Model of NMDA-Induced Oscillations

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Model of NMDA-Induced Oscillations

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Model of NMDA-induced oscillations, Abstract
The aim of this master thesis-project was to examine the NMDA-receptor part and the NMDA-induced oscillations in an existing mathematical model of a lamprey spinal cord neuron. Experimental studies have previously been performed in isolated lamprey spinal cords, and the results show that fictive swimming can be induced when placing the spinal cords in a physiological bath solution containing excitatory amino acids such as NMDA. Increasing the concentration of NMDA in the bath will give an increase in frequency of the neural oscillatory activity underlying fictive swimming. Also data from experiments imitating single-cell situations suggest the same behaviour.

However, in the original mathematical model that was examined in this work, an increased NMDA-concentration instead gave rise to a prolonged plateau-phase and a somewhat shortened hyperpolarized phase in the oscillatory activity pattern underlying fictive swimming, eventually leading to a decreased frequency. In order to examine the effects of different involved model parameters and try to make the model behave more similar to experimental data, two simplified models were implemented.

It was not easy to achieve a behaviour that corresponds well to biological data. The simulations, however, have given some insights. For example, it seems there should be some kind of hyperpolarizing force that is activated during the plateaus, and which can terminate them earlier. This force should be dependent on (and growing with) NMDA-concentration and also with increasing voltage. Another thought is that NMDA-saturation, which is not considered in the original model, could be taken into account.

Modell av NMDA-inducerade oscillationer, Sammanfattning
Syftet med detta examensarbete var att undersöka NMDA-inducerade oscillationer i en existerande matematisk modell av ett ryggmärgsneuron från nejonöga. Experimentella studier har tidigare utförts på isolerade ryggmärger från nejonögon, och resultaten visar att fiktiv simning kan induceras om ryggmärgen placeras i ett bad med fysiologisk saltlösning som innehåller excitoriska aminosyror, t.ex. NMDA. En ökad NMDA-koncentration i badet ger upphov till en ökad frekvens i den oscillatorande neurala aktiviteten som ger upphov till simningsrörelser i det intakta djuret. Även data från experiment som imiterar ett isolerat ryggmärgsneuron indikerar samma beteende.

I den ursprungliga matematiska modellen som undersöktes i detta examensarbete gav istället en ökad NMDA-koncentration upphov till en förlängd platå-fas och en något förkortad hyperpolariserad fas i den oscillatorande neurala aktiviteten som styr den fiktiva simningen, vilket för högre NMDA-koncentrationer därmed leder till en minskning av simningsfrekvensen. Två förenklade modeller implementerades för att kunna undersöka de involverade parametrarna och försöka få modellen att bete sig mer likt experimentella data.

Det var inte lätt att uppnå ett beteende som väl liknar biologiska data. Simuleringarna har ändå gett upphov till huvudsakligen två insikter. Det verkar som att det krävs någon form av hyperpolariserande kraft som aktiveras under platåerna och som kan terminera dessa tidigare. Denna kraft borde bero av (dvs öka med) NMDA-koncentrationen och membranpotentialen. En annan tanke är att man kan ta NMDA-mättnad i beaktande.
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1 Introduction
The aim of this master thesis-project was to examine the NMDA-receptor part and the NMDA-induced oscillations in an existing mathematical model of a lamprey spinal cord neuron. In experimental studies of fictive swimming in a lamprey spinal cord, it has been shown that fictive swimming can be induced when placing the spinal cord in a bath of NMDA. Most of the neurons continue to oscillate also when imitating a single-cell situation by adding tetrodotoxin (TTX) to the bath, which will remove the synaptic connections and block action potentials (Grillner S, Wallén P, 1985; Sigvardt et al., 1985). Experimental data from a piece of spinal cord (without TTX) in a bath of NMDA shows that with an increased NMDA-concentration in the bath, the frequency of fictive swimming increases (Brodin et al., 1985). If TTX is added to the bath, an increased NMDA-concentration will give an increased frequency of oscillations until a limit where it stays at the same level (Wang et al., 2006). These data could suggest that even in a single cell, the frequency of NMDA-induced oscillations would increase when increasing the NMDA-concentration. However, in the original mathematical model used in this work, an increased NMDA-concentration induces a prolonged plateau-phase while the hyperpolarized phase is shortened. In the model, the frequency of oscillations will eventually decrease when the prolongation of the plateau-phase overcomes the shortening of the hyperpolarized phase.

2 Background

2.1. The NMDA-receptor
General
The NMDA (N-methyl-D-aspartate)-receptor belongs to a family of receptors called ionotropic glutamate receptors. Ionotropic glutamate receptors have glutamate as their transmitter, and a cation-selective ionchannel. Other ionotropic glutamate receptors are AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and Kainate receptors (Dingledine et al., 1999). NMDA-receptors are permeable to Na⁺, K⁺-ions and Ca²⁺-ions. Their high permeability for Ca²⁺ is of special interest, since Ca²⁺ takes part in many functions in the central nervous system (CNS) such as creating changes in synaptic strength (synaptic plasticity).
NMDA-receptors have an important role in many of the normal activities in the CNS, for example during the neuronal development (Moghaddam, 2003), in the central synaptic transmission where information is exchanged between neurons, learning and establishment of memory which is believed to involve synaptic plasticity such as long term potentiation, LTP (Moser et al., 1998) where the strength of a synaptic connection is increased for a long time. Also some pathological conditions have been proposed to involve NMDA-receptors. NMDA-receptors play an important role in a process called excitotoxicity, in which too much glutamate is released. Excitotoxicity can eventually kill neurons as a result of overstimulation of their glutamate-receptors, which will lead to
too much inflow of Ca\textsuperscript{2+} into the cell. It is implied that excitotoxicity is involved in many pathological conditions for example epilepsy and in brain damage caused by ischaemia (Lynch DR and Guttmann RP., 2001). NMDA-receptors are possibly involved in some of the neurodegenerative diseases such as Alzheimers disease, Parkinsons disease and Amyotrophic Lateral Sclerosis (Dingledine et al., 1999). There are also some findings indicating that NMDA-receptors are somehow involved in some of the psychiatric disorders, for example depression (Kugaya A, Sanacora G, 2005) and schizophrenia (Javitt, 2007; Mohn et al., 1999).

Structure & subunits of the NMDA-receptor

It is believed that NMDA-receptors are multimers consisting of four subunits (Furukawa et al., 2005). A subunit (see Fig 1) contains three trans-membrane (TM) domains (TM1, TM3 and TM4) that span the membrane, and the M2 segment that does not go through the whole membrane but makes a loop in the transmembrane region (Dingledine et al., 1999). A subunit also has an amino-terminal (N-terminal) located in the extracellular space, a carboxyl-terminal (C-terminal) in the intracellular space and the S1S2 ligand-binding core (Dingledine et al., 2005).

Fig 1. General structure of a subunit from a ionotropic glutamate receptor.

Three main families of NMDA subunits have been identified: NR1, NR2 (A-D) and NR3 (A-B). There are eight NR1 subunits that comes from different splicing of one single gene, NR2 subunits can be coded by 4 different genes (A-D) and NR3 can be coded by 2 different genes (A and B). Functional NMDA-receptors consist of NR1 subunits and at least one type of NR2 subunit both of which are needed for the receptor to function. It is believed, although not fully examined, that most NMDA-receptors consist of four subunits, of which two are NR1 and the two others are NR2 of either same or different type (Paoletti and Neyton, 2007). Properties of functions such as kinetics, conductance and ion channel block will be different depending on which NR2 subunits the receptor contains (Sobolevsky et al., 2007). The NR3 subunit can only be expressed together with NR1 and NR2 subunits for a functional receptor. NR3A subunits are believed to modulate the current of ions flowing through the receptor, for example amplitude of
the current and Ca\(^{2+}\)-inflow (Dingledine et al., 1999). Single channel conductance, inflow of Ca\(^{2+}\) and Mg\(^{2+}\)-block are lower in the NMDA-receptors that contain the NR3 subunit (Cavara et al., 2008; Tong et al., 2008).

**NMDA-receptor binding-sites**

The NMDA-receptor requires for its opening binding of its transmitter glutamate and a coagonist glycine (Kleckner and Dingledine, 1998). Studies on mouse hippocampal neurons using voltage-clamp recordings in combination with mathematical models have indicated that two molecules of glutamate and two molecules of glycine must bind for activation of the ion-channel (Benveniste and Mayer, 1991).

![Fig 2. NMDA-receptor, permeability and recognition sites.](image)

The glycine binding-site is formed by the NR1 subunit (Lynch et al., 1994; Dingledine et al., 1999) and the glutamate-binding site is formed by the NR2 subunit (Lynch, 2001; Anson et al., 1998; Laube et al., 1997; Dingledine et al., 1999). If the NMDA-receptor contains two NR1-subunits and two NR2-subunits, this will give two binding-sites for glutamate and two binding-sites for glycine. Both the NR1 and all of the NR2 subunits contain an asparagine in a critical site called the “N-site” (because of asparagines label ‘N’). The “N-site” is located in segment M2. Replacing the asparagine with a glutamine will strongly reduce both the Ca\(^{2+}\)-permeability and the Mg\(^{2+}\)-block (see explanation below) sensitivity (Liu Yun and Zhang Juntian, 2000; Ishii et al., 1993). It is therefore believed that the M2 segment is the channels pore region.

