Mechanisms of excitability in the central and peripheral nervous systems

Implications for epilepsy and chronic pain

JENNY TIGERHOLM
The work in this thesis concerns mechanisms of excitability of neurons. Specifically, it deals with how neurons respond to input, and how their response is controlled by ion channels and other active components of the neuron. I have studied excitability in two systems of the nervous system, the hippocampus which is responsible for memory and spatial navigation, and the peripheral C–fiber which is responsible for sensing and conducting sensory information to the spinal cord.

Within the work, I have studied the role of excitability mechanisms in normal function and in pathological conditions. For hippocampus the normal function includes changes in excitability linked to learning and memory. However, it also is intimately linked to pathological increases in excitability observed in epilepsy. In C–fibers, excitability controls sensitivity to responses to stimuli. When this response becomes enhanced, this can lead to pain.

I have used computational modelling as a tool for studying hyperexcitability in neurons in the central nervous system in order to address mechanisms of epileptogenesis. Epilepsy is a brain disorder in which a subject has repeated seizures (convulsions) over time. Seizures are characterized by increased and highly synchronized neural activity. Therefore, mechanisms that regulate synchronized neural activity are crucial for the understanding of epileptogenesis. Such mechanisms must differentiate between synchronized and semi synchronized synaptic input. The candidate I propose for such a mechanism is the fast outward current generated by the A-type potassium channel ($K_A$).

Additionally, I have studied the propagation of action potentials in peripheral axons, denoted C–fibres. These C–fibres mediate information about harmful peripheral stimuli from limbs and organs to the central nervous system and are thereby linked to pathological pain. If a C–fibre is activated repeatedly, the excitability is altered and the mechanisms for this alteration are unknown. By computational modelling, I have proposed mechanisms which can explain this alteration in excitability.

In summary, in my work I have studied roles of particular ion channels in excitability related to functions in the nervous system. Using computational modelling, I have been able to relate specific properties of ion channels to functions of the nervous system such as sensing and learning, and in particular studied the implications of mechanisms of excitability changes in diseases.

Keywords: Dendritic excitability, synchronized synaptic input, multicompartment model, epilepsy, axonal excitability, silent C–fibres, Hodgkin-Huxley dynamics, conduction velocity, $K_A$
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**Soma**
The cell body of a neuron

**Dendritic tree**
The large tree–like structure connected to the cell body of a neuron

**Apical trunk dendrite**
The main dendrite, which originates from the soma of a pyramidal neuron

**Oblique dendrite**
The dendrite that branches from the apical trunk dendrite

**Tuft dendrites**
Distal dendrites at the end of the apical trunk dendrite

**Membrane potential**
The voltage across the cell membrane of a neuron

**Action potential (spike)**
A large transient depolarisation of the membrane potential generated in the soma when the cell is activated

**Back–propagating action potential**
The spread of the action potential from the soma throughout the dendritic tree

**Spike threshold**
Critical membrane potential threshold. Pass this threshold an action potential is generated
**Excitatory postsynaptic potential**  A temporary depolarisation of the membrane potential caused by the flow of positively charged ions into the cell

**Ion channel**  Pore–forming proteins that help establish and control the voltage gradient across the membrane by allowing the flow of ions down their electrochemical gradient

**Gating variable**  A variable that describes the opening state of an ion channel

**Steady state parameters**  Parameters that describe the opening state of an ion channel in steady state

**Synapse**  Functional connections between neurons by which the cells can activate each other

**K_A**  A–type potassium channel

**KChIP1**  K+ channel–interacting protein 1, which is an auxiliary subunit of K_A

**DPP6**  Dipeptidyl aminopeptidase–like protein 6, which is an auxiliary subunit of K_A

**PUFA**  Polyunsaturated fatty acids

**PKC**  Protein kinase C

**CaMKII**  Ca2+/calmodulin–dependent protein kinase II

**AA**  Arachidonic acid

**Ketogenic diet**  Diet consisting of high fat, adequate protein and low carbohydrate intakes
<table>
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<th>Term</th>
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<td>AMPA-receptor</td>
<td>An excitatory ionotropic receptor that gives rise to a depolarisation of the cell membrane potential</td>
</tr>
<tr>
<td>GABA-receptor</td>
<td>An hyperpolarising ionotropic receptor that gives rise to hyperpolarisation of the cell membrane potential</td>
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<tr>
<td>Hippocampus</td>
<td>An important brain structure located in the temporal lobe</td>
</tr>
<tr>
<td>CA1</td>
<td>An area in the hippocampus</td>
</tr>
<tr>
<td>CA3</td>
<td>An area in the hippocampus</td>
</tr>
<tr>
<td>Temporal lobe epilepsy</td>
<td>A form of epilepsy in which the seizures are generated in the temporal lobe</td>
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<td>EEG</td>
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Chapter 1

Scope of the thesis

One of the most fascinating features of neurons is their elaborate dendritic arbours. The vast majority of the synaptic input projects on the dendrites rather than on the soma. One important function of dendrites is therefore to integrate synaptic input, which is influenced by numerous ion channels embedded in the cell membrane. Certain pathological conditions, such as epilepsy and chronic pain conditions, can originate from dysregulations of ion channels mutations in ion channel genes (reviewed in Köhling, 2002; Drenth et al., 2007). The main purpose of this thesis is to explain how certain ion channels influence functional behaviours observed during epileptogenesis and to suggest candidates for modulatory substances that reduce epileptic activity. In the following part of this section, I will describe the main objectives of this thesis.

1.1 Objectives

- **Objective 1:** To study the ability of A–type potassium channels to selectively reduce synchronized synaptic input

  Epilepsy is a brain disorder in which a subject has repeated seizures (convulsions) over time. Seizures are characterized by increased and highly synchronized neural activity (McNamara et al., 1999; Chiu et al., 2006; Bragin et al., 1999; Ochi et al., 2007; Urrestarazu et al., 2006; Worrell et al., 2008). Therefore, mechanisms that regulate synchronized neural activity are crucial for an understanding of epileptogenesis. Such mechanisms must differentiate between synchronized and semi–synchronized synaptic input. The candidate I propose for such a mechanism is the fast outward positive current generated by the A–type potassium channel (K_A). K_A which reduces the excitability of the cell. A large K_A current may prevent the neuron from becoming activated by synchronized synaptic input. The first objective of my thesis is
CHAPTER 1. SCOPE OF THE THESIS

to investigate whether $K_A$ can suppress the cellular response to synchronized synaptic input while minimally affecting desynchronized input.

- **Objective 2:** To investigate whether $K_A$ can suppress fast ripple activity
  Fast ripples, high-frequency oscillations (200–1000 Hz), have been identified as possible signature markers of epileptogenic activity and may be involved in generating seizures (Chiu et al., 2006; Bragin et al., 1999; Ochi et al., 2007; Urrestarazu et al., 2006; Worrell et al., 2008). Importantly, fast ripples are characterized by abnormally synchronized population activity. Therefore, reducing cellular response to fast ripple input should help reduce epileptic activity. The second objective is accordingly to investigate whether $K_A$ can suppress fast ripple activity.

- **Objective 3:** Reversing nerve cell pathology by optimizing the concentration of modulatory substances targeting the $K_A$ channel
  In diseases of the brain, the distribution and properties of ion channels deviate from those of healthy control subjects. I studied three cases of ion channel alteration related to epileptogenesis and derived three pathological models of epilepsy. The third objective is to apply computational modelling and optimization to reverse pathological models and thereby restoring normal neural function. The input parameters for the optimizer were the relative concentrations of the modulatory substances KChIP1, DPP6, PKC and CaMKII.

- **Objective 4:** To reverse pathological models of epilepsy by ion channel alterations related to the ketogenic diet
  A ketogenic diet is an alternative treatment of epilepsy in children (Kossoff et al., 2011). The diet, rich in fat and low in carbohydrates, elevates the level of polyunsaturated fatty acids (PUFAs) in plasma (reviewed in Bough and Rho, 2007). These substances have therefore been suggested to contribute to the anticonvulsive effect of the diet. PUFAs modulate the properties of a range of ion channels, including K and Na channels. These changes have been hypothesized to be part of a mechanistic explanation of the ketogenic diet. The fourth objective is to study how ion channel alterations, due to the ketogenic diet, can reverse pathological models of epilepsy.

- **Objective 5:** To study the influence of the Perforant pathway on the suppression of synchronized synaptic input via the Shaffer collateral
  Highly synchronized neural firing, for instance sharp-wave activity in the hippocampus, has also been discussed in relation to normal neural activity, such
as memory consolidation (Buzsaki, 1998; Siapas and Wilson, 1998). During sharp–wave activity, highly synchronized input is mainly mediated by the Schaffer collateral pathway. Recent studies have shown interaction between different input pathways (Doiron et al., 2011; Pissadaki et al., 2010; Dudman et al., 2007), such as the Perforant and Shaffer collateral pathways. The Perforant pathway, projecting to the tuft, has relatively low direct influence on spike activity. It has therefore been assumed to have a modulatory function (Otani et al., 1995; Dudman et al., 2007). The fifth objective is to study the influence of the Perforant pathway on the cellular suppression of synchronized synaptic input via the Shaffer collateral.

- **Objective 6: To describe the alteration in excitability in C–fibres due to repetitive stimulation**
  
  Up to this point, I have studied the integration of synchronized input in the central nervous system. In this project, I have also studied the propagation of action potential in the peripheral axons, denoted C–fibres. These C–fibres mediate information about harmful peripheral stimuli from limbs and organs to the central nervous system and are therefore linked to pathological pain. If a C–fibre is activated repeatedly, their excitability is altered (Hallin et al., 1970; Serra et al., 1999; Weidner et al., 1999). The mechanisms of this alteration are unknown. However, in pathological conditions, such as chronic pain, this alteration in excitability displays deviate from that of healthy control subjects (Ørstavik et al., 2003; Krishnan and Kiernan, 2005) and may contribute to the pathology. The sixth objective is to study the mechanisms that generate the alteration in excitability in C–fibres due to repetitive stimulations.

### 1.2 List of papers included in thesis

- **Paper I**
  
  Erik Fransén, Jenny Tigerholm
  
  Role of A–type potassium currents in excitability, network synchronicity and epilepsy,
  
  Hippocampus, 20:877–887, 2010
  
  My contribution to this work was to improve the model, construct the fast ripple input, run the simulations, and conduct the analysis.

- **Paper II**
  
  Jenny Tigerholm, Erik Fransén,
  
  Reversing nerve cell pathology by optimizing modulatory action on target ion channels,
  
My contribution to this work was to construct the models of epilepsy, synchronized input, and modulatory substances. I implemented the optimization method and ran all simulations. I contributed to writing the paper.

- **Paper III**
  Jenny Tigerholm, Sara I Börjesson, Linnea Lundberg, Fredrik Elinder, Erik Fransén,
  Dampening of hyper–excitability in CA1 pyramidal neurons by polyunsaturated fatty acids acting on voltage–gated ion channels
  Submitted, 2012
  I contributed to the design of the project. I constructed the models of epilepsy, the models of synchronized input, and contributed to the models of modulatory substances. I ran all simulations and performed the analysis of the results. I contributed to writing the paper.

- **Paper IV**
  Jenny Tigerholm, Michele Migliore, Erik Fransén
  Integration of synchronous synaptic input in CA1 pyramidal neuron depends on spatial and temporal distributions of the input.
  Submitted, 2012
  I contributed to the design of the project and to writing the paper. I ran all simulations and analysed the results.

- **Paper V**
  Modelling activity–dependent changes of AP conduction of primary afferent C–nociceptors
  Jenny Tigerholm*, Marcus Petersson*, Otilia Obreja, Angelika Lampert, Richard Carr, Schmelz Martin, Erik Fransén
  *contributed equally
  I contributed to the design of the project and to writing the paper. I ran some of the simulations and analysed the results.
Chapter 2

Basics of neuroscience

Computational neuroscience is the application of computer modelling to elucidate biological phenomena in the nervous system. Hence, relevant biological concepts and computational modelling techniques must be understood to properly comprehend this interdisciplinary field. This chapter provides an overview of the basic underlying biological concepts relevant to this thesis and of the relevant biological assumptions. In the following chapter, a short introduction to the basic terminology and computational models used in this thesis will be provided. The knowledgeable reader can go directly to chapter 4.

The nervous system has been divided into two classes, central and peripheral. The central nervous system consists of the brain and spinal cord, while the remaining nerves and ganglia constitute the peripheral nervous system. The main function of the peripheral nervous system is to provide information from the limbs and organs to the central nervous system where it is processed.

2.1 Neurons – the building blocks of the brain

The morphology of neurons in the brain can vary significantly. However, all neurons have a cell body (soma) and many have a tree–like structure called a dendritic tree. Many neurons have a large dendritic tree, primarily divided into apical and basal dendrites. The ion concentrations inside and outside the membrane of a neuron differ, generating a voltage across the cell membrane called the membrane potential. One way to activate a neuron is by activating receptors sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA). The AMPA receptors are primarily located at the synapses, which are locations where neurons are connected with each other. When the AMPA receptor is activated, it opens and lets ions pass through the membrane. The flux of ions depolarizes the neuron, in what is known as an excitatory postsynaptic potential (EPSP). This depolarization travels
to the soma, where an “all or nothing” mechanism determines whether the neuron becomes activated. If the membrane potential is higher than a certain spike threshold, the neuron becomes activated. When a neuron becomes activated, sodium channels open in the soma. This generates a large transient depolarization (i.e., action potential or spike) that is typical of an activated neuron. Not only can a cell become activated by synapses, but it can also be inhibited. The typical central neurotransmitter that mediates the signal is gamma-aminobutyric acid (GABA). When GABA receptors are activated, the cell becomes hyperpolarized and thereby less excitable.

2.2 Ion channels

Innumerable ion channels are embedded in the cell membrane, enabling ions to pass through. The ion concentrations inside and outside the neuron differ. This induces a chemical gradient and electrical voltage across the membrane that drives the ions through the ion channels. When a neuron is resting, the inflow and outflow of ions are in equilibrium, but when the neuron is activated, the balance is disturbed by the opening of ion channels. Ion pumps can restore the balance by pumping back the ions that have been flowing through the channels. The next section will describe the characteristics of ion channels relevant to this thesis.

A–type potassium channel

The A–type potassium channel (KA) is a channel that is permeable to potassium ions, which cause the neuron’s excitability to decrease (Hoffman et al., 1997; Cai et al., 2004; Makara et al., 2009). KA is composed of four subunits that can either be identical or different. The KA current belongs to a group of threshold currents activated near the spike threshold and it produces the well–known action potential delay (Melnick, 2011). KA has a rapid activation (within milliseconds) and a semi–slow inactivation (within 30ms) (Hoffman et al., 1997). The dynamics of KA differ between the distal and proximal dendrites of CA1 pyramidal neurons in the hippocampus (Hoffman et al., 1997). Not only do the dynamics of KA differ in the dendritic tree, but the density of KA increases farther out on the dendritic tree (Hoffman et al., 1997). The auxiliary subunit DPP6 may contribute to the conductance gradient of the KA current, since dendritic recordings from mice lacking the DPP6 gene did not display increased KA conductance (Sun et al., 2011).

Other potassium channels

Other vital channels are permeable to potassium ions, for example, the KDR channel (KDR). Hodgkin and Huxley first demonstrated the importance of KDR in generating an action potential in 1952 (Hodgkin and Huxley, 1952). KDR is a semi–fast potassium channel, activated during depolarized potential, and its main function is to repolarize the membrane after an action potential (Hodgkin and Huxley, 1952).
Other channels, such as the hyperpolarization–activated cyclic nucleotide–gated channel and the h–channel (also referred to as If, IQ and HCN), are permeable to both sodium and potassium ions. The h–current belongs to a group of threshold currents that are activated near the spike threshold. The h–channel is sometimes referred to as the “pacemaker channel” because it helps generate rhythmic activity within groups of heart and brain cells (Luthi and McCormick, 1998).

### Sodium channels

Sodium channels are ion channels that are permeable to sodium ions. The major function of sodium channels is to generate action potentials. In central neurons, such as the CA1 pyramidal neurons, TTX–sensitive sodium currents are dominant. Peripheral C–fibres have three sodium channels that are relevant to this thesis: Na\textsubscript{V}1.7, Na\textsubscript{V}1.8, and Na\textsubscript{V}1.9. Their characteristics in the peripheral system will be further described in this section. Both the Na\textsubscript{V}1.7 and Na\textsubscript{V}1.8 channels are fast–activating sodium channels contributing to spike initiation (Blair and Bean, 2002). These channels differ, since the Na\textsubscript{V}1.8 channels generate the action potential while the Na\textsubscript{V}1.7 channels only help initiate the action potential (Blair and Bean, 2002). One reason why the Na\textsubscript{V}1.7 channels do not contribute to generating the action potential is that they are almost inactivated when the cells are resting. Furthermore, the Na\textsubscript{V}1.9 channels are slow sodium channels and are therefore also called persistent sodium channels. The Na\textsubscript{V}1.9 channels mainly influence the resting potential, since they are too slow to affect the action potential (Herzog et al., 2001).

