Cellular Simulations (2):

Analytic, Finite Difference, & Monte Carlo Approaches to Reaction-Diffusion Systems

NIH-NSF BBSI: Simulation and Computer Visualization of Biological Systems at Multiple Scales

June 17, 2005

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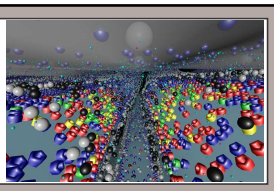
PITTSBURGH SUPERCOMPUTING CENTER

Biomedical Supercomputing Initiative

Counter-intuitive Insights from Monte Carlo Simulations of Synaptic Transmission



Supported by:
NIH R01 GM068630, P20 GM065805, P41 RR06009, & NIH/NSF 0234002

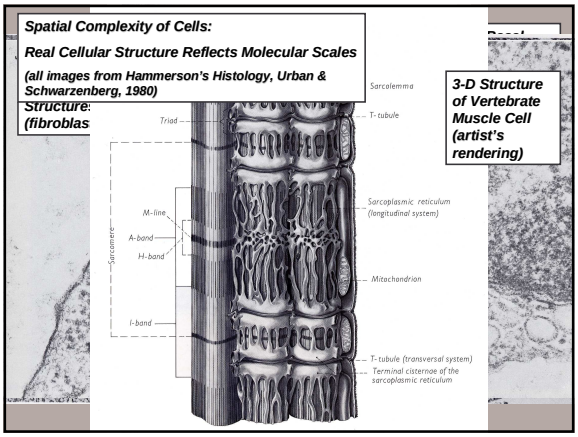
Spatial Complexity of Cells:

Real Cellular Structure Reflects Molecular Scales

(all images from Hammerson's Histology, Urban & Schwarzenberg, 1980)

Structure: (fibroblas

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



The Biological Challenge…

"[R]esults to date show a dizzying array of signaling systems acting within and between cells. … In such settings, intuition can be inadequate, often giving incomplete or incorrect predictions. … In the face of such complexity, computational tools must be employed as a tool for understanding." Fraser & Harland, Cell 100:41 (2000).

Requires: Stochastic methods applied to 3-D reaction-diffusion and cells-as-machines problems - largely embryonic due to the scope of necessary software development and computational scale.

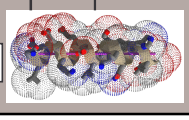
The Computational Challenge…

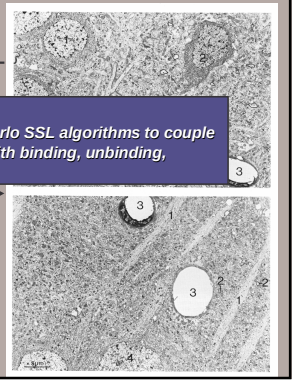
Cellular Structures
Molecular Locations
Mass Action Rate Constants

MCell & DReAMM
Build models and use Monte Carlo SSL algorithms to couple Brownian Dynamics diffusion with binding, unbinding, conformational changes,…

