of such a differentiation have not been clarified. However, we believe that differentiation between random and deterministic mechanism is relevant and nontrivial for two main reasons. First, determinism implies that the system’s behavior is reproducible and predictable, and that its mechanisms can be manipulated, for example, by changing the nonlinearity of one of its parameters (see Figure 12) by superfusion of an antiarrhythmic agent. Second, the demonstration of very complex activation patterns at BCLs that are within the range of the supernormal phase of excitability suggests that this behavior may have a role in the establishment of life-threatening cardiac arrhythmias involving the so-called “vulnerable period.” The existence of such a period has been known to physiologists and cardiologists for many years. Yet, the mechanism of increased vulnerability to arrhythmias at that particular phase with the cardiac cycle has not been adequately explained. Recently, Winfree provided topological arguments in favor of the importance of a particular overlap of voltage (and phase) gradient generated in the myocardium by each ventricular activation. Such an overlap can result in self-sustained rotating waves around a center (singularity) if an electrical stimulus of the appropriate characteristics (i.e., neither too weak nor too strong) and at the appropriate phase is applied. On the other hand, stimuli applied at phases different from, but close to, those unmasking the singular condition will result in unstable spiral waves (one to several extra systoles), which resemble the reentrant circuits recorded in the heart during multiple electrode mapping. It is thus obvious that both Winfree’s description of the topology of vulnerability to fibrillation and our analysis of the Purkinje fiber behavior under the conditions of supernormal excitability predict the existence of a singular phase in the cardiac cycle in which electrical activity becomes highly irregular (chaotic?). Recently, Chen et al provided partial experimental support for Winfree’s hypothesis, but the contribution of cardiac Purkinje fiber in the development of reentrant circuits or fibrillation was not clarified. Nevertheless, a recent experiment in Langendorff perfused pig hearts whose endocardium had been frozen by treatment with liquid nitrogen suggests that Purkinje fibers may indeed be involved in the establishment of ventricular fibrillation. In those studies, a thin rim of epicardium remaining after rapid endocardial freezing was capable of sustaining well-organized reentrant ventricular tachycardia. However, under those conditions, it was impossible to induce fibrillation. We therefore propose that the sensitivity to initial conditions in the electrical activity of Purkinje fibers may be important in determining a wide dispersion of activation timing (similar to the effects of Winfree’s singularity) and, consequently, in the establishment of highly irregular cardiac rhythms.

**Chaotic Responses and Sensitivity to Initial Condition**

In any given experiment, the time course of the APD restitution determines whether an abrupt transition from a high to a low stimulus frequency will be followed by a transient oscillation in a relevant variable (e.g., APD); or, if there is supernormal excitability, by chaotic responses which may persist for many beats. The demonstration of chaotic responses further requires a critical slope in the function describing the restitution of APD (see Figure 11). As demonstrated by numerical and graphic solution of the model equations, such a critical slope makes the system highly sensitive to the initial conditions, which are responsible for disordered behavior of many electrical, chemical, and mechanical deterministic systems. In the case of our model, the critical slope amplifies any initial differences in such a way that there need not be any sources of random noise to turn the system’s behavior from regular and predictable to irregular and highly unpredictable. Hence, from our results we can conclude that, under certain circumstances, Purkinje fibers can have a very high sensitivity to initial conditions and thus, for certain ranges of BCL and stimulus strength, their behavior may be chaotic.

Our simulation results predict also that, by reducing the critical slope in the restitution curve, the memory process reduces the sensitivity to initial conditions as well. Yet, since the time course of these effects would be quite slow, it is possible to demonstrate relatively long periods of “transient chaotic behavior” even in the presence of memory effects.

