

In silico stochastic simulation of Ca^{2+} triggered synaptic release

Andrea Bracciali

Pierpaolo Degano

Dipartimento di Informatica

Università di Pisa

{braccia,degano}@di.unipi.it

Enrico Cataldo

Marcello Brunelli

Dipartimento di Biologia

Università di Pisa

{ecataldo,mbrunelli}@biologia.unipi.it

Models of the neural function

- The **functional capabilities** of the nervous system arise from the **complex organization of the neural network**
- Models are needed to understand
 - the ways in which neural circuits **generate behavior**,
 - the ways in which **experience alters** the functional properties of circuits and therefore their behaviour (plasticity/memory),
 - ... (and many other issues).
- (some) Key elements are
 - the intrinsic biophysical/biochemical properties of the individual neurons
 - the pattern of the synaptic connections amongst neurons
 - the physiological properties of synaptic connections

Models of the neural function

Focus:

1. A model of a pre-synaptic calcium triggered release
 - Synapses: points of functional contact between neurons
 - Chemical synapses: presynaptic action potentials cause chemical intermediary (neurotransmitters) to influence postsynaptic terminal
 - Chemical synapses are plastic: modified by prior activity

Models of the neural function

Focus:

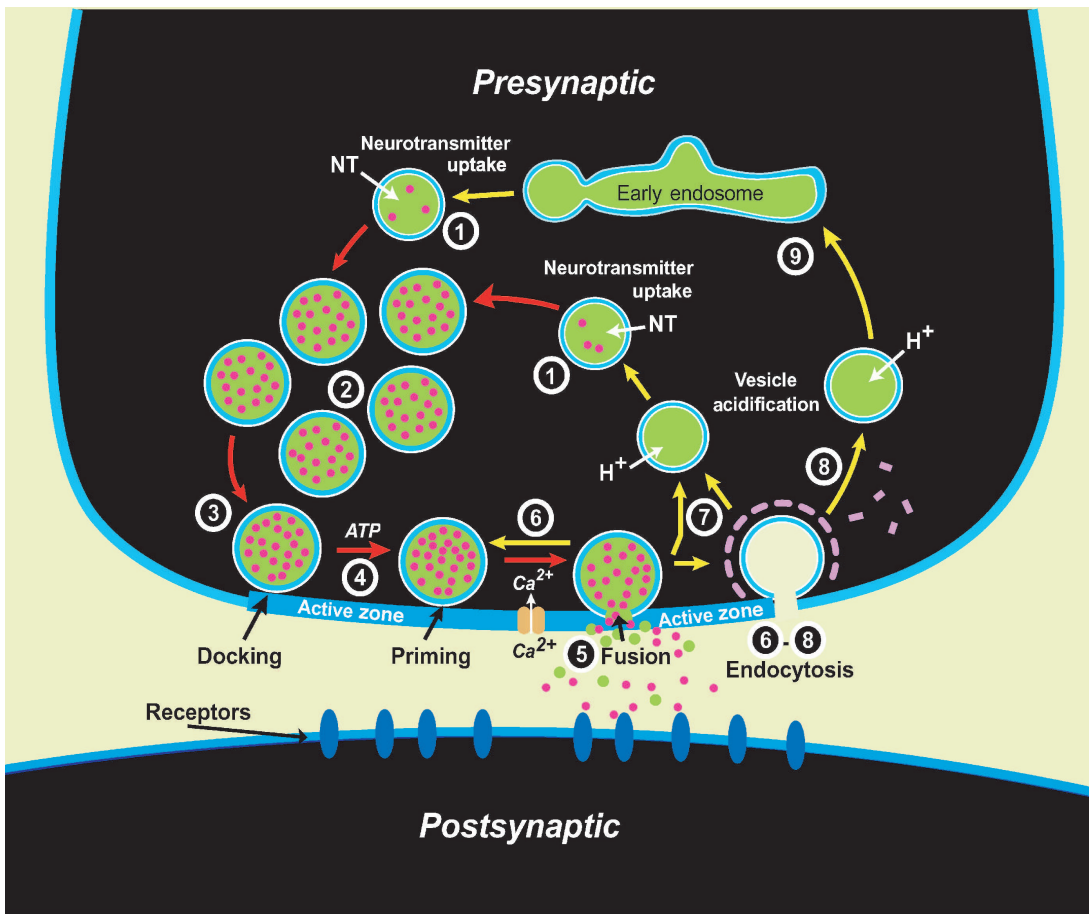
1. A model of a pre-synaptic calcium triggered release
 - Synapses: points of functional contact between neurons
 - Chemical synapses: presynaptic action potentials cause chemical intermediary (neurotransmitters) to influence postsynaptic terminal
 - Chemical synapses are plastic: modified by prior activity
2. A (first) stochastic model

Models of the neural function

Focus:

