

INTERFACING COMPUTER MODELS WITH REAL NEURONS: RESPIRATORY “CYBERNEURONS” CREATED WITH THE DYNAMIC CLAMP

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1. INTRODUCTION

The mechanism of respiratory rhythm generation remains an important unsolved problem. While experimental evidence has been collected about the location and distribution of respiratory neurons within the mammalian brainstem, only recently has the locus of rhythm generating neurons been discovered,¹ a functionally identified region of the brainstem known as the Pre-Bötzinger complex (pre-BötC). We've just begun to investigate the membrane and network properties of pre-BötC neurons underlying the generation and transmission of inspiratory rhythm.^{2,3,4,5}

We have proposed a pacemaker network model in which intrinsic membrane and synaptic conductances are responsible for inspiratory burst generation and synchronization of pacemaker neurons in the pre-BötC.^{3,4} We have been exploring these mechanisms through two approaches. Our first approach relies on experimental evaluation of intrinsic and synaptic currents in pre-BötC neurons present in our in vitro, rhythmic slices.^{1,2,5} Our second approach has been the use of mathematical models to evaluate hypotheses regarding the nature of the burst generating currents and synaptic coupling between neurons of the Pre-BötC.^{3,4} We present our recently developed approach that combines experimental and computational methods to allow direct interfacing of computer models with real neurons to explore cellular and network properties of the pre-BötC.

Within the last decade, a few investigators have made efforts to combine electrophysiology techniques with computational modeling, to develop a method of dynamically adding currents representing artificial chemical and electrical synapses into single neurons or small neural networks.^{6,7} Typically, the added current is calculated based on a mathematical model of a synapse or ionic conductance. These “dynamic

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clamp” methods have relied on proprietary software and operating systems that lack the speed and flexibility necessary to investigate a wide variety of conductance mechanisms. Our laboratory has developed a very high performance and extensible dynamic clamp system that we have dubbed Model Reference Current Injection (or MRCI, pronounced merci). We based MRCI on a free operating system (Linux) and on open source tools with the intention of making it inexpensive, very extensible, and freely available.

2. BIOLOGICAL PREPARATION

Our experimental preparation consists of thin ($\sim 300 \mu\text{m}$) slices of neonatal rat (P0-P4) brainstem containing the pre-BötC and sufficient neural circuitry to generate inspiratory network activity, recorded from the hypoglossal (XII) nerve rootlets contained in the slice.^{1,5} Using infrared differential interference contrast microscopy (IR-DIC) and whole-cell patch-clamp methods, we identify and record from inspiratory pacemaker neurons, interneurons projecting to XII motoneurons, and the XII motoneurons with axons in the hypoglossal rootlets.

In the pilot studies presented here, we typically targeted XII motoneurons to evaluate MRCI performance. Figure 1 diagrams the experimental setup, showing the relationship between the biological elements (whole-cell patch-clamped neurons), the patch-clamp amplifier, the acquisition system, and the computer used to calculate the artificial conductance. The dynamic clamp is very straightforward: membrane voltage is read and scaled appropriately, the value of the given modeled current is calculated based on the membrane voltage and instantaneously fed back out to the neuron. We have used both Axon Instruments (Axoclamp 1/Axopatch 1D) and HEKA amplifiers (EPC-9) in our experiments; MRCI incorporates adjustable scaling to accommodate different amplifiers.

