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

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Abstract

Background: Research in life sciences is benefiting from a large availability of formal description techniques and analysis methodologies. These allow both the phenomena investigated to be precisely modeled and virtual experiments to be performed *in silico*. Such experiments may result in easier, faster, and satisfying approximations of their *in vitro/vivo* counterparts. A promising approach is represented by the study of biological phenomena as a collection of interactive entities through process calculi equipped with stochastic semantics. These exploit formal grounds developed in the theory of concurrency in computer science, account for the not continuous, nor discrete, nature of many phenomena, enjoy nice compositional properties and allow for simulations that have been demonstrated to be coherent with data in literature.

Results: Motivated by the need to address some aspects of the functioning of neural synapses, we have developed one such model for the case of the *calyx of Held* synapse. Our stochastic model has been drawn from a kinetic model based on ODEs developed in literature. Experiments have confirmed the coherence of the two models. Our model overcomes some limitations of the kinetic one and, to our knowledge, represents the first process calculi based model of a presynaptic terminal. We have improved the expressiveness of the model, e.g. by embedding easy controls of element concentration time course. A sensitivity analysis has been performed for the basic and enhanced model with results that may help clarify the dynamics of vescicles and reaction rates.

Conclusions: The developed model points to specific presynaptic mechanisms. These can be further analysed, for instance by studying the presynaptic behaviour under repeated activations. Further models can be designed for other brain elements within the same framework. Taking advantage of the compositionality of our approach, one can assemble a more detailed neural model. In the long term, we are interested, in particular, in addressing models of synaptic plasticity, i.e. activity dependent change mechanisms, which are the bases of memory and learning processes.

More on the computer science side, we plan to follow some directions for improving the underlying computational model and the linguistic primitives it provides as suggested by the experiments carried out, e.g. by introducing a notion of (spatial) locality.

Background

During the last decades, the development of high-throughput technologies has produced a huge amount of information in the field of neurobiology, requiring the utilization of mathematical modeling to describe the complex dynamics of the biological processes and stimulating new collaborations among biologists, physicists and computer scientists [1, 2]. The building blocks of neural systems are the neurons, which are specialized eukaryotic biological cells able to communicate with each other at highly specialized contact sites called synapses. In general, each neuron consists of a somatic cellular body, on which a variable number of thin elongated structures called dendrites converge and from which a long single structure called axon emerges, branching in several synaptic terminals. The synaptic terminals of the transmitting neuron (the presynaptic element) send signals by releasing chemical molecules (neurotransmitters) to the dendritic part of the receiving neuron (postsynaptic term) [3].

The synapses are the places of functional contacts between neurons, where the information is stored and transmitted from one to another neuron. Synaptic transmission is a complex process not completely understood: "Our current knowledge concerning synaptic transmission in neuronal networks of the brain is comparable to a puzzle in which most of the pieces are still missing" [4]. Current knowledge on synapses is based on the analysis of a limited number of experimental synaptic models, which were chosen for their experimental accessibility.

One aspect of the synaptic transmission concerns the involvement of calcium ions in the presynaptic terminal control of transmitter release, which was hypothesized many years ago and more recently demonstrated [5]. The neurotransmitter release is mediated

by exocitosis of synaptic vescicles (small elements containing the neurotransmitters) located at the presynaptic so-called active zone [6]. The electrical signals (action potentials) arriving at the synaptic terminal induce the opening of the Ca^{2+} channels. The transient elevation of the internal Ca^{2+} concentration in the presynaptic terminal triggers synaptic vescicle exocitosis, and hence the neurotransmitter release (calcium-triggered-release hypothesis). Interestingly, chemical messengers (intracellular) and modulators (extracellular) regulate the relationship between action potential and release in a synaptic terminal, which is also altered by the repeated activity. All these things make the presynaptic terminal a kind of computational unit, which changes its output based on its previous activity and ongoing modulation.

