

Phase-locked rhythms in periodically stimulated heart cell aggregates

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GUEVARA, MICHAEL R., ALVIN SHRIER, AND LEON GLASS. *Phase-locked rhythms in periodically stimulated heart cell aggregates*. *Am. J. Physiol.* 254 (Heart Circ. Physiol. 23): H1-H10, 1988.—We have studied the effect of injecting a periodic train of current pulses into spontaneously beating aggregates of embryonic chick ventricular heart cells. Over a range of stimulation frequencies around the intrinsic frequency of an aggregate we find one action potential for each stimulus with a fixed latency from each stimulus to the subsequent action potential. For a stimulation frequency higher (lower) than the intrinsic frequency, this corresponds to overdrive (underdrive). At high frequencies of stimulation dropped beats occur leading to complex rhythms analogous to various Wenckebach rhythms observed clinically. At higher stimulation frequencies one can obtain a complete suppression of action potential generation. At low frequencies of stimulation, there are rhythms containing escape beats. Almost every rhythm seen bears a striking resemblance to some cardiac arrhythmia. We present a simple classification scheme that predicts the order of appearance of all the classes of rhythms experimentally observed as one changes the stimulation frequency. We propose that this scheme can be used generally to describe the behavior of other biological oscillators.

cardiac arrhythmias; Wenckebach; reverse Wenckebach; alternating Wenckebach; escape rhythm

NORMALLY, THE SINOATRIAL NODE acts as the pacemaker of the heart. As a result of the orderly spread of excitation, there is a relatively fixed interval between the activation times of any two cardiac cells. In a large number of different abnormal situations, this one-to-one synchronization between different regions of the heart is lost. Abnormal activation sequences and loss of one-to-one synchronization can be found in second and third degree atrioventricular heart block and in rhythms such as parasystole in which an ectopic pacemaker competes with the sinus pacemaker.

One approach in studying the complex situations associated with loss of one-to-one synchronization is to subject cardiac tissue to periodic stimulation and to analyze the behavior as stimulation frequency or amplitude is varied. In previous reports, we have described the effects of delivering isolated stimulus pulses as well as periodic trains of stimuli to spontaneously beating aggregates of embryonic chick ventricular heart cells (7-9, 11, 12). These reports, as well as others describing work carried out on intact hearts (3, 18, 19, 22, 26, 28) and in vitro preparations (15, 16, 27, 31, 32), have not compre-

hensively characterized the wide range of complex rhythms generated in response to periodic stimulation as frequency and amplitude of stimulation are varied. In this paper we provide an extensive description of these rhythms, as well as a scheme that predicts the exact order in which they are encountered as stimulation frequency is changed. This scheme is derived from a theory that predicts the existence of these rhythms from the phase-resetting behavior produced by the delivery of isolated stimulus pulses (7-11). Many of the patterns described below resemble rhythms seen in the intact heart during various cardiac arrhythmias. Experimental tracings additional to those shown here and a summary of analogous rhythms in the intact heart can be found in Ref. 9. We propose that the patterns we observe and the order in which they occur will be found in other biological oscillators.

METHODS

Aggregates of cells from the ventricles of 7-day-old embryonic chicks were prepared (12) following techniques similar to those employed by DeHaan and Fozzard (4). These aggregates beat spontaneously at an external potassium concentration of 1.3 mM, the value used here. The transmembrane potential of one cell in an aggregate was recorded using a microelectrode, and a periodic train of current pulses was injected through the same microelectrode. In some cases two microelectrodes were impaled into different cells in a given aggregate, one microelectrode to inject current and the other to record transmembrane potential. The electrical activity in two widely separated cells (100-200 μm) in an aggregate is virtually synchronous, both in spontaneously beating preparations (4, 12) and after delivery of a single stimulus (12). Since it is very difficult to maintain impalement of two microelectrodes in a vigorously contracting aggregate, the majority of our experiments were carried out with single electrode impalements. Currents were measured to the nearest 0.5 nA and are indicated in the figure captions.

Voltage and injected current waveforms were monitored on a digital oscilloscope (Nicolet model 206) and recorded on an FM instrumentation recorder at a tape speed of 3.75 in./s (Hewlett-Packard model 3964A; 3-dB frequency response at 3.75 in./s: DC-1,250 Hz) for later off-line analysis. An electronic stimulator (Frederick Haer, Pulsar 6i) with a 1- μs internal clock cycle time delivered a periodic train of 20-ms duration current pulses. Between periodic trains, stimulation was stopped,

stimulation parameters were changed, and enough time was allowed for the preparation to recover from the effects of stimulation. This time depends in a complex way on the stimulation frequency and amplitude, as well as on the total duration of stimulation. Since an aggregate will almost invariably recover its control rate of beating within a few seconds after the cessation of stimulation, we generally allowed 10–30 s between successive runs as a recovery period. The stimulator was then manually switched on at some random point in the spontaneous activity cycle of the aggregate. The protocol followed was generally as follows. At a fixed amplitude and duration of the current pulse, the time between stimuli (t_s) was incremented or decremented in steps of 50 ms or 100 ms and the coupling pattern set up between the electronic stimulator and the aggregate at each value of t_s noted. Then t_s was changed in steps of 10 ms to inspect ranges of t_s more finely. The above protocol would then be repeated at new current pulse amplitudes.

All of the experimental voltage traces in this report were obtained by playing back the tape-recorded signal to the digital oscilloscope through a low-pass filter (to avoid aliasing). The contents of the oscilloscope memory were then reconverted to an analogue signal and sent to an analogue X–Y plotter (Hewlett-Packard, model 7015B).

Each rhythm described below was seen in several aggregates; each voltage tracing or description is intended to be typical of the rhythm observed. A total of 23 aggregates were studied, with mean diameters (arithmetic mean of two diameters measured at right angles in the horizontal plane using a calibrated optical graticule placed in the microscope eyepiece) ranging from 90 to 250 μm . Of these aggregates, 18 were subjected to depolarizing stimulation and 5 to hyperpolarizing stimulation. In the results below, we concentrate on the response to depolarizing stimulation. Frequently, but not in all cases, the phase-resetting response of the aggregate (12) was also studied.

RESULTS

Principal phase-locking rhythms. Over a broad range of stimulation frequency and amplitude the electrical activity of the aggregate can be entrained in a 1:1 fashion by the stimulator. As the stimulation frequency or amplitude is changed, other periodic and nonperiodic rhythms are observed. The periodic rhythms consist of cycles containing N stimuli and M action potentials that repeat with a period of Nt_s . Such a rhythm is called an $N:M$ phase-locking rhythm, and the fraction N/M is called the phase-locking ratio. For t_s smaller than the intrinsic cycle length, there are $N:M$ rhythms with $N/M \geq 1$. As t_s is decreased, the ratio N/M increases in a monotonic fashion, leading to rhythms in which there are “skipped” or “dropped” beats. For t_s larger than the intrinsic cycle length, there can be “escape” beats, i.e., action potentials not immediately preceded by a stimulus pulse. In that case, $N/M (<1)$ decreases in a monotonic fashion as t_s increases. In the following sections we provide experimental evidence for the existence of many $N:M$ phase-locking rhythms, emphasizing dropped-beat

rhythms.

