Asymptotic analysis for vesicular release at neuronal synapses

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Séminaire Les probabilités de demain

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Outline

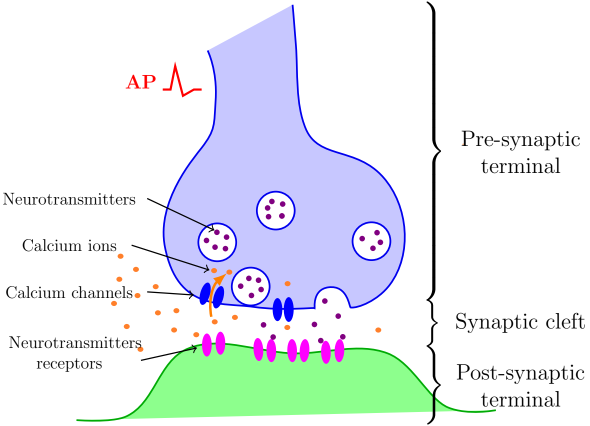
1. Overview of synaptic transmission at chemical synapses
2. Asymptotic analysis of the narrow escape problem at a cusp

1 Overview of synaptic transmission at chemical synapses

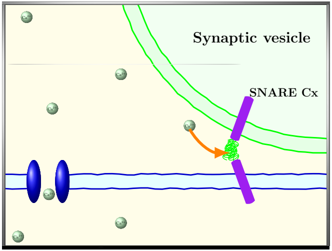
2 Asymptotic analysis of the narrow escape problem at a cusp

Functional organization of chemical synapses

10^{11} neurons in the human brain, each containing 10^3 synapses.



Modeling SNARE complex activation by calcium ions



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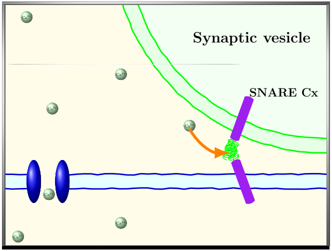
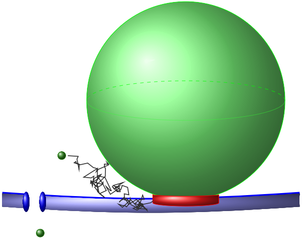


- Calcium ions: Brownian particles.
- Docked vesicle: a sphere tangent to the surface of the Active Zone.
- Binding on the SNARE Complex: a particle reaches the red cylinder between the vesicle and the pre-synaptic membrane.



1 Overview of synaptic transmission at chemical synapses

2 Asymptotic analysis of the narrow escape problem at a cusp

The narrow escape problem in a cusp

A Brownian particle is described by the stochastic equation

$$\dot{\boldsymbol{X}} = \sqrt{2D}\dot{\boldsymbol{w}}.$$

The first time to exit the domain $\bar{\Omega}$ through the small hole $\partial\bar{\Omega}_a$, starting from $\boldsymbol{x}$ is

$$\tau(\boldsymbol{x}) = \inf\{t > 0; \boldsymbol{X}(t) \notin \bar{\Omega} | \boldsymbol{X}(0) = \boldsymbol{x} \in \bar{\Omega}\}.$$

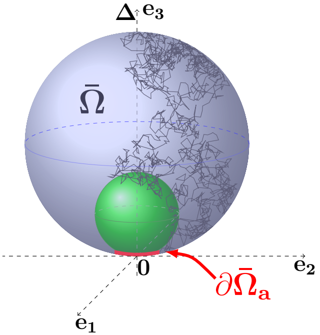
The mean first passage time

$$u(\boldsymbol{x}) = \mathbb{E}(\tau(\boldsymbol{x}))$$

is the solution of the mixed boundary value problem

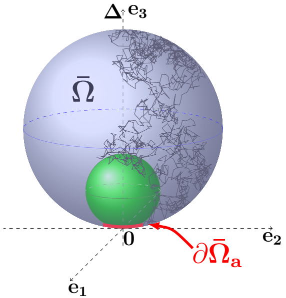
$$\begin{cases} D\Delta u(\boldsymbol{x}) = -1 \text{ for } \boldsymbol{x} \in \bar{\Omega} \\ \dfrac{\partial u}{\partial n}(\boldsymbol{x}) = 0 \text{ for } \boldsymbol{x} \in \partial\bar{\Omega} \setminus \partial\bar{\Omega}_a \\ u(\boldsymbol{x}) = 0 \text{ for } \boldsymbol{x} \in \partial\bar{\Omega}_a, \end{cases}$$

where $|\partial\bar{\Omega}_a| \ll |\partial\bar{\Omega}|$.



(Dynkin, 1961)

Reduction to a 2D problem



The problem is independent of θ in cylindrical coordinates $\boldsymbol{x} = (r, \theta, z)$.
It is equivalent to the following problem in Ω:

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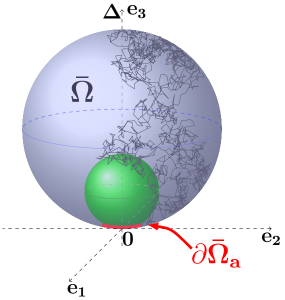
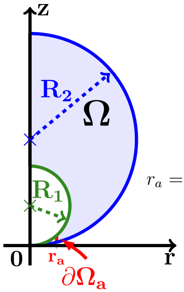




