Models of CaMKII Activation

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Abstract

Models of CaMKII activation
Calcium/calmodulin-dependent Kinase Type II (CaMKII) is a Ca$^{2+}$-activated enzyme that is highly abundant in the brain. It is central to the regulation of glutamatergic synapses and necessary for the induction of long-term potentiation (LTP), an activity-dependent strengthening of synapses that is thought to be the basis for some forms of learning and memory. CaMKII is activated by Ca$^{2+}$ elevations and its key property is its ability to remain persistently active, in the absence of increased [Ca$^{2+}$], for long periods of time. Existing computational models of CaMKII activation are surprisingly diverse and there is little general consensus on which processes should be included or how detailed the modelling needs to be.

This study focuses on properties of CaMKII activation models. Eight models are reviewed, three of these are implemented and tested against experimental data. The models are compared with respect to general and specific model features and biological realism. Finally, the influence of some common model assumptions are discussed and a general outline for a realistic model of CaMKII activation is proposed.

Sammanfattning

Modeller av CaMKII-aktivering
Calcium/calmodulin-beroende kinas typ II (CaMKII) är ett Ca$^{2+}$-aktiverat enzym som är närvarande i stora mängder i hjärnan. Det är centralt för regleringen av glutamatergiska synapser och nödvändigt för induktion av ”long-term potentiation” (LTP), en aktivitetsberoende ökning av synapsstyrka som betraktas som grund för vissa typer av inlärning och minne. CaMKII aktiveras av höjda Ca$^{2+}$-nivåer och dess viktigaste egenskap är förmågan att förblir aktiv, även i avsaknad av Ca$^{2+}$, under långa tidsperioder. Existerande beräkningsmodeller för CaMKII-aktivering är förvånansvärt olika och samstämmigheten om vilka processer som bör inkluderas, samt hur detaljerad modelleringen bör vara, är dålig.

Denna studie fokuserar på egenskaper hos modeller av aktivering av CaMKII. Åtta modeller gäis igenom varav tre implementeras och testas mot experimentella data. Generella och specifika drag hos modellerna samt överensstämmlighet med den biologiska verkligheten jämförs. Slutligen diskuteras effekterna av några vanliga modellantaganden och ett utkast för en realistisk modell av CaMKII-aktivering föreslås.
Preface

This report describes a Master’s project in Computational Neuroscience, performed in the SANS group at the Department of Numerical Analysis and Computer Science (NADA) at the Royal Institute of Technology (KTH) in Stockholm. Dr. Jeanette Hellgren-Kotaleski was supervisor and Prof. Anders Lansner was examiner.

The project consisted of three parts: a literature study on synaptic plasticity and models of CaMKII activation, implementation and testing of some of the models and a comparison of all the studied models on grounds of general model structure and biological realism.

This report has three parts. First, a rather extensive background section on enzyme kinetics, synaptic plasticity and the properties of the substances included in the CaMKII pathway. Second, a general overview of a number of models of CaMKII activation and third, a study and comparison of these models on the grounds of general model properties and biological realism. Also, a list of abbreviations and an overview of model characteristics are given in the appendices. A reader unfamiliar with basic enzyme kinetics and/or the CaMKII pathway is recommended to read the background chapter before the rest of the report.

All implementation and simulation has been performed with MATLAB 6.5.

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Chapter 1

Background

In this chapter, background information on basic enzyme kinetics and chemical species relevant for this project is given. Figure 1.1 shows the reactions studied in this project.

Figure 1.1: A schematic spine with the reactions studied in this project. Arrows labeled $P$ and $dP$ denote phosphorylation and dephosphorylation, respectively. Plain arrows denote binding.
1.1 Enzymes: kinetics and basic concepts

Readers who are unfamiliar with basic enzyme kinetics are recommended to read either this section or the enzyme kinetics chapter(s) in a standard biochemistry textbook (such as Stryer’s *Biochemistry, 4th ed.*) before continuing to later sections and chapters in the report. All equations in sections 1.1.1-1.1.3 are adapted from Stryer’s *Biochemistry, 4th ed.* [53].

1.1.1 Enzymes are biochemical catalysts

Enzymes are the catalysts of biological systems, i.e. they increase the rate of biochemical reactions. They are specific both in which reactions they catalyze and in their choice of substrates. Many enzymes are regulated, for example by phosphorylation, which is the reversible attachment of phosphoryl groups to specific serine (Ser) and threonine (Thr) residues. Phosphorylation is catalyzed by *kinases* and dephosphorylation is catalyzed by *phosphatases* (see section 1.7).

1.1.2 The free energy governs all biochemical reactions

A standard chemical reaction looks something like

\[ A + B \rightleftharpoons C + D \quad (1.1) \]

and the free energy change of this reaction is given by

\[ \Delta G = \Delta G^\circ + RT \log_\text{e} \frac{[C][D]}{[A][B]} \quad (1.2) \]

where \( \Delta G \) is the change in free energy, \( \Delta G^\circ \) is the standard free energy change, \( R \) is the gas constant, \( T \) is temperature in Kelvin and brackets denote molar concentrations of the different species.

A reaction can occur spontaneously only if \( \Delta G < 0 \), and a system is said to be at equilibrium if \( \Delta G = 0 \). The *equilibrium constant* is defined as

\[ \frac{k_f}{k_b} = K_{eq} = \frac{[C][D]}{[A][B]} = e^{-\Delta G^\circ/RT} \quad (1.3) \]

and \( k_f \) and \( k_b \) are the forward and backward rates of the reaction.

1.1.3 The Michaelis-Menten model of enzyme kinetics

The simplest model that accounts for the kinetic properties of many enzymes is the *Michaelis-Menten model*:

\[ E + S \rightleftharpoons_{k_2}^{k_1} ES \rightleftharpoons_{k_3}^{k_2} E + P \quad (1.4) \]
where E, S and P denote uncombined enzyme, uncombined substrate and product, respectively. ES denotes the concentration of the enzyme-substrate complex. The catalytic rate is

\[ v = k_3 [ES] \] (1.5)

and the rates of formation and breakdown of ES are

\[ v_f = k_1 [E] [S] \]
\[ v_b = (k_2 + k_3) [ES] \] (1.6)

In a steady state, when the rates of formation and breakdown are equal, we can define the Michaelis constant \( K_M \), and rewrite this as

\[ K_M = \frac{k_2 + k_3}{k_1} \]
\[ [ES] = \frac{[E] [S]}{K_M} \] (1.7)

Provided that the concentration of enzyme is much lower than the concentration of substrate, the concentration of uncombined substrate is nearly equal to the substrate concentration, then follows

\[ [E] = [E_T] - [ES] \]
\[ [ES] = [E_T] \frac{[S]}{[S] + K_M} \implies \]
\[ v = k_3 [E_T] \frac{[S]}{[S] + K_M} \] (1.8)

The maximal rate is reached when the concentration of substrate is much greater than \( K_M \) and the fraction \( \frac{[S]}{[S] + K_M} \to 1 \). Thus

\[ V = k_3 [E_T] \]
\[ v = V \cdot \frac{[S]}{[S] + K_M} \] (1.9)

The last equation is the Michaelis-Menten equation and \( V \) is the maximal rate (theoretically). \( V \) is sometimes denoted \( V_{max} \).

**Significance of \( K_M \) and \( V \) values**

As can be deduced from equation (1.9) \( K_M \) is the substrate concentration at which the reaction rate is half of its maximal value, i.e.

\[ S = K_M \iff v = \frac{V}{2} \] (1.10)
1.2 Synaptic plasticity

Long-lasting and activity-dependent changes in synaptic strength are considered as key properties of neurons to explain cellular and molecular mechanisms supporting the formation and storage of memories. So-called bidirectional synaptic plasticity is considered to be governed by calcium influx through NMDA receptors. The calcium signal then triggers a signal transduction cascade, involving calcium-dependent protein kinases and phosphatases (among others calmodulin dependent kinase type II (CaMKII) and calcineurin (CaN)), that induces LTP (long-term potentiation, an increase in synaptic strength) or LTD (long-term depression, a decrease in synaptic strength). There is strong evidence that LTP involves a postsynaptic process [41], which selectively enhances AMPA receptor-mediated transmission.
1.3 Dendritic spines

Dendritic spines are small protrusions from dendrites of various types of neurons, consisting of a head, with a volume of \( \sim 0.01 \text{-} 1 \text{ } \mu \text{m}^3 \), connected to its parent dendrite via a thin (\( \sim 0.1 \text{ } \mu \text{m} \)) neck [38]. Most spines receive an excitatory synapse on their head. The neck effectively limits diffusional exchange between the spine head and the dendrite (see section 1.5.2). The spine contains a wealth of different substances, and spine morphology is highly variable even on the same dendrite [45]. A simple illustration of a spine and the reactions studied in this project can be found in figure 1.1.

1.3.1 The Post-Synaptic Density (PSD)

The PSD is a scaffolding organelle located at the postsynaptic membrane. It contains a wealth of proteins important for signal transmission, among them scaffolding proteins, glutamate receptors, kinases and phosphatases. Many of these proteins are known to organize themselves into complexes that are thought to be important for synapse function, see [21], [29] and [45].

1.4 Glutamate receptors

The glutamate receptors can be divided into two categories: the ionotropic receptors that directly gate channels and the metabotropic receptors that gate channels indirectly, through second messengers.

The ionotropic glutamate receptors belong to a separate genetic family of ligand-gated channels. Two branches of the glutamate receptor-gene family, closely related to each other, include the AMPA and kainate receptors. A more distantly related branch of the family codes for the NMDA type of receptors [52]. The glutamate-gated channels are all multimeric proteins, thought to be composed of four subunits built up by three transmembrane \( \alpha \)-helices each.

1.4.1 NMDA receptors

NMDA receptors (NMDARs) are heteromeric ion channels, composed of NR1 and NR2 subunit proteins. The NR2 subunit has four subtypes (NR2A-NR2D), each of which gives the receptor distinct functional properties [69].

NMDARs are detectors of coincident activity

The NMDA receptor channel is unique in that it is permeable not only to \( \text{Na}^+ \) and \( \text{K}^+ \), but also to \( \text{Ca}^{2+} \), and that its opening depends on both membrane voltage and the presence of the transmitter. Thus, the NMDAR can act as a detector of coincident activity in the pre- and postsynaptic cells [52]. The channels open efficiently only when glutamate is released from the presynaptic terminal (i.e. when the presynaptic cell is active) and the postsynaptic cell is
strongly depolarized.

At membrane resting potential, the channel is blocked by Mg\textsuperscript{2+}. When the membrane is depolarized, Mg\textsuperscript{2+} is expelled from the channel by electrostatic repulsion. Channel opening produces a rise in \([\text{Ca}\textsuperscript{2+}]\) that is largely restricted to the dendritic spine onto which the active synapse terminates. The depolarization level that is needed to activate NMDARs usually requires the summation of multiple synaptic inputs. LTP induction is considered to depend on activation of NMDA receptors.

Proteins interacting with NMDA receptors

There are many substances that bind to and/or interact with NMDA receptors in different ways, either directly or via scaffolding proteins. Lists of these can be found in the articles by Husi et al. \[21\] and Tashiro and Yuste \[45\]. Interacting proteins include CaM, CaMKII, CaN and PP1. The NMDAR subunit NR2B can be phosphorylated by CaMKII (see section \[1.7.1\]) and this phosphorylation practically inhibits further CaMKII binding.

1.4.2 AMPA receptors

The AMPA receptor (AMPAR) is a heteromer composed of multiple subtypes of subunit proteins (GluR1-GluR4). In the adult hippocampus, receptors consisting of GluR1/GluR2 and GluR2/GluR3 are predominant \[18\]. The function of the AMPAR is regulated by the composition of individual receptors and/or the phosphorylation and dephosphorylation states of individual subunit proteins. Of particular interest are the residues serine 831 (Ser\textsuperscript{831}) and serine 845 (Ser\textsuperscript{845}) on the GluR1 subunit, as they can be phosphorylated by CaMKII/protein kinase C and protein kinase A, respectively.

Phosphorylation of AMPARs in LTP and LTD

The induction of LTP specifically increases phosphorylation of S-831 which increases the single channel conductance of GluR1 AMPARs \[18\]. The induction of LTD is accompanied by a decrease in the phosphorylation of Ser\textsuperscript{845}, which appears to be phosphorylated at resting potential. Phosphorylation of Ser\textsuperscript{845} increases the "open time" of the AMPAR \[9\] and is required for receptor incorporation in the membrane \[18\].

AMPA receptor trafficking

AMPA receptor subunits are synthesized and assembled in the rough endoplasmic reticulum (ER) and then inserted into the plasma membrane after crossing the Golgi. AMPARs undergo a constant trafficking between the plasma membrane and intracellular compartments. This process plays a key role in the regulation of the synaptic levels of AMPA receptors. Activation of CaMKII causes translocation of the GluR1 subunit to dendritic spines and synapses \[17\].
1.5. The Ca\(^{2+}\) ion - an intracellular messenger

AMPA receptor internalization can be influenced by both AMPA and NMDA receptors, but the nature of the stimuli determines the exact fate of the internalized AMPARs. Upon NMDAR activation, AMPA receptors are transported rapidly from the membrane and can be recycled (i.e. rapidly reinserted) [17], but after AMPAR activation, the receptors are probably transported away and degraded [18].

NMDAR-dependent AMPAR trafficking is regulated by PKA and accompanied by GluR1 phosphorylation and dephosphorylation at a PKA site [17]. PKA also exerts a differential modulatory effect on intracellular AMPA receptor trafficking upon AMPA- and NMDA-induced internalization. NMDA receptor induced AMPA receptor trafficking is accompanied by dephosphorylation followed by rephosphorylation of GluR1 AMPA receptor subunits at Ser\(^{845}\) by PKA. In contrast, PKA is without effect on AMPA-induced AMPA receptor cycling [18].

