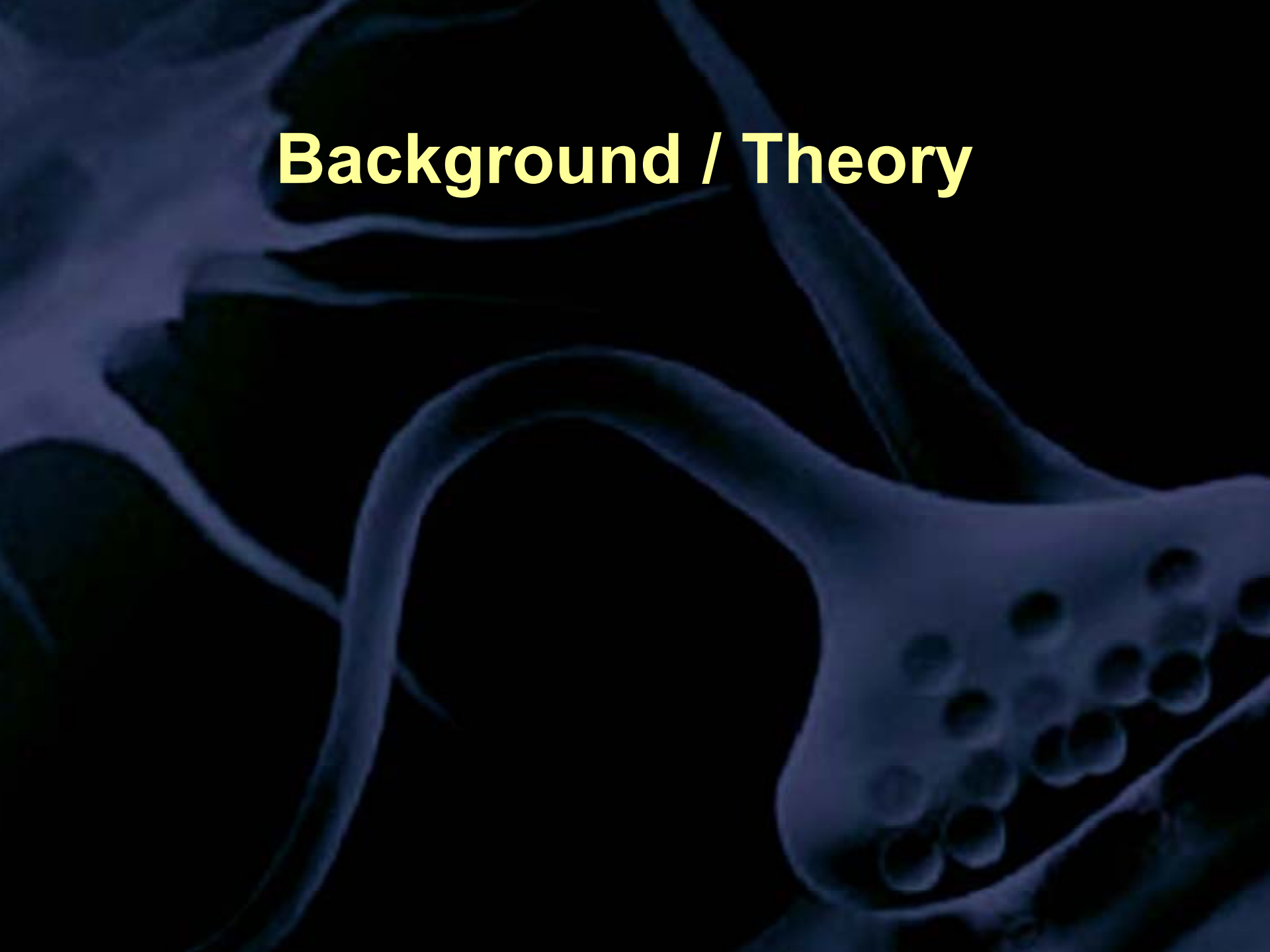
Regulation of Signal Strength by Presynaptic Mechanisms

9.013 / 7.68: Core Class

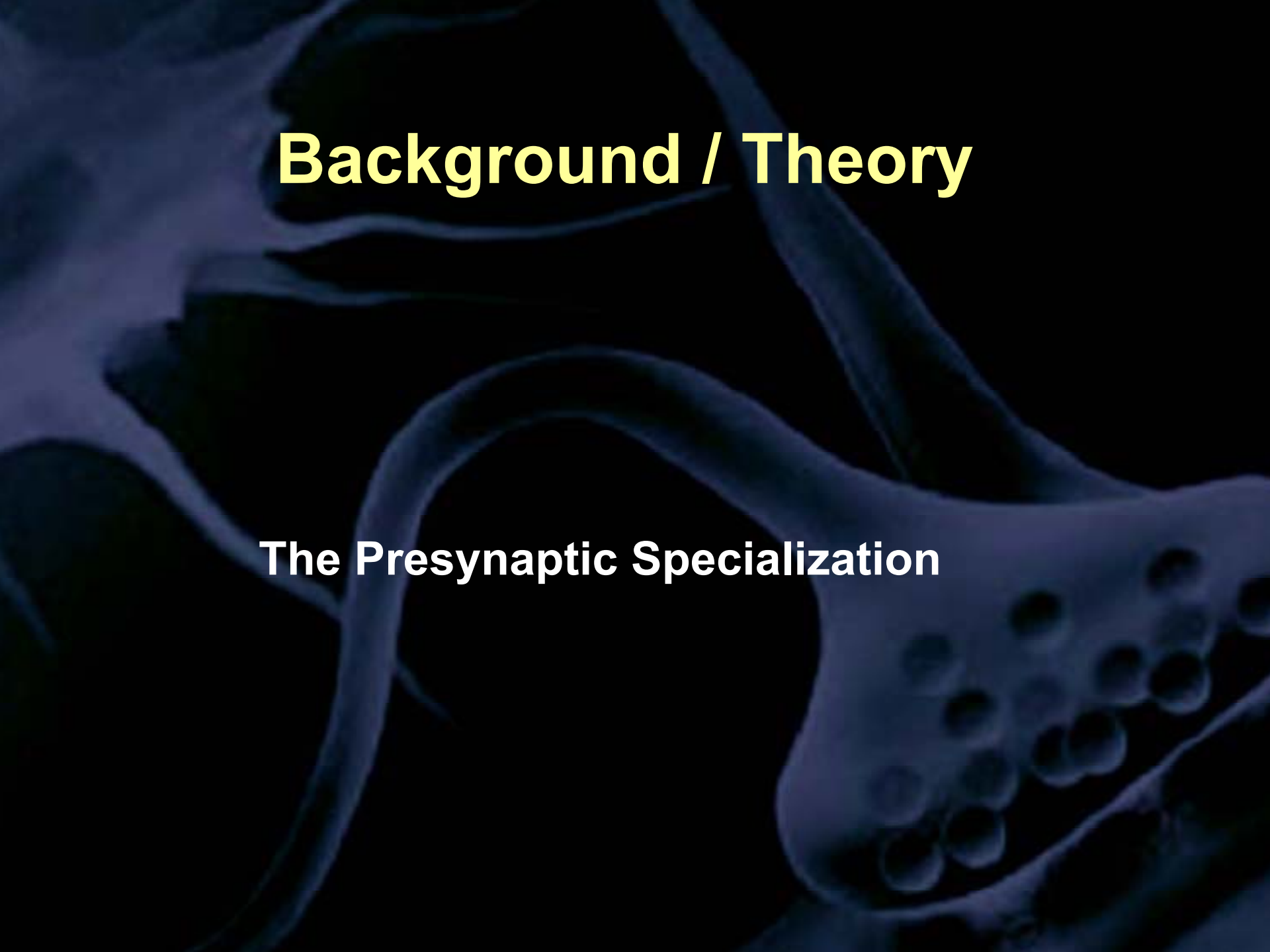
Sheng Lectures
Presynaptic Mechanisms

Nathan Wilson

Background / Theory



Background / Theory

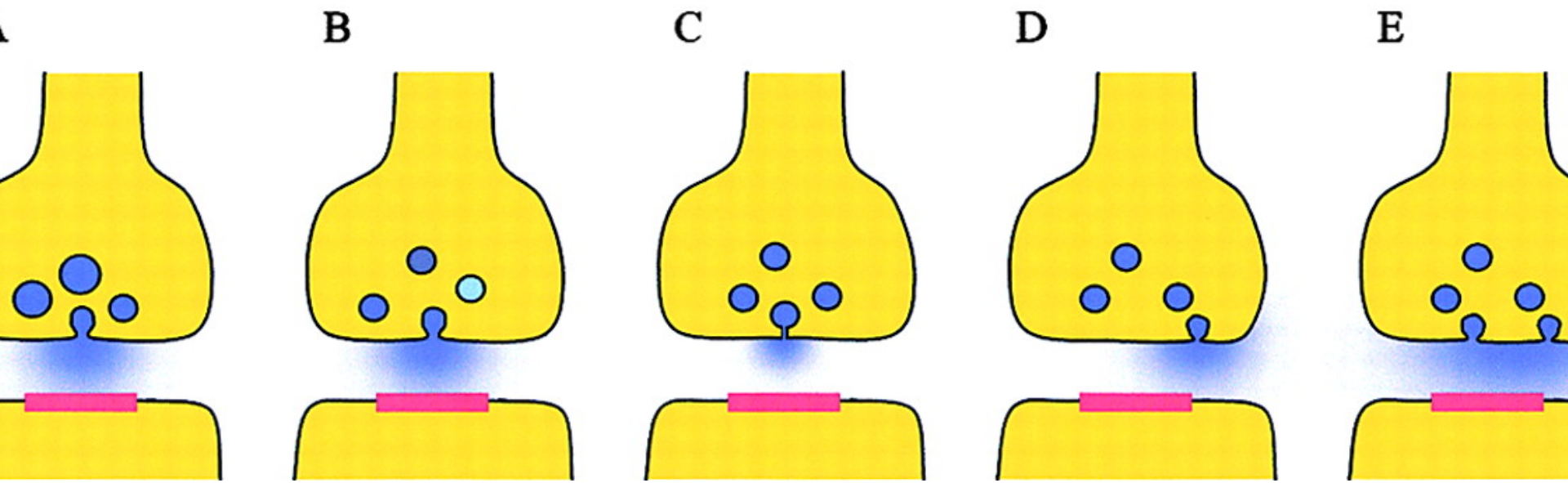


The Presynaptic Specialization

Background / Theory

Presynaptic Characteristics of Interest

Possible Explanations for Variability in a Synapse's Quantal Amplitude



Non-Uniform Volume

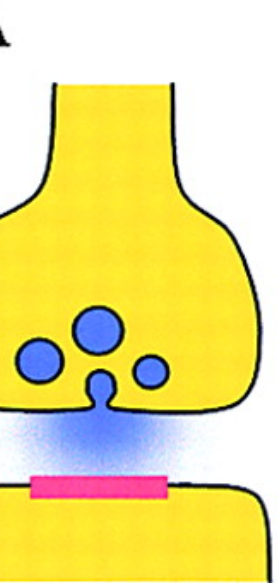
Non-Uniform Filling

Non-Uniform Flux

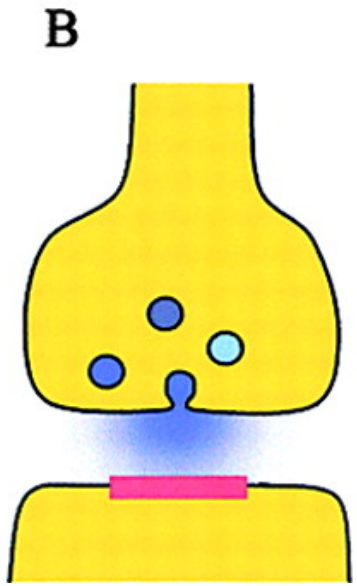
Non-Uniform Alignment of Release

Variable Number of Vesicles Fusing

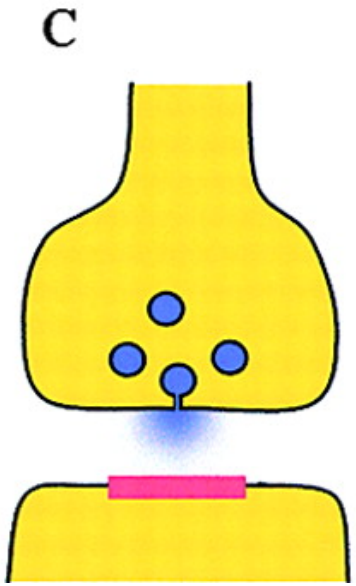
Possible Explanations for Variability in a Synapse's Quantal Amplitude



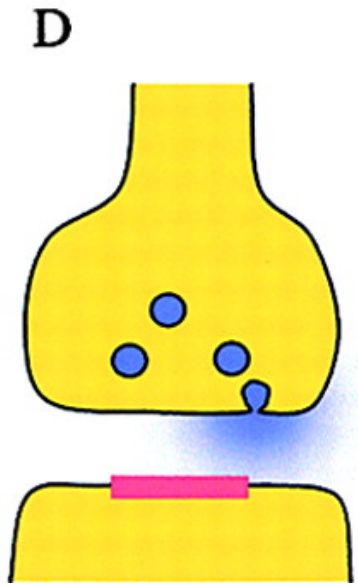
Non-Uniform Volume



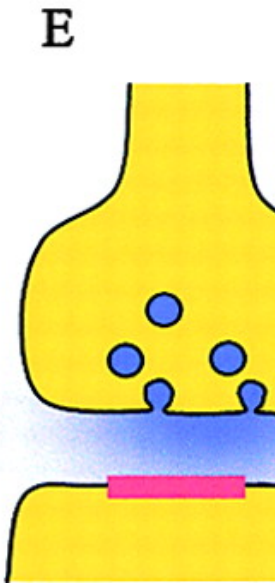
Non-Uniform Filling



Non-Uniform Flux

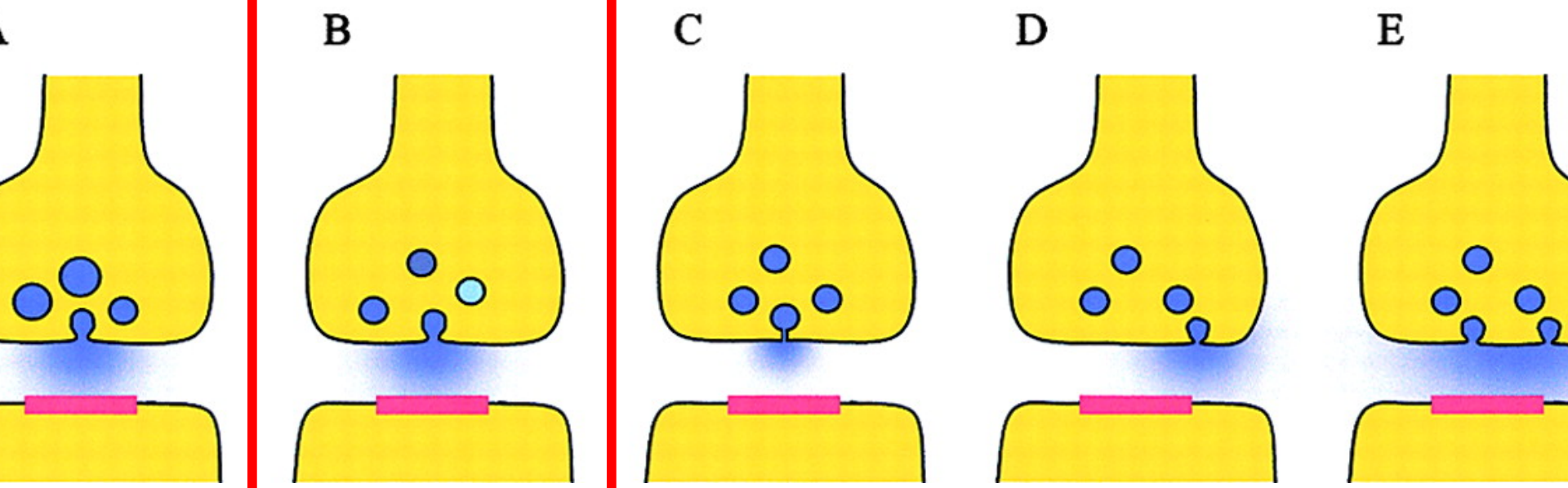


Non-Uniform Alignment of Release



Variable Number of Vesicle Fusing

Possible Explanations for Variability in a Synapse's Quantal Amplitude



Non-Uniform Volume

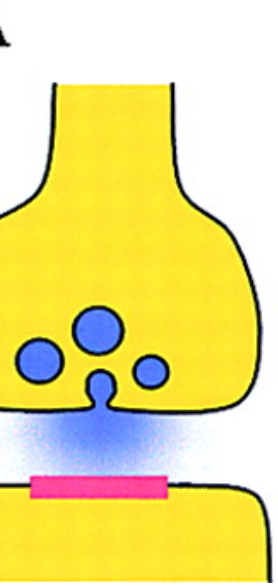
Non-Uniform Filling

Non-Uniform Flux

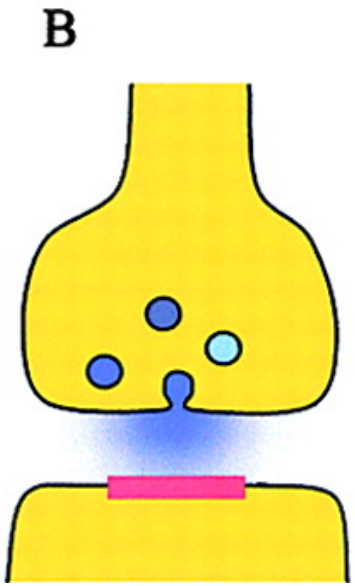
Non-Uniform Alignment of Release

Variable Number of Vesicle

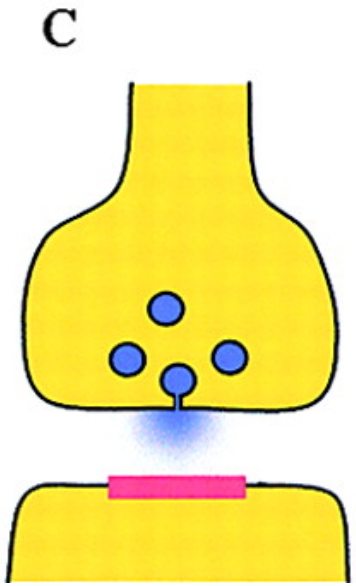
Possible Explanations for Variability in a Synapse's Quantal Amplitude



