Modeling Oscillatory Activity of Endocrine Cells

Richard Bertram

Department of Mathematics
and
Programs in Neuroscience and Molecular Biophysics
Florida State University, Tallahassee, FL.

FSU Biomedical Sciences, 2012

Coworkers and Collaborators

Pancreatic Islets

Arthur Sherman (NIH)
Leslie Satin (U. Michigan)
Matthew Merrins (U. Michigan)
Craig Nunemaker (U. Virginia)
Bernard Fendler (Cold Spring Harbor Lab)

Pituitary Cells

Joël Tabak (FSU)
Arturo Gonzalez-Iglesias (FSU)
Maurizio Tomaiuolo (U. Penn)
Lorin S. Milescu (U. Missouri)

Funding: NSF-DMS0917664 and NIH-DK043200

Why use mathematical modeling?

1. As an aid to the interpretation of experimental data.

2. Integration of multiple sets of experimental results.

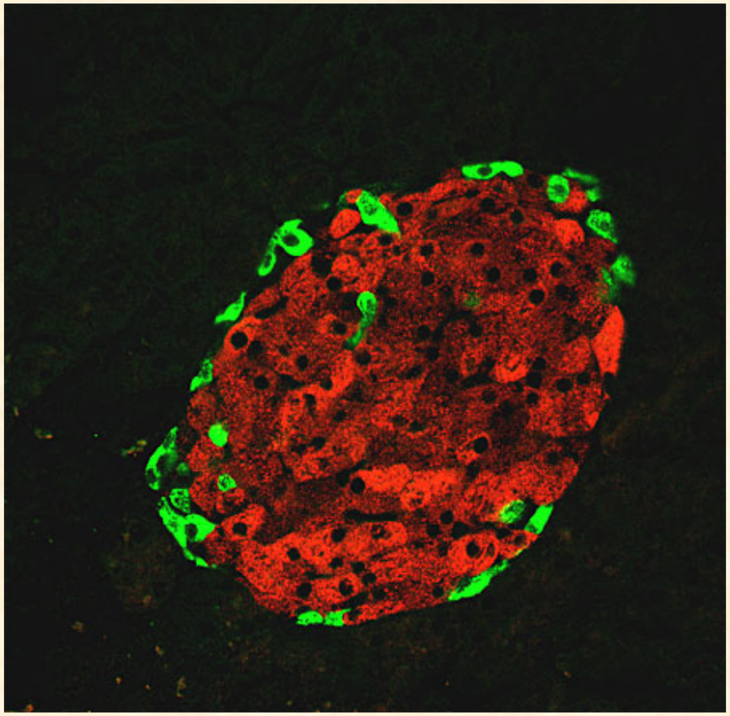
3. Great for finding holes in our knowledge.

4. Ideal for making predictions. This is a powerful tool for experimental design.

Project 1: Pancreatic Islets

What is an Islet of Langerhans?

Cluster of electrically couple hormone-secreting cells, located throughout the pancreas. The human pancreas has about 1 million islets.

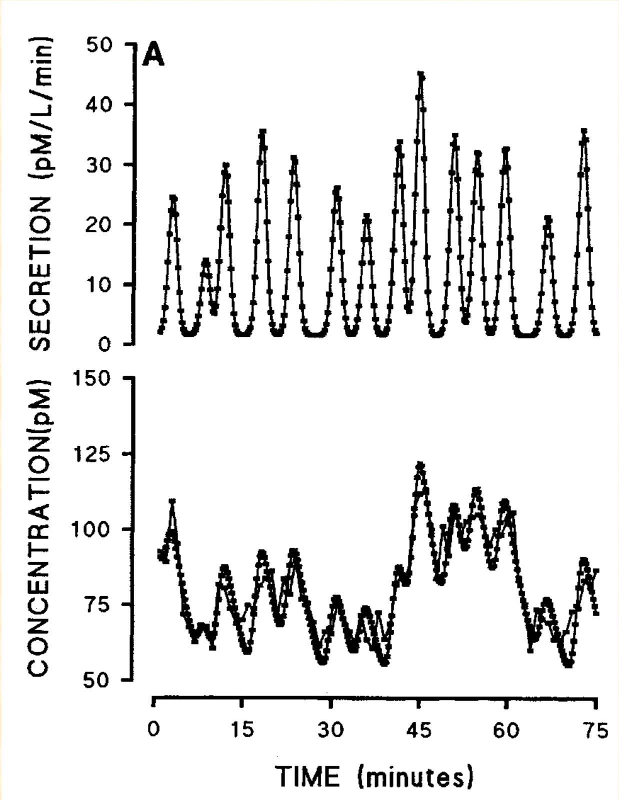


Courtesy of Rohit Kulkarni

Immunostained for glucagon (green) and insulin (red)

Insulin Secretion is Pulsatile

Porksen et al., AJP, 273:E908, 1997



deconvoluted

measured

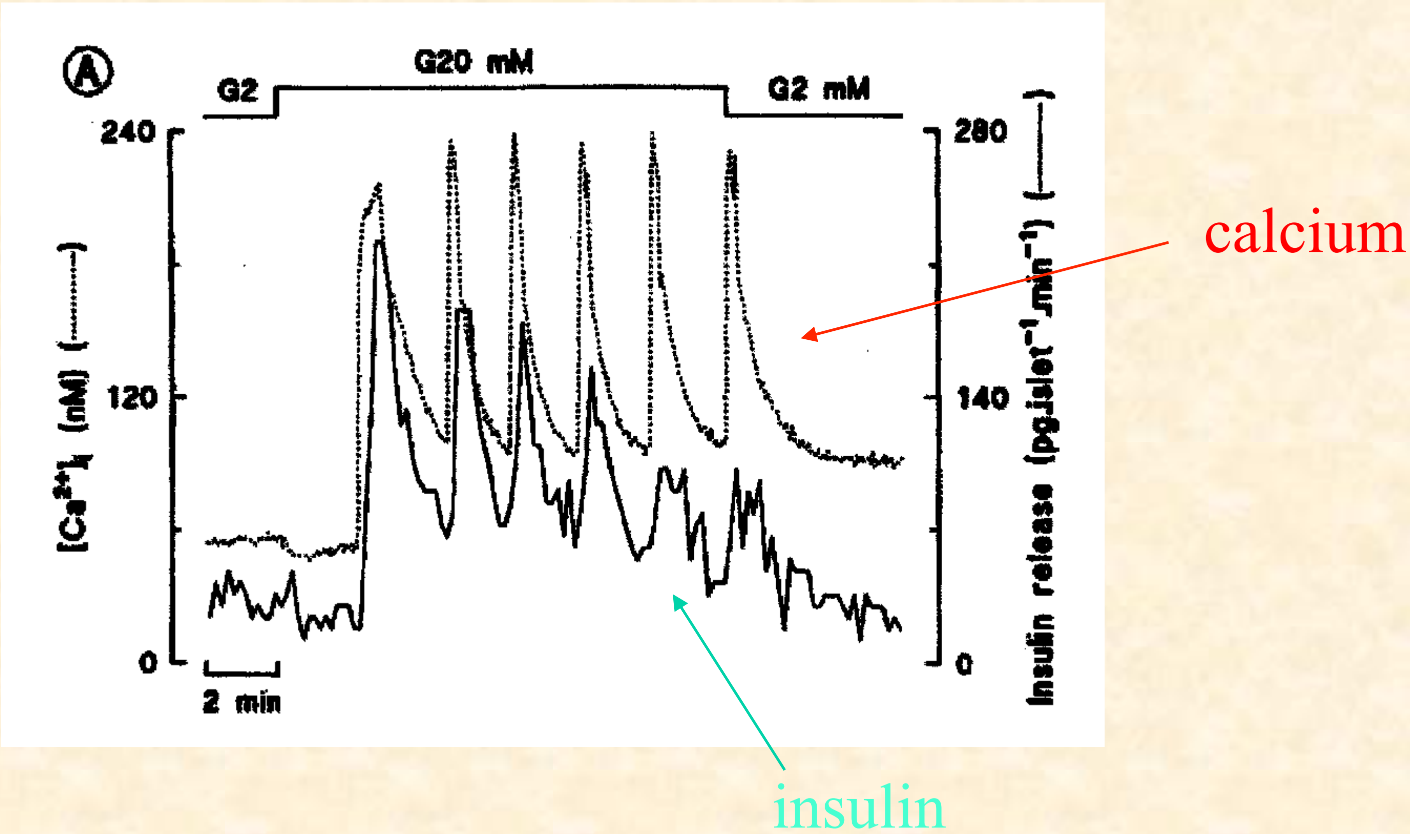
Peripheral insulin measurements in the blood of humans exhibits oscillations, suggesting that insulin is secreted in a pulsatile manner.

Central Question:

What is the mechanism for oscillations in insulin secretion from pancreatic β-cells?

Islets are Electrically Excitable

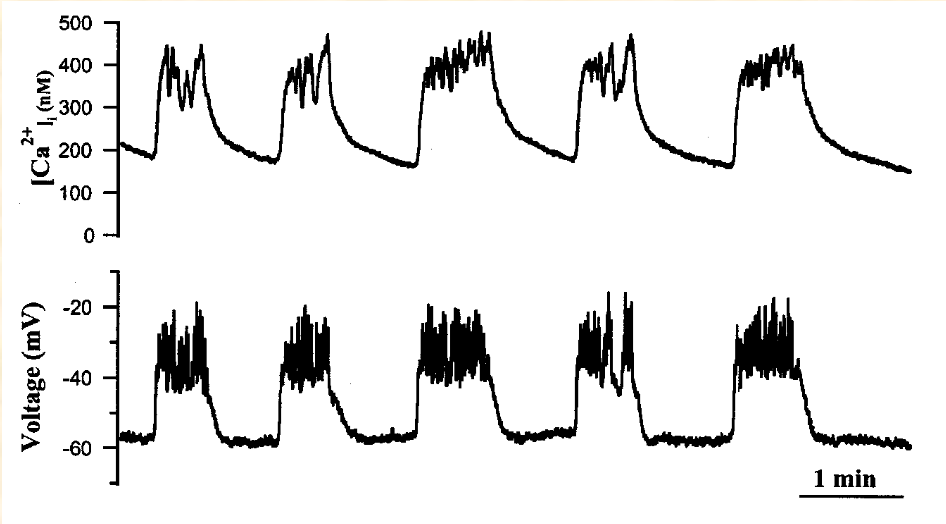
Islets are like nerve cells in that they produce electrical impulses. During an upstroke of an impulse Ca^{2+} enters the cells, causing insulin to be released.