1:1 rhythm. The electrical activity in any spontaneously beating aggregate can be made to lock onto the electrical activity of the electronic stimulator in a 1:1 fashion if the frequency of stimulation is not too much higher or lower than the intrinsic frequency of the aggregate. In 1:1 phase locking, there is one action potential associated with each stimulus, with a fixed latency from the beginning of each stimulus to the following action potential. Overdrive occurs when the stimulation frequency is higher than the intrinsic frequency, and underdrive occurs at stimulation frequencies lower than the intrinsic frequency. During overdrive, the stimulus falls during the period of diastolic depolarization (Fig. 1A), whereas during underdrive, it falls during the period of repolarization of the action potential (Fig. 1B).

During overdrive, before 1:1 phase locking becomes firmly established, a stabilization process occurs progressively over many cycles, accompanied by a gradual decrease in the slope of diastolic depolarization (Fig. 1A, *top trace*). When stimulation is stopped, “overdrive suppression” (23, 29) is seen; the intrinsic frequency of the aggregate is transiently reduced (Fig. 1A, *bottom trace*). In contrast, Fig. 1B (*bottom trace*) shows that once stimulation is stopped after a period of underdrive, the aggregate is found to be beating more quickly than before stimulation was started. This phenomenon of

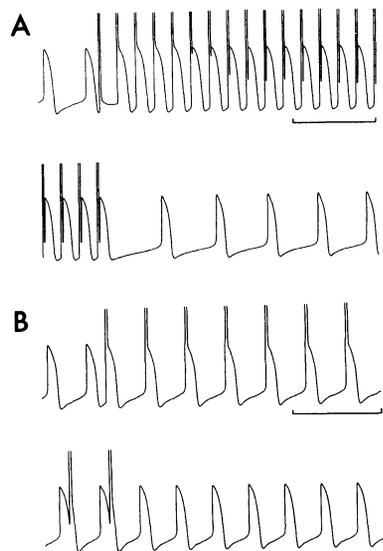


FIG. 1. Development of 1:1 phase locking. A: overdrive ($t_s = 220$ ms). *Top trace*: beginning of stimulation at a rate higher than spontaneous beat rate. Off-scale vertical deflections are stimulus artifacts that can be taken as markers of time during which current is injected. Due to the rather large sampling interval of digital oscilloscope (~ 1 ms), the upstroke phase of the action potential appears slower than in reality (see Fig. 1A of Ref. 12). *Bottom trace*: end of stimulation. Latency from stimulus to following action potential has achieved its steady-state value. There were 63 stimuli delivered in this stimulation run. Cessation of stimulation results in marked overdrive suppression; interbeat interval gradually recovers back to its control value (*top trace*). In this and all subsequent figures, unless otherwise stated, vertical calibration bar indicates range from -50 to 0 mV; horizontal calibration bar indicates 1 s. Pulse amplitude = 8 nA (aggregate 1: diameter = 114 μm). B: underdrive ($t_s = 450$ ms). *Top trace*: beginning of stimulation at a rate lower than spontaneous beat rate. *Bottom trace*: end of stimulation. Cessation of stimulation results in underdrive acceleration. Pulse amplitude = 40 nA (aggregate 1: diameter = 114 μm).

“underdrive acceleration” (31) is thus the reverse of the phenomenon of “overdrive suppression” shown in Fig. 1A. Sufficient time (generally 10–30 s) must thus be allocated between successive stimulation runs to allow recovery of the beat rate back to the control rate. The range of t_s over which 1:1 phase locking can be found increases with increasing stimulus amplitude. However, the response is asymmetric, in that one can obtain a greater degree of overdrive than of underdrive at a given pulse amplitude.

Rhythms between 1:1 and 3:2 zones. As stimulation frequency is increased, 1:1 phase locking is lost and rhythms are seen in which there is an increasing latency from stimulus to subsequent action potential culminating in a single “skipped” or “dropped” action potential (Fig. 2, B–D). Each cycle thus consists of $n + 1$ stimuli and n action potentials. These rhythms are analogous to the familiar Wenckebach rhythms observed in the intact heart (3, 18, 25, 26). In addition to the common rhythms shown in Fig. 2, rarer varieties have been occasionally observed. For example, at a value of t_s lying between values that produce 4:3 and 3:2 rhythms, a 7:5 rhythm

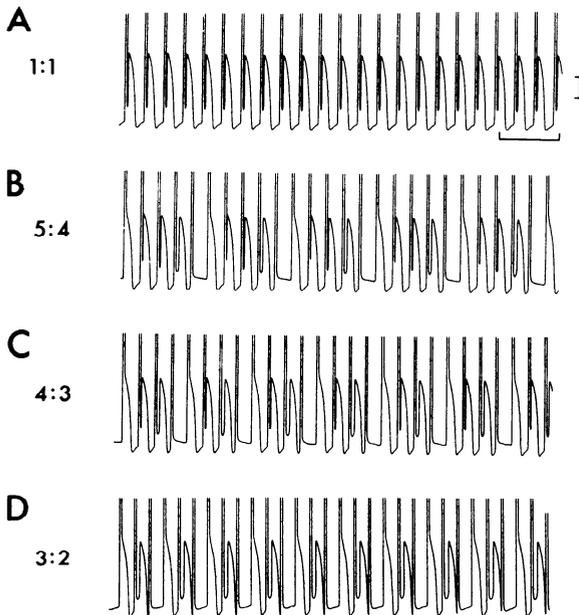
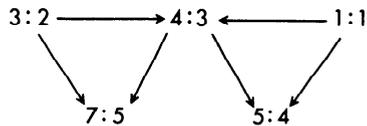


FIG. 2. Rhythms intermediate to 1:1 and 3:2 phase locking. A: 1:1 phase-locking pattern ($t_s = 360$ ms). B: 5:4 phase locking ($t_s = 310$ ms). C: 4:3 phase locking ($t_s = 300$ ms). D: 3:2 phase locking ($t_s = 270$ ms). Scheme at top and in subsequent figures represents sequence of patterns encountered as t_s is changed. All rhythms in A–D above were obtained by stimulating for a time long enough to allow transients to pass and a steady-state rhythm to be established. Current pulse amplitude remained constant. Within each $n + 1:n$ cycle in B–D, there is an increasing latency from stimulus to subsequent action potential, until a “skipped” or “dropped” beat occurs. Rhythms in B–D are analogous to Wenckebach rhythms. Spontaneous interbeat interval of this aggregate was ~ 490 ms. Pulse amplitude = 9 nA (aggregate 14: diameter = 181 μm). See Fig. 1 for calibration bars definitions.

consisting of an alternation of 4:3 and 3:2 cycles can be observed.

