Bistabilities and Annihilation Phenomena in Electrophysiological Cardiac Models

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We have investigated the oscillatory behavior of cardiac cellular elements simulated by two electrophysiological models: the van Capelle and Durrer (VCD) model and the sinoatrial node cell model of Yanagihara, Noma, and Irisawa (YNI). The VCD model behavior was examined systematically by using continuation-bifurcation analysis. Bifurcation diagrams were constructed as a function of Qit1, an intrinsic parameter of the model, which sets both maximum diastolic potential and depolarization threshold of the cell. The existence of stable high amplitude oscillations was evidenced between two Hopf bifurcation points (HB). Near each HB, a zone of bistability was detected. Close to the HB that corresponded to high values of Qit1, a high amplitude periodic stable state coexisted with a stable steady state. Close to the other HB, in a narrow range of lower Qit1 values, a relatively high amplitude periodic stable state coexisted with a low amplitude periodic stable state. There was no stable steady state in the latter bistability zone. Through the use of phase-plane representations and the determination of separatrices between the different attractor basins, we could deduce the conditions of timing, polarity, and strength needed for a pulse perturbation to send the system from one state to another and vice versa. The YNI model was analyzed by numerical simulation, and the oscillatory behavior of the sinoatrial node cell was explored while applying a depolarizing bias current of various strengths. Results were similar to those obtained from the VCD model in that there were two bistability regions for two different ranges of applied bias current. Depending on current intensity, annihilation of pacemaker activity could be achieved in both zones. However, the coexistence of two oscillatory stable states was never observed in the YNI model. From the behavioral similarities of these different models, we can conclude that bistabilities and annihilation phenomena can be found in transitional zones between quiescence and rhythmic activity. (*Circulation Research* 1990;66:1658–1672)

Biological oscillatory systems can display phase resetting or even annihilation of rhythmic activity when perturbed by external stimuli from their surroundings.1 Cardiac pacemaker cells share this peculiar behavior in that, for a given pulse duration and polarity, a stimulus of proper magnitude and timing can annihilate pacemaker activity. Annihilation of spontaneous rhythm has been observed experimentally in sinoatrial node cells,2 isolated Purkinje fibers,3 and depolarized ventricular muscle.4,5 Similar phenomena have been reported in simian mitral valve cells.6 In some cases, suppression of pacemaker activity is transient: a stimulus applied at the right phase in the pacemaker cycle leads to subthreshold oscillations that become progressively larger and eventually reach threshold. In other cases, damped oscillations result in complete suppression of rhythmic activity, which may be restored by the application of a second brief stimulus. These findings suggest the possible coexistence of two stable states in such spontaneously oscillatory systems.

Conceptually, processes underlying phase-response (phase-resetting) curves should be intimately linked to the annihilation phenomenon. In fact, according to Winfree’s theory,1 if a certain phase-resetting pattern is present in the biological system under study, then a stimulus featuring specific timing and intensity may abolish automatic activity. By using voltage perturbation methods, several theoretical investigations have confirmed the applicability of this topological theory,7–11 and the experimental demonstration of such behavior in squid axon, sinoatrial node, and Purkinje fibers5,11,12 lends further support to this concept.
In all these studies, the construction of phase-resetting curves was used to find "black holes" or "null spaces," where rhythmic activity of the pacemaker ceases.

The topological description of bistable behaviors in automatic cardiac cells can also be performed through the use of bifurcation analysis. This approach was carried out by Chay and Lee\textsuperscript{13} in ventricular myocardium by using the quantitative model of Beeler and Reuter.\textsuperscript{14} They found that a ventricular muscle fiber stimulated by depolarizing current can exhibit the coexistence of an oscillatory repetitive firing state and a time-independent steady state in two different domains of input variable values. As a result, the conditions of annihilation could be predicted.

The purpose of the present study was to describe the behavioral dynamics of a single cardiac pacemaker cell by using the van Capelle and Durrer (VCD) model\textsuperscript{15} and continuation and bifurcation techniques.\textsuperscript{16} In addition, since the VCD model yields only a qualitative expression of the cardiac cell oscillatory behavior, we performed numerical simulations by using the Yanagihara, Noma, and Irisawa (YNI) model,\textsuperscript{17} thus approaching in a more quantitative manner the function of membrane ion channels. From our results, we could classify the behavior of a cellular element and describe two zones where high amplitude oscillatory stable solutions coexisted either with steady-state or subthreshold stable solutions. The mathematical conditions that allow the pacemaker cell to be sent from a stable repetitive activity into a steady state, and vice versa, were delineated, thus providing the description of the very specific conditions underlying the annihilation of pacemaker activity.

Materials and Methods

\textit{van Capelle and Durrer Model}

For bifurcation analysis and certain numerical simulations, we have used the qualitative description of electrical activity of cardiac cell membrane designed by van Capelle and Durrer\textsuperscript{15} (see "Appendix"). This model comprises two variables, the transmembrane voltage (V) and the generalized inexcitability parameter (Y; referred to as the "activation variable" below). Two first-order nonlinear differential equations describe the behavior of V and Y for one given cell, pacemaker or nonpacemaker. The capacitive membrane current is supplied by an external current and an ionic transmembrane current, which is a weighted average (Y being the weighting factor) between two currents I_d(V) and I_i(V). I_d(V) is the current-voltage-relation for a maximally excitable membrane, displaying a region of negative slope conductance to make the regenerative process work. I_i(V), on the other hand, represents the current-voltage relation for a completely inexitable membrane. Both relations determine the pacemaker or nonpacemaker behavior of the cell under consideration.

To investigate the pacemaker and nonpacemaker capabilities of a cellular element, we choose a parameter in the VCD model,\textsuperscript{15} which is labeled Qit1 and is strongly related to the excitability property, as the continuation-bifurcation parameter. If we let f(V)=I_d(V)−I_i(V), then Qit1 is the maximum of a cubic function, which is a piece of f(V). We have previously demonstrated that, when Qit1 is lowered, the resting potential is reduced and the depolarization threshold level is decreased.\textsuperscript{18} Figure 1 shows the effects of such lowering of Qit1. In addition to altering the shape of the f function (Figure 1A), the decrease from −7 to −15 of the Qit1 value diminishes both the period and the maximum diastolic potential of the spontaneously beating element (Figure 1B). Therefore, Qit1 variations should be expected to induce corresponding variations in excitability and oscillatory behavior of the cell.

