THE MEMORY EFFECT AND PHASE RESPONSE OF MODEL SINOATRIAL NODE CELLS

A. C. F. Coster, B. G. Celler
Biomedical Systems Laboratory, School of Electrical Engineering, University of New South Wales, Sydney, Australia

Abstract- **Phase resetting of cells in response to a brief current pulse has been observed in a variety of nervous tissues including cardiac cells. External stimulation of the sinoatrial node is due to largely positive currents, depolarising the cells, except for some ACh interactions. Factors influencing this include the timing of the current injection, its polarity, magnitude and duration. Both prolongation and shortening of the interbeat interval can occur.**

The effect of current injection on cells of the sinoatrial node have been analysed using a model cell developed by [1]. This model defines the ionic currents that cause the spontaneous electrical activity of the cells.

***Keywords* - Phase response, sinoatrial node, memory effect**

I. PHASE RESETTING

The application of brief pulses of current to sinoatrial pacemaker cells may result in phase-dependent changes in the cell's cycle length. In general, these applied pulses do not affect the amplitude or the shape of the pacemaker cell action potential. The magnitude and direction of the phase shift depend on the timing as well as on the intensity and duration of the stimulus.

The effects of the external stimuli over the cell's cycle of activity can be summarised as a Phase Response Curve (PRC). This curve defines the phase shift of the discharge of the pacemaker cell with constant intrinsic cycle length as a function of phase at which an external stimulus is applied to the pacemaker action potential. In this study, the maximum downstroke gradient of the pacemaker action potential is taken to be the reference point, i.e. the point of zero phase. The phase ϕ is then defined as $\phi = t/\tau$, when t is the time of stimulus onset and τ is the period of the unperturbed cell cycle. The phase shift, $\Delta\phi$, is either an advance or a delay of the phase, lengthening or contracting the cell's cycle after the stimulus. In general, a pulse applied early in the cycle prolongs that cycle, as opposed to later in the cycle, whereupon it shortens it (for example [2], [3]). An approximation of the cardiac PRC is a linear function of $\Delta\phi$ with ϕ. The slope can be related to fundamental parameters of the oscillator.

Physiological data shows phase resetting in aggregates of ventricular cells [2], both embryonic and adult [4], as well as single sinus cells [5]. Simple Hodgkin-Huxley type [6] ionic models model this effect quite well. More complex models, however, also fit the PRC as well as matching other physiological measurements.

Physiological recordings of cells and their response to external stimuli usually take the form of responses to pulse trains. It has also been observed experimentally, that the period of the cell's cycle does not significantly change in those cycles post the stimulation period [5]. This leads to the hypothesis that there is no "memory" effect in the cell; that is, the spontaneous cycle length is unchanged after applying a single pulse to the system. If the cells do indeed have no "memory" of the perturbation in cycles post the stimulation cycle then it is possible to derive from these experiments the effect of only one such pulse.

When a stimulus is singly applied to the cell, only the cycle in which it is applied is affected. The cell's cycle appears to revert to its intrinsic length, frequency and shape in subsequent cycles after a single pulse.

This assumption may be used to derive a PRC for a single sinus cell [5]. Pulse trains were applied in different entrainment ratios to build up a picture of the phase response of the cell to a particular current stimulus. For 1:1 entrainment, the new cycle length is directly related to the phase of the applied stimulus:

$$\tau = (1 + \Delta\phi)\, T_{fr}$$

where τ is the new cycle length,
T_{fr} is the intrinsic cycle length, and
$\Delta\phi$ is the phase movement due to the stimulus (a fraction of the intrinsic cycle length).

If the stimulus period is too brief to fall within the maximum limit specified by the PRC, 1:1 entrainment is lost, and 2 or more current stimuli may fall within a single pacemaker period. Each can be assumed to act independently and sequentially. Thus in the case of 2 pulses in a single pacemaker cycle, the new cycle length becomes:

$$\tau = (1 + \Delta\phi_1 + \Delta\phi_2)\, T_{fr}$$

Fig. 1 shows the derived PRC.

