

ON THE PHASE-RESPONSE CURVES OF REPETITIVELY ACTIVE NEURONES

M. BARBI,* P. G. HAYDON,** A. V. HOLDEN*** and W. WINLOW
Department of Physiology, University of Leeds
Leeds LS2 9JT, U.K.

(Received November 3, 1983)

Abstract

The first transient phase response curves of repetitively active molluscan neurones are obtained. Electrically coupled neurones, that can be driven by maintained currents, are insensitive to brief current pulses. The transient phase response curves show considerable scatter even when synaptic transmission is blocked. First transient and steady state phase response curves are computed for a simple neural model, and the separation between the first transient and steady state curves increases as the amplitude of the perturbing pulse increases. As the magnitude of the perturbing pulse increases, the calculated steady state phase response curves change from Type 1 to Type 0, and so there is the possibility of the annihilation of repetitive activity by an appropriate perturbing pulse.

1. Introduction

Many neurones in the central nervous system exhibit an irregular, background discharge of action potentials. Mathematical models for this repetitive activity are reviewed in Holden (1976, 1982), and this activity is generated either as the response to synaptic inputs (the effect of the background activity of other neurones), or by pacemaker mechanisms intrinsic to the neurone, or by a mixture of both these endogenous and exogenous mechanisms.

Whatever the mechanisms that generate the background discharge, and whatever the functions that may be attributed to the background activity, it may be modified by synaptic inputs or experimentally applied currents. In chemical synapses the interaction of the transmitter with its receptor site causes conductance changes that allow ionic currents to flow through the sub-synaptic membrane; these synaptic currents may be inward or outward, or a sequence of inward and outward currents as in biphasic postsynaptic potentials. In electrical synapses and during electrical stimulation a current pulse flows across the membrane. An inward, depolarising current is usually considered to be excitatory, in that it moves the membrane potential away from its resting value towards threshold; however, the effect of a depolarising current pulse on a repetitively active cell depends on when the pulse is

*Istituto di Biofisica, C.N.R., Via Lorenzo 26, 56100 Pisa, Italy

**Department of Zoology, University of Iowa, Iowa City, Iowa 52242, U.S.A.

***To whom correspondence should be addressed

applied. Depolarising and hyperpolarising perturbations applied to a repetitively active cell can either advance or delay the occurrence time of the next action potential: if the average interspike interval of the unperturbed discharge is taken to represent the period of the repetitive activity, the effect of depolarising or hyperpolarising perturbations depends not only on their amplitude and duration, but also on their position within the unperturbed period, or phase (Best, 1979; Winfree, 1980).

In this paper we describe the effects of brief current pulse perturbations on the background activity of identified giant neurones of the freshwater gastropod mollusc, *Lymnaea stagnalis* (L.). We have previously described the background activity of these neurones and their responses to applied currents (Holden and Ramadan, 1981) and pharmacological agents (Holden and Winlow, 1982; Holden *et al.*, 1982).

2. Methods

The experimental methods and salines were as in Winlow and Benjamin (1976). Giant somata were identified on the basis of location, colour, size and electrophysiological properties (Winlow and Benjamin, 1976; Winlow *et al.*, 1982). The identified soma was penetrated by a single microelectrode, with a resistance of 10–20 Mohm, that was filled with the supernatant from a saturated solution of K_2SO_4 . A WPI M701 microprobe system was used both for recording signals and injecting current pulses: during current injection the membrane potential signal is biased, but the time of the perturbation and the times of action potentials may be accurately obtained. Signals were recorded on ultra-violet light sensitive paper using a Medelec FOR-4, with a paper speed that gave the period of the repetitive discharge greater than 5 cms. Measurement of the position of perturbations and action potentials was to the nearest mm, giving an accuracy of phase measurements of better than 2% (7.2°).

3. Results

In the *Lymnaea* isolated brain preparation in standard saline the same identified neurone may exhibit a variety of behaviours — it may be silent, perhaps endogenously active, or driven by synaptic inputs. However, the types of discharge pattern that are observed are consistent for given identified neurones. In these experiments we selected giant cells with a fairly regular discharge (coefficient of variation of interspike interval of background activity less than 10%), and in which patterned synaptic inputs were not apparent.

Solitary, non-coupled cells

Many of the giant neurones of *Lymnaea* are solitary, in that they are not electrotonically coupled to other cells: examples are the paired cells VV 1 and 2 (Holden and Ramadan, 1981) and the dopaminergic neurosecretory cell R.Pe.D.1 (Winlow *et al.*, 1982).