\textit{Continuation-Bifurcation Analysis}

We explored the phase transitions between automatic and nonautomatic states for a given cardiac cell by means of bifurcation analysis.\textsuperscript{19,20} This procedure consisted of including the VCD model in the continuation-bifurcation algorithm and of observing the behavior of the modeled cellular element while...
varying one parameter (Qit1, see below). Behavior was expressed as quiescent or rhythmic equilibrium solutions obtained after transient stages. The first step aimed at determining the quiescent-state domain, which corresponds to the steady-state solutions of the system, with the whole set of these solutions forming one (or several) branch(es) of stationary solutions. Let λ be a real parameter of the system. Since we want to look for the solutions expressing cell behavior in some domain of λ, we solve, as a function of λ, the nonlinear system expressing the existence of a stationary solution. As a result, λ is called the continuation parameter. In fact, the problem is reparameterized according to Keller’s techniques, and the solution is given as a function of a new parameter s (an arclength approximation). Then, the crucial step of the procedure features the detection of a Hopf bifurcation point (see Table 1 for definition of this and other terms), from which always emerges a periodic and a stationary branch of solutions. For the continuation of periodic solutions, Keller’s method enables us to solve the problem in a manner similar to that applied to the continuation of stationary solutions. Finally, results can be represented as bifurcation diagrams showing the resting potential level for the stationary solutions (quiescent state) and the amplitude of oscillations for the periodic solutions as functions of the continuation parameter λ. They can also be displayed as phase-plane representations of the orbit solution (for one given λ value), which illustrates the shape of the oscillatory cycle in a two-dimensional space (e.g., activation vs. membrane potential).

Qit1 was chosen as the continuation-bifurcation parameter because it plays a major role in the dynamic behavior of the cell (Figure 1 and “Appendix”). Bifurcation diagrams were constructed by using AUTO, a program for automatic analysis developed by Doedel. By varying Qit1, we could induce characteristic patterns and thus classify the possible behavior of the cell. The maximum amplitude of the action potential and the period were obtained as functions of Qit1. As a result, regions of stable and unstable steady-state and oscillatory behaviors, as well as attractor basins (i.e., the set of initial conditions that make the system converge to one type of behavior), were evidenced. The determination of unstable orbits was a cornerstone in describing attractor basins, since they can be identified as separatrices between stable states in a two-dimensional model such as the VCD model (which is not possible thus far in more sophisticated models). Indeed, these unstable orbits (separatrices) could be drawn in the Y-V phase-plane representation. Through this mathematical representation, the effects of depolarizing or hyperpolarizing pulses of various amplitudes and timings were accurately predicted.

Numerical Simulations of the VCD Model

To visualize dynamically the patterns obtained at phase transitions between oscillatory and steady-

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<th>Table 1. Glossary of Terms</th>
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<tr>
<td><strong>Annihilation</strong>: permanent stable extinction of spontaneous activity.</td>
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<td><strong>Stable steady-state attractor basin</strong>: set of initial conditions from which the system evolves to a stable steady-state point, which is also called a static attractor.</td>
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<td><strong>Equilibrium point or static attractor</strong>: for a particular setting of state variables, it is defined by the two coordinates (the generalized inexcitability parameter [Y], transmembrane voltage [V]) in the phase-plane representation of Figure 7A, and it corresponds to a quiescent state of the cell.</td>
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<td><strong>Stable limit cycle</strong>: periodic cycle of transformations to which a system evolves from a set of initial conditions or tends to return to after certain perturbations. Because it represents a periodic equilibrium, it is also called a periodic attractor. In the particular case represented in Figure 7A, the stable limit cycle corresponds to the solid line along which the cell periodically moves as a function of Y and V.</td>
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<td><strong>Periodic attractor basin</strong>: the inset (or domain) of initial conditions and perturbations that will end up at the stable limit cycle.</td>
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<td><strong>Separatrix</strong>: dividing boundary between two attractor basins.</td>
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<td><strong>Due to the two-dimensionality of the VCD model, they can be readily shown in the Y-V phase-plane representation; they correspond to unstable solutions in this model. We can imagine a separatrix as a sharp mountain ridge between two valleys corresponding to the attractor basins.</strong></td>
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<td><strong>Hopf bifurcation point (HB)</strong>: splitting of equilibrium solutions (corresponding to the quiescent state of the cell) in two branches of solutions, which are unstable stationary and unstable periodic around HB, and unstable stationary and stable periodic around HB.</td>
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<td><strong>Stable steady-state branch</strong>: set of solutions corresponding to stable equilibrium points (i.e., quiescent states of the cell).</td>
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<td><strong>Unstable steady-state branch</strong>: set of solutions corresponding to unstable equilibrium points, which have only a mathematical expression.</td>
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<tr>
<td><strong>Stable periodic branch</strong>: set of solutions corresponding to a stable oscillatory state of the cell.</td>
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<tr>
<td><strong>Unstable periodic branch</strong>: set of solutions corresponding to unstable oscillations, which are exactly located on the separatrices (in the VCD model). Since they are unstable, they cannot be visualized.</td>
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<td><strong>Periodic limit point or turning point</strong>: point located on a periodic branch where the directionality of the continuation parameter reverses.</td>
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state stable solutions and at regions close to Hopf bifurcation points, we solved the system numerically by using an automatically controlled step length algorithm based on a fourth-order Runge-Kutta method as previously reported (see “Appendix”). Thus, hysteresis loops between different stable states could be traced as a function of time.

**YNI Model**

For quantitative investigations, numerical simulations of the activity of single cardiac sinoatrial pacemaker cells were performed using the YNI model, as described previously. Briefly, membrane potential was reconstructed using equations that describe various voltage- and time-dependent membrane currents. Cell surface area was assumed to be 1 cm², and membrane capacitance was assumed to be 1 μF/cm². Specific currents included four time- and voltage-
dependent currents: 1) slow inward (calcium), 2) outward potassium, 3) fast inward sodium, and 4) hyperpolarization-activated. A voltage-dependent, but time-independent, leak current was also present. Numerical integrations were performed using a modified Euler method. 

No significant differences between results were obtained by using time steps of 0.1, 0.2, 0.5, and 1 msec. For time steps longer than 1 msec, results for numerical integrations differed somewhat from those using 0.1–1-msec time steps. Hence, a 1-msec time step was eventually chosen. Since, in this model, there is no “excitability parameter” such changes, in resting potential, pacemaker frequency, and excitability were achieved by applying bias depolarizing or hyperpolarizing current. For both polarities, studies were done at a current intensity at which oscillatory activity was just sustained (i.e., a further increase in current intensity would abolish spontaneous activity). Membrane voltage was perturbed by applying short (50-msec) depolarizing current pulses at a phase of 50% of the stable spontaneous cycle as reported in a previous study.

For our purposes, phase zero was defined as the moment in the simulations at which the membrane potential surpassed −20 mV during the upstroke of the action potential. Percent phase is then defined as a percentage of one complete cycle between the same phase zero on two successive action potentials. Plots of membrane potential versus time were used to indicate the effects of these perturbations.