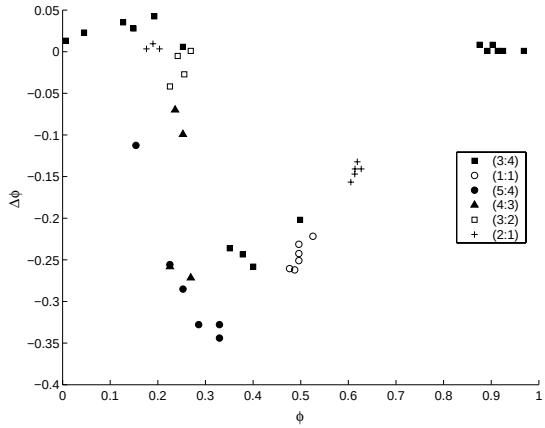

Fig. 1: A steady-state phase response curve ("derived PRC") for an isolated sinus nodal cell.

Redrawn from [5]. Pacemaker cycle length was 280ms. The curve was constructed using a subset of data obtained during repetitive stimulation of the cell. Pulses of 0.17nA in strength and 20ms in duration were used. Phase (ϕ) and phase shift ($\Delta\phi$) are expressed as fractions of the pacemaker intrinsic cycle. (Original figure expressed these as percentages). This figure has re-expressed the data [5], taking the maximum downstroke gradient of the beat to be zero phase. Symbols represent the different entrainment patterns obtained during repetitive stimulation.

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle The Memory Effect and Phase Response of Model Sinoatrial Node Cells	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Biomedical Systems Laboratory School of Electrical Engineering University of New South Wales Sydney, Australia	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
4

The biphasic nature of the PRC can be readily seen. Applying a depolarising stimulus to the cell up to approximately 0.2 of the way through the cell's cycle causes a small increase in the cycle length. After this however, the application of the pulse decreases the cell's cycle quite dramatically.

This also can be seen from PRCs of aggregates of cells (see for example [2], [4]).

II. Ionic Currents Contributing To Membrane Potential

The single cell SA node model used in this study utilises Hodgkin and Huxley formalisms [6] in modelling the cell membrane as a capacitance connected to a number of parallel pathways representing individual ionic current flows. The formulation used is that developed by Dokos [1]. The complete cell model consists of nine membrane currents that interact to generate spontaneous pacemaker activity. These currents are the L-type calcium current $i_{Ca,L}$, the T-type calcium current $i_{Ca,T}$, the fast Na^+ current i_{Na}, the delayed rectifying K^+ current i_K, the hyperpolarisation-activated current i_f, the Na-Ca exchanger i_{NaCa}, the Na-K pump i_p, the background Na^+ current $i_{b,Na}$ and the ACh-activated K^+ current $i_{b,K}$. The model also incorporates variations in extracellular and cytosolic ion concentrations, including Ca^{2+} sequestration by the sarcoplasmic reticulum. We refer the reader to [1] for a complete description of the model.

III. Simulations

Computer simulations of the electrical characteristics of the cell were performed using GENESIS, the Generic Neural Simulation System [7]; implementing each of the currents of the model [1] as well as the relevant concentrations.

The cell was modelled as a cylinder, 100μm in length, 8μm in diameter, yielding a surface area of $2613(\mu m)^2$. The capacitance of the cell was taken to be 32pF. Reference [1] outlined a set of stable initial parameters, and these were used to create an initialisation set whereby the cell could be initialised at any point along this stable cycle.

The timestep used for the simulation was between 0.025ms and 0.001ms. Current pulses were applied at different points along the cycle for a series of durations and magnitudes, and the simulation then continued for another 0.6s (thus ensuring a minimum of one further beat was recorded, bearing in mind that the period of the cell cycle is approximately 0.384s for this model).

