# A Systems Biology Approach to Model Neuronal Activity Mediated through Chemical Interactions

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## Abstract

Genome-scale metabolic networks have been widely used in many different applications to predict and analyze both cellular physiology and phenotype of specific cell models. These metabolic networks provide insight into energy usage, gene expression, and pathway architecture of cells. In the context of human brain cells, such metabolic networks not only reveal important information about the overall metabolism of neurons and astrocytes, but also poses the potential to incorporate with neuronal activity. That is, chemical synapses depend upon the concentrations of different types of neurotransmitters in the cell. This allows us to look into the relationship between the concentration of neurotransmitter and the specific electrical activity of these cells for the transmission of information. In this project, we used both systems biology and neurodynamics to model and predict the depletion rates of neurotransmitters due to synaptic release in both normal and Alzheimer neurons. These simulations were based on metabolic fluxes and electrical stimulation rates.

## 1 Introduction

Chemical synapses are the most important mechanism for the transmission of action potentials between neurons. It is the release of neurotransmitters that propagates these potentials by binding to membrane receptors in the postsynaptic neuron. Neurotransmitters are released from their storage vesicles by exocytosis, which is a process modulated by $Ca^{2+}$ ions. Although it would be reasonable to presume that an action potential passing down the axon inevitably and invariably results in an episode of neurotransmitter release, it is not necessarily the case as the probability of any one potential is extremely low [1].

Among all types of chemical synapses, acetylcholine-dependent synapses are the best understood as being the first one identified and characterized in terms of quantized release (vesicles) by Katz and colleagues [2]. According to their picture, a quantitative description of the process is a coin flipping problem. The number of release sites and the probability that a vesicle undergoes exocytosis just after the nerve impulse arrival determine the frequency and the amplitude of the transmission signal. Because release occurs in a probabilistic fashion, the precise number of quanta released varies randomly from stimulus to stimulus. Specifically, the probability of quanta released follows a binomial distribution (see the section "Methods" below) [3].

Acetylcholine (Ach) is produced in the cytosol of neurons by the enzyme choline

acetyltransferase (ChAT). This reaction has acetyl-CoA and choline as substrates and the rate at which it occurs in neurons depends on the rate at which the TCA cycle works in the mitochondria, where acetyl-CoA is produced and exported to the cytosol [5]. Once Ach is produced, it is transported and stored inside synaptic vesicles through an ATP-dependent reaction. When a vesicle fuses with the axon membrane, Ach is released into the synaptic cleft and passively diffuses towards the nicotinic ionic channels in the post-synaptic membrane. Neurons producing Ach for neurotransmission are termed "cholinergic".

Acetylcholine-mediated synaptic transmission is very important for understanding the molecular and metabolic mechanisms involved in the development of dementia and Alzheimer's disease [4]. Alzheimer's disease (AD) is characterized by several neuropathological features such as cerebral atrophy, neuritic plaques, and neurofibrillary tangles. In parallel with these pathological changes associated with AD, there was an increasing interest in changes in various neurotransmitter systems. Deterioration in neural pathways involving transmitters such as acetylcholine, serotonin, and dopamine were identified. An important finding was the report from several laboratories of a significant decrease in choline acetyltransferase activity, a biochemical marker for cholinergic neurons, in the postmortem brains of demented patients [6].

These findings were instrumental in the development of the cholinergic hypothesis of dementia and AD [7]. According to this hypothesis, the learning and memory deficits of dementia in the aged and in AD may be attributable to a decline in the cholinergic systems of the basal forebrain. A substantial body of literature suggested that the learning and memory deficits observed in patients suffering from AD may be at least partly attributable to degeneration of cholinergic projection. Cholinergic neurons have a unique metabolic capability that may potentially contribute to their vulnerability in AD and aging [8]. Aging and AD may be associated with decreased choline uptake in synthesizing neurons. Thus, deprived of choline as a precursor, cholinergic neurons autocannibalize the choline-containing membrane phospholipids, and as a result promote their own demise [9].