Figure 3 shows recordings from microelectrodes impaled within two cells in an aggregate, separated by a distance of $\sim 130 \mu\text{m}$. Note that during the 3:2 rhythm, the action potentials recorded in the two cells are virtually synchronous, with, for example, upstrokes occurring within about 60 μs of each other.

Rhythms between 2:1 and 3:2 zones. At values of t_s intermediate to those giving 3:2 and 2:1 phase locking, a 5:3 rhythm is observed in which 3:2 and 2:1 cycles alternate. This 5:3 rhythm may also be regarded as consisting of two 2:1 cycles terminated by one 1:1 cycle (Fig. 4C). During each 5:3 sequence the latency from the stimulus to the action potential for the first 2:1 cycle is greater than that for the second 2:1 cycle. This is evident in Fig. 4C starting with the first 2:1 cycle of this record. The 5:3 rhythm is the first member of a family of rhythms of the form $2n - 1:n$ ($n \geq 3$), which lie at values of t_s between those producing 5:3 and 2:1 rhythms. The next periodic $2n - 1:n$ rhythm encountered as t_s is decreased is a 7:4 rhythm (Fig. 4D). Since the latencies from stimulus to action potential of the 2:1 cycles decrease, such patterns bear a striking similarity to reverse Wenckebach rhythms (1, 26).

At a value of t_s slightly greater than the value at which the 5:3 rhythm is found, behaviors such as that shown in Fig. 4B occur. This 8:5 phase-locking rhythm can be thought of as a sequence of two consecutive 3:2 cycles followed by one 2:1 cycle. In this rhythm, there is a gradual increase in the latencies during the 3:2 cycles until a 2:1 cycle results. Other related rhythms follow the formula $3n + 2:2n + 1$ ($n \geq 3$). In the aggregate, these rhythms are much more rarely seen than the reverse Wenckebach rhythms when t_s is changed in 10-ms steps. The rather limited range of stimulation parameters over which these two classes of rhythms are seen in the aggregate might be connected with the finding that these rhythms have been only rarely described in the intact heart (3).

Rhythms between 2:1 and 3:1 zones. Decrease in t_s to just below the value of t_s at which 2:1 phase locking occurs gives rise to rhythms in which alternate action potentials are dropped with a monotonically increasing latency until there is a cycle in which an additional beat is dropped. The pattern can be periodic, producing $2n + 1:n$ ($n \geq 3$) phase-locking patterns (e.g., Fig. 5B shows a 7:3 rhythm). These rhythms are seen at values of t_s between those at which the periodic 2:1 and 5:2 phase-

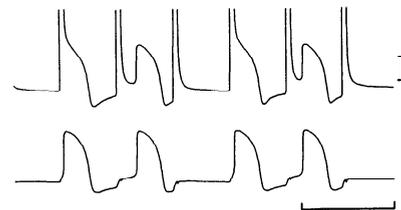


FIG. 3. Two-microelectrode recording of 3:2 phase-locking rhythm ($t_s = 300$ ms). Top trace is recording from electrode used also as stimulation electrode. Bottom trace is from a site $\sim 130 \mu\text{m}$ away. Note close similarity of both voltage tracings. Pulse amplitude = 23 nA (aggregate 22: diameter = 171 μm). Horizontal calibration bar indicates 500 ms. See Fig. 1 for vertical calibration bar definition.

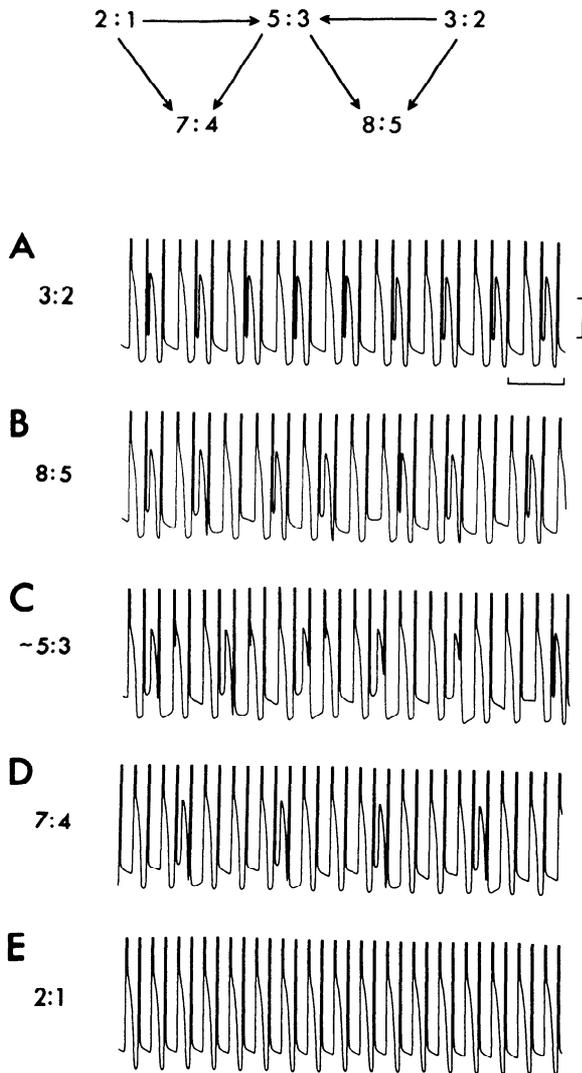


FIG. 4. Rhythms intermediate to 3:2 and 2:1 phase locking. A: 3:2 phase locking ($t_s = 300$ ms). B: 8:5 phase locking ($t_s = 290$ ms). Latency associated with last conducted beat of each 3:2 cycle increases until a 2:1 cycle results. C: aperiodic rhythm very close to 5:3 phase locking ($t_s = 270$ ms). A 5:3 rhythm would be seen were t_s to be increased by a few milliseconds. D: 7:4 phase locking ($t_s = 260$ ms). This is analogous to a reverse Wenckebach rhythm. Three 2:1 cycles are followed by a single 1:1 cycle. E: 2:1 phase locking ($t_s = 240$ ms). Pulse amplitude = 11 nA (aggregate 2: diameter = 105 μ m). See Figs. 1 and 2 for additional information.

locking patterns are found. Analogous rhythms observed clinically are called alternating Wenckebach rhythms (17, 20).

