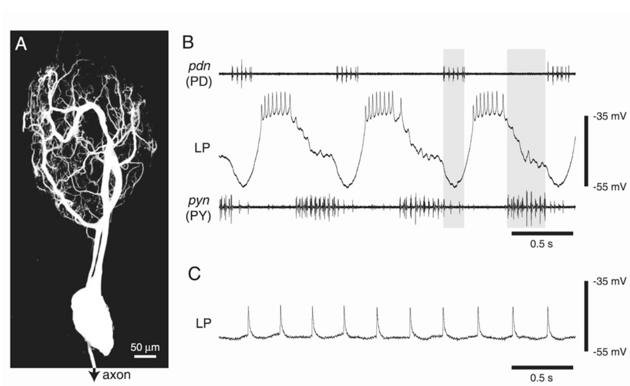
How do cells regulate their excitability? The density of channels, along with the gating properties of channels, determine a cell's degree of electrical excitability, its ability to respond to stimuli, the patterns of activity it will generate, its computational abilities generally. Somehow, there must be a feedback connection from a cell's electrical activity back to its membrane properties, in order to regulate the former.

Sources for this lecture:

Turrigiano, Abbott, Marder *Science* 264: 974-977 (1994).
LeMasson, Marder, Abbott *Science* 259: 1915-1917 (1993).
Turrigiano, LeMasson, Marder *J. Neuroscience* 15: 3640-3652 (1995).
Liu, Golowasch, Marder, Abbott *J. Neuroscience* 18: 2309-2320 (1998).

Taylor, Goaillard, Marder *J. Neuroscience* 29:5573-5586 (2009).
Grashow, Brookings, Marder *J. Neuroscience* 30:9145-56 (2010).

The lateral pyloric (LP) neuron, in the stomatogastric ganglion (STG) of the crab *Cancer borealis* is a motor neuron that controls the gastric mill of the crab. It is a complex neuron shown at left below. The rhythm that it generates depends on its own membrane properties and the circuit of the ganglion. The records at right below show the bursting pattern of an LP cell and extracellular recordings from two inhibitory inputs (PD and PY) which participate in generating the rhythm. If the inhibitory inputs are blocked with picrotoxin, the result is the limit cycle in C.



Cells in this ganglion regulate their membrane properties so as to produce a bursting pattern of activity, even when isolated from the rest of the ganglion. This lecture is about how that occurs..

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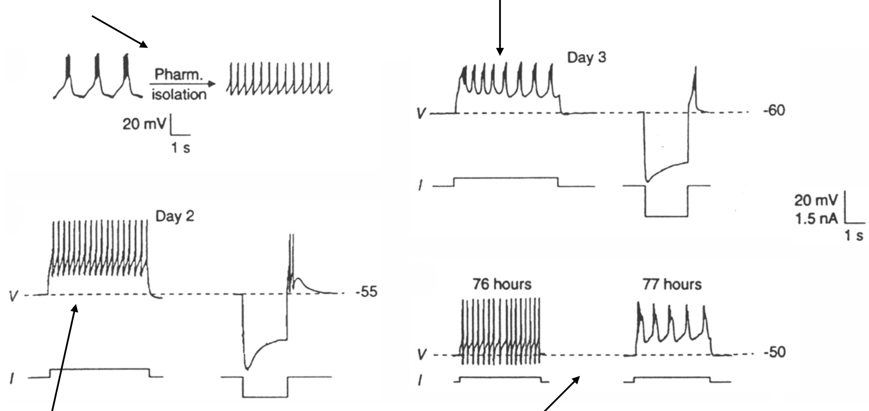
Stomatogastric ganglion neurons normally produce a rhythmic bursting, which depends on both their interconnections and their intrinsic properties

At some point, they begin to <u>burst</u> in response to depolarization.