## 2 Methods

Since metabolism plays an important role in the development of AD, it is plausible to think that this metabolism can be modeled and simulated using metabolic networks and systems biology to predict the phenotype of neurons via genome-scale models [10]. In this project, we aimed towards modeling the effects of deteriorated metabolism of AD neurons on the electrical properties of action potential being transmitted in cholinergic synapses. Using the Hodgkin Huxley equations, we were capable of connecting two neurons *in silico* using Python(x,y) and a mathematical framework found in literature. We found that AD neurons get "exhausted" early compared to normal neurons. Thus, we have incorporated Ach production and release into a common neurodynamic model to try to explain the physiological effects of neuronal metabolism on electric signal propagation.

### 2.1 Metabolic network analysis

Metabolic networks and interactions between neurons and astrocytes have been developed by Lewis et al [10]. Within this framework, Lewis and colleagues were capable of: (1) identifying key genes responsible for metabolic phenotypes in three different types of human neurons (glutamatergic, GABAergic, and cholinergic); and (2) characterizing relevant metabolic fluxes of these neurons in both normal individuals and patients with Alzheimer Disease. Based upon this model and the computed metabolic fluxes, the concentration of neurotransmitters inside each cell type and the rate of neurotransmission from presynaptic cells can be calculated. Since the metabolic fluxes determine the rate at which neurotransmitters build up in the neuron, we

expect to see different chemical synaptic interactions between neurons of different types (normal and AD). In order to analyze our metabolic network, we used the Constraint Based Reconstruction and Analysis (COBRA) Matlab toolbox [11]. Within this toolbox, all reactions in the metabolic network are described mathematically by a stoichiometric matrix, *S*, of size *m* x *n*, where *m* is the number of metabolites and *n* is the number of reactions, and each element is the stoichiometric coefficient of the metabolite in the corresponding reaction. The mass balance equations at steady state are represented as:

$$S \cdot v = 0$$

Where v is the flux vector. Maximum and minimum fluxes and reaction reversibility, when known, are placed on each reaction, further constraining the system as follows

$$v_{minimum} \leq v \leq v_{maximum}$$

At this point the model then can be analyzed with Flux Balance Analysis (FBA), which is a linear programming algorithm designed to find optimal states in metabolic networks [12]. This is shown in Figure 1 below.

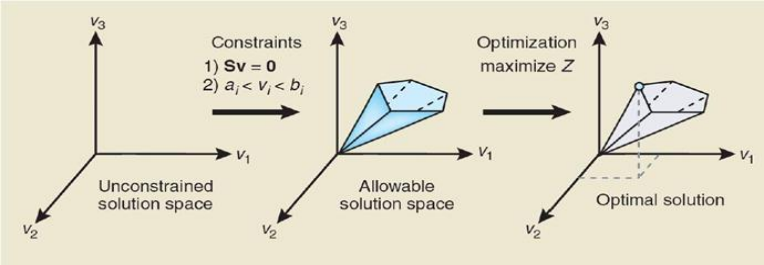



*Figure 1: Graphic representation of Constraint Based modeling and analysis. Here, Z represents the objective function to be optimized, which was chosen to be Ach production for this project. (Adapted from [10])*

## 2.2 Propagation of Action Potentials

We used Hodgkin Huxley model to account for action potentials and to generate a spiking signal in the presynaptic neuron by injecting an external current of 10 $\mu A/cm^2$ to induce synaptic release [13]. It is worth mentioning that the simulation time was 800 ms.

## 2.3 Excitatory synapse

The nature of the chemical synapse triggered by release of Ach is excitatory. Thus, the current generated by a receptor channel (nicotinic Ach channel) is given by

$$I_{syn} = g_{syn}(V - E_R)$$

Where $V$ is the membrane potential and $g_{syn}$the synaptic conductance and $E_R$ is the reversal potential of the ionic species involved [14]. For cholinergic synapses, it is usually assumed that $E_R$ is -5 mV.

As shown in the figure below, we have connected two neurons through a simple excitatory Ach synapse so that external current is injected only in the presynaptic neuron (normal or AD) and the postsynaptic action potential is recorded as a consequence of transmitter release.