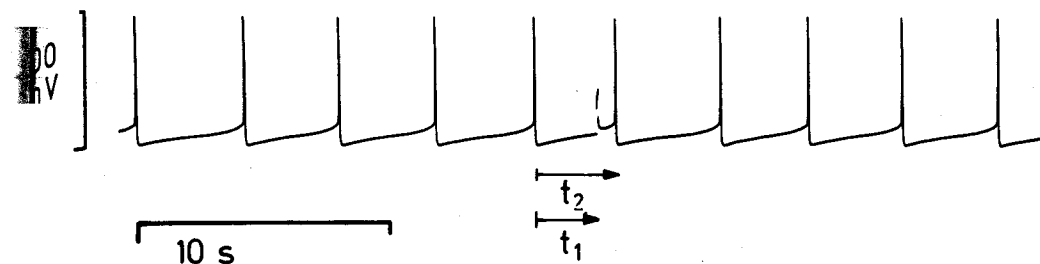


Fig. 1. Background activity of R.Pe.D.1 interrupted by a 30 ms, 0.5 nA depolarising pulse applied at a phase t_1/T , where T is the period of the unperturbed activity.

The background discharge of such solitary giant somata may be altered by intrasomatic current perturbations: this is illustrated in Fig. 1. Injection of a brief (less than 100 ms) current pulse can advance or delay the time to the next action potential. If the background discharge was strictly periodic, with a period T , then the application of a perturbing pulse t_1 ms after the preceding action potential would apply the perturbation at a phase $\phi = t_1/T$. If the next action potential will occur t_2 ms after the preceding action potential it would have been delayed by $\Delta\phi = t_2/T - 1$.

The background discharge of neurones is stochastic, rather than strictly periodic, and so the period T may be estimated by either the interspike interval preceding the perturbation (for a regular discharge), or by the mean interspike interval of the activity preceding the perturbation (for less regular discharge). Figure 2 a–d show the phase response curves for R.Pe.D.1 subjected to 30 ms duration current pulse perturbations: in Figure 2a the period T was estimated by the preceding interspike interval, while in Figure 2 b–d T was estimated by the mean pre-perturbation interspike interval. Use of the averaged interspike interval reduces, but does not abolish, the scatter in the phase response plot: this reflects the stochasticity of the background discharge.

The data points in the phase response plots for the depolarising perturbation (Fig. 2 a, b) fall into two regions: for phases from 0 to close to 0.5 the perturbing pulse has little consistent effect, and although the scatter is wide there is a tendency for the depolarising perturbation to delay the time of occurrence of the next action potential. For applied phases greater than about 0.5 each perturbation evoked an action potential: the points are distributed about a line with slope +1. These two regions are separated by a boundary so narrow that, given the variability of the background activity and the concomitant scatter in the phase response plane, it is indistinguishable from a discontinuity.

The phase response points for hyperpolarising perturbations do not show such a discontinuity — although there is scatter, the hyperpolarising perturbations tend to delay the next action potential. Thus the shape of the phase response plots can be