**Results**

*van Capelle and Durrer Model*

A bifurcation diagram representing the maximum amplitude of the action potential as a function of Qit1, the bifurcation parameter, is presented in Figure 2. Starting with relatively high values for Qit1 (right side of Figure 2) and decreasing progressively, a stable equilibrium potential (quiescent state represented by a solid line) approaches a Hopf bifurcation point (HB2; reached at about Qit1 = −4.91). Two unstable solutions emerge from that point: an unstable steady branch (dashed line) and an unstable periodic branch (open circles). The unstable periodic branch connects HB2 to a stable periodic limit point (PLP2; see the enlargement around this point in Figure 3A), defined as a critical parameter value where the directionality of the continuation parameter variation reverses. In this case, this point coincides with the transition from an unstable to a stable periodic state, from which begins a stable periodic branch (filled circles in Figure 2) that extends to much lower Qit1 values. A progressive decrease of the maximum diastolic potential (not shown) and maximum amplitude of the action potential as Qit1 is reduced characterizes the pattern of this stable oscillatory domain (Figure 2). For Qit1 values of about −22.46, a second Hopf bifurcation point (HB1) is attained after an abrupt fall of the maximum action potential amplitude through a relatively short unstable periodic branch. HB1 represents the junction with the unstable steady branch connected to HB2 and a stable steady branch corresponding to a higher equilibrium potential (i.e., a lower resting potential) than that obtained in the right part of the diagram for greater Qit1 values.

A magnification of the region around HB2 is displayed in Figure 3A. There is clear evidence of the coexistence of two stable states, a steady and a high amplitude oscillatory state, between the Qit1 values −4.91 and −4.84. HB2 and PLP2 are connected by unstable oscillatory states (open circles in Figure 3A) that behave as separatrices between periodic and stationary stable solutions (see below).

In Figure 3B, a time series describes a transit around a hysteresis loop as Qit1 is allowed to vary back and forth through the right zone of transition between the quiescent state and the large amplitude oscillatory state. The time course of the membrane potential follows several steps labeled 1–5. Each of these numbers, also plotted on the diagram of panel A, corresponds to a particular setting for Qit1 and to the end of the observation period for this particular setting. From 1 to 2, Qit1 was set to −4.85, and the same setting was used from 3 until 4. Thus, for an identical Qit1 value either a stable steady state or a stable oscillatory state was observed depending on the past history of the cell. It is noteworthy that this burst of repetitive activity could be obtained for relatively small fluctuations of Qit1 through the right-phase transition zone.

The presence of an overlap between two stable solutions is also demonstrated around HB1, as shown by the enlargement of the left portion of the bifurcation diagram presented in Figure 4A. In this case, however, both states are oscillatory: one corresponds to low amplitude oscillations; the other, to higher amplitude oscillations. The two states are connected by an unstable periodic branch extending between the two stable periodic limit points (PLP1 and PLP2; range of Qit1 values, −22.19 to −22.02). In contrast to the bistable zone near HB2 (Figure 3A), near HB1 (Figure 4A) the maximum diastolic potentials of the
oscillatory states (not shown) are reduced, and the maximum amplitude of the action potential of the higher amplitude oscillatory state is three times lower than near HB₂. Yet, just as in the HB₂ case, unstable periodic solutions play the role of separatrices between the two stable attractor basins, defined as the set of initial conditions from which the system evolves to the particular periodic (or nonperiodic) solution (see below).

In Figure 4, panels B–D represent the most characteristic steps of a hysteresis loop described around the left phase transition zone as Qit₁ varies back and forth through the left transition zone, near HB₂. The steps of the whole loop are labeled 1–5 as visualized in panel A. For the sake of simplicity, only steps labeled 3–5 in panel A are represented as time series in panels B, C, and D, respectively. As Qit₁ is described from −22.088 to −22.30 (step 2 to step 3; panel B), a very long transient decrease lasting about 35 cycles takes place before reaching the low amplitude stable oscillatory state. In addition to the amplitude decrease, there is also a significant decrease (about 40%) of the period. From step 3 to step 4 (panel C), changes of amplitude and period are quite small and are difficult to discern. It is noteworthy that the input parameter value for this particular step (Qit₁= −22.088) is the same as for step 2, which displayed higher amplitude oscillations. From step 4 to step 5 (panel D), a very long transient increase lasting more than 35 cycles evolved before attaining the higher amplitude stable oscillatory state (i.e., the pattern observed at the starting point of the hysteresis loop).

A diagrammatic representation of the period as a function of Qit₁ in the zone of high amplitude stable oscillations is illustrated in panel A of Figure 5. On the right side of panel A, the unstable periodic branch that originates from HB₂ reaches the limit point (O), which corresponds to the finite value of about 7.7 for the period. Subsequently, as Qit₁ is decreased, the period sharply decreases and then more gradually diminishes until PLP₂ is reached. Panel B of Figure 5 shows the period versus the Qit₁ plot corresponding to the enlargement of the left part of panel A. In this bistability zone, an overlap also exists between two ranges of period values, one corresponding to the relatively high amplitude stable oscillations and the other to the stable low amplitude oscillations (filled circles in Figure 5). The periods of the unstable branch (open circles in Figure 5) smoothly connect both stable branches.

Panels A–E in Figure 6 show several patterns obtained for successively decreasing values of Qit₁ in the domain to the right of PLP₁ (see Figure 3A) when a single just-suprathreshold stimulus is given to the cell. In Figure 6, panel A, the resting potential remains unchanged after a full repolarization. In panel B, an early hyperpolarization follows the action potential. In panel C, an early hyperpolarization is followed by damped oscillations. In panel D, the oscillations increase but do not reach threshold. Finally, in panel E, the threshold for automaticity is attained for a Qit₁ value that allows the stimulating perturbation to send the steady state into stable repetitive activity. This pattern corresponds to PLP₂ in Figure 3A. Thus, panels A–E of Figure 6 display the progressive changes of membrane potential, when the cell becomes more and more excitable just before becoming automatic at the phase transition zone corresponding to HB₂. At the right phase tran-
FIGURE 4. Panel A: Magnification of the left part of the bifurcation diagram. MAX AP, maximum of the action potential; Qiti, bifurcation parameter: PLP1 and PLP2, periodic limit points (the last stable periodic solutions); HB1, Hopf bifurcation point; ●, stable periodic orbit; ○, unstable periodic orbit. The vertical line shows that for the corresponding value of Qiti, there are two stable solutions; both are oscillating, but their amplitudes are different. The sequence of numbers 1–5 points out the circulation around a hysteresis loop depicted in panels B through D. Panels B–D: Hysteresis loop in the left bistability zone. Variations of membrane potential (V) versus time are shown as Qiti is set to different values through the transition zone near HB1. For sake of clarity, only the steps 2–5 of the hysteresis loop depicted in panel A are presented. In panel B, Qiti is decreased from −22.088 to −22.30 (steps 2 and 3 in panel A). In panel C, Qiti is increased from −22.30 to −22.088 (steps 3 and 4 in panel A). In panel D, Qiti is increased from −22.088 to −21.85 (steps 4 and 5 in panel A). Note that Qiti values were the same at steps 2 and 3. Initial conditions are as follows: step 1: Qiti = −21.85, V = −1.63, Y = −0.83, t f = 5.95; step 2: Qiti = −22.088, V = 1.23, Y = 0.77, t f = 35.15; step 3: Qiti = −22.30, V = −0.19, Y = 0.88, t f = 84.60; step 4: Qiti = −22.088, V = 8.98, Y = 0.80, t f = 134.10; and step 5: Qiti = −21.85, V = 8.09, Y = 0.87, t f = 184.60, where Y is the generalized instability parameter and t f is time elapsed.

