KTH Computer Science
and Communication

Dendritic and axonal ion channels supporting neuronal integration

From pyramidal neurons to peripheral nociceptors

MARCUS PETERSSON

Akademisk avhandling som med tillstånd av Kungliga Tekniska högskolan framlägges till offentlig granskning för avläggande av teknologie doktorsexamen tisdagen den 9e oktober 2012 kl 10.00 i sal F3, Lindstedtsv. 26, Kungliga Tekniska högskolan, Stockholm.

ISSN-1653-5723
TRITA-CSC-A 2012:09
ISRN-KTH/CSC/A--12/09-SE
ISBN 978-91-7501-475-3

Abstract

The nervous system, including the brain, is a complex network with billions of complex neurons. Ion channels mediate the electrical signals that neurons use to integrate input and produce appropriate output, and could thus be thought of as key instruments in the neuronal orchestra. In the field of neuroscience we are not only curious about how our brains work, but also strive to characterize and develop treatments for neural disorders, in which the neuronal harmony is distorted. By modulating ion channel activity (pharmacologically or otherwise) it might be possible to effectively restore neuronal harmony in patients with various types of neural (including channelopathic) disorders. However, this exciting strategy is impeded by the gaps in our understanding of ion channels and neurons, so more research is required. Thus, the aim of this thesis is to improve the understanding of how specific ion channel types contribute to shaping neuronal dynamics, and in particular, neuronal integration, excitability and memory. For this purpose I have used computational modeling, an approach which has recently emerged as an excellent tool for understanding dynamically complex neurophysiological phenomena.

In the first of two projects leading to this thesis, I studied how neurons in the brain, and in particular their dendritic structures, are able to integrate synaptic inputs arriving at low frequencies, in a behaviorally relevant range of $\sim$8 Hz. Based on recent experimental data on synaptic transient receptor potential channels (TRPC), metabotropic glutamate receptor (mGluR) dynamics and glutamate decay times, I developed a novel model of the ion channel current I_{TRPC}, the importance of which is clear but largely neglected due to an insufficient understanding of its activation mechanisms. We found that I_{TRPC}, which is activated both synaptically (via mGluR) and intrinsically (via Ca^{2+}) and has a long decay time constant (τ_{decay}), is better suited than the classical rapidly decaying currents (I_{AMPA} and I_{NMDA}) in supporting low-frequency temporal summation. It was further concluded that τ_{decay} varies with stimulus duration and frequency, is linearly dependent on the maximal glutamate concentration, and might require a pair-pulse protocol to be properly assessed.

In a follow-up study I investigated small-amplitude (a few mV) long-lasting (a few seconds) depolarizations in pyramidal neurons of the hippocampal cortex, a brain region important for memory and spatial navigation. In addition to confirming a previous hypothesis that these depolarizations involve an interplay of I_{TRPC} and voltage-gated calcium channels, I showed that they are generated in distal dendrites, are intrinsically stable to weak excitatory and inhibitory synaptic input, and require spatial and temporal summation to occur. I further concluded that the existence of multiple stable states cannot be ruled out, and that, in spite of their small somatic amplitudes, these depolarizations may strongly modulate the probability of action potential generation.

In the second project I studied the axonal mechanisms of unmyelinated peripheral (cutaneous) pain-sensing neurons (referred to as C-fiber nociceptors), which are involved in chronic pain. To my knowledge, the C-fiber model we developed for this purpose is unique in at least three ways, since it is multicompartmental, tuned from human microneurography (*in vivo*) data, and since it includes several biologically realistic ion channels, Na^+/K^+ concentration dynamics, a Na-K-pump, morphology and temperature dependence. Based on simulations aimed at elucidating the mechanisms underlying two clinically relevant phenomena, activity-dependent slowing (ADS) and recovery cycles (RC), we found an unexpected support for the involvement of intracellular Na^+ in ADS and extracellular K^+ in RC. We also found that the two major Na^+ channels ($Na_V1.7$ and $Na_V1.8$) have opposite effects on RC. Furthermore, I showed that the differences between mechano-sensitive and mechano-insensitive C-fiber types might reside in differing ion channel densities.

To conclude, the work of this thesis provides key insights into neuronal mechanisms with relevance for memory, pain and neural disorders, and at the same time demonstrates the advantage of using computational modeling as a tool for understanding and discovering fundamental properties of central and peripheral neurons.

Keywords: ion channels, computational modeling, simulations, dendrites, axons, TRP, hippocampus, C-fiber nociceptors, pain

Acknowledgments

My work was supported by the Swedish Research Council (Grant number: 621-2007-3774) and the National Institutes of Health (Grant number: MH061492-06A2). I have received generous travel support from Stockholm Brain Institute and the Knut and Alice Wallenberg Foundation.

I want to thank my supervisor Erik Fransén and co-supervisor Anders Lansner for the opportunity to write this thesis. I also want to express my deepest gratitude to our international collaborators Angelika, Martin, Motoharu, Otilia and Richard, with whom I have enjoyed many fruitful discussions and learned a lot about neuroscientific research. I am particularly grateful for the very rewarding internship weeks in the Schmelz laboratory.

I further want to acknowledge all the friendly people at CB who contributed to making my PhD journey enjoyable. The moral and intellectual support from Micke and Paweł was crucial. I have highly appreciated your company. I am very grateful to Paweł for thoroughly reading this thesis and for giving numerous suggestions on structure, content and language, and also to Jeanette and Örjan for useful feedback on various parts of the thesis. I additionally want to thank Örjan for never hesitating to spend time on providing excellent computer support and career advice, in spite of being a busy head of the department. I also want to thank Iman, Nalin and Simon for great company over the years, and Harriet for always smoothly sorting out administrative issues. I have also enjoyed the company of several colleagues not yet mentioned, including Alex, Bernhard, Cristina, David, Henrik, Johannes, Malin, Phil, Pierre, Pradeep, Ronald and Sten.

I'm lucky to have many close friends who enrich my life significantly, including Christian, Emad, Erik, Fredrik, Helena, Jeppe, Kalle, Nicke, Pepa and Togge. I hope to see you more often now that my studies are over. I'm also grateful to my family-in-law for always making me feel at home abroad. Jag vill förstås också tacka Mamma, Pappa, Samuel och Gabbie, för att ni alltid står beredda med trygghet, uppmuntran och humor, och för att varje gång vi ses blir en av årets höjdpunkter!

Finally, and most importantly, I want to thank you Khadeeja, my wife and soulmate, for contaminating me with your happiness, for your patience and sacrifices, for love and support. I would take my research far too seriously if you weren't there to fall asleep every time I talk about it. This thesis (or lullaby) is dedicated to you.

Contents

Chapter 1

Introduction

1.1 A brief introduction to neuroscience

Neuroscience refers to the study of the nervous system, which is generally divided into a central (*CNS*) and a peripheral (*PNS*) part. Neuroscientists study the function of the CNS and PNS on levels ranging from genes to behavior. The PNS includes e. g. pain-sensing nerve cells (*neurons*) in the skin, while the CNS includes the brain and the spinal cord. Today most research groups focus on the CNS and in particular the brain, which contains a complex network of approximately 100 billion neurons. However, neuroscientists also study the PNS, since its neurons are far from being well understood.

The purpose of a neuron is essentially to integrate its incoming input to produce an appropriate output. The neuronal cell membrane is equipped with a multitude of *ion channels* – pores in the cell membrane that are selective to specific ions – which could be thought of as the fundamental building blocks that allow neurons to integrate and communicate information. Due to the presence of ion channels (and ion pumps, as described in section 2.2), there is a voltage over the membrane referred to as the membrane potential (V_m), which in turn governs the behavior of many important types of ion channels.

A typical neuron consists of a *soma*, *dendrites* and an *axon*, as well as *synapses* receiving input from neighboring cells (see figure 1.1). There are many types of neurons in the nervous system, and although they share many features there are also important differences. The number of synapses of a single neuron varies between 1 to about 100 000 (Purves, 2008), which indicates the complexity of the human brain. The hippocampal CA1 pyramidal cell (see figure 3.2), for example, has around 30 000 excitatory and 1 700 inhibitory synapses (Megías *et al.*, 2001).

In the traditional view, signals arrive at synapses located on the dendrites and travel down to the soma where they are summed and may or may not trigger an all-or-none signal (the *action potential*, see section 2.2), which is conducted along the axon until it reaches its target, a synapse on another neuron. In recent years, however, a more complex view of neuronal signaling has

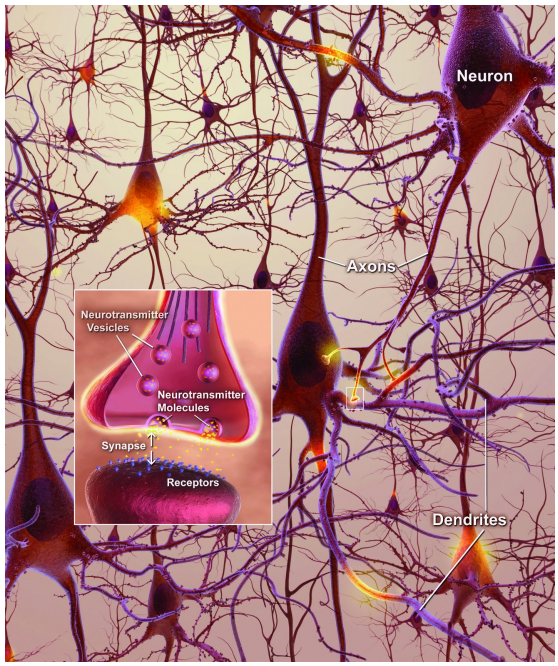

Figure 1.1. *Neurons of the brain. Image courtesy of the National Institute on Aging/National Institutes of Health.*

emerged. It is now clear that signals can also travel in the opposite direction, from the soma out to the dendrites. It has even been questioned if there really is any fundamental distinction between dendrites and axons, since dendrites may release transmitter substances, and axons may receive synaptic input (Stuart *et al.*, 2007, but see Brady *et al.*, 2009). Furthermore, it has been shown that dendrites, classically thought of as passive conductors, can in fact perform local computations of incoming signals (Larkum *et al.*, 2009). Similarly, it has been shown that axons are not simply signal transmitters, but can also to some extent perform signal processing (Segev and Schneidman, 1999; Bucher and Goaillard, 2011). Dendrites and axons will be discussed in more detail in the Chapter 2.

This thesis is partly focused on *pyramidal neurons*, which are abundant in cortical areas. However, the cellular mechanism that my work is focused on – the canonical TRP (transient receptor potential) current I_{TRPC} – is likely to be important for synaptic integration also in other neuron types. The cerebellar Purkinje cell is one such example, for which much progress has been made in characterizing the function of synaptically activated TRPC channels (Hartmann and Konnerth, 2008), the dopaminergic midbrain neuron being another (Bengtson *et al.*, 2004). Another neuron type in focus here is the pain-sensing *nociceptor* which is situated in the PNS. I will further discuss nociceptors in section 2.5.

1.2 Why study the nervous system?

From my point of view, there are two strong driving forces behind the progress of neuroscience, one being curiosity. It is within the human nature to be curious, and it should be safe to say that all people have questions on some level relating to the mechanisms of sensation, movement, emotion, or cognitive functions such as memory, intelligence and language. The second driving force is the more specific need to characterize neural disorders, such as addiction, Alzheimer's disease (AD), attention deficit hyperactivity disorder (ADHD), autism, brain tumors, chronic pain, Down's syndrome, dyslexia, Huntington's disease, major depression, multiple sclerosis, Parkinson's disease, schizophrenia, seizures and epilepsy, stroke, Tourette's syndrome, and ultimately to find ways of restoring normal function.

1.3 Why study ion channels?

For the nervous system to function well, it is important that ion channels perform their duties. Malfunctioning ion channels can cause neurological disorders – often referred to as "channelopathies" – such as epilepsy, migraine, movement disorders, pain, muscle diseases and perhaps also psychiatric and cognitive disorders (Kullmann, 2010; Lampert *et al.*, 2010; Catterall, 2012). From genetic knock-out studies of e. g. TRP channels it is clear that several types of ion channels can play important roles in physiology (Wu *et al.*, 2010, see also figure 2.4).

Thus, it is important to understand how ion channels contribute to physiological and pathophysiological mechanisms in order to ultimately find ways to treat patients. There are pharmacological (Camerino *et al.*, 2008) as well as non-pharmacological (see section 3.1 for discussion on deep brain stimulation) approaches to treating neurological disorders, but side effects are common. Conveniently, pharmacology can be used to specifically target ion channels. However, it takes years to identify drug targets and to develop drugs, and in order to reduce side effects it might be necessary to make use of synergistic effects resulting from certain drug combinations (for example and discussion, see Fairbanks *et al.*, 2009).

1.4 Why use computer models?

The use of computational modeling can facilitate an understanding of dynamically complex neurophysiological phenomena. A model can consist of a large network of connected morphologically complex neurons, each with multiple ion channel types. I expect that the use of computational modeling will significantly speed up the development of the type of drugs described above, reduce the number of animals used, and save development costs. The reason for this is that computer simulations are typically fast compared to experiments, and

might therefore prove to be an effective method for identifying targets. Computer simulations could also allow for quicker systematic searches for possible synergy effects. In light of this, it is not surprising that computational modeling projects, such as the large-scale Blue Brain Project, are receiving a lot of attention.

While many modeling groups today are working with large neuronal networks, there is also research (like this thesis) specifically aimed at understanding the dynamics of individual neurons. A strong motivation for using single-neuron models is that they enable a direct link to the search for pharmacological ion channel targets, as the model can contain several types of biologically realistic ion channels, expressed in varying densities along the neuron. In contrast, large network models often use more simplified neuronal dynamics, which makes the connection to pharmacology more difficult. That being said, to fully understand functioning and malfunctioning nervous systems, future models should ultimately be realistic on levels ranging from molecular levels to networks.

1.5 Aim of this thesis

In order to characterize and develop treatments for neural disorders, it is necessary to gain a deeper understanding of the role of ion channels in neuronal dynamics. Three important and partially overlapping concepts relating to neuronal dynamics are neuronal *integration* (how the neuron processes information by combining its inputs), *excitability* (how likely the neuron is to generate action potentials) and *memory* (the ability of the neuron to integrate temporally sparse input).

The aim of this thesis is to improve the understanding of how specific ion channel types shape neuronal integration, excitability and memory.

1.6 Scope of this thesis

This thesis is based on two major modeling projects:

1. The role of synaptically (via metabotropic glutamate receptors) and intrinsically (by calcium) activated TRPC channels in synaptic integration and subthreshold long-lasting depolarizations in pyramidal cell dendrites.

2. The role of axonal ion channels and pumps in activity-dependent excitability changes of peripheral nociceptive C-fiber axons.

Each project resulted in three papers, of which the relevance and main conclusions will be summarized below.

1.6.1 *Project 1:* Role of dendritic I_{TRPC} in synaptic integration and and cellular mnemonics

Dendritic integration of synaptic input is essential for information processing in the human brain. It has recently become clear that complex phenomena of dendritic integration are abundant and supported by a plethora of non-linear dendritic mechanisms. One of these mechanisms involve an excitatory synaptically and intrinsically activated current, working in parallel with the classical ionotropic AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) and NMDA (N-methyl-D-aspartic acid) currents in integrating synaptic inputs. This current, denoted I_{TRPC}, will be central in many parts of this thesis. I_{TRPC} is mediated by canonical transient receptor potential (TRPC) channels, which are activated (synaptically) downstream of group I metabotropic glutamate receptors (mGluR1/5), and (intrinsically) by intracellular calcium.

Papers I and II

An important difference between the ionotropic currents and I_{TRPC} is that the latter has a longer decay time. Thus, we hypothesized in Paper I and Paper II that I_{TRPC} plays an important role in the summation of low-frequency synaptic inputs. We chose to investigate frequencies below 10 Hz, a range commonly observed in single-unit recordings from awake behaving animals. To test the hypothesis, we extended a computational pyramidal cell model by constructing an mGluR1/5 model and the I_{TRPC} model, as described in Paper I. Our main source of data for tuning the model was electrophysiological recordings of synaptically activated I_{TRPC} currents in entorhinal pyramidal neurons. This data was provided by our experimental collaborators Motoharu Yoshida and Michael Hasselmo at Boston University.

In Paper I we found that I_{TRPC} can indeed support summation of low-frequency synaptic inputs, and by further exploring the dynamics of I_{TRPC} we found that the decay time constant (τ_{decay}) actually increases with increasing stimulus duration and frequency. In Paper II, written prior to Paper I, we tuned the model from current injection experiments rather than from synaptic stimulation experiments. One of the conclusions was that current injection experiments yield a significantly longer τ_{decay} than synaptic stimulation experiments. As will be discussed later in this thesis, this led us to focus on results from synaptic stimulation when conducting the work for Paper I. Both papers supported the hypothesis that I_{TRPC} plays an important role in low-frequency synaptic integration. In this thesis I will present further background and arguments in support of this and other conclusions in Paper I and II. I will also discuss the importance of the model, which I believe is the first computational model to incorporate a non-ionotropic excitatory synaptic current. It should be noted, however, that we did not include mechanisms downstream of mGluR1/5 other than the activation of I_{TRPC}, such as presynaptic effects or effects on other ion channels. Data for a detailed description of the intracellular signaling

network underlying I_{TRPC} activation is incomplete, and although we accounted for much of what is known about the activation mechanisms, our model should be considered functional. Details regarding the underlying biochemical pathways are not included in the model. Although it is of great importance to elucidate the I_{TRPC} activation mechanisms, this is not the aim of this thesis.

Despite the fact that the model tuning was based on data from entorhinal cortex, we assume that the conclusions regarding low-frequency summation are valid for other brain regions as well, such as the prefrontal cortex, hippocampus, subiculum, anterior cingulate cortex, amygdala, substantia nigra and cerebellum. This assumption is based on the observation that the mGluR-activated TRPC current is present all of these brain regions. Based on this, it is likely that I_{TRPC} plays an important role in behavior and disease, which I will argue and provide support for in various parts of this thesis.

In this study we did not use a network model, but focused on synaptic integration from the perspective of a single neuron. Ultimately, to get a complete picture of the importance of mGluR-activated TRPC currents, a further step should be taken, by including model neurons equipped with I_{TRPC} in network models. Conversely, I would argue that network models only including the quickly decaying AMPA and NMDA currents might become more realistic with the inclusion of a slowly decaying I_{TRPC} current.

Paper III

Another hypothesis based on the same model was tested in Paper III, in which we wanted to explore the ability of a neuron to hold a memory trace of events that occurred earlier (a few seconds) in time. Specifically, we wanted to test whether I_{TRPC} can be involved in supporting small-amplitude long-lasting dendritic depolarizations, which are commonly observed in experiments of CA1 hippocampal neurons, but not fully understood. We contributed to the understanding of these depolarizations by showing that (i) they may be intrinsically stable to weak excitatory and inhibitory synaptic input, (ii) the phenomenon is essentially located in distal apical dendrites, (iii) induction is facilitated if simultaneous input arrives at several dendritic branches and if calcium- and/or mGluR-evoked signals undergo summation (suggesting that both spatial and temporal synaptic summation might be required for the depolarization to occur), (iv) the existence of multiple stable states is possible but not likely, and we also show that (v) in spite of their small somatic amplitudes, these depolarizations may strongly modulate the probability of action potential initiation.

1.6.2 *Project 2:* Activity-dependent latency changes in nociceptor axons

In the second major project we studied mechanisms of peripheral pain. Unwanted pain is a large problem for patients around the world, and some types

of unwanted pain are associated with nociceptors in the PNS. Sadly, good treatment is lacking since the underlying mechanisms are not completely understood. In Papers IV, V and VI we cooperated with experimental researchers at the Universities of Heidelberg and Erlangen and at AstraZeneca to develop and use a computational nociceptor model. To my knowledge our cutaneous unmyelinated nociceptor (C-fiber) model is unique in at least three ways, since it is (i) multicompartmental, (ii) tuned from human microneurography (*in vivo*) data, and since it (iii) includes several biologically realistic ion channels, Na^+/K^+ dynamics, an Na-K-pump, morphology and temperature dependence.

Paper IV

The peripheral pain project has thus far resulted in three papers. In Paper IV we described how the abovementioned novel nociceptor model was built and tuned according to several criteria. We also used the model to gain insights into the mechanisms underlying a phenomenon called activity-dependent slowing (ADS). A better understanding of the mechanisms underlying ADS can improve our understanding of normal as well as pathological nociceptor mechanisms (since ADS patterns are changed in certain pathological pain states), and can be useful for developing pharmacological pain treatment. Specifically, the simulation data presented in the paper suggested that intracellular Na^+ accumulation might play an important role in ADS.

Paper V

In this paper we used the model to explore the mechanisms underlying another phenomenon, called recovery cycles, which is similar to ADS in the sense that, if understood, it can improve our understanding of physiological and pathophysiological nociceptor function. Specifically, we contributed to the understanding of C-type nociceptor recovery cycles by providing support for previous hypotheses regarding the role of afterdepolarizations. In addition, we found strong support for a previously underestimated (even rejected) hypothesis that extracellular K^+ plays a role in recovery cycles. We were also able to show that two major sodium channels differ in how they respond to afterdepolarizations and therefore differ in how they contribute to recovery cycles. Finally, our simulations suggested that intracellular Na^+ accumulation might contribute to the activity-induced transition from subnormal to supernormal conduction.

Paper VI

Mechano-sensitive (CM) and mechano-insensitive (CMi) C-fiber nociceptors differs in axonal propagation properties. In the final paper we wanted to study why these differences exist. This question is important since the two nociceptor types differ in how they contribute to normal and pathological pain. To address the question we used data measured with several types of experimental protocols. Among other things we found that, in terms of ion channel density, CM fibers might have less K_{dr} but more $Na_V1.7$ than CMi fibers.

1.6.3 Thesis outline

I will start by discussing aspects of the biological background that are important to be familiar with in order to understand the discussion in subsequent chapters (Chapter 2). Following this, I will describe the models and methods used to test the hypotheses (Chapter 3). The focus will be on describing the model components that are unique to this study, but also more general concepts of computational neuroscience and multicompartment modeling in particular will be discussed. I will then explain and elaborate on the relevance of insights gained in the published work, and also present several complementary simulation results and conclusions (Chapter 4). Finally, I will discuss future work (Chapter 5).

Details of Papers I-VI will be discussed throughout Chapters 2-5, but not necessarily repeated. Hence, to fully appreciate the discussion, the reader is advised to study the papers while reading the chapters.

1.7 List of papers included in this thesis

Paper I: *Petersson ME*, Yoshida M, Fransén EA (2011) Low-frequency summation of synaptically activated transient receptor potential channel-mediated depolarizations, *European Journal of Neuroscience,* 34, 578-593.

Paper II: *Petersson ME*, Fransén E (2009) TRPC channels activated by group I mGluR in Entorhinal pyramidal neurons support integration of low frequency (<10 Hz) synaptic inputs, *BMC Neuroscience*, 10(Suppl 1):P26

Paper III: *Petersson ME*, Fransén E (2012) Long-lasting small-amplitude TRP-mediated dendritic depolarizations in CA1 pyramidal neurons are intrinsically stable and originate from distal tuft regions. *European Journal of Neuroscience (in press).*

Paper IV: Tigerholm J*, *Petersson ME**, Obreja O, Lampert A, Carr R, Schmelz M, Fransén E (2012) Modelling activity-dependent changes of axonal spike conduction in primary afferent C-nociceptors. *Submitted.*

** authors contributed equally*

Paper V: Tigerholm J*, *Petersson ME**, Obreja O, Lampert A, Carr R, Schmelz M, Fransén E (2012) Modelling post-spike excitability changes in peripheral C-fibers. *Manuscript in preparation.*

** authors contributed equally*

Paper VI: *Petersson ME*, Obreja O, Lampert A, Carr R, Schmelz M, Fransén E (2012) C-type peripheral nociceptors differ in axonal ion channel densities. *Manuscript in preparation.*

1.8 Contributions to papers

Papers I and II: I implemented the model, ran the simulations, analyzed the results (from simulations and *in vitro* experiments), and conceived the idea of using a pair-pulse protocol for studying the dynamics of the model. I contributed to the literature search (including the meta-analysis concerning decay times of I_{TRPC} from experimental data), and to designing the model and writing the papers.

Paper III: I conceived the idea of studying the SUBTLE phenomenon, implemented the model, ran the simulations, analyzed the results, did most of the literature search and wrote most of the paper.

Papers IV and V: I contributed to conceiving the project, developing the model, running simulations, analyzing results and writing the manuscript. I share the first-authorship on both these papers since I equally contributed to model development, simulations and analysis. Among other things, I was responsible for implementing I_{pump}, I_{leak}, I_{KNa} and the Na^+/K^+ dynamics. Furthermore, I found the role of intracellular sodium accumulation in activity-dependent slowing, and the role of extracellular potassium and $Na_V1.7$ in recovery cycles.

Paper VI: Based on background discussions together with the other authors, I designed the simulation and analysis approach, ran all simulations, did all analysis and wrote most of the manuscript.

Chapter 2

Biological background

In this chapter, my intention is to give the reader an introduction to concepts of particular importance for understanding the methods, results and discussions in subsequent chapters. I will first describe relevant brain regions and basic concepts relating to the electrical and chemical signalling of nervous system. I will subsequently describe the transient receptor potential (TRP) ion channels, and especially highlight a synaptically evoked current that is mediated by these ion channels (I_{TRPC}). I will also discuss recent progress in the field of dendritic functioning, since my work may have implications for how these structures integrate synaptic inputs. Finally, I will discuss nociceptor axons and their role in unwanted pain.

2.1 Brain regions

Some areas of the human brain have relatively clear functions, such as the visual cortex dealing with visual information processing, the motor cortex controlling the muscles, or the thalamus serving as a relay station for most incoming information. Other areas, such as the prefrontal cortex, are associated with more than one function and thus more difficult to study and understand.

In Paper III we explored a phenomena described in CA1 pyramidal neurons, situated in the *hippocampus* (see figure 2.1). The hippocampus is a brain structure with a well-recognized importance for functions of memory and spatial navigation (Bird and Burgess, 2008).

Another brain region in focus of this thesis is called the *entorhinal cortex* (EC). The EC is situated in the medial temporal lobe and serves as the main interface between hippocampus and neocortex. The EC plays a role in olfactory information processing, but in recent years more attention has been given to its role in various aspects of memory and spatial navigation. Specifically, the EC has been shown to be involved in complex spatial navigation (Frank *et al.*, 2000; Fyhn *et al.*, 2004; Jacobs *et al.*, 2010) as well as short-term and working memory (Otto and Eichenbaum, 1992; Suzuki *et al.*, 1997; Esclassan *et al.*, 2009).

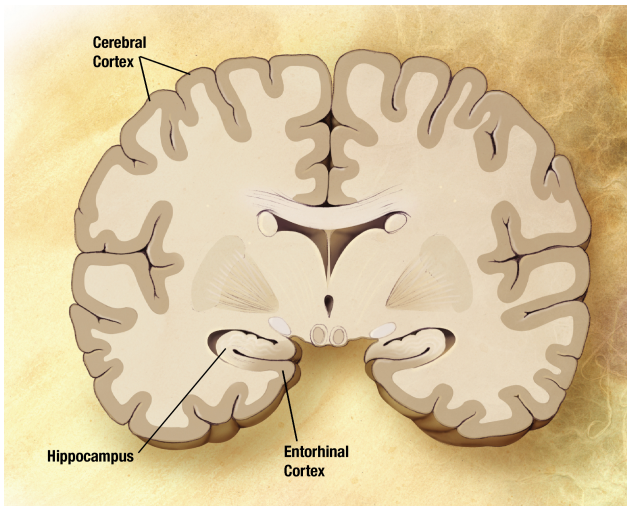

Figure 2.1. *Coronal section of the human brain. Image courtesy of the National Institute on Aging/National Institutes of Health.*

2.2 Electrical and chemical signaling

Electrical and chemical signaling in the nervous system occurs both in parallel and in series. For example, a synaptically transferred signal is typically a combination of chemical and electrical events occurring in series (e. g. neurotransmitter $\rightarrow$ transmembrane current), while a postsynaptic response can consist of parallel changes in concentrations of intracellular substances on the one hand, and electrical changes of the membrane on the other. In the work leading to this thesis I investigated both electrical and chemical signaling, and below I will introduce some of the most important concepts.

2.2.1 Membrane, resting and action potentials

As mentioned in the introduction, due to the presence of ion channels and pumps in neuronal membranes, there is an electrical potential difference – the *membrane potential* V_m – across the membrane. V_m is generated since there is a difference in ionic concentrations inside and outside the cell (due to the ion pumps, see below), and because the membrane is selectively permeable to some ions (due to the ion channels). The consequence is that, on the one hand, there is a chemical force counteracting the concentration differences, and the ion-selective channels allow ions to travel down this chemical gradient. On the other hand, the resulting ion movement causes an imbalance in electrical charge, and thereby an electrical force opposing the chemical force. An electrochemical equilibrium is reached, resulting in the *resting potential* V_{rest}.

One of the most well-recognized neuronal signals is called the *action potential* (AP), or simply the "spike", and appears as a change in V_m. AP initiation

occurs in the vicinity of the soma (in pyramidal neurons) or in the axon (in C-fibers), and requires the membrane to be depolarized enough to cross a certain threshold. An excitatory synaptic signal which is subthreshold will not by itself evoke an AP and therefore not reach the synapses of downstream neurons. Nevertheless, the nature of subthreshold signals is important to study because, for a neuron (in CNS) to decide whether or not it should generate an AP, it must perform sophisticated subthreshold computations of current and previous synaptic inputs. These computations are normally referred to as *synaptic integration*.

2.2.2 Synaptic integration

According to Gulledge *et al.* (2005), "synaptic integration is the complex process by which synaptic responses interact to promote or inhibit action potential generation". Similarly, Magee (2000) defines it as "the combination of voltage deflections produced by a myriad of synaptic inputs into a singular change in membrane potential". He further explains that there are three basic elements involved in the integration: the amplitude of the synaptic signal (excitatory postsynaptic potential, EPSP), spatial summation and temporal summation (of the EPSPs). In this thesis I study synaptic integration in pyramidal neurons from the perspective of a single neuron. Specifically, I study the dynamics of ion channels contributing to spatial and temporal summation.

2.2.3 Ion channels

Ion channels are pores in the cell membrane that are selective to specific ions. Their opening and closing, and thus the ion flux, is governed by changes in V_m or concentrations of surrounding molecules. In response to such changes, the ion channel proteins undergo morphological changes. In dendrites and axons there are typically several types of ion channel currents. Many are gated by V_m (e. g. I_{Na}, $I_{K,fast}$, $I_{K,slow}$, $I_{Ca,L}$, $I_{Ca,T}$, $I_{Ca,N}$, I_h) or calcium (e. g. $I_{KCa,fast}$, $I_{KCa,slow}$, I_{CAN}), while others are gated by both V_m and transmitter substances such as glutamate (I_{NMDA}, see below). The distribution of these and other ion channels along the dendrites is often non-uniform, and both distribution and function may vary among different neuron types (Migliore and Shepherd, 2002). Importantly, much data is still lacking when it comes to the distribution and function of dendritic ion channels. Not only dendrites, but also axons are endowed with a large variety of ion channels. Below I will describe a few ion channels, both dendritic and axonal, that are of special interest in this thesis. Figure 2.2 shows a simplified overview of the types of ion channels included in the models used in this thesis.

