Spatial Complexity of Cells:

Real Cellular Structure Reflects Molecular Scales

"structures" (fibroblasts)

3-D Structure of Vertebrate Muscle Cell (artist’s rendering)

The Computational Challenge...

Cellular Structures
Molecular Locations
Mass Action Rate Constants

Cellular Structures
Molecular Mechanisms
Molecular Structure-

Constan...
Various Software Packages

- FormZ, XVoxTrace, NWGrid, Mesquite, LaGrit, VTK, OpenDX, NETGEN, ParaView

**DReAMM**
- Design, Render & Animate MCell Models

**MCell**
- General Monte Carlo Simulator of Microcellular Physiology

**Where are the bottlenecks?**

**Skipping over ...**
- Brownian Dynamics Random Walk (Grid-free)
- Monte Carlo Probabilities for: Unimolecular Transitions
  - Bimolecular Associations
  - Numerical Accuracy
  - Run-time Optimizations

**Vertebrate Neuromuscular Junction (NMJ)**

**Engineering Issues:**
- Motor Programs
- Final Common Path
- Impedance Mismatch
- 1:1 Transmission
- Motor Unit Size & Recruitment
- Synaptic Latency & Jitter

**Neuromuscular Physiology**

**Individual Experimental (Lizard) maps**

**Simulate mepsca...**

**Neuromuscular Physiology**

**Engineering Issues:**
- Impedance Mismatch, Quantal Analysis & Variability?
Junctional Folds Decrease mepc Amplitude

Planar NMJ Model

<table>
<thead>
<tr>
<th>Fold spacing</th>
<th>Depth</th>
<th>Peak current</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACHE active</td>
<td>μm</td>
<td>n=4</td>
</tr>
<tr>
<td>No folds</td>
<td>0</td>
<td>7.36 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.29 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>5.48 ± 0.1</td>
</tr>
<tr>
<td>ACHE inactive</td>
<td>μm</td>
<td>n=4</td>
</tr>
<tr>
<td>No folds</td>
<td>0</td>
<td>9.56 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>9.27 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>7.38 ± 0.04</td>
</tr>
</tbody>
</table>

Testing Prediction for Untreated mepc t;
Simultaneous Broadband EC and VC Recordings

Skipping over ...  
- Parameter Fitting for NMJs:  
  - Untreated; ACHE Inhibited; ACHE Inhibited + AChR Blockade  
  - Predicted Untreated mepc t.

mepc t, and ACh Exocytosis

Averaged Experimental mepcs
Broadband immersion (BI) and enhanced high-speed voltage-clamp (VC) recordings

Predicted Effects of Fusion-Pore Expansion Rate
Lizard, 18ºC, Planar NMJ model

Temperature Sensitivity of mepcs is Mostly Governed by Offsetting Effects on Channel Gating

Experimental mepcs (Lizard, VC and EC)

<table>
<thead>
<tr>
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<th>Value</th>
<th>Change for 1ºC Increase</th>
<th>Change for 1ºC Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACHE Site Density</td>
<td>1000 molecules</td>
<td>-80%</td>
<td>-60%</td>
</tr>
<tr>
<td>EC Electrode Diameter</td>
<td>50 μm</td>
<td>-30%</td>
<td>-20%</td>
</tr>
<tr>
<td>EC Electrode Pressure</td>
<td>100 kPa</td>
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<td>-10%</td>
</tr>
<tr>
<td>EC Electrode Temperature</td>
<td>37ºC</td>
<td>-10%</td>
<td>-5%</td>
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</table>

Highly Nonlinear Sensitivity of mepc t, to ACHE Parameters

Temperature Sensitivity of mepcs is Mostly Governed by Offsetting Effects on Channel Gating

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Patient followed from birth:

- Progressive weakness and impaired neuromuscular transmission without early degenerative endplate changes typically associated with SCCMS
- Prolonged, low amplitude synaptic currents at early and late stages
- Atypical, initially mild (focal) ultrastructural changes progressed over time
- Novel C-to-T substitution in exon 8 of the δ subunit of AChR: serine to phenylalanine mutation in the second transmembrane domain (M2) that lines the ion channel
- AChR numbers not significantly reduced
Spatio-Temporal Correlations and Synaptic Noise

Synaptic Topology & Current Variability

3D reconstruction of vertebrate neuromuscular junction

Open AChR Channels

Type I Variability (single ACh release site)

100 simulations

Type II Variability (multiple ACh release sites)

~1000 simulations

Open AChR Channels

Time (μs)
A model of voltage-gated $\text{Ca}^{2+}$ channel activity

$D_{\text{Ca}} = 6 \times 10^4 \text{ cm}^2/\text{sec}$

$\alpha = 0.06(\text{mV})^{-2} + 4(\text{mV})^{-1}$

$\beta = 1.2(\text{mV}) + 3(\text{mV})^2 + 1$
Conclusions and Predictions

1. Can a model of an entire active zone simultaneously reproduce the known CRR, distribution of release times and average release probability? Yes

2. If a model can be found, what does it predict for the number of Ca\(^2+\) binding sites per vesicle and what constitutes a vesicle fusion event? ~30-40 binding sites, ~6 of which must be bound simultaneously to trigger fusion

Future Directions

- CRR changes during development and reinnervation. Can this be explained by spatial changes that occur?

- Multiple action potentials can induce short-term plastic changes. Can this be predicted using our model of vesicle fusion?

Developers & Collaborators

MCe/DRaAMM Development:
  - Tom Bartol, Rex Kerr, Terry Sejnowski (Salk Institute)
  - Jack Chang, Boris Kaminsky (PSC)

Microphysiology:
  - John Pattillo (PSC)
  - Steve Merinov (U. Pittsburgh)
  - Tom Deerinck, Mark Ellisman (UCSD)
  - Will Ford (U. Pittsburgh, Cal Tech)
  - Chris Gomez (U. Minnesota)
  - Deanna Nachreiner (BBSI)
  - Jordan Torok (BBSI)
  - Nick Morsillo (U. Pittsburgh, BBSI)
  - Evan Kepner (U. Pittsburgh)