Gilon et al., JBC, 268:22265, 1993

Islets Have Characteristic Patterns of Electrical Activity

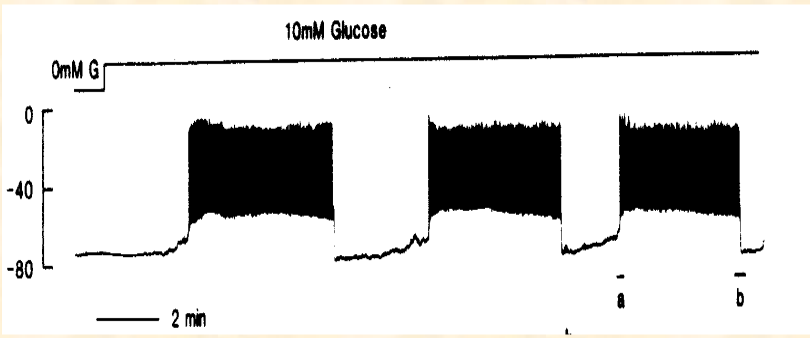
Fast Bursting Oscillations



Simultaneous fast Ca^{2+} and voltage measurements from a mouse islet in 11.1 mM glucose. From Zhang et al., *Biophys. J.*, 84:2852, 2003

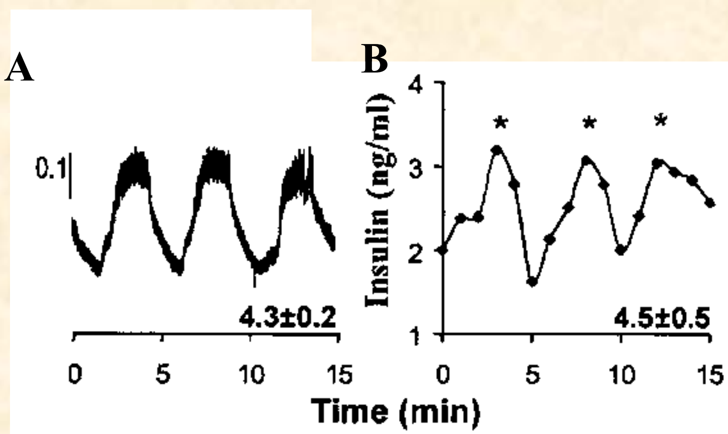
Slow Bursting Oscillations

Slow oscillations of Ca^{2+} and voltage from an islet...



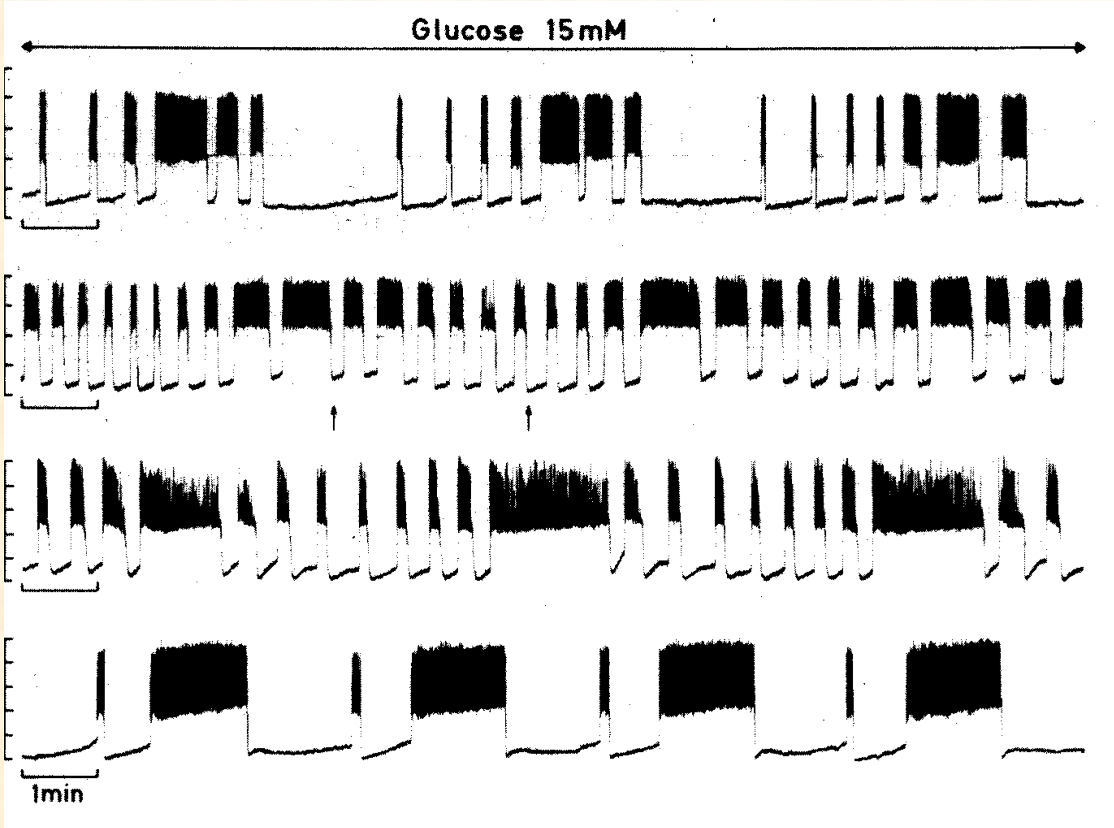
Smith et al., *FEBS Lett*., 261-187, 1990

Zhang et al., *Biophys. J*., 84:2852, 2003



…have period similar to slow insulin oscillations measured from a mouse in vivo (Nunemaker et al., Diabetes, 54:3517, 2005)

Compound Bursting Oscillations

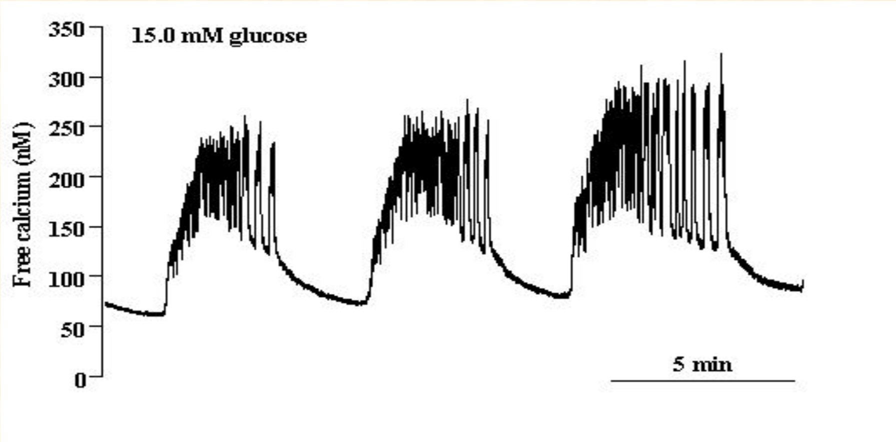


Henquin et al., Eur. *J. Physiol*., 393:322, 1982

Bursting oscillations superimposed on a slow wave of activity

More Evidence of Compound Oscillations

Measurements of intracellular Ca^{2+} also reveal compound oscillations.



Compound Ca^{2+} oscillations in an islet
(Zhang et al., *Biophys. J.*, 84:2852, 2003)

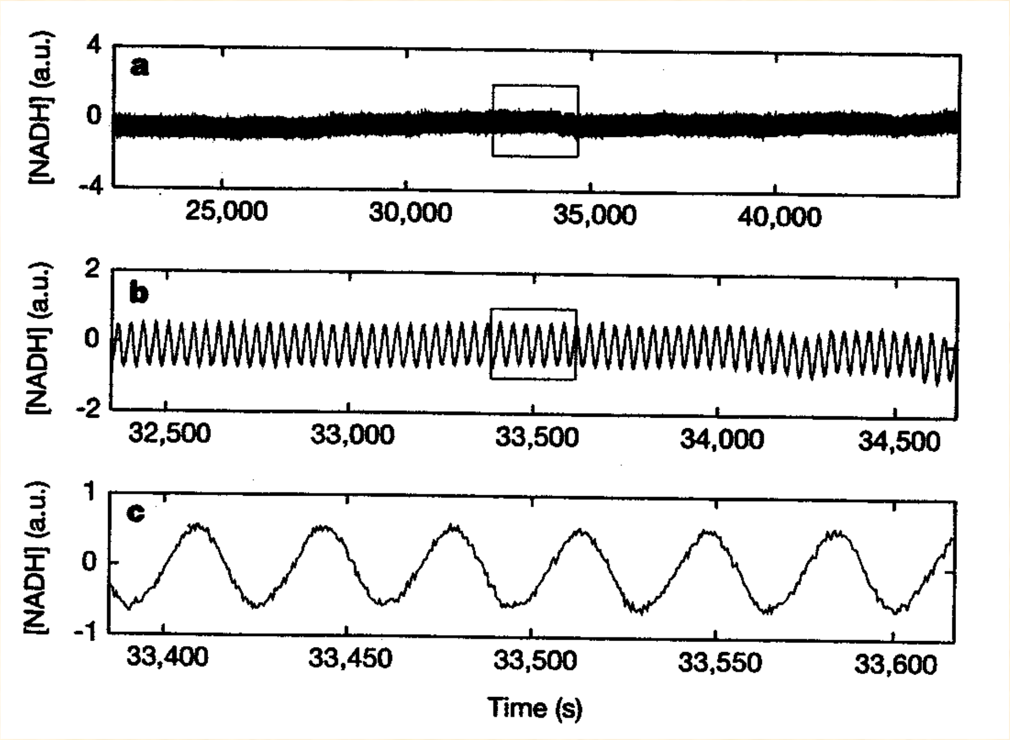
Goal: Develop a Mathematical Model That Can Reproduce the Various Patterns of Activity