TRP channels

I_{CAN}, listed above, is a calcium-activated non-selective current, sometimes denoted I_{NCM} (calcium-sensitive nonspecific cation current). It is non-selective

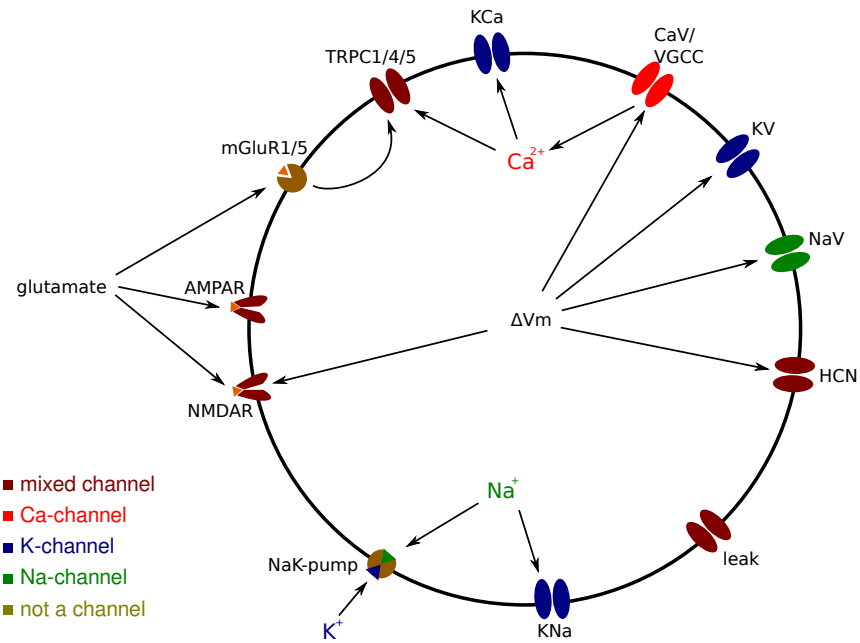

Figure 2.2. *Simplified overview of membrane elements (ion channels, Na-K-pump and mGluR1/5 receptor) included in this thesis. Arrows indicate activation mechanism or effect. For simplification, many mechanisms are not shown, including: (1) Currents (all elements except mGluR1/5) affect the membrane potential V_m; (2) Currents mediate ions (which can have significant effect on ionic concentration, here only shown for VGCC and $[Ca]_{in}$); (3) Ion channel currents are affected by reversal potentials that depend on ionic concentrations; (4) mGluR1/5 activates TRPC not directly but via a PLC pathway which can also be triggered by acetylcholine; (5) $[Ca]_{in}$ levels can also be increased via the stores in the endoplasmic reticulum.*

in the sense that more than one type of ion may pass through it. The molecular counterpart of what mediates the current (the ion channel/s) could differ among different cells, but is thought to be a transient receptor potential (TRP) channel. In the brain stem the current is believed to be mediated by TRPM (melastatin TRP) channels (Crowder *et al.*, 2007). In cortical areas, however, there is much evidence pointing to *TRPC (canonical TRP)* channels being the main candidates. I will devote section 2.3 to describing properties of TRP channels in general and TRPC channels in particular.

Voltage-gated sodium channels

In our C-fiber model we included three types of *voltage-gated sodium channels* ($Na_V1.7$, $Na_V1.8$ and $Na_V1.9$, all preferentially expressed in nociceptors), and excluded six ($Na_V1.1$-1.6). Among $Na_V1.1$-1.6, it is unclear which channels play important roles in unmyelinated nociceptors. The specific roles of various Na_V channels in nociceptor physiology is an active area of investigation, as exemplified by a large number of reviews in recent years (Lai *et al.*, 2003; Cummins *et al.*, 2007; Dib-Hajj *et al.*, 2007; Hargus and Patel, 2007; Rush *et al.*, 2007; Dib-Hajj *et al.*, 2009a,b, 2010; Lampert *et al.*, 2010). Among other things, it has been shown that $Na_V1.8$ has a more depolarized activation threshold than $Na_V1.7$. While both $Na_V1.7$ and $Na_V1.8$ contribute to the action potential in nociceptive C-fibers, $Na_V1.9$ might rather be involved in determining the resting potential (Herzog *et al.*, 2001). On a functional level, it has been shown that $Na_V1.7$ mutations can dramatically change the sensitivity to painful stimuli (Yang *et al.*, 2004; Cox *et al.*, 2006; Fertleman *et al.*, 2006 and aforementioned reviews). See section 4.2 for further discussion on the specific contribution of each sodium channel in our C-fiber model.

Voltage-gated potassium channels

The family of *voltage-gated potassium channels* (K_V) is very large, and rather than specifically referring to the genetic types (as for Na_V channels) it is often more useful to describe them according to their function. In the model used for Papers IV, V and VI we included three common K_V channels. K_{dr} (delayed rectifier) can be thought of as the classical Hodgkin-Huxley K_V channel, with no (or very little) inactivation. K_A (A-type) typically refers to a channel with fast inactivation, while K_M (M-type) is a slower channel.

Voltage-gated calcium channels

There are a number of different *voltage-gated calcium channels* (denoted VGCC or Ca_V) in the nervous system, such as $Ca_{V,L}$, $Ca_{V,N}$ and $Ca_{V,T}$ (Catterall *et al.*, 2005). The L-type VGCC is in focus of this thesis, as will be described in section 3.2.

Hyperpolarization-activated channels

Although voltage-gated ion channels typically activate with depolarization, there are also *hyperpolarization-activated ion channels* (denoted HCN or simply h). HCN channels are widespread in the nervous system, serving important roles in pyramidal neurons (Larkum *et al.*, 2009) as well as in nociceptors (Emery *et al.*, 2012; Weng *et al.*, 2012).

Sodium-activated channels

There are several reports showing that *sodium-activated potassium channels* (K_{Na}) might play a role in both CNS (Schwindt *et al.*, 1989; Bhattacharjee *et al.*, 2005; Budelli *et al.*, 2009) and PNS (Safronov and Vogel, 1996; Bischoff *et al.*, 1998; Tamsett *et al.*, 2009; Nuwer *et al.*, 2010). In fact, it has been suggested that the importance of $[Na]_{in}$ for K^+ channels is comparable to that of $[Ca]_{in}$ (Bhattacharjee and Kaczmarek, 2005).

Leak channels

Most ion channels are activated by changes in V_m and/or concentrations of various substances (e. g. Ca^{2+}, Na^+, glutamate and GABA). However, there are also *leak channels*, which are typically thought of as having a constant conductance. In section 3.3 I will discuss the leak currents included in the C-fiber model.

2.2.4 Ion pumps

While ion channels allow selected ions to flow down their electrical and concentration gradients, *ion pumps* actively move ions in the opposite direction (against the ionic gradients). While ion channels are responsible for generating electrical events (such as the action potential), ion pumps are responsible for maintaining appropriate intra- and extracellular ionic concentrations. Given the importance of Na^+ and K^+ in neurophysiology, it is not surprising that one of the most important pumps – the Na-K-pump (sometimes called the Na-K-ATPase or simply the Na^+ pump) – is dedicated to pumping these ions. In addition to maintaining ionic concentrations, it also produces a hyperpolarizing current (since more Na^+ is pumped out than K^+ is pumped in). It is also interesting to note that mutations in pump genes have been implicated in pain (De Fusco *et al.*, 2003).

2.2.5 mGluR and synaptic transmission

Synaptic transmission is a major form of neuronal communication. Synapses are in principle either inhibitory or excitatory. Inhibitory signals decrease, while excitatory increase the probability of an AP being generated. For the majority of excitatory synapses in the brain, glutamate serves as the neurotransmitter,

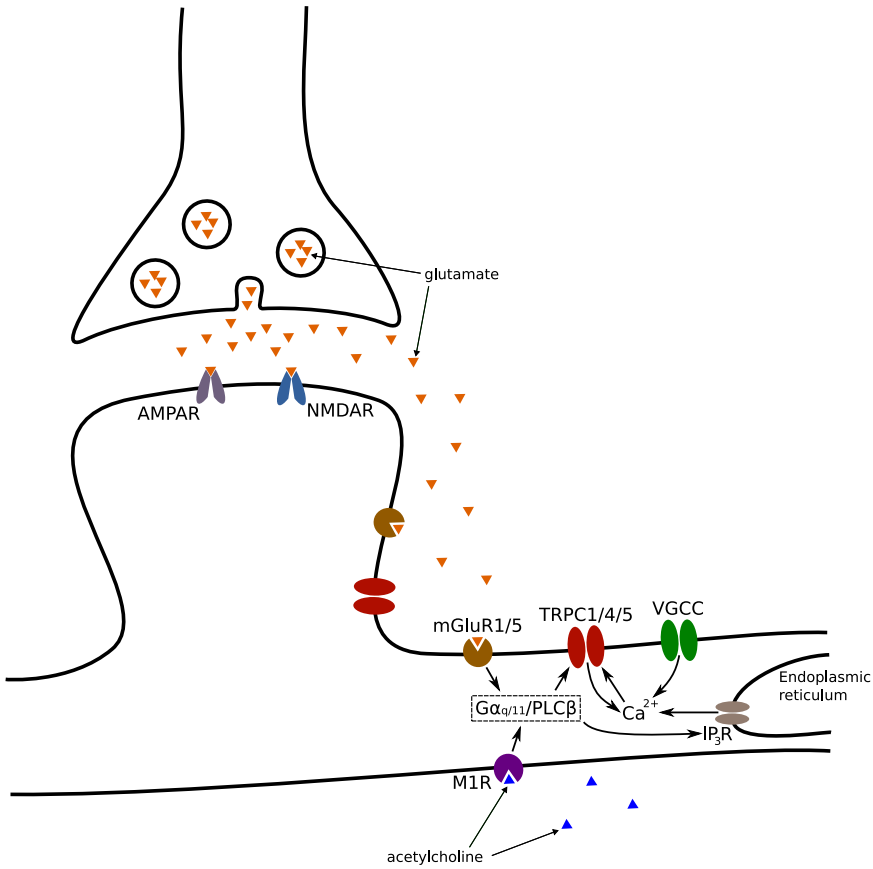

Figure 2.3. *Schematic drawing of ionotropic (AMPA and NMDA) and metabotropic (mGluR1/5) receptors at an excitatory synapse. At the arrival of a presynaptic action potential, glutamate is released and binds to AMPA, NMDA and mGluR1/5 receptors. mGluR1/5, as well as TRPC channels, are located peri- and extrasynaptically. The $G\alpha_{q/11}/PLC$ pathway is triggered by activation of mGluR1/5 activation, or activation of the muscarinic (acetylcholine) M1 receptor. This pathway in turn leads to activation of TRPC channels (although the exact mechanisms of this is unclear), which are also dependent on Ca^{2+}. Sources of Ca^{2+} that may be involved are: voltage-gated calcium channels (VGCC), release from the endoplasmic reticulum (via IP_3 receptors), and the TRPC channels themselves.*

while inhibitory synapses are typically mediated by GABA (γ-aminobutyric acid).

Postsynaptic currents at glutamatergic synapses, which are mediated by either AMPA, NMDA or mGluRs (as was indicated in figure 2.2 and more fully illustrated in figure 2.3), depolarize the dendritic membrane at the site of synaptic input. The depolarization then spreads to other parts of the dendritic tree and may be observed in the soma as an excitatory postsynaptic potential (EPSP).

Glutamate receptors are either ionotropic or metabotropic

The ionotropic receptors AMPA and NMDA form transmembrane pores and, when bound to glutamate, allow for ionic current flows that depolarize the membrane. The AMPA current is relatively fast and gives rise to an EPSP with a decay time constant of around 5 ms (Destexhe *et al.*, 1994b). The NMDA current is slower, with an EPSP decay time constant of around 150 ms.

In contrast to the ionotropic glutamate receptors, the metabotropic glutamate receptors do not form transmembrane pores and hence cannot directly produce an EPSP. Instead, they trigger intracellular pathways that may subsequently lead to the activation of ion channels. Resulting EPSPs are typically slower than ionotropic EPSPs.

Group I mGluR receptors: mGluR1 and mGluR5

Metabotropic glutamate receptors are abundant in the brain (Fotuhi *et al.*, 1994; Stuart *et al.*, 2007; Olive, 2009; Niswender and Conn, 2010) and have several important functions (Anwyl, 1999; Benarroch, 2008). mGluR receptors are involved in mechanisms relating to e. g. fear (Schulz *et al.*, 2001; Rudy and Matus-Amat, 2009), working memory (Hayashi *et al.*, 2007) and synaptic plasticity (Anwyl, 1999; Clem *et al.*, 2008; Gladding *et al.*, 2009; Niswender and Conn, 2010). Several reviews have implicated mGluR receptors as future targets for pharmacological treatment, since they may be involved in a range of neurological diseases (Anwyl, 1999; Benarroch, 2008; Ferraguti *et al.*, 2008; Olive, 2009; Niswender and Conn, 2010).

mGluR receptor subtypes are divided into three groups: I, II and III. According to Knöpfel and Uusisaari (2008), effects following the activation of group I are mainly postsynaptic, whereas effects of group II and group III are mainly presynaptic (though they are present in postsynaptic structures in some brain regions, see Stuart *et al.*, 2007) and will not be discussed in this thesis. Group I mGluR, which includes subtypes mGluR1 and mGluR5, couple to the PLC (phospholipase-C) pathway via activation of G-proteins. Mechanisms downstream of mGluR1/5 activation are diverse and include regulation of NMDA currents, calcium-gated, voltage-gated and inwardly rectifying potassium channels, calcium channels (Knöpfel and Uusisaari, 2008) and TRPC channels (Congar *et al.*, 1997), the latter being in focus of this thesis. Unlike AMPA and NMDA receptors, mGluR1/5 receptors are not located at the synaptic cleft, but peri-

and extrasynaptically (at least in hippocampal and Purkinje neurons, see Lujan *et al.*, 1996; Mateos *et al.*, 2000), which has led to discussions about "spillover-glutamate" effects in synaptic transmission (see e. g. Knöpfel and Uusisaari, 2008), with synaptic cooperativity as one consequence. mGluR1/5 receptors are present in several different brain regions (Olive, 2009).

2.2.6 Second messengers and intracellular signaling

Molecular signaling within neurons is important for neuronal function, but harder to study than the electrical activity of the membrane. Much of the information within the neuron is carried by second messengers (molecules relaying signals within cells), several of which are important in this thesis. Examples of common second messengers are PIP_2 (phosphatidylinositol bisphosphate), DAG (diacylglycerol) and IP_3 (inositol trisphosphate), which are all involved in the signaling following mGluR1/5 activation. Specifically, mGluR1/5 leads to activation of G-proteins which in turn activates the PLC enzyme. PLC then cleaves PIP_2 into DAG and IP_3.

Calcium

One of the most important second messengers is the calcium ion, which has an impact on nearly every aspect of cellular life (Berridge *et al.*, 2003; Clapham, 2007). An interesting fact about Ca^{2+} is that its intracellular concentration is typically very low ($\sim 100\,\mathrm{nM}$) compared to the extracellular ($\sim 2\,\mathrm{mM}$) concentration (Clapham, 2007). Furthermore, Ca^{2+} ions may be highly localized, restricted to microdomains. Ca^{2+} not only plays a crucial role in the activation of TRPC channels, but is also to some extent conducted when these channels are open (Ramsey *et al.*, 2006). The role of Ca^{2+} in TRPC activation will be further described in section 2.3.

Sodium

In recent years it has become clear that intracellular sodium constitutes an important cellular signal, in both CNS and PNS (Rose, 2002; Bhattacharjee and Kaczmarek, 2005; Kelly and Rose, 2010). In CNS, large $[Na]_{in}$ elevations can occur in the hippocampal and cerebellar dendrites (Rose, 2002; Bhattacharjee and Kaczmarek, 2005). $[Na]_{in}$ directly affects processes that it binds to, such as the sodium-activated potassium channel, the sodium-calcium exchanger and the sodium-potassium pump. In addition to these direct effects, $[Na]_{in}$ also affects the electrochemical gradient that drives current through sodium channels, the importance of which we showed in Paper IV.

2.2.7 Studying the nervous system

The field of neuroscience is very broad, and in order to make proper assumptions anyone who studies it needs to be aware of its many levels. For example, psychologists who study the development of AD often need to be familiar with

genetic risk factors such as APOE-ϵ4, and how to interpret data from fMRI (functional magnetic resonance imaging) studies. Similarly, cellular neurophysiologists need to understand not only cell physiology, but also the neuron's role in network dynamics, and the network's role in behavior. The work in this thesis involves assumptions on levels ranging from genes to behavior, showing that computational neuroscience is not an exception.

Animal studies

For ethical reasons, there are many restrictions on how the human brain, in particular, can be studied with invasive techniques. Some insights can be gained from using non-invasive electromagnetic measurement techniques such as fMRI, EEG (electroencephalography) and MEG (magnetoencephalography). Unfortunately, these techniques cannot give a complete picture of the neural mechanisms underlying a certain behavior, and studying the human brain is hence problematic. Fortunately, the human nervous system has many features in common with less complex animals that are easier to study. Important examples of neuroscientists that benefited from working with animals are Hodgkin and Huxley who studied the squid, Hubert and Wiesel who studied the cat, and Kandel who studied a sea snail called Aplysia. They were all awarded the Nobel Prize for their discoveries. The work in this thesis concerns cellular neurophysiology, a subfield of neuroscience that to a large extent relies on rodent studies. In cellular neurophysiology we aim to understand how neurons integrate their input signals and subsequently transmit signals to other neurons. For this purpose I used computational modeling, a technique often used in parallel with experimental techniques. I will give an introduction to computational modeling in Chapter 3.

In vitro

In vitro, in Latin literally meaning "within the glass", is a term which in neuroscientific research usually refers to an experiment conducted using brain slices or in a cell culture. Two common experimental protocols for studying single neurons in brain slices are voltage clamp and current clamp, and these are often used in combination with application of pharmacological blockers. Most of the knowledge we have about ion channels and receptors comes from such studies. The data in figure 1 of Paper I comes from *in vitro* experiments performed by our experimental collaborator Motoharu Yoshida, who induced synaptic activation of EC neurons, with and without mGluR1/5 blockers, and measured the response in the soma in order to study the properties of the TRPC channel. It is possible to do similar recordings out in the dendrites, but it is relatively difficult (the diameter of dendritic branches is much smaller than that of the soma) and therefore rarely done. Computational modeling, however, with which electrical activity at any part of the neuron can be monitored, can well complement this type of *in vitro* experiment.

It should also be mentioned that the concentration of Ca^{2+} can be mea-

sured, using optical techniques where Ca^{2+} is bound to a fluorescent dye. Since Ca^{2+} plays a very important role as an intracellular messenger, it is beneficial and common to study its dynamics for a range of different research questions, including those relating to TRPC channels.

In vivo

In vivo, meaning "within the living", refers to an experiment performed in a living animal. In some *in vivo* experiments, single-unit activity (spiking) is recorded, with the purpose of enhancing our understanding of the neural code (Buzsáki, 2004). This is however complicated by the fact that neurons are not point sources, but rather generate complex spatio-temporal patterns of electrical activity. Furthermore, extracellular recordings do not reveal much about the underlying single-neuron dynamics, especially in the subthreshold domain. It is therefore exciting to learn about the recent development of *in vivo* measurement techniques, which give insights into, for example, Ca^{2+} dynamics and dendritic activity during behavior. Murayama and coworkers recently succeeded in using *in vivo* Ca^{2+} imaging to show that dendritic spikes exist in awake rats (Murayama *et al.*, 2009), and that dendritic activity correlates with the strength of subsequent hindlimb movement (Murayama and Larkum, 2009). Harvey *et al.* (2009) have been able to record subthreshold membrane dynamics in an awake rat performing a visuospatial task in a virtual reality environment, and Lütcke *et al.* (2010) have reported the development of an optical technique enabling Ca^{2+} transients to be recorded from individual apical dendrites in awake and behaving rats. A review by Scanziani and Hausser (2009) discusses several aspects of optical techniques, both *in vitro* and *in vivo*.

Microneurography is an *in vivo* technique of particular interest for this thesis, by which extracellular action potentials can be recorded from single human C-fibers (Schmelz and Schmidt, 2010). In a typical experiment, the peripheral end of a C-fiber (e. g. in the foot) is naturally (touch, heat) or electrically stimulated so that an AP is evoked. The AP travels (along the leg) and is recorded at a proximal location (upper leg) by the insertion of a needle. Propagation latencies can thus be recorded and generate important insights about the membrane excitability in healthy subjects as well as in patients.

2.3 Transient receptor potential (TRP) channels

2.3.1 Introduction

The transient receptor potential (TRP, often pronounced "trip") ion channel family consists of channels with a great diversity in activation mechanisms and selectivity. There are 28 channel protein (or subunit) members expressed in humans. A single defining functional characteristic among TRP channels has not yet been found, but one can generally describe them as calcium-permeable

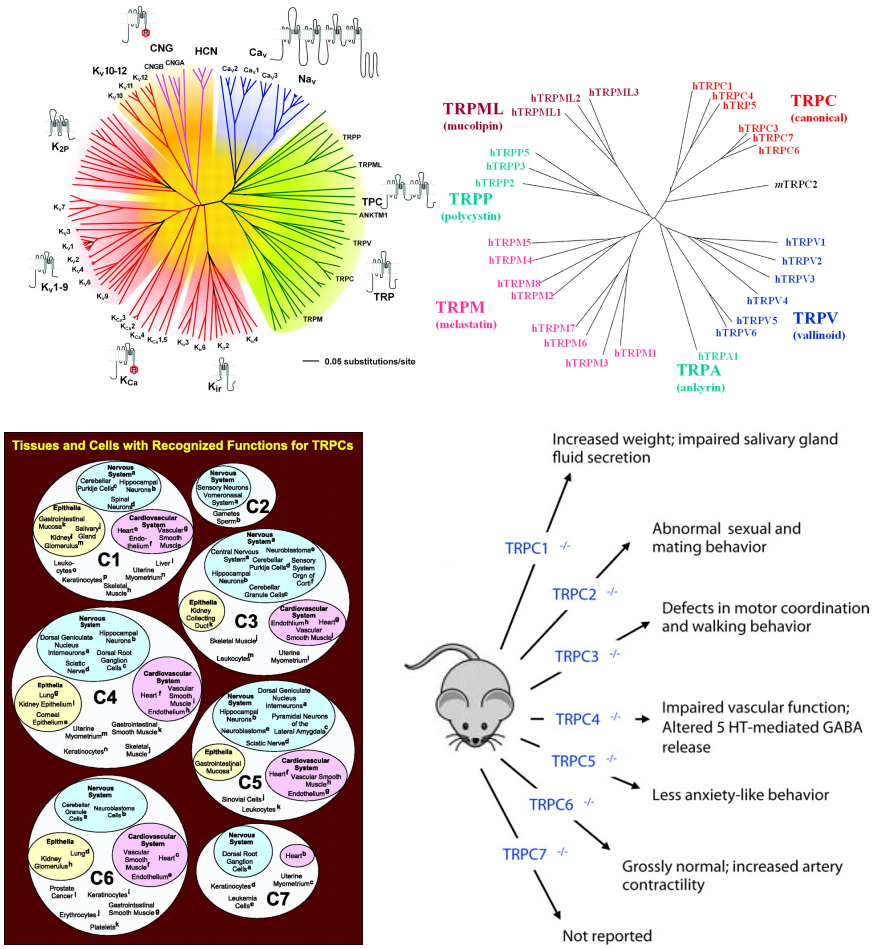

Figure 2.4. *TRP channels constitute a large portion of the human ion channels (top left, from Yu* et al.*, 2005), and are divided into six major subfamilies (top right, from Nilius* et al.*, 2007). TRPC ion channels are expressed in a variety of tissue and cell types (bottom left, from Abramowitz and Birnbaumer, 2009). Genetic deletion experiments show that TRPCs are important on the behavioral level (bottom right, from Wu* et al.*, 2010).*

cation channels with polymodal activation properties (Ramsey *et al.*, 2006). On the structural level, a common characteristic is that the subunit proteins have six transmembrane segments. The TRP channels play critical roles in sensory physiology of the peripheral nervous system, where they contribute to vision, taste, olfaction, hearing, touch and thermo- and osmosensation (Voets *et al.*, 2004; Venkatachalam and Montell, 2007; Damann *et al.*, 2008; Talavera *et al.*, 2008), and also play important roles in nociceptive signaling (Chung *et al.*, 2011). Furthermore, they enable individual cells to sense their local environment, including changes in growth cues (TRPC1/3/5), vascular tone (TRPC3/6) and synaptic activity (TRPC1/3/4/5). Intriguingly, recent studies have shown that infrared detection is mediated by TRPA1 in snakes (Gracheva *et al.*, 2010) and by TRPV1 in vampire bats (Gracheva *et al.*, 2011). The list of cellular functions that TRP channels give rise to can be made very long, and the importance of their role in calcium signaling has been particularly emphasized (Clapham *et al.*, 2001; Minke, 2006; Birnbaumer, 2009).

2.3.2 The origin of the name

While other cation-selective channels are classified according to their selectivity or ligand function, TRP channels are classified as a group on the basis of gene sequence homology, due to the complexity in their properties (Moran *et al.*, 2004). In analogy with this (and unlike major ion channel families such as the voltage-gated sodium, potassium and calcium channels), the TRP family is not named according to the essential function of its members. Rather, the origin of the name is related to how the family was discovered. In 1969 a Drosophila (fruit fly) mutant was identified that had a transient rather than sustained response to prolonged illumination (Cosens and Manning, 1969). It was given the name "transient receptor potential" (TRP) by Minke *et al.* (1975). Several years passed before it was realized that the responsible gene was actually encoding a transmembrane protein (Montell and Rubin, 1989), or more specifically an ion channel (Hardie and Minke, 1992). The TRP-channel research field was only slowly developing during the first two decennia (Minke, 2006), but in recent years it has grown substantially as it has become clear that the TRP ion-channel family, with its 30 members, is very important for both peripheral and central cells of the mammalian nervous system.

2.3.3 TRP channels are expressed in most tissue types

TRP channels (see figure 2.4) are expressed in almost all mammalian tissue types, and all known TRP channel types are expressed in the nervous system (Nilius *et al.*, 2007; Squire, 2009). The TRPC channels, which mediate the I_{TRPC} current of Papers I, II and III, are expressed to various extents in different brain regions. Strübing *et al.* (2001) studied the subcellular localization of TRPC1 in embryonic hippocampal neurons and found that it was distributed throughout the cell (soma, dendrites and axon), but not at synaptic structures. The subcellular localization in adult neurons is unknown but suggested to differ

from that in embryonic neurons (Blair *et al.*, 2009). von Bohlen und Halbach *et al.* (2005) showed that TRPC1 and TRPC5 are ubiquitous in mammalian medial temporal lobe structures and suggested that these proteins may therefore play an important role in neural plasticity, learning and memory. Fowler *et al.* (2007) showed that TRPC4 and TRPC5 are expressed in several regions of the brain. See the review by Abramowitz and Birnbaumer (2009) for additional references on TRPC expression.

2.3.4 The TRPC subfamily

The TRP channels are divided into six subfamilies: TRPC, TRPV, TRPM, TRPA, TRPP and TRPML (channels from a seventh subfamily, TRPN, are not expressed in mammals but only in lower vertebrates and invertebrates). The TRPC (C denoting "canonical" or sometimes "classical") channels, which were the first to be discovered, serve important physiological functions (figure 2.4). In spite of this, they are the least characterized (Minke, 2006), as many questions remain regarding their physiological functions and gating mechanisms. Like all members of the TRP family, the TRPC subunit proteins assemble as homo- or heterotetramers to form ion channels (Desai and Clapham, 2005; Schaefer, 2005; Villereal, 2006; Venkatachalam and Montell, 2007). The current belief is that each subunit contributes to a shared selectivity filter and ion-conducting pore similar to that seen in potassium channels (Ramsey *et al.*, 2006). There are seven TRPC subunits, TRPC1-7, though TRPC2 is not expressed in humans. Alternative splice variants have been observed for all subunits except TRPC5 (Li *et al.*, 2005). Based on sequence alignments and functional comparisons, TRPC subunits are sometimes grouped into: TRPC1, TRPC2, TRPC3/6/7 and TRPC4/5.

Members within a TRPC subunit group tend to form heterotetrameric ion channels together, although channels can consist of proteins from different subunit groups as well. Strübing *et al.* (2001) showed that this may have important functional consequences, as they compared the magnitude of single channel conductances and current-voltage relations between pure TRPC1 and TRPC5 homomeric channels with TRPC1/5 heteromeric channels, and found that it differed. Muscarinic receptor-activated TRPC5 homomers had a single channel conductance of 38 pS and an inwardly rectifying current (i. e. the channel passes inward current more easily than outward current), while TRPC1 homomers did not conduct currents at all. TRPC1/5 heteromers had a single channel conductance of 5 pS, and an outwardly rectifying current similar to that of NMDA channels. The implication of their finding is quite important, namely that TRPC heteromers have different biophysical properties from TRPC homomers, which extends the functional repertoire of TRPC channels substantially as the number of possible subunit combinations is large. This has been pointed out in several reviews, such as: Pedersen *et al.* (2005); Schaefer (2005); Villereal (2006); Venkatachalam and Montell (2007). Neurons may benefit from this TRPC subunit promiscuity, but for scientists studying the resulting currents it becomes problematic, which I will discuss further in section 4.1.

Although many questions regarding the functional properties of TRPC channels still remain to be resolved, some fundamental characteristics have been identified (in addition to their structural similarities):

- They are nonselective cation channels (like all other TRP channels). In other words, they can conduct sodium, potassium as well as calcium.
- They are weakly voltage-dependent (Squire, 2009).
- They can be activated downstream of the PLC pathway (Montell, 2005; Pedersen *et al.*, 2005).
- They may be located and regulated within spatially confined calcium microdomains (Ambudkar, 2006).
- They may play important roles in both sodium (Eder *et al.*, 2005) and calcium signaling.

2.3.5 The mGluR1/5-activated TRPC current – I_{TRPC}

As mentioned above, it is known that TRPC currents can be activated in many different ways. Here, I will focus only on what is known about the aspects relating to the TRPC-mediated current – I_{TRPC} – which is generated in response to synaptic activation of mGluR1/5 (or non-synaptic activation of M1). This current has been known and studied for several years, and has been reported in several regions of the brain, including hippocampal CA1 (Congar *et al.*, 1997), entorhinal cortex (Egorov *et al.*, 2002; Fransén *et al.*, 2006; Yoshida *et al.*, 2008; Zhang *et al.*, 2011), cerebellum (Kim *et al.*, 2003), substantia nigra pars compacta (Bengtson *et al.*, 2004), amygdala (Faber *et al.*, 2006), anterior cingulate cortex (Zhang and Séguéla, 2010), subiculum (Yoshida and Hasselmo, 2009) and prefrontal cortex (Sidiropoulou *et al.*, 2009).

I_{TRPC}, which is an inward current leading to a depolarization of the membrane potential (V_m), has essentially been studied either in its subthreshold or suprathreshold form of appearance. In its subthreshold form, I_{TRPC} mediates a slow afterdepolarization (sADP) similar to the EPSPs seen in response to AMPA or NMDA activation. In its suprathreshold form, I_{TRPC} mediates plateau potentials. During plateau potentials, which sometimes have a graded nature (Egorov *et al.*, 2002; Fransén *et al.*, 2006; Yoshida *et al.*, 2008), the neuron generates persistent trains of action potentials at a stable frequency during a long period of time. Both sADPs and plateau potentials have been implicated as being important for proper mnemonic functioning, since these two phenomena allow the activity of a neuron to outlast the original input stimulus (Egorov *et al.*, 2002; Yan *et al.*, 2009).

I_{TRPC} is activated downstream of $G\alpha_{q/11}$, a G-protein pathway which in turn is activated in response to activation of either mGluR1/5 or the muscarinic M1 receptor, as illustrated in figure 2.3. Consequently, the current has been studied by activation or inactivation of either mGluR1/5 or M1. Both types of studies are relevant for the work in this thesis, but the mGluR1/5 induced

mechanisms are of special interest. It should be mentioned that several names have been used to refer to what is now believed to be a very similar (or identical) current, including I_{sADP} (Caeser *et al.*, 1993; Yan *et al.*, 2009), I_{NCM} (Shalinsky *et al.*, 2002), I_{CAN} (Fransén *et al.*, 2006), "Alonso-current" (Hasselmo and Stern, 2006) and mGluR-EPSC (Reichelt and Knöpfel, 2002).