sion (Figure 3A), for a range of Qiti values between −4.84 and −4.91, we are dealing with cells whose behavior can be of two coexisting types: steady state and high amplitude periodic. Accordingly, they were labeled as stationary-periodic transition cells. In these cells, a subthreshold voltage pulse induces damped oscillations while a suprathreshold pulse gives rise to a self-sustained rhythmic activity (Figure 6F). This behavior is similar to the so-called triggered activity observed in experimental preparations. The mathematical explanation of this kind of behavior is given below through the description of the attractor basins in the Y-V phase-plane representation.

The activation versus the voltage phase-plane representation of Figure 7A illustrates a stable limit cycle (expressing the stable oscillatory behavior of the cell) and a stable singular point (S in panel A, which corresponds to the stable quiescent state of the cell) for a stationary-periodic transition cell featuring a Qiti value equal to −4.84538. This representation shows the activation and membrane potential values assumed by the system while it oscillates (e.g., the stable limit cycle) or keeps silent (e.g., the steady-state point). The smaller orbit (dashed line in Figure 7A) included within the stable limit cycle is both an unstable periodic solution and a separatrix between two attractor basins: the steady state basin and high amplitude periodic attractor basin. As a result of this geometrical structure, we can define a “robust” zone above the dashed line, where any hyperpolarizing or depolarizing pulse driving the membrane potential into this area (even outside the stable limit cycle) cannot annihilate rhythmic activity. Below the dashed line, a “fragile” zone extends to the lower part of both superimposed stable limit cycle and separatrix. Any depolarizing or hyperpolarizing pulse pulling membrane voltage into the area encompassed by the separatrix annihilates rhythmic activity. This is exemplified in Figure 7B where the membrane potential versus the time tracing features the behavior of the cellular element when a perturbation is applied at a time corresponding to point 7 on the stable limit cycle shown in Figure 7A. The initial four action potentials correspond to the stable limit cycle before the perturbation. Due to the vicinity of the stable limit cycle and the separatrix at point 7, a very tiny brief depolarizing pulse (+0.1) applied at this phase in the cycle (indicated by the arrow in Figure 7B) is sufficient to send the stable repetitive rhythm state into the stable steady state after some damped
oscillations. However, if the depolarizing pulse is large enough (+9.5) to reach point 8, the system remains in the domain of attraction of the stable repetitive mode (simulation not shown).

The same kind of phenomena are found at points 0, 1, and 2 in Figure 7A. The corresponding time course of the repetitive activity annihilation is plotted in Figure 7C. A +6 depolarizing pulse denoted by the arrow annihilates automatic activity. In the phase-plane representation of Figure 7A, this pulse extends from point 0 to point 2. If point 2 is reached, the membrane potential falls into the steady-state basin encompassed by the separatix. As a result, the system becomes quiescent after some damped oscillations. In contrast, a +5 depolarizing pulse starting at point 0 and reaching point 1 does not annihilate the repetitive firing state (simulation not shown). Thus, point 0 can be considered as a less “fragile” position than point 7 with respect to annihilation.

The effects of hyperpolarizing pulses are illustrated in Figure 7D. A −74 hyperpolarizing pulse applied at a time corresponding nearly to the peak of the action potential annihilates periodic activity. Annihilation is readily explained by considering the phase plane of Figure 7A, where this pulse is represented by the line extending from 3 to 5. Because the membrane potential falls into the steady-state attractor basin, annihilation ensues. On the contrary, a −65 hyperpolarizing pulse starting at point 3 and reaching point 4 is unable to annihilate periodic activity; hyperpolarizing further to −84 (point 6) allows the system to remain in the stable oscillatory attractor basin and to maintain its self-sustained rhythmic activity (simulations not shown).

In the right bistability zone (enlarged in Figure 3A), the strength of attraction for each of the two stable states depends on the relative size of attraction domains, which in turn depends on the Qit1 values set in this overlap range. Figure 8 shows the stable and unstable limit cycles and singular points corresponding to the steady-state orbits on the Y-V phase-
Figure 7. Panel A: Phase-plane representation (generalized inexcitability parameter $|Y|$ vs. transmembrane voltage $|V|$) of a stationary-periodic transition cell for bifurcation parameter (Qitl) = -4.84358. The solid orbital tracing represents the stable limit cycle; the dashed orbital tracing represents the unstable periodic solution and separatrix between the two attractor basins: one is defined by the stable limit cycle, and the other is defined by the stable steady state (S). Any pulse starting from the stable limit cycle and reaching the zone above the dashed line does not stop rhythmic activity (robust zone). Below the dashed line some depolarizing or hyperpolarizing pulses can get through the separatrix and fall into the steady-state attractor basin (fragile zone). The different horizontal lines, dots, and numbers illustrate the various related possibilities. Dots 1–8 indicate perturbations applied at times corresponding to the points on the stable limit cycle. Panel B: Annihilation obtained through a depolarizing pulse. Membrane potential (AP) versus time is shown. The initial high amplitude oscillations correspond to the self-perpetuating stable limit cycle shown in the phase-plane representation of Figure 7 without any applied perturbation. At the time indicated by the arrow, corresponding to the dot 7 on the limit cycle of panel A, a tiny depolarizing perturbation applied at this phase time annihilates rhythmic activity. Initial conditions are $V(0)=-7.2509$, $Y(0)=0.4762$, $V$(depolarizing pulse) = +0.1, applied when $V=-7.2509.$ Panel C: Annihilation obtained through a depolarizing pulse. AP versus time is shown. The initial large oscillations correspond to the stable limit cycle represented in panel A. A depolarizing pulse (+6) applied at the phase time indicated by the arrow and corresponding to the pathway from dot 0 to dot 2 in panel A sends the stable repetitive activity into the steady state. Initial conditions are as follows: $V(0) = -9.6765$, $Y(0) = 0.7053$; depolarizing pulse applied when $V = -9.6765$. Panel D: Annihilation obtained through a hyperpolarizing pulse. AP versus time is shown. The large amplitude oscillations correspond to the limit cycle shown in panel A. At the time indicated by the arrow that corresponds nearly to the maximum amplitude of the AP and to the pathway from dot 3 to dot 5 in panel A, a hyperpolarizing pulse (-74) suppresses rhythmic activity. Initial conditions are as follows: $V(0) = 75.79$, $Y(0) = 0.65$; hyperpolarizing pulse applied when $V(0) = 75.79$.

plane representation for three different Qitl values. For values of Qitl greater than -4.84 (not shown), the steady state is the only attractor. As Qitl decreases, the steady-state attractor basin delineated by the unstable limit cycle (i.e., the separatrix) decreases and shrinks onto the steady-state point (S in Figure 8). For values of Qitl less than -4.91, the stable limit cycle is then the only attractor. It is clear from these diagrams that annihilation should be much easier to achieve in Figure 8A than in Figure 8C and should eventually depend on suitably setting Qitl within a relatively narrow range of values. Indeed, in this bistability zone the likelihood of annihilation seems appreciable only within the very small range of Qitl values where the steady-state attractor basin (corresponding to a silent state) is large compared with the area included in the stable limit cycle (corresponding to a self-sustained rhythmic activity).