1. A model of a pre-synaptic calcium triggered release
 - Synapses: points of functional contact between neurons
 - Chemical synapses: presynaptic action potentials cause chemical intermediary (neurotransmitters) to influence postsynaptic terminal
 - Chemical synapses are plastic: modified by prior activity
2. A (first) stochastic model
3. A computational (process-algebra based) approach
 - formal
 - executable
 - compositional

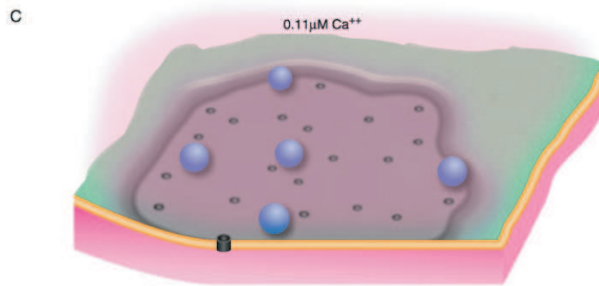
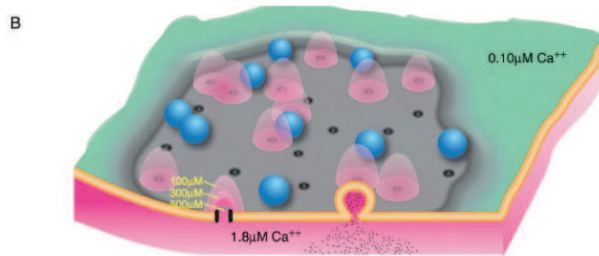
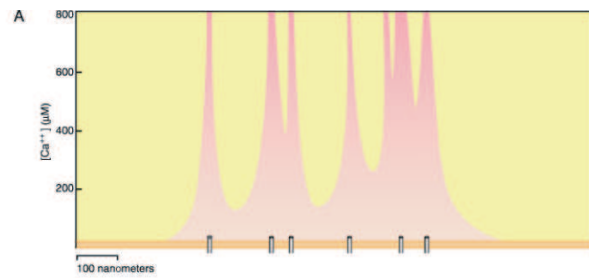
Presynaptic calcium triggered release



1. Calcium gradient
2. Vesicle activation (exocytosis)
3. Neuro-transmitter release
4. Calcium extrusion
5. Vesicle recharging
6. ...
7. Neuro-transmitter reception
8. ...

From: Sudhof TC. The synaptic vesicle cycle. *Annu Rev Neurosci.* 27:509-47, 2004.

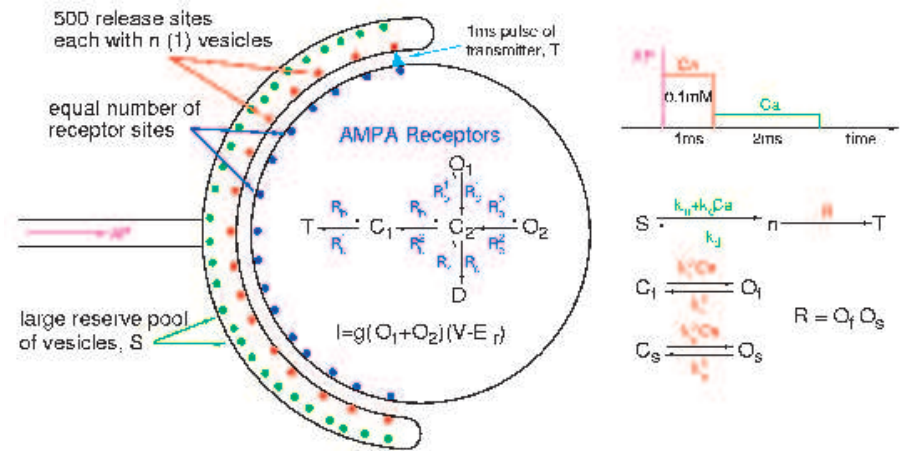
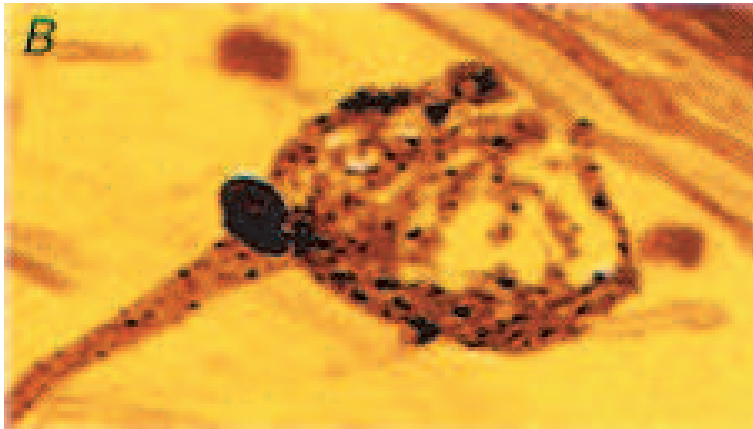
Presynaptic calcium concentration profile



- Microdomains of Calcium concentrations near open channels
- trigger the exocytosis of synaptic vesicles.
- Calcium concentration during release is *not homogeneous*
- unless subsequently in not effective concentrations.
- *Uncaging*: An experimental method capable to induce spatially homogeneous Calcium elevation in the presynaptic terminal. Applied to the synapse *Calyx of Held*.