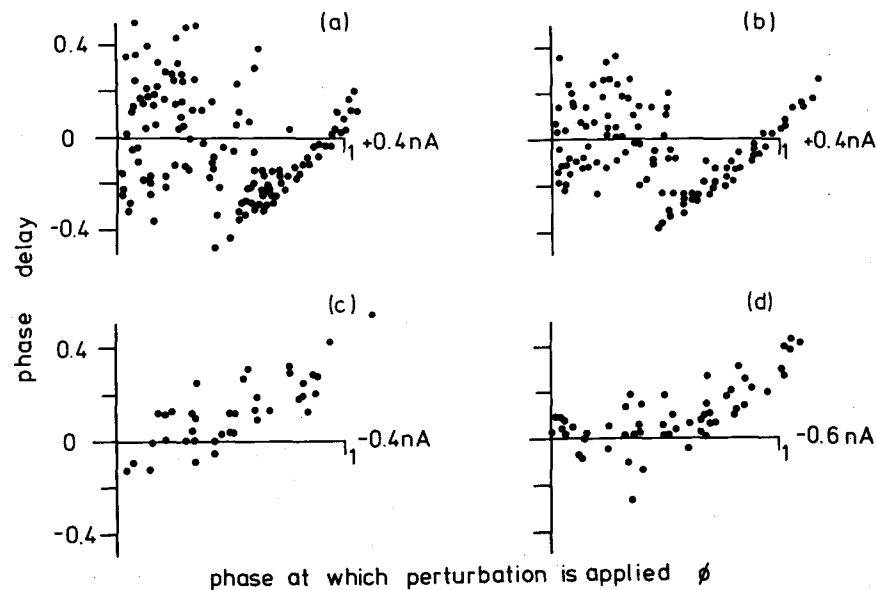


Fig. 2. First transient phase response plots for R.Pe.D.1 subjected to 30 ms duration perturbation. (a, b): 0.4 nA depolarising perturbation; in (a) the period T is estimated by the interspike interval that immediately preceded the perturbation, in (b-d) T is estimated by the average of the interspike intervals that preceded the perturbation. (c) -0.4 nA and (d) -0.6 nA hyperpolarising perturbations.

changed by changes in the amplitude and polarity of the perturbing pulses, from that with a discontinuity to a smooth curve with an average slope close to 0.

Coupled neurones

Many neurones of *Lymnaea* occur in groups of cells of similar morphology that are electrically coupled — an example is the pleural D group (Haydon and Winlow, 1982), and some giant somata are electrotonically coupled to other giant neurones or cell groups — L. and R.Pe.D.4 are electrically coupled. Current injected into the soma of such a cell will spread, via the low resistance connections, to the other coupled cells. Figure 3 shows the phase resetting characteristics of L.Pe.D.4: the effect of the 30 ms depolarising current pulse is independent of the phase at which it is applied, and is weak. However, the cell can be driven by maintained currents.

Solitary neurones in low Ca^{2+} saline

The scatter in the phase response plots is partly due to variability produced by synaptic inputs — chemical synaptic transmission may be blocked by bathing the

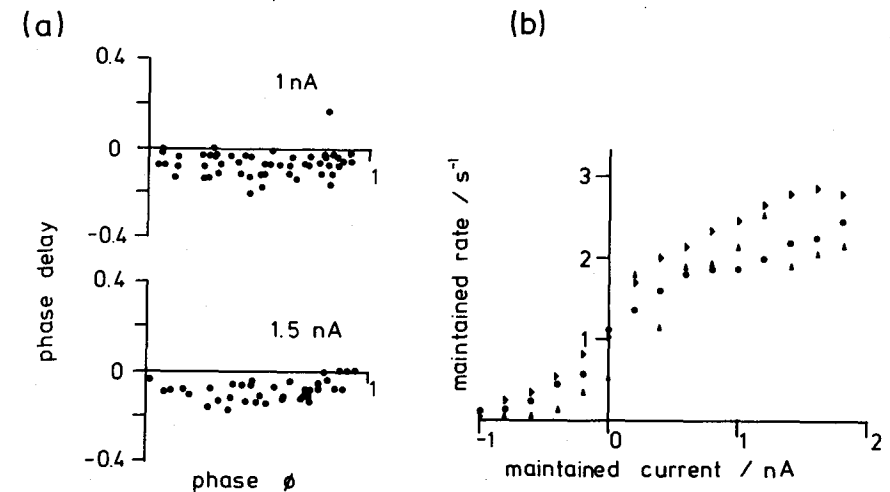


Fig. 3. (a) First transient phase response plots for L.Pe.D.4 (an electrically coupled neurone) with a 1 and 1.5 nA, 30 ms depolarising current pulse. (b) Maintained discharge rate of L.Pe.D.4 plotted against maintained current. Each point is obtained from 1 minute of adapted discharge.

isolated brain preparation in a Ca^{2+} -free saline. Phase response plots for a neurone in Ca^{2+} -free, Mg^{2+} -substituted saline are shown in Figure 4: even when the depolarising current pulse evokes an action potential when it is applied at a phase between 0.5 and 1 there is no discontinuity in the phase response plot.

4. Numerical simulations

The properties of molluscan somata are complex (Adams *et al.*, 1980; Holden and Winlow, 1983), but it is possible to represent the dynamics of these neurones by simple models. Since the different giant neurones of *Lymnaea* differ markedly in their discharge patterns and action potential trajectories (Winlow *et al.*, 1982), a simple model can only represent a caricature of the behaviour seen in experiments.