IV. Simulation Results

A. Simulated and Experimentally Derived PRCs

Simulations were performed using the Dokos model, perturbing the cell using varying pulse amplitudes and durations, and applying the stimulus during different phases of the cell cycle. The stimulation pulses had magnitudes ranging from 0.01nA to 5nA and durations of 0.1ms to 20ms.

Does the derived PRC [5] above, match that of the simulated cell, exposed to only single pulses?

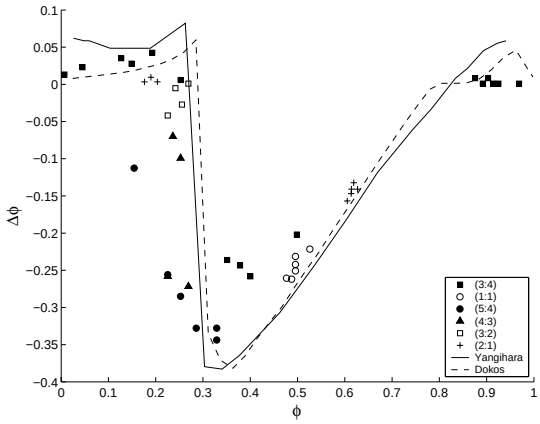

Fig. 2. Comparison of derived PRC [5] and two simulation experiments. The simulated stimulus in the Dokos model was a current pulse of 0.4nA magnitude depolarising the cell for 1.25ms, and $1.1\mu A/cm^2$ for 20ms for the Yanagihara model [5]. The experiment applied 0.17nA for 20ms. Note that the experimental data [5] and Yanagihara simulation data [5] has been adjusted for phase, as their zero phase was at the peak of the action potential, whereas we take the maximum downstroke gradient to be zero phase here.

It is actually possible to obtain a fit of the general characteristics of the PRC with a simpler ionic model. Previously, simulations were made of the Yanagihara model ([8], [5]).

Although the specific cell geometry used in the experiments and simulations of [5] is unknown, on comparing the PRC with those derived from our simulations, the overall shape of the PRC was matched. This can be seen in Fig. 2, which shows an overlay of the experimentally derived PRC with the two simulations.

The fact that the much simpler Yanagihara model produces a PRC that closely resembles the PRC of the model points to the fact that the four, time and voltage independent currents in the Yanagihara model play a major role in shaping the PRC. They are a slow inward current, a fast sodium current, a hyperpolarisation activated current and a potassium current. There is also a time independent leakage current. These as well as other ionic currents are present in the Dokos model. Whilst the simpler model seems to characterise the PRC quite well, the Dokos model also fits data from voltage and patch clamp experiments and is capable of accurately reproducing membrane current kinetics yielding an accurate reconstruction of the basic sinoatrial node action potential waveshape. For a comparison of the Dokos and Yanagihara models (as well as other ionic models) we refer the reader to [9]. In extending the study of the effect of brief depolarising pulses on the pacemaker cell's cycle, we chose to implement the more complex model.

B. No Memory Effect

It has been observed experimentally, that the period of the cell's cycle does not significantly change in those cycles post the stimulation period [5]. This leads to the hypothesis that there is no "memory" effect in the cell; that is, the spontaneous cycle length is unchanged after applying a single pulse to the system.

Indeed, the theory [5] used to "derive" the experimental PRC of Fig. 1 and 2 depend upon this. To investigate, we performed simulations recording the membrane voltages of a

cell exposed to a depolarising pulse and also a control, which was unperturbed. We then plotted the tracings of the membrane potential of these two cells, as well as a third trace, shifting the phase of the control trace to match the phase shift of the perturbed cell.

It was found that over the entire range the simulation experiments performed in this study that subsequent cycles of the perturbed cell matched the period and shape of the control in cycles post the stimulation cycle. Examples of this are shown in Fig. 3 and 4. In this case, the applied pulse was 1nA for 1ms. The stimulus was applied in a series of simulations over a range of onset phases. Fig. 3 shows the membrane voltage as a function of time for the cell showing a control trace, the perturbed cell's trace and a "shifted" trace, where the control trace has been offset by the amount of phase resetting of the perturbed cell. The shifted and perturbed traces overlap completely in cycles subsequent to that in which the pulse was applied. Fig. 4 shows the I-V limit cycle for the cell, the total membrane current versus the membrane voltage. Again both the control and perturbed traces are shown for comparison. The systems move clockwise in time. Upon application of the pulse, the perturbed trace deviates from the control limit cycle, but returns to it in subsequent cycles.