Between the values of t_s at which the periodic 5:2 and 3:1 rhythms are seen, one can find rhythms consisting of several 3:1 cycles periodically interrupted by a single 2:1 cycle such as a 8:3 rhythm (9). These rhythms can be periodic, producing $3n - 1:n$ ($n \geq 3$) phase-locking rhythms. These patterns are similar to the 7:4 rhythm described above and the reverse Wenckebach rhythm in that there are isolated cycles that have one less dropped beat than the immediately preceding and following cycles. However, we are not aware of any previous clinical or experimental reports of this class of rhythms in the cardiac literature. This may not be surprising, since these rhythms are seen much less frequently in the aggregate

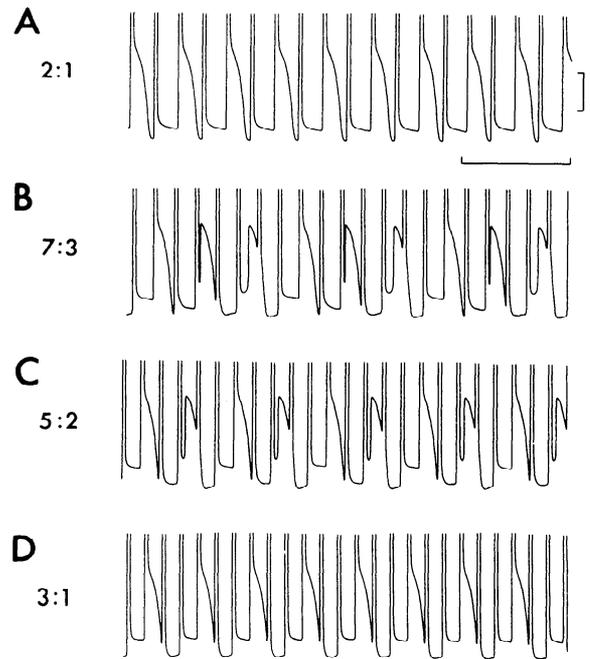
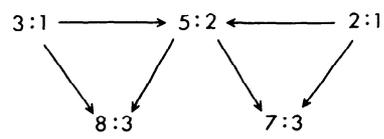


FIG. 5. Rhythms intermediate to 2:1 and 3:1 phase locking. A: 2:1 phase locking ($t_s = 220$ ms). B: 7:3 phase locking ($t_s = 190$ ms). This is analogous to an alternating Wenckebach rhythm. Two 2:1 cycles are followed by one 3:1 cycle. C: 5:2 phase locking ($t_s = 170$ ms). There is alternation of 2:1 and 3:1 cycles. D: 3:1 phase locking ($t_s = 160$ ms). Pulse amplitude = 11 nA (aggregate 2: diameter = 105 μ m). See Figs. 1 and 2 for additional information.

than the alternating Wenckebach rhythm when t_s is changed in steps of 10 ms.

n:1 ($n \geq 3$) rhythms. Decreasing t_s to a value below that which results in 3:1 phase locking produces rhythms analogous to high-grade block. Since it is rather difficult to visualize what is happening during these $n:1$ rhythms due to the presence of stimulus artifact we show a 5:1 rhythm (Fig. 6) from a two-microelectrode experiment. This figure shows transmembrane potential recorded from one microelectrode while current is injected via a second microelectrode impaled in the same aggregate. The average rate of action potential generation does not change appreciably during the various $n:1$ rhythms seen as t_s is decreased. As the pulse amplitude increases the order n of the highest order $n:1$ pattern observed during periodic stimulation decreases. In fact, for a sufficiently high pulse amplitude, $n:1$ rhythms with n greater than about 4 are not seen, even at the highest possible frequency of stimulation. Instead, with high-frequency, high-amplitude stimulation, one finds $n:0$ rhythms as described below.

More complex periodic rhythms occur at values of t_s between those at which $n:1$ and $n + 1:1$ rhythms are seen. For example, a 7:2 rhythm consisting of alternating 3:1 and 4:1 cycles is often seen between 3:1 and 4:1

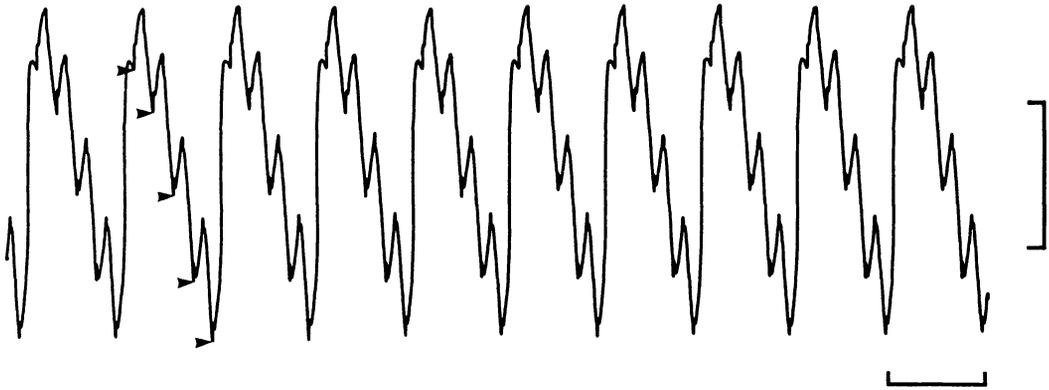


FIG. 6. 5:1 phase locking ($t_s = 40$ ms). \blacktriangleright , Times of onset of five consecutive stimuli. This recording is taken from a two-microelectrode experiment. Horizontal calibration bar: 200 ms. Pulse amplitude = 30 nA (aggregate 23; diameter = 171 μ m). See Fig. 1 for vertical calibration bar definition.

rhythms. At a fixed stimulus amplitude, the range of t_s over which a particular $n:1$ ($n \geq 1$) rhythm is maintained diminishes with increasing n . In fact, for n greater than about 10, the rhythms observed tend to be nonperiodic, with an apparently random skipping back and forth between adjacent $n:1$ and $n + 1:n$ cycles.

1:0 rhythm. For a current pulse of sufficiently high amplitude, 1:0 phase locking can be seen when the stimulation frequency is also sufficiently high. Once again we show the result from a two-microelectrode experiment to better visualize what is happening during a 1:0 rhythm (Fig. 7). As is generally the case with periodic stimulation, a transient is seen before the steady-state pattern is set up. During 1:0 phase locking, the current pulse depolarizes the membrane, which then repolarizes back to the same voltage just before the onset of each pulse. Thus the membrane potential repeatedly traverses and retraverses the same small range of potentials on the repolarizing limb of the action potential. The activity is periodic in time with period t_s .

During 1:0 phase locking, no visible sign of periodic contractile activity is discernible in the aggregate. The suppression of action potential generation during 1:0 phase locking cannot be attributed to injection of a constant current, since the current declines back to zero between pulses. However, in all cases of 1:0 phase-locking, the current amplitude was sufficiently high so that injection of a constant current of that magnitude also produced an apparent standstill of periodic mechanical activity. Resumption of spontaneous cyclic mechanical and electrical activity after a period of 1:0 phase locking

is immediate; indeed, the rate of action potential generation in the recovery period immediately after the release of stimulation is greater than in control.

Note that 1:0 phase locking can only be obtained at a rather high-current amplitude (≥ 20 nA). This is not the case for the other patterns described in this paper, which can all be seen at lower current amplitudes.

