

Sussex Research

Models wagging the dog: are circuits constructed with disparate parameters?

Thomas Nowotny, Attila Szucs, Rafael Levi, Allen I. Selverston

Publication date

01-01-2007

Licence

This work is made available under the **Copyright not evaluated** licence and should only be used in accordance with that licence. For more information on the specific terms, consult the repository record for this item.

Citation for this work (American Psychological Association 7th edition)

Nowotny, T., Szucs, A., Levi, R., & Selverston, A. I. (2007). *Models wagging the dog: are circuits constructed with disparate parameters?* (Version 1). University of Sussex. <https://hdl.handle.net/10779/uos.23312492.v1>

Published in

Neural Computation

Link to external publisher version

<https://doi.org/10.1162/neco.2007.19.8.1985>

Copyright and reuse:

This work was downloaded from Sussex Research Open (SRO). This document is made available in line with publisher policy and may differ from the published version. Please cite the published version where possible. Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners unless otherwise stated. For more information on this work, SRO or to report an issue, you can contact the repository administrators at sro@sussex.ac.uk. Discover more of the University's research at <https://sussex.figshare.com/>

Models wagging the dog: Are Circuits Constructed with Disparate Parameters ?

Thomas Nowotny, Attila Szücs, Rafael Levi, Allen I. Selverston

Institute for Nonlinear Science, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0402, tnowotny@ucsd.edu

Abstract

In a recent paper Prinz et al. (Nature Neurosci. 7, 1345-52 (2004)) have addressed the fundamental question, whether neural systems are built with a fixed blueprint of tightly controlled parameters or in a way in which properties can vary largely from one individual to another, using a database modeling approach. In this article we examine the main conclusion that neural circuits indeed are built with largely varying parameters in light of our own experimental and modeling observations. We critically discuss the experimental and theoretical evidence including the general adequacy of database approaches for questions of this kind and come to the conclusion that the last word for this fundamental question has not yet been spoken.

Introduction

A major factor in the success of determining the precise synaptic connectivity of invertebrate circuits has been the ability to identify individual neurons. The advantage of being able to work with identifiable neurons was recognized early on by those working on *Aplysia* and lobster ganglia (Arvanitaki and Chalazonitis 1958; Kandel et al. 1967; Otsuka et al. 1967) who gave names to individual neurons based on their physiological and anatomical properties. Although the identified neurons always behaved consistently, with small variations attributed to the dissection or the physiological techniques used, little thought was given to how different populations of ion channels were combined to produce the characteristic voltage output of each cell. When neural circuits were established between identified cells, similar questions relating to the variability of identified synapses were not addressed either.

Are identified neurons made up of the same type and density of ionic channels or are the channels arranged differently, but in a way that gives each neuron its desired properties? Are the synapses within a particular circuit the same or different for each animal but balanced in a way that insures that the circuit performs equivalent operations from animal to animal? These two fundamentally different ways of building cells and circuits would have important implications for biologists as well as for modelers.

A new modeling study based on a three neuron circuit from the lobster pyloric central pattern generator (CPG) (Prinz et al. 2004), raises important issues directly related to this question. It is well known that when constructing conductance based models with different ionic channels and synaptic types, extreme sensitivity to parameter values is common, even in relatively simple systems. Thus the method used to assign these values becomes a critical matter when trying to “tune” the circuit. Using a unique method to

address this problem, Prinz et al (2004) developed a large database by computing and evaluating the output of thousands of circuits over a wide range of parameter values and selecting those sets, which produced outputs that matched the biological pattern. The outcome of this modeling work suggested that the values of the neuronal and synaptic parameters in the pyloric CPG could in fact be combined in many different ways, each being able to generate equivalent pyloric patterns. These results have extremely important implications for how neural circuits might be assembled in general. But they also raise some questions that would be troubling for research on small CPG circuits.

There are many reasons to be initially skeptical of the idea that ion channels and other cell and circuit properties can, by some unknown mechanism, be mixed and matched but still generate the same functional pyloric pattern. One should, however, never dismiss an interesting idea without probing it carefully. Therefore, we have tested several predictions based on this hypothesis and examined the details of the “database approach”, that led to it. On the circuit level, we carried out a series of experiments by blocking a single channel type in all neurons in the intact pyloric circuit. We reasoned that if there were disparities in the conductance of this channel, there would also be variations in the effects of its selective blocking. We repeated the same manipulations in the “equivalent circuit models” which did include different values for the conductance of the blocked channel. On the single cell level we compared the variability of isolated cell dynamics in experiments and models and tested the sensitivity of the models to manipulations to assess the explanatory power of a database approach.

A Simple Test for the Consistency of Parameters in the Pyloric CPG

The results in (Prinz et al. 2004) obtained with a database approach using the pyloric subset model, suggest that the entire neural circuit produces similar firing patterns even if the parameters of the intrinsic voltage-dependent properties vary over a wide range – in fact over several orders of magnitude. Hence, there are several equally acceptable combinations of cellular and synaptic parameters that can produce equivalent firing patterns. One particular voltage-gated current may be very strong in an identified neuron from one animal but be very weak in the same neuron from a different animal. The desired network output would then come about as a result of correct combinations of cellular and synaptic properties, i.e., strong hyperpolarizing currents would balance strong depolarizing currents. This kind of balancing requires compensatory mechanisms not only at the network level but also in the individual neurons. Developing muscle cells in *Xenopus* for example compensate for the overexpression of exogenous Na^+ channels by upregulating the expression of at least two endogenous K^+ channels (Linsdell and Moody 1994). One way to see if such a possibility exists for the pyloric system is to remove one of the currents and observe the behavior of the neuron. We chose to do this with the transient potassium current I_A , which has long been known to be one of the most important voltage-gated currents in shaping the voltage output of single neurons as well as the patterned activity of circuits containing those neurons (Tierney and Harris-Warrick 1992). Neurons with such fast potassium currents would be expected to display strong changes in their behavior following blocking of the I_A . Therefore if we assume that the same type of neuron from different animals may indeed have very different maximal conductances for I_A , then the compensatory currents, e.g., the H-current which has been