Sodium channels, due to their vital function, are implicated in numerous pathological conditions, such as chronic pain and epilepsy (reviewed in Wada, 2006; Köhling, 2002). All three sodium channels, Na\textsubscript{V}1.7, Na\textsubscript{V}1.8, and Na\textsubscript{V}1.9, have been implicated in chronic pain (reviewed in Wada, 2006). In chronic pain disorders, sodium channels can be both upregulated and downregulated (reviewed in Wada, 2006). The interplay between sodium channels likely generates the hyperexcitability associated with chronic pain. Mutations in the Na\textsubscript{V}1.7 channel gene can generate a gain as well as a loss of function. A gain–of–function mutation has been found to generate peripheral pain disorders (reviewed in Fischer and Waxman, 2010), while a loss–of–function mutation has been found to generate a rare disorder that strongly reduces the patient’s ability to feel physical pain (reviewed in Fischer and Waxman, 2010).

### Ion pumps

Embedded in the membrane are proteins, called ion pumps, that transport ions across the membrane. Ion pumps differ from ion channels, since they transport ions against the concentration gradient. The ion pumps transport the ions back after the neuron has been activated, thereby preparing the neuron for reactivation.
In thin axons, where ion accumulation can occur easily due to the small volume, ion pumps may play an important role in excitability (De Col et al., 2008).
Chapter 3

Basics of computational neuroscience

Computational neuroscience combines two of the most intriguing disciplines, computational modelling and neuroscience. One pioneer in the field was Lapicque, who described the excitability of axons in 1907 (reviewed in Brunel and Van Rossum, 2007). These observations were later developed into the integrate–and–fire model of a neuron (Brunel and Van Rossum, 2007), which is among the simplest models, describing a neuron with one equation. Despite this, it is still frequently used in large–scale network models, since it is not computationally demanding and still captures many of the integration properties of a neuron (Brunel and Van Rossum, 2007). In 1952, Hodgkin and Huxley introduced a model describing the generation of action potential in an axon (Hodgkin and Huxley, 1952) by mathematical components. The models used in this thesis are derived from the original Hodgkin and Huxley model. The purpose of this chapter is to introduce the basic terminology and computational models used in this thesis.

3.1 Hodgkin and Huxley models of rectification in cell membranes

Hodgkin and Huxley described the electrical current across the membrane using a gate model (Hodgkin and Huxley, 1952). They identified two ionic currents, a potassium and a sodium current, which they described as a voltage–dependent conductance and a potential driving force. Each ion has a particular direction and amplitude of flux through the membrane, which is called the reversal potential and modelled as a voltage source.

The membrane is a lipid bilayer and has electrical properties similar to those of a capacitor. Therefore, a capacitor was added to the circuitry to generate the appro-
priate functional behaviour. Finally, a small leak current was added to increase the realism of the model. The original circuitry as described by Hodgkin and Huxley in 1952 is shown in figure 3.1. It differs little from the modern model, except that more components have been added, such as ion pumps and synapses.

3.2 Compartment models

Hodgkin and Huxley constructed their model based on experiments performed on a squid axon. During the experiments, they inserted a silver thread through the axon to generate an isopotential axon (Hodgkin and Huxley, 1952). Therefore, the original model of the cell membrane was a single-compartment model. In single-compartment models, we assume that the neuron consists of one isopotential compartment. These models are usually used to describe experimental results recorded in the soma or in large-scale network models. No phenomena that require spatial distribution, such as dendritic integration, can be studied using single-compartment models.

A multicompartment model adds spatial attributes to single-compartment models. There are two methods for incorporating spatial distribution into the basic Hodgkin–Huxley model. The first is to discretize the dendritic tree into compartments (see Figure 3.2). This is computationally demanding and mainly applied if the use of an elaborated dendritic tree is significant to a given study. The second method is to discretize the dendritic tree into equivalent electrical compartments. This is less computationally demanding but may not be suitable for studying dendritic filtering.
3.3 Hodgkin–Huxley and multistate models of ion channels

In both the Hodgkin–Huxley and multistate ion channel models, the current through the channel is modelled as the conductance multiplied by the driving force of the ion. The driving force depends on the concentration of intracellular and extracellular ion. For example, if the concentrations of ions inside and outside the neuron are equal, no ions would flow through the channel, regardless of its conductance. The Hodgkin–Huxley and multistate models differ in how the conductance of the channel is modelled. In the Hodgkin–Huxley model, ion channel conductance is based on “gates”, which can be more or less open. Their dynamics are described by a steady–state curve and a time constant. The conductance is calculated as the product of the activation levels of the gates and the maximum conductance. In the Hodgkin–Huxley model, the gates are independent from each other, this is not the case in multistate model. Thus, in a multistate model, many more transitions and states can occur. A multistate model is much more computationally demanding to run, so it is only used if a particular transition is needed for the functional behaviour.
3.4 Error sources in ion channel models

Ion channel models are critical elements for describing membrane dynamics. In this thesis, I have used Hodgkin and Huxley’s ion channel model. The parameters needed to construct the model are generated from an electrophysiological experiment. Therefore, to understand the limitations of ion channel models, one must understand the limitations of electrophysiological experiments. Two important limitations of electrophysiological experiments are temperature and lack of modulatory substances. Some experiments are performed at room temperature, so the kinetics of the channel differ significantly from those at body temperature. This difference can be compensated for by a temperature coefficient \( Q_{10} \), translating ion channel kinetics between different temperatures. \( Q_{10} \) represents the factor by which the time constant of the steady state curves changes by a change in temperature of ten degrees. However, \( Q_{10} \) values vary significantly between systems and experiments. For example, for the K\( _A \) channel the range in \( Q_{10} \) value can be as great as 3–7.2 (Huguenard et al., 1991; Nobile et al., 1997). To compensate for the temperature difference will therefore reduce the precision of the model. Another error source is that several modulatory substances may be missing from the \( \textit{in vitro} \) preparation; modulatory substances can alter the steady–state activation and inactivation as well as their kinetics.

The two most commonly used systems for studying ion channel dynamics are expression systems and brain slices. An expression system is an artificial or natural cell that has the ability to transcribe and translate a genetic sequence. If the gene coding for an ion channel is added to an expression system, the cell will express the ion channel on the cell surface. In an expression system, the ion channel can be studied without interference from other ion channels. Native ion channels can be constructed by different subunits, coded by different genes. In expression systems, however, a single gene is usually expressed, so the channels may not be equivalent to the native channels studied in brain slices. In brain slice experiments, an animal brain is used to create a thin slice of brain tissue. Recordings of brain slices usually provide no information about what specific subunits constitute the channels.
Chapter 4

Introduction

In this thesis, I describe how nerve cells in the central and peripheral nervous systems can process inputs. The main focus in the CA1 pyramidal neuron is on the integration of synaptic input from the dendritic input site to the soma, where spike initiation occurs. Along this spatial path, through the dendritic tree, ion channels play a key role in shaping the EPSP. It is technically difficult to experimentally measure this integration, since the distal dendrites are very thin. I therefore studied dendritic integration using a detailed multicompartment model with a wide range of ion channels.

The main focuses in my thesis have been on dendritic integration of synchronized input in pyramidal cells. However, in paper V, I have also studied the propagation of action potential in the peripheral axons, denoted C–fibres. These C–fibres mediate information about harmful peripheral stimuli from limbs and organs to the central nervous system and are therefore linked to pathological pain. If a C–fibre is activated repeatedly, their excitability is altered (Hallin et al., 1970; Serra et al., 1999; Weidner et al., 1999). The mechanisms for this alteration are unknown and in this thesis, I have studied then influence of ion channels on this alteration.

In the next two sections I will give an introduction to the two main objectives in this thesis: integration of synchronized input and sensory processing.

4.1 Dendritic integration of synchronized synaptic input

In this thesis, I have focused on the dendritic integration, within a couple of few milliseconds, of highly synchronized input. Some researchers criticize the use of highly synchronized input, because they claim that it is physiologically impossible for the brain to generate input with such high temporal precision. Studies of highly synchronized input are accordingly claimed to be irrelevant, since such input does
not occur during normal brain activity. Several lines of evidence contradict this claim. The most convincing evidence is that cell activity within milliseconds have been recorded from many cortical and sub-cortical areas in a variety of species (Takahashi and Sakurai 2009; Ylinen et al. 1995; Azouz and Gray 2003; Gray 1994). Even though it may be difficult to understand how such high precision can occur, one cannot deny that it does in fact occur. Notably, in this thesis, I disregarded the mechanism by which the brain generates the synchronized activity and only focused on the integration of the input.

Synchronicity is commonly viewed as a network phenomenon. However, even though synchronicity manifests itself as simultaneous spikes throughout the network, the single neuron (or part of a dendrite) constitutes the point of convergence. Synchronized neural activity is associated with several vital cognitive processes (reviewed in Uhlhaas and Singer 2006). However, changes in synchronicity is are also associated with cognitive disorders, for example, schizophrenia, epilepsy, autism, Alzheimer’s disease, and Parkinson’s (reviewed in Uhlhaas and Singer 2006). For this reason, mechanisms that regulate synchronized neural activity are crucial for an understanding of the brain and neurological diseases. In epilepsy, synchronicity is elevated (McNamara et al. 1999; Chiu et al. 2006; Bragin et al. 1999; Ochi et al. 2007; Urrestarazu et al. 2006), so I hypothesized that reducing the cellular response to synchronized input would help in reducing network synchronicity. Such mechanisms must differentiate between synchronized and semi–synchronized input, as these two different types of neural input depolarize the cell differently. Synchronized input generates a large and rapid depolarization, whereas semi–synchronized input generates a smaller and slower depolarization (see Figure 4.1).

The candidate mechanism I propose is the A–type potassium channel ($K_A$). The $K_A$ current is a fast outward positive current that reduces cell excitability (Hoffman et al., 1997). A large $K_A$ current could therefore prevent a neuron from being activated by synchronized synaptic input. One objective of my thesis is to investigate whether $K_A$ can suppress the cellular response to synchronized input, while minimally affecting desynchronized input.

In the next two sections, I will further describe the implications of synchronized input in epilepsy as well as memory and learning.

**Synchronous brain activity and cognitive functions**

Extracellular electrical recording is a widely used method for measuring synchronized brain activity. Electroencephalography (EEG) and local field potentials (LFP) measure the electrical activity induced by the activity of a mass of neurons. Both these methods record the combined activity of many neurons and cannot isolate the contributions of individual neurons. EEG and LFP, which measure population activity, can only detect activity if the neuron population exhibits some
degree of synchronicity. If the neural activity is uncorrelated, all the cells’ activity, in sum, is cancelled out and the population activity becomes zero. Synchronized brain activity, recorded by EEG or LFP, is classified by its frequency, i.e., alpha (8–12 Hz), beta (13–30 Hz), gamma (30–200 Hz), delta (0–3 Hz), and theta (4–7 Hz) (reviewed in Uhlhaas and Singer, 2006).

Large depolarizations (sharp waves) have been recorded in the hippocampus during slow-wave sleep and quiet waking (Buzsáki, 1986; Buzsaki, 1998; Maier et al., 2003; Siapas and Wilson, 1998; Worrell et al., 2008). Superimposed on the sharp waves are high-frequency oscillations, called ripples (see Figure 4.2) (Maier et al., 2003). This activity has been associated with memory consolidation (Buzsaki, 1998; Siapas and Wilson, 1998), i.e., the transfer of memory from the hippocampus to the neocortex. During sharp waves, dendritic sodium spikes can be generated and presumably play a role in the dendritic integration and plasticity processes (Kamondi et al., 1998). Due to the high synchronicity of the input, a rapid and large enough depolarization is generated to reach the spike threshold in the dendrites. The $K_A$ channel has been proposed as a candidate for suppressing dendritic spikes, since pharmacologically blocking $K_A$ increases the probability of dendritic spike generation (Gasparini et al., 2004; Makara et al., 2009).

It is widely believed that learning and memory depend on the long-term alteration of synaptic conductance. During a phenomenon called long-term potentiation, the synaptic conductance can be altered from an hour up to days, weeks, and even months (reviewed in Sjöström et al., 2008). Synchronized input induces a large
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Figure 4.2: Frequency components of sharp wave–ripples in vitro. A, raw data from a recording in CA1 pyramidal cell layer. Lower traces show the same recordings after application of a high-pass filter (B), band-pass filter (C) and low pass-filter (D). Isolation of high-frequency components reveals unit activity. Note that units do not discharge on each cycle of the ripple oscillations (compare high-pass vs. band-pass filtered signal). Low pass filtered traces shows the underlying sharp wave

depolarization that can remove the magnesium block of the NMDA channel and thereby allow long–term potentiation. Thus, dendritic sodium spikes are efficient at inducing long–term potentiation, since a dendritic spike generates a large depolarization. This could explain why K_A is involved in learning and synaptic plasticity [Ramakers and Storm 2002; Chen et al. 2006; Lockridge and Yuan 2011].

Moreover, when an action potential is generated in the soma, it will propagate back through the dendritic tree. The back propagation of an action potential transmits the information regarding the effectiveness of the synaptic input back to the synapses located in the dendrites. This creates an association between the synaptic input and its impact on action potential generation. Large back propagation has been found to induce long–term potentiation (reviewed in Sjöström et al. 2008).

Moreover, synchronized input leads to an increase in synaptic conductance via long–term potentiation. This in turn generates more synchronized activity and thereby
a further increase in synaptic conductance. This process would be autogenerative if it were not controlled by mechanisms other than long–term potentiation (reviewed in Sjöström et al., 2008). Due to this potential positive feedback, comparisons have been made between mechanisms of memory and those of epilepsy (Beenhakker and Huguenard, 2009) and chronic pain (Ji et al., 2003). In Papers I–III, I have studied the cellular integration of synchronized input implicated in epileptogenesis. In the next section, I will provide an introduction to these papers.

**Synchronous brain activity and epilepsy**

Epilepsy is a major neurological disease with a prevalence rate of 5–10 per 1000 individuals (Sander and Shorvon, 1987; Semah et al., 1998). The predominant symptoms of epilepsy are recurrent spontaneous seizures. Patients can experience seizures during conditions of stress and fatigue or when they are calm or at rest. The uncertainty regarding the triggering conditions of seizures is very stressful for the patient. Therefore, the period immediately preceding the seizure, i.e., the preictal period, has been the subject of significant research seeking to construct algorithms that can help predict seizures. Epileptic seizures can be divided into two types: partial and general. Partial seizures affect only certain parts of the brain, while general seizures cause disturbances throughout the brain. Mesial temporal lobe epilepsy is a common form of epilepsy caused by partial seizures located in the olfactory cortex, amygdala, and hippocampus. Though the underlying mechanisms of seizures are unknown, many studies have investigated preictal activity in an attempt to identify the triggering mechanisms of seizures. Fast ripples, i.e., high–frequency oscillations (200–1000Hz), have been identified as possible signature markers of epileptogenic activity and may be involved in generating seizures (Chiu et al., 2006; Bragin et al., 1999; Ochi et al., 2007; Urrestarazu et al., 2006). In fact, fast ripples have only been recorded in brain structures capable of generating epileptic seizures (Engel et al., 2009).

The analysis of fast ripples versus normal brain ripple activity helps us understand the pathological behaviour of fast ripples. The amplitude and frequency of ripples and fast ripples indicate that the activity is initiated in CA3 pyramidal cells and becomes prominent in CA1 cells (Lasztoczi et al., 2004; Dzhala and Staley, 2004; Ylinen et al., 1995). Fast ripples can reach frequencies up to 1000Hz, while individual neurons can fire at only a few hundred Hertz for an extended period (Staley, 2007). This implies that fast ripples are the combined effect of a population of neurons. A hypothesis explaining the occurrence of fast ripples has been proposed by Foffani et al. (2007). The higher frequency arguably occurs when two populations of neurons oscillating at lower frequencies are combined. For example, consider two populations of neurons oscillating at 200Hz; if one population is delayed by 2.5 ms, the combined oscillating frequency of both populations would be 400 Hz.

Another hypothesis of how fast ripples occur is that they are a pathological de-
violation of ripples. When fast ripple activity is generated, synchronicity increases followed by an increase in frequency (Lasztoczi et al., 2004). These results suggest that fast ripples may be generated from normal ripples subject to higher synchronicity (Engel et al., 2009; Foffani et al., 2007).