The Dual Oscillator Model

Central Hypothesis

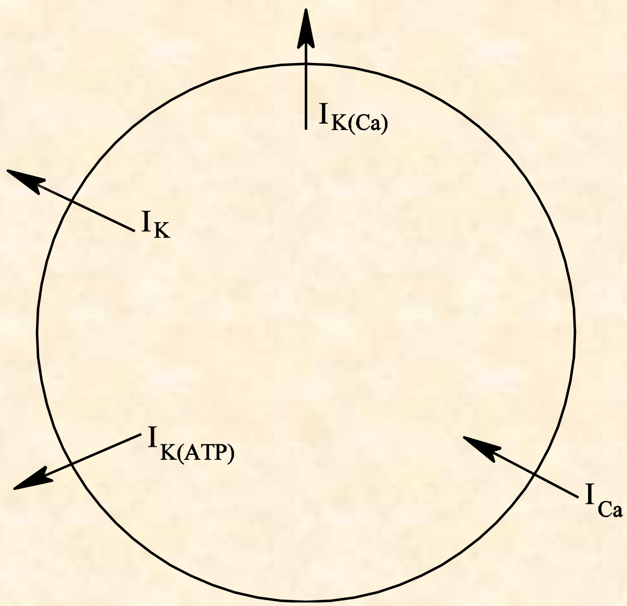
Fast, slow, and compound oscillations can all be produced by a mechanism that includes Ca^{2+} feedback onto ion channels (driving the fast oscillation consisting of a burst) and glycolytic oscillations (driving the slow oscillation that modulates the bursts).

Glycolytic Oscillations in Yeast



Dano et al., Nature, 402:320-322, 1999

Electrical Component of the DOM



[redacted]

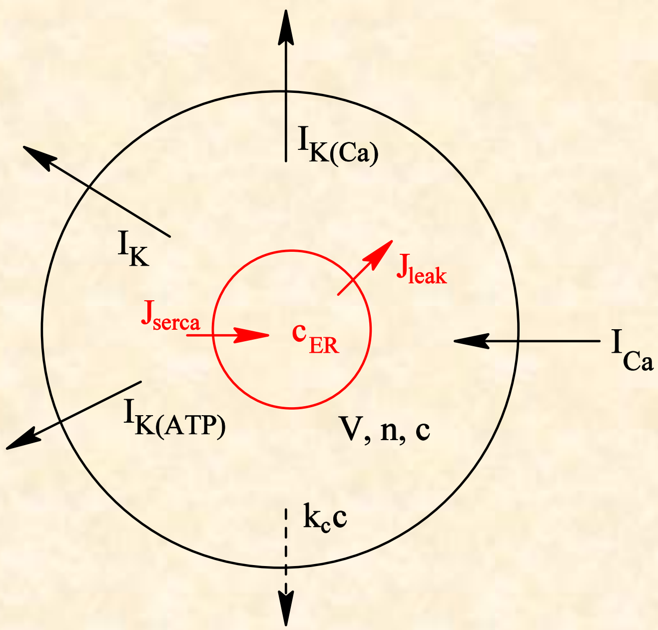
$$\dot{V} = -(I_{Ca} + I_K + I_{K(Ca)} + I_{K(ATP)})/C_m$$

$$\dot{n} = (n_\infty(V) - n)/\tau_n$$

Voltage equation reflects Kirchoff's current law

Second equation describes dynamics of the K^+ activation variable *n*. This depends on the voltage.

Electrical/Calcium Components of the DOM



[redacted]

$$\dot{V} = -(I_{Ca} + I_K + I_{K(Ca)} + I_{K(ATP)})/C_m$$

$$\dot{n} = (n_\infty(V) - n)/\tau_n$$

$$\dot{c} = f(J_{leak} - J_{serca} - \alpha I_{Ca} - k_c c)$$

$$\dot{c}_{ER} = f_{ER}\left(V_{cyt}/V_{ER}\right)(J_{serca} - J_{leak})$$

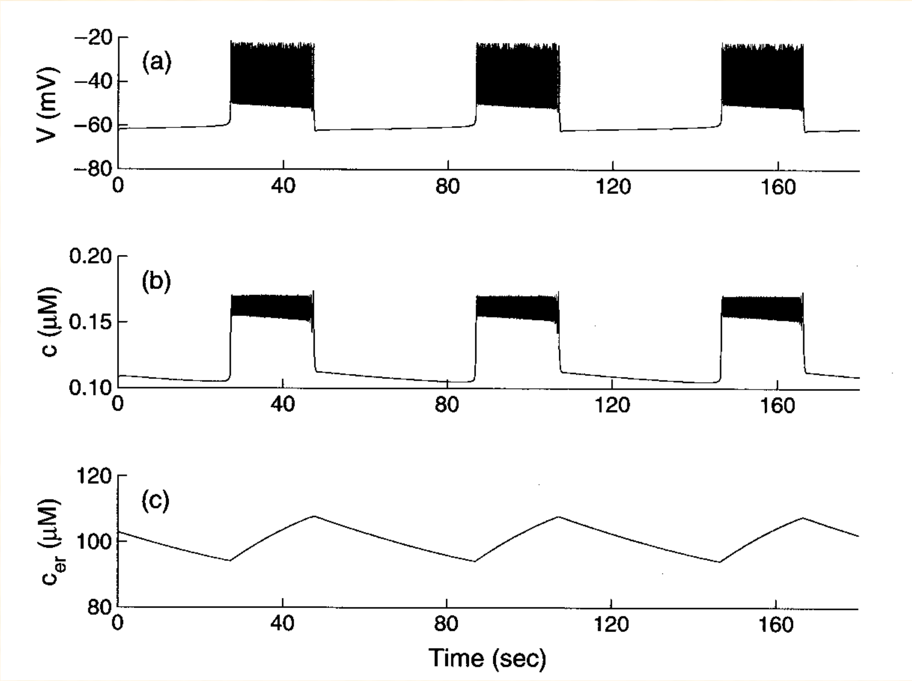
ER is the Endoplasmic Reticulum

Ca^{2+} enters the cell through L-type Ca^{2+} channels. The free cytosolic Ca^{2+} activates K(Ca) channels. Thus, there is mutual feedback between the electrical and Ca^{2+} components.

Fast Oscillations with the DOM

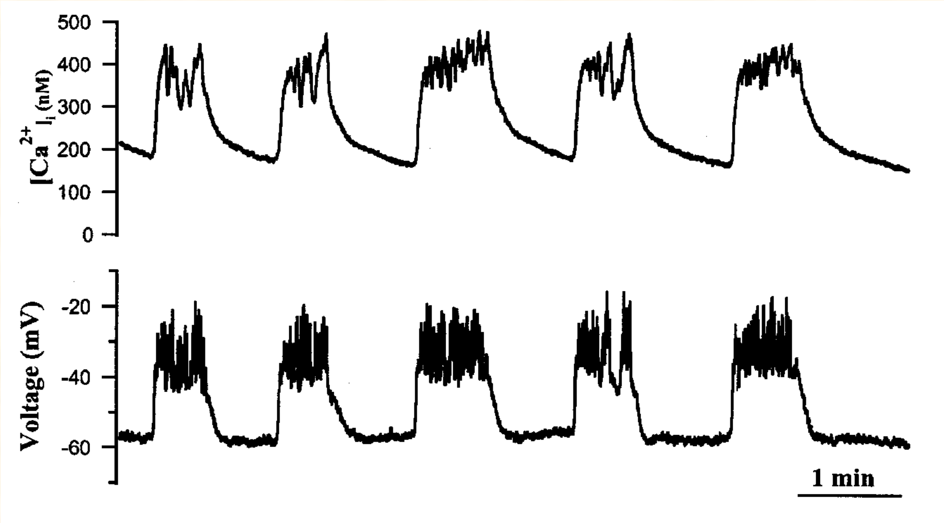
When glycolysis is non-oscillatory, the DOM produces fast bursting oscillations, due to the electrical/calcium components of the model.

Bertram and Sherman, BMB, 66:1313, 2004



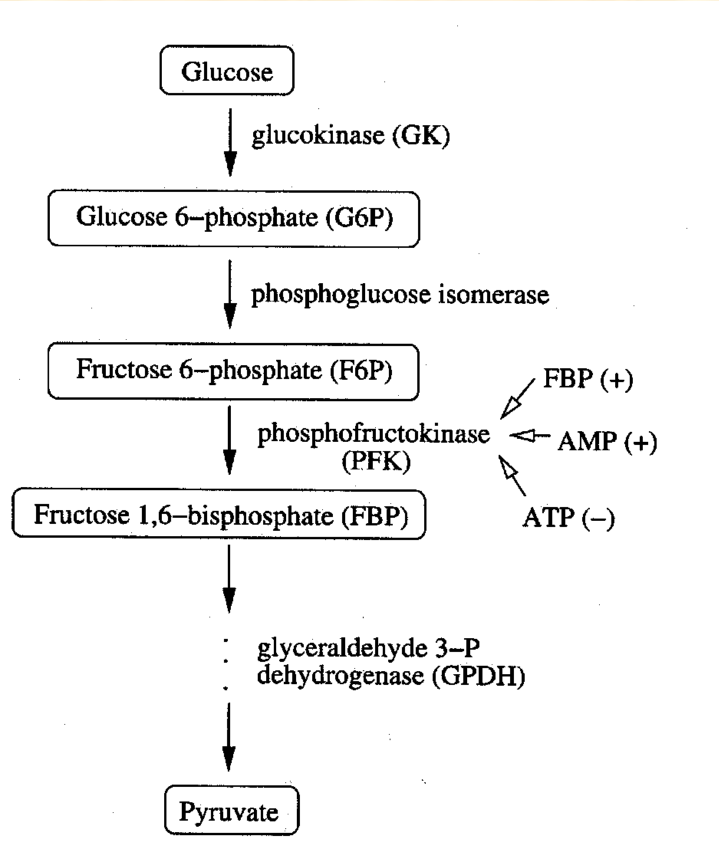
The ER acts as a slow Ca^{2+} filter, setting the period of bursting through its interaction with the cytosol.

