NUMERICAL SIMULATIONS OF THE ACH - DRIVEN SYNAPSE AT THE NEUROMUSCULAR JUNCTION

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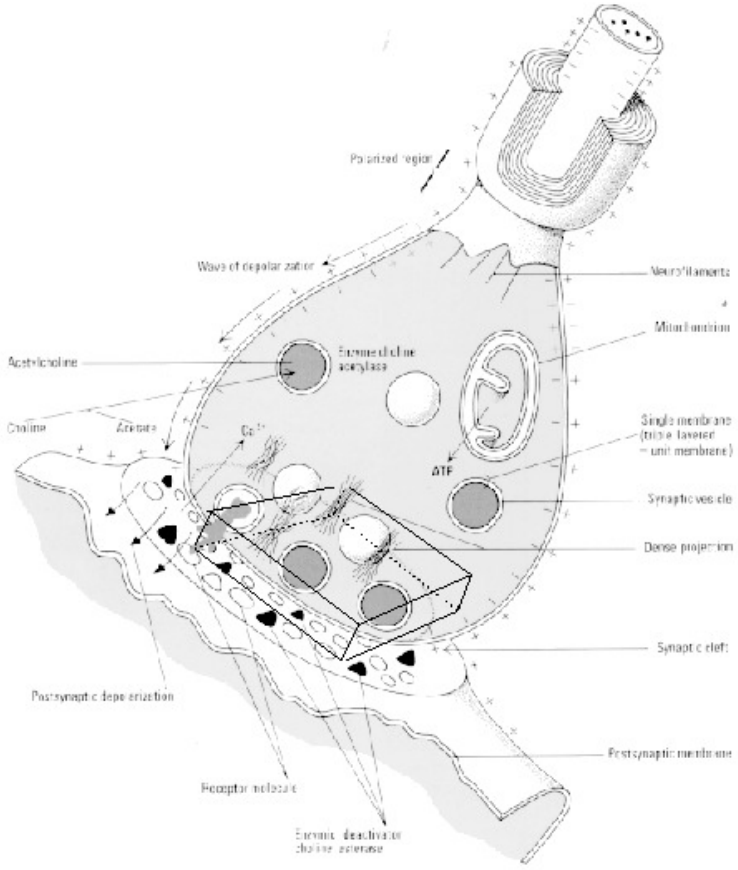
A stochastic model for single quantal release in the ACh-driven synapse is studied with the help of Monte Carlo simulations. The model incorporates release, diffusion, binding/unbinding, and removal of neurotransmitter. The AChR receptor cycle includes five states, among them two desensitized ones. When the receptor diffusion on the postsynaptic membrane depends on the receptor configuration, both desensitization and reorganization processes are observed, in quantitative agreement with experimental data.