Proteins interacting with AMPA receptors

AMPA receptor localization at the postsynaptic membrane of excitatory synapses is dynamically regulated. Targeting of receptors to synapses is thought to be mediated through interaction of AMPA receptor subunits with scaffolding proteins. For a list of some directly interacting proteins and further details, see Gomes et al. [18] and Tashiro and Yuste [45].

1.5 The Ca\(^{2+}\) ion - an intracellular messenger

The resting level of Ca\(^{2+}\) in a cell is typically somewhat lower than 100nM, which is several orders of magnitude less than the extracellular Ca\(^{2+}\) level. Thus, a rise in [Ca\(^{2+}\)] (Ca\(^{2+}\) concentration) – achieved by the opening of calcium channels, or release from intracellular stores – can be used as a signal. Calcium concentration regulates many processes in cells, on many different timescales: for instance synaptic transmission and muscle contraction in skeletal and cardiac muscles. Prolonged exposure to high levels of Ca\(^{2+}\) is toxic and can end in cell death (excitotoxicity).

1.5.1 Ca\(^{2+}\) regulation in general

Calcium enters the cell via several kinds of calcium channels and via NMDA channels. It can be stored in and released from intracellular stores such as buffers and the sarcoplasmic reticulum (SR), and is removed from the cell by a Ca\(^{2+}\)/H\(^{2+}\) ATPase, a Ca\(^{2+}\) pump driven by ATP hydrolysis, and a 3Na\(^{+}\)/Ca\(^{2+}\) exchanger driven by the electrochemical Na\(^{+}\) gradient across the membrane [37]. Calcium concentration can also be directly and indirectly influenced by itself through processes as Ca\(^{2+}\) pump regulation, calcium-induced-calcium release from the SR and phosphorylation of NMDA receptors by CaMKII (see below).
1.5.2 Ca$^{2+}$ regulation in dendritic spines

Calcium is known to be crucial for the induction of LTP and LTD. In these cases, NMDA receptors are considered to be the important Ca$^{2+}$ sources. Sabatini et al. [38] have shown that spines are specialized in several ways:

- Spines have low Ca$^{2+}$ buffer capacity (20 units → 5% of the Ca$^{2+}$ entering the spine remains unbound [19]).
- Ca$^{2+}$ diffusion across the spine neck is negligible.
- The spine head functions as a separate compartment, allowing localized Ca$^{2+}$ buildup (which is crucial for synaptic plasticity).
- The kinetics of NMDAR opening primarily determines the time course of synaptically evoked [Ca$^{2+}$] transients. NMDAR-mediated transients in unperturbed spines reach $\sim$12 µM at depolarizing potentials.
- Spine Ca$^{2+}$ returns to resting levels with a time constant of $\sim$12 ms.
- Ca$^{2+}$ resting level in the spines is low; [Ca$^{2+}$]$_0$ = 70 ± 29 nM at resting potential.
- Clearance of Ca$^{2+}$ from the cytoplasm is too rapid to allow Ca$^{2+}$ accumulation during low-frequency stimulation (LFS).
- The 3Na$^+$/Ca$^{2+}$ exchanger is probably responsible for most of the Ca$^{2+}$ efflux from spines.

A discussion of Ca$^{2+}$ measurement in spines and commentary on the Sabatini results can be found in the article by Helmchen [19].

1.5.3 Ca$^{2+}$ thresholds for induction of LTP and LTD

Cormier et al. [12] found that the [Ca$^{2+}$] needed to induce plasticity was $\sim$ 180 nM, while LTD was induced in the range of 180-500 nM and LTP was induced when [Ca$^{2+}$] $>$ $\sim$540 nM.

1.6 Calmodulin

Many proteins are regulated by calmodulin (CaM), which is a small protein that can bind up to four calcium ions. It consists of two structurally similar domains, each containing a pair of helix-loop-helix calcium-binding motifs (also called EF-hands). However, the affinities of all Ca$^{2+}$ binding sites are not equal; CaM has two high-affinity and two low-affinity sites (the Ca$^{2+}$-binding sites in the C-terminal end have a tenfold higher affinity ($K_d \approx 0.2$ µM) than those in the N-terminal end ($K_d \approx 2$ µM) [48]). Positive cooperativity is observed between sites within a domain.
Mostly, CaM-binding proteins bind CaM with four Ca\(^{2+}\) ions (CaM fully loaded with Ca\(^{2+}\) will in this report be denoted CaM*) and become activated, but there are also cases where CaM is bound without Ca\(^{2+}\) and activates the protein when Ca\(^{2+}\) is bound. Yet another variety are the proteins that bind calcium-free calmodulin and release it when it binds calcium [33]. In many enzymes that are activated by CaM, an autoinhibitory part overlapping the CaM binding site is dislocated upon the binding of CaM, leading to enzyme activation.

### 1.6.1 Integration of Ca\(^{2+}\) signals by CaM

In rat sensory neurons, Millikan et al. [33] found that CaM was able to "integrate" Ca\(^{2+}\) signals in the sense that

- CaM activation peaked after the maximum of the Ca\(^{2+}\) signal, with maximum activation a half second after the calcium influx ended
- CaM activation did not immediately fall to baseline when the Ca\(^{2+}\) level did, but fell to a slowly decaying plateau persisting for tens of seconds

However, in this case Ca\(^{2+}\) remained in the cytosol for a couple of seconds (i.e. Ca\(^{2+}\) clearance was not as rapid as in spines).

### 1.6.2 Ca\(^{2+}\) binding to CaM is affected by Mg\(^{2+}\)

Mg\(^{2+}\) binds to CaM and competes with Ca\(^{2+}\) binding [31], but the affinity for Mg\(^{2+}\) is at least thousandfold lower [48]. Both Mg\(^{2+}\) and CaM-binding proteins affect the affinity of CaM for Ca\(^{2+}\), as reported by Stemmer and Klee [42]. Moderate (i.e. millimolar) Mg\(^{2+}\) concentrations increase the affinity while high concentrations decrease the affinity.

### 1.6.3 Ca\(^{2+}\) binding to CaM is affected by CaM-binding peptides

The presence of Mg\(^{2+}\) and CaM binding peptides (short protein fragments containing the CaM-binding sequence of CaN subunit A) increases the affinity for Ca\(^{2+}\) in a complex way, but in general by at least 10-fold for each of the four binding sites [42].

### 1.7 Kinases and phosphatases

The human genome encodes \(\sim 500\) kinases and \(\sim 150\) phosphatases [10]. They regulate protein function by phosphorylation and dephosphorylation, respectively (addition or removal of a phosphoryl group). Two special subclasses are serine-threonine kinases and phosphatases, who phosphorylate and dephosphorylate serine and threonine residues. CaMKII is the dominant kinase in the hippocampal area; it is present in 20 times higher concentration than the other kinases [20].
1.7. Kinases and phosphatases

1.7.1 CaMKII

CaMKII (calmodulin-dependent kinase type II) is one of the major constituents of the post-synaptic density (PSD) [41] and is thought to have both structural and functional importance. It is activated by binding of CaM* and its key property is autophosphorylation, the ability of its subunits to phosphorylate neighbouring subunits.

Initially, CaMKII is bound to F-actin [28] distributed in the cytoplasm, but it translocates to the PSD upon binding of CaM* [40]. The half-maximal time for translation ranges from around 20 s (α-CaMKII) to 280 s (β-CaMKII) and translation time can thus be regulated in the neurons by the ratio of α- to β-CaMKII. PSD-bound CaMKII is nearly immobile during glutamate stimulation [40].

Structure

CaMKII has 8-12 nearly identical subunits, arranged in two hexameric rings. Each subunit consists of a catalytic domain, an autoinhibitory domain, a variable segment and a self-association domain. The catalytic domain contains the ATP- and substrate-binding sites, as well as sites for interaction with anchoring proteins.

CaMKII states

CaMKII subunits can be said to have four different states of activation, as well as an inactive ground state: bound to CaM*, trapped, autonomous and capped. See figure 1.2.

- The activation in the bound state lasts only as long as CaM* is bound.
- The trapped state, or CaM* trapping on CaMKII subunits, is an effect of the thousandfold decreased off rate of CaM* after phosphorylation at Thr286/287 by a neighbouring subunit that is already active.
- An autonomous CaMKII subunit is phosphorylated at Thr286/287 (by a neighbouring subunit) and has in addition lost its bound CaM* (i.e. the activity is still high, but has become CaM*-independent).
- Capped CaMKII, finally, is also phosphorylated at Thr305/306. Having a trapped CaM* prevents capping, and capping prevents rebinding of CaM*.

Capping of CaMKII appears to be both an intrasubunit and an intersubunit reaction, and capped CaMKII has less catalytic activity than autonomous CaMKII or no catalytic activity, according to some [11, 26]. Kubota and Bower claim that capped CaMKII has the same activity as autonomous CaMKII [25]. Holmes claims [20] that the activity of autonomous and capped subunits is about 40% of the activity of bound and trapped subunits.
Chapter 1. Background

1.7. Kinases and phosphatases

Figure 1.2: CaMKII states. $CII_s^X$ denotes CaMKII subunit in state X, C indicates calcium/calmodulin dependent transitions, P denotes phosphorylation and D denotes dephosphorylation.

CaMKII isoforms

There are four genes for different CaMKII isoforms: $\alpha$, $\beta$, $\gamma$ and $\delta$. Additional isoforms can also be generated by alternative splicing of all four isoforms. The $\alpha$ and $\beta$ isoforms are the predominant isoforms in the brain, and the enzyme can contain both $\alpha$ and $\beta$ subunits.

$\beta$-CaMKII has a larger affinity for CaM than $\alpha$-CaMKII (CaM$_{50}$ for autophosphorylation is 15nM for $\beta$-CaMKII and 130 nM for $\alpha$-CaMKII [7]) and has a relatively larger activation at all frequencies [13]. Also, regulation of $\alpha$-CaMKII transcription levels is strongly influenced by NMDAR activity, while $\beta$-CaMKII transcription levels are insensitive to NMDAR activity and sensitive to AMPAR activity [47]. For other CaMKII interactions with NMDA, see section 1.7.1

It is thought that the cell fine-tunes its response to changing Ca$^{2+}$ signalling levels by regulating the relative amount of $\alpha$-CaMKII to $\beta$-CaMKII. The $\alpha/\beta$ ratio has been shown to change as most ~5-fold after 24 hours as a response to changing intensities of Ca$^{2+}$ signalling [47].

Under resting conditions, $\beta$-CaMKII but not $\alpha$-CaMKII are bound to F-actin ([28 and [40], respectively) distributed in the cytoplasm.

Activation and function

Under basal conditions, the kinase has almost no catalytic activity since the autoinhibitory domain of each subunit inhibits its own catalytic domain. Each subunit can be activated by the binding of a single CaM* to that subunit. This simple form of activation lasts only as long as the increase in [Ca$^{2+}$].

When the increase in [Ca$^{2+}$] is sufficient to cause CaM* to bind simultaneously to two neighbouring units, one subunit can phosphorylate the other at residue threonine 286 (Thr$^{286}$, for $\alpha$-CaMKII) or threonine 287 ($\beta$-CaMKII). The occurrence of two neighbouring subunits with bound CaM* is sparse, so this is a slow process. Further phosphorylation only requires binding of one CaM* (to a neighbouring site to one already phosphorylated) and proceeds much faster. Autophosphorylation of CaMKII by itself shows a strong dependence on [Ca$^{2+}$],
with a Hill coefficient close to 5 (with no phosphatases present [6]).

Immobilized CaMKII has been shown to reach 60-80 percent autonomous activity, and have a half-maximal activation at a CaM concentration of 25-80 nM (CaM_{50} for pure β-CaMKII and pure α-CaMKII, respectively) [13]. Brocke et al. report CaM_{50} values of 15 nM and 130 nM, respectively [7].

**CaMKII in the PSD is dephosphorylated by PP1**

An autonomous subunit remains active even after [Ca^{2+}] levels return to baseline. Thus, phosphorylation results in persistent activity; a biochemical “memory trace” of the previous [Ca^{2+}] elevation. Its activity will remain until it is dephosphorylated. In the PSD, CAMKII is dephosphorylated only by PP1 [29]. When CaMKII autophosphorylation is balanced by PP1 dephosphorylation, it has an even stronger dependence on [Ca^{2+}], with a Hill coefficient close to 8 [6], compared to a Hill coefficient of 5 without PP1.

**CaMKII frequency sensitivity**

De Koninck and Schulman [13] have shown experimentally that CaMKII activation in itself is dependent on the frequency of stimulation, i.e. that oscillation frequency and duration is translated to different levels of activity. They immobilized CaMKII inside PVC tubing and subjected it to pulses of Ca^{2+} and CaM of varying amplitude. No phosphatases were present. Activation at 1 Hz (with pulses of 100 nM CaM and saturating amounts of Ca^{2+} and ATP, 500 µM and 250 µM respectively) produced only ~ 6-7 % activity after 100 pulses, while stimulation at 4 Hz produced ~ 60 % activation after the same number of pulses. Naturally, also the pulse length had influence on the sensitivity, with the frequency response shifted to lower frequencies with longer pulse lengths.

The frequency sensitivity of β-CaMKII has also been demonstrated to be modulated by alternative splicing [3]. As the splice variants are differentially expressed among individual neurons, this provides cells with a possibility to modulate their sensitivity to oscillating Ca^{2+} signals.

Frequency sensitivity has also been replicated by several models, by among others Coomber [11], Dupont et al. [16] and Kubota and Bower [25].

