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# Locomotor network modeling based on identified zebrafish neurons

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**Abstract**

The larval zebrafish generates a discrete set of locomotor maneuvers, each with distinctive bending patterns and tail-beat frequencies (TBFs). It is not known how these locomotor patterns are generated. We had previously shown that aspects of the locomotor repertoire could be modeled with a simple 30 segment replicated serial model that simulated the larval spinal cord. This model, however, conflicted with known features of the spinal circuitry and was not able to produce the natural whole-cord activity patterns. We present here three new more realistic CPG models which incorporate anatomical and neurotransmitter features of identified zebrafish spinal interneurons. These whole-cord models were able to produce oscillatory rhythms across the range of natural TBFs in ways that the simpler model could not.



*Keywords:* CPG; Swimming; Locomotion; Zebrafish; Spinal cord; Brainstem; Motor control

## 1. Introduction

Understanding the operation of the spinal neural networks that underlie locomotor rhythms is a challenge with both theoretical and clinical implications. While a number of models have been put forth to explain the operation of spinal central pattern generators or CPGs [5,13,24] there is still much uncertainty. In the case of the lamprey CPG, there is an indeterminate number of cell types in each segment of lamprey spinal cord [4] and so there may be as yet unidentified neurons that contribute to the CPG. In the case of the *Xenopus* tadpole, there seem to be fewer cell types, yet even in this apparently simpler system, there are still diverse views on the precise mechanisms of rhythm generation [1,18]. The situation is more complex in mammals, but the application of new molecular techniques promises to accelerate progress across species [17].

In spite of these uncertainties, there is much common ground. *Excitatory ipsilateral descending neurons* (termed *EINs* in lamprey) are believed to provide an excitatory drive that activates both AMPA and NMDA receptors in both Xenopus and lamprey [7,26] and possibly in all vertebrate spinal CPGs. It is also well accepted that a commissural glycinergic inhibitory interneuron is central to the generation of the alternating spinal activity that underlies undulatory swimming in lower vertebrates [5]. Potentially homologous cell types are present in zebrafish [15]. Given the highly conserved nature of spinal cell types stretching from agnathans to amphibians [9], it is plausible that there is a canonical rhythm generation mechanism that is largely conserved across the vertebrate sub-phylum.

Because the larval zebrafish CNS is transparent and well-suited for genetic analysis and manipulation, there is considerable interest in understanding both its development [19] and functional organization [10,21,23]. The larval spinal cord is believed to have about 15 distinct types of spinal interneurons [14], and the neurotransmitter phenotypes have recently been determined [15]. Two cell types, the MCoD and large CiD cells, are known to be active during swimming and escape behaviors respectively [23], but for most cell types their functional roles remain to be determined. This diverse array of spinal cell types is almost certainly involved in generating the extensive locomotive repertoire of the larval zebrafish [2,3,20,25], but there is also an array of brainstem neurons whose spinal projec-

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doi:10.1016/j.neucom.2005.12.068

tions presumably shape the output of these spinal networks [11,12,22].

In this report, we significantly extend our previous model [16] by incorporating neurons that implement key anatomical and phenotypic features of individually identified zebrafish spinal interneurons. By modulating synaptic strengths, we were able to recreate, in an anatomically more realistic architecture, the range of oscillator frequencies or TBFs normally exhibited by larval zebrafish, with some characteristics not explicitly seen in the 2-cell model calculation.

## 2. Methods

We used 6-cell and 8-cell models comprised of excitatory and inhibitory Hodgkin–Huxley neurons. Details on ionic conductances, synaptic time constants and other modeling parameters are the same as in our original 2-cell model calculations [16]. There we used a simple 2-cell segmental model, in which each hemi-segment's cell made a reciprocal inhibitory (glycine-like) connection to the contralateral hemi-segment. Each cell also made a recurrent, self-excitatory glutamate (NMDA and AMPA) synapse. We used the *NEURON* modeling program to integrate the differential equations and the statistical program "R" to do the spiking data analysis. The calculations were done in Pentium-4 or a 16-node Itanium cluster computers. In some simulations, the segmental oscillators are replicated to create a chain of 30 identical segments connected in series via a descending, ipsilateral excitatory synapse. Oscillatory activity is triggered by a brief asymmetric current injection to the cells of the first segment. To vary the strength of excitation (or inhibition), all excitatory (or inhibitory) synapses are varied en masse. The consequences of varying synaptic strength was assessed by measuring oscillator or tail-beat frequency (TBF) for each hemi-segment.