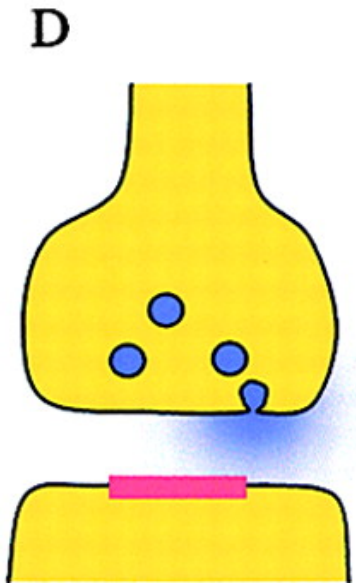
Non-Uniform Volume



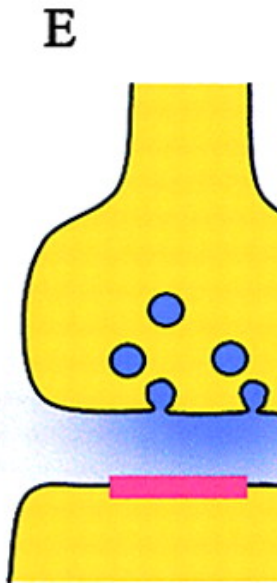
Non-Uniform Filling



Non-Uniform Flux

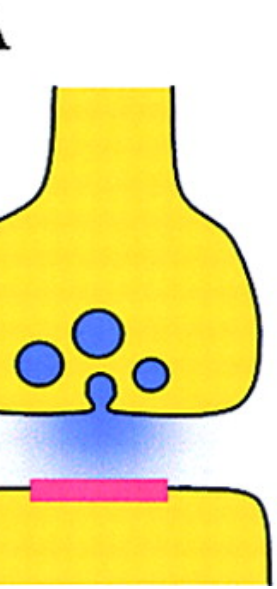


Non-Uniform Alignment of Release

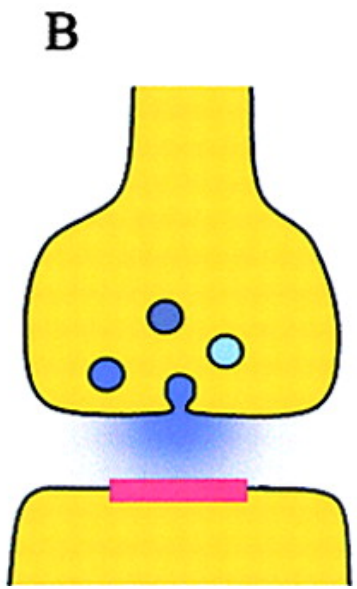


Variable Number of Vesicle

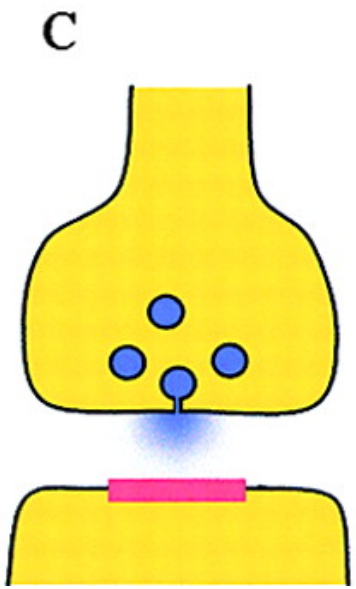
Possible Explanations for Variability in a Synapse's Quantal Amplitude



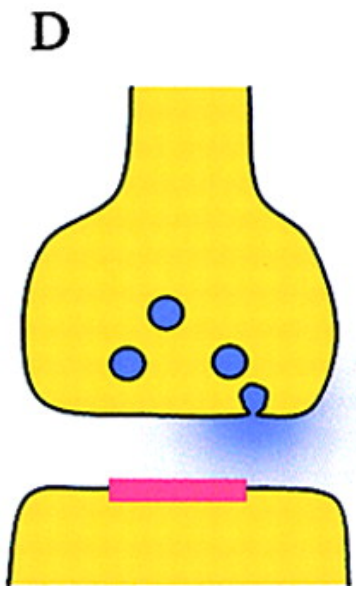
Non-Uniform Volume



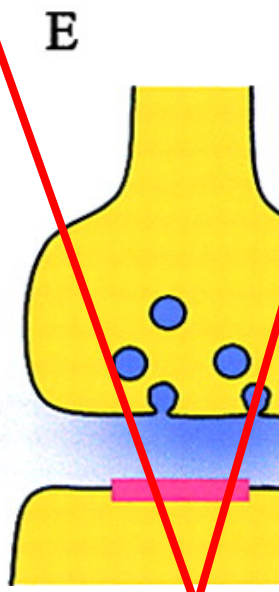
Non-Uniform Filling



Non-Uniform Flux

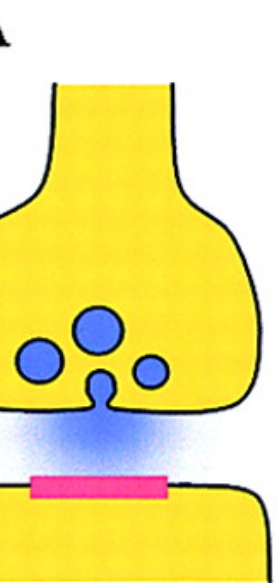


Non-Uniform Alignment of Release

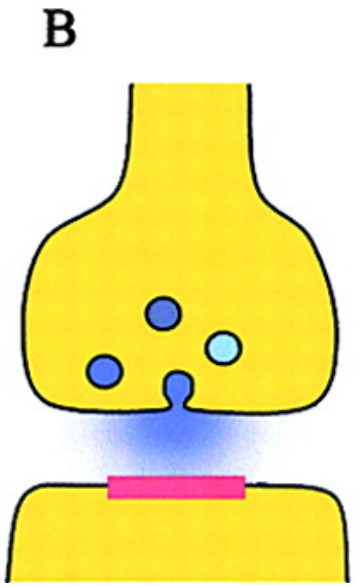


Variable Number of Vesicle Fusing

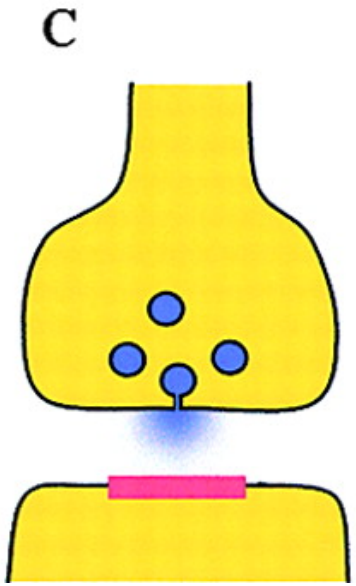
Possible Explanations for Variability in a Synapse's Quantal Amplitude



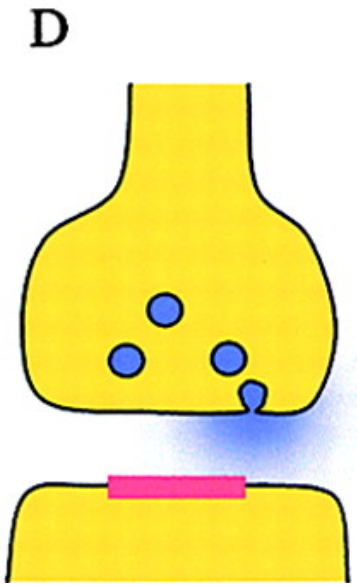
Non-
Uniform
Volume



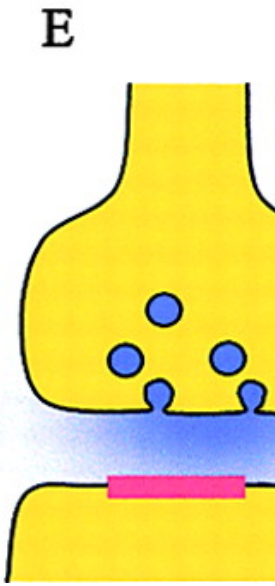
Non-
Uniform
Filling



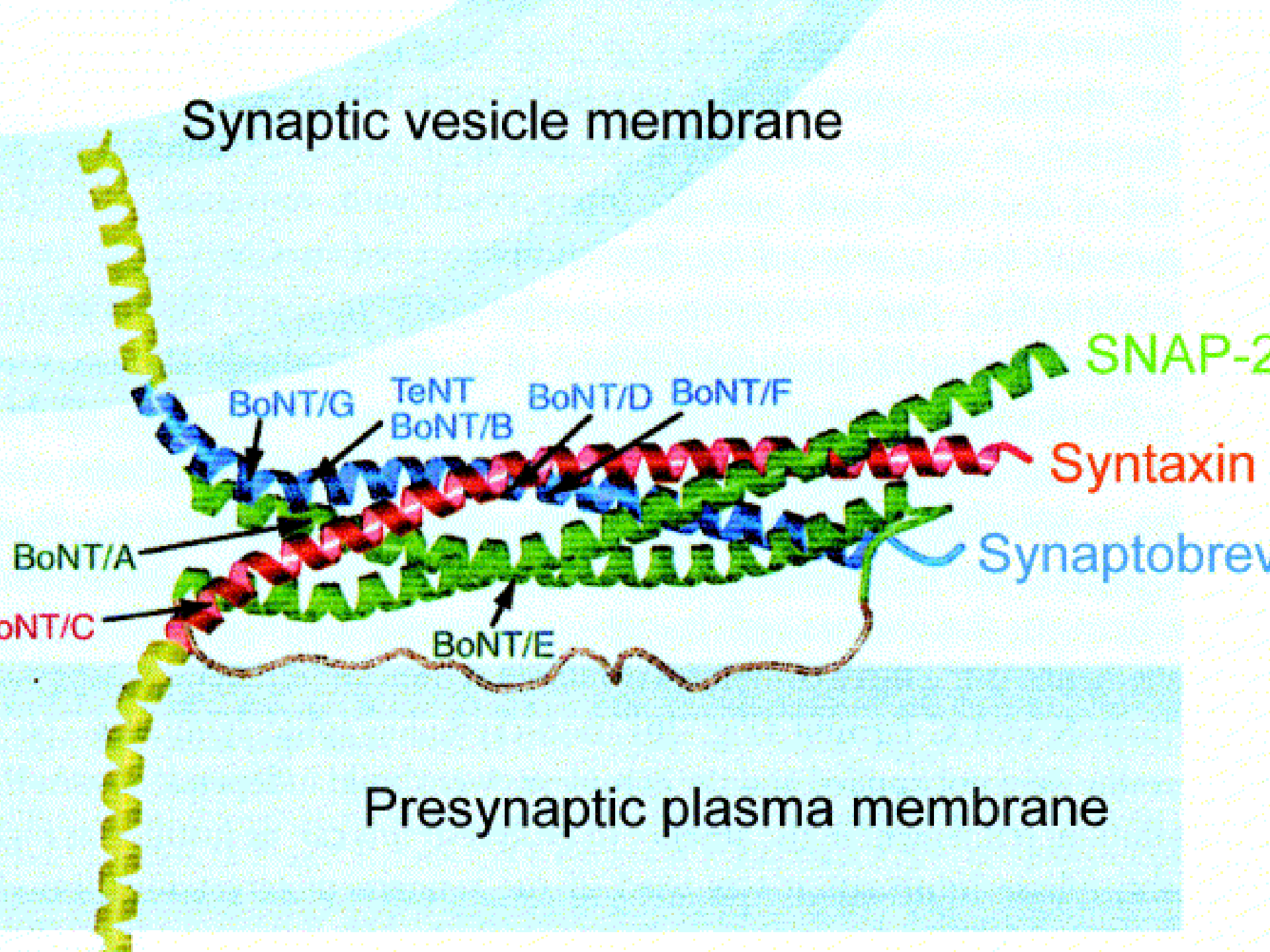
Non-
Uniform
Flux



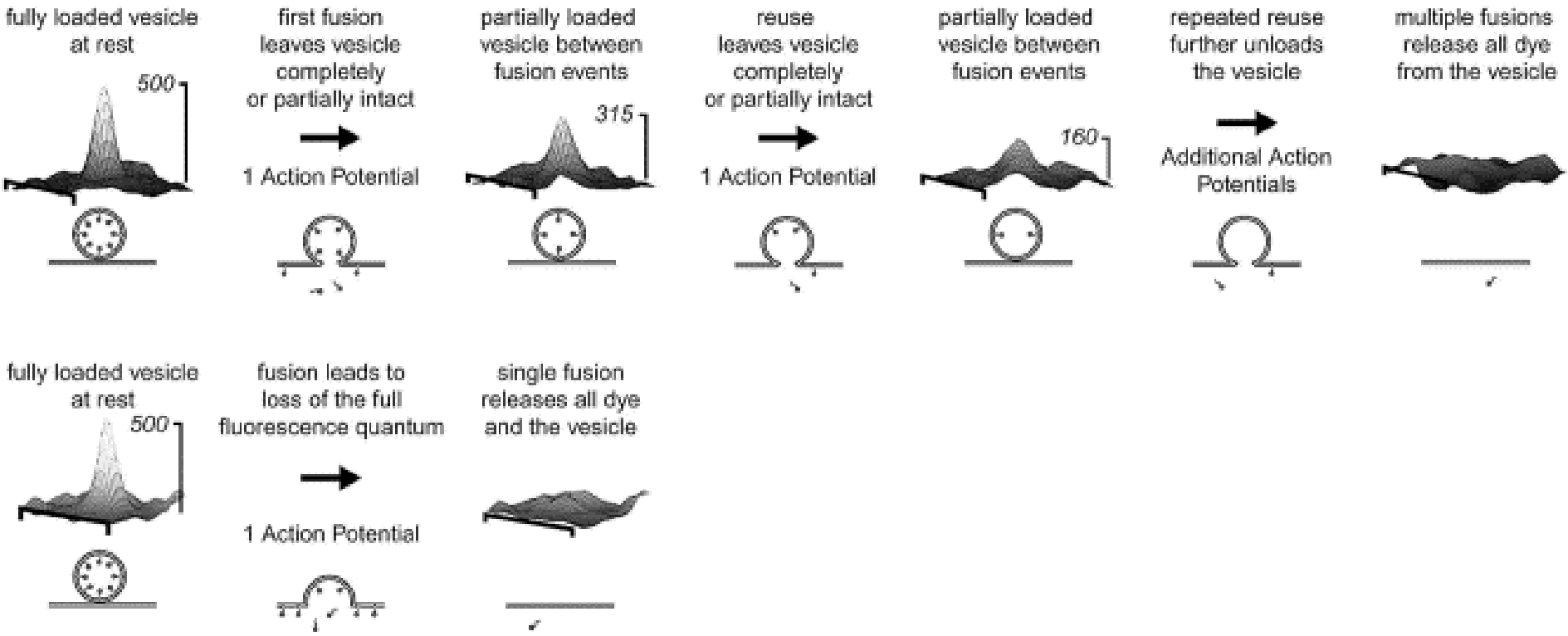
Non-
Uniform
Alignment
of
Release



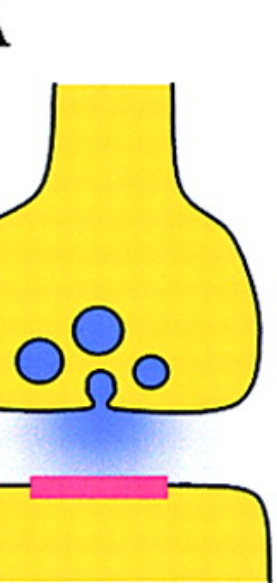
Variable
Numbe
of
Vesicle
Fusing


Synaptic vesicle membrane
SNAP-2
BoNT/G
TeNT
BoNT/B
BoNT/D
BoNT/F
Syntaxin
BoNT/A
Synaptobrev
oNT/C
BoNT/E
Presynaptic plasma membrane

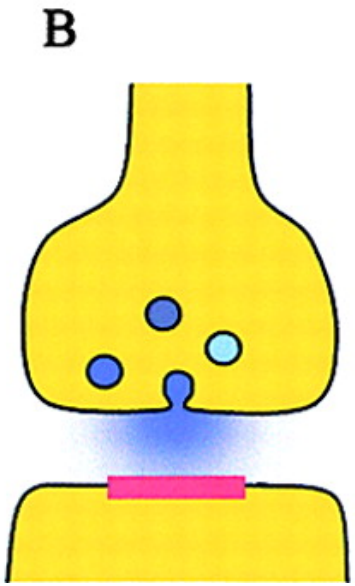
(At Least) Two Modes of Fusion



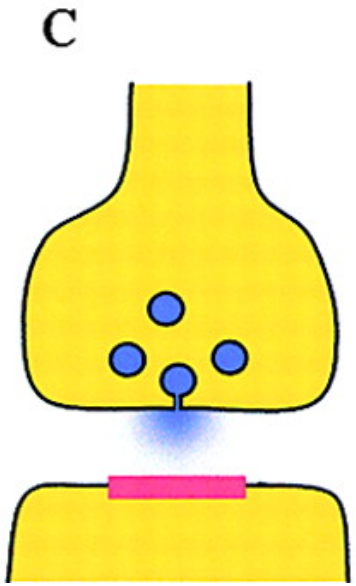
Possible Explanations for Variability in a Synapse's Quantal Amplitude



