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Synchronization in Chains of Pacemaker Cells by Phase Resetting Action Potential Effects

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Abstract. Interactions between pacemaker cells in a chain were calculated according to a "phase-reset" model. It is based on effects of action potentials in the cells on the cycle lengths of neighbouring cells. These effects were defined for each cell by a latency-phase curve (LPC), giving the latency time (L) until the onset of the next action potential in that cell, as a function of the phase (ϕ) at which a neighbour cell fired an action potential. Neighbour cells with simultaneous action potentials did not influence each others cycle length. We investigated how stable synchronization depends on the shape of the LPC's of the pacemaker cells and on chain length. Three types of interactive behaviour were distinguished. First, anti-phase synchrony, in which neighbouring cells fired with large phase differences with respect to the synchronized period P_s. Second, asynchrony, in which the periods of the cells did not become equal and constant. Third, in-phase synchrony, in which the phase differences between the neighbouring cells were zero or much smaller than the synchronized period P_s, depending on the differences between the intrinsic periods. Asynchrony and anti-phase synchrony may be seen as cardiophysiological arrhythmias, while in-phase synchrony represents the physiological type of synchrony in the heart. In-phase synchrony appeared to be strongly favoured by LPC's, which have a no-effect (refractory) part at early phases, a lengthened latency (or phase delay) part at intermediate phases and a shortened latency (or phase advance) part at late phases in the cycle. Such LPC-shapes are commonly found in preparations of cardiac pacemaker cells. When the pacemaker cells were identical, the synchronized period P_s during in-phase synchrony was equal to their intrinsic period P_i^*. For different intrinsic periods, P_s was equal to the intrinsic period of the fastest cell if the LPC's contained a sufficiently long initial no-effect period at early phases and a shortened latency part at late phases. When, on the other hand, such cell chains had a linear gradient in their intrinsic periods, "action potentials" started from the fast end and traveled along the chain. The propagation of an action potential wave slowed down as it reached the slower cells. When the gradient in the intrinsic periods was too steep, only the intrinsically fast end of the chain developed synchrony. Surprisingly, by making the intrinsic gradient somewhat irregular such a chain of cells could be made to exhibit areas of cells that were locally synchronized at different frequencies. These model results show that the concept of phase resetting provides a useful framework to understand interactions between pacemaker cells by action potential effects.

1 Introduction

Regular spontaneous impulse generation in the sinus node involves electrical interactions between the individual pacemaker cells. Pacemaker cell synchronization in this structure is often considered as a result of "premature" triggering of action potentials in the slower cells by action potentials occurring in the faster cells. The concentric activation pattern is then explained by postulating the existence of a centre of "true" pacemaker cells, surrounded by "latent" pacemaker cells with lower intrinsic frequencies (cf. Bonke, 1978; Bleeker et al., 1980). Although this view seems consistent with the normal synchronized condition in the sinus node, it is not specific enough to predict the establishment or the loss of synchrony in a population of coupled pacemaker cells. More detailed concepts in terms of the properties of the individual pacemaker cells are required to understand the stability – or, in

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disease – the instability of the synchronized condition of the population. In the present study we present a model based on such concepts, which allowed us to relate the behaviour of a population of coupled pacemaker cells with the responsiveness of the single cells to action potentials in neighbouring cells.

This model may be considered as an extension of the simple two-oscillator model described before by Van Meerwijk in Ypey et al. (1982a). The interactions are effects of action potentials in any cell on the length of the cycles of their neighbouring cells. In between the action potentials (i.e. during the "diastolic depolarizations") neighbouring cells do not influence each other. In that respect the model is based on conventional assumptions (cf. Sano et al., 1978; Ikeda, 1982).

Not only cycle shortening effects are allowed, but cycle lengthening effects of action potentials on neighbouring cells may also be incorporated. Cycle shortening effects of action potentials are familiar to cardioelectrophysiologists as premature triggering of a slow pacemaker cell by a faster neighbour cell. Cycle lengthening effects by depolarizing stimuli like artificial current pulses or natural action currents from neighbour cells may seem paradoxical, but do occur in sinus node (Sano et al., 1978) and in embryonic heart pacemaker cell preparations (Scott, 1979). In addition, we investigated the influence of the refractory period on the interactive behaviour of pacemaker cells.

Interaction models of two coupled pacemaker cells are simple enough to be represented by analytical expressions (Ikeda, 1982) or by a graphical procedure (Ypey et al., 1982a). However, interactions between pacemaker cells in larger populations are often too complex for such a representation. We, therefore, developed a computer program to calculate the successive action potential intervals of all cells, resulting from the successively occurring action potential effects in the population. The effects of all action potentials of neighbour cells on cycle length were defined for each cell by a so-called latency-phase-curve (LPC). In this curve the latency time to the next action potential of the cell is given as a function of the phase (ϕ) of its cycle at which a neighbour action potential occurred. Such a curve is one of the three representations of phase resetting effects used to characterize the influence of an action potential on the neighbour's cell cycle (cf. Ypey et al., 1982a, b). Hence, the interactions between pacemaker cells in our model are called phase resetting interactions (for further explanation see the model description).

The present model study concerns the interactive behaviour of pacemaker cells in a chain. We chose this cell geometry as a logical extension from the earlier study on two coupled pacemaker cells by Ypey et al. (1982a). A further extension – towards a two-dimensional geometry – has already been used in a study on localized pacemaker suppression (Ypey et al., 1982b).

Since interactions between the diastolic depolarizations of the cells were not incorporated in the model, the following question could be answered in this study: what kind of action potential effects on neighbour cells are required to obtain stable synchronized conditions in a population (i.e. a chain) of coupled pacemaker cells. Phase resetting concepts provide a straightforward answer to this question. Although this study is primarily directed towards an understanding of cardiac pacemaker cell interactions, the results may apply to other pacemaker structures as well, present for example in the nervous system or in the walls of hollow visceral organs.

2 Model Description

The computer model consists of a chain of N coupled pacemaker cells ($2 \leqq N \leqq 200$). It is a special version of the computer model for a two-dimensional population of pacemaker cells described recently in Ypey et al. (1982b). In the present study we used a different representation of the phase resetting interactions between the cells. Therefore, we briefly explain the model again.

Each cell i of the chain is characterized by an intrinsic period (P_i^*) and a so-called latency-phase-curve (LPC). As explained in Fig. 1, this curve defines the effect of an action potential in a neighbour cell on the waiting time (latency L) until the next expected action potential. The phase ϕ_i of an unperturbed cell i is defined as:

$$\phi_i = T/P_i^* \tag{1}$$

in which T is the time since the previous action potential of that cell ($0 \leqq T \leqq P_i^*$). A cell i fires an action potential at $\phi_i = 1$ (is equal to $\phi_i = 0$). If a neighbour cell's action potential would not affect the cycle length of cell i at any phase ϕ_i, the latency $L_i(\phi_i)$ – also scaled according to P_i^* – is given by the diagonal D in Fig. 1B. If neighbour action potentials shorten the cycle of a cell, then L-values will be found below D (Fig. 1B). On the other hand, cycle lengthening effects of neighbour action potentials result in L-values above D (Fig. 1B).

