Structure of large cells in crab cardiac ganglion – a computational study

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INTRODUCTION

- The cardiac ganglion (CG) of the crab *Cancer Borealis* coordinates the rhythmic contractions of a single heart muscle. The ganglion consists of 9 cells, 5 large motor cells (LCs) and 4 small endogenous pacemaker cells (SCs)[1]. (Fig.1)
- We report an improved 3-compartmental LC model that is thought to more accurately represent biology. The three compartments are: soma, neurite, spike-initiation zone (SIZ).
- The new LC model was used to investigate variations in maximal densities that preserved LC responses seen in experiments.
- Using a rejection sampling technique, we aim to embed the LCs that pass the criteria above into a network model and predict covariations among a wider parameter space, which modulate the single cell and network output.

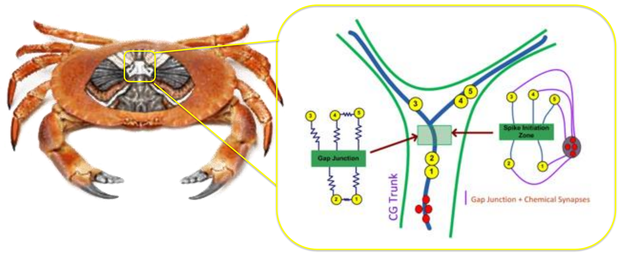

Fig.1 Cardiac ganglion with 5 LCs and 4 SCs.

METHODS

Single Cell Model

- We used the same ion channel models as those by Samarth at el.[1] Based on that, we assigned realistic dimensions to each compartment, measured from published photos of the specimen.[2] A passive neurite segment is also added, which we found to be essential for reconstructing the difference in waveforms at the soma and the spike initiation zone (SIZ).
- The soma + neurite model is tuned to pass the passive property test and the current characterization test using data from the Schulz Lab, including 8 different voltage clamp and current clamp experiments. The full model is tuned to match the intact LC current clamp data.
- The membrane potential is governed by:

$$(Soma)\ C\frac{dV}{dt} = -I_{CaT} - I_{CaS} - I_{CAN} - I_{NaP} - I_{KA} - I_{Kd} - I_{BKKCa} - I_{SKKCa} - I_{Leak}$$

$$(SIZ)\ C\frac{dV}{dt} = -I_{Na} - I_{Kdr} - I_{Leak}$$

Ion channel	Maximum Conductance Density (S/cm²)
CaT	0.00031
CaS	0.00013
CAN	0.000105
NaP	0.00019
KA	0.0025
Kd1 (soma)	0.0009
Kd2 (soma)	9.00E-05
KCa	0.011
SKKCa	0.000879
Leak (All segments)	0.00015
Na	0.5
Kdr (SIZ)	0.22

Table.1&2 Parameters and equations of the nominal model

F = Faradays constant
R = Gas constant
V = Membrane voltage
[Ca²⁺] = intracellular Calcium concentration