Fast Oscillations in Islets



Simultaneous fast Ca^{2+} and voltage measurements from a mouse islet in 11.1 mM glucose. From Zhang et al., *Biophys. J.*, 84:2852, 2003

Glycolytic Component of the DOM



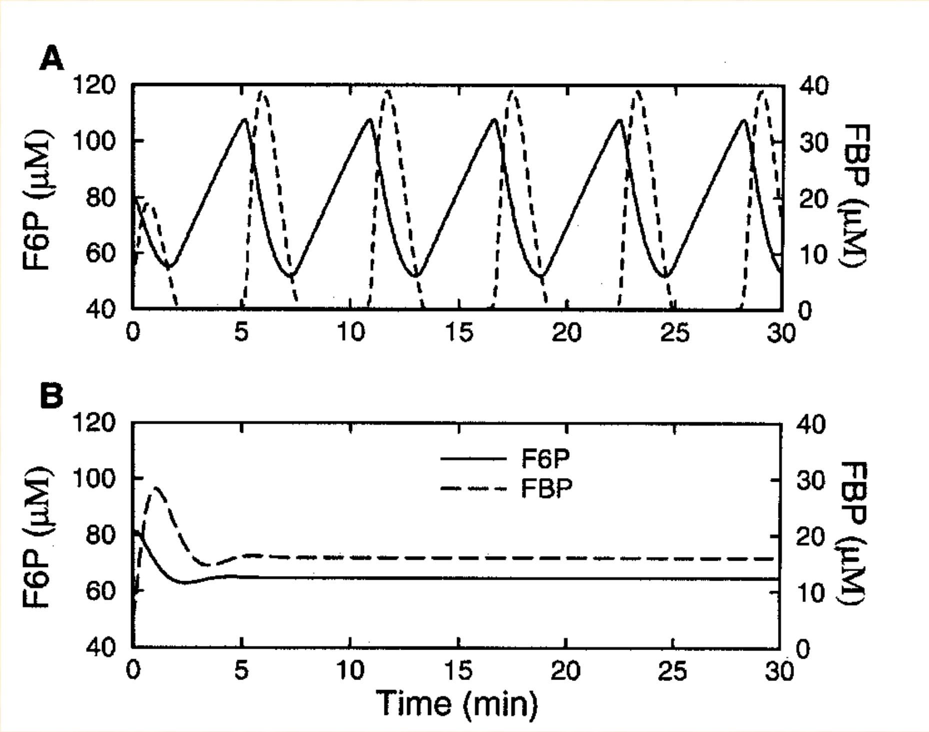
$$\frac{d\,F6P}{d\,t} = \lambda(J_{GK} - J_{PFK})$$

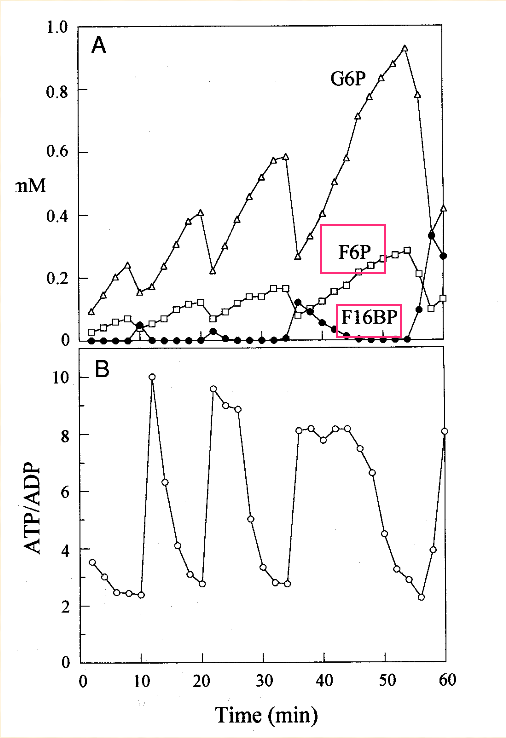
$$\frac{d\,FBP}{dt} = J_{PFK} - 0.5J_{GPDH}$$

Key feature: The product FBP feeds back positively onto the allosteric enzyme PFK (phosphofructokinase). Leads to oscillations due to substrate depletion.

Glycolytic Oscillations Produced if Glucokinase Rate is in the Right Range

Solid-F6P, Dashed-FBP





(A) Intermediate J_{GK}
(B) High J_{GK}

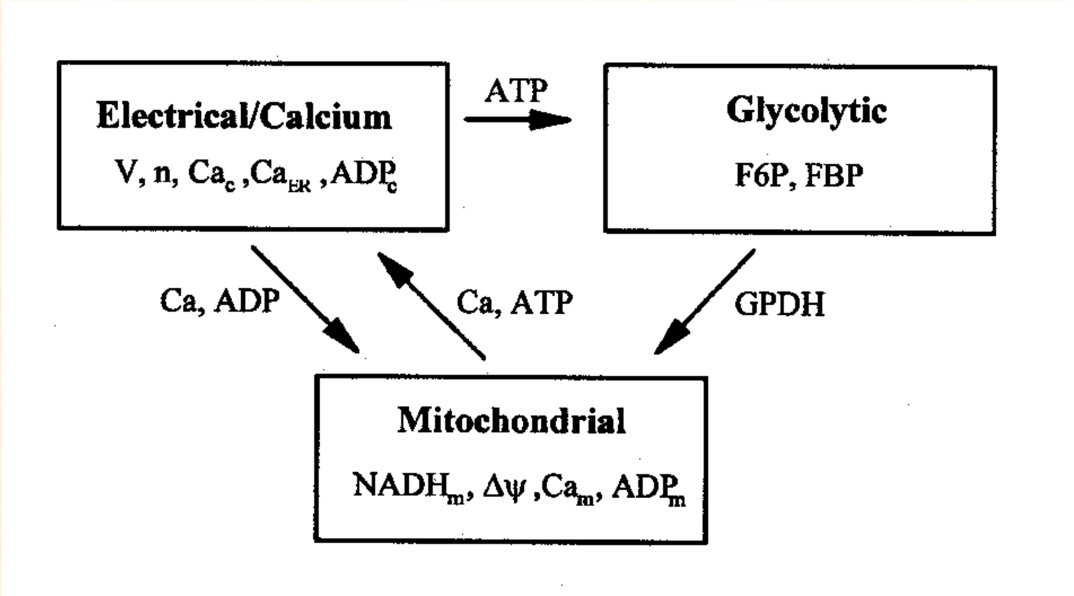
Bertram et al., BJ, 87:3074, 2004

Glycolytic oscillations in muscle extracts (Tornheim, *Diabetes*, 46:1375, 1997)

Mitochondrial Component

Includes equations for mitochondrial NADH concentration, inner membrane potential, Ca^{2+} concentration, and ADP/ATP concentrations.

Final 3-compartment model:



Bertram et al.,
BJ, 92:1544, 2007

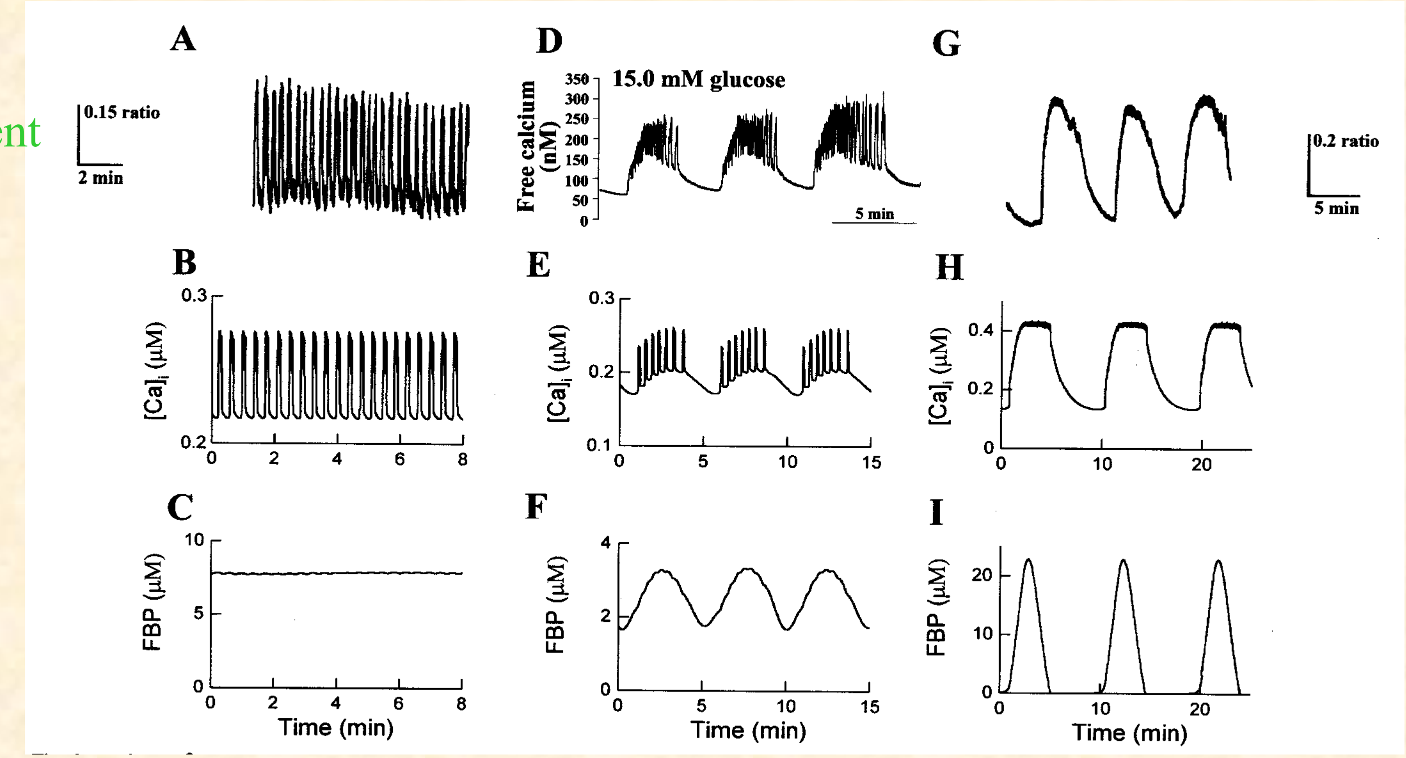
The Three Types of Activity can be Reproduced by the Model