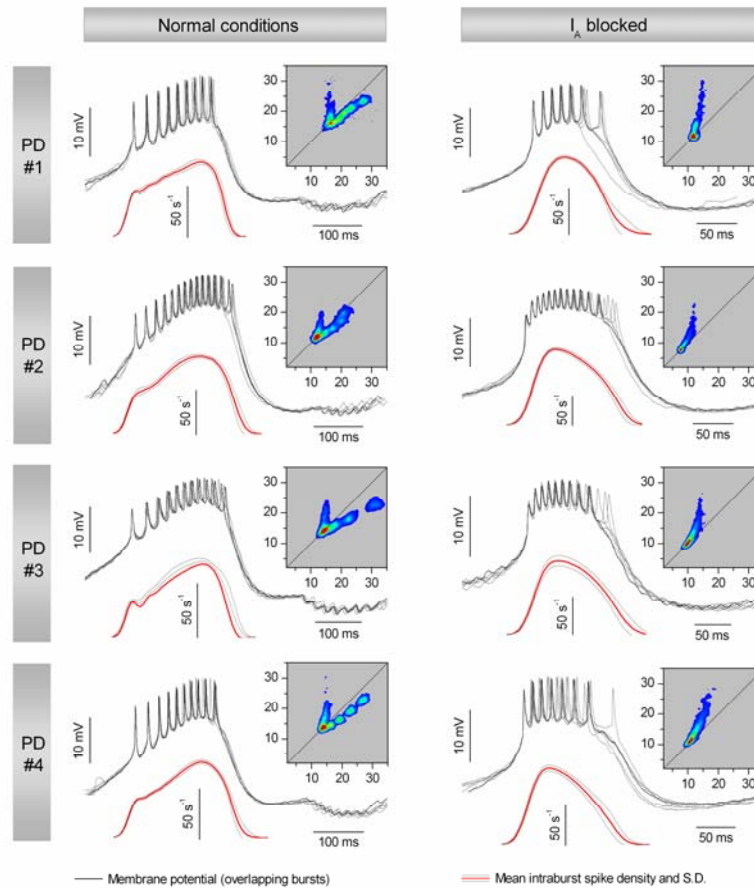


Fig. 1 4-aminopyridine induces consistent changes in the voltage output and spike dynamics of the PD neuron. Voltage waveforms of PD neurons from four different preparations (arranged in 4 rows) are displayed before (left) and during (right) the application of the A-current blocker 4-AP (4 mM). There are 6 overlapping bursts in each panel. Red traces with gray lines running on both sides are the average intraburst spike density functions (\pm S.D.) calculated from ~ 100 successive bursts of each neuron. In control all PD neurons are accelerating type bursters while 4-AP turns them into decelerating type ones. Color-coded maps are joint interspike interval (ISI) density plots showing the serial dependence of ISIs within the bursts of the PDs neurons. While in control conditions all the neurons display the characteristic PD signature (V-shaped and clustered maps, compare Szűcs et al. 2003), the joint ISI maps during 4-AP indicate a different behavior: rapid onset of the burst with gradually increasing ISI durations. The analysis reveals very similar dynamics in the PD neurons from different animals both in normal conditions and when they are exposed to 4-AP (modified from (Szűcs and Selverston 2006)).

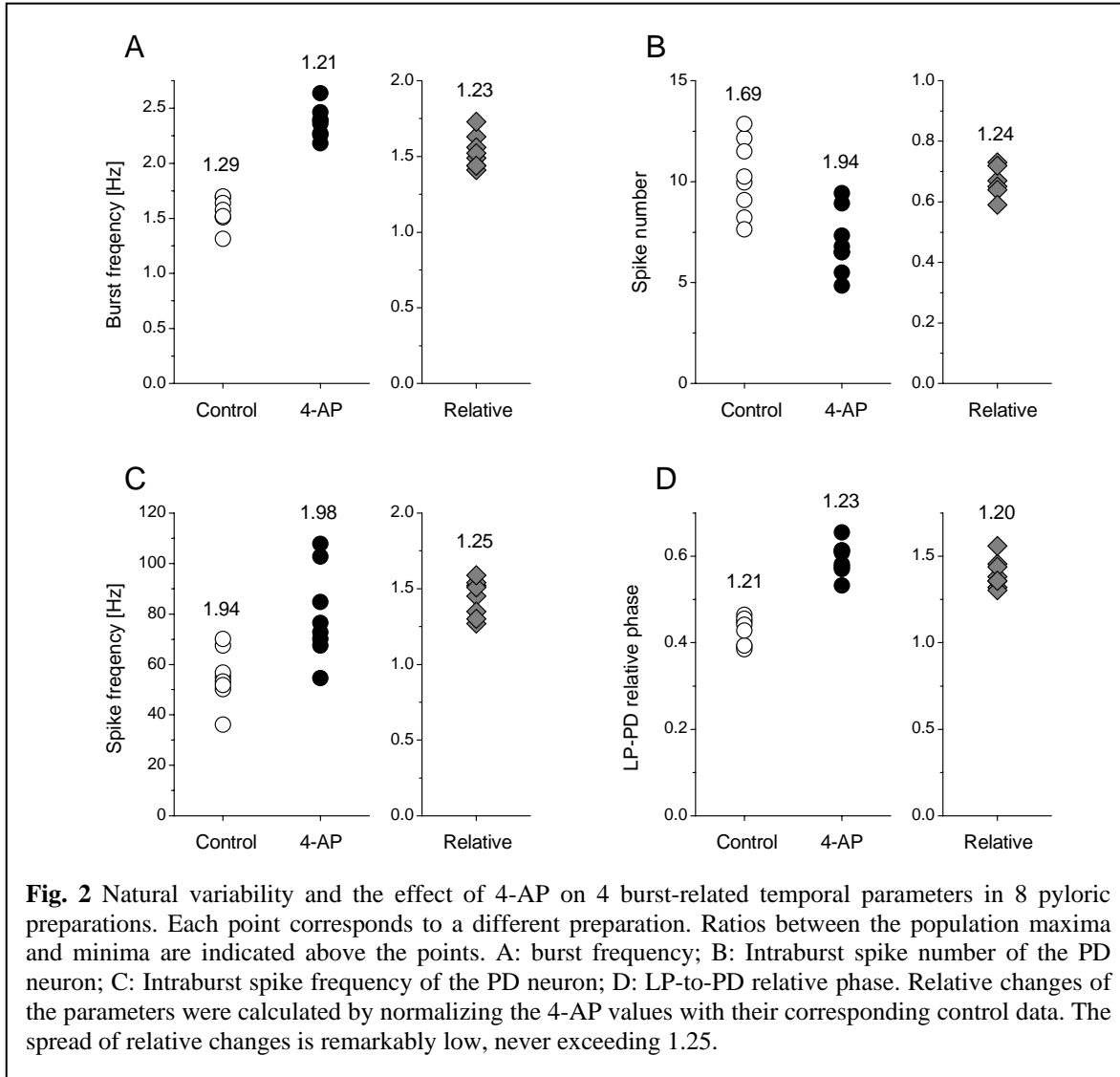
suggested by others (MacLean et al. 2003), will also vary over a wide range. A total blocking of an identified cell's A-current in different preparations should result in populations of neurons with zero conductance in I_A but with very different conductances in the compensatory type voltage-gated currents. Blocking the I_A would therefore unbalance the entire pyloric circuit. Accordingly, the activity pattern of single pyloric cells as well as the overall pyloric pattern should vary widely among different preparations.

