The Molecular Machine for Neurotransmitter Release

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Neural Circuits Underlie Brain Function

pyramidal neurons

interneuron

interneuron
Neural Circuits Underlie Brain Function

Synapses: the basic computational units of the brain

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Neural Circuits Underlie Brain Function

Although synapses differ in properties, all synapses operate by the same principle.

Bernard Katz - Nobel Prize, 1970
All Synapses Operate by the Same Principle

An action potential invades the presynaptic nerve terminal
All Synapses Operate by the Same Principle

An action potential invades the presynaptic nerve terminal

Presynaptic Ca$^{2+}$-influx triggers neurotransmitter release
An action potential invades the presynaptic nerve terminal

- Presynaptic Ca\textsuperscript{2+}-influx triggers neurotransmitter release

- Neurotransmitters bind to postsynaptic receptors & elicit an electrical signal
All Synapses Operate by the Same Principle

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Approach:
Synaptic function is measured electrophysiologically via excitatory or inhibitory postsynaptic currents (EPSCs or IPSCs)
An action potential invades the presynaptic nerve terminal.

- Presynaptic Ca^{2+}-influx triggers neurotransmitter release.
- Neurotransmitters bind to postsynaptic receptors & elicit an electrical signal.

Synaptic transmission is rapid = \textbf{1-5 ms}.
- Key step is neurotransmitter release.
All Synapses Operate by the Same Principle

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- key step is neurotransmitter release

Three basic processes enable rapid release
Three Processes Govern Neurotransmitter Release

1. Synaptic vesicle fusion
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2. Ca\(^{2+}\)-triggering of fusion
   • Very fast: ≈0.1 msec
   • Cooperative: ≈5 Ca\(^{2+}\)-ions
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When I started my lab in 1986, neurotransmitter release fascinated me because of its importance, inexplicable speed, and precision – but not a single synapse component had been molecularly characterized.

Now – 25 years later – a molecular framework that plausibly explains release in molecular terms has emerged …
A Neurotransmitter Release Machine Mediates Fusion, Ca\(^{2+}\)-triggering & Ca\(^{2+}\)-Channel Tethering
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Let's start at the beginning
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1993 Model of Synaptic Membrane Fusion Machinery

Hata et al., Nature 1993
Based on three convergent observations:

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Munc18 is not by-stander but central actor in membrane fusion

Munc18 is **absolutely essential** for vesicle fusion whereas individual SNAREs are not
Munc18 KO: Normal synapse formation, normal postsynaptic receptors, no presynaptic release

Spontaneous synaptic activity in the cortex

control

Munc18 KO

Verhage et al., Science 2000
Spontaneous synaptic activity in the cortex

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Munc18 KO: Normal synapse formation, normal postsynaptic receptors, no presynaptic release

A model for Munc18 function based on lots of subsequent work …

Verhage et al., Science 2000
SNARE/SM Protein Complex Assembly Drives Fusion

1. Partial trans-SNARE/SM-Complex Assembly (Chaperones: CSPs + synucleins)
2. Full Assembly of trans-SNARE/SM-Complexes & Fusion-Pore Opening
3. Fusion-Pore Expansion & Conversion of trans- to cis-SNARE/SM Complexes
4. SNARE/SM-Complex Disassembly & Vesicle Recycling (Chaperone: NSF)
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Synaptic cleft

Presynaptic terminal

SNAP-25

Munc18

Synaptobrevin

Syntaxin

SV

complete cis-SNARE/SM complexes

complete trans-SNARE/SM complexes

SNARE/SM protein Cycle
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SNARE/SM protein Cycle

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Synaptobrevin
Syntaxin
SNAP-25
Munc18
Synaptic cleft

Complete cis-SNARE/SM complexes
Partial trans-SNARE/SM complexes
Complete trans-SNARE/SM complexes

fusion pore
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Disease implications
Heterozygous de novo Munc18 mutations cause Ohtahara syndrome (epileptic encephalopathy)
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Reinforces the importance of Munc18
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SNARE/SM protein Cycle

SNARE terminal

Synaptobrevin

Syntaxin

SNAP-25

Munc18

Synaptic cleft

complete cis-SNARE/SM complexes

complete trans-SNARE/SM complexes
- α-Synuclein aggregates accumulate in Parkinson’s Disease
- Loss of α-synuclein or CSPα promotes neurodegeneration
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- Loss of α-synuclein or CSPα promotes neurodegeneration

Let me illustrate …
Deletion of Synucleins Causes Age-Dependent Impairment of SNARE-Complex Assembly

