modeling methodology forum

Discontinuities in phase-resetting experiments

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GLASS, LEON, AND A. T. WINFREE. *Discontinuities in phase-resetting experiments.* Am. J. Physiol. 246 (Regulatory Integrative Comp. Physiol. 15): R251–R258, 1984.—The effects of perturbing an on-going biological oscillation with a single brief stimulus are considered. If the time from some observable event before the stimulus to the next event after the stimulus is plotted as a function of the phase of the stimulus, then there may be discontinuities in this plot. The discontinuities reflect the size of the stimulus and the topological properties of the biological oscillation. The implications for experiments are discussed.

biological oscillator; integrate-and-fire model; limit cycle oscillation

IN MANY BIOLOGICAL OSCILLATIONS, one event is readily observable, for example, the eclosion of fruit flies, the initiation of an action potential in a spontaneously firing neural or cardiac preparation, the onset of inspiration, and the start of mitosis in cells growing in tissue culture. After a perturbation of the oscillator, the timing of subsequent repeats of the event is generally altered: the prior rhythm eventually reasserts itself, only with its phase reset relative to an unperturbed control. The amount of resetting generally depends both on the magnitude and manner of perturbation and on the phase in the oscillation when the perturbation begins.

Studies of phase resetting of biological rhythms go back at least to the middle of the last century. In 1868, Hering and Breuer showed that lung inflation can either advance or delay the time of the next inspiration, depending on the phase of the respiratory cycle at which the inflation pulse was delivered (7). Somewhat later, Mines showed that an electric pulse delivered to the heart can send the heart into fibrillation if the pulse is delivered at a certain vulnerable phase of the cardiac cycle but that the same pulse would temporarily reset the rhythm if delivered at other phases (38). Brown and Eccles and co-workers (8, 12) made a detailed experimental study of phase shifts of the heartbeat in cats induced by vagal stimulation. In the intervening years, there have been literally hundreds of experimental studies of phase resetting in diverse systems. These studies provide data on functional control of biological rhythms. As well, phase-resetting experiments can be used as independent tests of hypotheses and models for generation of biological rhythms derived from other experiments (e.g., voltage clamp, single-cell recording, and lesion studies).

The basic experimental paradigm for phase-resetting experiments is to present a single stimulus to an ongoing rhythm. The effect of this stimulus on subsequent repeats of the observable event is determined as a function of the phase of the cycle at which the stimulus was delivered. The experiment is repeated for stimuli of varying strength. This paper was motivated by trying to understand the theoretical implications of the following experimental results: *1*) in some systems, stimuli of critical magnitude delivered at a critical phase of an oscillation can abolish the oscillation or delay the reappearance of the next event for several times the intrinsic period of the oscillation (13, 20, 23, 24, 29, 30, 38, 42–43, 53, 56, 57, 59, 60); and *2*) if the time from the event before a stimulus to an event after a stimulus is plotted as a function of the phase of the stimulus, there can sometimes seem to be discontinuities (2, 3, 8, 12, 23–28, 36, 39, 41, 45, 47–52, 60, 63).

The main point of this paper is to show how the topological properties of mathematical models of biological oscillators are related to the experimental observation of discontinuities in phase-resetting experiments. Although some of the results of the analysis are contained elsewhere (16, 32, 33, 53, 59, 60), a short description of this topic directed toward experimentalists has not appeared previously. Our hope is that this paper will stimulate experimentalists to collect data that can be used to distinguish between the different classes of models presented or to suggest alternatives.

Section I describes the experimental paradigm and our notation. Sections II and III described the effects of perturbation on timing in several different models for biological oscillators.

I. TERMINOLOGY

A great many different researchers have conducted phase-resetting experiments and reported their results in different notations. Ours follows. Suppose a periodically recurring event as indicated in Fig. 1. We call the events "firings," because we have in mind neural or

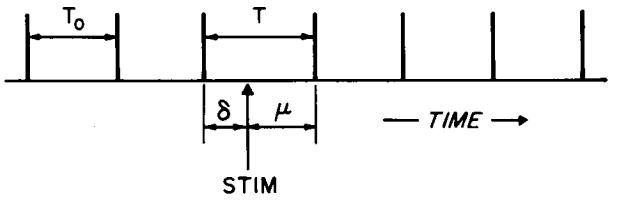

FIG. 1. Schematic representation of experimental paradigm for phase-resetting experiments. *Heavy vertical bars*, observable events of oscillator, with intrinsic interval between successive events of T_0. Stimulus (STIM) is delivered at time δ after event. After time μ, oscillation resumes with time T_0 between events.