The 2-cell model was first expanded into a 6-cell segmental model (3 -cells per hemi-cord) by adding in both an excitatory and an inhibitory neuron, one per hemi-cord. The excitatory neuron descended ipsilaterally for 13 segments, giving off mixed excitatory (NMDA and AMPA) synapses to all cells within each hemisegment it passed through. The inhibitory neuron projected contralaterally and bifurcated to send an axon both rostrally and caudally for four segments giving off inhibitory synapses onto all cells in each hemi-segment to which it projected. The third neuron in each hemi-segment is a "slave" motoneuron that has no spinal outputs, but instead acts as a readout cell from which action potentials are recorded as discrete events (each time the membrane voltage moves positive to 0 mV). The motoneuron firing rate was used to calculate TBF. In further simulations, two cell types posited to participate in other spinal CPGs were incorporated by adding a fourth cell type to each hemi-segment (8-cell model), as detailed in the results and figure legends. To characterize the behavior of the 6-cell and 8-cell models, synaptic weights of the AMPA, NMDA and glycine synapses were automatically varied over large ranges.

## 3. Results

Our earlier 2-cell model was able to produce the range of oscillator or TBF normally exhibited by larval zebrafish, and when replicated into a 30-segment model produced neural outputs that were consistent with the kinematic patterns of the larval trunk, at least for some sets of parameters [16]. To carry out a more detailed evaluation between the 2-cell and 6–8-cell models, we first performed a more complete analysis of the original 2-cell/30-segment model. We had anticipated that each segment of the 30-segment model might fire in a coordinated fashion, i.e. following the preceding segment after a brief delay. Although this had been observed with certain parameter sets [16], this was not always the case.

The sets of synaptic weights that gave stable oscillatory patterns over a broad range of TBFs in the 2-cell model gave identical results in the first segment of the 30-cell model (Fig. 1A; the two are formally identical): sustained oscillations at frequencies ranging from 15 to 80 Hz were observed. But in downstream segments, these parameters produced stable rhythms only in certain regions of the frequency phase space, as illustrated for segments #8 and #15 (Figs. 1B,C). In the outlined region towards the center of each parameter space (where indicated by asterisks), a rhythm was often observed initially but broke down over time. When the AMPA synaptic strength was increased by 100-fold, segment #1 still yielded a continuous range of values producing stable rhythms (Fig. 1D). With this increased AMPA value, the more caudal segments showed a more complete "filling" of the parameter space, in comparison to the lower AMPA-strength simulations, as is shown for segments #8 and #15 (Figs. 1E and F), but there will be still regions with irregular or failed alternation (as indicated by the asterisks). We next evaluated the effects of incorporating identified zebrafish neurons into the model.

Identified zebrafish spinal interneurons project for multiple segments, sometimes for half the length of spinal cord, depending on cell type. To evaluate their possible contributions to locomotor rhythm generation requires a model representing the 30 segments of the zebrafish spinal cord so that the axonal projection distances can be incorporated. The first modification was to "split" the artificial "dual-function" neuron of the original model into an inhibitory and an excitatory cell type based on zebrafish identified neurons (Fig. 2A). The excitatory Circumferential Descending (CiD) spinal interneuron was chosen based on its ipsilateral descending axon (which projects on average 13 segments caudally) and its excitatory (vglut2-positive) phenotype [14,15]. CiD is the sole excitatory element of the CPG in our 6-cell model and plays a role comparable to the lamprey EIN neuron. The Commissural Bifurcating Longitudinal (CoBL) neuron was chosen for its inhibitory (glycinergic) phenotype and its commissural