Hindmarsh and Rose (1982) have proposed such a simple model for the behaviour of *Lymnaea* neurones: this model is an extension of the Bonhoeffer-van-der-Pol approach of FitzHugh (1969). The model consists of two coupled differential equations:

$$\begin{aligned} dx/dt &= -a(f(x) - y - z) \\ dy/dt &= b(f(x) - q e^{rx} + s - y), \end{aligned} \quad (1)$$

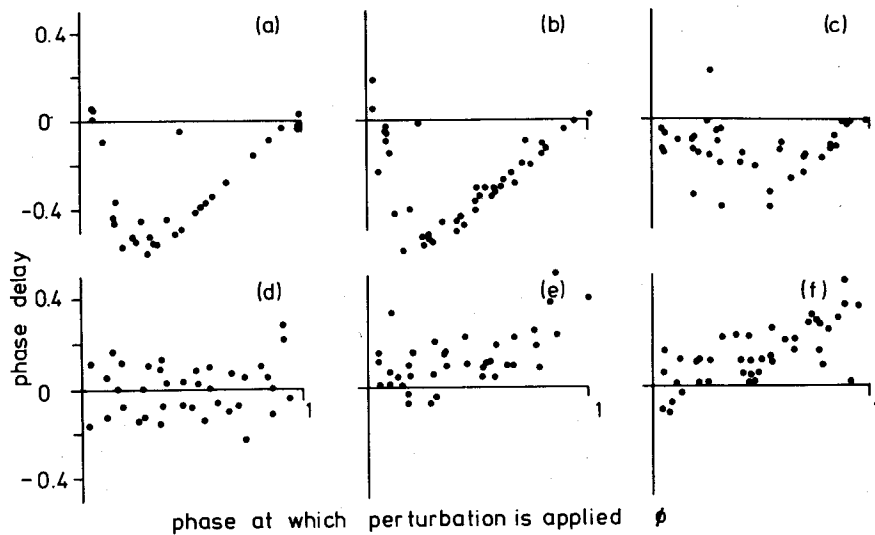


Fig. 4. First transient phase response plots for L.Pe.D.1 (a solitary neurone) in 0 Ca^{2+} , 6 mM Mg^{2+} snail saline, with 30 ms current pulses of (a) 0.4 (b) 0.3 (c) 0.25 (d) 0 (e) -0.25 and (f) -0.3 nA.

where x represents the membrane potential, z the applied current and the nonlinear function $f(x)$ is given by the cubic polynomial

$$f(x) = c x^3 + d x^2 + e x + h,$$

with $a = 5.4 \text{ Vs}^{-1}$, $b = 30 \text{ s}^{-1}$, $c = 1.7 \times 10^{-5}$, $d = -10^{-3}$, $e = -10^{-2}$, $h = -0.1$, $q = 0.024$, $r = 0.088$, $s = 0.046$. These numerical values are taken from Hindmarsh and Rose (1982), who obtained them by fitting the voltage-dependence of the peak inward and steady-state outward currents seen under voltage clamp. Numerical solutions of (1) using these values reproduce the voltage trajectory both during the action potential and during the interspike interval for a giant neurone in the visceral ganglion; some problems of this model are discussed in Game (1982, 1983) and Barbi and Holden (1984).

We have numerically investigated the behaviour of this system when it is driven into periodic activity by a constant current z , and perturbed by brief current pulses. For z greater than -0.026 nA the system has an elementary stable limit cycle.

We have perturbed the periodic solution to $z = 0.033$ nA, with a current pulse of duration 0.015 s and amplitude μ nA. The effect of the perturbation is characterised by the phase response curve, a plot of phase shift $\Delta \phi$ against the phase ϕ at which the perturbation is applied ("old" phase). A different way of plotting the results would be

by a phase transition curve, a plot of "new" phase ($\phi - \Delta \phi, \text{mod } 1$) against ϕ : see Kawato, 1981; Winfree, 1980. A first transient phase response curve is obtained by measuring the phase shift of the action potential immediately after the perturbation, and the steady state phase response curve is obtained by measuring the phase shift of the n -th action potential after the perturbation, with $n \geq 2$ (but $n = 2$ was always sufficient).

