Chapter 7
The More We Look, the More Biological Variation We See: How Has and Should This Influence Modeling of Small Networks?

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Abstract Models of the small neuronal networks from invertebrates, especially rhythmically active central pattern generators, have not only been useful experimental tools for circuit analyses but also been instrumental in revealing general principles of neuronal network function. This ability of small network models to illuminate basic mechanisms attests to their heuristic power. In the 20 years since the first CNS meeting, theoretical studies, now supported abundantly by experimental analyses in several different networks and species, have shown that functional network activity arises in animals and models even though parameters (e.g., the intrinsic membrane properties (maximal conductances) of the neurons and the strengths of the synaptic connections) show two to fivefold animal-to-animal variability.

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In our own work, presented at CNS 2010, we reviewed experiments, using the leech heartbeat CPG, which explore the consequences of animal-to-animal variability in synaptic strength for coordinated motor output (Fig. 7.1). Our experiments focused on a set of segmental heart motor neurons that all receive inhibitory synaptic input from the same four premotor interneurons (Norris et al. 2011) (Fig. 7.1A). These four premotor inputs fire in a phase progression and the motor neurons also fire in a phase progression because of differences in synaptic strength profiles of the four inputs among segments (Fig. 7.1B). Our experiments showed that relative synaptic strengths of the different premotor inputs to each motor neuron vary across animals yet functional output is maintained. Moreover, animal-to-animal variations in strength of particular inputs do not correlate strongly with output phase. We measured the precise temporal pattern of the premotor inputs, the segmental synaptic strength profiles of their connections onto motor neurons, and the temporal pattern (phase progression) of those motor neurons all in single animals and compiled a database of 12 individual animals (Fig. 7.1C1, 2). We analyzed input and output in this database and our results suggest that the number (four) of inputs to each motor neuron and the variability of the temporal pattern of input from the CPG across individuals weaken the influence of the strength of individual inputs so that correlations are not easily detected. Additionally, the temporal pattern of the output, albeit in all cases consistent with heart function, varies as much across individuals as that of the input. It seems then that each animal arrives at a unique solution for how the network produces functional output. This work has been supplemented by dynamic clamp analysis of pharmacologically isolated heart motor neurons using synaptic input patterns derived from the 12 individual of our database that further support these conclusions (Wright and Calabrese 2011a, b).

All the observations summarized above have contributed to the growing consensus that to understand a neuronal network through biophysical modeling, we must construct populations of models with multiple sets of parameter values corresponding to parameters from different individuals (Prinz 2010; Marder 2011; Marder and Taylor 2011). Thus the computational effort needed to produce a state-of-the-art biophysical model is vastly increased. The situation is clearly still fluid, and the reaction in the modeling community has ranged from a continued pursuance "ideal parameter sets" or sticking to averaged values for parameters to what Prinz (2010) calls ensemble modeling, where multiple functional instances are identified and examined. We have not come to this situation smoothly but by fits and starts, and the purpose of this chapter is to highlight two papers that were presented at CNS 1993 that seem now dated but indeed presage this understanding.