Role of I_{TRPC} in behavior and disease

There is data which strongly indicates that TRPC channels are important in behavior. Riccio *et al.* (2009) studied TRPC5 knock-out mice and observed that fear levels in response to aversive stimuli were decreased in comparison with control mice. Interestingly, they also showed that, in amygdala neurons, the amplitude of mGluR1/5-mediated postsynaptic responses was decreased. This is direct support for the hypothesis in Paper I and Paper II, namely that mGluR1/5-mediated TRPC currents are important for synaptic transmission. Taken together, it also implies that the phenomenon we study is important on the behavioral level. The same conclusion can be made from a study by Sidiropoulou *et al.* (2009), in which they showed that the I_{TRPC}-mediated depolarization is modulated by dopamine and cocaine experience. They discuss that, as a consequence, I_{TRPC} may be important for cognitive phenomena such as attention, working memory, long-term memory and addiction. Additional support for the role of TRPC channels in working memory comes from experimental studies of entorhinal pyramidal cells (Egorov *et al.*, 2002; Hasselmo and Stern, 2006).

Several reviews have stressed the important role that TRPC channels may play in disease (e. g. Abramowitz and Birnbaumer, 2009; Selvaraj *et al.*, 2010). Not only the TRPC5 protein has been knocked out in experiments, but also most of the other TRPC subtypes. As can be seen in figure 2.4 (lower right), these studies have implicated that TRPC channels may play important roles in behavior and disease. Other reviews have discussed not only TRPC channels but TRP channels in general, such as Nilius *et al.* (2007); Venkatachalam and Montell (2007) and all reviews included in the 2007 special issue on "TRP Channels in Disease" of Biochimica et Biophysica Acta (Nilius, 2007).

Activation mechanisms

Although the mechanisms for I_{TRPC} activation have been studied for many years, they are still not clear. Key questions relate to the molecular mechanisms for I_{TRPC} activation, the role of intracellular Ca^{2+}, and the subunit composition of the ion channels. A few recent papers have produced several relevant insights.

Yan *et al.* (2009) showed with a gene knock-out technique that, in cortical pyramidal cells, $G\alpha_{q/11}$ is required for generating the M1R/TRPC-mediated sADP, and the PLC (or more specifically, PLCβ1) pathway contributes strongly. Experimental techniques involving the use of HEK293 (human embryonic kidney) cells are common for studying how certain combinations of membrane proteins (like ion channels) behave when expressed in isolation. In the same

study, Yan *et al.* expressed T-type voltage-gated calcium channels (VGCC) together with TRPC5 in HEK293 cells and obtained an sADP. Importantly, the sADP was absent if the Ca^{2+} source (they used T-type VGCCs, but pointed out that any Ca^{2+} source may be able to do the job) was excluded, indicating that Ca^{2+} is required for generating the sADP. When overexpressing TRPC5 or TRPC6 in cortical pyramidal cells they saw an enhancement of the sADP, and thus concluded that I_{TRPC} mediate the sADP.

Zhang *et al.* (2011) showed, in EC pyramidal cells, that PLC (or more specifically, PLCβ) activation is required for generating the I_{TRPC} current via muscarinic receptors, that decreases in PIP_2 levels and permissive Ca^{2+} levels are required, and that IP_3 receptors may be involved in activation I_{TRPC}. Further, they found that I_{TRPC} was reduced after application of TRPC4/5-disrupting peptides, and that the I_{TRPC} I-V curve is similar to I-V curves of TRPC heteromers involving TRPC1, TRPC4 and TRPC5, indicating that these subunits form the ion channels responsible for mediating the I_{TRPC} current. This hypothesis is supported by studies showing that these three subunits are indeed present in the rodent EC (von Bohlen und Halbach *et al.*, 2005; Fowler *et al.*, 2007).

Gross *et al.* (2009) expressed TRPC5 channels in HEK293 cells and observed that intracellular Ca^{2+} either from stores or from VGCCs can lead to activation of TRPC5. They argued that the activation of TRPC5 by calcium is fast (and hence direct), essential and even sufficient. In contrast to many other studies, they showed that inhibition of the PLC pathway does not affect this Ca^{2+} activation.

Furthermore, Blair *et al.* (2009) used HEK293 cells to express TRPC5 homomers. They showed that Ca^{2+} plays an important role in controlling the amplitude of agonist-activated TRPC5 currents, while stressing the fact that Ca^{2+} affects numerous steps before channel activation (including receptor desensitization, PLC activation and PKC activation), indicating that it is a complex process. Like Yan *et al.* (2009), they found that increases in Ca^{2+} by VGCCs (L-type, in this study) can trigger TRPC5 activation.

Kanki *et al.* (2001) expressed TRPC5 channels in frog eggs (Xenopus oocytes) and showed that IP_3 receptors are important for TRPC5 activation, which is partially supported by the study by Zhang *et al.* (2011) described above. It should be noted, however, that this is a debated topic (see e. g. reviews by Vazquez *et al.*, 2004; Plant and Schaefer, 2005; Birnbaumer, 2009).

Taken together, these reports suggest that

- the $G\alpha_{q/11}$ pathway (including PLCβ, at least in EC pyramidal cells) is involved in I_{TRPC} activation,
- an increase in cytosolic Ca^{2+} is required for I_{TRPC} activation,
- activation of IP_3Rs or VGCCs can contribute to the increase in Ca^{2+}, and that,
- in EC pyramidal cells, it is likely that I_{TRPC} is mediated by channels formed by TRPC1/4/5 subunits.

I_{TRPC} is poorly characterized and often absent in experimental observations

Although shown to be of great importance (see e. g. Batchelor and Garthwaite, 1997; Riccio *et al.*, 2009; Sidiropoulou *et al.*, 2009), mGluR-mediated currents such as I_{TRPC} are not always seen in experiments. This may be due to a number of reasons. In some systems the current does not exist. It may also be the case that Ca^{2+}-activated potassium channels are activated in response to the Ca^{2+} influx, leading to a hyperpolarization that partially or completely occludes the depolarization caused by I_{TRPC}. It is furthermore likely that I_{TRPC} is sometimes lumped together with the much more well-recognized I_{NMDA}, since their time scales partially overlap. Their sum may then simply be interpreted and referred to as an "NMDA current", although "NMDA-like current" or "slow synaptic current" would be more appropriate descriptions. I_{TRPC} may also be hard to see in experiments if, for example, a low temperature is used, since the probability of synaptic glutamate release is then reduced, with the consequence that little glutamate reaches perisynaptic and extrasynaptic regions (where mGluR1/5 is situated). An important problem related to identifying I_{TRPC} is that it may require activation of VGCCs, in turn requiring depolarization caused by activation of ionotropic synaptic receptors. VGCCs will not be sufficiently activated if the synaptic stimulus-induced membrane depolarization is too weak. For the same reason, it is problematic to attempt to study I_{TRPC} by simply applying blockers of AMPA and NMDA. It would be much better to block the TRPC channels themselves in order to study their influence on synaptic signaling. Such blockers are being developed (Zhang *et al.*, 2011), but are still not widely used. Blocking mGluR1/5 is another option, but since these receptors act on intracellular pathways that have many other downstream targets than TRPC such manipulation does not give full insight into the nature of I_{TRPC}. In addition, the relative contribution of mGluR1 and mGluR5 is still unclear, though it has been argued that the two receptors have a synergistic effect (Gee *et al.*, 2003). Finally, there is also the option to knock out the genes coding for e. g. TRPC5 channels (Riccio *et al.*, 2009), but in such studies it is difficult to control for the possibility that compensatory mechanisms are triggered or that homomeric TRPC channels are formed in the absence of the TRPC5 partner.

2.4 Dendrites in CNS

It has in recent years become clear that dendritic processing is complex. This has been reviewed by e. g. Segev (1998); Reyes (2001); Häusser and Mel (2003); London and Häusser (2005); Magee and Johnston (2005); Sidiropoulou *et al.* (2006); Johnston and Narayanan (2008); Spruston (2008), and in the book devoted to dendrites (Stuart *et al.*, 2007). In this section I will mention a few interesting studies pointing to this complexity, and also discuss spatio-temporal summation of synaptic input. Finally, I will discuss what is known about the role of dendrites in disease.

2.4.1 Dendrites extend the neuronal computation toolbox

The role of dendrites in neuronal computation was poorly understood for a long time. One of the first important insights came from a modeling study by Agmon-Snir *et al.* (1998), who demonstrated a function of auditory neurons that could not be carried out without the nonlinear synaptic integration provided by the dendrites. They compared a point-neuron model with a bipolar neuron model and showed that detection of sound localization was improved in the latter. The auditory neuron is supposed to respond (generate an action potential) only if input arrives simultaneously from both the left and right ear, and achieves this by receiving synaptic inputs from the two ears on different dendritic branches (Segev, 1998; London and Häusser, 2005). Agmon-Snir *et al.* (1998) showed that, if two inputs arrive on the *same* dendrite, the voltage deflection will be so large that the driving force ($V_m - E_{syn}$) for the synaptic currents will be small, resulting in sublinear summation. If the two inputs arrive on *different* dendrites and summed in the soma, however, the sublinearity will be less severe, thus increasing the probability of reaching the spike threshold. If a simple bipolar neuron can achieve this kind of algorithmic computation, then what are for example pyramidal or Purkinje neurons capable of, considering the complexity in morphological structure of these neurons?

In addition to being morphologically complex, dendritic trees are equipped with a plethora of ion channels, as mentioned in section 2.2. The presence of these active conductances supports phenomena such as back-propagation of action potentials, local increases in Ca^{2+} concentrations and modulation of synaptic potentials (Schiller and Schiller, 2001).

Dendritic functional compartmentalization

Katz *et al.* (2009) recently showed, using electron microscopy in combination with computational modeling, that hippocampal CA1 pyramidal neurons do not integrate synaptic inputs by simply summing them in the soma. Rather, local regenerative spikes are generated in branches of the dendritic tree and these signals may in turn propagate and undergo computation in a second stage, which is similar to what Losonczy and Magee (2006) argued for. In line with these results from CA1 neurons, Polsky *et al.* (2004) showed that a similar two-layer computation may exist in neocortical pyramidal neurons, supported by dendritic spikes. Research on dendritic spikes has increased in recent years, and a study by Larkum *et al.* (2009) could perhaps be considered to be a landmark study. They argued for a "unifying principle" of synaptic integration (in pyramidal cells), which involves spatially confined regions of the dendritic tree supporting different kinds of dendritic spikes (see also Larkum and Nevian, 2008). According to this hypothesis, sodium spikes can be generated throughout the dendritic tree, whereas initiation of calcium spikes are limited to a zone on the apical trunk, and NMDA spikes can only be initiated at distal regions of the dendritic tree. An interesting phenomenon emerging from these properties is a kind of

chain-reaction, as discussed by Williams and Wozny (2009). Inputs arriving at distal parts of the tree may be translated into NMDA spikes, which in turn may induce calcium spikes and finally an axonal action potential. These studies are very interesting, as they suggest that the neuron processes information not only in the soma, but also in spatially confined distal parts of the dendritic tree. I recommend reviews by Häusser and Mel (2003) and Magee and Johnston (2005) for further discussions on functional dendritic compartmentalization. Furthermore, it has been suggested that neuronal plasticity can exist specifically for individual dendritic branches (see Frick *et al.*, 2004; Losonczy *et al.*, 2008, and reviews by Frick and Johnston, 2005; Sjöström *et al.*, 2008).

Dendritic spatio-temporal summation

Under what circumstances does spatial summation occur in a pyramidal cell? Let us first look at a few numbers. As discussed above, a number of studies have suggested that sections of the dendritic tree (e. g. a branch) may function as computational subunits. Larkum *et al.* (2009) showed that dendritic spikes are local events, which do not spread to neighboring tuft branches. In line with this, Polsky *et al.* (2004) showed that effective spatial summation of two adjacent synaptic inputs occurs only when the distance between these is less than $40\,\mu m$, which suggests this number to be an estimation of the length of a dendritic functional compartment. As an estimate of the density of excitatory inputs to a pyramidal neuron, one can measure the density of spines. According to Stuart *et al.* (2007), CA1 neurons are "very spiny", averaging 2.5 spines/μm, while pyramidal cells of the visual cortex are "much less spiny", averaging only about 1.5 spines/μm. Thus, pyramidal neurons have approximately $2\ spines/\mu m$. The dendritic functional compartment would thus have *80 synapses*. It has further been suggested that only *10 %* of the neurons in the brain are active (Shoham *et al.*, 2006), which could mean that only every tenth presynaptic terminal actually receives input. Furthermore, it is important to consider that, even if a neuron generates a spike which propagates to a presynaptic terminal, it is not guaranteed that the signal reaches the postsynaptic terminal, since the probability of vesicle release may be low. Borst (2010) has recently suggested that, in contrast to previous estimates of typical release probability, which he believes are high ($\sim 50\,\%$), actual release probability *in vivo* may be close to *10 %*. If we now multiply all these numbers we get an estimate of how many synaptic inputs arrive at a functional dendritic compartment: $80 \cdot 0.1 \cdot 0.1 = 0.8 \approx 1$. Admittedly, these numbers may be in the lower range. Nevertheless it is likely that, under certain circumstances, no spatial summation will occur since only a few synapses receive input. It is of course possible, as hypothesized by Larkum and Nevian (2008), that synapses are functionally clustered on dendritic branches, meaning that functionally related input may arrive on the same dendritic segment. On the other hand, a recent study by Jia *et al.* (2010) shows that synapses involved in a certain class of synaptic inputs (afferent sensory inputs with the same orientation preference) may be widely dispersed over the dendritic tree, and not grouped together on a specific dendritic branch.

From this reasoning, one may suspect that spatial summation is limited in cases when input is spatially sparse. So, what about temporal summation? Can temporally sparse input be summed? Is it even likely that input arrives at low frequencies in a physiologically realistic situation? The latter question has a relatively straightforward answer. The code with which neurons communicate – the neural code – is still poorly understood but, as described in section 2.2, it is actually possible to measure the spike output of single neurons (single-unit activity) in living and behaving animals. Looking at single-unit recording data from studies using a wide range of tasks (spatial orientation, working memory, categorization, recognition), recordings from various brain regions (hippocampus, entorhinal cortex, prefrontal cortex, amygdala) in various species (rodent, non-human primate, human), one finds that spike frequencies during behavior are typically low, around 8 Hz (see Paper I and II for details). This indicates that the dendritic branch receiving such input may need to possess mechanisms allowing for temporal summation of such low frequencies. In Chapter 4 I will argue that I_{TRPC} is one such mechanism.

2.4.2 Dendrites and diseases

Although most studies of dendritic function are performed in the normal brain, there also research aimed at associating certain neurological, psychiatric and developmental disorders with changes in dendrites (Stuart *et al.*, 2007). Epilepsy, Alzheimer's disease and schizophrenia are examples of disorders which have been associated with dendritic abnormalities. Other dendritic abnormalities which may play a role in neurological diseases are structural changes such as loss of spines, changes in spine size and shape, reduced dendritic branching patterns and shortened dendritic length. Unfortunately, there is a lack in knowledge of how these structural changes correlate with changes in neuronal function such as synaptic integration, plasticity, excitability and firing behaviors, thus motivating the need for more research. Besides structural changes, disorders relating to ion channels and receptors are likely to be important.

2.5 Axons in PNS

The basic role of an axon is to transmit action potentials from one end (e. g. the soma of a pyramidal cell, or the peripheral end of a nociceptor) to the other (synaptic connection with another neuron). However, like dendrites, axons may also to some extent perform signal processing (Segev and Schneidman, 1999; Bucher and Goaillard, 2011), such as signal enhancement (activity-induced frequency increase due to axonal supernormality, see e. g. Weidner *et al.*, 2002) and conduction block (propagation failure, see e. g. Obreja *et al.*, 2011).

2.5.1 Nociceptor axons

Nociceptor neurons are specialized in responding to noxious stimulus, such as pressures and temperatures extreme enough to cause tissue injury, as well as

toxic molecules and inflammatory mediators (Dubin and Patapoutian, 2010). The nociceptors can be over 1.5 m long, reaching from the skin (e. g. on the feet) to the spinal cord, and are in fact the largest cells in the body (Hogan, 2010). The axon constitutes the main part of the nociceptor. The soma, situated in a dorsal root ganglion (DRG), is attached to the axon via a T-junction. Both ends (peripheral and central) of the parent axon are split into several daughter branches. Nociceptor neurons are not said to have dendrites, although one could argue that, functionally, the nociceptor axon acts as both dendrite (receivers) and axon (transmitter).

2.5.2 C-type nociceptors

Most nociceptor axons are unmyelinated and referred to as C-fibers (see figure 2.5), while some are myelinated and referred to as Aδ-fibers (Dubin and Patapoutian, 2010). The slowly conducting C-fibers, for which we built a computational model in Paper IV, tend to have a small DRG soma (Harper and Lawson, 1985). Importantly, activity in these fibers corresponds well to perceived pain (Raja *et al.*, 1988). C-fibers are also thought to be involved in pathological pain, as will be discussed below.

Nociceptive C-fibers are typically divided into mechano-sensitive (CM) and mechano-insensitive (CMi) fibers, and are sometimes further divided according to their heat sensitivity; CM: C-MH and C-M; CMi: C-H and C-M_iH_i. C-M_iH_i fibers, often referred to as *silent* or *sleeping nociceptors*, are not active under normal conditions but only after intense mechanical stimulation (Schmidt *et al.*, 2000), under inflammatory conditions (Schmidt *et al.*, 1995) or during itch (Schmelz *et al.*, 1997), and have therefore received particular attention in recent years. A further discussion on the differences between CM and CMi fibers can be found in Paper VI.

2.5.3 Nociceptors and diseases

According to the International Association of the Study of Pain (IASP), pain is defined as follows:

> *pain* is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"

There are various types of pain, which can be categorized as nociceptive, inflammatory, dysfunctional or neuropathic pain (Costigan *et al.*, 2009). While normal (functional) pain is important for humans to be aware of potential danger, there are also many examples of unwanted pain (allodynia and hyperalgesia). Unwanted pain can have a strong negative influence on the life quality of affected patients, and also results in large healthcare costs. Pain problems are typically thought to arise from problems in the CNS, while the influence of PNS is often underestimated (Serra, 2009). In fact, many types of unwanted pain

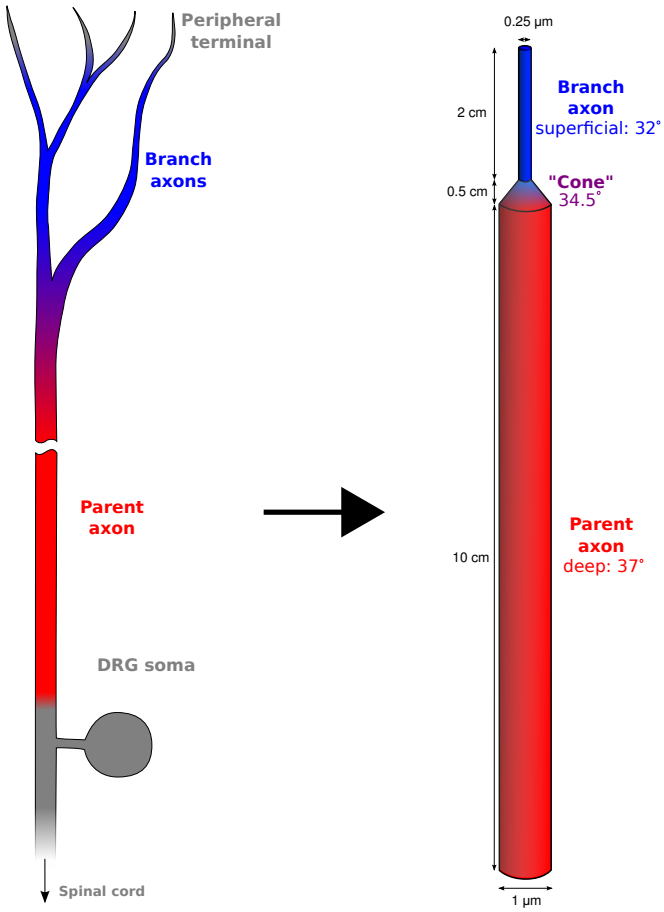

Figure 2.5. *C-fiber nociceptor. Left: cartoon of actual nociceptor. Right: simplification used for our computational model, which includes a branch (blue) and parent (red) axon, but not peripheral terminals, DRG soma or central parts connecting to spinal neurons (gray).*

Figure 2.6. *Pain pathway, as illustrated by Descartes (Traite de l'homme, 1664).*

(e. g. diabetic neuropathic pain, erythromelalgia, phantom limb pain and injury-evoked peripheral sensitization) are associated with PNS, and good treatment is lacking since the underlying mechanisms remain to be completely understood (Baron, 2006; Gold and Gebhart, 2010). Interestingly, there is strong evidence supporting that abnormal C-fiber excitability plays a role in neuropathic pain (Ørstavik *et al.*, 2003; Bostock *et al.*, 2005; Ochoa *et al.*, 2005; Serra *et al.*, 2011; Schmidt *et al.*, 2012; Serra *et al.*, 2012).

Chapter 3

Models and methods

In this chapter I will introduce and review the relevance of computational modeling and in particular multi-compartment modeling. I will also describe the details of the models built and utilized in this thesis, along with simulation protocols and analysis methods.

3.1 Computational neuroscience

Computational neuroscience is an approach in which computer models are used to simulate and understand the function of the nervous system. Computer modeling provides an excellent tool for dealing with complex systems such as the brain, neurons and biomolecular pathways. Hypotheses emerging from experiments can be evaluated, and novel hypotheses can be formulated, thereby guiding future experiments. Computer models are also useful as platforms that integrate experimental data from different settings, as well as assumptions and insights that may not be explicitly stated in the experimental literature. Even before simulations are initiated, a consequence and bonus of this is that conceptual ideas and assumptions will need to become explicit and clarified, which may reveal important questions.

3.1.1 The Hodgkin and Huxley model and beyond

The work by Hodgkin and Huxley (1952), for which they received the Nobel Prize in 1963, is considered by many to be among the most important contributions to the field of neuroscience. Their model – a mathematical description of how two voltage-dependent membrane conductances can give rise to the action potential – provided a simple yet quantitative explanation of a phenomenon which had previously been poorly understood, and thereby another dimension in our understanding of neuronal function. It is thus a good example of how a mathematical theory is useful, or even necessary, to understand and describe certain complex phenomena. The model by Hodgkin and Huxley was the first to incorporate the fact that neuronal membranes have separate, ion-selective and

voltage-dependent membrane conductances (i. e. ion channels). Their model included Na^+ and K^+ channels, and although simplified when compared to the biophysical reality, it was possible to simulate the basic components of an action potential. The concept of time- and voltage-dependent gating parameters n, m and h was introduced, together with descriptions of corresponding rate constants α and β. Ion channel research took a big leap forward, and this framework has been widely utilized and has largely influenced most contemporary conductance-based models.

The classical Na^+ and K^+ channels are essential for neuronal function, but so are many other channels that currently lack a satisfactory description. Thus, much more research is required to fully understand the function of ion channels. Many laboratories today use computational models to study ion channels, often in combination with experimental techniques.

3.1.2 Choosing an abstraction level

When modeling single neuron dynamics, a proper level of abstraction must be chosen, ranging from detailed compartment models to black-box models (Herz *et al.*, 2006). For several reasons it is desirable to have a reduced complexity. With fewer dimensions it is more feasible to achieve a mathematical understanding of modeling results. Reduced models are also less computationally demanding and may therefore allow for more rigorous sensitivity analyses, in turn leading to a better understanding of the roles and importance of various parameters. On the other hand, models should not be too simple, especially considering the risk of losing the connection to experiments and pharmacological development.

In the first project leading to this thesis I studied the influence of dendritic ion channel properties on synaptic integration. For this purpose, it is necessary to use quite detailed models with realistic dendritic filtering, and we therefore decided to use *multicompartmental modeling*. Multicompartment models are well suited for studying the spatiotemporal dendritic integration of synaptic inputs, and allow for modeling of non-uniformly distributed ion channels. I chose to use a multicompartment model published by Poirazi *et al.* (2003), which I describe in section 3.2 below. The Poirazi model is one of the most detailed pyramidal neuron models, and includes calcium dynamics, an important feature when studying the calcium-dependent TRPC current.

In the second project, where we studied the influence of axonal ion channels on action potential propagation in peripheral C-fibers, a similarly detailed abstraction level was used, with multiple ion channel types and a large number of compartments. The main motivation behind this choice was that the purpose of that project was to have a direct connection to specific ion channels as well as to AP propagation along a morphologically realistic axon.

3.1.3 Multicompartment modeling

Multicompartment modeling theory, which is based on cable theory, was developed by Rall (1959). This theory provides a discrete solution to the cable equation, by which the membrane potential (V_m) at any point in the dendritic tree can be calculated as a function of time (Johnston and Wu, 1995). Multicompartment models are typically built such that they capture the passive electrical properties of a neuron, incorporating for example a diameter which changes throughout the dendritic tree, affecting both the membrane time and length constants (τ_m and λ_m). When building a model, the morphological structure of a neuron is often first extracted using a staining technique. Each of these sections are then divided, or discretized, into small compartments. These compartments are individually modeled as electrical circuits and coupled with resistive components. The electrical circuit of each compartment may be simple (passive) and only consist of one resistive and one capacitive component in parallel, mediating the current flowing between the inside and outside of the cell membrane. In this case, the resistive current represents the flow of ions through pores (e. g. ion channels) in the membrane, while the capacitive current represents the thin bilipid membrane layer. However, compartments may also be more complex (active) and contain a multitude of ion channels, ion pumps, receptors, synaptic currents, biochemical reactions, etc., and the complexity may vary among different sections of the neuron.

Insights gained using multicompartment models

Multicompartment neuron models have been used for a variety of research questions. They have proven especially useful for studying dendritic integration phenomena relating to ion channels and receptors as well as arising nonlinear phenomena such as dendritic spikes and backpropagating action potentials. Here I will describe a few examples of studies and areas of research where multicompartment models have been successfully used and produced valuable neuroscientific insights.

Passive properties of the dendrites are important for the electrical behavior of neurons. Thanks to compartment models, several consequences of the detailed geometry of the dendritic tree can be understood (Segev and London, 2000). For example, since EPSPs evoked in distal thin dendrites will experience a large current sink when traveling to the soma, voltage attenuation is stronger than for an EPSP of similar amplitude traveling in the soma-dendrite direction, as shown by Rall and Rinzel (1973). This and other insights gained from modeling passive (and active) dendritic trees are outlined in the book by Stuart *et al.* (2007), who also emphasizes that passive (not only active) properties need to be taken into account to understand the mechanisms of dendritic integration.

By using the CA1 cell model which this thesis is partly based on, Poirazi *et al.* (2003) were able to predict that distal dendrites can act as functional computational subunits. The outputs of these subunits, which individually summate inputs with a sigmoidal activation function, then sum linearly in the

soma. The model predictions were later experimentally confirmed (Polsky *et al.*, 2004; Losonczy and Magee, 2006).

Another successful example of multicompartment modeling comes from research on deep brain stimulation (DBS), a neuroprosthetic technique developed to help patients suffering from neurological movement and affective disorders such as chronic pain, Parkinson's disease, tremor and dystonia. In DBS, electrical stimulation is applied via stimulation electrodes that are implanted into a certain region of the brain, thereby affecting the neural activity such that the patient is relieved of some symptoms. The most successful application of DBS so far has been to help patients with Parkinson's disease by stimulating structures in the basal ganglia. The technique works well but side effects are common. Since the underlying mechanisms are not fully understood, computational models are used to gain further insights, thereby improving the technique. McIntyre and coworkers have contributed to the improvement of DBS techniques by coupling detailed multicompartment models to models of the electrical field induced by the DBS electrode (Miocinovic *et al.*, 2006; McIntyre *et al.*, 2007; Miocinovic *et al.*, 2007). In a recent study on Parkinson's patients they showed that side effects were reduced if stimulus parameters predicted by computational models were used, as compared with typical clinically used stimulus parameters (Frankemolle *et al.*, 2010). Their models were patient-specific, in the sense that model parameters were individually chosen for each patient, based on data from structural imaging, surgical targeting coordinates and microelectrode recordings. Their study provides a powerful demonstration of how computational models can be used for clinically relevant research questions. Note that a morphologically detailed model might be necessary for capturing the behavior of a neuron in the spatially complex electrical field that results from a DBS electrode (Herz *et al.*, 2006), as well as for other types of extracellular stimulation techniques.

Some theoretical insights from dendritic modeling which have been experimentally validated are described in a review by Segev and London (2000) and include synaptic boosting, coincidence detection, the function of spines, background activity, and motion-sensitive tangential cells. Similarly, a review by Kath (2005) exemplifies the usefulness of dendritic modeling by discussing a number of studies relating to synaptic efficacy, membrane resistance, intracellular resistivity, the dependency of back-propagating action potentials on dendritic morphology and ion channel properties, the effect of dendritic structure on firing patterns, and coincidence detection tuned by dendritic branching patterns, among other things. More examples can be found in a review by Herz *et al.* (2006), describing how modeling predictions (Jaeger *et al.*, 1997) regarding a net inhibitory synaptic current underlying *in vivo* spike patterns of Purkinje cells were subsequently confirmed in an experimental study (Jaeger and Bower, 1999). See also the review by Sidiropoulou *et al.* (2006) for examples of dendritic modeling studies. Finally, as the distribution of ion channels is often non-uniform along the dendritic tree, thus complicating the analysis, modeling is often used to increase the understanding of experimental results (see review by Migliore and Shepherd, 2002).

3.2 The dendritic I_{TRPC} model

In order to study how the synaptically activated TRPC current (I_{TRPC}) affects dendritic integration, we used a multicompartment model of a pyramidal neuron. We tuned the I_{TRPC} dynamics to replicate experimental data provided by our collaborator Motoharu Yoshida, who recorded somatic responses in rodent entorhinal cortex following synaptic stimulation, as described in Paper I. In this section I will describe and discuss the various components of the model.

3.2.1 The Poirazi model

Development of a new detailed multicompartment pyramidal neuron model with biophysically realistic properties and several ion channels is a large project outside the scope of this thesis. Instead, I decided to use a morphologically detailed (see figure 3.2) model developed by Poirazi *et al.* (2003), which is available for download in the ModelDB online database.

The model contains 17 types of ion channels as well as calcium dynamics, which is of high importance for the mechanisms we studied. The model represents on of the most well-studied pyramidal neuron: the hippocampal CA1 pyramidal neuron. It is a state-of-the-art compartment model, taking into account a range of physiological properties. The details of how the model was tuned can be found in the supplementary material published together with their 2003 paper (Poirazi *et al.*, 2003). Briefly, they tuned the internal parameters of the ion channels, many of which have a nonuniform distribution along the dendritic tree, both from studies primarily concerned with individual ion channels properties and from studies primarily concerned with dendritic physiology and synaptic integration. Several validation studies were performed, including testing the effect of the hyperpolarization-activated I_h current on input resistance and steady-state voltage propagation, and properties of back-propagating APs and dendritic spikes. The model also captured the spatial summation properties in both the apical trunk and thin dendrites that had been identified in experiments by Cash and Yuste (1999). In addition to this, as already mentioned, predictions made with the model were confirmed in subsequent experimental studies. Furthermore, the Poirazi model serves as a well-established model in the community, and has been frequently referred to and cited by several leading scientists in the field of dendritic research.

3.2.2 Extending the Poirazi model by adding I_{TRPC}

In order to adjust the Poirazi model to suit the exploration of our research questions, I developed and added three major model components: glutamate concentration dynamics, a mGluR1/5 and TRPC. In figure 3.1 these model components are presented in a schematic overview, and in the following sections I will describe them in detail.

