Background / Theory
Background / Theory

The Presynaptic Specialization
Background / Theory

Presynaptic Characteristics of Interest
Possible Explanations for Variability in a Synapse’s Quantal Amplitude

- Non-Uniform Volume
- Non-Uniform Filling
- Non-Uniform Flux
- Non-Uniform Alignment of Release
- Variable Number of Vesicles Fusing
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A: Non-Uniform Volume
B: Non-Uniform Filling
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D: Non-Uniform Alignment of Release
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Possible Explanations for Variability in a Synapse’s Quantal Amplitude

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Possible Explanations for Variability in a Synapse's Quantal Amplitude

A Non-Uniform Volume  B Non-Uniform Filling  C Non-Uniform Flux  D Non-Uniform Alignment of Release  E Variable Number of Vesicles Fusing
(At Least) Two Modes of Fusion

**Kiss-and-Run Fusion**
- **a**
  - Fully loaded vesicle at rest
  - First fusion leaves vesicle completely or partially intact
  - Partially loaded vesicle between fusion events
  - Reuse leaves vesicle completely or partially intact
  - Partially loaded vesicle between fusion events
  - Repeated reuse further unloads the vesicle
  - Multiple fusions release all dye from the vesicle

**Full Quantal Loss**
- **b**
  - Fully loaded vesicle at rest
  - Fusion leads to loss of the full fluorescence quantum
  - Single fusion releases all dye and the vesicle
  - 1 Action Potential
  - 1 Action Potential
Possible Explanations for Variability in a Synapse’s Quantal Amplitude

A: Non-Uniform Volume
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Clever Methods

Method 1:
Capacitance
(Direct Sensing of Transmitter Release)
Capacitance Technique

(A) -70 mV
-80 mV

$I_{\text{Pre}}$

200 pA
2 ms

(B)
Clever Methods

Method 2:

Amperometry

(Direct Sensing of Transmitter Release)
Westerink, *Neurotoxicity*, 2004
Measurement of Fusion Modes via Amperometry

Burgoyne and Barclay, *Trends in Neurosci.*, 2003
Clever Methods

Method 3:

FM Imaging

(Fluorescent Staining of Active Vesicles)
Clever Methods

Method 4:

Synaptophysinorins

(pH-Sensitive Visualization of Fusion Events)
Regulation

Molecular Regulation of Fusion Process
Regulation of the Fusion Pore

- The rate of fusion pore expansion is regulated by:
  - Intracellular Ca2+
Regulation of the Fusion Pore

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  - synaptotagmin I/IV
  - dynamin
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  – synaptotagmin I/IV
  – dynamin

• Shift of the mode of exocytosis to “kiss-and-run” by:
  – High extracellular Ca2+
  – Staurosporine (kinase inhibitor) (?)
  – Phorbol esters (e.g., PMA, PKC activator)
  – Munc-13
Fusion pore can be regulated

(A) Spikes from control and phorbol ester (PMA)-treated cells;

(B) spikes from control non-transfected and from Munc18(R39)-expressing cells;

Fisher et al., *Science*, 2001
Regulation

Molecular Regulation of Fusion Process
Regulation

Implications for Synaptic Transmission
Quantal size is regulated by the fusion pore in small vesicles of dopaminergic neurons

Receptors Differ in Sensitivity to the Timecourse of Release of Neurotransmitter Quanta

Concentration timecourse (normalized)

16 ms

NMDAR

100 ms

100 pA

GABA\textsubscript{A}R

200 pA

AMPAR

10 ms

50 pA
Regulation

Implications for Synaptic Transmission
Regulation

Implications for Development of Neural Networks
During Development of Neural Networks, Transmission Changes from “Silent” Type to Functional

Immature: Silent Transmission

Synaptic Release Causes NMDA Receptor-Mediated Current

Mature: Functional

Synaptic Release Causes (NMDA + AMPA) Receptor-Mediated Current
During Development of Excitatory Signaling in Hippocampal Circuits, Transmission Changes from a Slow, “Silent” Type, to a Fast, Functional Type

**Immature:**
Silent Transmission

- Synaptic Release Causes NMDA Receptor-Mediated Current

**Mature:**
“Functional Transmission”

- Synaptic Release Causes (NMDA + AMPA) Receptor-Mediated Current

What’s Different?
Summary of presynaptic neurotransmitter release maturation

"AMPA-quiet" restricted release

Functional unrestricted release

$I_{\text{synaptic}}$

FM1-43
Early glutamatergic synapses exhibit "silent" transmission consisting mainly of NMDA currents.
Later in development, NMDA currents are joined by AMPA currents.
[Glu]

Functional flux

“AMPA-quiet” flux

\[ I_{\text{NMDA}} \]

\[ I_{\text{AMPA}} \]

\[ I_{\text{TOTAL}} \]

restricted release

unrestricted release

restricted release
Regulation

Implications for Development of Neural Networks
What is the Fusion Pore?
What is the Fusion Pore?
Possible Explanations for Variability in a Synapse’s Quantal Amplitude

Non-Uniform Volume

Non-Uniform Filling

Non-Uniform Flux

Non-Uniform Alignment of Release

Variable Number of Vesicles Fusing
Regulation of Signal Strength by Presynaptic Mechanisms

9.013 / 7.68: Core Class

Sheng Lectures
Presynaptic Mechanisms

Nathan Wilson