No glycolytic oscillations | With glycolytic oscillations | With glycolytic oscillations

Experiment

Model

Model

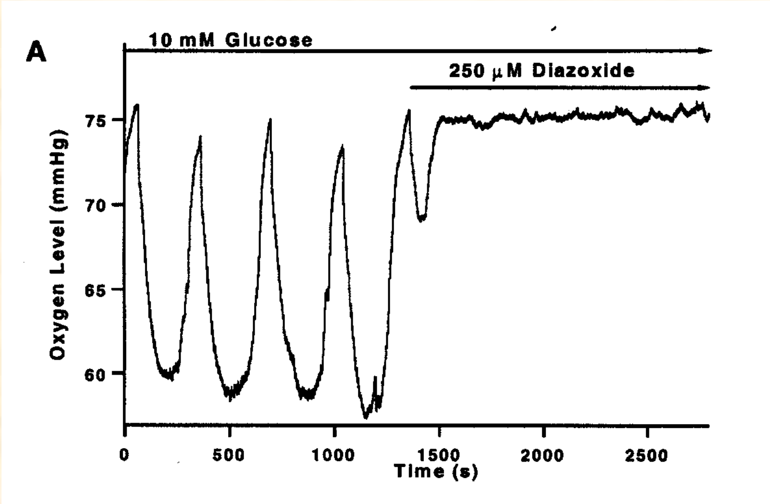


Bertram et al., *Am. J. Physiol.*, 293:E890, 2007

Who's Driving? Are Metabolic Oscillations the Driver or the Passenger?

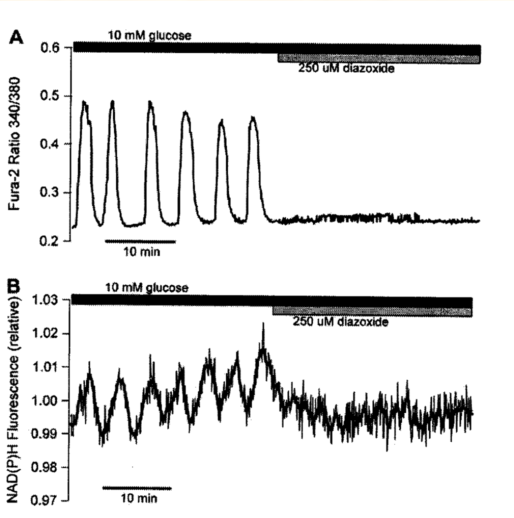
Diazoxide hyperpolarizes the β-cells by activating K(ATP) ion channels, terminating electrical activity.

Oxygen



Kennedy et al., *Diabetes*, 51:S152, 2002

NADH



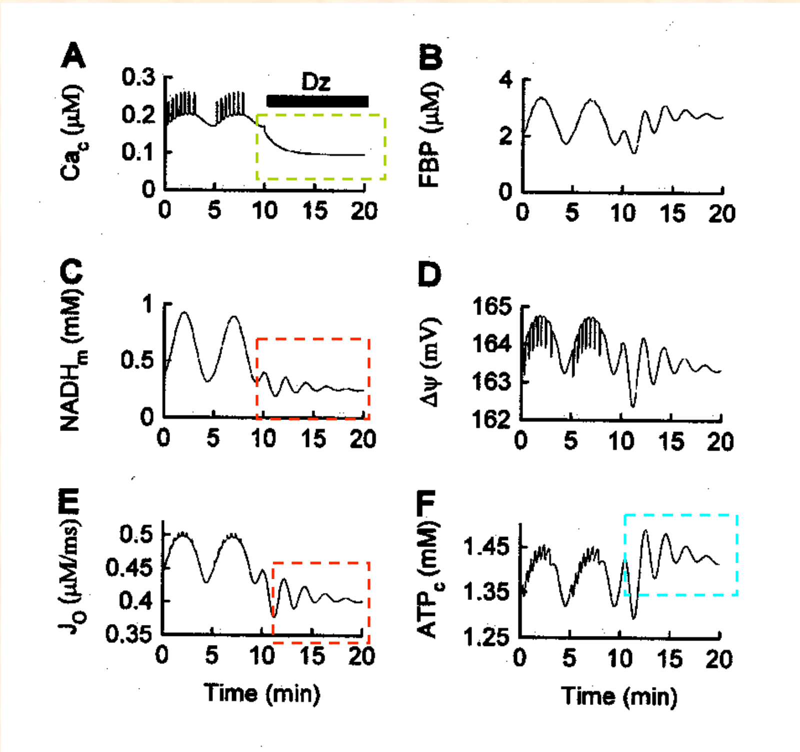
Bertram et al., BJ, 92:1544, 2007

Kennedy's Conclusion

Slow oscillations in metabolic variables are driven by Ca^{2+} feedback. That's why they stop when Ca^{2+} is constant at a low value. Metabolic oscillations are the passenger!

Kennedy Data Consistent with the DOM; The Hyperpolarization Drains the Fuel!!

Opening K(ATP) channels with diazoxide (Dz) can terminate the oscillations in glycolysis, and thus the metabolic oscillations. Explains O_2 recordings from Kennedy's lab and our own NAD(P)H data.

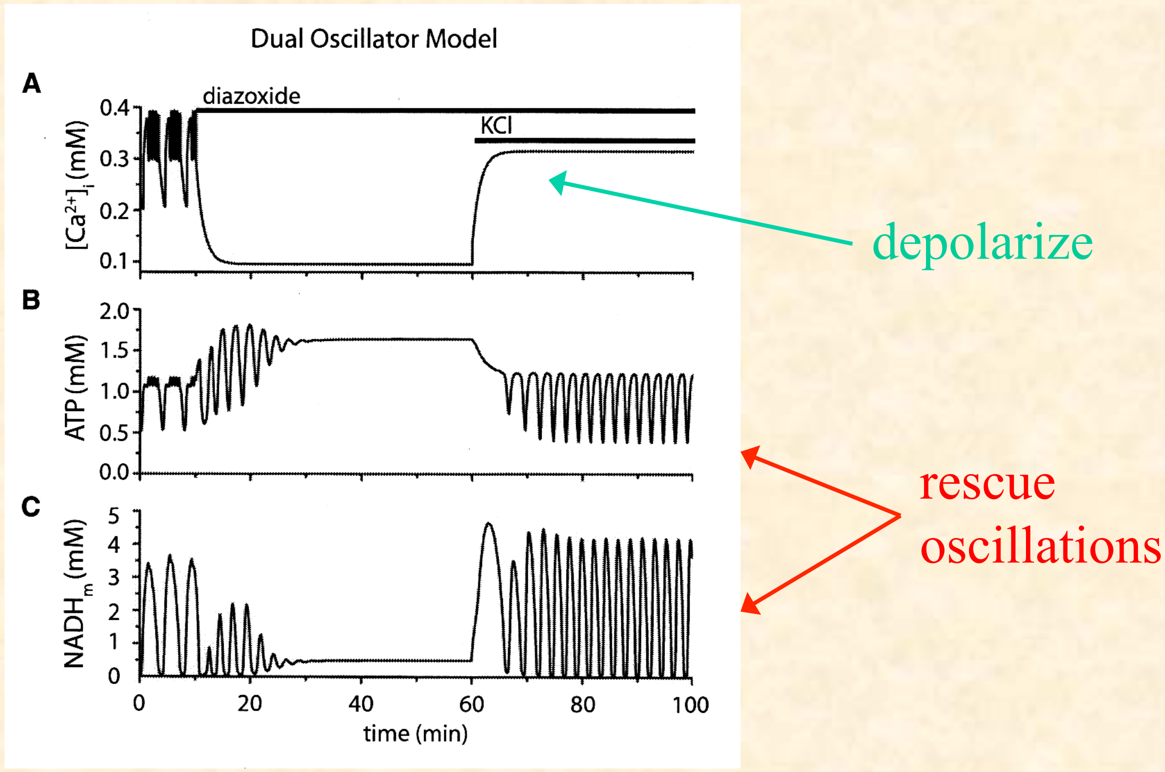


Bertram et al., BJ, 92:1544, 2007

1. Dz hyperpolarizes cell
2. Cytosolic Ca^{2+} concentration is reduced
3. Ca^{2+} pumps don't need to work as hard, so less ATP is utilized.
4. Cytosolic ATP level increases
5. The ATP inhibits PFK, terminating metabolic oscillations

The "Killer" Prediction

The DOM, and no other model, predicts that depolarizing the cell can restart metabolic oscillations.

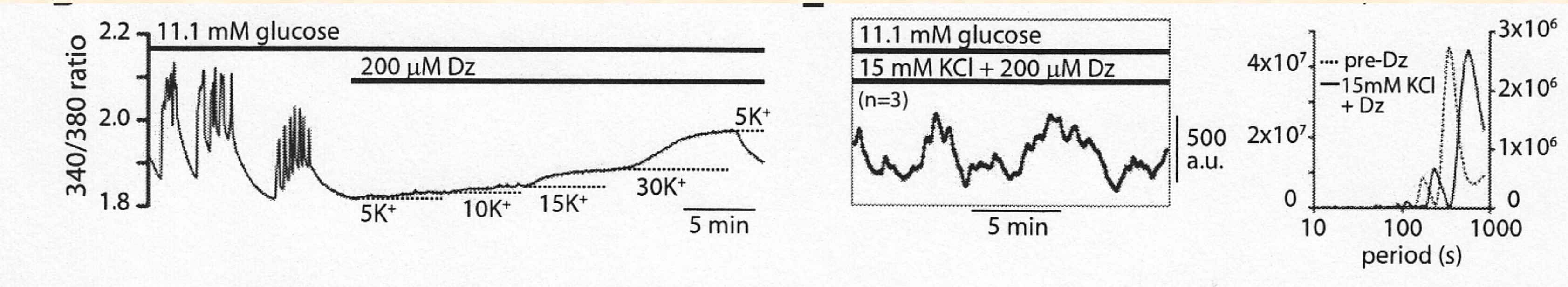


Merrins et al., BJ, 99:76, 2010

The "Killer" Experiment

After some lobbying, the Satin lab tested the model prediction…

Calcium concentration NADH autofluorescence



Merrins et al., BJ, 99:76, 2010

Oscillations in NADH that were eliminated by hyperpolarization with Dz were rescued by depolarization with KCl, as predicted.