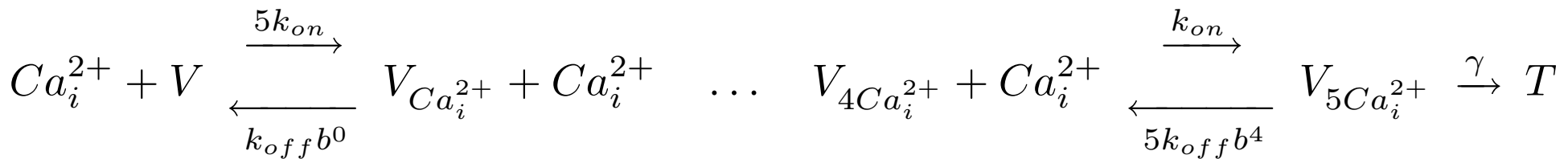
From: Zucker RS, Kullmann DM, Schwartz TL. Release of Neurotransmitters. In: From molecules to networks - An introduction to cellular and molecular neuroscience. Elsevier pp 197-244 2004.

Calyx of Held: deterministic model



From: www.cs.stir.ac.uk/bpg/research/syntran.html

By means of the uncaging method, a 5-step model of release has been defined based on concentrations, [SN00N]:



where $k_{on} = 9 \times 10^7 M^{-1} s^{-1}$, $k_{off} = 9500 s^{-1}$, $\gamma = 6000 s^{-1}$ and $b = 0.25$

have been defined by experimental fitting (complex).

Calyx of Held: stochastic model of calcium uncaging

Deterministic model: unsuitable for small concentrations and volumes, e.g.

- if $[Ca^{2+}] = 10 \mu M$, in a volume of $60 nm^3$ there is a single free ion;
- the assumption that binding of Ca^{2+} to vesicle does not affect the $[Ca^{2+}]$ concentration not adequate
(with vesicle diameter $\sim 17 - 22 nm$, $V = 60 nm^3$ few Ca^{2+} ions).

Calyx of Held: stochastic model of calcium uncaging

Deterministic model: unsuitable for small concentrations and volumes, e.g.

- if $[Ca^{2+}] = 10 \mu M$, in a volume of $60 nm^3$ there is a single free ion;
- the assumption that binding of Ca^{2+} to vesicle does not affect the $[Ca^{2+}]$ concentration not adequate
(with vesicle diameter $\sim 17 - 22 nm$, $V = 60 nm^3$ few Ca^{2+} ions).

Stochastic model: actual quantities and stochastic rate constants:

$$c = k \quad \text{1st order}$$

$$c = k/(NA \times V) \quad \text{2nd order}$$

Calyx of Held: stochastic model of calcium uncaging

Deterministic model: unsuitable for small concentrations and volumes, e.g.

- if $[Ca^{2+}] = 10 \mu M$, in a volume of $60 nm^3$ there is a single free ion;
- the assumption that binding of Ca^{2+} to vesicle does not affect the $[Ca^{2+}]$ concentration not adequate (with vesicle diameter $\sim 17 - 22 nm$, $V = 60 nm^3$ few Ca^{2+} ions).

Stochastic model: actual quantities and stochastic rate constants:

$$c = k \quad \text{1st order}$$

$$c = k/(NA \times V) \quad \text{2nd order}$$

Calyx [SF06CTR]:

- a vast “parallel” arrangement of active zones (3-700)
- each one with up to 10 vesicles
- clustered in groups of about 10 in a volume with a diameter of almost $1 \mu m$.
- action potential activates all the active zones.

Calyx of Held: stochastic model of calcium uncaging

Deterministic model: unsuitable for small concentrations and volumes, e.g.

- if $[Ca^{2+}] = 10 \mu M$, in a volume of $60 nm^3$ there is a single free ion;
- the assumption that binding of Ca^{2+} to vesicle does not affect the $[Ca^{2+}]$ concentration not adequate (with vesicle diameter $\sim 17 - 22 nm$, $V = 60 nm^3$ few Ca^{2+} ions).

Stochastic model: actual quantities and stochastic rate constants:

$$c = k \quad \text{1st order}$$

$$c = k / (NA \times V) \quad \text{2nd order}$$

Calyx [SF06CTR]:

- a vast “parallel” arrangement of active zones (3-700)
- each one with up to 10 vesicles
- clustered in groups of about 10 in a volume with a diameter of almost $1 \mu m$.
- action potential activates all the active zones.