The NMDA-receptor is blocked by Mg\(^{2+}\)-ions that bind to a binding-site, probably situated deep inside of the channel-pore (Cull-Candy and Brickley, 2001), while the channel is open. At resting membrane potential and physiological concentrations of Mg\(^{2+}\), no ions can flow through the NMDA-receptor, due to the binding of Mg\(^{2+}\)-ions inside of the channel pore. At depolarization of the membrane potential, the Mg\(^{2+}\)-ion can release from its binding site, and allow for other ions to flow through the ion-channel.
The NMDA-receptor also has recognition-sites for Zn\textsuperscript{2+}, polyamines and H\textsuperscript{+}, which can modulate its behaviour in different ways. The modulation of NMDA-receptors by polyamines has been examined using the polyamines spermine and spermidine. The effect has been shown to be somewhat complex and can be either inhibitory or potentiating, depending on concentration of the polyamines. At low concentrations, polyamines will have a potentiating effect on the NMDA-receptor. This effect is probably induced via two different mechanisms of which one will increase the receptors affinity for the agonist glycine, while the other, independent of glycine-concentration, will potentiate the ion-channel in some way. At high concentrations, polyamines will block the ion-channel in a voltage-dependent manner, indicating involvement of a binding-site inside of the channel-pore. H\textsuperscript{+} has an inhibitory effect on the NMDA-receptor, making NMDA-function very pH-dependent. Zn\textsuperscript{2+} also inhibits the receptor (McBain et al., 2001). Reducing and oxidizing agents also modulate NMDA-receptor behaviour. Experiments using reducing agent DTT and oxidizing agent DTNB have shown DTT to potentiate and DTNB to inhibit NMDA-receptor-mediated synaptic potentials in the hippocampus (Tauck DL, 1992). These compounds are not endogenous, but there are some speculations about endogenous red-ox-agents that can affect NMDA-receptors as well, for example NO (McBain and Mayer, 1994).

**Distribution of subunits in the CNS**

The NR1-subunit exists in its different splice-variants everywhere throughout the CNS, consistent with the belief that it is probably always a part of each functional NMDA-receptor. Distribution of the other subunits (NR2, NR3) may vary. Expression of the different subunits in various places is not static but will change during development (Watanabe et al., 1992). Since, as earlier described, properties and functions of NMDA-receptors are believed to depend on which subunits they consist of, NMDA-receptors functions may vary in different places in the CNS, depending on which subunit-compositions are the most commonly existing in the place. Experiments using immunoblotting-techniques with antibodies for NR2A- and NR2B-subunits have shown that the NR2B-subunit protein in rat is most commonly expressed in parts of the forebrain such as the cerebral cortex, hippocampus and olfactory bulb. NR2B-subunits also exist to some extent in the striatum, colliculus and the brainstem (Loftis and Janowsky, 2003; Wang et al., 1995). Expression of the NR2A-subunit is most common in the hippocampus and cerebral cortex. It has also been found in somewhat lower concentrations in colliculus, striatum, cerebellum and in the olfactory bulb (Wang et al., 1995). The NR2C-subunit is mostly expressed in the cerebellum, and has been found in the rat lumbar spinal cord (Goebel and Poosch, 1999; Lynch and Guttmann, 2001; Yun and Juntian, 2000). NR2D-subunits are not very common in the brain but are predominantly expressed in the spinal cord (Yun and Juntian, 2000). The highest concentration of NR3A-subunits has been found in the colliculi and in hypothalamus (Goebel and Poosch, 1999).


2.2 Mg\(^{2+}\)-block of NMDA-receptors

A unique property of NMDA-receptors compared to the other ionotropic glutamate receptors is that for current to flow through the channel both the binding of its agonists and a depolarization of the membrane potential is needed. Therefore, sometimes the NMDA-receptor is called a coincidence-detector. This voltage-dependence depends on block by extracellular Mg\(^{2+}\)-ions, which will bind/unbind in a voltage-dependent manner (Nowak et al., 1984). Even intracellular Mg\(^{2+}\) can bind to and block NMDA-receptors. Amino-acid substitutions at different sites inside of the channel have been shown to affect the internal and external Mg\(^{2+}\)-block to varying extent. A finding suggests that extracellular and intracellular Mg\(^{2+}\) bind to different sites inside of the channel pore (Kupper et al., 1996). The sensitivity for Mg\(^{2+}\)-block by NMDA-receptors is different depending on what subunits the receptor contains. Receptors that contain NR2A or NR2B-subunits have shown a larger sensitivity to Mg\(^{2+}\)-block than receptors with NR2C- and NR2D subunits (Ishii et al., 1993; Dingleidine et al., 1999; Yun and Juntian, 2000).

The Mg\(^{2+}\)-unblock, at least in NR2A and NR2B-containing NMDA-receptors, has been shown to be non-instantaneous and to consist of different components, with one fast component and also one or several slower components as reaction to membrane potential changes. After depolarization of the membrane potential due to activation of AMPA-receptors, a fast transient rise in conductance followed by a longer period of elevated conductance has been observed (Vargas-Caballero and Robinson, 2003; Kampa et al., 2004; Vargas-Caballero and Robinson, 2004).

There are some conceptual models to describe different blocking-mechanisms: 

**Open channel block:** With the open-channel block, the blocking ion can only bind/unbind to its site when the ion-channel is in its open state (Ascher and Nowak, 1998). The blocking-model used for the magnesium-block in this thesis-work is one similar to the open channel block model.

**Sequential block:** In sequential block models, the blocking ion will prevent the channel from closing while it is bound, making it impossible for the ion to get trapped inside of the channel (Antonov and Johnson, 1996; Neher and Steinbach, 1978; Adams, 1976).

**Trapping-block:** In trapping-block models, the channel can close while the ion is inside it, and the agonist(s) can unbind. This will lead to trapping of the ion inside of the channel. For the trapped ion to release, it will be required that the agonists bind again and that the membrane potential is depolarized. This trapping-block scheme has been proposed to model the magnesium-block of NMDA-receptors (Benveniste and Mayer, 1995; Sobolevsky and Yelshansky, 2000; Blanpied et al., 1997). Depolarization of the membrane potential can be caused for example by activation of AMPA-receptors. A modification of the trapping-block scheme with the closing-rate of the receptor being faster when the channel is blocked, a so called asymmetric trapping block (ATB) model, has also been suggested for the magnesium-block (Vargas-Caballero and Robinson, 2004).
2.3 $\text{Ca}^{2+}$ and $K_{\text{Ca}}$-channels

$\text{Ca}^{2+}$ from outside enters the cell through NMDA-channels, AMPA-channels, low voltage-gated Ca$^{2+}$-channels and high voltage-gated Ca$^{2+}$-channels. It is then transported away from the cell-cytosol by pumps, buffers etc. In some cases this will give an oscillation-pattern in Ca$^{2+}$-concentration inside of the cell if NMDA-receptors are enough activated. This is the case in the lamprey spinal cord (see below).

![NMDA-oscillation-pattern, voltage and [Ca$^{2+}$].](image)

Ca$^{2+}$ inside of the cell can activate $K_{\text{Ca}}$-channels. There are several different types of $K_{\text{Ca}}$-channels, some of which are not only dependent on intracellular concentration of Ca$^{2+}$ but are also voltage-dependent, while others show no voltage-dependence. The opening of $K_{\text{Ca}}$-channels will have a hyperpolarizing effect on the membrane potential when K$^+$ flows out of the cell.

2.3.1 NMDA-induced oscillations in the lamprey spinal cord can be divided into four phases

When applying TTX to a piece of spinal cord from lamprey thereby blocking the Na$^+$-channels, the synaptic connection between neurons that comes from Na$^+$-mediated spikes is removed. TTX is therefore used to simulate single-cell situations. In many of the neurons, oscillations can still be observed when TTX is added. These NMDA-induced TTX-resistant oscillations look similar in shape to oscillations without TTX, except from that they do not have spikes coming from Na$^+$-channels on their plateau.
An NMDA-induced oscillation can be said to have 4 phases (see Fig 4).
1) *Slow depolarization* due to a lowered concentration of Ca\(^{2+}\) in the cell, which leads to the force from the hyperpolarizing K\(_{\text{Ca}}\)-channels getting weaker.
2) A level in the membrane potential is reached where the Mg\(^{2+}\)-block is relieved, causing many NMDA-channels to be opened. This will give a *fast depolarization*. Na\(^+\) and Ca\(^{2+}\) flows into the cell. The largest part of the depolarization is due to inflow of Na\(^+\), and only to a smaller extent due to inflow of Ca\(^{2+}\).
3a) Voltage-gated K\(^+\)-channels are opened and even some K\(_{\text{Ca}}\)-channels, because of Ca\(^{2+}\) that has started to enter the cell. These two will counteract the NMDA-channels and a balance between these two forces is reached, so that the *membrane potential stays on a plateau*.
3b) A gradual accumulation of Ca\(^{2+}\) inside the cell due to Ca\(^{2+}\) entering through NMDA-channels and voltage-gated Ca\(^{2+}\)-channels will start to activate K\(_{\text{Ca}}\)-channels, K\(^+\) begins to flow out from the cell giving a slow hyperpolarizing effect on membrane potential.
4) This Ca\(^{2+}\) will eventually open enough K\(_{\text{Ca}}\)-channels so that the membrane potential reaches a value where many NMDA-channels again are closed because of Mg\(^{2+}\)-block. This gives a *fast repolarization* (Wallén and Grillner, 1987).