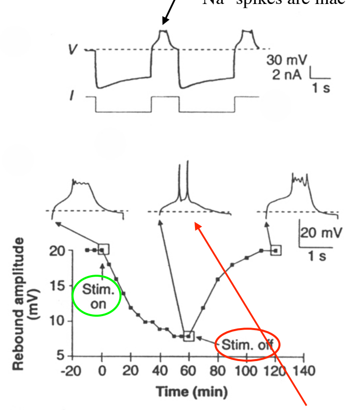
When isolated in culture, neurons are deprived of Ca^{++} and Na^{+} channels (on the dendrites) and are silent (day 1, not shown). Later, when depolarized, they produce a tonic discharge

The transition can happen suddenly

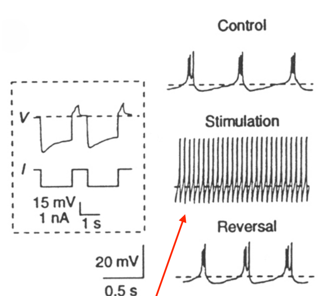
Turrigiano et al, 1994

The mode of activity is regulated, to move the cells into bursting activity

Rebound bursts evoked by anode-break; a Ca^{++} spike, Na^{+} spikes are inactivated



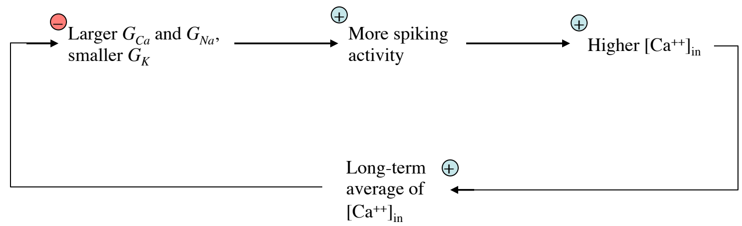
Amplitude of the Ca^{++} plateau is reduced by stimulation at a high rate. Na^{+} spikes return



In a different cell, 1 hour of stimulation suppressed bursting completely and reversibly

Turrigiano et al, 1994

The hypothesis: cells regulate the **average Ca^{++} concentration** in their cytoplasm. When $[Ca^{++}]$ is too low, Ca^{++} currents are increased and K^+ currents are decreased, and vice-versa.



In support of this idea, intracellular BAPTA (a calcium chelater) prevents adjustments

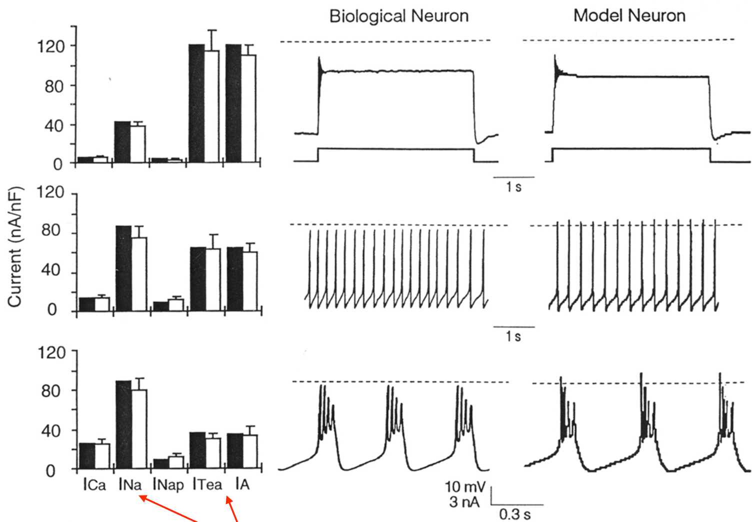
Voltage clamp analysis shows a number of channels present in the membrane

Table 1. Equations describing the activation and inactivation properties of the ionic currents of the model STG neuron