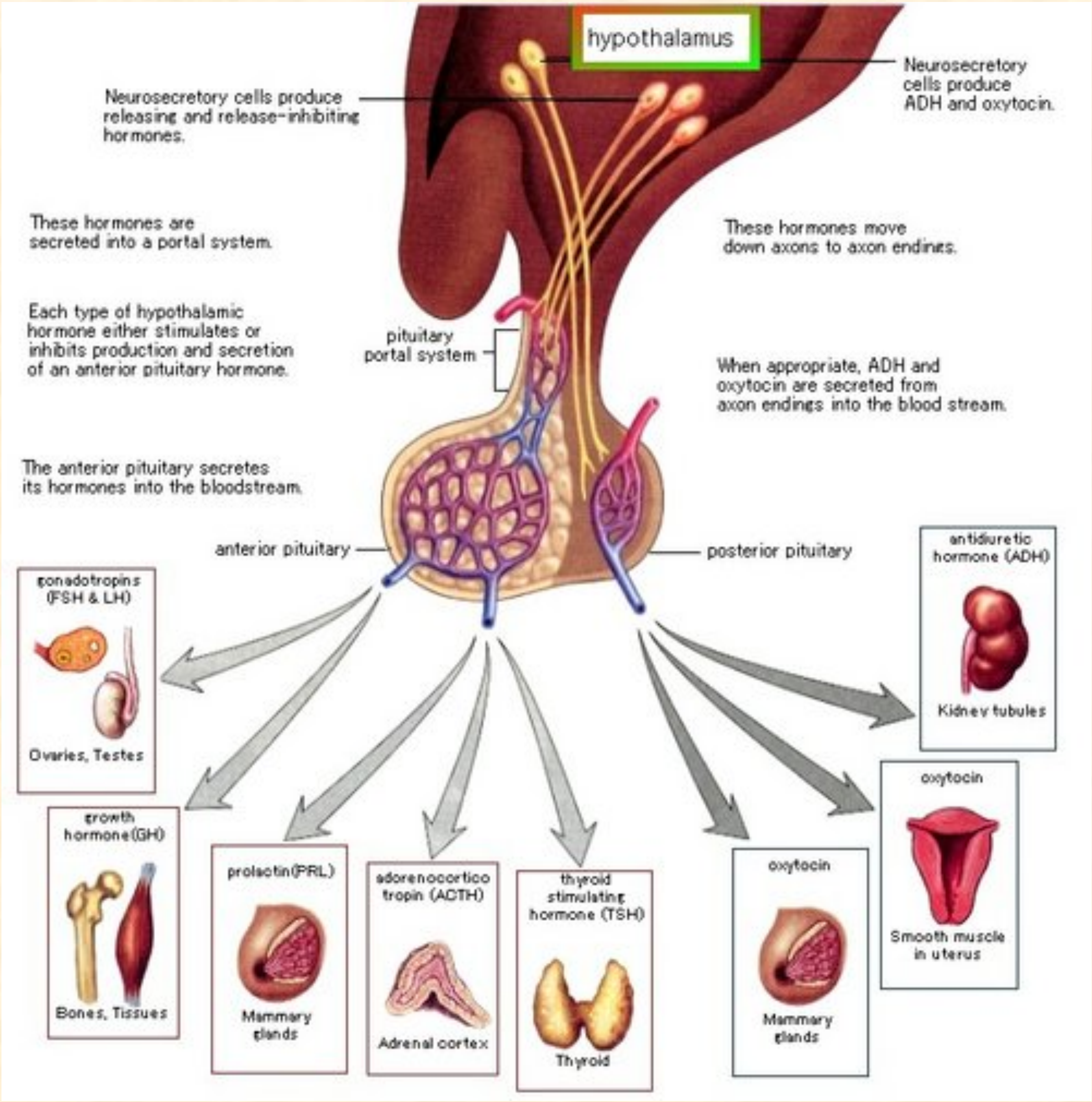
Take Home Messages

1) A mathematical model can help with the interpretation and re-interpretation of experimental data.

2) Making predictions and then testing them is a great way to challenge your model, and thus your understanding of the system.