Molecular Diffusion
Molecular Mechanisms
~~Molecular Structure~~

QM/MM
Δt ~10⁻¹⁵ s

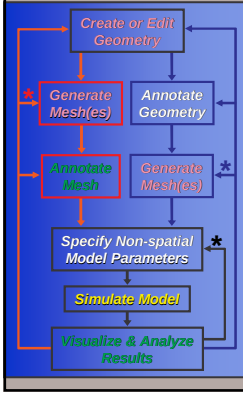




The Multiscale Computational Challenge

Problem/ Method	Typical Application	Software Examples	Resolution (Scale)	Spatial Realism	Stochastic Realism	Time Step	Time Scale	Serial/ Parallel	Computer Time
Networks of Reactions/ Sets of ODEs	Metabolic or signaling pathways	VCell, ECell Gepasi XPPAUT	N/A (cell)	N/A	none	ms	ms - hrs	serial	minimal
Excitation/ Compartmental Circuit	Nerve signaling	NEURON GENESIS NEOSIM	µm - mm (cell - multicell)	low - medium	none	ms	ms - hrs	usually serial	usually low
Reaction Kinetics/ Stochastic	Gene regulation/ transcription	BioSpice StochSim XPPAUT MCell	N/A (cell)	N/A	high	ms	ms - hrs	serial	low
3-D Reaction Diffusion/ Finite Element	Flow models, calcium dynamics	VCell FIDAP Kaskade	<µm (cell)	medium-high	none	µs - ms	µs - sec	either	low - high
3-D Reaction Diffusion/ Monte Carlo	Micro-physiological processes	MCell ChemCell	nm – mm (subcell - cell)	high	high	ps - ms	µs - sec	either	low - high
Macromolecular Machinery/GNM	Collective dynamics	GNM ANM	Å - 100 nm (complexes)	high	none	N/A	<ns - µs>	N/A (analytic)	minimal
Diffusion in Potential Field/Poisson-Nernst-Planck	Electrostatic interactions, ion channels	UHBD Delphi CHARMM	Å - nm (membrane proteins)	high (implicit solvent)	none	N/A	<ns - µs>	parallel	low - medium
Macromolecular Motions/Brownian Dynamics (BD)	Conformational dynamics (in flow fields)	CHARMM GROMOS UHBD	Å - nm (macro-molecules)	high (implicit solvent)	high	5 - 10 fs	<ns - µs>	parallel	medium - high
Molecular Structure/ Molecular Dynamics (MD)	Conformational dynamics & free energies	AMBER CHARMM GROMOS	Å (macro-molecules)	exact (explicit solvent)	exact	1 - 2 fs	<ns - µs>	parallel	very high
Transition Dynamics/ Quantum-Molecular Mech. (QM/MM)	Enzyme reactions (make/break bonds)	DYNAMO (AMBER CHARMM)	Å (molecules)	exact (explicit solvent)	exact	1 - 2 fs	<ns - µs>	parallel	very high
Molecular Structure/ *Ab initio* simulations	Solution of the Schrodinger equation	Gaussian98	< Å (electrons - atoms)	exact	exact	N/A	N/A	parallel	highest

Microphysiological (Spatially Realistic) Modeling Pipeline

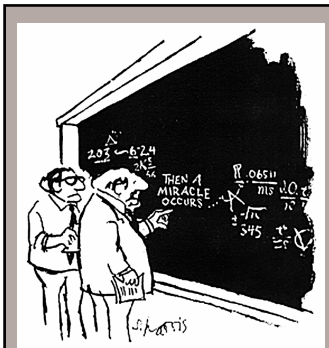


Various Software Packages
e.g., FormZ, XVoxTrace, NWGrid, Mesquite, LaGrit, VTK, OpenDX, NETGEN, PSC-VB

DReAMM
Design, Render & Animate MCell Models

MCell
General Monte Carlo Simulator of Microcellular Physiology

Where are the bottlenecks?



"I think you should be more explicit here in step two."

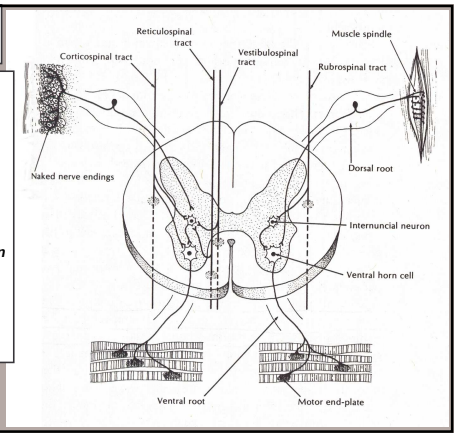
Skipping over …

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

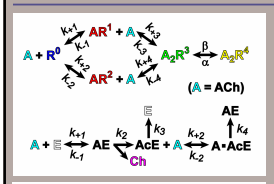
Vertebrate Neuromuscular Junction (NMJ)

Engineering Design Issues:

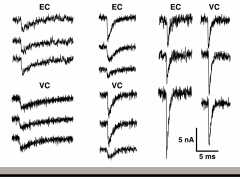
- *Motor Programs*
- *Final Common Path*
- *Impedance Mismatch*
- *1:1 Transmission*
- *Motor Unit Size & Recruitment*
- *Synaptic Latency & Jitter*

