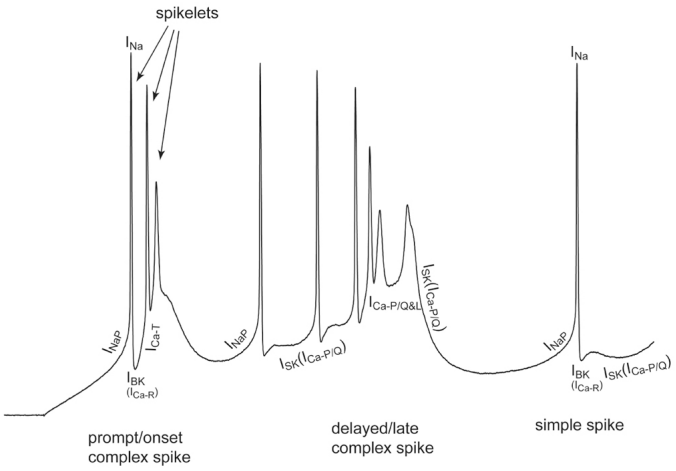
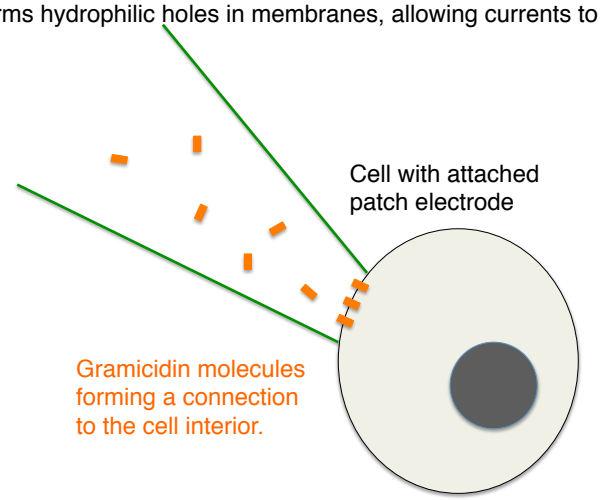
The channels contributing to complex action potentials

from: Y Kim and LO Trussell *J. Neurophysiol.* 97:1705-25 (2007).

Reading: Hille, Chapters 3,4,5, 9.

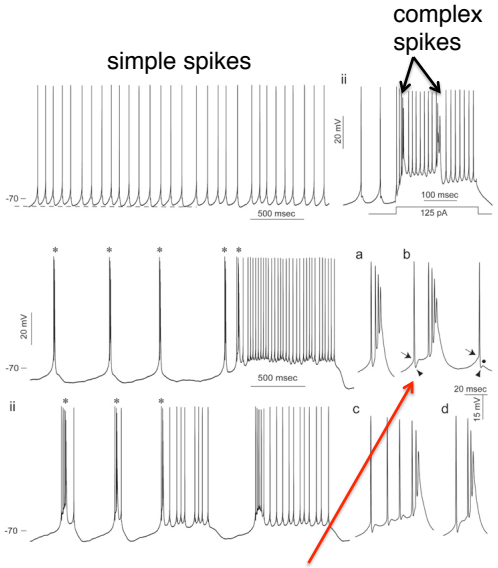


Perforated patch recording was used to record from cartwheel neuron somas. This method allows access to the membrane potential without disturbing the contents of the cell. It is based on gramicidin, a molecule that forms hydrophilic holes in membranes, allowing currents to flow.



Cartwheel neurons in a part of the auditory system have spike trains that are a mixture of simple and complex spikes (bursts). The cases at right are spontaneous activity. Note

1. Simple and complex spikes
2. Spontaneous shifts in the minimum potential, usually depolarization in association with trains of spikes.
3. Both isolated (onset) and delayed complex spiking. Expanded views of complex spikes at right.



Simple spikes have a two-part afterpotential consisting of a fast hyperpolarization and an afterdepolarization (ADP). Complex spikes seem to "take off" from the ADP.