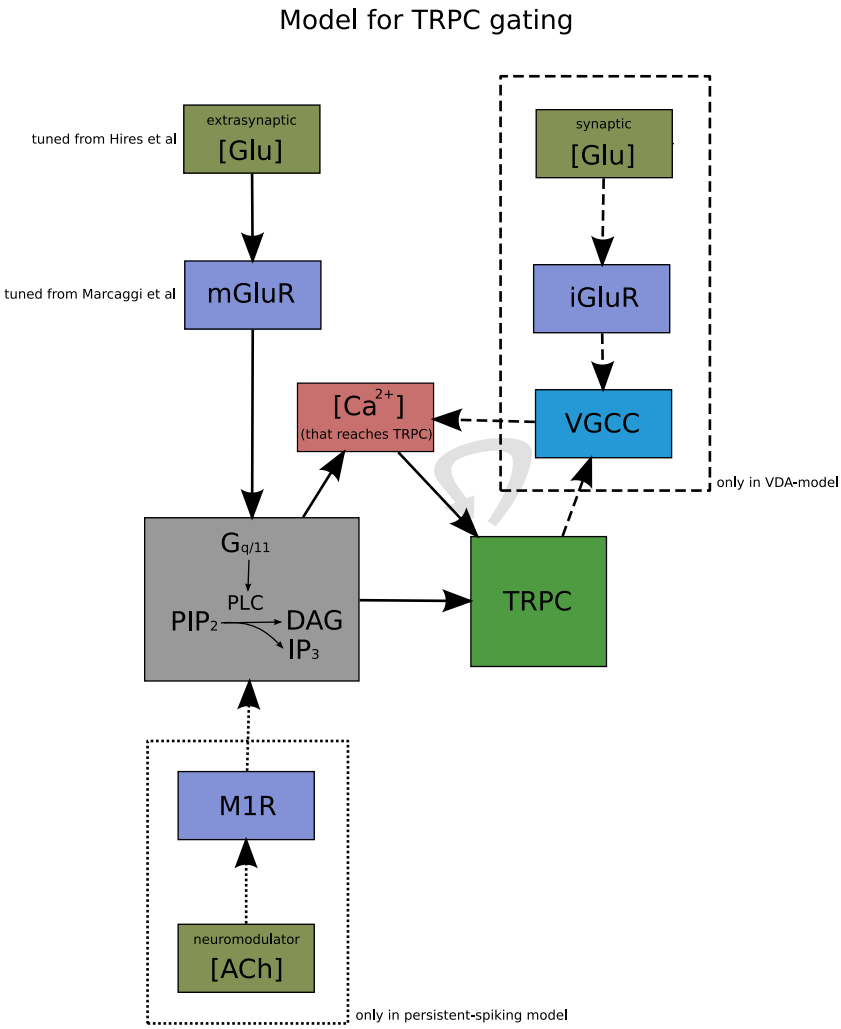

Figure 3.1. *Model for I_{TRPC} activation. The $G\alpha_{q/11}/PLC$ pathway, which is triggered by activation of mGluR1/5 (or M1R, in the persistent-spiking model), leads to TRPC (I_{TRPC}) activation. Although the mechanisms are not clear, it is believed that this activation partially depends on Ca^{2+} released from stores. Moreover, voltage-gated calcium channels (VGCC, opened by depolarization caused by AMPA and NMDA activation) may contribute to further increases in Ca^{2+}, an effect we included in the VDA model, but not in the VIA model. See the text for details.*

3.2.3 Glutamate concentration

In the Poirazi model there is no explicit module implemented for modeling the dynamics of the glutamate concentration released at the synapse. The research regarding neurotransmitter concentration profiles is still relatively incomplete, as pointed out in a recent review by Scimemi and Beato (2009). In Paper II we modeled the glutamate concentration as a simple square pulse lasting 100 ms. As the maximum frequency never exceeded 10 Hz in that study, we did not need to consider summation of glutamate since triggering of synaptic inputs were always separated by at least 100 ms (1/10 Hz). From the study in Paper II we concluded that the glutamate pulse shape had a significant effect on the simulation results, and we thus decided to construct a more accurate glutamate model for the subsequent work in Paper I.

The glutamate model in Paper I is based on data from a recent experimental study indicating that the glutamate concentration following a synaptic input has a rather slow decay, several hundred milliseconds, and undergoes summation (Hires *et al.*, 2008). It should be noted that, due to limitations in the temporal resolution, the glutamate concentration dynamics found in that study might not be suitable for modeling glutamate receptors in the synaptic cleft (AMPA and NMDA), but is more favorable to use when modeling extrasynaptic receptors activated by glutamate spillover, such as mGluR1/5 (Scimemi and Beato, 2009). I thus designed the glutamate model in Paper I to replicate a slowly decaying glutamate concentration, allowing summation at low frequencies. This model, represented by the "extrasynaptic [Glu]" box in figure 3.1, is described in detail in the method section of Paper I.

3.2.4 Metabotropic glutamate receptor

In both Paper I and Paper II we wanted to simulate realistic synaptic activation of mGluR1/5. An important problem we became aware of was the lack of data available for describing the dynamics of mGluR mechanisms, which was rather surprising, considering how important mGluR1/5 is for neuronal function, and considering how well the excitatory ionotropic receptors currents mediated by AMPA and NMDA can be modeled (Destexhe *et al.*, 1994b). However, the ionotropic receptors are more straightforward in the sense that they are directly connected to a synaptic ion channel current. As a consequence, to model the ionotropic synaptic currents, including the chain of events following synaptic stimulation (glutamate release and removal, receptor binding, channel opening) is relatively simple since it is easy to measure the currents. For metabotropic receptors the situation is more complex, as the corresponding chain of events also involves activation of intracellular pathways before the current flow is initiated. This may be one reason why mGluR-mediated currents are rarely included in compartment models, which I will further discuss in section 4.1.

We based our mGluR1/5 model on a $GABA_B$ model by Destexhe and Sejnowski (1995). The $GABA_B$ receptor is similar to mGluR1/5 in the sense that it is a synaptically activated metabotropic receptor which activates G-protein

cascades, although downstream pathways and targets are different. The kinetic reactions describing mGluR1/5 activation can be presented in several steps. First, the mGluR1/5 receptor binds to the transmitter substance T (concentration of glutamate):

$$R_0 + T \underset{k_2}{\overset{k_1}{\rightleftarrows}} R \tag{3.1}$$

$$\frac{dR}{dt} = k_1 T R_0 - k_2 R \tag{3.2}$$

R_0 represents the fraction of receptors that are not bound to glutamate and R the fraction of activated receptors. k_1 and k_2 are rate constants. Since the sum of R_0 and R must be one the equation becomes:

$$\frac{dR}{dt} = k_1 T(1 - R) - k_2 R \tag{3.3}$$

For tuning the rate constants k_1 and k_2 I used data from a recent study by Marcaggi *et al.* (2009), which is the first study to report a detailed description of mGluR dynamics. The authors provided a detailed scheme for mGluR1 activation, but our simple model was able to capture the essential dynamics, as described in Paper I. By applying square glutamate concentration pulses they could show that the deactivation time constant of R is seemingly independent of glutamate concentration (and estimated it to 50 ms). This is in line with equation 3.3, which similarly implies that the deactivation is independent of glutamate (when $T = 0$ at the end of the square pulse $\frac{dR}{dt}$ is only dependent on R). The activation time constant, on the other hand, decreases exponentially with glutamate concentration in their study. To model R I therefore had to choose a value of T, preferably as physiologically realistic as possible. Unfortunately a detailed characterization of the spatio-temporal profile of synaptically released glutamate does not exist (Scimemi and Beato, 2009). A few suggestions of extrasynaptic T exist, however, which I will discuss next.

3.2.5 Choosing a value of glutamate concentration

In the synaptic cleft T is in the order of 1 mM, but peri- and extrasynaptically (i. e. outside the synaptic cleft, where mGluR1/5 receptors are located) the value is probably smaller. Hires *et al.* (2008) observed very small T transients, in the order of 1 μM. Their technique, however, has a limited temporal resolution (as discussed in their paper) and the large initial glutamate transients may not be captured (Marcaggi *et al.*, 2009). Previous mGluR1/5 models have assumed 10 μM to be a reasonable value, but this assumption has not been convincingly motivated (Fiala *et al.*, 1996; Steuber and Willshaw, 2004; Steuber *et al.*, 2006). By using a model of extrasynaptic glutamate diffusion, Rusakov and Kullmann (1998) predicted that extrasynaptic glutamate reaches at least 50 μM, whereas Dzubay and Jahr (1999) estimated the value to lie within the range from 160 to 190 μM. We chose the value of 200 μM when tuning our model, which is

in line with the two lastly mentioned studies and it also corresponds to full activation of the receptor (Marcaggi *et al.*, 2009). Furthermore, 200 μM is the value chosen by Marcaggi *et al.* for the major part of their kinetic analysis. Finally, an additional argument for physiological values of T being larger than just a few μM is that extracellular glutamate in the intact mammalian brain is thought to be in an interval of 0.5 to 5 μM at rest (Featherstone and Shippy, 2008).

3.2.6 G protein-mediated cascade as a black box

Next, the mGluR1/5-triggered activation of the G protein-mediated cascade was modeled. Although it is known that $G\alpha_{q/11}$/PLC activation and PIP_2 cleavage is involved, kinetic data is lacking for the chain of events taking place between mGluR1/5 activation and TRPC activation, so we decided to model this in a black-box fashion. Specifically, we introduced x, the concentration of an unknown metabolite that reaches and activates TRPC, and calculated it as:

$$x = C_1 \frac{R^n}{R^n + C_2} \tag{3.4}$$

We chose to use $n = 4$ in line with Destexhe and Sejnowski (1995). Values of C_1 and C_2 were selected in the TRPC tuning process described in Paper I. As described in Paper I, x is not independent of Ca^{2+}, and may partly consist of Ca^{2+} released from stores. This is illustrated in figure 3.1 by the arrow drawn from the (gray) PLC box to the (red) Ca^{2+} box.

3.2.7 TRPC gating dynamics

Previous models of the TRPC current I_{TRPC}, often referred to as I_{CAN} (calcium activated non-specific cation current), have focused on the role of calcium as activator (Destexhe *et al.*, 1994a; Fransén *et al.*, 2002, 2006), but to my knowledge there are not any other models that consider mGluR1/5 as activator. We therefore developed a novel mGluR1/5-activated I_{TRPC} model, as described in Paper I. In this and the following section, I will discuss the various components of that model. In line with previous work on I_{CAN} (Destexhe *et al.*, 1994a), I modeled I_{TRPC} according to:

$$I_{TRPC} = \bar{g} m f(V_m) \tag{3.5}$$

where $\bar{g}$ is the maximal conductance and $f(V_m)$ describes the voltage dependence (see below). m denotes the gating parameter and was modeled simply as having two states, open and closed, with rate constants α and β, which follows the classical Hodgkin and Huxley formalism:

$$open \underset{\alpha}{\overset{\beta}{\rightleftarrows}} closed \tag{3.6}$$

Destexhe *et al.* (1994a) let β be a constant and α depend on intracellular calcium concentration ($[Ca]_{in}$) according to:

$$\alpha = K_\alpha [Ca]_{in}^2 \tag{3.7}$$

Due to the lack of data for the exact Ca^{2+} dependence of I_{TRPC} I simplified the equation by omitting the exponent from equation 3.7. Next, I modified the equation to account for the influence of mGluR1/5, by adding the factor x described in the previous section. Now it is important to note that I in Paper I evaluated two models in parallel. The more complex model, in which calcium from voltage-dependent calcium channels (VGCCs) affects I_{TRPC}, is called the *VDA* (voltage-dependent activation) model. In the *VIA* (voltage-independent activation) model, I_{TRPC} does not depend on calcium from VGCCs, but activation of mGluR1/5 ($x > 0$) is sufficient for opening the TRPC channel. In both models we assumed that the mGluR1/5-mediated activation signal x (described in the previous section) produces a shift in the calcium dependence. An argument for assuming a shift is that the voltage dependence of other TRP channels appears to be shifted in response to important activating factors. Specifically, the voltage dependence of TRPM8 is shifted by menthol or cooling, and the voltage dependence of TRPV1 is shifted by capsaicin (the active component of chili pepper) or heating (Voets *et al.*, 2004). Furthermore, the x-induced shift in calcium activation we proposed for TRPC activation is in line with how PIP_2 has been suggested to affect TRPM4 (Nilius *et al.*, 2006). However, there are important differences between TRPC and TRPM4 channels. PIP_2 activates the latter while it appears to inhibit the former (Otsuguro *et al.*, 2008). The shift in our model is implemented simply by adding x to the Ca^{2+} concentration:

$$\alpha = K_\alpha (x + [Ca]_{in} - Ca_{th}) \tag{3.8}$$

In equation 3.8 I also added Ca_{th}, representing a calcium threshold, which the sum of $x + [Ca]_{in}$ needs to exceed for the TRPC channel to open. Consequently, the current is zero at rest. This calcium threshold represents both a calcium buffer which may become saturated, and a non-linear dose dependency of $[Ca]_{in}$ on the TRPC channel. $[Ca]_{in}$ in equation 3.8 represents Ca^{2+} originating from VGCCs. In the VIA model, where we assume that this VGCC-mediated Ca^{2+} is not required for I_{TRPC} activation, we let $[Ca]_{in} = Ca_{th}$ so that 3.8 simply becomes:

$$\alpha = K_\alpha x \tag{3.9}$$

It should be noted that x in equation 3.9 implicitly includes the increase in intracellular calcium, which is mediated by IP_3 receptors. This equation is also used in Paper II, since that I_{TRPC} model is similar to the VIA model described here. A minor difference exists, however, between the VIA model of Paper I and Paper II. In the latter we assumed $[Ca]_{in} > Ca_{th}$ at rest, resulting in non-zero I_{TRPC} at rest.

In figure 3.1 I illustrate the I_{TRPC} model components described here. The three boxes marked by the dashed rectangle are not included in the VIA model,

but only in the VDA model. The reader may note the feedback loop present in the VDA model, arising since the I_{TRPC}-mediated depolarization activates VGCC, which results in increased calcium, in turn activating I_{TRPC}. The feedback loop is important for Paper III. Furthermore, the acetylcholine-activated muscarinic receptor (M1) components is marked with a dotted rectangle. These components are not central to this thesis but worth mentioning since they are important for figure 7 in Paper I, which shows that the cell model is capable of producing persistent spiking.

For tuning the dynamics of the TRPC model in Paper I we used data from synaptic-stimulation experiments. For Paper II, however, we tuned the model according to data from current-injection experiments, for which much longer time scales for I_{TRPC} activation were observed. While a typical decay time from a TRPC-mediated EPSP following synaptic stimulation is around 300 ms, decay times following current injection can be $> 3\,000$ ms.

3.2.8 TRPC voltage dependence

In 2001 it was reported that TRPC channels can have an NMDA-like voltage dependence (Strübing *et al.*, 2001). The result has been replicated many times, and seems to appear only for heteromers (e. g. TRPC1+TRPC5) and not for homomers (e. g. TRPC5+TRPC5). The NMDA-like voltage dependence was originally thought to be caused by a magnesium block, just like for the NMDA channel. However, experiments have shown (e. g. Faber *et al.*, 2006) that the NMDA-like voltage dependence persists even after magnesium is removed, so the mechanism remains elusive. It should also be mentioned that the TRPC voltage dependence has been indicated to undergo changes during the activation cycles (Obukhov and Nowycky, 2008).

In both Papers I and II, I incorporated the voltage dependence, i. e. the $f(V_m)$ term in equation 3.5, such that the model replicates recent data from I_{TRPC} activation in the EC (Zhang *et al.*, 2011), as described in Paper I.

3.2.9 Modified calcium channels

Studies have indicated that calcium from the L-type voltage-gated calcium channel contribute to TRPC activation in EC pyramidal neurons (Egorov *et al.*, 2002). Among the four VGCC-L subtypes ($Ca_V1.1$-1.4) $Ca_L1.3$ stands out as it is activated at lower voltages, whereas the other three subtypes have more depolarizeds activation thresholds (Lipscombe *et al.*, 2004). In the original Poirazi model, the VGCC-L channel has a high activation threshold, and hence it does not activate in response to subthreshold synaptic inputs. I therefore shifted the VGCC-L voltage dependence (i. e. the half-activation value $V_{1/2}$) into a more low-threshold range, as described in Paper I.

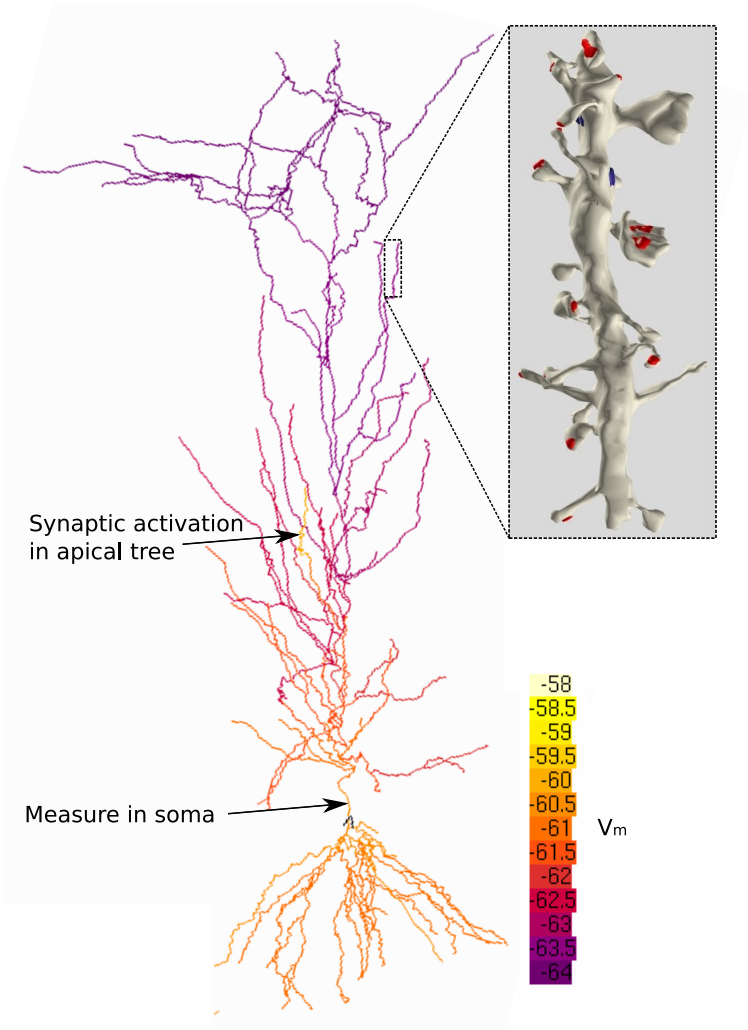

Figure 3.2. *Model of pyramidal neuron with detailed morphology. In simulations for Paper I and II synaptic stimulation was applied in a dendritic branch of the apical tree. The membrane potential, which is color-coded, was measured in the soma in order to analyze synaptic integration properties. Note that the membrane at the branch receiving synaptic input is more depolarized than its neighboring branches. The inset figure (upper right) is a 3D illustration of a CA1 pyramidal branch (not from the same study as the full neuron to the left), reconstructed from serial electron microscopy (http://synapses.clm.utexas.edu/index.asp).*

3.2.10 Synaptic stimulation

As described in section 2.2, the electrical properties of neurons can be studied using electrophysiological techniques. Our experimental collaborator measured somatic potentials in EC pyramidal cells in response to synaptic stimulation. To tune the TRPC model, I applied synaptic inputs in the dendritic tree as illustrated in figure 3.2, in order to mimic these experimental recordings. Synaptic stimulation evokes glutamate transients, which couple to ionotropic (AMPA and NMDA) and metabotropic (mGluR1/5) receptors in parallel, subsequently evoking transmembrane currents. I then measured the resulting change in somatic membrane potential – the EPSP. The details of the stimulation protocols are explained in Papers I and II.

3.2.11 Calculating the decay time constant – τ_{decay}

Since we were interested in studying the effects of the TRPC channel on temporal summation, it was natural to study the decay time of the TRPC current. It is therefore worth mentioning how the EPSP decay time constant τ_{decay} was measured. In the strict sense, τ_{decay} can only be measured if the decay can be approximated to follow an exponential function, as described in equation 3.10, where $EPSP_{max}$ is the maximal EPSP value:

$$EPSP(t) = EPSP_{max} e^{(-\frac{t}{\tau_{decay}})} \tag{3.10}$$

The EPSP (or sADP) following activation of TRPC channels typically has a relatively slow rise, in the order of 100 ms, and a wide peak (see figures 5A and 9B in Paper I). It is therefore not obvious how to measure τ_{decay}, as the process is clearly not a simple exponential decay. In Paper I we defined it as the time from the peak to the time where $e^{-1} = 37\,\%$ of $EPSP_{max}$ remains. However, this measure was not appropriate to use in the VDA model, as that EPSP consists of two peaks (figure 5A in Paper I). I therefore instead chose to use the pair-pulse protocol (PPP) shown in figure 6 of that paper, to get an indirect measure of τ_{decay}. The rationale for using the PPP is that we could thereby measure what actually remained of the signal after the end of the stimulus, which was the purpose of calculating the decay time constant in that study where temporal summation was in focus. Thus, the main advantage of PPP over τ_{decay} is that it is not sensitive to the shape of the peak.

3.3 The C-fiber axon model

In Papers IV, V and VI we developed and used a C-fiber nociceptor model. We wanted the model to be directly comparable with microneurographic experiments of human C-fibers. These experiments are used to assess propagation properties in branch and parent axons, but do not assess the peripheral sensory terminals, the DRG soma or the central branches. Accordingly, the model was constructed to include branch and parent axons, but not peripheral sensory

terminals, the DRG soma and the central branches, as illustrated in figure 2.5. The model was designed to be used for a variety of investigations, and includes a realistic geometry and temperature profile. As described in the papers, it has multiple K^+ and Na^+ channels ($I_{Na_V1.7}$, $I_{Na_V1.8}$, $I_{Na_V1.9}$, I_h, I_{Kdr}, I_{KM}, I_{KA}, I_{KNa}, I_{leak}), Na^+/K^+concentration dynamics and a pump current (I_{pump}). Most channels were borrowed (though modified) and are standard Hodgkin and Huxley-type models. Parameters (in particular the maximal conductance values $\bar{g}_X$) were fitted to replicate experimental data, including human activity-dependent slowing and recovery cycle data. In the remaining part of this section I will relate our model to previous models, explain why it is novel, and describe how I implemented the models for the Na^+/K^+concentration dynamics, I_{pump}, I_{KNa} and I_{leak}.

3.3.1 Brief review of previous nociceptor models

Several models of peripheral C-type nociceptors were developed before ours. Typically these models consists of a single DRG soma compartment (Scriven, 1981; Herzog *et al.*, 2001; Baker, 2005; Sheets *et al.*, 2007; Kouranova *et al.*, 2008; Maingret *et al.*, 2008; Choi and Waxman, 2011; Gurkiewicz *et al.*, 2011), and therefore cannot be directly used for studying propagating action potentials, so the connection to microneurographic recordings is problematic. Furthermore, these models are limited in the sense that they *either* include concentration dynamics and a pump current (Scriven, 1981), *or* a realistic set of ion channels (but note that none of these included the sodium-activated potassium current). In addition to the single-compartment C-fiber models listed above, there is also an A-type (myelinated) multicompartment nociceptor model, developed by Amir and Devor (2003).

3.3.2 Novel model

To my knowledge, our model is the first cutaneous C-fiber axon model with several types of ion channels, a pump current and Na^+/K^+ dynamics. Our model is also novel in the sense that it has a realistic morphology and temperature profile, and ion channel conductances tuned to replicate human data. I will discuss the relevance and implications of this in Chapter 4.

3.3.3 The Na^+/K^+ dynamics

Na^+/K^+ dynamics is important to include because it affects ion channels via reversal potentials (E_{Na} and E_K) and gates (of I_{pump} and I_{KNa}). It is not very common to explicitly model Na^+ and K^+ concentrations in compartment models, and it is therefore worth discussing here. We used the equations and parameters of Scriven (1981). In equation 3.11, where F is the Faraday's constant, it is demonstrated how changes in intracellular sodium $[Na]_{in}$ relates to the sum of trans-membrane sodium currents I_{Na} (measured in current per area) and the diameter D of the C-fiber (for a segment of length L). The equation for

potassium is identical. It is assumed that the ions mix instantaneously. With intracellular volume $Vol_{in} = \pi L(D/2)^2$ and membrane area $A_{membrane} = \pi LD$ we get:

$$\frac{d[Na]_{in}}{dt} = \frac{-I_{Na}A_{membrane}}{Vol_{in}F} = \frac{-I_{Na}\pi LD}{\pi L(\frac{D}{2})^2 F} = \frac{-4I_{Na}}{DF} \tag{3.11}$$

The corresponding equation for periaxonal concentration $[Na]_{sp}$ is slightly more complicated, since we also have to account for the diffusion barrier (with permeability D_{Na}) to the extracellular space, in which the sodium concentration $[Na]_{out}$ is assumed to be constant. The volume of the periaxonal space is $Vol_{sp} = \pi L((D/2+\theta)^2-(D/2)^2) = \pi L((D\theta/2+\theta^2)$ and the sodium flux leaving by diffusion through the barrier with area $\pi L(D+2\theta)$ is $j_{Na} = D_{Na}([Na]_{out} - [Na]_{sp})\pi L(D+2\theta)$.

$$\frac{d[Na]_{sp}}{dt} = \frac{I_{Na}A_{membrane}/F + j_{Na}}{Vol_{sp}} =$$

$$= \frac{I_{Na}\pi LD/F + D_{Na}([Na]_{out} - [Na]_{sp})\pi L(D+2\theta)}{\pi L(D\theta/2+\theta^2)}$$

which can be simplified to

$$\frac{d[Na]_{sp}}{dt} = \frac{I_{Na}D/F + D_{Na}([Na]_{out} - [Na]_{sp})(D+2\theta)}{(D/2+\theta)\theta} \tag{3.12}$$

Scriven (1981) assumed that $D \gg \theta$, so that equation 3.12 can be simplified to:

$$\frac{d[Na]_{sp}}{dt} = \frac{I_{Na}/F + D_{Na}([Na]_{out} - [Na]_{sp})}{\theta} \tag{3.13}$$

In his model, the ratio $\theta/D = 14.5\,nm/0.75\,\mu m = 0.019$ was small enough to allow for this simplification. In our model however, the ratios for the branch and parent axon were $29\,nm/0.25\,\mu m = 0.116$ and $29\,nm/1\,\mu m = 0.029$ respectively, which raises the question whether the simplification in equation 3.13 is adequate, particularly for the branch axon. I will return to this question in section 4.2.

3.3.4 The Na-K-pump current

To model the Na-K-pump, we again used the equations by Scriven (1981) as a starting point:

$$\begin{cases} I_{pump} & = I_{Na,pump} + I_{K,pump} \\ I_{K,pump} & = \frac{a}{(1+b_1/[K]_{sp})^2(1+b_2/[Na]_{in})} \\ I_{Na,pump} & = -rI_{K,pump} \end{cases} \tag{3.14}$$

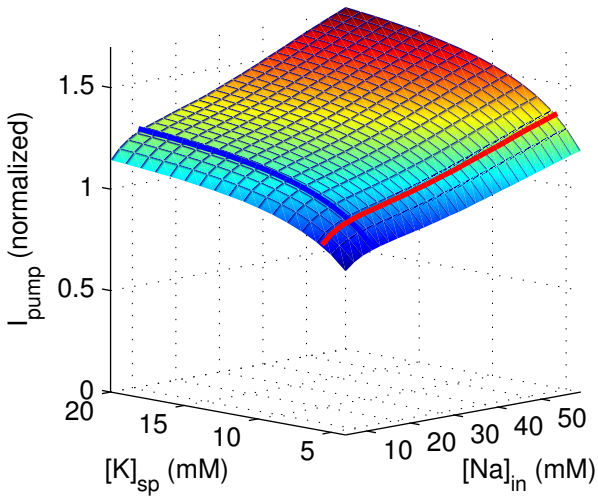

Figure 3.3. *I_{pump} depends on both periaxonal (extracellular) potassium $[K]_{sp}$ and intracellular sodium $[Na]_{in}$. Red and blue lines show how I_{pump} depends on one concentration if the other is kept fixed.*

Coupling ratio

In older literature, the ratio r was subject to investigation (Mullins and Brinley, 1969; Thomas, 1972). Scriven chose to use data by Mullins and Brinley (1969), which suggested that the ratio was dependent on the sodium concentration as $r = c[Na]_{in} + d$. In more recent experimental literature it is accepted that $r = 3/2$ (Rakowski *et al.*, 1989; Glynn, 1993; Glitsch, 2001; Hamada *et al.*, 2003; Gadsby *et al.*, 2009; Krishnan *et al.*, 2009). Hence I chose to use $r = 3/2$ when implementing our model, implying that three Na^+ ions are transported out while two K^+ ions are transported in.

Na^+ and K^+ dependence

Figure 3.3 shows I_{pump} as a function of sodium and potassium concentrations. I improved the Scriven model further by incorporating recent sodium-dependence data by Hamada *et al.* (2003), who recorded I_{pump} from small dorsal root ganglia (in rat) and found the following biphasic sodium dependence, which I used to replace the term $1/(1 + b_2/[Na]_{in})$ in equation 3.14:

$$\frac{1.62}{1 + (6.7/([Na]_{in} + 8))^3} + \frac{1}{1 + (67.6/([Na]_{in} + 8))^3} \tag{3.15}$$

The potassium dependence in equation 3.14 is described by $b_1 = 1\,\text{mM}$, which Scriven took from an experimental study on C-fibers (Rang and Ritchie, 1968). I am not aware of any more recent estimates of b_1 in C-fibers.

Voltage dependence

There is evidence supporting that I_{pump} has a voltage dependence such that it decreases with hyperpolarizations. Scriven (1981) did not include such voltage dependence in his C-fiber pump model. However, recent data suggests that a slight voltage dependence exists in C-fibers, although the situation is complicated by the fact that two pump isoforms are present (Hamada *et al.*, 2003). One of these isoforms ($\alpha 1\beta 1$) is voltage dependent, while the other ($\alpha 3\beta 1$) is not (Hamada *et al.*, 2003; Dobretsov and Stimers, 2005), and the voltage dependence seems to be modulated by the level of intracellular sodium. Since the voltage dependence is rather weak in the V_m range relevant to our studies, we neglected it. In future studies, when more detailed data is available, we could consider implementing a more complex model for the pump current by, for example, building on the multi-state model by Chapman *et al.* (1983).

3.3.5 The K_{Na} current

It is not very common to include the sodium-dependent potassium current I_{KNa} in compartment models. Fortunately for our studies, however, there is a quantitative study of I_{KNa} performed in small DRG neurons (Bischoff *et al.*, 1998). When designing their study of neurons in the central nervous system, Wang *et al.* (2003) used this DRG-data to construct a simple I_{KNa} model according to:

$$I_{KNa} = \bar{g}_{KNa} w (V_m - E_K)$$

where $\bar{g}_{KNa}$ is the maximal conductance, E_K is the potassium reversal potential, and w is the gating parameter, modeled according to:

$$w = \frac{0.37}{1 + (38.7/[Na]_{in})^{3.5}}$$

The model by Wang *et al.* (2003), which we used in our studies, does not include kinetics but is assumed to be instantaneously activated by increases/decreases in $[Na]_{in}$. This was not problematic in our studies, since changes in $[Na]_{in}$ only occurred on a rather long time scale as stimulation frequencies were low (< 2 Hz). However, if using the C-fiber model to study faster processes (e. g. many pulses given at high frequency) one should consider introducing time constants in the equations above, which has been done in other models (see e. g. Huss *et al.*, 2007).