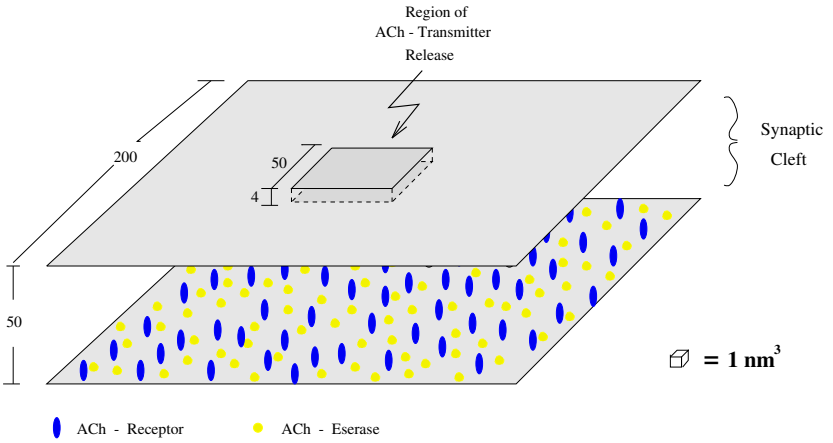
1. Introduction

We developed a stochastic lattice model describing the area affected by one quantum release of ACh-transmitter in the neuromuscular junction synapse (NMJ). The level of description is more detailed than a continuum diffusion - reaction but does not yet account for the detailed kinetics of the chemical processes. Our aim was twofold: 1) Implement a realistic, experimentally valid simulation of a small portion of the synapse and 2) a quantitative understanding of the desensitization and adaptation processes generated by a frequent discharge of a vesicle. A schematic picture of the modeled region within the NMJ-synapse can be seen below as a rectangular insert. The NMJ forms the connection between nerve and muscle and is one of the best known chemical synapses. The neurotransmitter acetylcholine (ACh) is released in quanta by vesicle fusion. The transmitter diffuse into the synaptic cleft and combines either with an ACh-receptor (AChR) or is split into acetyl and choline by ACh-esterase (AChE). The ACh receptor has a complicated configuration with two binding sites. When both binding sites are occupied, the receptor changes its configuration. As a result, a Ca^{2+}-selective ion channel opens, depolarizing the postsynaptic membrane. Such a miniature synaptic potential can be directly measured.



2. Lattice Configuration

The space-time grid was chosen small enough ($1nm \times 10^{-8}$ sec) to realistically describe the diffusion of each neurotransmitter molecule releases by a single vesicle. The three dimensional lattice consisted of $200 \times 200 \times 50$ lattice points corresponding to the horizontal and the vertical coordinates, respectively. The lattice configuration is shown below:



The lattice contains

- $\rho(AChR) = 10^4 \frac{AChR}{\mu m^2} \longrightarrow$ 4000 AChR-molecules distributed randomly on the postsynaptic membrane.
- $\rho(AChE) = 2.5 \cdot 10^5 \frac{AChE}{\mu m^2} \longrightarrow$ 10000 AChE-molecules distributed randomly one layer above the postsynaptic membrane.
- $N(\frac{ACh}{Vesicle}) = 10000$

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3. Stochastic Dynamical Rules

Once the transmitter is released on the square region at the top of the lattice, one defines the following probabilistic dynamics:

(i) **Diffusion of ACh–Transmitter**
Each individual ACh-molecule performs a random walk with nearest neighbor exclusion (maximum one ACh-molecule per site).

(a) Monte Carlo Step: for all released particles do:
- choose at random one of the 6 neighbor sites,
- go to the selected position if site is free.

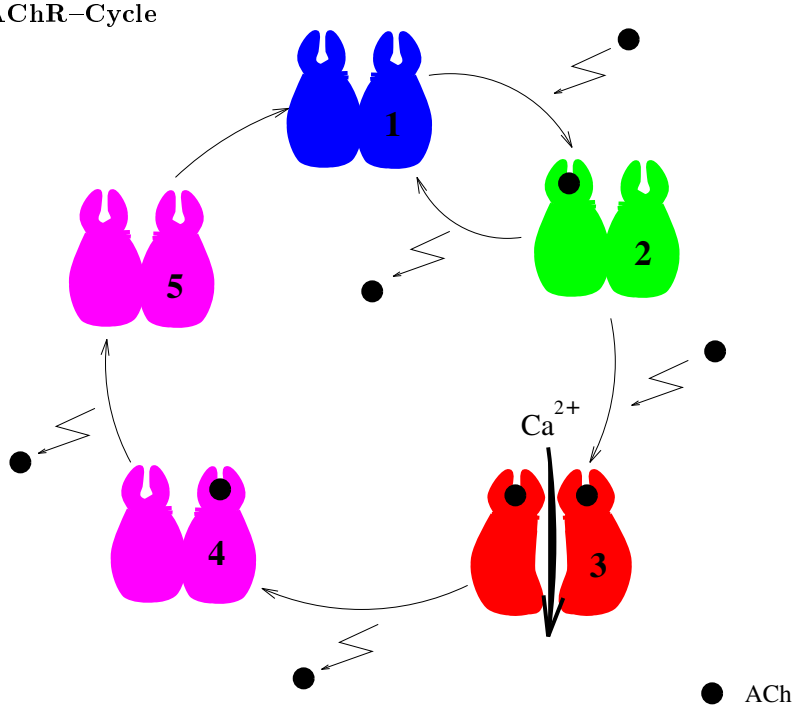
(b) The diffusion constant for ACh is about $D_{ACh} \approx 10^{-6} \frac{cm^2}{s}$. A lattice constant of $1nm = 10^{-7}cm$, about the size of an ACh-molecule, leads to the time scale [1] [2]:

$$\Delta t = \frac{1}{6}\frac{h^2}{D} \approx \frac{10^{-14}cm^2}{10^{-6}\frac{cm^2}{s}} = 10^{-8}s$$

(ii) **ACh–AChE–Interaction**
If an ACh-molecule hits an ACh-esterase it is destroyed with probability 1.

(iii) **ACh–AChR–Interaction**
If an ACh-molecule choose to jump to a site occupied by an ACh-receptor, then the current state of the receptor determines the next move.

(iv) **The AChR–Cycle**

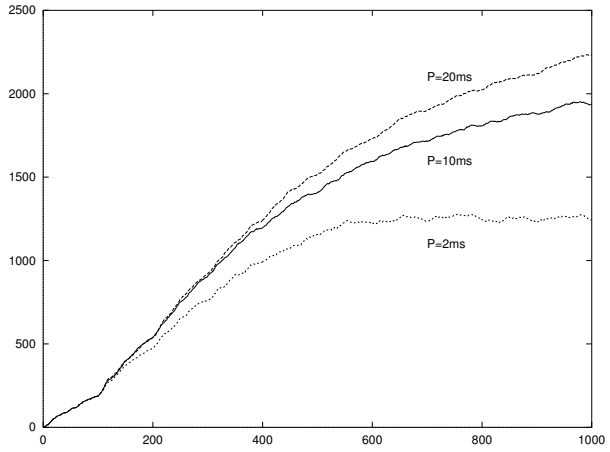


The model includes five different AChR-states. Among them, the only state with an open ionic channel is state **3** $(AChR + 2ACh)$ [3], and contributes to the miniature (post)synaptic potential. All other states can be measured indirectly only. The binding (**1**→**2** and **2**→**3**) occurs with probability 1 when ACh and AChR find themselves on the same lattice site. The transition **3**→**4** (the probability that ACh leaves an AChR) is proportional to the mean opening time of the ion channel $(1ms)$. This is also valid for the transition **2**→**1** and **4**→**5**. Relaxation from state **5** into state **1** is controlled by a refractory parameter $\mathbf{P_{des}}$, which defines the mean life time of the desensitive state. Biological experiments have shown that the upper limit for the duration of the desensitive state is about $30ms$ [4]. We did simulations for different parameter values of $\mathbf{P_{des}}$ (see below).