Looking Back

At CNS 1993 two papers were presented and book chapters written in "Computation in Neurons and Neural Systems" edited by Frank H. Eeckman were inspired by work on invertebrate CPGs (LoFaro et al. 1994; Skinner et al. 1994). These papers
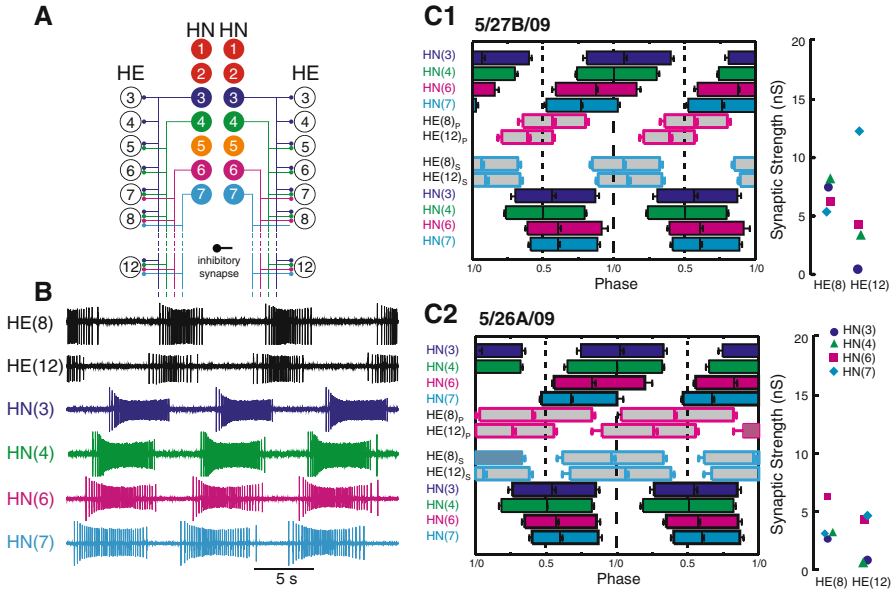
Fig. 7.1 (**A**) Bilateral circuit diagram from the heartbeat control system of medicinal leeches including all the identified heart (HN) interneurons of the core CPG showing the inhibitory connections from the heart interneurons of the leech heartbeat CPG onto heart (HE) motor neurons in the first 12 midbody segmental ganglia. The ipsilateral HN(3) and HN(4) front premotor interneurons and the ipsilateral HN(6) and HN(7) middle premotor interneurons provide input to heart motor neurons (HE(3)–HE(12)) (Norris et al. 2007a). The *large filled circles* are cell bodies and associated input processes. *Lines* indicate cell processes and *small filled circles* indicate inhibitory chemical synapses. Connections among the interneurons of the CPG are not indicated. Standard colors for the heart interneurons are used in the rest of the figure. (**B**) There are two coordination modes (peristaltic and synchronous) of the heart motor neurons and heart interneurons one on either body side that switch sides regularly (Norris et al. 2006, 2007b). Simultaneous extracellular recordings are shown of ipsilateral HN(3), HN(4), HN(6), and HN(7) premotor interneurons (inputs) (standard colors) and HE(8) and HE(12) motor neurons (outputs) (*black*) in peristaltic (p) coordination mode—similar recordings, not shown, were made in the synchronous (s) coordination mode. (**C1, 2**) Complete analysis of input and output temporal patterns and synaptic strength profiles for two different animals from our sample of 12. Summary phase diagram (temporal patterns of inputs and outputs) of the premotor interneurons (standard colors) and the HE(8) and HE(12) motor neurons in both the peristaltic (boxes outlined in *pink*) and synchronous (boxes outlined in *light blue*) coordination modes for two different preparations. Phase diagrams were determined from recordings like in (**B**). The segmental synaptic strength profiles of the inputs were determined in the same preparations by voltage clamping each of the motor neurons (HE(8) and HE(12)) and performing spike-triggered averaging of IPSCs, and are shown to the *right* of each phase diagram. Standard colors are used. Animals are specified by the day on which they were recorded; letters accompany the designation of day, if more than one animal was recorded on that day. Note that both the temporal patterns (both input and output) and synaptic strength profiles vary between the two animals as in the rest of the sample of 12 animals. Adapted from Norris et al. (2011)

reflect the time in which they were written yet they point to the present day. They illustrate the limits of our technological ability to model small neuronal networks and the naiveté of our theoretical understanding of what a realistic neuronal network model was. They also illustrate how the then novel technique of dynamic current clamping would be brought to bear in future studies of small networks. Using these papers as a starting point, I will discuss how my own thinking and that of the field has evolved since then. In both these papers, parameter variation in reduced models of half-center oscillators (oscillatory networks with reciprocal inhibition between two neurons (or groups of neurons)) is shown to lead to interesting changes in network activity.

In the first case, a two-cell network was modeled with one cell an inherent burster and the other not, the presence of I_h is shown to be critical for the non-bursting neuron to assume an integer bursting ratio smaller than 1:1 as the level of injected current in the non-bursting neuron is adjusted. The theoretical analysis was motivated and augmented by electrophysiological experiments in the crustacean stomatogastric nervous system (STN) focusing on the well-characterized pyloric CPG. The LP neuron in the isolated STN is capable of plateau production: it is not an autonomous bursting neuron but it is engaged in reciprocal inhibitory connections to the bursting PD neurons. Normally these cells produce alternating bursts but with appropriate hyperpolarizing current injection into the LP neurons they assume a 6:1 (−3.6 nA) or 12:1 (−5.1 nA) burst ratio. The theoretical analysis modeled each neuron using the Morris–Lecar formalism (Morris and Lecar 1981) tuned so that the PD neuron was spontaneously oscillatory (a burster) whereas the LP neuron was silent in the absence of input from the PD but plateau forming. The LP neuron was additionally given the I_h current. A two-cell network was then constructed with reciprocal inhibitory synapses, thus forming a half-center oscillator, with one cell (PD) an inherent burster and the other (LP) not. The presence of I_h in the non-bursting LP neuron was shown to be critical for it to assume an integer bursting ratio smaller than 1:1 as the level of injected current in the non-bursting neuron was adjusted.