### 3 Methods

The aim here is to use this biological information to try to understand how the frequency and shape of NMDA-induced oscillations in the mathematical model will be affected when different parameters are varied. A special focus is to try to make the oscillations more similar to oscillations obtained in experiments where NMDA-concentration has been varied.

In this work two software-tools have been used for simulations, GENESIS and XPPAUT (described below). Plotting the curves and analyzing the results have been done in MATLAB. The NMDA-concentration, Ca\(^{2+}\)-permeability, Mg\(^{2+}\)-block, and K\(_{\text{Ca}}\)-activation have been examined and varied to understand their role for the oscillations.

#### 3.1 GENESIS

Second edition, Springer-Verlag, New York (1998)) is a software-tool that is used to implement neuron-models and run simulations. In the present work, a whole-cell implementation of the model in GENESIS was used although only the part responsible for NMDA-induced oscillations was examined. The cell has a soma, an initial segment and 8 compartments, 3 levels of dendrites (primary, secondary and tertiary dendrites).

3.2 XPPAUT

XPPAUT (Ermentrout, 2002) (Bard Ermentrout, Simulating, Analyzing, and Animating Dynamical Systems: A Guide to XPPAUT for Researchers and Students, SIAM 2002, Philadelphia, USA.) is a software-tool that solves ODE-equations. XPPAUT was used for a simplified implementation of the model where only the NMDA-channels, voltage-dependent $K^+$-channels and $K_{Ca}$-channels are taken into consideration. Also an even more simplified version with only NMDA-channels and $K_{Ca}$-channels was used in XPPAUT. Some of the tests were not possible to do with the GENESIS-model since there are so many other things affecting the result. Therefore, those tests were done only in XPPAUT.

3.3 Original model

The NMDA-channel-part of the model is based on the article: Computer simulations of N-methyl-D-aspartate receptor-induced membrane properties in a neuron model. (Brodin et al., 1991).

3.3.1 Membrane potential

The change in membrane potential is modeled using the equation:

$$\frac{dV}{dt} = g_L (E_L - V) + g_{NMDA} [NMDA] p (E_{NMDA} - V) + g_{KCa} [Ca] (E_{KCa} - V)$$

$V$ = resting membrane potential
$g_L$ = conductance for leak-channels
$E_L$ = reversing potential for leak-currents
$g_{NMDA}$ = conductance for NMDA-channels
$E_{NMDA}$ = reversing potential for NMDA-channels
$p$ = number of open NMDA channels
$g_{KCa}$ = conductance for $K_{Ca}$-channels
$E_{KCa}$ = reversing potential for $K_{Ca}$-channels

$g_{NMDA(total)}$ is linearly proportional to $[NMDA]$, where $[NMDA]$ is a constant value.
3.3.2 Calcium permeability

Fig 5. Ca\(^{2+}\) in the model is divided into Ca\(_{\text{NMDA}}\) entering through NMDA-channels and CA\(_{\text{HVA}}\) entering through voltage-gated Ca\(^{2+}\) channels.

Ca\(^{2+}\) entering the cell is divided into Ca\(^{2+}\) entering through NMDA-channels (Ca\(_{\text{NMDA}}\)) and Ca\(^{2+}\) entering through voltage-gated Ca\(^{2+}\) channels (CA\(_{\text{HVA}}\)). This is modeled using two separate Ca\(^{2+}\)-pools in the cell-model, one Ca\(_{\text{NMDA}}\)-pool with Ca\(^{2+}\) from Ca\(_{\text{NMDA}}\) and one CA\(_{\text{HVA}}\)-pool with Ca\(^{2+}\) from CA\(_{\text{HVA}}\).

The time-dependent concentration of the Ca\(^{2+}\) coming from NMDA-channels (Ca\(_{\text{NMDA}}\)) in the cell at a time \(t\) is described by the equation:

\[
d[Ca]/dt = B*I_{\text{CONMDA}}[Ca]/\tau
\]

\(B\) = a constant that transforms current into concentration

\(I_{\text{CONMDA}}\) = Ca\(^{2+}\) that is entering the cell through NMDA-channels

\([Ca]\) = Ca\(^{2+}\)-concentration inside of cell

\(\tau\) = decay constant of Ca\(^{2+}\)

Ca\(^{2+}\)-concentration inside of the cell depends on the rate of influx through NMDA-channels, \(B*I_{\text{CONMDA}}\) and a decay-constant, \(\tau\) which decides the rate in which Ca\(^{2+}\) is transported away from the intracellular medium.

3.3.3 \(K_{\text{Ca}}\)-activation

\(K_{\text{Ca}}\)-activation is divided into \(K_{\text{Ca}}\) channels activated by Ca\(^{2+}\) from the Ca\(_{\text{NMDA}}\)-pool, \(K_{\text{Ca}}\)\(_{\text{NMDA}}\)-channels and those activated by Ca\(^{2+}\) from the CA\(_{\text{HVA}}\)-pool.

The \(K_{\text{Ca}}\)\(_{\text{NMDA}}\)-channels in the original model are activated described by the function \(Ca_{\text{conc}}/(Ca_{\text{conc}}+K)\), where \(K\) is a constant =5e-7.
3.3.4 Mg$^{2+}$-block

The Mg$^{2+}$-block in NMDA-receptors is in this model assumed to be a so called open channel block, which means that Mg$^{2+}$-ions can only bind to the channel when it is in its open state. This is described by some equations that are supposed to represent an open-channel block-scheme in which channels have three states: open, closed or blocked (Brodin et al., 1991; Ascher and Nowak, 1988; Woodhull, 1973). In the open channel block-model, the channel is either closed, open or blocked, it can not be both blocked and open or blocked and closed. The channel can go from closed to open state/open to closed and from open to blocked/blocked to open. It is not possible for the channel to go from the blocked state to closed, which would maybe illustrate trapping of the ion inside of the pore. This would require the channel to be able to be closed/blocked or blocked/open. Another model has been proposed by (Vargas-Caballero and Robinson, 2004) where the trapped states in which the channel can be closed and blocked are also included in the scheme.

The original open channel block-scheme can be written as: (Ascher and Nowak, 1998)

\[
\text{Closed} \quad \xleftrightarrow{\alpha} \quad \text{Open}
\]

\[
\text{Open} + \text{Mg}^{2+} \xrightarrow{\beta} \text{Blocked}
\]

Fig 6. Open channel block-scheme.

However, in this model, channels are assumed to be always in their open state, since agonists are always available in enough concentration when it simulates a situation of bath-applied NMDA. Therefore, only the simulation from unblocked to blocked state is done, i.e. the lower line in the scheme.

The blocking reaction is described by the equation:

\[
\frac{dp}{dt} = \alpha_p*(1-p) - \beta_p*p,
\]

where

\[
p = \text{the fraction of NMDA-channels that are in their open state.}
\]

\[
\alpha_p = \alpha_A*e^{(E/\alpha_c)}
\]

\[
\beta_p = \beta_A*[Mg]*e^{(-E/\beta_c)}
\]

$\alpha_p$ is a rate constant for unblocking.

$\beta_p$ is a rate constant for blocking.
Values of rate constants used in the original model:
\[ \alpha_A = 700 \text{ s}^{-1} \]
\[ \alpha_C = 17 \text{ mV} \]
\[ \beta_A = 5.6 \text{ mM}^{-1}\text{s}^{-1} \]
\[ \beta_C = 17 \text{ mV} \]
\[ [\text{Mg}] = 1.8 \]
\[ E = \text{membrane potential} \]

Fig 7. Mg\(^{2+}\)-block probability-curve with original values on parameters.

### 3.4 Two simplified models

In order to examine some mechanisms, two simplified models were used. The most simplified has NMDA-channels and K\(_{\text{Ca}}\)-channels.

The equation used to describe membrane potential changes in this model is:
\[ \frac{dv}{dt} = 1000(p(v) \cdot g_{\text{nm}da} \cdot (0-v) + g_{\text{kca}} \cdot \text{act}_{\text{kca}}(C) \cdot (v_{\text{rest}} - v)) \]

The other model has also K\(_{\text{A}}\)-channels added and its membrane potential is described by the following equation:
\[ \frac{dv}{dt} = 1000(p(v) \cdot g_{\text{nm}da} \cdot (0-v) + g_{\text{kca}} \cdot \text{act}_{\text{kca}}(C) \cdot (v_{\text{rest}} - v) + g_k \cdot \text{act}_{k}(v) \cdot (v_{\text{rest}} - v)). \]

for the two equations above,
\[ p(v) \] is a function describing the Mg-block,
\[ \text{act}_{\text{kca}}(C) \] is a function describing activation of K\(_{\text{Ca}}\)-channels and
\[ \text{act}_{k}(v) \] the activation of voltage-gated K-channels.

The equation used to describe the changes in [Ca] is:
\[ \frac{dc}{dt} = i \cdot p(v) \cdot g_{\text{nm}da} \cdot (0-v) - C/\tau \]

In the model without K\(_{\text{A}}\)-channels, the initial value of C(0) is set to 20 and in the model with K\(_{\text{A}}\)-channels, it is set to 0.

The same Mg-block-equations as in the original GENESIS-model have been used (see chapter 3.3.4, Fig. 7).
4 Results

Four main things have been examined in this work. In section 4.1 the following are investigated:
1. The frequency range in the original model, i.e. the range of NMDA-concentration within which oscillations will occur for the different models tested.
2. The role of Ca\(^{2+}\)-dynamics, inflow etc.
3. How oscillations are affected by assumed different K\(_{\text{Ca}}\)-activation-mechanisms.
4. The role of different shapes of the Mg-block curve for the oscillations.