I_{ion}	x^p	x_∞	τ_x (msec)
I_{Na}	m^3	$\frac{0.3463}{1+0.0086e^{-\frac{V}{9.93248}}} + \frac{0.75187}{1+1.11022e^{-\frac{V}{9.93248}}} + 0.162947$	$3.002 + \frac{4.073}{1+\exp((V+24.18)/2.592)}$
	h	$\frac{0.93854475}{1+144209.656e^{\frac{V}{5.08668603}}} + 0.02804584$	$9.434 + \frac{11.7}{1+\exp((V+1)/5.317)}$
I_{CaS}	m^2	$\frac{1}{1+\exp((V+24.75)/-5)}$	$20 + \frac{50.2}{\exp((V+20.25)/1)}$
	h	$\frac{45}{40+[Ca^{2+}]}$	$\frac{1}{0.02}$
I_{CaT}	m	$\frac{1}{1+\exp((V+20)/-1.898)}$	$18.51 - \frac{3.388}{\exp((V-6.53)/9.736)+\exp((V+12.39)/-2.525)}$
	h	$\frac{1}{1+\exp((V+55.27)/6.11)}$	$20.23 + \frac{40}{\exp((V+23.48)/-9.976)+\exp((V+5.196)/10.84)}$
I_{Kd}	m^4	$\frac{1}{1+\exp((V+24.19)/-10.77)}$	$25.049 + \frac{25}{1+\exp((V+25.84)/6.252)}$
	h_1	$0.3 + \frac{1-0.3}{1+\exp((V+15.87)/5.916)}$	$550 + \frac{954.9}{\exp((V+10.8)/-15)}$
	m^4	$\frac{1}{1+\exp((V+23.32)/-10)}$	$100 + \frac{550}{\exp((V+15)/12.46)}$
I_{NaP}	m^3	$\frac{1}{1+\exp((V+32.7)/-18.81)}$	$3.15 + \frac{0.8464}{\exp((V+0.8703)/-6.106)}$
I_{CAN}	w	$(0.0002*[Ca^{2+}]^2)/(0.0002*[Ca^{2+}]^2+0.05))$	$(40/(0.0002*[Ca^{2+}]^2+0.05))$
$I_{SKK(Ca)}$	w	$(0.0001*[Ca^{2+}]^2)/(0.0001*[Ca^{2+}]^2+0.1))$	$(4/(0.0001*[Ca^{2+}]^2+0.1))$
$I_{BKK(Ca)}$	a	$\frac{[Ca^{2+}]}{(1+\exp((V-15+0.08*[Ca^{2+}])/-15))*(1+\exp((V+5+0.08*[Ca^{2+}])/-9))*(2+[Ca^{2+}])}$	$\frac{1}{0.4}$
	b	$\frac{7}{5+[Ca^{2+}]}$	$\frac{1}{0.2}$

METHODS Cont.

Rejection Sampling Technique – in two phases

- Phase 1: For each parameter set selected, G_{leak} was varied from 7e-5 S/cm2 to 30e-5 S/cm2 in steps of 1e-5 S/cm2 and input resistance was calculated. If the input resistance was within the acceptable range (2.63-7.43 MΩ), the responses to square pulse injections (50ms 6nA) for the Pre-TEA, Post-TEA and Post TTX cases were saved. The response to a stimulus protocol (Fig.3) when clamped at -40mV was also saved for each case. The parameter set was deemed 'acceptable' if it satisfied the following conditions:
  a) The duration of the Pre-TEA response to current injection should be less than 120 ms, with a the peak not higher than -22mV.
  b) The duration of the Post-TEA response should be between 255-667 ms, with its peak greater than -15mV.
  c) For the Post TTX response, the peak should decrease at-least 15 mV from its Post TEA response, and the duration should reduce to less than 120ms.
  d) Stimulus protocol response should have an r^2 value of at least 0.8 or higher when compared to an biological response.
- Phase 2: Add a biologically realistic neurite and SIZ to the passing soma from phase 1. The cells will be presented with continuous stimulation from simulated small cell input with the expectation of bursting periodically. For each case the following items need to be checked for accuracy:
  a) Spikes per burst, higher than 4 but lower than 15
  b) Burst interval, and Synchrony between cells

RESULTS

A. Selection of Large Cells based on Ligated Soma Experiments

- In Fig.2, the upper panel lists the published[1,3] and unpublished experiment recordings, and the lower panel lists the simulation results from current model. For the first 4 pairs, we used the soma + neurite model, where the soma was receiving the stimulus protocol. The biological plots were also from ligated somas. For the last 2 pairs we used the full model, with the SIZ receiving the injection, as the biological plots are from somas in intact networks.
- We found that the various shapes of post-TEA responses were determined by the interplay of three currents in the soma: NaP, CaS and SKKCa.
- As shown in the mini-panels below, if GNaP is increased a lot (4x), it will dominate the other two and produce a "fat" depolarization.
- If GNaP is not significantly increased (2x), the activation of SKKCa after CaS will quickly terminate the depolarization, resulting a sharp response.
- If GNaP has a value between those above (3x), the large depolarization can happen early enough to open CaS and then SKKCa, producing a "valley".