Figure 5 shows the steady state and first transient phase response curve for this model: as the amplitude μ of the perturbation increases in absolute value, the steady state phase response curves change from Type 1 to Type 0. A Type 1 phase response curve has an average slope of zero, and the corresponding phase transition curve has an average slope of one; a Type 0 phase response curve has an average slope of minus one, and the corresponding phase transition curve has an average slope of zero. A change from a Type 1 to Type 0 steady state phase response curve implies that an

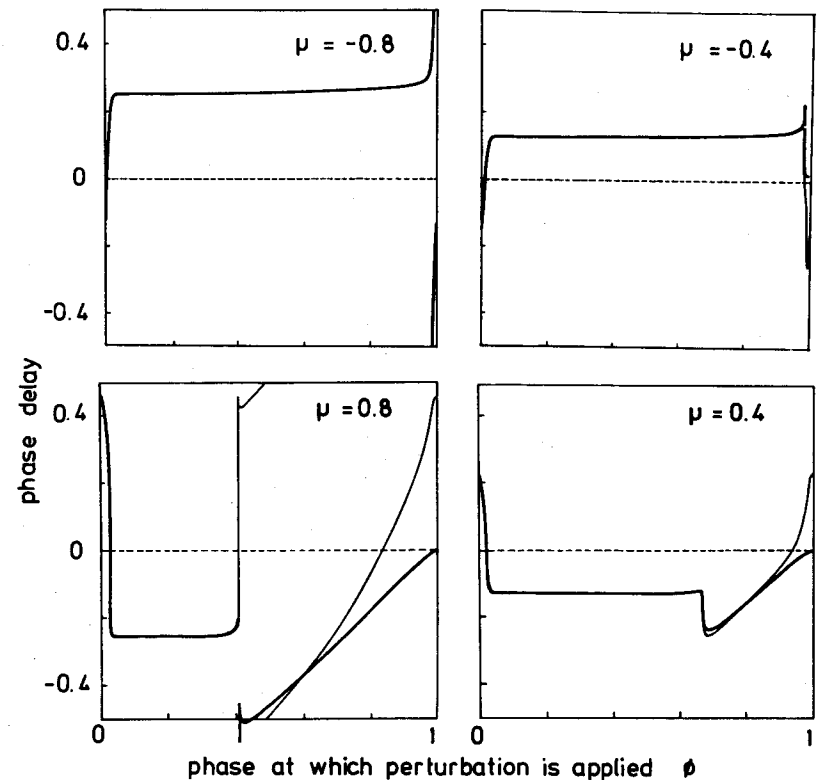


Fig. 5. First transient (thick lines) and steady state (thin lines) phase response curves from equation (1), with $z = 0.033$ nA, pulse duration 0.015 s and $\mu = -0.8, -0.4, 0.4, 0.8$ nA.

appropriately timed perturbation of the appropriate amplitude could annihilate the periodic activity.

There are technical problems in the evaluation of these steady state phase response curves. The closed locus of the representative state points at the end of a perturbation intersects in two points the $dx/dt = 0$ isocline of the system: close to one of these intersections sample trajectories after a perturbation of given amplitude can be very different. It is necessary to confirm precisely the continuation of the new phase as a function of the old phase across the phase ϕ^* corresponding to this intersection.

The system (1) is two-dimensional, and so the null space is a single point: the change in the phase response curve from Type 1 to Type 0 occurs at a single value of the amplitude μ of the perturbation. For this critical amplitude annihilation of repetitive activity will only occur (in the absence of noise) at one precise phase. However, the excitation system based on a full description of the ionic currents measured under voltage clamp will be of high order, and so annihilation of repetitive activity in a neurone should be produced by a range of perturbation phases and amplitudes.

In our experimental results, the first transient phase response curves were estimated. In any real experimental estimation of phase response curves, transient rather than steady state phase response curves will be estimated. The first transient phase response curve is the simplest to estimate, and choice of the first transient phase response curve avoids the slow time constant processes, such as adaptation, that are not considered in the model (1).

The first transient phase response curves of this model have been obtained by defining the time of the event (the action potential) by the time at which $dx/dt = 0$ with $d^2x/dt^2 < 0$; this local maximum naturally corresponds to the peak of the action potential. For precision, we have also accepted as events local maxima that occur during the perturbation.

The first transient phase response curves are shown in Figure 5, by the thick lines.

As the amplitude of the perturbation moves in the depolarising direction the separation between the first transient and the steady state phase response curves increases.

5. Discussion

In this paper we have presented the results of experiments as well as numerical simulations. A problem in the interpretation of the experimental results is produced by the variability of neuronal discharge. The period T may be estimated by the average period, or by the measurement of the interspike interval that immediately precedes the perturbation. Variability introduces an ambiguity into both the concept and measurement of phase: during a longer than average interval, it is possible to apply a perturbation at a phase greater than 1.

The calculated steady state phase response curve for $\mu = \pm 0.8$ nA are of Type 0; however, the rapid change in $\Delta\phi$ with ϕ that occurs in a narrow range of phase angles would make difficult to ascertain this in a real system.