Section 4.1.5 shows results from the XPPAUT-model with K\(_{\text{A}}\)-channels, where a hyperpolarizing force which is dependent of NMDA-concentration and which activates at the plateau-voltage levels has been added in form of adding a NMDA-dependence to the voltage-activated K-channels. In section 4.1.6, the dependence of membrane resting potential and what happens when changing the membrane potential by injecting current has been examined to some extent. Some parts of this have been done in the model with NMDA and K\(_{\text{Ca}}\)-channels and in the GENESIS-model.

Section 4.2 shows results where the same things that were previously examined in the XPPAUT-models (section 4.1) are confirmed with the GENESIS-model, with some exceptions. Ca\(^{2+}\) inflow has not been separately simulated in the GENESIS-model. Not all K\(_{\text{Ca}}\)-activations that were examined with XPPAUT could be examined with GENESIS because oscillations could not always be obtained in the GENESIS-model due to the complexity of this model involving many other ion-channels.

The last section in the results-chapter shows results where NMDA saturation has been taking into consideration.

4.1 Results in XPPAUT-models

Two original models have been implemented in XPPAUT. One is a simple model in order to be able to test things without involving too many parameters, this model has only K\(_{\text{Ca}}\) and NMDA-channels. In biological lamprey-neurons, K\(_{\text{A}}\)-channels also are present and play an important role. These channels have been added to the other model, which consists of K\(_{\text{Ca}}\), K\(_{\text{A}}\) and NMDA-channels. This is the only difference between the two models, and also that the initial value of [Ca] which is 0 in the model with K\(_{\text{Ca}}\), K\(_{\text{A}}\) and NMDA-channels is set to 20 in the model with no K\(_{\text{A}}\)-channels. Results from the model with only K\(_{\text{Ca}}\) and NMDA-channels will be presented first and after that, results of the same thing from the model with K\(_{\text{A}}\)-channels will be presented.
4.1.1 Frequency range

**Model without K\textsubscript{A}-channels**

In the first set of simulations, the NMDA-activation was varied.

![Graph showing voltage-oscillations at different NMDA-concentrations](image)

Fig 8. Voltage-oscillations at different NMDA-concentrations in the XPPAUT-model with only K\textsubscript{CA} and NMDA-channels.

The NMDA-span within which oscillations occur is 2.39-6.27. However, the oscillations at NMDA=5.00 and lower do not look so much like the natural oscillations and can possibly be some oscillations that can only be obtained in the XPPAUT-model since it has so few channels (see Fig. 8).

![Graph showing frequency, duration of plateau-phase and hyperpolarized phase](image)

Fig 9. Frequency, duration of plateau-phase and hyperpolarized phase in relation to [NMDA].

The frequency of oscillations will first increase with [NMDA] and then decrease. The plateau-phase will, to a beginning, get shorter and then starts to be prolonged. The hyperpolarized phase does not actually get shorter, but the rate in which it prolongs is not as fast as it is for the plateau-phase, so the result for the shape of oscillations is that
the plateau-phase gets much longer in comparison with the hyperpolarized phase above NMDA=6.00.

Model with K\(_A\)-channels
As mentioned earlier, K\(_A\)-channels exist in the biological lamprey-model and play an important role. Therefore, in the other XPPAUT-model K\(_A\) -channels have been included. The K\(_A\)-channels have a K-activation-curve looking like the figure below. The K\(_A\)-channels are voltage-dependent only and activated following the function:

\[ k_{activation} = mk \cdot hk \]

where,

\[ mk(v) = \frac{1}{1 + e^{\frac{(V_0 - V)}{-10.6}}} \]

and

\[ hk(v) = \frac{1}{1 + e^{\frac{(V_0 - 9.3)}{11.7}}} \].

![K-activation used in the XPPAUT-model with K\(_A\)-channels.](image)

Fig 10. K-activation used in the XPPAUT-model with K\(_A\)-channels.

![Voltage oscillations, Hill-K\(_A\)-activation.](image)

Fig 11. Voltage oscillations, Hill-K\(_A\)-activation.
When $K_A$-channels are included in the XPPAUT-model, the amplitude of the oscillations is lower compared to the amplitude in the model without $K_A$-channels, since another hyperpolarizing force is added in form of the $K_A$-channels. The concentrations at which oscillations arise are also a little higher. In this model the amplitude also is slightly affected by differences in NMDA-concentration, with a higher NMDA-concentration giving a higher amplitude. This is maybe because the $K_A$ channels are dependent only of membrane potential and not of Ca-concentration or NMDA-concentration. $K_A$-channels will only activate at a certain membrane potential regardless of NMDA-concentration. Therefore the membrane potential gets more depolarized with a higher NMDA-concentration resulting in a higher excitatory drive.

The frequency of oscillations also here will first increase when increasing the NMDA-concentration and then decrease with generally bigger oscillations, both the plateau and hyperpolarized phase.

The span within which oscillations are obtained is NMDA=3.18-7.38, which is a little larger span than without the $K_A$-channels. Maybe, this means that the reason why oscillations do not occur at too low or too high [NMDA] is because there is not enough hyperpolarizing force. When another hyperpolarizing force is added in form of $K_A$-channels they will help to hyperpolarize the membrane potential even at NMDA-concentrations where not enough $K_{Ca}$-channels are activated, either because of too little Ca inflow or because the activation of $K_{Ca}$-channels has reached its maximum level or is too slow.

**4.1.2 Effects of Ca$^{2+}$ inflow**

As stated earlier in chapter 3.3.2, the changes in Ca-concentration with time is described by the equation: $\frac{d[Ca]}{dt}=B*i_{Ca_{NMDA}}*[Ca]/\tau$, where the rate of decay of Ca from the cell-cytosol can be varied by changing the parameter $\tau$. Some plots were made to see how Ca inflow is related to [NMDA], Mg-block and the $\tau$-parameter at different membrane potentials. [Ca] at steady-state for the lowest, some middle and the highest membrane potential of oscillations was calculated for [NMDA] values of 0-10 with a step of 1.

**Varying the $\tau$-parameter**

Effects on Ca-inflow when varying the value of the $\tau$-parameter to a higher and a lower value was examined.
[Ca]-dependence of [NMDA] at different values of \( \tau \)-parameter. \( V_m = -40 \) mV, NMDA=[0 1 2 3 4 5 6 7 8 9 10].

Ca\(^{2+}\)-concentration in the cell is linearly proportional to the NMDA-concentration. A lower value of \( \tau \) (\( \tau = 1.5 \)) will give a decrease in Ca\(^{2+}\)-concentration, because of the faster rate of decay. A higher \( \tau \) (\( \tau = 3 \)) gives the opposite effect with higher concentrations of Ca\(^{2+}\) inside of the cell.

The figure shows the dependence at a membrane potential of \(-40 \) mV, which is somewhere in the middle of the oscillations. The same kind of measurement was also done at membrane potentials of \(-67 \) mV and \(-19 \) mV, which are the highest and lowest membrane potentials of oscillations. These measurements gave the same qualitative results.

*Varying the slope of the Mg\(^{2+}\)-block-curve*

A steeper and a flatter shape of the Mg-block-curve was examined.
A steeper shape of the Mg-block-curve (= lower value of \( \alpha_c \)) will give a larger difference between \( Ca^{2+} \)-concentrations at the different membrane potentials. Also, oscillations have a larger amplitude (-69 mV- (-7 mV)) compared to oscillations with a more flat shape of their Mg-block-curve. \([Ca]\) was calculated at the lowest (-69 mV), highest (-7 mV) and one in the middle (-30 mV) of membrane potentials of the oscillations. The highest \([Ca]\) of the ones measured was 43.39 and obtained at -30 mV, and the lowest \([Ca]\) at -69 mV was 0.73. \([Ca]\) at the highest membrane potential (-7 mV) was 13.86 (Values taken from the NMDA-concentration of 10). That the highest \([Ca]\) was not obtained at the highest membrane potential which one could maybe expect is because the membrane potential of -7 mV is too close to the reverse-potential for \([Ca]\) in the model.

The larger difference between the lowest (0.73) and the highest (43.39) \( Ca^{2+} \)-concentration in the model with a steep Mg-block depends on that oscillations take place between a bigger lowest and highest membrane-voltage, but also on how much a certain change in membrane potential will affect the fraction of NMDA-channels that open. The later depends on how the Mg-block-curve looks. With this steep shape, a change in membrane potential will block/unblock a larger fraction of NMDA-channels than it will with a more flat shape.
A flat Mg-block will give oscillations between a smaller span of voltage ((-55mV) – (-37 mV)), a smaller fraction NMDA-channels blocked/unblocked with a similar change in voltage and less variation between the highest and lowest values of Ca-concentration (16.3134 at -55 mV/[NMDA] = 10 and 35.3519 at -37 mV/[NMDA] = 10).

4.1.3 Effects of different $K_{Ca}$-activations

**General mechanisms of $K_{Ca}$-activation**

In the simplified XPPAUT-model, oscillations occur from NMDA and $K_{Ca}$-channels as two opposing forces. As seen in Fig 3, Ca inflow through NMDA-channels is linear to NMDA-concentration. In the XPPAUT-models, Ca will activate $K_{Ca}$-channels instantly with no time delay.