4. Desensitization

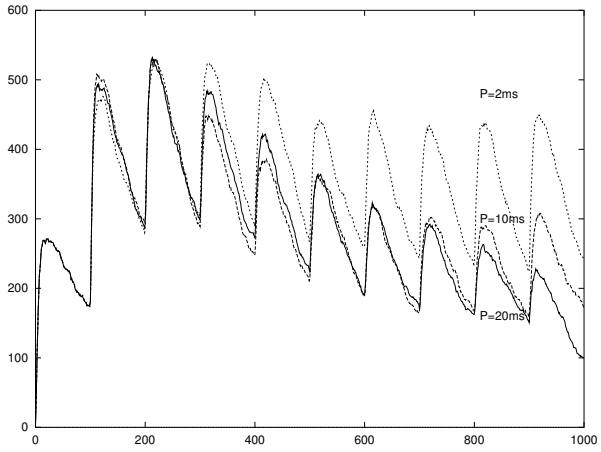
A high density of transmitter on the postsynaptic side leads to a decreased pool of free receptors. This effect, *desensitization*, means that by a periodic stimulation of the presynaptic neuron, the postsynaptic response will fade in time. The magnitude of desensitization depends strongly on the mean life time of the desensitive state $\mathbf{P_{des}}$, on the frequency, and on the duration of the stimulus. The time dependence of the total number of desensitive states **4** + **5** are shown below for two frequencies $(\frac{1Vesicle}{ms}, \frac{1Vesicle}{10ms})$ and three desensitization parameters $\mathbf{P_{des}}(2ms, 10ms, 20ms)$.

Desensitization at $f = \frac{1Vesicle}{ms}$



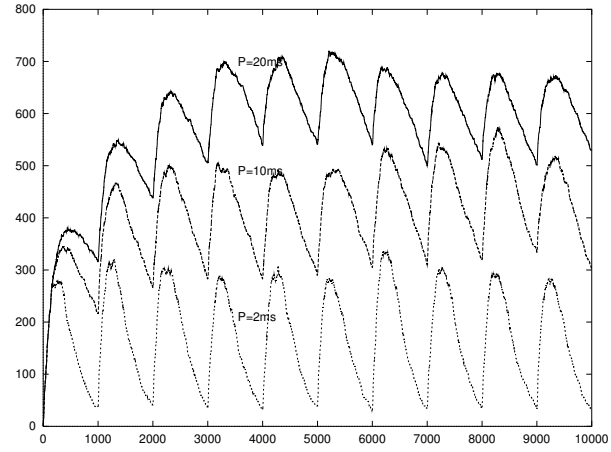
$t \quad [10^{-2}]ms$

Signal transduced



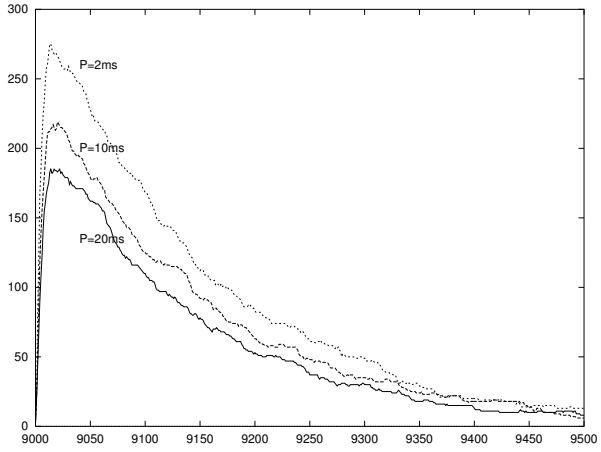
$t \quad [10^{-2}]ms$

Desensitization at a frequency of $\frac{1Vesicle}{10ms}$



$t \quad [10^{-2}]ms$

Signal transduced after 10 Vesicles



$t \quad [10^{-2}]ms$

5. Synaptic Reorganization

Long term potentiation (LTP), the biological basis of memory, is probably controlled by many, diverse pathways. We considered here a simple physical pathway related to receptor diffusion. Experiments clearly show that in the NMJ the AChR receptors diffuse on the postsynaptic membrane, albeit much slower than the neurotransmitters. A form of synaptic reorganization based on the coupling between diffusion, transverse potential gradients, and charged receptors has been proposed [5]. The mechanism proposed here is rather different and is based on the additional **assumption** that only the first two AChR states contribute to receptor diffusion,

the 'fat' states 3,4, and 5 have a negligible diffusion constant. This leads to the additional rules:

- The diffusion rule: The diffusion constant for state **1**, state **2** AChR is $D_{AChR} \approx 10^{-9} \frac{cm^2}{s}$. AChR moves in average every 1000^{th} time-step.
- The '*no-walk-rule*': Open ion channels (AChR in state **3**) and desensitive AChR (states **4** and **5**) do not move.