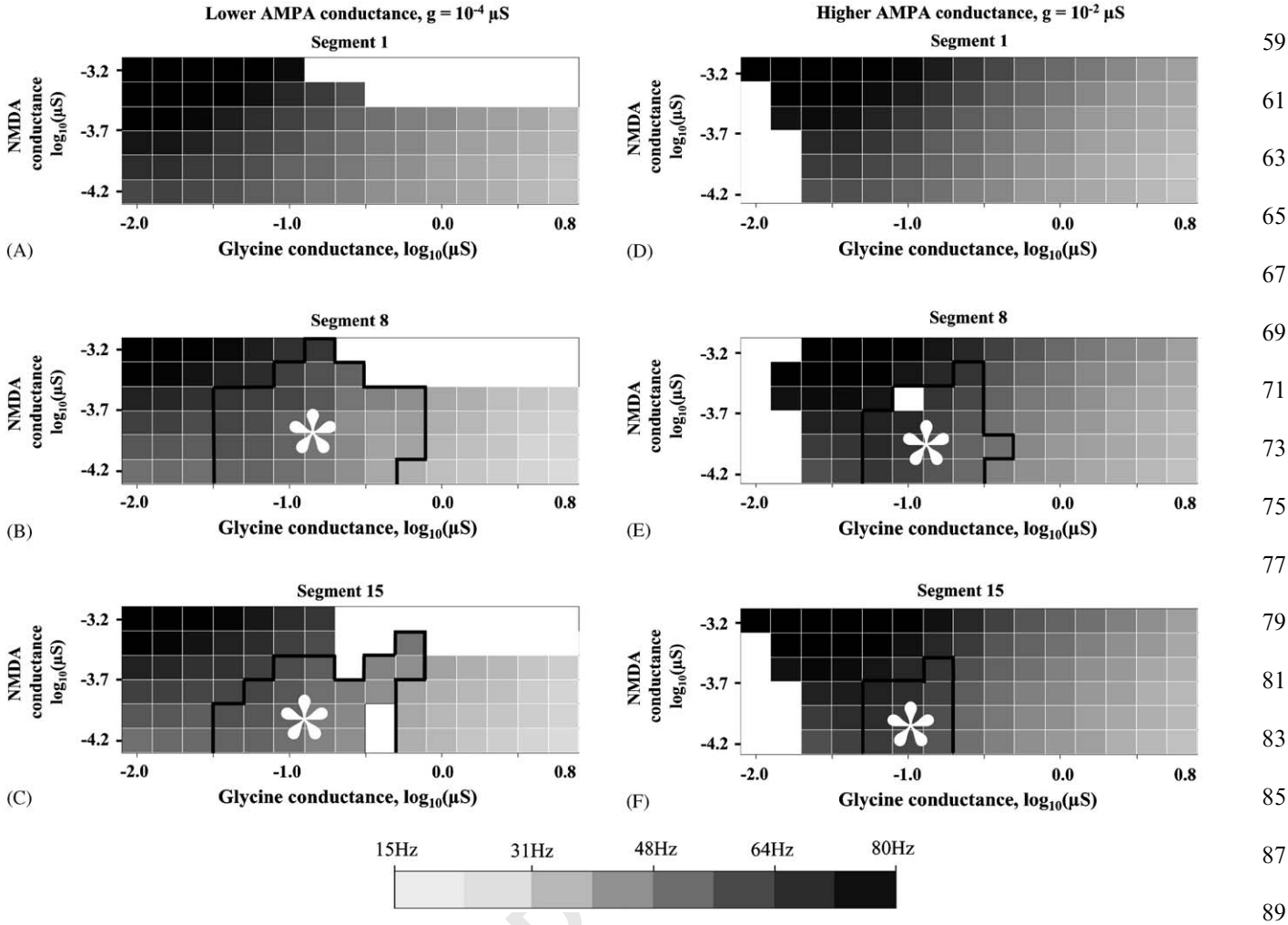


Fig. 1. Disrupted rhythms are seen in caudal segments of the 2-cell, 30-segment model at certain oscillation frequencies. Varying combinations of glycine and NMDA synaptic strengths produced oscillator frequencies ranging from 15 to 80 Hz, as indicated by the grey scale. In the open (white) regions of the plots there was no rhythmic firing. In the regions inside the solid lines (indicated by asterisks) there was partial rhythm disruption: normal firing epochs were periodically disrupted. At the lower AMPA strength (B,C) disruptions were greater than at the higher AMPA strength (E,F).