This study was naïve in that simplified neuron models were used and only one parameter was considered in determining how burst ratios less than 1:1 could be achieved—considering the desktop computational ability available at the time it is hardly surprising that simplified neuron models were used and other parameters were not also analyzed. The study was forward-looking in that it was clearly tied to an interesting experimentally observed phenomenon—frequency de-multiplication—and implicated a specific ionic current as producing the phenomenon. The real implications of this tenuous step were seen in strong experimental, modeling, and hybrid analysis with dynamic clamp that followed and which led to fundamental insights into how fast and slow rhythms in neuronal networks can interact not only in the crustacean STN (Bartos and Nusbaum 1997; Bartos et al. 1999) but also in general (Marder et al. 1998).

In the second case, again a half-center oscillator was formed between two oscillatory Morris–Lecar model neurons (Morris and Lecar 1981)—and the mechanisms promoting the transitions during alternate "bursting" were explored. The most

interesting aspect of the analysis was a determination of the effect of synaptic threshold on the period of the half-center oscillator's activity. The main finding was that there was a middle range where period was prolonged and relatively insensitive to synaptic threshold but fell of sharply on either side of this range. This theoretic analysis was given some experimental backing by forming a half-center oscillator between pharmacologically isolated leech heart interneurons using artificial inhibitory synapses implemented with dynamic clamp. The hybrid half-center oscillators again showed a period maximum at a middle range of synaptic threshold with period falling off on either side.

Like the previous study, this one was naïve in that simplified neuron models were used and only one parameter was considered in determining how burst period was controlled in a half-center oscillator. The study was very forward-looking in that it introduces the profound interaction of theory and experiment that is possible when hybrid systems are created with dynamic clamp. This analysis planted the seed for more sophisticated and systematic hybrid systems analysis of half-center oscillators that have defined the role of currents like I_h and low-threshold Ca current in producing half-center oscillations (Sorensen et al. 2004; Olypher et al. 2006). Other studies have used modeling and dynamic clamp and similar techniques to more fully explore the synaptic dynamics of mutually inhibitory neurons in controlling network period (Mamiya and Nadim 2004; Nadim et al. 2011). Yet more interesting and germane to the current interest in how neuronal and synaptic variability affects circuit performance are more recent hybrid system analyses of half-center oscillators that employ ensemble modeling and database techniques to systematically explore the parameter space of the half-center oscillator and make an attempt to make sense of animal-to-animal variability in neuronal properties that confront all experimentalists (Grashow et al. 2009, 2010; Brookings et al. 2012).

Concluding Thoughts

Models of neuronal networks essentially consist of differential equations that describe the dynamics of state variables, e.g., membrane potential (V_m) and the gating variables of voltage-gated conductances and the variables controlling activation of synaptic conductances. Embedded in these equations are a number of parameters, including maximal conductances, half-activation voltages and time constants of channel gates, and parameters controlling synaptic dynamics. Some of these parameters are considered free, or variable between instances, while the remaining parameters are fixed. For example, in the pioneering work of Prinz et al. (2003, 2004), only maximal conductances were considered free parameters. Indeed maximal conductances have been shown to be quite variable among animals (Bucher et al. 2005; Schulz et al. 2007; Goaillard et al. 2009; Tobin et al. 2009; Norris et al. 2011; Roffman et al. 2012). But it is clear that the other parameters mentioned will also show animal-to-animal variability though these have not been as widely studied

(Marder et al. 2007; Marder 2011). Even with powerful computing resources, it is not possible or desirable to consider all instances of a model. Making a model then involves deciding on a neuronal structure (single or multiple compartments), network connectivity, descriptive equations (often derivatives of the Hodgkin–Huxley formalism), which parameters are free, and the range over which each may vary. These decisions will all be driven by the data available and by the investigators' intuition for which parameters are likely to be significant in controlling neuronal activity. In short, the ability to consider multiple instances of a model does not free one from making a good model, and making a good model requires detailed knowledge of the system and judgment about what details can be ignored and parameters fixed.

There are many pertinent issues which models of small networks can still help clarify many interesting issues. Although some studies have suggested that variability in cellular intrinsic properties becomes less important when neurons are embedded in networks (Grashow et al. 2010; Brookings et al. 2012), others suggest that the interaction network topology and neuronal dynamics are critical (Gaiteri and Rubin 2011). Moreover, we know that networks are subject to frequent environmental perturbations and that neuromodulation plays an important role in pattern generation in many networks. Nevertheless, the question of how networks can produce functional output despite perturbations and modulatable parameters and yet not crash has barely been addressed especially at the experimental level (Grashow et al. 2009; Marder and Tang 2010; Tang et al. 2010). The ensemble modeling approach to address such questions is likely to expand as we move forward, despite the caveat expressed above, especially given the ever-increasing computational capabilities available. The analysis experimental and computational of small neuronal networks like invertebrate CPGs is likely to lead the way in this endeavor for several years to come.

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