It must be noted that the Ca^{2+} signals were unaccessible to direct measurements up to few years ago. Theoretical and functional studies have suggested that calcium acts on presynaptic vescicles by a local huge and short-lived elevation of its concentration. The locality and rapidity of the concentration variation render the study of this phenomenon not approachable with the conventional microscopic imaging techniques. Among the methods envisaged to overcome this limitations, one very fruitful is the so-called reverse approach, in which Ca^{2+} uncaging is induced in the presynaptic element. The uncaging method induces spatially homogeneous Ca^{2+} elevation, implying that measuring the Ca^{2+} fluorescent indicator gives an indication of the real Ca^{2+} sensed by the vescicles. This experimental method has been applied to the study of the large synapse of the auditory tract of the central nervous system, called calyx of Held. Moreover, it has been possible to build a minimal kinetic model for the process of the Ca^{2+} triggered vescicle release. Also, one can infer local Ca^{2+} signal waveform which is compatible with the experimental data on the time course and amplitude of release [7,8].

Most of the models treating the calcium triggered release issues present some methodological limitations. These models, and among them the calyx of Held model, use differential equations to describe the time course of $[Ca^{2+}]$, the Ca^{2+} concentration ($mole \times liter^{-1}$) interacting with the synaptic vescicles. This approach implies that $[Ca^{2+}]$ is continuous, while it is clearly not [9]. For example, with a Ca^{2+} concentration of 10 μM in a volume of 60 nm^3 there is a single free ion. Another common assumption is that the binding of the Ca^{2+} to the release sensor of the vescicle does not affect the $[Ca^{2+}]$ concentration [9]. Also this assumption is not properly adequate: considering that the dimensions of the vescicle diameters range in the interval 17-22 nm, in a volume of 60 nm^3 there could be few Ca^{2+} ions, and when some of them bind to the vescicle sensors, the number of calcium ions could change substantially. In these cases, the use of a

stochastic approach appears to be much more appropriate.

The deterministic and the stochastic approach are the main formalisms to mathematically describe the time evolution of a chemical system which is spatially homogeneous. In the first approach, the time evolution of the average molecular populations of the chemical species present in a given volume are described by a system of coupled, first order differential equations, known as reaction rates equations. The second approach must be utilized when the fluctuation in the molecular population levels are important, for example when the numbers per unit volume of the molecular species involved are small. In the stochastic approach, the system is described by the so-called "master equation", which usually is intractable. A stochastic simulation algorithm has been proposed in [10] to overcome these difficulties.

Recently, stochastic techniques have been also adopted in computer science to model quantitative aspects of interactive systems within concurrency theory. Concurrency theory aims to model the behaviour and the structure of systems composed of autonomous computational entities, which dynamically interact one with another, possibly reconfiguring the system itself. At the beginning, stochastic models have been used to study performance/time related properties, e.g. [11]. The strong analogies between concurrent and living systems, "cells as computation" [12], has fostered the development of Systems Biology [13,14], a systemic approach to living system modeling. According to this metaphor, cells, molecules and biological "active" components, i.e. those capable of exhibiting a behaviour, are assimilated to computer processes, the computational units of a concurrent software system. Then, biological interaction corresponds to process communication. By communicating, processes may exchange information or synchronise themselves, i.e. they interact one with another. Finally, a biological experiment, or biological activity in general, has then a direct correspondence into computation. That is, biological processes can not only be simulated by in silico experiments, but also it is possible to formally reason about their computational models and infer properties of interest. Process calculi are a formalism to describe such models: systems are compositionally described in terms of suitable abstractions of their component behaviour. Several process calculi whose "operators" are oriented to describing different aspects of biological interaction, have been proposed e.g. [15–18]. Some of these calculi have been equipped with stochastic semantics in order to study the quantitative evolutions of systems, e.g. [16, 19–21]. This approach benefits from conjugating the abstract and compositional algebraic models, the possibility of precisely describe their semantics and formally reasoning about them, and the quantitative
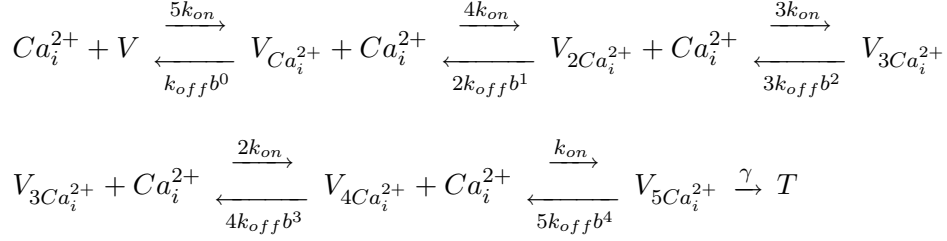
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analysis provided by stochastic semantics. Executable implementations of the calculi and analysis tools are provided.