As Ca enters, $K_{Ca}$-channels will successively open and hyperpolarize the membrane potential, leading to the membrane potential staying on a plateau for a time depending on $[\text{NMDA}]$ and the $K_{Ca}$-activation used. When enough $K_{Ca}$-channels have been activated, the membrane potential will reach a voltage-level where the Mg-block again closes many NMDA-channels, which will lead to a fast hyperpolarization of the potential.

![Different $K_{Ca}$-activations](image)

**Fig 14.** Different $K_{Ca}$-activations that have been tested. A. Linear $k_{ca}(ca)=tc*ca$, $tc=0.06$, B. Hill $(ca)=ca^4/(ca^4+k^4)$, $k=10$, C. $k_{ca}(ca)=ca^2/k^2$, $k=11$ and D. Aldair $k_{ca}(ca)=aldair$

The simplified model in XPPAUT is suited to examine different $K_{Ca}$-activations since the only two forces involved are NMDA and $K_{Ca}$. Ca inflow is linear proportional to NMDA and $K_{Ca}$-activation is instantaneous. The four different $K_{Ca}$-activations in Fig.14 were tested in this simplified XPPAUT-model.

**Investigations using the model without $K_A$-channels**

In the XPPAUT-model without $K_A$-channels, the amplitude of oscillations is higher than in the model with $K_A$-channels, and the amplitude is not affected by changing the NMDA-
concentration. The only hyperpolarizing force involved here is dependent only of Ca-concentration, which depend of NMDA-concentration. The span within oscillations is obtained is NMDA = 2.39-6.27.

**Linear K\textsubscript{Ca}-activation**

The model with a linear K\textsubscript{Ca}-activation (see Fig 14 A) is maybe not so realistic since Ca\textsuperscript{2+} does not get saturated and it acts as if there were unlimited numbers of K\textsubscript{Ca}-channels in the cell. K\textsubscript{Ca}-activation will just follow with [NMDA] and [Ca\textsuperscript{2+}] and reach very high values.

Fig 15. Linear K\_Ca activation, voltage oscillations, tc=0.06.

Fig 16. Linear K\_Ca activation, Ca oscillations, tc=0.06.
Fig 17. Linear $K_{Ca}$-activation, $K_{Ca}$-activation.

Fig 18. Frequency, duration of plateau-phase and hyperpolarized phase in relation to [NMDA].

When a linear $K_{Ca}$-activation is used, the span within which oscillations occur is very large (NMDA=0.004). Voltage-oscillations at different NMDA-concentrations seem to look the same in shape, and there are very small changes in frequency. The shape of these oscillations does not look so much like the typical shape of the natural NMDA-oscillations. Chances in the concentrations of Ca-oscillations are much bigger at higher NMDA-concentrations. The oscillations start earlier in time when using lower NMDA-concentrations. That it takes longer time for the initial oscillation to start with a higher [NMDA] is because membrane potential first rises to a high voltage (almost 0 mV, which is the reversal potential for NMDA in the model), and then it takes some time to accumulate the amount of Ca needed to activate enough $K_{Ca}$-channels to hyperpolarize it. However when oscillations already started, the bigger differences in Ca is compensated in the way that the Ca-oscillations become more spiky in their shape at higher NMDA and can therefore keep the same frequency.

**Hill-like $K_{Ca}$-activation**

The Hill-like $K_{Ca}$-activation (see Fig 14 B) is more realistic compared to the linear (Fig 14 A). At low $[Ca^{2+}]$, the KCa-activation is not so sensitive to changes in Ca, then it takes a
steep shape where the activation is increasing strongly with changes in \([\text{Ca}^{2+}]\), then plans out to give again a less sensitive activation at high values of \([\text{Ca}^{2+}]\). This outplanning illustrates that \([\text{Ca}^{2+}]\) becomes saturated and can not open more \(K_{\text{Ca}}\) - channels at a \([\text{Ca}^{2+}]\) around 20-25.

![Diagram](image)

Fig 19. Voltage, Ca, and \(K_{\text{Ca}}\)-activation at NMDA=2.

At low \([\text{NMDA}]\) (\([\text{NMDA}] < 2.12\)), the membrane potential will stay on a plateau at 0 mV (see Fig 19) which is the reversal-potential for NMDA in the model. This is probably because too little Ca flows in and \(K_{\text{Ca}}\)-activation will be in the lowest part of the the \(K_{\text{Ca}}\)-activation-graph, where almost no \(K_{\text{Ca}}\)-channels are activated (Fig 14B). When continuing to increase \([\text{NMDA}]\) up to 2.38 (where it takes the shape as in Fig 20), membrane potential will stay on plateaus at lower and lower voltage-values. More and more Ca flows in with the \([\text{NMDA}]\) increase, but not enough to hyperpolarize the potential so much to bring it to the level where enough Mg-block is relieved to hyperpolarize it back to the resting potentials.
Fig 20. Voltage-oscillations at different NMDA-concentrations in XPPAUT-model without $K_A$ channels.

Fig 21. Ca-oscillations at different NMDA-concentrations in XPPAUT-model without $K_A$ channels.
Fig 22. Frequency, duration of plateau-phase and hyperpolarized phase in relation to [NMDA] (NMDA-span:2.39-6.27)

The paragraphs below describe what is seen in figures 20 and 21.

At NMDA-concentration of 2.38, Ca\(^{2+}\) will take a value of around 8.72, and the voltage will stay on a half-depolarized level of -35 mV. Around 35% of K\(_{Ca}\)-channels are here activated, which seems to be not enough to press down the membrane potential back to the resting potential -70 mV.

If changing the tau-parameter from 2.000 to 2.005, which should give a slower decay and a greater amount of Ca\(^{2+}\) accumulated, oscillations will start to occur. This gives a very small change in Ca and K\(_{Ca}\)-activation.

At NMDA=2.39, oscillations start. There is only a very small difference in Ca\(^{2+}\) between this concentration and NMDA=2.39. It seems that a certain fraction of K\(_{Ca}\)-channels must be activated and there is some limit at maybe 35%.

Between NMDA-concentration of 2.39-4.0, the frequency of oscillations increases with increased NMDA-concentration. In this span, Ca\(^{2+}\) oscillates with a small amplitude (=maybe 2 or something) around concentration of 9, which correspond to the steepest part of the K\(_{Ca}\)-activation-graph with a K\(_{Ca}\)-activation of around 40-50%.

The fastest oscillations occur at NMDA=4.0, with Ca\(^{2+}\)-oscillations between 9-11.

From NMDA=5.0 –6.0, Ca-oscillations have higher and higher amplitude in the interval of 10-19. In the K\(_{Ca}\)-activation-graph, this corresponds to the steepest part and also the upper part when it plans out. These bigger Ca-oscillations seems to prolong voltage oscillations as well, maybe because since it takes more time to transport this greater amount of Ca\(^{2+}\).

At NMDA=5.0 – 6.0 somewhere, oscillations changes in shape, and start to look more similar to the natural oscillations.

NMDA=6.27 is the value just before voltage-oscillations stop. At this concentration Ca oscillates between a great interval of 11-23. This gives rise to a very long plateau-phase in the voltage-oscillations, indicating that a big fraction of K\(_{Ca}\)-channels are opened but at this NMDA-concentration it is still hard for them to press down the voltage, and changes in membrane potential can only activate very few more channels.
At NMDA=6.28, voltage will stay at -35 mV. Ca\(^{2+}\) will stay at 23, which correspond to fraction of maybe 96% opened K\(_{Ca}\)-channels. This fraction of open K\(_{Ca}\)-channels seems to be still not enough to fully hyperpolarize the membrane potential.

Ca-oscillations get spikier with increased NMDA, which to some degree compensate for the greater amount of Ca that has to be transported. However, when the difference between the two values that Ca oscillates between, the spikiness can not compensate, and oscillations become slower. The fastest oscillations therefore occur when Ca oscillate between two values somewhere in the middle of the kca_activation-graph, and have a little spiky but not too spiky shape.

**Ca\(^2\)/K\(^2\) K\(_{Ca}\)-activation**

A model with a K\(_{Ca}\)-activation with the equation Ca\(^2\)/K\(^2\), where K is a constant and functions as a normalization-constant, was tested (Fig 14 C) in order to get the behaviour of oscillations of the Hill-like model but without the decreased frequency of oscillations when the K\(_{Ca}\)-activation reaches its max-value. However, this model is not so realistic, since similar to the situation with the linear model, Ca does not get saturated and K\(_{Ca}\)-channels are infinited.

![Graphs showing voltage-oscillations with different NMDA values](image)

Fig 23. Ca\(^2\)/K\(^2\) K\(_{Ca}\)-activation, K=11, voltage-oscillations.
Fig 24. \( \frac{Ca^2}{K^2} K_{Ca} \)-activation, \( K = 11 \), Ca-oscillations.

Fig 25. \( \frac{Ca^2}{K^2} K_{Ca} \)-activation, \( K = 11 \), \( k_{Ca} \)-activation.

Fig 26. Frequency, duration of plateau-phase and hyperpolarized phase in relation to [NMDA].
With this \( \text{K}_\text{Ca} \)-activation, an increased NMDA-concentration leads to a shorter plateau-phase and a longer hyperpolarized phase. Maybe this is because \( \text{K}_\text{Ca} \)-activation too soon reaches too high levels and does not get saturated. This is the opposite pattern to the original GENESIS-model, but will also lead to an increase in frequency first and after that the frequency starts to decrease.