Pyloric burst freq., control	Hz	1.56 ± 0.13
Pyloric burst freq. with 4-AP	Hz	2.37 ± 0.14
Change in pyloric burst freq.	%	$+52.6 \pm 10.8$
PD number of spikes, control		10.3 ± 1.7
PD number of spikes with 4-AP		6.9 ± 1.3
Change in number of spikes	%	-32.4 ± 5.6
PD intrab. spike freq. , control	Hz	54.9 ± 9.2
PD intrab. spike freq. with 4-AP	Hz	78.9 ± 15.3
Change in PD intrab. spike freq.	%	$+43.6 \pm 11.0$
LP-PD relative phase, control		0.42 ± 0.03
LP-PD relative phase, 4-AP		0.59 ± 0.03
Change in LP-PD relative phase	%	$+41.7 \pm 9.0$

Table 1 4-AP induces consistent effects in the gross burst parameters and in the spike patterns of the PD neuron from different preparations (n=8). The intraburst spike frequency was calculated by dividing the number of spikes with the duration of the bursts. The LP-PD relative phase was calculated by dividing the intervals between the LP and PD burst onset times with the PD burst cycle period.

To test this possibility we performed a set of experiments with bath applied 4-aminopyridine (4-AP), a potent and specific blocker of I_A in lobster pyloric neurons (Tierney and Harris-Warrick 1992), and examined how consistent the effects are. At the network level, 4-AP had the obvious effect of speeding up the frequency of the pyloric rhythm by $52.6 \pm 10.8\%$ (n=8). This is consistent with the general expectation that the removal of a hyperpolarizing current, without altering other currents, will result in a net depolarization of the neurons at the same time. Application of 4-AP did not disrupt the phase-relationship between the bursts of the pyloric neurons and the overall regularity of the three-phasic rhythm. At the single neuron level, 4-AP affected the voltage output and spike patterns of the neurons in a cell-specific manner with the PD neuron showing the most profound changes. The key question here is how much variability is observed after the A- currents are eliminated? Remarkably, the changes in burst shape and interspike interval (ISI) pattern for the PD neuron in 4-AP were very reproducible and characteristic (Fig. 1, Table 1). In fact, 4-AP turned the accelerating type (ISIs decreasing) PD neuron into a decelerating type neuron. As shown in the table, the standard deviations of the changes in the statistical parameters were consistently small. Consequently, 4-AP produces very similar effects in pyloric neurons from different animals. These effects are consistent and reproducible not only in the network parameters but also in the parameters describing the spike dynamics of single neurons. Usually, burst timing based gross parameters are used to characterize the activity of oscillatory networks. These gross parameters of neural activity are the functionally most important ones and, hence, are understandably very similar among different animals. However, even the more subtle parameters, like the interspike interval-based metrics and spike density are also consistent. The pyloric neurons apparently tune their biophysical properties in a way that makes the bursts of the individual neurons remarkably similar across different animals. Actually this is one of the reasons why the identification of such neurons is straightforward for the experienced neurophysiologist. As we and other investigators

have previously shown, synaptic and neuromodulatory factors not only induce consistent changes to the overall burst pattern of the pyloric circuit but they also do the same consistently to the burst waveforms of the individual cells (Szűcs et al. 2005; Szűcs et al. 2003). When inspecting the experiments individually, we did not notice any clear



differences in the changes in the circuit characteristics listed summarily in table 1. In particular, there were no indications that the remaining variability, as indicated by the non-zero standard deviations, was due to differences in the effect of blocking I_A rather than due to the a priori variability of the quantities in the intact circuits.

Figure 2 demonstrates the range of 4 parameter values before and during 4-AP application for the 8 preparations. Natural variations of the burst frequency, PD spike number, PD intraburst spike frequency as well as the LP-to-PD relative phase are reflected by the 1.3 to 1.9-fold variability in the scatter plots (max/min ratios of the populations are indicated in each plot). A closer look at the burst frequency data (Fig. 2A) reveals that the population mean frequency is increased by 4-AP, but the dispersion of the data points remains very similar. The ratios are 1.29 and 1.21 for the control data

and the 4-AP data, respectively. Relative changes of the burst frequency of each pyloric circuit are displayed in a similar format and we find low dispersion of the data (1.23 ratio). Following the same procedure for the other three temporal parameters, we find that 4-AP clearly shifts the populations, but the variability does not change significantly in either case. Remarkably, max/min ratio for the relative changes is always close to or less than 1.25. These values as well as the max/min ratios of the raw data are much less than those reported by Golowasch or Schulz for maximal conductance densities of selected K-currents. Looking at the original data (before normalization) we find somewhat greater variability in the PD intraburst spike number and spike frequency (close to 2-fold variability). Nonetheless, 4-AP does not increase the variability of these parameters either.