In this context, motivated by addressing some aspects of the functioning of neural synapses, we have developed a stochastic model of the calcium triggered release in the calyx of Held synapse. Our work starts from a deterministic model presented in [7], from which we have derived a suitable stochastic model. This has subsequently been formalised by means of a representation based on Pi-calculus [22], in which the behaviour of Ca^{2+} and vescicles has been described and composed to form the synaptic terminal. Model development has benefited from the above mentioned features, like modular design, abstract representation of the component functioning and stochastic interpretation of the system dynamics. Then, in silico experiments have been carried out by means of the stochastic Pi-calculus simulator SPiM [23], which represents one of the most complete and expressive simulation environment for stochastic calculi currently available. Obtained results are coherent with those from the deterministic models and with others in literature. Moreover, the expressiveness of the model has allowed us to easily modulate calcium waves, helping us to identify some critical points of the model and the parts of the overall process that seem to play a more relevant role. Beyond the experiments here reported, our approach appears to be interesting, in the long term, for the development of more comprehensive stochastic models of synaptic functioning. Moreover, the experiments carried out so far have been given interesting insights about the linguistic mechanisms that could usefully extend the expressiveness of the calculus.

The model and its development are described in the next section Results and Discussion, where we also report on some of the experiments done. Perspectives of the approach are discussed in Conclusions.

Results and Discussion

We have applied a stochastic approach, based on the algorithm introduced in [10], to describe the calcium triggered release mechanisms studied in the model system of the synapse calyx of Held. Our starting point was the following phenomenological kinetic model, described in [7]. Vescicles are activated through a process of five calcium binding steps and eventually released with a given kinetic rate constant. This process is determined by a cooperativity factor b. Vescicles are represented as V, the intracellular calcium as Ca_i^{2+}, the released vescicles as T and the kinetic rate constant relative to vescicle release is $\gamma = 6000\ s^{-1}$. The values of the other constants of the model are $k_{on} = 9 \times 10^7\ M^{-1}s^{-1}$, $k_{off} = 9500\ s^{-1}$ and $b = 0.25$:
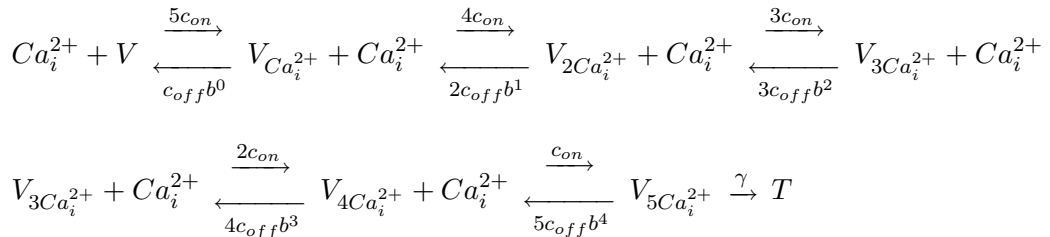
$$Ca_i^{2+} + V \underset{k_{off}b0}{\overset{5k_{on}}{\rightleftharpoons}} V_{Ca_i^{2+}} + Ca_i^{2+} \underset{2k_{off}b1}{\overset{4k_{on}}{\rightleftharpoons}} V_{2Ca_i^{2+}} + Ca_i^{2+} \underset{3k_{off}b2}{\overset{3k_{on}}{\rightleftharpoons}} V_{3Ca_i^{2+}}$$

$$V_{3Ca_i^{2+}} + Ca_i^{2+} \underset{4k_{off}b3}{\overset{2k_{on}}{\rightleftharpoons}} V_{4Ca_i^{2+}} + Ca_i^{2+} \underset{5k_{off}b4}{\overset{k_{on}}{\rightleftharpoons}} V_{5Ca_i^{2+}} \overset{\gamma}{\rightarrow} T$$