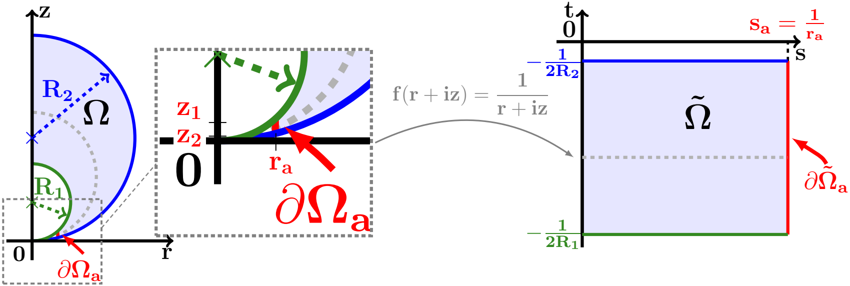
$$|\partial\Omega_a| = \varepsilon \ll R_1,$$

$$r_a = \sqrt{2\frac{R_1R_2}{R_2-R_1}\varepsilon}\,(1+o(1))\,.$$

The problem is independent of θ in cylindrical coordinates $\boldsymbol{x} = (r, \theta, z)$.
It is equivalent to the following problem in Ω:

$$\begin{cases} \dfrac{\partial^2 u}{\partial r^2}(r,z) + \dfrac{1}{r}\dfrac{\partial u}{\partial r}(r,z) + \dfrac{\partial^2 u}{\partial z^2}(r,z) \\ \qquad\qquad = -\dfrac{1}{D} \text{ for } (r,z) \in \Omega \\ \dfrac{\partial u}{\partial n}(r,z) = 0 \text{ for } (r,z) \in \partial\Omega \setminus \partial\Omega_a \\ u(r,z) = 0 \text{ for } (r,z) \in \partial\Omega_a\,. \end{cases}$$

Conformal mapping of domain Ω



Boundary value problem for $v(s,t) = u(r,z)$, where $f(r+iz) = \frac{1}{r+iz} = s + it$:

$$\begin{cases} (s^2+t^2)^2 \Delta v(s,t) + \dfrac{s^2+t^2}{s}\left(\dfrac{\partial s}{\partial r}\dfrac{\partial v}{\partial s}(s,t) + \dfrac{\partial t}{\partial r}\dfrac{\partial v}{\partial t}(s,t)\right) = -\dfrac{1}{D} \text{ for } (s,t) \in \tilde{\Omega} \\ \dfrac{\partial v}{\partial n}(s,t) = 0 \text{ for } (s,t) \in \partial\tilde{\Omega} \setminus \partial\tilde{\Omega}_a \\ v(s,t) = 0 \text{ for } (s,t) \in \partial\tilde{\Omega}_a. \end{cases}$$

The inner solution near the absorbing boundary

Scaling:

$$\zeta = s\sqrt{2R\varepsilon} = s\sqrt{\tilde{\varepsilon}}, \quad \left(R = \frac{R_1 R_2}{R_2 - R_1}\right)$$
$$Y(\zeta, t) = v(s, t),$$

and a regular expansion of Y in power of $\tilde{\varepsilon}$

$$Y(\zeta, t) = Y_0(\zeta, t) + \tilde{\varepsilon} Y_1(\zeta, t) + \tilde{\varepsilon}^2 Y_2(\zeta, t) + \ldots$$

gives the expansion for the equation in the mapped domain:

$$\frac{1}{\tilde{\varepsilon}^2}\left[\zeta^4 \frac{\partial^2 Y_0}{\partial t^2}\right] + \frac{1}{\tilde{\varepsilon}}\left[\zeta^4 \frac{\partial^2 Y_1}{\partial t^2} + \zeta^4 \frac{\partial^2 Y_0}{\partial \zeta^2} + 2\zeta^2 t^2 \frac{\partial^2 Y_0}{\partial t^2} - \zeta^3 \frac{\partial Y_0}{\partial \zeta} + 2\zeta^2 t \frac{\partial Y_0}{\partial t}\right] = O(1).$$

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Leading order term $O(\tilde{\varepsilon}^{-2})$:
Using the boundary conditions,

$$\frac{\partial Y_0}{\partial t}(\zeta, t) = 0.$$

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Leading order term $O(\tilde{\varepsilon}^{-2})$:
Using the boundary conditions,

$$\frac{\partial Y_0}{\partial t}(\zeta, t) = 0.$$

Second order term $O(\tilde{\varepsilon}^{-1})$:

$$\zeta^4 \frac{\partial^2 Y_1(\zeta, t)}{\partial t^2} + \zeta^4 \frac{\partial^2 Y_0(\zeta)}{\partial \zeta^2} - \zeta^3 \frac{\partial Y_0(\zeta)}{\partial \zeta} = 0.$$

Integrating over t and using the boundary conditions, we obtain

$$Y_0(\zeta) = A\left(1 - \zeta^2\right),$$

and

$$v(s, t) = A\left(1 - s^2 \tilde{\varepsilon}\right).$$

Computation of A using the divergence theorem

$$v(s,t) = A\left(1 - 2R\varepsilon s^2\right).$$

The constant A is determined from the divergence theorem

$$\int_{\bar{\Omega}} \Delta u = \int_{\partial\bar{\Omega}} \frac{\partial u}{\partial n}$$

$$-\frac{|\bar{\Omega}|}{D} = \int_{\bar{\Omega}} \Delta u = \int_{\partial\bar{\Omega}} \frac{\partial u}{\partial n} = 2\pi\sqrt{2R\varepsilon}\int_0^{\varepsilon} \frac{\partial u}{\partial r} dz = -4\pi A\varepsilon.$$

Thus

$$A = \frac{|\bar{\Omega}|}{4\pi D\varepsilon}.$$

The leading order term of the mean first passage time outside of the boundary layer is obtained by setting $s = 0$. It is independent of the initial position:

$$\tau = \frac{|\bar{\Omega}|}{4\pi D\varepsilon}.$$

In the boundary layer, the leading order term of the mean first passage time is:

$$v(s,t) = \frac{|\bar{\Omega}|}{4\pi D\varepsilon}\left(1 - 2R\varepsilon s^2\right).$$

Summary

- We computed the leading order term of the mean first passage time to a small ribbon located between two tangent spheres.
- The mean first passage time is constant outside of a boundar layer near the cusp, and is well approximated by a Poisson process.
  (*Schuss et al.*, *PNAS* 2007)

Next steps of the project:

- We built a model of the Active Zone, and investigated the influence of channels and vesicular organization on the release probability.
- We combined our previous result on the mean first passage time and the model of the Active Zone to build a model of the pre-synaptic terminal.
- This approach allows us to replace a model initially described using a system of PDE, with a system of ODE coupled to a Markov chain.
- We could the realize fast stochastic simulations using a Gillespie algorithm.