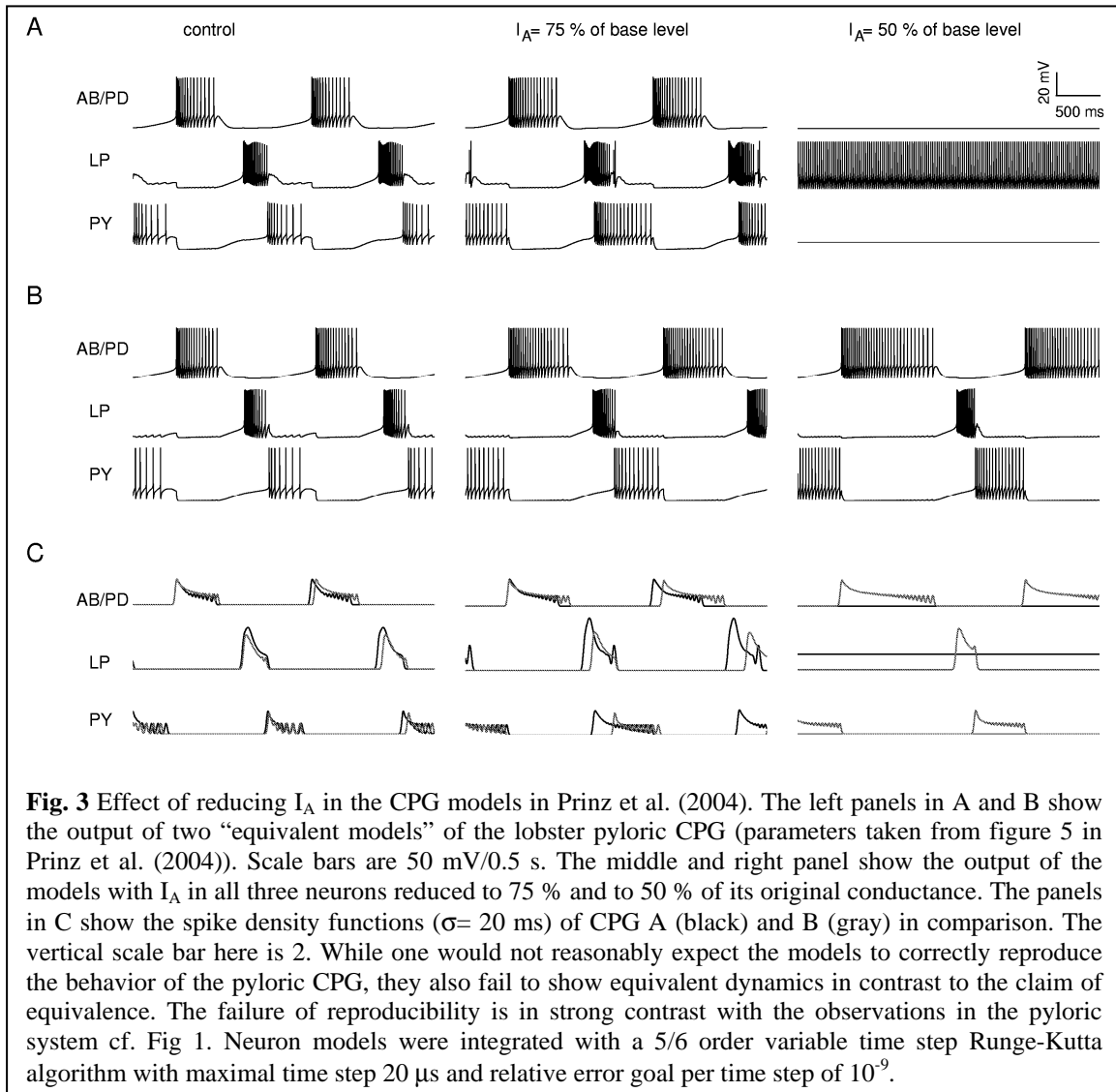


Fig. 3 Effect of reducing I_A in the CPG models in Prinz et al. (2004). The left panels in A and B show the output of two "equivalent models" of the lobster pyloric CPG (parameters taken from figure 5 in Prinz et al. (2004)). Scale bars are 50 mV/0.5 s. The middle and right panel show the output of the models with I_A in all three neurons reduced to 75 % and to 50 % of its original conductance. The panels in C show the spike density functions ($\sigma = 20$ ms) of CPG A (black) and B (gray) in comparison. The vertical scale bar here is 2. While one would not reasonably expect the models to correctly reproduce the behavior of the pyloric CPG, they also fail to show equivalent dynamics in contrast to the claim of equivalence. The failure of reproducibility is in strong contrast with the observations in the pyloric system cf. Fig 1. Neuron models were integrated with a 5/6 order variable time step Runge-Kutta algorithm with maximal time step $20 \mu\text{s}$ and relative error goal per time step of 10^{-9} .

The observed consistency of the pyloric CPG with respect to removal of I_A can now be compared to corresponding results with the model used by Prinz et al. (2004). Figure 3 shows two control patterns that would meet their criteria for identification as pyloric rhythms (the same models as shown in Fig. 5 of (Prinz et al. 2004)). If the I_A values of the

three neurons are different in the two models, one would expect a uniform reduction of this conductance to produce different effects in each model. As expected, a small reduction in I_A already shows noticeable differences in frequency between the two models whereas a larger reduction of 50% leads to completely different behavior for each. For complete removal of I_A , both models cease bursting in sharp contrast to the real system, which continues almost normal activity. Compare the AB/PD model trace in Fig. 3 A & B with the effects of complete I_A removal from PDs shown in Fig. 1.

The pyloric CPG has many regulatory mechanisms and internal feedback loops driving it to a stable pattern even when some of its components are severely disrupted. Could this make the experimentally observed changes consistent in spite of very disparate parameter sets? While one should not exclude this possibility from the outset, there is no evidence that regulatory mechanisms can act in acute preparations. Furthermore, one would expect feedback mechanisms to stabilize the normal physiological pattern rather than the deviation from it. Instead of large, but consistent, changes one would expect to see no, or very slight changes overall. Along the same lines, one would expect the presumably functionally more important properties like frequencies and duty cycles to be