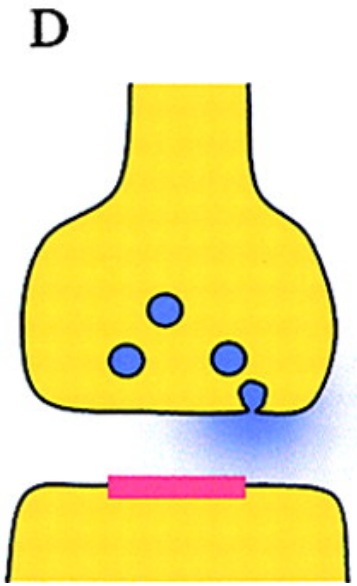
Non-
Uniform
Volume



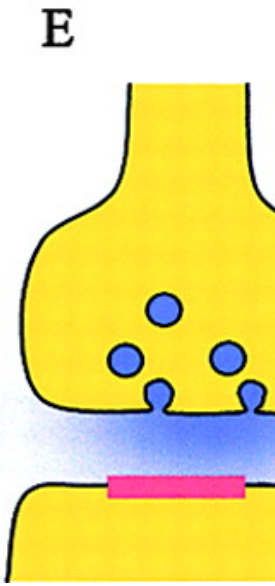
Non-
Uniform
Filling



Non-
Uniform
Flux



Non-
Uniform
Alignment
of
Release



Variable
Numbe
of
Vesicle
Fusing

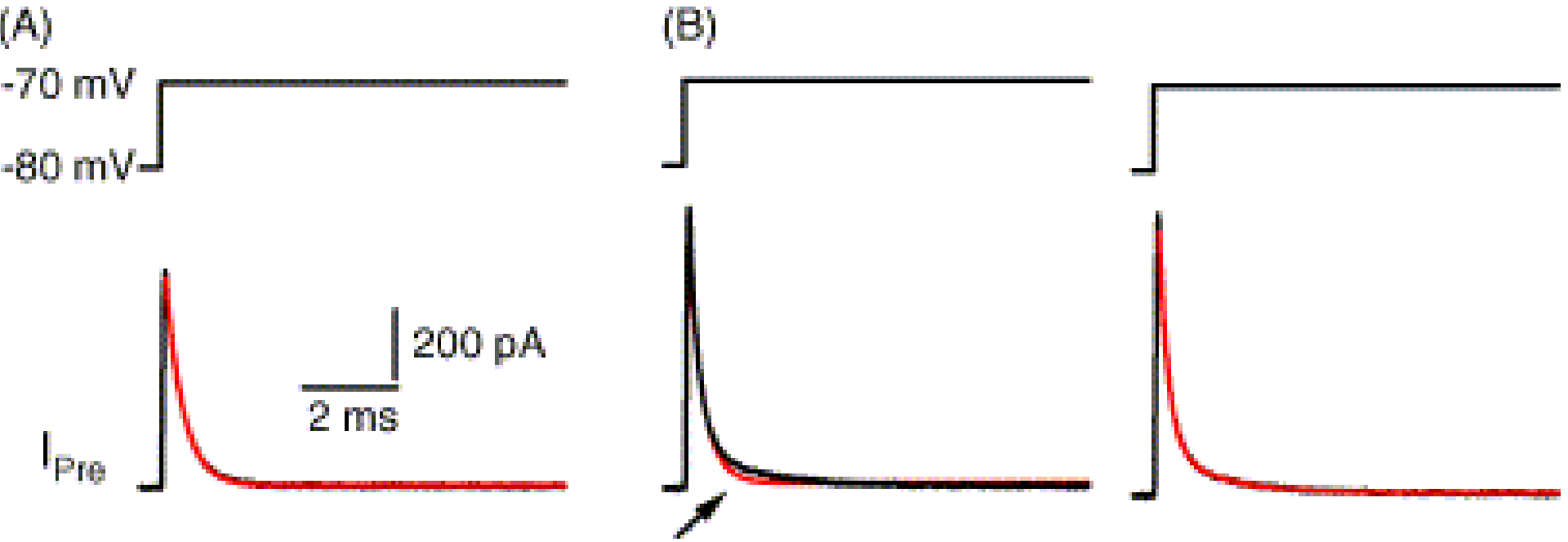
Clever Methods

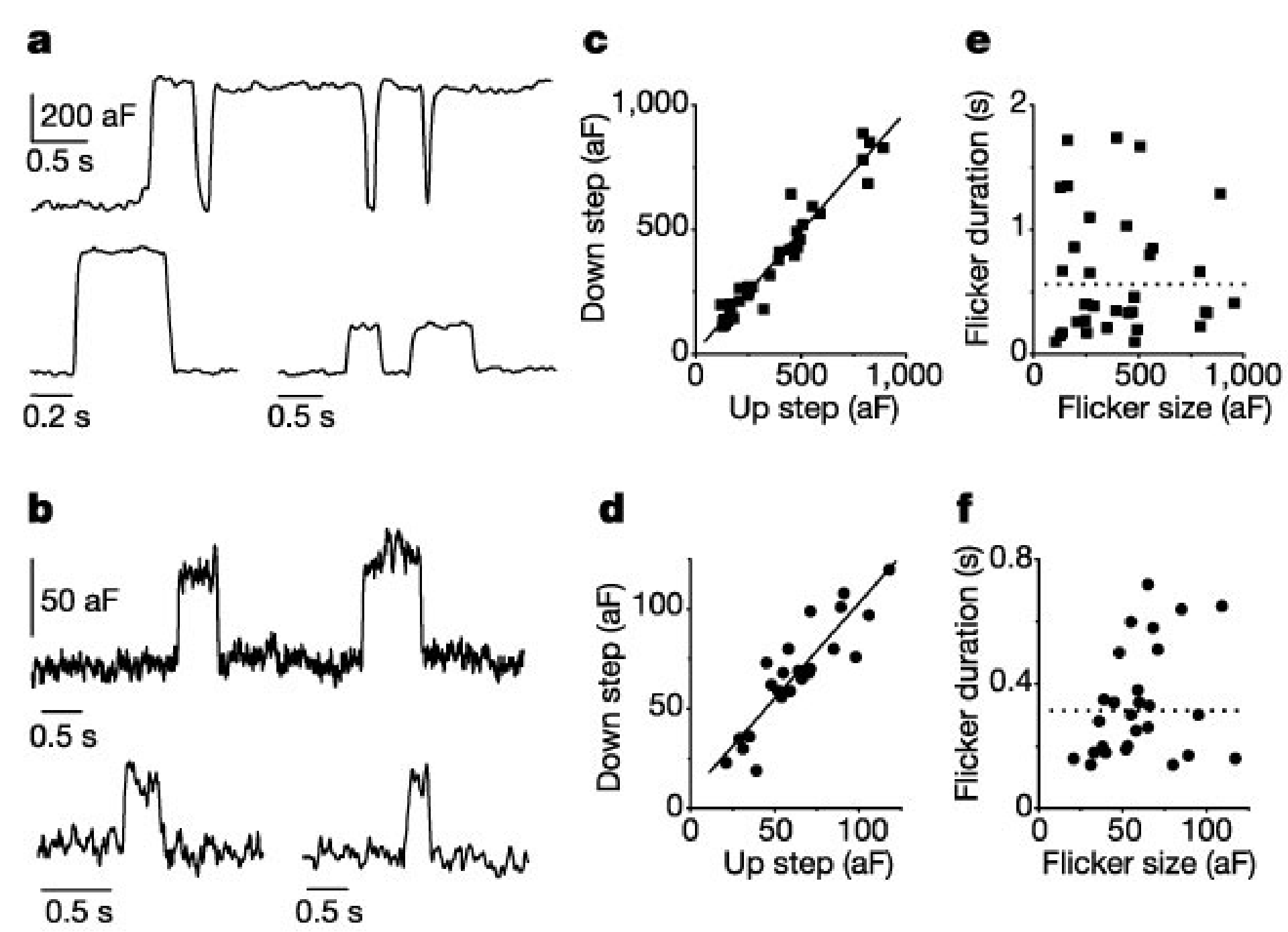
Method 1:

Capacitance

(Direct Sensing of Transmitter Release)

Capacitance Technique



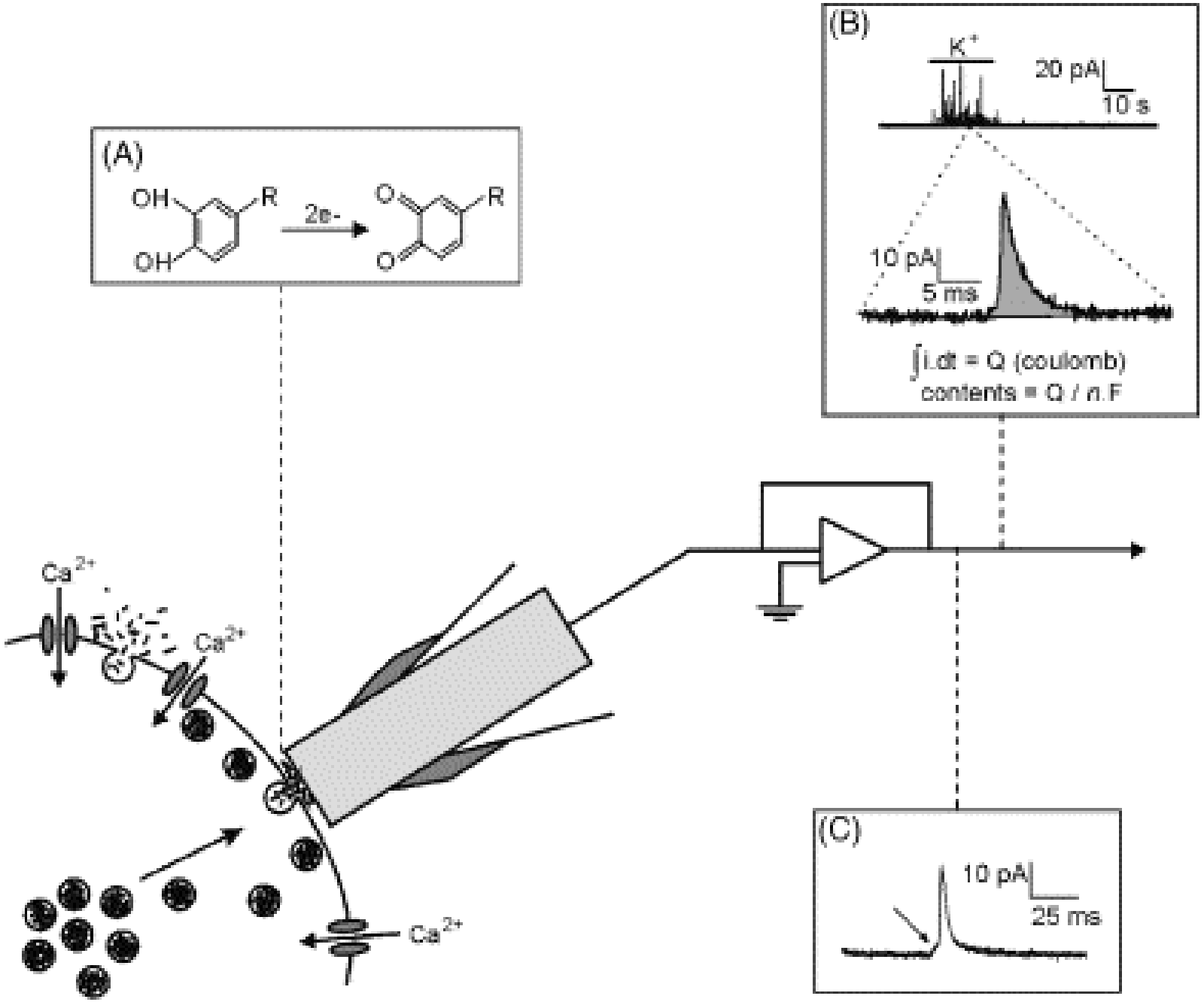

a
200 aF
0.5 s
0.2 s
0.5 s
b
50 aF
0.5 s
0.5 s
0.5 s
c
Down step (aF)
1,000
500
0
0
500
1,000
Up step (aF)
d
Down step (aF)
100
50
0
0
50
100
Up step (aF)
e
Flicker duration (s)
2
1
0
0
500
1,000
Flicker size (aF)
f
Flicker duration (s)
0.8
0.4
0
0
50
100
Flicker size (aF)

Clever Methods

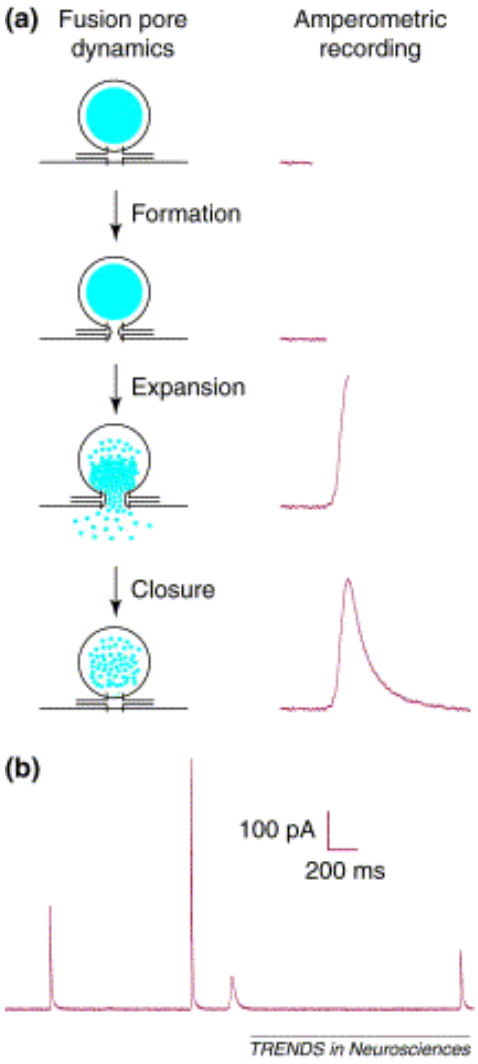
Method 2:

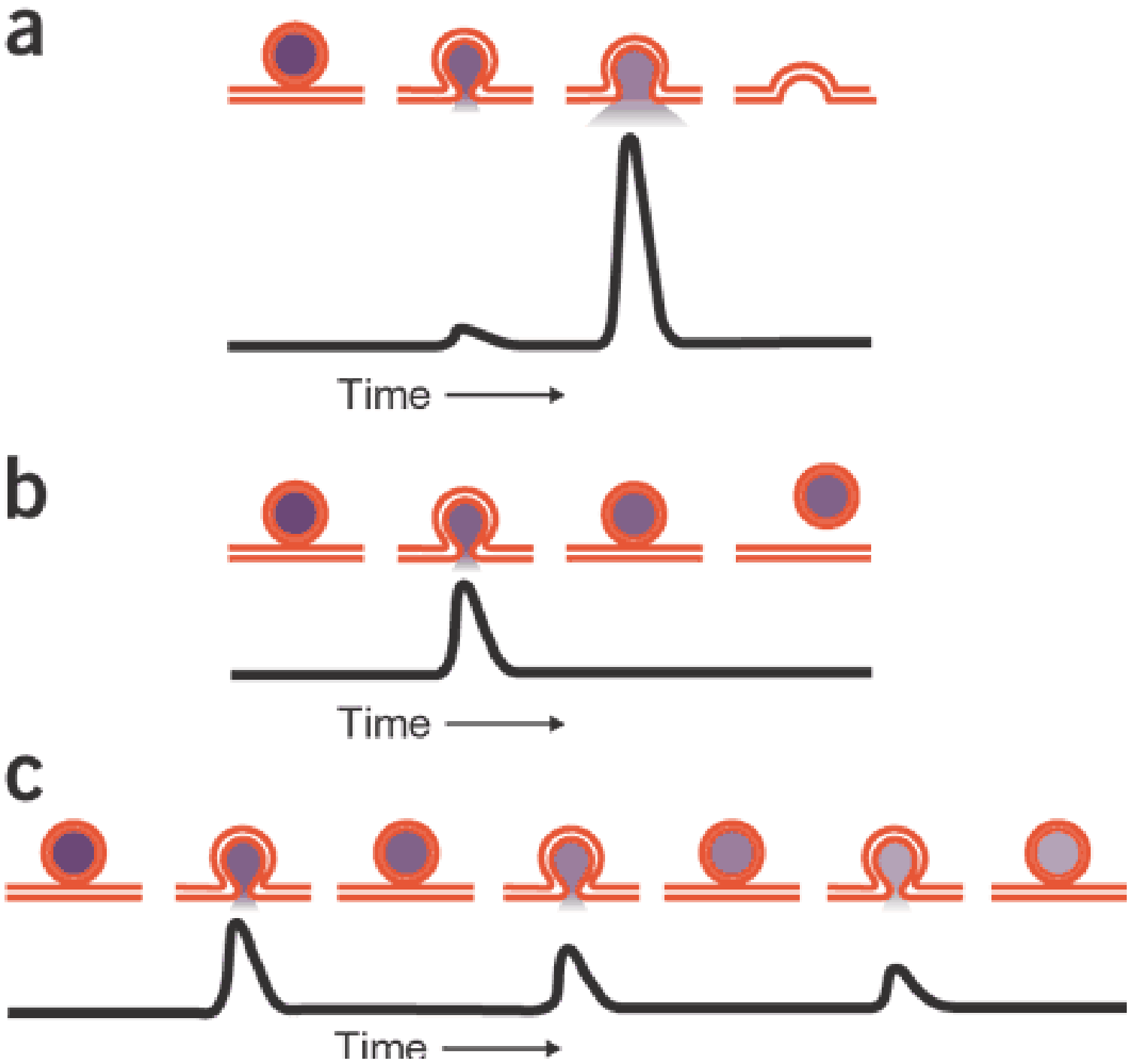
Amperometery

(Direct Sensing of Transmitter Release)


(A)
OH
OH
R
2e-
O
O
R
(B)
K+
20 pA
10 s
10 pA
5 ms
∫i.dt = Q (coulomb)
contents = Q / n.F
Ca2+
Ca2+
Ca2+
(C)
10 pA
25 ms