The parameters of the kinetic model were computed by a fitting of the experimental data. These had been obtained by means of elevating the intracellular presynaptic $[Ca^{2+}]$ in a controlled, homegeneous and step-like manner [7].

Step-like calcium uncaging

The previous equations have been transformed by utilising the relationship between the stochastic rate constants (c) and deterministic rate constants (k) [24]. For reactions of the first order, $c = k$. For reactions of second order, the relationship becomes $c = k/(NA \times V)$, where NA represents the Avogadro's number and V the volume of the reaction. Hence, in order to determine the values of the stochastic rate constants, we need to estimate the value of V. Spatially, the calyx of Held is organized as a "parallel" arrangement of a large array of active zones, ranging from 300 to almost 700 [25]. Active zones, each containing up to 10 vescicles, are clustered in groups of about 10 of them, in a volume having a diameter of almost 1 μm. Each action potential activates all the active zones. Such particular morpho-functional organization of this synapse has allowed us to model a subunit of the presynaptic element, consisting of a cluster of 10 active zone, each containing 10 vescicles, in a volume of 0.5 10 $^{-15}$ *liter*. With this volume estimate, we have obtained the following values for the stochastic constants:
$c_{on} = 9 \times 10^7 / (6.02 \times 10^{23} \times 0.5 \times 10^{-15}) \ s^{-1} = 0.3 \ s^{-1}$, $c_{off} = 9500 \ s^{-1}$, $\gamma = 6000 \ s^{-1}$ and $b = 0.25$, and the following numbers of Ca^{2+} ions: 300, 3000 and 6000, corresponding to molar concentrations $[Ca^{2+}]$ of 1, 10 and 20 μM. The equations of the stochastic model are (note that, according to [7], we represent vescicle fusion as the production of the released vescicle T, abstracting, for simplicity, from the actual quantities involved in the final step of the release process):

$$Ca_i^{2+} + V \underset{c_{off}b0}{\overset{5c_{on}}{\rightleftharpoons}} V_{Ca_i^{2+}} + Ca_i^{2+} \underset{2c_{off}b1}{\overset{4c_{on}}{\rightleftharpoons}} V_{2Ca_i^{2+}} + Ca_i^{2+} \underset{3c_{off}b2}{\overset{3c_{on}}{\rightleftharpoons}} V_{3Ca_i^{2+}} + Ca_i^{2+}$$

$$V_{3Ca_i^{2+}} + Ca_i^{2+} \underset{4c_{off}b3}{\overset{2c_{on}}{\rightleftharpoons}} V_{4Ca_i^{2+}} + Ca_i^{2+} \underset{5c_{off}b4}{\overset{c_{on}}{\rightleftharpoons}} V_{5Ca_i^{2+}} \overset{\gamma}{\rightarrow} T$$