Escape-beat rhythms. As t_s is increased to a value beyond that at which 1:1 phase locking can be maintained, escape rhythms are seen in which there are spontaneously generated escape beats not immediately preceded by a stimulus (Fig. 8). Cycles of the form $n:n + 1$ result in which for every n stimuli there are $n + 1$ action potentials. The most commonly seen mode of periodic escape behavior is $n:n + 1$ phase locking. For example, 3:4 (Fig. 8B), 2:3 (Fig. 8C), and 1:2 (Fig. 8E) phase-locking rhythms are easily seen. More complex $N:M$ phase-locking rhythms with $M \neq N + 1$ can also be observed. For example, alternation of a 2:3 with a 1:2 rhythm produces a 3:5 rhythm (Fig. 8D).

During the course of any of the 3:4 cycles shown in Fig. 8B, there is a progressive decrease in the latency from stimulus to subsequent action potential that precedes the occurrence of the escape beat. This is the opposite of the behavior seen in the $n + 1:n$ rhythms that occur when 1:1 phase locking is lost in response to high-frequency stimulation (Figs. 2 and 3). On the other hand, one can also see rhythms in which there is a progressive increase in the latencies associated with the nonescape beats (9). In summary, escape rhythms are in many ways the low-frequency counterpart of the various

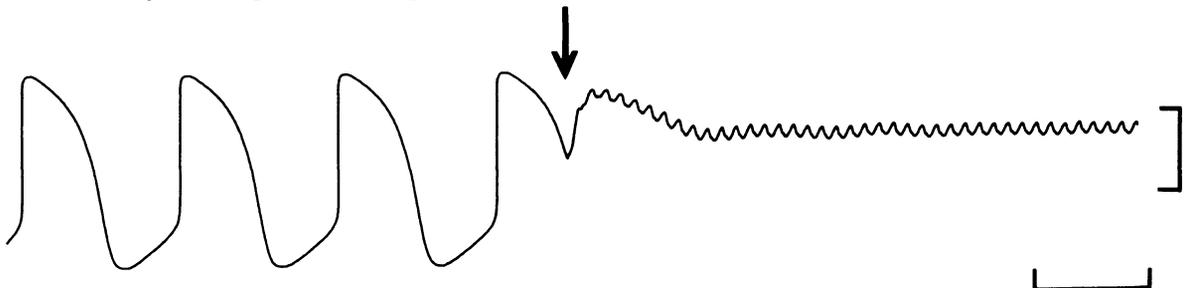


FIG. 7. 1:0 phase locking. \downarrow , Start of stimulation ($t_s = 25$ ms). In steady state, 1:0 phase locking is seen. This voltage waveform was recorded using a second microelectrode; it thus shows true membrane potential, free from stimulus artifact. Horizontal calibration bar: 200 ms. Pulse amplitude = 30 nA (aggregate 23; diameter = 171 μ m). See Fig. 1 for vertical calibration bar definition.

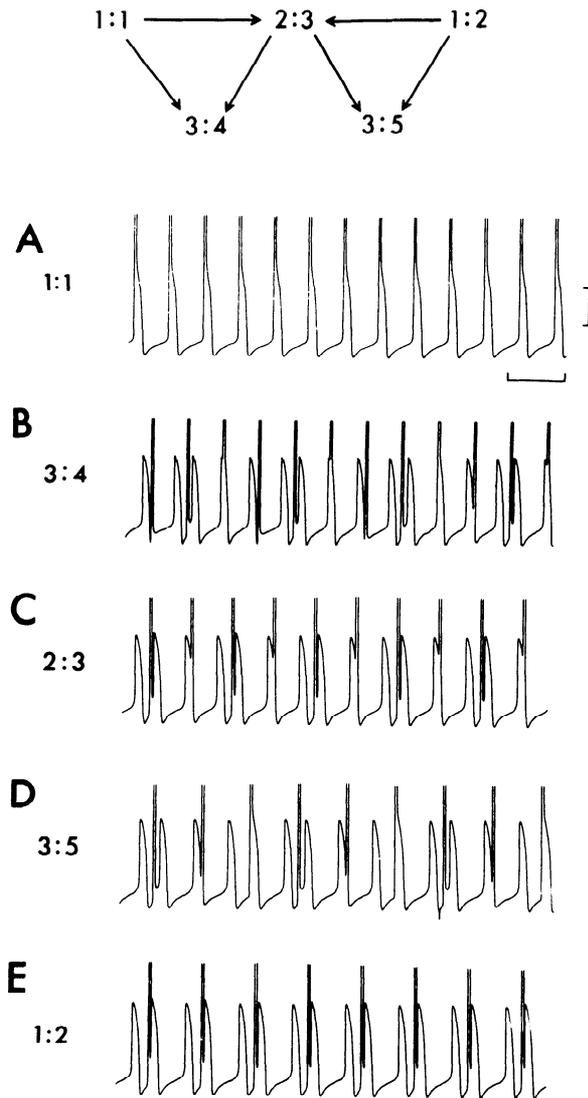


FIG. 8. Escape-beat rhythms. *A*: 1:1 phase locking ($t_s = 550$ ms). *B*: 3:4 phase locking ($t_s = 660$ ms). *C*: 2:3 phase locking ($t_s = 720$ ms). *D*: 3:5 phase locking ($t_s = 840$ ms). This rhythm can be regarded as an even mixture of 2:3 and 1:2 cycles. *E*: 1:2 phase locking ($t_s = 920$ ms). Pulse amplitude = 9 nA (aggregate 14: diameter = 181 μ m). See Figs. 1 and 2 for additional information.

dropped beat rhythms that occur at high stimulation frequencies (Figs. 2–6); instead of dropped beats, there are extra beats.

Nonperiodic rhythms. In the above, with one exception (Fig. 4C), we have shown periodic rhythms. However, in our experiments, rhythms are often observed in which there is no obvious low-order periodicity. These rhythms are observed at values of t_s intermediate to those at which phase-locked rhythms are found; an increase or decrease in t_s often results in a transition from aperiodicity to periodicity. In some situations the rhythms appear to be “close” to a low-order phase-locked rhythm. One such example was already shown in Fig. 4C. The behavior shown in Fig. 9A, in which there is an occasional dropped beat, appears to be analogous to “millisecond” Wenckebach (5). In contrast, in Fig. 9B there is an occasional escape beat. Similar phenomena are observed with stimulation parameters near those which give rise