**CaMKII translocation to the PSD**

Shen and Meyer [40] have shown that, upon Ca^{2+} influx through NMDA receptors and subsequent binding of CaM, β-CaMKII rapidly dissociates from F-actin and translocates to the PSD (where it is thought to increase synaptic strength by phosphorylating PSD ion channels and signaling proteins). CaM binding but not autophosphorylation was needed for translation to occur, although autophosphorylation plays an indirect part as the affinity of CaMKII
for CaM is increased hundred-fold upon autophosphorylation. This is thought to prolong the association of CaMKII with the PSD. Dosemeci et al. [15] have shown that accumulation of CaMKII in the PSD is reversed within 30 minutes upon removal of glutamate and Ca$^{2+}$.

**Binding of phosphorylated CaMKII to the NMDAR**

After it is exposed by binding of CaM*, the catalytic domain of a CaMKII subunit can also bind to the NMDA receptor subunit NR2B with high affinity. This will cause the CaMKII subunit to remain active also after releasing CaM*. Phosphorylation of the NR2B subunit residue Ser$^{1303}$ by CaMKII (with a very low turnover rate, $\sim 10$ min$^{-1}$) inhibits almost all CaMKII/NR2B interaction. The NR2B-CaMKII complex has a dissociation time of several minutes [43].

Phosphatase activity may, paradoxically, increase CaMKII/NR2B interaction if Ser$^{1303}$ is more dephosphorylated than CaMKII. The identity of the phosphatase that dephosphorylates Ser$^{1303}$ is not known [43].

**A structural role for CaMKII in the PSD**

It has been proposed by Lisman and Zhabotinsky [29] that CaMKII binding to the NMDA receptor organizes a structural process that leads to the incorporation of AMPA-receptor-binding proteins into the PSD, and to the subsequent anchoring of additional AMPA receptors, accumulating AMPARs in the postsynaptic membrane.

It is further suggested that these scaffolding proteins create an isolated and limited pool of PP1 in the PSD and prevent PP2A, which is able to dephosphorylate CaMKII in solution but apparently not in the PSD [28], from dephosphorylating CaMKII. This would allow the local phosphatase pool to become saturated by highly phosphorylated kinase.

Saturation of the local phosphatase pool would provide a solution to the "protein turnover problem": keeping the highly phosphorylated state although part of the highly phosphorylated kinase pool is replaced by newly transcribed, unphosphorylated protein. It would also allow bistability, which is thought to be a property required for synaptic plasticity [27], [28], [29], [51]. Bradshaw et al. [6] have shown that the CaMKII-PP1 system indeed is bistable.

**CaMKII phosphorylation of AMPARs**

Besides the role CAMKII has in AMPAR trafficking, it is also known that activated CaMKII phosphorylates GluR1 subunits of already existing AMPARs on the Ser$^{831}$ residue [2]. This increases their singel-channel conductance (which has the possible range of 9 to 28 pS, depending on the conductance state of the channel) by $74 \pm 6$ % [14]. Presence of CaMKII does not increase the mean
1.7. Kinases and phosphatases

open probability of the channel [14]. An LTP induction protocol produces a mean increase in single channel conductance of 84 ± 20 % [4].

CaMKII regulation by Zn$^{2+}$

According to Lengyel et al. [26], α-CaMKII can during presence of Zn$^{2+}$ be converted into a form wholly or partly unable to respond to CaM*. It can also be autophosphorylated (on Thr$^{286}$, Thr$^{306}$ and Ser$^{279}$) by Zn$^{2+}$, although not as efficiently as by CaM*. Releasable Zn$^{2+}$ is present in mossy fiber terminals in the hippocampal CA3 region. Thus, CaM* activation is, strictly speaking, not the only activation mechanism for CaMKII (but by far the most usual and relevant one).

CaMKII clustering

In intact adult brain, CaMKII molecules containing α-CaMKII have been shown to self-organize into clusters upon prolonged [Ca$^{2+}$] elevation (after 1–2 minutes). It is considered to be a mechanism for protection against excessive protein phosphorylation and is reversible [44]. This would give a large decrease in effective CaMKII concentration and also, as the possibility of CaMKII to bind CaM remains, a large decrease in the concentration of free CaM (due to continued trapping).

Experimental behaviour of CaMKII and modelling this behaviour

According to Bradshaw et al. [6], the CaMKII-PP1 system is an ultrasensitive switch, i.e. is cooperative to Ca$^{2+}$. They examine this cooperativity by letting α-CaMKII, in a solution of CaM, ATP and differing amounts of Ca$^{2+}$, autophosphorylate for 5 minutes. The resulting autonomy, for different [Ca$^{2+}$], is fitted to the Hill equation. See figure 1.3. They also investigate the resulting autonomy with the same procedure when PP1 is included.

1.7.2 Calcineurin (PP2B)

Structure

Calcineurin (CaN, or PP2B) is a so-called serine/threonine phosphatase; a protein phosphatase that dephosphorylates serine and threonine residues on proteins. It consists of two subunits, called Calcineurin A and Calcineurin B, that are inseparable unless the protein is denatured. Calcineurin A binds one CaM* and Calcineurin B can bind up to four Ca$^{2+}$ ions; it contains four EF-hands (Ca$^{2+}$-binding motifs) and its calcium binding parts are structurally similar to those found in calmodulin. Thus, CaN can be activated by calcium in two different ways; partly by binding of CaM*, partly by binding of Ca$^{2+}$ directly to the B subunit [24], [42].
Chapter 1. Background

1.7. Kinases and phosphatases

Figure 1.3: CaMKII autophosphorylation with 1 µM CaMKII and 50 µM CaM. The Hill number is 4.9, the half-maximal Ca\(^{2+}\) value is 1.4. All values taken from Bradshaw et al.

Activation and function

CaN activation by CaM* is highly cooperative; it has a Hill coefficient of 2.8-3.1, indicating that binding to at least three of the four Ca\(^{2+}\) binding sites of CaM is necessary for activation [42]. Calcium binding directly to CaN B decreases the K\(_m\) (the half-maximal concentration) without changing the V\(_{max}\) (the maximum catalytic rate) of CaN, while CaM* binding increases the V\(_{max}\) but not the K\(_M\). In the presence of Ca\(^{2+}\) but not CaM, CaN has an activity that is ~10% of the maximal activity obtained when both are present.

CaN is involved in regulation of PP1 activity (see section 1.7.3).

CaN in LTD and LTP

Using a fluorescent indicator for CaN activity to study the role of CaN in LTD-induction in neurons of rat visual cortex, Yasuda et al. [50] found that low-frequency stimulation (LFS) during at least 3 minutes is necessary to activate CaN, and that \(\theta\)-burst stimulation (five \(\theta\)-bursts of 100Hz stimuli, this is known to induce LTP according to [50]) fails to significantly activate CaN. They also confirm that not only the Ca\(^{2+}\) concentration but also the period during which it is in the optimal range has consequences for the activation of CaN. Jouveneau et al. [22] found that CaN is involved in depotentiation of LTP, but not in LTD.
1.7.3 Protein Phosphatase 1 (PP1)

PP1 is a member of the same family of serine/threonine phosphatases as PP2B (CaN). It interacts with over 50 regulatory subunits of target proteins in different parts of the body and it is thought that the large diversity of PP1 function is due to the interaction with these targets [10]. There are many inhibitors of PP1; for instance Inhibitor-1 (I-1) and DARPP-32 (DARPP-32 is an isoform of I-1 highly expressed in part of the brain). Tables of PP1 interactors and inhibitors can be found in Cohen [10].

PP1 regulation in the PSD

PP1 is the only phosphatase that dephosphorylates CaMKII in the PSD [29]. I-1 and DARPP-32 are both converted to PP1 inhibitors through phosphorylation by PKA (PKA activation is caused by cAMP), and in the PSD, PP1 and PKA are kept in close proximity by the A-Kinase-anchoring protein Yotiao. Phosphorylated I-1/DARPP-32 is dephosphorylated – thereby reactivating PP1 – by CaN. For a full list of PP1 interactors, see Cohen [10].

1.8 Reactions included or excluded in models

Models of CaMKII activation differ by the reactions that are included. All include Ca binding to CaM and subsequent CaM* binding to CaMKII, but with different levels of detail. Some include PP1 or/and PP1-regulators such as I1 and CaN. All possible reactions involving Ca\(^{2+}\), CaM, CaMKII and CaN (except for Ca\(^{2+}\) binding directly to CaN) can be seen in figure 2.2 with rate constants taken from [20].

Some models include PP1, others only contain dephosphorylation rates. CaMKII activation states (trapping, autonomy, capping) might be explicitly included or simplified to one ‘activated’ state. Some models also include activation of PKA, which actually is activated by another pathway (the cAMP pathway). Often, CaM binding of Ca\(^{2+}\) is simplified to one step, dependent on the fourth power of [Ca\(^{2+}\)]. Ca\(^{2+}\) kinetics in the spine are also often considerably simplified.

For a full discussion of model characteristics, see chapters 3 and 5. For a condensed overview of models, see the tables in appendix B.

1.9 Producing realistic LTP/LTD curves

Castellani et al. [9] found, with a more abstract calcium-dependent model of LTP/LTD, that the following conditions were necessary for realistic LTP/LTD curves:

- That the activity level of phosphatases rises at lower calcium concentration than the activity level of kinases
- That, at high calcium concentration, the activity level of kinases is higher than the activity level of phosphatases

These conditions would give a threshold calcium level for LTP/LTD induction. However, Castellani et al. did not study Ca$^{2+}$-dependent inhibition of phosphatase.
1.9. Producing realistic LTP/LTD curves
Chapter 2

Models

In this chapter I will give an overview of all models I have studied, and point out some relevant results from the respective articles. Results from my simulations with the models by Dupont et al. [16], Holmes [20] and Zhabotinsky [51] will be presented in chapter 4. There are also tables with overviews of model characteristics in the appendices.

Rationale for the choice of models to study

There are many models that, in some way, include CaMKII activation. This made it necessary to chose only some of the models, and to try to find good criteria for which models to choose. The limited availability of time and computer resources made it necessary to choose “small” models of CaMKII activation, i.e. models that contain a limited number of chemical species. This was the main reason not to include the well-known model by Bhalla and Iyengar [5]. Also, I have tried to use recently published models – the oldest model I have studied was published in 1998. One of the reasons for this is that new and more accurate analysis methods are continuously developed, making new and more accurate data available which makes it possible to make better approximations for model parameters such as rate constants. The third concern was the level of detail in the CaMKII activation process. CaMKII has several activation states, but many models (especially models where CaMKII activation is not the focus) only contain one active state. I have chosen to include both more and less detailed models, and to implement two more detailed models (Holmes [20] and Dupont et al. [16]) and one more abstract model (Zhabotinsky [51]) for comparison.

2.1 D’Alcantara et al.

The article by d’Alcantara et al. [1] introduces one full model, with interdependent activation and inhibitory pathways together with two simplified models. I will here only present the full model.
2.1. D’Alcantara et al.

2.1.1 Aim

The aim of d’Alcantara et al. is to examine if a “realistic model using reported parameter values” can simulate the activity of either LTD or LTP, as expressed by the activity levels of AMPARs.

2.1.2 Input signal

The input signal is Ca\(^{2+}\) concentration, given directly (i.e. not simulated as an influx from NMDA channels or voltage-gated calcium channels (VGCCs)).

2.1.3 The model

The model includes Ca binding to CaM (a Hill equation with \(n_H = 4\)), CaMKII activation by CaM* and CaMKII autophosphorylation on Thr\(^{286}\) (only the trapped state), CaMKII dephosphorylation by PP1, CaN activation by CaM*, DARPP-32/I1 phosphorylation by PKA and dephosphorylation by CaN, PP1 inactivation by phosphorylated DARPP-32/I1 and AMPAR phosphorylation by CaMKII and PKA (of the GluR1 subunit at Ser\(^{831}\) and Ser\(^{845}\)) and dephosphorylation by PP1 and CaN. The model has three AMPAR states: the fully dephosphorylated, the naive state with only Ser\(^{845}\) phosphorylated, and the fully phosphorylated state.

LTP is in this model assumed to correspond to phosphorylation of Ser\(^{831}\), while LTD is assumed to correspond to Ser\(^{845}\) dephosphorylation. AMPAR “activity” is a general variable, and thus not dependent on whether insertion of new AMPARs or an increase in AMPAR conductance is assumed to be the relevant AMPAR strengthening mechanism.

2.1.4 Results of d’Alcantara et al.

The steady-state configuration of AMPARs in different states was found to depend on Ca\(^{2+}\) concentration, or rather the relative increases in phosphatase and kinase activity.

The indirect modulation of PKA by Ca\(^{2+}\) (via the cAMP pathway) was found to have no effect on AMPAR depression at low Ca\(^{2+}\) concentrations, but amplified the amplitude of the AMPAR potentiation occurring at higher Ca\(^{2+}\) concentrations (the cAMP pathway was not included; PKA modulation was estimated by letting PKA phosphorylation rates depend linearly on CaM* concentration).
2.2 Coomber

2.2.1 Aim
Coomber’s aim [11] is to construct and investigate a detailed model of CaMKII based on experimental findings and concepts from earlier modelling studies.

2.2.2 Input signal
The input signal is fluctuations in [Ca$^{2+}$] caused by presynaptic spikes (the reference level of Ca$^{2+}$ is set to zero for convenience). The time evolution of [Ca$^{2+}$] is specified as a dual-exponential function, with the time to peak $\sim 10$ ms and time to decay to baseline $\sim 1$ s. Peak amplitude is 1 $\mu$M.

