The Effect of Non-uniform Acetylcholine Receptor Protein Distribution in Neuromuscular Junctional Folds

Deanna R. Nachreiner
Replicating Known mEPC Variability and Decay: MCell Simulations of Synaptic Transmission

Deanna R. Nachreiner¹,³, Jordan A. Torok¹,⁴, John M. Pattillo², and Joel R. Stiles²,⁵

¹ Bioengineering and Bioinformatics Summer Institute, Center for Computational Biology and Bioinformatics, University of Pittsburgh, Pittsburgh, PA 15261
² Biomedical Initiative, Pittsburgh Super Computing Center, Pittsburgh, PA 15213
³ Department of Bioengineering, The Pennsylvania State University, University Park, PA 16802
⁴ Department of Biology, Bucknell University, Lewisburg, PA 17837
⁵ Mellon College of Science, Carnegie Mellon University, Pittsburgh, PA 15213
Presentation Overview

- Review of synaptic transmission and the Neuromuscular Junction (NMJ)
- Review of MCell & DREaMM and their applications
- Creation of the initial model and the problems encountered
- Introduction to individual experiments and proposed solutions to problems
- Individual results
- Individual conclusions
The Importance of Neurotransmission

- "Neurotransmission: passage of signals from one nerve cell to another via chemical substances or electrical signals." (www.hyperdictionary.com)
- Responsible for cellular communication and thousands of physiological processes
- Core area of research because of the close relationship with drug development and disease prevention
  - Better understanding of the mammalian NMJ will lead to treatments for diseases like Slow-Channel Congenital Myasthenic Syndrome (SCCMS) – a disease stemming from mutations of AChR proteins with symptoms like extreme muscle weakness and fatigue

The Neuromuscular Junction

- Well-described, widely studied synapse between a nerve and a muscle cell

http://academic.wsc.edu/faculty/jatodd1/351/ch6outline.html
The Neuromuscular Junction

- **Synaptic Transmission**

- Action potentials propagate down the nerve and trigger the release of synaptic vesicles filled with acetylcholine (ACh)

- When a vesicle binds to the presynaptic membrane ACh is released and diffuses across the synaptic cleft (animation)

  http://harveyproject.science.wayne.edu/development/nervous_system/cell_neuro/synapses/release.html
The Neuromuscular Junction

- **Synaptic Transmission**
  - ACh molecules migrate through the folds of the postsynaptic membrane and bind to their receptor proteins (AChRs)
  - Binding of ACh to AChRs leads to the opening of transmembrane ion channels and the production of small individual currents called **miniature endplate currents (mEPCs)**
  - As AChR channels close and ACh is broken down by acetylcholinesterase (AChE), the minis decay due to lack of binding events

http://www.mcell.psc.edu/recon.html
The Neuromuscular Junction

- Through wet lab experiments done on the NMJ we know:
  - There is a large variability in mEPC amplitude
  - There is a large variability in mEPC decay time
  - There is a first order mEPC decay phase

- We hypothesize that NMJ architecture plays an important role in the shaping of mEPC amplitude and decay phase

- Unfortunately, it has become difficult to investigate this concept experimentally due to the spatial, temporal and chemical specifics of interacting structures and diffusing molecules
A Solution: NMJ Simulations

- Integrate mechanics, kinetics and stochastic behaviors at the molecular level with the structural organization and function at the cellular level
- Achieve this through programs like MCell (a Monte Carlo cellular simulation engine)
- Visualize data output from MCell with programs like DReAMM (Design, Rendering, and Animation of MCell Models)
Mcell and DReAMM Review

- Spatially realistic physiological simulations of synaptic transmission at the subcellular scale
- Reads its own Model Description Language (MDL)
- Uses probability-based, random walk and reaction algorithms in combination with meshes (corresponding to cellular objects) to accurately simulate diffusion within a cell and intercellular space
- Can output data files to DReAMM, which uses Open DX to build spatially realistic, 3D images and animations
The Neuromuscular Junction: A Spatially Realistic Model

- From 60 serial Electron Micrographs of a rat neuromuscular junction, a 3D mesh was created:

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The Neuromuscular Junction: A Spatially Realistic Model

- **Important Characteristics of the Model:**
  - Uniform AChR distribution throughout the folds
  - Uniform kinetics at each AChR
The Neuromuscular Junction: A Spatially Realistic Model

- Kinetics of ACh binding are governed by the equation:

\[ A + R^0 \xrightarrow{K_{-1}} AR^1 + A \xrightarrow{K_{+1}} A + R^0 \]

\[ AR^2 + A \xrightarrow{K_{+2}} A_2R^3 \xrightarrow{\beta} A_2R^4 \]

\[ (A = ACh) \]
The Neuromuscular Junction: A Spatially Realistic Model

The 2 bind sites for ACh on AChRs

www.zoology.ubc.ca/.../skeletal_muscle.html
The Neuromuscular Junction: A Spatially Realistic Model

Procedure:
- Ran MCell simulations on the model replicating synaptic transmission and ACh diffusion in the NMJ
- Plotted data output in a graphing program to analyze mini amplitude and decay time variability, as well as the shape of the decay phase

Results:
- Variability in mEPC amplitude was produced
- Variability in mEPC decay time was not produced
- Decay phase was second order
Many different things could be contributing to these inconsistencies in the model.