Latency changes are considered to be a consequence of instantaneous "phase-resetting" effects of (nearest) neighbour action potentials. In our model the state of the oscillator is completely defined by its phase ϕ. Therefore, a latency (and the corresponding cycle) shortening is equivalent to an advance of the state of the oscillating cell to a later phase in the cycle

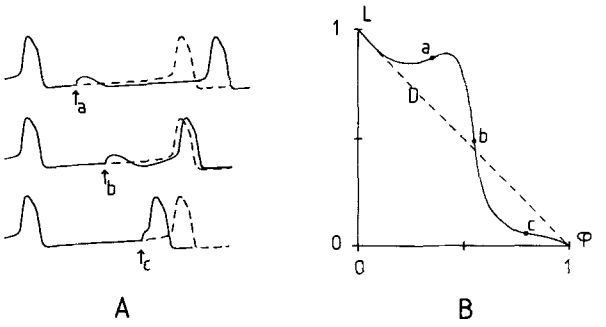

Fig. 1A and B. The latency-phase-curve (LPC) as a representation of phase resetting effects of action potentials in neighbour pacemaker cells. **A** Exemplar records from a pacemaker cell, which undergoes phase resetting effects from neighbour cell action potentials. The dashed records are unperturbed cycles of the cell. The continuous records illustrate the changes in the cycle length when a neighbour action potential occurs (at the arrows). L denotes the latency time from the onset of the (nearest) neighbour action potential to the onset of the next action potential of this cell. **B** A latency-phase-curve (LPC) as may be obtained from a cell like that in **A**. ϕ denotes the phase of the cell, at which the neighbour action potential occurs. The resulting latency L (scaled to the intrinsic period) is given on the vertical axis. D is for diagonal

(phase advance). A lengthening of the latency (and of the corresponding cycle) is equivalent to a reset of the state of the oscillator to an earlier phase, so that the action potential becomes delayed (phase delay). We use latency-phase-curves throughout this study instead of phase-reset-curves (PRC) as in Ypey et al. (1982b), since this formalism allows convenient comparison of the behaviour of long chains with that of "two-cell chains" (Ypey et al., 1982a). In addition, LPC's are more familiar to cardiophysiologists, as they are similar to sino-auricular function (SAF) curves, which represent latency changes of sinus node action potentials caused by single current pulses (Strauss et al., 1973). However, LPC's can be easily converted to PRC's according to the relationship:

$$\phi_i' = [P_i^* - L_i(\phi_i)]/P_i^* \qquad (2)$$

in which ϕ_i' is the new phase to which cell i is reset by a neighbour action potential occurring at the old phase ϕ_i.

The model accounts for action potential effects only. Thus, cells at phases other than $\phi_i = 1$ do not influence the cycle length of their neighbours.

Before starting the calculations, each cell i was assigned an intrinsic period P_i^* and a phase $\phi_i (0 < \phi_i < 1)$, randomly selected from a homogeneous distribution with boundaries 0 and 1. Linear gradients in P_i^* or in its reciprocal value (intrinsic frequency) could be introduced and made either smooth or irregular through a random factor. The normalized LPC, which was shared by all cells, was described by at least 3 (L, ϕ)-values, with $L = 1$ at $\phi = 0$ and $L = 0$ at $\phi = 1$ (see Fig. 2a; note the scaling to P_i^*). At intermediate ϕ-values the latency L was obtained by linear interpolation.

The interactions were calculated on a PDP 11/70 computer with the following steps:

1) Find the cell (j) with the smallest latency $L_j = (1 - \phi_j) P_j^*$.

2) Set the time T_c of the common clock to $T_c = T_c + L_j$.

3) Define $\phi_j = 0$ and $\phi_i = (\phi_i P_i^* + L_j)/P_i^*$ for all $i \neq j$.

4) Reset $\phi_{j-1} (1 < j \leqq N)$ and $\phi_{j+1} (1 \leqq j < N)$ according to their respective LPC.

5) Repeat sequence 1 to 4 until a prescribed number M of cycles is reached.

Time was kept in double precision (17 significant digits). All other variables were in single precision (8 significant digits). Since rounding errors in ϕ_i and ϕ_j during the successive steps accumulate, latencies $-5 \times 10^{-7} < L_j < 0$ occasionally occurred and were made zero.

3 Results

3.1 Chains of Phase Advancable Pacemaker Cells

Premature triggering of a pacemaker cell by an action potential of an intrinsically faster neighbour pacemaker cell seems a naturally occurring condition in a population of synchronized pacemaker cells like the sinus node (Bleeker et al., 1980). Such a premature triggering can be considered as a phase advancing effect through the action potential of the faster cell on the cycle of the slower neighbouring cell. This view opens the question, what kind of phase advancing properties of pacemaker cells are required for the establishment of synchrony in a population of pacemaker cells with given intrinsic frequencies.

We investigated this problem by determining the interactive behaviour in a chain of pacemaker cells for different shapes of the commonly shared phase advancing LPC. Since we expected stronger synchronization conditions for larger populations of cells, we also compared chains of different length. For simplicity, chains of cells with identical intrinsic periods $P_i^* = 1$ were considered first.

Figure 2a illustrates an example of a very simple advancing LPC, defined by three points only. Two of these points have coordinates $(L = 1, \phi = 0)$ and $(L = 0, \phi = 1)$. Location of the third point somewhere within the triangle formed by the diagonal D and the L- and ϕ-axis allows easy variation of the shape of the advancing LPC. By systematically varying the coordinates of this third point of maximal advance and observing the resulting interactions within the chain, we could relate certain types of interactive behaviour