2.2.3 The model
Coomber [11] simplifies his model by reducing the number of subunits to four, and grouping rotationally symmetric configurations as one configuration. Each modeled CaMKII subunit can have twelve different states; inactive, bound to CaM*, phosphorylated and bound to CaM* (trapping), phosphorylated (autonomous) and three capped states (phosphorylated on Thr$^{286}$ and phosphorylated on Thr$^{305}$ or/and Thr$^{306}$ or just phosphorylated at Thr$^{305/306}$). Subunits that are only phosphorylated at Thr$^{305/306}$ are considered inhibited. The five last states consist of a phosphorylated state with a bound molecule of phosphatase (i.e. subunits that will become dephosphorylated).

Dephosphorylation by a CaMKII-specific phosphatase is included. Nearest-neighbour phosphorylation is assumed to be unidirectional. Coomber also assumes a "reference concentration" of basal Ca$^{2+}$, equal to zero. The concentration of ATP is assumed to be much larger than the concentration of CaMKII and CaMKII-specific phosphatase (1mM; as far as I know, this is the only model including an explicit concentration of ATP).

2.2.4 Coomber’s results
The model exhibited frequency sensitivity; high-frequency stimulation (50-100 Hz) was needed for a high level (>70%) of kinase activity, while low-frequency (<2 Hz) stimuli resulted in minimal activity. Generally, HFS promoted kinase activation and LFS promoted kinase inhibition. This was not dependent on a limiting concentration of CaM, but due to the preferential stimulation of phosphatase activity. The system exhibited a frequency stimulation threshold for switching behaviour (i.e. persistent CaMKII activity in the absence of Ca$^{2+}$).

The influence of the strength of CaM trapping by a phosphorylated subunit was examined by varying the off rate of CaM from the subunit and simulating 100 Ca$^{2+}$ spikes. The largest difference was seen at low Ca$^{2+}$ spike frequency, and
the fraction of trapped subunits converged to $\sim 0.7$ with increasing frequency. The fraction of inhibited subunits peaked at $\sim 1$ Hz, the maximum value was 0.4-0.5 depending on the off rate.

Capping
In simulations, Coomber found that the maximal level of CaMKII inhibition could be as high as 50 percent (with LFS), and that the inactivation could last as long as 20 minutes (with HFS). These levels were influenced by the assumed rate of dephosphorylation, but the result that LFS selectively autophosphorylates inhibitory sites was conserved. In both cases this could have a substantial effect on subsequent CaMKII activation; prior LFS could prevent the induction of LTP by HFS. The amount of inhibition of CaMKII could also affect the threshold for induction of LTP.

2.3 Dupont et al.

2.3.1 Aim
The aim of Dupont et al. is to construct a simple model that captures the frequency sensitivity of CaMKII to Ca$^{2+}$ signals as previously reported by De Koninck and Schulman [13].

2.3.2 Input signal
The input signal is Ca$^{2+}$ concentration, given directly.

2.3.3 The model
Dupont et al. [16] have developed a simple model, without dephosphorylation, that captures the frequency sensitivity of CaMKII. It is a deterministic model with fixed CaM concentration (i.e. a fixed, inexhaustible CaM pool) and five CaMKII states (inactive, bound, "phosphorylated", trapped and autonomous – capping is not included). The non-standard "phosphorylated" state is a trapped state, but with only CaM bound to the trapping subunit. The number or location of CaMKII subunits is not specified; instead of neighbour-dependent autophosphorylation Dupont et al. use a nonlinear (empirically based) rate of phosphorylation based on the ratio of active subunits:

$$V_A = K_A \cdot ((c_B W_B)(c_B W_B + c_P W_P + c_T W_T + c_A W_A))$$
$$K_A = K_A' \cdot (a \cdot T + b \cdot T^2 + c \cdot T^3)$$

(2.1)

where $c_X$ is the relative activity of state X, $W_X$ is the amount of CaMKII in state X, $K'_A$ is the phenomenological rate constant, a-c are constants that were adjusted for a good fit to experimental data from De Koninck and Schulman [13] and T is the fraction of CaMKII in any of the active states. The activities
2.3.4 Results of Dupont et al.

Dupont et al. show that their model is able to replicate the steady-state behaviour and frequency sensitivity of CaMKII as reported by De Koninck and Schulman.

2.4 Holmes

2.4.1 Aim

Holmes’ aim [20] is to estimate levels and durations of CaMKII activity following LTP-inducing tetani.

2.4.2 Input signal

The input signal is presynaptic voltage (leading to glutamate release and the subsequent influx of Ca\(^{2+}\) through NMDA channels).

2.4.3 The model

Holmes [20] models a fully reconstructed dentate granule cell with compartments. The mechanisms included are stimulation of synapses and subsequent Ca\(^{2+}\) influx through NMDA receptor channels, Ca\(^{2+}\) diffusion, pumping and binding to buffers (but CaM is assumed to be the only buffer in this system), reactions for Ca\(^{2+}\) binding to CaM, CaM\(^*\) binding to CaMKII and CaN, and CaMKII transition steps (trapped, autonomous, capped) including all possible transitions between the various complexes (also CaM with less than 4 Ca\(^{2+}\) was allowed to bind to CaMKII and CaN).
2.4. Holmes Models

Note that there is a large difference between the values for Ca$^{2+}$-CaM interaction that Holmes uses and the value commonly used in the Hill equation. CaM* dissociation from a trapping subunit is dependent on [Ca$^{2+}$] (see figure 2.4). CaM and CaM-Ca$_x$ diffusion is also included. The model is split into two parts: one deterministic (for all reactions except for CaMKII transition steps) and one stochastic. Autonomous CaMKII rebinding of CaM* and subsequent transition back to the trapped state is modeled as a deterministic process. See figures 2.2 and 2.3 for details.

**Dendritic spine part of the model**

The spine head in the model has four spine-head compartments, four spine-neck compartments and twelve dendritic compartments. The dendritic spine is assumed to be a long-thin spine with cylindrical dimensions of 0.55 x 0.55 $\mu$m for the head and 0.1 x 0.73 $\mu$m for the neck. CaMKII is considered to be restricted to the outer 50 nm of the spine head (corresponding to PSD-bound CaMKII).

\[
\begin{align*}
\text{CaMKII} & \xrightarrow{2700} \text{Ca} \\
\text{CaMKII} - \text{CaM} & \xrightarrow{0.2} \text{CaM} \\
\text{CaMKII} - \text{CaMCa} & \xrightarrow{2700} \text{CaMCa} \\
\text{CaMKII} - \text{CaMCa}_2 & \xrightarrow{22.5} \text{CaMCa}_2 \\
\text{CaMKII} - \text{CaMCa}_3 & \xrightarrow{4.5} \text{CaMCa}_3 \\
\text{CaMKII} - \text{CaMCa}_4 & \xrightarrow{4.5} \text{CaMCa}_4
\end{align*}
\]

Figure 2.2: Holmes’ reaction scheme, deterministic part. All values (units are $\mu$M and s) were taken from Holmes [22].

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaMKII → Ca</td>
<td></td>
</tr>
<tr>
<td>CaMKII → CaM</td>
<td>0.2</td>
</tr>
<tr>
<td>CaMKII → CaMCa</td>
<td>2700</td>
</tr>
<tr>
<td>CaMKII → CaMCa2</td>
<td>22.5</td>
</tr>
<tr>
<td>CaMKII → CaMCa3</td>
<td>4.5</td>
</tr>
<tr>
<td>CaMKII → CaMCa4</td>
<td>4.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaM → CaN → CaM</td>
<td>5000</td>
</tr>
<tr>
<td>CaMCa → CaN → CaMCa</td>
<td>3750</td>
</tr>
<tr>
<td>CaMCa2 → CaN → CaMCa2</td>
<td>1250</td>
</tr>
<tr>
<td>CaMCa3 → CaN → CaMCa3</td>
<td>100</td>
</tr>
<tr>
<td>CaMCa4 → CaN → CaMCa4</td>
<td>100</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{Autonomous} & \xrightarrow{33.3} \text{Trapped}
\end{align*}
\]
2.4. Holmes Models

Figure 2.3: Holmes’ reaction scheme, stochastic part. The probability of a transition in time interval $\Delta t$ is $1-e^{-k\Delta t}$. All values were taken from Holmes [20].

Figure 2.4: The calcium-dependent dissociation rate of CaM* from a trapping subunit, calculated from an equation given in Holmes’ article [20].
2.4.4 Holmes’ results

The level of CaM trapping was found to be strongly dependent on the frequency of the input and the magnitude of the calcium signal. Total activity of CaMKII decayed slowly, and decay was further slowed if the activity was induced with a repeated tetanus rather than a single one. When NMDA receptor desensitization was included and intervals between tetani were short, the maximal activity and number of bound subunits were significantly altered.

Disallowing the binding of Ca to all CaMKII-CaM\textsubscript{Ca} and CaN-CaM\textsubscript{Ca} was shown to increase the total activation approximately twofold, compared to the "normal" case.

2.5 Kubota and Bower

2.5.1 Aim

Kubota and Bower \cite{25} aim to construct a detailed biophysical model based solely on the kinetics of purified enzymes and first show that the model replicates the frequency sensitivity of CaMKII as reported by De Koninck and Schulman, secondly investigate which the mechanisms responsible for this sensitivity are.

2.5.2 Input signal

The input signal is Ca\textsuperscript{2+} concentration, given directly.

2.5.3 The model

The model of Kubota and Bower \cite{25} includes Ca\textsuperscript{2+} binding to CaM, CaMKII activation by CaM*, CaMKII phosphorylation on Thr\textsuperscript{286} and subsequent CaM\textsuperscript{*} trapping, CaMKII autophosphorylation, CaMKII capping. The association rates of CaM from CaMKII were modeled as functions of [Ca\textsuperscript{2+}].

Ca\textsuperscript{2+} binding to CaM is modeled by the Adair-Klotz equation \cite{25} that considers the cooperation between sites:

\[
CaM^* = CaM_T \cdot \frac{0.2 \cdot Ca + 0.32 \cdot Ca^2 + 0.0336 \cdot Ca^3 + 0.00196 \cdot Ca^4}{4 + 0.8 \cdot Ca + 0.64 \cdot Ca^2 + 0.0448 \cdot Ca^3 + 0.00196 \cdot Ca^4}
\]

(2.2)

where CaM\textsuperscript{*} is CaM\textsubscript{Ca} concentration, CaM\textsubscript{T} is total CaM concentration and Ca is Ca concentration.
2.5. Kubota and Bower

In the model, the number of CaMKII subunits is four (to reduce model complexity, just as in Coomber’s model). CaMKII states are modeled deterministically, with terms of the kind \( P_{ij^1j^2j^3j^4}^{(i=0,1,2, j=0,1)} \), where the superscripts denote the phosphorylation state of the first through fourth subunit (\( i=0,1,2 \); 1 denotes phosphorylation on Thr\(^{286} \) and 2 denotes phosphorylation on Thr\(^{305/306} \)). The corresponding subscripts denote if CaM* is bound (=1) or not (=0). The equations are on the form

\[
\frac{d}{dt} P_{0001}^{0000} = k_{n1} \cdot P_{0001}^{0000} + k_{n1} \cdot P_{0100}^{0001} + k_{n1} \cdot P_{0010}^{0001} + k_{n10} \cdot P_{0001}^{0001} - (CaM^* \cdot k_{1} + CaM^* \cdot k_{1} + CaM^* \cdot k_{1} + CaM^* \cdot k_{10}) \cdot P_{0001}^{0000} - k_{3} \cdot P_{0000}^{0000}
\]

and configurations with the same rotational symmetry are contained in the same term. Interautophosphorylation is assumed to be unidirectional. Capped states are considered to be active. Dephosphorylation of CaMKII is assumed to be performed solely by PP1 and to follow the scheme

\[
PP1 + S_p \rightleftharpoons PP1S_p \rightarrow PP1 + S
\]

i.e. the Michaelis-Menten model, where \( S_p \) denotes phosphorylated CaMKII subunit and \( S \) denotes dephosphorylated CaMKII subunit.

2.5.4 Results of Kubota and Bower

Kubota and Bower found that their model was able to reproduce both the basal enzyme kinetics of CaMKII, in comparison with data from Brocke et al. [7], and the experimentally established frequency sensitivity of CaMKII as reported by De Koninck and Schulman [13].

Frequency sensitivity

Frequency sensitivity was simulated both in the absence of phosphatase, as in the experiment by De Koninck and Schulman, and with PP1. No qualitative difference was observed, but overall phosphorylation was slightly reduced in the presence of phosphatase. Frequency sensitivity could be modeled with as little as 2 subunits. Kubota and Bower also claim that the phenomenon “frequency sensitivity” should be split in two parts; transient and asymptotic frequency sensitivity. Transient frequency sensitivity is predicted to be an intrinsic property of CaMKII, while asymptotic frequency sensitivity is seen to depend on the interplay between CaMKII and phosphatase. See Kubota and Bower [25] for details.
2.6 Lundh

2.6.1 Aim

Lundh’s aim [30] is to study the Ca\textsuperscript{2+}-dependence of phosphorylation of synapsin I and its consequences for short-term facilitation.

2.6.2 Input signal

The input signal is Ca\textsuperscript{2+} influx governed by Hodgkin-Huxley dynamics.

2.6.3 The model

The model by Lundh [30] includes concentrations of synapsin I, cAMP, CaM and Ca\textsuperscript{2+}, and focuses on phosphorylation of synapsin I, not CaMKII activation. Some Ca\textsuperscript{2+} dynamics are included (a Ca\textsuperscript{2+} current and an ATP-driven Ca\textsuperscript{2+} pump, but not Ca\textsuperscript{2+} buffering). Adenylyl cyclase (AC), phosphodiesterase (PDE) and CaMKII are present as rate constants (i.e. for instance CaM trapping by CaMKII is not considered). Ca\textsuperscript{2+} binding to CaM is modeled as a four-step process. The cytosolic CaM concentration is 10 µM.