Current	p	m_∞	h_∞	τ_m	τ_h
I_{Na}	3	$\frac{1}{1+\exp\left[\frac{-V-25.5}{5.29}\right]}$	$\frac{1}{1+\exp\left[\frac{V+48.9}{5.18}\right]}$	$1.32-\frac{1.26}{1+\exp\left[\frac{-120-V}{25}\right]}$	$0.67\cdot\left[\frac{1}{1+\exp\left[\frac{-62.9-V}{10}\right]}\right]\cdot\left[1.5+\frac{1}{1+\exp\left[\frac{V+34.9}{3.6}\right]}\right]$
I_{Nap}	3	$\frac{1}{1+\exp\left[\frac{-V-26.8}{8.2}\right]}$	$\frac{1}{1+\exp\left[\frac{V+48.5}{4.8}\right]}$	$19.8-\frac{10.7}{1+\exp\left[\frac{-26.5-V}{8.6}\right]}$	$666-\frac{379}{1+\exp\left[\frac{-33.6-V}{11.7}\right]}$
I_{Ca1}	3	$\frac{1}{1+\exp\left[\frac{-V-27.1}{7.18}\right]}$	$\frac{1}{1+\exp\left[\frac{V+30.1}{5.5}\right]}$	$21.7-\frac{21.3}{1+\exp\left[\frac{-68.1-V}{20.5}\right]}$	$105-\frac{89.8}{1+\exp\left[\frac{-V-55.0}{16.9}\right]}$
I_{Ca2}	3	$\frac{1}{1+\exp\left[\frac{-V-21.6}{8.5}\right]}$		$16-\frac{13.1}{1+\exp\left[\frac{-V-25.1}{26.4}\right]}$	
I_{KCa}*	4	$\frac{[Ca]}{[Ca]+3}\cdot\frac{1}{1+\exp\left[\frac{-V-28.3}{12.6}\right]}$		$90.3-\frac{75.1}{1+\exp\left[\frac{-V-46}{22.7}\right]}$	
I_{Kd}	4	$\frac{1}{1+\exp\left[\frac{-V-12.3}{11.8}\right]}$		$7.2-\frac{6.4}{1+\exp\left[\frac{-V-28.3}{19.2}\right]}$	
I_A	3	$\frac{1}{1+\exp\left[\frac{-V-27.2}{8.7}\right]}$	$\frac{1}{1+\exp\left[\frac{V+56.9}{4.9}\right]}$	$11.6-\frac{10.4}{1+\exp\left[\frac{-V-32.9}{15.2}\right]}$	$38.6-\frac{29.2}{1+\exp\left[\frac{-V-38.9}{26.5}\right]}$
I_{As}	3	$\frac{1}{1+\exp\left[\frac{-V-24.3}{9.4}\right]}$	$\frac{1}{1+\exp\left[\frac{V+61.3}{6.6}\right]}$	$13.3-\frac{9.0}{1+\exp\left[\frac{-V-50.3}{11.8}\right]}$	$9821-\frac{9269}{1+\exp\left[\frac{-V-69.9}{4.6}\right]}$
I_h	1	$\frac{1}{1+\exp\left[\frac{V+78.3}{6.5}\right]}$		$272-\frac{-1499}{1+\exp\left[\frac{-V-42.2}{8.73}\right]}$	

Turrigiano et al. 1995

The model reproduces the activity of real neurons, when given conductance values equal to those measured in the three activity states of neurons in culture.



Early: inactivating spikes in response to current steps

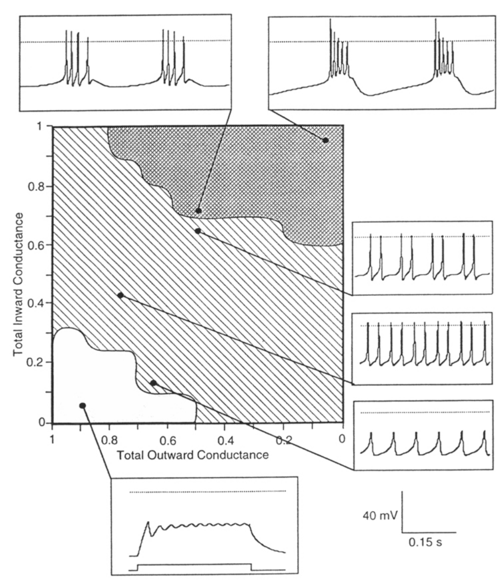
Later: tonic discharge in response to a steady current

Finally: bursting in response to a steady current

Note increase in Ca/Na and decrease in K currents.

Turrigiano et al. 1995

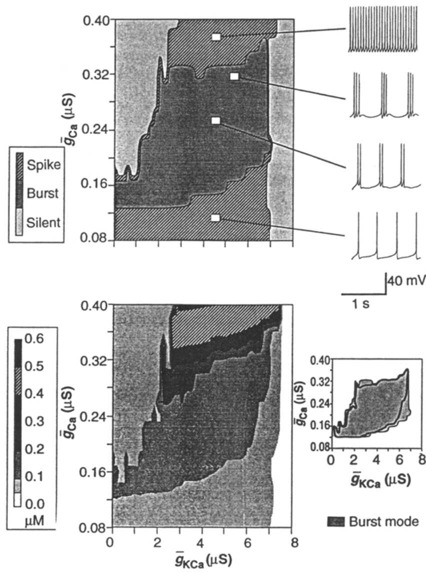
The nature of the activity produced by the model depends on the balance of inward (Ca^{++} and Na^{+}) and outward (K^{+}) currents



Turrigiano et al. 1995

Most important:

The type of activity shown by the cell . . .