A third hypothesis of how fast ripples occur involves networks of pyramidal cells connected by gap junctions, where the cytoplasm of the cells is directly connected. This allows molecules and ions to pass between cells. The direct connections between pairs of axons, caused by gap junctions, may generate high synchronicity. This would arise because activity in one axon leads to activity in the connected axon as well, generating a cascade of activity. Experimental evidence indicates that gap junctions do exist in pyramidal cells and interneurons in the hippocampus (Church and Baimbridge, 1991; Hamzei-Sichani et al., 2007; Bartos et al., 2001). In the case of pyramidal cells, gap junctions have been found between the axons (Schmitz et al., 2001). If the chemical synapses are blocked, it is still possible to observe a population frequency as high as 200 Hz (Draguhn et al., 1998). It is hypothesized that axo–axonal gap junctions in pyramidal cells can generate high population frequencies when spontaneous action potentials are generated in the axons (Traub and Bibbig, 2000). For this and other reasons, gap junctions may play a role in epileptogenesis.

Fast ripples are characterized by abnormally highly synchronized population activity. Therefore, reducing cellular response to highly synchronized synaptic input would be beneficial for reducing epileptic activity. In this thesis, I have therefore investigated whether $K_A$ can suppress highly synchronized synaptic input. $K_A$ generates a fast outward positive current that reduces the excitability of the cell. A large $K_A$ current could therefore prevent the neuron from becoming activated by synchronized synaptic input. Based on pharmacological and genetic studies, $K_A$ currents have been implicated in epileptogenesis. In this section some of these experimental studies will be discussed.

A–type potassium channels and epilepsy

Singh et al. (2006) discovered a $K_A$ gene mutation in a patient with temporal lobe epilepsy. The mutation caused a frame shift that changed an amino acid coding codon to a stop codon; as a result, the mutated gene lacked its last 44 amino acids. To investigate the dynamics of the mutated $K_A$ gene, the gene was injected into an expression system. The mutated $K_A$ had the same steady–state curves as did the wild type, but the current density was reduced. This study suggests that this downregulation of the $K_A$ current could be the cause of the patient’s temporal lobe epilepsy. Moreover, if $K_A$ was knocked out, instead of being downregulated, this also resulted in increased seizure susceptibility to convulsant stimulation (Barnwell et al., 2009). Interestingly, the Kv4.2 knockout was associated with 100% mortality.
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during status epilepticus, versus 25% in the control group. Barnwell et al. (2009) conclude that $K_A$ does not contribute to initiating the seizure but instead regulates the seizure threshold. However, a general problem with the use of knockouts is developmental complications, such as seizures, which preclude conclusions. A better technique would be to use blockers to isolate the behaviour of a specific ion channel. When $K_A$ is blocked by a selective blocker, seizures were also induced (Juhng et al., 1999). These three experimental results all suggest that a deficiency in $K_A$ may increase seizure susceptibility.

As a consequence of seizures, neurons can be relocated to abnormal positions, becoming what are called heterotopic cells. An animal model of this kind of cortical malformation is provided by methylazoxymethanol (MAM), as heterotopic cells in MAM–exposed rats lack a functional $K_A$ current (Castro et al., 2001). This may contribute to the spontaneous seizures found with cortical malformations (Castro et al., 2001). $K_A$ deficiency may be involved in inducing seizures and can also aggregate over time. Moreover, Kv 4.2 is downregulated after seizures (Francis et al., 1997; Tsaur et al., 1992), which might create a vicious circle: seizures caused by low $K_A$ currents will further reduce the $K_A$ current, which will increase the susceptibility to new seizures (Juhng et al., 1999). Seizures also cause stress reactions that initiate many processes that may contribute to downregulating $K_A$. However, in an animal model that mimics a stress response, without triggering a seizure, Kv 4.2 gene expression could be measured; the result indicated that downregulation of Kv 4.2 was not the result of stress reactions alone (Francis et al., 1997).

Lamotrigine is an antiepileptic drug that affects $K_A$ channels. Lamotrigine is not specific and affects other channel types as well. Interestingly, Lamotrigine enhances the $K_A$ current in the neocortex (Zona et al., 2002), whereas it reduces it in the hippocampus (Huang et al., 2004). This might explain why some patients with epilepsy display improved status, whereas others find that Lamotrigine aggravates pre–existing seizures and triggers new seizure types (Guerrini et al., 1998). The experimental data presented in this section indicate that $K_A$ downregulation may be involved in generating seizures. Since seizures themselves contribute to $K_A$ downregulation, this increases the susceptibility to new seizures even more. Upregulation of $K_A$ could stop this progressive downregulation and prevent a patient from relapsing. To my knowledge, no currently existing antiepileptic drug selectively targets the $K_A$ channel. However, the drug industry has recently become interested in the $K_A$ channel and a Belgian company, Devgen, has published several patent applications pertaining to the discovery and development of $K_A$ modulators. Interestingly, some of the patents are for possible use in the treatment of epilepsy (Castle, 2010).

Reducing hyperexcitability in models of epilepsy

In diseases of the brain, the distribution and properties of ion channels deviate from those of healthy control subjects. I have studied three cases of ion channel alteration
related to epileptogenesis and derived three pathological models of epilepsy. In papers II and III, I apply computational modelling and optimization to reverse the pathological models by restoring normal neural function. The input parameters to the optimizer were the relative concentrations of modulatory substances. Many substances can modulate the \( K_A \) channel, such as auxiliary proteins (An et al., 2000; Maffie and Rudy, 2008), kinases (Hoffman and Johnston, 1998; Varga et al., 2004), and PUFAs (Xu et al., 2008). In this thesis, I have modelled substances that modulate the \( K_A \) channel, so I will briefly describe the modulatory substances relevant to this thesis. Five important proteins that modulate \( K_A \) channels will be further described.

\( K_v \) channel–interacting proteins (KChIP) form complexes with \( K_A \) and modulate its kinetic properties (An et al., 2000; Maffie et al., 2008). KChIPs also upregulate the cell surface expression of \( K_A \) channels, thereby increasing the \( K_A \) current (An et al., 2000; O’Callaghan et al., 2003). Another protein that modulates \( K_A \) dynamics by forming complexes is the dipeptidyl aminopeptidase–like protein (DPPX). The time constants of activation and inactivation of \( K_A \) are significantly reduced by all splice variants of DPPX (Nadal et al., 2006). The activation curve of \( K_A \) is also shifted by all splice variants of DPPX (Nadal et al., 2006).

Several protein kinases also modulate \( K_A \), for example, the cAMP–dependent protein kinase A (PKA) and protein kinase C (PKC) (Hoffman and Johnston, 1998). PKA and PKC reduce the \( K_A \) current by altering the inactivation of the \( K_A \) channel.

In paper III, I have investigated the affect of polyunsaturated fatty acids (PUFA) influence of models of epilepsy. PUFAs are elevated during ketogenic diet (Bough and Rho, 2007), which is an alternative treatment of epilepsy. The ketogenic diet mimics the same condition as during fasting but it less extreme. It has been known since the early 1920s that patients with epilepsy could control their seizures by fasting (Kossoff et al., 2011). During fasting, the primary energy source is fat stored in the body, which metabolizes to ketone bodies. Ironically, this treatment did not become frequently used until the 1990s, after a Hollywood movie (“Do no harm”) increased patient awareness of it. The resistance from the pharmacological industry and research community has been significant, and several myths and misconceptions persist regarding the diet (Kossoff et al., 2011). The treatment has today been modified from fasting to the ketogenic diet, which consists of high fat, adequate protein, and low carbohydrate intakes. The ketogenic diet mimics the conditions that occur during fasting but is less extreme. It has mainly been used as the final alternative when no medical treatment has been successful or if medical options are limited due to low patient age. Between 10 and 15\% of patients become completely seizure free and 50\% reduce their seizure incidence by 50\% or more (Vining et al., 1998). The most astonishing fact about this treatment is that after a few years on the diet, many remain seizure free even after discontinuing the diet (Vining et al., 1998).
The mechanisms responsible for the anticonvulsant effect of the diet are still a mystery, despite its long history. In the early 1920s, the hypothesis was that fasting cleansed the body of toxins responsible for the seizure. Today, no single mechanism has been widely accepted as generating the anticonvulsant effect; instead, several contributing mechanisms of the diet may explain the success. Both ketone bodies and fatty acids are elevated during the ketogenic diet (reviewed in Bough et al., 2007) and have been postulated to contribute to the reduction of seizures. PUFAs have been studied extensively due to their beneficial effects on the heart (reviewed in Boland et al., 2008). In my thesis, I have studied the effects of PUFAs on ion channels to identify what alterations caused by the ketogenic diet are beneficial in reducing epileptic activity.

4.2 Sensory processing and peripheral pain

Nociceptors are sensory nerve endings that can be activated if the body tissue is exposed to any potentially harmful stimulus. They convey pain signals to the central nervous system via the axon, which is also called a nerve fibre. In this thesis, I have modelled a C-fibre, which is an unmyelinated fibre conducting a slow, dull, long-lasting pain. C-fibres average 0.2–1.5 µm in diameter, and are therefore too thin to allow recording of intracellular membrane potential. Therefore, most data on C-fibres are obtained by velocity measurements or intracellular recordings in the soma. C-fibres can be activated by one specific or several different types of physiological stimuli, such as cold, heat, and mechanical stimuli. However, some C-fibres cannot be activated by any physiological stimulus and are therefore called silent C-fibres. During experiments, silent C-fibres can be activated by very high electrical stimuli, the injected current generating an action potential in the axon and not by activating receptors in the nerve ending. One of the first recordings of a silent C-fibre was made in a study of the response behaviour of fine nerve fibres innervating the knee joint capsule (reviewed in Michaelis et al., 1996). The study demonstrated that if the knee joint is acutely inflamed, the silent C-fibres “awaken” and can be activated by physiological stimuli. The most interesting result of the study was that the silent C-fibres can be activated in the absence of mechanical stimulation, probably as a consequence of the inflammation. More recent studies have confirmed that C-fibres can become activated by inflammatory agents (reviewed in Michaelis et al., 1996).

Moreover, various forms of hyperalgesia, which is characterized by increased sensitivity to pain, are caused by damage to nociceptors or peripheral nerves. One way of experimentally inducing hyperalgesia is by injecting capsaicin into the skin,
which leads to intense pain in the tissue surrounding the injection site. Simone et al. [1989] and LaMotte et al. [1991] studied the contribution of silent C-fibres to sensitization after capsaicin injections. The study found, consistent with the results of Schaible and Schmidt [1988], that the silent C-fibres became sensitive to physiological stimuli such as mechanical pressure or heat. The most interesting result of the study was that silent C-fibres exhibited prolonged bursting discharges for several minutes after the injection, which could explain the pain experienced by the subject.

When studying action potential conduction in C-fibres, the fibre is activated by electrical or other physiological stimuli in the skin. The action potential is recorded extracellularly at a more distal part of the C-fibre and the latency is measured (see Figure 4.3). One functional behaviour of a C-fibre is that after an action potential has propagated along it, the propagation of the next action potential will change due to altered excitability (see Figure 4.4). Two changes in excitability have been measured that are relevant to this thesis: one is more prominent in the 400–1000 ms interval [Hallin et al., 1970; Obreja et al., 2010] and the other in the 20–200 ms interval [Weidner et al., 1999; Serra et al., 2011] after the action potential.

The slower of the above mentioned excitability changes displays increased propag-
4.2. SENSORY PROCESSING AND PERIPHERAL PAIN

Figure 4.4: Different patterns of activity-induced conduction slowing in the mechano-unresponsive C-fibers, in pig. Repetitive electrical stimulation at ascending frequencies increases conduction latencies in all three units: the cold nociceptor (cold noci, open star) slows marginally during the low-frequency protocol (~Hz) and only slightly more, reaching a steady-state (plateau) during the higher-frequency (2Hz) stimulation. A sympathetic-like efferent (symp-like, open circle) slows very little during both electrical protocols. Characteristic reversal of conduction latency changes during stimulation at higher frequencies (2Hz) is shown. The silent nociceptor (silent, filled square) slowed the most in both stimulation protocols. (Obreja et al., 2009)

...tion latency with repetitive stimulation and is therefore called activity-dependent slowing (ADS). In Figure 4.4, different patterns of ADS are shown for different classes of C-fibres. Silent C-fibres, which are considered to play a role in inflammatory pain (reviewed in Michaelis et al., 1996), display the greatest ADS of the C-fibre classes. The most convincing hypothesis suggests that slowing is due to the slow inactivation of the Na\textsubscript{V1.8} sodium channel (De Col et al., 2008). The decreased velocity during ADS is viewed as an indirect indication of excitability and is altered in several chronic pain conditions (Serra et al., 2011; Ørstavik et al.)
In patients with erythromelalgia, a rare condition characterized by painful hot extremities, ADS is significantly increased in silent C-fibres. This may seem to be a contradiction, since neuropathies are associated with hyperexcitability, while an increase in ADS is associated with reduced excitability. However, C-fibre behaviour is complex and all measurements of action potential propagation are indirect measurements of excitability. In this thesis, we have therefore developed a computational model to describe complex behaviours such as this in silent C-fibres.

A second functional behaviour indicates altered excitability in the 20–200 ms interval after an action potential has propagated along the C-fibre (Weidner et al., 1999; Serra et al., 2011) (see Figure 4.5). This interval is referred to as the supernormal phase. During this phase, the excitability increases as does the action potential velocity. The magnitude and time course of the supernormal phase can differ between fibre classes, also dependent on pre-existing ADS (Weidner et al., 2003; Bostock et al., 2003).

In several pain conditions, the supernormal phase is altered (Krishnan and Kiernan, 2005). In some neuropathies, the supernormal phase decreases (Krishnan and Kiernan, 2005), while in others it can increase (Park et al., 2009). In this thesis, I have tried to explain the mechanisms that generate the supernormal phase in order to explain pathological behaviours such as those described above.
Figure 4.5: Recording latency recovery cycles from single C-fiber. (A) Latencies of conditioned (filled circles) and unconditioned (open circles) test action potentials while conditioning-test delay was reduced from 400 to 2 ms, as shown below. Impulse rate was increased from 0.25 Hz first to 0.5 Hz and then to 1.0 Hz. (B) Recovery cycle plotted as percentage change in latency as a function of conditioning-test delay, showing prolonged phase of supernormality. (Serra et al., 2011)
Chapter 5

Review of computational models in epilepsy research

A large part of modelling consists of simplifying the problem. A given biological system must be understood if we are to know what parts of a particular problem can be safely ignored. In many cases, the underlying mechanisms of the biological system are unknown and it is therefore difficult to construct a model. One approach to solving this problem is to generate several alternative models based on hypotheses as to the underlying mechanisms and to analyse their consistence with experimental data. This approach has been used to describe the transition from the normal to the ictal states in epilepsy (Albert, 1991; Wong et al., 2007).

Epilepsy is characterized by spontaneous recurrent seizures. Therefore, a first approach to modelling epilepsy would be to model a seizure. During a seizure, the brain activity has a rich repertoire of characteristics that makes it difficult to model. Accordingly, many models focus instead on specific questions regarding the seizures. When do seizures occur? After being diagnosed with epilepsy, what is the probability of remission after three years? How do certain antiepileptic drugs affect cell excitability? The next chapters will describe some of the models that are currently used to study epilepsy.

5.1 Seizure prediction algorithms using probabilistic state models

The uncertainty of when a patient will have a seizure is stressful for the patient. Therefore, many studies have attempted to learn how to predict seizures. One of the first models for predicting seizures was based on a Poisson process (Milton et al., 1987). This model could predict the number of seizures a patient would experience over a given period, and for 50% of patients, seizure occurrence was
CHAPTER 5. REVIEW OF COMPUTATIONAL MODELS IN EPILEPSY

RESEARCH

indistinguishable from the Poisson predictions. Certain patients experienced extended periods without seizures followed by shorter periods with several seizures. In a second study, this kind of cyclical seizure behaviour was modelled using a two–state Markov mixture model (Albert [1991]) consisting of two states with transition probabilities. Each state had its own frequency distribution corresponding to a Poisson distribution. This Markov mixture model could predict seizures in some of the problematic patients for whom the Poisson process model failed (Albert [1991]).

The two models described above are all stochastic dynamic models that attempt to mimic a functional behaviour without considering the actual underlying mechanisms. This approach can be effective in developing a model with a specific behaviour but might not help us understand the underlying mechanisms of seizures.

5.2 Modelling of remission in epilepsy using probabilistic models

Following a medical epilepsy diagnosis, many patients experience remissions and relapses. Berg et al. (2004) used a Markov process to describe the remissions and relapses children experienced after being diagnosed with epilepsy. This model is similar to the previously described Markov model, but instead of predicting seizure probability, it predicts the probability of being in remission or relapse. Berg et al. (2004) used two models: one with three states and one with seven states. The three states in the first model are initial (i.e., no remission), remission, and relapse. In the model, the patient can move between states two and three, but not back to the initial state. In the seven–state model, the patient can never revisit a state, hence the large number of states.

Figure 5.1 from Berg et al. (2004), shows the probability of being in remission or relapse. The probability of the patient never experiencing any remission is 10%. After diagnosis, the patient’s probability of experiencing remission and relapse changes rapidly, and the system reaches steady state after five years.