As the goal of this model was to study phosphorylation of synapsin and its consequences for short-term facilitation (rather than CaMKII activation), the results and the equations will not be included here. The interested reader is referred to Lundh [30]. However, it is noteworthy that Lundh is the only one except for Holmes who has an explicit four-step model of Ca\textsuperscript{2+} binding to CaM.

2.7 Okamoto and Ichikawa

2.7.1 Aim

The aim of Okamoto and Ichikawa [34] is to investigate the functional importance of CaMKII trapping of CaM\* in the neuronal structure (several neighbouring spines are modelled, as well as their mother dendrite).

2.7.2 Input signal

The input signal is Ca\textsuperscript{2+}, given directly for each spine.

2.7.3 The model

The model includes Ca\textsuperscript{2+}, Ca\textsuperscript{2+} binding to CaM and CaM\* binding to CaMKII, phosphorylation and dephosphorylation of CaMKII on Thr\textsuperscript{286/287}. Capping is not included. Buffering of Ca\textsuperscript{2+} and CaM\* (i.e. binding to other proteins than CaM or CaMKII, respectively) is included but not discussed. CaMKII is considered to have 10 subunits.
2.7. Okamoto and Ichikawa Models

Phosphorylation is modeled as

\[
K(n) \xrightarrow{G(n)} K(n+1)
\]

\[
G(n) = k_a \gamma^2 (N-n)(N-n-1) + k_b \gamma^2 n(N-n)
\]

\[
\gamma = \frac{[\text{Ca}^{2+}/\text{CaM-S}]}{\sum_{n=0}^{N}(N-n)[K(n)]}
\]  

(2.5)

where \(K(n)\) denotes CaMKII holoenzyme of \(N\) subunits, of which \(n\) are phosphorylated (and \(N-n\) are dephosphorylated). \([\text{Ca}^{2+}/\text{CaM-S}]\) denotes the concentration of dephosphorylated CaMKII subunit with bound CaM*.

Dephosphorylation is modeled as

\[
K(n) \xrightarrow{R(n)} K(n-1)
\]

\[
R(n) = \frac{nV_D}{K_D + \sum_{n=0}^{N} n[K(n)]}
\]  

(2.6)

This gives the following set of dynamical equations

\[
\frac{d}{dt}[K(n)] = G(n-1)K(n-1) - (G(n) + R(n))[K(n)] + R(n + 1)K(n + 1)
\]

\[
\frac{d}{dt}[\text{Ca}^{2+}/\text{CaM} - S] = -\sum_{n=0}^{N} (G(n) - R(n))[K(n)] + k_2[\text{Ca}^{2+}/\text{CaM}][S] - k_{-2}[\text{Ca}^{2+}/\text{CaM} - S]
\]

\[
\frac{d}{dt}E = \sum_{n=0}^{N} (G(n) - R(n))[K(n)] - k_s((E - [\text{Ca}^{2+}/\text{CaM} - S] - [\text{Ca}^{2+}/\text{CaM} - B]) - [\text{CaM}]_d)
\]

\[
\frac{d}{dt}[\text{CaM}]_d = -k_d([\text{CaM}]_d - (E - [\text{Ca}^{2+}/\text{CaM} - S] - [\text{Ca}^{2+}/\text{CaM} - B]))
\]

(2.7)
and algebraical equations

\[ E = [Ca^{2+}/CaM - S] + [Ca^{2+}/CaM - B] + [Ca^{2+}/CaM] + [CaM] \]

\[ [S] = \sum_{n=0}^{N} (N - n)[K(n)] - [Ca^{2+}/CaM - S] \]

\[ [Ca^{2+}/CaM] = \frac{-a + \sqrt{a^2 - 4b}}{2} \]

\[ a = [Ca^{2+}/CaM - S] + K_3 + C_B - \frac{[Ca^{2+}]^4E}{K_1^4 + [Ca^{2+}]^4} \]

\[ b = \left( \frac{[Ca^{2+}]^4E}{K_1^4 + [Ca^{2+}]^4} - [Ca^{2+}/CaM - S] \right)K_3 \]

\[ [Ca^{2+}/CaM - B] = \frac{[Ca^{2+}/CaM]C_B}{K_3 + [Ca^{2+}/CaM]} \]

where E is the total concentration of non-trapped CaM, B denotes other CaM-binding proteins and S denotes (unphosphorylated) CaMKII subunit.

Although it is not immediately apparent, the CaMKII part of this model is quite similar to the CaMKII part of Zhabotinsky’s model (see section 2.8).

### 2.7.4 Results of Okamoto and Ichikawa

The model by Okamoto and Ichikawa is the only model containing more than one spine, of the models I have studied. The model assumes diffusional transport of CaM between spines and the parent dendrite. Okamoto and Ichikawa show that CaMKII trapping of CaM* can lead to a ”winner-take-all” competition between spines for CaM, as trapping increases the affinity for CaM* and thereby promotes further trapping.

### 2.8 Zhabotinsky

#### 2.8.1 Aim

Zhabotinsky’s aim \[51\] is to investigate if the CaMKII-PP1 system can operate as a bistable switch, i.e. to investigate if CaMKII autophosphorylation can be bistable in a wide range of [Ca\(^{2+}\)] and whether such bistability can play a role in LTP.

#### 2.8.2 Input signal

The input signal consists of simple Ca\(^{2+}\) pulses given by an exponential equation with given maximal amplitude and time constant.
2.8. Zhabotinsky Models

2.8.3 The model

Zhabotinsky’s model \[51\] contains concentrations of CaMKII — unactivated or phosphorylated on from one to ten subunits, giving a total of eleven different CaMKII concentrations — PP1 and inhibitor-1. PKA and CaN are included as rate constants in some reactions (see below) and are thus treated as unlimited. CaM is implicitly included in the \( \text{Ca}^{2+} \) activation of CaMKII. The fraction of CaMKII subunits bound to \( \text{Ca}^{2+} / \text{CaM} \) is given by

\[
F = \frac{(\text{[Ca}^{2+}]/K_{H1})^4}{1 + ([\text{Ca}^{2+}]/K_{H1})^4}
\]

(2.9)

in contrast to Holmes, who treats all the \( \text{CaM} \alpha \chi \) steps explicitly. The per-subunit rates \( \nu_1 \) of the first phosphorylation step (which requires binding of \( \text{CaM}^* \) at two neighbouring sites) and \( \nu_2 \) of the following steps are, consequently:

\[
\nu_1 = 10k_1P_0 \cdot F^2
\]

(2.10)

\[
\nu_2 = k_1 \cdot F
\]

(2.11)

where \( P_0 \) is the concentration of unactivated (unphosphorylated) CaMKII.

2.8.4 Phosphorylation

Zhabotinsky claims that phosphorylated subunits are phosphorylated at random, resulting in a random distribution of phosphorylated and unphosphorylated subunits in the holoenzyme, and assumes that all distinguishable configurations of subunits in the holoenzyme with a given number of phosphorylated subunits exist with equal probabilities. The effective number of autophosphorylating pairs, \( w \) is given by

\[
w_i = w_{10-i} = \frac{\sum^i j m_j}{\sum m_j}
\]

(2.12)
and $\nu_2$ is multiplied with the corresponding $w$ to give the total rate of phosphorylation, $V_i = \nu_3 w_i P_i$:

$$
\begin{align*}
\frac{dP_0}{dt} &= -\nu_1 + \nu_3 P_1 \\
\frac{dP_1}{dt} &= \nu_1 - \nu_3 P_1 - \nu_2 + 2\nu_3 P_2 \\
\frac{dP_2}{dt} &= \nu_2 P_1 - 2\nu_3 P_2 - 1.8\nu_2 P_2 + 3\nu_3 P_3 \\
\frac{dP_3}{dt} &= 1.8\nu_2 P_2 - 3\nu_3 P_3 - 2.3\nu_2 P_3 + 4\nu_3 P_4 \\
\frac{dP_4}{dt} &= 2.3\nu_2 P_3 - 4\nu_3 P_4 - 2.7\nu_2 P_4 + 5\nu_3 P_5 \\
\frac{dP_5}{dt} &= 2.7\nu_2 P_4 - 5\nu_3 P_5 - 2.8\nu_2 P_5 + 6\nu_3 P_6 \\
\frac{dP_6}{dt} &= 2.8\nu_2 P_5 - 6\nu_3 P_6 - 2.7\nu_2 P_6 + 7\nu_3 P_7 \\
\frac{dP_7}{dt} &= 2.7\nu_2 P_6 - 7\nu_3 P_7 - 2.3\nu_2 P_7 + 8\nu_3 P_8 \\
\frac{dP_8}{dt} &= 2.3\nu_2 P_7 - 8\nu_3 P_8 - 1.8\nu_2 P_8 + 9\nu_3 P_9 \\
\frac{dP_9}{dt} &= 1.8\nu_2 P_8 - 9\nu_3 P_9 - \nu_2 P_9 + 10\nu_3 P_{10} \\
\frac{dP_{10}}{dt} &= \nu_2 P_9 - \nu_3 10 P_{10}
\end{align*}
$$

(2.13)

where $P_i$ is the concentration of holoenzymes with $i$ phosphorylated subunits, $\nu_3$ is the rate of dephosphorylation, and the total rate of dephosphorylation of holoenzymes with $i$ phosphorylated subunits is $V_{-i} = \nu_3 i P_i$.

### 2.8.5 Dephosphorylation

PP1 is deactivated by binding to phosphorylated inhibitor-1. The per-subunit rate of dephosphorylation is given by

$$
\nu_3 = \frac{k_2 e_p}{K_M + \sum_{i=1}^{10} i P_i}
$$

(2.14)

where $e_p$ is the concentration of PP1 not bound to phosphorylated inhibitor-1 (I1P). Inhibitor-1 is phosphorylated by PKA and dephosphorylated by activated CaN. Zhabotinsky has CaN activation modeled as a Hill equation with $n_H = 3$ (opposed to Holmes who has the whole range of CaNCaM to CaNCaMCa4 but who does not really use CaN for anything). The interaction between PP1 and
inhibitor-1 is expressed as:
\[
\frac{de_p}{dt} = -k_3Ie_p + k_4(e_{p0} - e_p) \\
\frac{dI}{dt} = -k_3Ie_p + k_4(e_{p0} - e_p) + \nu_{PKA}I_0 - \nu_{CaN}(\frac{[Ca^{2+}]/K_{H2}}{33})^3I
\] (2.15)

where \(e_{p0}\) is the total concentration of PP1, \(I\) is the concentration of free \(I\)P and \(I_0\) is the concentration of free inhibitor-1 which is treated as constant (which means that \(I\) may well exceed \(I_0\)).

### 2.8.6 Zhabotinsky’s results

Zhabotinsky shows that, if the total concentration of CaMKII subunits is significantly higher than the phosphatase Michaelis constant, two stable steady states of CaMKII autophosphorylation can be found over a wide range of intracellular \(Ca^{2+}\) concentrations. Also, he finds that the levels of CaMKII necessary to produce this bistability are in the same range as the values found in the postsynaptic density.

However, Zhabotinsky’s model does not include diffusion or CaM binding dynamics and he treats the supply of CaM as unlimited. Several other authors have pointed out that the level of CaM might be important, see Okamoto and Ichikawa [34] and further discussion in section 5.4.

### 2.9 Lisman and Zhabotinsky

The Lisman-Zhabotinsky model [29] is built on Zhabotinsky’s model (see the previous section), no relevant new simulations are performed.

#### 2.9.1 Aim

The aim of Lisman and Zhabotinsky is to use Zhabotinsky’s model as a framework for a hypothesis of the mechanism of AMPAR addition to the membrane.

#### 2.9.2 Input signal

The input signal is the same as in Zhabotinsky’s model (in the previous section, section 2.8).

#### 2.9.3 The model

It is proposed that phosphorylated CaMKII enhances transmission through an assembly process that leads to addition of AMPARs to the membrane. CaMKII is thought to be dephosphorylated only by PP1, and only the PP1 that is held in the PSD by scaffolding proteins. This restriction means that the relevant
biochemical compartment is the volume of the PSD within which the concentration of CaMKII subunits is 100-200 µM [29] (this is roughly equivalent to Holmes – see section 2.4 – who has CaMKII restricted to the outer 50 nm of the spinehead, equivalent to a concentration of CaMKII subunits of 110 µM). This would make it easier to saturate the local phosphatase pool and thus make it easier to keep CaMKII in an activated state even at basal [Ca$^{2+}$].

### 2.9.4 AMPAR addition to the membrane

Phosphorylated CaMKII is known to bind to the NMDA receptor and α-actinin (an actin-binding protein) binds to α-CaMKII. Another actin-binding protein, called protein 4.1, is known to be in a complex with the protein SAP97. Both are major binding partners for the GluR1 subunits of AMPARs and form sites where AMPARs can anchor (see Lisman and Zhabotinsky [29] and references therein). The key link, proposed by Lisman and Zhabotinsky, is that actin filaments could bind to α-actinin and form a binding site for the protein 4.1, allowing actin filaments to link the NMDAR-CAMKII-α-actinin complex to the AMPAR-SAP97-4.1 complex. Lisman and Zhabotinsky [29] also list some experimental evidence for this link.

**About the role of AMPAR insertion**

Benke et al. [4] found that the increase in AMPAR single channel conductance resulting from phosphorylation could be sufficient to explain the enhanced AMPAR efficacy, without the need for AMPAR insertion, and that if new-inserted AMPARs exist, they have to be in the high-conductance state [4], [14].
Chapter 3

Comparison of models

In this chapter I will discuss some important general model features and relate these to the studied models.