We have performed a series of simulations which confirmed the results obtained with the deterministic model: high sensitivity of vescicles to calcium concentrations [7]. Moreover, while several other synapses require a calcium concentration in the range of 100-300 μM for triggering vescicle releases [6], it is known that the local calcium concentration can be much lower than 100 μM in the calyx of Held [7]. Our results also confirm this result, by showing that concentrations as low as 1, 10 and 20 μM are able to deplete the releasable pool in a few milliseconds. In the following we will report a sample of our results. Each one consists of three pictures reporting the time course of Ca^{2+}, the logarithmic scale of it, which shows intermediate states of calcium binding, and a focus on vescicle activation (Vstar) and release (T), respectively. Figure 1 shows simulation results for the following parameters: $V = 100$; $Ca^{2+} = 6000$; $c_{on} = 0.3$; $c_{off} = 9500$; $\gamma = 6000$; $b = 0.25$. It can be observed that the pool of vescicles is 80% depleted within 3 ms, coherently to the experimental findings [7].
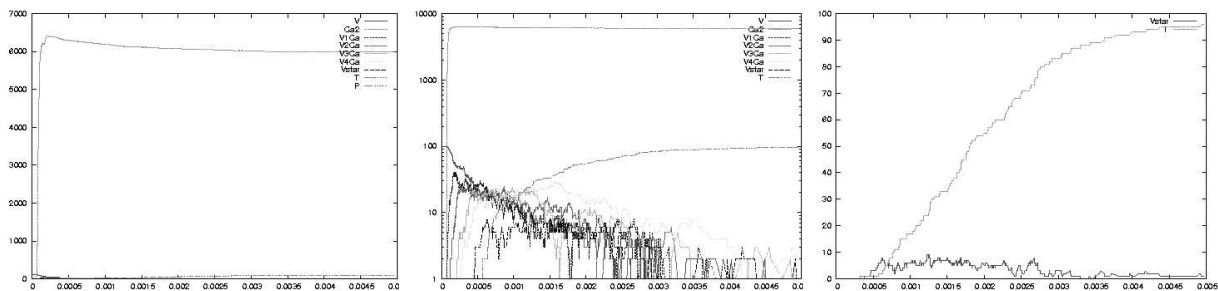

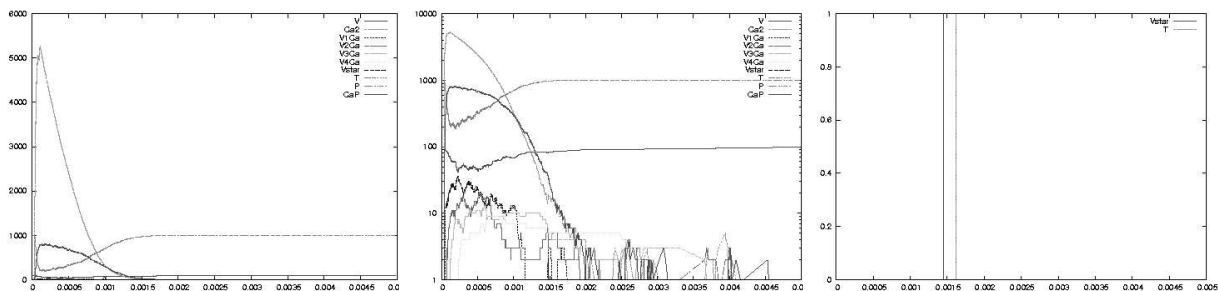Figure 1: Step-like calcium uncaging (V=100; Ca=6000; C$_{on}$=0.3; C$_{off}$=9500; γ=6000; b=0.25).

Figure 2: Wave-like calcium uncaging (V=100; Ca=6000; C$_{on}$=0.3; C$_{off}$=9500; γ=6000; b=0.25).

Wave-like calcium uncaging

The experiments and models on Ca^{2+} uncaging [7] showed a high sensitivity of vescicle release in response to a uniform elevation of $[Ca^{2+}]$ in the range 10 μM. It was not clear whether very short $[Ca^{2+}]$ elevations are sufficient to induce a release similar to that induced during an action potential. A recent experimental work [26] has addressed this

issue. A spatially uniform and very rapidly decaying $[Ca^{2+}]$ transient, obtained by Ca^{2+} uncaging in the presence of added Ca^{2+} buffers, was induced in the presynaptic element of a calyx of Held synapse. This short-lived elevation of calcium concentration has been revealed to be able to trigger vescicle release. We have introduced in our model a simple mechanism of calcium extrusion utilized in a previously developed model [27], adapting the rate constants to fulfill our needs:

$$Ca_i^{2+} + P \xrightarrow{c_1} \underset{c_2}{\overset{}{\rightleftharpoons}} CaP \xrightarrow{c_3} Ca_o^{2+}$$
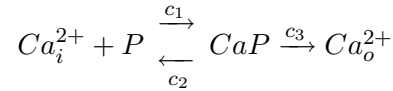
where Ca_o^{2+} is the extruded calcium, P is an abstraction of a pumping mechanism, $c_1 = 8 \ s^{-1}$, $c_2 = 25 \ s^{-1}$ and $c_3 = 10000 \ s^{-1}$. We have obtained a simulated calcium wave lasting about 1 ms and with a half width 0.5 ms, conforming to the experimental requirements [26]. On the left side of Figure 2, it can be seen a calcium wave lasting about 1 ms with a half width of about 0.5 ms and a peak value of about 6000, corresponding to a peak calcium concentration of 20 μM. In the right side of the same figure, the release of one vescicle can be observed. Considering that a whole presynaptic element can be made of about 70 of our simulated clusters, this implies that a single action potential, and accordingly a single calcium wave, is able to release a significant amount of vescicles. This is also along the line of the experimental findings [7, 8, 26].

Bio-processes

In order to provide a grasp of the synapse formal models based on Pi-calculus, we briefly sketch some of its parts. The language models interaction as pairs of input/output actions over the same communication channel (?c/!c). These atomic actions can be composed in a sequence (!c;!c) or in alternative choices (?c or ?d) so as to form a process (p()= ...). Processes can run in parallel (p()|q()). Figure 3 reports some excerpts from our model. Initially, a command sets the duration of the simulation (here 0.005s), then some of the stochastic parameters are defined. Communication channels can be (dynamically) created by means of the new command and have associated a stochastic rate (this and the current quantities of reactants determine the probability of a reaction "happening" through the channel). One calcium ion (ca()) can interact with a vescicle (v()) over the channel vca with rate con5=1.5 (beyond being able to do other things). After this communication, ca() disappears (it becomes the null process ()) and v() becomes v_ca(), which represents the binding of the two. This realises a second order reaction. First order reactions are modeled as interactions with a single dummy molecule (so as not to alter stochastic dynamics). For instance, v_ca() can then either accept