**Aldair \( \text{K}_\text{Ca} \)-activation**

Aldair \( \text{K}_\text{Ca} \)-activation (see Fig 14 D) is steep at the beginning and then flackenes out and becomes saturated at \([\text{Ca}]=15\) approximately.

![Fig 27. Aldair K_Ca-activation, voltage-oscillations.](image)

![Fig 28. Aldair K_Ca-activation, ca-oscillations.](image)
For the Aldair-model, activation is fast at low [Ca] and then plans out. Therefore, as long as [Ca] is placing the activation in the lower part of the graph, oscillations will get a shorter and shorter plateau when increasing [NMDA]. When [Ca] gets too high, the activation of more K\textsubscript{Ca}-channels goes slower and a prolongation of the plateau and also the hyperpolarized phase will be seen. For the frequency this gives the same result as in most of the models, first increased and then decreased.

**A summary of observations in the different K\textsubscript{Ca}-activation-models**

For all the K\textsubscript{Ca}-activation models that were tested except for the linear, the fastest frequency of voltage-oscillations occurred when Ca oscillated between two values with not so great difference. The values between which Ca-oscillations occur in all of the models increased with nmda-concentration and therefore frequency of oscillations will increase until the level is reached where either K\textsubscript{Ca} is beginning to become saturated and activation is slow (Hill, Aldair) or too high (Ca\textsuperscript{2}/K\textsuperscript{2}). In the linear model, frequency is near the same for all NMDA-concentrations.

Fig 29. Frequency, duration of plateau-phase and hyperpolarized phase in relation to [NMDA].

Fig 30. Relation plateau/total cycle for the four k_Ca-activations at different levels of [NMDA].
The graph in Fig 30 shows % of the time of the total cycle that the membrane potential is on the plateau. It was made to examine the different \( K_{\text{Ca}} \)-activations effect on the time the membrane potential will stay in its plateau-phase in relation to the whole cycle at different [NMDA].

With the **linear** \( K_{\text{Ca}} \)-activation, [NMDA] does not affect the plateau/total cycle % much. Probably this is because a change in [NMDA] is counteracted with a linear proportional change in \( K_{\text{Ca}} \)-activation.

In the **Hill-like** \( K_{\text{Ca}} \)-activation, plateau/total cycle is decreased when increasing [NMDA] to a certain level and then it is again increasing. The NMDA-concentrations with the lowest plateau/total cycle are them with also a high frequency which corresponds to the steepest part of the \( K_{\text{Ca}} \)-activation-graph. At these concentrations a small change in membrane potential will activate many \( K_{\text{Ca}} \)-channels which can hyperpolarize the membrane fast and give a short plateau. The hyperpolarized phase at these concentrations is not so much varied, so the plateau/total cycle decrease is mainly because of a shorter plateau. Then at higher [NMDA] the plateau/total cycle goes up again. This is at the upper part of the \( K_{\text{Ca}} \)-activation-graph where many channels are activated and activation is pretty slow. The plateau/total cycle is almost the same as for low [NMDA] but both the plateau and the hyperpolarized phase are here longer, and frequency slower.

With the **Ca\(^2+\)/K\(^2+\)-activation**, \( K_{\text{Ca}} \)-activation is not saturated and soon goes from low to high, leading to oscillations with large plateau/total cycle for the low [NMDA] but which fast gets low, because of oscillations with a long hyperpolarized phase and a very short plateau.

**Aldair K\(_{\text{Ca}}\)-activation** is steep at the beginning and then flackenes out. At low [NMDA] levels it will therefore activate many channels, leading to oscillations with a short plateau-phase. At higher [NMDA], activation is slower and the plateau gets longer since it can not activate as many channels. This looks a little like the Hill-activation in that at high [NMDA] it will give very slow oscillations with both a long hyperpolarized phase and a long plateau, since a large fraction of \( K_{\text{Ca}} \)-channels are activated, and activation is slow.
**Investigations using the model with $K_A$-channels**

In the following chapter, the simulations that were earlier made in the XPPAUT-model with NMDA and $K_{Ca}$-channels are repeated with the model in which $K_A$-channels have been added.

**Linear $K_{Ca}$-activation**

When $K_A$-channels are added, the model will give oscillations that increase in amplitude when [NMDA] increases. The highest amplitudes are the same as for the model without $K_A$-channels, around -70 -20 mV. The $K_A$-channels decide the amplitude more than the $K_{Ca}$-channels since they activate earlier. $K_A$-channels are not dependent of NMDA but only of the membrane potential, and when [NMDA] is higher it gets time to reach higher amplitude before $K_A$-channels can activate enough.

Because of higher amplitude and in general larger oscillations, the frequency of oscillations will decrease with [NMDA] in the model with $K_A$-channels, which can be compared to the model without $K_A$-channels where frequency is almost not affected by changes in [NMDA] at all.

That it takes longer time from the initial values for oscillations to start to occur with [NMDA] could be because the membrane potential first goes up higher and then it takes longer time for Ca to accumulate enough to hyperpolarize the membrane. Span for oscillations $1.5<[NMDA]<70$. For [NMDA] lower than 1.5, there are still oscillations but with a very low amplitude.

![Voltage-oscillations, linear $K_{Ca}$, $g_k=20$.](image)

Fig 31. Voltage-oscillations, linear $K_{Ca}$, $g_k=20$. 
Hill-like $K_{Ca}$-activation

The differences between the voltage-oscillations in the model with $K_A$-channels added compared to the simplified model are that the amplitude is somewhat lower, and can also be a little affected by [NMDA], although not as much as in the model with linear $K_{Ca}$-activation. Another difference is that the frequencies are generally faster with the $K_A$-channels, which is because the $K_A$-channels help hyperpolarizing the membrane potential, and gives generally shorter plateau-phases. This can be seen when changing the $g_K$-parameter to a higher value, when oscillations will have very short plateaus. In shape and the general pattern the oscillations are similar to the $K_{Ca}$/NMDA-oscillations. Frequency first increase and then decrease, and also the shape changes at high [NMDA].
Fig 33. Voltage-oscillations, Hill K\textsubscript{Ca}-activation.

Span for oscillations is $3.17<\text{[NMDA]}<7.39$. The [NMDA] span for oscillations is at a little higher concentrations in general compared to the model with no K\textsubscript{A}-channels. This is probably because another hyperpolarizing force is added and the system demands more NMDA to compensate for this.

$Ca^{2+}/K^2$ K\textsubscript{Ca}-activation

As in the model with a linear K\textsubscript{Ca}-activation, amplitude is much affected by [NMDA], and at low [NMDA], oscillations with a low amplitude can still be seen. In shape and other behaviour the results are similar to the model without K\textsubscript{A}-channels, except for the plateau-phases, that are in general shorter.
Fig 34. Voltage-oscillations, $\text{Ca}^2+/\text{K}^2$-activation, $K=10$.

Fig 35. $\text{K}$-activation, $\text{Ca}^2+/\text{K}^2$-activation, $K=10$.

The span for oscillations is here $2.21<\text{[NMDA]}<21.98$. The upper limit in the NMDA-span for oscillations is a little lower compared to the simplified model (22.7). Maybe because the plateau is too short and oscillations do not appear at all.

**Aldair $K_{\text{Ca}}$-activation**

With the Aldair-activation the span for oscillations is $3.39<\text{[NMDA]}<5.83$. For the Aldair $K_{\text{Ca}}$-activation, oscillations start at much lower $\text{[NMDA]}$ (=0.1) in the model without the $K_{\text{X}}$-channels. These oscillations have longer plateaus. With a too low $\text{[NMDA]}$ for oscillations to occur in this model the membrane potential will stay more hyperpolarized (around $-55$ mV) than for example in the model with Hill-like $K_{\text{Ca}}$-activation in which the membrane potential first goes up and then down to stay at
around −40 mV. This maybe indicates that the $K_{Ca}$-activation in the Hill-model is not enough to hyperpolarize the membrane potential, while in the Aldair the $K_{Ca}$-activation at this low [NMDA] and the $K_{A}$-channels gets too strong compared to the NMDA. This seems also to be consequent with the Aldair-activation-graph, which activates many channels at low [Ca]. Also when oscillations stop at NMDA=5.84, membrane potential is staying at a higher voltage, indicating that the reason oscillations stop is that activation of more $K_{Ca}$-channels is not enough, corresponding to the part of the activation graph where activation is slow. The reasons for oscillations to start and stop seem to be the opposites for the Aldair $K_{Ca}$-activation compared to the $Ca^{2+}/K^{+}$-model, which can also be seen in the $K_{Ca}$-activation-graph, which is somewhat the opposite.

(Hill-like $K_{Ca}$-activation without saturation and $K=10$, NMDA-span=6.18-12.62.)
4.1.4 Effects of changing the slope of the Mg\(^{2+}\)-block

With a steep shape of the Mg-block-curve, a small change in membrane potential will give a larger difference in the fraction of opened NMDA-channels, which will give larger differences even in Ca that flows into the cell. This gives both a higher depolarized voltage since more NMDA-channels open and a lower hyperpolarized voltage since more Ca is accumulated and can activate more K\(_{Ca}\)-channels. These bigger oscillations will take longer time and therefore get a slower frequency.