Close to HB1 (Figure 4A), within a range of Qitl values between -22.02533 and -22.1979, the behavior of cellular elements is characterized by two coexisting states. Both are periodic, but one consists of relatively high amplitude oscillations, and the other consists of low amplitude oscillations. Thus, we are dealing with biperiodic transition cells, whose behavior can never be stationary at any stage within this zone. This is demonstrated in the $Y-V$ phase-plane representation of Figure 9B. As a matter of fact, for a Qitl value of -22.088, we find two stable limit cycles (outer and innermost solid orbital lines) defining two periodic attractor basins centered on the largest and the smallest orbits. The middle orbital
Figure 8. Generalized inexcitability parameter (Y)–transmembrane voltage (V) phase-plane representations of a stationary-periodic transition cell for three values of the bifurcation parameter (Qitl): panel A, Qitl = -4.843442; panel B, Qitl = -4.84358; and panel C, Qitl = -4.902. Solid tracings, stable limit cycles; dashed tracings, unstable orbits (separatrices); S, stable steady-state solution. Note the shrinkage of the steady-state attractor basin from panel A to panel C as Qitl is decreased.

Figure 9. Behavior of biperiodic transition cell detected in the left phase transition zone illustrated in generalized inexcitability parameter (Y)–transmembrane voltage (V) phase-plane representations for three different values of the bifurcation parameter (Qitl) comprised in the overlap region: panel A, Qitl = -22.03488; panel B, Qitl = -22.088; and panel C, Qitl = -22.1928. Points 1–3 in panel B indicate perturbation points. The solid orbital tracings correspond to two stable limit cycles of different amplitudes. Dashed orbital tracings represent the unstable periodic solution and separatrix between the two attractor basins: one, relatively high amplitude periodic; the other, low amplitude periodic. The corresponding frequencies are also different (see Figure 5B). There is no stable steady state in this zone.

As a result, robust zone areas become smaller. It is noteworthy that, in this case, the changes of attractor basin areas are opposite and symmetrical to those observed in the right transition zone when Qitl values decrease. Thus, as in the right bistability zone, the shape variations of attractor basins resulting from Qitl changes modulate the possible displacements from one stable state to another.

YNI Model

Since the results with the VCD model demonstrated the existence of regions in which it was possible to annihilate spontaneous pacemaker activity, a more physiologically relevant model of the
cardiac pacemaker cell was used to further investigate this phenomenon. Prior studies with the YNI model indicated that it could mimic many of the phase-related phenomena seen experimentally. Therefore, we used this model to search for those conditions under which pacemaker activity could be eliminated by a single current pulse of proper timing and intensity. The results shown above for the VCD model indicate that only under conditions in which the separatrix had been enlarged by the proper selection of membrane parameters was it possible to obtain bistable behavior. Indeed, under conditions where no bias current was applied to the normal YNI model, we failed to find proper conditions of stimulus timing and intensity necessary to eliminate pacemaker behavior (not shown). Indeed, similar unsuccessful attempts were previously reported on embryonic chick ventricular heart cell aggregates by several authors. However, by applying bias hyperpolarizing or depolarizing current, changes could be effected in the system kinetics such that the character of the response to brief depolarizing current responses could be altered.

Application of bias hyperpolarizing current brought the YNI cell to a condition similar to that found near HB2 in the VCD model, that is, near a Hopf bifurcation point. This is illustrated in the sequence of tracings shown in Figure 11A. The top tracing shows pacemaker activity when a hyperpolarizing bias current of 0.460 μA was applied. This bias current produced a marked increase in pacemaker cycle length from the control value of 318 msec (not shown) to 866 msec. As illustrated in the bottom three tracings of Figure 11A, application of a single depolarizing current pulse (50-msec duration) at a phase of 50% of the cycle induced a variety of behaviors, depending on stimulus intensity. At a stimulus intensity of −0.02 μA (second tracing of Figure 11A), the current pulse induced a marked prolongation (to 1,565 msec) of the cycle during which it was applied. The following cycle was briefer (861 msec), and then activity returned to normal. When pulse intensity was increased to −0.08 μA, a single pulse at a phase of 50% produced small oscillations, which damped with time and led to permanent stable extinction of pacemaker activity (third tracing of Figure 11A), as determined by continuous recording during 30 seconds after application of the pulse (not shown). Further increasing pulse intensity to −0.20 μA produced a marked shortening (to 507 msec) of the cycle during which the pulse occurred, and then activity returned to normal. These results are similar to those found in the VCD model near HB2 (see Figure 7) in that both small and large voltage changes produced transient activity that returned to the limit cycle, whereas intermediate voltage changes drove the system to the annihilation of pacemaker activity.
Similar simulations were used to investigate the effects of brief current pulses when depolarizing bias current was applied to the YNI model. The first tracing of Figure 11B shows control activity when a depolarizing bias current of \(-0.8365\) \(\mu\)A was applied. Pacemaker cycle length decreased from the control value of 318 msec (not shown) to 205 msec. The bottom three tracings of Figure 11B show the effects of single current pulses of different amplitude applied at a phase of 50% of the pacemaker cycle. A depolarizing current pulse of \(-0.08\) \(\mu\)A (second tracing of Figure 11B) induced a transient decrease in both maximum diastolic potential and maximum amplitude of the action potential, which gradually returned to control values during successive beats. When current pulse amplitude was increased to \(-0.20\) \(\mu\)A (third tracing of Figure 11B), the pulse led to damped oscillations, which progressed to complete annihilation of pacemaker activity during 30 seconds of simulation time. Further increasing current amplitude to \(-0.80\) \(\mu\)A (third tracing of Figure 11B) produced only a voltage transient with pacemaker activity returning immediately to control values. These results are similar to those obtained with hyperpolarizing current pulses and suggest that a separatrix may exist between an oscillatory stable state and a quiescent stable state such that a stimulus of proper amplitude can annihilate pacemaker activity. However, they differ from the results obtained with continuation techniques for the VCD model. Those results (Figures 9 and 10) indicate that pulses applied near HB, should not annihilate pacemaker activity totally but should reduce the amplitude of pacemaker oscillation to a low value, since biperiodic transition cells were found in this region in the VCD model. In contrast, the results obtained with the YNI model suggest that, in the two bistability zones found in this model, the cell is behaving as a stationary-periodic transition cell.