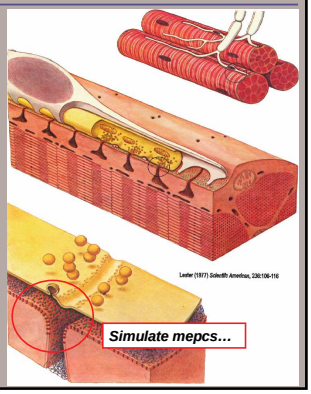


Neuromuscular Physiology

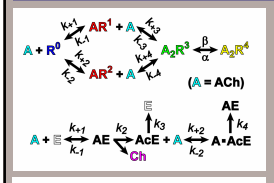


Individual Experimental (Lizard) mepcs

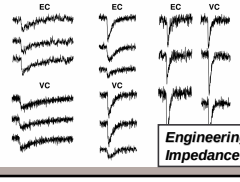


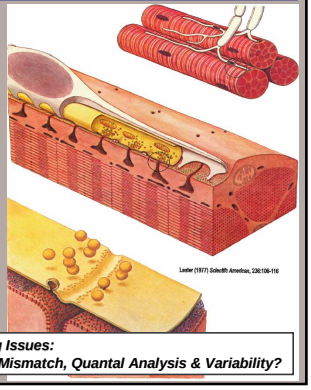


Neuromuscular Physiology



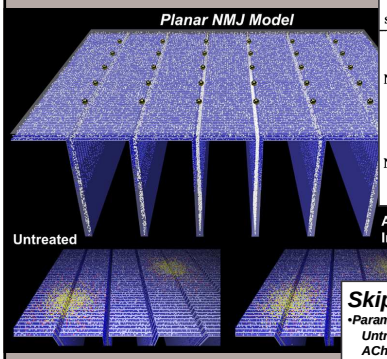
Individual Experimental (Lizard) mepcs





Engineering Issues:
Impedance Mismatch, Quantal Analysis & Variability?

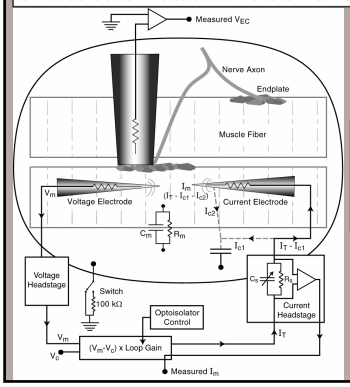
Junctional Folds Decrease mepc Amplitude



Fold spacing	Fold depth	Peak current
	AChE active	
μm	μm	nA
No folds	0	7.36 ± 0.09
1.0	0.5	6.29 ± 0.1
0.29	0.8	5.48 ± 0.1
	AChE inactive	
No folds	0	9.98 ± 0.15
1.0	0.5	9.27 ± 0.17
0.29	0.8	7.38 ± 0.04

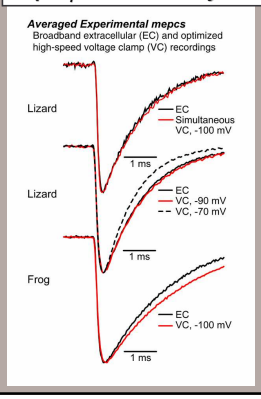
Skipping over …
- *Parameter Fitting for NMJs: Untreated; AChE Inhibited; AChE Inhibited + AChR Blockade*
- *Predicted Untreated mepc t_r*

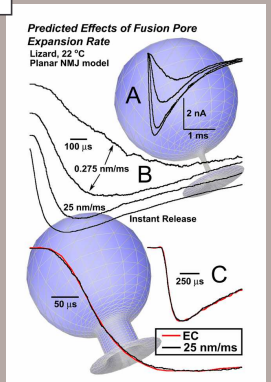
Testing Prediction for Untreated mepc t_r: Simultaneous Broadband EC and VC Recordings



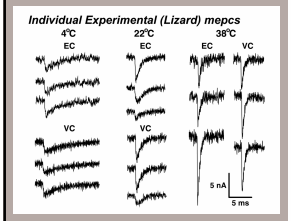
Skipping over …
- *Quantitative Bandwidth Determination*
- *Effect of EC Electrode Diameter and Pressure*
- *mepc Alignment and Averaging*

mepc t_r and ACh Exocytosis





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



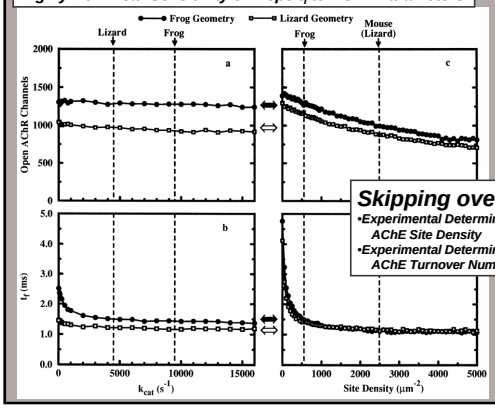
Experimental mepcs (Lizard, VC and EC)

	A_c	t_r	t_f
Change for 10° Increase (17° - 27°) (Arrows Normalized to Equal Size)	↑ (38%)	↓ (24%)	↓ (39%)

Simulated mepcs (Planar NMJ Model)