cardiac pacemaker cells. To simplify notation, let us restrict attention to instantaneous firings and instantaneous stimuli; our conclusions are similar but more awkwardly expressed in the more realistic case of finite, even long, durations. The interval of time (T_0) between successive firings is assumed constant in the absence of stimulation. A stimulus is administered at a *time* δ after one such firing. As a consequence, the successive firing times are altered. Assume that after the first poststimulus event, firing repeats at its previous interval T_0. We designate by μ the time from the stimulus to the next firing. The sum δ plus μ, the time from the firing immediately before the stimulus to the firing next after stimulation is T

$$T = \delta + \mu \tag{1}$$

The basic experiment entails measuring either T or μ as a function of δ for several different strengths of the perturbing stimulus. It is not common to present experimental results in this format. A diversity of other formats have been used (e.g., see catalog of p. 111, Ref. 60). A normalized version of this format best serves the purposes of the present paper: to facilitate comparison between different experimental preparations, we normalize each quantity to the unperturbed interval (T_0) observed immediately before the perturbation in that preparation. Dividing by T_0 we have

$$\tau = \phi + \theta \tag{2}$$

where the normalized T (the perturbed interval taking the unperturbed interval as the unit of time) is denoted by τ. The normalized δ is called the old phase (ϕ) and the normalized μ is called the cophase (θ). ϕ ranges between 0 and 1, equivalent to the full circle ranges 0–360° or 0–2π familiar in other contexts. T may be very long so that τ and θ, unlike ϕ, may exceed 1.

The usual consequence of stimulation is to produce a phase difference ($\Delta\phi$) between the perturbed oscillator and an unperturbed control oscillator. In terms of the quantities defined above, this $\Delta\phi = 1 - \tau$. The dependence of $\Delta\phi$ on ϕ for any fixed stimulus is commonly called the phase-response curve (PRC).

Another convenient representation emphasizes not the phase difference, but the new phase (ϕ')

$$\phi' = \phi + \Delta\phi = \phi + 1 - \tau \tag{3}$$

Its dependence on ϕ is called the phase-transition curve (PTC) [also called the phase-resetting curve (60)]. Just before the stimulus the oscillator had reached phase ϕ; just after, it appears to resume from new phase ϕ'. In the event of a long delay, ϕ' should be considered modulo 1 [e.g., 1.4 (mod 1) = 0.4 = −0.6 (mod 1)].

We compute $\Delta\phi$ and ϕ' from the first firing time. Under our approximation that the interval τ between firings after perturbation remains the same as before perturbation, the values of $\Delta\phi$ and ϕ' computed from the first firing are the same as asymptotic values long after perturbation. The possibility that after resumption of the firing, there are transient changes in the firing rate for several cycles until a new equilibrium is established has been discussed in some detail (32). In this case, call τ_i the normalized time from the firing before the stimulus to the ith firing. Then $\phi_i' = \phi + 1 - \tau_i$ (mod 1) and $\phi' = \lim_{i\to\infty} \phi_i'$. Since the implications of such transient behavior for the analysis of discontinuities in phase-resetting experiments have already been discussed by Kawato (32), we do not deal further with this situation.

We prefer the "new phase" notation to the "phase shift" notation, because it avoids a possible source of ambiguity in reporting experimental results. This ambiguity arises, because, as noted above, it is often convenient to measure the change of phase only after several cycles have elapsed after the perturbation. In this circumstance a phase delay of $\Delta\phi = -0.1$ is indistinguishable from the complementary phase advance, $\Delta\phi = +0.9$. The difference between delay and advances lies only in the immediate aftermath of perturbation, frequently obscured by diverse experimental artifacts. In the case of neural pacemakers it may also happen that several expected firings are missed before the rhythm is reestablished. In this circumstance we would measure $\tau > 2$ and $\Delta\phi < -1$ (see *Eq. 2*). But phase values are commonly reported modulo 1 and might be reported either positive or negative as noted above. Moreover when, as ϕ is varied the phase difference crosses some value (e.g., +0.5 or −0.5), the reported value is commonly increased or decreased by 1. Thus a large advance (+0.49) may be plotted adjacent to a large delay (− 0.50), giving the appearance of discontinuity or an unmeasurably steep slope.

II. PERTURBATIONS OF INTEGRATE-AND-FIRE MODELS

One caricature for many kinds of biological oscillator is called the "integrate-and-fire" model. It has been invoked in reference to the activity of pacemaker neurons and of cardiac pacemaker cells (1, 34, 46), circadian eclosion of insects (60, p. 403–406), flashing of fireflies (9), circadian timing of sleepiness in humans (11, 61, 62), the timing of cell division and nuclear division (31, 37, 54), and respiratory rhythms (4, 6, 44). In the corresponding models a quantity called the "activity" rises toward a threshold (linearly in Fig. 2). When the threshold is reached, an event "firing" is triggered. Activity then decays back toward a lower threshold (instantaneously in Fig. 2) then again begins to rise.