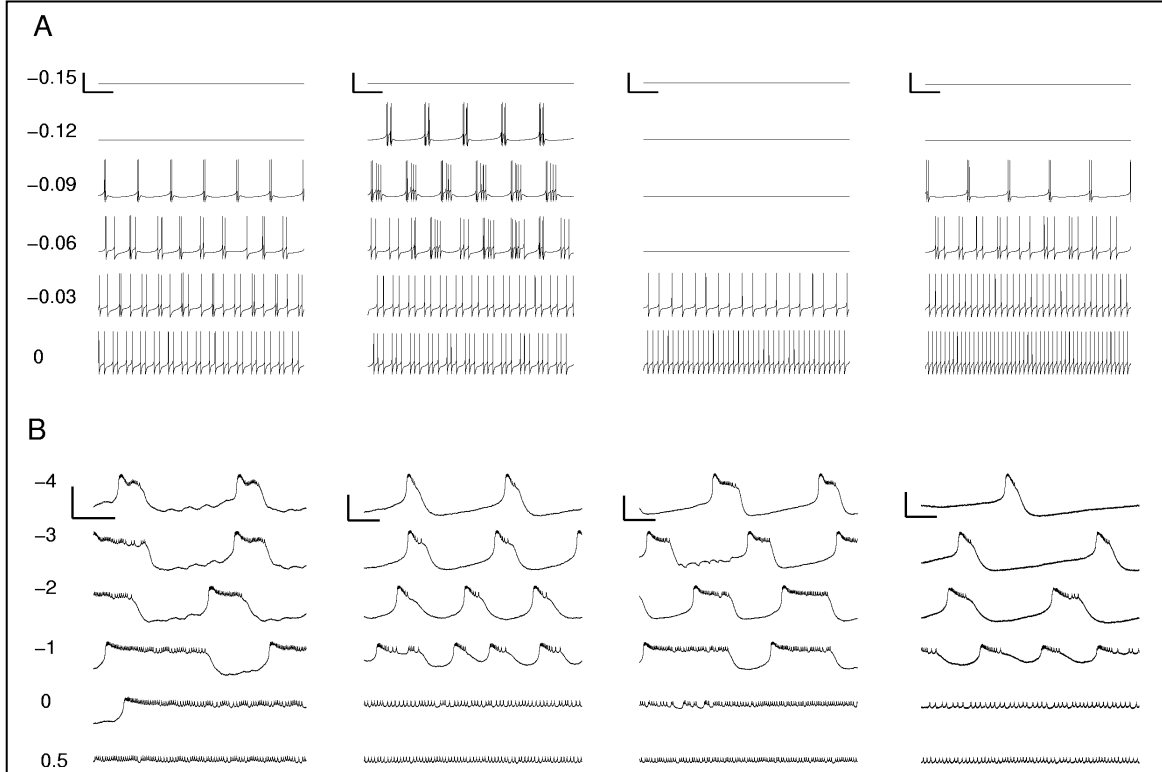


Fig. 4 Models and data of isolated LP neurons. A) models LP2 through LP5 from Prinz et al. (2004) at 6 levels of current injection. Current injections range from -0.15 nA to 0 nA (control) in steps of 0.03 nA. Scale bars are $1s / 50$ mV. B) Data from isolated LP neurons (PD and VD neurons were killed by photoablation, $10 \mu M$ PicROTOXIN) in 4 preparations of the stomatogastric ganglion of the lobster. Current injections into the soma through a separate sharp electrode were -4 nA to 0.5 nA as noted next to the graphs. Scale bars are $1s / 25mV$. Taken the chaotic nature of the LP neuron and the expected experimental variability in the isolation procedure, the data appears, at least qualitatively, rather consistent across preparations. The models, on the other hand, appear to differ quantitatively *and* qualitatively from each other. Models were integrated with the same algorithm as in Figure 3.

stabilized, but not details like the ISI signature of each neuron.

When are models equivalent?

The apparent discrepancies between the two circuit models with respect to blocking the I_A current led us to a more fundamental question: When can one consider two models equivalent or similar enough to be in the same relationship to each other as the identified cells of two different animals? To approach this question we compared the variability between the dynamics of the models that are equivalent with respect to the criteria in (Prinz et al. 2004) to the variability of dynamics observed in isolated cells of the lobster stomatogastric ganglion. Figure 4 illustrates our findings. The panels show the membrane potential of 4 “equivalent models” (A) and four different isolated LP neurons (B) in response to 6 typical levels of DC current injection. Clearly, the LP cells show a noticeable variability but their overall bifurcations from slow, short bursting over long, irregular bursting, to irregular spiking and eventually to fast, tonic spiking are very typical. It is important to note that, as in the case of the network, regulatory mechanisms such as feedback loops are not likely to shape the dynamics of the isolated neurons, because the natural system has never been exposed to such conditions making it unlikely that a special mechanism to control the activity of the LP neuron in isolation would have evolved. Therefore, it seems fair to compare the results from isolation experiments to the behavior of single model neurons. The model neurons reproduce the irregular spiking for 0 nA current injection, the criterion on which they were selected, but otherwise do not match the data, nor each other, very well. Apparently the models chosen from the database were not yet quite adequate. Assuming there are models in the pool of tested models with matching dynamics over a wide range of stationary current injections, would this requirement restrict their parameters to similar values? And, if not, are there other criteria that would do so?

One might argue that in order to address this question one just needs to go into the database of models and select with increasingly restrictive criteria to answer this question easily. This assumes that all possible models are covered by the database and conversely that all parameter sets in the database constitute in some sense equivalent and viable “model entities”. To gain some insight into this question we analyzed a subset of models from the pyloric neuron model database of (Prinz et al. 2003a).

Database approaches

At first sight, it is an intriguing and bold idea to map out the complete parameter space of a model in a database approach and observe all of the dynamical regimes, especially the bifurcations. Furthermore, this approach seems to offer a completely new wealth of information on how typical certain dynamical properties of models are; for example by allowing statements about how many of all “equivalent LP models” have a large I_A conductance, etc. After careful inspection our enthusiasm was, however, somewhat damped by the following observations:

Conductance-based models of the Hodgkin-Huxley type are fairly complex, containing on the order of tens of parameters. Even if one restricts the free parameters to maximal conductances only, one is easily left with five to ten parameters for a given neuron. This already raises some skepticism on the feasibility of a complete database. Furthermore, experience with this type of model has demonstrated that they are highly

sensitive to the values of some parameters, see Fig. 4 for an example. Parameter mismatches often lead not only to dynamics inappropriate for the neurons being described but also inappropriate for neurons in general. Two common and very surprising, examples from conductance-based models are metastable depolarized states or lack of stability in the hyperpolarized regime. Because of the sensitivity of the models to some of the parameters (and their combinations), a complete survey of the parameter space obviously needs to be done with appropriately small increments in each of the parameters. This quickly increases the computational cost. For example, if we take just 1000 different values for each of, say, 5 parameters, we have to compute the dynamics of 10^{15} models which might take on the order of 10^{16} seconds on a modern PC, i.e., about 3 billion years. On the other end of the spectrum of possibilities, if we take only five different values for each of five parameters we will not cover any relevant portion of the parameter space (compare the gray panels in figure Fig. 4 to the wealth of different dynamics in the remaining panels). Continuing this approach to the network level makes the combinatorial complexity even more daunting.

Figure 5 illustrates a few further difficulties of brute force database approaches. The region in which a parameter change is relevant to the neuron dynamics is *a priori* not known. In the example of Fig. 5 it is only meaningful to vary g_{Na} between 0 and about 220 mS/cm². For all values beyond this range the dynamics of the model neuron is insensitive to changes in g_{Na} . Following (Prinz et al. 2003b) in counting the number of model neurons with a certain property, e.g., all neurons that are tonic spikers, one will count basically the same model several times, for $g_{Na} = 300, 400, \text{ and } 500 \text{ mS/cm}^2$. The wealth of different models between $g_{Na} = 20 \text{ and } 90 \text{ mS/cm}^2$, however, will go unnoticed. The head count of models with given properties apparently strongly depends on the choice of parameter values examined (both in terms of spacing and total range) and thus could become a highly ambiguous statement. Inspecting the data manually, like we did here for illustration purposes, does not resolve this problem because the relevant range of values of, e.g., the sodium conductance, will likely depend in a non-trivial way on the values of all the other parameters.

Furthermore, given the different ways parameters enter into models it even seems unclear whether linear, logarithmic or other increments in parameter values might be appropriate. A simple example illustrates this basic problem: For simplicity, let us assume that we are looking for sets of three parameters with the property $p_1 \cdot p_2 \cdot p_3 \leq 1$. If we sample the three parameters on a logarithmic scale, e.g. from $\{0, 0.1, 0.2, 0.5, 1, 2, 5\}$, we get the result that 82.8 % of parameter sets have this property. If we sample from a linear scale, like $\{0, 1, 2, 3, 4, 5\}$, however, we obtain a count of 42.6 % of the parameter sets with this property. The percentage count almost entirely depends on our prior choice of the sampling method.