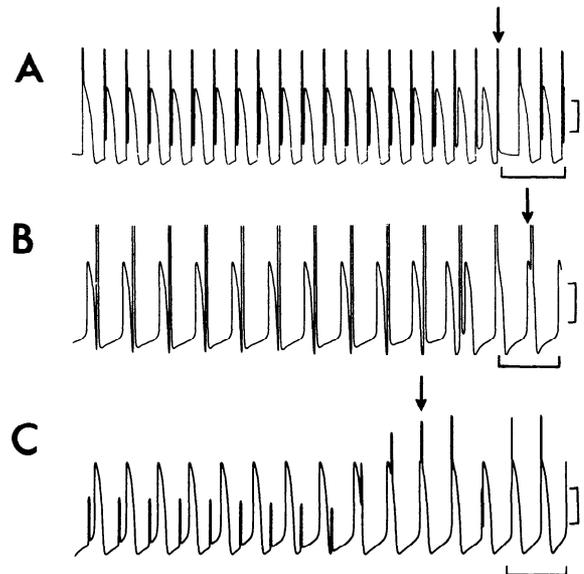


FIG. 9. Nonperiodic rhythms. *A*: “millisecond Wenckebach” rhythm close to 1:1 phase locking ($t_s = 360$ ms). First action potential shown is first beat of a 20:19 cycle. Beat to beat increment in latency from stimulus to following action potential was ≤ 5 ms for all but the last three beats of the cycle. \downarrow , Blocked stimulus. Pulse amplitude = 5 nA (aggregate 11: diameter = 132 μ m). *B*: rhythm close to 1:1 phase locking ($t_s = 600$ ms). Note progressive gradual increase in phase in cycle at which stimulus falls preceding the occurrence of the escape beat (\downarrow). Pulse amplitude = 9 nA (aggregate 14: diameter = 181 μ m). *C*: nonperiodic rhythm corresponding to “quasiperiodic” dynamics ($t_s = 500$ ms). Long stretches containing 1:1 cycles are occasionally interrupted by a single 2:1 cycle. \downarrow , Blocked stimulus of a 2:1 cycle. Pulse amplitude = 2 nA (aggregate 5: diameter = 95 μ m). See Fig. 1 for calibration bars definitions.

to the other phase-locked rhythms shown in this paper. In each case there is an occasional “extra” dropped or escape beat. At very low stimulus amplitudes, the current pulses have little effect on the spontaneous activity. In these situations there is a nonperiodic rhythm in which there is a slowly progressing shift of the phase in the cycle at which the stimulus falls (Fig. 9C).

Response to hyperpolarizing periodic stimulation. We have described above the response of the aggregate to a periodic train of depolarizing current pulses. However, many of the rhythms described above have also been seen in response to hyperpolarizing stimulation. For example, one can readily see 1:1 (Fig. 10A) and $n + 1:n$ (Fig. 10B) rhythms. Note that the aggregate in Fig. 10A is overdriven, but that this is in response to a hyperpolarizing input. Hyperpolarizing pulses are also able to produce $n:1$ and 1:0 rhythms (9). However, a much smaller current amplitude is needed to produce 1:0 phase locking with a hyperpolarizing input than with a depolarizing input, and the membrane potential remains in the pacemaker and not the plateau range of potentials (9).

DISCUSSION

Phase-locking rhythms. In the preceding we have described a rich diversity of rhythms set up between the injected current pulse train and the action potentials in spontaneously beating aggregates of embryonic chick ventricular cells. In our work, the response to periodic

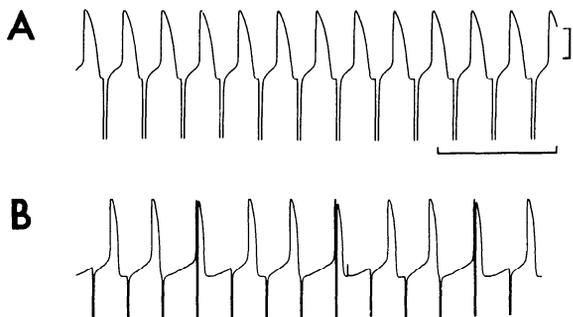


FIG. 10. Rhythms seen in response to periodic hyperpolarizing stimulation. A: 1:1 phase locking ($t_s = 330$ ms). Aggregate is being overdriven by a hyperpolarizing pulse train, since its intrinsic interbeat interval is ~ 345 ms. Pulse amplitude = 14 nA (aggregate 16: diameter = 223 μm). B: 4:3 phase locking ($t_s = 590$ ms). Note increase in latency from stimulus to subsequent action potential as cycle progresses. Horizontal calibration bar: 1s (A), 2s (B). Pulse amplitude = 36 nA (aggregate 16: diameter = 223 μm). See Fig. 1 for calibration bars definitions.

stimulation is a function of two parameters, the period of the stimulation (t_s) and the amplitude of the stimulus pulse. The existence of these rhythms and the order in which they appear as t_s changes can be predicted by the simple construction depicted in Fig. 11. Starting from seed values 1/0, 1/1, and 1/2 (top line of Fig. 11) we generate a new line of phase-locking ratios by adding the numerators and denominators of adjacent entries in the top line. Thus the second line in Fig. 11 is 1/0, 2/1 $[(1 + 1)/(0 + 1)]$, 1/1, 2/3 $[(1 + 1)/(1 + 2)]$, 1/2. The ratios in the third line are generated in a similar way from those in the second line, and so on. The resulting sequences, read from left to right, correspond to the phase-locking rhythms predicted to exist as t_s increases. All of the $N:M$ rhythms shown in Fig. 11 with the exception of the five in the lowest line with $M \geq 10$ have been seen experimentally, in the indicated order.

It is remarkable that a simple numerical construction can be used to predict the sequence in which complex dynamical patterns appear in this study. This construction can be generated from theoretical work (7–10, 15), which is based on consideration of the effect on the beat rate of the aggregate produced by injecting a single isolated stimulus pulse of sufficiently low amplitude at various phases of its spontaneous cycle. Once this “phase-resetting” effect of an isolated stimulus has been characterized, the mathematical technique of iteration can then be applied to predict the rhythms expected during periodic stimulation. The major physiological assumption underlying application of this technique is that a stimulus delivered as part of a pulse train has the same effect as if only that stimulus were to be delivered.

Figure 12 shows the predictions of the theory. Since the response of an aggregate to delivery of a single pulse of a given amplitude varies widely from aggregate to aggregate, depending on its size, beat rate, and other as yet undetermined factors (7, 9, 12), the computations carried out in Fig. 12 assume a typical evolution in the phase-resetting response as stimulus amplitude is increased. This typical response was obtained by examining the phase-resetting response of many aggregates (see Ref. 7 for further details). The range of the stimulus amplitude parameter, A , in Fig. 12 is thus different from

aggregate to aggregate; however, the maximum value in Fig. 12 corresponds to a pulse amplitude of 5–10 nA. We have previously shown that there is excellent agreement between theory and experiment in any given aggregate (see e.g., Fig. 3 of Ref. 11).