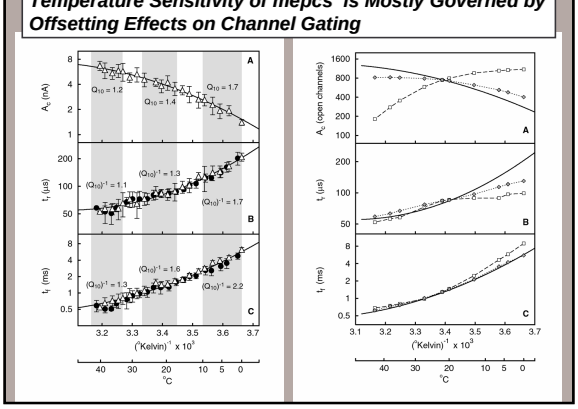
Kinetic Parameter	Value at 22°C	Relative Change for 2-fold Increase in Kinetic Parameter		
k_-	18365 s^{-1}	↓	↓	↓
α	1670 s^{-1}	-	↓	↓
k_{cat}	4500 s^{-1}	-	↓	-#
k_+E	1.50×10^8 $M^{-1}s^{-1}$	-	↓	-#
fusion pore expansion rate	25 nm·ms^{-1}	-#	+#	-
D	3.36×10^{-6} cm^2s^{-1}	↓	↓	-
N	5045 molecules	↑ (3.6x)	-	-
γ	55 pS	↑ (2.7x)	n/c	n/c
k_+	8.03×10^7 $M^{-1}s^{-1}$	↑	-	-
β	36729 s^{-1}	↑	↑	↑

Highly Nonlinear Sensitivity of mepc t_f to AChE Parameters



Skipping over …
- *Experimental Determination of AChE Site Density*
- *Experimental Determination of AChE Turnover Number*

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

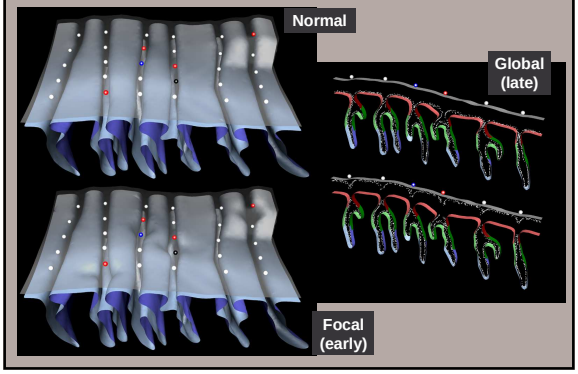


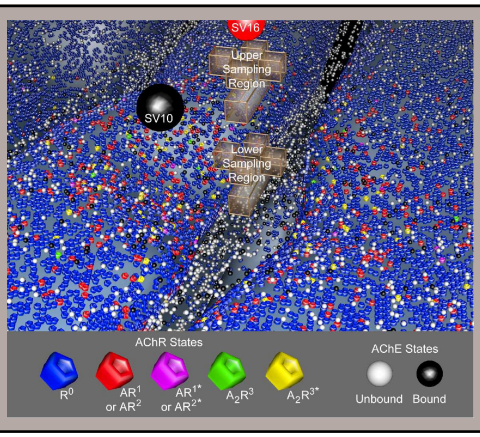
Synaptic Pathophysiology in a Novel Form of Slow Channel Congenital Myasthenic Syndrome (SCCMS)

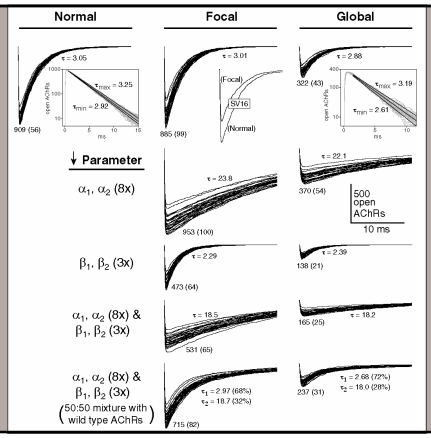
Patient followed from birth:

- *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*

Synaptic Pathophysiology in a Novel Form of Slow Channel Congenital Myasthenic Syndrome (SCCMS)