**Sensitivity to Initial Conditions, Asynchronous Firing, and Reentry**

The analysis of the experimental data (see Figure 11B) as well as the numerical results in Figures 12 and 13B suggest that, in the presence of chaotic behavior, a 1-msec change in the initial condition can produce a very large change in the quality of the outcome. This suggests that under the appropriate conditions of stimulus timing and strength, two healthy neighboring cells in a conducting pathway,
connected end-to-end, and differing by only 1 msec in their activation times, can behave extremely asynchronously in relation with each other, even if all other parameters (intrinsic APD, excitability, upstroke velocity, etc.) are identical in both cells. Consequently, local reexcitation may possibly occur. It is probably true that when some degree of asynchrony begins to appear, the effects of both electrotonic interactions between the two cells and the memory process affecting the restitution of APD would tend to counteract the irregular behavior. Yet it is conceivable that a critical balance of electrotonus plus memory versus sensitivity to initial conditions will allow the establishment of reentrant activity in the absence of a circuitous pathway. Such a reexcitation would be analogous to the recently demonstrated reflection phenomenon in Purkinje fibers, but in the present case, no intervening inexcitable tissue or high resistance pathway would be necessary.

At this point, we can only speculate about the relevance of our results to the mechanisms of life-threatening arrhythmias. However, it is interesting that the circumstance in which our experimental and model results predict high sensitivity to initial conditions and chaos are quite consistent with at least two general ways used by many authors to trigger ventricular tachycardia or fibrillation. In some cases, single or double premature stimuli are applied after several basic stimuli at a slower BCL. According to our results, the timing of these premature stimuli would be that at which chaotic responses should occur. Thus, it would not be necessary to attribute the initiation of the arrhythmia to an as yet undetermined “dispersion of refractoriness.” Instead, it can be argued that the highly irregular arrhythmia may be initiated by a small group of cells having identical electrophysiological properties and just a small difference in their respective activation times. Because of a very high sensitivity to initial conditions, a premature stimulus in the critical range may lead to chaotic responses and produce a “dynamic dispersion of refractoriness” which would increase in magnitude with each subsequent discharge. If the initial premature stimulus is unable to induce tachycardia or fibrillation, by the time of the second stimulus, asynchronous behavior would have already taken place and the stage for the establishment of unidirectional block and reentry would be set. In short, even in the absence of an initial dispersion of refractoriness, sensitivity to the initial conditions would allow for the development of highly irregular arrhythmias. Recently, Akhtar et al have suggested that the most effective protocol for inducing ventricular tachycardia secondary to bundle branch reentry consists in applying an initial premature stimulus followed by a second stimulus at a briefer coupling interval. Such a protocol is consistent with our hypothesis because it provides for a situation in which the sensitivity to initial conditions would be exaggerated. Other investigators induce ventricular fibrillation in their experiments by gradually increasing the stimulus rate, which is also compatible with our model results. Indeed, our numerical analysis would predict that the critical frequency for the onset of ventricular fibrillation should correspond to the range at which Purkinje fibers become sensitive to the initial conditions, and thus, the successive chaotic activation wave fronts ongoing in those tissues would generate and amplify the asynchrony necessary for the establishment of reentry and fibrillation.

Sensitivity to initial conditions can also be important in determining the factors leading to spontaneous block or instability of a reentrant tachycardia. Results by Simson et al in experimental reentrant tachycardias already have suggested that the stability of the arrhythmia is related to the slope of the excitability curve. In fact, the computer model used by Simson et al was very similar to ours, and their graphic analysis (their Figure 1) used very similar arguments to those used by us in the establishment of alternation of APD (our Figure 9). Finally, a recent experimental study in the Purkinje-muscle junction has suggested that reentrant activity in that model may also show sensitivity to initial conditions similar to that demonstrated here. Further, in vivo and in vitro studies using protocols derived from nonlinear dynamics theory to analyze behavior in models of reentry should shed some light on the relevance of our hypothesis to the development and maintenance of reentry.

Acknowledgments
We appreciate the helpful suggestions of Dr. Paco Lorente during the experiments and of Dr. Mario Delmar in the preparation of the manuscript. The comments of Dr. Arthur T. Winfree and Dr. Robert F. Gilmour Jr. are also much appreciated. We thank Wanda Coombs for skillful technical assistance and LaVerne Gilbert for secretarial and administrative assistance. For the presentation of this work, Dr. Chialvo was awarded the first prize in the competition for the Young Investigator Awards of the North American Society of Pacing and Electrophysiology (NASPE) held in Toronto, Canada, in May 1989.

References


**KEY WORDS** • supernormality • Purkinje fibers • chaos • nonlinear dynamics