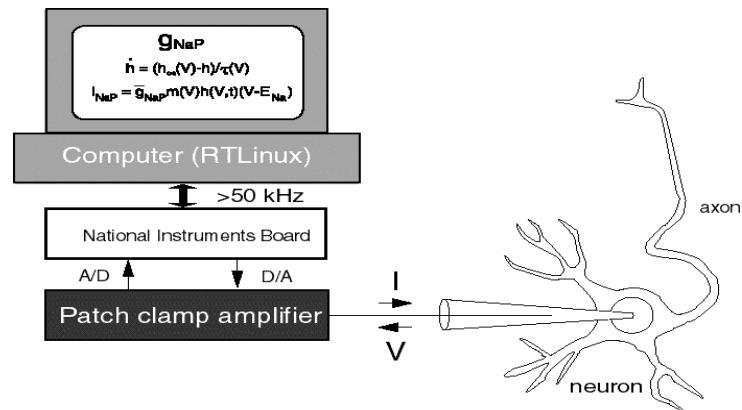


Figure 1. Simplified overview of MRCI. The real neuron, on the right, is held under whole-cell recording conditions by the patch-clamp amplifier (HEKA EPC-9 or Axon Axopatch 1D) and the membrane voltage is sampled into the computer running MRCI through a National Instruments data acquisition board (both A/D and D/A capable). The voltage is scaled appropriately and the model current calculated and injected back into the cell. Computational throughput rates of $>50 \text{ kHz}$ are possible with MRCI.

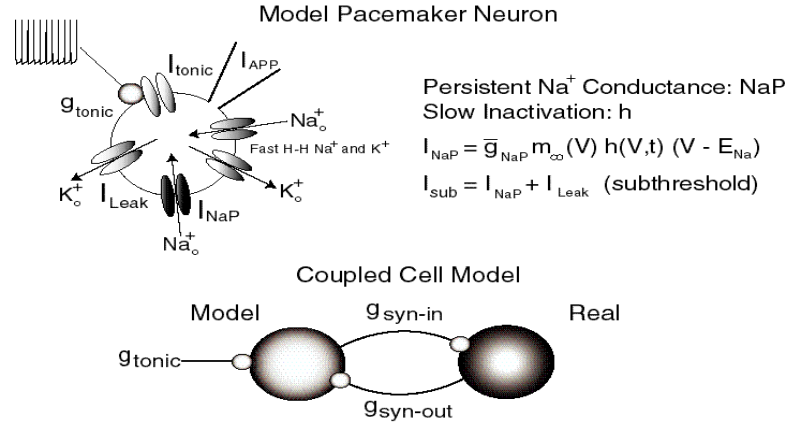


Figure 2. The model upon which MRCI is based. Our model includes a single compartment and various simulated currents/conductances including: Hodgkin-Huxley type fast Na⁺ and K⁺ currents, a K⁺ dominated leak current (I_{Leak}), tonic inputs (I_{tonic}), an applied current as through a patch-pipette (I_{app}), and our putative burst-generating current, I_{NaP} . In addition, a simulated cell within the MRCI computer can be connected to a real neuron with artificial excitatory synapses either unidirectionally or reciprocally through synaptic conductances g_{syn} fed into the real cell (g_{syn-in}) and connecting the cell to the model ($g_{syn-out}$).

3. MODEL

Details of the pacemaker neuron and network model upon which MRCI is based can be found in our earlier papers.^{3,4} Presented below are the relevant conductances for the single cell model and the model we use for synaptic coupling experiments (see Fig. 2). The single cell model is based on a bursting pacemaker model that incorporates a slowly inactivating I_{NaP} (persistent sodium current) acting as a burst generating and burst terminating conductance. In addition to I_{NaP} , the model includes fast Hodgkin-Huxley-like action potential generating sodium (I_{Na}) and potassium (I_K) currents, a linear leak current (I_{Leak}), tonic excitatory inputs ($I_{tonic-e}$), and the ability to apply a holding or pulse current (I_{app}). MRCI allows us to “endow” an *in vitro* neuron with any of these intrinsic or synaptic currents, whether or not the neuron innately possesses these properties. In an alternative version, a real-time model of a pacemaker neuron is coupled to an *in vitro* neuron via modeled reciprocal excitatory synapses (fast glutamatergic).⁴

4. SOFTWARE

MRCI depends on real-time, meaning that the system acquires membrane voltage and injects calculated current at highly reliable timing intervals. A real-time operating system is guaranteed that the timing requirements of the application will be met at relatively high rates of acquisition. Most operating systems have a variable latency before a task is scheduled for handling by the hardware, a latency of up to several hundred milliseconds. This varies greatly depending on the priority given to the application by the operating

system. This variability renders non-real-time operating systems inappropriate for the high speed sampling that neural data acquisition requires. To address this problem and to allow for accurate sampling and control of the patch-clamp amplifier, MRCI was developed on Real-Time Linux (RTLinux, <http://www.rtlinux.org>), on a Pentium II based computer (266 Megahertz). RTLinux is a modified version of the free Linux operating system that allows the MRCI application to run in **hard** real-time while the rest of the operating system runs when MRCI is idle. This design choice allows us to develop mission-critical real-time applications with the convenience of a free and robust development environment⁸ while still allowing real-time adjustment of model parameters and data logging. MRCI allows data acquisition and current injection rates of up to 50 KHz with high reliability, speeds sufficient to allow simulation of the full range of conductances in our model, including fast Hodgkin-Huxley ionic currents.