3.3.6 The leak currents

We included two leak currents in the model, one for K^+ ($I_{K,leak}$) and one for Na^+ ($I_{Na,leak}$). Since $I_{Na,leak}$ is not always included in compartment models I will here motivate why we included this current. While the molecular identity of $I_{K,leak}$ is quite well studied (Enyedi and Czirják, 2010) and its presence in neurons well accepted, $I_{Na,leak}$ has not been given the same attention. Recently,

however, researchers have begun to elucidate its molecular identity and impact on neuronal function (Lu *et al.*, 2007; Ren, 2011). Furthermore, compared to many neurons in the central nervous system, C-fibers have a quite depolarized resting potential $V_{rest} \approx -55\,\mathrm{mV}$. (This might be related to the fact that the activation of $\mathrm{Na_V}1.8$, which is the major action-potential generator, has a rather depolarized voltage dependence compared to $\mathrm{Na_V}$ channels of the CNS, as described in section 2.2). One could speculate that a relatively depolarized V_{rest} reflects that positive currents (I_{pump} and K^+ currents open at rest) do not dominate over negative currents (Na^+ currents open at rest) as much as in more hyperpolarized neurons. Consequently, $I_{Na,leak}$ might play a greater role in C-fibers than in many other neurons. Note, however, that also voltage-dependent sodium currents, in particular $I_{Na_V1.9}$, might be large at rest and thus contributing to determining V_{rest} (Herzog *et al.*, 2001). Note also that, for this kind of reasoning, one should also consider components not present in our model, such as calcium, chloride and other pumps/transporters.

3.4 Software used in this thesis

All simulations were run with the *NEURON* software (version 7.0), developed by Hines and Carnevale (2001) for the purpose of studying both neuronal networks and individual neurons with properties including complex branching morphology, multiple channel types, inhomogeneous channel distribution, ionic diffusion, and the effects of second messengers. It builds on the cable theory of Rall which I briefly outlined in section 3.1. NEURON is one of the most popular software programs used in the field of computational neuroscience, with more than 700 papers reporting work employing it as of April 2008, according to Hines *et al.* (2009). *MATLAB* (version R2011a) was used for simulation initiation and analysis. *Engauge Digitizer* was used for extracting data from published experiment results.

Chapter 4

Results and discussion

As stated in the introduction, the aim of this thesis is to improve the understanding of how specific ion channel types shape neuronal integration, excitability and memory, with the long-term goal of contributing to the characterization and development of treatments for neural disorders. For this purpose, I developed and employed the computational models described in the previous chapter. In this chapter, I will summarize, explain and elaborate on the relevance of the insights gained from the simulation results of Papers I-VI, and also present and discuss several complementary simulation results. Essentially, this thesis is based on two major projects, where I investigated:

1. the role of synaptically (via metabotropic glutamate receptors) and intrinsically (by calcium) activated TRPC channels in integration and subthreshold long-lasting depolarizations in pyramidal cell dendrites (Papers I, II, III), and

2. the role of axonal ion channels and pumps in activity-dependent excitability changes of peripheral nociceptive C-fiber axons (Papers IV, V, VI).

Both projects have resulted in several novel insights into the dynamical properties of neurons, and particularly the role of ion channels in shaping neuronal integration, excitability and memory. It is further common to both projects that the models developed were multicompartment models with several types of ion channels, and that they are expected to be useful in future modeling studies. Notably, there are also many differences between the projects, as the first is focused on the hippocampus and the entorhinal cortex, brain regions that are considered to be functionally distant from sensory inputs as well as motor outputs, while the second project deals with the very first neuron in a long chain of neurons that constitute the nociceptive pathway. The projects also differ in terms of specific ion channels involved.

In sections 4.1 and 4.2 below I will discuss the two projects individually. Following this, I will in section 4.3 discuss concepts and insights relating to both.

4.1 I_{TRPC} in synaptic and dendritic integration

I will begin this section by discussing results relating to the role of the TRPC current I_{TRPC} in low-frequency synaptic integration (Papers I and II), and then continue by describing stable and subthreshold long-lasting depolarizations, for which I_{TRPC} plays a central role (Paper III). I will end the section with a more general discussion about why it is important to study I_{TRPC} and include it in computational modeling studies.

4.1.1 Synaptic transmission beyond AMPA and NMDA: slow synaptic mGluR/TRPC currents

Depending on the nature of an input arriving at a synapse, different strategies may be used by the neuron to integrate and respond to the input. Naturally, if a short train of high-frequency synaptic input arrives, it may be beneficial for the neuron to be equipped with a fast mechanism that is highly sensitive to inputs on a short time scale. If, on the contrary, inputs arriving with low frequency are to be processed, it may be necessary for the neuron to possess slow mechanisms of integration. For example, in certain working memory tasks (e. g. delay-match-to-sample), sensory inputs may arrive separated by silent intervals in the range of seconds, and the subject should respond if the current input is identical to the preceding input. It has been suggested that single neurons, due to intrinsic ion-channel mechanisms outlasting the duration of the input, may be able to perform such calculations (Marder *et al.*, 1996; Fransén *et al.*, 2002). In this work, I have studied a mechanism that might be particularly important in supporting the integration of low-frequency synaptic inputs. It is mediated by a cascade of events that starts with the activation of group I metabotropic glutamate receptors (mGluR1/5), and ends with a membrane depolarization caused by I_{TRPC}, as described in previous chapters.

In Papers I and II I investigated the hypothesis that I_{TRPC} is important for integrating low-frequency synaptic input. I will here discuss the major conclusions and present additional data relating to decay time constants. I will also present figures supporting the sensitivity analysis of Paper I.

Conclusion 1:
I_{TRPC} is important for low-frequency synaptic integration

As described in Paper I and in section 2.4, spike frequencies during behavior are low, around 8 Hz, implying that typical synaptic inputs arrive with frequencies in the same range. With current experimental techniques it is not possible to directly record the electrical activity of a dendritic branch from a behaving animal. It is therefore useful to employ computer simulations for studying synaptic integration. Ionotropic receptors, including AMPA and NMDA, are well characterized and incorporated in most compartmental models. However, as we discussed in Paper I, their kinetics are typically fast and corresponding currents decay rapidly after each synaptic input, and they will hence not allow

for effective temporal summation of frequencies below 10 Hz, at least not under circumstances where spatial summation is limited (see section 2.4). We therefore turned our attention to mGluR-mediated currents, and the slow I_{TRPC} in particular. In contrast to the AMPA and NMDA currents, mGluR-mediated currents are rarely included in compartmental models and, to my knowledge, a synaptically evoked I_{TRPC} current has never been taken into account.

Although there is strong evidence suggesting that I_{TRPC} is important for neuronal communication, I have not encountered a systematic study (experimental or computational) of how it contributes to synaptic integration. With the models I implemented it was possible to study the relative importance of the two major classes (ionotropic and metabotropic) of synaptic currents in supporting synaptic integration. We showed in figures 5, 10 and 15 of Paper I that, in contrast to ionotropic receptor currents, the mGluR-evoked current I_{TRPC} is well suited for supporting temporal summation of low-frequency synaptic inputs.

Even though brain slice experiments show that mechanisms of mGluR1/5 and TRPC may be important for synaptic function, this does not guarantee that they are critical on a behavioral level. It is therefore important to note that *in vivo* studies have indeed shown that mGluR1/5 receptors are critical on the behavioral level. These *in vivo* studies have utilized both pharmacological block of mGluR1/5 (Naie and Manahan-Vaughan, 2004; Balschun *et al.*, 2006; Hayashi *et al.*, 2007; Mikami *et al.*, 2007; Gregory and Szumlinski, 2008) and mGluR1/5 knock-outs (Niswender and Conn, 2010). These results are interesting, but note that mGluR1/5 receptors also couple to other channels in addition to TRPC. TRPC channels have been less studied, but as mentioned earlier, a recent TRPC knock-out study suggests that I_{TRPC} has strong effects on the behavioral level (Riccio *et al.*, 2009). Furthermore, the hypothesis that I_{TRPC} is involved in the integration of low frequencies is supported by results from Purkinje cells (Batchelor and Garthwaite, 1997). Taken together, these observations suggest that it is important to develop and include I_{TRPC} in computational models, in particular when studying low-frequency dynamics. Moreover, our finding that mGluR1/5-activated TRPC channels are important for the summation of inputs arriving at physiologically relevant frequencies may be one contributing factor to why behavior is affected by mGluR1/5 and TRPC block and knock-outs.

Conclusion 2: τ_{decay} depends on frequency and duration of synaptic input

As explained above, we were interested in studying synaptic integration of low frequencies, and in particular the contribution of TRPC. Naturally, in order for synaptic input to be effectively summed, some portion of every input-driven signal must remain until the arrival of the next. For example, if excitatory synaptic input is delivered at 5 Hz (corresponding to an inter-stimulus interval of 200 ms), then the decay time of the post-synaptic depolarization needs to be longer than 200 ms to allow for effective summation. For the purpose of our project it was therefore natural to study the decay time constant of the

depolarization resulting from activation of TRPC channels, denoted τ_{decay}.

For both the VIA and VDA model, we measured τ_{decay} of the EPSP following a synaptic stimulus train of varying frequency and number of pulses (see e. g. figures 5, 11 and 16 of Paper I). As described in the paper, we found that τ_{decay} (or ISD_{R10}, for the VDA model) increases with increasing stimulation duration and frequency. This result may seem trivial at a first glance, since a long pulse train of high frequency naturally will produce a large-amplitude depolarization, not only immediately at the end of the stimulus pulse train, but also after some delay. It should therefore be stressed that a change in τ_{decay} actually reflects a change in the decay characteristics – the curve form – of the depolarization. Note that, if the decay would follow a simple exponential, τ_{decay} would not be affected by the increase in depolarization amplitude (resulting from long pulse trains of high frequency). In summary, I_{TRPC} provides a mnemonic capacity whereby stimulus frequency and intensity combine to affect the longevity of the synaptically evoked depolarization.

Conclusion 3: τ_{decay} depends on glutamate concentration

The finding that τ_{decay} changes with stimulus parameters was intriguing and led us to further explore the phenomenon. As reported in Paper I, we found a linear relation between τ_{decay} and the maximal glutamate concentration: $\tau_{decay} = 91.4 + 103 \cdot max([Glu])$. Additionally, we found a linear relation between τ_{decay} and the maximal TRPC current $max(I_{TRPC})$: $\tau_{decay} = 144 + 4.07 \cdot 10^3 \cdot max(I_{TRPC})$, I_{TRPC} with unit mA/cm^2 (data not shown). It follows that $max(I_{TRPC})$ is linearly correlated with $max([Glu])$. The maximum glutamate concentration appears to be an important determinant of the TRPC current, and the fact that glutamate can summate is therefore important for our conclusions, as will be discussed in the sensitivity analysis below.

Conclusion 4: Pair-pulse protocol suitable for studying τ_{decay}

It is easy to realize the problem of obtaining a direct measurement of τ_{decay} if the pulse shape is more complicated than a simple exponential. The shape of an EPSP peak is typically not sharp and may have an unwanted influence on the τ_{decay} measure, as discussed in section 3.2. What we actually wanted to measure was the ability of the pulse train to provide a depolarization that affects subsequent pulses. It was therefore natural to attempt to utilize a pair-pulse protocol (PPP, described in section 3.2) in order to get a more indirect measurement of τ_{decay}. PPP is often used in studies of short-term potentiation, a phenomenon that has been intensively studied for many years. The details of the PPP used in our study were described in figure 6 of Paper I. We also introduced ISD_{R10}, defined as the largest inter-stimulus delay possible for obtaining more than 10 % amplification of a test pulse amplitude when compared to control.

To validate that ISD_{R10} was a suitable measure of τ_{decay}, we showed in figure 14B of Paper I that a linear relationship between ISD_{R10} and τ_{decay} exists in the VIA model. Note that it was even more complicated to directly measure or even define τ_{decay} in the VDA model since the EPSP here consists of two peaks, the fast being unique for the VDA model since it originates from activation of ionotropic receptors. We concluded that the PPP could be successfully utilized in our study of decay time constants (because of the linear relationship with τ_{decay}).

Conclusion 5:
Similar results obtained with VIA and VDA

As described in Paper I there is a discrepancy among experimental studies aimed at understanding the activation of TRPC channels. While some studies report that activation of voltage-gated calcium channels is necessary for TRPC activation, others report that it is not. We developed two models, VIA and VDA, representing the two possible cases. Certain differences were found in the results, such as a saturation in both average membrane potential (figure 15, Paper I) and relative pulse train amplification R_{PT} (figure 16, Paper I) for frequencies above 3 Hz present only in the VDA model. However, the two models were in agreement on the major findings (*Conclusions 1* and *2*). We thus concluded that the two models essentially gave similar results.

Model verification

As discussed in Paper 1 and in other sections of this thesis, our modeling results were in good agreement with experimental data. Specifically, we have found that:

- mGluR1/5-activated TRPC channels supports summation of low-frequency synaptic input down to 1 - 2 Hz. In entorhinal cortex our experimental collaborator has observed summation for 2 Hz stimulus trains (Yoshida, unpublished observations), and Sidiropoulou *et al.* (2009) showed that 2 Hz is sufficient for activating TRPC in prefrontal cortex. Furthermore, in our model a single synaptic stimulus is sufficient for TRPC activation in line with our experimental data as described in Paper I, and other studies such as the one by Faber *et al.* (2006), who studied amygdala pyramidal neurons. Also in the hippocampus it has been shown that one pulse is enough for mGluR1/5 activation (Charpak and Gähwiler, 1991). It should be noted, however, that in other systems it seems like more than one synaptic stimulus pulse is required for I_{TRPC} activation (e. g. Kim *et al.*, 2003).

- I_{TRPC} is modulated by the glutamate concentration. This has also been shown in experiments, e. g. by Reichelt and Knöpfel (2002), who applied a glutamate uptake blocker and observed facilitated activation of the mGluR1/5 mediated current.

- The amplitude of I_{TRPC} increases with increasing number of inputs, in line with experimental observations (see e. g. Congar *et al.*, 1997; Faber *et al.*, 2006).
- I_{TRPC} kinetics can be modified by stimulus parameters, as discussed above. This finding is to some extent supported by experimental data, which I will discuss in depth in the meta-analysis below.
- With a modified version of our TRPC model it was possible to induce persistent spiking following synaptic input, which has also been shown in experiments (Egorov *et al.*, 2002).

τ_{decay} – a closer look

A major part of Paper I was devoted to studying the decay time constant of the I_{TRPC}-evoked EPSP – τ_{decay}. Here we will take a closer look at the data and discuss the results. As already discussed, we showed that τ_{decay} depends on the stimulus parameters. I wanted to know if the decay time constant of I_{TRPC} itself – $\tau_{decay,iTRPC}$ (measured at the site of synaptic input) – also has this stimulus-parameter dependency. In figure 4.1 (left) I show that $\tau_{decay,iTRPC}$ has a similar dependence on stimulus parameters as τ_{decay} (see figure 11 in Paper I), but lies in the range 120 - 160 ms whereas τ_{decay} lies in the range 195 - 250 ms. In line with the results discussed above, a linear relation was found also between $\tau_{decay,iTRPC}$ and the maximal glutamate concentration: $\tau_{decay,iTRPC} = 38 + 79 \cdot max([Glu])$, data not shown.

In figures 14A and 16D of Paper I we showed that the value of ISD_{R10} increases with increasing stimulus number (N) and frequency (f). ISD_{R10} was plotted versus N, and f was color coded. As an alternative one may choose to plot the same data in 3D, which I have done in figure 4.1 (right). The top figure contains data from the VIA model. One observation is that pulse trains of 1 or 2 Hz do not provide enough depolarization to allow for 10 % amplification of the subsequent test pulse. However, it should be noted that the ISD_{R10} measure is in one sense thresholded at 100 ms, since I did not use inter-stimulus delays $ISD < 100$ ms. A shorter ISD would correspond to a stimulus frequency above 10 Hz, which is outside the interval we studied (1 - 10 Hz). In the VDA model (bottom) ISD_{R10} does not reach as high values as in the VIA model, but saturates at less than 800 ms.

Sensitivity analysis

In Paper I we presented a sensitivity analysis but did not include a graphical presentation of the data. I will here show examples of data supporting the conclusions made in the paper. As described in Paper I, the steady-state summation of V_m, and to a lesser extent the decay time constant, is affected when the maximum TRPC conductance ($\bar{g}$) is varied from 50 to 200 % of its original value. The data is presented in figure 4.2. The left figure (based on figure 11A in Paper I) shows τ_{decay} plotted versus f for varying N. There is only a minor

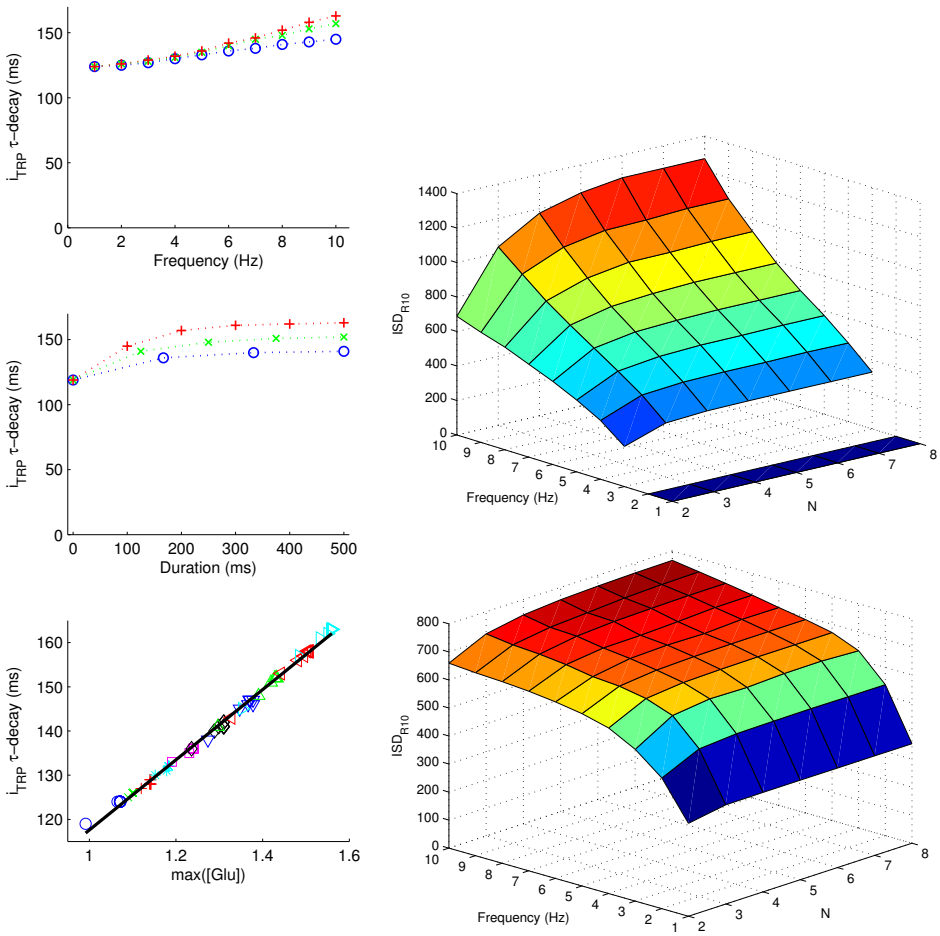

Figure 4.1. *(Left) I_{TRPC} decay time constant ($\tau_{decay,iTRP}$) increases with increasing frequency (top; blue: $N = 2$; green: $N = 3$; red: steady-state) and stimulus duration (middle; blue: $f = 6$ Hz; green: $f = 8$ Hz; red: $f = 10$ Hz). It has a linear dependence on maximum glutamate (bottom). This can be compared to figure 11 of Paper I, which is similar but with τ_{decay} instead of $\tau_{decay,iTRP}$. (Right) ISD_{R10} increases with increasing frequency and stimulus duration in both the VIA model (top) and the VDA model (bottom), as shown in figures 14 and 16 of Paper I. In the VDA model ISD_{R10} does not reach as high values as in the VIA model, but saturates at less than 800 ms.*

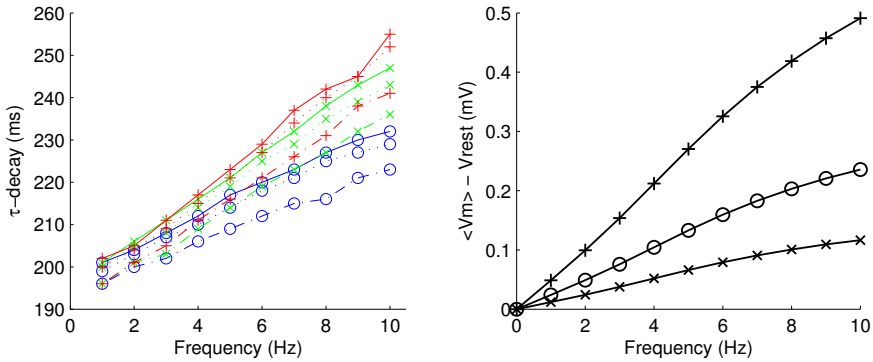

Figure 4.2. *Sensitivity analysis for the VIA model, varying the maximum TRPC conductance ($\bar{g}$). (Left) τ_{decay} increases with increasing frequency. $\bar{g}$ is set to 50 (solid line), 100 (dotted line, identical to figure 11A of Paper I) and 200 % (dash-dotted line) of its original value. (Right) Steady-state summation increases with frequency. $\bar{g}$ is set to 50 (cross), 100 (circle, similar to figure 10B of Paper I) and 200 % (plus) of its original value.*

variation ($< 5\,\%$) in τ_{decay} for the three values of $\bar{g}$ used. In the right figure (based on figure 10B in Paper I) it is shown that the steady-state average V_m is strongly dependent on the value of $\bar{g}$.

As shown in figure 11C of Paper I, glutamate summation is essential for the ability of τ_{decay} to depend on stimulation parameters. In figure 4.3 I present data from simulations where the glutamate summation parameter K_{glu} is set to zero, resulting in a model of glutamate concentration that reaches the same amplitude for every new synaptic input, regardless of input history. In the top figures it can be seen that τ_{decay} does not vary much with varying f or N. In the bottom figures it can be seen that the steady-state TRPC current still increases with increasing f, as does the resulting average V_m. It should be noted that setting K_{glu} to zero may not be consistent with the physiological observation, since it has been shown in at least one study that glutamate does indeed undergo summation for frequencies in the range studied here (Hires *et al.*, 2008).

Additional insights

The model of Paper II has many features in common with Paper I. Paper I could in fact be seen as an extension of Paper II. Differences exist in how the models were tuned, however, and the consequences of these will be discussed here. As already mentioned, one insight from Paper II was that the shape of the glutamate pulse has a strong effect on the I_{TRPC} kinetics. Another important insight was that decay time constants of I_{TRPC} are longer from experiments using somatic current injection and subsequent spike production, than those using synaptic stimulation.

The model in Paper II is similar to the VIA model of Paper I but has slower kinetics and a different glutamate model. The slower kinetics originates from

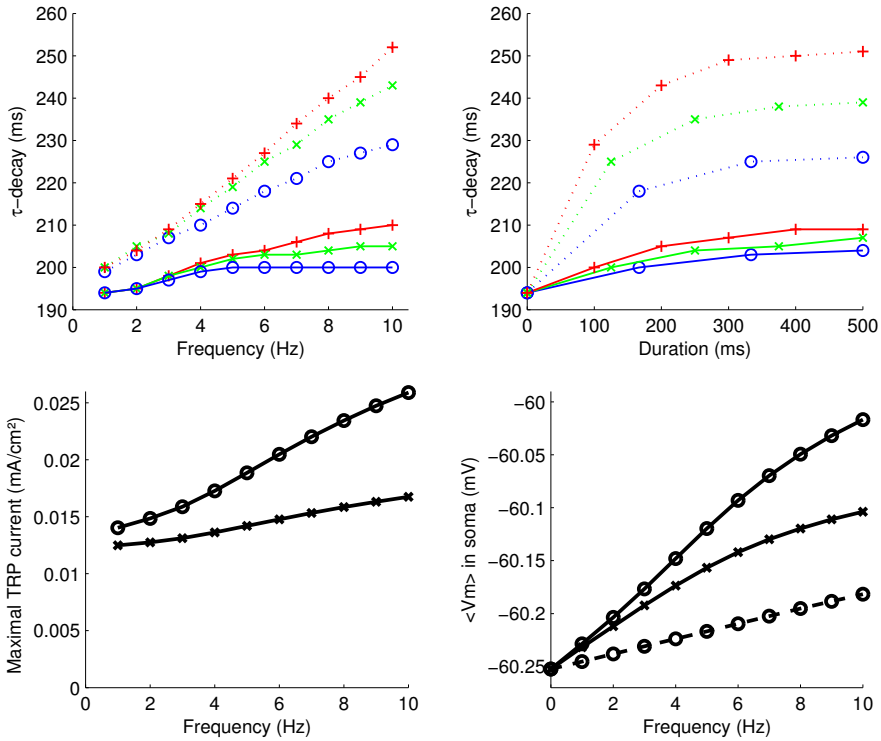

Figure 4.3. *Sensitivity analysis for the VIA model – impaired glutamate summation. τ_{decay} only slightly increases with frequency (top left) and duration (top right) when $K_{glu} = 0$ (solid) compared to $K_{glu} = 0.8$ (dashed, default as in figure 11 of Paper I). (Bottom, left) max(I_{TRPC}) increases less with frequency when $K_{glu} = 0$ (cross) compared to $K_{glu} = 0.8$ (circle, identical to figure 10A of Paper I). (Bottom, right) Steady-state summation increases less with frequency when $K_{glu} = 0$ (cross) compared to $K_{glu} = 0.8$ (circle, solid, identical to figure 10B of Paper I). Dashed line shows, for comparison, the case of summation when only ionotropic receptors are included.*

the fact that the model was designed to study the channel dynamics during current injection rather than synaptic stimulation, which I will discuss below. Development of the glutamate model in Paper II preceded that of Paper I and should be considered less accurate. The major conclusion in Paper I was that I_{TRPC} may be able to support summation of low-frequency synaptic input. It is therefore important to note that the same conclusion can be made from simulations in Paper II, as shown in figure 1 of that paper. Another important conclusion from Paper I is that I_{TRPC} decay time constants are affected by stimulus parameters. This phenomenon was also present in the model used for Paper II, though the effect was much smaller. A plausible explanation for this difference is that the glutamate concentration did not summate in Paper II. Taken together, we conclude that the results of Paper I are robust.

Current injection versus synaptic stimulation

I will here discuss the consequences of the fact that the model in Paper II was tuned from experiments using current injection, whereas the models in Paper I were tuned from experiments using synaptic stimulation. First it should be said that synaptic stimulation represents the physiological reality of mGluR-activated TRPC currents more accurately than current injection. Neurons communicate mainly via presynaptic transmitter release (in this case glutamate), which activates mGluR1/5 and subsequently TRPC. On the contrary, square pulse current injection in the soma combined with pharmacological (agonist) activation of mGluR1/5 is relatively far from the physiological reality of synaptically evoked TRPC currents (it may however represent back-propagating action potentials). Nevertheless, current injection provides one way of studying transient TRPC currents, and an advantage is that it is experimentally better constrained and more frequently used. The difference in which the two stimulation protocols affect I_{TRPC} has previously been studied experimentally with focus on persistent spiking (Yoshida *et al.*, 2008), but when it comes to the subthreshold domain I have not encountered any such systematic study. It was therefore highly interesting to find, by doing the meta-analysis described below, that the values of τ_{decay} resulting from current injection experiments are clearly longer than those from synaptic stimulation. However, it is hard to really compare the data since there were no data points from current injection with durations of less than 250 ms, so this finding should be considered preliminary (experiments using both protocols with identical stimulus durations would be clarifying). In Paper I we focused on relatively short stimuli, typically up to 500 or 1 000 ms. It was therefore natural to tune the model from synaptic stimulation experiments since that is where most data on this transient time scale exist. In Paper II on the other hand, we included stimulus durations up to values of 10 000 ms.

There may be many explanations as to why current injection yields larger values of τ_{decay} than synaptic stimulation. Since I_{TRPC} is strongly dependent on calcium, one explanation could be that the source of calcium from current injection may have a slower decay. Also note that a prediction from Paper I is that

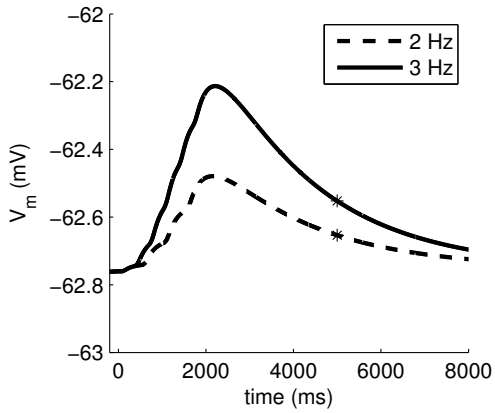

Figure 4.4. *In the model of Paper II, which is tuned from current injection experiments, τ_{decay} (marked with asterisk) is longer than in the models of Paper I. Figure shows V_m trace for synaptic stimulation, frequency of 2 and 3 Hz respectively, applied for 2 000 ms.*

τ_{decay} is strongly dependent on the agonist (glutamate, in the case of synaptic stimulation) concentration. In the current injection case, the mGluR1/5 agonist used (e. g. DHPG) may be applied at relatively high concentrations, possibly contributing to long decay time constants. One can also compare data from synaptic stimulation and current injection with data from mGluR1/5-agonist pressure-ejection experiments. One such experiment was performed by Bengtson *et al.* (2004), who found that currents from pressure injection had similar amplitudes, but much slower decay kinetics (mean 382 %) when compared to currents from synaptic stimulation. The authors used 100 μM DHPG, but it would also be interesting to investigate if a dose-response effect on τ_{decay} appears if the DHPG concentration is varied.

EPSP time course comparison

The components in the model of Paper II are similar to the VIA model of Paper I, though with slower kinetics. To illustrate the slow kinetics of this model I present in figure 4.4 two examples of membrane voltage traces following synaptic input applied for 2 000 ms at a frequency of 2 and 3 Hz respectively. The decay time constants are practically identical (approximately estimated to 3 000 ms) in this case, and much longer than the decay time constants in Paper I.

Meta-analysis of *in vitro* τ_{decay} measurements

Few experimental studies have systematically varied synaptic stimulus parameters (e. g. frequency and duration) in order to study the mGluR1/5-activated I_{TRPC}, and I am not aware of any review looking into experimental time constants of I_{TRPC} from different experiments. I thus decided to do a meta-analysis

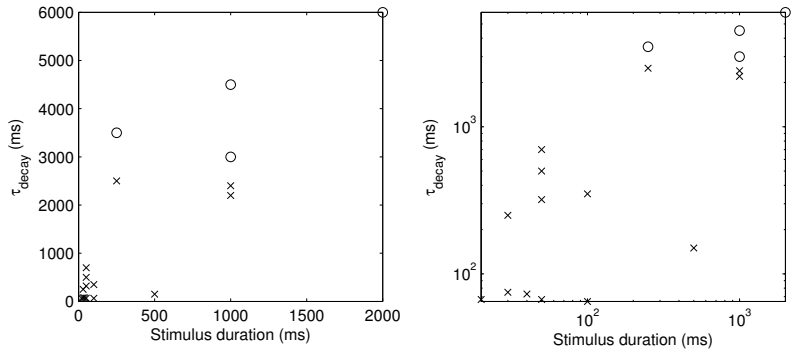

Figure 4.5. *Result of meta-analysis. Values of τ_{decay} from experiments using synaptic stimulation (crosses) and current injection (circles) are plotted versus stimulus duration, on normal (left) and logarithmic (right) scales.*

of time constants measured from variety of experiments, to get an idea of typical time constant values, and the result is plotted in figure 4.5, where τ_{decay} is plotted versus stimulus duration. Data points are taken both from experiments using current injection (Egorov *et al.*, 2002; Fowler *et al.*, 2007; Zhang *et al.*, 2011) and from experiments using synaptic stimulation (Congar *et al.*, 1997; Partridge and Valenzuela, 1999; Kim *et al.*, 2003; Bengtson *et al.*, 2004; Faber *et al.*, 2006; Hartmann *et al.*, 2008; Sidiropoulou *et al.*, 2009). These studies were performed on neurons from various regions of the brain: entorhinal cortex (pyramidal cells), hippocampus (CA1 pyramidal cells), prefrontal cortex (pyramidal cells), lateral amygdala (pyramidal cells) and cerebellum (Purkinje cells). Different frequencies were used in the synaptic stimulation experiments, ranging from 50 to 300 Hz, and stimulus durations varied from 20 to 6 000 ms.