A change from Type 1 to Type 0 phase response curves implies that the periodic activity may be annihilated, or that there is a stable equilibrium as well as a stable periodic solution to the excitation equations. These two stable solutions will be separated by an unstable periodic solution, that generally emerges from the stable equilibrium solution at a subcritical Hopf bifurcation (Holden and Winlow, 1983).

Annihilation of regular repetitive activity has been seen in axonal membrane (Guttman *et al.*, 1980), and the possibility of annihilation could allow appropriately timed, brief synaptic currents to act as gating pulses, switching on or off a repetitively active neurone. However, this is an unlikely function, as the range of overlap of stable periodic and equilibrium solutions of excitation equations close to subcritical Hopf bifurcation points is much narrower than the range of parameters where periodic solutions are the only stable solutions. Further, neuronal variability would make such control unreliable.

Acknowledgements

This work was partially supported by the C.N.R., grant number 90701, by the British Council, and used equipment provided by the (U.K.) S.E.R.C., and was carried out as part of a C.N.R.-British Council progetto bilaterale.

References

- Adams, D. J., Smith, S. J. and Thompson, S. H. (1980). Ionic currents in molluscan soma. *Ann. Rev. Neurosci.* **3**, 141-167.
- Barbi, M. and Holden, A. V. (1984). The phase resetting characteristics of endogenously active neurones. Proc. Conf. Mathematics in Biology and Medicine, Bari, June 1983. *Lecture Notes in Biomathematics*. Springer-Verlag: Berlin.
- Best, E. N. (1979). Null space in the Hodgkin-Huxley equations — a critical test. *Biophys. J.* **27**, 87-104.
- FitzHugh, R. (1969). Mathematical models of excitation and propagation in nerve in: *Biological Engineering*, ed. H. P. Schwan. McGraw-Hill: New York.
- Game, C. J. A. (1982). BVP models of nerve membrane. *Nature* **299**, 375.
- Game, C. J. A. (1982). BVP models: an adjustment to express a mechanism of inactivation. *Biological Cybernetics* **44**, 223-229.
- Guttman, R., Lewis, S. and Rinzel, J. (1980). Control of repetitive firing in squid axon membrane as a model for a neurone oscillator. *J. Physiol.* **305**, 377-395.
- Haydon, P. G. and Winlow, W. (1982). Multipolar neurones of *Lymnaea stagnalis*. I. Multiple spike initiation sites and propagation failure allow neuronal compartmentalization. *J. Comp. Physiol.* **147**, 503-510.
- Holden, A. V. (1976). Models of the Stochastic Activity of Neurones. *Lecture Notes in Biomathematics* **12**. Springer-Verlag: Berlin.
- Holden, A. V. (1982). The mathematics of excitation in: *Biomathematics in 1980*, ed. L. M. Ricciardi and A. C. Scott. North-Holland: Amsterdam.

- Holden, A. V. and Ramadan, S. M. (1981). The response of a molluscan neurone to a cyclic input: entrainment and phase-locking. *Biological Cybernetics* **41**, 157-163.
- Holden, A. V. and Winlow, W. (1982). Bifurcation of periodic activity from periodic activity in a molluscan neurone. *Biological Cybernetics* **42**, 189-194.
- Holden, A. V. and Winlow, W. (1983). Neuronal activity as the behaviour of differential system. I.E.E.E. Trans. S.M.C. (in press).
- Holden, A. V. and Winlow, W. (1983). Neuronal activity as the behaviour of differential system. I.E.E.E. Trans. S.M.C. **13**, 711-719.
- Hindmarsh J. L. and Rose, R. M. (1982). A model of the nerve impulse using two first order differential equations. *Nature* **296**, 162-164.
- Kawato, M. (1981). Transient and steady-state phase response curves of limit cycle oscillators. *J. Math. Biol.* **12**, 13-30.
- Winfree, A. (1980). *The Geometry of Biological Time*. Springer-Verlag: Berlin.
- Winlow, W. and Benjamin, P. R. (1976). Neuronal mapping in the brain of the pond snail, *Lymnaea stagnalis* (L) in: *Neurobiology of Invertebrates, Gastropoda Brain*, ed. J. Salánki. Akademiai Kiado: Budapest.
- Winlow, W., Holden, A. V. and Haydon, P. G. (1982). Characterization of *Lymnaea* neurones by determination of action potential trajectories. *J. Exp. Biol.* **99**, 207-221.