This kind of modelling describes the clinical development after a patient is diagnosed with epilepsy. The results of these models imply that the brain can enter into various states, some of which are more likely to generate seizures. Describing epileptogenesis can help experimentalists identify what they are looking for in an experiment; for patients, it can be comforting to have a description of the likely clinical development.
5.3 EEG modelling using lumped or mean field deterministic models

Lumped models, in many cases, do not model single cells at all but rather a neural population. One of the first such models is that of Wilson and Cowan (Wilson and Cowan, 1972). It models two populations of neurons, one excitatory and one inhibitory, allowing brain oscillations to be studied. In an EEG model, epileptic activity was explained by impaired GABAergic synaptic inhibition (Wendling et al., 2002). The model consists of four populations of neurons: pyramidal cells, excitatory interneurons, and two populations of inhibitory interneurons. The two inhibitory interneuron populations differ in that one projects on the soma of the pyramidal populations and the other on the dendrites. As the EEG is one of the most used techniques for studying epileptic activity, lumped models are excellent for modelling synchronized oscillations as recorded by an EEG.

5.4 Detailed multicompartment models

A detailed multicompartment model has the morphology of a real cell and therefore has many compartments. Such models have mainly been used in this thesis. Detailed compartmental models of neurons are usually computationally demanding,
and for this reason are rarely used for large neural networks. One exception to this is the work of the Lansner group at KTH, where super-computers are used to simulate millions of multicompartment neurons [Lundqvist et al., 2011]. Multicompartment models are usually used to study the behaviour of single cells or small networks. The study of dendritic integration is a large field in which detailed neural models are used.

An excellent example of a model used to explain experimental data is a compartmental model of the effect of Lamotrigine [Poolos et al., 2002]. An in vitro experiment demonstrated that Lamotrigine differentially affects excitability in dendrites and the soma. Since h–channel density differs between the dendrites and the soma, Poolos et al. (2002) set out to test whether this effect was due to Lamotrigine’s modulation of the h–channel. The study had demonstrated that Lamotrigine shifts the h–channel’s activation curve and thereby increases the current through the channel. The model indicated that a 10mV shift in the h–channel activation curve is enough to produce the reduction of excitability measured in the experiment. A similar model was used in a study of mossy cell loss and mossy fibre sprouting [Santhakumar et al. 2005]. Seizures can alter the brain structure by inducing mossy cell loss and mossy fibre sprouting. Mossy cells are specialized cells that play a role in regulating excitability in the hippocampus. Mossy fibre sprouting refers to an increase in synapses in mossy cells. Loss of mossy cells reduces the network excitability while mossy fibre sprouting increases network excitability. Using experimental techniques, it is complicated to separate the effect of mossy cell loss from that of mossy fibre sprouting, but modelling allows the alterations to be studied individually. The model indicated that even a low level of mossy fibre sprouting would be sufficient to create a hyperexcitable network despite mossy cell loss [Santhakumar et al. 2005].
Chapter 6

Review of models of C–fibre and peripheral nerve cells

Modelling of ion channels and cell membranes started with research regarding axons (Hodgkin and Huxley [1952]). Hodgkin and Huxley developed the first model describing action potential generation due to the dynamics of sodium and potassium channels. Today, the most advanced cell models are those of central nervous system neurons, whereas models of the peripheral nervous system are less detailed. As far as I'm aware, there is no other detailed multicompartment model of C–fibres than ours.

Some efforts have been made to model myelinated axons. For example, a model of sensory neurons with spatial distribution has been developed (Amir and Devor [2003]), including spatial components consisting of both an axon and a soma. In the model, the concentrations of sodium and potassium ions are kept as constants. This is a reasonable assumption for the myelinated axon, since the action potential is generated only between the myelinated sections (i.e., in the internodes), so fewer potassium and sodium ions accumulate. This is not the case in unmyelinated axons, in which the action potential propagates along the whole axon.

There are, however, a few models describing the soma of peripheral nerve cells (Sheets et al. [2007], Herzog et al. [2001], Maingret et al. [2008], Kovalsky et al. [2009]). Ion channel dynamics, particularly of the sodium channels, have been modelled with high precision (Sheets et al. [2007], Herzog et al. [2001], Maingret et al. [2008]), sometimes even using multistate techniques (Gurkiewicz et al. [2011]). As these cell models have mainly been developed to describe the in vitro recording measurements performed in the soma, they lack three properties with respect to in vivo recordings of action potential propagation. First, they obviously lack the spatial distribution. Second, despite the fact that ion channel dynamics alter substantially
CHAPTER 6. REVIEW OF MODELS OF C–FIBRE AND PERIPHERAL NERVE CELLS

depending on temperature, these dynamics are modelled at room temperature. Third, during electrophysiological recordings of nerve cells, the holding potential is usually hyperpolarized (-80 to -70 mV); accordingly, existing models of peripheral somas are hyperpolarized, whereas \textit{in vivo}, the cells are actually fairly depolarized (-63 to -41 mV) \cite{Fang2005}. A different resting potential leads to altered ion channel activation. For example, the Na$_V$1.7 channel is almost completely inactivated at depolarized resting membrane potentials compared with hyperpolarized resting membrane potentials \cite{Blair2002}.
Chapter 7

Models used in this thesis

In this thesis, I have studied the influence of ion channels on dendritic integration of synchronized synaptic input in pyramidal neurons as well as on axonal propagation of action potentials in C–fibres. Distal dendrites and C–fibres are both too thin to allow experimental measurement of intracellular membrane potentials. I have therefore studied dendritic integration and axonal propagation of action potentials in a detailed multicompartment model with a wide range of ion channels.

7.1 Computational models for studying dendritic integration of synchronized synaptic input

In Papers I–V, I have studied dendritic integration of synchronized synaptic input in CA1 pyramidal neurons, which represent the principal cells in the CA1 region of the hippocampus. The CA1 pyramidal cells have been studied extensively due to their implication in both memory and epileptogenesis, and synchronized brain activity (see chapter 4).

Cell models of CA1 pyramidal neurons

In my thesis, I have used several models developed by Migliore et al. (Migliore et al., 1999; Poolos et al., 2002; Migliore et al., 2008) for studying a hippocampal CA1 pyramidal neuron. All these cell models are detailed multicompartment models (200–600 compartments) with three ion channels, i.e., sodium, K_D, and K_A; some versions of these models include an h-channel. All channels are modelled based on Hodgkin–Huxley kinetics (see chapter 3). Four different morphologies have been used in Papers I–IV. As a control model, I used a model developed by Poirazi’s group (Poirazi et al., 2003). Compared with Migliore’s models, Poirazi’s model includes calcium dynamics and a larger range of ion channels.
CHAPTER 7. MODELS USED IN THIS THESIS

Figure 7.1: The different models of the temporal distribution of the synchronized input. A. The temporal distribution for the Exponential distribution input for the synchronization levels 0%, 70% and 50%. B. The temporal distribution for the Normal distribution input for the synchronization levels 0ms, 5ms and 10ms. C. The temporal distribution for the Deterministic normal distribution input for the synchronization levels 0ms, 5ms and 10ms.

Models of synchronous synaptic input

For all papers, I have used the built-in model of a synapse in NEURON, which is a two state kinetic scheme synapse, described by a rise time and a decay time constant. For more information, please refer to the documentation of NEURON.

I have generated synaptic input with different levels of synchronization in the temporal distribution. Three different models for the generation of synchronized synaptic input have been used. The different temporal features of the input are shown in Figure 7.1.

Exponential distribution model (Paper I)

Ten synaptic inputs were included in the neuron model, representing the activated synapses at a particular period of time within a restricted part of the dendrite. For each synapse, I generated exponentially distributed synaptic events at a rate of 12 Hz. The different levels of synchronization were modelled according to Charcos Lloréns and Fransén (2004). Different inputs were separated with an exponential distribution within a time window, and the relative size of the window in relation to the base frequency of 12 Hz determines the degree of synchronization. For instance, if the frequency of the input is 12 Hz, the time window between the cycles is on average 83 ms (T₀ in the Figure 7.2). If the synchronization level is 90% (T_W/T₀
7.1. COMPUTATIONAL MODELS FOR STUDYING DENDRITIC INTEGRATION OF SYNCHRONIZED SYNAPTIC INPUT

![Diagram showing time-window for synaptic input summation. Ten inputs, the frequency of which is synchronized at 70%. The time-window for the ten inputs is T0 – TW. Note that both the occurrence of synaptic input at any time-window and the intervals between windows are stochastic, reflecting the inherent unpredictability of neural firing.]

Figure 7.2: Time–window for synaptic input summation. Ten inputs, the frequency of which is synchronized at 70%. The time–window for the ten inputs is T0 – TW. Note that both the occurrence of synaptic input at any time–window and the intervals between windows are stochastic, reflecting the inherent unpredictability of neural firing.

in the Figure 7.2 the synaptic input should in average use 10% of 83 ms. If the synchronization level is 100%, all the synaptic inputs are simultaneous. Figure 7.2 shows the temporal distribution of the input for the synchronization levels 100%, 70% and 50%.

Normal distribution model (Papers II–IV)

For this model of synaptic input, a normal distribution was used to generate the temporal distribution instead of the exponential distribution previously described. The standard deviation of the normal distribution, expressed in ms, was chosen as the measurement of the synchronization level. Figure 7.1 shows the temporal distribution of the input for the synchronization levels 0 ms, 5 ms and 10 ms. Both this model and the previous model are stochastic models, which to some degree serves as a test of the robustness of the models. Small alterations to the temporal distribution of the input should not significantly alter the functional behaviour.

Deterministic normal distribution model (Papers III–IV)

For this model, I used a temporal normal distribution and defined the temporal distribution of the input to represent a normal distribution. The advantage with this model, compared with the previous two, is that it is deterministic and therefore the need for repeated simulations required in the former models is eliminated. This model was mainly used during optimization, since it is less computationally demanding than the previous two models. Figure 7.1 shows the temporal distribution of the input for the synchronization levels 0 ms, 5 ms and 10 ms.
Model of fast ripple input

During a fast ripple event, a neuron population is activated synchronously at high frequency. In my work, I focus on the activity during a fast ripple event and not on the mechanisms underlying fast ripples. By simulating the input to a pyramidal cell during a fast ripple event, I can study the ability of $K_A$ to reduce the neural response.

Fast ripple events have abnormally high frequencies. This implies that this is a population frequency rather than an individual neuron frequency, since CA1 pyramidal cells cannot fire at the fast ripple frequency for an extended period. As far as I know, the activity of individual neurons during fast ripples has not been recorded. I therefore derived two fast ripple models from two hypotheses of how fast ripples occur, the first based on an *in vitro* study and the second on an *in vivo* study. Both models are further described in the section below.

**Fast ripple model: spontaneous synchronized burst–type discharges**

The CA3 neurons projects onto the CA1 neurons (Bragin et al., 2002), which are the cells I study in this thesis. The CA3 neurons can activate CA1 synapses with burst–like behaviour; however, the mechanisms generating CA3 burst–type activity have not been established. The burst of input generates a burst–type activity in the CA1 cells. Fast ripple activity may occur when these burst–type discharges are synchronized (Dzhala and Staley, 2004). The second fast ripple model is based on data from Volodymyr et al. (2004), who recorded spontaneous burst–type neural discharges from brain slices. The interspike interval (ISI) was measured using cell–attached recordings of CA1 and CA3 pyramidal cells. To generate fast ripple oscillation, the extracellular potassium was increased. At times of high potassium concentration, the ISI decreased and the cell delay approached zero. The fast ripple input was generated by ten synapses that were activated according to the ISI measured at times of high potassium concentration. The ten input time series were evenly distributed with a time delay of 0.15 ms. For the control case, the ISI was from normal extracellular potassium and the delay was 2.5 ms. Figure 7.3 shows the temporal distribution of the synaptic input.

**Fast ripple model: synchronized ripples**

When fast ripples are generated, the neurons are first synchronized and then the frequency increases (Lasztoczi et al., 2004). We therefore hypothesized that fast ripples may be a synchronized variant of ripples (Engel et al., 2009). Conversely, if the increased synchronization is suppressed, this may reduce the fast ripple activity. As mentioned before, the activity of individual cells during fast ripples has not been established, so we do not know how synchronized the cells are. However, ripples have been studied more thoroughly, so I could construct a model of ripples. By increasing the synchronization of the input in the ripple model, I generated a fast ripple model, featuring synchronized ripples. Only 11% of the neurons participate.
7.2. COMPUTATIONAL MODELS FOR STUDYING ACTION POTENTIAL VELOCITY CHANGES IN C–FIBRES

Figure 7.3: Model 1: Spontaneous synchronized burst-type discharges. The top three graphs represent spontaneous burst–type discharges of individual neurons. The left column represents fast ripple activity and the right column is the control. The vertical marks represent time points of synaptic input. The bottom graphs represent the summation of all ten inputs.

In each ripple (Ylinen et al., 1995; Buzsaki et al., 1992). I focused on the neurons constituting the core of the ripple and therefore used a frequency of 50Hz, adding ten synaptic input series were at this frequency. The ten input time series were evenly distributed with a time delay of 0.3ms; the time delay of the control was 1.1ms (Ylinen et al., 1995). Figure 7.4 shows the temporal distribution of the synaptic input.

7.2 Computational models for studying action potential velocity changes in C–fibres

In clinical studies, the change in action potential conduction velocity can be measured, but not explained by ion channel properties. Ion channel properties are highly relevant for drug development. Therefore, computational models are needed to bridge the gap between the functional behaviour of action potential conduction velocity and ion channel dynamics. For the result to be relevant for drug development, the models of ion channels must be detailed enough to enable different pharmacological alterations in the model. For this purpose, we have developed a detailed model with nine specific ion channels and with cell morphology designed to enable measurement of the propagation of action potentials.
The C-fibre model has been developed in collaboration with Dr. Schmelz’s research group in Mannheim, which comprises an electrophysiological, an animal, and a clinical laboratory. We have received valuable information and constructive criticism from this group, input that has been invaluable in constructing the model. In this section, I will describe the C-fibre model and its underlying assumptions.

**Morphology**

The C-fibre model consists of three sections: the branch (400 compartments), the cone (100 compartments), and the parent section (2000 compartments) (see Figure 7.5). The branch section represents the distal superficial axon, and the cone and parent sections the proximal parts of the C-fibre axon. When an action potential travels from the branch axon directly to the parent axon, it propagates poorly due to the large difference in axon diameters. The biology of the transition between the branch and the parent has not been fully understood. The cone section has been implemented in the model to facilitate the propagation from the branch to the parent section. Two known differences between the distal and proximal parts of the axon are the diameter and the temperature. The branch axon is more superficial in the skin and is therefore lower in temperature than the parent, which is at body
7.2. COMPUTATIONAL MODELS FOR STUDYING ACTION POTENTIAL VELOCITY CHANGES IN C–FIBRES

**Figure 7.5:** Morphology of the C-fibre. Showing the spatial distribution: branch (2 cm, 32°C), cone (0.5 cm, 34.5°C) and parent axon (10 cm, 37°C).

Both these features were implemented in the model (see Figure 7.5).

**Ion channel models**

In C-fibre research, sodium channels have been studied more than potassium channels. We therefore used existing models of the three sodium channels: the $\text{Na}V_{1.7}$ (Sheets et al., 2007), $\text{Na}V_{1.8}$ (Maingret et al., 2008), and $\text{Na}V_{1.9}$ (Herzog et al., 2001) sodium channels. The model also includes the following five ion channels: $K_A$ (Sheets et al., 2007), $K_M$, $K_{DR}$ (Sheets et al., 2007), sodium-dependent K, and h-channel. Furthermore, we have converted the kinetics of the ion channel from room temperature to body temperature kinetics.

**Concentration model**

When modelling CNS neurons, the intracellular concentration of sodium is usually kept constant. This assumption is justified for neurons, which have a relatively large volume relative to the cell membrane surface. For thin axons, ions can accumulate more easily. In the model, we have implemented the intracellular ion concentrations as a variable depending on the ionic flow through the membrane. The axons are tightly packed in bundles in the body; the volume between the axons is called the periaxonal space. In the C-fibre model, we have modelled the periaxonal space as a separate volume in which the ion concentration can be altered due to action potential activity.

**Implementing the model**

The purpose of the C-fibre model is to study two functional behaviours: ADS and the supernormal phase. The main challenge of developing the model was the lack of intracellular membrane potential data. The axons of C-fibres are too thin to measure the membrane potential intracellularly, but somatic intracellular recordings have frequently been made. We used the dynamics and conductances observed in the soma as a starting point. An optimizer, the Hook and Jeeves algorithm was used to find conductance parameters that generated ADS. We also wanted to incorporate several other functional behaviours, such as the supernormal phase and experimental pump block data. In addition, we had data on conductance velocity, action potential width, and functional behaviour of certain channels. When several
constraints were incorporated in the cost function, the optimizer could not find a solution, because it would reach points where attempting to improve one constraint would lead to another constraint suffering. Therefore, we mainly hand-tuned most of the parameters in the C-fibre model.