An $N:M$ phase-locking rhythm will be found for any combination (τ, A in Fig. 12) of stimulus parameters such that the point (τ, A) lies in the interior of the corresponding tongue-shaped $N:M$ phase-locking zone of Fig. 12 (τ is normalized stimulation period, and A is stimulus amplitude parameter.) For any oscillator, provided that the stimulus amplitude is sufficiently low, there is the general theoretical result that, as t_s (or equivalently, τ) is increased, all possible $N:M$ phase-locking zones will be encountered so long as N and M are relatively prime.¹ In addition, as t_s is increased, the ratio N/M decreases monotonically from infinity to zero, attaining any rational number over some range of t_s . The range of t_s over which a particular $N:M$ rhythm will be seen at a given amplitude depends on both the values N and M . For example, when t_s is changed in steps of 10 ms, we can always find in our experiments values of t_s at which the major zones of Fig. 12 are encountered. Other more complex rhythms described above (e.g., 7:3, 8:5, 7:4) are associated with zones covering smaller areas in the (τ, A) parameter space (Fig. 12); these rhythms are not always experimentally seen. Theoretical analysis indicates that such rhythms should be seen in all aggregates if the increment in t_s is sufficiently small. However, the limited time of impalement in a given aggregate precludes the analysis of the parameter space at such fine increments.

In addition, in the case of high-frequency stimulation, the same organization in Fig. 11 arises for the case of periodic stimulation of spatially distributed systems (such as the atrioventricular node), in which conduction velocity of a premature beat decreases with increasing prematurity of stimulation (3, 8, 18). Thus the organization shown in Fig. 11 is expected to be broadly applicable to the description of the dynamics of periodically stimulated cardiac tissue and, indeed, a wide variety of excitable cells, not simply the spontaneously active, effectively isopotential model system we have been studying. In the remainder of this manuscript we discuss the various rhythms observed experimentally and show that a unified perspective to the etiology of diverse arrhythmias is indicated.

1:1 phase-locking zone: overdrive and underdrive. A wide variety of cardiac tissues can be underdriven by a periodic train of depolarizing stimuli (7, 9, 11, 16, 22, 25, 27, 31). This apparently paradoxical result occurs when the current pulse falls early in the cycle (Fig. 1B, *bottom trace*), during which a delay in the timing of the onset of the next spontaneously generated action potential is produced (10, 12, 15, 16, 31, 32). In similar fashion, overdrive of a pacemaker using hyperpolarizing stimu-

¹ The technical definition of sufficiently low is that the plot of new phase vs. old phase (the phase transition curve) is a monotonic type 1 curve. The iterative technique assumes that following injection of any stimulus there is a rapid relaxation of the trajectory back to the limit cycle (7, 11, 12). The $N:M$ rhythms expected are with $N, M \geq 1$; in addition, a 1:0 rhythm is predicted to exist.

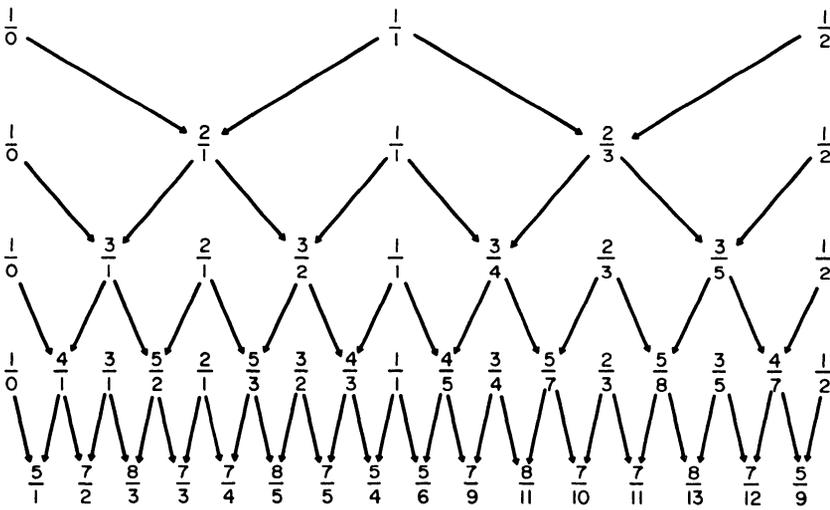


FIG. 11. Scheme for generating phase-locking ratios. See text for further description.

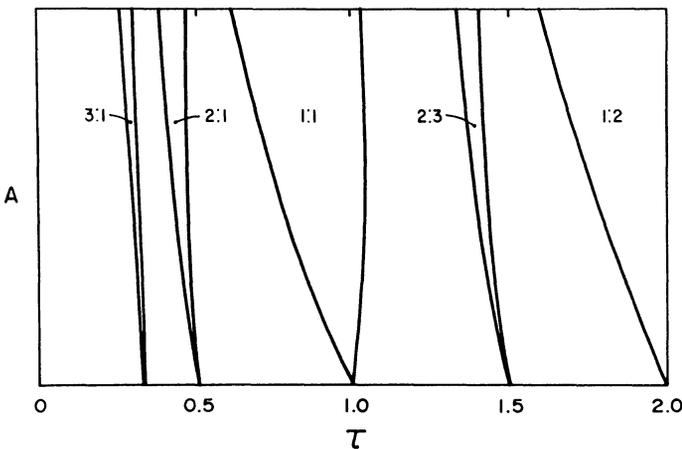


FIG. 12. Principal phase-locking zones. Theoretically computed zones as a function of normalized stimulation period, τ , and stimulus amplitude parameter, A . Parameter τ is given by t_s/\bar{T} , where \bar{T} is the intrinsic period of aggregate. Parameter A is discussed further in text.

lation (Fig. 10A) is possible (14, 15, 19).

When the sinoatrial rate is sufficiently high and there is patent conduction of the cardiac impulse to all regions of the heart, the activity of subsidiary pacemakers is not manifest. It is commonly thought that this silence occurs because subsidiary pacemakers are overdrive suppressed to the point where they are no longer spontaneously oscillating (29). However, our experiments suggest that 1:1 phase-locking at a stimulation rate faster than the intrinsic rate of the subsidiary pacemaker is an alternative mechanism by which activity in a subsidiary focus could be concealed.

At a given value of the pulse amplitude, the aggregate can be overdriven to a greater degree than it can be underdriven. This observation fits in well with the fact that subsidiary ventricular pacemakers have an intrinsic rate slower than that of supraventricular pacemakers, and that they are therefore normally overdriven by the supraventricular input. The range of t_s over which a 1:1 rhythm can be found increases as the stimulus amplitude increases; similar findings have been previously described in both spontaneously active and quiescent cardiac tissues (8, 9, 22, 27, 28).

After overdrive, there is a transient decrease in the

intrinsic frequency of the aggregate, "overdrive suppression" (Fig. 1A); after underdrive, underdrive acceleration is seen (Fig. 1B). Both overdrive suppression and underdrive acceleration serve to make narrower the range of t_s over which a 1:1 rhythm occurs than it would be in their absence (11, 31).