A cluster of 10 active zone each one with 10 vesicles in $V = 0.5 \cdot 10^{-15} \text{ liter}$

Calyx of Held: stochastic model of calcium uncaging

The obtained stochastic model:

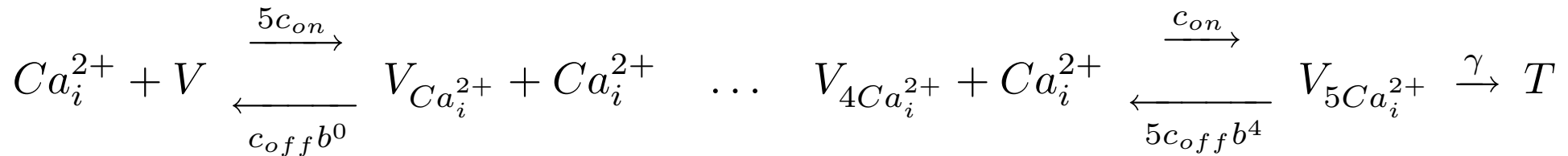
$$c_{on} = 9 \times 10^7 / (6.02 \times 10^{23} \times 0.5 \times 10^{-15}) \text{ s}^{-1} = 0.3 \text{ s}^{-1},$$

$$c_{off} = 9500 \text{ s}^{-1},$$

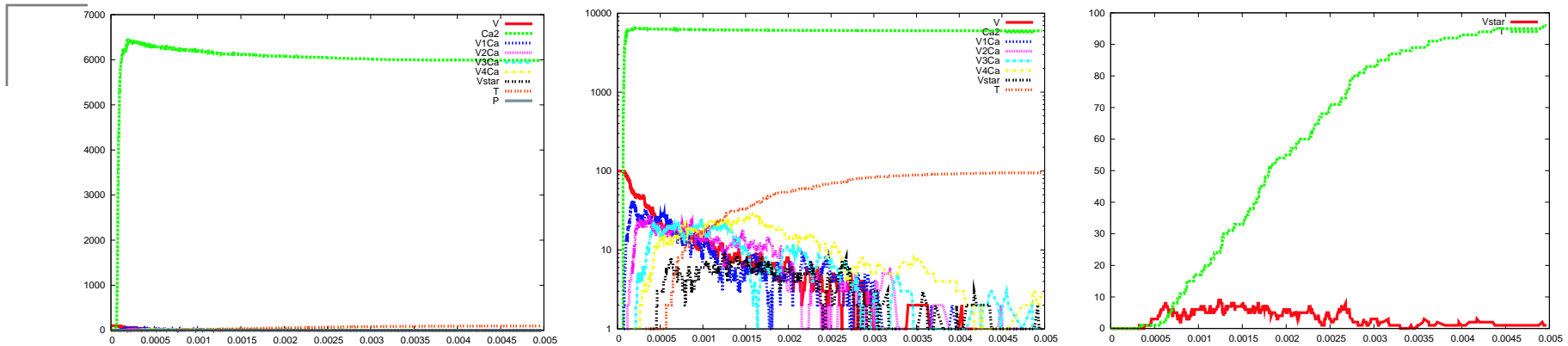
$$\gamma = 6000 \text{ s}^{-1}$$

$$b = 0.25.$$

Ca^{2+} ions: 300, 3000 and 6000, corresponding to molar concentrations $[Ca^{2+}]$ of 1, 10 and 20 μM .