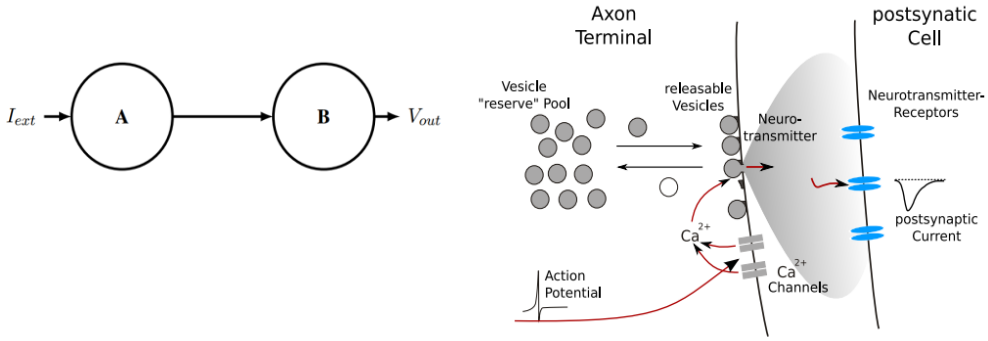



*Figure 2: (Left) Schematic representation of excitatory synapse. In our model, neuron A is either a normal or an Alzheimer neuron. (Right) Graphic representation of a chemical synapse. (Adapted from [14] and [15])*

Channel dynamics of the corresponding nicotinic receptors in the postsynaptic neuron are modeled using the following equations [16]

$$\frac{dr}{dt} = \alpha_r[T](1-r) - \beta_r r$$

$$[T] = \frac{[T]_{max}(t)}{1 + e^{-\frac{V_{pre}-V_p}{K_p}}}$$

$$I_{syn} = g_{Ach} r (V_{post} - E_p)$$

Where $Vpre$ and $Vpost$ are the pre and postsynaptic voltages, respectively, $g_{Ach}$ is the conductance of the nicotinic channel, $r$ is the fraction of open channels (0 to 1) and $[T]$ is the concentration of Ach.

### 2.4 Depression through vesicle depletion

To account for neurotransmitter exhaustion, we used the simplest vesicle depletion model [17].This model assumes that at each active zone, only a limited number of Ach-filled vesicles are available for release. Refilling of this release pool takes time (depends on Ach production rate which is given by the metabolic network), hence it will be progressively depleted during repetitive stimulation. Mathematically, this model can be written as

$$\frac{dn(t)}{dt} = \frac{1-n(t)}{\tau_r} - \sum_j \delta(t - t_j) \cdot p \cdot n(t)$$

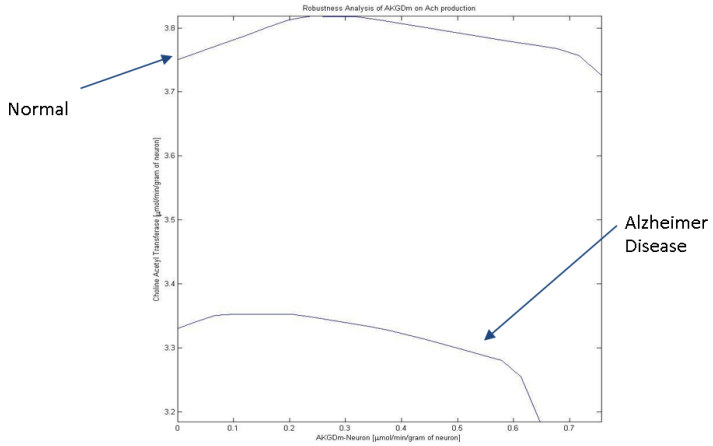
Where $n(t)$ is the occupancy of the release pool (bounded between 0 and 1). The first term on the right makes sure the release pool is refilled with a time constant $\tau_r$ (which depends on the metabolic flux of Ach production). The second term implements the release of vesicles each time a presynaptic action potential arrives (at times $t_j$, $j$=1..N).