other calcium bindings or degradate back to an unbound vescicle by communicating with the dummy `Dv_ca()` through `bvca`. `Dv_ca()` restores `ca()` and itself.

```
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;()
        ...
        or ?cp;()

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

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

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

Figure 3: The calyx of Held SPiM code.

So far, one can easily realise how the system has been described by specifying simple atomic behaviours, basically corresponding to chemical reactions, and then composing them together. The parametric process `w(cnt:int)` allows us to suitably modulate the calcium wave. After a stochastic delay, if its parameter is positive then it replicates 80 copies of itself, with the parameter decreased, and 80 parallel copies of `ca()`, otherwise it dies. This realises an exponential growth, which can be controlled in its rapidity and quantity by the delay rate and the parameter. Finally, the initial state can be populated specifying how many molecules of each specie are present (one wave, 1000 pumping molecules, 100 vescicles and the needed dummy molecules). The SPiM produces the dynamics reported in the figures.

Parameter sensitivity analysis

For both models (step-like and wave-like calcium), we performed a parameter variation study (sensitivity analysis). The sensitivity analysis is a standard procedure in modeling and is performed with the aim of evaluating the robustness of the model, i.e. the tolerance of the release process to parameter variation, and to identify, if any, critical parameters. We have run simulations for different values of the number of vescicles (reference value 100, other values: 10, 50, 200 and 500), the number of calcium ions Ca^{2+} (reference values 300, 3000 and 6000, other values: 12000, 18000, 24000), the stochastic coefficients c_{on} (reference value 0.3, other value: 0.1, 0.2, 0.4 and 0.5), c_{off} (reference value 9500, other values: 5500, 7500, 11500 and 13500), b (reference value 0.25, other values: 0.1, 0.2, 0.3 and 0.4) and γ (reference value 6000, other values: 2000, 4000, 8000
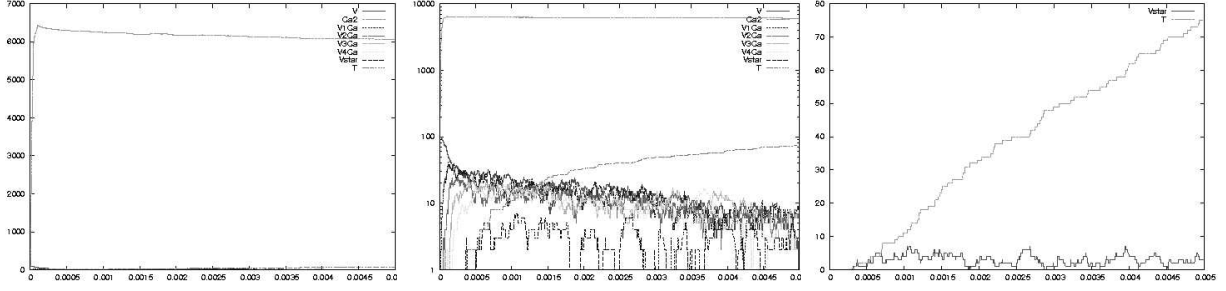
Figure 5: Parameter variation: b = 0.4 (step-like case).

and 10000). One of the results of this analysis is that the forward coefficient c_{on} seems to have a critical role: higher values of this coefficient correspond to a faster release, in the step-like case, and to a switch from no-release to a consistent release, in the wave-like case. Due to lack of space, a selection of analysis results is reported below.
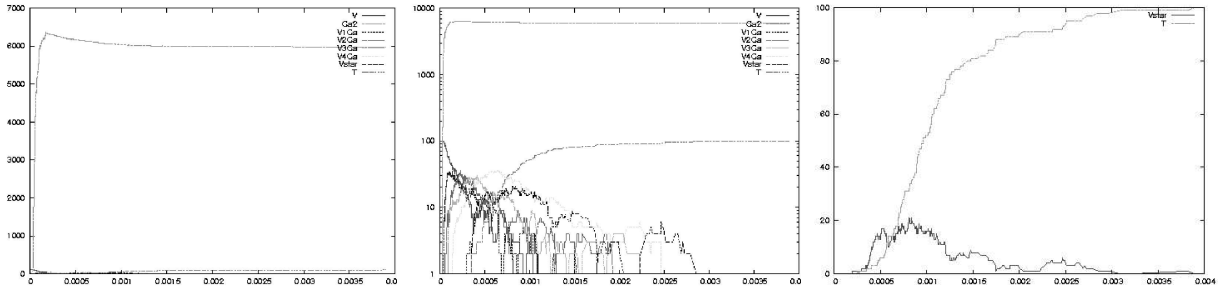

Figure 4: Parameter variation: c$_{on}$ = 0.5 (step-like case).

For the step-like case, Figure 4 and 5 report runs for $c_{on} = 0.5$ and $b = 0.4$, respectively. In Figure 4 we observe a high increase of the release rate, while in Figure 5 we observe a lower and more uniform release rate, both compared to the reference case (Figure 1). A simple variation of these coefficients changes the dynamics of the release processes in an unpredictable manner, producing in one case a variable and decreasing rate of release (see the change in the slope of the curve in between 1 and 1,5 ms, Figure 4) and in the other case a stationary rate