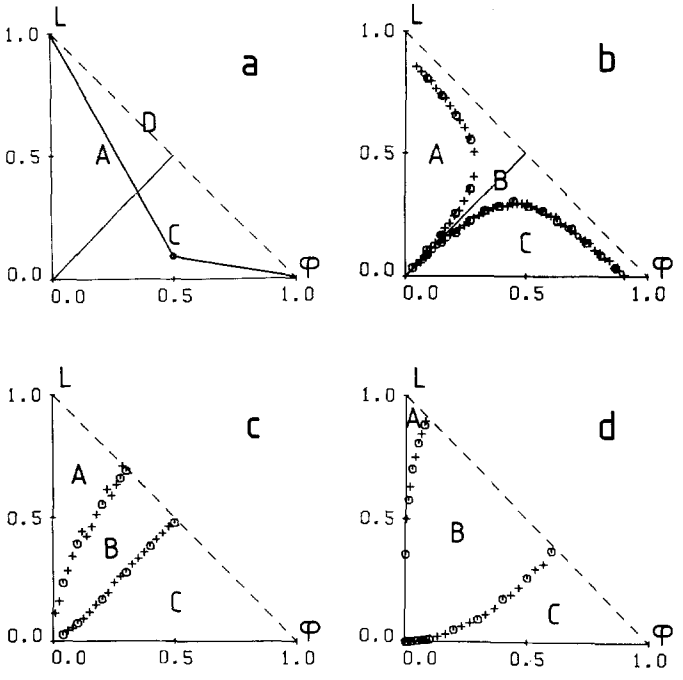

Fig. 2a–d. Three different modes (A, B, C) of pacemaker cell interaction in chains of 2–11 cells. The LPC's exhibited latency shortenings (phase advancings) only and had a linearized shape like the example drawn in **a**. Two of the three points of the LPC were fixed ($L=1, \phi=0$; $L=0, \phi=1$), whereas the third point (determining the maximal latency shortening) was varied systematically throughout the $L-\phi$-plane. Depending on the location of this point in certain $L(\phi)$-areas a specific mode of interaction resulted. A indicates anti-phase synchrony, B asynchrony and C in-phase synchrony. Data points ○ were obtained from a single set of initial randomly chosen ϕ_i. Each point + was obtained with a different set of initial random ϕ_i. Accuracy in the vertical direction is plus or minus 0.005 (absolute). **a** Two equal cells always synchronize (no area B). In-phase synchrony (C) occurs for maximal latency shortenings below the perpendicular from the origin to the diagonal D. Anti-phase synchrony (A) occurs above this perpendicular (and below D). **b** Two unequal cells ($P_1^*=1$ and $P_2^*=1.1$) may synchronize in anti-phase (area A), in-phase (area C) or may fire in asynchrony (area B) depending on the location of the maximal latency shortening of the LPC in the $L-\phi$-plane. **c** Three identical pacemaker cells may develop anti-phase synchrony (area A), asynchrony (B) or in-phase synchrony (C), depending on the location of the point of maximal latency shortening in the $L-\phi$-plane. **d** In a chain of 11 identical pacemaker cells asynchrony area B has increased, mainly at the expense of area A (compare **c**)

to the shape of the LPC. These different types of interactive behaviour were visualized by indicating the transitions between them in the $L-\phi$-plane, resulting in a $L(\phi)$-"map" (see e.g. Fig. 2).

3.1.1 Two-Cell Chains. For "chains" of two identical cells ($N=2$) we found two types of synchronization, as predicted by the graphical method given in Ypey et al. (1982a). When the maximal advance point of the LPC was located in area C (Fig. 2a), synchronization resulted with period $P_s=P_1^*=P_2^*$. Then the action potential of cell 2 fell at $\phi_1=0$ and that of cell 1 at phase $\phi_2=0$. This type of synchronization, in which the firing time differences between neighbour cells are zero or much smaller than the synchronized period will be called "in-phase" synchronization throughout this paper (cf. Kawato, 1979). When, on the other hand, the maximal advance point was chosen in area A (Fig. 2a), a different type of synchrony was obtained. The synchronization period P_s was then smaller than the intrinsic periods ($P_1^*=P_2^*$) and the difference in firing times was equal to $0.5P_s$. This type of synchronization, in which the firing time difference between two neighbour cells amounts to approximately half of the synchronized period, is called "anti-phase" synchronization (cf. Kawato, 1979).

Finally, defining the maximal advance point of the LPC exactly on the perpendicular to the diagonal D from the origin ($L=0, \phi=0$) resulted in a continuation of any initially defined phase difference. Then the periods were equal and constant, but smaller than $P_1^*=P_2^*$. Thus, this perpendicular forms the sharp boundary between the areas A and C of anti-phase and in-phase synchronization respectively.

Pairs of cells with different intrinsic periods ($P_1^*<P_2^*<1.2P_1^*$) showed a new feature in their interactive behaviour, namely that asynchrony could result. This behaviour was also represented in a plot like Fig. 2a by considering such a plot as applying to the normalized LPC of the two cells. Again, an anti-phase area A and in-phase area C were found (Fig. 2b). However, synchronization now required a minimum latency shortening (or phase advance). Whenever the top of the LPC was located within area B (Fig. 2b), the fast cell could not cause the required latency shortening in the slow cell and asynchrony resulted. Since synchrony was maintained, mainly by the advancing effects of the faster cell on the slower cell, phase differences now occurred during in-phase synchrony, as in anti-phase synchrony. In addition, the synchronized period P_s was smaller than that of the fastest cell (P_1^*). During anti-phase synchrony the firing time differences between the cells were no longer equal to half the synchronized period, when P_1^* and P_2^* were different.

3.1.2 Chains of Three or more Phase Advancable Cells. For three or more identical cells there also exists an anti-phase area A and an in-phase area C. However, in contrast to pairs of equal cells (Fig. 2a) areas A and C were not contingent. As in pairs of cells with different P_i^* (Fig. 2b), an asynchrony area B had arisen between A and C (Fig. 2c and d). If the maximal advance point of the LPC was chosen within this area B, synchronization could not be obtained.