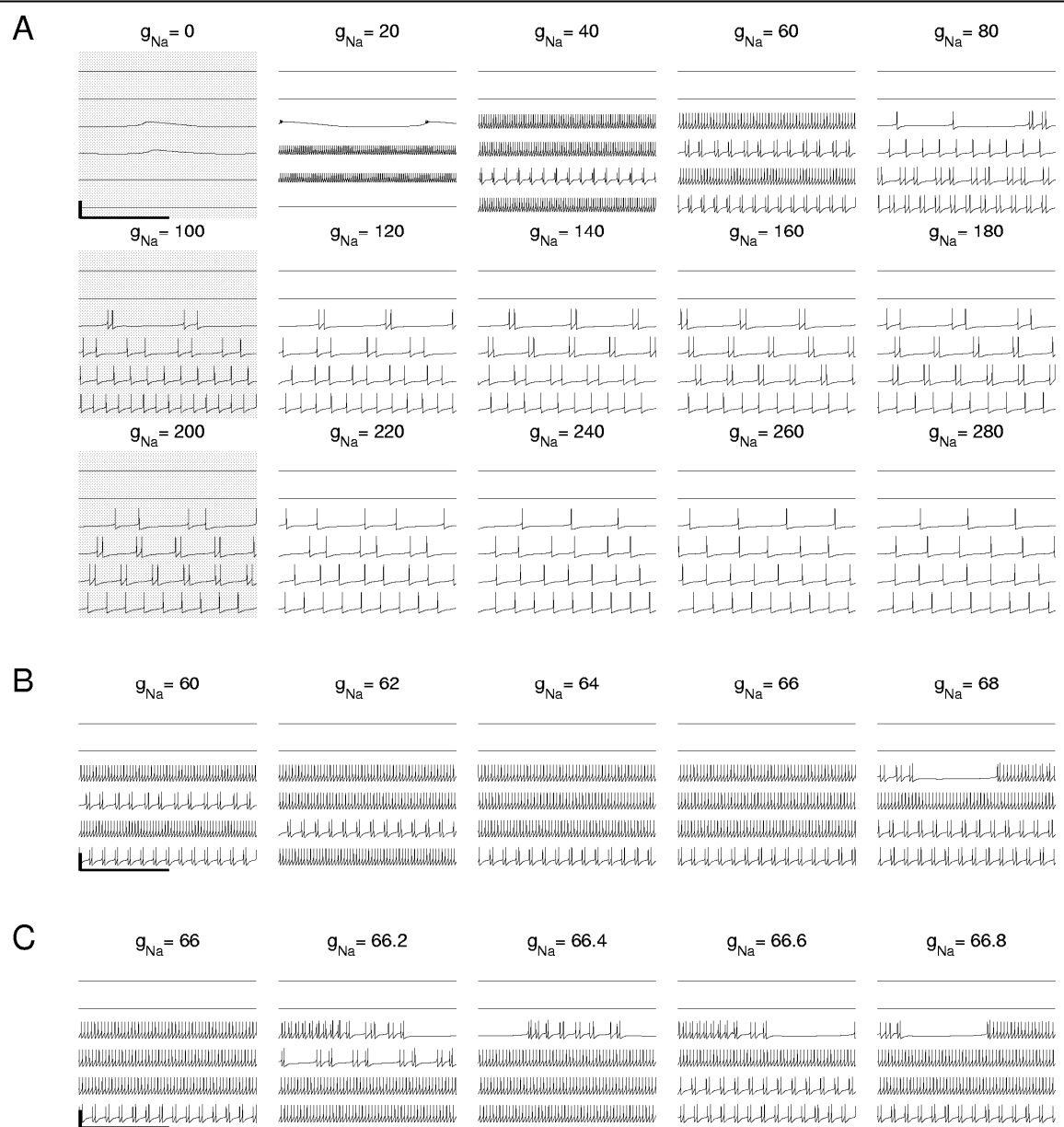


Fig. 5 Illustration of the principle difficulties of a database approach. A) Each panel shows 6 membrane potential traces of the model LP5 of Prinz et. al. (2004) with a given value for the Na conductance as noted above the panels. The 6 traces correspond to DC current injection from -0.125 to 0 nA in increments of 0.025 nA (top to bottom). Gray panels are models that were examined in the database of Prinz et al. (2003), the other panels were not. Relevant bifurcations in the neuron dynamics (transitions from silent to fast tonic spiking to slower tonic activity and eventually irregular bursting) were not included into the database. The remaining models for even stronger Na currents (not shown here) are all comparatively similar and need not have been examined. B) The 10 fold finer resolution in the parameter values shown in panel A still reveals further transitions in the activity patterns. Panel C) shows another 10 fold increase in resolution from panel B. The model appears to be so sensitive to this parameter that even this resolution does not suffice to clearly resolve the transition between fast tonic spiking and irregular bursting. It seems that the necessary resolution to really cover the whole dynamics of a neuron model is very fine whereas, on the other hand, this resolution is only necessary in very small but *a priori* unknown regions. A brute force database approach leads to either incomplete observations or to an explosion in computational cost (see main text). Neuron models were integrated with the same procedure as before. Correct integration was controlled in some examples with a linear Euler algorithm of time step 10^{-6} ms which revealed no relevant deviation.

Another dangerous pitfall is the assumption that, if the relevant range of

parameter values were tightly constrained by additional knowledge, counting models with a given property becomes more meaningful. A simple back-of-the-envelope calculation shows that if we assume we know the relevant parameter range to $\pm 10\%$ accuracy, e.g., we know that 6 parameters have relevant values between 0 and 1 ± 0.1 , then the parameter space volume examined varies between $0.9^6 \approx 0.531$ and $1.1^6 \approx 1.772$. Therefore, one could easily obtain a result where the fraction of models having a given property varies between 90 % and 27 % depending whether one sampled with the lower or upper value for the parameter range. The problem becomes even more aggravated for higher dimensional parameter spaces.

With respect to the models that are overlooked by too coarse parameter sampling, one could argue that parameter regions for which the models are highly sensitive to their parameters and initial conditions (like the models for between $g_{Na} = 20$ and 90 mS/cm^2 above) are not relevant for describing neural systems, which need to be robust and reliable. It has, however, been observed that neurons can have chaotic regimes (Elson et al. 1999; Ren 1997; Schiff et al. 1994) and it is known that self-organized systems often approach such instable parameter regions for greater flexibility (Bak et al. 1988; Bertschinger and Natschlager 2004; Kauffman and Johnsen 1991). In this light, disregarding models solely based on the observation of sensitive and/or irregular dynamics appears to be somewhat presumptuous.

The approach of systematically mapping the dynamics resulting from all possible parameter combinations might be better-suited for models that are fairly insensitive to the parameters in question or for which the structure of the bifurcations is well known and simple enough such that sampling only a few points in every known dynamical regime will permit the identification of the dynamics over wide regions of the parameter space. Both of these limitations are probably not true for any Hodgkin-Huxley type model (Izhikevich 2006).

A different, more important function of building databases of model neurons can be to identify potentially useful models for other modeling purposes or to gain a *general overview* over possible model behaviors. As such, it can help circumvent tedious hand-adjustments of models and become a valuable part of the portfolio of modeling tools used in neuroscience.