Results

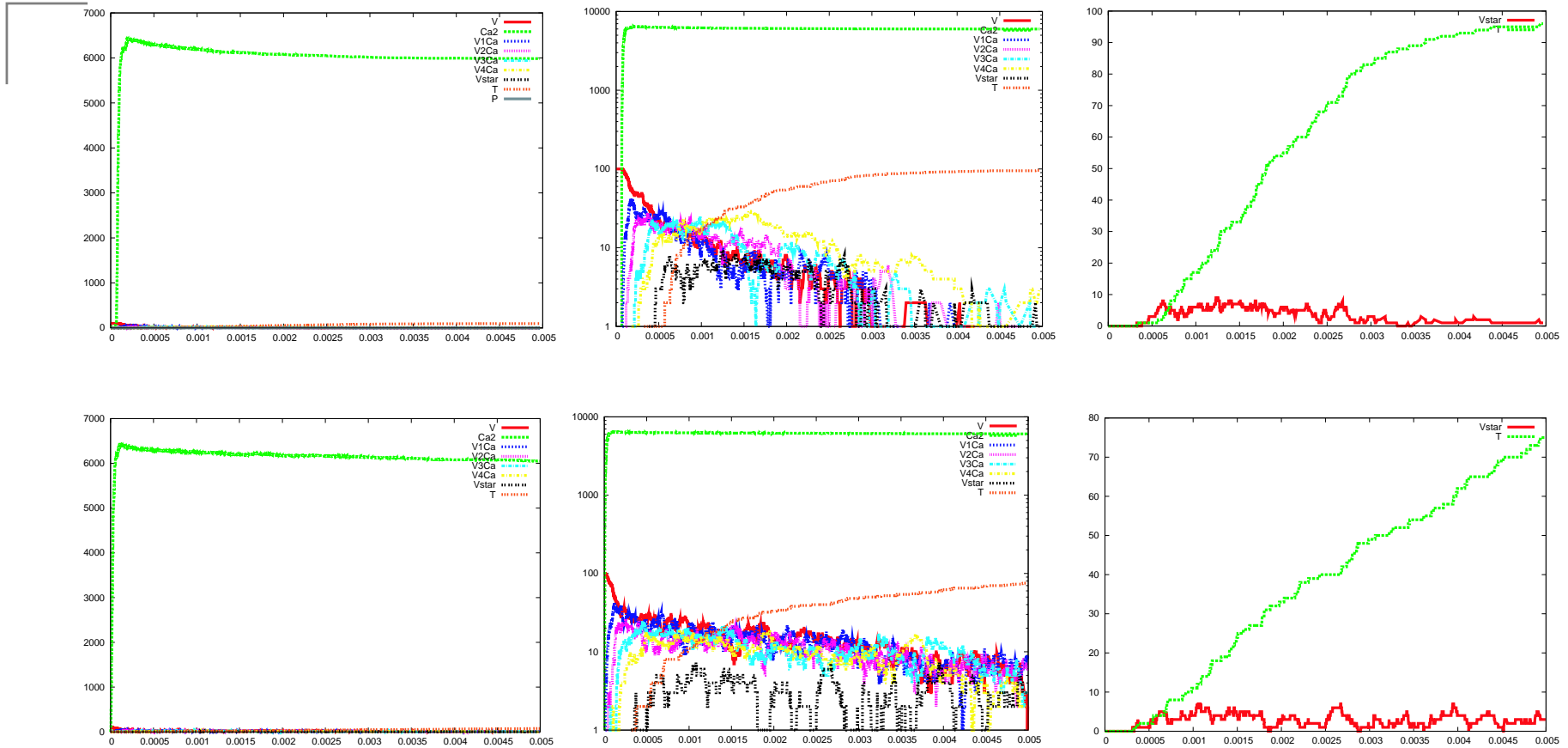


Step-like calcium uncaging, $V = 100$, $Ca^{2+} = 6000$.

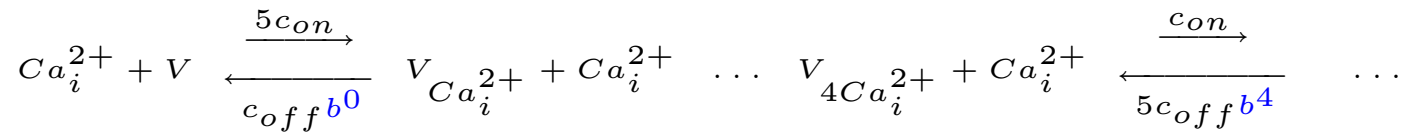
Results are coherent with literature, [SN00N], e.g.

- High sensitivity of vesicles to Ca^{2+} concentration
- Calyx of Held triggers vesicle release with concentrations lower than $100 \mu M$ (usual values for other synapses are $100 - 300 \mu M$). In the figure $6000 Ca^{2+}$ correspond to $20 \mu M$.