Suppose the perturbation temporarily increases the activity or decreases the upper threshold; when the (instantaneous) perturbation ends, activity or threshold reverts immediately to the prior level. Such a perturbation advances the oscillator by prematurely putting it above threshold. But if applied too early, the boost may not exceed threshold and there will be no advance. Thus resetting is only a piecewise continuous function of stim-

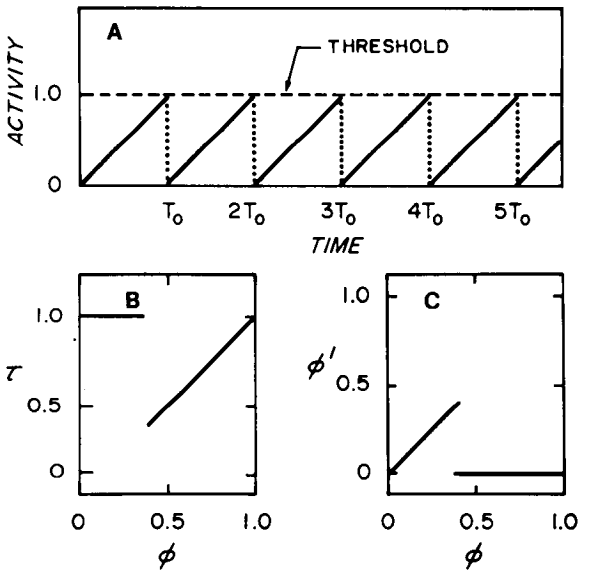

FIG. 2. *A*: simple integrate-and-fire model for biological oscillator activity linearly rises to threshold. Event occurs every time threshold is reached, whereupon activity resets to zero. *B*: plot of perturbed cycle length (τ) as function of phase of stimulus when threshold in *A* is instantaneously lowered by an amount 0.6 at different phases of cycle. If activity is greater than or equal to threshold at instant threshold is lowered, event occurs and activity is reset to zero. *C*: plot of new phase (ϕ') vs. ϕ (phase-transition curve) derived from *B*, using *Eq. 3*.

ulus parameters, and discontinuities in the plot of τ vs. ϕ are expected (3, 9, 58). Figure 2*B* shows the perturbed cycle length τ vs. ϕ for the model of Fig. 2*A* with a stimulus that instantaneously lowers the threshold by 0.6. Note that the stimulus has no effect before $\phi = 0.4$; then it abruptly causes an advance of 0.6 cycle, which declines as the stimulus is offered nearer and nearer to spontaneous firing. A larger perturbation makes the discontinuity larger and places it correspondingly earlier in the cycle. Figure 2*C* is a plot of ϕ' vs. ϕ. In this situation ϕ' and θ are not continuous or differentiable on the unit circle.

Many modifications of the simple integrate-and-fire models have been made to provide closer agreement with experiment or to study the properties of the model subject to periodic inputs: *1*) introduction of two phases, for example, by having a finite time for relaxation to zero in Fig. 2 [models for fireflies (9) and respiration (4, 6, 44)], *2*) nonlinear increases and decreases in activities [models for phase locking of neural and cardiac pacemakers (34) and respiration (4, 6)], and *3*) periodic modulation of the thresholds [models for entrainment of respiration (4, 44), the circadian rhythm (11, 61, 62), and neural and cardiac oscillators (1, 46)].

Despite these many modifications, if one maintains the central idea of integrate-and-fire models (that firing is an all-or-none phenomenon initiated once an activity reaches a fixed threshold), one will observe discontinuities of varying sizes, whose magnitude and location depend on stimulus magnitude, in the plot of τ vs. ϕ (Fig. 2*B*). To our knowledge, close analysis of integrate-and-fire models using phase-resetting data has been performed in only three situations: the mitotic oscillator in *Physarum* (54), firefly flashing (9), and the crayfish stretch receptor's oscillation (39).

III. PERTURBATIONS OF LIMIT CYCLE MODELS

Although integrate-and-fire models have provided convenient and readily understandable idealizations of biological oscillators, inconsistencies between the predictions of integrate-and-fire models and experimental data have been observed.

1) Experimentalists have observed that a particular selection of stimulus magnitude and phase of application can lead to a long delay (longer than twice the intrinsic period) or even abolition of the oscillation. This has been found from perturbation of an oscillating squid neuron (20), in spontaneously oscillating Purkinje fibers (23, 24) and sinoatrial node cells (27), in the circadian activity rhythms of mosquitoes (43), in water regulation of plant sprouts (29, 30), in the rhythmic metabolism of glucose in yeast (56), and in the circadian eclosion rhythm in *Drosophila* (57, 60) and in *Sarcophaga* (42). Such long delays are inconsistent with integrate-and-fire models.

2) The PTC and PRC are often found to be continuous, whereas this is impossible in integrate-and-fire models.

Suppose alternatively that the biological rhythm is generated by a stable limit cycle oscillation. A stable limit cycle is a periodic solution of a differential equation that is attracting in the limit $t \rightarrow \infty$ from all points in the neighborhood of the cycle. To illustrate this concept, Fig. 3 shows a schematic phase plane illustration of a limit cycle in a two-dimensional ordinary differential equation. A limit cycle is further said to be globally attracting if from all initial conditions, except for a set of measure zero (e.g., a point or line in a 2-dimensional phase space), the limit cycle is approached in the limit $t \rightarrow \infty$. All points that approach the limit cycle as $t \rightarrow \infty$ are said to be in the basin of attraction of the limit cycle. In this paper we consider phase resetting of globally attracting limit cycles in two-dimensional ordinary differential equations. We do not consider the added complications from phase resetting in higher dimensions or in functional or partial differential equations.