Acknowledgements

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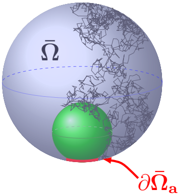
Ofir Shukron
Pierre Parutto

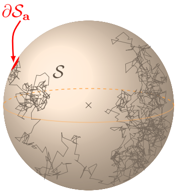
Comparison with MFPT in other geometries



$$\tau = \frac{|\bar{\Omega}|}{4\pi D\varepsilon}$$

Surface of the hole: $|\partial\bar{\Omega}_a| = 2\pi\sqrt{2R}\varepsilon^{3/2}$.

(**Guerrier et al.**, *MMS*, 2015)

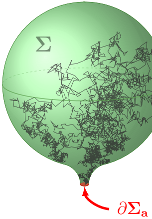


Small hole on a sphere:

$$\tau = \frac{|\mathcal{S}|}{4Da}.$$

Surface of the hole: $|\partial\mathcal{S}_a| = \pi a^2$.

(**Singer et al.**, *J. Stat. Phys.*, 2006)



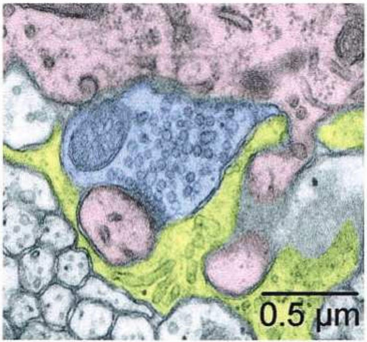
Small hole at the end of a funnel-shaped cusp:

$$\tau = \frac{|\Sigma|\sqrt{R}}{Da^{3/2}}.$$

Surface of the hole: $|\partial\Sigma_a| = \pi a^2$.

(**Holcman et al.**, *MMS*, 2012)

The Climbing Fiber to Purkinje cell synapses

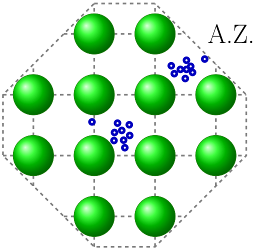


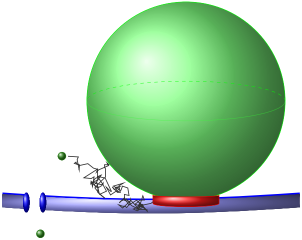
Serial Electron Microscopy section of Climbing Fiber (CF) synapses.
Blue: CF pre-synaptic terminal
Pink: Purkinje cell.
Yellow: Astrocytes.
(**Xu-Friedman et al.**, *J Neuroscience* 2001.)

- We observe several vesicles in the terminal.
- Some vesicles are docked to the pre-synaptic membrane.
- They are docked at the Active Zone, where calcium channels are also located.

Modeling the Active Zone

Active Zone: a dense region apposed to the post-synaptic neuron where calcium channels and docked vesicles are located.





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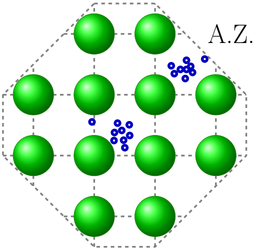
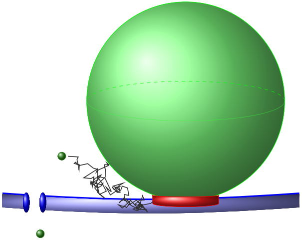
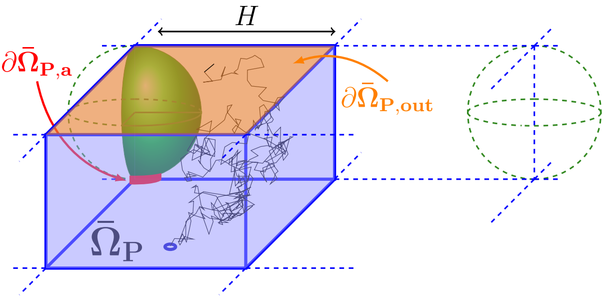




Model:

- Vesicles are spheres located on a square lattice. There radius is 20 nm. (**Xu-Friedman et al.**, *J Neuroscience* 2001.)
- Distance between vesicles: between 60 and 150 nm. (**Rollenhagen et al.**, *Cell Tissue Res* 2006.)
- Channels can be uniformly distributed or clustered.