This phenomenon was investigated for a range of stimulation pulses, magnitudes ranging from 0.01nA to 5nA and durations of 0.1ms to 20ms. These were applied at points distributed along the whole cycle.

IV. Discussion

The comparison of the model with the experimentally derived PRC [5] shows that the overall shape and attributes of the PRC are similar. This gives credence to both the Dokos model, as well as the assumptions made in [5] that the cell cycle is unaltered by small depolarising pulses after the initial cycle in which the perturbation is applied.

Indeed, when stimulations were performed with the model, the cell cycle returned to its original characteristics of cycle length and shape for cycles past that in which it was perturbed. The only effect was to possibly alter its phase. This was true for the entire range of perturbation characteristics investigated. This is indeed the hypothesis used in [5] to derive a PRC from results of pulse trains of differing entrainment ratios.

As can be seen from the examples presented, (see Fig. 3), the greatest deviation from the limit cycle occurs when the pulse is applied near the maximum upstroke of the beat. This does not, however, mean that the largest phase resetting ensues at this onset phase. Rather, this is nearer mid-phase, (~0.2-0.3 depending on the particular stimulus), see for instance Fig. 2, when the cell membrane voltage is only gently rising.

The existence of a single, stable limit cycle for this system also indicates that other, more abstract models for the sinoatrial node can also represent some important features. See for instance models based on Bonhoeffer-van der Pols formalisms (for example [10]).