3.1 Ca\(^{2+}\) and CaM dynamics

Surprisingly few models contain a multi-step binding scheme for Ca\(^{2+}\) to CaM. Since binding dynamics of CaM surely will influence transient CaMKII activation, at least LFS and/or low-amplitude Ca\(^{2+}\) signals should not be modeled without full CaM dynamics. However, the rate constants that are used in Holmes’ model are strongly deviant from the corresponding value used in the Hill equation (\(K_{Hill}\) is \(\sim 9\) times larger than the equivalent value in Holmes model, see below) and the values reported by Vetter and Leclerc [48], see section 1.6. An example of CaM dynamics (rate constants were taken from Holmes [20]) with a 4Hz Ca\(^{2+}\) signal is given in figure 3.1. The Ca\(^{2+}\) signal in all simulations presented in this chapter is a square pulse with a duration of 200 ms and an amplitude of 1 \(\mu\)M.

The dynamics of Holmes’ four-step CaM model will also be considerably altered by the presence of CaM-binding proteins. CaM dynamics with the same conditions as in figure 3.1 but with CaN included, are plotted in figures 3.2 (3-fold more CaN than CaM) and 3.3 (3-fold less CaN than CaM). Notice especially the interesting difference in the dynamics of CaM-Ca\(^{3+}\) when the CaN concentration is altered (a close-up is given in figure 3.4). For reference, the concentration of CaM-Ca\(^{4+}\) with the same 4Hz signal when one uses simplified binding (the Hill equation or the Adair-Klotz equation) is given in figure 3.5. The Adair-Klotz equation takes cooperativity of Ca\(^{2+}\) binding to CaM into consideration but still gives the same kind of simplified dynamics as the Hill equation. Note the relatively large difference (\(\sim 5\)-fold) between the Hill equation and the Adair-Klotz equation.
3.1. Ca\(^{2+}\) and CaM dynamics

Comparison of models

![Graphs showing dynamics of CaMCa states](image)

Figure 3.1: The dynamics of all CaMCa\(_x\) states with a 4Hz Ca\(^{2+}\) signal of 1 \(\mu\)M Ca\(^{2+}\) in 200 ms. Total CaM concentration was 10 \(\mu\)M. All rate constants were taken from Holmes [20].

As is evident from the previous figures (3.1-3.4), the presence of CaM-binding protein alters both shape and amplitude of the resulting CaMCa\(_x\) concentrations in Holmes' model. The frequency of the input also has some influence: in figure 3.6 a comparison is given between CaMCa\(_x\) concentrations in the presence of much and little CaN (30 \(\mu\)M and 3.3 \(\mu\)M respectively, in comparison to 10 \(\mu\)M of CaM) with the same Ca\(^{2+}\) signal but a frequency of 1 Hz.

There are some experimental arguments for and against the usefulness of the Hill equation: Burgoyne et al. [8] found, in measurements of CaM behaviour at different [Ca\(^{2+}\)], that CaM bound no distinguishable amount of Ca\(^{2+}\) below Ca\(^{2+}\) concentrations of \(\sim 200\) nM (i.e. no Ca\(^{2+}\) is bound at resting levels, so there should not be any CaM* present at all, contrary to the Hill equation approach), and that the half-maximal occupancy occurred at \(\sim 1\) \(\mu\)M. This is in accordance with the value commonly used, but \(\sim 9\) times larger than the resulting value of \((\prod_{i=1}^{4} \frac{k_{b_i}}{k_{f_i}})^{1/4}\) for Holmes' model (the exact value is 8.8). Burgoyne et al. also calculate that in order to have complete loading of CaM by Ca\(^{2+}\) within 1 ms, [Ca\(^{2+}\)] has to rise above 10 \(\mu\)M. Thus, the lower [Ca\(^{2+}\)], the less rapid is the loading of CaM by Ca\(^{2+}\). If substantial "integration" of Ca\(^{2+}\) by CaM (section 1.6.1) is evident, simplified binding will likewise not account for this.

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3.1. Ca$^{2+}$ and CaM dynamics

Comparison of models

3.1.1 A simple test of correction of Holmes’ CaM constants

The simplest way to examine the effects of the difference of the Ca$^{2+}$-CaM steps is to multiply the forward rate constants with the fourth root of the difference, which is approximately 8.8. The corresponding result to figure 3.1 is shown in figure 3.7. Note that the difference between CaMCA$_4$ in Holmes’ model and CaMCA$_4$ from the Hill equation is much smaller (but the level of CaMCA$_4$ is still 5-fold lower than with the Hill equation) and that the agreement with the Adair-Klotz equation is rather good. Also, the general "shape" of the CaMCA$_4$-curve is more "Hill-like" than with Holmes’ original model.

To continue the comparison, also the forward rate constants for Ca$^{2+}$ binding to CaN-CaMCA$_x$ were multiplied with the same correcting factor (to satisfy the demand for thermodynamic equilibrium) and simulations corresponding to figures 3.2 and 3.3 were performed. The results are shown in figures 3.8 and 3.9 respectively. It can be seen that the differences between the states with more and less CaM-binding proteins are more pronounced, but that the shape of the curve in the four-step case still is approximately like that of the Hill equation. Note that the level of available CaM is drastically lowered in the case of limiting CaM. The behaviours of the other CaMCA$_x$ are also altered, but this is hard

Figure 3.2: The dynamics of all CaMCA$_x$ states with a 4Hz Ca$^{2+}$ signal of 1 µM Ca$^{2+}$ in 200 ms, in the presence of a CaM-binding protein (CaN). Total CaM concentration was 10 µM and total CaN concentration was 30 µM. All rate constants were taken from Holmes [20], thus all CaMCA$_x$ can bind to CaN.

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3.1. Ca\textsuperscript{2+} and CaM dynamics

Comparison of models

Figure 3.3: The dynamics of all CaMCA\textsubscript{x} states with a 4Hz Ca\textsuperscript{2+} signal of 1 µM Ca\textsuperscript{2+} in 200 ms, in the presence of a CaM-binding protein (CaN). Total CaM concentration was 10 µM and total CaN concentration was 3.3 µM. All rate constants were taken from Holmes [20], thus all CaMCA\textsubscript{x} can bind to CaN.

Figure 3.4: Close-up of [CaMCA\textsubscript{3}] from the previous figure
Figure 3.5: *The concentration of CaM-Ca* given by the Hill equation (solid lines) and the Adair-Klotz equation (dotted lines), with the same conditions as in the previous two figures.*

...to interpret since there will be effects from Holmes’ assumption that CaN can bind all CaM-Ca$_x$.
3.2 Trapping of CaM* and capping of CaMKII

It has been speculated that trapping of CaM* has the functional role of depriving the other CaM-dependent pathways of CaM. Thus, trapping can for instance lower the level of PP1 activity by preventing activation of CaN (that dephosphorylates the PP1 inhibitor I1P). Trapping also seems to protect CaMKII subunits from capping. Capping, on the other hand, prevents previously autonomous subunits from reuptake of CaM* (i.e. ensures that CaM* becomes available to the other CaM pathways). If any other CaM-dependent reactions than CaMKII activation are included in a model (for instance PP1 regulation), then both trapping and capping should be included. None of the studied models includes both capping and full PP1 regulation. I consider the lack of all CaMKII activation states to be one of the most important deficiencies of the Zhabotinsky model.

3.3 Feature span

Since Ca^{2+} is involved in so many cellular processes, it is hard to cut out a number of pathways and claim that they are a self-sufficient system. Nevertheless, I think most of the studied models contain too few processes. If one considers CaMKII and PP1 to be a system, one should also add the PP1 regulation pathway that competes with CaMKII both for CaM* and for the level of Ca^{2+}.
3.3. Feature span Comparison of models

Figure 3.7: The dynamics of all CaMCa$_x$ states with a 4Hz Ca$^{2+}$ signal of 1 µM Ca$^{2+}$ in 200 ms. Total CaM concentration was 10 µM. All rate constants were taken from [20] and the forward rate constants for Ca$^{2+}$ binding to CaM were multiplied with 8.8.

phosphorylation. None of the implemented models are complete from this point of view, but the Zhabotinsky model comes closest.
Figure 3.8: The dynamics of all CaMCax states with a 4Hz Ca\(^{2+}\) signal of 1 µM Ca\(^{2+}\) in 200 ms. Total CaM concentration was 10 µM and total CaN concentration was 30 µM. All rate constants were taken from [20] and the forward rate constants for Ca\(^{2+}\) binding to bound and unbound CaM were multiplied with 8.8.
3.4 Implementation

I have found that in general, published models are hard to implement since important rate constants often are missing, or units are not given. Simple de-
3.5. A simple test

Comparison of models

terministic models like Zhabotinsky’s model and the model of Dupont et al. also are much easier to implement and solve than Holmes’ model where communication between the deterministic and stochastic parts is quite extensive. Multi-configuration models like Coomber’s or Kubota and Bower’s generate a large number of equations which would make them hard to implement (and then the number of subunits is still much lower than in reality) as well as harder to handle and interpret. Although, as the most interesting phenomena often demand long simulations, simple methods might be the only ones that are useful if the more complex models demand too much computational resources.

From this point of view I prefer Holmes’ model of the three I’ve implemented, since it is the least simplified but still complex enough to feel realistic. Given the large number of equations in the other quite realistic models by Coomber or Kubota and Bower, it might also be the easiest to use of the more realistic models - especially if one wants to have a model with the full number of CaMKII subunits (where configurational modelling becomes practically impossible - with 10 subunits and 12 possible states, as in Coomber’s model, \( \sim 10^{12} \) equations would be needed).

3.5 A simple test

To examine the absolute difference between the models, I have run simulations with the same \( \text{Ca}^{2+} \) signal but the respective "original" values of CaMKII etc given in the articles. The \( \text{Ca}^{2+} \) signal is given in figure 3.10 and the resulting activities (in percent of respective maximal activity) are given in figure 3.11. The values of all active states in Holmes’ model and Dupont et al.’s model were added without weighting.

One can see that Holmes’ model and Zhabotinsky’s model are quite alike, but the Dupont model has much less activity. This result is not surprising if it is compared to the frequency sensitivity tests in the next chapter: the only model with much dissociation of CaM* is Dupont’s model.
3.5. A simple test

Comparison of models

Figure 3.10: The Ca$^{2+}$ signal

Figure 3.11: The respective activities obtained with the different models. Dupont: 1 µM CaMKII, 0.1 µM CaM. Holmes: 100 CaMKII molecules, 100 µM CaM (and 286 CaN molecules). Zhabotinsky: 20 µM CaMKII, 0.05 µM PP1, 0.1 µM II
3.5. A simple test

Comparison of models
Chapter 4

Comparisons with experimental data

In this chapter, results from simulations with the models of Dupont et al. [16], Holmes [20] and Zhabotinsky [51] are reported. Comparisons with experimental results from Bradshaw et al. [6] and De Koninck and Schulman [13] are made.

4.1 Dupont et al

4.1.1 Implementation

I implemented this model in Matlab 6.5. A fourth-order Runge-Kutta method was used to integrate the equations. The stability varied considerably with Ca concentration (a thousandfold decrease in the timestep length was required when the Ca concentration was increased from 5 to 50 $\mu$M).

4.1.2 Comparison with experimental data

The Dupont model was explicitly developed to embody the frequency sensitivity characteristics reported by De Koninck and Schulman [13]. A thorough comparison with these data can be found in [16]. I have tested to run the model with the same 1 Hz and 4 Hz stimuli described in the article (which was also used by De Koninck and Schulman). The resulting frequency sensitivity is clearly evident (see figure 4.1).

I thought it would be interesting to examine if the model’s behaviour agreed also with data from Bradshaw et al. [6], so I made steady-state simulations with 50 $\mu$M CaM, varying Ca$^{2+}$ concentrations and 1 $\mu$M CaMKII. All relative activities were considered equal to 1. Values of constants are given in the figure legend of figure 4.2. If one assumes that the ”phosphorylated state” $W_P$ is equal to the ”autonomy” measured by Bradshaw et al., the agreement is rather
4.2 Holmes

4.2.1 Implementation

The whole model (complete dentate granule cell) was not implemented, due to the demands on computational resources. Instead the implemented volume was chosen to correspond to the outermost compartment in the spine head in Holmes’ model (which is the only compartment containing CaMKII) and concentrations of CaMKII and CaN (which are given as “number of molecules” in Holmes’ article) were computed. MATLAB was used for the implementation. Just as in Holmes’ model [20], one deterministic and one stochastic part was modeled. A fourth-order Runge-Kutta method was used and the timestep was 1 µs.

4.2.2 Comparison with experimental data

I have compared the result of steady state simulations of Holmes’ model with experimental results from Bradshaw et al. [6]. They examine the Ca$^{2+}$ dependence of CaMKII autophosphorylation.
4.2. Holmes Comparisons with experimental data

I have performed steady state simulations of Holmes’ model with Ca$^{2+}$ concentrations ranging from 0.1 to 50 µM, with 100 µM CaM and 10.97 µM CaMKII (which is equivalent to 100 10-subunit CaMKII molecules with the volume equal to the volume of the outermost compartment Holmes uses). The closest corresponding values in Bradshaw et al. [6] are 50 µM CaM and 5 µM CaMKII. Bradshaw et al. recorded their results after 5 minutes, so I ran the simulations for the same time. Regrettably, I do not have the resources to run multiple simulations and average the results; the results presented below are from single simulations.

Bradshaw et al. performed their experiments at 0°C to avoid capping, while Holmes’ model includes capping. Thus, I modified the model so that it no longer included capping. No other rate constants were adjusted.

For comparison with the experimental values I use the equation

\[ A = A_{\text{max}} [\text{Ca}^{2+}]^{n_H} / ([\text{Ca}^{2+}]^{n_H} + \text{Ca}_{1/2}^{2+ n_H}) \]  

(4.1)
4.2. Holmes Comparisons with experimental data

from Bradshaw et al. [6], where $A$ is percent autonomy and $A_{max}$ is maximum autonomy, with their reported values of $n_H$ (the Hill coefficient) and $Ca^{2+}_{1/2}$ (the half-maximal $Ca^{2+}$ value).