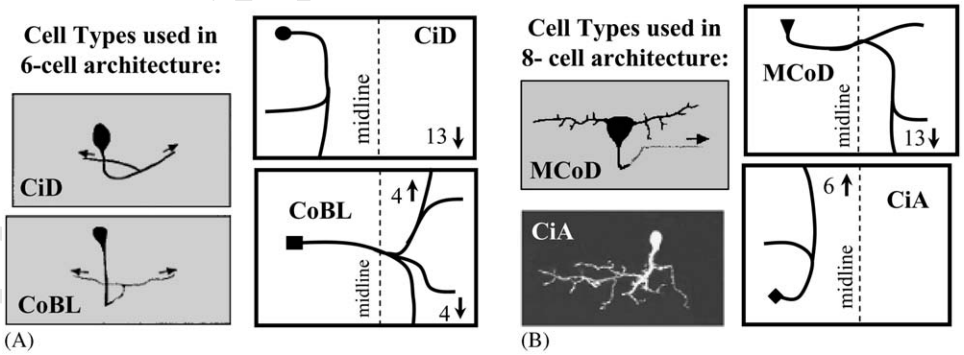


Fig. 2. Identified spinal interneurons from zebrafish. (A) The six-cell model includes 3 cells on each side: the CiD-like and CoBL-like interneurons and a readout "motoneuron". The interneurons make output synapses onto every cell in each hemi-segment they project to, which is indicated in the diagrams to the right; numbers indicate the number of segments projected to either rostrally (up) or caudally (down). (B) To produce the 8-cell segmental models, a fourth cell type was added to each hemi-segment. The CiD, CoBL, MCoD cell morphology silhouettes were adapted from Hale et al. (2001), while the CiA cell morphology silhouette was adapted from Higashijima et al. (2004).

projection which ascends and descends for 4 segments. CoBL is analogous to some members of the lamprey CC interneuron class. The third cell per hemi-segment is a "slave" motoneuron, which completes the "6-cell" per segment model, creating a 180-cell, whole-cord model. In addition, we evaluated the effects of separately adding in

two additional cell types, either the MCoD or CiA neurons, to generate two distinct 8-cell models (Fig. 2B). This allowed us to evaluate the operation of several alternative zebrafish spinal networks using more realistic anatomical features.

We first compared the performance of the 6-cell and 8-cell models within the parameter space originally used in the 2-cell model. We found that while some parameter sets yielded stable, alternating rhythms, there were quite large regions in this parameter space where the model failed (Figs. 3A–C); these bad regions were in fact more extensive than seen with the original 2-cell model. These failures were not due, however, to the models being intrinsically incapable of producing the desired TBFs, because when AMPA values were increased 100-fold, all of the models yielded stable rhythms across the full frequency range (Figs. 3D–F). In comparing these results with the 2-cell (30 segment) model, we find that extension to a 6-cell model, with realistic intersegmental projection lengths, provided more reliable frequency-generation performance with not large gaps located "within" the synaptic-weight parameter space where many values produce stable rhythms (asterisked regions in Fig. 1B,C; lacking in Fig. 3D). Furthermore, incorporation of two additional cell types (MCoD and CiA) for which there are putative homologues in other species, also yielded broad ranges of parameters where stable frequencies could be generated (Figs. 3E, F). Of these competing models, the 6-cell model might be considered most robust in strict terms of frequency generation, but this is only one performance measure. For this given parameter space, there are other aspects of the whole-cord activity patterns (still to be evaluated) that may prove central to producing trunk kinematics appropriate to the larval locomotor repertoire. One of the 8-cell models, or other testable architectures, may prove superior in such measures.