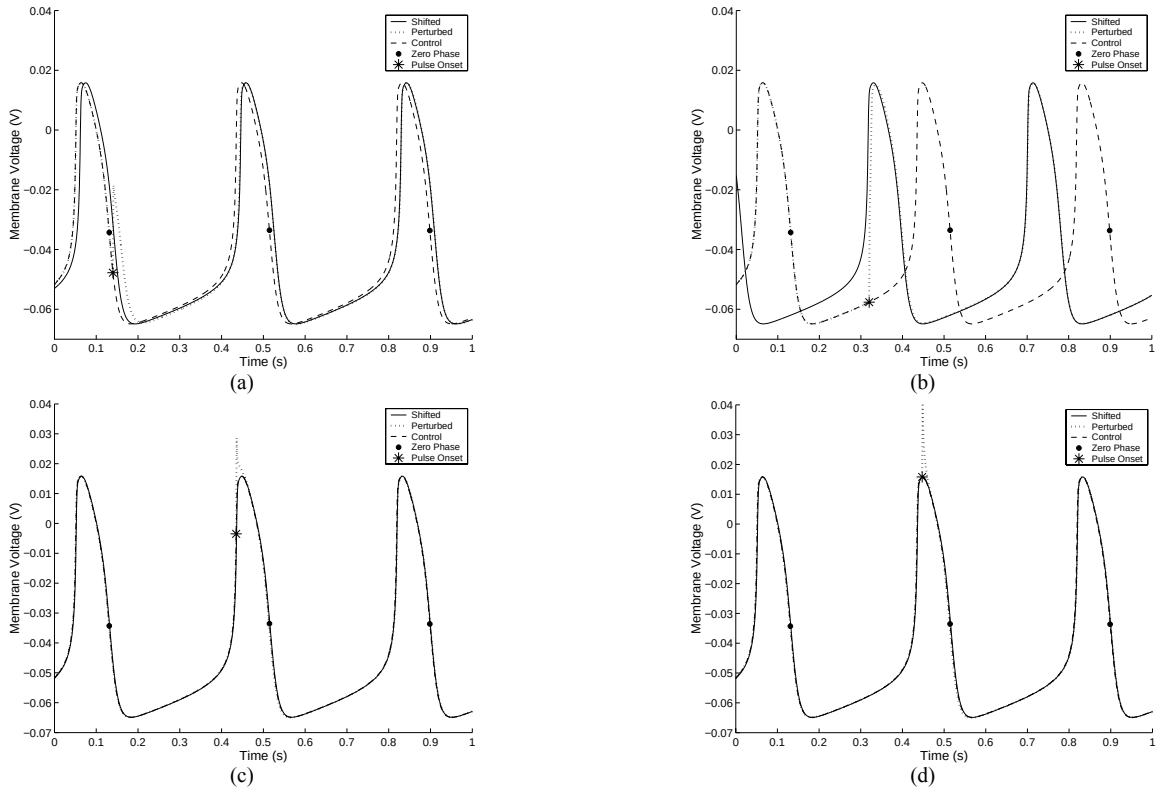

Fig. 3 The Membrane Voltage trace for the cell showing a control trace, the perturbed trace. The applied pulse was 1nA for 1ms at the phases indicated. A "Shifted Control" trace is also shown for comparison, where the control trace has been shifted by the amount of phase resetting of the perturbed cell. Note that the shifted control and perturbed traces overlap completely in cycles subsequent to that in which the pulse was applied. Phases for the plots are approximately: (a) 0.02, (b) 0.5, (c) 0.79 – the maximum upstroke of the beat, (d) 0. 83 – the peak of the beat.