## 3 Results

### 3.1 Metabolic flux

The first thing we evaluated was the *in silico* sensitivity of the metabolic network to changes of the flux in the TCA cycle in order to evaluate the optimal flux of Ach production in both the normal and Alzheimer Disease neuron. When performing Flux Balance Analysis in Matlab, we

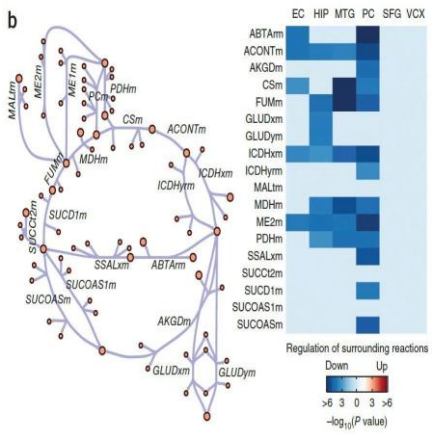
set the ChAT (choline acetyltransferase) reaction to be the objective function (Z). We constrained the flux through the TCA cycle to different values by constraining the flux through the rate-limiting reaction α-keto-glutarate dehydrogenase (AKDH).




It can be clearly seen the contrasting differences between the Ach production of normal neurons and that of AD neurons. Thus the production of Ach in AD neurons is reduced due to its inherent metabolic differences with respect to normal metabolism. The average Ach production rates of both cells was estimated to be as follows (with units $\mu mol * g^{-1} * min^{-1}$).

| Reaction flux | Normal | Alzheimer |
|---|---|---|
| Acetylcholine production | 3.82 | 3.35 |
| Vesicle production | 1.27 | 1.11 |

We also compared the fluxes through the TCA cycle in both computational neurons against experimental data [10].




| Reaction | Normal | Alzheimer |
|---|---|---|
| 'ACONTm' | 0.325785714 | 0.075228571 |
| 'AKGDm' | 0.0971 | 0 |
| 'CSm' | 0.325785714 | 0.075228571 |
| 'FUMm' | 0.325785714 | 0.075228571 |
| 'ICDHxm' | 0 | 0 |
| 'ICDHyrm' | 0.325785714 | 0.075228571 |
| 'MDH' | 1.93E-13 | 0 |
| 'MDHm' | 0.325785714 | 0.075228571 |
| 'SUCD1m' | 0.325785714 | 0.075228571 |
| 'SUCOAS1m' | 0 | 0 |
| 'SUCOASm' | -0.291585714 | -0.038964286 |

The experimental data is based on microarrays that allowed researchers to determine which reactions (genes) were down-regulated in AD patients. As can be seen from our table on the right, the metabolic network is capable of predicting the same results.

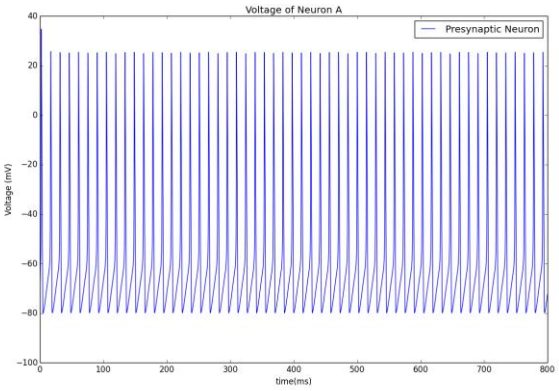
### 3.2 Chemical Synapse Model

After calculating the fluxes, our next step was to integrate this into the chemical synapse and then incorporate it in the simulation. First of all, we estimated the recovery constant $\tau_r$ for each type of cell using the Ach production rates obtained with the COBRA toolbox.