Measured by SNARE co-immuno-precipitation

Burre et al., Science 2010
α-Synuclein overexpression in synuclein KO neurons

α-Synuclein Catalyzes SNARE-Complex Assembly

Measured as SDS-resistant SNARE complexes

α-Synuclein levels
(normalized)

Burre et al., Science 2010
α-Synuclein overexpression in synuclein KO neurons

α-Synuclein overexpression in synuclein KO neurons

α-Synuclein catalyzes SNARE-complex assembly

Measure: SDS-resistant SNARE complexes

α-Synuclein protects against some forms of neurodegeneration

SNARE-complex dysfunction may contribute to Parkinson’s disease

Burre et al., Science 2010
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At the same time as we were studying synaptic fusion, we systematically characterized synaptic vesicle proteins. This approach led (among others) to the discovery of synaptotagmin, the Ca\(^{2+}\)-sensor for neurotransmitter release.
Systematic Analysis of Synaptic Vesicle Proteins Identifies Synaptotagmin-1

Südhof and Jahn, Neuron 1991
Systematic Analysis of Synaptic Vesicle Proteins Identifies Synaptotagmin-1

Ca\textsuperscript{2+}-binding to Syt1 C2-domains induces lipid- and SNARE-binding

Südhof and Jahn, Neuron 1991
Systematic Analysis of Synaptic Vesicle Proteins Identifies Synaptotagmin-1

How does synaptotagmin-1 bind Ca\(^{2+}\), and what is its physiological significance?
Synaptotagmin-1 is a Synaptic Vesicle Ca\(^{2+}\)-Binding Protein

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Ca²⁺-binding to Syt1 C²-domains induces lipid- and SNARE-binding

Ca²⁺-binding to Syt1 C2-domains induces lipid- and SNARE-binding.

Does knockout of Syt1 impair Ca²⁺-triggered release?
Synaptotagmin-1 is Essential for Ca$^{2+}$-Triggered Neurotransmitter Release

Fast Ca$^{2+}$-triggered release is gone ...

Geppert et al., Cell 1994
Synaptotagmin-1 is Not Essential for Sucrose-Stimulated Neurotransmitter Release

Hypertonic sucrose stimulates release by a Ca\textsuperscript{2+}-independent mechanism

Synaptotagmin is ONLY required for Ca\textsuperscript{2+}-triggered fusion

Geppert et al., Cell 1994
Synaptotagmin-1 is a synaptic vesicle Ca$^{2+}$-Sensor

Essential for Ca$^{2+}$-Triggered Vesicle Fusion

• Synaptotagmin-1 is a synaptic vesicle Ca$^{2+}$-binding protein
• Synaptotagmin-1 is essential for fast Ca$^{2+}$-triggered release

However, synaptotagmin does not act alone - it needs an accomplice = complexin
A Neurotransmitter Release Machine Mediates Fusion, Ca\textsuperscript{2+}-triggering & Ca\textsuperscript{2+}-Channel Tethering

McMahon et al., Cell 1995; Chen et al., Neuron 2002
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Complexin is an essential activator of synaptotagmin-1 that is evolutionarily conserved – an example
Nomastella Complexin Functions in Mouse Neurons

*Nematostella vectensis* (cnideria)

Encodes synapto- tagmins & complexins

Yang et al., unpublished
Nomastella Complexin Functions in Mouse Neurons

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complexin

*Mus musculus*

Yang et al., unpublished
Nomastella Complexin Functions in Mouse Neurons

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**EPSCs**

Yang et al., unpublished
Synaptotagmin-1 is a Synaptic Vesicle Ca$^{2+}$-Sensor Essential for Ca$^{2+}$-Triggered Vesicle Fusion

- Synaptotagmin-1 is a synaptic vesicle Ca$^{2+}$-binding protein
- Synaptotagmin-1 is essential for fast Ca$^{2+}$-triggered release
- Synaptotagmin-1 uses complexin as essential co-activator

This is where we stood in 1995
We had – together with others – identified the major components of the synaptic vesicle membrane fusion machinery and described a candidate Ca^{2+}-sensor for fusion.
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However: Many doubted that SNARE & SM proteins ‘do’ membrane fusion, others suggested that synaptotagmin is a scaffold but NOT a Ca\textsuperscript{2+}-sensor for fusion, and we had no idea how Ca\textsuperscript{2+}-influx is localized to the site of vesicle fusion.
Remainder of the talk:
How we addressed the issues of Ca\(^{2+}\)-triggering of fusion and of Ca\(^{2+}\)-influx

Major question: Does Ca\(^{2+}\)-binding to synaptotagmin-1 really trigger fast release?
Ca\textsuperscript{2+}-binding to Syt1 C\textsubscript{2}A-domains induces lipid- and SNARE-binding

Architectures of Synaptotagmin-1 Ca\(^{2+}\)-Binding Sites

**Ca\(^{2+}\)-binding sites of Synaptotagmin-1 \(C_2\)A-domain**

Design mutations that shift the Ca\(^{2+}\)-affinity of synaptotagmin-1 during SNARE- or phospholipid binding.