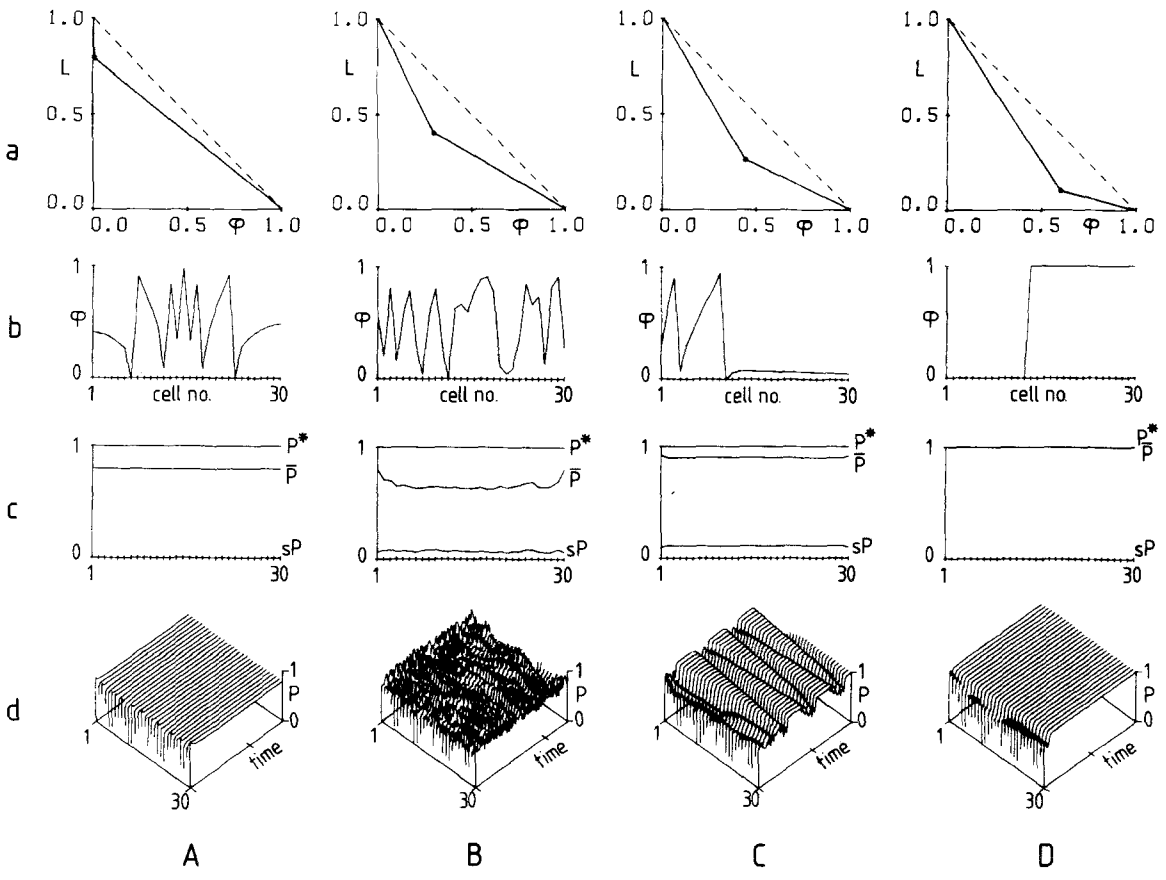

Fig. 3A–D. Different types of interactive behaviour in a chain of 30 identical pacemaker cells for different shapes of LPC's, exhibiting latency shortening effects only. From top to bottom are shown: the LPC's used in the simulations (Aa–Da); the resulting phase relationships (Ab–Db); the mean period $\bar{P}$ of the last 40 intervals of each cell, their standard deviation sP and the intrinsic periods P^* (Ac–Dc); the interspike intervals of all cells as function of time T, indicating a quick establishment of synchrony (Ad and Dd). **A** Location of the point of maximal latency shortening at very small phases in the LPC (Aa) results in anti-phase (Ab) synchrony (compare area A in Fig. 2d). **B** Shifting the point of maximal latency shortening towards a larger phase in the LPC (Ba) results in a loss of synchrony (compare area B in Fig. 2d). The phase relationship remains chaotic (Bb) and the periods P do not become constant (Bc, d). **C** Location of the top of the LPC at a still larger phase (Ca), close to a transition towards in-phase synchrony (cf. Fig. 2d), may give rise to an alternating kind of interactive behaviour. As a result the phase relationship (Cb) at the end of the calculations reflects nearly in-phase synchronization at cell numbers > 12, whereas a nearly anti-phase relationship is found at the smaller cell numbers. **D** Location of the point of maximal latency shortening at large phases (compare area C in Fig. 2d) gives rise to in-phase synchronization. As a result, all phases (Db) and all periods P have become identical (Dc, d)

The action potential intervals P_i of the pacemaker cells did not become constant (cf. Fig. 3Bd and 3Cd) and the phase-relationship between the cells remained irregular (Fig. 3Bb).

A comparison of Fig. 2a–d shows, that the sizes of the areas A, B, and C depend on chain length. Asynchrony area B grew with increasing chain length, mainly at the expense of anti-phase area A. The latter area (A) was already small in 11-cell chains (Fig. 2d), but had become very small in 30-cell chains (cf. Fig. 3Aa). This was related to an increasing tendency of the outer cells to escape from the (synchronous) center. It is therefore noteworthy that by closing the chain into a ring a much larger area A resulted. Different runs with the same chain of cells, but with different random initial ϕ_i, showed that the boundaries between B and C and particularly between A and B were somewhat diffuse and depended on the initial values of ϕ_i, especially for short chains.

During type A synchrony P_s was again smaller than the intrinsic values P_i^* (cf. Fig. 3Ac) and considerable phase differences occurred between neighbouring cells (Fig. 3Ab). The differences in firing times of neighbour cells, however, were not equal to $0.5P_s$, as in the case of two equal cells. During type C synchronization (Fig. 3D) all phases ϕ_i became the same (Fig. 3Db) and the synchronized period P_s became equal to P_i^* (Fig. 3Dd). In general, both type A and type C synchrony were established from the initial random phases within a few cycles (Fig. 3Ad and Dd). When, however, the maximal advance of the LPC was defined close to the diagonal D or to the boundary between A and B or C and B (cf. Fig. 2), long lasting transients could be observed.

Moving the top of the LPC from the middle of B towards C or to A indicated that the tendency to develop entrainment increased in either direction. This is illustrated in Fig. 3C, where the maximal advance point was chosen in B close to the border between B and C. As a result the phase differences between the cells were not constant. At regular times, a varying part of the chain seemed to exhibit type C synchrony (Fig. 3Cb). These cells showed interval fluctuations (Fig. 3Cd), as if they periodically escaped from type C synchrony towards intervals shorter than P_i^*. A similar phenomenon has been recognized during mutual synchronization of two heart cell aggregates and electronic pacemaker cell models (Ypey et al., 1980, 1982a), and was called "periodic synchrony" or "almost-synchrony". However, the chain of Fig. 3C showed an additional feature, since the escape from type C synchrony travels back and forth along the chain, bouncing at the open ends (Fig. 3Cd).

So far the intrinsic periods have all been taken to be equal. Introduction of small gradients, or a random distribution in P_i^* with differences in P_i^* not exceeding a few percent, showed that the results were only slightly different. The main difference was that the LPC had to reflect a minimal latency shortening to allow both type A and type C synchrony, as was required during synchronization of two unequal cells (Fig. 2b).

An important conclusion to be drawn from these simulations is that in-phase synchrony, which is the type of synchrony in the heart required physiologically is favoured by LPC's with their largest latency shortening at large ϕ-values in the cycle. This is a property of many LPC's measured for heart pacemaker cells (Scott, 1979; Jalife et al., 1980; Guevara et al., 1981; Ypey et al., 1982a). LPC's with the largest latency shortening at small ϕ-values do not exist, due to the presence of a refractory period in excitable cells.

3.2. *Chains of Phase Advancable Cells with a Refractory Period*

When excitable cells produce an action potential as a response to a current pulse, they do not fire a second action potential if they are stimulated by the same pulse during or shortly after the action potential. Both pacemaker and non-pacemaker cells exhibit such a refractory period. However, in pacemaker cells it may occur that this stimulus does not directly trigger a second action potential in this refractory period, but that it still influences the time course of the diastolic depolarization, thereby affecting the latency time to the next spontaneously occurring action potential. Stimuli with a duration smaller than or equal to that of the action potential and applied during the first part of the refractory period do not in general influence the latency (cf. Ypey et al., 1982a).