Comparison of [Ca] ε [0.1,0.3] and region of bursting

is strongly correlated with the average [Ca^{++}] in the soma

So: controlling [Ca^{++}]$_{in}$ is sufficient to regulate the cell's activity type.

LeMasson et al. 1993

A model for the control of membrane conductances by long-term intracellular calcium:

Each conductance is determined by a first-order equation

$$\tau_i \frac{d\bar{g}_i}{dt} = f_i\left([Ca^{++}]_{in}\right) - \bar{g}_i$$

where $\bar{g}_i$ is the all-gates-open conductance of the i^{th} channel and τ_i is a time constant, set to 50 s in the model.

The function $f_i([Ca^{++}]_{in})$ is the target toward which the first-order system moves $\bar{g}_i$. It is determined by the calcium concentration according to

$$f_i\left([Ca^{++}]_{in}\right) = \frac{G_i}{1 + e^{\pm\left([Ca^{++}]_{in} - C_T\right)/A}}$$

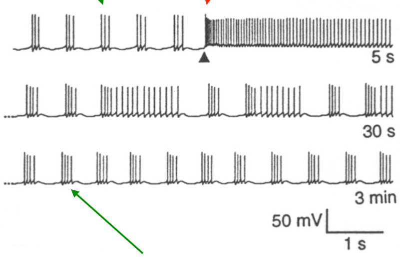
C_T is the calcium concentration target (say 0.2 mM, see the previous slide). A is a sensitivity parameter, set to 0.05 mM. The sign of the exponent is + for Ca^{++} and – for K^+, in order to provide negative feedback (high calcium should reduce calcium conductance).

LeMasson et al. 1993

Behavior of a model cell

Normal bursting

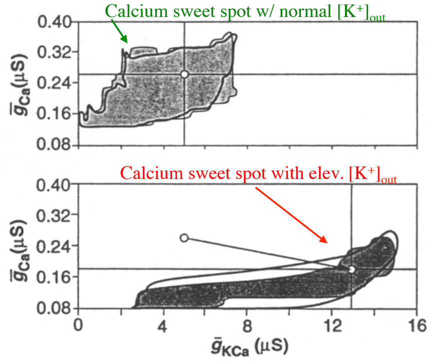
$[K^+]_{out}$ is increased (E_K goes -80 to -65 mV) producing tonic discharge



Model finds its way back to bursting

Calcium sweet spot w/ normal $[K^+]_{out}$

Calcium sweet spot with elev. $[K^+]_{out}$



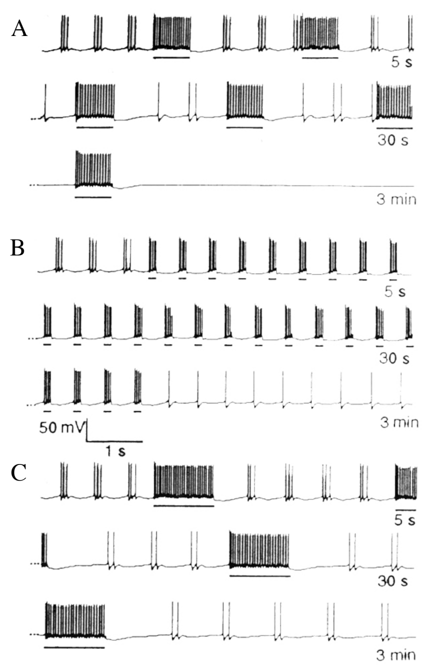
LeMasson et al. 1993

Three more tests of the model, in which equal total amounts of electrical stimulation are applied to the cell to force its activity.