Measurement of Fusion Modes via Amperometry



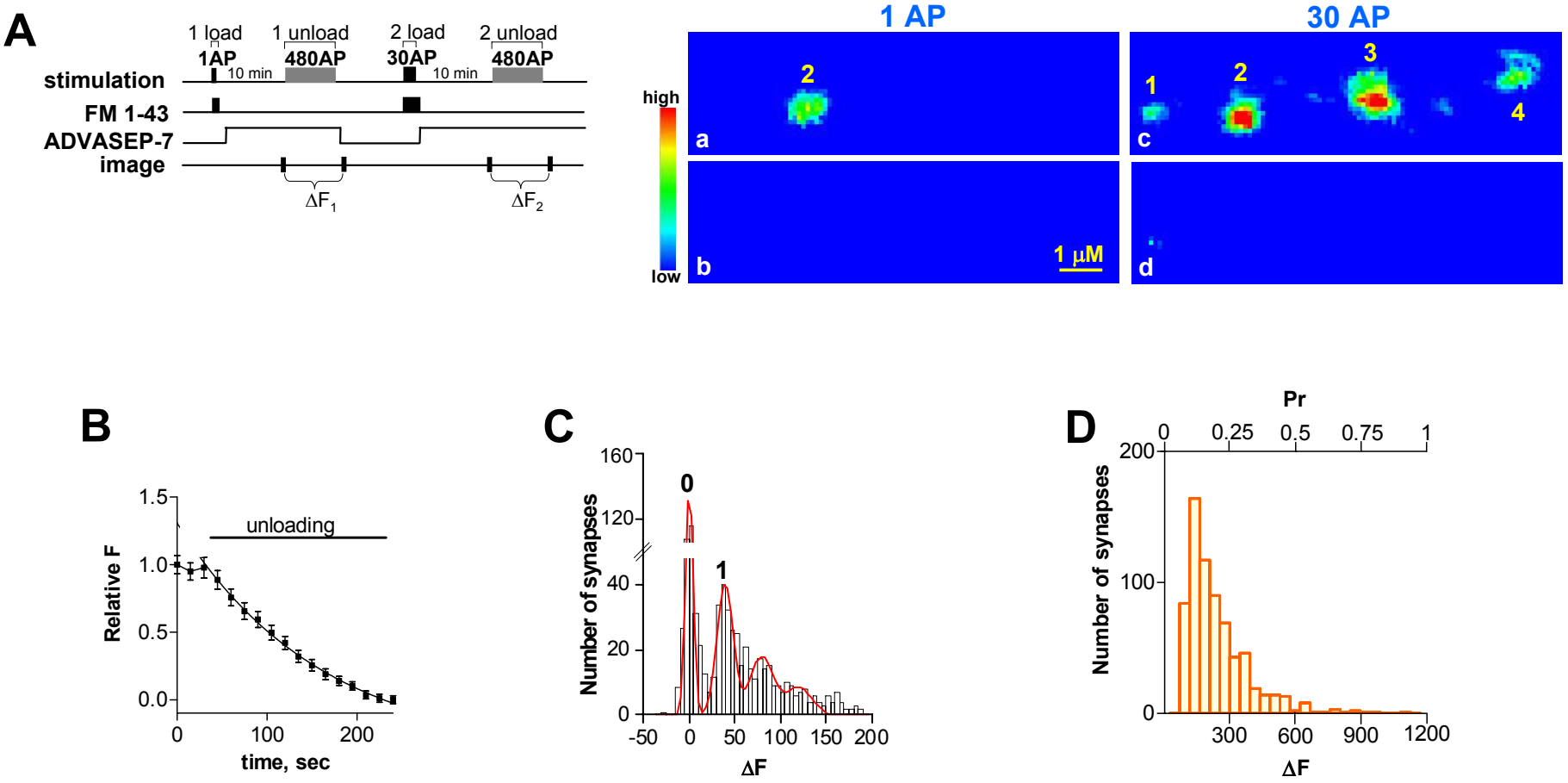

a
Time
b
Time
c
Time

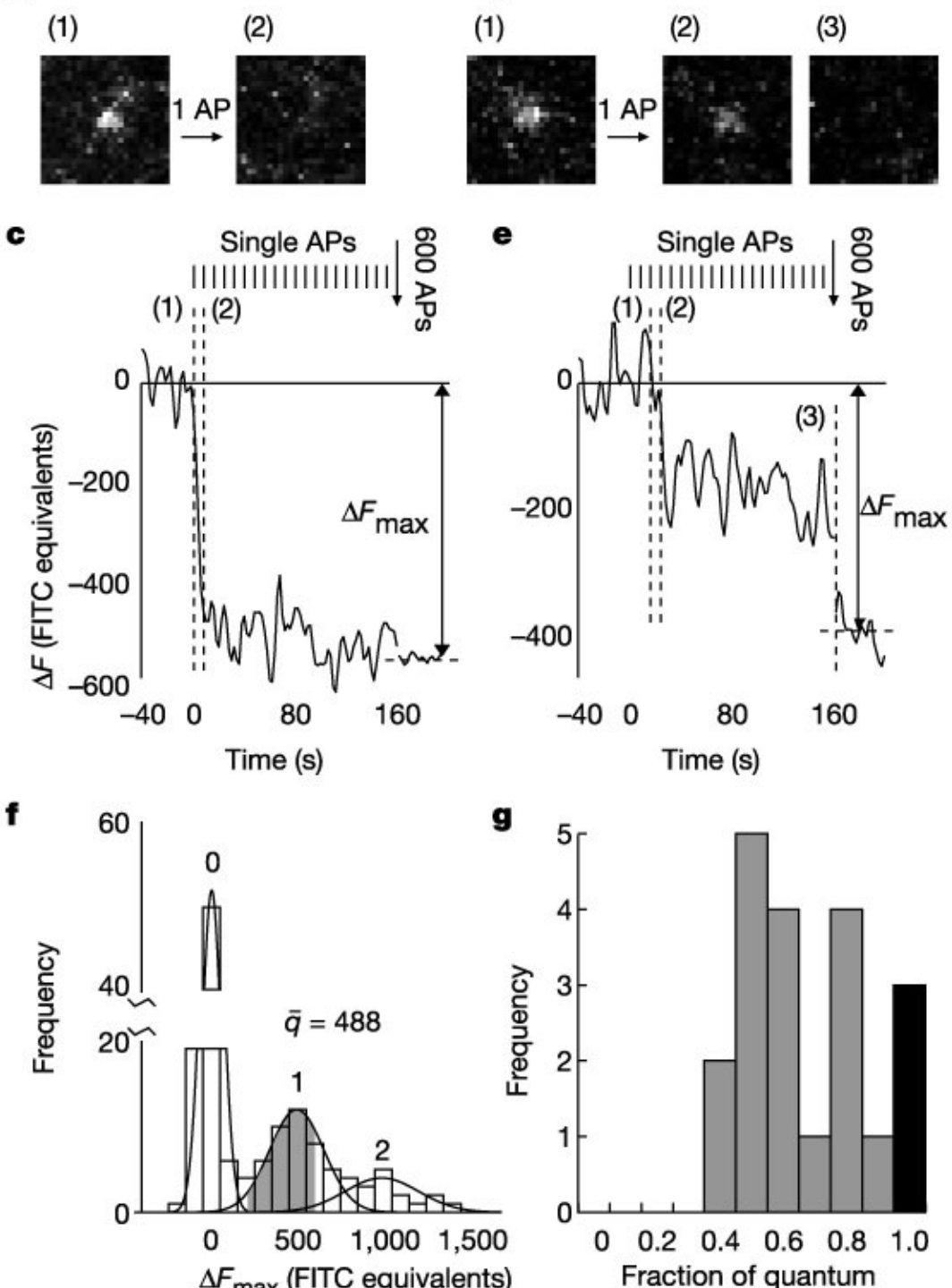
Clever Methods

Method 3:

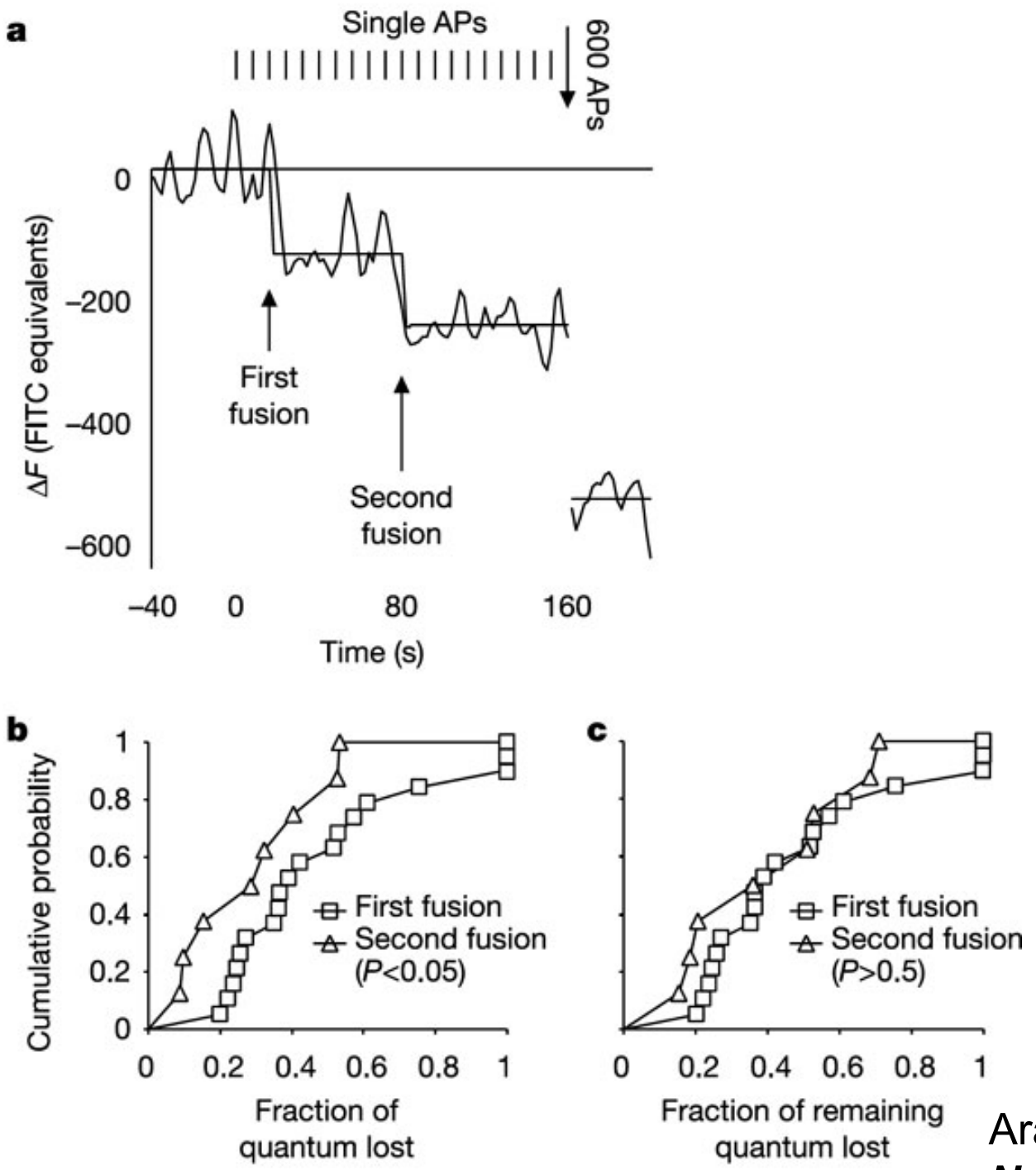
FM Imaging

(Fluorescent Staining of Active Vesicles)


A
1 load
1 unload
2 load
2 unload
1AP
10 min
480AP
30AP
10 min
480AP
stimulation
FM 1-43
ADVASEP-7
image
ΔF1
ΔF2
1 AP
30 AP
high
low
a
b
c
d
1 μM
1
2
3
4
B
unloading
Relative F
time, sec
C
Number of synapses
ΔF
0
1
D
Pr
Number of synapses
ΔF



Aravanis et al.,
Nature, 2003



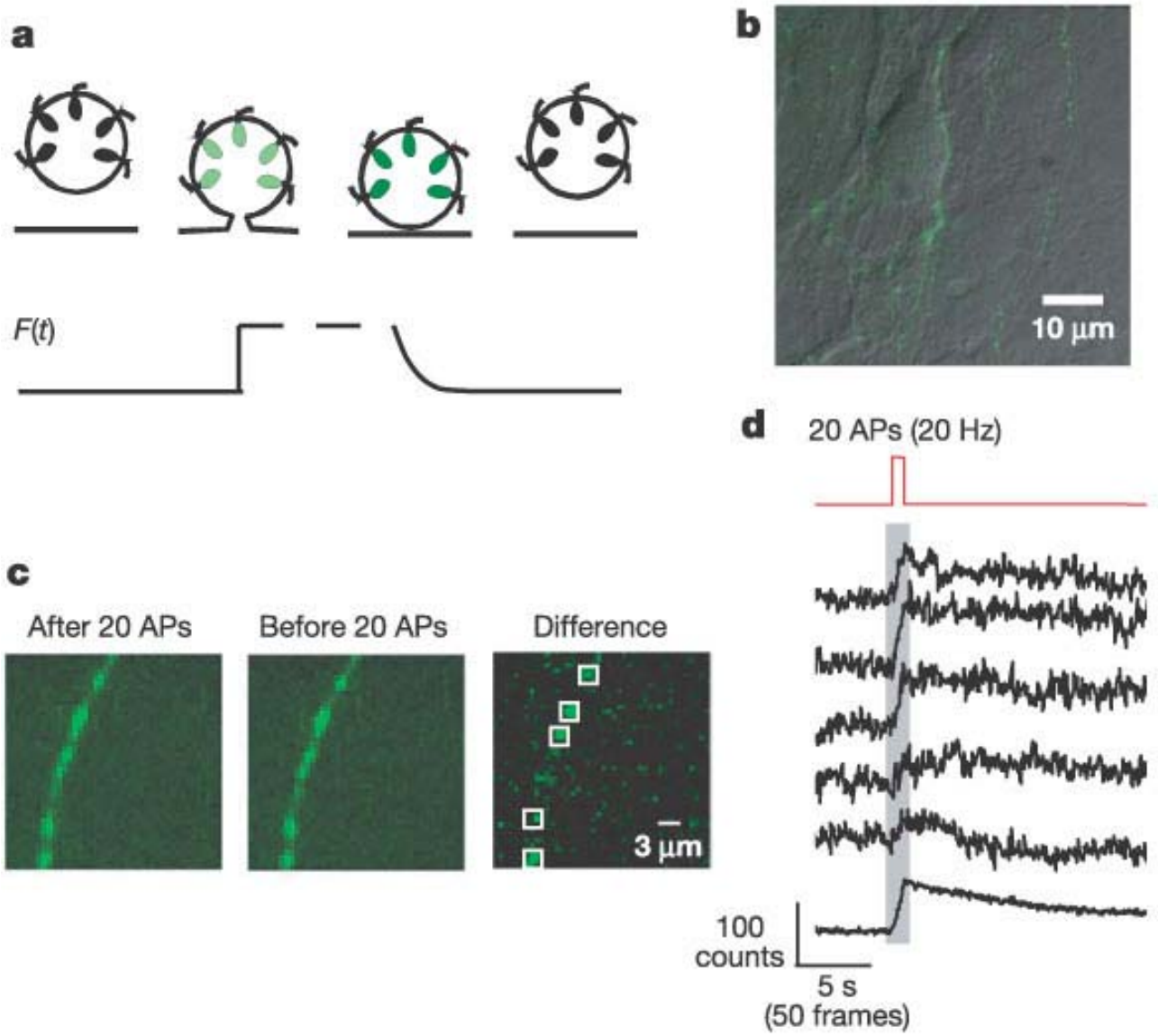
Aravanis et al.

Clever Methods

Method 4:

Synaptophluorins

(pH-Sensitive Visualization of Fusion Events)

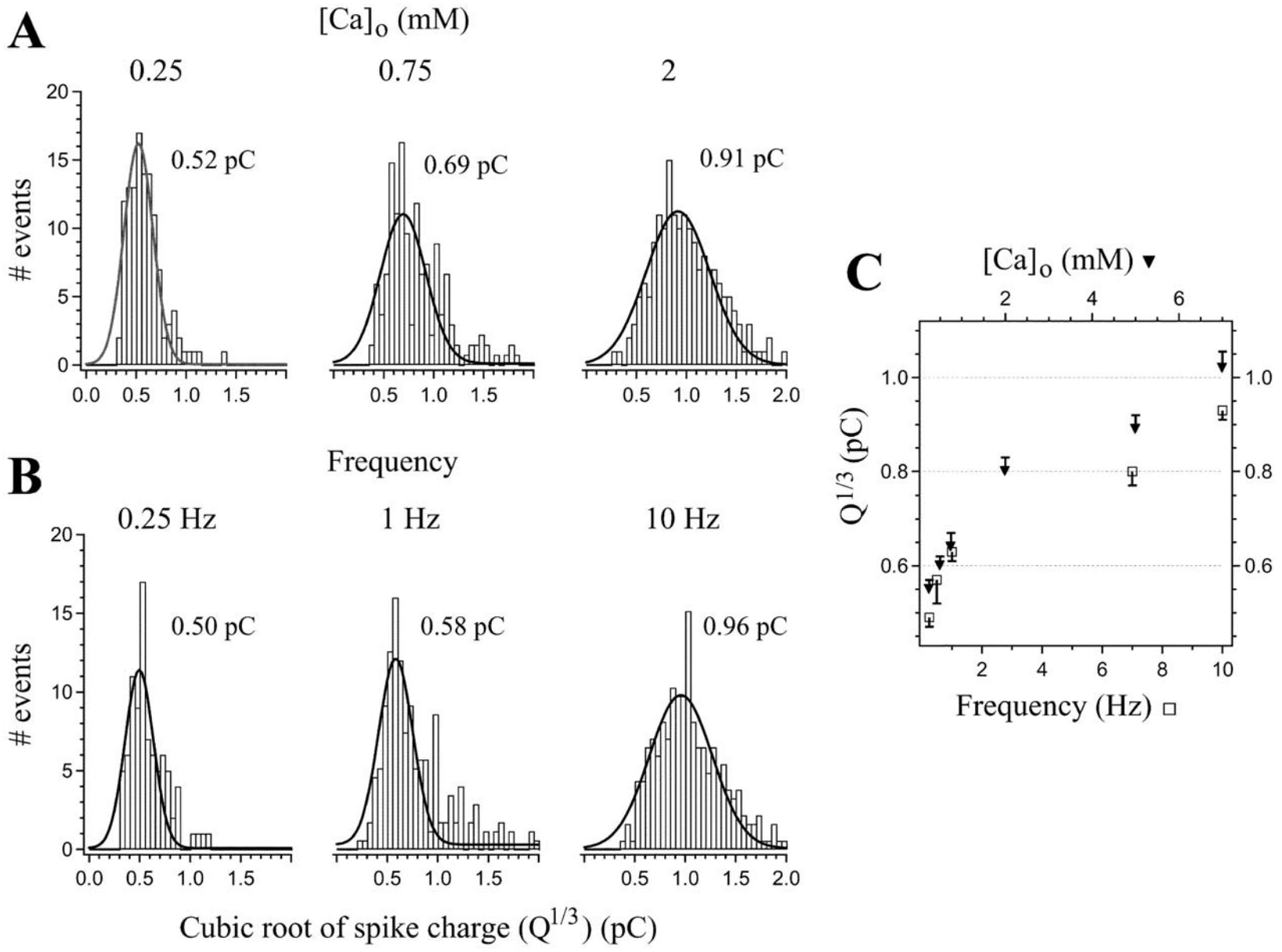

a
F(t)
b
10 µm
c
After 20 APs
Before 20 APs
Difference
3 µm
d
20 APs (20 Hz)
100
counts
5 s
(50 frames)