From our calculations, we rounded up the time constants to be 600 ms for normal cells and 300 ms for AD neurons. This value was incorporated in the equation of section 2.3 of this report. From the literature, we found that the value of $p$ can be determined using a binomial distribution (experimental data) and that a common value for this probability is 0.8 [18].

$$\frac{dn(t)}{dt} = \frac{1 - n(t)}{\tau_r} - \sum_j \delta(t - t_j) \cdot p \cdot n(t)$$

The input signal in the presynaptic neuron looks like this (Hodgkin-Huxley neuron with a current of 10 $\mu A * cm^{-2}$




We solved this differential equation numerically using Python(x,y). We implemented this in the presynaptic neuron for both the normal and AD case. This is the plot we obtained:

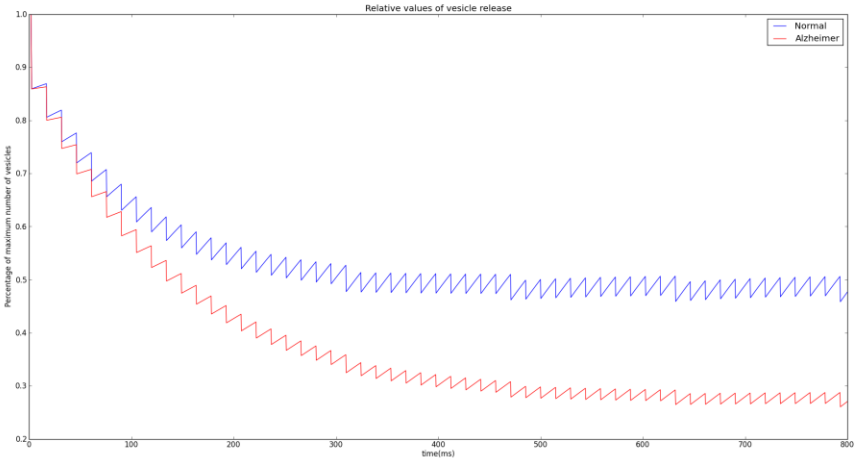



*Figure 3: Vesicle release $n(t)$ (bounded 0-1) for two Hodgkin-Huxley neurons, normal (blue) and Alzheimer (AD). The presynaptic action potentials have a frequency of 66 Hz which was achieved injecting an external current of 10 $\mu A * cm^{-2}$*

This successfully showed, as expected, that AD neurons cannot refill their vesicles as normal

neurons do. With these results, we proceeded to evaluate two things: 1) the excitatory post-synaptic potentials (EPSPs) triggered in the postsynaptic neuron and 2) the action potentials. For the first of these, we modify the transmitter release equation so that it could account for vesicle depletion as follows

$$[T](t) = \frac{[T]_{max} * n(t)}{1 + e^{-\frac{V_{pre} - V_p}{K_p}}}$$

Where $n(t)$ is the time-dependent value obtained previously. Now, we were finally ready to evaluate and plot spikes in the post synaptic neuron by inducing these two different patterns of vesicle release.

The first thing plotted were the EPSPs, we noted that the postsynaptic neuron has exponentially decaying EPSPs when connected to a presynaptic AD neuron whereas EPSPs remain unaffected when connected to a normal vesicle releasing neuron.

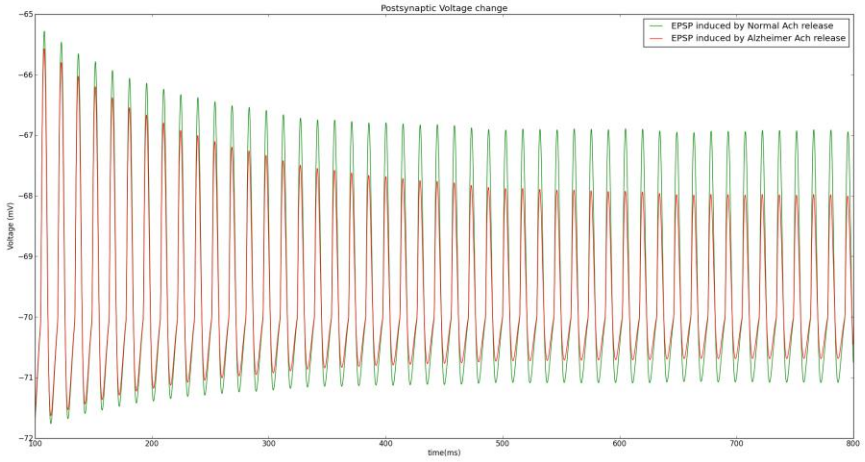



*Figure 4: EPSPs induced in the postsynaptic neuron when connected to a presynaptic normal (green) or to an AD (red) neuron. Note the decaying amplitude of these EPSPs as time elapses, which is a reflection of vesicle exhaustion happening in the presynaptic cell as action potentials are continuously arriving to the axon.*

