Hybrid Experiments: Linking Real-Time Simulations to *In Vitro* Electrophysiology Experiments

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• Membrane Dynamics and Ion Channels
• Cellular Level Electrophysiological Modeling
• Dynamic Clamp - model + experiment
• Science: Dynamic Clamp Applications
• Engineering: Performance and Implementation
• Can this be applied to other areas of “dynamical biology?”
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Response of Pyramidal Cell after Serotonin
... are everywhere

pancreatic beta cell

motoneuron

atrial myocyte
... are everywhere

pancreatic beta cell

motoneuron

atrial myocyte
...are everywhere

pancreatic beta cell

motoneuron

atrial myocyte
... are everywhere

- Pancreatic beta cell
- Motoneuron
- Atrial myocyte
Functional Role of APs

- **Cardiac**: heart rate, propagation of contraction, excitation-contraction coupling

- **Neural**: sensory information, pattern generation, motor commands

- **Endocrine**: secretion (hormones, metabolic regulation, insulin)
HH Animation

exit to show animation (stupid flash)
HH Model

\[ C \frac{dV}{dt} = -I_{Na} - I_{K} - I_{Leak} + I_{stimulus} \]
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\[ I_{Na} = g_{Na} m^3 h (V - E_{Na}) \]

\[ \frac{dm}{dt} = \alpha_m(V) (1 - m) - \beta_m(V) m \]

\[ \frac{dh}{dt} = \alpha_h(V) (1 - h) - \beta_h(V) h \]
HH Model

\[ C \frac{dV}{dt} = -I_{Na} - I_K - I_{Leak} + I_{stimulus} \]

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\[ \frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V)m \]

\[ \frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V)h \]
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Since then ...

- Nobel prize (with Eccles, 1963)
- Dynamical systems approaches to modeling single electrophysiological models is “standard practice”
- Modeling articles found in all major neuroscience and cardiac electrophysiology journals (Neuron, Nature Neuroscience, J Neuroscience, J Neurophysiology, AJP Heart, Circulation Research, etc.)
Today: More complex, yet same basic concepts

Demir et al., 1999
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Models and Experiments

usually separate exercises
Models and Experiments

usually separate exercises

(Butera et al., 1995)
Integrating Simulation and Experiment
Dynamic Clamp: Computer-Generated Conductances in Real Neurons

ANDREW A. SHARP, MICHAEL B. O'NEIL, L. F. ABBOTT, AND EVE MARDER
Departments of Biology and Physics, and Center for Complex Systems.
Brandeis University, Waltham, Massachusetts 02254
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Common Applications

• Virtual (Functional) Single Ion Channel Insertion (or Deletion)

• Processing of synaptic input

• Coupling of two cells with virtual coupling
Before: characterize and simulate

Common way to characterize ion channels:

1. Obtain I-V curve under voltage clamp and control solution

2. Obtain I-V curve in presence of pharmacological agent that blocks a single ion channel

3. Subtract 2 from 1, difference describes that ion channel

4. Functionality? Dynamics are tested via simulation. But now ...
After: virtual ion channel insertion

The same ion channel kinetics determined from voltage-clamp experiments can be inserted into a real cell. **Protocol:**

1. Measure dynamics (ex. action potential) in control solution

2. Block ion channel in question pharmacologically - observe *dynamic consequences* of lack of ion channel

3. While pharmacological block is still in place, *reintroduce* ion channel dynamics via dynamic clamp
Rapid Modeling

\[ I_A = \bar{g}_A m^4 \left( w h_1 + (1 - w) h_2 \right) (V - E_K) \]

Dynamic clamp mimicked the effects of 4-AP and 5-HT

(Sakurai, Darghouth, Butera, and Katz; *J. Neurosci.*, 2006)
This has Pharma Applications - “Channelopathies”

“Rapid prototyping of the functional consequences of voltage-gated ion-channel expression”
Common Applications

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Input Processing

- A single neuron may receive synaptic input from many (10-1000+ in some cases) neurons
- Input occurs via conductance changes (activated by binding events of neurotransmitter)
- Dynamic clamp can test this by enabling a conductance input of the form:

\[ I_{syn} = g(t)(V - E_{syn}) \]
Input Processing

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\[ I_{\text{syn}} = g(t)(V - E_{\text{syn}}) \]

measured

specified time course

updated every 50 us
\[ I_{syn} = g(t)(V - E_{syn}) \]

Raikov, Preyer, and Butera; J. Neurosci. Meth. 2004
\[ I_{syn} = g(t)(V - E_{syn}) \]

Common Applications

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Theory Motivated Experiments

Coupled oscillator theory has had a great impact on studies of neuronal synchrony .... but .... 100 times as many theoretical papers as there are validative experiments!
Neuronal Synchronization

- Fundamental to understanding:
  - information representation in sensory processing
  - generation of stable motor patterns
- Key: interplay between intrinsic and synaptic (coupling) kinetics
Two Real Coupled Neurons

Uncoupled

Phase Locked

Mostly Phase Locked

File=15 Date=02/04/07 g=0.03 t=15.0

File=18 Date=02/04/07 g=0 t=0.0

File=15 Date=02/04/07 g=0.03 t=15.0

File=25 Date=02/04/07 g=0.01 t=20.0

Preyer and Butera, unpublished
Ex: Inhibitory synaptic kinetics alter synchrony modes

Phase Difference vs. Synaptic Rate

Fast kinetics lead to anti-phase synchrony

Slower kinetics lead to in-phase synchrony

(Fig 8 van Vreeswijk et al. 1994)
Some results ...
(work in progress)

- Anti-phase, in-phase and bistable solutions present
- Transition between phases gradual as synaptic time constant is increased
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Around and Around ...