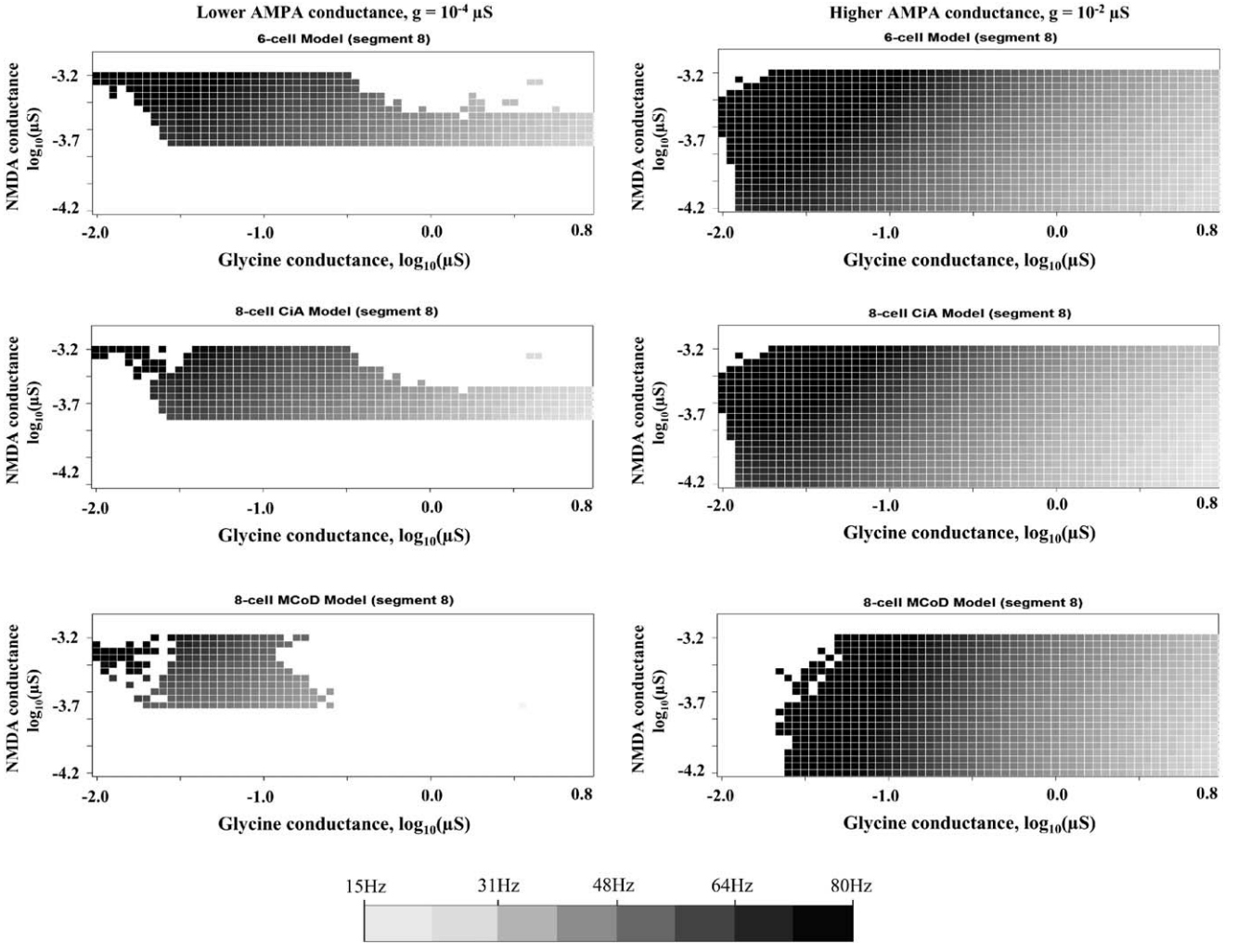


Fig. 3. Frequency responses of the 6-cell and 8-cell models. For all 3 models, at the high-AMPA level, there was a larger and more continuous region of parameter space in which alternating, regular firing pattern was observed. The presence of contiguous regions of parameter space over which stable oscillations are produced represents a regime over which CPG or oscillator frequency can potentially be modulated and thereby provides a potentially robust mechanism for generating larval TBFs.

## 4. Discussion

Simulation of three distinct neural architectures, which seem plausible candidate architectures for the zebrafish spinal CPG, reveals that each can generate alternating rhythms over the broad range of TBFs exhibited by zebrafish larvae. The incorporation of intersegmental connections, spanning numerous segments, is a necessary step towards more realistic modeling because spinal interneuron classes with purely nearest-neighbor coupling have not (to our knowledge) been described for any vertebrate animal. Thus, the true neurodynamics at play in the living spinal cord must be able to operate within such anatomical constraints. While the specific identified neurons chosen may not be correct, the choices do, to a significant extent, "bracket" an anatomical space within which most remaining zebrafish spinal interneurons fall. There are about 15 distinct interneuron types in the larval spinal cord [14,15], and relatively few with the required axonal projection pattern and phenotype to serve the CPG roles played by the lamprey EIN and CC interneurons. For example, the zebrafish VeMe cell is sufficiently similar to CiD, in terms of projection distance, that it would likely support the activity patterns produced in our model. But because there is no physiological data available for VeMe, the CiD cell is (currently) the more appropriate choice.

Synaptic weights are just one of a number of parameters that can be varied to produce different frequencies of rhythm generation. Ionic conductances, e.g., can be modulated to alter intrinsic network frequencies [4,5]. Nonetheless, large numbers of descending neurons are involved in swimming and escape behaviors [8,12], and based on axonal arborization patterns [1] an increased synaptic output of the reticulospinal system along the full length of cord seems a plausible hypothesis. Thus, the large increase in excitatory synaptic strength required to produce burst swim frequencies ($>45$ Hz), does not (necessarily) imply modulation of the strength of individual synapses, but might well be produced by a brainstem population code in which there is a greater number of AMPA/NMDA synapses active during, for example, the more vigorous bouts of burst swimming.

In this paper we have shown that increasing the complexity of the models in an effort to better represent the available neuronal data does lead to quantitatively different results, and in some instances the 2-cell to 8-cell models calculations lead to qualitatively different results. More work improving and bracketing the physiologically realistic properties and parameter ranges of the neuronal models should lead to better predictions of the neurodynamics used in the generation of larval locomotor behaviors.

Support for this research from CIRCS is gratefully acknowledged.

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