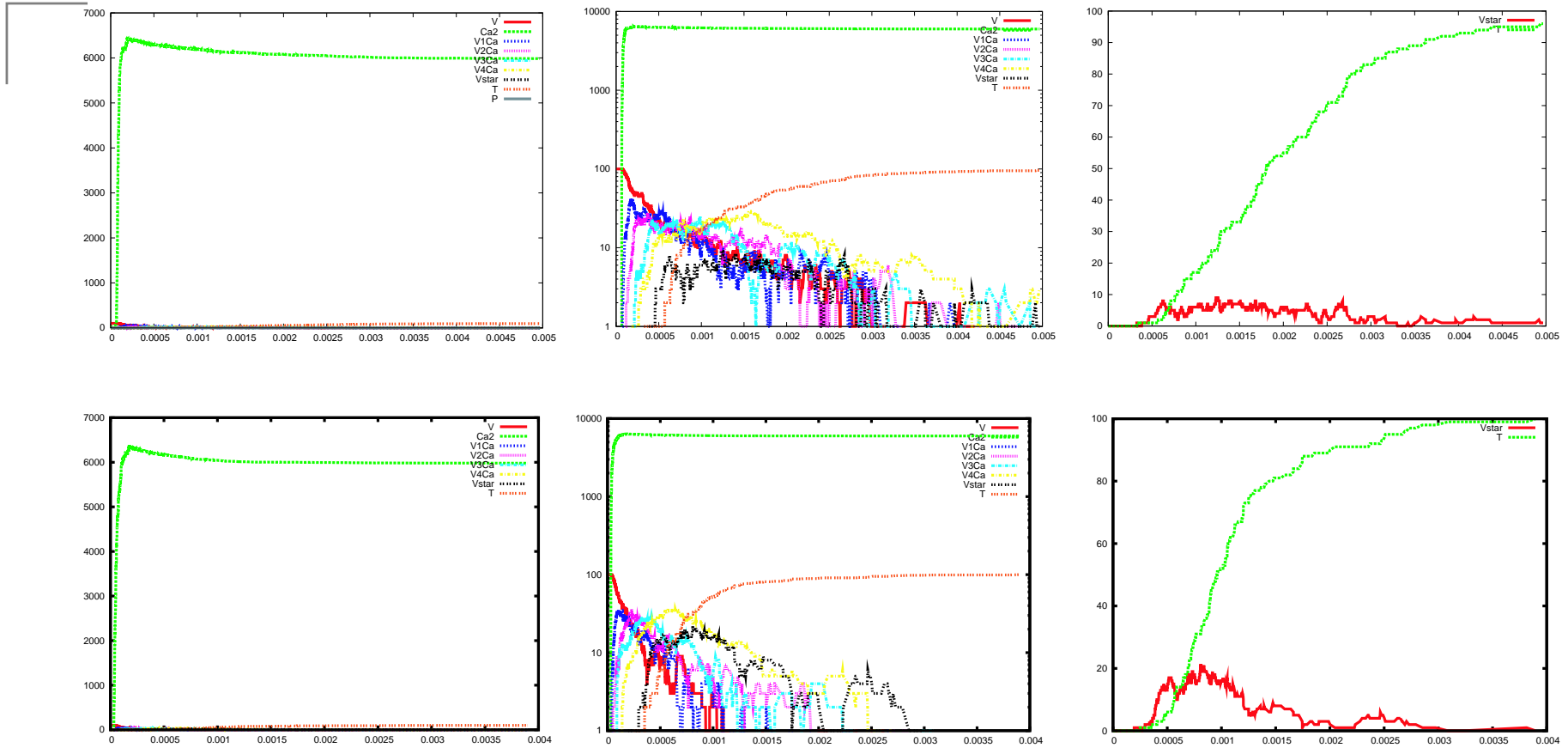
Results



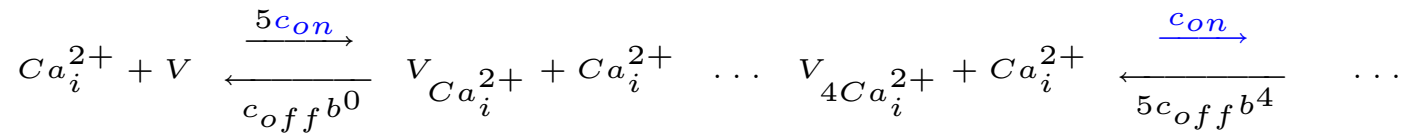
Variation of $b = 0.4$ (was $b = 0.25$): lower and more uniform release rate



Results



Variation of $c_{on} = 0.5$ (was $c_{on} = 0.3$): increase of the release rate



Cell as computation [RS02N]

A process-algebra approach [recall previous Degano's talk]

- interaction as communication
- processes [molecules, ions, proteins, vesicles, ...] defined as sequential, parallel, choice composition of communications
- stochastic reaction rates - associated to each communication - determine the next most probable interaction ...
- – Gillespie algorithm: $\text{rate} \times \# \text{ Processes ready to interact}$ –
- ... and the system evolves.

A dialect of Pi-calculus as modeling language

the SPiM interpreter [PC2004BC] as programming language/execution environment

Model implementation — overview

```
directive sample 0.005 1
```

```
val con5 = 1.5
```

```
val b = 0.25
```

```
val coff5 = 47500.0 * b * b * b * b
```

```
new vca@con5:chan
```

```
ca() = do ?vca;()
```

```
or ?v2ca;()
```

```
...
```

```
v() = !vca; v_ca()
```

```
v_ca() = do !bvca; v()  
or !v2ca; v_2ca()
```

```
Dv_ca() = ?bvca; ( ca() | Dv_ca() )
```

```
run 6000 of ca()
```

```
run 100 of v()
```

```
run 1 of (Dv_ca() | Dv_2ca() | ... )
```

Model implementation — overview

directive sample 0.005 1

val con5 = 1.5

val b = 0.25

val coff5 = 47500.0 * b * b * b * b

new vca@con5:chan

ca() = do ?vca;()

or ?v2ca;()

...

v() = !vca; v_ca()

v_ca() = do !bvca; v()
or !v2ca; v_2ca()

Dv_ca() = ?bvca; (ca() | Dv_ca())

run 6000 of ca()

run 100 of v()

run 1 of (Dv_ca() | Dv_2ca() | ...)