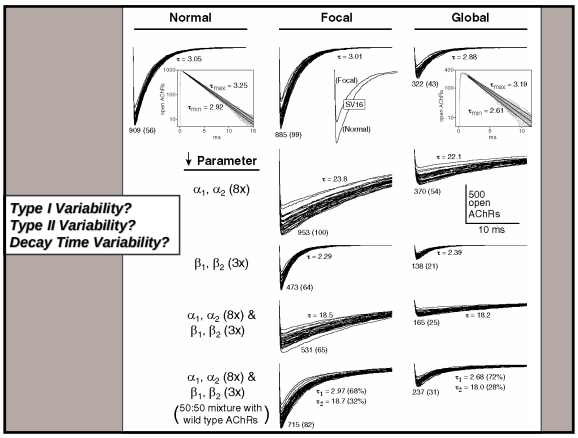


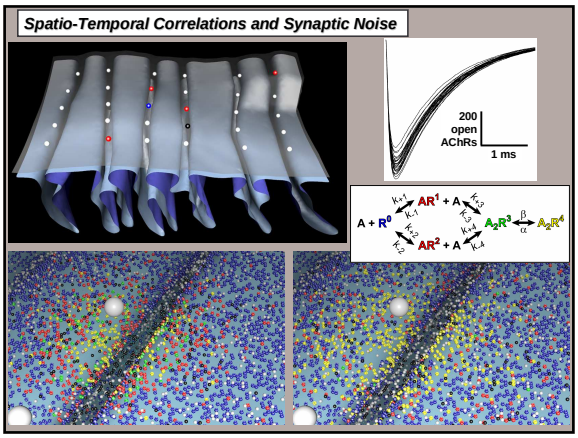


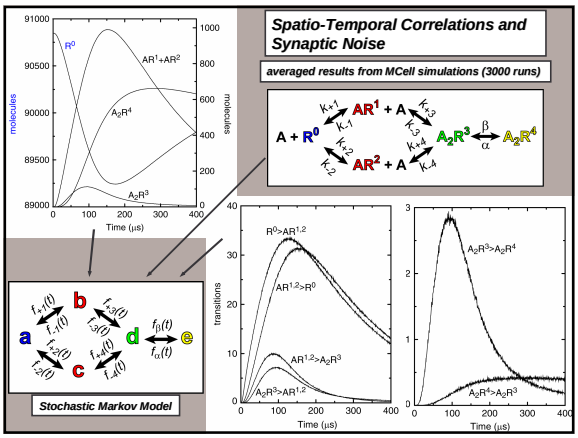


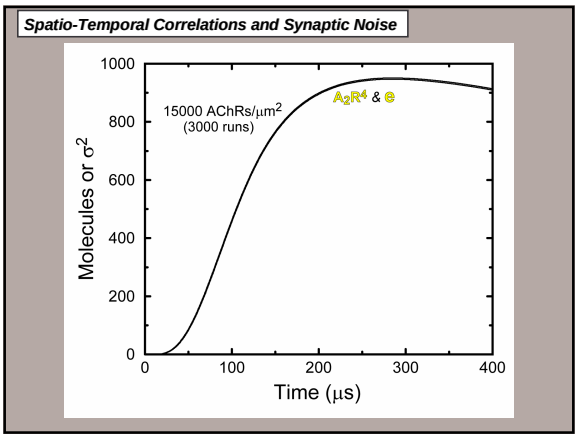
Synaptic Pathophysiology in a Novel Form of Slow Channel Congenital Myasthenic Syndrome (SCCMS)

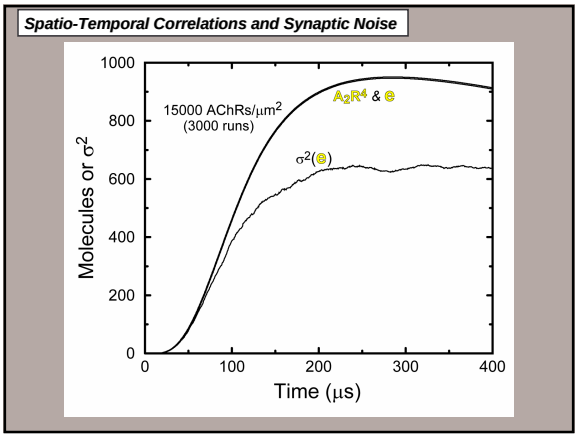
- *Typical of SCCMS, mutant neurotransmitter receptors showed dramatically slowed deactivation (ion channel closing rate)*
- *However, simulations of synaptic signals predicted an additional novel slowing of receptor activation (ion channel opening rate)*
- *Based on model predictions, opening rate was measured and found to be decreased - likely explains unique early course of disease in this patient*