In this section we report on the role in synchronization of such a no-effect period in the LPC, using pacemaker cell chains of variable length. As in the previous section the point of maximal advance in the LPC was varied systematically. The no-effect period was $0.2P_i^*$.

3.2.1 Two Coupled Phase Advancable Cells with a No-Effect Period. The introduction of a no-effect period in the LPC strongly influenced the ability of the cells to become synchronized. Figure 4a shows this influence for a "chain" of two equal cells. Again areas A and C

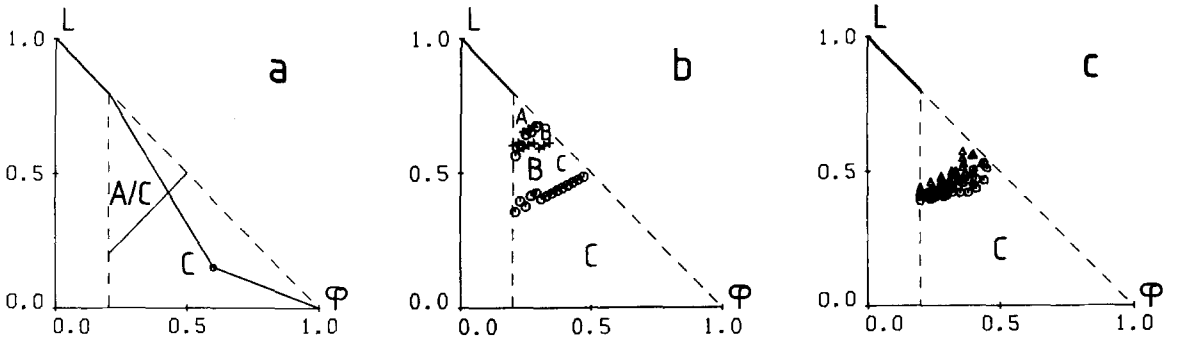

Fig. 4a–c. Interactive behaviour of chains of identical pacemaker cells with phase advancing LPC's having a no-effect period of $0.2P_i^*$ (see LPC-example drawn in **a**). **a** Pairs of cells never interact in asynchrony. They synchronize in-phase, when the point of maximal latency shortening is located in area C. In area A/C in-phase as well as anti-phase synchrony may occur depending on initial ϕ_i. **b** An example of interactive modes of three coupled pacemaker cells. The data points ○ and + were obtained with two different sets of random initial ϕ_i. Three coupled cells do not synchronize, when the point of maximal latency shortening is located in area B (cf. Fig. 2c). As the boundaries between the areas A, B and C depend very much on initial ϕ_i, these results mainly serve to illustrate the existence of interaction mode B (asynchrony). This has been indicated in the figure by a small, respectively, large B and C for either set of data. The accuracy of the L-coordinates of ○ and + is plus or minus 0.015 (absolute). **c** Coupling of 11 or 30 pacemaker cells in a chain leads to in-phase synchrony, when the top of the LPC is located within area C. Transitions from in-phase synchrony towards one of the other two types of interaction (B or A) are indicated by ○ (5 sets of different initial ϕ_i, 30 cells) and △ (idem, 11 cells). Accuracy as in **b**

were found, in which anti-phase (with synchronized period $P_s < P_i^*$) and in-phase synchronization (with $P_s = P_i^*$) occurred, respectively. These two areas were separated by the same perpendicular as in Fig. 2a, where the two coupled cells lacked a no-effect period. However, by the introduction of a no-effect period the occurrence of anti-phase synchronization no longer depended on the location of the variable third point of the LPC only. Depending on the initial phase conditions in-phase synchronization could be found as well, when the LPC had its point of maximum phase advance in A. Thus, with unknown random initial phases ϕ_1 and ϕ_2, the occurrence of either synchronized state is possible.

Similar results were obtained, when the intrinsic periods were unequal. In that case in-phase synchronization occurred at or slightly above the intrinsic frequency of the fastest cell. In addition, areas A and C in the $L(\phi)$-map (scaled to P_i^*) no longer extended to the diagonal D, since synchronization now required a minimal phase advance of the slower cell, as in the case of two cells without a no-effect period (Fig. 2b).

These results are in perfect agreement with predictions from the graphical procedure to describe interactions of pacemaker cells on the basis of their respective LPC's (Ypey et al., 1982a). For example, application of this graphical procedure to LPC's with their maximal phase advance in area A also yields two stable phase relationships, each with its own synchronized period P_s.

3.2.2 Chains of Three or More Phase Advancable Cells with a No-Effect Period. Increasing the number of identical pacemaker cells in a chain from two to three resulted in a new property, as was the case for cells without a no-effect period. Again, the cells were unable to synchronize from random initial phase differences when the point of maximal advance in the LPC was located at certain $\phi < 0.5$. This property is illustrated in Fig. 4b as the appearance of an area B between A and C. However, the sizes of these areas A, B, and C depended much more on initial ϕ_i-values than was the case for phase advancing LPC's without a no-effect period (cf. Fig. 2c). Depending on initial ϕ_i-values, it could also happen that areas A and B were completely absent.

The dependency of location and size of areas A, B, and C on the initial (randomly chosen) ϕ_i-values became less apparent for longer cell chains ($N \geqq 10$). In that case area A could no longer be found as a contiguous area, but appeared in very small patches only. Figure 4c gives an impression of the size and location of area C for two different cell chain lengths (with $N = 11$ and $N = 30$, respectively). Five sets of randomly chosen initial ϕ-values were used with each chain length. Since patches of type C synchrony occurred as well within area B, only those transitions towards type C synchronization are given that mark the border of a contiguous area C. Additional irregularity in this border line results from the different sets of (random) initial ϕ-values.

As it did without a no-effect period (see Fig. 2c and d) type C synchrony occurred when the maximal advance in the LPC fell in the lower right corner of the $L(\phi)$-map. However, area C now no longer depended strongly on chain length (Fig. 4b and c). When the maximal advance fell in the upper left corner of the $L(\phi)$-map (but at $\phi > 0.2$) synchrony could in general not be obtained. On the other hand, occasionally type A or even type C synchrony could be observed at specific locations in this upper left corner. Similar results were obtained for chains of cells in which the intrinsic periods were (slightly) different. In that case again a minimal advance was required to obtain synchrony, comparable to two cells without a no-effect period (Fig. 2b).

Two main conclusions can be drawn. First, cells with a no-effect (refractory) period and interconnected in a chain, synchronize more easily in-phase than cells without such a no-effect period. Second, an increase in chain length does not drastically reduce the abilities of the cells to become synchronized in-phase when an initial no-effect period is present in the LPC's of these cells.