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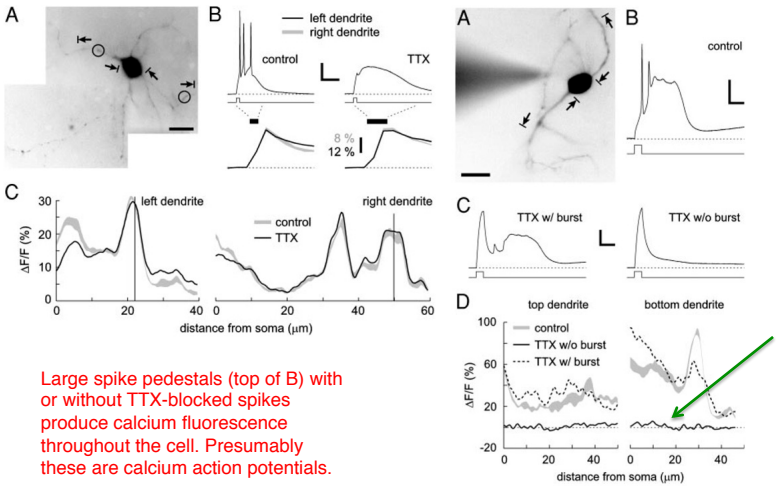
The complex spiking is correlated with calcium entry, shown by calcium imaging. When there is a complex spike or a broad depolarization in the presence of TTX, there is a calcium signal and vice versa.
Note that the calcium entry spreads through the cell, suggesting calcium channels in the dendrites.

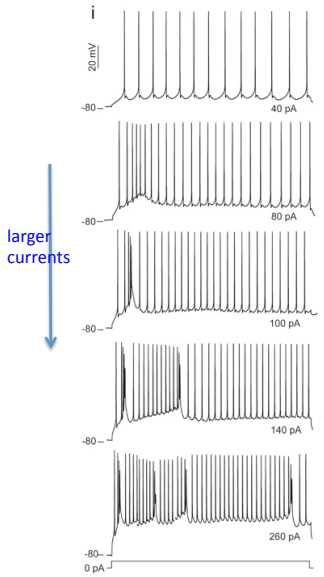


Large spike pedestals (top of B) with or without TTX-blocked spikes produce calcium fluorescence throughout the cell. Presumably these are calcium action potentials.

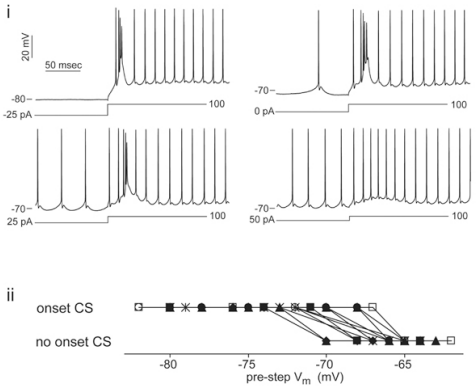
If TTX blocks the pedestal, the calcium signal disappears.

Molitor and Manis, 2003

Current stimulation evokes both onset and delayed complex APs



Pre-hyperpolarization increases the likelihood of getting an onset complex spike.



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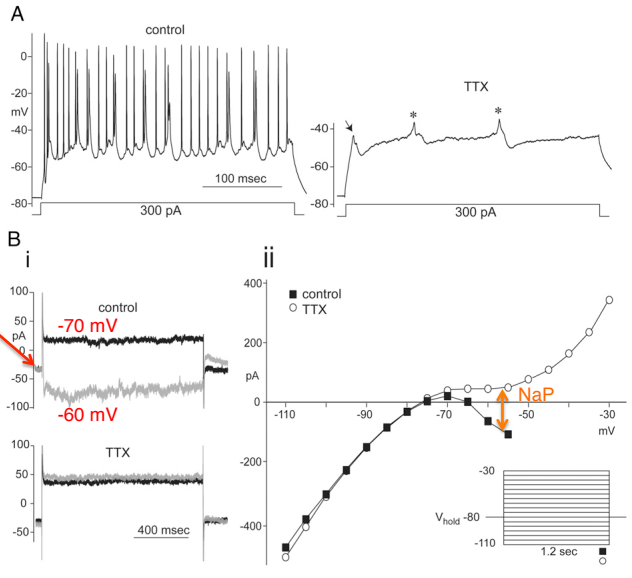
Tetrodotoxin (TTX) blocks sodium channels.

The fast action potentials go away in TTX, revealing what will turn out to be calcium action potentials.

(side issue) TTX also reveals the presence of *persistent sodium channels* (NaP, in the -60 mV voltage clamp), which do not inactivate. These are a commonly-seen small conductance in neurons. Shown are voltage-clamp currents.