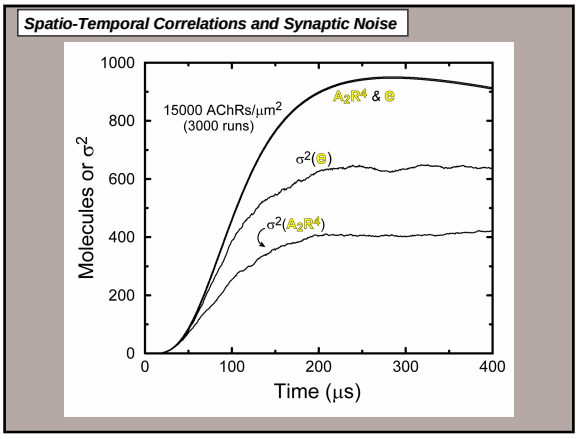

Normal
Focal
Global
Parameter
α1, α2 (8x)
β1, β2 (3x)
α1, α2 (8x) & β1, β2 (3x)
α1, α2 (8x) & β1, β2 (3x) (50:50 mixture with wild type AChRs)
Type I Variability?
Type II Variability?
Decay Time Variability?
500 open AChRs
10 ms


Spatio-Temporal Correlations and Synaptic Noise
200 open AChRs
1 ms

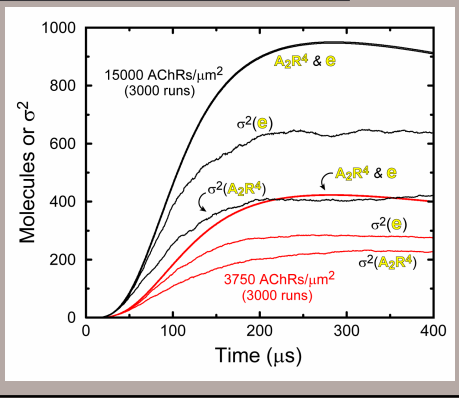

Spatio-Temporal Correlations and Synaptic Noise
averaged results from MCell simulations (3000 runs)
Stochastic Markov Model
Time (μs)
transitions
molecules


Spatio-Temporal Correlations and Synaptic Noise
15000 AChRs/μm2
(3000 runs)
A2R4 & e
Molecules or σ2
Time (μs)

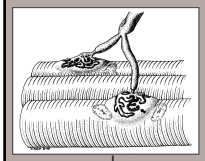

Spatio-Temporal Correlations and Synaptic Noise
15000 AChRs/μm2
(3000 runs)
A2R4 & e
σ2(e)
Molecules or σ2
Time (μs)


Spatio-Temporal Correlations and Synaptic Noise
15000 AChRs/μm2
(3000 runs)
A2R4 & e
σ2(e)
σ2(A2R4)
Molecules or σ2
Time (μs)

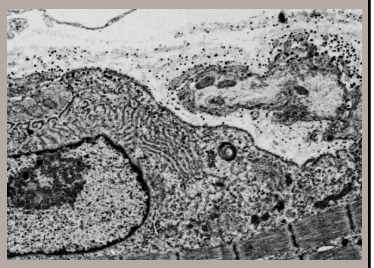
Spatio-Temporal Correlations and Synaptic Noise