Close to the target, the splitting probability



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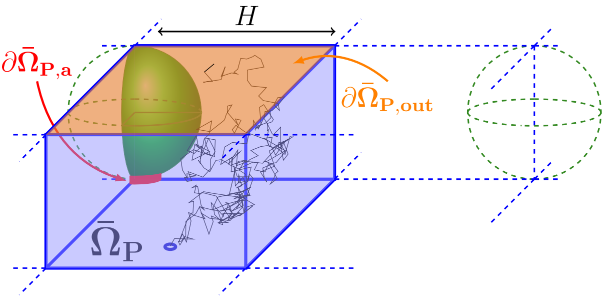
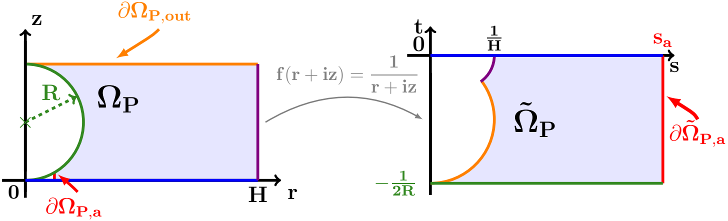


Splitting probability: probability to reach the red target $\partial\bar{\Omega}_{P,a}$ before reaching the orange boundary $\partial\bar{\Omega}_{P,out}$

$$\begin{cases} \Delta p_s(\boldsymbol{x}) = 0 & \text{for } \boldsymbol{x} \in \bar{\Omega}_P \\ p_s(\boldsymbol{x}) = 1 & \text{for } \boldsymbol{x} \in \partial\bar{\Omega}_{P,a} \\ p_s(\boldsymbol{x}) = 0 & \text{for } \boldsymbol{x} \in \partial\bar{\Omega}_{P,out} \\ \dfrac{\partial p_s}{\partial n}(\boldsymbol{x}) = 0 & \text{for } \boldsymbol{x} \in \partial\bar{\Omega}_P \setminus \left(\partial\bar{\Omega}_{P,a} \cup \partial\bar{\Omega}_{P,out}\right). \end{cases}$$

Restriction to a 2D problem

Our previous results motivate the restriction of the analysis to the domain Ω_P:



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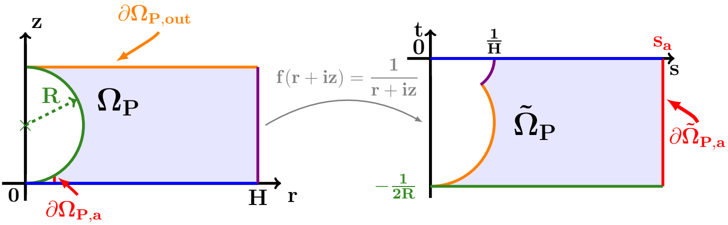


In cylindrical coordinates, we get:

$$\begin{cases} \Delta p_s + \dfrac{1}{r}\dfrac{\partial p_s}{\partial r}(r,z) = 0 \text{ for } (r,z) \in \Omega_P \\ p_s(r,z) = 1 \text{ for } (r,z) \in \partial\Omega_{P,a} \\ p_s(r,z) = 0 \text{ for } (r,z) \in \partial\Omega_{P,out} \\ \dfrac{\partial p_s}{\partial n}(r,z) = 0 \text{ for } (r,z) \in \partial\Omega_P \setminus \left(\partial\Omega_{P,a} \cup \partial\Omega_{P,out}\right). \end{cases}$$

Using our previous mapping method and the boundary condition at $\partial\Omega_{P,a}$ we obtain:

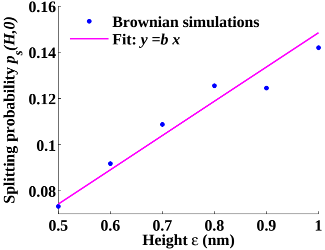
$$p_s(r,0) = 1 - A\left(1 - \frac{2R\varepsilon}{r^2}\right).$$

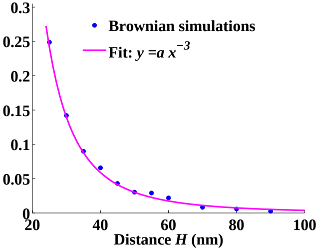
Numerical approximation of the splitting probability

We express the splitting probability as a function of $p_s(H,0) = p(\varepsilon, R, H)$:

$$p_s(r,0) = 1 - \frac{1 - p(\varepsilon, R, H)}{1 - \dfrac{2R\varepsilon}{H^2}} \left(1 - \frac{2R\varepsilon}{r^2}\right).$$

We determine $p(\varepsilon, R, H)$ using Brownian simulations:





$\Rightarrow p(\varepsilon, R, H) = \alpha \frac{R^2 \varepsilon}{H^3}$, α is fitted numerically using Matlab. We get:

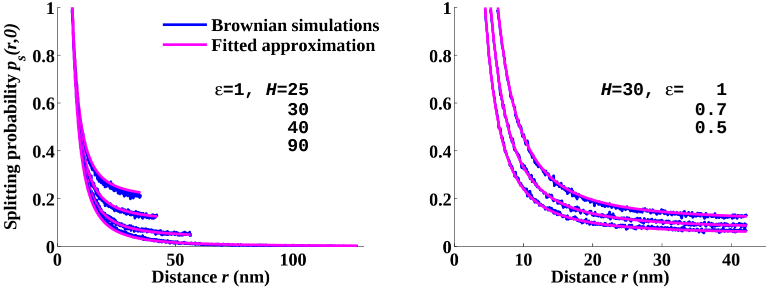
$$p_s^{approx}(r,0) = 1 - \frac{1 - 9.8\dfrac{R^2\varepsilon}{H^3}}{1 - \dfrac{2R\varepsilon}{H^2}} \left(1 - \frac{2R\varepsilon}{r^2}\right), \quad R \leq H,\ 0 \leq r \leq H.$$

Comparison between the splitting probability and Brownian simulations

We observe a nice agreement between Brownian simulations and the asymptotic formula:

$$p_s^{approx}(r,0) = 1 - \frac{1 - 9.8\,\frac{R^2\varepsilon}{H^3}}{1 - \frac{2R\varepsilon}{H^2}}\left(1 - \frac{2R\varepsilon}{r^2}\right),$$