**Discussion**

**Limitations of the Study**

The aim of the present study was to classify the behavior of an isolated cardiac excitable element by using the VCD\(^{15}\) model and continuation-bifurcation analysis techniques. In addition, the YNI model\(^{17}\) was used to demonstrate the possible occurrence, in a quantitative membrane ionic model, of annihilation phenomena similar to those observed in the VCD model. From the results reported here, annihilation in stationary-periodic transition cells is understood as a permanent stable extinction of spontaneous activity, which depends on the shape of the respective domains of oscillatory and steady stable states (the attractor basins) and on the polarity, timing, and magnitude of the applied stimuli. In this sense, the annihilation phenomenon reported here has a mechanism different from that described previously on the basis of topological arguments.\(^{1}\) As a matter of fact, topological studies\(^{1-12}\) using stimulus strength–coupling interval maps have demonstrated that there is only one combination of stimulus strength and coupling interval that can give rise to annihilation in certain conditions: the black hole should be an infinitesimal point capable of being expanded in the heart, due to some facilitating factors.

Uncovering the coexistence of stable large amplitude oscillation and stable steady state was expected in the VCD model because this model derives from that of FitzHugh\(^3\) (the so-called Bonhoeffer–van der Pol model), which can be regarded as an approximation of a two-dimensional projection of the four-dimensional Hodgkin-Huxley model,\(^{15}\) and because theoretical works have demonstrated that such a topology can occur in the Hodgkin-Huxley model.\(^7,11\) However, we cannot infer from our results that annihilation phenomena should be easily observed in every kind of cardiac tissue as well as in all models of cardiac pacemaker activity. Indeed in the past, such findings have been difficult to obtain experimentally and in mathematical models.\(^{10}\) Extinction of spontaneous activity could not be achieved in normal Purkinje fibers\(^3\) or in embryonic chick ventricular heart cell aggregates\(^{28-30}\) or in ionic models such as the Purkinje cell model\(^9\) and the embryonic chick ventricular cell model.\(^30\) In some modeling and experimental studies, an applied bias depolarizing current is required to obtain this phenomenon.\(^2,7,9,11,13\)

Reiner and Antzelevitch\(^{10}\) attempted to mimic a depressed state in a modified version of the Bristow and Clark\(^{32}\) sinus node model by introducing a bias depolarizing current and reducing the slow inward current and the delayed rectifier current; a transient annihilation could be achieved only under these conditions. In the present study, the issue lies in how representative the VCD model is for cardiac tissue since this model is very simplified compared with other ionic models (see "Appendix"). As was reported by van Capelle,\(^{15}\) the VCD model was primarily designed to simulate arrhythmias usually observed in depressed myocardium such as triggerable focal and circus movement tachycardias. Due to the large size of sheets or cables used in these simulations, the ionic equations were drastically reduced while retaining some basic electrophysiological properties. In so doing, the study was restricted to elements with a low upstroke velocity, "which are more representative of nodal or depressed myocardial cells than of healthy myocardium or Purkinje fibers."\(^{15}\) We can bring together these VCD model features with the above-mentioned requirements for annihilation, which consist in applying a bias depolarizing current and/or inducing some degree of depression. In the same view, we cannot generalize the YNI sinus node model behavior to the behavioral features of all cardiac pacemaker cells. As a matter of fact, annihilation could be obtained in this model only after applying a bias current (see "Results"); every attempt of using the standard sinus node model was unsuccessful. Hence, our current findings do not agree with the previous experimental report from this laboratory that annihilation is possible in the sinoatrial node.\(^5\) The
reason for this discrepancy might be that the sinoatrial node preparations used in the experimental report were obtained from peripheral areas of the kitten sinus node, which featured slower spontaneous activity and greater maximum diastolic potentials than cells in the compact region of the node. From these considerations, we can deduce that some particular conditions prevailing in the cells or tissue under study may facilitate the extinction of automatic activity. On the other hand, the relative narrowness of the parameter value range where annihilation can be obtained in theoretical studies may be related to the difficulties in observing this phenomenon in experimental works.

The Parameter \( \text{Qit1} \)

To bring about rhythmic oscillatory potentials in a resting cardiac cell modeled by the VCD model, we used \( \text{Qit1} \) as the bifurcation parameter instead of an externally applied bias depolarizing current. In most experimental and theoretical reports, \( \text{Qit1} \) a steady depolarizing current has been applied to produce spontaneous rhythmic activity. In fact, as outlined by Chay and Lee,13 any parameter in the model that affects the maximum diastolic potential can be used as a bifurcation parameter. In experimental settings, various interventions that influence maximum diastolic potential, such as variations of external ionic concentrations of \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) or \( \text{K}^+ \), or reduction of the \( \text{Na}^+-\text{K}^+ \) pump activity caused by ischemia make all cardiac cells susceptible to automatic firing. Indeed, during acute phases of myocardial ischemia, significant current may flow between injured depolarized cells and normal tissue.33 However, in many instances, automatic mechanisms are neither mediated by external current flow nor induced by other external interventions. Furthermore, the level at which diastolic depolarization occurs may not be a satisfactory basis for discriminating between normal and abnormal rhythmic activity.4 Therefore, we can assume that some factor intrinsic to the cell does exist, a factor that can induce automaticity without any extrinsic influence. As a result, we chose \( \text{Qit1} \), an intrinsic parameter of the model, rather than an external current flowing from neighboring cells.15 Indeed, we have previously demonstrated that \( \text{Qit1} \) plays a major role in setting the current-voltage relation for a maximally excitable membrane in such a way that the regenerative process can work repetitively18,22; both maximum diastolic potential and threshold levels are decreased when \( \text{Qit1} \) is lowered. Conceptually, the difference may be appreciable even though the general findings are similar.

Indeed, we found a range of \( \text{Qit1} \) values between two stable periodic limit points \( \text{PLP}_1 \) and \( \text{PLP}_2 \) (see Figures 2–4), where high amplitude rhythmic potentials were present. When \( \text{Qit1} \) was decreased from \( \text{PLP}_2 \) to \( \text{PLP}_1 \), maximum diastolic potential progressively decreased, and the oscillatory activity of the system increased. This is somewhat similar to the experimental and theoretical observations reported by Gutman et al11 and the theoretical findings obtained by Chay and Lee13 in the Beeler and Reuter model. In the latter study, as the bias depolarizing current was increased, the system’s oscillation period followed a similar path, decreasing first very rapidly from a homoclinic orbit and then more gradually until a second homoclinic orbit was found at the opposite end of the diagram. Yet in the VCD model, we did not discover any homoclinic orbit in contrast with the above-mentioned report.13 Instead, in the left part of the bifurcation diagram corresponding to a markedly depolarized region, there were two overlapping oscillatory stable states showing two different periods. In regard to the action potential amplitude, we observed a significant reduction of this parameter as the cell became more depolarized, which agrees with the Hodgkin-Huxley case,11 but not with the Beeler and Reuter model results.13 However, the YNI model agrees with the VCD model concerning amplitude and period changes obtained by varying \( \text{Qit1} \): by increasing the bias depolarizing current, both action potential amplitude and cycle length decreased (Figure 11).