**3 Ca\(^{2+}\)**

**C\(_2\)A- Domain**

**2 Ca\(^{2+}\)**

**C\(_2\)B- Domain**

Ca\(^{2+}\)-binding to Syt1 \(C_2\) domains induces lipid- and SNARE-binding.
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Design mutations that shift the Ca\(^{2+}\)-affinity of synaptotagmin-1 during SNARE- or phospholipid binding
Adjacent C2A-Domain Mutations (D232N & R233N) Differentially Alter Synaptotagmin-1 Ca\(^{2+}\)-Affinity

**D232N mutant Syt1**

- Ca\(^{2+}\)-dependent binding of native brain Syt1 to liposomes
- Ca\(^{2+}\)-dependent co-IP of native brain Syt1 with SNARE complexes

**R233Q mutant Syt1**

- Ca\(^{2+}\)-dependent binding of native brain Syt1 to liposomes

Effect on Ca\(^{2+}\)-triggered neurotransmitter release?
Synaptotagmin-1 is a Ca\textsuperscript{2+}-Sensor for Synaptic Vesicle Fusion

D232N *increases* Ca\textsuperscript{2+}-dependent SNARE binding

R233Q *decreases* Ca\textsuperscript{2+}-affinity during phospholipid binding

Formally proved that Ca\textsuperscript{2+}-binding to synaptotagmin-1 triggers neurotransmitter release
Synaptotagmin-1 is a Ca$^{2+}$-Sensor for Synaptic Vesicle Fusion

- Synaptotagmin-1 is a synaptic vesicle Ca$^{2+}$-binding protein
- Synaptotagmin-1 is essential for fast Ca$^{2+}$-triggered release
- Synaptotagmin-1 uses complexin as essential co-activator
- Ca$^{2+}$-binding to Synaptotagmin-1 triggers fast release

However, mammals express 16 synaptotagmins!
Two Classes of Synaptotagmins Bind Ca\textsuperscript{2+}

Syt1, Syt2, Syt7, and Syt9

Syt3, Syt5, Syt6, and Syt10

Eight other synaptotagmins do not bind Ca\textsuperscript{2+}

Which synaptotagmins are Ca\textsuperscript{2+}-sensors for fast release?
Syt1, Syt2, and Syt9 Rescue Syt1 KO Phenotype

Rescue of Syt1 KO neurons

Syt1, Syt2, Syt5, Syt6, Syt7, Syt9, Syt10

Xu et al., Neuron 2007
Syt1, Syt2, and Syt9 Rescue Syt1 KO Phenotype

Syt1, Syt2, and Syt9 selectively rescue fast release in Syt1 KO neurons, but with distinct properties – whereas Syt7 does NOT rescue.
Two Classes of Synaptotagmins Bind Ca$^{2+}$

Two new issues:
1. Why does the Syt1 KO have a phenotype if Syt2 and Syt9 can compensate?
2. Why doesn’t Syt7 function in release if it is so similar to other ‘blue’ synaptotagmins?
Quantitation of Synaptotagmin mRNA Levels in Single Hippocampal Neurons: Syt2 and Syt9 are Absent

Bacaj et al., Neuron 2013
Quantitation of Synaptotagmin mRNA Levels in Single Hippocampal Neurons: Syt2 and Syt9 are Absent

Syt2 & Syt9 are not expressed but Syt7 is highly expressed

What does Syt7 do? Recall the initial KO results …
Synaptotagmin-1 is Essential for Ca$^{2+}$-Triggered Neurotransmitter Release

Some residual Ca$^{2+}$-triggered release remains in synaptotagmin-1 KO neurons
Synaptotagmin-7 Deletion Impairs Remaining Ca\(^{2+}\)-Triggered Release in Syt1 KO Neurons
Two Classes of Synaptotagmins Bind Ca$^{2+}$

All blue synaptotagmins function in synaptic vesicle fusion but exhibit different Ca$^{2+}$-triggering kinetics – function also in neuroendocrine/hormone secretion, mast cell degranulation etc.

What about red synaptotagmins? Focus on Syt10 …
Synaptotagmin-10 Co-Localizes with IGF-1 in Olfactory Bulb Neurons

Cao et al., Cell 2011
Synaptotagmin-10 Knockout Impairs Depolarization-Induced IGF-1 Secretion

Cao et al., Cell 2011

Loss of IGF-1 secretion decreases neuron size and synapse numbers – rescue with IGF-1
Syt10 KO Decreases Total Synaptic Responses & Capacitance of Neurons - Rescue with IGF-1

Syt10 is a Ca\(^{2+}\)-sensor for IGF-1 exocytosis – does Syt10 use complexin as a co-factor?