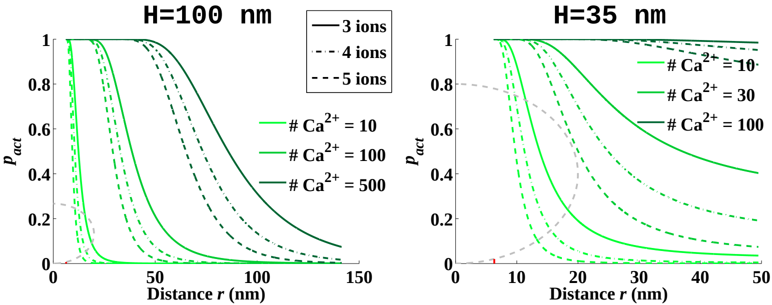
for different values of H and ε:



Estimation of the vesicular release probability

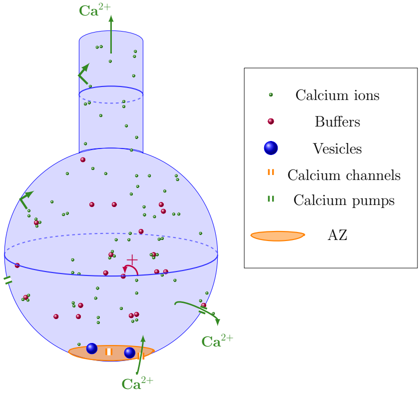
We compute the probability $p_{act}(r, N)$ that T calcium ions bind the target, when N ions enter through a channel at distance r:

$$p_{act}(r, N) = 1 - \sum_{k=0}^{T-1} \binom{N}{k} p_s(r)^k \left(1 - p_s(r)\right)^{N-k}.$$



- High crowding of vesicles is associated with high release probability.
- A synapse with high release requires a nm precision of channel location. It can be compensated by channel clustering.

Modeling calcium dynamic in the pre-synaptic terminal



The terminal is a sphere (head) connected to a cylinder (thin neck).

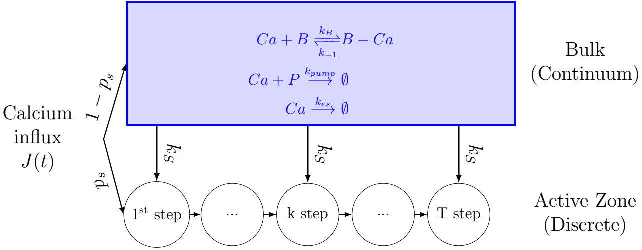
Calcium ions are Brownian particles.

They enter through calcium channel located at the AZ.

They can bind and unbind buffer molecules: specific proteins that regulate calcium concentration in the terminal.

They leave the terminal through calcium pumps, or through the end of the neck.

A Markov model coupled to mass action equations



- We model the activation of the SNAREs at the Active Zone using a Markov chain, with rates that depend on the density of ions.
- The arrival to small holes (buffers, pumps, targets) is well approximated by Poisson processes, with rates the inverse of the mean first passage time.
- It allows to derive a system of ordinary differential equations for the density of ions in the terminal

A Markov chain to describe target activation at the Active Zone

For each target i, the probabilities to have k particles bound, $p_k^i(t),\ 0 \leq k \leq T$ are solution of the following system of equations:

$$\begin{cases} \dfrac{dp_0^i(t)}{dt} &= -\,\phi(t)p_0^i(t) \\ \dfrac{dp_k^i(t)}{dt} &= \phi(t)\left(p_{k-1}^i(t) - p_k^i(t)\right) \\ \dfrac{dp_T^i(t)}{dt} &= \phi(t)p_{T-1}^i(t), \end{cases}$$

and with initial conditions $p_k^i(0) = \delta_{k=0}$,

and normalization condition $\sum_{k=0}^{T} p_k^i(t) = 1$.

$$\phi(t) = \sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l, t) + k_{Target} N_f(t),$$

$J^i(\boldsymbol{x}^l, t)$ represents the flux fraction of particles arriving at target i, coming from a calcium channels located at $\boldsymbol{x}^l$,
$k_{Target} N_f(t)$ represents the binding of calcium ions coming from the terminal.

The mass action equations for the ions in the bulk

The number of free particles in the bulk N_f and the number of buffered ones N_b satisfies:

$$\begin{cases} \frac{dN_f}{dt} =k_{-1}N_b - k_B(B_{tot}-N_b)N_f(t) + \left(l_V - \sum_{l=1}^{l_V} p_s(\boldsymbol{x}^l) \right) J(t) \\ \qquad - \left(k_{pump}N_p + k_{es} + k_{Target} \left(N_{Dock} - \sum_{i=1}^{N_{Dock}} p_T^i(t) \right) \right) N_f(t) \\ \qquad + T \sum_{i=1}^{N_{Dock}} \left(\sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l, t) + k_S N_f(t) \right) p_{T-1}^i(t) \\ \frac{dN_b}{dt} = -k_{-1}N_b + k_B(B_{tot}-N_b)N_f. \end{cases}$$

Influx of ions entering the bulk

Total number of free sites.

Release of bound ions in the bulk after vesicular fusion.

Parameters estimation

The arrival time of a Brownian particle to a small target is well approximated by a Poisson process, with rate the inverse of the mean first time ($k_X = \frac{1}{\bar{\tau}_X}$).
(*Schuss et al.*, *PNAS* 2007)

- Mean binding time to a buffer:

$$\bar{\tau}_B = \frac{|\Omega_h|}{4\pi(D + D_B)r_{buff}}.$$

(*Holcman et al.*, *SIAM Rev* 2014)

- Mean escape time to a pump:

$$\bar{\tau}_{pump} = \frac{|\Omega_h|}{4Dr_{pump}}.$$

(*Holcman et al.*, *SIAM Rev* 2014)

- Mean escape time through the neck:

$$\bar{\tau}_{es} = \frac{|\Omega_h|}{4Dr_{neck}} + \frac{l_{neck}|\Omega_h|}{D\pi r_{neck}^2} + \frac{l_{neck}^2}{2D}.$$