Summary and Conclusions

Modeling is an extremely useful tool when handling large amounts of neurophysiological data from complex, nonlinear systems. If it goes hand in hand with experimental approaches it can help explain the experimental findings as well as generate new hypotheses. Models can also provide consistency tests and testbeds for principles that may underlie the observations. All models in neuroscience are, however, by necessity phenomenological in nature and a tight connection to experimental observations is therefore indispensable. Without direct experimental foundation, models can be fairly ambiguous because they are - unlike theories in physics - not constrained by clear fundamental principles nor are all specific details, e.g., values of parameters, precisely known. Furthermore, the very nature of biological systems is to vary between animals, which might not allow to build universal models of biological systems in the way one can in chemistry or physics. This exact premise led Prinz et al (2004) to the intriguing idea of

circuits being assembled in very different ways with very different components across animals.

Does existing data support the idea that individual neurons and entire circuits can be made with such variable combinations of parameters? If each circuit is assembled *de novo*, from one animal to another, so that each one is unique, we would have to take the following into consideration:

Regulatory mechanisms

Assuming that neurons and circuits in every animal can be considerably different, there must be a mechanism that regulates neuron and synapse properties to lead to a successful activity pattern. Furthermore, once this is achieved, individual biophysical and synaptic properties have to be maintained in the face of continuous protein turnover and activity-dependent changes (Turrigiano 1999). While potential regulatory mechanisms have been suggested on the single cell level (Golowasch et al. 1999; LeMasson et al. 1993; Liu et al. 1998), it remains rather unclear how this could be realized for synapses on the network level.

The action of neuromodulators.

When CPG circuits are exposed to neuromodulators delivered artificially or by stimulation of the neurons which contain them, they produce characteristic changes in ongoing motor patterns that are consistent from animal to animal (Harris- Warrick and Marder 1991). A large body of evidence exists that indicates that the modulators bind to particular receptors on specific identified neurons and that they activate second messenger systems which are also cell specific (Hempel et al. 1996). These actions in turn lead to specific changes in membrane conductances and alterations in the biophysical properties of the neurons and synapses in a circuit. Would consistent effects of neuromodulatory action be possible if the types and distribution of ion channels in identifiable neurons were inconsistent? One could argue that regulatory mechanisms and specific neuromodulator actions are tuned carefully to allow consistent effects in spite of highly variable substrates. But our experiment with 4AP also showed very consistent effects of blocking I_A channels, a perturbation that the system has never been and would not be exposed to in natural circumstances.

The fact that neuromodulators always produce consistent results is neither a paradox nor a conundrum that can be dismissed and relegated to future research (Marder and Goaillard 2006). Modulators have reproducible effects on networks because each has specific target cells to which they bind and specific second messenger pathways and phosphorylation sites that are affected. Of course modulators exist within the framework of the entire system so it is not surprising that they can produce variable results when other modulators or sensory inputs are present. But a single modulator applied to a ganglion in its standard experimental condition will always produce consistent effects. This would be extremely unlikely if the target channel had a thirty fold density range.

Feedback and descending control mechanisms.

For a CPG to be effective in the control of a behavior it must be able to respond to sensory inputs. Such sensory feedback may impinge directly onto CPG circuitry or it may

be in the form of commands from higher centers after different sensory inputs have been integrated and decisions made about how to respond. These feedback pathways are specific to particular neurons. Can feedback control mechanisms provide consistent results if the target neurons are inconsistent?

Time constants of channels.

The mixing and matching of channels in a neuron in a way that produces the same overall physiological properties could be done in theory if it was only the effective polarity of the response that mattered, i.e., a little too much inward current could be offset with a corresponding amount of an outward current. But ionic currents also have different activation and inactivation curves as well as different kinetics, ranging from transient to persistent. These factors would make it almost impossible to mix ionic currents in a way that would achieve similar actions in a wide enough dynamic regime because not only would the polarity have to be compensated for, the balancing currents would have to possess identical activation/inactivation curves and kinetic properties as well.

Additional experimental evidence.

(Baro et al. 1997) examined the expression of Shal channels in identified neurons in the pyloric CPG and found that it is typical for each identified cell type with small variations between cells of the same type. They also found rather small variability in the maximal conductance of I_A channels for each identified cell in contrast to the results in (Golowasch and Marder 1992). Furthermore in a recent work (Schulz et al. 2006) it has been shown that there is a certain amount of variability in some channels while other are more controlled. This is not unexpected as parameters which have a large impact on the system dynamics need to be more tightly controlled than others which have less impact. In particular, Schulz et al. (2006) found in LP neurons g_{Kd} in the range of 0.09-0.12 $\mu\text{S/nF}$, g_A in 0.05-0.16 $\mu\text{S/nF}$, and g_{KCa} in 0.2-0.6 $\mu\text{S/nF}$, which corresponds to maximally 3 fold differences. This is less variability in parameters than postulated from the models discussed above but more than most experimenters would have expected.

Implications for the concept of identified cells

Identifiability of neurons has been a cornerstone of the success of invertebrate CPG “circuit chasing”. Identifiable neurons have identical physiological properties, the same connections to other identifiable cells, a similar morphology, and identical biochemical and molecular signatures. The application of molecular biological techniques to the study of CPGs will depend on the assumption that different types of neurons have consistent cellular, synaptic channel compositions and receptor properties (Callaway 2005; Kiehn and Kullander 2004; Wulff and Wisden 2005). The implications of disparate parameter compositions occurring in the same neuron type, on the contrary, would make such molecular approaches less applicable for pharmacological development because channel specific drugs would affect each cell of the same type, differently.

Another interesting observation in the recent work of Schulz et al. (2006) is that the identified neurons, though having a fairly wide range of certain parameters, are well separated in parameter space. In this way, wide ranges of parameter values might be consistent with the original idea of identified cells: Each identified cell is characterized by a more or less wide region in parameter space. If the action of neuromodulators and

other neuroactive substances were sufficiently robust, this could still allow for consistent effects on the neuronal activity. How much variability would be sustainable in this view remains an interesting open research direction.

The voltage output of a neuron is not a simple algebraic sum of channel populations but a complex nonlinear computation that includes both biophysical and anatomical factors. The novel methodology of using large-scale databases developed by Prinz et al (2003, 2004) is an exciting approach that incites the thinking of how neural systems are composed and maintained. One has to remember, though, that modelers, by necessity, have to make assumptions and simplifications, which may greatly influence the modeling results. This is aggravated by the fact that we do not know, which aspects of the observed circuit and neuron dynamics really matter. The fact that we observe very typical ISI signatures in the neurons of every preparation is suggestive but does not prove that ISI properties are indeed important. It might be, for example, that the A-current in the PD neuron is critically important to achieve reliable phase shift during the action of a specific endogenous neuromodulator such as dopamine and only as a 'side-effect' the A-current also reshapes the burst of the PD neuron and produces those nice V-shaped and clustered ISI signatures. In a model the modeler will have to choose which properties of the system (bursting frequencies, bursting phases, ISIs, ISI signatures, spike shape, etc.) are important to model and which can be neglected. When drawing conclusions from models the limitations due to these assumptions and simplifications have to be very clear in mind.