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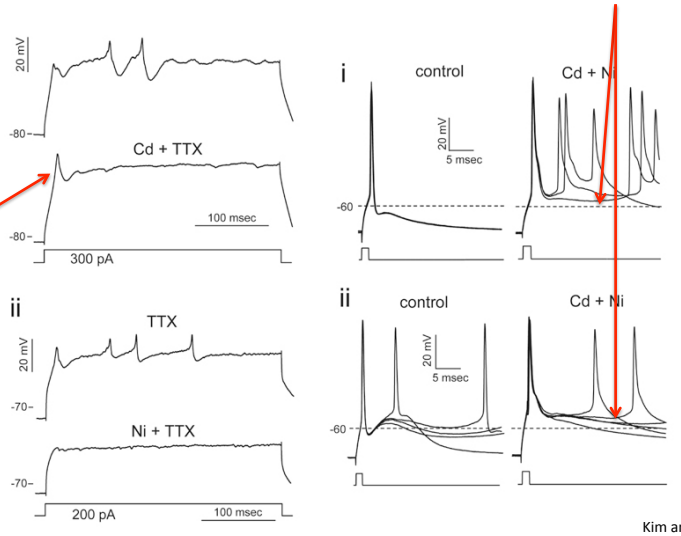
As expected, non-specific Ca channel blockers (Cd^{++} and Ni^{++}) block the calcium action potentials.

But curiously, this block can actually lead to less repolarization after action potentials.

Note that the onset spike is somewhat resistant to Cd block



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The effects of calcium block on repolarization seems to relate to the behavior of potassium channels.

The varieties of potassium channels (some of them):

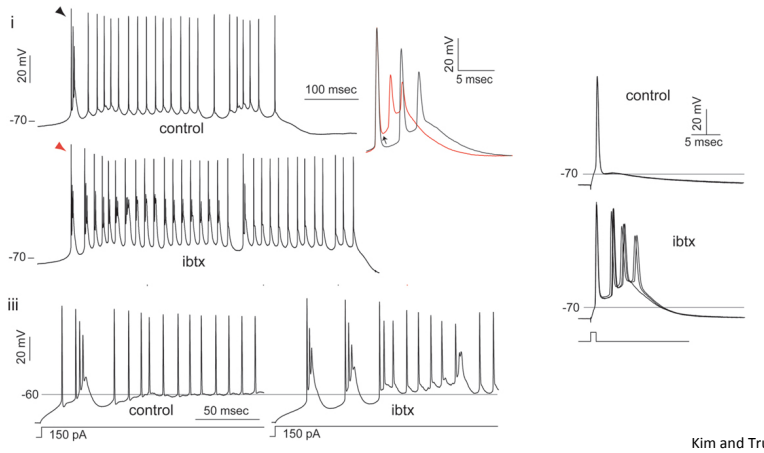
1. *Voltage gated* – K_V like the delayed rectifier of the HH model. Some of these also have inactivation gates. These repolarize action potentials and limit the spiking rate during excitation.
2. *Calcium dependent - K(Ca)* There are two varieties of these:

   BK – gated by both *V* and *Ca.* Important for repolarization and for activity-dependent sensing.

   SK – gated by *Ca* only. Produce afterhyperpolarization (AHP) and help govern stability after bouts of activity (e.g. between bursts).
3. *H channels* – non-specific channels that are related to K channels. These have only an inactivation gate.
4. *Inward rectifier* – Non-V-gated channels whose conductance is often controlled by intracellular second messengers.
5. *Tandem pore domain* – contribute to the resting potential.

Repolarization in cartwheel cells depends on calcium-dependent potassium channels K(Ca), which are less activated when calcium currents are decreased. Of course there are V-dependent K channels as well.

The data below show the effects of iberiotoxin, an antagonist of *BK* channels. Note more complex spikes, faster bursting, and bursting in previously stable cells.



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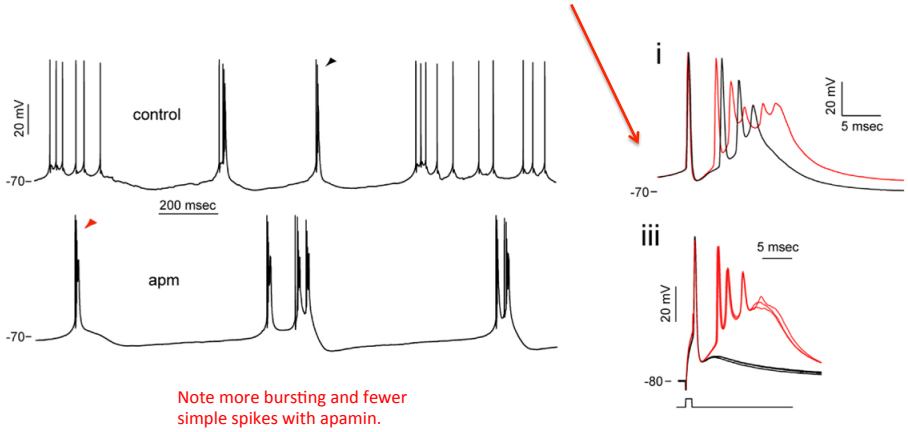
Apamin, which blocks the other variety of K(Ca) channel, *SK*, has a similar effect. SK channels are not voltage-gated (only Ca gated), so they are less important for repolarizing the action potential, but they are important for afterhyperpolarization, as in i) and iii) below.