(*Holcman et al.*, *SIAM Rev* 2014)

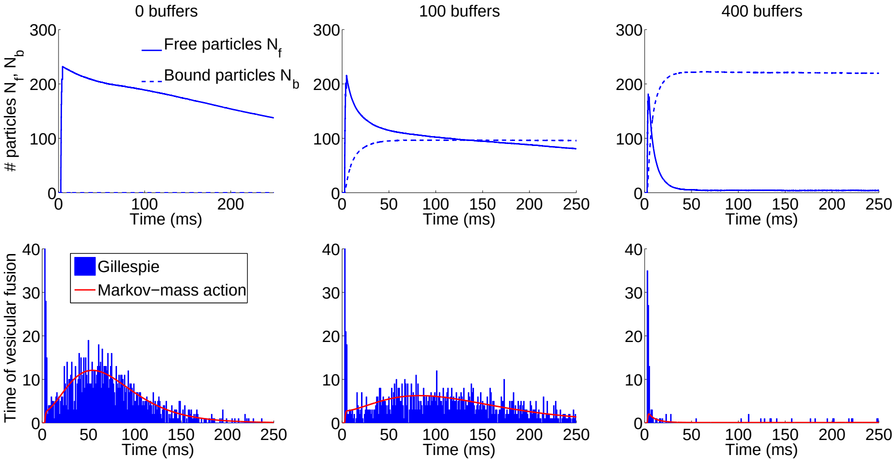
- Mean binding time to a SNARE complex:

$$\bar{\tau}_{Target} = \frac{|\Omega_h|}{4\pi D\varepsilon}.$$

(*Guerrier et al.*, *MMS* 2015)

The unbinding rate from buffers k_{-1} is extracted from litterature.
(*Meinrenken et al.*, *J. Physiol* 2003)

Distribution of release time for a uniform channel distribution



Calcium entry: for $0 \leq t \leq 5$ ms.

Outside of the AZ: mean arrival time to the SNARE Complex: $\dfrac{|\bar{\Omega}|}{4\pi D\varepsilon} \approx 4\,\text{sec}\,.$

Mean time to escape the AZ: $\bar{\tau} = \frac{2R^2}{D} \approx 4\mu s$.
Mean time to bind the SNARE from the AZ: $< 7\mu s$.

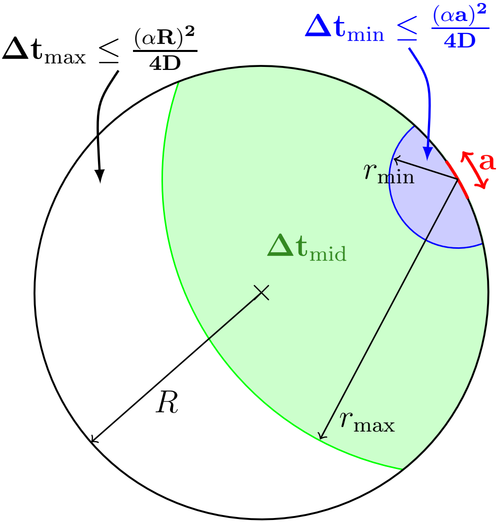
Reaction-diffusion equations for calcium in the pre-synaptic terminal

The reaction-diffusion equation of the density of calcium ions $M(\boldsymbol{x},t)$, the density of buffers with ($B^{(1)}$) and without ($B^{(0)}$) bound calcium ions, and the density of targets (SNARE machinery) with j bound particles $S^{(j)}$ are:

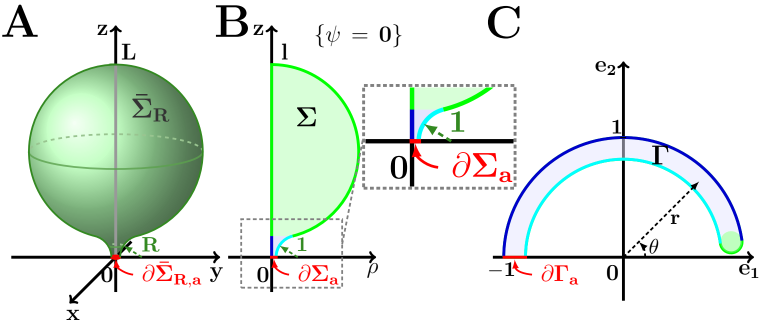
$$\begin{cases} \dfrac{\partial M(\boldsymbol{x},t)}{\partial t} =& D\Delta M(\boldsymbol{x},t) - k_0 M(\boldsymbol{x},t)B^{(0)}(\boldsymbol{x},t) + k_{-1}B^{(1)}(\boldsymbol{x},t) \\ & - k_S M(\boldsymbol{x},t)\displaystyle\sum_{j=0}^{T-1} S^{(j)}(\boldsymbol{x},t) + T k_S M(\boldsymbol{x},t) S^{(T-1)}(\boldsymbol{x},t) \\ \dfrac{\partial B^{(0)}(\boldsymbol{x},t)}{\partial t} =& D_B \Delta B^{(0)}(\boldsymbol{x},t) - k_0 M(\boldsymbol{x},t)B^{(0)}(\boldsymbol{x},t) + k_{-1}B^{(1)}(\boldsymbol{x},t) \\ \dfrac{\partial B^{(1)}(\boldsymbol{x},t)}{\partial t} =& D_B \Delta B^{(1)}(\boldsymbol{x},t) - k_{-1}B^{(0)}(\boldsymbol{x},t) + k_0 M(\boldsymbol{x},t)B^{(1)}(\boldsymbol{x},t) \\ \dfrac{\partial S^{(0)}(\boldsymbol{x},t)}{\partial t} =& - k_S M(\boldsymbol{x},t) S^{(0)}(\boldsymbol{x},t), \\ \dfrac{\partial S^{(j)}(\boldsymbol{x},t)}{\partial t} =& k_S M(\boldsymbol{x},t)\left[S^{(j-1)}(\boldsymbol{x},t) - S^{(j)}(\boldsymbol{x},t)\right], j = 1..T-1 \\ \dfrac{\partial S^{(T)}(\boldsymbol{x},t)}{\partial t} =& k_S M(\boldsymbol{x},t) S^{(T-1)}(\boldsymbol{x},t). \end{cases}$$

$\Rightarrow$ Analytical and numerical difficulties due to the particular organization of the Active Zone.