Initial set-up (creation of communication channels)

Model implementation — overview

```
directive sample 0.005 1
```

```
val con5 = 1.5
```

```
val b = 0.25
```

```
val coff5 = 47500.0 * b * b * b * b
```

```
new vca@con5:chan
```

```
ca() = do ?vca;()
```

```
or ?v2ca;()
```

```
...
```

```
v() = !vca; v_ca()
```

Second order reaction

```
v_ca() = do !bvca; v()  
or !v2ca; v_2ca()
```

```
Dv_ca() = ?bvca; ( ca() | Dv_ca() )
```

```
run 6000 of ca()
```

```
run 100 of v()
```

```
run 1 of (Dv_ca() | Dv_2ca() | ... )
```

Model implementation — overview

```
directive sample 0.005 1
```

```
val con5 = 1.5
```

```
val b = 0.25
```

```
val coff5 = 47500.0 * b * b * b * b
```

```
new vca@con5:chan
```

```
ca() = do ?vca;()
```

```
or ?v2ca;()
```

```
...
```

```
v() = !vca; v_ca()
```

```
v_ca() = do !bvca; v()  
or !v2ca; v_2ca()
```

```
Dv_ca() = ?bvca; ( ca() | Dv_ca() )
```

```
run 6000 of ca()
```

```
run 100 of v()
```

```
run 1 of (Dv_ca() | Dv_2ca() | ... )
```

First order reaction (via single, dummy processes)

Model implementation — overview

```
directive sample 0.005 1
```

```
val con5 = 1.5
```

```
val b = 0.25
```

```
val coff5 = 47500.0 * b * b * b * b
```

```
new vca@con5:chan
```

```
ca() = do ?vca;()
```

```
or ?v2ca;()
```

```
...
```

```
v() = !vca; v_ca()
```

```
v_ca() = do !bvca; v()  
or !v2ca; v_2ca()
```

```
Dv_ca() = ?bvca; ( ca() | Dv_ca() )
```

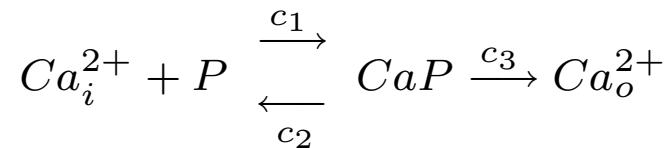
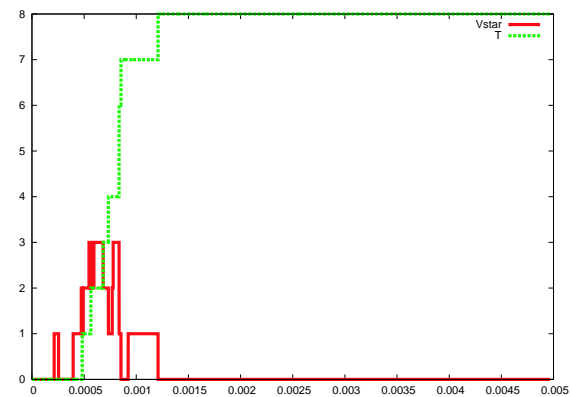
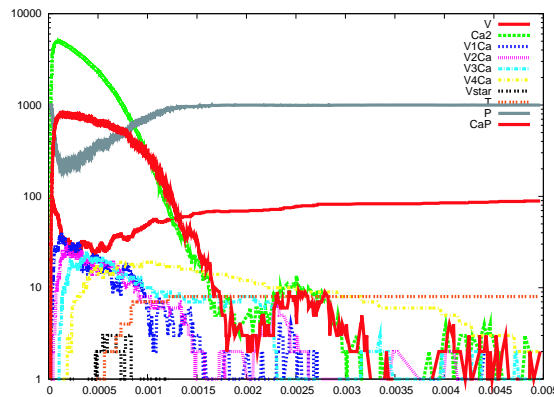
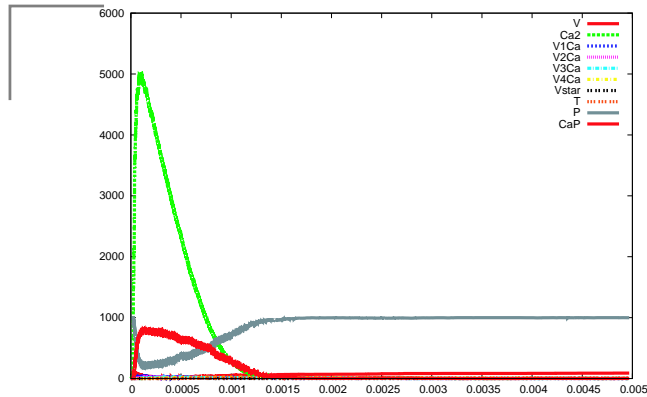
```
run 6000 of ca()
```

```
run 100 of v()
```

```
run 1 of (Dv_ca() | Dv_2ca() | ... )
```

Setting initial conditions (quantities)