Project 2: Pituitary Cells

Hypothalamus and Pituitary



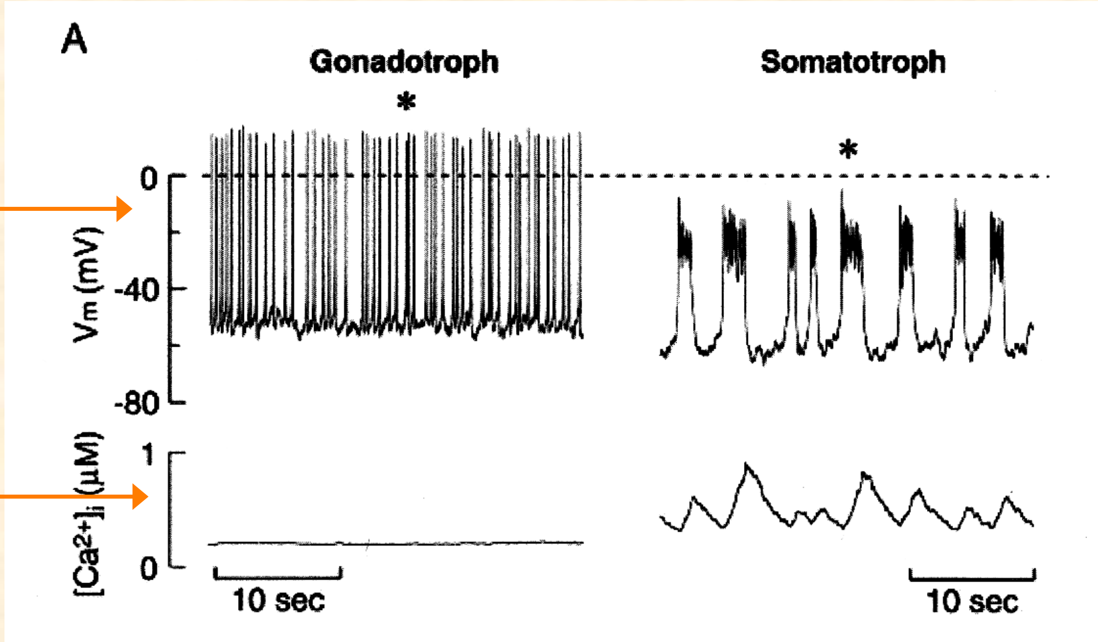
Pituitary Cells are Electrically Active

Spontaneous activity of two pituitary cell types

Van Goor et al., J. Neurosci., 2001:5902, 2001

tonic spiking

calcium level
is low

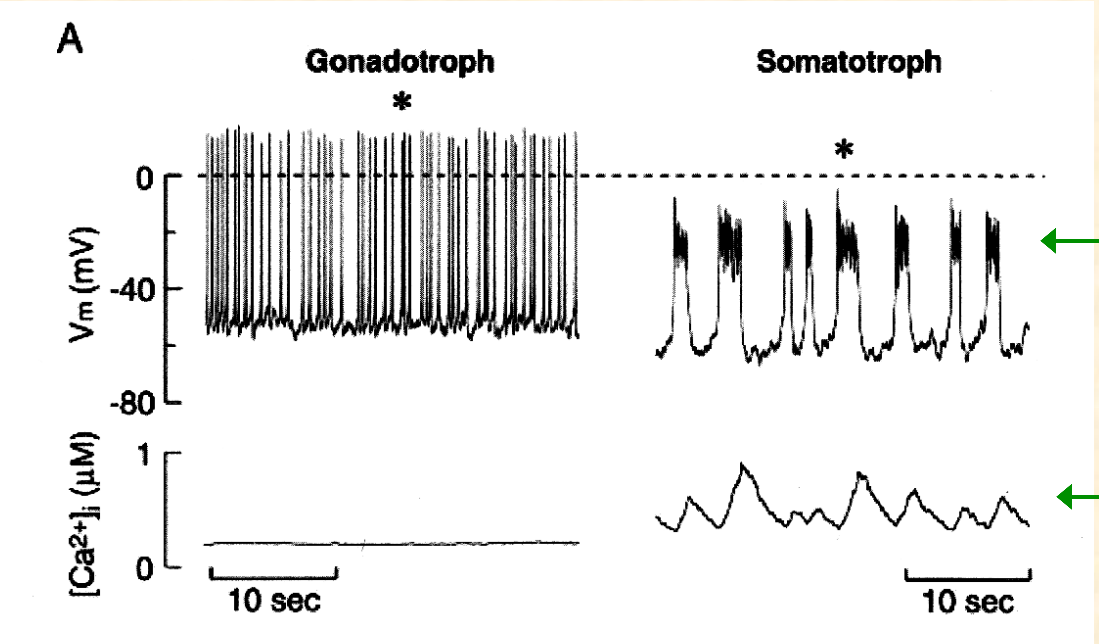


little basal hormone release

Pituitary Cells are Electrically Active

Spontaneous activity of two pituitary cell types

Van Goor et al., J. Neurosci., 2001:5902, 2001



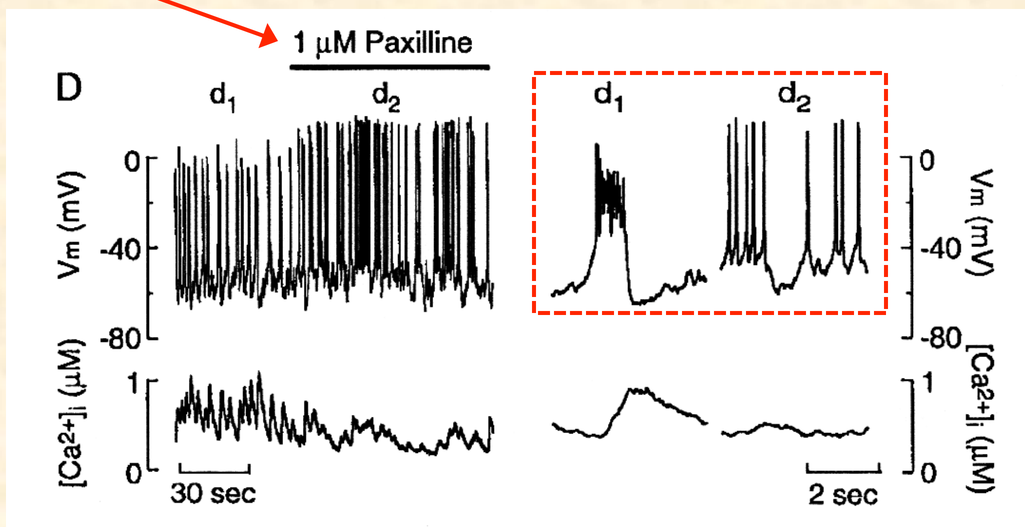
bursting

elevated calcium

substantial basal hormone release

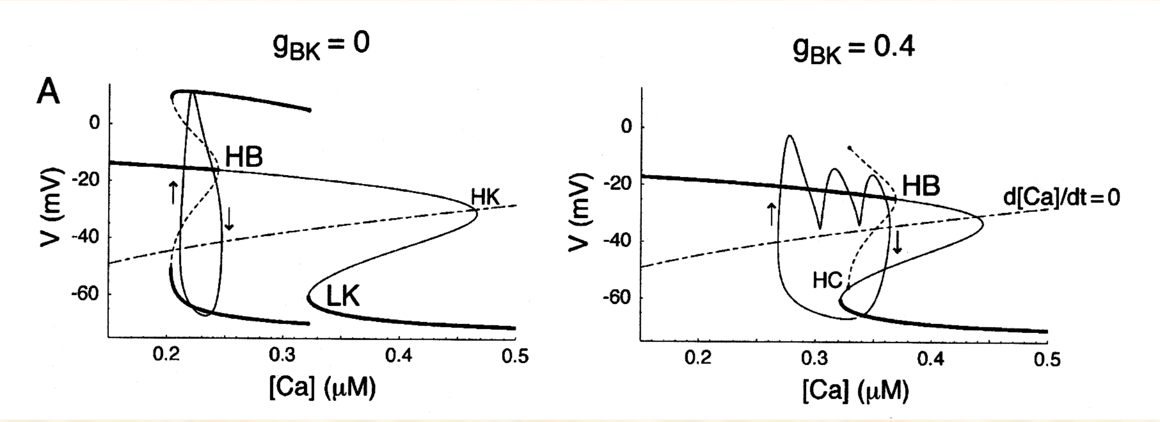
Blocking Large-Conductance K(Ca) Channels (or BK Channels) Converts Bursting to Spiking

blocker

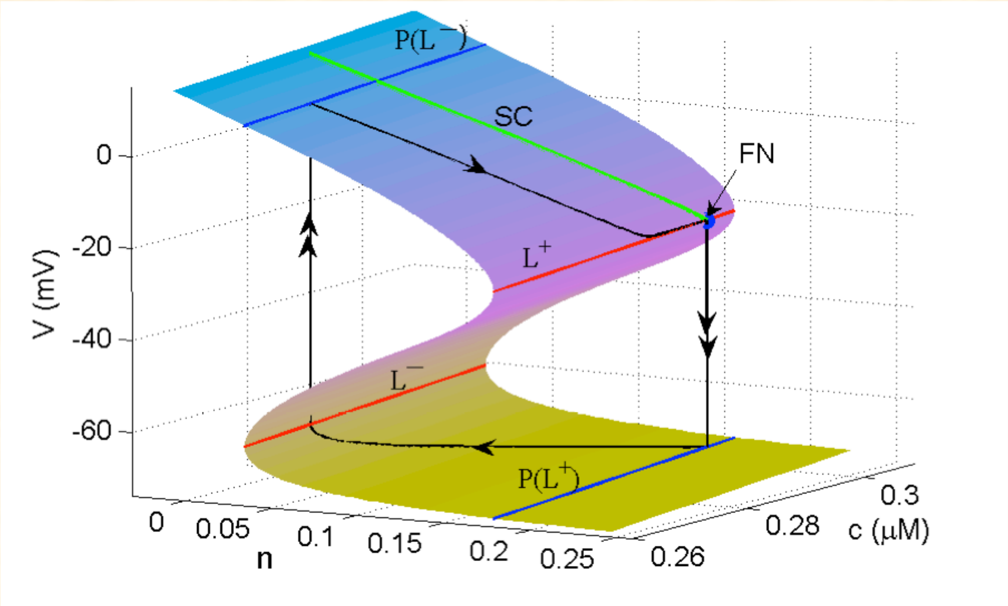


Van Goor et al., J. Neurosci., 2001:5902, 2001

Why???

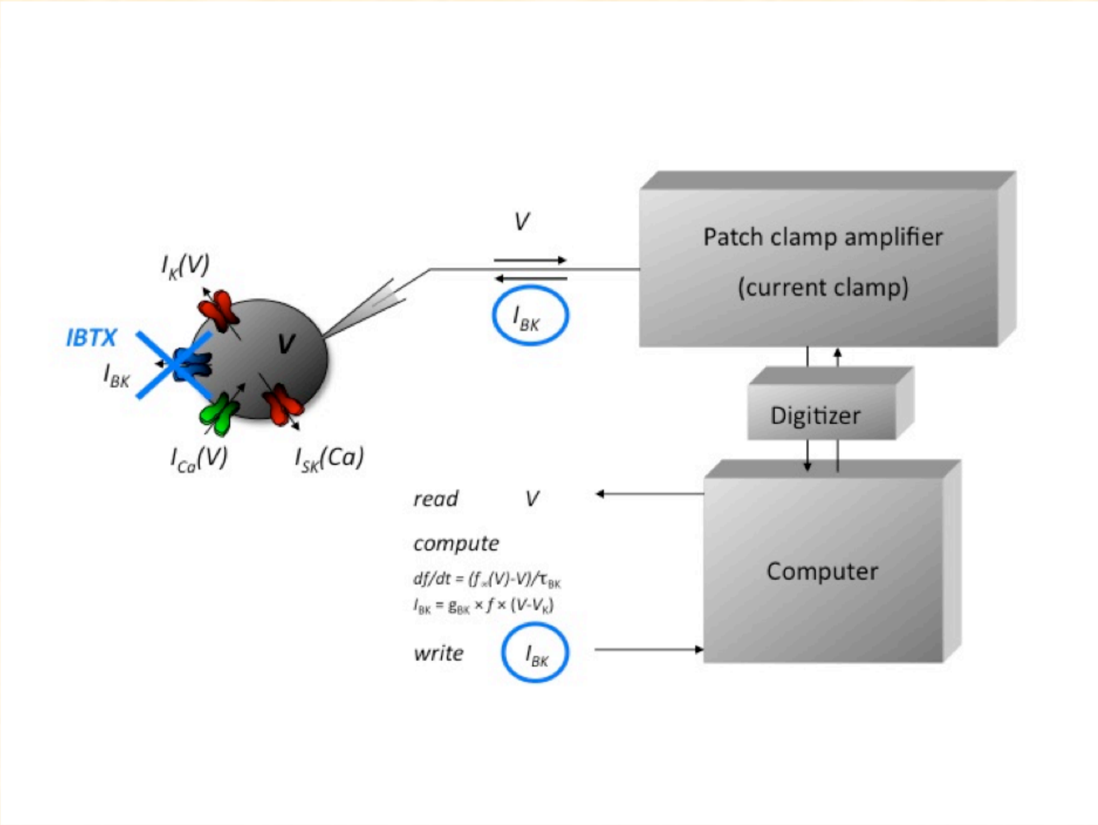


Tabak et al.,
J. Comp. Neurosci.,
22:211, 2007



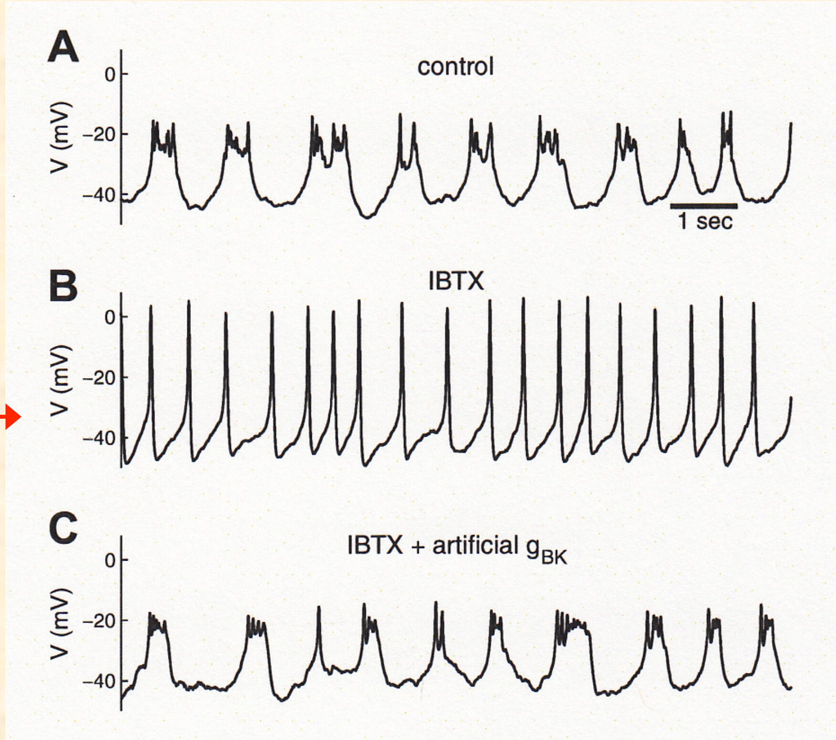
Teka et al.,
J. Math. Neurosci.,
1:12, 2011