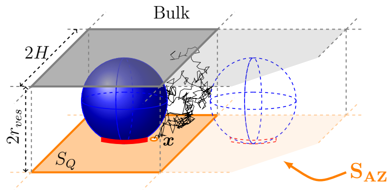
How to choose Δt for Brownian simulations



Funnel-shaped cusp



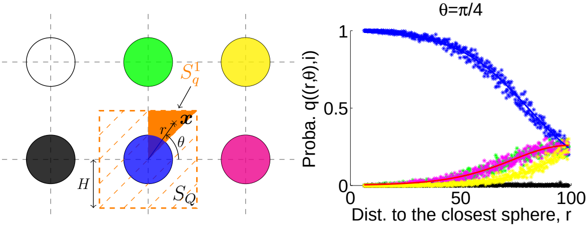
Model of target site organization at the AZ



Probability that a particle reaches a target before leaving the boundary layer on an infinite AZ full of vesicles, starting from $\boldsymbol{x}$:

$$p_s(\boldsymbol{x}) = 1 - \frac{1 - 9.8\dfrac{r_{ves}^2\varepsilon}{H^3}}{1 - \dfrac{2r_{ves}\varepsilon}{H^2}}\left(1 - \frac{2r_{ves}\varepsilon}{r(\boldsymbol{x})^2}\right).$$

We estimate using Brownian simulations the probability $q\left(\boldsymbol{x}, i\right)$ to reach specifically target i, and fit the results using Matlab.



Flux fraction to vesicle i coming from a channel at $\boldsymbol{x}$: $J^i(\boldsymbol{x}, t) = J(t)p_s(\boldsymbol{x})q(\boldsymbol{x}, i)$.

A Markov chain to describe target activation at the AZ

Transition probability from $k-1$ to k bound particles, for l_V channels located at $(\boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})$:

$$\begin{aligned} Pr^i\{k, t+\Delta t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}\} =& Pr^i\{k-1, t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}\}\phi(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})\Delta t \\ &+ Pr^i\{k, t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}\}\left(1-\phi(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})\Delta t\right), \end{aligned}$$

where $\phi(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) = \sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l, t) + k_S N_f(t)$ is the flux of particles arriving to the target.

For each target i, the Markov chain for $p_k^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) = Pr^i\{k, t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}\}$ is

$$\begin{cases} \dfrac{dp_0^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})}{dt} = -\phi(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})p_0^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) \\ \dfrac{dp_k^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})}{dt} = \phi(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})\left(p_{k-1}^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) - p_k^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})\right) \\ \dfrac{dp_T^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})}{dt} = \phi(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})p_{T-1}^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}), \end{cases}$$

with initial conditions $p_k^i(0, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) = \delta_{k=0}$,

and normalization condition $\sum_{k=0}^{T} p_k^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) = 1$.

The mass action equations for the ions in the bulk

The number of free particles in the bulk N_f and the number of buffered ones N_b satisfies:

$$\begin{cases} \dfrac{dN_f}{dt} = k_{-1}N_b - k_0(B_{tot} - N_b)N_f(t) + \left(l_V - \sum_{l=1}^{l_V} p_s(\boldsymbol{x}^l)\right) J(t) \\ \qquad - \left(k_p N_p + k_a + k_S \left(N_{Dock} - \sum_{i=1}^{N_{Dock}} p_T^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})\right)\right) N_f(t) \\ \qquad + T \sum_{i=1}^{N_{Dock}} \left(\sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l, t) + k_S N_f(t)\right) p_{T-1}^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) \\ \dfrac{dN_b}{dt} = -k_{-1}N_b + k_0(B_{tot} - N_b)N_f. \end{cases}$$

Influx of ions entering the bulk

Total number of free sites.

Probability density function $f_{\tau_T^i, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}}$ of the release time for target i, τ_T^i:

$$f_{\tau_T^i, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}}(t) = \frac{dp_T^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})}{dt} = \left(\sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l, t) + k_S N_f(t)\right) p_{T-1}^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})$$

$\Rightarrow T \sum_{i=1}^{N_{Dock}} \left(\sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l, t) + k_S N_f(t)\right) p_{T-1}^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})$: release of bound ions in the bulk after a vesicular release event.

The mass action equations for the ions in the bulk

The number of free particles in the bulk N_f and the number of buffered ones N_b satisfies:

$$\begin{cases} \dfrac{dN_f}{dt} = k_{-1}N_b - k_0(B_{tot}-N_b)N_f(t) + \left(l_V - \sum_{l=1}^{l_V} p_s(\boldsymbol{x}^l)\right)J(t) \\ \qquad - \left(k_pN_p + k_a + k_S\left(N_{Dock} - \sum_{i=1}^{N_{Dock}} p_T^i(t,\boldsymbol{x}^1,..,\boldsymbol{x}^{l_V})\right)\right)N_f(t) \\ \qquad + T\sum_{i=1}^{N_{Dock}}\left(\sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l,t) + k_SN_f(t)\right)p_{T-1}^i(t,\boldsymbol{x}^1,..,\boldsymbol{x}^{l_V}) \\ \dfrac{dN_b}{dt} = -k_{-1}N_b + k_0(B_{tot}-N_b)N_f. \end{cases}$$

Influx of ions entering the bulk

Total number of free sites.