To discuss phase resetting of limit cycle oscillators some additional concepts are needed. Assume the limit cycle has period T_0 and that we start with initial conditions $x(t = 0) = x_0$, with x_0 being an arbitrary point on the limit cycle. Set the phase of x_0 to be zero. Then the phase of the point $x(t)$ is defined to t/T_0 (mod 1). Thus a phase $\phi(0 \leq \phi < 1)$ can be assigned to every point on

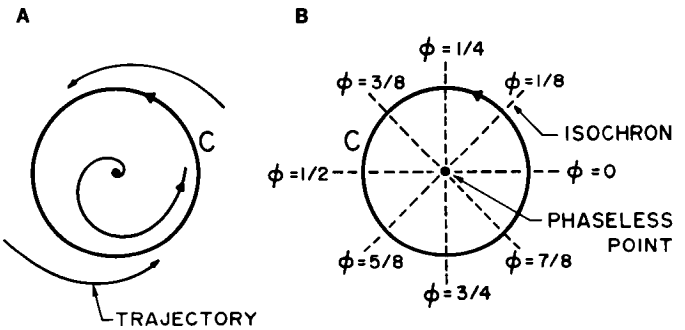

FIG. 3. *A*: schematic representation of phase plane of limit cycle oscillator. All points, except for singular phaseless point, attract to limit cycle (C) as $t \rightarrow \infty$. *B*: for simple model in *Eq. 4*, isochrons (see text, *dashed lines* in *right-hand panel*) are straight lines that approach arbitrarily closely to singular point. For *Eq. 4* limit cycle is circle with radius = 1.

the limit cycle. The latent phase of points in the basin of attraction of the cycle can now be defined. Let $x(t = 0)$, $x'(t = 0)$ be the initial conditions of a point on the cycle and a point not on the cycle, respectively, and $x(t)$, $x'(t)$, be the coordinates of the trajectories at time t. If $\lim d[x(t), x'(t)] = 0$, where d is the Euclidean distance, then the latent phase $t \to \infty$ of $x'(t = 0)$ is the same as the phase of $x(t = 0)$. A locus of points all with the same latent phase is called an isochron (60). An isochron is a smooth curve crossing the trajectories in the attractor basin of the limit cycle (Fig. 3*B*). The state point on any trajectory in the attractor basin of the limit cycle passes through all the isochrons at uniform rate. Thus the isochrons are very close together wherever the time derivatives are small. In particular, they all come arbitarily close together at any stationary state (critical point or fixed point) and therefore necessarily also along any singular trajectory leading to a stationary state. Near any such singular trajectory a small displacement may shift the oscillator across many isochrons to a distinctly different latent phase. We call the locus of stationary states and the attracting sets of these stationary states the phaseless set. Except for the phaseless set, one and only one isochron passes through each point of the attractor basin of the limit cycle.

The effect of a stimulus is to shift a state point on the limit cycle, at some isochron (ϕ) to a new point in phase space lying on some new isochron (ϕ') generally not on the limit cycle. If stimuli are administered at all phases of the cycle, then the locus of new states reached immediately after the stimulus in all those experiments will be a displaced image of the limit cycle. We call this closed curve C′, the shifted cycle, Fig. 4*A*. From one of these initial conditions (after the stimulus), the oscillator follows the corresponding trajectory back toward the limit cycle.

Phase changes continuously along C′ except wherever C′ cuts across the phaseless set. Supposing continuity, one can circumnavigate C′, advancing through one full cycle of ϕ, while counting the net number of times ϕ' advances through a cycle. Call this integer the winding number or topological degree of C′. Recall that the plot of ϕ' vs. ϕ is the PTC. After a sufficiently slight perturbation, the shifted cycle, C′, scarcely differs from the limit cycle, so ϕ' scarcely differs from ϕ and its degree is still 1. We call this type 1 resetting. A perturbation that changes the degree to 0 would be said to inflict type 0 resetting (Fig. 4*A*). (Note that the PRC plots $\phi' - \phi$ vs. ϕ. Therefore for type 1 resetting the degree of the plot of $\phi' - \phi$ vs. ϕ is 0, whereas for type 0 resetting the degree is −1.)

Given the kinetics of a free-running oscillator, we can construct its isochrons. Given the kinetics of the oscillator while subjected to a stimulus, we can construct the shifted cycle for each stimulus strength. Given a shifted cycle and the isochrons, we can construct the PTC. Its grossest feature, the integer degree of resetting, is immediately obvious from the way the shifted cycle lies on the isochrons. Note, however, that if the shifted cycle intersects the phaseless set, the degree of the PTC is not defined. We now consider three examples of phase resetting of two-dimensional oscillators.