Complex phase-locking rhythms. Between the 1:1 and 3:2 zones, rhythms similar to the familiar Wenckebach rhythms are observed. Although Wenckebach rhythms typically occur in the atrioventricular node, they have also been described in virtually all other regions of the heart, either spontaneously active or quiescent (9, 24). Note that the mechanism most commonly invoked to explain Wenckebach rhythm, decremental conduction, is not operative in the aggregate; the Wenckebach patterns we observe arise simply from the electrophysiological properties of the cell membrane (Fig. 3). The Wenckebach patterns seen in the aggregate are "atypical," since there are approximately equal increments in the latency from stimulus to following action potential, but with the largest increments occurring toward the beginning or end of the cycle (e.g., Fig. 9A). However, the so-called atypical Wenckebach rhythms of atrioventricular block are more common clinically than the "typical" variety (18, 24).

Between the 3:2 and 5:3 zones one finds rhythms such as the 8:5 rhythm shown in Fig. 4B, which have been predicted from theoretical arguments (3), but which have not been previously observed clinically or experimentally. The observation of this rhythm and others rarely noted (7:3, 8:3, 3:5) is a striking confirmation of the general approach and relevance of the theoretical construction in Fig. 11.

Between the 5:3 and 2:1 zones there is a class of rhythms analogous to reverse Wenckebach rhythms previously observed in the atrioventricular node (1, 13, 26) and in several other cardiac tissues (see Ref. 9 for references). The origin of the reverse Wenckebach phenomenon in the atrioventricular node has been the subject of some dispute. It has been interpreted as being possibly due to "atrioventricular dissociation in the presence of 2 to 1 atrioventricular block" (21). Indeed, if we consider the aggregate to represent a subsidiary junctional pacemaker, with the periodic stimulation representing a reg-

ular supraventricular input to that pacemaker, then such a rhythm could indeed be interpreted as being due to an occasional escape from 2:1 entrainment of the junctional pacemaker (see Fig. 4D, for example).

Between the 2:1 and 5:2 phase-locking zones there are rhythms similar to alternating Wenckebach rhythms seen in other cardiac tissues. Alternating Wenckebach rhythms were first described in cases of atrioventricular block in which alternate ventricular beats are dropped with a monotonically increasing P-R interval until either usually three (alternating Wenckebach type B) or four (alternating Wenckebach type A) beats are dropped (17). The type A pattern was not seen in the aggregate; only the type B pattern was observed. The mechanism most widely accepted as an explanation of these rhythms is block occurring at two or more levels as a result of horizontal dissociation in the conduction pathway, with an $n + 1:n$ Wenckebach pattern of block at one level and a 2:1 pattern of block at the other (17, 20). Our experiments provide a clear example that alternating Wenckebach (type B) patterns can occur in the absence of multiple levels of block.

Several of the rhythms that have been experimentally observed in this preparation (e.g., 8:5, 7:4, 8:3, 7:3) have not been previously described or have been only rarely described in clinical or experimental work. Moreover, many rhythms analogous to high-grade block and escape rhythms are theoretically predicted and experimentally observed. We have shown that a simple scheme provides the means to organize the many diverse rhythms described in all of the experiments to date.

Nonperiodic dynamics. Based on purely theoretical arguments, it is expected that nonperiodic dynamics should be found at some stimulation frequencies over the range of stimulus amplitudes used here (7–11). This can be appreciated intuitively from the fact that as the stimulus amplitude decreases toward zero, the range of t_s over which any phase-locking rhythm will be seen decreases toward zero. The phase-locking rhythms seen at higher amplitudes are then replaced by nonperiodic rhythms similar to that shown in Fig. 9C. These rhythms correspond theoretically to a particular class of nonperiodic rhythms arising out of “quasiperiodic” dynamics (7–10). However, there are fundamental methodological problems in identifying any experimentally obtained rhythm as quasiperiodic (see Ref. 9 for further discussion of this point). As stimulus amplitude decreases, the probability of encountering a quasiperiodic pattern for a randomly chosen combination of t_s and stimulus amplitude increases, theoretically attaining a probability of one at zero stimulus amplitude. The nonperiodic rhythms observed in the aggregate at low-stimulus amplitude and which we associate with quasiperiodic dynamics resemble those seen in some instances of complete atrioventricular heart block, in which “P waves progress sequentially relative to QRS and more through QRS” (2).

Other rhythms, not described above, can be seen in the periodically stimulated aggregate at higher pulse amplitudes (7–9, 11). Among these other rhythms is a nonperiodic “chaotic” rhythm that is not the same as the nonperiodic “quasiperiodic” rhythm described here.

1:0 phase-locking rhythm. Before it was experimentally found in a biological oscillator, the existence of 1:0 phase locking was theoretically predicted from the response of such an oscillator to single isolated pulses (10, 27). In the limit where t_s is equal to the duration of the stimulus pulse, we achieve a condition equivalent to constant current injection. It is well known in many cardiac preparations (e.g., Ref. 27) that a constant depolarizing current of sufficiently large magnitude will maintain the potential at the plateau level and generate tonic contraction. When t_s is increased beyond the pulse duration, the contribution of both passive membrane properties (i.e., the time constant) and active membrane properties (i.e., ionic currents) determine the voltage excursions during and after current injection. During each cycle of a stable 1:0 rhythm there is presumably an oscillation in both the active and passive properties of the membrane. The relative contributions of these components is difficult to ascertain, since they vary continuously during each stimulus cycle.

The only other report of which we are aware that demonstrated 1:0 phase locking in a cardiac oscillator also employed heart cell aggregates (32). However, in that report no experimental tracings were shown, 1:0 phase locking was only obtained occasionally, and it was stated that the experimental conditions necessary for obtaining 1:0 phase locking (termed “rapid repetitive phase delay”) were unclear. In another even earlier experimental study, attempts were made to obtain 1:0 phase locking, but were unsuccessful (27). We have also not been able to obtain 1:0 phase locking in some aggregates. We believe that this was due, in those instances, to saturation in the current-carrying capacity of the microelectrode. In all such cases, injection of a constant current of the largest magnitude available failed to abolish spontaneous activity. Thus the conditions necessary for 1:0 phase locking to be seen with a depolarizing stimulus seem to be both a sufficiently large current amplitude and a sufficiently high stimulation frequency. In contrast, with hyperpolarizing stimulation we were able to obtain 1:0 phase locking in all aggregates assayed. This may be due to the fact that a much smaller stimulus current is needed to overcome the much smaller currents flowing during spontaneous diastolic depolarization than to overcome those flowing during repolarization. The existence of 1:0 phase locking might have important implications, since it shows that it is possible to suppress spontaneous activity given the proper combination of stimulation parameters.

Implications for other cardiac preparations. We have described systematically a wide variety of complex rhythms in a virtually isopotential spontaneously active cardiac preparation. Many of these rhythms can also be seen in aggregates made quiescent by elevating the external potassium concentration. Although most of these patterns have been described clinically and experimentally in distributed cardiac systems, our study is the first to unify a body of complex, apparently divergent data. Theoretical work predicts the existence of these patterns and the order in which they will appear. We thus believe that the construction shown in Fig. 11 forms a framework

within which it will be possible to analyze other periodically driven excitable systems and to categorize certain classes of arrhythmias, such as block and parasystole, encountered in the intact human heart.

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