A few observations can be made. First, the values of τ_{decay} from synaptic stimulation are quite consistent with the values used for tuning the model in Paper I, based on data from entorhinal neurons ($\sim 200-300$ ms). Second, τ_{decay} appears to increase with increasing stimulus durations, which is in line with the prediction made in both Paper I and Paper II (as mentioned earlier), thus suggesting that our models predictions are consistent with data. Third, there is a large variation in the data set, indicating that I_{TRPC} behaves differently in different experimental settings, which was also discussed above. Below I offer a few possible sources of this variation. Note, however, that the difference in τ_{decay} from current injection and synaptic stimulation data was discussed in depth above and will hence not be specifically discussed here.

Variation of mechanisms among systems studied

First, some of the variation discussed above can be explained by the simple fact that the neuron types investigated varies among different studies. Neurons are specifically adapted for the task they are involved in. As a consequence, entorhinal layer III pyramidal neurons are different from entorhinal layer V

neurons and from hippocampal CA1 neurons, and probably even less related to the neocortical prefrontal neurons, not to mention how different they are from the neurons of the cerebellum. Also the experimental protocols typically vary among studies, with respect to e. g. the species being used (it is often a rat, as in the studies included in figure 4.5, but sometimes a mouse), synaptic stimulation intensity, type of blocker and the temperature of the brain slice. The temperature, which affects the synaptic release probability (Kovalchuk *et al.*, 2000), is typically outside the physiological range, for various reasons. Furthermore, ACh may be spontaneously released in slices (possibly modulating the TRPC channels), which is often not controlled for. In addition to this, the researchers behind each study have different backgrounds and motives, and may therefore use different conditions or techniques, sometimes without explicitly stating them when describing the study.

The TRPC channel itself comes in different flavors, with varying subunit composition. While TRPC1/4/5 subtypes appear to constitute the most important heteromers in the entorhinal cortex, TRPC3/6 subtypes appear to be dominant in the cerebellum. The exact subunit composition is rarely controlled for in experiments. In line with this, what is often referred to as mGluR1/5-receptor activation includes activation of either mGluR1 or mGluR5 or both together, and their individual contribution and effect are often not fully explored. In addition, both mGluR1 and mGluR5 undergo alternative splicing (six splice variants for mGluR1 and two for mGluR5 have been identified, Niswender and Conn, 2010). Other complicating factors relate to the intermediate mechanisms coupling mGluR1/5 activation to TRPC activation, such as the PLC pathway. There are several PLC isozymes and although PLCβ is believed to be dominant, other isozymes may play a role (P Séguéla, personal communication). Also the effects of PLD (phospholipase D) remain to be explored.

Furthermore, it is believed that Ca^{2+} from more than one source can be involved in TRPC activation, which of course substantially adds to the complexity considering the great complexity in Ca^{2+} dynamics, with its numerous sources and downstream effects. The TRPC channel itself may also conduct Ca^{2+} ions when open, as shown in figure 2.3.

I_{TRPC} may be an important regulator of neuronal function

I_{TRPC} is affected by many modulatory substances. TRPC can be activated following activation of muscarinic acetylcholine (ACh) receptors (see figure 2.3), which mediate most of the effects of ACh in the brain (Purves et al., 2008). Hasselmo and Stern (2006) proposed the ACh-mediated "Alonso current", later attributed to TRPC channels (Zhang *et al.*, 2011), as the mechanism underlying working memory for novel information, but also stressed its role in encoding long-term memory. While the synaptically activated mGluR1/5 receptors have a transient effect on TRPC channels, ACh could be seen as affecting TRPC on a slower time scale as it appears to be important for processes of learning and memory (Hasselmo and Bower, 1993; Hasselmo and Giocomo, 2006; Hasselmo, 2006).

Another mechanism that can affect I_{TRPC} on a slow time scale is glutamate uptake. We showed in Paper I that the TRPC dynamics is strongly dependent on the maximal glutamate concentration. Also experiments have shown the influence of glutamate uptake on TRPC (Congar *et al.*, 1997; Reichelt and Knöpfel, 2002). Yet another example of a modulatory mechanism is receptor trafficking. It was recently shown that the amount of mGluR5 at the cell surface is likely to be regulated *in vivo* (Wang *et al.*, 2009), indicating subsequent regulation of I_{TRPC}.

Finally, the complex activation of TRPC channels should be described in light of the family they belong to (TRP). When the TRP research was still in its infancy, Montell *et al.* (2002) wrote that:

> "TRP cation channels display an extraordinary assortment of selectivities and activation mechanisms, some of which represent previously unrecognized modes for regulating ion channels. Moreover, the biological roles of TRP channels appear to be equally diverse and range from roles in pain perception to male aggression."

The observations above, together with the strong links between TRPC and Ca^{2+} suggest that TRPC channels are important regulators of neuronal function, but since the mechanisms of mGluR1/5-mediated TRPC currents are complex, they need to be studied more systematically to be fully understood and appreciated. I predict that computational modeling will be an important tool when performing these systematic studies, since it will be necessary to integrate several functional levels.

4.1.2 Stable subthreshold dendritic depolarizations

It is commonly assumed that the V_m of neurons approaches V_{rest} when there is a lack of incoming inputs. In Paper III I explored a phenomenon which raises questions regarding this assumption. Specifically, I found that the neuron can rest at a potential separate from V_{rest}, due to a phenomenon that I termed *SUBTLE* (SUBThreshold stable and Long-lasting synaptically Evoked dendritic depolarization). Interestingly, I actually ran into this phenomenon by accident, and at first it appeared to me as a bug in the code. After realizing that it was not a bug I started to search the experimental literature for anything resembling the SUBTLE. I did find several examples of subthreshold long-lasting small-amplitude depolarizations, and also talked to experimental colleagues who confirmed that it is not uncommon however quite unexplored. Thus, I started to explore the phenomenon and compare it to experimental findings.

Main findings

In short, I found support for the hypothesis posed by Fraser and MacVicar (1996) that an interplay between CAN (i. e. TRP) and VGCC currents can result in small-amplitude depolarizations in hippocampal CA1 neurons. As described in Paper III, I_{TRPC} is activated in response to synaptic input, both via

activation of mGluR receptors and by calcium from VGCC currents (activated by AMPA/NMDA/TRPC-mediated depolarizations). So, if for example a certain part of the dendritic tree has elevated calcium levels (and/or high densities of VGCC and/or TRP channels), I_{TRPC} will be strong and keep the membrane depolarized, in a state which I hereafter will refer to as a *local SUBTLE upstate*. In addition to providing support for the hypothesis posed by Fraser and MacVicar (1996) we contributed to the understanding of these long-lasting subthreshold depolarizations in several ways. In the following sections I will discuss the main questions addressed in our study concerning the SUBTLE phenomenon:

1. Is it stable?
2. Where in the neuron does it occur?
3. Is spatial and/or temporal summation necessary for induction?
4. Will it affect neuronal integration?
5. Is multistability possible?

Conclusion 1:
The SUBTLE is intrinsically stable

In previous studies of small-amplitude subthreshold depolarizations in hippocampal CA1 neurons it has been shown that they can be long-lasting (up to 60 s, El-Hassar *et al.*, 2011), but stability (robustness) to perturbating stimuli has not been tested. Thus, we used the model to test whether the SUBTLE can be stable to excitatory and inhibitory input. We found that it was indeed stable to both synaptic inputs and current injections. In figure 3A of Paper III we illustrated the stability of the SUBTLE by simply applying synaptic input, while we applied current injections in figures 3B-D. Before and after induction we applied short (duration = 5 ms) depolarizing (t = 500 and 2500 ms) and hyperpolarizing (t = 1000 and 3000 ms) current injections. For the depolarizing current injections, it can be seen that the sum of all ionic membrane currents I_{ionic} (black trace in figure 3D of Paper III) is changing in an outward (positive/hyperpolarizing) direction, thereby forcing V_m to return to its stable value when the current injection is released. Likewise, the hyperpolarizing current injections cause I_{ionic} to change in an inward (negative/depolarizing) direction so that stability is maintained. Thus, the SUBTLE is stable to short current injections. In the same figure we also showed that longer (100 ms) hyperpolarizing current injections can disrupt the SUBTLE state. This is because the temporary hyperpolarization causes a decrease in calcium inflow (due to decreased VGCC currents), resulting in deactivation of I_{TRPC}.

The long-lasting depolarization can be thought of as a memory trace, since the state of the neuron depends on what happened a few seconds earlier. The idea of single neurons maintaining this kind of stable memory trace is intriguing, and supported by work in neuron types other than CA1, showing that intrinsic

(network-independent) bi-/multistability is indeed possible: entorhinal pyramidal neurons (Egorov *et al.*, 2002); motoneurons (Hounsgaard and Kiehn, 1993); thalamocortical neurons (Hughes *et al.*, 1999); mitral cells (Heyward *et al.*, 2001); Purkinje cells (Loewenstein *et al.*, 2005). Perhaps the mnemonic bi-/multistability maintained by single neurons is complementary to network-level stable states, such as those suggested in attractor memory network modeling studies (Lansner, 2009).

Conclusion 2: SUBTLE is located in distal dendrites

In our study we argued that the local SUBTLE upstates are more likely to occur in distal dendrites than in other parts of the CA1 neuron. Three observations support this argument:

First, the depolarization caused the by the TRP current is quite strong but decreases with distance from the local SUBTLE upstate. Hence, if the VGCC-Ca^{2+}-TRP feedback loop occurs too close to the soma, action potentials will be generated and per definition disrupt the SUBTLEness.

Second, several dendritic branchlets contribute to making a SUBTLE stable while axial current loss makes single branchlets unstable. So oblique dendritic branches, which are typically quite small, are not likely to support the generation of local SUBTLE upstates, unless equipped with a high density of VGCC (as explored in the paper) and/or TRP channels (as explored below). The distal apical tuft dendrites, on the other hand, typically consist of a few large branches, and are therefore well-suited to support SUBTLE states.

Third, Poirazi *et al.* (2003) found that, in order to replicate experimental data, the VGCC-density profile needs to be such that the apical tuft dendrites has a higher VGCC density than the apical trunk (which in turn has a higher VGCC density than the oblique dendrites). Assuming that the VGCC profile suggested by Poirazi *et al.* is a reasonably good approximation of the actual VGCC profile in CA1 neurons, their study supports our hypothesis that SUBTLE upstates are typically located in the distal apical tuft (the probability of SUBTLEs increases with increased VGCC density, as shown below and in Paper III).

Conclusion 3: Spatiotemporal summation facilitates induction

We found that induction (onset of the SUBTLE) is facilitated if simultaneous input arrives at several dendritic branches, and if calcium- and/or mGluR-evoked signals undergo summation, suggesting that both spatial and temporal synaptic summation might be required for the depolarization to occur. When choosing parameter values it is important to consider the difference between factors relating to induction and those relating to maintenance (i. e. factors assuring that the SUBTLE is sustained). Examples of inductive factors are the number of pulses (N) and frequency of the synaptic input, the density/weight

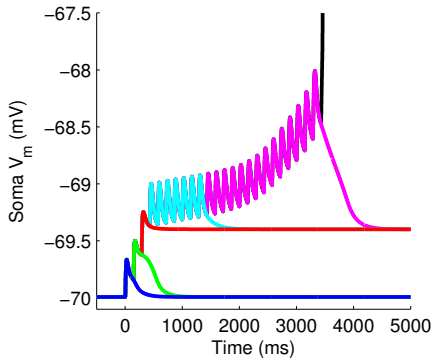

Figure 4.6. *Simulation identical to that of figure 2 in Paper III, but with $N = 1$ (blue), 2 (green), 3 (red, as in Paper III), 10 (cyan), 24 (magenta) and 25 (black).*

of ionotropic and metabotropic receptors and number and location of synaptic inputs. I want to stress here that the model is quite robust to changes in parameters relating to induction. In figure 5 of Paper III we showed that the choice of synaptic location is not crucial, and here in figure 4.6 I show that N is not critical as long as $N \geq 3$ (as discussed and shown in another way in the paper). It does not matter if we apply 10 or even 24 pulses, although we see that the subthreshold stable state is not reached for $N \geq 25$. Also note that, just like in Paper I, the summation saturates for $N > 4$. The main factors relating to maintenance are $\bar{g}_{VGCC}$, $\bar{g}_{TRPC}$ and Ca_{th}. The first factor was explored and discussed in Paper III, and the latter two will be discussed below.

Conclusion 4:
SUBTLE can affect neuronal integration

Since the effect of the SUBTLE is quite small in the soma, it is not obvious why neurons are equipped with this mechanism. We therefore wanted to test if the SUBTLE could affect future input. To do so, we applied synaptic input to an oblique dendrite before and after the SUBTLE was induced, and found that the SUBTLE could strongly modulate the excitability, specifically by leading to action potential triggering, as shown in figure 7 of Paper III. It is also reasonable to assume that the SUBTLE can increase the probability of other types of regenerative events such as NMDA spikes and calcium spikes (Antic *et al.*, 2010), although I have not yet explored this idea.

Conclusion 5:
Multistability cannot be ruled out

As shown in the paper, it is certainly possible to tune/design the model such that multistability can occur, in particular by local increases in the VGCC density. However, this multistability requires manipulation of VGCC and/or

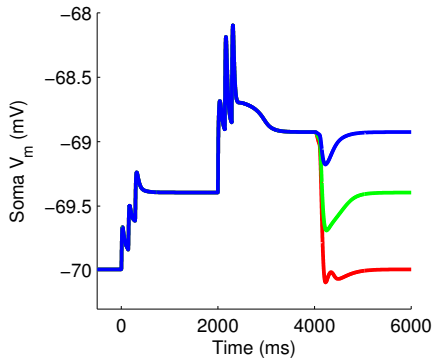

Figure 4.7. *As in figure 6C of Paper III, I here applied synaptic stimulation in an oblique branch with $\bar{g}_{VGCC}$ increased by a factor 15, subsequently to stimulating the tuft. Here I additionally apply inhibitory input of varying synaptic weight (1: blue; 3: green; 5: red) to the same oblique branch. The weak synaptic input does not disrupt the stable state, while the intermediate synaptic weight causes a switch to the intermediate stable state, and the strong synaptic weight causes V_m to return to V_{rest}.*

TRP channels, and the parameter ranges found were quite small (i. e. fine-tuning was necessary), suggesting that multistability is not likely to occur. It would, however, be interesting to explore multistability in a model with various gradients of channel densities, as such gradients are known to exist (Migliore and Shepherd, 2002; Nusser, 2009). That being said, I have not encountered experimental data in support for the presence of multiple subthreshold states. It is nevertheless fascinating to speculate about the potential consequences of such multistability. Even if only e. g. 5 different dendritic branchlets would each have a local SUBTLE upstate to enter, the neuron as a whole would have $2^5 = 32$ states. I will further explore aspects of multistability below.

Stability explored in a multistable model

In figure 3A of Paper III we tested stability to synaptic inhibitory input in the default model, which has a single stable state/equilibrium. Here in figure 4.7 I show simulations from the model where I manipulated $\bar{g}_{VGCC}$ (figure 6C in Paper III) such that a second stable state/equilibrium could be induced. The figure shows that, depending on the strength of the inhibitory synaptic weight, V_m either (1) returns to the highest (most depolarized) stable state (both the apical tuft and oblique branch in local SUBTLE upstates), or (2) falls down one level to the intermediate stable state (only tuft in local SUBTLE upstate) or (3) falls even further down to V_{rest}.

Insights gained from increasing $\bar{g}_{TRPC}$

In figure 6A of Paper III we showed that the SUBTLE can occur in oblique dendrites if $\bar{g}_{VGCC}$ is locally increased by a factor 20. We also mentioned that

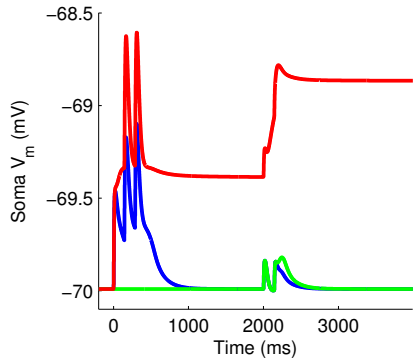

Figure 4.8. *Two separate oblique branches (here denoted O1 and O2) with $\bar{g}_{VGCC}$ increased a factor 10 were stimulated (O1 followed by O2). For the default value $\bar{g}_{TRPC} = 5\,S/cm^2$ (blue), no local SUBTLE upstate is induced, while for $\bar{g}_{TRPC} = 8.2\,S/cm^2$ (red) two local SUBTLE upstates are induced. When O1 stimulation is not preceding O2 stimulation (green), there is no SUBTLE.*

it is sufficient to increase $\bar{g}_{VGCC}$ with a factor 10 if $\bar{g}_{TRPC}$ is increased by a factor 1.64 in the whole dendritic tree, but did not show this data in the paper. Here I show the data in figure 4.8, and explore the case in further detail by adding a (test) stimulus in a separate oblique branch subsequent to the initial (conditioning) stimulus. Two conclusions can be drawn from the figure. First, as mentioned above, increases in either $\bar{g}_{TRPC}$ or $\bar{g}_{VGCC}$ are facilitatory for SUBTLE maintenance, and can even allow for multistability. Second, when comparing the two cases where $\bar{g}_{TRPC}$ is increased we see that the test stimulus only leads to a response (i. e. a stable subthreshold depolarization) if preceded by a conditioning stimulus. This could further be compared to figure 7 in Paper III, where a test stimulus applied to an oblique branch gave action potential outputs, but only if preceded by a conditioning SUBTLE-inducing apical tuft stimulus. Taken together, it seems natural to suggest that the SUBTLE phenomenon could play a role in integrating stimulus on time scales in the range of seconds (without necessarily triggering a spike as in figure 7, but also by jumping between various stable states).

Insights gained from decreasing $\bar{g}_{TRPC}$

In Paper III we showed that the SUBTLE is intrinsically stable and does not decay over time. This is in contrast to the subthreshold potentials found by Fraser and coworkers (Fraser and MacVicar, 1996; Fraser *et al.*, 2001), which decayed after a few seconds. In the modeling work of Paper III we chose parameter values that gave a robust model, in the sense that it was not sensitive to changes in the number of pulses, synaptic locations (figure 5 in Paper III) or other induction factors. I show in figure 4.9 that V_m decays also in the model when $\bar{g}_{TRPC}$ is slightly decreased.

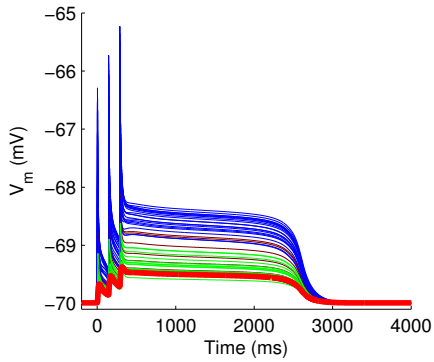

Figure 4.9. *When reducing the TRPC conductance to $\bar{g}_{TRPC} = 4.7\,S/cm^2$ in the default model (i. e. default VGCC densities) of Paper III, the depolarization decays towards V_{rest} in all compartments (color coding same as in figure 2B in Paper III; red: soma; brown: apical trunk; green: oblique dendrites; blue: apical tuft) of the dendritic tree.*

Simulating elevated levels of acetylcholine

As discussed in other parts of this thesis, acetylcholine (ACh) activates TRPC channels via muscarinic receptors. It is also known that blocking of these receptors (by e. g. applying the drug scopolamine) can cause memory loss in humans. ACh affects several ion channels in addition to TRPC, but one could make a simplification in our cell model and assume that ACh only affects I_{TRPC}. This is done in figure 4.10, where elevated ACh is assumed to decrease the calcium threshold while increasing $\bar{g}_{TRPC}$. Input is applied to two separate apical tuft branches. Interestingly, depending on the input location, different stable states are reached. In the soma the difference in V_m from rest is very small in both cases: $\Delta V_m = 0.200$ (left) and 0.225 mV (right). However, if one would apply subsequent synaptic input to either of the two tuft branches, the output response would differ strongly (i. e. spiking or not spiking) depending on if the input arrives to the branch in its SUBTLE upstate or the branch at rest. In line with this reasoning, I speculate that the presence of branch-specific subthreshold long-lasting depolarizations might play a role in novelty detection, since the integration of synaptic input on a certain dendritic branch depends on whether identical input has arrived in a recent past. It should be noted, however, that the model used for these simulations is less robust than the default model, and the results are quite preliminary. These ideas could be tested and explored more carefully in future research.

4.1.3 Relevance of the implemented I_{TRPC} model

I suggested above that computational modeling should be a useful tool for learning more about the mGluR1/5-mediated I_{TRPC}. In this thesis I have also argued that, conversely, the current may be important to include when building models

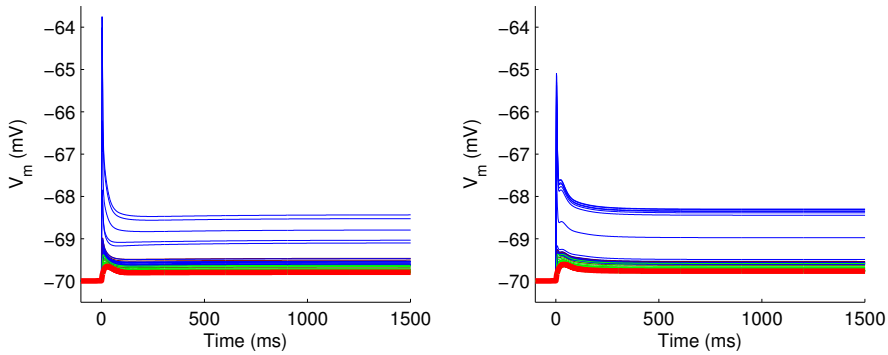

Figure 4.10. *Elevated ACh levels were simulated by decreasing the calcium threshold to $Ca_{th} = 1.03$, removing the mGluR1/5 contribution and increasing the TRPC conductance to $\bar{g}_{TRPC} = 8.9$. I selected three separate branches of the apical tuft and applied a single synaptic input. In 2 out of 3 branches it was possible to generate local SUBTLE upstates (as shown in the two figures) while in the third branch V_m decayed to V_{rest} (not shown). Color coding is same as in figure 4.9.*

on different levels, a discussion I will continue here. Multicompartment models typically do not include effects of mGluR activation. The only two exceptions I have encountered are the Purkinje cell work by Steuber and colleagues who studied the mGluR effects on the K_{Ca} channel (Steuber and Willshaw, 2004; Steuber *et al.*, 2006), and a model of slow calcium oscillations in lamprey spinal cord neurons by Hallen *et al.* (2004). Neither of these two studies included I_{TRPC}. From a broader perspective we should also acknowledge the fact that active dendrites have only been appreciated in the recent 15 years or so (Johnston and Narayanan, 2008). Consequently, dendritic models have classically only included passive cable properties and no ion channels.

To my knowledge, our I_{TRPC} model is the first of its kind. The idea of implementing it came from experimental work where an mGluR agonist was applied to show that the current, which indeed has an important role in persistent spiking, may also be observed in its subthreshold form as a depolarizing slow afterdepolarization (Al-Yahya *et al.*, 2003). Since I_{TRPC} is not well characterized experimentally, the process of implementing it required a time-consuming literature search, including studies of the spatiotemporal glutamate profile and the dynamics of the mGluR1/5 receptor. We have hitherto focused on using the model for studying the dynamics of I_{TRPC} and its role in synaptic and dendritic integration in a single cell. We have however not tested the potential role of I_{TRPC} in a network of neurons, which would provide insights into functional correlates of the channel at a higher level of neural system description. See chapter 5 for further discussion of future work.

Computational modeling studies are good complements to *in vitro* experiments (as discussed in sections 1.4 and 3.1), and insights can be gained that would be hard to optain from experiments. Above I have presented results from a large number of simulations, which systematically tested the influence of stim-

ulation parameters. One insight was that the decay time constant τ_{decay} of the I_{TRPC}-evoked depolarization depends on the maximal glutamate concentration, and that it increases with increasing stimulus duration and frequency. I demonstrated in figure 4.3 that the latter phenomenon is strongly reduced if glutamate summation is disabled. A similar test would not be feasible in an experiment. Furthermore, I evaluated two different I_{TRPC} activation models, based on two different hypotheses. As described earlier, similar results were obtained, except that the VDA model (but not the VIA model) showed saturation (of both average steady-state membrane potential and relative pulse train amplification). It would therefore be interesting to conduct an experiment which mimics the model simulations, and see if the results are more compatible with the VIA or the VDA model, thereby supporting either model. The work in this thesis also suggests that it would be insightful to use a paired-pulse protocol for testing the results predicted here in an experimental setting. Finally, without the use of a model it would have been hard to address the questions of Paper III.

4.2 Insights gained from C-fiber simulations

As already mentioned, it is problematic to make electrophysiological recordings from peripheral C-fibers. The axon, which is the major electrically functional part of these neurons, is so small ($\sim$1 μm) that intracellular measurements are hard (but see recent developments by Vasylyev and Waxman, 2012). Thus, experiments on C-fiber axons are often done using extracellular stimulation and recordings *in vivo*, which provides only indirect information about membrane properties. To some extent, a model can bridge the gap between extracellular measurements and membrane targets (ion channels) suitable for pharmacological pain treatment.

4.2.1 Novel modeling approach

In Paper IV we presented, to my knowledge, the first multicompartmental model of a cutaneous unmyelinated (C-type) peripheral nociceptor with ionic conductances tuned to replicate human microneurography data. The details were described in the paper and in section 3.3. The model has hitherto been successfully used to gain insights into the mechanisms of activity-dependent slowing (Paper IV), recovery cycles (Paper V) and to understand the differences between CM and CMi fibers (Paper VI), and it has also been useful in many group discussions, since several ideas not yet published have emerged. The intention, however, is to use the model in projects with higher clinical relevance, by testing and generating new hypotheses relating to neuropathic pain. In other words, the long-term goal is to contribute to the development of pain treatment (such as channel-specific drugs).

4.2.2 Activity-dependent slowing

The activity-dependent slowing (*ADS*) protocol, in which stimulus frequencies typically range from 0.125 to 2 Hz, is used to measure slow changes in membrane dynamics. One reason for using the ADS protocol is that the amount of slowing is strongly correlated with the degree of supernormality, which is associated with a putatively painful increase in C-fiber output frequencies (Weidner *et al.*, 2000, 2002; Bostock *et al.*, 2003). On the other hand, ADS is also correlated with conduction failures (Obreja *et al.*, 2011), which could be thought of as a self-inhibition mechanism (Sun *et al.*, 2012). ADS is also correlated with mechanical activation threshold (De Col *et al.*, 2012). The ADS pattern has also been used to gain insights regarding diabetic pain (Ørstavik *et al.*, 2006) as well as a painful inherited disease called erythromelalgia (Ørstavik *et al.*, 2003). Another reason for using the ADS protocol is that it can be used for dissociating different types of C-fibers (Serra *et al.*, 1999; Weidner *et al.*, 1999), which is important since fibers vary in how they contribute to pathological pain (Handwerker, 2010). Furthermore, since C-fiber classes are similar in pig and human (Obreja *et al.*, 2010), the ADS protocol suits well for testing the effect of pharmacological compounds *in vivo* animal experiments (Obreja *et al.*, 2012).

Mechanisms of ADS

Until a study by De Col *et al.* (2008), it was believed that the ADS in C-fibers was due to activity-induced hyperpolarization, resulting from increased pump activity (caused by accumulation of $[Na]_{in}$). However, the work by De Col et al. showed that activity-induced inactivation of Na_V channels is likely to have a greater influence on ADS that the activity-dependent hyperpolarization. Further support for this Na_V-inactivation hypothesis was provided by Snape *et al.* (2010), who reported an activity-dependent threshold increase (which corresponds well to ADS) in DRG somas. From this finding it became clear that increased pump activity is not necessary for the phenomena to occur, since the volume of the DRG soma is too large for $[Na]_{in}$ to accumulate. Our computational study (Paper IV) provided additional support for the hypothesis that the magnitude of Na_V currents plays a role, but highlighted the possibility of an activity-induced reduction in Nernst potential due to $[Na]_{in}$ accumulation, which is similar to what was suggested in studies on rat vagus nerves (Endres *et al.*, 1986) and tortoise olfactory nerves (Bliss and Rosenberg, 1979). In the following section I will discuss in more detail the insights gained from simulations into the role of individual membrane currents during the ADS protocol.

4.2.3 Currents during ADS protocol

The membrane currents included in the model can roughly be divided into two major groups:

- *Action potential currents:* $I_{Na_V1.8}$, $I_{Na_V1.7}$, I_{Kdr}, I_{KA}
- *Resting currents:* I_h, I_{pump}, I_{leak}, I_{KM}, I_{KNa}, $I_{Na_V1.9}$

During the model development we evaluated a large number of combinations of channel densities (or more precisely the maximal conductances $\bar{g}_X$) of these currents. I will here discuss the roles and behavior of the currents. Even though certain channel densities (in particular $\bar{g}_{Na_V1.9}$ and $\bar{g}_{KA}$) are quite small and almost without significance in the current model, I will nevertheless discuss them, since it is likely that they will play more important roles in future models.

During the typical ADS protocol, 360 pulses are applied at a frequency of 2 Hz. Figure 4.11 shows the relative contribution of all currents to the total current for the 1st and 360th action potential (AP) in the ADS protocol, and figure 4.12 shows the corresponding absolute currents plotted on top of each other. The purpose of these plots is to show how the influence of all currents changes during the different phases of the APs. The corresponding V_m traces are shown in figure 4.13. The time span (400 ms) in the top figures is chosen such that the resting currents are revealed. We see that the major resting currents are I_h, I_{pump}, I_{leak}, I_{KM}, and for the 360th AP also I_{KNa} (due to the accumulation of $[Na]_{in}$). The center and bottom figures show the currents during the AP (time span 10 ms) and AP upstroke (time span 0.6 ms) respectively. We see that the major AP currents are $I_{Na_V1.8}$, $I_{Na_V1.7}$, I_{Kdr} and to some extent I_{KA}. These figures also show how the relative influence of each current vary during the course of the AP. Note e. g. that the impact of $I_{Na_V1.7}$ is restricted to the AP upstroke while $I_{Na_V1.8}$ is more long-lasting (in accordance with Blair and Bean, 2002), and that the onset of the K^+ currents (I_{Kdr} and I_{KA}) is later than the onset of Na^+ currents (in accordance with the classical AP model of Hodgkin and Huxley). Next, to gain a deeper understanding of the development of the currents during the ADS protocol, I will show and explain the individual changes of all currents.