A (modular) extension: Wave-like uncaging



```

...
val c1 = 8.00

new cp@c1:chan

ca() = do ?vca;()
      or ?v2ca;()
      ...
      or ?cp;()

p() = !cp; ca_p()

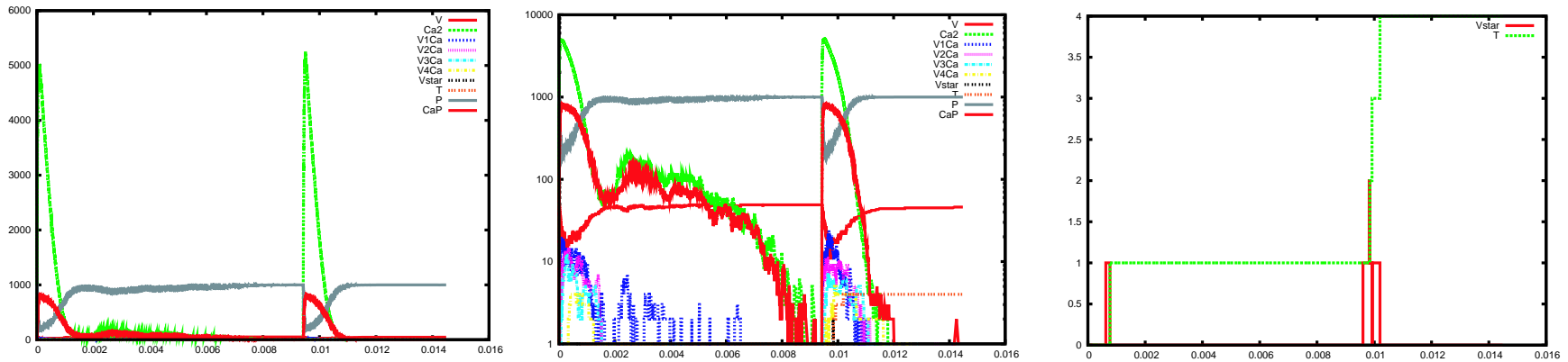
ca_p() = do !cpout; p()
        or !cpback; ( p() | ca() )
    
```

```

...
w( cnt : int ) =
    do delay@40000.0;
    if 0 <= cnt
    then ( 80 of ca() | 80 of w(cnt - 1) )
    else ()
    or !void; ()

run 1 of w(1)
run 1000 of p()
run 100 of v()
run 1 of (Dv_ca() | Dv_2ca() | ... )
    
```

Further ongoing developments [CMBS07]



- addressing plasticity: 2nd wave sensitive to residual calcium

- Moreover, compartmentalisation of the pre-synaptic terminal,

- ...

Conclusions

- A first stochastic model for (pre-)synaptic terminal
- A formal, executable, modular, verifiable computational model

Conclusions

- A first stochastic model for (pre-)synaptic terminal
- A formal, executable, modular, verifiable computational model
- further studying compartmentalisation

Conclusions

- A first stochastic model for (pre-)synaptic terminal
- A formal, executable, modular, verifiable computational model

- further studying compartmentalisation
- further pursuing the modular construction of one (more) synapses - post-synaptic terminal, ...

Conclusions

- A first stochastic model for (pre-)synaptic terminal
- A formal, executable, modular, verifiable computational model

- further studying compartmentalisation
- further pursuing the modular construction of one (more) synapses - post-synaptic terminal, ...
- studying linguistic and semantic suitable constructs, e.g. expressing locality, dynamically varying rates, ...

Conclusions

- A first stochastic model for (pre-)synaptic terminal
- A formal, executable, modular, verifiable computational model
- further studying compartmentalisation
- further pursuing the modular construction of one (more) synapses - post-synaptic terminal, ...
- studying linguistic and semantic suitable constructs, e.g. expressing locality, dynamically varying rates, ...
- devising qualitative/predictive analysis techniques

Conclusions

- A first stochastic model for (pre-)synaptic terminal
- A formal, executable, modular, verifiable computational model
- further studying compartmentalisation
- further pursuing the modular construction of one (more) synapses - post-synaptic terminal, ...
- studying linguistic and semantic suitable constructs, e.g. expressing locality, dynamically varying rates, ...
- devising qualitative/predictive analysis techniques
- making available results in an accessible (graphical) form.

End of the talk

References

[SN00N] Schneggenburger N. and Neher E. “Intracellular calcium dependence of transmitter release rates at fast central synapse”, Nature 46:889-893, 2000.

[SF06CTR] Schneggenburger N. and Fortsythe I.D. “The Calyx of Held”, Cell Tissue Res 326:311-337, 2006.

[RS02N] Regev A. and Shapiro E. “Cellular Abstractions: Cells as Computation”, Nature 491:343, 2002.

[CMSB] Bracciali A., Brunelli M., Cataldo E. and Degano P. “Expressive Models for Synaptic Plasticity”, CMSB 2007. To appear.

[PC2004BC] Philips A. and Cardelli L. “A Correct Abstract Machine for the Stochastic Pi-Calculus”, Bioconcurr 2004, ENTCS.

Andrea Bracciali
Pierpaolo Degano

Dipartimento di Informatica
Università di Pisa
{braccia,degano}@di.unipi.it

Enrico Cataldo
Marcello Brunelli

Dipartimento di Biologia
Università di Pisa
{ecataldo,mbrunelli}@biologia.unipi.it