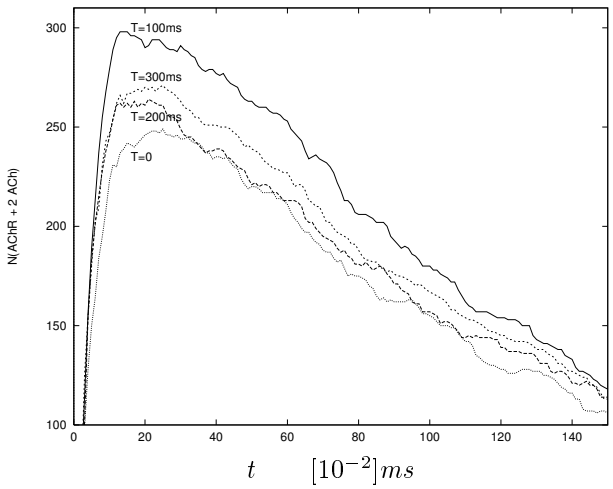
Since in our model the transmitter is released always from the central little square, the postsynaptic region beneath it will contain the highest concentration of neurotransmitter, hence most AChR receptors in higher states. By keeping this concentration high, free receptors moving into this region will be also 'trapped', leading to an accumulation of receptors under the vesicle. It is conceivable that such a facilitation process happens also on the presynaptic side, so that vesicles fuse with the presynaptic membrane near the same position.

6. Signal Transduction after 'Training' the Synapse

To test the influence of AChR-accumulation the synapse was 'trained' by a periodic signal with a frequency of 1 Vesicle per 10 ms for 100, 200 and 300 ms. After the training, the synapse was kept quiet for an appropriate period so that almost all AChR returned to rest (state **1**). When the timing of the activation signal and the relaxation rate of the state **5** are properly chosen, the response increases about 30% compared to the synapse with randomly distributed receptors. This matches the maximal synaptic efficiency increase observed in LTP. During this 'waiting time' **T** (which was $70ms$) the AChR starts to diffuse again. Hence, the synaptic organization is temporary. We are considering at present questions regarding the typical 'training' times, the typical 'forgetting' time, and the minimal activity needed to sustain a structured synapse.

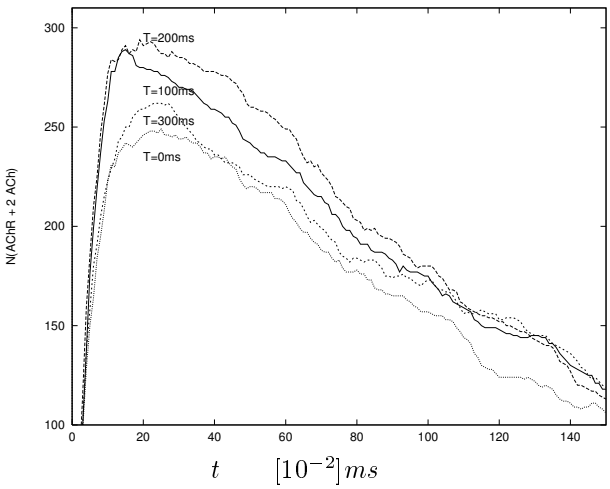
Signal after Training

$P_{des} = 20ms$



Signal after Training

$P_{des} = 2ms$



7. Numerical Methods

The model described above was implemented in C-language, as an Xlib-based graphics program. A single vesicle release event in one-to-one correspondence to the biological reality requires about 1 CPU hour on a HP-735-99 machine *versus* 4 *ms* biological time. The simulation of receptor diffusion (events happening on a 10^3 larger time-scale) requires special methods, described in the R. U.'s Diplomarbeit.

8. References

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