Two Types of Bistabilities

As already observed in the Beeler and Reuter model,13 the VCD model showed two Hopf bifurcation points around which bistability zones could be evidenced. The right one (Figure 3A) extends from \( \text{HB}_2 \) to \( \text{PLP}_2 \), whereas the left lies between \( \text{PLP}_1 \) and \( \text{PLP}_2 \) (Figure 4A). The right bistability zone corresponds to the coexistence of a high amplitude oscillatory state with a singular point represented by a stable steady state. In contrast, the left bistability zone features a more depolarized membrane potential and the coexistence of two oscillatory states differing significantly in period and amplitude and characterized by specific stable limit cycles and attraction basins in the Y-V plane representation (Figures 9 and 10). Such a pattern is very similar to the behavior, termed birhythmicity, recently observed in coupled biochemical oscillators.35,36 Experimental findings suggest that it might also occur in cardiac tissue.5 However, the latter bistability pattern was not found in the Beeler and Reuter model,13 in which oscillatory states always coexisted with steady states in bistability regions. In this aspect, our results with the YNI model (Figure 11) were more consistent with those reported for the Beeler and Reuter model. We did not investigate the entire parameter space in this model, but the results with single current pulses show that there are also two bistability zones in this model, both of which are stationary-periodic.

In the VCD model, considering the right part of the general bifurcation diagram (Figure 2) and starting from the right, we found that the progressive decrease of \( \text{Qit1} \) induced a gradual lowering of maximum diastolic potential, as well as the successive characteristic responses to a single stimulus (Figure 6). By decreasing further \( \text{Qit1} \) and giving a supathreshold stimulus, the system was sent from the steady state through the separatrix into the domain.
of attraction of the stable repetitive activity. Therefore, along these intermediate steps, the cell became more and more excitable, showing precordial patterns in the early diastolic phase and eventually gaining a bistable behavior for Qit1 values just below that corresponding to PLP3.

Conditions for Annihilation

Previously reported studies,1,2,7-11,13 based on topological representations, have pointed out that annihilation of pacemaker activity depends on some critical combination of polarity, phase timing, and magnitude of an applied perturbation. Instead, in the situation here explored, it is necessary to drive the membrane voltage into the area surrounded by the separatrix (Figures 7 and 8) to obtain the annihilation of pacemaker activity. This area corresponds to a finite range of the activation (Y) and membrane potential (V) parameter values, and as a result, annihilation requires a proper range of magnitude and phase timing together with an adequate polarity of the applied stimulus. Thus, due to size and shape variations, the probability of inducing annihilation appears strongly dependent on the relative area encompassed by the separatrix defining the steady-state attractor basin. Hence, it can be suggested that failures to obtain annihilation in experimental settings,2,3 as well as in mathematical models,10 might be related to the extent of the steady-state attractor basin relative to the stable limit cycle. The two-dimensionality of the VCD model allows us to show easily why a proper range of stimulus strength and timing values is needed to achieve annihilation (Figure 7), while in more sophisticated models5,13 a reduction of dimensionality is required to achieve this topographical representation. The choice of a proper polarity is also mandatory; values of stimulus strength and phase timing cannot be the same if depolarizing or hyperpolarizing pulses are used to send stable repetitive activity into the steady state. Phase and polarity dependence have also been demonstrated for annihilation in the YNI model.37 This requirement was demonstrated previously in theoretical works7,10,13 and in experimental preparations2 through scanning the pacemaker cycle by brief current pulses and then plotting the corresponding phase-resetting curves. An optimal duration of the pulse perturbation is also likely to be a contributing factor, as suggested by experimental findings already reported.2 In such experiments, annihilation was obtained for a 30-msec current pulse, whereas no cessation of rhythmic activity could be achieved by 10- and 50-msec pulse durations. The influence of this parameter in the VCD model was not further explored.

Hysteresis Phenomena

The coexistence of stable states near the two Hopf bifurcation points implies hysteresis phenomena. Figure 3B illustrates the expression versus time of such a phenomenon when Qit1 is allowed to fluctuate around the phase-transition zone. Consequently, small fluctuations of some parameter governing the cyclic regenerative process of this kind of transitional cell (stationary-periodic) would be able to bring about burst-like repetitive intermittent activities. Likewise, Figure 4 shows a circulation around a hysteresis loop featuring the other kind of transitional cell (biperiodic), but in this case the transients are much longer than in the right transition zone when Qit1 values are changed in a stepwise manner. In addition, the biperiodic transition cell cannot evolve as high amplitude oscillations as the stationary-periodic type for the largest stable limit cycle.

Conclusions

The results of the present study may contribute to the elucidation of the behavior of cardiac pacemakers, especially the interactions of multiple pacemakers within the heart and the interactions between pacemaker cells and their environment. In particular, it has been suggested that vagal discharge would be able to modulate the sinoatrial node function by resetting spontaneous pacemaker activity2 or inducing localized or temporary inhibition. The present study might also be a step toward understanding the mechanism of life-threatening arrhythmias such as ventricular fibrillation. As a matter of fact, Winfree38 has recently proposed that the possibility of inducing “black holes” (i.e., “singular points” that correspond to some unique conditions able to entail cessation of rhythmic activity) somewhere in the heart by some internal or external impulse might be the underlying cause of the generation of rotating waves. Then, these rotating waves might be transformed into many scattered smaller waves and thus into fibrillation. Accordingly, the experimental demonstration of both singular points and oscillatory rotating waves during the time course of such processes (i.e., some kind of multistability involving space and time) might be a basic clue to understanding this lethal arrhythmia.