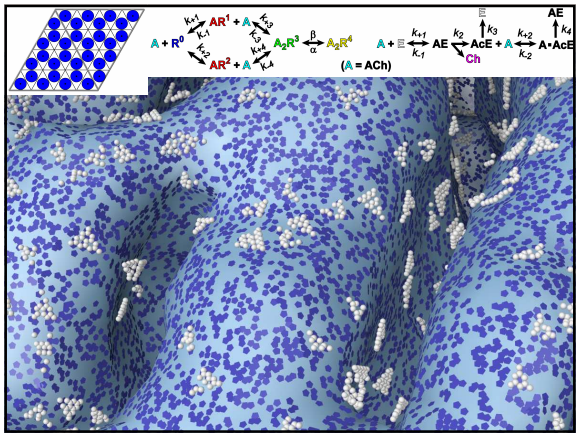
Synaptic topology & current variability

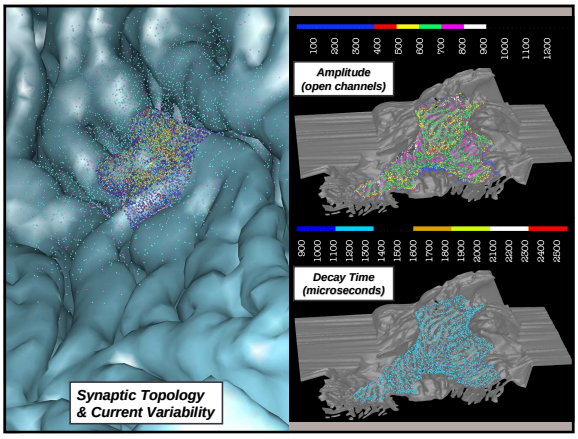


3-D reconstruction of vertebrate neuromuscular junction

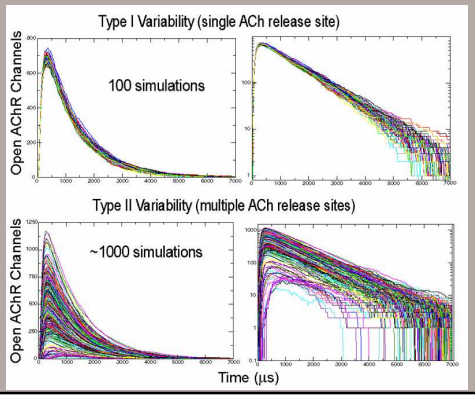


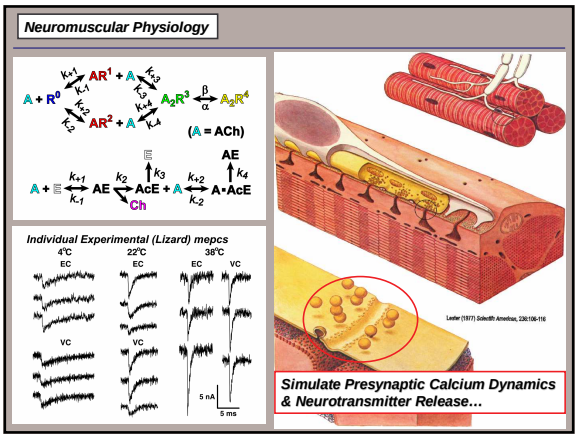
PSC's Biomedical Supercomputing Initiative – An NIH Resource Center