3.3 Chains of Pacemaker Cells with Phase Delaying Interactions

Latency-phase curves of cardiac pacemaker cells often show a phase delay part after the no-effect part of the refractory period and before the phase advance part at late phases in the cycle (Jalife et al., 1980; Guevara et al., 1981; Ypey et al., 1982a). Hence, we investigated phase delaying interactions between cells within a chain and their ability to synchronize. Three different types of pacemaker cell chains were compared. First, chains of cells with LPC's exhibiting phase delays only (Fig. 5a); second, chains of cells with a no-effect part followed by a phase delay part (Fig. 5b); third, chains of cells with the most physiological type of LPC, having a no-effect part at early phases, a phase delay at intermediate phases and a phase advance part at late phases in the cycle (Fig. 5c). As before, the no-effect (refractory) period had a (fixed) duration of $0.2 P_i^*$. All 11 cells within a chain had identical intrinsic periods $P_i^* = 1$. The shape of the phase delay part of the LPC was systematically varied by changing the location of the point of maximal delay in the L-ϕ-plane in a similar way as in the previous sections. Boundaries between areas of maximal delay locations

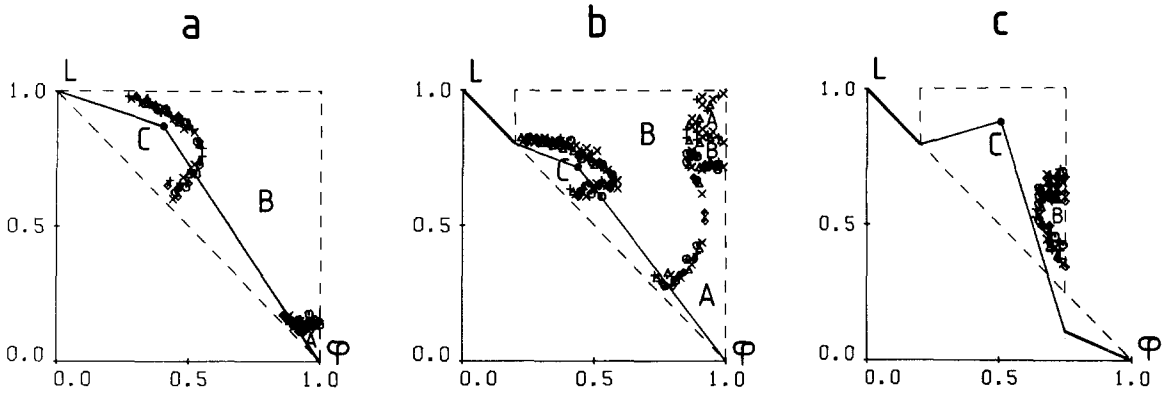

Fig. 5a–c. Different modes (A, B, C; meaning as in Fig. 2) of interactive behaviour in a chain of 11 coupled identical pacemaker cells, having LPC's with a phase delay. Examples of three types of LPC's are drawn in **a–c**. The shapes of these LPC's were varied by changing the point of maximal latency lengthening in the L-ϕ-plane. Accuracy of the L-coordinates of all data points is plus or minus 0.015 (absolute). **a** Interaction modes (A, B, C) when the LPC exhibits phase delays only. At the coordinates, indicated by any of the 5 symbols (each related to a different set of initial random ϕ_i), transitions were observed in the interactive behaviour from one mode to one of the other two modes. Note, that the order of the different modes in the L-ϕ-plane is reversed on the ϕ-axis in comparison with that of Fig. 2d, where the LPC exhibited latency shortenings only. **b** Interaction modes (A, B, C), in case the delaying LPC exhibits an initial no-effect period lasting $0.2P_i^*$. **c** Interaction modes (B, C) for a LPC having an initial no-effect period (at $0 \leqq \phi \leqq 0.2$), a given phase advance part (at $0.75 \leqq \phi < 1$) and a variable delay at intermediate phases. The coordinates of the point of maximal latency lengthening were varied within the trapezium formed by the interrupted lines. In-phase synchrony (C) resulted, unless this point was located within asynchrony area B

exhibiting different types of interaction (synchrony and asynchrony) were obtained for five different sets of randomly chosen initial ϕ_i.

Figure 5a shows the synchronization conditions for such a chain of cells, which exhibit phase delaying interactions by neighbour action potentials only. Three areas of maximal delay locations were found in the L-ϕ-plane corresponding to the three types of interactions reported before. In-phase synchrony (area C) was found, when the maximal delays were located at early phases. Then the synchronization period was equal to the intrinsic period $P_i^* = 1$. Anti-phase synchrony (area A) occurred when the maximal delay was located at late phases. In this type of synchrony the synchronization period was larger than P_i^*, consistent with the occurrence of phase delays only in the LPC. Asynchrony (area B) was found for in between location of the maximal delay. The boundaries were slightly dependent on the initial phase values. Occasionally in-phase synchronization could be found with the point of maximal delay outside area C in Fig. 5 (not shown). Usually this resulted from suppression of the activity of some of the pacemaker cells by repetitive delaying interactions (cf. Ypey et al., 1982b).

The introduction of an initial no-effect period in the LPC's of the cells reduced area C, but increased area A considerably (Fig. 5b). In addition, asynchrony and anti-phase synchrony no longer occurred within single solid areas B and A, respectively. However, area C strongly increased again after the introduction of a phase advance part in the LPC, as illustrated in Fig. 5c. Asynchrony now could be found only in a small area B, where the transient in the LPC from maximal delay to maximal advance was relatively steep. In addition this area B became still smaller (and even vanished, giving rise to type C synchronization only), when the phase advancing interactions were made stronger (larger maximal advance at smaller phase). Omission of the initial no-effect period from these LPC's slightly favoured in-phase synchrony as well.

From these simulations we draw two main conclusions. First, a phase delay part in the LPC only favours in-phase synchrony if the point of maximal delay occurs at $\phi < 0.5$. Second, a phase delay part in the LPC favours in-phase synchrony much more, when it is followed by a phase-advance part.

As before, these conclusions and observations were not essentially changed, when slightly different intrinsic periods were chosen.

3.4 Wave Propagation and Synchronization in Pacemaker Cell Chains

Action potential fronts in the sinus node move with different speeds in different directions from the centre to the periphery (Bleeker et al., 1980). In this section we show that the local propagation speed of an impulse in a tissue will depend on the distribution of intrinsic periods.