Investigations using the model without K\(_A\)-channels

Two different Mg-blocks were examined in the simplified XPPAUT-model, one with a steeper shape of the graph compared to the original model and one with a flater shape. This can be varied changing the value of the parameter alfa\(_C\). The parameter alfa\(_A\) has been changed only to place the graphs at the same half-activation, for making it easier to compare the results. The values of the parameters were changed in both directions, as much as possible where oscillations could still arise. The K\(_{Ca}\)-activation used was the Hill-like K\(_{Ca}\)-activation with K=10.
Fig 38. Mg-block curves for different values of parameters: alfa_a, alfa_c that were used in the XPPAUT-model without K_A-channels.

The values of parameters used were alfa_C=10, alfa_A=3088 for the steep curve. For the flat curve, the values were alfa_C=30, alfa_A=279.

At voltage levels under the half-activating (around -35 mV) the flat Mg-block will have a larger fraction of NMDA-channels opened.

**Steep Mg-block**

The steep Mg-block (alfa_C=10) will give oscillations with a higher amplitude compared to the normal Mg-block oscillations (see Fig 20). This is because a small change in membrane potential will unblock/block a larger fraction of NMDA-channels.

The [NMDA]-span for oscillations is here 4.43-6.05, compared to 2.39-6.27 with the normal Mg-block. With too low [NMDA] (=4.42) the voltage stays at a depolarized potential. Probably the amount of Ca that can flow into the cell is too small to activate enough K_Ca-channels. Membrane potential will therefore approach 0 which is NMDAs reversal potential in the model. That this model demands a little higher [NMDA] (4.45 compared to 2.39) seems realistic when looking at the open channel probability-graph, since the steep Mg-curve has fewer channels opened at the resting potential. Therefore, a larger amount of NMDA will be needed to open some channels and get away from the very low part of the graph. The shape of the oscillations looks very much the same as with the normal Mg-block/Hill K_Ca-activation with the plateaus at high [NMDA] having this different shape compared to them with a low [NMDA]. The voltage-span for these oscillations is between approximately (-70mV{-5}) , which means almost the whole probability-graph is used (Fig 38).
Fig 39. Voltage-oscillations, steep Mg-block, XPPAUT-model without K_A channels.

Fig 40. Frequency, duration of plateau-phase and hyperpolarized phase in relation to [NMDA].

The pattern of the frequency with increased [NMDA] is the same with first an increasing frequency and then a slower. However, frequencies with the steep Mg-block are much slower than them with the normal, maybe because of the higher amplitude everything will take longer time. Also for the duration of the plateau-phase and the hyperpolarized phase, the pattern looks very much the same as in the model with the normal Mg-block.

Flat Mg-block
The flat Mg-block (alfa_C=30) will give oscillations with a lower amplitude compared to the normal Mg-block oscillations. A change in membrane potential will block/unblock only a small fraction of NMDA-channels, also giving smaller changes in the amount of Ca flowing in. This gives generally smaller oscillations in both Ca and voltage. Maybe because of the lower amplitude and smaller oscillations, frequencies with the flack Mg-block are very fast compared to them with the normal.
The [NMDA]-span for oscillations is here 3.67-5.52, compared to 2.39-6.27 with the normal Mg-block. The shape of the oscillations looks a little different to both the normal and the steep Mg-block-oscillations. These oscillations seem to have the same shape at different [NMDA].

With the flat Mg-block, oscillations will be in the voltage-span (-55 mV to -37)). This means only the lower part of the probability-graph is used, and p never becomes more than around 40-50% of NMDA-channels opened.

Also here, the shape of the frequency-, plateau-phase- and hyperpolarized phase-graphs are similar to the ones with the normal Mg-block. An increased [NMDA] gives first an increased frequency and then a slower. Frequencies with the flat Mg-block are much faster than them with the normal. Also for the duration of plateau-phase and the
hyperpolarized phase, the pattern looks very much the same as in the model with the normal Mg-block.

![Graph showing the plateau/total cycle for Mg-blocks with different [NMDA] concentrations.](image)

**Fig 43. Steep and flat Mg-block, plateau/total cycle.**

The plateau/total cycle graph for the steep Mg-block does not look much different from the one with a normal Mg-block. The pattern is the same with a part with not too low and not too high [NMDA] giving the shortest plateau-phases. Also the plateau/total cycle graph for a flat Mg-block does not vary much from the one with the normal and the steep Mg-block, except that it is a little more extreme. The lowest plateau/total cycle % is also here obtained at [NMDA] not too low or too high, where the frequencies also are the highest.

**Investigations using the model with K_A-channels**

In the model with K_A-channels, a little steeper shape of the curve could be used and still give oscillations. The parameters used for the steep Mg-block were alfa_c=7, alfa_a=15000.

For the flat Mg-block the parameters were alfa_c=25 and alfa_a=360. No oscillations could arise with alfa_c having a value larger than 25.
Steep Mg-block
As with the normal Mg-block, amplitude is lower and increases with [NMDA], frequency is faster in general when K_A-channels are included. The NMDA-span for oscillations is here 1.67-7.22.

Fig 44. Mg-block curves for different values of parameters: alfa_a, alfa_c that were used in XPPAUT with K_Ca, K_A and NMDA-channels.

Fig 45. Voltage-oscillations, steep Mg-block, alfa_c=7, alfa_a=15000.
**Flat Mg-block**

The NMDA-span for oscillations with a flat Mg-block is 5.02-6.25.

![Fig 46. Voltage-oscillations, flat Mg-block, alfa_c=25, alfa_a=360.](image)

4.1.5 NMDA- and voltage-dependent hyperpolarizing force

One attempt to get away from the behaviour with the prolongation of plateaus that seems to often follow from increased [NMDA] in the models was to add some kind of current that is both activated at the voltage-levels of the plateaus, and also dependent on [NMDA]. Therefore an NMDA-dependence was added to the $K_A$-channels in the XPPAUT-model.

Three different models with this NMDA-dependent current were tested. In this simulations a $g_k=90$ was used instead of $g_k=20$ in the other simulations with the XPPAUT-model with $K_A$-channels.

1) **A simple model with a linear $K_{Ca}$-activation and a linear NMDA-dependence of the $K_A$-current**

First a simple model was tested with both a linear $K_{Ca}$-activation described by the equation $kca(ca)=tc*ca$ with $tc=0.05$ and a linear NMDA-dependence of the $K_A$-current described by the equation $act_k(v)=(gnmda*0.1)*mk(v)*hk(v)$.

This model gave as the other models with linear $K_{Ca}$-activation a very large NMDA-span for oscillations. The frequency was not affected very much with concentration of NMDA, but there is a little increase of frequency with NMDA (see Fig 47).
2) **A model with a linear \(K_{Ca}\)-activation and a quadratic NMDA-dependence of the \(K_A\)-current**

Since in the NMDA-receptor two molecules of NMDA must bind to the receptor to open it, a model was tested where the NMDA-dependence of the \(K_A\)-current was described by the function \(\text{act}_k(v) = (0.1 \cdot g_{nmda}^2) \cdot m_k(v) \cdot h_k(v)\).

In this model, frequency did increase with [NMDA], but the amplitude was very much affected, which can be seen in Fig 48.
3) A model with a Hill-like $K_{Ca}$-activation and a linear NMDA-dependence of the $K_{A}$-current

Also a model with the Hill-like $K_{Ca}$-activation was tested, since this seems to be the most realistic of the $K_{Ca}$-activations. The value of the $K$-parameter was set to 10. NMDA was as in model 1 linearly dependent of the $K_{A}$-current with the equation $\text{act}_k(v) = (0.1 \cdot gnmda) \cdot mk(v) \cdot hk(v)$.

The results here were similar as in the Hill-like regular model, with an initial increase of frequency, but then larger oscillations and a decreased frequency (see Fig 49).

4.1.6 Oscillations dependence of membrane resting potential

Some simulations were done to compare the model to experimental data obtained in the article: Wallen P, Grillner S. N-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey., where among other things, the effect on oscillations of injecting current has been examined. In the article they have injected positive current and negative current. In the XPPAUT-model it is not possible to inject current, but instead, the membrane resting potential was set to a higher and a lower value. In the article the result of injecting a positive current was an earlier onset of the depolarization and a longer plateau-phase. Injecting negative current showed opposite results, the depolarization peak was reached later, and the time when potential stayed on the plateau shorter. Injecting negative current gave a higher amplitude and a slower frequency.
When using a higher resting potential, more NMDA-channels are activated from the beginning, but still more NMDA is needed to start oscillations. Oscillations with a higher resting potential have a lower amplitude and faster frequency compared to them obtained with a lower resting potential.

This result looks somewhat similar to the results with a steep Mg-block-curve, which is maybe not so strange since when starting at a lower resting potential it will be placed in the part of the Mg-block-graph where very few channels are activated. This leads to a higher concentration of NMDA needed to initiate the oscillations. The high \([\text{NMDA}]\) can depolarize the membrane more and therefore amplitude gets bigger.

In comparison with the experimental data obtained in the article, the results with the XPPAUT-model seem to generally follow the same pattern. A more negative resting potential gives oscillations of higher amplitude and slower frequency, and a more positive resting potential gives oscillations of lower amplitude and faster frequency.
4.2 Confirmation of the results in the GENESIS-model

4.2.1 Frequency range

The span within which oscillations are obtained in the GENESIS model is NMDA=0.69-0.75. This is a much more narrow span than the one in the XPPAUT-models, where many currents that are present in the GENESIS-model are not included. Therefore the XPPAUT-models will give oscillations even at more unrealistic NMDA-concentrations compared to the GENESIS-model.