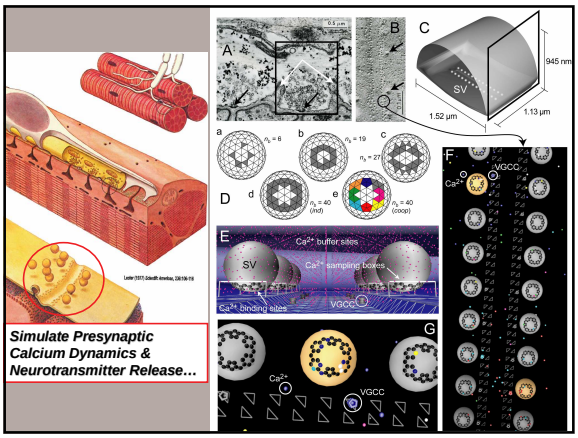


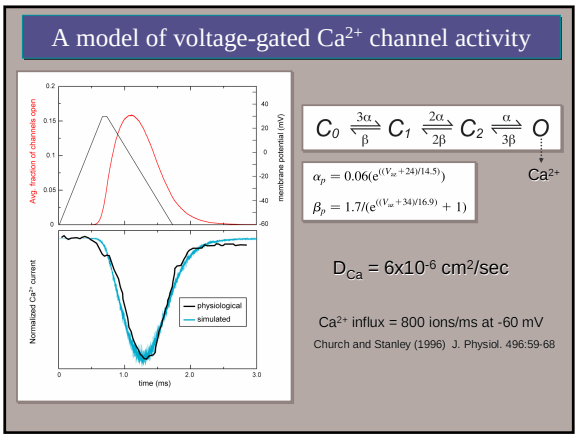


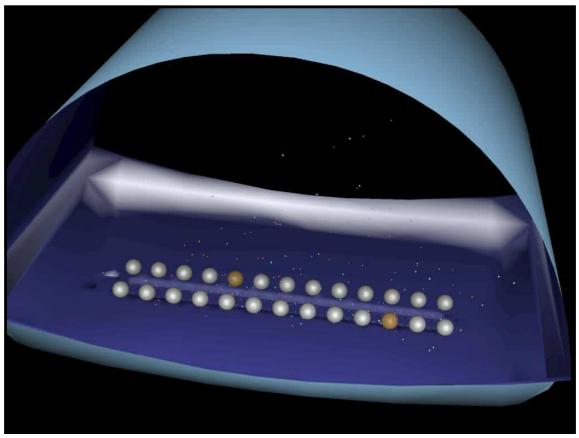
Synaptic Topology & Current Variability

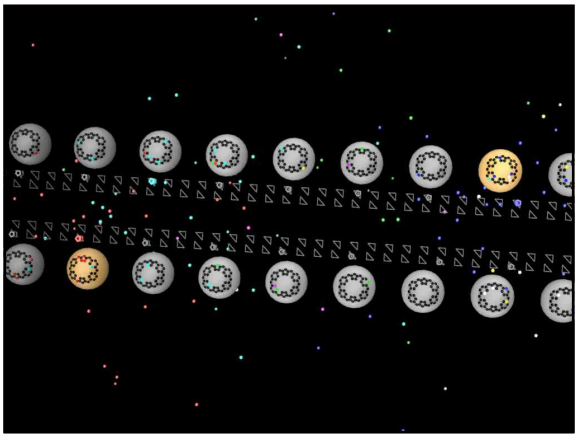


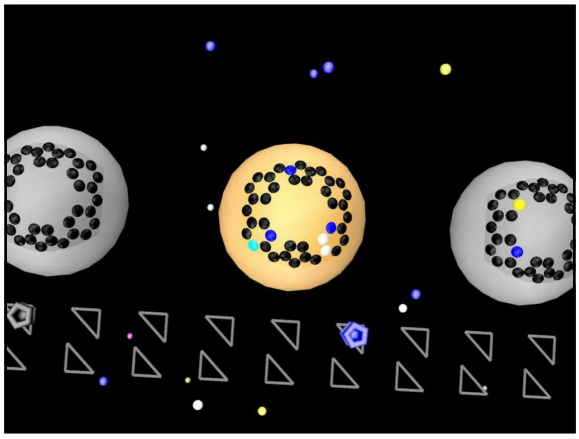

Neuromuscular Physiology
(A = ACh)
Individual Experimental (Lizard) mepcs
4°C
22°C
38°C
EC
VC
5 nA
5 ms
Simulate Presynaptic Calcium Dynamics & Neurotransmitter Release…


Simulate Presynaptic Calcium Dynamics & Neurotransmitter Release…
945 nm
1.52 µm
1.13 µm
SV
VGCC
Ca2+ buffer sites
Ca2+ sampling boxes
Ca2+ binding sites

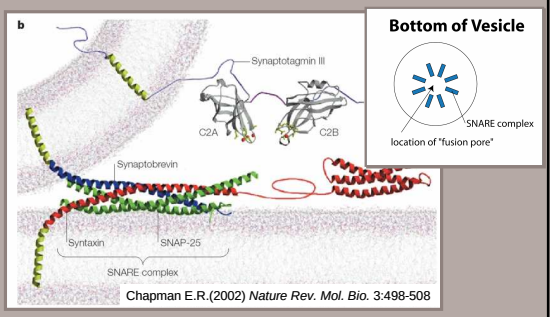

A model of voltage-gated Ca2+ channel activity
Avg. fraction of channels open
membrane potential (mV)
Normalized Ca2+ current
physiological
simulated
time (ms)
C0 ⇌ C1 ⇌ C2 ⇌ O
Ca2+
DCa = 6x10-6 cm2/sec
Ca2+ influx = 800 ions/ms at -60 mV
Church and Stanley (1996) J. Physiol. 496:59-68







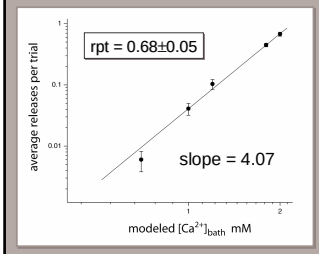
Is this number of binding sites feasible?

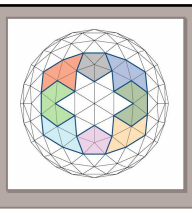


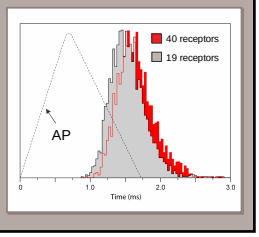
Chapman E.R.(2002) *Nature Rev. Mol. Bio.* 3:498-508

There may be as many as 40 binding sites per vesicle

40 binding sites, subgroups of 5







If binding sites are subdivided into groups of five, then **40** are necessary with **3 groups of 2** required for fusion.

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

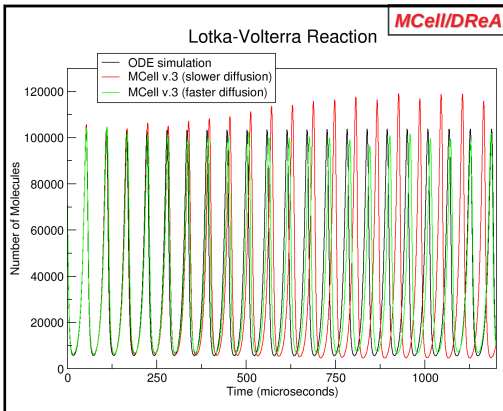
2. **If a model can be found, what does it predict for the number of Ca^{2+} binding sites per vesicle and what constitutes a vesicle fusion event?**

   ~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?**

MCell/DReAMM v.3



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PSC's Biomedical Supercomputing Initiative – An NIH Resource Center