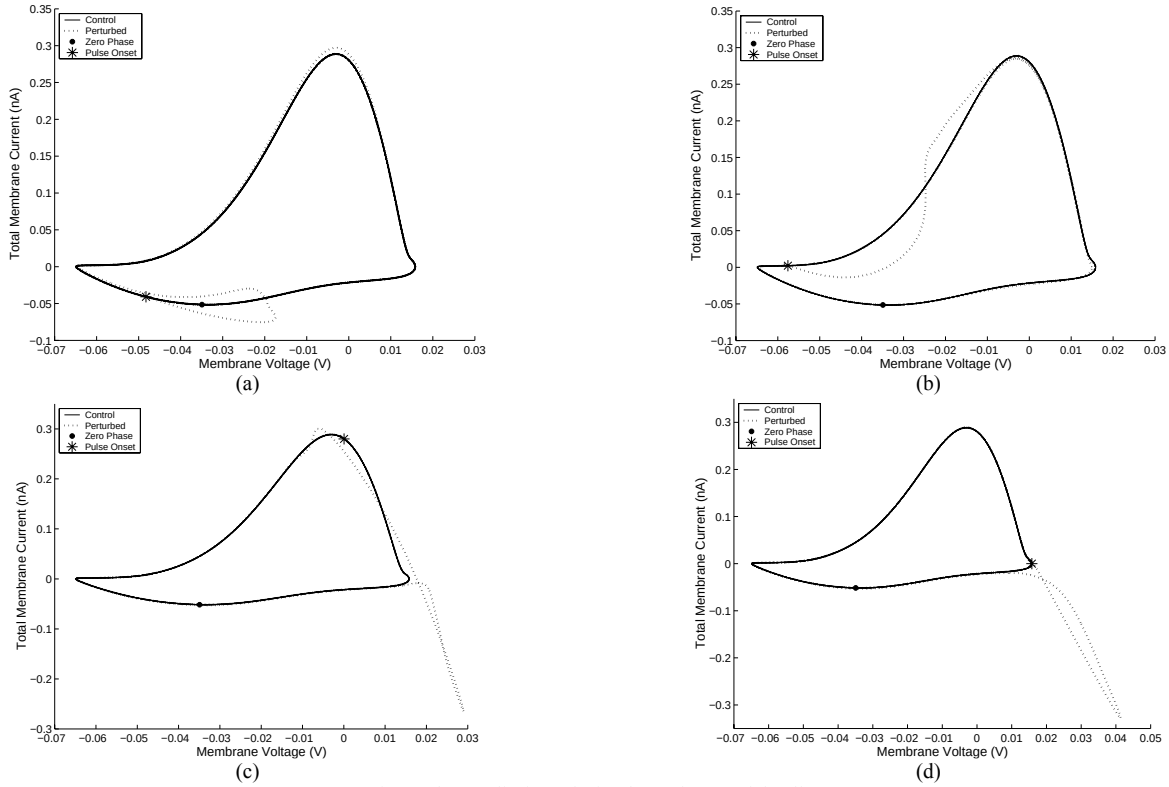

Fig. 4 The I-V limit cycle for the Dokos model cell.
The applied pulse was 1nA for 1ms at the phases indicated. Both the control and perturbed traces are shown for comparison. The traces move clockwise in time. Upon application of the pulse, the perturbed trace deviates from the control limit cycle, but returns to it in subsequent cycles. Phases for the plots are approximately: (a) 0.02, (b) 0.5, (c) 0.79 – the maximum upstroke of the beat, (d) 0.83 – the peak of the beat. Note the different scales for the figures.

V. Conclusion

Our simulations of the Dokos model of a sinoatrial node cell have shown that the cells cycle in a stable limit cycle. When perturbed, they exhibit phase-resetting behaviour where the cycle shifts in phase but in no other characteristic. This concurs with the hypothesis of "no memory" of the cells, used to derive phase response curves for particular single pulses from data recorded from series of such pulses.

We have also shown that whilst the greatest deviation from the cell's normal limit cycle occurs near the maximum upstroke of the beat, this does not lead to the greatest phase resetting. Indeed, the greatest phase resetting effect appeared to occur during the gradual increase in membrane potential subsequent to the beat.

References

[1] Dokos, S., B. Celler, N. Lovell "Ion Currents Underlying Sinoatrial Node Pacemaker Activity: A New Single Cell Mathematical Model." *J Theor Biol* vol. 181(3): 245-272, 1996.

[2] Clay, J. R., M. R. Guevara, A. Shrier "Phase Resetting of the Rhythmic Activity of Embryonic Heart Cell Aggregates." *Biophys J* vol. 45: 699-714, 1984.

[3] Abramovich-Sivan, S. and S. Akselrod "A single pacemaker cell model based on the phase response curve." *Biol Cybern* vol. 79(1): 67-76, 1998.

[4] Guevara, M. R., L. Glass, A. Shrier "Phase Locking, Period-Doubling Bifurcations, and Irregular Dynamics in Periodically Stimulated Cardiac Cells." *Science* vol. 214: 1350-1353, 1981.

[5] Anumonwo, J. M. B., M. Delmar, A. Vinet, D. C. Michaels, J. Jalife "Phase Resetting and Entrainment of Pacemaker Activity in Single Sinus Nodal Cells." *Circ Res* vol. 68: 1138 – 1153, 1991.

[6] Hodgkin, A. L. and A. F. Huxley "A quantitative description of membrane current and its application to conduction and excitation in nerve." *J Physiol (Lond)* vol. 117: 500 – 544, 1952.

[7] Bower, J. M. and D. Beeman *The Book of GENESIS: Exploring Realistic Neural Models with the GEneral NEural SImulation System*, 2nd Ed. TELOS/Springer-Verlag, 1998.

[8] Yanagihara, K., A. Noma, H. Irisawa "Reconstruction of sino-atrial node pacemaker potential based on the voltage clamp experiments." *Jpn J Physiol* vol. 30: 841 – 857, 1980.

[9] Dokos, S., N. H. Lovell, B. G. Celler "Review of ionic models of vagal-cardiac pacemaker control." *J Theor Biol* vol. 192(3): 265-274, 1998.

[10] Sato, S., S. Doi, T. Nomura. "Bonhoeffer-van der Pol Oscillator Model of the Sino-Atrial Node: A Possible Mechanism of Heart Rate Regulation." *Meth Inform Med* vol. 33: 116-119, 1994.