Finally, we evaluated the action potentials. It was really interesting to see how the presynaptic AD neuron gets exhausted of Ach before the normal neuron and thus, the action potentials in the postsynaptic neuron stop early in the simulation.

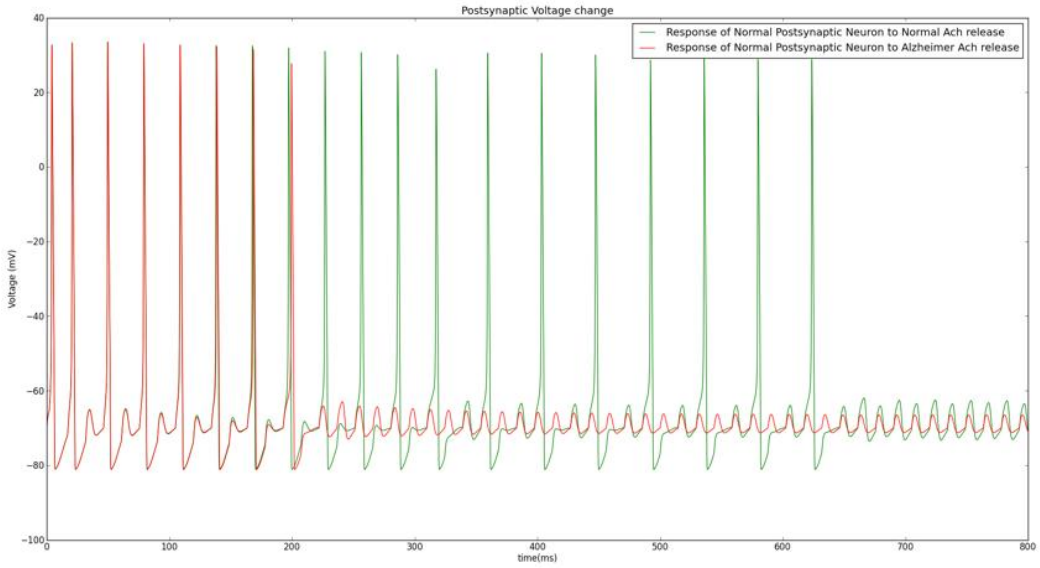



*Figure 5: Action potentials triggered in the postsynaptic neuron for the two presynaptic alternatives. Note how both cases reach neurotransmitter exhaustion but it happens earlier in the AD neuron (red). The simulation time was 800 ms.*

# 4 Conclusions

In this project, we have built a simple computational model that accounts for neurotransmitter release in Hodgkin-Huxley neurons. We incorporated a systems biology approach to successfully account for the metabolic cost and dynamics of Ach production. From our last plot, it was clear that metabolism plays a key role in the electrical properties of the synapse.

The last plot shows how AD neurons get exhausted faster than normal neurons when excited with continuous action potentials. This showed that:

1. The difference in acetylcholine production between normal and AD neuron determines the enrichment of the synaptic vesicles.
2. The availability of neurotransmitters in pre-synaptic release pool affects the post-synaptic membrane voltage response.
3. The membrane conductance in the receptor channel can determine if an action potential is fired.

Therefore, we've showed how a simple difference in metabolism can affect the nature of the electrical synapse. This implies that synaptic plasticity will also be affected in the long term (explaining the loss of short-term memory in AD patients from a metabolic and neurodynamic perspective). That's something worth exploring in future work. Other than that, we would also like to expand into small neural networks and look at how AD neurons affect neuronal interaction and information transmission in a network scale.

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