Here we pointed out some problems that come with a database approach, including the arbitrary choice of range, step, and type (logarithmic, linear, etc.) of parameter sampling, the high sensitivity and non-linearity of Hodgkin-Huxley type neuron models, the ambiguities in assessing the accuracy and adequacy of models and the difficult interpretation of "model counting" results. All this again stresses the need to work closely with experimental data. There is a danger that modeling becomes a substitute for experimental work while making assumptions that are not or can not be obtained experimentally. To our mind, the important biological question of how consistent circuit and neuron parameters really have to be remains wide open.

Bibliography

- Arvanitaki A and Chalazonitis N. Modal configurations of the activity of different neurons emanating from a common center. *J Physiol (Paris)* 50: 122-125, 1958.
- Bak P, Tang C, and Wiesenfeld K. Self-organized criticality. *Physical Review A* 38: 364-374, 1988.
- Baro DJ, Levini RM, Kim MT, Willms AR, Lanning CC, Rodriguez HE, and Harris-Warrick RM. Quantitative single-cell-reverse transcription-PCR demonstrates that A-current magnitude varies as a linear function of *shal* gene expression in identified stomatogastric neurons. *J Neurosci* 17: 6597-6610, 1997.
- Bertschinger N and Natschlager T. Real-time computation at the edge of chaos in recurrent neural networks. *Neural Comput* 16: 1413-1436, 2004.
- Callaway EM. A molecular and genetic arsenal for systems neuroscience. *Trends Neurosci* 28: 196-201, 2005.

Elson RC, Huerta R, Abarbanel HD, Rabinovich MI, and Selverston AI. Dynamic control of irregular bursting in an identified neuron of an oscillatory circuit. *J Neurophysiol* 82: 115-122, 1999.

Golowasch J, Casey M, Abbott LF, and Marder E. Network stability from activity-dependent regulation of neuronal conductances. *Neural Comput* 11: 1079-1096, 1999.

Golowasch J and Marder E. Ionic currents of the lateral pyloric neuron of the stomatogastric ganglion of the crab. *J Neurophysiol* 67: 318-331, 1992.

Harris- Warrick RM and Marder E. Modulation of neural networks for Behavior. *Ann Rev Neurosci* 14: 39-57, 1991.

Hempel CM, Vincent P, Adams SR, Tsien RY, and Selverston AI. Spatio-temporal dynamics of cyclic AMP signals in an intact neural circuit. *Nature* 384: 166-169, 1996.

Izhikevich EM. *Dynamical systems in neuroscience: The geometry of excitability and bursting*. Cambridge, MA: The MIT Press, 2006.

Kandel ER, Frazier WT, Waziri R, and Coggeshall RE. Direct and common connections among identified neurons in *Aplysia*. *J Neurophysiol* 30: 1352-1376, 1967.

Kauffman SA and Johnsen S. Coevolution to the edge of chaos: coupled fitness landscapes, poised states, and coevolutionary avalanches. *J Theor Biol* 149: 467-505, 1991.

Kiehn O and Kullander K. Central pattern generators deciphered by molecular genetics. *Neuron* 41: 317-321, 2004.

LeMasson G, Marder E, and Abbott LF. Activity-dependent regulation of conductances in model neurons. *Science* 259: 1915-1917, 1993.

Linsdell P and Moody WJ. Na^+ channel mis-expression accelerates K^+ channel development in embryonic *Xenopus laevis* skeletal muscle. *J Physiol (Lond)* 480: 405-410, 1994.

Liu Z, Golowasch J, Marder E, and Abbott LF. A model neuron with activity-dependent conductances regulated by multiple calcium sensors. *J Neurosci* 18: 2309-2320, 1998.

MacLean JN, Zhang Y, Johnson BR, and Harris-Warrick RM. Activity-independent homeostasis in rhythmically active neurons. *Neuron* 37: 109-120, 2003.

Marder E and Goaillard JM. Variability, compensation and homeostasis in neuron and network function. *Nat Rev Neurosci* 7: 563-574, 2006.

Otsuka M, Kravitz EA, and Potter DD. Physiological and chemical architecture of a lobster ganglion with particular reference to Gamma-aminobutyrate and glutamate. *J Neurophysiol* 30: 725-752, 1967.

Prinz AA, Billimoria CP, and Marder E. Alternative to hand-tuning conductance-based models: construction and analysis of databases of model neurons. *J Neurophysiol* 90: 3998-4015, 2003a.

Prinz AA, Billimoria CP, and Marder E. Alternative to hand-tuning conductance-based models: construction and analysis of databases of model neurons. *J Neurophysiol* 90: 3998-4015, 2003b.

Prinz AA, Bucher D, and Marder E. Similar network activity from disparate circuit parameters. *Nature Neuroscience* 7: 1345-1352, 2004.

Ren W, Hu, S.J., Zhang, B.J., Wang, F.Z., Gong, Y.F., Xu, J.X. Period-adding bifurcation with chaos in the interspike intervals generated by an experimental pacemaker. *International Journal of Bifurcation Chaos* 7: 1867-1872, 1997.

Schiff SJ, Jerger K, Duong DH, Chang T, Spano ML, and Ditto WL. Controlling chaos in the brain. *Nature* 370: 615-620, 1994.

Schulz DJ, Goaillard JM, and Marder E. Variable channel expression in identified single and electrically coupled neurons in different animals. *Nat Neurosci*, 2006.

Szücs A, Abarbanel HD, Rabinovich MI, and Selverston AI. Dopamine modulation of spike dynamics in bursting neurons. *Eur J Neurosci* 21: 763-772, 2005.

Szücs A, Pinto RD, Rabinovich MI, Abarbanel HD, and Selverston AI. Synaptic modulation of the interspike interval signatures of bursting pyloric neurons. *J Neurophysiol* 89: 1363-1377, 2003.

Szücs A and Selverston AI. Consistent dynamics suggest tight regulation of biophysical parameters in a small network of bursting neurons. *J Neurobiol* in press, 2006.

Tierney AJ and Harris-Warrick RM. Physiological role of the transient potassium current in the pyloric circuit of the lobster stomatogastric ganglion. *J Neurophysiol* 67: 599-609, 1992.

Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci* 22: 221-227, 1999.

Wulff P and Wisden W. Dissecting neural circuitry by combining genetics and pharmacology. *Trends Neurosci* 28: 44-50, 2005.