Note more bursting and fewer simple spikes with apamin.

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To refine the description of this system, consider the electrophysiological families of calcium channels.

Varieties of calcium channels:

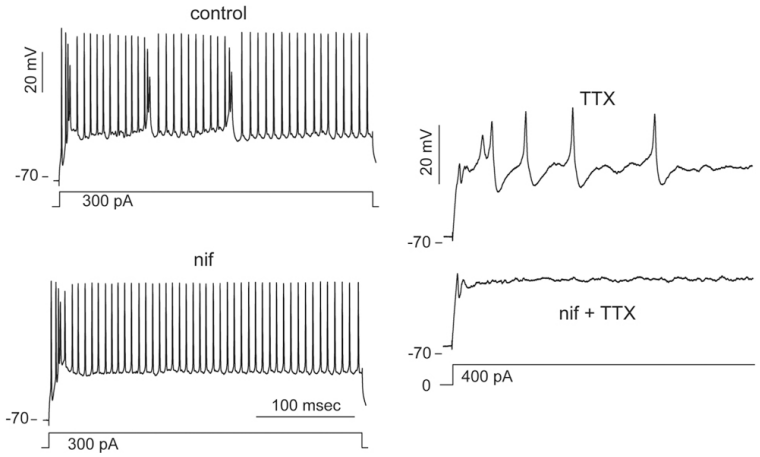
L-type – high threshold (>-30 mV), slow V inactivation, Ca^{++} inactivation.

P/Q, N, R – high threshold (>-20 mV), weak V inactivation, Ca^{++} inactivation.

T-type – low threshold (>-70 mV), strong V inactivation, no Ca^{++} inactivation.

These types were originally identified on voltage-clamp criteria, but have subsequently been associated with specific genes, with multiple genes for each type. They differ in pharmacology and in their localization.

Block of L-type channels (nifedipine) eliminates the delayed bursts, but not the onset burst.



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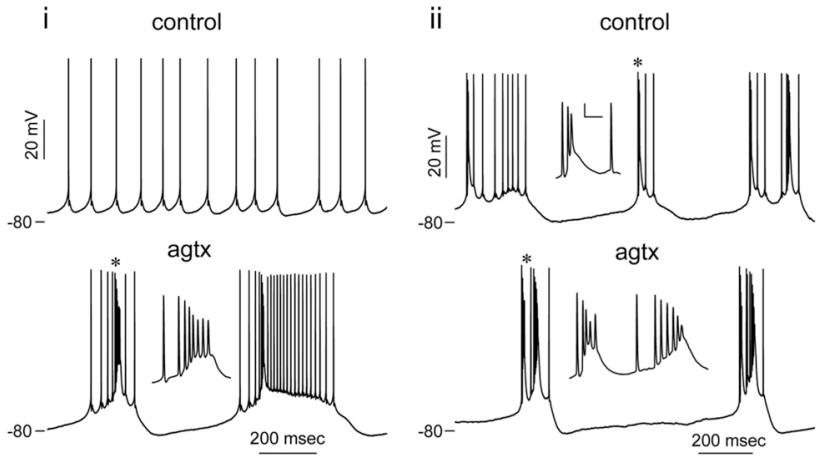
Block of P/Q channels (agatoxin) produces a mixture of effects. Again, onset spikes are not affected. The bursts become stronger, probably because of a K-channel effect and tend to have more spikes per burst, but delayed bursts are less likely.



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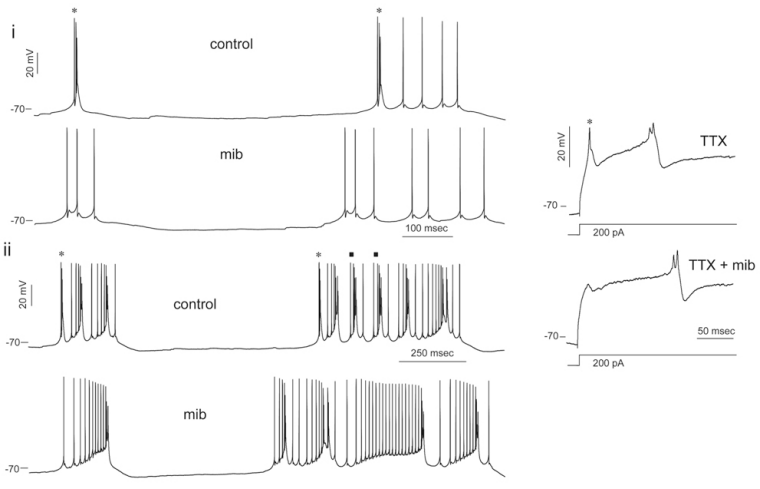
Block of T-type channels (mibefradil) weakens or eliminates the onset burst, without affecting delayed bursts.