Our goal was to fix these problems by using different methods, in hope of producing a realistic model in the end.
Tying it all together

- **Goals of the project:**
  - Correct the shape of the mEPC decay phase
  - Increase variability in decay time
  - Maintain achieved variability in amplitude

- **Focus:**
  - Increasing the physiological realism of the previous model’s postjunctional folds, by creating a non-uniform distribution of AChRs in keeping with experimental data
More Background Information

- **Understanding the postjunctional folds**
  - Mammalian folds extend anywhere from 0.8-1 μm downward and increase the surface area of the postjunctional membrane 3-7 fold.
  - Microscopic data revealed the top 25-30% of the folds looked thicker and "fuzzy," while the bottoms remained normal.
    - **EM Studies** – thickness = high protein density.
    - **Freeze Etch Studies** – high protein density = closely packed intramembrane particles, most likely AChRs.
  - Statistical analysis of grain distribution as a function of depth shows that there are clearly 3 distinct regions.

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More Background Information

- **Identifying AChRs – α-Bungarotoxin (BGT) Experiment**
  - BGT is a snake venom that binds irreversibly to the ACh bind sites on an AChR molecule
  - Fluorescently-labeled BGT was introduced and observed in the mouse NMJ
    - Results = about 15-20,000 sites of fluorescence per square micron at TOPS of folds
    - Since AChRs have 2 ACh bind sites = 7,500–10,000 AChRs
    - AChRs at bottoms of folds are 10-100 fold LESS DENSE than at the top

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[Image: fluorescent α-BGT at acetylcholine bind sites on AChRs in the postjunctional folds]

Salpeter, et. al

Hypothesis

- NMJ Architecture is theorized to play an important role in the shaping of mEPC amplitude and decay phases

- Problems with second-order characteristics of the decay phase are most likely attributed to late openings of AChR channels

- Uniform AChR distribution in the folds is not consistent with physiological data
Hypothesis

It is therefore hypothesized that the addition of a spatial AChR gradient to the model may correct the shape of the decay phase and may also partially correct the variability of decay times.
Methods

- Using Open DX on the previously made model
  - Designated a number of clipping sites on the surface of the postjunctional membrane
  - Assigned a radius to each point where the spheres will be centered
Methods

Creating the tops of the folds:

- Sphere radius was set to 0.3 µm to mimic the depth of the “thickness” seen 25-30% of the way down in the folds
Methods

- Creating the middle section of the folds:
  - The same clip points were used and sphere radius was reset to 0.6 \( \mu \text{m} \) to mimic statistical grain distribution data.
Methods

- The remainder of the model makes up the “bottom” of the folds
  - No receptors were populated here so it is ok to include the rest of the ultrastructure
  - By giving each mesh object a different color in DReAMM, they can be viewed simultaneously:
Methods

- Each piece (mesh element) was then converted to an MDL (Model Description Language) file to be read by MCell.

- The separate MDL files for each piece were modified to include statements that controlled the individual mesh AChR densities:

```plaintext
ADD_EFFECTOR {
    STATE = AChR.R
    DENSITY = 10000 (7250, 0)
    ELEMENT = ALL_ELEMENTS
    POLE_ORIENTATION = POSITIVE_FRONT }
```
Methods

First simulation set:

- Top = 10,000 AChRs µm²
- Middle = 7,250 AChRs µm²
- Bottom = 0 AChRs µm²

Trials were done with AChE both active and inhibited (simulating the use of an anticholesterase drug) to produce interesting mEPC amplitude and decay phase data.
Results

- **AChE Active**: Non-uniform Distribution (0.3 microns)

  **mEPC Amplitude**

  Mean amplitude matched that of original data and variability of mEPC amplitude was preserved
Results

- **AChE Inactive**: Control vs. Non-uniform Distribution
  
  mEPC Decay Phase

First order characteristics definitely improved...
Results

- To try to increase first order characteristics even further, the top clipping radius was reset to 0.25 μm holding the middle radius at 0.6 μm.

- mEPCS were then re-simulated and more results were generated.
Results

- **AChE Active**: Non-uniform Distribution (0.25 µm)

  mEPC Amplitude

Again, mEPC mean amplitude and variability were maintained
Results

- **AChE Inactive:** Non-uniform Distributions 0.3 µm vs. 0.25 µm

  mEPC Decay Phase

Little improvement in first order characteristics can be seen from the 0.3 µm to the 0.25 µm simulations...
Results

To attempt to completely correct the decay phase and to see the effect of decreased receptor density on mEPC amplitude, two new sets of simulations were run:

- Top = 10,000 AChRs µm²
- **Middle = 0 AChRs µm²**
- Bottom = 0 AChRs µm²

- Top = 7,250 AChRs µm²
- **Middle = 0 AChRs µm²**
- Bottom = 0 AChRs µm²

- Both simulations were run with AChE active and inhibited
Results

- **AChE Active**: No Middle Receptors and Top @ 10,000 mEPC Amplitude

AChRs at Full Density to 0.25 microns without Middle Receptors

Mean amplitude and variability were preserved again
Results

- **AChE Inactive:** Control vs. No Middle Density and Top @ 10,000 mEPC Decay Phase

First order characteristics were drastically improved
Results

- **AChE Active**: No Middle Receptors and Top @ 7,250

  mEPC Amplitude

Mean amplitude was slightly decreased here, however variability was preserved
Results

- **AChE Inactive**: Control vs. No Middle Density and Top @ 7,250 mEPC Decay Phase

First order characteristics were almost completely restored to the decay phase.
Conclusions

- Introducing a physiologically accurate AChR gradient to the model *did* lead to a more correct mEPC decay phase, leading us to believe that late openings of AChR channels *were* responsible for this problem in the original model.

- mEPC mean amplitude and variability were *unaffected* by the initial AChR gradient; it was not until drastic removal of AChR molecules that even slight variations in mean amplitude could be seen.
Conclusions

- The non-uniform distribution of AChRs did not induce the expected variability in decay time, meaning that there must be other biophysical factors contributing to this variability that were not tested here.
References

- www.TristanRichards.com
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