The result shows clear differences between the behaviour of the model and the behaviour of CaMKII: the autonomy response in ‘real life’ is more graded while the simulation results are almost all-or-none. See figure 4.3. And more important: the number of autonomous subunits actually decreases with increasing $[Ca^{2+}]$ (see figure 4.4). This is probably due to the rebinding of CaM$Ca_4$ to autonomous subunits (which gives a transition back to the trapped state). This mechanism, together with the drastic decrease in the off rate of CaM from trapping subunits, produces near total trapping at high $[Ca^{2+}]$.

![Figure 4.3: CaMKII autophosphorylation comparison between simulation results with Holmes’ model and experimental results from Bradshaw et al. Holmes’ model: 100 $\mu$M CaM and 100 CaMKII molecules (equivalent to 10.97 $\mu$M). Bradshaw et al: 50 $\mu$M CaM and 5 $\mu$M CaMKII.](image)

However, if one compares the total amount of trapping with the experimental values, the agreement is not worse than that of the other models (figure 4.5).

If capping is included, the autonomous state in this model gets the character of "transition state": there can be much trapping and successively much capping, but there is never much autonomy. See figure 4.6. The model exhibits some frequency sensitivity, but nowhere near as much as the Dupont model. See figures 4.7 and 4.8.
4.3 Zhabotinsky

4.3.1 Implementation

The full model was implemented in MATLAB. A fourth-order Runge-Kutta method was used and the timestep was 1 µs.

4.3.2 Comparison with experimental data

Bradshaw et al

A comparison of simulation data from Zhabotinsky’s model with experimental data from Bradshaw et al. [6] is given in figure 4.9. Concentrations: CaMKII=1 µM, PP1=1.25 µM. Experimental values were \( n_H = 8.6 \pm 2.5 \) (Hill number) and \( C_{a_h} = 3.1 \pm 0.6 \) (half-maximal Ca-value). Simulation results correspond to \( C_{a_h} = 4.55 \) and \( n_H \approx 6.1 \).

De Koninck & Schulman

It has been proposed that limiting CaM and trapping of CaM is important for the ability of CaMKII to function as a frequency detector. Therefore, it is interesting to see if Zhabotinsky’s model, that has a very simplified CaMKII activation and an unlimited supply of CaM, exhibits any frequency sensitivity. There are, to my knowledge, no experiments on frequency sensitivity in the

Figure 4.4: Fraction of the respective maximum activity of trapped and autonomous subunits during the simulations (Holmes’ model). Note the unrealistic decrease in the number of autonomous subunits with increasing \([Ca^{2+}]\).
4.3. Zhabotinsky Comparisons with experimental data

Figure 4.5: Total amount of trapped subunits (Holmes’ model) during the simulations, compared to the experimental ‘percent max. autonomy’ of Bradshaw et al. Experimental values were chosen within the boundaries of the standard deviation to give as good agreement as possible ([Ca]_{50}=1.3, n_H=5.8).

presence of PP1, but the model by Kubota and Bower exhibited a frequency sensitivity that only changed a little when PP1 was added.

The Zhabotinsky model gives yet another opportunity: to investigate if and how the frequency sensitivity is changed when inhibition of PP1 is added. Figures 4.10 and 4.11 show the frequency sensitivity with I1. There is practically no difference between the two cases, i.e. frequency sensitivity with and without I1, at least not at the relatively low concentrations I have examined (data not shown).
4.3. Zhabotinsky Comparisons with experimental data

Figure 4.6: CaMKII activation in Holmes’ model, split into bound, trapped, autonomous and capped. The Ca$^{2+}$ signal was 50 µM continuously in 200 seconds. The transition character of the autonomous state is evident.

Figure 4.7: CaMKII activation in Holmes’ model, split into bound, trapped, autonomous and capped. The Ca$^{2+}$ signal was pulses of 50 µM for 200ms, at a frequency of 1 Hz for a total duration of 106 s. 100 µM CaM and 100 CaMKII molecules, no CaN.
4.3. Zhabotinsky Comparisons with experimental data

Figure 4.8: CaMKII activation in Holmes’ model, split into bound, trapped, autonomous and capped. The Ca\(^{2+}\) signal was pulses of 50 µM for 200ms, at a frequency of 4 Hz for a total duration of 30 s. 100 µM CaM and 100 CaMKII molecules, no CaN.

Figure 4.9: Comparison of steady-state phosphorylation of Zhabotinsky’s model with experimental data from Bradshaw et al. Concentrations: CaMKII=1 µM, PP1=1.25 µM. Experimental values were \(n_H = 8.6 \pm 2.5\) (Hill number) and \(Ca_h = 3.1 \pm 0.6\) (half-maximal Ca-value). Simulation results correspond to \(Ca_h = 4.55\) and \(n_H \approx 6.1\).
4.3. Zhabotinsky Comparisons with experimental data

Figure 4.10: Frequency sensitivity of Zhabotinsky’s model with I1 regulation of PP1 activity. Only the concentration of fully active CaMKII is shown (1 Hz and 4 Hz pulses of 50 μM Ca\(^{2+}\), 20 μM CaMKII, 0.05 μM PP1 and 0.1 μM I1).

Figure 4.11: Frequency sensitivity of Zhabotinsky’s model with I1 regulation of PP1 activity. The total weighted sum of CaMKII activity is shown, in percent of maximal activity (1 Hz and 4 Hz pulses of 50 μM Ca\(^{2+}\), 20 μM CaMKII, 0.05 μM PP1 and 0.1 μM I1).
4.3. Zhabotinsky Comparisons with experimental data
Chapter 5

Model assumptions revisited

This chapter contains specific discussion on model features, assumptions and their consequences. A general concluding discussion on modelling of CaMKII activation can be found in chapter 6.

5.1 The well-stirred cell assumption

Assuming that all involved species are equally distributed in the modeled volume (i.e. that there are no spatial variations in concentration) is referred to as the well-stirred cell assumption. This means that diffusion-related effects are neglected, as well as spatially related differences in concentration. The validity of the assumption differs from situation to situation, but in situations where diffusion is important (for instance in larger volumes), the assumption is generally not valid, likewise when the number of molecules is small.

One way to model processes where diffusion is included is to use compartmental modelling, where the simulated volume is divided into relevant subvolumes and the diffusional exchange between neighbouring subvolumes is computed for each timestep (A relevant subvolume could in the current context be the PSD, where among others CaMKII, NMDARs and AMPARs are anchored). A general discussion of modelling of complex biological systems can be found in Weng et al. [49].

5.2 Simplified Ca\textsuperscript{2+} binding to CaM

Holmes is the only one who includes all CaM-Ca binding steps in a CaMKII activation model (he even goes as far as allowing all different CaM\textsubscript{CaX} \textsubscript{X=0,...,4} to bind to both CaMKII and CaN). The standard way otherwise seems to be
5.3 Diffusion of CaM and CaM*  

Model assumptions revisited

to assume that the binding of Ca$^{2+}$ to CaM is always at equilibrium, and use a simplified binding scheme where

$$CaMCa_4 = CaM_{Tot} \cdot \frac{Ca^4}{Ca^4 + [Ca^{2+}]_{1/2}}$$  \hspace{1cm} (5.1)

This is the Hill equation, with $n_H=4$.

This does not completely reproduce CaM dynamics (see sections 1.6 and 3.1); the simulated concentration and dynamics of CaM* will precisely follow those of the Ca$^{2+}$ signal, as shown in the previous chapter, section 3.1. In steady-state simulations this might not be so important but otherwise it will have effects on for instance the level of trapping.

5.3 Diffusion of CaM and CaM*

In cells, almost all CaM* is bound. The Holmes model includes diffusion of CaM*, and CaM diffusion between spines is the key assumption in the Okamoto-Ichikawa model. As even Ca$^{2+}$ is safely sequestered in the spine (at least for $\sim 1$ s [38]), how valid are these assumptions?

5.3.1 Diffusion of CaM

According to Kim et al. [23], diffusion of CaM is slowed down by a factor of 2.2 when $[Ca^{2+}]$ rises above resting levels (giving a $\tau_D$ of 1.6 ms as opposed to $\tau_D=0.73$ ms at resting levels), and at least part of this slowing is due to interactions with CaMKII.

These experiments were carried out in intact cells (HEK-293 type) with a concentration of CaMKII in the nanomolar range. Given the large number of calmodulin-binding species in the spine, and the fact that especially CaMKII is present in much larger concentration ($\sim 10$ µM in the PSD) than in the experiment, there would likely be a much more pronounced slowing effect on CaM diffusion when Ca$^{2+}$ levels rise. Thus, it is questionable if CaM diffusion between spines in reality is fast enough to allow the kind of winner-take-all competition between spines for CaM that Okamoto and Ichikawa describe.

5.3.2 Diffusion of CaM*

According to Persechini and Stemmer [36], the mean distance of diffusion for CaMCa$_X$, before it loses all bound Ca$^{2+}$, is $\sim 0.1$ µm in presence of excess targets. Diffusion of CaMCa$_X$ can therefore not be an important Ca$^{2+}$-bearing process.
5.4. CaM levels

The short diffusion distance of CaM* means that there can be – depending on the distribution of CaM and targets of CaM – transient local elevations of CaM* (also depending on if only CaM-Ca4 or all CaM-Cax are bound by targets). Different pathways of CaM* can then, at least indirectly, influence each other.

5.4 CaM levels

Persechini and Cronk [35] have examined the relation between concentrations of Ca2+ and CaM* in intact human embryonic kidney 293 (HEK-293) cells. They found that the concentration of CaM-binding proteins was ~2-fold higher than the concentration of CaM, and that the maximal level of CaM* was 45 nM. HEK 293 cells have been found to be considerably similar to neurons and cells of neuronal lineage [39] so these results may be relevant also for neurons. Also, the level of Ca2+ required to produce half-maximal concentration of free CaM* was 20-fold less in the cell than the concentration required to half-saturate pure CaM in vitro, and below a [Ca2+] of 0.2 µM there was no evident CaM activation [35], [8].

5.4.1 CaM distribution in the spine

As is illustrated by the results of Okamoto and Ichikawa [34], CaM will most likely not be evenly distributed in the cell. Instead, the concentration of CaM will be highest where the binding of CaM is most efficient (i.e. where the most CaM is bound compared to the amount of CaM that is released).

5.4.2 Limiting CaM

According to Persechini and Stemmer [36], CaM concentration in the cell is limiting, i.e., CaM concentration is significantly below the total concentration of its targets. This would make the assumption in the Zhabotinsky model [51], that the CaM concentration is unlimited and constant, improper. The Zhabotinsky model, though, better captures the experimental behaviour of the CaMKII-PP1 system (examined in [6]) but on the other hand, in that experiment there are no competing CaM pathways or targets. Persechini and Stemmer also found that the behaviours of CaM binding systems with and without excess CaM can be qualitatively different.

If the CaM concentration is limiting, CaM trapping provides a mechanism to detect the frequency of calcium spikes [11]. Limiting CaM also means that the \( \alpha/\beta \) ratio of CaMKII heteromers becomes important, since autophosphorylation is dependent on CaM affinity. Intersubunit phosphorylation of \( \beta \)-CaMKII then can become dependent on CaM* binding to its lower-affinity \( \alpha \)-CaMKII neighbours and vice versa (see [7] for further discussion). Also, the as yet unresolved issue of uni- or bidirectional intersubunit phosphorylation might not be as triv-
ial as it has been thought to be (by, among others, Holmes [20]) if the CaM affinity of neighbours is different and the neighbours are unevenly distributed.

5.5 Modelling CaMKII configurations

Given that a CaMKII molecule consists of 8-12 subunits of at least two possible isoforms and that each of these subunits will be in one of five different states, the number of possible configurations to model is somewhat daunting from a computational point of view. Mainly, there are two ways to choose between: stochastic (using, for instance, a matrix with numbers indicating the state of each subunit) or deterministic (using one concentration for each configuration included in the model).

5.5.1 Simplifications

Often, modellers prefer a deterministic approach and this will cause the model to become increasingly complex (and computationally heavy) with each state included in the model. The most obvious simplification is to take advantage of the fact that the CaMKII molecule is rotationally symmetric and adjust the rate constants for each configuration. Also, the existence of isoforms is usually ignored (see discussion in section 5.7). Another simplification is to assume that the number of CaMKII subunits is constant (often, it is assumed to be equal to 10), and yet another, used by for instance Coomber [11] and Kubota and Bower [25], is to reduce the number of subunits. Models have also been simplified by using one “activated” CaMKII state instead of several different states (for instance Zhabotinsky [51]).

5.6 CaMKII capping

Only some models ([11], [20], [25]) include CaMKII capping, phosphorylation on Thr\(^{305/306}\) that inhibits CaM\(^*\) binding and leads to an autonomous form with decreased [26] or inhibited [11] activity (according to Kubota and Bower [25] capped subunits are active, but they only cite unpublished data). There seems to be no general agreement on how active capped subunits are, or the importance of capping. Based on Coomber's finding, that prior LFS could prevent LTP induction by HFS by giving rise to substantial amounts of capping, capping probably should be included in a model that studies both LTP and LTD.