A. Long stimulus bouts decrease bursting

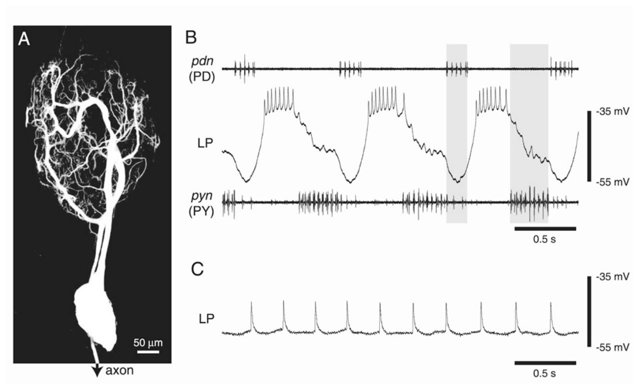
B. Short rapid bursts

C. Long, infrequent stimulation



LeMasson et al. 1993

Electrical activity of cell (LP) and two important inhibitory synaptic inputs (PD and PY) are shown at right. If the inhibition is blocked (picrotoxin), the cell gives the limit cycle in C, showing the importance of network interactions in producing the bursts.



The model in previous slides postulates a very simple kind of regulation, but real neurons have many more parameters to adjust. A few channel parameters seem to be regulated by mRNA, in that mRNA levels are correlated with channel conductances, but most are not, so how can regulation be accomplished? Perhaps there are electrophysiological constraints that simplify regulation? How wide is the parameter range that allows proper electrical activity?

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Consider a model of the LP cell. The models has 4 compartments (below) with 12 ion channels (those at right plus 2 leaks).

The model includes Ca^{++} accumulation, to drive the K(Ca) channel and has separate channels for the axon and the soma/ dendrites.

There are three inhibitory synaptic inputs, driven by the pyloric rhythm.

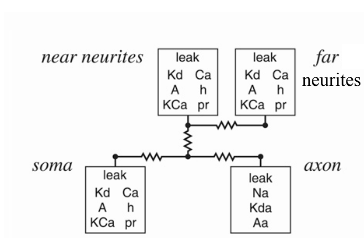

Table 1. Equations governing the voltage dependence and kinetics of currents in the LP model

		m_∞	τ_m	h_∞	τ_h
I_{Kd}	m^4	$\mathrm{lgc}\left(\frac{v+25}{17}\right)$	$120-113.8\cdot\mathrm{lgc}\left(\frac{v+46.1}{18.1}\right)$		
I_{Af}	m^3h	$\mathrm{lgc}\left(\frac{v+14.5}{18.1}\right)$	3	$\mathrm{lgc}\left(-\frac{v+68.1}{4.5}\right)$	$119.4-100.1\cdot\mathrm{lgc}\left(\frac{v+1.8}{4}\right)$
I_{As}	m^3h	$\mathrm{lgc}\left(\frac{v+21.0}{22.8}\right)$	$10.3-5.3\cdot\mathrm{lgc}\left(\frac{v-5.6}{4}\right)$	$\mathrm{lgc}\left(-\frac{v+55.0}{4.8}\right)$	$\frac{1}{253.4^{-1}\cdot\mathrm{lgc}\left(\frac{v+9}{11.1}\right)+250.5^{-1}\cdot\mathrm{lgc}\left(-\frac{v+92}{16}\right)}$
I_{Ca}	m^3h	$\mathrm{lgc}\left(\frac{v+15.2}{15.6}\right)$	$1.8+2.3\cdot\mathrm{lgc}\left(\frac{v+40.2}{20.7}\right)$	$\frac{1}{1+[Ca]_i/12.57}$	0
I_{KCa}	mh	$\frac{\mathrm{lgc}\left(\frac{v+5.5}{8}\right)}{1+\left([Ca]_i/1.43\right)^{-5}}$	$499-494\cdot\mathrm{lgc}\left(\frac{v-\left[-51.9-2.7\ln([Ca]_i)\right]}{10}\right)$	$\frac{1}{1+\left([Ca]_i/7.2\right)^{5/4}}$	11.85
I_h	m	$\mathrm{lgc}\left(-\frac{v+84.3}{6.4}\right)$	$\frac{46.9}{\mathrm{lgc}\left(+\frac{v-29.7}{19.4397}\right)+\mathrm{lgc}\left(-\frac{v+206.2}{19.4397}\right)}$		
I_{pr}	m	$\mathrm{lgc}\left(\frac{v-v_{1/2,pr}}{5}\right)$	6		
I_{Na}	m^3h	$\frac{1}{1+\frac{0.083^{-1}\cdot\exp\left(-\frac{v+48.73}{16.480}\right)}{0.333^{-1}\cdot\mathrm{linoid}\left(\frac{v+25.84}{9.155}\right)}}$	$\frac{1}{0.333^{-1}\cdot\mathrm{linoid}\left(\frac{v+25.84}{9.155}\right)+0.083^{-1}\cdot\exp\left(-\frac{v+48.73}{16.480}\right)}$	$\frac{1}{1+\frac{1.332^{-1}\cdot\mathrm{lgc}\left(\frac{v+13.76}{5}\right)}{19.028^{-1}\cdot\exp\left(-\frac{v+28.76}{10}\right)}}$	$\frac{1}{19.028^{-1}\cdot\exp\left(-\frac{v+28.76}{10}\right)+1.332^{-1}\cdot\mathrm{lgc}\left(\frac{v+13.76}{5}\right)}$
I_{Kda}	m^4	$\frac{1}{1+\frac{21.312^{-1}\cdot\exp\left(-\frac{v+47.3}{68.28}\right)}{26.639^{-1}\cdot\mathrm{linoid}\left(\frac{v+38.77}{8.535}\right)}}$	$\frac{1}{26.639^{-1}\cdot\mathrm{linoid}\left(\frac{v+38.77}{8.535}\right)+21.312^{-1}\cdot\exp\left(-\frac{v+47.3}{68.28}\right)}$		
I_{Aa}	m^3h	$\left[0.0761\cdot\exp\left(\frac{v+102.41}{32.996}\right)\cdot\mathrm{lgc}\left(-\frac{v+5.98}{29.98}\right)\right]^{1/3}$	$1.710+5.453\cdot\mathrm{lgc}\left(-\frac{v+62.76}{20.85}\right)$	$\left[\mathrm{lgc}\left(-\frac{v+55.9}{15.015}\right)\right]^4$	$6.276+13.555\cdot\mathrm{lgc}\left(-\frac{v+52.5}{16.550}\right)$