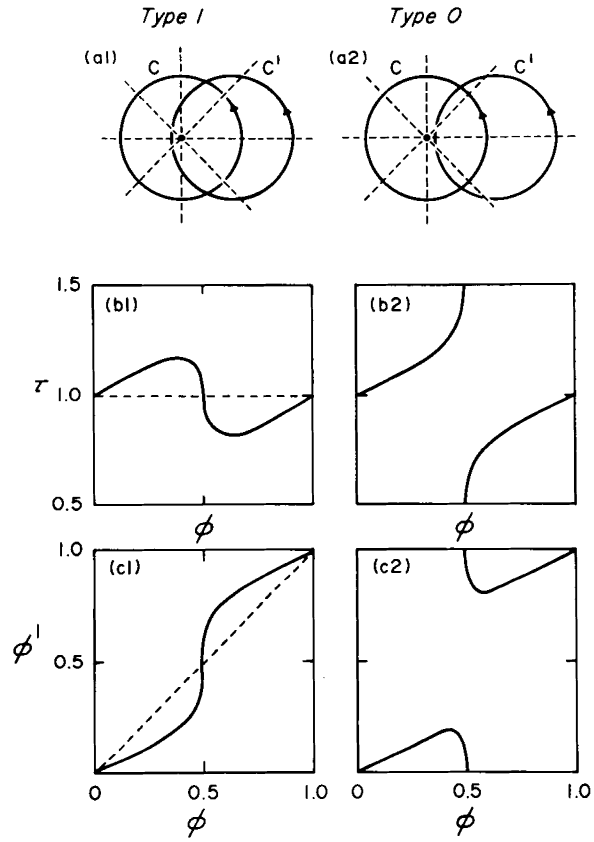

FIG. 4. *A*: schematic representation of effects of perturbing limit cycle oscillator such as that depicted in Fig. 3 and *Eq. 4*. Perturbations, in this case horizontal translation, result in shifted cycle, C′. If shifted cycle encloses singular point, there is type 1 phase resetting (a1). If shifted cycle does not enclose phaseless point, there is type 0 phase resetting (a2). Isochrons are represented by *dashed lines*. *B*: perturbed cycle length (τ) as function of phase of stimulus (ϕ) for type 1 (b1) and type 0 (b2) phase resetting. *C*: schematic representation of phase-transition curve for type 1 (c1) and type 0 (c2) phase resetting. *B* and *C* are related through *Eq. 3*.

A. Radial isochron clock. We consider a simple equation for a limit cycle oscillator of unit period that enables explicit computation of the effects of perturbations. This model, the radial isochron clock (RIC), has been proposed as a simplified model for the circadian rhythm (57), cardiac oscillator (17), and neural oscillators (21). The equations of the RIC are

$$\begin{aligned} d\phi/dt &= 1 \\ dr/dt &= kr(1 - r) \end{aligned} \qquad (4)$$

Except for the singular point at the origin, trajectories pass through every point in the (r, ϕ) plane, leading toward the attracting cycle at $r = 1$.

Now consider a discrete stimulus of magnitude M that displaces the oscillator a distance M to the right. From the new initial condition immediately after stimulation a trajectory leads back to the attracting cycle at a rate that depends on k. In the case of $k \to \infty$, relaxation back to the cycle is instantaneous after perturbation. This case was analyzed by Winfree (57, 59) with additional consideration of implications for entrainment (17, 21). We assume that the oscillator initiates a firing every time its state crosses the half line $\phi = 0$ (17). Thus the perturbed interval τ is simply $\tau = 1 + \phi - \phi'$ (*Eq. 3*).

Note the two distinctly different types of PTC: type 1 resetting (PTC degree of 1) occurs in response to small stimuli ($M < 1$), but type 0 resetting (PTC degree of 0) occurs in response to stimuli exceeding strength $M = 1$. With stimuli of strength $M = 1$ one would measure a PTC that has a discontinuity, but only at that exact strength. In general, for two-dimensional limit cycle oscillators topologically equivalent to Fig. 3*A*, one expects to find type 1 resetting for small stimuli and type 0 resetting for sufficiently large stimuli (60).

In this paper we generalize the previous analysis in two ways. First we let k be a finite positive number. Then we alter the triggering locus to initiate firing only if r is larger than some positive r_0 inside the limit cycle.

Because $d\phi/dt$ does not depend on r, the plot of ϕ' vs. ϕ for any given M remains the same as under the prior assumptions. However, the plot of τ vs. ϕ is markedly changed in regions of (ϕ, M) that move the oscillator inside the circle r_0. In particular, if the stimulus results in a new (r, ϕ) coordinate from which the trajectory may pass $\phi = 0$ one or more times at amplitudes still less than r_0, then one or more firings will be missed. To determine the exact boundaries of (r, ϕ) regions in which n firings will be missed, we need to follow backward the trajectory through ($\phi = 0$, $r = r_0$). Its successive crossing of the radius $\phi = 0$ are given by

$$r_n = r_0/[r_0 + (1 - r_0)e^{nk}] \qquad (5)$$

In Fig. 5 the spiral zone bounded by line segments (r_0, r_1) and (r_1, r_2) contains initial conditions after which one firing is skipped, because the oscillator will cross the $\phi = 0$ axis once before triggering amplitude is exceeded (zone 1, Z_1). From Z_2, two firings will be skipped, and so on. In general in Z_n, $\tau = 1 + \phi - \phi' + n$. Figure 6 shows T vs. ϕ for $k = 10$ and $r_0 = 0.98$ for two stimulus magnitudes, $M = 0.94$ and $M = 1.04$. The discontinuities of exactly one period correspond to crossing the boundary of a spiral zone in Fig. 4 as the stimulus timing changes. These discontinuities are not artifacts of conventions for defining the phase angle. Despite the discontinuities in τ, the PTC is continuous and differentiable on the unit circle. Thus the PTCs corresponding to Fig. 6, *A* and *B*, are topologically equivalent to the graphs in Fig. 4 (c1 and c2), respectively.