Regulation

Molecular Regulation of Fusion Process

Regulation of the Fusion Pore

- The rate of fusion pore expansion is regulated by:
  - Intracellular Ca^{2+}

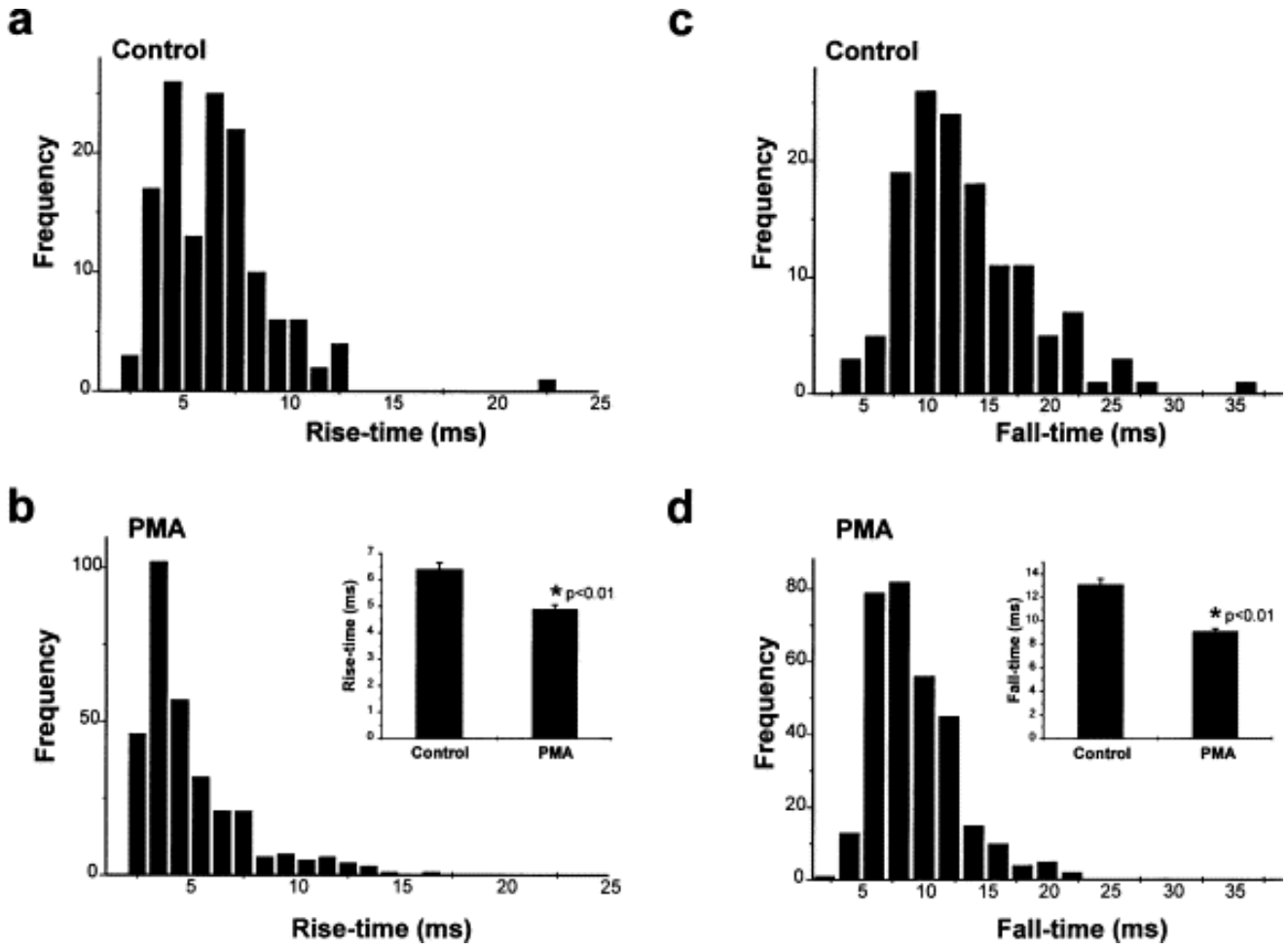

A
[Ca]o (mM)
0.25
0.75
2
0.52 pC
0.69 pC
0.91 pC
events
B
Frequency
0.25 Hz
1 Hz
10 Hz
0.50 pC
0.58 pC
0.96 pC
events
Cubic root of spike charge (Q1/3) (pC)
C
[Ca]o (mM) ▼
Q1/3 (pC)
Frequency (Hz) □

Regulation of the Fusion Pore

- The rate of fusion pore expansion is regulated by:
  - Intracellular Ca^{2+}

Regulation of the Fusion Pore

- The rate of fusion pore expansion is regulated by:
  - Intracellular Ca2+
  - Phorbol esters (e.g. PMA, via PKC pathway)


a
Control
Frequency
Rise-time (ms)
b
PMA
Frequency
Rise-time (ms)
Rise-time (ms)
Control
PMA
*p<0.01
c
Control
Frequency
Fall-time (ms)
d
PMA
Frequency
Fall-time (ms)
Fall-time (ms)
Control
PMA
*p<0.01

Regulation of the Fusion Pore

- The rate of fusion pore expansion is regulated by:
  - Intracellular Ca2+
  - Phorbol esters (e.g. PMA, via PKC pathway)

Regulation of the Fusion Pore

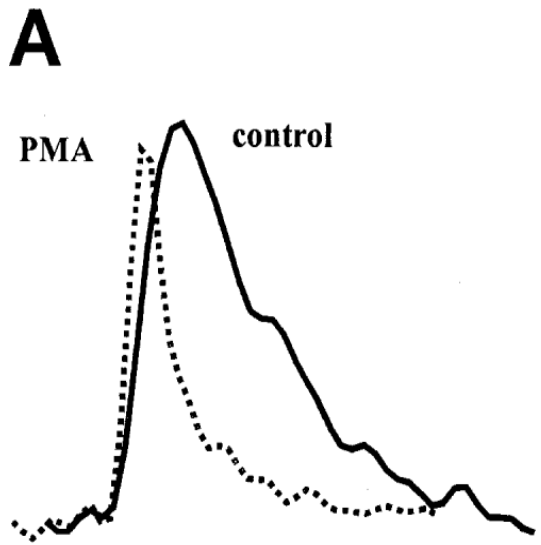
- The rate of fusion pore expansion is regulated by:
  - Intracellular Ca2+
  - Phorbol esters (e.g. PMA, via PKC pathway)

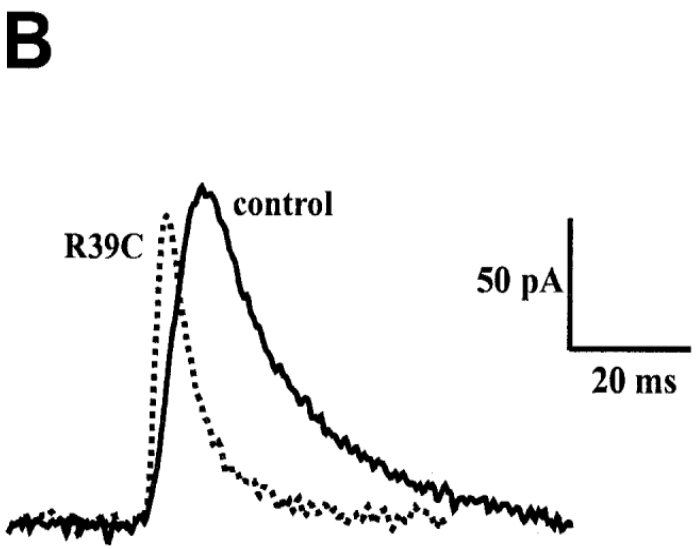
- Fusion pore open time is regulated by
  - synaptotagmin I/IV
  - dynamin

Regulation of the Fusion Pore

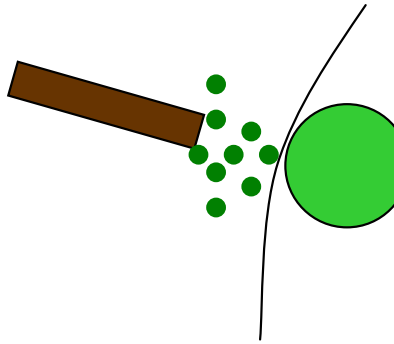
- The rate of fusion pore expansion is regulated by:
  - Intracellular Ca2+
  - Phorbol esters (e.g. PMA, via PKC pathway)
- Fusion pore open time is regulated by
  - synaptotagmin I/IV
  - dynamin
- Shift of the mode of exocytosis to “kiss-and-run” by:
  - High extracellular Ca2+
  - Staurosporine (kinase inhibitor) (?)
  - Phorbol esters (e.g., PMA, PKC activator)
  - Munc-13

Fusion pore can be regulated





Carbon Fibre Amperometry

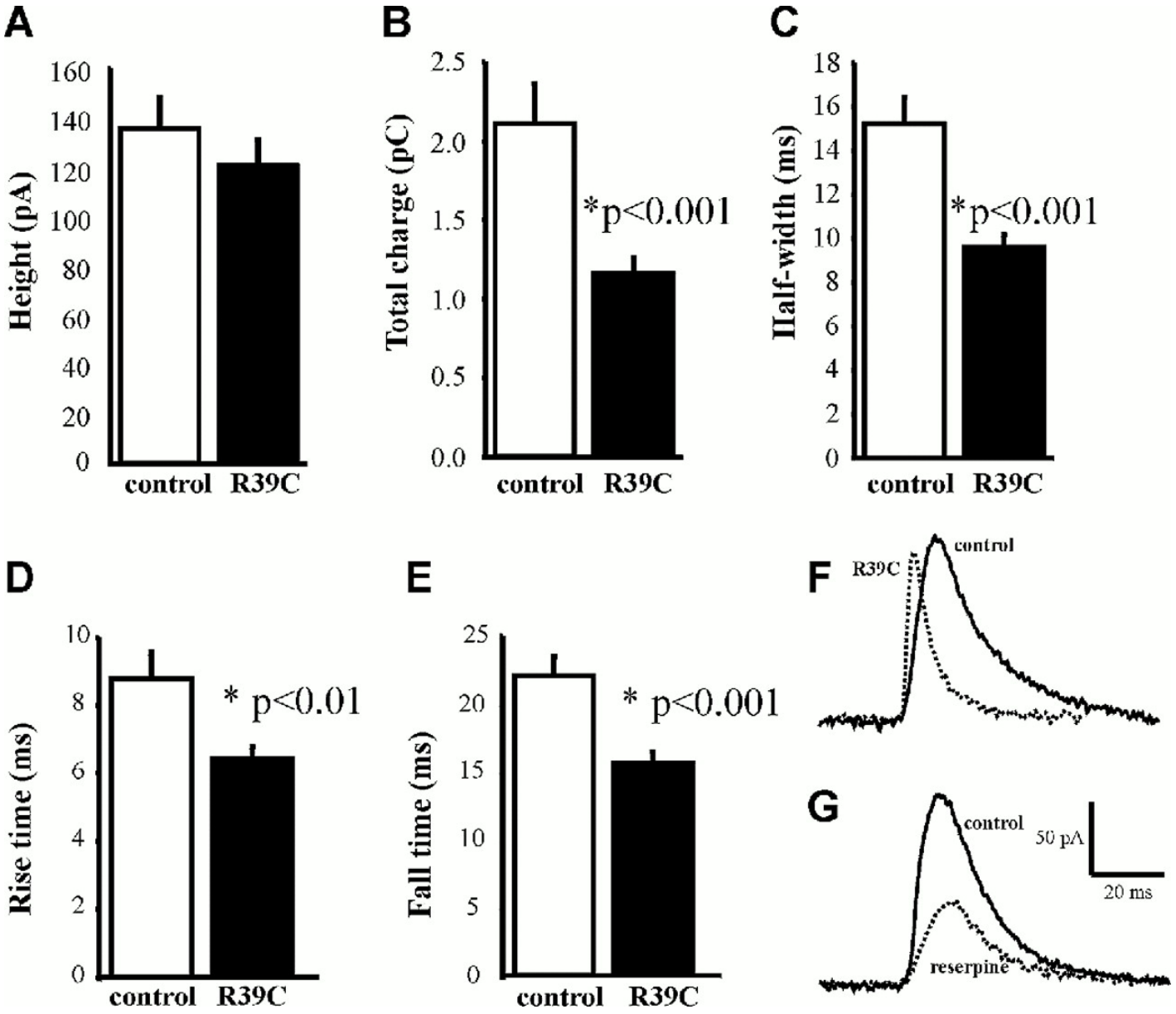


Catecholamine

Chromaffin Cells

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

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


A
Height (pA)
160
140
120
100
80
60
40
20
0
control
R39C
B
Total charge (pC)
2.5
2.0
1.5
1.0
0.5
0.0
*p<0.001
control
R39C
C
Half-width (ms)
18
16
14
12
10
8
6
4
2
0
*p<0.001
control
R39C
D
Rise time (ms)
10
8
6
4
2
0
* p<0.01
control
R39C
E
Fall time (ms)
25
20
15
10
5
0
* p<0.001
control
R39C
F
R39C
control
G
control
50 pA
20 ms
reserpine

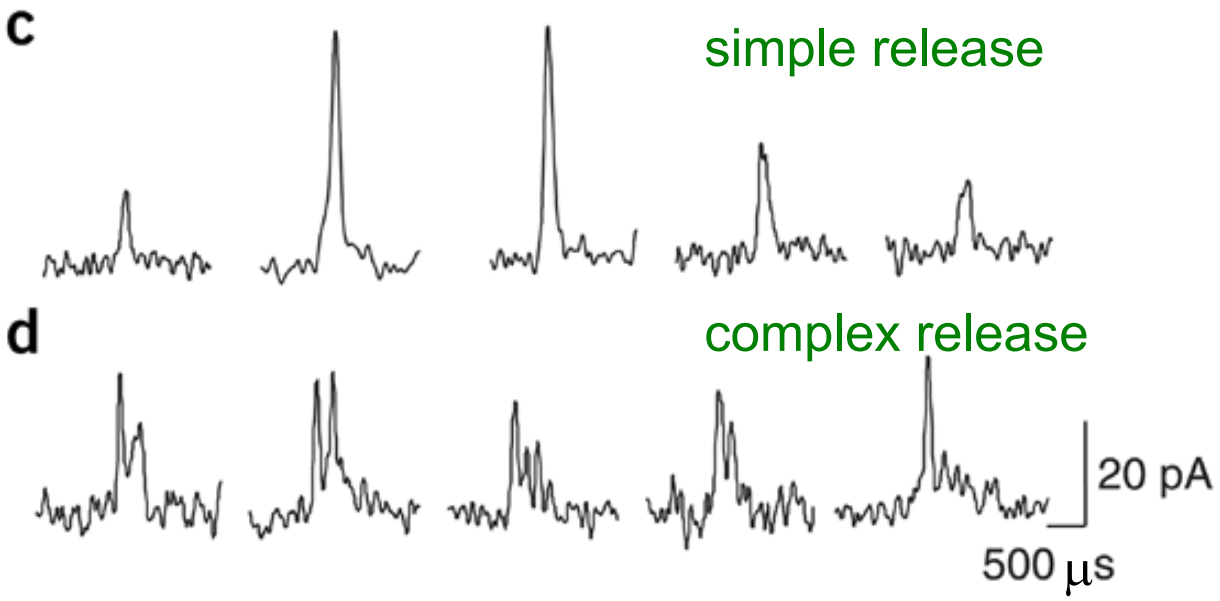
Regulation

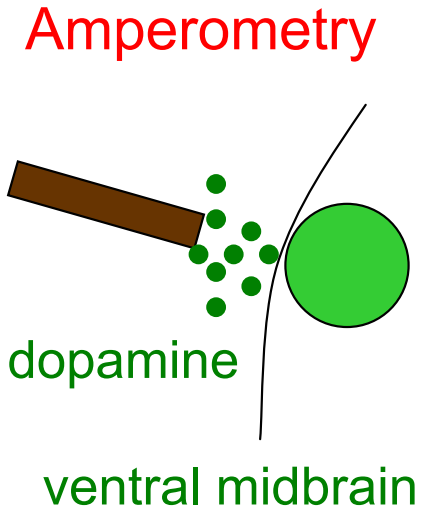
Molecular Regulation of Fusion Process

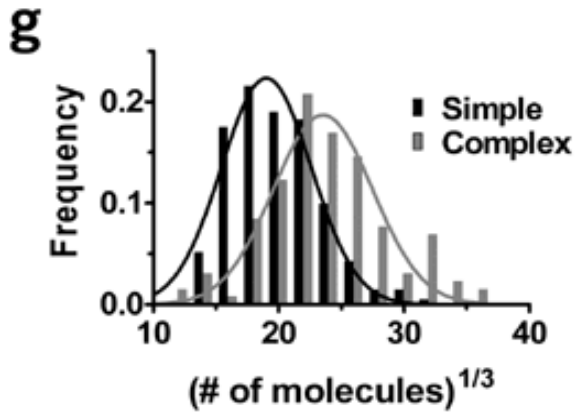
Regulation

Implications for
Synaptic Transmission

Quantal size is regulated by the fusion pore in small vesicles of dopaminergic neurons