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The analysis proceeds by generating ~600,000 models with random variation of the parameters as in the table at right.

The models were tested for *acceptability* based on

1. Input conductance
2. Spontaneous activity
3. Activity in the presence of synaptic inputs.

The ranges of acceptable parameters are given below.

Table 2. Range of parameters used in random sampling of parameter space

	Parameter	Minimum	Maximum	Units
Soma and neurites	E_{leak}	−23	−13	mV
	$\bar{g}_{leak}$	0.001	0.002	μS/nF
	$\bar{g}_{Kd}$	0	0.2	μS/nF
	$\bar{g}_{A}$	0	0.5	μS/nF
	$\bar{P}_{Ca}$	0	6	$\mu m^3/(ms \cdot nF)$
	$\bar{g}_{KCa}$	0	1	μS/nF
	$\bar{g}_{h}$	0	0.02	μS/nF
	$\bar{g}_{pr}$	0	0.008	μS/nF
	$v_{½,pr}$	−55	−35	mV
Neurites	$\bar{g}_{synAB}$	0	0.06	μS/nF
	$\bar{g}_{synPD}$	0	0.06	μS/nF
	$\bar{g}_{synPY}$	0	0.02	μS/nF
Axon	$E_{leak,ax}$	−7	+3	mV
	$\bar{g}_{leak,ax}$	0.2	0.45	μS/nF
	$\bar{g}_{Na}$	0	600	μS/nF
	$\bar{g}_{Kd}$	0	74	μS/nF
	$\bar{g}_{Aa}$	0	100	μS/nF

For each model, each parameter was drawn independently from a uniform distribution with the given bounds. $\bar{g}_x$ is the maximal conductance of any current x, E_{leak} values are leak reversal potentials in the indicated compartments, $\bar{P}_{Ca}$ is the maximal permeability of the (nonohmic) calcium conductance, and $v_{½,pr}$ is the half-activation potential of the proctolin-activated conductance. During sampling, the capacitances and axial resistances were fixed. See Results, Production of LP model population, for an explanation of how these ranges were chosen.

Table 3. Bounds on properties used to define the population of admissible LP model neurons

Property	Lower bound	Upper bound
Input conductance (nS)	36	132
Resting membrane potential (mV)	−47.5	−32.5
Resting spike rate (Hz)	13.1	30.6
Phase of burst onset (%)	32.0	44.0
Phase of burst offset (%)	61.7	74.9
Spike rate in burst (spikes/cycle)	16.3	30.2
Slow-wave amplitude (mV)	12.5	27.5
Peak slow-wave potential (mV)	−47.5	−32.5
ISI coefficient of variation in burst	0	0.25

In most cases, the bounds were chosen to contain the central ~85% of the experimental data points (see Fig. 4, compare dashed lines, histograms). For more details, see Results, Production of LP model population. ISI, Interspike interval.