In Fig. 3D it was already illustrated that chains of equal cells develop in-phase synchrony with zero phase differences at a synchronization period $P_s = P_i^* = 1$. Even with very small phase advances in the LPC the action potentials of these cells coincided

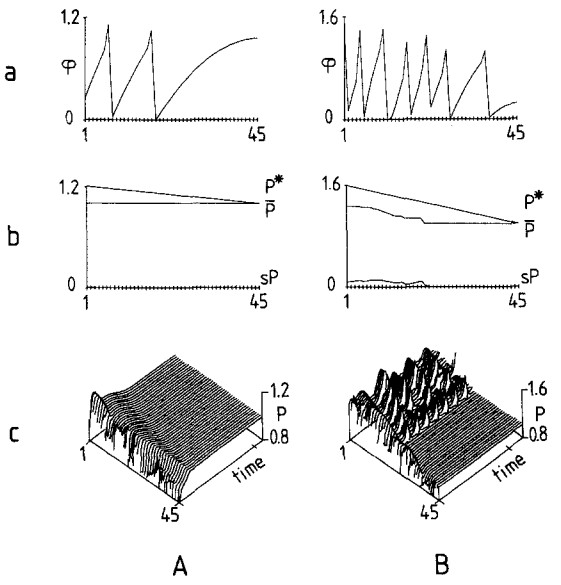

Fig. 6A and B. Complete (**A**) and incomplete synchrony (**B**) in a chain of 45 pacemaker cells, haveing a linear gradient (Ab and Bb) in their intrinsic periods P_i^*. The LPC had a no-effect period of $0.2P_i^*$ and exhibited latency shortenings at $\phi > 0.2$ only. **A** Wave propagation when all the cells are in synchrony. The point of maximal latency shortening of the LPC was choosen in area C (cf. Fig. 4c). The transitions in ϕ (scaled from 0 to P_i^*) indicate the position of an action potential wave front (Aa). The wave fronts originate from the fastest cell (No. 45), while the synchronization period of all cells is equal to P_{45}^* (Ab, c). The average period $\bar{P}$ and its standard deviation sP were obtained from the last 40 cycles of each cell (Ab). **B** Synchrony is incomplete, when the gradient in P_i^* is too steep (Bb). Only the fast cell terminal part of the chain shows synchrony. The slower cells are unable to become synchronized with the faster cells (Bb, c). As a result the phase relationships between the slower cells (Ba) are not constant, as indicated by the variations in P_i (Bb, c)

exactly after an initial transient towards synchrony. This implies that the action potential "propagates" at infinite speed in any direction along the chain, in spite of the very weak coupling between the cells. This contra-intuitive conclusion illustrates that action potential propagation in a cell chain not only depends on passive membrane properties and coupling strength between the cells, but – in contrast to propagation in non-pacemaker tissue – that the automaticity of the cells itself does play an important role as well. This is further exemplified in Fig. 6A, where action potential propagation is shown in a chain of 45 pacemaker cells with a linear gradient in their intrinsic period P_i^* (Fig. 6Ab). The LPC's of these cells had an initial no-effect part of $0.2P_i^*$, followed by a phase advance part. The results were the same when a phase delay part was introduced between the no-effect and the phase-advance part.

Figure 6Ac shows that the cells synchronized within 5–20 cycles to a period P_s equal to the intrinsic period of the fastest cell ($P_s = P_{45}^* = 1$). This is also illustrated in Fig. 6Ab, showing that the mean period ($\bar{P}$) of all cells was equal to the intrinsic period of the fastest cell ($P_{45}^* = 1$), while the standard deviation (sP) in $\bar{P}$ was zero. This rate dominance by the fastest cell occurred whenever a sufficient phase advance was included in the LPC.

In order to become synchronized, the slowest cell (No. 1) had to undergo a phase advance of $(P_1 - P_s)/P_1$ during each cycle. With increasing cell number and, therefore, with smaller intrinsic periods, these phase advances became less within the chain, resulting in the phase relationship given in Fig. 6Aa. The sudden phase transitions in this graph indicate the position of the action potential fronts in the chain at that instant. Successive plots of this phase relationship showed that these action potential fronts moved from the fastest cell (No. 45) to the slowest cell (No. 1). Since the distance between the action potential fronts becomes less as the front moves from left to right, it follows that the "speed of propagation" becomes slowed down. Thus, the speed of action potential propagation in the chain depends on the difference between the local intrinsic frequency and the synchronized frequency. Therefore, if action potential effects are the major interactions in pacemaker cell synchronization in the sinus node, we may conclude that the speed of action potential propagation in this tissue will depend on the spatial distribution of intrinsic periods as well.

The establishment of a smoothly propagating action potential wave along a chain of pacemaker cells may be hindered if the intrinsic period gradient is too steep in relation to the amplitude of the phase advance part of the LPC. This is illustrated in Fig. 6B, where the cells have the same scaled LPC as in Fig. 6A, but follow a steeper gradient in P_i^*. These cells were not able to establish synchrony all along the chain. However, the cells at the fast end of the chain, up to a certain cell number, were able to establish synchrony at a $P_s = P_{45} = 1$. The cells at the slow end of the chain did not establish synchrony, but the interactions between these cells apparently were such, that their intervals varied more or less in a periodic way. This observation suggests that arrhythmia's with periodically fluctuating intervals may arise in the sinus node if the intrinsic frequency of a cluster of pacemaker cells gets increased too much for some reason.

3.5 Local Areas of Synchrony in a Chain of Pacemaker Cells

In an attempt to simulate more realistic pacemaker cell interactions we did a remarkable observation. After introducing sufficient irregularity into the linear intrinsic period gradient of Fig. 6Ab, we found that

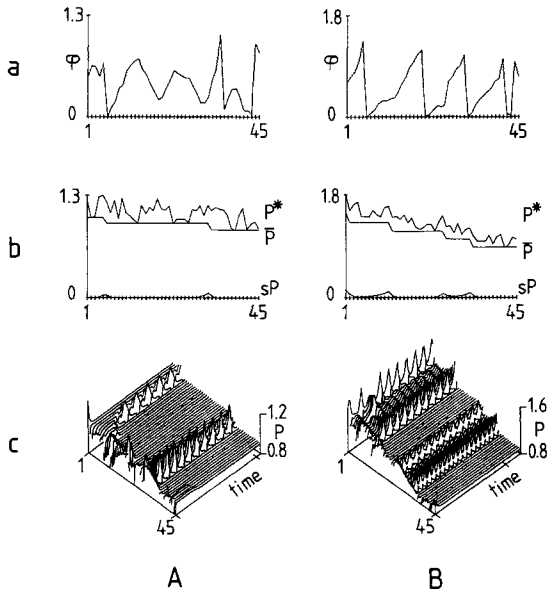

Fig. 7A and B. Local areas of synchrony in a chain of pacemaker cells with an irregular gradient in intrinsic periods P_i^*. The LPC's of the cells are as in Fig. 6. **A** Three local areas of (nearly exact) synchrony for the irregular gradient given in Ab. Within each local area the cells have a constant period P_i (Ab, c). Between the plateau's in P_i, cells exhibit periodically changing intervals. The phase relationship (Aa; scaled from 0 to P_i^*) is irregular and indicates different "sources", from which wave fronts originate in either direction along the chain. **B** Increasing the steepness of the gradient (as in Fig. 6Bb), while maintaining an irregularity in P_i^* (Bb), may increase the number of local area's of synchrony. The phase relationship (Ba) indicates, that wave fronts move from the faster towards the slower cells. Since the areas are synchronized at different frequencies, occasionally propagation will not take place at the crossings

the cells no longer established synchrony all along the chain (Fig. 7A). Instead, local areas of synchrony could develop, separated by asynchronous cells exhibiting more or less periodic interval fluctuations. This happened when the irregularity in the intrinsic periods P_i^* was comparable to the maximum advance of the LPC and provided that the LPC did not exhibit both phase delays and phase advances.