Na^+ currents

In figure 4.14 (top panel) I have plotted V_m versus time during the high-frequency (2 Hz) ADS protocol, and also the corresponding voltage-dependent Na^+ currents (lower panels), versus time (left) and V_m (right). The data is color-coded according to the time in order to facilitate the interpretation of the phase plots (currents vs. V_m). A few observations can be made from this figure. The amplitude of $I_{Na_V1.8}$ is much larger than that of $I_{Na_V1.7}$ (while $I_{Na_V1.9}$ is very small, as mentioned above). The reason for this is not only that the channel density of $Na_V1.7$ is lower, but also that $I_{Na_V1.7}$ suffers from a relatively strong inactivation at the resting potential V_m=-55 mV. The figures also show that the amplitude of $I_{Na_V1.8}$ and to a lesser extent $I_{Na_V1.7}$ decreases with activity (i. e. during the ADS protocol). As explained in Paper IV, this is because the reversal (Nernst) potential E_{Na} decreases, as a result of the activity-dependent $[Na]_{in}$ accumulation. The reason why $I_{Na_V1.7}$ decreases less (24 % reduction) than $I_{Na_V1.8}$ (40 % reduction) is that $Na_V1.7$ to a larger degree is released from inactivation during the pulse train due to the activity-dependent hyperpolarization (the $Na_V1.7$ fast inactivation parameter actually doubles, measured before APs). A prediction from this observation (differential activity-induced

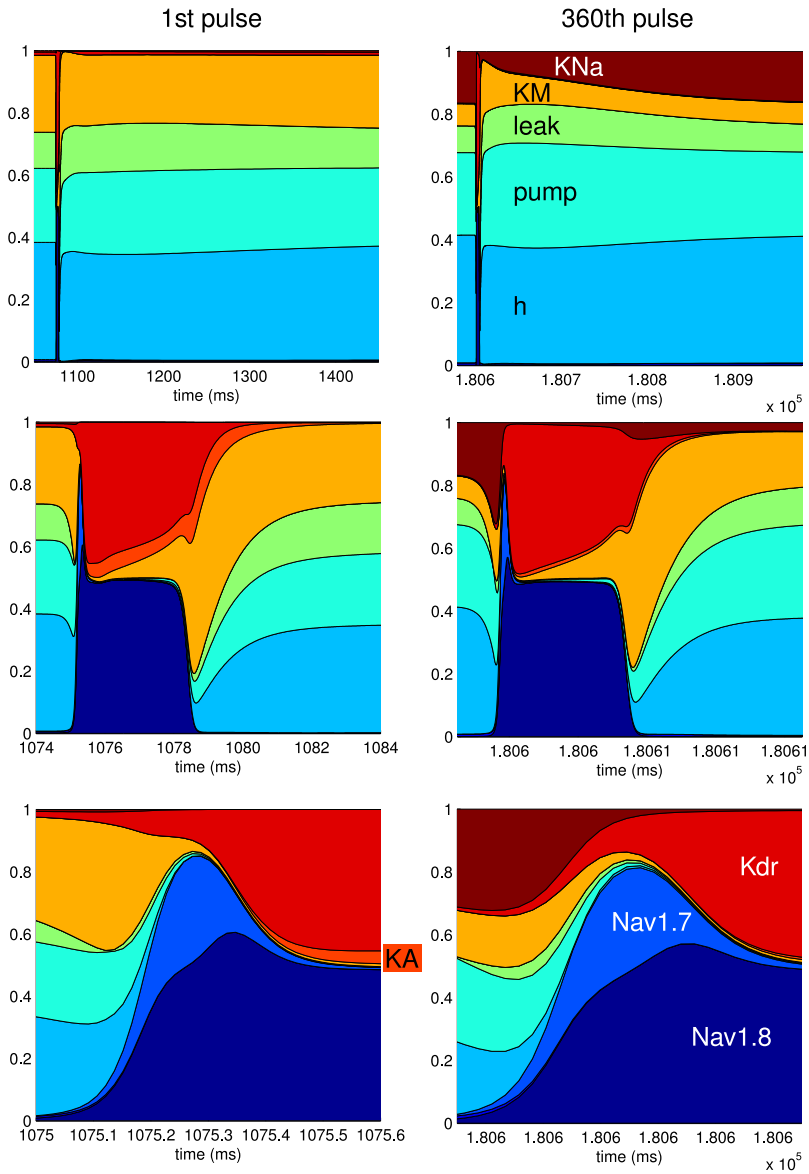

Figure 4.11. *The relative influence of currents on a long time scale (top), during the AP (center) and AP upstroke (bottom), for the 1^{st} and 360^{th} AP of the ADS protocol. See text for details.*

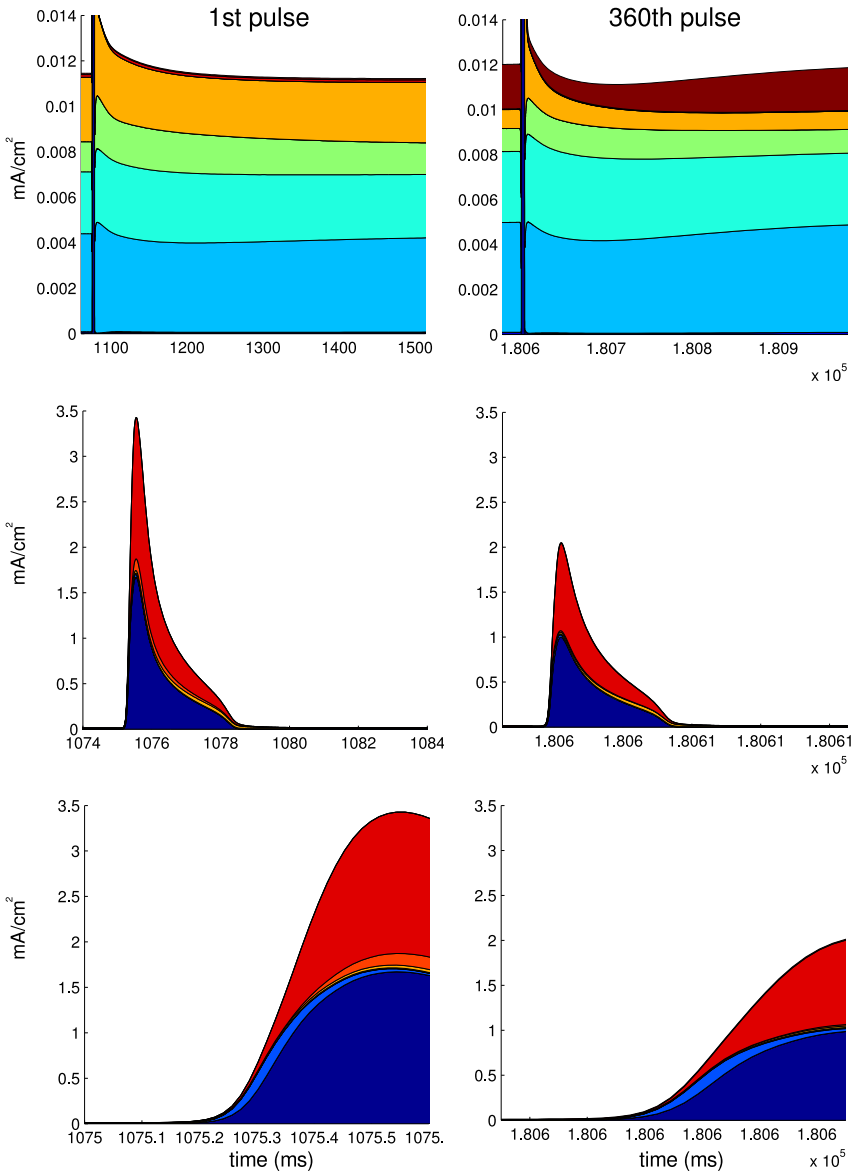

Figure 4.12. *Currents (mA/cm^2) on a long time scale (top, where y-axis is truncated at $0.014\, mA/cm^2$ for clarity), during the AP (center) and AP upstroke (bottom), for the 1^{st} and 360^{th} AP of the ADS protocol. See text for details.*

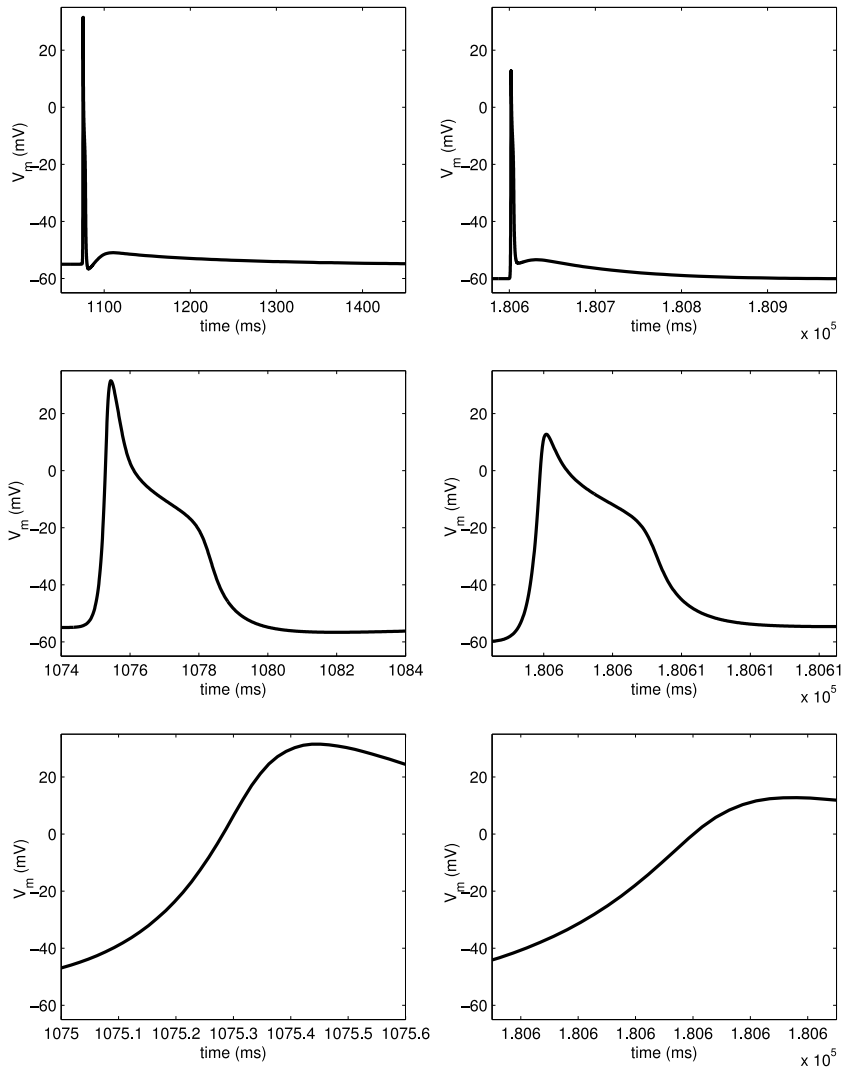

Figure 4.13. *APs plotted on a long (top), medium (center) and short (bottom) time scale, for the 1^{st} and 360^{th} pulse of the ADS protocol. See text for details.*

inactivation) is that if the ratio $\bar{g}_{Na_V1.7}/\bar{g}_{Na_V1.8}$ would be increased, the model would show less ADS (since $I_{Na_V1.7}$ would have a larger influence in generating the AP). Below I will show that this prediction holds. In contrast to $I_{Na_V1.7}$ and $I_{Na_V1.8}$, $I_{Na_V1.9}$ actually increases with activity, since the effect of the hyperpolarization-induced release from inactivation is stronger than the effect of E_{Na} reduction.

The above reasoning exemplifies the benefits of using computational modeling to gain insights into complex neuronal systems. For instance, based on the observation that the activity-induced reduction of $I_{Na_V1.8}$ is stronger than that of $I_{Na_V1.7}$, one could speculate about the reasons why mammals are actually equipped with the $Na_V1.7$ channel. For single action potentials $I_{Na_V1.7}$ has a limited role (since it is largely inactivated, as discussed above), but after activity it might play a more important role in ensuring that the AP is generated (due to the hyperpolarization-induced release from inactivation), even though $I_{Na_V1.8}$ is reduced. This is consistent with findings that humans lacking $I_{Na_V1.7}$ are insensitive to pain (Cox *et al.*, 2006).

Furthermore, $I_{Na_V1.7}$ might affect the generation of AP bursts. In some models that we have evaluated, the AP has been followed by a prominent AHP (afterhyperpolarization), while others have shown a prominent ADP (afterdepolarization). These two extremes strongly influence the behavior of $I_{Na_V1.7}$ in opposite directions. While an AHP leads to a dramatic increase in $I_{Na_V1.7}$, which presumably facilitates the generation of AP bursts, an ADP would reduce of $I_{Na_V1.7}$.

K^+ currents

Figure 4.15 shows the K^+ currents during the ADS protocol. A few observations can be mentioned. The AP currents I_{KA} and I_{Kdr} decrease significantly with activity, since activation gates as well as driving potentials ($V_m - E_K$) are reduced due to the activity-dependent reduction in AP peak (in turn resulting from reduction of AP Na^+ currents due to reduced E_{Na}). To analyze the resting currents I_{KM} and I_{KNa} it is more informative to look at figure 4.11, which shows that I_{KNa} increases with activity (due to the activity-dependent $[Na]_{in}$ accumulation) while I_{KM} is decreases (due to the activity-dependent hyperpolarization).

Mixed currents

Figure 4.16 shows those currents that mediate both Na^+ and K^+ ions (I_{pump}, I_h and I_{leak}). I_{pump} simply depends on $[Na]_{in}$ and $[K]_{sp}$, and therefore increases with activity (due to the $[Na]_{in}$ accumulation). The I_h and I_{leak} plots do not provide much information, and like for the other resting currents (I_{KM} and I_{KNa}) it is more informative to look at figures 4.11 and 4.12, showing that I_h and I_{leak} does not change much.

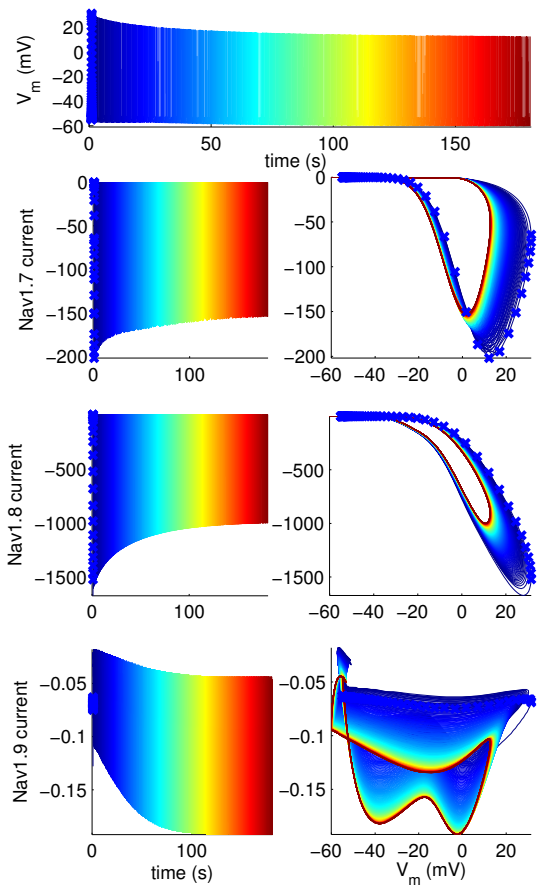

Figure 4.14. *V_m and Na_V currents ($\mu A/cm^2$) measured in the parent axon. See text for details.*

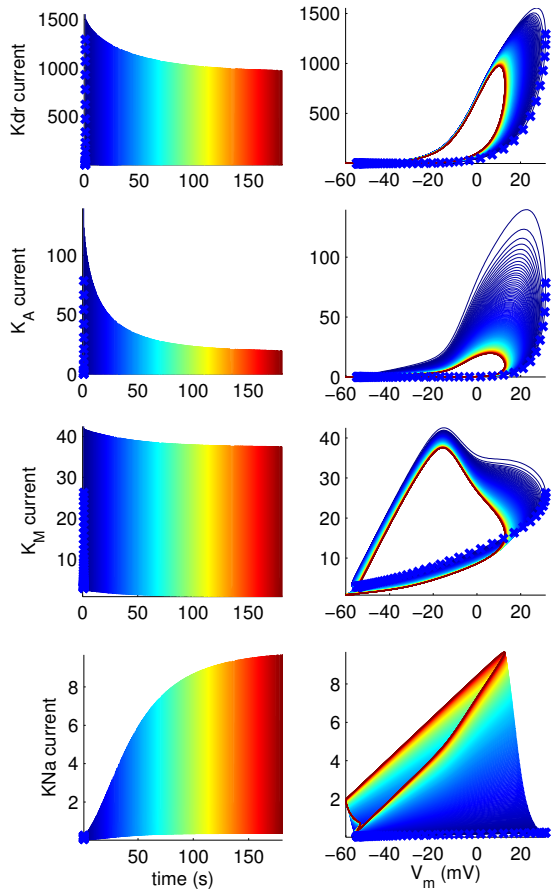

Figure 4.15. *K^+ currents ($\mu A/cm^2$) measured in the parent axon. See text for details.*

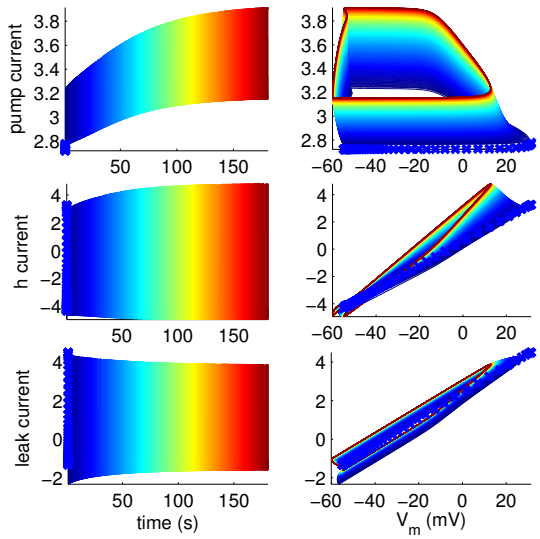

Figure 4.16. *Currents (expressed in $\mu A/cm^2$) mediating both Na^+ and K^+ ions, measured in the parent axon. See text for details.*

Channels contributing to Na^+ accumulation

Since we found in Paper IV that a major factor determining the amount of ADS in the model is the accumulation of $[Na]_{in}$ it is interesting to study the contributing Na^+ sources. In addition to the three sodium currents ($I_{Na_V1.7}$, $I_{Na_V1.8}$, $I_{Na_V1.9}$), there is also Na^+ influx via I_h and I_{leak}, while I_{pump} mediates the sodium efflux. In figure 4.17 I have plotted the integral of these currents during the high-frequency ADS protocol. Somewhat surprisingly, we see that I_h is the major contributor to Na^+ influx, followed by $I_{Na_V1.8}$ and I_{leak} ($I_{Na_V1.7}$ and $I_{Na_V1.9}$ does not contribute significantly). The importance of $I_{Na_V1.8}$ is perhaps more intuitive (since it is the major action-potential current) than the importance of I_h and I_{leak} (which are small in amplitude compared to $I_{Na_V1.8}$). The reason why so much Na^+ is mediated by I_h is that, due to its hyperpolarizing voltage dependence, its gate opens during the ADS protocol (since the membrane undergoes activity-dependent hyperpolarization). This is shown in the figure 4.18 (the gray area between pulse 359 and 360 is larger than the gray area between pulse 1 and 2). Because the gate opens during the pulse train, the current increases (in spite of the reduction in E_{Na}, which also affects I_{leak}).

4.2.4 Recovery from ADS

After the ADS pulse train (360 pulses at 2 Hz) it takes time for the neuron to return to steady state. In figure 4.19 I plot V_m, E_{Na} and E_K after the pulse train, which has resting values -55.0, 69.0 and 81.6 mV respectively. Just at the

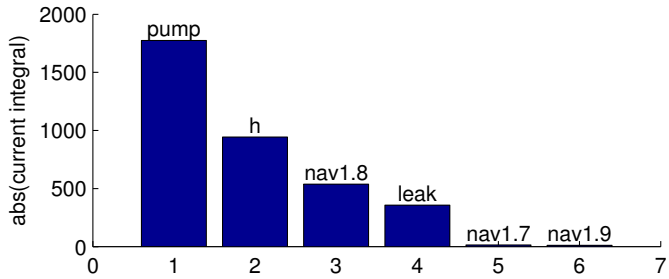

Figure 4.17. *Integral of sodium-mediating currents during the high-frequency ADS protocol (360 pulses at 2 Hz). Only Na^+ components were included (e. g. only $I_{Na,leak}$ and not $I_{K,leak}$). Currents measured from parent axon.*

end of the pulse train, E_K is already close (-78.6 mV) to its resting value, while V_m (-59.5 mV) and E_{Na} (31.9 mV) are further from rest. It takes approximately 2 minutes before the neuron has returned to its resting state, which could be considered a long time, since the AP duration is only a few milliseconds. The figure also shows that E_{Na} and V_m are quite closely correlated during the recovery from ADS. It could be interesting to further explore these events, and e. g. test the excitability (current injection threshold) of the membrane at various time points during the recovery.

4.2.5 Recovery cycles

While the ADS protocol is used to assess relatively slow changes in neuronal properties (e. g. membrane potential, ion channel states, ion concentrations), the recovery cycle (RC) protocol is used to assess the pattern of excitability following the action potential on a shorter time scale, and it can also be used to investigate pathological changes in human nerve fibers (Krishnan *et al.*, 2009).

In addition to generating knowledge about e. g. afterpotentials of single APs, results from human RC experiments has also triggered ideas on peripheral contrast enhancement (intensification of painful signals), which is correlated with the degree of ADS (Weidner *et al.*, 2002). Two important concepts relating to RC curves are *supernormality* and *subnormality*, which typically means that the current threshold (for generating an AP) for a certain stimulus is lower (supernormality) or higher (subnormality) than that of a preceding stimulus. In an axon, however, excitability is typically measured in terms of AP propagation latency. Supernormality then means that one AP causes a subsequent AP to propagate faster than the first. Many studies have shown a strong correlation between supernormal threshold and supernormal velocity (Bucher and Goaillard, 2011).

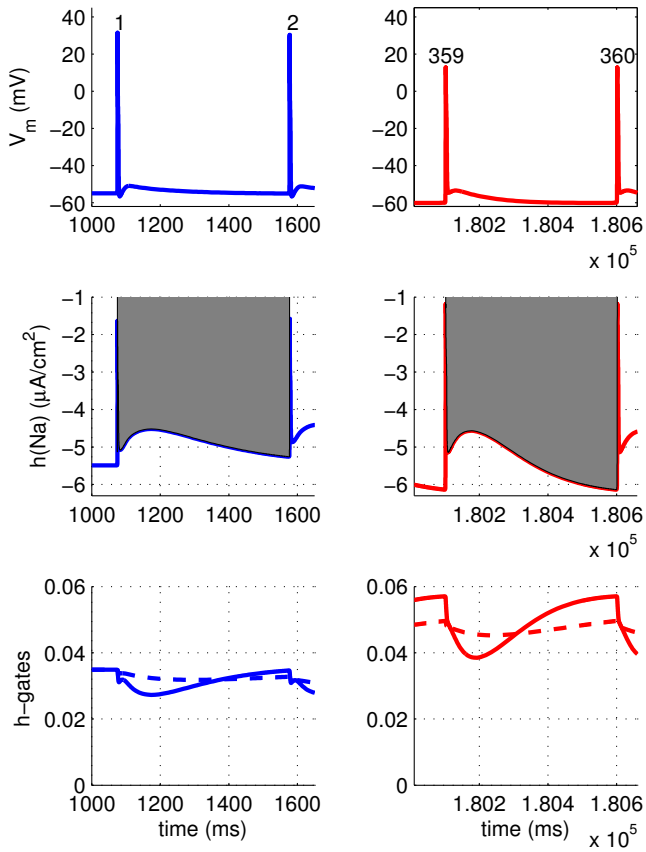

Figure 4.18. *Na^+ component $I_{Na,h}$ and activation gates of I_h plotted during pulse 1 and 2 (blue) as well as 359 and 360 (red) of the high-frequency ADS protocol (360 pulses at 2 Hz), measured from parent axon. Top: V_m; Centre: $I_{Na,h}$; Bottom: activation gates.*

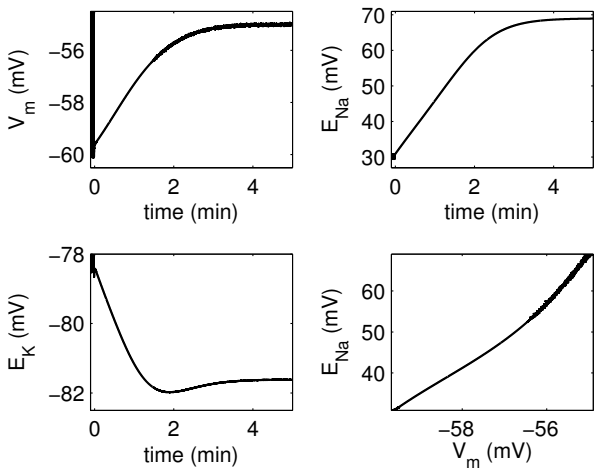

Figure 4.19. *Recovery from ADS. V_m and reversal potentials measured in parent starting at a time point at the of the ADS protocol.*

Mechanisms of supernormality (SN)

Currently, a widely accepted hypothesis regarding the mechanisms underlying supernormality in peripheral unmyelinated C-fibers is that an afterdepolarization appears as the membrane hyperpolarizes (Weidner *et al.*, 2002; Bostock *et al.*, 2003). In Paper V we found support for the idea of an emerging ADP, but also observed that it does not simply lead to an enhancement of the action currents ($I_{Na_V1.7}$ and $I_{Na_V1.8}$) and thereby supernormality (SN). Rather, while $I_{Na_V1.8}$ is enhanced by the ADP, $I_{Na_V1.7}$ is actually reduced due to its strong inactivation at relatively depolarized potentials. As described in the paper, we also found support for a contribution of periaxonal/extracellular potassium ($[K^+]_{sp}$) to supernormal latency shifts, a hypothesis previously rejected on the basis of conditioning prepulses (Bostock *et al.*, 2003). Many previous studies have assumed that SN is based on an ADP, and essentially argued about the origins of this ADP. It is therefore of interest that we found that an SN-inducing $[K]_{sp}$ increase is not necessarily manifested as an ADP. Finally, our simulations suggested that $[Na^+]_{in}$ accumulation might contribute to the activity-induced transition from subnormality to supernormality.

4.2.6 Parameter-variation analysis and CM versus CMi

As emphasized in Paper VI, it is important to understand the differences between mechano-sensitive (CM) and mechano-insensitive (CMi) nociceptive peripheral C-fibers, as the latter is more involved in inflammatory responses and may be involved in pathological pain. We therefore wanted to explore what differs between the two fiber types, and tested the hypothesis that the ob-

served differences in propagation properties can be explained by differences in membrane resting potential and/or differences in axonal ion channel and pump densities. To do so, I varied these parameters and ran stimulation protocols identical to protocols used in human and pig *in vivo* experiments, which have demonstrated several differences in the two fiber types (see Paper VI for details).

In the study I found, among other things, the following:

- Compared to CMi fibers, CM fibers are likely to have less K_{dr} channels, more $Na_V1.7$ channels and more Na-K-pump (in terms of channel/pump densities).

- ADS and activity-induced conduction failure tendency was clearly correlated, which is consistent with porcine *in vivo* data and could thus be taken as a supporting argument for the model's validity.

- In terms of AP Na_V channels, individual differences (across a population of humans) in AP propagation properties are more likely to be caused by variation in $Na_V1.7$ than variation in $Na_V1.8$. This suggestion was based on the experimentally observed negative correlation between CV and ADS. We also discussed the possibility of using model-based patient-specific pharmacological treatment in the future.

- Differences in ion channel conductances are not likely to explain the observed differences in CM and CMi current thresholds.

- We also speculated that enhanced slow $Na_V1.7$ inactivation might contribute to erythromelalgia pain, which would be in contrast to previous hypotheses. The model prediction of increased $Na_V1.7$ levels in CM fibers is thus in a sense also supported by the observation that erythromelalgia patients have particular problems with normal touch. These ideas should be tested in further simulations and experiments.

Below I will discuss some of the insights gained from parameter-variation simulations, in more detail than in the paper. These insights go beyond an identification of CM and CMi differences, and help to understand how various ion channels shape the membrane excitability.

Activity-dependent slowing

Figure 4.20 shows how changes in channel (and pump) densities affect the ADS pattern and $[Na]_{in}$ accumulation. The sensitivity analysis gives insight into the roles that each of the currents play in the model. In addition, it has proven useful in model tuning. Although further analysis is necessary to make strong conclusions, I will here provide a few observations and preliminary conclusions:

- To a large extent, a change that causes an increase in intracellular sodium also causes a strong ADS. This is consistent with our findings in Paper IV.

- According to the prediction described above, the ratio $\bar{g}_{Na_V1.7}/\bar{g}_{Na_V1.8}$ should be negatively correlated with the amount of ADS. We see that this prediction holds, as a decrease in $\bar{g}_{Na_V1.7}$ or increase in $\bar{g}_{Na_V1.8}$ leads to increased ADS. A second reason behind the positive effect of $\bar{g}_{Na_V1.8}$ on ADS is that a larger $I_{Na_V1.8}$ gives stronger Na-accumulation.

- ADS decreases with reduced $\bar{g}_{Kdr}$. One reason for this is related to the role of I_{Kdr} during AP repolarization. With a small $\bar{g}_{Kdr}$, V_m during the repolarization phase is increased, leading to stronger inactivation of $I_{Na_V1.8}$ and consequently a smaller $I_{Na_V1.8}$ integral during the AP (particularly when V_m repolarizes from +10 to -20 mV), which gives a reduced sodium accumulation and thereby reduced ADS. A 20 % reduction of $\bar{g}_{Kdr}$ causes a large reduction in the $I_{Na_V1.8}$ integral (9.1 % for a single AP; 11.5 % for the full 360-pulse ADS-protocol), and consequently a large reduction in total Na accumulation (7.3 %). An additional reason for the effect of I_{Kdr} on ADS is that I_{Kdr} contributes to activity-dependent hyperpolarization (see below), which activates I_h (a current that strongly contributes to sodium accumulation).

- ADS decreases with increased $\bar{g}_{pump}$, since I_{pump} is responsible for pumping out sodium.

- ADS increases with $\bar{g}_h$, which is expected considering that I_h significantly contributes to the accumulation of sodium.

- An increased $\bar{g}_{KNa}$ or decreased $\bar{g}_{KM}$ leads to increased ADS, which might be explained, as can be seen below, by the fact that both changes lead to increased activity-dependent hyperpolarization, presumably leading to an increased I_h and thereby increased ADS.

One way of describing the shape of the ADS curve is to measure the amount of slowing after 50 pulses and divide by the total slowing (Obreja *et al.*, 2010). In the figure I have plotted this measure, denoted S_{50}, against changes in channel/pump densities. Apparently, $\bar{g}_{pump}$, $\bar{g}_{Na_V1.8}$, $\bar{g}_h$ and $\bar{g}_{KNa}$ has the largest influence on S_{50}. While these results might not provide any interesting physiological insights, they can be used for tuning the model to better replicate human experimental data.