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This shows the general nature of the results, six examples of acceptable models.

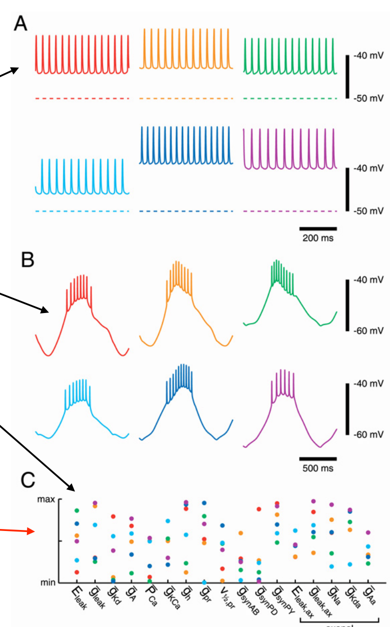
A - spontaneous activity (no synaptic inputs)

B - activity driven by synaptic inputs

C - the parameters of each model (color coded)

The number of acceptable models was 1304.

Note the wide range of parameters in acceptable models!



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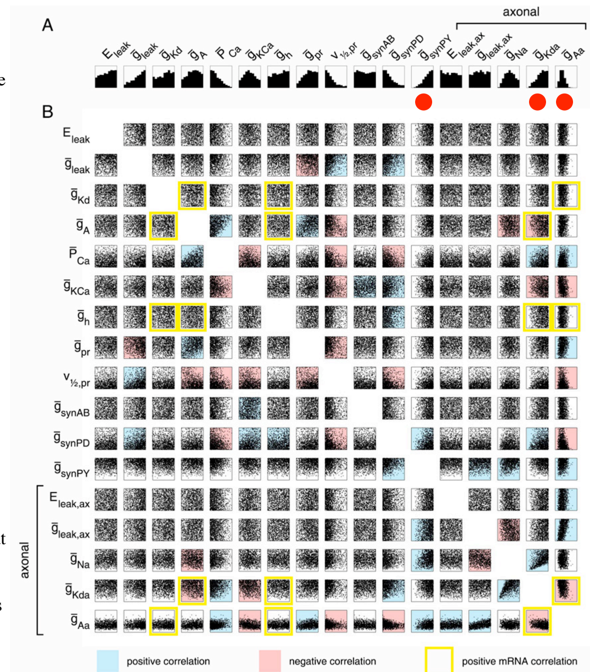
Very little structure is apparent in the parameter values of the successful models.

Only a few parameters (e.g. the red circles) had limited parameter values.

Correlation between pairs of parameters was seen in a few cases (blue and red). Strongest for gKda and gNa. (These correls are too weak to be seen experimentally).

The parameters with mRNA correlation in a previous study are shown in yellow.

The models seemed to form a compact set in that lines between pairs of acceptable models usually passed through parameter values that gave acceptable models or were connected by lines drawn through other acceptable points. The space is not convex, however.



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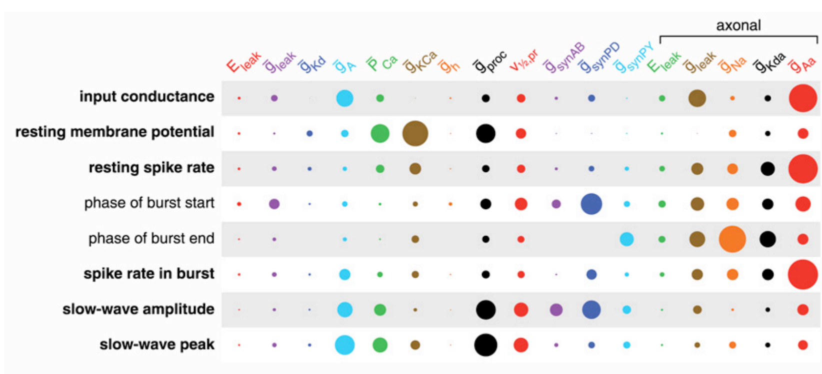
This shows the strength of each model parameter on the major electrophysiological properties of the models (and the experimental data). Note the large role of the A-conductances.



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