The synchronized periods of these areas in general increased towards the slow end of the chain. The number of synchronized period plateau's could be increased by increasing the steepness of the mean linear intrinsic period gradient (Fig. 7B). The period of one local area of nearly perfect synchrony was equal to the intrinsic period of the fastest cell in that area. This cell then could act as a focus from which the action potential propagated. The irregularity in the distribution of intrinsic periods within one area could also result in an irregular activation pattern, even when the cells were in perfect local synchrony. This observation suggests that inhomogeneities in the intrinsic properties of pacemaker cells in pacemaker tissues may result in multiple foci of impulse initiation.

4. Discussion

The results of the present study demonstrate that pacemaker cell interactions by phase resetting effects of action potentials are sufficient to cause stable in-phase synchrony in a chain of coupled pacemaker cells, provided a latency-phase curve (LPC) of a physiologically acceptable shape is chosen. It is also possible to obtain travelling action potential waves along a chain of synchronized cells, if there is a gradient in the intrinsic frequencies. This one-dimensional activation pattern [and its two-dimensional analogon in a previous model-study (Ypey et al., 1982b)] resembles that in the sinus node. Therefore, we conclude that diastolic interactions, which were not present in our model, are no absolute requirement for stable synchrony in a population of pacemaker cells. The extent to which diastolic interactions play a role in pacemaker cell synchronization has still to be shown. These interactions, for example, may play a supportive role in the synchronization of pacemaker cells, dependent on the strength of coupling between the cells.

Some of the results of this study are relevant to the question: what determines the synchronized frequency of a population of pacemaker cells with different intrinsic frequencies like the sinus node? The classical view on this problem may be explicitly formulated as follows: 1) pacemaker cells interact by action potential effects and 2) an action potential in one pacemaker cell causes a premature (i.e. phase advanced) impulse in a neighbour cell, provided it arrives not too early in the cycle of that cell (i.e. not in its refractory period). Such interactions were considered to result in a synchronized firing of the coupled pacemaker cells at the frequency of the fastest cell. This view is known as the "classical pacemaker hypothesis" (cf. De Haan, 1982). De Haan and Hirakow (1972), Jongsma et al. (1975), Scott (1979), and Ypey et al. (1979) have refuted this hypothesis in coupling experiments on tissue cultured pacemaker cells or cell aggregates, obtained from embryonic chick or neonatal rat hearts. Thus, one or both of the two basic assumptions of the classical pacemaker hypothesis (see above) are inadequate. Indeed, evidence is available (Ypey et al., 1979, 1980), that besides action potential effects, diastolic interactions play a role in pacemaker cell synchronization, especially at strong electric coupling. In addition, it has been shown that action potential effects may cause not only premature but also delayed impulses (Sano et al., 1978). The first finding may restrict the value of the classical pacemaker hypothesis to weak coupling.

The latter finding requires an extension of the classical pacemaker hypothesis by including latency lengthening effects in it. Ikeda's model for pairs of coupled cells (1982) and the present phase reset model of a chain of coupled pacemaker cells also include this extension.

In-phase synchrony is favoured by a physiological type of LPC, including an initial no-effect period, followed by a phase delay and a phase advance part, respectively. Phase relationships between neighbour cells during synchrony then are often such that follower cells (i.e. cells which fire slightly later in time) do not have any back effect on the leading cells (i.e. cells firing just before their neighbours) because their impulses fall within the no-effect period of the leading cells. In that case the net effect of the action potential interactions during synchrony is an accelerating effect of the fastest cell on the slower cells to its own intrinsic frequency. This has been observed for our model (Fig. 6A) and is consistent with the classical pacemaker hypothesis. However, the phase differences between the cells during synchrony could also be such that the follower cells fire in the phase delay part of the LPC of the leading cells. In that case the slower cells will not only be accelerated by the faster cells, but the faster cells will also be slowed down by the slower cells. Then, synchrony will be at a frequency intermediate to the intrinsic frequencies of the fastest and the slowest cell. With our model such observations were made for "two-cell chains" only. Intermediate synchronization frequencies have also been observed in coupling experiments on pairs of pacemaker cells or cell aggregates (De Haan and Hirakow, 1975; Ypey et al., 1979). However, it is still unclear whether these intermediate frequencies arose from phase resetting action potential effects or from diastolic interactions. Experimental separation of action potential effects and diastolic interactions is required to determine the contribution of both types of interaction to the synchronized condition. For example, intermediate synchronization frequencies for coupled pacemaker cells, which lack phase delaying responses to action currents from neighbour cells would indicate a substantial role of diastolic interactions in pacemaker cell synchronization.

The observation, that synchronization in a chain may depend on the initial phases the cells start to interact with (cf. Fig. 4) may be of importance for the understanding of arrhythmia's like fibrillation. A perfectly healthy heart may start to fibrillate when "phase differences" are imposed on the cells of the myocardium by electric shocks. When coronary circulation is kept intact, fibrillation may appear as a stable condition with high rates of discharge of the cells at large phase differences until these phase differences are removed by another electric shock (defibrillation). In order to be able to compare pacemaker cell interaction to non-pacemaker cell interaction by action potential effects, the "phase" of non-pacemaker cells like myocardial cells may be thought of as the time since the last action potential, so that latency-phase-curves (LPC's) can be constructed for these cells as well. Such curves characterize the recovery of excitability of the cells after an impulse and have been used in the reversed form (as "velocity-phase-curves") to study fibrillation (Moe et al., 1964). Then anti-phase synchrony and asynchrony in our simulations may be equivalent to fibrillation of the myocardium, since in both of these cases the mean discharge frequency can be considerably higher than the in-phase synchronization frequency. From this we predict that neighbour cells in the myocardium can discharge in anti-phase during fibrillation, i.e. with nearly alternating activity between neighbour cells (cf. Herbschleb et al., 1982). In addition, the results of the present paper suggest how the tendency to loose in-phase synchrony depends on the shape of the "excitability curve" (the LPC) of the cells.

Our results on a one-dimensional pacemaker cell structure (a "chain") may contribute to a better understanding of cellular interactions in three-dimensional cell structures like the sinus node and the myocardium. The present study is a first step towards a phase reset model of cellular interactions in such complex cell geometries.

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