(Sharp et al., 1993)
The Need to Keep a Beat ...

measure V
integrate variables
calculate, output
\( t = t + dt \)
The Need to Keep a Beat ...
Desktop OSs Make Poor Metronomes
Desktop OSs Make Poor Metronomes
Two approaches

- **RTOS** (Real-Time Operating System): a PC operating system designed for high-priority low-latency tasks (RT-Linux, RTAI, QNX)

- **Embedded Target**: Dedicated processor (no OS) that does the computing, external PC programs and controls it
With all these approaches, surely the dynamic clamp technique is now widely used ...
With all these approaches, surely the dynamic clamp technique is now widely used ... Not Really ...
Papers Published

Research Papers vs. Methods

Year:
- 1993: 2 papers
- 1994: 1 paper
- 1995: 2 papers
- 1996: 5 papers
- 1997: 4 papers
- 1998: 4 papers
- 1999: 5 papers
- 2000: 6 papers
- 2001: 7 papers
- 2002: 8 papers
- 2003: 10 papers
- 2004: 14 papers
- 2005: 16 papers
- 2006: 20 papers
Technological Bottlenecks

- Current solution platforms (RTOS or embedded) were designed by engineers or computer scientists, for a similar audience - end-user ignorant!

- All solutions have some aspect that is time or cost intensive to the end user

- Technology Adoption Life Cycle
Technology Adoption Life Cycle

Innovators

Early Adopters

Pragmatists

Traditionalists

adapted from G.A. Moore (1991)
Open Ended R&D Questions

Measurement Noise?
Numeral Accuracy?

Flexibility?
Model size?
Ease of use?

How fast?
How accurate is timing?

(Sharp et al., 1993)
• Simple numerical methods for gating variables are used due to time constraints

• Most popular: Exponential Euler (EE)

• Is it “better” than Euler?

• Does measurement error (noise) in $V_m$ matter?
Goal: Error Estimate

• Desired an estimate of error in a single computational cycle for a voltage-dependent gating variable

• Error should be in terms of model and experimental parameters

• Basis: many folks use “exponential Euler” as a shortcut when speed is of the essence

• Exp Euler: First order quasi-steady-state assumption w.r.t. voltage
### The Players

<table>
<thead>
<tr>
<th></th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta t)</td>
<td>time step (msec)</td>
</tr>
<tr>
<td>(\tau)</td>
<td>gating variable time-constant (msec)</td>
</tr>
<tr>
<td>(\delta)</td>
<td>voltage measurement error (mV)</td>
</tr>
<tr>
<td>(d)</td>
<td>steady-state voltage-dependent activation “slope factor”</td>
</tr>
</tbody>
</table>

\[
\frac{dx}{dt} = \frac{x_\infty(V) - x}{\tau}
\]

\[
x_\infty(V) = \frac{1}{1 + \exp\left(\frac{V - V_1}{d} \frac{2}{2}\right)}
\]
The Result

\[ |e|_{\text{ExEuler}} \leq (e^{\frac{\Delta t}{\tau}} - 1)\left(\frac{\delta}{4|d|} + \frac{\Delta t}{\tau}\right) \]

\[ |e|_{\text{Euler}} \leq (e^{\frac{\Delta t}{\tau}} - 1)\left(\frac{\delta}{4|d|} + \frac{\Delta t}{2\tau}\right) \]

• This is for local error during a single time-step

• Two important ratios: \( \delta/|d| \) and \( \Delta t/\tau \)

• Limiting Cases:
  
  No measurement noise (\( \delta=0 \)) EE bound is twice as large as E
  
  Noise dominates (\( \delta/|d| >> \Delta t/\tau \)) method does not matter
  
  Best case (\( \delta=0 \) and \( \Delta t << \tau \)) one-step error scales with \( \Delta t^2 \! \)

(Butera & McCarthy, 2004)
Putting it into practice

(Butera & McCarthy, 2004)
Numerical Validation Procedure

- Reference Simulation (high-order, low-error)
- At each point in reference simulation, take one time-step of E or EE
- Compare this new value with the next time-step of the reference simulation
- 10 kHz time step, gating variables have $\tau$ of 0.1, 10, and 10000 ms
- $d$ estimated from figure at left

(Butera & McCarthy, 2004)
The Bounds Work

\[ \Delta t / \tau = 0.01 \]

\[ \Delta t / \tau = 1 \]

(Butera & McCarthy, 2004)
ExpEuler is over-rated

- Others have suspected as much (high performance simulations)
- Steep slope factors magnify measurement noise effect on error bound
- Dclamp speed (relative to kinetics) always matters, higher measurement noise decreases the error improvement gained by speeding up
- Unresolved: \( \Delta t \) noise effects (jitter)
- Unresolved: are higher order methods worth it?
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Summary

- A field of “biological dynamics” with an accessible “mode of interaction” (the membrane) and fast dynamics (50 us or faster time constants) amenable to real-time interaction

- Technique is known, but obstacles exist to ease of implementation and use by end users

- Very little research done on performance analysis (numerical or computational)

- Still untapped as a tool for marrying nonlinear dynamics theory with an experimental setting
Other real-time measurement and stimulation modalities?

- Measurement: Fluorescent imaging (calcium, cAMP, voltage ...), 2 photon
- Stimulation: optical release of caged compounds (creating “fluxes”)
thanks

Ivan Raikov, M.S.

Amanda Preyer, Ph.D.

✦ NIH
✦ NSF
✦ James S. McDonnell Foundation

› Jianxia Cui (postdoc)
› Dave Christini (Cornell)
› John White (BU, Utah)
Xtra Stuff
More Things That Work

PC w/Real-Time Linux

Custom DSP board with 2 channels ADC/DAC