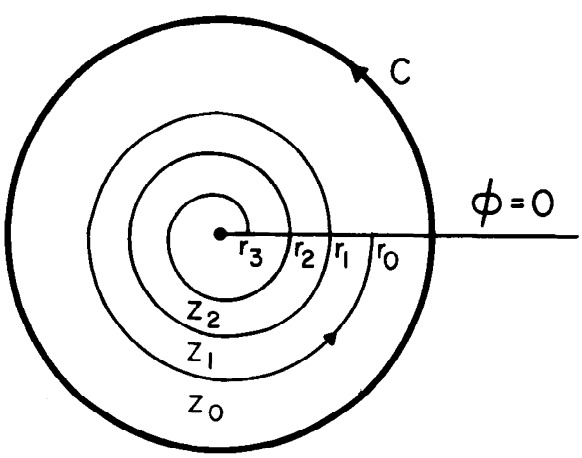

FIG. 5. Phase plane for limit cycle with finite relaxation back to limit cycle and threshold (r_0) that must be exceeded at $\phi = 0$ to initiate event. Point in spiral zone Z_n will cross $\phi = 0$ n times before initiating event. Points r_0, r_1, r_2, ... all lie on common trajectory and are given by *Eq. 5*.

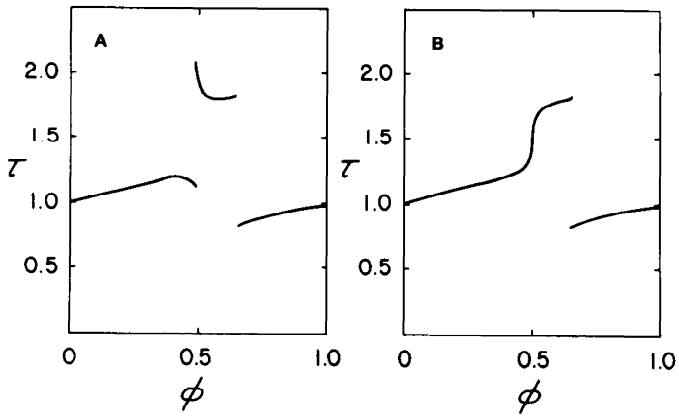

FIG. 6. Plot of τ vs. ϕ for *Eq. 4*, with finite threshold of $r_0 = 0.98$ at $\phi = 0$ needed to initiate event and $k = 10$. Effects of perturbation with magnitude of 0.94 (*A*) and 1.02 (*B*).

B. A model with three stationary states. Recent theoretical studies (10) of phase resetting in ionic models of cardiac cells have shown that some of the qualitative features of the dynamics can be approximated by assuming a two-dimensional limit cycle oscillation surrounding three unstable steady states (Fig. 7*A*). The referee pointed out that the phase plane in Fig. 7*A* also arises in mathematical models of the Belousov-Zhabotinsky reaction (40, Fig. 9A) and of continuous stirred-tank chemical reactors (55, Fig. 6G). The isochrons and phase-resetting behavior of such systems will now be described.

In Fig. 7*A*, there are three stationary points (two unstable nodes and a saddle point). Two singular trajectories (separatrices) lead from the nodes to the saddle point. Since any initial condition on the separatrices does not approach the limit cycle in the limit $t \rightarrow \infty$, the latent phase is not defined on the separatrices. The three steady states and the separatrices constitute a phaseless set, as defined above.

We propose (without proof) the following structure (Fig. 7*B*) for the isochrons for the phase plane shown in Fig. 7*A*.[1] The isochrons all approach every point of the phaseless set arbitrarily closely: they wrap around it an unlimited number of times (16). Latent phase is thus discontinuous across this locus. Any stimulus whose shifted cycle, C′, cuts across this locus produces a PTC discontinuous at the separatrix and is therefore of indeterminate degree.

A coarse sampling of C′ in an experiment with few initial ϕ values might suggest a PTC resembling Fig. 8*A*. In this example, the size of its apparent discontinuity depends on the sampling. In Fig. 8*B* we sketch the result of much finer sampling of τ near the locus where C′

[1] In the basin of attraction of the limit cycle, isochrons are continuous, and they cannot branch or cross. The isochrons in Figs. 3*B* and 7*B* are topologically equivalent (homeomorphic). We conjecture that in the basin of attraction of any 2-dimensional limit cycle, the isochrons will always be homeomorphic to the isochrons shown in Figs. 3*B* and 7*B*.