5. MRCI EXPERIMENTS

Our initial experiments included protocols in which we injected I_{NaP} into XII motoneurons that do not spike during inspiratory network activity, to show that I_{NaP} is sufficient to elicit pacemaker-like bursting without the addition of more complex currents other than those present in the cell. Fig. 3 shows a neuron that exhibited no rhythmic activity until I_{NaP} was added, and then burst like a pre-BötC pacemaker. A non-pacemaker neuron, even when synaptically driven by the inspiratory network, will tonically spike when depolarized above action potential threshold. Conversely, pacemaker neurons generate bursts of increasingly shorter duration and higher frequency when depolarized until they tonically spike.

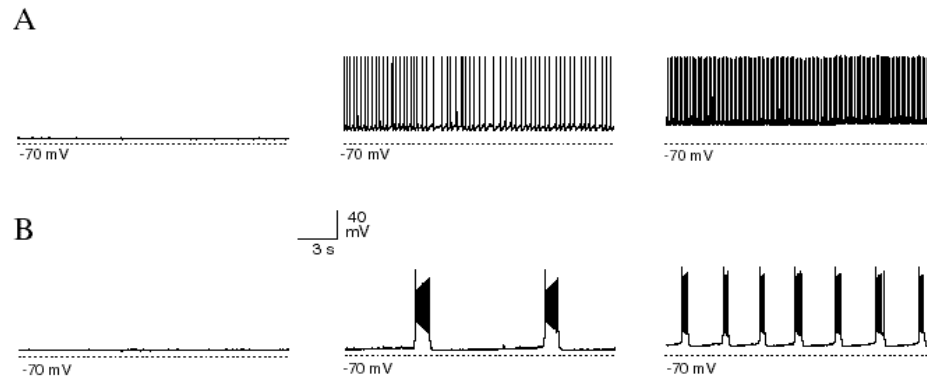


Figure 3. An example of a "mundane" motoneuron to which I_{NaP} has been added to generate pacemaker-like rhythmic bursts. In panel A (before the addition of I_{NaP} with MRCI), from left to right, as the cell is progressively depolarized with I_{app} it begins to spike tonically and does not burst. Panel B (after the addition of I_{NaP}) shows that as the cell is depolarized, the motoneuron bursts with increasing frequency (decrease in burst duration and spikes/burst) as the cell is further depolarized.

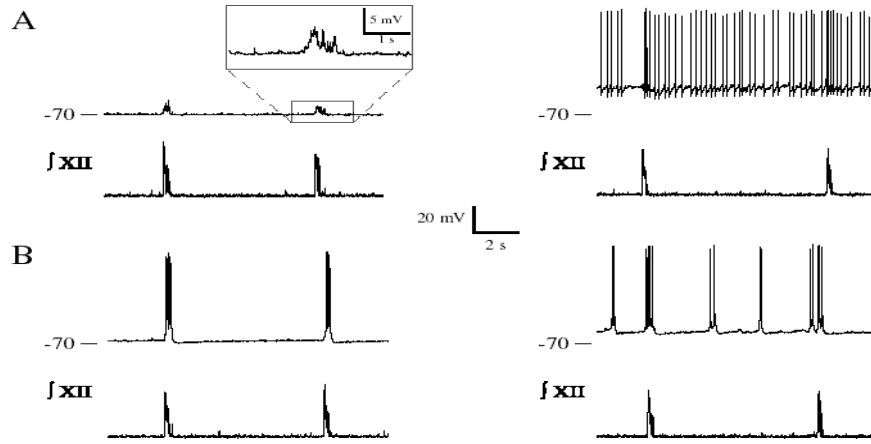


Figure 4. A synaptically driven neuron before and after MRCI added I_{NaP} . In A, the cell is depolarized and exhibits tonic spiking, with bursts occurring only during synaptic drive. In B, with the addition of I_{NaP} , the cell now discharges ectopic bursts upon depolarization. Note: at the left in both A and B, the membrane potential is the same showing an enhancement of synaptic input with the addition of I_{NaP} . Center scale bars apply for all traces except the expanded subthreshold burst in A.