of release. This kind of experiments are of interest when addressing the problem of the variations in the release rate, one of the still obscure phenomena which have been observed about vescicle release. These variations have been explained by the recruitment of new vescicles within the same active zone or by a different sensitivity to calcium ions of the vescicle belonging to same cluster. Our kind of analysis might give some contributions to the debate on the interpretation of these controversial experimental data [7, 8, 26].

For the wave-like case, Figure 6 and 7 report runs for $c_{on} = 0.1$ and $c_{on} = 0.5$, respectively. It is interesting that in Figure 6 no release appears, whereas in Figure 7 a significant release (up to 8 vescicle in 1 ms) can be observed. These two different

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behaviours are obtained with the same (shape, peak value and duration) calcium wave. Hence the same very short lived calcium wave can be not enough or, on the contrary, even too much for inducing vescicle release. This is certainly a significative findings for the interpretation of recent experimental results, in which most of the attention has been directed, so far, only to the issue of calcium concentration variation [26]. A modulation of this coefficient might be one of the way in which the synapse can store information, increasing or reducing the vescicle release rates (and ultimately it might be relevant for memory and learning).
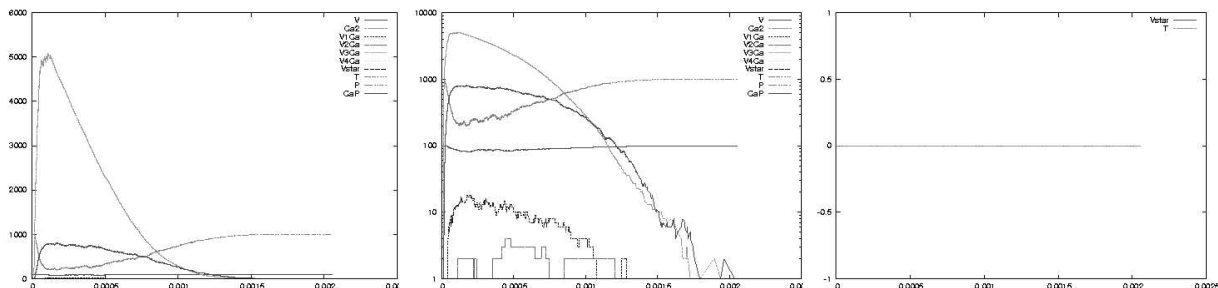

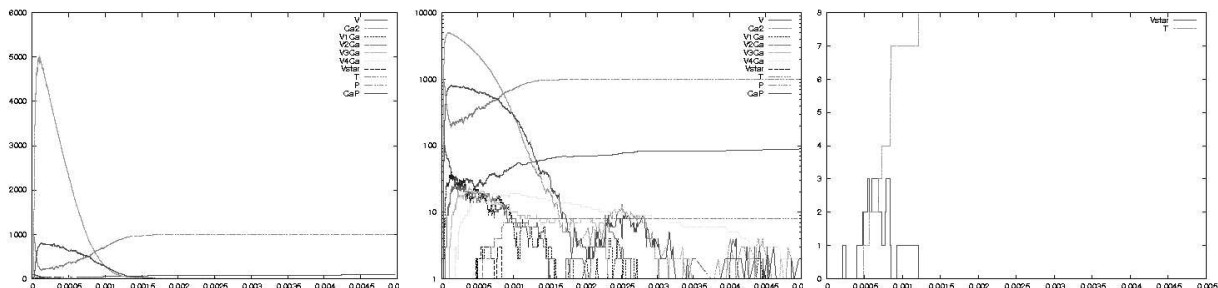Figure 6: Parameter variation: $c_{on} = 0.1$ (wave-like case).

Figure 7: Parameter variation: $c_{on} = 0.5$ (wave-like case).

Conclusions

The presented results are encouraging about the validity of the stochastic approach in studying the synaptic processes, which consist of many discrete-like events and involve arrays of vescicles and hundreds of different molecules. Many of these molecules have roles in the process of synaptic transmission which still are not fully understood [6]. We have studied a part of the process (the release) by using data of a simplified experimental model, in which the concentration of Ca^{2+} was controlled and homogeneous, so that the issue of spatial locality can be not considered. The current model can be extended through several directions. For instance, the use of a single uniform well-mixed cluster of releasable vescicles is correct when the process is studied in short interval of time. When events take place during long-lasting neural activity, more details on vescicle trafficking

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and cluster compartimentalisation should be introduced. Moreover, the vescicle release is regulated by many intracellular signal pathways, which influence the number and the speed of recruitment of the release-competent vescicles [28]. Embedding these processes in the model might shed further light on the ways the nervous system processes and stores information. In order to support these, and others, developments the underlying process calculus might be extended, too. Surely, the problem of expressing locality, already addressed by several calculi, could be valuably addressed within a stochastic viewpoint.

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