Appendix

van Capelle and Durrer model (VCD model)

The membrane model used in the present study was previously described by van Capelle and Durrer15 (F.J.L. van Capelle, personal communication) as follows:

\[
SC(dV/dt) = -S[YI_1(V) - (1-Y)I_0(V)] + I_{ex} \quad (1)
\]

We do not include the relative size of the cell (S) and the external current flowing from neighboring cells (I_{ex}) because we deal with a single cellular element. Thus, Equation 1 becomes

\[
C(dV/dt) = -[YI_1(V) + (1-Y)I_0(V)] \quad (2)
\]

Equation 2 is then coupled to the following equation:

\[
T(dY/dt) = Y_s(V) - Y \quad (3)
\]

The two state variables are the transmembrane voltage (V) and the generalized inexcitability parameter (Y). Y can be considered as summing up the whole
behavior of the \( m, n, \) and \( h \) gates of the Hodgkin-Huxley model and is allowed to assume any value between 0 (maximal excitability) and 1 (complete inexcitability). \( C \) denotes the membrane capacitance, and \( T \) denotes the time constant of the activation/inactivation process. The capacitive membrane current is supplied by an ionic transmembrane current, which is a weighted average between the two currents: \( I_0(V) \) (current-voltage relation for a maximally excitable membrane) and \( I_1(V) \) (current-voltage relation for a completely inexcitable membrane). For computational purposes, we set \( f(V) = I_0(V) - I_1(V) \), \( g(V) = I_1(V) \), and \( h(V) = Y_e(V) \). Then the model can be expressed as

\[
\frac{dV}{dt} = -C^{-1} [g(V) + (1 - Y) f(V)]
\]

\[
\frac{dY}{dt} = T^{-1} [h(V) - Y]
\]

\( f(V) \) is composed of a central cubic function between two straight lines, \( g(V) \) is a piecewise linear function, and \( h(V) \) is linear between the 0 and 1 levels. \( f(V) \) is written as follows:

- for \( V < v_f 1 \), \( f(V) = Q_i f_1 + a f_1 (V - v_f 1) \)
- for \( v_f 1 \leq V \leq v_f 2 \), \( f(V) = Q_i t_1 + A (V - v_t 1 - B) (V - v_t 1) \)
- for \( V > v_f 2 \), \( f(V) = a f_2 (V - v_n a) \)

with

\[
a f_1 = 3 A (v_f 1 - v_t 1) (v_f 1 - v_t o p)
af_2 = 3 A (v_f 2 - v_t 1) (v_f 2 - v_t o p)
A = 2 (Q_i t_1 - i_t o p) / (v_t o p \cdot v_t 1)^3
B = 3 / 2 (v_t o p - v_t 1)
\]

where \( a f_1, v_t 1, Q_i t_1, i_t o p, v_n a \) are independent parameters and \( (v_t 1, Q_i t_1) \) and \( (v_t o p, i_t o p) \) are the respective coordinates of the maximum and the minimum of the cubic central part of \( f(V) \). \( v_f 1, Q_i f_1, v_f 2, A, B, \) and \( a f_2 \) are easily adjusted to connect the three parts of \( f(V) \) in a continuous and differentiable function.

\( g(V) \) and \( h(V) \) are piecewise linear functions:

- for \( V < v_g 1 \), \( g(V) = a g_1 (V - v_k) - s p 12 \)
- for \( v_g 1 \leq V \leq v_g 2 \), \( g(V) = Q_i g_1 + a g_2 (V - v_g 1) - s p 12 \)
- for \( v_g 2 \leq V \leq v_g 3 \), \( g(V) = Q_i g_2 + a g_3 (V - v_g 2) - s p 12 \)

where \( a g_1, a g_2, \) and \( a g_3 \) are the slopes for the linear pieces of \( g(V) \), and \( v_g 1, v_g 2, Q_i g_1, Q_i g_2, v_k, s p 12, i_150, \) and \( v_h \) are constants. The constants corre-

sponding to the initial values assumed during the continuation process were as follows:

\[
\begin{align*}
C^{-1} & = 20 \\ v_f 1 & = -14.28 \\ Q_i g_1 & = 2 \\
T^{-1} & = 1 \\ Q_i f_1 & = -15.83 \\ v_g 2 & = 70 \\
a f_1 & = 2 \\ v_f 2 & = 60.24 \\ Q_i g_2 & = 15 \\
v_t 1 & = -5 \\ A & = 0.0011178 \\ v_k & = -10 \\
Q_i t_1 & = -7 \\ B & = 82.5 \\ s p 12 & = -2 \\
v_t o p & = 50 \\ a f_2 & = 2.24 \\ i_150 & = 40 \\
i_t o p & = -100 \\ v_g 1 & = 0 \\ v_h & = 10 \\
v_n a & = 100
\end{align*}
\]

Because the functions used in continuation-bifurcation analyses must be twice differentiable continuously, the original \( f(V), g(V), \) and \( h(V) \) functions were replaced by 5th splines around the angular point of these functions and their derivatives, thus providing the regularized functions \( f_r, g_r, h_r \).

\( f_r \) regularized from \( f \) is given by the following:

\[
\sum_{V(V f_1 - e, V f_1 + e), f_r(V) = a g_1 (V - v_g 1)}^{V(V f_2 - e, V f_2 + e), f_r(V) = a g_2 (V - v_g 2)}
\]

\( g_r \), regularized from \( g \) is given by the following:

\[
\sum_{V(V g_1 - e, V g_1 + e), g_r(V) = a g_3 (V - v_g 2)}^{V(V g_2 - e, V g_2 + e), g_r(V) = a g_4 (V - v_g 3)}
\]

\( h_r \), regularized from \( h \) is given by the following:

\[
\sum_{V(V h - e, V h + e), h_r(V) = a g_5 (V - v_g 3)}^{V(V h - e, V h + e), h_r(V) = a g_6 (V - v_g 4)}
\]

where \( e \) is the width of the regularization interval and \( a_1, b_1, c_1, d_1, \) and \( e_1 \) are constants. The constants used in the regularized functions were as follows:

\[
\begin{align*}
a_0 & = -6.44 \times 10^{-7} \\ b_1 & = -2.10 \times 10^{-3} \\ d_2 & = 0.47 \\
a_1 & = 2 \\ b_3 & = 7.84 \\ d_3 & = 0 \\
a_2 & = -6.16 \times 10^{-2} \\ c_0 & = -2.68 \times 10^{-4} \\ d_4 & = -7.92 \\
a_3 & = -0.46 \\ c_1 & = 0.19 \\ d_5 & = 0 \\
a_4 & = 2.09 \times 10^{-3} \\ c_2 & = -5.36 \times 10^{-2} \\ e_0 & = 9.37 \times 10^{-4} \\
a_5 & = 7.69 \\ c_3 & = 0 \\ e_1 & = 2.50 \times 10^{-2} \\
b_0 & = 1.91 \times 10^{-6} \\ c_4 & = 0.89 \\ e_2 & = 0.19 \\
b_1 & = 2.24 \\ c_5 & = 0 \\ e_3 & = 0 \\
b_2 & = 0.06 \\ d_0 & = 2.38 \times 10^{-3} \\ e_4 & = -3.12 \\
b_3 & = -0.47 \\ d_1 & = 0.25 \\ e_5 & = 0
\end{align*}
\]
Direct Numerical Integration Scheme

The automatically controlled step length algorithm used in our study is based on a fourth-order Runge-Kutta method with Merson error estimates, as developed in the DA02A Harwell subroutine. At each step (h), the new step length is computed by means of the formula: h(new) = h(old) (0.9w^0.5), with w = (1/ε) max_{s,1}(|e_i|/y_i), where ε is the accuracy requirement, (e_i)_{1s,1s} is the error estimate, (y_i)_{1s,1s} is the state vector, and n is the dimension of the problem.

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