The C–fibre model compared with existing models

In the C–fibre model, we included usually neglected details, for example, the accumulation of intracellular sodium. When modelling CNS neurons, the intracellular sodium concentration is usually kept constant.

Another feature that distinguishes the C–fibre model from most of the existing models of peripheral neurons is the temperature. Most ion channel experiments treating the peripheral nervous system are performed at room temperature (Sheets et al., 2007; Herzog et al., 2001; Kovalsky et al., 2009). Therefore, most ion channel models use room-temperature kinetics, even though temperature significantly alters ion channel kinetics, which can result in different functional behaviour. We have therefore converted the kinetics of the ion channels and the pumps to be consistent with body temperature. To translate the time constants of the ion channels, we used the temperature coefficient ($Q_{10}$), which represents the factor by which the time constant changes for every change in temperature by ten degrees. For instance, if the $Q_{10}$ is 3 for a specific ion channel, the time constant in room temperature is approximately 7 times larger than in body temperature.

7.3 Optimization algorithms used in this thesis

In diseases of the brain, the distribution and properties of ion channels display deviations from healthy control subjects. In Paper II, I studied three cases of ion channel alteration related to epileptogenesis, and derived three pathological models of epilepsy. The main objective of the research was to apply computational modelling and optimization to reverse the pathological models and restore normal neural functions. The input parameters to the optimizer were the relative concentrations of the modulatory substances KChIP1, DPP6, PKC and CaMKII. To identify the relative concentrations of the modulatory substances, I used a pattern search method developed by Hook and Jeeves. It is a simple direct search method, which only uses the output returned by the model to guide the search and belongs to the family of pattern search algorithms (Hooke and Jeeves, 1961). The algorithm searches in various directions in the input parameter space. If a specific direction in the parameter space reduces the cost function, the optimizer will continue taking steps in that direction. When a direction is not successful in reducing the cost function, the optimizer will search for a new alternative direction in the input parameter space that does reduce the cost function. If no directions reduce the cost function, the step will be reduced and new directions will be evaluated. The Hook
and Jeeves algorithm has been used frequently in the thesis due to its simplicity and stable convergence.\cite{Kolda2003, Torczon1997}.

### Programs used in this thesis

The simulations were performed using the NEURON simulation environment.\cite{Hines1997} NEURON is a simulator that is used for cell and network models; the code is open source and is available at http://www.neuron.yale.edu/neuron. An advantage over other tools is NEURON’s adaptive time step. When the system is changing slowly, the time step will be long, but when the system is changing rapidly, the time step is shortened. In our simulations, there are very small system changes for long periods of time, so the adaptive time step reduces the simulation time significantly. In NEURON, the cell morphology is separated from the compartmental structure. The dendrites are divided into compartments, which in turn are divided into segments. This allows the user to increase the number of segments without changing the number of compartments. MATLAB was used to create model input and to analyse the output. MATLAB is a tool for manipulating matrices; it is compact and its powerful notation is ideally suited for data analysis.

### In vitro recordings in pyramidal cells

We have recently begun to experimentally investigate the potential suppression of synchronized input by $K_A$ (Silberberg, Planert, Tigerholm, Fransén). The idea behind the experiment was to reproduce the simulation of differential suppression of the synchronous input in vitro. For experimental convenience, the current injections were moved from the dendrites to the soma, since it is more difficult to perform membrane potential recordings and injections on thin dendrites than on the relatively large soma.

Before the experiment, I modelled the input on the soma in order to investigate the implications for the suppression of $K_A$. If synaptic input with the same fast dynamics as recorded in the dendrites were used (reviewed in Magee et al., 2000) (as opposed to the slow kinetics in the soma), selective suppression was obtained. I could therefore use the model to design the optimal parameters, which would lead to a successful experiment. There are three main reasons why the suppression of highly synchronized input by $K_A$ is lower in the soma. The first is the lower conductance of $K_A$, the second is that the dynamics of $K_A$ is altered in the soma (Hoffman et al., 1997) and the third that the integration through a large dendritic tree does not occur. The current through this synapse model was used as input in the in vitro experiment. The in vitro experiment was performed by Gilad Silberberg and Henrike Planert and for a description of the experiment please refer to Appendix – Experimental procedures.
Chapter 8

Results and discussions

In this thesis, I describe how nerve cells in the central and peripheral nervous systems can process inputs. The main focus in the CA1 pyramidal neuron is on the integration of synaptic input from the dendritic input site to the soma, where spike initiation occurs. Along this spatial path, through the dendritic tree, ion channels play a key role in shaping the EPSP. It is technically difficult to experimentally measure this integration, since the distal dendrites are very thin. I therefore studied dendritic integration using a detailed multicompartment model with a wide range of ion channels.

8.1 $K_A$ and synchronized synaptic input

One objective of my thesis is to investigate whether $K_A$ can reduce the cellular response to highly synchronized neural while minimally affecting desynchronized input. In this section, I will present results concerning the ability of $K_A$ to selectively reduce the cellular response to highly synchronized input. The results presented in this section were tested in an in vitro experiment, see section 8.2.

$K_A$ selectively reduces the cellular response to synchronized input (Papers I and IV)

According to conventional views, and consistent with basic biophysics, synchronized input is more efficient in activating a neuron, since it generates a larger depolarization than does less synchronized input. During a large depolarization, the neuron would be closer to the somatic spike threshold and therefore more easily activated. However, when the EPSP is travelling from the input site to the soma, it is modulated by numerous ion channels. These ion channels have unique properties and can therefore generate neural integration properties not predicted by passive biophysics. I will present the results of the model and synaptic input configuration
applied in Paper IV. In this configuration, I provided synaptic input to five oblique dendrites, on each of which five synaptic inputs were placed (see Figure 8.1), for a total of 25 synaptic inputs. The synchronization level is measured as the temporal standard deviation of the synaptic input, which is generated from a normal distribution (Figure 8.1). Thus, a smaller standard deviation generates a higher synchronization level of the synaptic input.

As expected, the general trend was that more synchronized activity led to the generation of more spikes. However, note the reduced spike activity in the 0–2 ms interval (see Figure 8.2), which corresponds to highly synchronized synaptic input. In conclusion, the results indicate that $K_A$ can reduce highly synchronized input while minimally affecting lower levels of synchronized input.

The results remain consistent when different cell models are used (Migliore et al., 1999, Poolos et al., 2002, Migliore et al., 2008, Poirazi et al., 2003). In modelling synchronicity, I have used stochastic models, both Exponential and normal distribution based, as well as deterministic models. All model results led to the same
8.1. $K_A$ AND SYNCHRONIZED SYNAPTIC INPUT

Figure 8.2: Spike response diagram for input at different synchronicity levels. (A-C) The left column shows simulation results with a synchronicity value of 0 ms, middle column of 4 ms, and right column of 8 ms. (A) Temporal distribution of synaptic inputs. Each bar corresponds to one synaptic input. The 5 inputs are superimposed in the left panel. (B) The membrane potential (EPSP) at one of the input sites. (C) The membrane potential at the soma. (D)

...conclusion, i.e., that $K_A$ could suppress the cellular response to highly synchronized synaptic input. Four cell morphologies were used in the various papers, as well as a control model developed by Poirazi et al., 2003. Compared with the cell models mainly used in this thesis, the Poirazi model includes calcium dynamics and a larger range of ion channels.

The selectivity of $K_A$ originates from its combination of fast activation and slow inactivation (Paper I)

To understand how $K_A$ can selectively reduce synchronized input, two simulations using different levels of synchronized input were compared. Figure 8.3 shows the results of these simulations. The $K_A$ current is significantly higher with the synchronized than the semi–synchronized input (see Figure 8.3 at 6 ms). Since EPSPs from synchronized versus semi–synchronized input have different depolarization shapes, $K_A$ can differentiate between them. When $K_A$ is not present, synchronized
CHAPTER 8. RESULTS AND DISCUSSIONS

Figure 8.3: Activation of \( K_A \) by synchronized versus semi-synchronized input. Synchronous input (100%), shown in red, activates \( K_A \) more than semi-synchronous input (80%), shown in black. A: Membrane potential in the soma. Inset shows initial slope of EPSP more clearly. B: Current through \( K_A \) at input site. C: Activation of \( K_A \) at input site. D: Inactivation of \( K_A \) at input site.

input generates a large and rapid depolarization, whereas semi–synchronized input generates a smaller, slower depolarization. Larger depolarization, generated by synchronized input, activates the neuron more effectively than does semi–synchronized input. However, when \( K_A \) is present this is not the case. When the membrane potential increases rapidly, the \( K_A \) channel has time to activate but does not have time for inactivation. This results in a large \( K_A \) current, which is an outward positive current making the cell less excitable and thereby preventing the cell from becoming activated. Semi–synchronized input generates a smaller, slower depolarization thereby making it less effective in activating the \( K_A \) channel. Semi–synchronized inputs produce a slow increase in membrane potential giving \( K_A \) time to inactivate, which results in a smaller \( K_A \) current. This result indicates that the inactivation time constant may play a crucial role in the ability of \( K_A \) to suppress synchronized input.

One major difference between various synchronization levels is the slope (derivative) of the rising phase of the EPSP. To further analyse the influence of the derivative
on the differential activation of $K_A$, I conducted voltage-clamp simulations providing triangular ramps as inputs to the location of the synapse (see Figure 8.4). To enable comparisons of cases using different derivatives, I kept the area of the triangular voltages constant, as higher potentials and longer times always generate more activation and more current. As can be seen in Figure 8.4, higher rising derivatives generate higher $K_A$ activation. These differences can be seen in both the current’s amplitude and integral. In general, inputs of higher synchronicity produce compound EPSPs with higher derivatives, which, as demonstrated, activate $K_A$ to a greater degree.

**Higher levels of synchronized input are suppressed when the input is placed on distal dendrites (Paper IV)**

Single cells can perform complex dendritic integrations due to their elaborate dendritic tree structure with numerous ion channels. Since neurons have large dendritic trees, I wanted to study whether the cellular suppression of synchronized input depends on the spatial location of the input. CA1 pyramidal neurons have a large branched dendritic tree. Connected to the soma is a long thick dendrite, called the apical trunk dendrite. Several so-called oblique dendrites emerge from the main apical trunk dendrite. In Paper IV, I demonstrated that when the input was placed on distal oblique dendrites, higher levels of synchronized input could

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**Figure 8.4:** Sensitivity to voltage slope. The top graph shows voltage clamp command of different initial slope but same area. The bottom graph shows the corresponding current through the $K_A$ channel. Note the difference in the peak current at 25 ms. The legend indicates the integral of the current for each protocol.
be suppressed than when input was placed on proximal oblique dendrites (see Figure 8.5). Interestingly, when the input was placed on the apical trunk dendrite, $K_A$ could not selectively suppress highly synchronized input at all (see Figure 8.5), which is consistent with experimental observations (Gasparini et al., 2004). I further demonstrated that the apical trunk possesses a longer time constant than do the oblique dendrites, possibly due to the larger diameter of its trunk (Trommald et al., 1995). The suppression by $K_A$ originates from its fast activation and its slower inactivation. When input is located on the apical trunk dendrite, the membrane depolarizes more slowly and the $K_A$ channel has time to inactivate, thereby generating a smaller $K_A$ current.

Since pyramidal cells have few synapses on the apical trunk dendrite (Andersen et al., 1980), this result may not be biologically relevant. However, it is still of interest, since most patch–clamp experiments on dendrites are performed on apical trunk dendrites, and my results indicate that apical trunk dendrites integrate input significantly differently from oblique dendrites.

The suppression of synchronized input can be modulated (Papers I and IV)

In Paper I, I varied the conductance of $K_A$ and thereby altered the dendritic integration of the synchronized input. If the conductance of $K_A$ is reduced, its ability to suppress highly synchronized input is reduced as well. In Paper IV, I confirmed that finding in a larger study, in which I examined whether the conclusions also applied to different spatial locations of the input. Neurmodulators such as PKC (Hoffman and Johnston, 1998), CaMKII (Varga et al., 2004), and acetylcholine (Nakajima et al., 1991) modulated the $K_A$ current. Furthermore, activity–dependent mechanisms of intrinsic excitability, depending on, for example, the back–propagating action potential, can open voltage–gated Ca–channels. The resulting calcium influx can lead to $K_A$ channel internalization (Tsubokawa et al., 2000; Kim et al., 2007), thereby downregulating the $K_A$ current and the suppression of highly synchronized input. This suggests modulatory substances or intrinsic excitability mechanisms can regulate the cellular response to synchronized input via the $K_A$ channel.

Discussion

In this section, I have described how the $K_A$ channel can selectively reduce synchronized input. As mentioned in Papers I and IV, lower efficacy of the most synchronized input was found in the study by Branco et al. (2010). Their data supported the role of NMDA receptors for the lower efficacy of the most synchronized input, as did the data of González et al. (2011).

In Papers I and IV, $K_A$ channels are demonstrated to play the major role in suppressing highly synchronized input; however, the impact from the NMDA receptor
8.1. $K_A$ AND SYNCHRONIZED SYNAPTIC INPUT

Figure 8.5: More suppression of high synchronicity levels for distal than for proximal locations on the dendrites. The figure illustrates the spike activity for different synchronicity levels when the input was moved from proximal to distal locations on the dendrites. We show the spike probability when timing was randomly chosen from a normal distribution (black) and repeated 100 times. We also show the number of spikes generated when the inputs were sampled so to obtain a normal distribution (blue). Each arrow in the schematic figure corresponds to five synaptic inputs. A. The input was placed on the five most proximal obliques (black arrows) and then systematically moved out to more distal oblique dendrites (blue and red arrows). The position of the input on the oblique dendrites was 10 $\mu$m from the apical trunk. The curves show from the bottom to the top the spike activity for proximal to distal oblique dendrites for different synchronicity levels. B. The input was placed close to the apical trunk on five distal obliques (black arrows) and then systematically moved to the distal end of the obliques (blue and red arrows). The synapses were placed on the same five obliques and moved out to the distal end of the obliques. The curves show from the bottom to the top the spike activity for proximal to distal position on the oblique dendrite for different synchronicity levels. The distance from the apical trunk was 0 $\mu$m, 30 $\mu$m, 60 $\mu$m and 90 $\mu$m (from top to bottom). C. On five neighboring locations on the apical trunk, five synaptic inputs were placed. The synaptic input is moved out along the apical trunk. The figure shows spikes produced when the input locations were moved from proximal (bottom) to distal (top) locations. D. Summary showing the highest synchronicity levels which the neuron can suppress for different spatial locations. The oblique dendrites are numbered from 1 to 10 starting from the soma.
has not been studied. The differences between our findings and Gasparini et al., (2004) may be explained by differences in resting membrane potential. In Paper IV, we demonstrated that selective suppression is strongest in the case of hyperpolarized membrane potential. In a study by Gasparini et al., (2004), on which González et al. (2011) build, the membrane potential was depolarized by changing the ionic composition of the extracellular medium. Both the NMDA receptor and $K_A$ may contribute to the reduction of synchronized input. The fast activation of $K_A$ will, as demonstrated in previously in the thesis, effectively reduce the amplitude of the EPSP, preventing the NMDA receptor from being released from its Mg block. $K_A$ will therefore be associated with the fast AMPA component of the EPSP and may modulate the effect of a subsequent slower NMDA component.
8.2 IN VITRO RECORDINGS SUPPORT THE SUPPRESSION OF SYNCHRONIZED INPUT

8.2 In vitro recordings support the suppression of synchronized input

In the previous chapter, computational simulation indicated that $K_A$ can selectively reduce the cellular response to highly synchronized synaptic input. I wanted to test the model prediction in an in vitro experiment. We therefore recently initiated collaboration with Silberberg and Planert in which we began experimentally investigating the suppression of synchronized input by $K_A$. In the experiment, we tried to mimic the simulation described in the previous section. In the model simulation, I placed a synchronous synaptic input on a distal dendrite. Experimentally, it is technically difficult to inject into or record from distal dendrites, so the experiment was performed in the soma.

Table 8.1: in vitro experiment testing the cellular response to two levels of synchronized input, 100% and 98%. The experiment was performed by Silberberg and Planert.

<table>
<thead>
<tr>
<th>Input</th>
<th>cell 1</th>
<th>cell 2</th>
<th>cell 3</th>
<th>cell 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synchronized (#spikes)</td>
<td>10</td>
<td>21</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Semi–synchronized (#spikes)</td>
<td>15</td>
<td>29</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Reduction(%)</td>
<td>33</td>
<td>28</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

In the model, I recorded the current induced in the soma by the synaptic input on dendrites, and the current was used in the experiment. Two types of currents were injected, representing synchronized and semi–synchronized synaptic input. The membrane potential was measured in the pyramidal cells during the experiment. The results are presented in Table 8.1. During synchronized input, cellular responses decreased by 10–33% compared with semi–synchronized input. This result supports the simulation prediction.