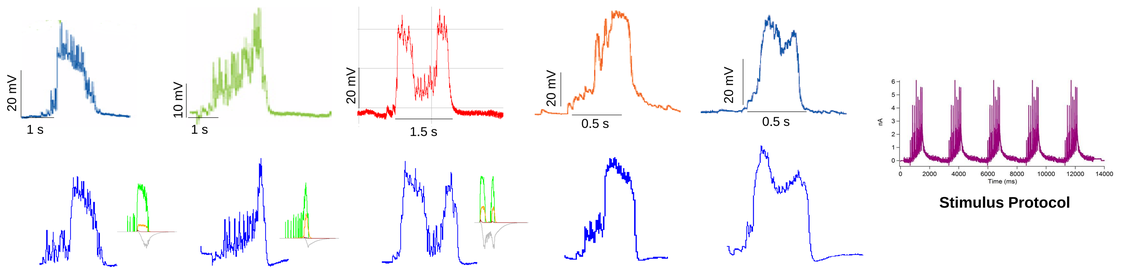

Fig.2 Post-TEA simulation using stimulus protocol

B. Selection of Large Cells based on Intact network experiments

- To further improve biological accuracy, SIZ and neurite sections were attached to passing single compartment somas to get 3-comp cells.
- LCs with 3-compartments each now need to pass the network level criteria of # of spikes/burst, burst frequency, etc.
- This may require addition of CaT and kCa channels to the neurite – being investigated presently. Initial results in Fig. 4

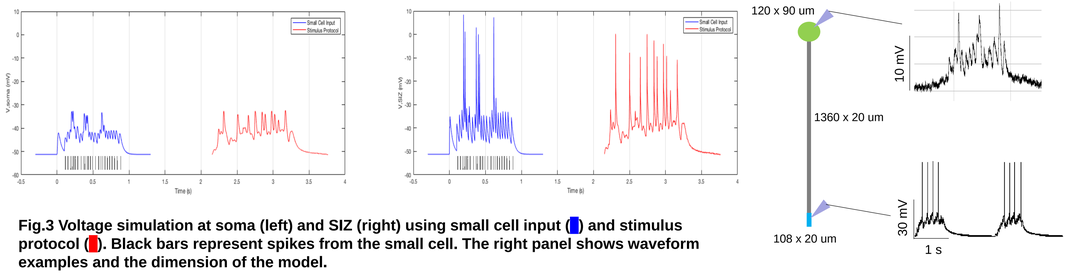

Fig.3 Voltage simulation at soma (left) and SIZ (right) using small cell input (▮) and stimulus protocol (▮). Black bars represent spikes from the small cell. The right panel shows waveform examples and the dimension of the model.

FUTURE WORK

A. Finalize tuning process for viable LCs, and then automate the procedure

- For this experiment viable cells were developed by incrementally adjusting parameters until they matched biological data. Once finalized, this will be automated in order to build a collection of viable cells for network implementation, similar to how the ligated soma cells were generated.

B. Determine covariations for "viable" LCs

- There is a tremendous amount of pre-TEA variations observed in the intact network recordings, whose underlying mechanism are yet unknown. We plan to study covariations in the parameters by using this set of cells.

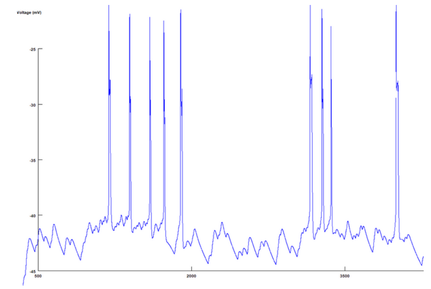

Fig.4 An example of an intact cell with burst intervals present

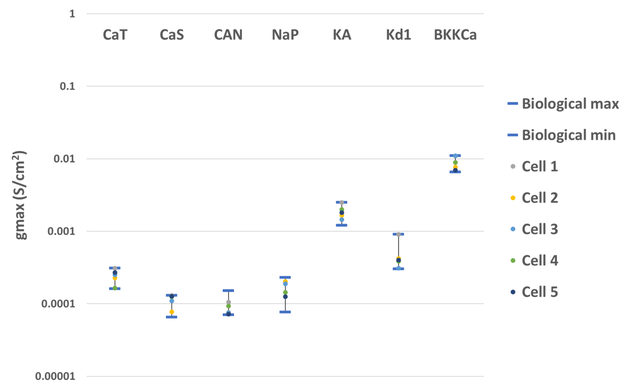

Fig.5 An example of 5 random LCs spanning across the parameter space

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