We Use the Dynamic Clamp to Test our Theories on Pituitary Cells

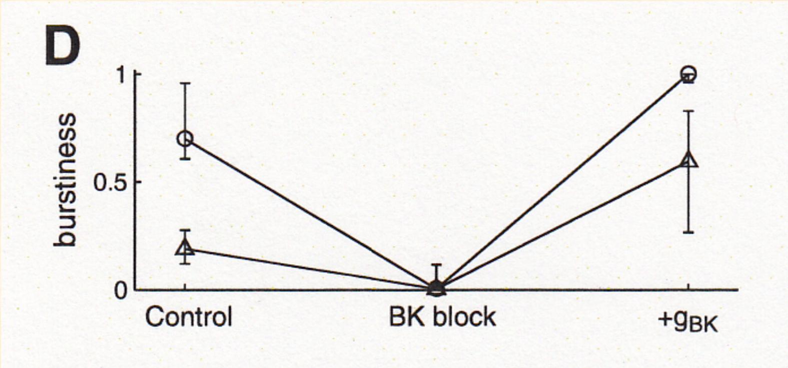


Blocking BK Current with Iberiotoxin Converts Bursting to Spiking

Block BK



Tabak et al., J. Neurosci., 31:16855, 2011

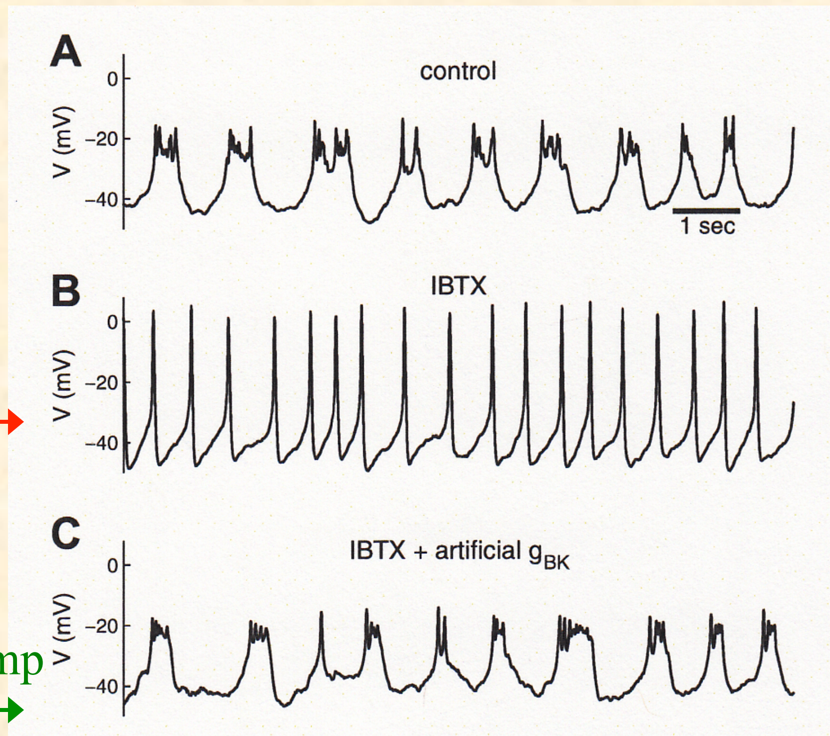


circle: bursters
triangle: spikers

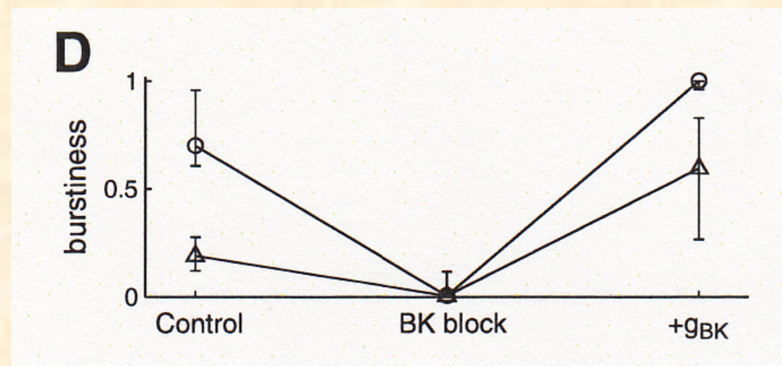
Adding BK Current with D-Clamp Converts Spiking to Bursting

Block BK

Add BK
back
with D-clamp



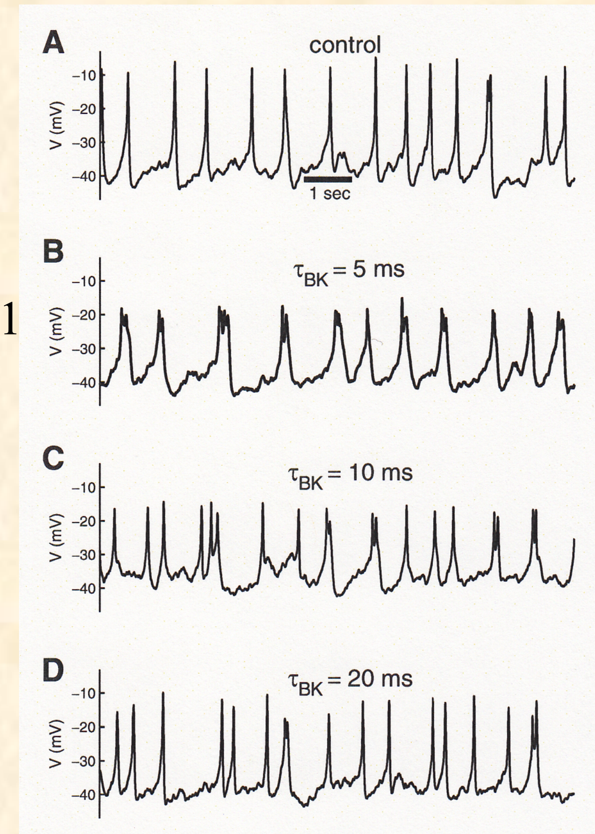
Tabak et al., J. Neurosci., 31:16855, 2011



Model and Analysis Predict That BK Current Rescues Bursting Only If It Activates Quickly

We tested this prediction on pituitary cells using D-clamp…

Tabak et al., J. Neurosci., 31:16855, 2011

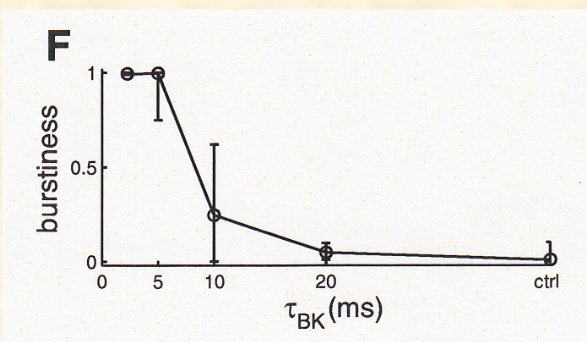


Tonic spiking cell

Rapidly activating BK added

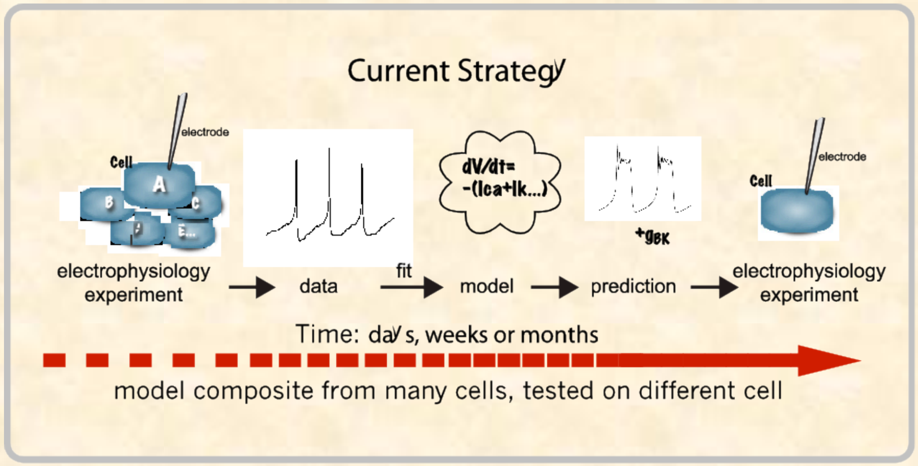
Slower activation

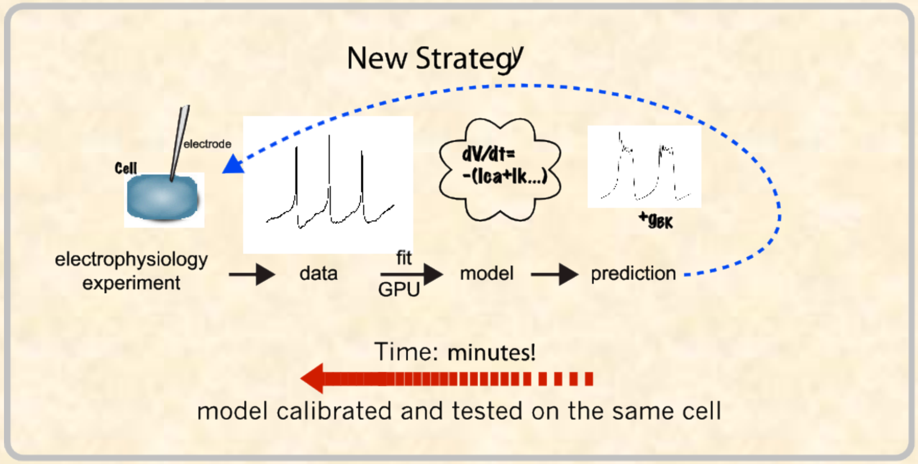
Even slower



Prediction Validated!

In the Future…





Thank You!