Figure 8.6 shows traces of the injected current and the cellular response in terms of membrane potential. To avoid the effect of wash–out protein, which might also modulate $K_A$, stimuli representing synchronized (100%) and semi–synchronized (98%) inputs were alternated. Despite the significantly higher amplitude of the current in the synchronized case, the spike activity was lower. When the current corresponding to the semi–synchronized input was injected again, cellular activity reverted to the previous state. This indicates that cell excitability did not change during the experiment. The triggering of some spikes in the synchronized case was delayed compared with the semi–synchronized case; this is a well–known effect of
the $K_A$ current (Melnick 2011). This supports our interpretation that the $K_A$ current reduces the synchronized input.

In this section, I have tested the prediction that $K_A$ can suppress highly synchronized synaptic input. I am fortunate to have had the opportunity to test a prediction, derived from the results of a computational model, in an in vitro experiment. The outcome of the in vitro experiment showed that current injections corresponding to highly synchronized input generated fewer action potentials than did semi–synchronized input, which supports the prediction. This result needs to be complemented by additional recordings in more cells, since so far only four cells have been recorded. I am still awaiting the control experiment in which blocking $K_A$ should lead to a loss of differential suppression.
8.3 Ability of K₄ to suppress synchronized input and the implication for cognitive functions

CA1 pyramidal cells receive input from several pathways. Two of the major pathways are the Schaffer collateral and the perforant pathways. The Schaffer collaterals project from the CA3 to the oblique dendrites and the perforant pathway projects from the entorhinal cortex to the most distal dendrites called tuft dendrites (see Figure 8.7). Previously in the thesis, I have examined the integration of synchronized input placed on the oblique dendrites, representing input from the Schaffer collaterals. In this section, I will describe the implications for cognitive processing of the ability of K₄ to suppress synchronized input. Finally in this section, I will discuss how the perforant pathway can interact with Schaffer collateral input and thereby modulate the cell integration of synchronized synaptic input.

Figure 8.7: A schematic figure of the pyramidal cell and the placement of the synaptic input. For a given simulation, 5 oblique dendrites were selected based on the nature of the test. On each oblique dendrite, five synaptic inputs were added; represented in the figure with blue arrows. Additional input was also placed on 11 distal tuft dendrites (green arrows).

K₄ suppression of dendritic sodium spikes (Paper IV)

The dendritic integration properties of CA1 pyramidal neurons, related to dendritic sodium spike generation, have been discussed recently (Gasparini and Magee, 2006; González et al., 2011; Makara et al., 2009). Dendritic sodium spikes occur mainly
when the neuron receives highly synchronized synaptic input (Gasparini et al., 2004; Losonczy et al., 2008). Dendritic sodium spikes are common and more frequent than somatic spikes especially during the sharp wave–ripple complex (Kamondi et al., 1998). These dendritic sodium spikes can generate a two-step integration process (Losonczy et al., 2008). The input is first integrated locally in the dendrite, where a dendritic spike can be generated. Subsequently, a second integration step occurs in the soma, where a somatic spike can be generated. In vivo, dendritic sodium spikes are strongly suppressed and do not always generate spikes in the soma (Kamondi et al., 1998), as is the case in vitro (Gasparini et al., 2004). I have examined the suppression of dendritic sodium spikes in Paper IV. In that study, I investigated the influence of the spatial location of synaptic input on dendritic spike propagation. At distal input sites, dendritic sodium spikes were generated but did not invade the apical trunk dendrite (see Figure 8.8). Both my results and those of experimental studies (Gasparini et al., 2004; Makara et al., 2009) indicate that $K_A$ plays an important role in suppression of dendritic sodium spikes.

**Influence of input to tuft dendrites on dendritic integration (Paper IV)**

Previously in the thesis, I examined highly synchronized input projecting via the Schaffer collateral pathway. In this section, I examine how the input, via the perforant path, influences the CA1 pyramidal cell’s integration of synchronized synaptic input via the Schaffer collateral pathway. The perforant pathway mediates not only excitatory input, but also inhibition of the distal dendrites (Ang et al., 2005). Input from the entorhinal cortex interacts with the Schaffer collateral input and affects the spike output in CA1 cells (Takahashi and Sakurai, 2009; Dudman et al., 2007; Ang et al., 2005). Since the perforant pathway has a relatively small direct influence on spike activity, this pathway is postulated to have a modulatory function (Otani et al., 1995; Dudman et al., 2007). I therefore placed either excitatory or inhibitory input on the tuft dendrites to examine whether the suppression of synchronized synaptic input via the Schaffer collateral pathway was affected. The dendritic integration of synchronized input was strongly influenced by the input on the tuft (Figures 8.8 and 8.9). Both the suppression of dendritic sodium spikes (see Figure 8.8) and the generation of somatic spikes (see Figure 8.9) were reduced when excitatory input was placed on the tuft dendrites, particularly at higher synchronization levels. Therefore, input on the tuft may regulate the CA1 cell’s dendritic integration of synchronized input and the propagation of dendritic sodium spikes. The circuitry linking CA1, CA3, and the entorhinal cortex has led to the postulate that CA1 neurons compare direct sensory information with memory traces from associative hippocampal networks (Lisman, 1999). Our results indicate that if input from the Schaffer collateral and perforant pathways coincides, this would reduce the suppression of both dendritic and somatic spikes.
8.3. ABILITY OF $K_A$ TO SUPPRESS SYNCHRONIZED INPUT AND THE IMPLICATION FOR COGNITIVE FUNCTIONS

Figure 8.8: Generation of dendritic spikes is influenced by the spatial location of the input, conductance of $K_A$ and excitatory input to the tuft. Each figure in A, B, C and D show if a dendritic spike was generated in the apical trunk (50 mm from the soma) for different input configurations. Along top to bottom different traces correspond to distal to proximal input sites (A and C the radial axis and B and D the somato–dendritic axis). A and B show the spikes activity when the conductance of $K_A$ was reduced by 25% (green curves) compared with control (blue curves). C and D show the spikes activity when the excitatory input was added to the tuft (black curves) compared with control (blue curves). Note that distal dendrites become more similar to proximal dendrites if the conductance of $K_A$ is reduced or synaptic input was added to the tuft.

Discussion

In this section, I have mainly presented the results of Paper IV, which concerns the dendritic integration of synchronized input. The main implication of Paper IV is that the ability of $K_A$ to modulate the cellular response to synchronized input may affect memory and learning. It is widely known that memory and learning are dependent on the strengthening of synapses (Sjöström et al., 2008). Long–term potentiation is one such mechanism that strengthens synapses and can be induced by large EPSP and/or the back propagation of action potentials (Sjöström et al., 2008). $K_A$ selectivity reduces large, rapid EPSP generated by synchronized input. The amplitude of the compound EPSP is reduced by $K_A$, thereby activating other voltage–dependent processes, such as calcium channels and NMDA receptors.
**Figure 8.9:** Excitatory input placed on the tuft modulates the dendritic suppression of synchronized input. The figure illustrates the spike activity for different synchronicity levels of the input when additional excitatory synaptic input was placed on the distal parts of the tuft and activated with a frequency of 100 Hz. Each arrow corresponds to five synaptic inputs. The input was placed close to the apical trunk on the five distal oblique dendrites (black arrows) and then systematically moved outward to the distal end of the obliques (blue and red arrows). The green arrows correspond to excitatory synaptic input on the tuft. The synapses were placed on the same five obliques and moved out to the distal end of the obliques. The distance from the apical trunk was 0 µm, 10 µm, 20 µm, 30 µm, 40 µm, 50 µm, 60 µm, 70 µm, 80 µm, and 90 µm (from top to bottom). The left curves (blue) represent the control and the right curves (black) have tuft inputs set to the same input strength as the input to the obliques. The middle curves (blue) have tuft input set to 10% of the synaptic strength of the oblique dendrites.

Therefore, $K_A$ activation will reduce the probability of triggering Ca and NMDA spikes associated with learning and memory.

Furthermore, dendritic sodium spikes generate significant depolarization and can therefore generate long-term potentiation. A dendritic sodium spike generates a rapid and large EPSP, which has the features needed to activate $K_A$ efficiently. Both our results and experimental studies (Gasparini et al., 2004; Makara et al., 2009) indicate that $K_A$ plays an important role in modulating dendritic sodium spikes. Functionally, dendritic sodium spikes have been implicated in memory processing,
8.3. ABILITY OF K_A TO SUPPRESS SYNCHRONIZED INPUT AND THE IMPLICATION FOR COGNITIVE FUNCTIONS

since they occur during sharp wave–ripple complex activity (Kamondi et al., 1998). This is supported by the fact that more dendritic sodium spikes are generated if a rat is exposed to an enriched environment (Makara et al., 2009).

Furthermore, K_A has been implicated in long–term potentiation (Ramakers and Storm, 2002; Chen et al., 2006) demonstrated that K_A can reduce the back–propagating action potential and thereby prevent long–term potentiation. Our understanding is somewhat different, in that we suggests that K_A may also be involved in affecting the amplitude of the forward–propagating dendritic EPSP/dendritic spike, and thereby potentially determine whether or not a somatic spike is generated.

Limitations of the model

In this section, I have examined the implications of the CA1 pyramidal cell dendritic integration of synchronized input. Highly synchronized activity can occur during the sharp wave–ripple complex. I have not developed synaptic input corresponds to the input during a sharp wave–ripple complex; instead, I have focused on one specific important feature, i.e., highly synchronized input, during a sharp wave–ripple complex. A major reason for this is that the mechanisms that generate ripples are not yet understood. It is debated whether sharp waves are generated by excitatory input from CA3 (Ylinen et al., 1995) or whether gap junctions generate high–frequency oscillation (Draguhn et al., 1998). Moreover, by focusing on synchronized excitatory input, I have omitted the inhibitory input, which plays an important role in generating a sharp wave–ripple complex.

Furthermore, calcium dynamics were not included in the CA1 cell model. Since calcium dynamics play an important role in generating long–term potentiation (Sjöström et al., 2008), it would be interesting to study the ability of K_A to reduce dendritic spikes in a cell model including calcium dynamics. Thus, the ability of K_A to suppress somatic spikes has been confirmed in a control model (Poirazi et al., 2003; Paper II) but not the suppression of dendritic spikes.
8.4 Ability of $K_A$ to suppress synchronized input and the implications for pathological activity

Pathological hypersynchronous activity, i.e., fast ripples, has been identified as a possible signature marker of epileptogenic activity that may be involved in generating seizures (Chiu et al., 2006; Bragin et al., 1999; Ochi et al., 2007; Urrestarazu et al., 2006). The ability to reduce hypersynchronous activity is therefore of great importance. Epilepsy has mainly been studied using network models (Soltesz and Staley, 2008), and seizures are ultimately a network phenomenon. In this study, I have taken a different approach by examining how network activity affects a single neuron. I hypothesize that if the cellular response to hypersynchronous input is reduced, this would beneficially reduce epileptic activity.

Fast ripples modelled as spontaneous synchronized burst–type discharges (Paper I)

According to Dzhala and Staley (2004), spontaneous synchronization of burst patterns is the mechanism underlying fast ripple generation. In their study, they measured spontaneous bursts in hippocampal CA1 and CA3 pyramidal cells. During fast ripple activity, the pyramidal cells reduced their ISI relative to control conditions; in addition, the delay between the bursts approached zero.

Figure 8.10 shows a reduction in spike activity when $K_A$ is present in the fast ripple model. Importantly, $K_A$ does not reduce activity in the event of normal spontaneous burst–type discharges, but suppresses fast ripple activity. When $K_A$ is not present in the model, fast ripple input generates high spike activity. The current through the $K_A$ channel is high at both the beginning and end of a fast ripple. In the middle, the current is low because the $K_A$ is not released from inactivation since the membrane potential is depolarized between burst of inputs. Even though $K_A$ did not fully prevent the cell from becoming activated by fast ripple activity, reduced activity can be important in a recurrent network. In this type of network, $K_A$ could convert a positive to a negative feedback loop by reducing the number of produced spikes.

Fast Ripples Modelled as a Synchronous Ripple (Paper I)

The model was based on data from an in vivo study by Ylinen et al. (1995), in which they measured several cells during ripple activity. Using their data, I constructed a ripple input model, modelling the fast ripple as a ripple of enhanced synchronicity. However, as ripples have been studied fairly thoroughly, I was able to construct a model of ripples. By increasing the synchronicity in the input to the ripple model, I generated a fast ripple model based on ripples with increase synchronization level. During ripple activity, only 11% of the neurons participate in each ripple (Ylinen...
8.4. ABILITY OF $K_A$ TO SUPPRESS SYNCHRONIZED INPUT AND THE IMPLICATIONS FOR PATHOLOGICAL ACTIVITY

Figure 8.10: Model 1: $K_A$ reduces response to fast ripple input. The first column represents the membrane potential measured in the soma. The second column represents the current through the $K_A$ channel measured at the location where the synaptic input was added. The third column represents gating variables of the $K_A$ channel measured at the location where the synaptic input was added. The black line represents activation and the red line inactivation. At five different distances from the soma ten synaptic input time series were added. The input time series for fast ripple model had the ISI: 3.8, 4.5, 4.9, 6.8, 7.2, 8.6, 9, 10, 10, 10 and for control: 5, 7, 9, 10, 10, 10, 10, 10, 10, 10. In the fast ripple case the ten synaptic time series were evenly distributed with the distance 0.15ms and for control 2.5ms. A: Fast ripple with no $K_A$ present. B: Fast ripple with $K_A$ present. C: Control. Note the reduction in spike activity in the fast ripple model when $K_A$ is present.

et al., 1995; Buzsaki et al., 1992). I focused on the neurons making up the core of the ripple and therefore used a frequency of 50Hz, adding ten synaptic input series with a frequency of 50Hz. The ten input time series were evenly distributed with a time delay of 0.3ms; the time delay of the control was 1.1ms (Ylinen et al., 1995).

Figure 8.11 shows that $K_A$ can prevent the cell from becoming activated by fast–ripple–generated input. The same figure shows the gating variable of $K_A$. Slow inactivation is unable to keep up with the variation in the membrane potential during a fast ripple, resulting in increased current. As an extension, I increased the input frequency to 100Hz, and $K_A$ still prevented the cell from becoming activated by fast–ripple–generated input.
Discussion

Both models, i.e., of spontaneous synchronized burst–type discharges and synchronous ripples, indicated that $K_A$ could reduce spike activity during fast ripple activity. Fast ripples have been suggested to be involved in generating seizures (Engel et al., 2009). By suppressing fast ripples, $K_A$ could prevent the activity from spreading, possibly preventing a seizure. This mechanism is in contrast to that of many antiepileptic drugs (Soltesz and Staley, 2008; Dichter and Brodie, 1996), which downregulate cell excitability by targeting sodium channels or GABA receptors. These antiepileptic drugs affect the cell during normal brain activity, thereby causing significant side effects. $K_A$, on the other hand, mainly affects the cell when it is exposed to abnormally high synchronized input. In healthy individuals, $K_A$ may serve to protect against fast ripple activity. In some patients
with epilepsy, this protection may be downregulated or dysfunctional, leading to increased susceptibility to seizures. Other patients may have functional $K_A$, but the cell is exposed to abnormally high synchronized input due to other factors. In both these cases, upregulation of the $K_A$ current would be beneficial in reducing epileptogenic activity. Both the pharmacological treatment (Zona et al., 2002) and ketogenic diet upregulate the $K_A$ current (Xu et al., 2008). Our results suggest that the beneficial effect is due to the reduced cellular response to highly synchronized input instead of a general downregulation of excitability.

A major limitation of the fast ripple model is that inhibitory synaptic input is omitted. If inhibitory synaptic input was added to the model, fast ripples might be even more suppressed. In the model, between the fast ripples, the membrane does not return to the resting membrane potential, so the $K_A$ channel will be slightly more inactivated for the next ripple. If inhibitory synaptic input were added to the model, the membrane would more easily return to the resting membrane potential or even lower, which could increase the $K_A$ current.

Another limitation of the model is that it does not include calcium dynamics, which may play a role when an input sequence is delivered and calcium can be accumulated intracellularly.
8.5 Modulation of ion channels to functionally reverse models of epilepsy

In the previous chapter, I demonstrated that $K_A$ can alter the neural integration of synchronized input by suppressing dendritic and somatic spikes. In normal conditions, the $K_A$ channel can reduce the cellular response to hypersynchronous synaptic input. However, in pathological conditions, the distribution and dynamic properties of numerous ion channels will deviate from those of healthy control subjects, so the $K_A$ current may not be strong enough to suppress the cellular response to hypersynchronous synaptic input. I then asked whether the upregulation of $K_A$, or any other channel, could alter the cellular response of a pathological cell such that it resembled a healthy control cell. To study this, I derived models of epilepsy by implementing alterations of ion channels linked with epileptogenesis (Papers II and III).