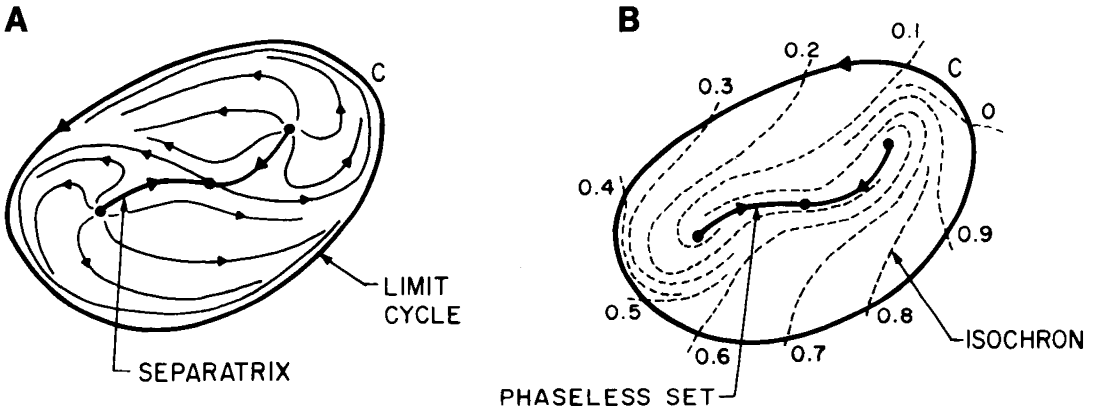

FIG. 7. Schematic representation of model of limit cycle oscillator with 3 steady states (*heavy points*). In terminology of qualitative theory of differential equations, central steady state is called saddle point and 2 other steady states are unstable nodes. Trajectory that leads from node to saddle point is called a separatrix. *A*: trajectories for this model system. *B*: conjectured structure for isochrons for this model. All isochrons continue to wind as tight spirals around phaseless set and approach arbitrarily close to phaseless set [(16); cf. Fig. 3]. Note that although the phase planes are not topologically equivalent, isochrons are.

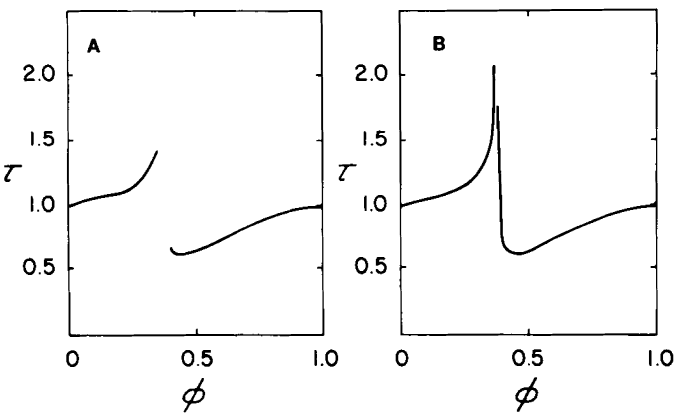

FIG. 8. Schematic plot of perturbed cycle length vs. ϕ for model shown in Fig. 7 for perturbation that leads to shifted cycle that intersects in 1 point phaseless set in Fig. 7*B*. Events are initiated when trajectory crosses isochron $\phi = 0$ in neighborhood of intersection of isochron and C. *A*: comparatively crude determination. *B*: finer determination. As a result of intersection of shifted cycle with phaseless set, perturbed cycle length exponentially increases to infinity, but resolution of this singularity depends sensitively on noise in system and fineness of experimental probing.

crosses the phaseless set. Note that a small change of ϕ can result in passing through many successive cycles of ϕ': τ may approach infinity. In terms of trajectories, this is because trajectories through states near the separatrix go almost to the central steady state and linger there for a long time before finally diverging toward the limit cycle. The PTC corresponding to Fig. 8 can be found by applying *Eq. 3*. The PTC is not continuous because the jumps in τ do not span an integer number of cycles. If the PTC were measured very finely it would show very sensitive dependence to the phase of stimulus presentation in the neighborhood of the discontinuity.

C. Models with nested limit cycles. In some biological systems one can abolish an oscillation by delivering perturbations over some range of stimulus phases and amplitudes (5, 20, 23, 24, 53, 60). A second perturbation will restart the oscillation. In two dimensions such behavior can be modeled by having two nested limit cycles

An equation giving this behavior is (60, p. 156)

$$\frac{d\phi}{dt} = 1 + \epsilon(1 - r)$$
$$\frac{dr}{dt} = k(1 - r)\,(r - \tfrac{1}{2}) \qquad (6)$$

where k and ϵ are constants. Here, there is a stable limit cycle at $r = 1$ and an unstable limit cycle at $r = \frac{1}{2}$. All points with $r < \frac{1}{2}$ approach the origin $r = 0$ in the limit $t \to \infty$. Thus the phaseless set is two dimensional. The PTCs are accordingly neither type 1 nor type 0: they have a finite gap in which phase is not defined for the same range of stimulus sizes. Diagrams of the isochrons and trajectories in this case are given elsewhere (60, p. 156) and will not be reproduced here.