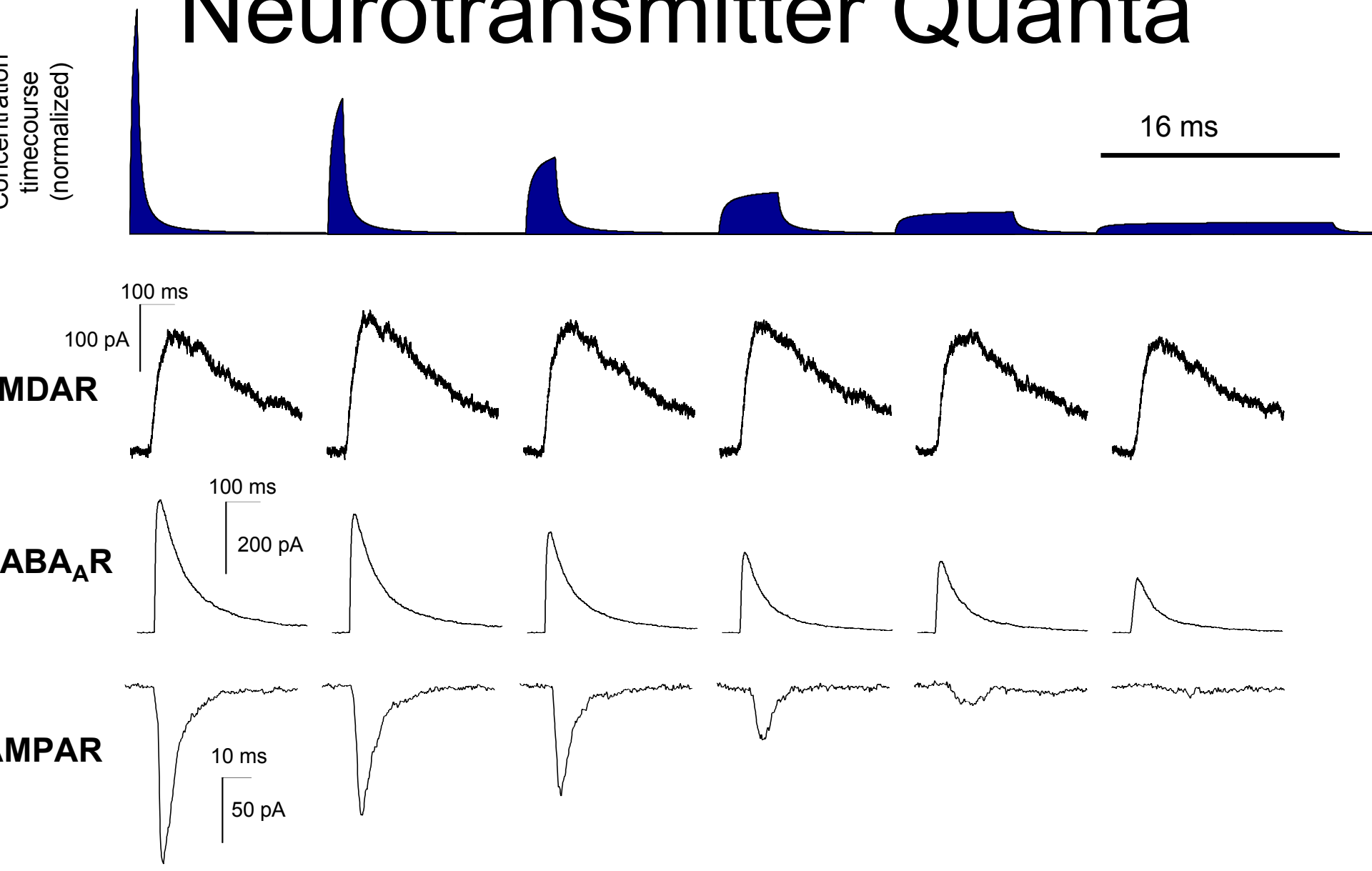






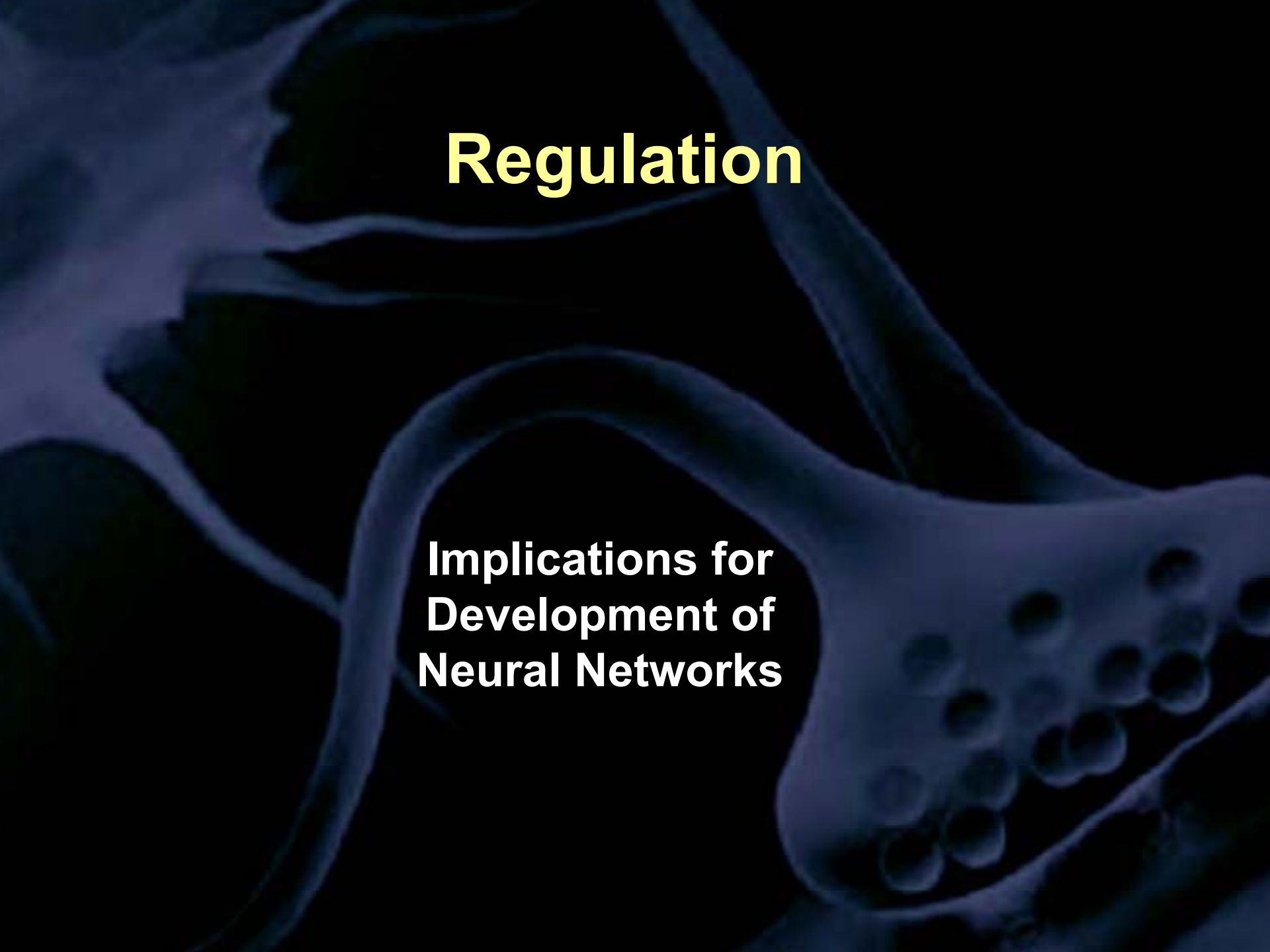
Staal, Mosharov, Sulzer
(Nat Neurosci, 2004)

the Timecourse of Release of Neurotransmitter Quanta



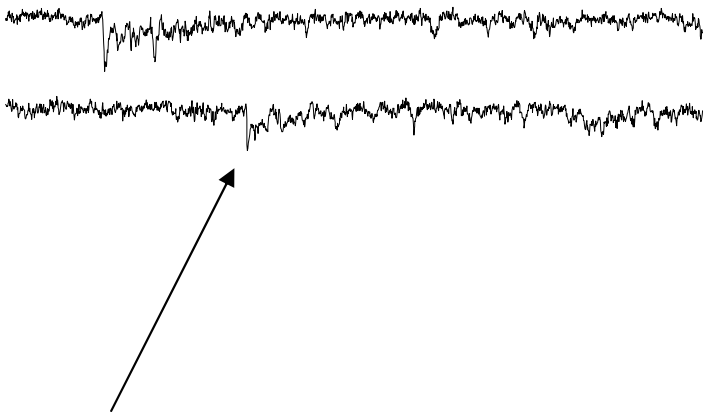
Regulation

Implications for
Synaptic Transmission


Regulation
Implications for
Development of
Neural Networks

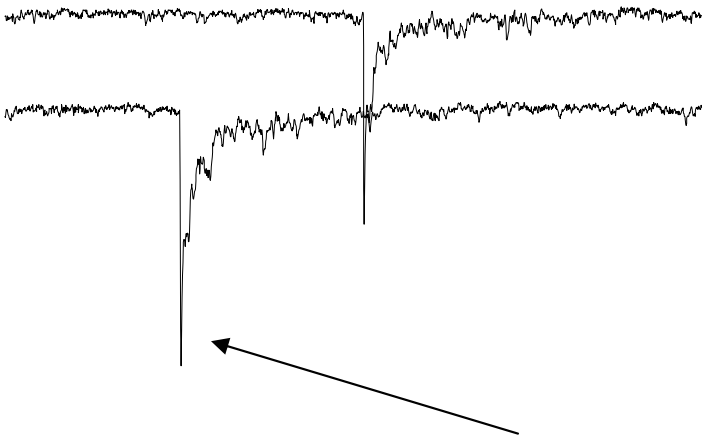
During Development of Neural Networks, Transmission Changes from "Silent" Type to Functional

Immature:
Silent Transmission



Synaptic Release Causes NMDA Receptor-Mediated Current

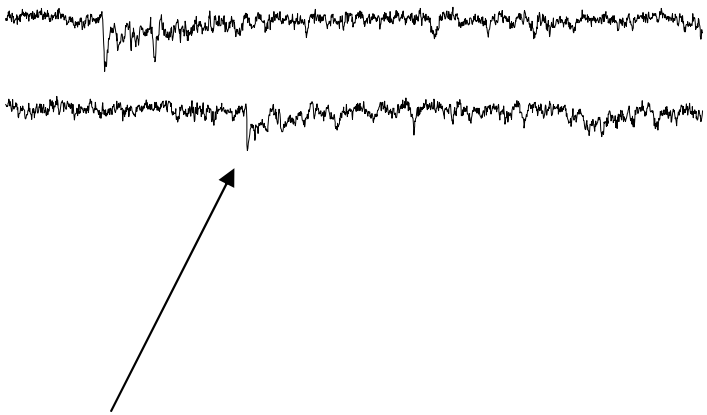
Mature: Functional



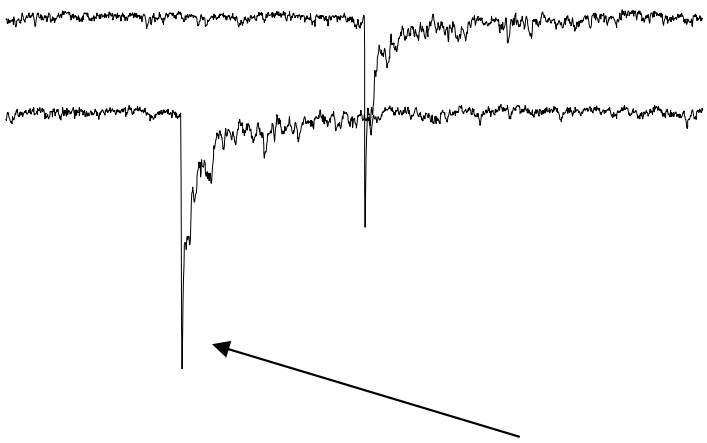
Synaptic Release Causes (NMDA + AMPA Receptor-Mediated Current

During Development of Excitatory Signaling in Hippocampal Circuits, Transmission Changes from a Slow, "Silent" Type, to a Fast, Functional Type

Immature:
Silent Transmission



Mature:
"Functional Transmission"



Synaptic Release Causes NMDA Receptor-Mediated Current

Synaptic Release Causes (NMDA + AMPA Receptor-Mediated Current

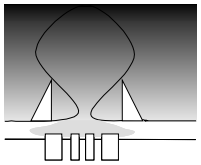
What's Different?

Summary of presynaptic neurotransmitter release maturation

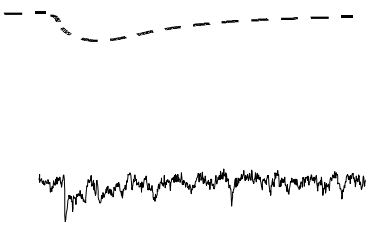
$I_{synaptic}$

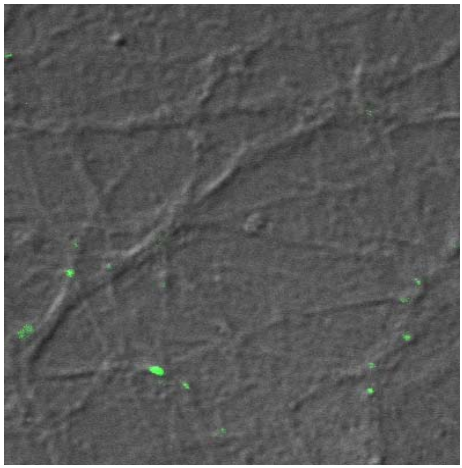
FM1-43

"AMPA-quiet"

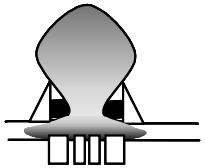


restricted release

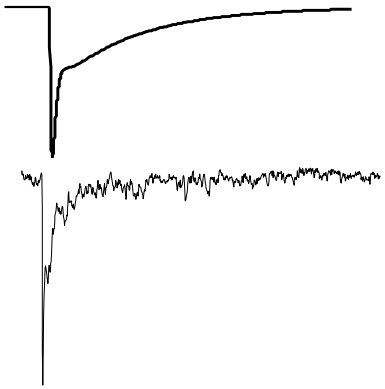


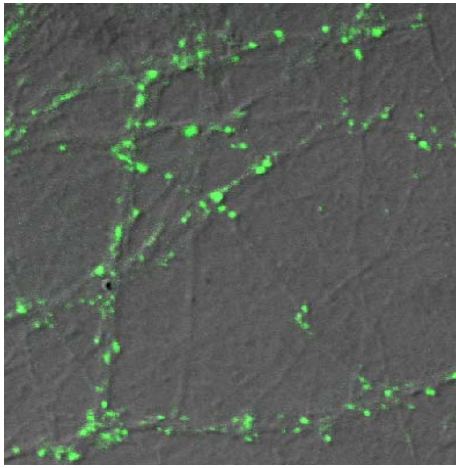


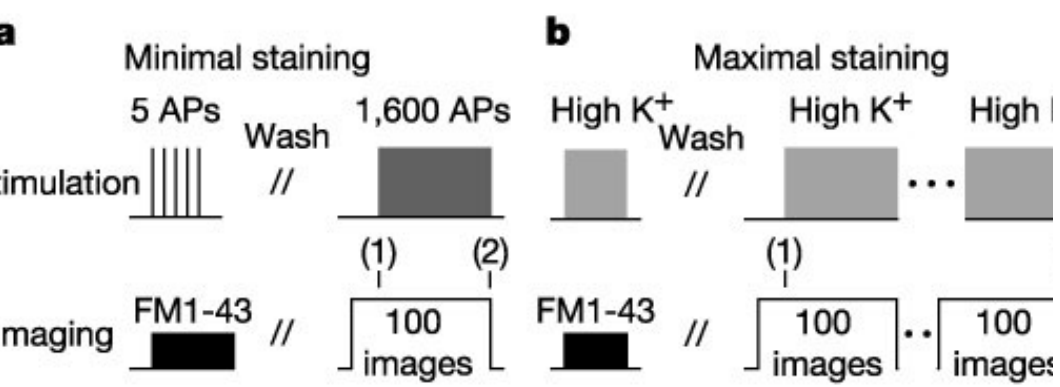
Functional



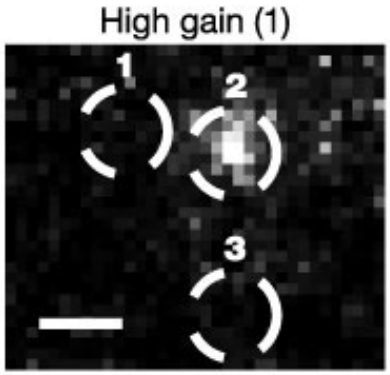
unrestricted release

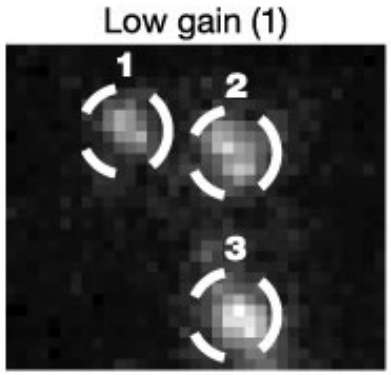


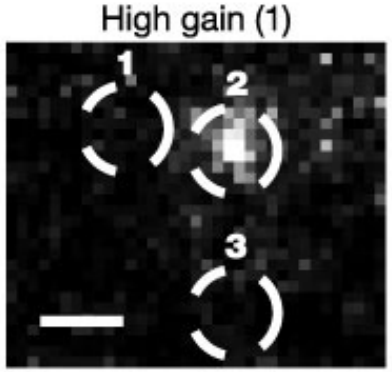



a
Minimal staining
5 APs
Wash
1,600 APs
timulation
(1)
(2)
maging
FM1-43
100 images
b
Maximal staining
High K+
Wash
High K+
High
(1)
FM1-43
100 images
100 images