Fig 52. Oscillations at different NMDA-concentrations in GENESIS.

The tendency when increasing the NMDA-concentration within this span, is that the plateau-phase is prolonged and the hyperpolarized phase is shortened. This pattern will give a decreased frequency of oscillations when NMDA-concentration has reached a level when the prolongation of the plateau-phase has overcome the shortening of the hyperpolarized phase.
In the GENESIS-model, the pattern when increasing NMDA-concentration is that the plateau-phase gets longer and the hyperpolarized phase gets shorter. The frequency will increase at first. When the shortening of the hyperpolarized phase can not compensate for the long plateaus, this will result in a decreased frequency. This pattern was not so clear in any of the XPPAUT-models. In the XPPAUT-models, the hyperpolarizing phase does not really get shorter but its prolongation will not follow in the same rate of the prolongation for the plateau-phase. However, the effect for the pattern when increasing NMDA looks the same, but is more characterized and clear in the GENESIS-model.

**4.2.2 Effects of different $K_{Ca}$-activations**

In the GENESIS-model, only the Hill-like and the linear $K_{Ca}$-activations have been examined. It was very difficult to get oscillations in the GENESIS-model when varying the $K_{Ca}$-activation-graph. This might be because of the many different ion-currents involved in this model, and how they can affect each other.

The oscillations seem to decrease in frequency with increased [NMDA]. However it is not easy to say, because of the difficulties to get oscillations at all.
To see if the oscillations are really NMDA-oscillations and not coming from Ca that enter through voltage-gated CA_HVA-channels, a simulation was run where these channels were blocked.

Because it was not so easy to get oscillations in this model it is difficult to say if they follow the pattern increased/decreased frequency. The oscillations at NMDA=7 are Ca_HVA-oscillations and not NMDA-oscillations.

4.2.3 Effects of changing the slope of the Mg\(^{2+}\)-block

The results of changing the value of Mg-block parameters are the same in the XPPAUT-model as in the GENESIS-model.

A steeper Mg-block curve gives 1) Higher amplitude of oscillations 2) bigger difference between the lowest and the highest value of Ca\(^{2+}\)-concentration. 3) Lower frequency. A flater Mg-block curve gives 1) Lower amplitude of oscillations 2) smaller difference between the lowest and the highest value of Ca\(^{2+}\)-concentration 3) Higher frequency.
Fig 57. Probability-curve, for values alfa_C=15 (steep) and alfa_c=30 (flat), used in GENESIS-model.

In the model, a steep shape of the Mg-blockcurve compared to the normal values will give a slower frequency and a higher amplitude of oscillations, while a flack shape will give an increased frequency and a lower amplitude. Changing the Mg-block to a flack shape does not change the general pattern that the plateau phase is prolonged and hyperpolarized phase is shortened with increasing NMDA-concentration. With the steep shape it was difficult to see since the NMDA-span with oscillations is too small.

Fig 58. Voltage-oscillations obtained with a steep Mg-block-curve, alfa_C=15, alfa_A=929, NMDA=0.81.
4.2.4 Oscillations dependence of membrane resting potential

As earlier described for the XPPAUT-model in chapter 4.1.6, some simulations were done to compare the model to experimental results shown in the article: Wallen P, Grillner S. N-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey., 1987. This was also done with the GENESIS-model.

In the GENESIS model, oscillations were obtained at membrane resting potentials between -0.061 and -0.072. It seems that NMDA-concentration must be higher when resting potential is more hyperpolarized. The frequency of oscillations was lower and the amplitude higher at more negative resting membrane potential. This result seems to be similar to results with the XPPAUT-model and also to the results from the article.

Fig 59. Voltage-oscillations obtained with a flat Mg-block-curve, alfa_C=30, alfa_A=279, NMDA =0.48/0.51/0.54.

Fig 60. Some oscillations obtained with a higher resting potential -0.68, and a lower resting potential -0.72.
4.2.5 Oscillations induced by injection of current

In the XPPAUT-model it is not possible to inject current, but this could be done with the GENESIS-model. As in the previous chapter, this was made to compare the model with experimental data shown in the article “N-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. Wallén P, Grillner S., 1987”.

Fig 61. With an NMDA-concentration of 0.76, hyperpolarizing current was injected from -0.005 nA to -0.04 nA.

Hyperpolarizing current was injected from levels ranging from -0.005 nA to -0.04 nA in an NMDA-concentration of 0.76. The current was injected after approximately 2 seconds.

Even when injecting hyperpolarizing current, as compared to setting resting potential to a lower value, it seems that, the frequency decreases when the amount of hyperpolarizing current is increased. The amplitude is not so much affected by the amount of current injected, but with a small tendency to increase with amount of current. The hyperpolarized phase is prolonged when increasing the amount of current. Even though the plateau-phase is shortened, the prolongation of the hyperpolarized phase is larger leading to a decreased frequency.
Fig 62. Oscillations with a flater Mg-block-curve, hyperpolarizing current was injected from -0.03 nA to -0.08 nA.

Also some simulations were done using a flater Mg-block-curve. Hyperpolarizing current was injected from levels -0.03 nA to –0.08 nA in an NMDA-concentration of 0.57. Also here, the current was injected after approximately 2 seconds. The results when injecting hyperpolarizing current using a flack and a steep Mg-block very much follows the same pattern as the results with the original Mg-block. The frequency is decreasing with the amount of current injected due to a longer hyperpolarized phase which overcomes the shortening of the plateau-phase. Also, the amplitude is somewhat lower when using the flater Mg-block curve.

4.2.6 NMDA saturation

In the original model, the NMDA saturation was not taken into consideration. Since it is believed that two molecules must bind to the NMDA-receptor to open it, a model was tried where \( \frac{\text{nmda}^2}{x}/(1+\frac{\text{nmda}^2}{x}) \) was used.

Fig 63. Voltage-oscillations with an NMDA-function that can get saturated. 
\( \frac{\text{nmda}^2}{x}/(1+\frac{\text{nmda}^2}{x}) \), \( x=0.5 \).
The pattern of oscillations is the same with a prolonged plateau-phase and a shortened hyperpolarized phase as NMDA-concentration is increased. Simulations have also been done with a value of \(x=1\) and \(x=2\), where the NMDA-span within which oscillations occurred varied.

5 Discussion

The results are here generalized to try to understand how a model in which an increased NMDA-concentration will give an increased frequency could be accomplished. In general, it was difficult to obtain a model which behaves similar to experimental data, by varying parameters which represent different aspects of \(K_{Ca}\)-activation and Mg-block. We first look at the \(K_{Ca}\)-activation. In the linear \(K_{Ca}\)-activations (linear and \(Ca^2/k^2\), see Fig 14 A, B), the frequency will increase a little with NMDA-concentration, but the oscillations resemblance to natural oscillations is not so good since the model do not account for saturation-mechanisms making the NMDA-span nearly infinite. Also, the oscillations shape is not similar to natural oscillations.

In the saturation models (Hill and Aldair, see Fig 14 C,D), the problem is that naturally saturation-curves often have an S-shape, which will lead to oscillations that have a high frequency in the middle of the graph, and when the graph levels out, their frequency decreases with a longer plateau-phase, due to too little inflow of Ca.

With these models, the general behaviour of oscillations is first an increase of frequency and then after that, the oscillations change their shape and there is a decrease of frequency. The oscillations that look more like the natural oscillations are those who occur at the higher concentrations of NMDA, and when frequency starts to decrease. If taking out these spans, the tendency with a prolonged plateau-phase as in the original GENESIS-model is seen in the models with both the Hill and Aldair \(K_{Ca}\)-activations.

Changing the Mg-block does not affect the plateau/cycle relation. A steep Mg-block will give higher amplitude with generally slower oscillations and a flat Mg-block-curve will give smaller amplitude and faster frequency of oscillations.

Adding \(K_A\)-channels to the XPPAUT-model does not change the general results with \(K_{Ca}\)-activation and Mg-block. The only difference between results from this and the model without \(K_A\)-channels is that in this model the amplitude is affected by \([NMDA]\) with an increased amplitude with higher \([NMDA]\), and amplitudes are generally lower.

Only when explicitly trying to achieve the wanted behaviour by making the \(K_A\)-current dependent on NMDA, a model where increased NMDA lead to increased frequency could be obtained although also the amplitude were considerably affected. However, this has no biological background, and the model also has the problem with no saturation.
6 Conclusions

The simulations have shown two ways of trying to solve the problem with a longer plateau-phase as a result of higher NMDA-concentration, leading to the following recommendations for changes of the model:

-NMDA-saturation

Since it is believed that two molecules must bind to the NMDA-receptor to open it, a model was tried where \((\text{nm}da^2/x)/(1+(\text{nm}da^2/x))\). Taking NMDA-saturation into consideration does not affect the general pattern.

-NMDA-dependent/voltage-dependent hyperpolarizing force

From the other simulations it seems that in order to get the behaviour of an increased frequency and no effect on the amplitude, and no prolongation of the plateau-phase when increasing the NMDA-concentration, it would need some kind of force that is growing with NMDA-concentration and is activated on the plateau.

One way to get this is to make the K_A-channels dependent on NMDA-concentration. The effect of this was tried in the XPPAUT-model, with some of the different K_Ca-activations that was earlier simulated. However, none of those gave a satisfying result.

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