Probability density function $f_{\tau_T^i,\boldsymbol{x}^1,..,\boldsymbol{x}^{l_V}}$ of the release time for target i, τ_T^i:

$$f_{\tau_T^i,\boldsymbol{x}^1,..,\boldsymbol{x}^{l_V}}(t) = \frac{dp_T^i(t,\boldsymbol{x}^1,..,\boldsymbol{x}^{l_V})}{dt} = \left(\sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l,t) + k_SN_f(t)\right)p_{T-1}^i(t,\boldsymbol{x}^1,..,\boldsymbol{x}^{l_V})$$

$\Rightarrow T\sum_{i=1}^{N_{Dock}}\left(\sum_{l=1}^{l_V} J^i(\boldsymbol{x}^l,t) + k_SN_f(t)\right)p_{T-1}^i(t,\boldsymbol{x}^1,..,\boldsymbol{x}^{l_V})$: release of bound ions in the bulk after a vesicular release event.

Solving the coupled Markov equations

For each target i, and a channel distribution $(\boldsymbol{x}^1, .., \boldsymbol{x}^{l_V})$, the flux of arriving particle is

$$g^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) = \sum_{l=1}^{l_V} p_s(\boldsymbol{x}^l) q(\boldsymbol{x}^l, i) J(t) + k_S N_f(t).$$

Probability to have k bound ions at target i at time t ($0 \leq k \leq T - 1.$):

$$p_k^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) = \frac{1}{k!} \left(\int_{t_0}^t g^i(u, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) du \right)^k \exp\left(- \int_{t_0}^t g^i(u, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) du \right).$$

Distribution of release time:

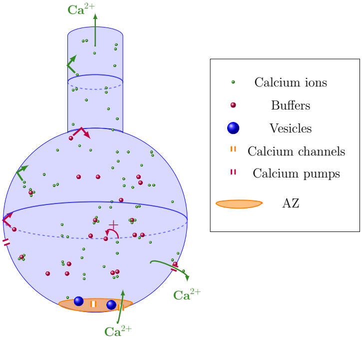
$$p_T^i(t, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) = \exp\left(- \int_{t_0}^t g^i(u, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) du \right) \sum_{k \geq T} \frac{1}{k!} \left(\int_{t_0}^t g^i(u, \boldsymbol{x}^1, .., \boldsymbol{x}^{l_V}) du \right)^k.$$

Brownian simulations

Ion trajectories are modeled as independent Brownian particles:

$$\dot{\boldsymbol{X}}_k = \sqrt{2D}\dot{\boldsymbol{w}}_k, \text{ for } k = 1..N,$$

simulated using the Euler scheme: $X(t+\Delta t) = X(t) + \sqrt{2D\Delta t}\,\xi,\ \xi \sim \mathcal{N}(0,1)$.



- Particles are reflected on the boundary according to the classical Snell-Descartes reflection principle.
- Absorbing part of the boundary: the end of the neck and the pumps.
- Binding on buffers: when the particle hits the small sphere $\partial B(r_{buff})$.
  Unbinding probability:
  $P(\tau_{ub} \in [t, t+\Delta t]) \approx k_{-1}\Delta t$.
- AZ organization: as previously described.
  Influx of ions: calcium current computed using a Hodgkin-Huxley model.
  Release of a vesicle: T particles bound to the target trigger vesicular fusion.

$\Rightarrow$ The small holes require a very small time step for simulations, which leads to never ending simulations.

Application: mean time to bind the SNARE complex.

Outside of the boundary layer:
Mean arrival time of a Brownian particle to the SNARE Complex:

$$\tau = \frac{|\bar{\Omega}|}{4\pi D\varepsilon} \approx 4\,\text{sec},$$

with:

- Calcium diffusion coefficient $D = 20\mu m^2.s^{-1}$ (**Biess et al.**, *PLoS Comput. Biol.*, 2011)
- Volume of the pre-synaptic terminal $|\bar{\Omega}| = 1\mu m^3$ (**Xu-Friedman et al.**, *J. Neurosci.*, 2001)
- Height of the ribbon $\varepsilon = 0.001\mu m$.

In the boundary layer:

Mean time spent in the boundary layer: $\bar{\tau} = \frac{(2r_{ves})^2}{2D} \approx 4\,10^{-3} ms$.

Mean time to bind the target $< 7\,10^{-3} ms$.

Distribution of ions on targets at the end of the transient regime, for a uniform channel distribution

Fraction of ions coming from one channel, reaching a target :

$$
\begin{aligned}
F_{ions} &= \int_{S_{AZ}} p_s(\boldsymbol{x}) q(\boldsymbol{x}, i) f(\boldsymbol{x}) d\boldsymbol{x} \\
&= \frac{r_{ves}\varepsilon}{N_{Dock} H^2} \left[\pi \ln \left(\frac{2H}{\sqrt{2 r_{ves} \varepsilon}} \right) + \left(\frac{9.8 r_{ves}}{H} - 2(K+1) \right) \right] + O(\varepsilon^2 \ln(\varepsilon)).
\end{aligned}
$$

Mean probability that k particles are bound at time t, $0 \leq k \leq T-1$

$$
p_k^i(t) = \int_{S_{AZ}^{l_V}} \frac{1}{k!} \left(\int_{t_0}^{t} g^i(u, \vec{\boldsymbol{x}}) du \right)^k \exp \left(- \int_{t_0}^{t} g^i(u, \vec{\boldsymbol{x}}) du \right) f(\vec{\boldsymbol{x}}) d\boldsymbol{x}^1 \ldots d\boldsymbol{x}^{l_V},
$$