Pathological models of epilepsy (Papers II and III)

The first pathological model involves an enhanced sodium current (Castro et al., 2001; Biervert et al., 1998), which was represented in the model by increasing the transient sodium conductance. The second model of epilepsy addressed the down-regulation of the $K_A$ current (Vreugdenhil et al., 1996), represented by reducing the $K_A$ conductance. The third case is related to the properties of $K_A$ in a patient with temporal lobe epilepsy and was implemented using the measured biophysical properties of $K_A$ (Rüschenschmidt et al., 2004). All three pathological alterations resulted in a hyperexcitable cell in general, particularly in the case of highly synchronized input (see Figure 8.12, 0–2 ms).

Functional correction of pathological models (Papers II and III)

In Papers II and III, I focused on improving the functional behaviour of the neuron by modelling substances that target ion channels. Many substances modulate the $K_A$ channel, such as auxiliary proteins (An et al., 2000; Maffie and Rudy, 2008), kinases (Hoffman and Johnston, 1998; Varga et al., 2004), and PUFAs (Xu et al., 2008). In Papers II and III, I implemented several modulatory substances, altering their relative concentrations until the pathological model’s cellular response to synchronized input was consistent with that of the control model (no pathological alterations). To reverse pathological changes, in Paper II, I used a numerical optimizer to search for improved models. I chose the pattern search method developed by Hook and Jeeves (1961). The optimization method will be discussed in depth in section 8.7. In the next section, I will focus on the results and their implications.
8.5. MODULATION OF ION CHANNELS TO FUNCTIONALLY REVERSE MODELS OF EPILEPSY

PUFA modulation of ion channels to functional reverse models of epilepsy (Paper III)

The ketogenic diet has been a successful treatment of epileptic seizures since the 1920s, even though its underlying mechanisms have remained a mystery. Ion channels are altered by the ketogenic diet; in particular, the ATP–dependent potassium channel and various sodium channels have been thought to play an important role (reviewed in Bough and Rho 2007). One reason for the mystery regarding the underlying mechanisms of the diet is the complex metabolism of ketone bodies and PUFA. In an animal model used to study the anticonvulsant effects of PUFA, mice were fed with DHA and EPA, which are the metabolized forms of n–3 PUFA (Yang et al. 2011). Surprisingly, this study found no anticonvulsant effect, which was explained by the lack of interaction between n–6 PUFA. To explain

Figure 8.12: Increased excitability induced by pathological channel changes. The number of spikes produced for different levels of synchronicity are shown. Spiking in the control case is shown in blue and pathological cases are shown in red. Top trace shows the spike activity produced by the reduced $K_A$ model, the middle shows the increased Na model and the bottom trace the TLE model. Note the increased activity for all three pathological models to highly synchronized input (<2ms) and to lower levels of synchronized input (>10ms).
CHAPTER 8. RESULTS AND DISCUSSIONS

the modulation of ion channels by the ketogenic diet, Xu et al. (2008) chose a different approach. They used cerebral spinal fluid from children on a ketogenic diet as the extracellular solution in an expression system, investigating the alteration of potassium channels before and during the ketogenic diet. This was the starting point of the project resulting in Paper III. In collaboration with Fredrik Elinder and Sara Börjesson, we started a project to investigate how alteration related to the ketogenic diet could reduce the hyperexcitability in cell models.

In the next part, I will discuss four ion channel alterations linked with the ketogenic diets and their implications for hyperexcitability.

Sodium channels
PUFAs can modulate the Na channels by shifting the voltage dependence of channel inactivation (Vreugdenhil et al., 1996). If the steady–state inactivation curve of the sodium channel could be shifted, the pathological cell models’ response to highly synchronized input could be restored. However, the response to lower levels of synchronized input was also suppressed (see Figures 8.13 and 8.14). If both the steady–state activation and inactivation were instead shifted, the pathological models became more hyperexcitable. Therefore, a more selective modulation of only the inactivation is preferable. Indeed, the general pattern of PUFA modulation of Na channels is dominated by the effect on Na channel inactivation (Vreugdenhil et al., 1996).

Potassium channels
When PUFAs induced shifts in the K_A channels’ voltage dependence (Xu et al., 2008) activation was implemented in the pathological model the cellular response to highly synchronized input could be reduced without affecting lower levels of synchronized input (see Figures 8.13 and 8.14). Since K_A activation and inactivation are strongly coupled, I also shifted both steady–state activation and inactivation. With a double shift, the pathological model could still be reversed such that the spike output resembled that of the control model (i.e., no pathological alterations) (see Figure 8.15). A shift in the K_DR channel’s steady–state activation curve was unable to reverse any of the pathological models.

Hyperpolarizing shift of the resting membrane potential
In Paper III, I investigated whether a hyperpolarizing shift of the resting membrane potential would reduce the hyperexcitability to highly synchronized input (see Figures 8.13 and 8.14). This shift could be generated by several channels affecting the resting potential, such as the m–current, h–current, and K_ATP current. K_ATP alteration during the ketogenic diet has previously been discussed (Bough and Rho, 2007; Kim et al., 2007). I have demonstrated that the hyperpolarization of the membrane potential, potentially caused by the K_ATP channel, reduces the cellular response to highly synchronized input while minimally affecting the
8.5. MODULATION OF ION CHANNELS TO FUNCTIONALLY REVERSE MODELS OF EPILEPSY

Figure 8.13: Effects of PUFAs on the decreased $K_A$ current pathology model. (A–C) The spike activity for the functionally corrected model when (A) $K_{ATP}$ is modulated by shifting the steady-state activation curve ($-5.4$ mV), (B) Na is modulated by shifting the steady-state inactivation curve ($-15.4$ mV) and (C) the resting membrane potential was shifted ($-4.2$ mV). (D) The intervals of the shift of the steady-state curves or resting membrane potential where the model was functionally corrected (generated zero spikes for synchronicity level 0 ms and 15 spikes for synchronicity level 2 ms).

Discussion

In Paper III, I modelled the effects of PUFAs on excitability in a CA1 pyramidal cell. The underlying rationale was to explore a possible mechanism for the ketogenic diet used in treating epilepsy. Altogether, my results indicate that small alterations in either steady-state curves or resting membrane potential are effective cellular response to semi-synchronized input. One interesting question is whether the $K_{ATP}$ channel is upregulated during the ketogenic diet. It is well known that the ketogenic diet can increase ATP levels (Pan et al., 1999), and since the $K_{ATP}$ channel is blocked by ATP, this would reduce the current. However, during the ketogenic diet, concentrations of ketone bodies increase (Yellen et al., 2007), and they have demonstrated to upregulate the $K_{ATP}$ (Yellen et al., 2007). Since the experimental data are contradictory, the question remains unanswered.
in reducing hyperexcitability, particularly with highly synchronized input. It was an unexpected finding that a shift in the resting potential reduced the pathological excitability without leading to a decrease in general excitability. In epilepsy research, the term “hyperexcitable” is used as a synonym for “pathological”. The term “hyperexcitable” is a general term not specific to the particular input provided to the cell. I have instead studied hyperexcitability to highly synchronized input, which is more relevant to epileptic activity. The results presented in Paper III, concerning the modulation of the \( K_{DR} \) channel, exemplify the difference. Shifting the steady-state activation of \( K_{DR} \) could reduce the excitability for all synchronization levels, but no selective reduction of high synchronicity levels could be obtained. If the \( K_{DR} \) channel were upregulated to reduce epileptic activity, it would generate severe side effects, since it not only reduces the pathological hypersynchronous input, but also lowers levels that are important for numerous functions in the body.

The combined effects on several ion channels imply that beneficial effects can be
8.5. MODULATION OF ION CHANNELS TO FUNCTIONALLY REVERSE MODELS OF EPILEPSY

Achieved using lower concentrations of the modulator. These simulations highlight the possible usefulness in epilepsy treatment of a cocktail of pharmacological compounds, each with high specificity and targeting a different ion channel. The rationale for targeting ion channels is their significance for drug development. Ion channels represent the second largest target for drugs, after G-protein-coupled receptors [Overington et al. 2006]. Despite this fact, the drug industry has not yet fully exploited ion channels as drug targets, and in the future, interest in ion channels is likely to increase [Dunlop et al. 2008].

We have developed an optimization procedure for functionally correcting a pathological model by modulating ion channels. This procedure can be used to test potential drug targets at an early stage of drug development. We suggest that the
optimization procedure be applied not only to neurons, but also to other organs
with excitable cells, such as the heart and pancreas, where channelopathies are
found (Li et al. 2009).
8.6. MODELLING OF ACTION POTENTIAL CONDUCTION IN PERIPHERAL NERVE CELLS

8.6 Modelling of action potential conduction in peripheral nerve cells

The main reason why the model of a silent C–fibre was developed was to study various pain conditions due to peripheral axon hyperexcitability. Pain is a very subjective concept that is difficult to measure. We therefore focused on modelling action potential conduction, which is altered in several pain conditions (Serra et al., 2011; Ørstavik et al., 2003; Kiernan et al., 2005; Kanai et al., 2006). To experimentally determine the processes underlying changes in action potential conduction, measurements of membrane potential would be ideal. Unfortunately, intracellular recordings are difficult since these fibres are thin and hard to locate in the tissue they innervate. Therefore, we set out to identify the processes underlying activity-dependent changes in single unmyelinated nerve fibres using computational modelling. In paper V, we have mainly focused on ADS and recovery cycles. In the next part of this section I will describe the result from the C-fibre modelling project.

Activity–dependent slowing during repetitive stimulation (Paper V)

In the model, action potential propagation along the C-fibre and latency changes were measured (see figure 8.16). During repeated stimulation (2 Hz), the propagation latency increased from 212 ms to 289 ms generating a 36% reduction in propagation latency (see figure 8.16). This can be compared to the experimentally obtained value for ADS in humans, 36.7% (Obreja et al., 2010), and in pigs, 30.1% (Obreja et al., 2010).

We also conducted a simulation with a lower frequency protocol, figure 8.16. The resulting ADS magnitude after 0.125 Hz, 0.25 Hz and 0.5 Hz (max ADS 8%) corresponds well to experimental results (Obreja et al., 2010).

Accumulation of sodium contributes to activity–dependent slowing

The simulations performed show that the concentration of intracellular sodium is likely to play a central role in ADS. In Figure 8.17, the concentration of intracellular sodium and extracellular potassium is illustrated during the ADS protocol. Intracellular sodium accumulation is considerable because the diameter and thus volume of the axon is small and this results in a substantial reduction in the reversal potential for sodium during the ADS protocol 8.17. To examine the extent to which this change in reversal potential of sodium contributes to ADS, the concentrations of both intracellular sodium and extracellular potassium were clamped to their initial values. As can be seen in Figure 8.17 clamping the ionic concentrations more or less abrogates ADS and the response latency stays constant. To further examine the contribution of each sodium channel to the supernormal phase, the sodium reversal potential was held constant separately for the individual Na
CHAPTER 8. RESULTS AND DISCUSSIONS

Figure 8.16: Activity–dependent slowing during the high–frequency protocol. (A) the membrane potential at the beginning of the repetitive stimulation and (C) and the end. (B) latency during repetitive stimulation (2 Hz). (D) The latency during repetitive stimulation normalized with the initial latency.) Relative latency changes for both the high–frequency (top: 300 pulses at 2 Hz, 60 pulses at 0.25 Hz) and low–frequency (bottom: 20 pulses at 0.125 Hz, 20 pulses at 0.25 Hz, 30 pulses at 0.5 Hz, 20 pulses at 0.25 Hz).

subtypes 1.7 and 1.8. The results are illustrated in Figure 8.17 and suggest that the increase in intracellular sodium is largely attributable to \( \text{Na}_V \text{1.8} \) Na current. With the reversal potential for \( \text{Na}_V \text{1.8} \) held constant the amount of slowing is drastically reduced, while clamping reversal potential for \( \text{Na}_V \text{1.8} \) results in an approximate 30% reduction in ADS.

Discussion
There are several hypotheses regarding the generation of ADS. One of the most accepted hypothesis is based upon slow inactivation of sodium channels, particularly \( \text{Na}_V \text{1.8} \) since it is the largest sodium current (De Col et al., 2008; Blair and Bean, 2002) and is responsible for generating the action potential. Our hypothesis builds upon accumulation of intracellular sodium, which would reduce the reversal potential of sodium channels and thereby affect the propagation velocity.

Based on two experiments, slow inactivation of \( \text{Na}_V \text{1.8} \) has been the most accepted hypothesis. The first experiment showed that activity-dependent decrease of the
8.6. MODELLING OF ACTION POTENTIAL CONDUCTION IN PERIPHERAL NERVE CELLS

Figure 8.17: Activity-dependent slowing is induced by accumulation of intracellular sodium. (A) The relative latency during the high-frequency protocol (2 Hz), control (blue) and with clamped reversal potentials (black). (B) Intracellular concentration of sodium (green) and periaxonal potassium concentration (black). (C) Reversal potential of sodium. (D) The latency normalized with the initial latency when different channels reversal potential was constant, Nav1.7 (green) and Nav1.8 (red) and control (blue).

Excitability could be measured in the soma of a peripheral nerve cell (Snape et al., 2010). The alteration in excitability could not be explained by any increase in intracellular sodium due to the large volume of the soma. The second experiment showed that when extracellular sodium was reduced, the ADS increased (De Col et al., 2008). Functionally, this result would exactly match our findings as reduced sodium driving force results in increased slowing. Both, increased intracellular or decreased extracellular sodium will have these consequences. However, reduction of extracellular sodium has to be regarded as an artificial experimental condition whereas increase of intracellular sodium concentration in the fine axons is in accordance with the physiological processes. Physiologically, decreased axonal excitability based on increase of intracellular sodium concentration might be regarded as negative feedback mechanism that prevents neurons excessive discharge under metabolically problematic conditions.
Figure 8.18: The slowing/speeding for different interspike intervals. The frequency is 2 Hz and the inter spike interval vary between 10–250 ms. (A) The membrane potential for different inter spike intervals. The upper graphs represent the membrane potential in the beginning of the branch axon and the lower at the end of parent axon. (B) The slowing/speeding for different inter spike intervals.

Recovery cycles (Paper V)

Simulations were performed using stimulus pulses at variable inter-stimulus intervals and at different base frequencies (Figure 8.18). Changes in membrane potential at the stimulation site (top) and at the end of the parent axon (bottom) are shown in Figure 8.18. In Figure 8.18, the effect of repetition frequency on the recovery cycle is shown. The overall trend of the change in latency of the second pulse relative to the first pulse corresponds well to experimental findings (Weidner et al., 2002; Bostock et al., 2003). In particular, subnormality (decreased conduction velocity) is observed at small ISI values followed by a supernormal phase for intermediate ISIs (peak around 40 ms; experimental range of peak 20-200 ms) and a second phase of subnormality for large ISI values. An increase in the magnitude of the supernormal phase was also observed with increasing repetition frequency.

The ion channels generating the supernormal phase

To elucidate the mechanisms behind generating the supernormal phase, relative
8.6. MODELLING OF ACTION POTENTIAL CONDUCTION IN PERIPHERAL NERVE CELLS

Figure 8.19:
Induced super normal phase due to accumulation of intracellular sodium. The figure shows the supernormal phase during the RC2. The frequency is 2 Hz and the inter spike interval 50 ms for A and B. (A) the supernormal and when the concentrations of sodium and potassium was clamped to the initial value (black curve). (B) Supernormal phase when clamping the reversal potential for the Nav 1.7 (green) or Nav 1.8 channel (red). (C) Supernormal phase for one double pulse when the initial value of the intracellular sodium concentration was varied. (D) Supernormal phase for one double pulse when the resting potential was altered with a current injection (blue) and by altering the leak conductance (blue).
CHAPTER 8. RESULTS AND DISCUSSIONS

changes in the conduction velocity of 2 action potentials initiated at a 50 ms interval was examined (8.19). The involvement of ionic concentrations was confirmed by clamping both sodium and potassium concentrations to their initial values and this abrogated and relative changes in conduction speed (Figure 8.19). To further study the role of intracellular sodium, simulations were performed using different intracellular sodium concentrations with the result that values of intracellular Na greater than ca.15 mM were linked with reduced conductance velocity (Figure 8.19). Based on this results we propose that increased intracellular sodium concentration constitutes a necessary condition for the appearance of a supernormal phase. Changes in intracellular sodium concentration lead to a change in the reversal potential of sodium and to subsequent reductions in the magnitude of sodium currents. To further study the role of specific NaV subtypes, the sodium reversal potential was clamped for NaV1.7 or NaV1.8 individually (Figure 8.19). A complex relationship then manifests between Na channel subtypes and the supernormal phase with clamped NaV1.8 leading to a pronounced supernormal phase and clamped NaV1.7 tending toward a reduction in conductance velocity.