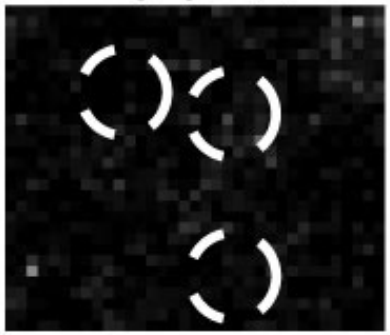
a
Minimal staining
5 APs
Wash
1,600 APs
Stimulation
(1)
(2)
Imaging
FM1-43
100 images
b
High K+
Wash
FM1-43

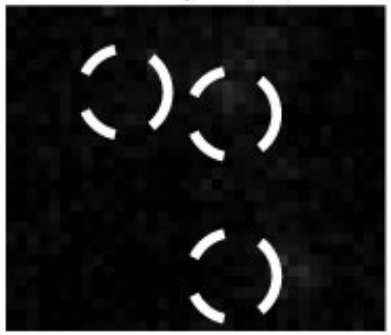

High gain (1)
1
2
3

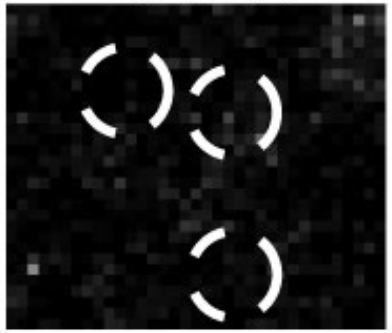

Low gain (1)
1
2
3


High gain (1)
1
2
3

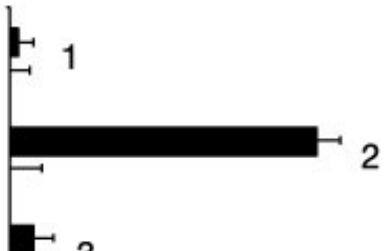

Lo
1

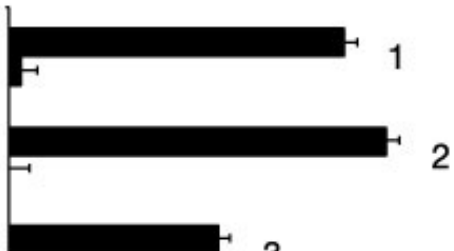

High gain (2)

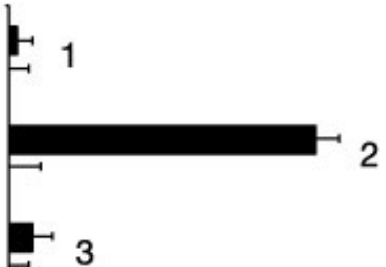

Low gain (2)

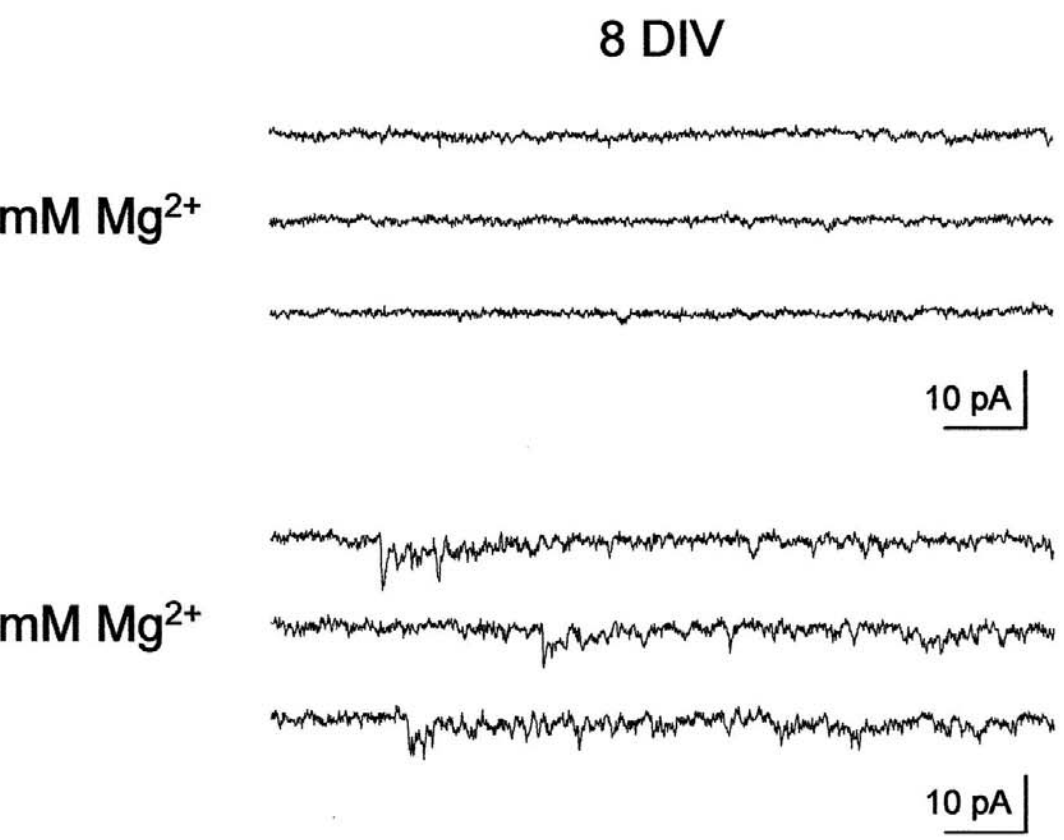

High gain (2)


Lo

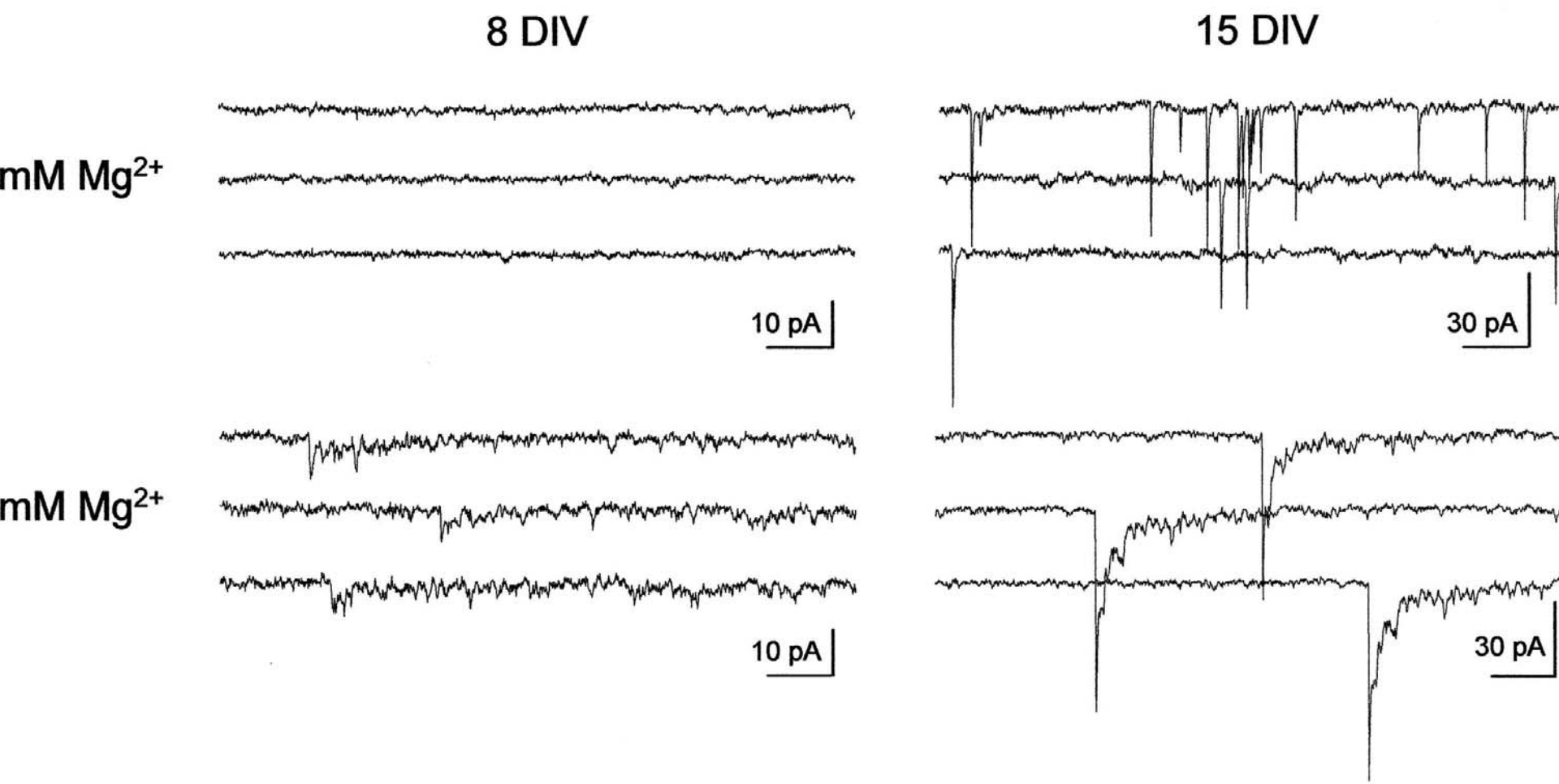

1
2
3


1
2
3

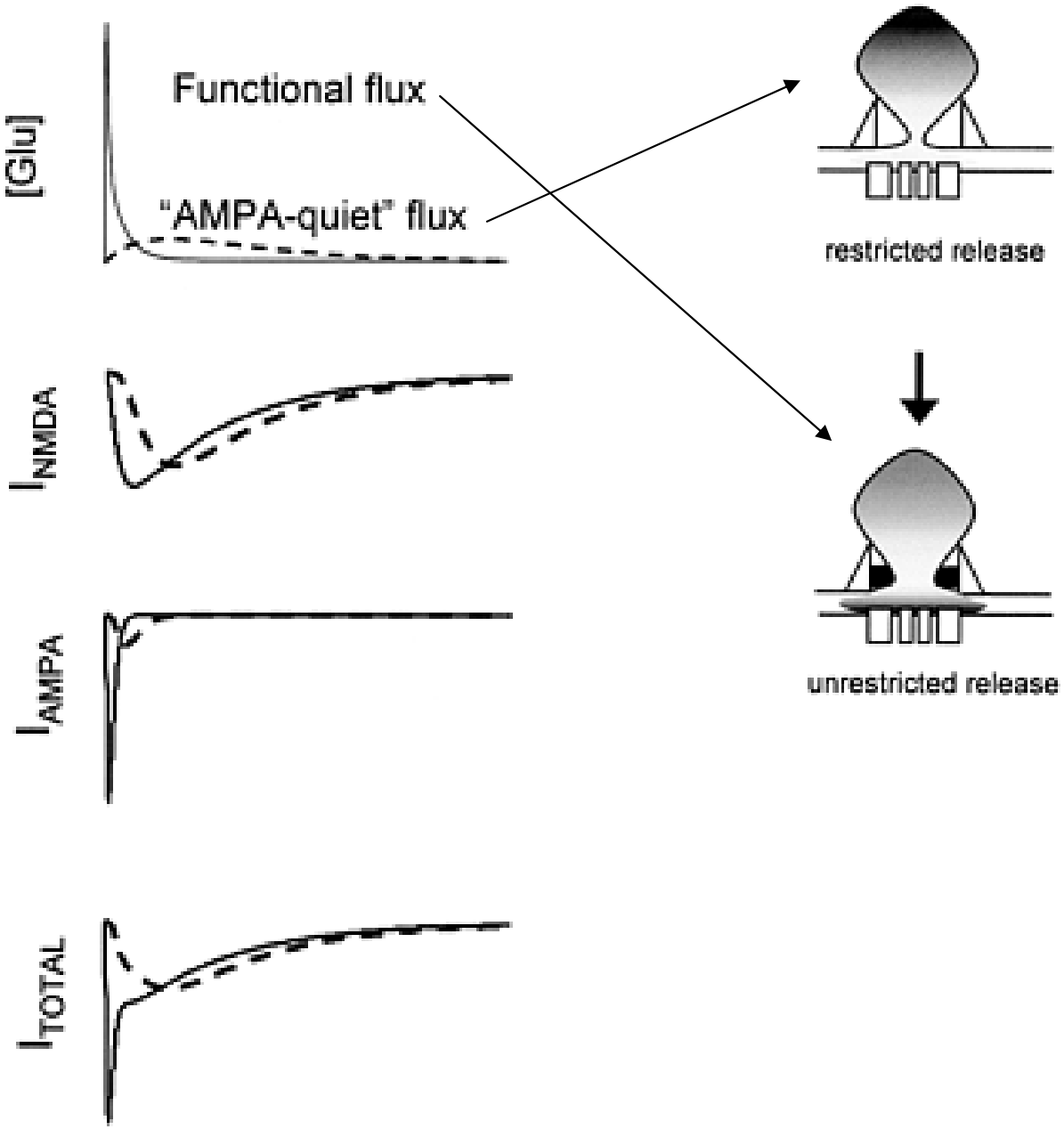

1
2
3

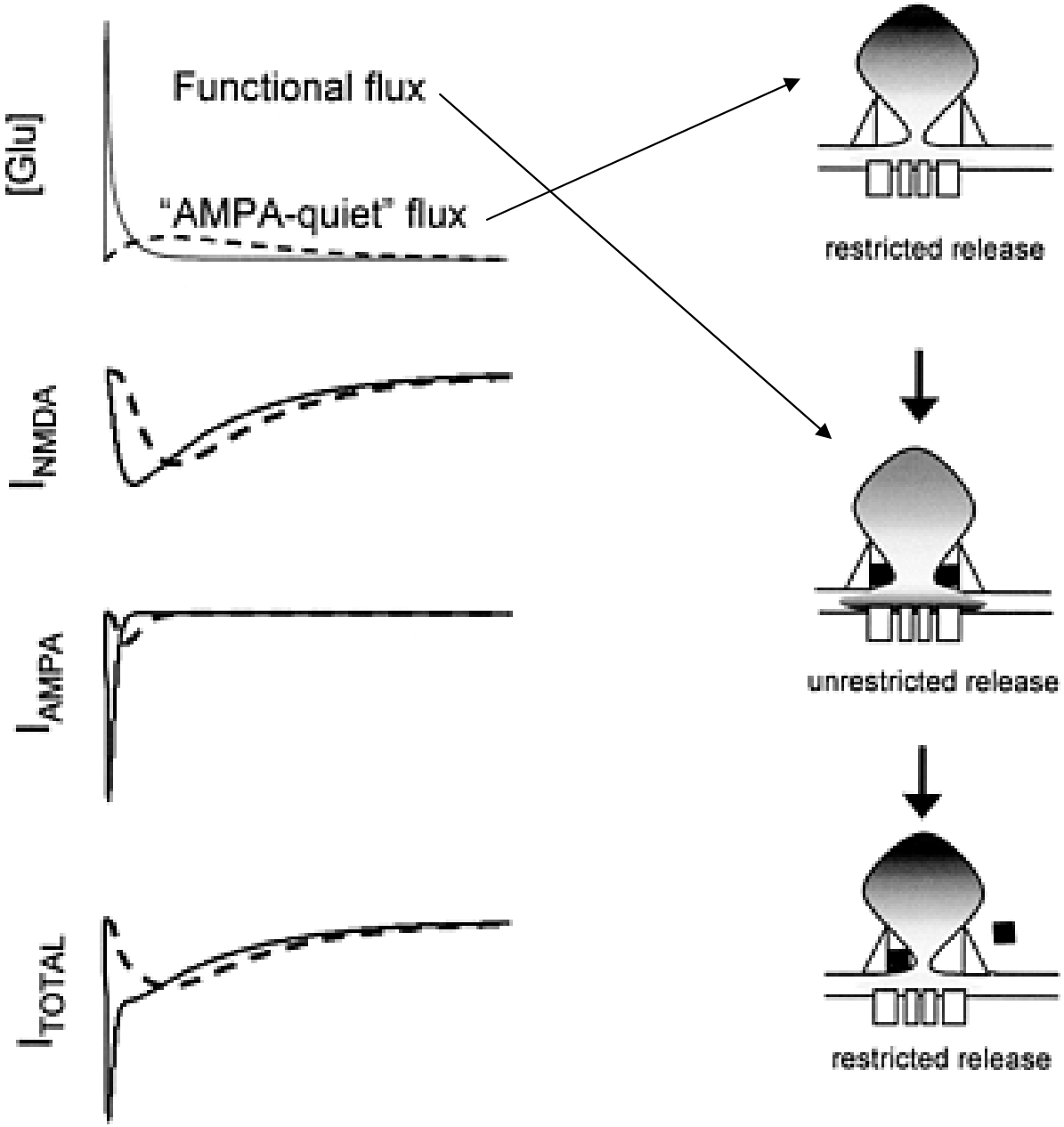


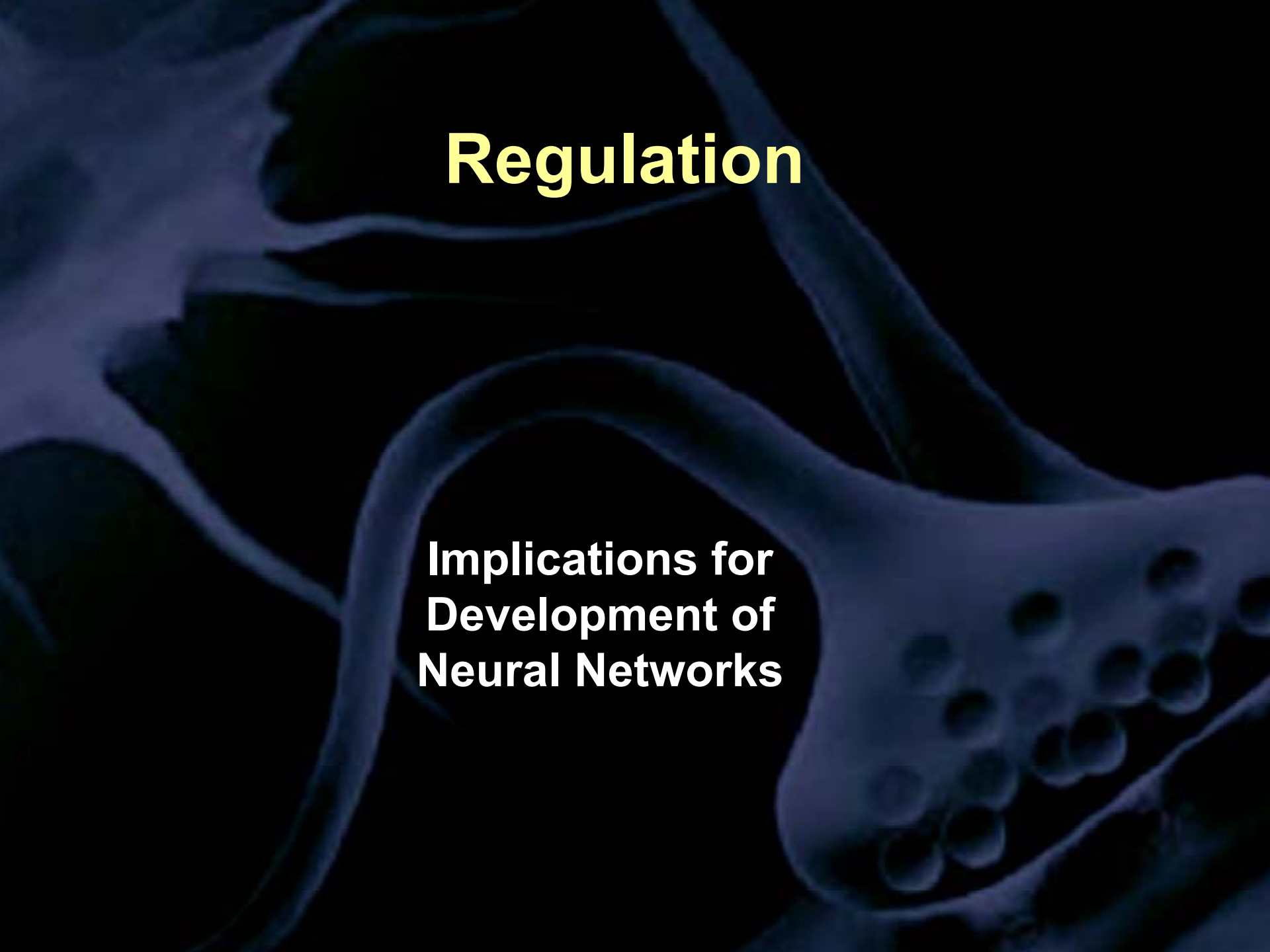
Early glutamatergic synapse
exhibit “silent” transmissio
consisting mainly of
NMDA currents.