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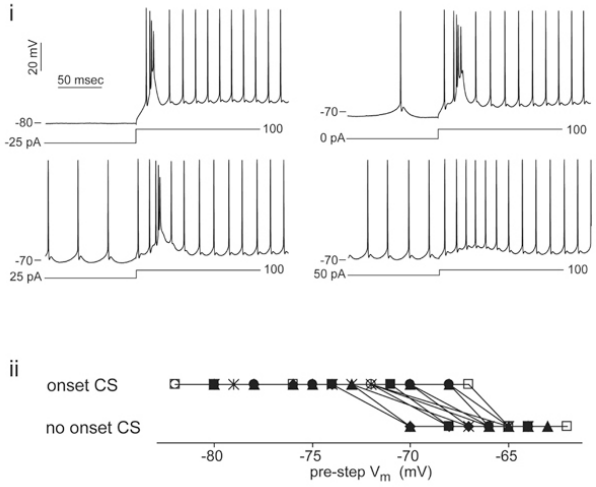
A second evidence pointing to T-channel involvement in the onset burst is the fact that pre-hyperpolarization increases the likelihood of getting an onset complex spike. T channels are inactivated by depolarization, so the pre-hyperpolarization is necessary to remove the inactivation.



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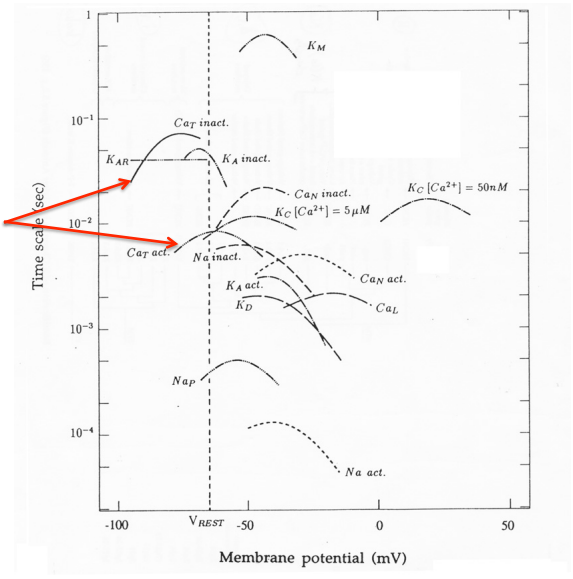
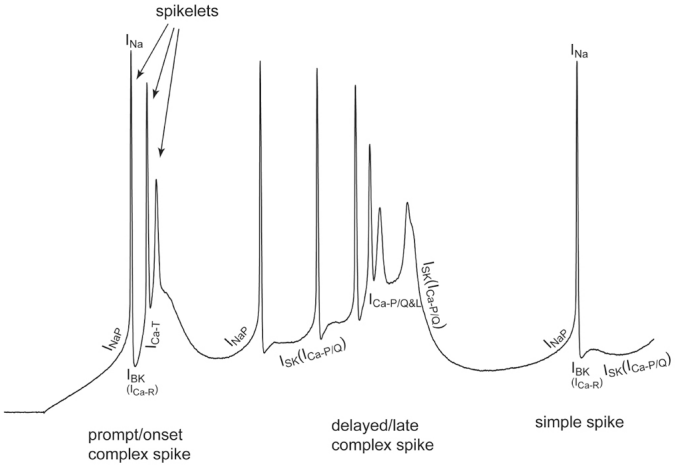
The diagram at right shows the voltage range over which channels typically activate or inactivate (abscissa) and the time scale (ordinate).

Note that the only channels that activate at low potentials (below V_{REST}) are the T-type Ca channel and inactivation of the A-type K channel.

(H channels, not shown, also activate below V_{REST})



A summary of the spontaneous spike bursting in the cartwheel cell. This is definitely not a minimal model. Note that some channels that are present (e.g. K_V channels) are not explicitly shown, but certainly contribute.



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