Discussion
The most accepted hypothesis today proposes that passive properties of the membrane potential generate the after–depolarisation (Bostock et al., 2003). After–depolarisation has been measured in hyperpolarized myelinated axons (Barrett and Barrett, 1982) and is the foundation on which this hypothesis is built upon. More convincing evidence was presented in a study by Bowe et al. (1987), where the after–depolarisation was correlated with reduction in excitability with very similar dynamics as measured during recovery cycle protocol. Our model results are consistent with the interpretation that the after–depolarization is correlated with ADS during the repetitive stimulations. However, the main contribution to supernormal phase originates from reduced reversal potential of potassium after an action potential (see Paper V).

Furthermore, in the study of Moalem-Taylor et al. (2007), current injections were used to depolarize the membrane and study the effect on the supernormal phase. Their findings that supernormal phase increases with depolarization was interpreted as a support for the hypothesis of the ADP as the mechanism of the supernormal phase. However, our study shows that shifting the membrane potential also leads to a change in intracellular ion concentration, and we further show that this change is the determining factor. Finally, as both ADS and supernormal phase depends on changes of intracellular sodium, a possible mechanism explaining the relationship between the supernormal phase and ADS may have been identified.
8.7 Parameter search in detailed multicompartment models

Parameter search methods have been used frequently in this thesis to identify parameters in detailed compartmental models. Parameter search is a tool for identifying “unknown” and “free” parameters. An unknown parameter, such as the conductance of a specific ion channel, is actually known to have a specific value, though this specific value is in fact unknown. Therefore, the specific model value of an unknown parameter cannot be defined arbitrarily. Free parameters are parameters that can have a range of values, such as the concentrations of modulatory substances. Such parameters may therefore be assigned parameter values more freely. Identifying the correct value of an unknown parameter is much more time consuming than simply assigning a free parameter a plausible value that satisfies a functional behaviour.

When developing detailed compartmental models, the number of unknown parameters is large and the constraints are few. This is especially true for multicompartment models of neurons, incorporating multiple ion channels (Migliore et al., 2008; Poirazi et al., 2003; Larkum et al., 2009). Until recently, the traditional approach has been to tune the model parameter manually (Van Geit et al., 2007). There are two drawbacks to this approach: first, it requires considerable effort and, second, the researcher has a natural tendency to tune the model subjectively. The most commonly used parameter search methods for identifying parameters in detailed multicompartment models are evolutionary and genetic algorithms (Hendrickson et al., 2011; Van Geit et al., 2007; Vanier and Bower, 1999). One disadvantage of both these methods is that they require a large number of iterations to reach convergence. A comparison of various parameter search methods (Vanier and Bower, 1999) identified the genetic algorithm as the most effective algorithm for multicompartment models. However, approximately 6000 iterations were required to reach convergence. For the problems presented in this thesis, neither genetic nor evolutionary search algorithms could be applied, due to the excessive simulation time required. In the following section, I will describe the results of the parameter search projects forming part of this thesis.

Functionally reverse models of epilepsy by parameter search methods (Paper II)

My second project as a PhD student was to identify a parameter search method suitable for detailed compartmental models. The goal of the project was to identify parameters that generated a specific spike output. In Paper II, I developed pathological models of epilepsy with the main objective of correcting the pathology by reducing the excess spiking, particularly at high synchronization levels (see Figure 8.20, red curve). In the control model (no pathological alteration), the cellular response to highly synchronized input (0-2 ms) is suppressed (see Figure 8.20, blue curve). If the spike activity in the pathological model is excessively suppressed, it will not generate spikes for any input, regardless of synchronization.
Figure 8.20: Schematic figure showing the procedure to functionally correct a pathological model. The pathological models generate excess spiking for both high and low levels of synchronicity. To functionally correct the pathological model the spike generation for different synchronicity levels should be similar to the control model, for high synchronicity levels in particular. If the activity in the pathological model is suppressed too much the cell will not respond to any synchronicity level of the input and therefore be classified as pathological. A shows the spike activity for different synchronicity levels of the input for the control (blue) and pathological model (red). B Schematic figure defining the interval of PUFA within which the pathological model is functionally corrected.

level. Therefore, there may be an interval within which the pathological model suppresses highly synchronized input, while still generating spikes for less synchronized input (see Figure 8.20). We decided to examine whether the modulatory features found in various subtypes of the auxiliary proteins KChIPs and DPPs (An et al., 2000; Maffie and Rudy, 2008) would reverse the pathological models. Not only does the current of the $K_A$ channel depend on the subtype composition of its auxiliary proteins, but also its kinetic properties are altered following, for example, phosphorylation. I therefore extended my analysis to include modulatory alterations of PKC (Hoffman and Johnston, 1998) and CaMKII (Varga et al., 2004) and the lipid modulator arachidonic acid (AA) (Angelova and Müller, 2006). Thus, the relative concentrations of these modulator substances, i.e., KCHIP1, DPP6, PKC, AA, and CaMKII, were controlled by a numerical optimizer, which compared model output with a predefined neural output representing a normal physiological response.

The next step was to construct the cost function, which as a starting point was
8.7. PARAMETER SEARCH IN DETAILED MULTICOMPARTMENT MODELS

defined as the difference in spike output between the pathological and control models (no pathology). In Paper 1, I used Exponentially distributed inputs, whereby a minimum of 20 simulations had to be generated for every synchronization level to achieve a stable mean output. Running the simulation 20 times was time consuming and most optimization algorithms require a large number of iterations before they converge. I therefore changed the model of the temporal distribution of the input to a deterministic instead of a stochastic model, which reduced the simulation time significantly. However, the applied optimizers (i.e., Hooks and Jeeves and evolutionary algorithms) could still not converge; to understand why, we have to consider the behaviour of a neuron. Whether or not an action potential is generated depends on the result of a threshold phenomenon. If the number of generated action potentials is the only input to the cost function, the output would be a flat surface with valleys where the cost function is low. If the starting point of the search is somewhere in the flat space, the optimizer will not find any meaningful search direction to follow and will therefore not converge. To solve this problem, I instead used the peak membrane potential at the input site as a predictor of action potential generation in the soma. This generates a smoother surface in which the optimizer can navigate and thereby a faster path to convergence. When the cost function was altered and the randomness eliminated, the choice of optimization method was not as crucial. I therefore used the Hook and Jeeves optimization method, since it is a robust direct search method [Kolda et al., 2003; Torczon et al., 1997].

Figure 8.21 shows the number of spikes generated in each iteration, for different synchronization levels. For one parameter, the cost function was a convex surface, with only one minimum (see figure 8.22).

For a multi-dimensional search with three modulatory substances used as free parameters, the surface of the cost function had several local minima. When solving classical optimization problems, the global minimum is viewed as the best solution, since all criteria are incorporated in the cost function. In a project like the present one, there are additional conditions not usually incorporated in the cost function. For example, the solution should also be robust in the sense that small parameter alterations should not lead to a pathological model. I therefore chose the three solutions with the lowest cost function values and then performed a sensitivity and robustness analysis to identify the best overall solution, see next section for more information about the analysis.
Figure 8.21: Presentation of the optimization procedure. A. Result of the optimization procedure. The starting point of the optimization is the pathological model (red) and the goal is the control model (blue). For illustration, spike activity for each iteration during the optimization is also shown (dashed lines). Actual solution found is indicated in green. Each colour in A, C, D corresponds to the same iteration cycle. B–D illustrates the optimization cycle. B. Dendrite membrane potential produced in a control case (blue) and a pathological case (red). The difference in peak EPSP amplitude at the input site for the first and second input cycles were used to calculate the cost function value. C. The cost function value for each iteration during the optimization. D. The relative concentration of DPP6 for each iteration of the optimization.

Parameter search methods used in the C–fibre model (Paper IV)

In the C–fibre model, we developed a spatial implementation of a C–fibre as a detailed compartmental model. In tuning this compartmental model, the membrane potential obtained from intracellular recordings was an important model constraint. However, as the C–fibre is too thin to preform intracellular recordings, intracellular recordings of the membrane potential have instead frequently been made in the soma of the C–fibre. As a starting point, we therefore assumed that the channel conductance and channel dynamics were the same as in the soma. We used the Hook and Jeeves optimization method to find the conductance levels that would generate ADS (see Figure 8.23).
8.7. PARAMETER SEARCH IN DETAILED MULTICOMPARTMENT MODELS

Figure 8.22: Local minimum. The cost function for different values of the relative concentration of KChIP1. The allowed interval for KChIP1 is from 0–2 a.u. Observe that it only exists one minima for the allowed interval.

Figure 8.23: Improved activity-dependent slowing by optimization. The conductances of the three ion channel: Nav 1.9, Nav 1.8, Nav 1.7 and membrane resting potential was chosen as input parameter to the optimizer.
Considerable effort was made to reduce the model complexity and simulation time. The simulation running time of the full ADS protocol is approximately 3 min, which is extremely long for a detailed compartmental model. Therefore, both the morphology and the input protocols were simplified. However, most of the simplified models did not make correct predictions compared with those of the original model. The output of the most successful simplified model is shown in Figure 8.23. This model version used a slightly simplified morphology, reducing simulation time by a factor of seven. The ADS curve was just one criterion of the model, but the other constraints could not be fully incorporated into the cost function. Therefore, manual tuning was used in parallel with parameter search methods.

How do we know we have found the right parameters? (Paper II)

Vanier et al. (1999) compared the effectiveness of various parameter search methods. Interestingly, different parameter search methods arrived at different solutions, leading to the question of how we are able to know whether we have identified the right parameters. The correct identification of an unknown parameter is the value representing the global minimum of the cost function. For many models, this is too computationally demanding to establish. In Paper II, I addressed this problem by first identifying the three solutions with the lowest cost function and subsequently selecting one manually, by means of additional constraint and robustness analyses (see section 8.8). To generate multiple solutions, I ran the optimizer several times using starting points far from previously identified solutions, repeating this exercise until the optimizer found only previously identified solutions. In my experience, this is an effective approach if the parameter interval is well defined and relatively small.

A third approach is to generate predictions from the model and then test them experimentally. No matter what the outcome of the experiment, this will in some way improve or strengthen the model. During my research work, we have also tried to establish close collaborations with experimentalists, since I believe this to be the only way to generate valid and relevant models. In this thesis, one model prediction has been tested and confirmed experimentally. In Paper I, my model predicted that the A-type potassium channel could suppress highly synchronized input. This prediction was tested in an electrophysiological experiment in a layer of five pyramidal cells. The model applied in Paper I–IV further predicted that the $K_A$ channel would reduce highly synchronized input without reducing lower levels of synchronized input. This prediction was supported in an in vitro experiment, constructed in collaboration with Silberberg and Planert.

Discussion

In conclusion, I used parameter search methods to identify ion channel conductance levels and channel kinetics. Parameter search methods have been used frequently
8.7. PARAMETER SEARCH IN DETAILED MULTICOMPARTMENT MODELS

in recent years in identifying conductance parameters, whereas it is unusual to alter the ion channel kinetics. The experimental recordings of ion channel characteristics have several sources of error (see section 3.4). Therefore, ion channel characteristics can be altered consistently with experimental data to improve the model.
8.8 Sensitivity and robustness analysis

When a model has been derived, the uncertainty in the parameter values can be analysed by means of sensitivity analysis. In the next sections, I will discuss the conclusions drawn from the use of sensitivity analysis.

Variance–based sensitivity analysis \cite{Saltelli2004} illustrates the relative influence of various parameters on a specific behaviour of the model. In Paper II, I used variance–based sensitivity analysis to examine the influence of the modulatory substances on spike generation at different synchronization levels. The modulatory substance, DPP6, shifted both the steady–state activation and inactivation as well as altered the inactivation time constant. Using variance–based sensitivity analysis, I could identify that the shift in steady–state activation was contributing the most to reversing the pathological model (see Figure 8.24).

Variance–based sensitivity analysis is a useful method, but measures the influence
8.8. SENSITIVITY AND ROBUSTNESS ANALYSIS

The relative concentration of KChIPI

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The intervall where the model is functionally correct

Figure 8.25: Robustness analysis. The figure shows the cost functions minima for relative concentration of a modulatory substance (KChIP1). The width of a minima measured by two methods. The horizontal line shows the interval of the relative concentration when the cost function is less than 25 a.u. The vertical line shows the interval were the model is classified as functionally corrected.

only around a specific point in the model. In other words, parameter influence on the model may change if the model is slightly altered. Most model parameters are nonlinearly dependent on a specific behaviour of the model. Therefore, variance-based sensitivity analysis has been used mostly to study the influence in a finished model and not used as a tool for developing models.

When designing a model, all the constraints should be fulfilled while achieving an adequate level of parameter robustness. In Paper II, I examined the robustness of a reversed pathological model. I measured how much the relative modulatory substance could be changed before the model became pathological again. I defined a model as pathological if it did not reduce highly synchronized input or did not generate spikes at any synchronization level. This is a better approach than defining robustness as a small change in the cost function, as this will not indicate whether the model has become pathological. The interval in which the model was functionally corrected was defined as the width of the solution’s minima (see Figure 8.25). Some solutions’ minima were very wide, but still did not represent a robust model, because the width was not symmetrical. For example, one parameter could be increased by 20% but decreased by only 5%. I therefore defined a robustness score that decreased by a factor of one if any small (i.e., <10%) alteration led to a pathological model. This was a useful method for finding robust models.
Chapter 9

Future work

In this thesis, I have studied the effects of ion channels on excitability linked with pathological conditions. A major reason why I examined ion channels is that they are the targets of numerous medical drugs. In my projects, I have been able to link ion channels with pathological conditions. In some of my projects, I have even modelled substances that alter ion channels, and considered their effect on a pathological condition. Targeting the proper combination of such modulatory substances would be of interest in drug development.

I have provided a mechanism, the $K_A$ channel, that may reduce the synchronized input implicated in epileptic activity. In an $in vitro$ experiment, we demonstrated that highly synchronized input is suppressed compared with semi–synchronized input. We are planning another $in vitro$ experiment in which $K_A$ is blocked. The outcome of this experiment could demonstrate whether $K_A$ is responsible for reducing synchronized input.

In the future, I would like to test whether $K_A$ can reduce both the highly synchronized and fast–ripple inputs to a network model. The network should include pyramidal cells and inhibitory interneurons. In a recurrent network, small effects at the single cell level can be significant for overall network activity.

In Paper IV, I examined the dendritic integration of highly synchronized input. The study predicts that $K_A$ can selectively reduce the cellular response to highly synchronized input and thereby possibly modulate long–term potentiation. As a next step, I would like to test this prediction in a network model incorporating synaptic plasticity. One important feature of the network is that the cell models need to be relatively detailed, since the ability of $K_A$ to reduce highly synchronized input is more prominent in distal dendrites. In simplified models, the suppression of highly synchronized input by $K_A$ would be reduced or non–existent.
CHAPTER 9. FUTURE WORK

The purpose of developing the C–fibre model was to study functional behaviours linked with chronic pain. Studying pathological conditions using a computational model calls for a control model. The control model contributed to my research by proposing the mechanisms underlying two functional behaviours, ADS and the supernormal phase. As a next step, I would like to implement a pathological version of the C–fibre model in a fashion similar to the way I implemented the epilepsy models. In the pathological model of hyperexcitable C–fibre, I would like to implement the modulatory substances that can make the pathological model output resemble that of the healthy control model.
Parasagittal slices (300 µm thick) were obtained from young rats (PN 14–18) in accordance with the guidelines of the Stockholm municipal committee for animal experiments. Slices were cut in an ice-cold extracellular solution, kept in 35 °C for 30 minutes, and then moved to room temperature before recordings. Whole-cell patch recordings were obtained from neocortical and striatal neurons at a temperature of 35 ± 0.5 °C. Recorded neurons were selected visually using IR–DIC microscopy (Zeiss Axioskop, Oberkochen, Germany). The extracellular solution (both for cutting and recording) contained (in mM) 125 NaCl, 25 glucose, 25 NaHCO3, 2.5 KCl, 2 CaCl2, 1.25 NaH2PO4, 1 MgCl2. Recordings were amplified using multiclamp 700B amplifiers (Molecular Devices, CA, USA), filtered at 2 KHz, digitized (5–20 KHz) using ITC–18 (Instrutech, NY, USA), and acquired using Igor Pro (Wavemetrics, OR, USA). Patch pipettes were pulled with a Flaming/Brown micropipette puller P-97 (Sutter Instruments Co, Novato, CA) and had an initial resistance of 5–10 MΩ, containing (in mM) 110 K-gluconate, 10 KCl, 10 HEPES, 4 ATP, 0.3 GTP, 10 phosphocreatine, and 0.4–0.5 % biocytin. Liquid junction potential (10 mV) was not corrected in any of the recordings. Recordings were performed in current–clamp mode, with pipette capacitance and access resistance compensated for throughout the experiments. Data was discarded when access resistance increased beyond 35 MΩ. Various current traces representing currents invoked by synaptic input were injected in order to test neuronal responses to input with varying degrees of synchrony. The recorded voltage response was then analyzed in terms of discharge responses (produced number of action potentials) as well as in terms of membrane potential pattern.