5.6.1 The role of capping in frequency detection

Coomber claims that capping promotes the ability of CaMKII to act as a frequency detector. The reason is that the large time constant of CaM\(^*\) dissociation from CaMKII prevents the discrimination of more fine-grained LFS, and that capping would allow synaptic activity to be encoded as a balance between active and inhibited subunits.
5.6.2 The maximal level of capping

The maximal possible level of capping can be influenced by the assumed phosphorylation direction(s). If one assumes, as Coomber does, that capping is due to phosphorylation (on Thr$_{305/306}$) by a neighbouring active (non-capped) subunit, this clearly places a geometric constraint on the maximal level of capping: every subunit that becomes inhibited must have an active neighbour. As phosphorylation is random, for a ten-subunit enzyme, the minimum maximal level of capping is 60% if bidirectional phosphorylation is allowed, or 50% if phosphorylation is unidirectional. If phosphorylation occurred in a completely orderly fashion, where subunit nr 2 phosphorylates subunit nr 1 and then itself gets phosphorylated by subunit nr 3 and so on, the maximal levels would be 100 or 90%, respectively. Both of these cases are naturally unlikely; the maximal observed level will probably lie between these two extremes.

5.7 CaMKII isoforms behave differently

The properties of α-CaMKII homomers have generally been much more studied than the properties of β homomers or α/β heteromers. The result of this is that many models might incorporate only α-CaMKII characteristics which do not fully correspond to CaMKII behaviour in real life.

As can be seen in section 1.7.1, α-CaMKII and β-CaMKII have rather different affinities for CaM, which means that all CaMKII molecules will not have exactly the same behaviour towards CaM and that these differences can be rather large. Also, the α/β ratio determines how fast translocation to the PSD occurs. α/β ratios differ between parts of the brain; for example in rat forebrain, CaMKII is present in α-CaMKII homomers and α/β heteromers in the ratio 2:1 (~50% α homomers and 50% α/β heteromers [7]).

5.8 Dual CaN regulation

As mentioned in section 1.7.2, calcineurin is regulated both by Ca$^{2+}$ and CaM*. The regulatory subunit of CaN, Calcineurin B, can bind up to four Ca$^{2+}$ ions directly, and binding of Ca$^{2+}$ partly stimulates CaN (to ~10% of its maximal phosphatase activity [42]). This fact is not included in any model I have studied so far. Partial stimulation could be expected to have some effect on PP1 activity when CaM* is limiting during high levels of Ca$^{2+}$, for instance when CaMKII trapping of CaM is substantial.
5.9 "Constant" and/or "unlimited"?

5.9.1 CaM

As mentioned before, CaM is probably limiting in the cell. It is unclear if diffusion of CaM can be so efficient that CaM can be counted as unlimited in the dendritic spine, but recalling the number of CaM-binding species in the cell, it seems implausible. Limiting CaM also provides the CaM-CaMKII pathway with qualities that are considered to be crucial or at least very important, and enables crosstalk (through competition for CaM) between the CaMKII pathway and other pathways. Thus, a realistic model would need a limiting concentration of CaM. Allowing the CaM concentration to be constant would, at least during prolonged stimuli, have the same effect as allowing it to be non-limiting. See also sections 5.3, 5.4 and 5.4.2.

5.9.2 ATP

ATP is the phosphate group donor. Most models (except Coomber [11]) simply assume an unlimited supply of ATP, and experimental studies have ATP included in the buffer in unlimiting quantities. I have not found any articles on the subject.

5.9.3 PKA

PKA is activated by another pathway than the CaMKII pathway, the cAMP pathway. Production of cAMP is regulated by adenylyl cyclase (AC) and phosphodiesterase (PDE), and there is an enrichment of Ca\(^{2+}\) sensitive AC's in regions exposed to high free intracellular Ca\(^{2+}\) concentrations by NMDA channels and VGCC's [46]. Thus, Ca\(^{2+}\) dynamics in the spine certainly will influence PKA activity, thereby influencing PP1 regulation. Setting the PKA activity to an arbitrary constant value will skew PP1 activation, how much is unclear. In most of the models studied here, PP1 activity is crucial for LTD induction and has some effects also on LTP induction. The extent of variation in PKA activation should at least be examined before a constant value of PKA can be assigned.

5.9.4 CaN

The Zhabotinsky model assumes a Hill equation (with \(n_H = 3\)) for activation of CaN. However, using this assumption one will probably (especially in combination with Hill equation activation for CaM) overestimate CaN activation (in the same way as CaM activation is overestimated, see chapter 3 section 3.1 and figures therein), especially for low-frequency stimuli (which are considered to activate CaN over CaMKII and thereby, according to some, induce LTD). See section 3.1. To overestimate CaN activity in the range of frequencies where it is considered to be most important - thereby underestimating PP1 inhibition - could be a serious disadvantage in the study of synaptic plasticity.
5.10  **Stochastic versus deterministic**

At physiological concentrations, especially in as small volumes as a dendritic spine, the actual concentrations might be equivalent with a small number of molecules. The approximation of concentration as a continuous variable loses credibility, and stochastic simulations (Markov processes) will be needed. Exactly where to draw the line depends, of course, on how much error one is willing to accept. On the other hand, stochastic simulations tend to demand more computational resources and, additionally, should be run several times and the results averaged, which further increases the workload. For simulations of phenomena that have long time-scales, this is a serious disadvantage and might even make the choice of a stochastic model impossible. A compromise might be to use a model that has both deterministic and stochastic parts, such as Holmes’ model [20].
5.10. Stochastic versus deterministic Model assumptions revisited
Chapter 6

Discussion

This chapter contains some concluding general discussion on modelling of CaMKII activity and comparison with experimental results. Specific discussion on model features can be found in chapter 5.

6.1 Model diversity and unknown mechanisms

Models of CaMKII activation are surprisingly diverse, and there is disagreement on the importance of several features of the CaMKII pathway, such as trapping and limiting CaM concentration. The mechanism(s) that induce the change in synaptic plasticity during and after synaptic activity are not well known, but many models seem too simple to give accurate predictions of the mechanisms of synaptic plasticity. Further work with more complex models will be needed.

6.2 The implemented models

Considering that synaptic plasticity is a complicated phenomenon, it is maybe not surprising that I, among the implemented models, prefer Holmes’ model. Its main advantages are the detailed CaM-CaMKII-CaN interaction and the stochastic CaMKII activation part (which can be very intuitively modelled as a matrix of subunits). Nevertheless, the lack of regulation of dephosphorylation is the model’s most serious drawback. However, this lack is easier to improve than it would be to add three other CaMKII activation states to Zhabotinsky’s model (then one would end up with something like Coomber’s model or Kubota’s and Bowers’ model, which would lead to a definitive need to diminish the number of modeled subunits). Using a model with too few subunits, even though it may be shown to have the correct qualitative behaviour, could lead to subtle effects on the levels of trapping and capping (which are neighbour-dependent). To use a model without capping, regardless of if capped subunits are considered active or not, will definitely have effect on both trapping levels and autonomy levels and probably also on the availability of CaM to other pathways. Using too simplified
Ca\(^{2+}\) signals and kinetics will probably both overestimate and underestimate the level of activation of CaMKII, depending on the circumstances.

### 6.3 Replication of experimental data

All of the implemented models exhibit some of the experimentally established CaMKII frequency sensitivity. There are large differences, though, between the amount of frequency sensitivity: only the Dupont model shows a large difference between the 1 Hz and 4 Hz signals. As the models have some differences in how CaMKII activity is defined and what activation states are included, comparison with the experimental values of Bradshaw et al. \[6\] is not straightforward. It can be seen, though, that even a simple model can show qualitative agreement with experimental data. It would have been interesting to have PP1 included in Holmes’ model and be able to examine how well the models behaviour would agree with data on the CaMKII-PP1 system. As it is, it is hard to relate the fixed dephosphorylation rate in Holmes’ model to a concentration of PP1.

The fact that a good model should be based on enzyme kinetics at body temperature (instead of the 0 °C that Bradshaw et al. performed their experiment at) also makes interpretation of their results in comparison to the simulation results difficult. (Of course, one would expect that all reactions would run slower at lower temperature, which would make more simulation activity – compared to the experimental results – probable.) There is also very little discussion on the general effect on the system of lowering the temperature so much.

### 6.4 The role of CaMKII isoforms

To the best of my knowledge no models, so far, have included isoforms of CaMKII. As \(\alpha\)- and \(\beta\)-CaMKII have very different affinities for CaM (see section \[1.7.1\]), interesting effects on for instance trapping (which is neighbour-dependent) could be expected in heteroenzymes (CaMKII molecules consisting of both \(\alpha\) and \(\beta\) subunits), as the states of low-affinity subunits become dependent on the states of high-affinity subunits.

### 6.5 My recommendations for a model

I would like to present my conclusion as a recommendation of model features to include, grouped after priority:
6.5. My recommendations for a model

Discussion

Definitely

- Membrane voltage
  *Needed for implementation of NMDARs, and eventually for VGCC’s*

- Ca\(^{2+}\) influx through NMDA channels
  *Since there is a general consensus on NMDA channels being the important source of Ca\(^{2+}\)*

- Fast Ca\(^{2+}\) clearance and low endogenous buffer levels
  *As reported by Sabatini et al. [38] for spines*

- Four-step Ca\(^{2+}\) binding to CaM
  *Because the dynamics might be too simplified otherwise, which will risk an apparent under- and overestimation of the Ca\(^{2+}\) available to the system. The best way might be to try both a four-step model and a Hill activation model to see if the difference is important. Another possibility is to use a two-step model based on the fact that CaM has two low-affinity and two high-affinity binding sites for Ca\(^{2+}\)*

- CaN, II and PP1 (with active PKA)
  *As inhibition of PP1 is Ca\(^{2+}\)-activated and a competing process for CaM\(^*\)*

- Stochastic CaMKII activation steps (as in Holmes’ model [20])
  *The easiest way to implement neighbour-dependent reactions for a full holoenzyme*

- Capping included
  *Capping seems to alter the nature of the autonomous state according to simulations with Holmes’ model*

Examine the effects and add if necessary

- Activation of PKA
  *Because PKA is activated by another, at least partly Ca\(^{2+}\)-dependent pathway*

- NMDAR interaction with CaMKII (including phosphorylation)
  *Alters the NMDAR activity level and allows CaMKII to be active without CaM\(^*\), interaction between NMDAR and CaMKII is slow and may lower the effective level of CaMKII for the rest of the substrates*

Further possible extensions

- Compartmental model
  *To investigate the effects of diffusion*

- Diffusion of Ca\(^{2+}\), CaM and CaM\(^*\)
  *To see how large the role is for Ca\(^{2+}\)-dependent activation outside the immediate vicinity of the Ca\(^{2+}\) release*
6.5. My recommendations for a model

- Diffusion of CaMKII in combination with NMDAR interaction and AMPAR phosphorylation
  
  *Since AMPARs and NMDARs are kept close by scaffolding proteins, the likelihood for AMPAR phosphorylation after CaMKII dissociation from NMDARs might be an important addition.*

- Diffusion of remaining substances
  
  *To see if there are any effects*

- Voltage-gated Ca\(^{2+}\) channels
  
  *As these Ca\(^{2+}\) channels also are activated by depolarizations and the additional Ca\(^{2+}\) might be important near threshold levels*

- Ca\(^{2+}\) release from internal stores
  
  *To investigate if this contribution is important*

- Phosphorylation of AMPARs
  
  *As an "output" variable, to compare to other more abstract models, and as a target for CaMKII activity*

- Mg\(^{2+}\) interaction with CaM
  
  *As Mg\(^{2+}\) also is important for NMDAR function*

- CaMKII isoforms
  
  *Which may have substantial influence on trapping*
Bibliography

Articles


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**Books**


Appendix A: Abbreviations

α-CaMKII a CaMKII isoform

β-CaMKII a CaMKII isoform

AC adenyl cyclase

AMPA α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPAR AMPA receptor

ATP adenosine-tri-phosphate; phosphate group donor

[Ca$^{2+}$ ] calcium concentration

CaM calmodulin

CaM* calmodulin with four Ca$^{2+}$ bound

CaMKII calmodulin dependent kinase type II

CaN Calcineurin (PP2B)

DARPP-32 Dopamine and cAMP regulated phosphoprotein Mr. 32000; an I1 isoform

GluR1 A subunit of the AMPA receptor

I1; I-1 inhibitor-1

I1P Phosphorylated inhibitor-1

LFS Low-frequency stimulation, around 1Hz (used to induce LTD)

LTD long-term depression
<table>
<thead>
<tr>
<th>Abbreviations</th>
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<tbody>
<tr>
<td><strong>LTP</strong> long-term potentiation</td>
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<tr>
<td><strong>NMDA</strong> N-methyl-D-aspartate</td>
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<tr>
<td><strong>NMDAR</strong> NMDA receptor</td>
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<tr>
<td><strong>PDE</strong> phosphodiesterase</td>
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<tr>
<td><strong>PKA</strong> protein kinase A</td>
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<tr>
<td><strong>PKC</strong> protein kinase C</td>
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<tr>
<td><strong>PP1</strong> protein phosphatase 1</td>
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</tr>
<tr>
<td><strong>PP2B</strong> protein phosphatase 2B (calcineurin)</td>
<td></td>
</tr>
<tr>
<td><strong>PSD</strong> the post-synaptic density</td>
<td></td>
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<tr>
<td><strong>Ser</strong> serine residue</td>
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<tr>
<td><strong>SR</strong> the sarcoplasmic reticulum (a Ca(^{2+}) storage organelle)</td>
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<tr>
<td><strong>STDP</strong> spike-timing dependent plasticity (bidirectional plasticity)</td>
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<tr>
<td><strong>Thr</strong> threonine residue</td>
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<tr>
<td><strong>VGCC</strong> voltage-gated Ca(^{2+}) channel</td>
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### Appendix B: Overview of models

#### Implemented models

<table>
<thead>
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<th>Model Type</th>
<th>Dupont</th>
<th>Holmes</th>
<th>Zhabotinsky</th>
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<td>-</td>
<td>X</td>
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## Other models

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