Cao et al., Cell 2011
Complexin Depletion Impairs Synaptotagmin-10 Dependent IGF-1 Secretion

IGF-1 secretion measured at different extracellular Ca$^{2+}$ concentrations

Implications for synaptotagmin function

Multiple Pathways of Ca$^{2+}$-Triggered Exocytosis Controlled by Different Synaptotagmins

Diverse non-redundant synaptotagmins use the same complexin-dependent mechanism for different Ca$^{2+}$-dependent membrane fusion reactions
Synaptotagmins Are Universal Ca\textsuperscript{2+}-Sensors for Ca\textsuperscript{2+}-Triggered Vesicle Fusion

- Synaptotagmin-1 is a synaptic vesicle Ca\textsuperscript{2+}-binding protein
- Synaptotagmin-1 is essential for fast Ca\textsuperscript{2+}-triggered release
- Synaptotagmin-1 uses complexin as essential co-activator
- Ca\textsuperscript{2+}-binding to Synaptotagmin-1 triggers fast release
- Other synaptotagmins perform analogous functions in Ca\textsuperscript{2+}-triggered release with complexin as co-factor
Three Processes Govern Neurotransmitter Release

1. Synaptic vesicle fusion
2. Ca\(^{2+}\)-triggering of fusion
   - Very fast: \(~0.1\) msec
   - Cooperative: \(~5\) Ca\(^{2+}\)-ions
3. Localized Ca\(^{2+}\)-influx

These studies thus established synaptotagmins as Ca\(^{2+}\)-sensors for membrane fusion and generalized their functions in most if not all Ca\(^{2+}\)-dependent fusion reactions

What about Ca\(^{2+}\)-influx?
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Without localized Ca\(^{2+}\)-influx at the active zone, action potentials and release become uncoupled, and release is desynchronized and decelerated.

The importance of localized Ca\(^{2+}\)-influx cannot be overestimated – like in real estate, location is everything!
A Neurotransmitter Release Machine Mediates Fusion, Ca\textsuperscript{2+}-triggering & Ca\textsuperscript{2+}-Channel Tethering
RIM is the central component of the active zone

A Neurotransmitter Release Machine Mediates Fusion, Ca\textsuperscript{2+}-triggering & Ca\textsuperscript{2+}-Channel Tethering
Deletion of RIM Severely Impairs Release

Is release impaired because of a defect in Ca\(^{2+}\)-influx?

Kaeser et al., Cell 2011; Deng et al., Neuron 2011; Han et al., Neuron 2011
RIM Deletion Decelerates & Desynchronizes Release: Renders Release Sensitive to Slow Ca^{2+}-Buffers

Kaeser et al., Cell 2011; Deng et al., Neuron 2011; Han et al., Neuron 2011
RIM Deletion Decelerates & Desynchronizes Release: Renders Release Sensitive to Slow Ca\textsuperscript{2+}-Buffers

- Consistent with impaired Ca\textsuperscript{2+}-influx → measure the role of RIM in Ca\textsuperscript{2+}-influx directly

- Effect of slow Ca\textsuperscript{2+}-buffers
Measurement of Ca\textsuperscript{2+}-Transients in Hippocampal Neurons

Neuron filled with:
Tracer = Alexa594 +
Ca\textsuperscript{2+}-indicator = Fluo5F

Kaeser et al., Cell 2011
Measurement of Ca$^{2+}$-Transients in Hippocampal Neurons

Neuron filled with:
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Ca$^{2+}$-indicator = Fluo5F

Axon with presynaptic terminals

Kaeser et al., Cell 2011
RIM Deletion Impairs Presynaptic Ca\(^{2+}\)-Influx

Kaeser et al., Cell 2011
RIM Deletion Impairs Presynaptic Ca\textsuperscript{2+}-Influx

Kaeser et al., Cell 2011
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RIM also mediates synaptic vesicle docking, enable synaptic plasticity, and activates Munc13 for vesicle priming.
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   - Cooperative: ~5 Ca^{2+}-ions

3. Localized Ca^{2+}-influx
A Neurotransmitter Release Machine Mediates Fusion, Ca\(^{2+}\)-triggering & Ca\(^{2+}\)-Channel Tethering Functionally, the fusion, Ca\(^{2+}\)-triggering, and active zone complexes form a single interacting nanomachine mediating fast transmitter release.
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