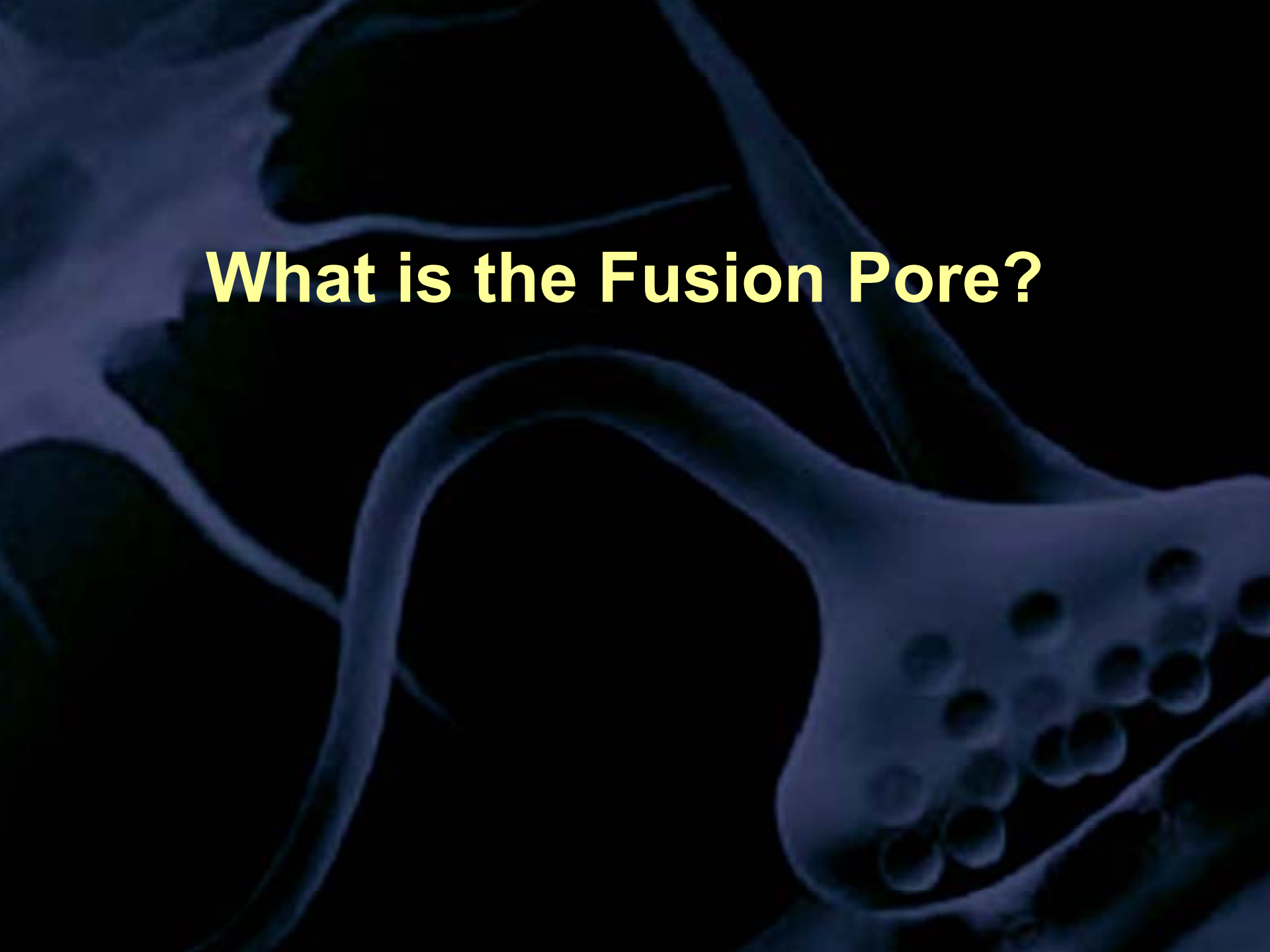


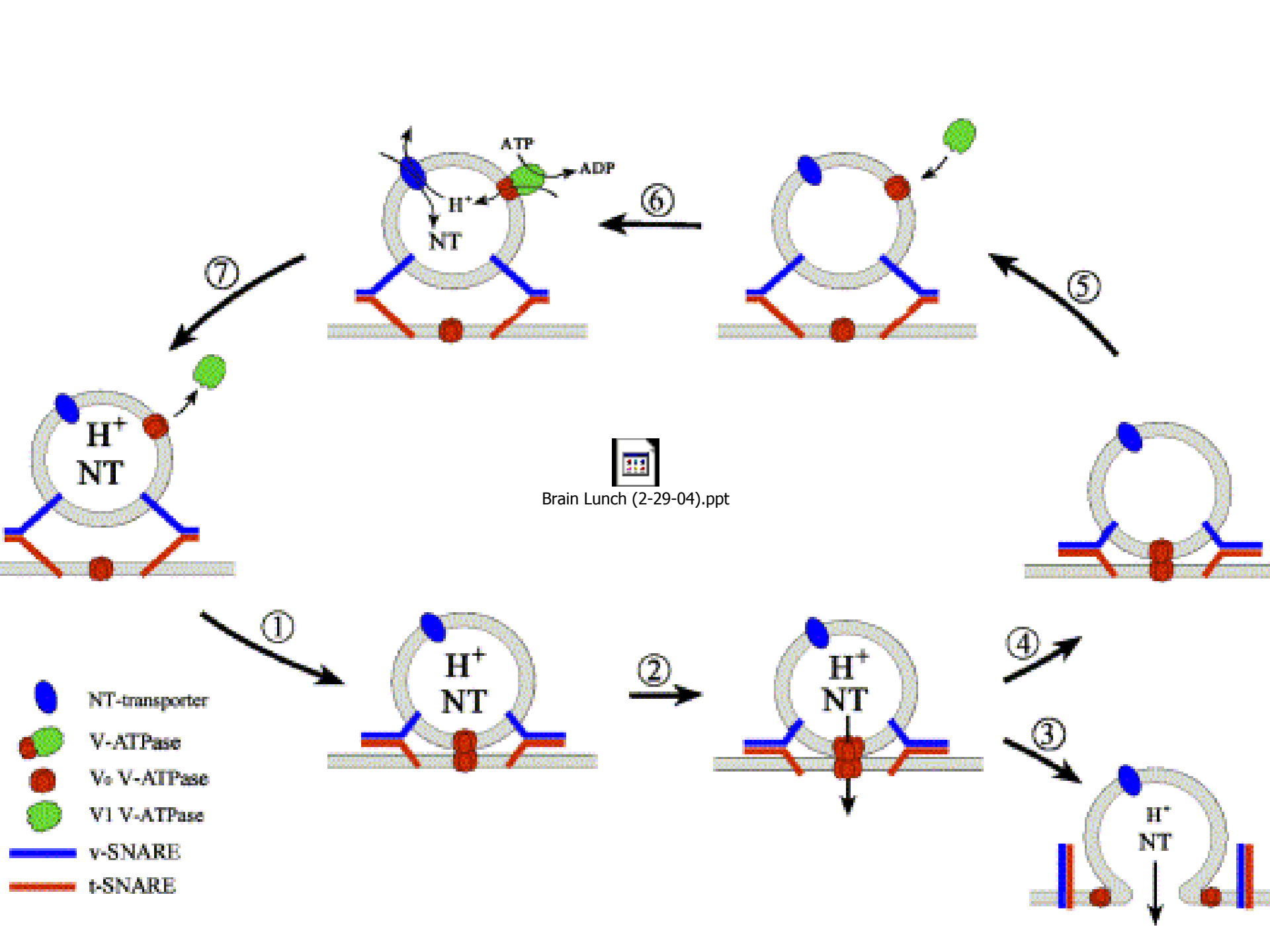
Later in development, NMDA currents are joined by AMPA currents.

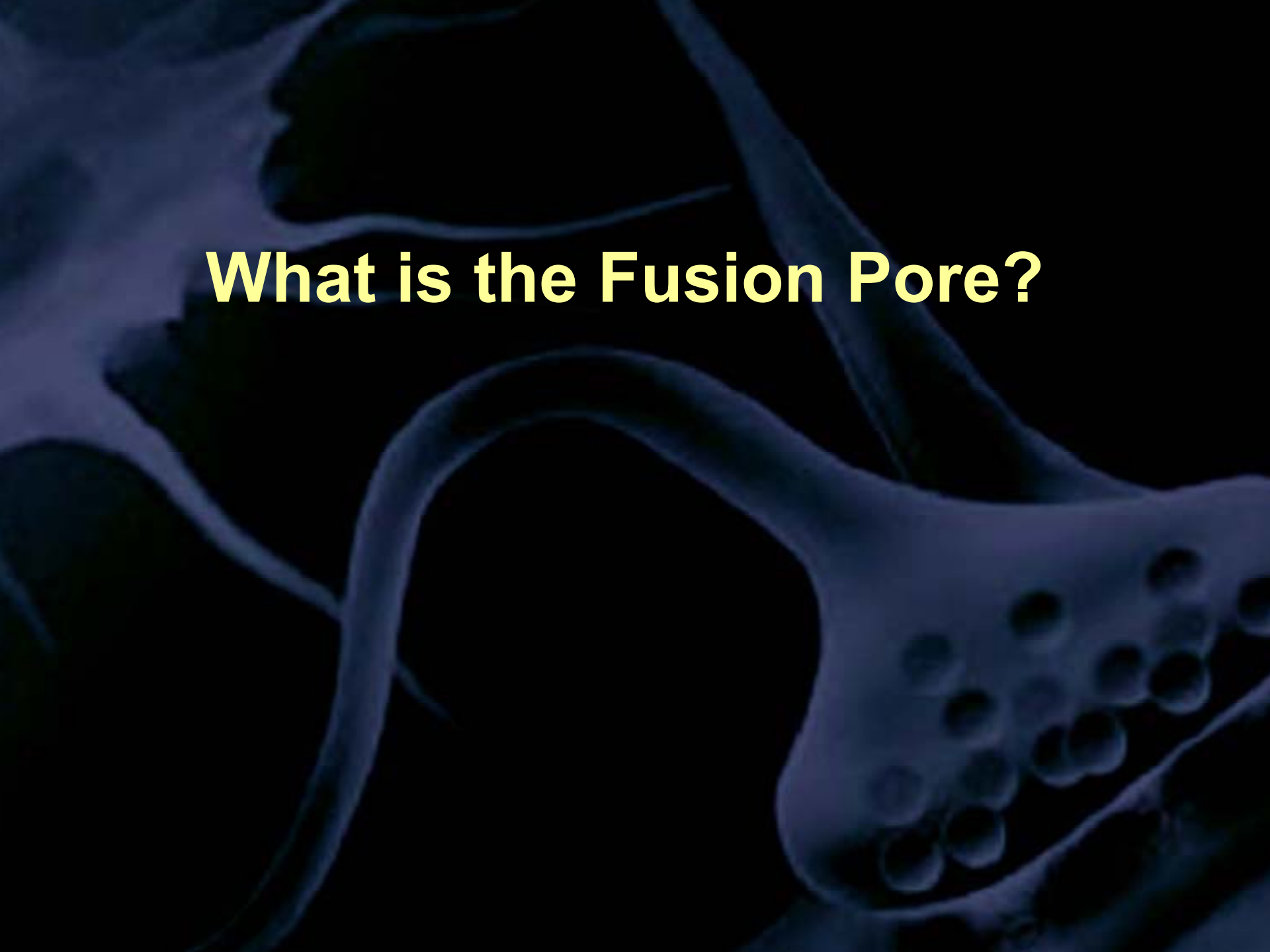

[Glu]
Functional flux
"AMPA-quiet" flux
restricted release
unrestricted release
I_{NMDA}
I_{AMPA}
I_{TOTAL}


[Glu]
Functional flux
"AMPA-quiet" flux
I_{NMDA}
I_{AMPA}
I_{TOTAL}
restricted release
unrestricted release
restricted release


Regulation
Implications for
Development of
Neural Networks

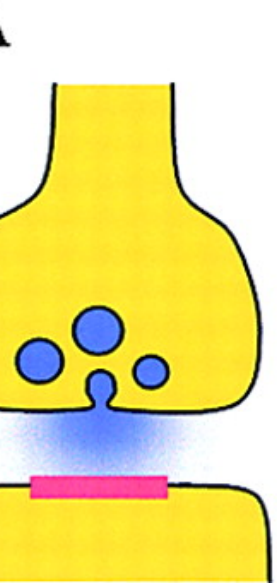

What is the Fusion Pore?


ATP
ADP
H+
NT
⑥
⑤
⑦
H+
NT
Brain Lunch (2-29-04).ppt
①
H+
NT
②
H+
NT
④
③
H+
NT
NT-transporter
V-ATPase
Vo V-ATPase
V1 V-ATPase
v-SNARE
t-SNARE

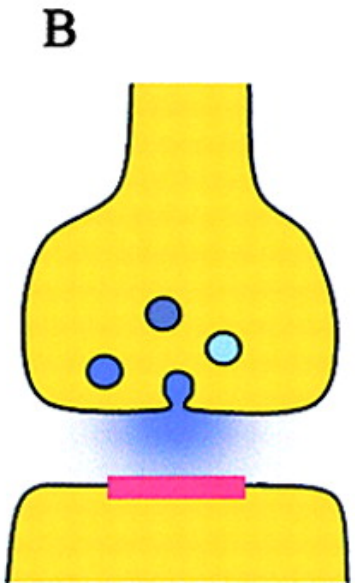

What is the Fusion Pore?


Summary

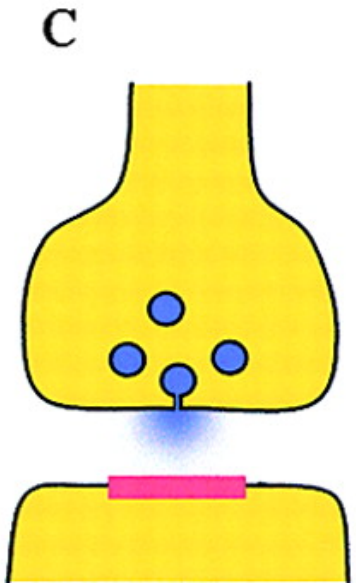
Possible Explanations for Variability in a Synapse's Quantal Amplitude



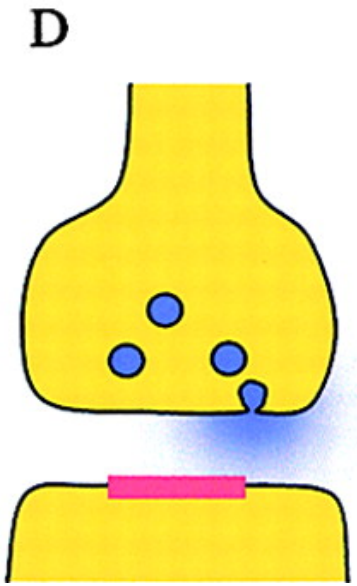
Non-
Uniform
Volume



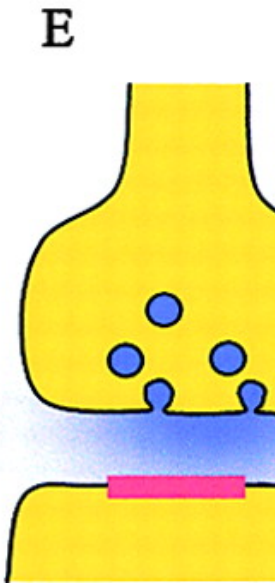
Non-
Uniform
Filling



Non-
Uniform
Flux



Non-
Uniform
Alignment
of
Release



Variable
Numbe
of
Vesicle
Fusing

Regulation of Signal Strength by Presynaptic Mechanisms

9.013 / 7.68: Core Class

Sheng Lectures
Presynaptic Mechanisms

Nathan Wilson