The topological degree of the PTC is defined only if the shifted cycle does not intersect the phaseless set. If the shifted cycle does intersect the phaseless set, the topological degree of the PTC is not defined. Kawato has shown that in the cases in which the PTC is defined, it must be of either degree 1 or degree 0 for two-dimensional limit cycles (32, 33). For the examples in section III, *B* and *C*, a finite range of stimulus strengths will lead to a PTC in which the topological degree is not defined.

IV. DISCUSSION

One of the basic methods for studying biological oscillations is to subject the oscillation to brief stimuli delivered at different phases of the cycle. We have described phase resetting observed for several simple mathematical models of biological oscillations and shown that topological properties of experimentally observable functions do depend sensitively on the class of models proposed. Consequently a recognition of the different predictions of the theoretical models can lead to refinement and reexamination of experiments in physiology.

As an illustration, consider the respiratory oscillator. Proposals have been made that the respiratory rhythm can be generated by limit cycle oscillations (4, 14), but integrate-and-fire models are much more common (4, 6,

44). Experimental predictions from the two classes of model do differ. If the respiratory rhythm is generated by a limit cycle oscillator, then it should be possible to find critical stimuli that will perturb the rhythm to a locus near the phaseless set and lead to an arrest of the respiratory oscillator. From a clinical context, such a stimulus could lead to a prolonged apnea and might even underlie respiratory arrest in infant apnea (and perhaps sudden infant death syndrome). However, such critical stimuli have not been demonstrated in experimental preparations. In experimental respiratory physiology it is not common to plot the perturbed cycle length as a function of phase of a stimulus such as lung inflation. However, since a discontinuous termination of inspiration by lung inflation is postulated by Bradley et al. (6), we anticipate that a plot of perturbed cycle length vs. the phase of lung inflation would have one or more discontinuities for a range of inflation amplitudes if contemporary integrate-and-fire models are correct. Careful studies of the continuity properties are warranted in view of the difference between predictions between the different classes of models.

A second situation in which a knowledge of the topological structure of a biological oscillator is important is in the analysis of the effects of periodic inputs to the oscillator. It has long been recognized that the PTC can be used to predict the effects of periodic stimulation of biological oscillators (17, 19, 41, 48, 49). It has recently been suggested that changes (bifurcations) in dynamics that occur as the stimulation parameters are varied depend on topological, continuity, and differentiability properties of the PTC (15). There are differences between the dynamics predicted from discontinuous PTCs and continuous PTCs with steep slopes. Thus studies using piecewise linear PTCs as models of pacemaker response, such as have been carried out by Segundo and co-workers (35, 39, 49–52) and Ikeda et al. (22), lead to different topological structures than studies using more realistic PTCs.

Although theoretical predictions of the different classes of models do differ, the extent to which experimental studies can resolve the differences remains to be clarified. Some effects, such as temporary respiratory arrest in response to a stimulus of critical amplitude, are dramatic and could be easily observed if present. Other measurable properties, such as the perturbed cycle length and phase-locking structure, show very large changes over very small changes in the stimulus and are difficult to measure experimentally. Moreover a small amount of noise present in the experimental system adds further complication to experiments, making resolution of fine features difficult. A further problem is that for some parameter ranges, continuous limit cycle oscillations may well approximate discontinuous integrate-and-fire models. Such a situation has been postulated for the mitotic oscillation in *Physarum* (54) and in respiratory rhythmogenesis (44).

We have shown that discontinuities in the plot of perturbed cycle length as a function of phase of stimulus can arise for many different reasons in mathematical models. Consider the following:

1) In integrate-and-fire models discontinuities are always expected for all stimulus magnitudes.

2) In limit cycle models an apparent discontinuity may arise as a consequence of regions of phase space in which isochrons are closely packed together. In this case the plots of perturbed cycle length vs. phase will have a large slope that would be difficult to resolve experimentally. Such situations have been found in mathematical models in the Hodgkin-Huxley equations and the Bonhoeffer-van der Pol equations (5, 17, 18, 48). Best (5) has also shown a discontinuity of the sort discussed in section III*C* in the Hodgkin-Huxley equations.

3) If there is type 0 phase resetting, then discontinuity is always expected in the plot of τ vs. ϕ. If the limit cycle is very strongly attracting, then the discontinuity is approximately equal to the unperturbed cycle length.

4) If there are three critical points in the phase space, one expects to find a complex behavior over a finite range of parameter values. As discussed, the discontinuity may be poorly resolved without finely detailed experimental probing.

This discussion shows that the discontinuities in phase-resetting experiments reflect the topological characteristics of the oscillator. To analyze these topological properties it is necessary to collect extensive data concerning the effects of a single stimulus on subsequent events. For ranges of stimulus phase in which the timing of subsequent events is very sensitive to the phase of the stimulus, it is necessary to probe the effects of stimuli over as fine a range as possible.

The model presented in Section III*B* was motivated by computations by J. Clay, M. R. Guevara, and A. Shrier, whom we thank for many helpful discussions. We have also benefitted from a discussion with J. Guckenheimer concerning the topology of isochrons.

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