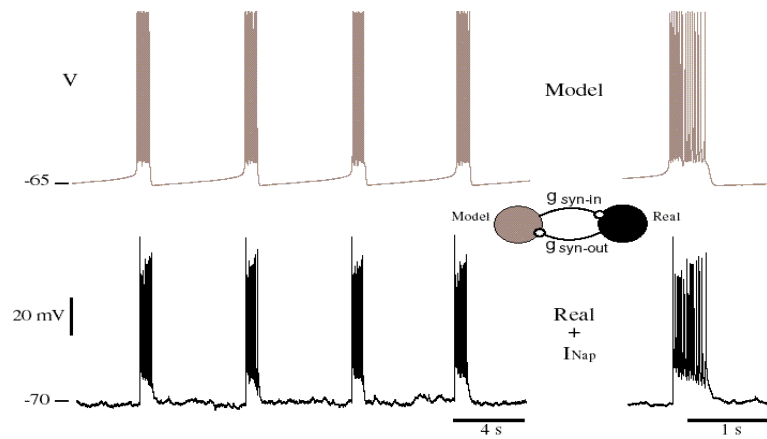


Figure 5. Coupling of a simulated bursting neuron to an MRCI generated burster. A modeled pacemaker (top panel) coupled to a real neuron (bottom panel) with added I_{NaP} by excitatory synapses (as in the inset) provides an ideal network to test model predictions about coupling strength and its effect on bursting. The inset indicates that these neurons are reciprocally connected with artificial excitatory synapses. The faster time scale traces on the right show the characteristic decrease in spike firing rate during the burst.

We have also added I_{NaP} to cells receiving rhythmic synaptic drive from the respiratory network. Fig. 4 is an example of a synaptically driven motoneuron that (top panel) shows, tonic spiking when depolarized and only bursts when driven by the network. In the lower panel, the neuron shows ectopic bursting when comparably depolarized. Additionally, at the same membrane voltage, after adding I_{NaP} with MRCI the synaptic drive is sufficient to induce a burst. This synaptic enhancement phenomenon has been previously described^{9,10} and suggests that even sparsely distributed I_{NaP} is sufficient to amplify synaptic input and change the behavior of the neuron.

In our most recent experiments we use MRCI to simulate a respiratory pacemaker neuron and couple that modeled cell with a real neuron to which we've added I_{NaP} via MRCI. We found that by varying the synaptic coupling strength between the model neuron and the real neuron we can synchronize the neurons and determine how coupling strength and connectivity affect pacemaker activity. We have previously shown that increased coupling strength slows pacemaker network bursting.⁴ Figure 5 shows the resulting activity when the model cell is coupled to the real neuron with it's added burst generating current..

6. CONCLUSIONS

We have developed a dramatically improved, freely available, and extensible version of the dynamic clamp using open source software and relatively common laboratory hardware. We built MRCI from the ground up as a bridge between experiment and model to test our model-driven hypotheses about burst generating currents, synaptic enhancement in bursting neurons, and coupling in pacemaker neuronal networks. Our experiments show that MRCI scales from very slow currents to very fast currents (e.g., we have reintroduced fast Na^+ and K^+ conductances in cells that have been denuded of these currents) and performs at very high sampling rates, more than adequate for the majority of modeled synapses and ionic conductances. MRCI is modular and extensible using the C programming language. We use a robust, reliable, and open source operating system to insure that MRCI can be altered to fit many needs.

MRCI is under continual development and we hope to build a strong user community to facilitate improvement and extension of the system. We are currently planning on adding a more user-friendly interface as well as more extensive features. For more information or to download MRCI software please visit the project's home page at <http://users.ece.gatech.edu/~rbutera/software/mrci.html>.

7. REFERENCES

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