Activity-dependent hyperpolarization

Figure 4.21 shows that the amount of activity-dependent hyperpolarization, hereafter referred to as *ADH*, varies in a 1 mV range when channel/pump densities are varied ± 20 %. Specifically, increased (decreased) $\bar{g}_h$ and $\bar{g}_{KM}$ leads to less (more) ADH, while the opposite is true for $\bar{g}_{KNa}$, $\bar{g}_{Kdr}$ and $\bar{g}_{Na_V1.8}$. The reason why increased resting currents I_{KM} and I_{KNa} have opposite effects on ADH can be understood from the results above (while I_{KM} decreases during the pulse train, I_{KNa} increases). So when the ratio $\bar{g}_{KM}/\bar{g}_{KNa}$ increases, there is more of a decreasing outward current (I_{KM}), and therefore less ADH. The

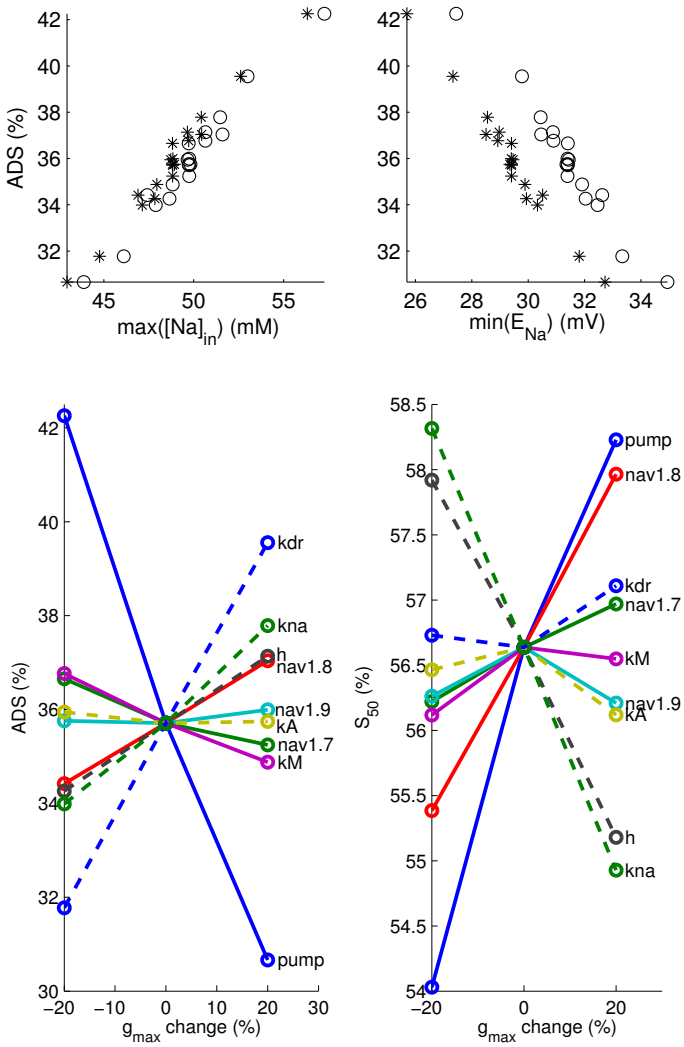

Figure 4.20. *Varying channel/pump densities – effect on ADS. Top: Total amount of ADS is correlated with $[Na]_{in}$ and E_{Na} in both the branch (circles) and parent (star) axons. Bottom: Total ADS (left) and the slowing after 50 pulses divided by the total slowing (S_{50}, right) plotted against channel/pump density changes.*

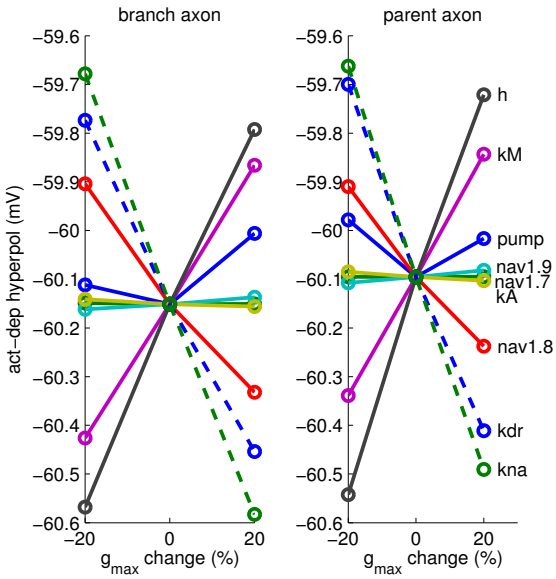

Figure 4.21. *Varying channel/pump densities – effect on activity-dependent hyperpolarization (AHP). V_m before initiation of the 360^{th} AP was measured in the branch (left) and parent (right) axons.*

reason why increased $\bar{g}_{Na_V1.8}$ and $\bar{g}_{Kdr}$ leads to increased ADH is likely due to the fact that they both contribute to Na^+ influx and thereby increased I_{KNa}.

The influence of the pump ($\bar{g}_{pump}$) is more complicated, since apparently both increases and decreases of $\bar{g}_{pump}$ reduces ADH. This complex behavior might be explained by the fact that an increased pump current leads to less Na^+ accumulation and therefore less I_{KNa} (i. e. less ADH). However, a decreased $\bar{g}_{pump}$ (obviously) also results in a reduction of I_{pump}, which is a hyperpolarizing current (i. e. less ADH). This effect should be more carefully studied in further analysis. Also I_h should be examined more carefully before drawing any conclusions about the origins of its relatively large negative influence on ADH. On the one hand, I_h is a large inward current which slightly increases during the ADS protocol (thus it makes sense that an increase in $\bar{g}_h$ leads to a reduction in ADH). On the other hand an increased $\bar{g}_h$ also leads to increased Na^+ accumulation and therefore more I_{KNa} (i. e. more ADH).

Conduction velocity

We can see in figure 4.22 that the three key players in determining the conduction velocity (CV) are $I_{Na_V1.8}$, $I_{Na_V1.7}$, and I_{Kdr}. It is not surprising that $I_{Na_V1.8}$ and $I_{Na_V1.7}$ are important, as they are the major AP generators. It is also reasonable that I_{Kdr} (negatively) affects AP conduction, since it counteracts the depolarization during AP upstroke thereby reducing the excitability.

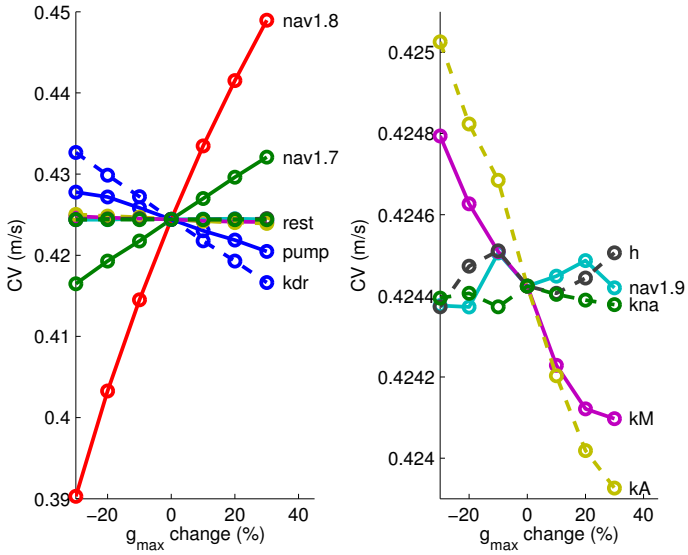

Figure 4.22. *Varying channel/pump densities – effect on conduction velocity (CV). For clarity, the currents that have a minor influence on CV (which are denoted 'rest' in left figure) are plotted again (zoomed in) to the right.*

Excitability (rheobase and chronaxie)

A common way of measuring excitability in experiments is to apply current injections of varying duration to find the corresponding threshold I_{th} for generating action potentials. I did this in our model axon and show the result in figure 4.23. The threshold follows a typical exponential decrease. The rheobase (defined as I_{th} for long durations) was 0.16 nA, and the chronaxie (defined as the duration for which I_{th} is twice rheobase) was 1.5 ms. I am not aware of specific rheobase measurements from human C-fibers, but the best estimate is probably that of Mogyoros *et al.* (1996), who measured from human peripheral sensory axons (but did not dissociate C-fibers and A-fibers) and found an average chronaxie of $\sim$0.7 ms. Thus, the chronaxie in our model might be too large.

The figure also shows how channel and pump densities affect excitability. I_{th} was measured for short (0.01, 1, 2 and 5 ms) pulses as well as for pulses long enough to generate rheobase measurements. Relative I_{th} (values divided by I_{th} of the default model expressed in %) is plotted for increases in each of the channel and pump conductances. For short pulses (0.01 ms, lower left) the effect of density changes on excitability are quite similar to the effect on CV (figure 4.22 above), which is an intuitive result.

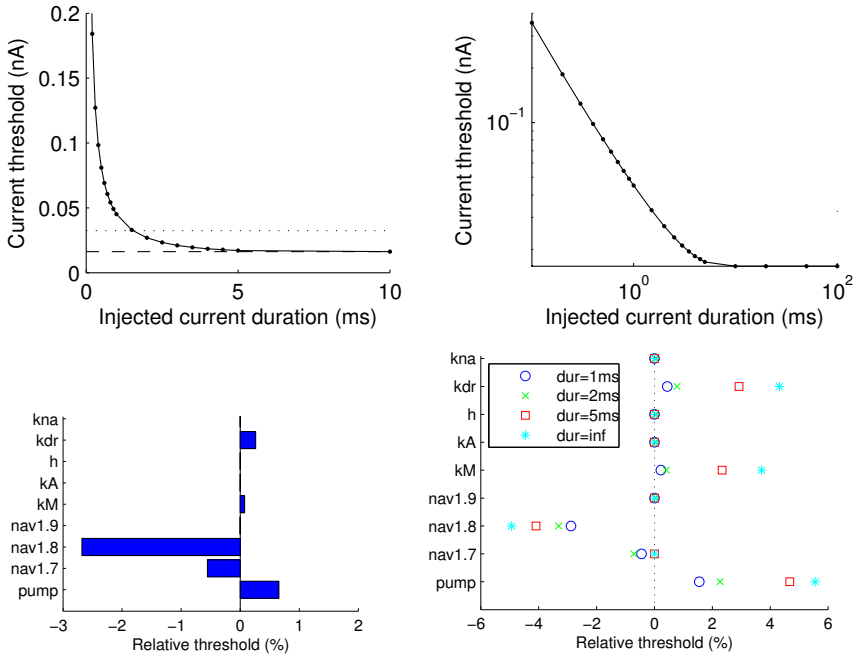

Figure 4.23. *Threshold current I_{th}: (Top) Rheobase=0.16 nA; Chronaxie=1.5 ms. (Bottom left) Effect on I_{th} from increases in channel/pump densities, for a stimulus duration of 0.01 ms. (Bottom right) Same but varying durations (1 ms, 2 ms, 5 ms and ∞).*

Robustness

Another insight from the sensitivity analysis is that the default model is relatively stable since none of the ±20 %-parameter changes lead to AP bursting or pre-slowing conduction failures. The maximal ion channel conductances were not based on direct measurements, since intraaxonal human *in vivo* recordings are not yet possible. If (or when) more detailed experimental data becomes available, the model should be updated. Sensitivity analysis could then be a useful tool to identify which parameters have the strongest effect on the model outcome, and more experimental effort should thus be made to find more accurate parameter values. Sensitivity analysis could also be used to address the question of how specific ion channels affect AP properties such as height, width and afterpotential magnitudes and durations.

Limitations

To test the hypothesis in Paper VI, I used a simple and traditional (univariate) sensitivity-analysis approach where one parameter at the time is varied while the others are kept at their default values. An obvious limitation of this approach is that it does not yield any information of how combined (multivariate) parameter changes would affect the various measurements. Another limitation is that only a local region of the parameter space is explored. As described by Taylor *et al.* (2009), there are alternative approaches that overcome these issues. However, such approaches typically involves generating a huge number of models, and since the protocols used in Paper VI are computationally expensive (the ADS protocol takes ∼9 h) this was outside the scope of that paper.

4.2.7 Control simulations

Control test of model concentration dynamics

In section 3.3 I argued that the simplification in the equation for Na^+/K^+ concentration dynamics, which we inherited from Scriven (1981), might not be accurate. Here in figure 4.24, I show the effect of this simplification. The black curves show the main results (ADS and RC) for the default model used in Papers IV, V and VI, where we used equation 3.13. The red curves demonstrate the similarity of the results when equation 3.12 (i. e. without simplification) is used. Both ADS and supernormal phase are slightly stronger. In conclusion, the assumption holds, as the errors are small and does not qualitatively affect the published results.

Control test of pump potassium dependence

As mentioned in section 3.3, detailed data on the Na-K-pump potassium dependence is lacking. I therefore ran ADS and RC simulations with b_1 reduced from 1 to 0.5. There was no significant change in results (not shown).

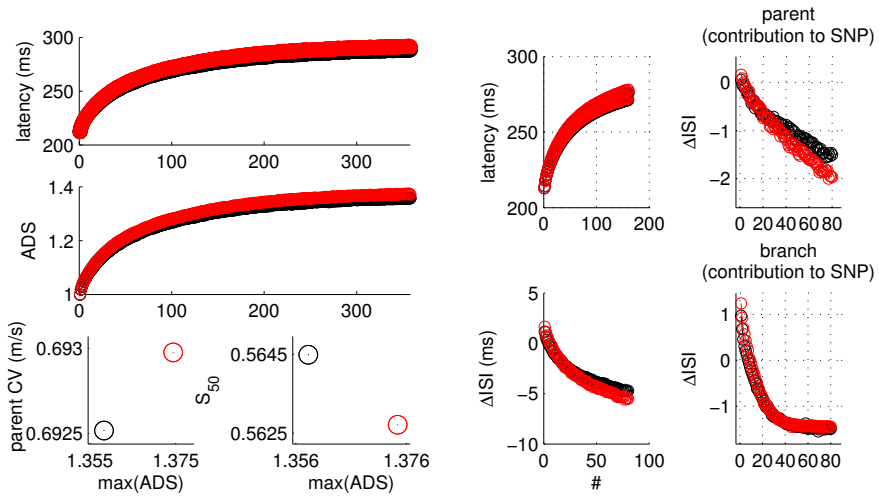

Figure 4.24. *Control test of assumption validity. ADS (left) and supernormal phase (right) plotted for default model (black) and for more accurate concentration dynamics (red).*

4.3 Additional remarks

4.3.1 Resting membrane potential V_{rest}

An important concept in cellular neurophysiology is *the resting potential* (V_{rest}). According to the textbook view of neuronal function, every neuron has a V_{rest} which is approached by V_m if there is no recent activity (such as synaptic input for pyramidal neurons, or generator potentials in the case of peripheral neurons). However, from the results mentioned above we can conclude that it is not always that simple. More specifically, I showed in section 4.1 and in Paper III that there can be more than one stable subthreshold state at which the (CA1 hippocampal) neuron can rest. Furthermore, I showed that V_m in peripheral nociceptor axons can change (hyperpolarize) substantially during low-frequency (2 Hz) activity, and that it takes a long time ($\sim$2 minutes) to reach V_{rest} again.

Furthermore, rodent *in vivo* measurements have shown that there is a large variation (between -63 and -41 mV) in V_{rest} among nociceptive C-fibers (Fang *et al.*, 2005). Since V_{rest} strongly affects neuronal excitability and dynamics, it is possible that some of the variability (in e. g. activity-dependent slowing, see Weidner *et al.*, 1999) observed among human C-fibers can be explained by variations in V_{rest}.

4.3.2 Excitability

Another important concept is *excitability*, i. e. how close a neuron is to its AP threshold (or how likely it is to generate APs). We have shown that mGluR-activated TRPC channels can contribute to temporal summation of synaptic

input (Paper I and Paper II) and subthreshold long-lasting depolarizations (Paper III), both of which brings V_m closer to spike threshold. Excitability is an important concept also in pain research, since neuropathic pain is associated with hyperexcitability. In Papers IV and V we explored how the excitability of C-fibers, measured as conduction velocity and latency shifts, changes with activity. In future studies it would be interesting to use the model to specifically look into axonal mechanisms related to hyperexcitability.

4.3.3 Contrast enhancement mechanisms

One type of computation that neurons can perform is *contrast enhancement* (or filtering), i. e. that the response to certain input frequencies is enhanced. As shown in Paper I, mGluR-activated TRP channels can allow for such contrast enhancement, since high (but not low) frequencies within the 1-10 Hz interval led to effective temporal summation in V_m. In Papers IV, V and VI we simulated subnormal and supernormal latency shifts (explained below), which can also be thought of as contrast enhancement mechanisms, as discussed by Weidner *et al.* (2002).

4.3.4 Elevated concentrations as cellular memory code

The importance of ion channel dynamics in shaping neuronal activity is rarely underestimated, while the role of ionic and transmitter concentrations receives less attention. Somewhat surprisingly, many conclusions in this thesis are actually related not only to ion-channel dynamics, but also to intra- and extracellular concentrations. In Paper I we showed that extrasynaptic glutamate levels can contribute to the decay dynamics of postsynaptic depolarizations, and in Paper III we showed that elevated intracellular Ca^{2+} is an important component in the maintenance of stable and long-lasting subthreshold depolarizations. Similarly, in Papers IV and V we found significant functional contributions of intracellular Na^{+} and extracellular K^{+}, to activity-dependent slowing and supernormal latency shifts respectively.

4.3.5 General insights

Multicompartment models with many types of dynamically complex ion channels are challenging to work with. One general insight resulting from the work leading to this thesis is that, for parameter tuning, automatic search methods are not necessarily better than "hand tuning" in my experience, neither in terms of time consumption or in terms of insights gained into the ion channel model dynamics. Although it was a surprisingly challenging and time-consuming task to develop the C-fiber model, I appreciate the experience, since it gave me unique insights and a good understanding of how the various ion channels affect each other. In this context I also want to echo the words of Panayiota Poirazi (who built the CA1 model described above) in her chapter of the book 'Hippocampal Microcircuits':

> "... despite a common belief that compartment models can be 'tuned' to replicate almost anything and should thus be interpreted with caution, in our experience it proved to be extremely difficult to find a single set of parameters which lie within physiological limits and reproduce all ... validation experiments without compromising the model's robustness"

Another general insight is that, as the type of questions addressed in this thesis are very closely related to those addressed in electrophysiological experiments, it is very important to have continuous interactions with experimental researchers. Not only is their expertise invaluable for properly approaching the hypotheses, but it is also crucial to have a continuous modeler-experimentalist dialogue in order to reformulate and update hypotheses as new results unfold.

Chapter 5

Future work

There are many ways to further improve the accuracy of the models used for this thesis, and there are also additional tests and analyses that could be performed, which I will describe below.

5.1 I_{TRPC} model

5.1.1 Model improvements

Due to the lack of experimental data, the calcium dependence in our I_{TRPC} model is currently phenomenological rather than tuned from experimental results. Gross *et al.* (2009) reported that homomeric TRPC5 channels appear to be directly activated by calcium in a dose-dependent matter. We could tune our model to replicate their data, but at least three things are then important to consider. First, I_{TRPC} is likely to be mediated by heteromeric TRPC1/4/5 channels (Zhang *et al.*, 2011), which have different biophysical properties than TRPC5 homomers (Strübing *et al.*, 2001). Second, Gross *et al.* used genetically engineered HEK293 cells, which may have different properties than the pyramidal cells that we study. Third, even if we know the exact calcium dependence of I_{TRPC} the problem remains that we do not know what the absolute levels of calcium should be in our model cell. The reader should note that we had to modify the L-type VGCC in the Poirazi model to even get a significant increase in calcium, so this part of our model can be considered phenomenological as well.

To better represent the dynamics of mGluR1/5 activation, we could in NEURON implement the 9-state kinetic scheme suggested by Marcaggi *et al.* (2009) (for mGluR1), and compare it to the current model. It is however important to note that the computations would then be significantly slowed down since such a detailed model is computationally demanding, and that the exact activation mechanism of I_{TRPC} is still not clear. In relation to this, we could also attempt to evaluate effects downstream of mGluR1/5 activation other than I_{TRPC} (see section 2.2), although this is challenged by the lack of data. Additionally, presy-

naptic effects could be added, such as a model of presynaptic calcium levels or presynaptically located metabotropic glutamate receptors, in order to get a more complete understanding of how I_{TRPC} contributes to synaptic integration.

As already pointed out, there is a lack of data regarding the exact mechanisms of I_{TRPC} activation. It is known that the PLC pathway is involved, but details remain elusive. However, experimental laboratories such as those of Séguéla, Clapham and Andrade focus on investigating these mechanisms. Our model could be updated when new data becomes available.

The current cell model is rather complex, with its detailed morphology and numerous ion channels. It would be insightful to evaluate a reduced model, by decreasing the number of compartments and ion channels. An advantage would be that more sophisticated techniques for analyzing dynamical systems, such as phase plane analysis, could be used to understand more about the phenomena underlying our results. By reducing the morphological complexity one could study the importance of spatial effects, and by systematically removing certain ion channels one could study the influence of these. Finally, it would be relevant to evaluate how our mGluR-activated I_{TRPC} model functions in the recently published refined CA1 model from the Poirazi group (Pissadaki *et al.*, 2010).

5.1.2 The role of I_{TRPC} in synaptic integration

The sensitivity analysis of Paper I was done in order to investigate the influence of parameters thought to be potentially important for the results presented there. One could also attempt to do a more rigorous sensitivity analysis by varying a larger number of parameters, individually or in combinations (Saltelli, 2004).

Furthermore, it would be interesting to use the model for investigating the role of I_{TRPC} on spatial summation of synaptic inputs, especially considering that this synaptically evoked current is mediated by channels located both peri- and extrasynaptically, in contrast to the ionotropic synaptic currents which are mediated by channels limited to the synaptic cleft. I_{TRPC} may thereby contribute differently to spatial summation than AMPA and NMDA currents, though the effect is hard to predict. It could also be noted that mGluR1/5 receptors are activated by extrasynaptic ('spillover') glutamate, and Marcaggi and Attwell (2005) showed that mGluR1-mediated EPSCs (excitatory postsynaptic currents) in Purkinje cells (probably mediated by TRPC3, see Kim *et al.*, 2003) may be strongly activated when synapses located close to each other are activated. They found a synergistic effect on this EPSC when activating adjacent synapses. In line with the evidence for compartmentalization of the dendritic tree (see section 2.4), this suggests that the spatial patterns of synaptic input plays a role in neuronal processing. One may speculate that I_{TRPC} has an important contribution to these phenomena, and computational modeling serves as a well-suited tool for exploring them.

I mentioned in section 4.1 that synaptic stimulation and current injection experiments yield different results regarding the decay time of I_{TRPC}, and suggested possible explanations to this. One could investigate this in further detail

by using the model to test ideas about the discrepancy in results from the two different experimental techniques. So far I have only tested the synaptic stimulation protocol, but a current injection protocol would be straightforward to implement by applying a current-clamp at the soma. Results from these two protocols could be compared, particularly to see if the effects on dendritic calcium concentration dynamics differ.

5.1.3 Mechanisms contributing to slow V_m decay

In section 4.1 I mentioned that subthreshold long-lasting depolarizations typically decay in experimental recordings, while in our simulations it remains stable. It should in this context be noted that I_{TRPC} does not undergo inactivation, so a non-decaying depolarization is not unthinkable. Indeed, suprathreshold forms of the calcium-TRP plateau potential can last for minutes (Egorov *et al.*, 2002). Nevertheless, in future studies the model could be used to test various hypotheses on mechanisms underlying the decay, such as activation of K_V, K_{Ca} or K_{Na} channels or inactivation of Na or Ca channels.

5.2 C-fiber model

5.2.1 Model improvements

Several aspects of the C-fiber model could be improved. The $Na_V1.9$ channel in our current model is essentially without influence on the neural dynamics, since $\bar{g}_{Na_V1.9}$ is very small. This is in contrast to experimental reports showing that $Na_V1.9$ plays an important physiological role, especially as a threshold current and in inflammatory pain (see Dib-Hajj *et al.*, 2010 and references therein). In future studies the model could be used to specifically study the role of $Na_V1.9$. The first step could be to introduce a tuning criterion that forces $\bar{g}_{Na_V1.9}$ to increase relative to other conductances. I predict that, since $I_{Na_V1.9}$ increases with activity (as shown in figure 4.14), it will counteract the activity-dependent hyperpolarization.

We could also add layers of complexity such as:

- Calcium dynamics, including voltage-gated calcium channels and calcium-activated potassium channels, which could be important for simulating high-frequency pulse trains.
- Temperature-dependent conductance values (i. e. not only temperature-dependent rate constants). We could also let the Na^+ channels have temperature-dependent shifts in their voltage-activation curves (Zimmermann *et al.*, 2007).
- Na-K-pump voltage dependence, as discussed in section 3.3.
- Components of the neuron other than the branch and parent axons (i. e. the gray areas of figure 2.5: peripheral terminal, DRG soma, central part of the axon).

5.2.2 Additional questions to address

Choi and Waxman (2011) recently used computational modeling to study how $Na_V1.7$ and $Na_V1.8$ differentially contribute to membrane excitability, in a DRG soma. Our model could be used for a similar study, and since our axon model has a spatial extent we could additionally introduce density gradients. We could for instance test the functional impact of the experimentally observed difference in $\bar{g}_{Na_V1.7}/\bar{g}_{Na_V1.8}$ ratios in peripheral versus central parts of the axon (Pinto *et al.*, 2008).

Furthermore, the model could be used to test phenomena linked more closely with neuropathic pain, such as spontaneous C-fiber activity (Schmidt *et al.*, 2012; Serra *et al.*, 2012) and $Na_V1.7$ mutations (Sheets *et al.*, 2007; Dib-Hajj *et al.*, 2010; Lampert *et al.*, 2010; Gurkiewicz *et al.*, 2011), and perhaps the model could also be integrated into spinal network models.

We could further use the approach of constructing a large population of models, each with a unique conductance, instead of just having a single model that captures the mean behavior (Marder and Taylor, 2011). This would potentially capture interesting variability in microneurography data.

List of Figures

Abbreviations

ACh	acetylcholine
ADH	activity-dependent hyperpolarization
ADP	afterdepolarization
ADS	activity-dependent slowing
AHP	afterhyperpolarization
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
AP	action potential
CAN	calcium-activated non-specific
CM	mechano-sensitive C-type nociceptor
CMi	mechano-insensitive C-type nociceptor
CNS	central nervous system
CV	conduction velocity
DAG	diacylglycerol
DBS	deep brain stimulation
DRG	dorsal root ganglion
EC	entorhinal cortex
EPSP	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
IP_3	inositol trisphosphate
ISD	inter-stimulus delay
K_A	A-type potassium

K_M	M-type potassium
K_V	voltage-gated potassium
K_{dr}	delayed-rectifier potassium
K_{Na}	sodium-activated potassium
mGluR	metabotropic glutamate receptor
Na_V	voltage-gated sodium
Na-K-ATPase	sodium potassium pump
NMDA	N-methyl-D-aspartic acid
PIP_2	phosphatidylinositol bisphosphate
PLC	phospholipase-C
PNS	peripheral nervous system
PPP	pair-pulse protocol
RC	recovery-cycle
sADP	slow afterdepolarization
SN	supernormality
SUBTLE	SUBThreshold stable and Long-lasting synaptically Evoked dendritic depolarization
TRP	transient receptor potential
TRPC	classical or canonical TRP
V_m	membrane potential
V_{rest}	resting membrane potential
VDA	voltage-dependent activation
VGCC	voltage-gated calcium channel
VIA	voltage-independent activation

Bibliography

Abramowitz, J. and Birnbaumer, L., Physiology and pathophysiology of canonical transient receptor potential channels., *The FASEB journal*, 23(2):297–328, **2009**.

Agmon-Snir, H., Carr, C. E. and Rinzel, J., The role of dendrites in auditory coincidence detection, *Nature*, 393(6682):268–272, **1998**.

Al-Yahya, E., Hamel, E., Kennedy, T., Alonso, A. A. and Egorov, A., Persitent activity in entorhinal cortex neurons induced by muscarinic and metabotropic glutamate receptor activation and its dependence on trp channels., in *Soc. Neurosci. Abstr.*, 377.5., **2003**.

Ambudkar, I. S., Ca2+ signaling microdomains:platforms for the assembly and regulation of TRPC channels., *Trends in pharmacological sciences*, 27(1):25–32, **2006**.

Amir, R. and Devor, M., Electrical excitability of the soma of sensory neurons is required for spike invasion of the soma, but not for through-conduction., *Biophysical journal*, 84(4):2181–2191, **2003**.

Antic, S. D., Zhou, W.-L. L., Moore, A. R., Short, S. M. and Ikonomu, K. D., The decade of the dendritic NMDA spike., *Journal of neuroscience research*, 88(14):2991–3001, **2010**.

Anwyl, R., Metabotropic glutamate receptors: electrophysiological properties and role in plasticity., *Brain research. Brain research reviews*, 29(1):83–120, **1999**.

Baker, M. D., Protein kinase C mediates up-regulation of tetrodotoxin-resistant, persistent Na+ current in rat and mouse sensory neurones., *The Journal of physiology*, 567(Pt 3):851–867, **2005**.

Balschun, D., Zuschratter, W. and Wetzel, W., Allosteric enhancement of metabotropic glutamate receptor 5 function promotes spatial memory., *Neuroscience*, 142(3):691–702, **2006**.

Baron, R., Mechanisms of disease: neuropathic pain–a clinical perspective., *Nature clinical practice. Neurology*, 2(2):95–106, **2006**.

Batchelor, A. M. and Garthwaite, J., Frequency detection and temporally dispersed synaptic signal association through a metabotropic glutamate

receptor pathway., *Nature*, 385(6611):74–77, **1997**.

Benarroch, E. E., Metabotropic glutamate receptors: synaptic modulators and therapeutic targets for neurologic disease., *Neurology*, 70(12):964–968, **2008**.

Bengtson, C. P., Tozzi, A., Bernardi, G. and Mercuri, N. B., Transient receptor potential-like channels mediate metabotropic glutamate receptor EPSCs in rat dopamine neurones., *The Journal of physiology*, 555(Pt 2):323–330, **2004**.

Berridge, M. J., Bootman, M. D. and Roderick, H. L., Calcium signalling: dynamics, homeostasis and remodelling, *Nature Reviews Molecular Cell Biology*, 4(7):517–529, **2003**.

Bhattacharjee, A., von Hehn, C. A., Mei, X. and Kaczmarek, L. K., Localization of the Na+-activated K+ channel Slick in the rat central nervous system., *The Journal of comparative neurology*, 484(1):80–92, **2005**.

Bhattacharjee, A. and Kaczmarek, L. K., For K+ channels, Na+ is the new Ca2+., *Trends in neurosciences*, 28(8):422–428, **2005**.

Bird, C. M. and Burgess, N., The hippocampus and memory: insights from spatial processing, *Nat Rev Neurosci*, 9(3):182–194, **2008**.

Birnbaumer, L., The TRPC class of ion channels: a critical review of their roles in slow, sustained increases in intracellular Ca(2+) concentrations., *Annual review of pharmacology and toxicology*, 49:395–426, **2009**.

Bischoff, U., Vogel, W. and Safronov, B. V., Na+-activated K+ channels in small dorsal root ganglion neurones of rat., *The Journal of physiology*, 510 (Pt 3):743–754, **1998**.

Blair, N. T. and Bean, B. P., Roles of tetrodotoxin (TTX)-sensitive Na+ current, TTX-resistant Na+ current, and Ca2+ current in the action potentials of nociceptive sensory neurons., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(23):10277–10290, **2002**.

Blair, N. T., Kaczmarek, J. S. and Clapham, D. E., Intracellular calcium strongly potentiates agonist-activated TRPC5 channels., *The Journal of general physiology*, 133(5):525–546, **2009**.

Bliss, T. V. and Rosenberg, M. E., Activity-dependent changes in conduction velocity in the olfactory nerve of the tortoise., *Pflügers Archiv : European journal of physiology*, 381(3):209–216, **1979**.

Borst, J. G., The low synaptic release probability in vivo., *Trends in neurosciences*, 33(6):259–266, **2010**.

Bostock, H., Campero, M., Serra, J. and Ochoa, J., Velocity recovery cycles of C fibres innervating human skin., *The Journal of physiology*, 553(Pt 2):649–663, **2003**.

Bostock, H., Campero, M., Serra, J. and Ochoa, J. L., Temperature-dependent double spikes in C-nociceptors of neuropathic pain patients., *Brain*

: a journal of neurology, 128(Pt 9):2154–2163, **2005**.

Brady, S., Colman, D. R. and Brophy, P., *Subcellular Organization of the Nervous System: Organelles and Their Functions*, Elsevier, **2009**, ISBN 9780080920832.

Bucher, D. and Goaillard, J.-M. M., Beyond faithful conduction: short-term dynamics, neuromodulation, and long-term regulation of spike propagation in the axon., *Progress in neurobiology*, 94(4):307–346, **2011**.

Budelli, G., Hage, T. A., Wei, A., Rojas, P., Jong, Y.-J. I. J., O'Malley, K. and Salkoff, L., Na+-activated K+ channels express a large delayed outward current in neurons during normal physiology., *Nature neuroscience*, 12(6):745–750, **2009**.

Buzsáki, G., Large-scale recording of neuronal ensembles., *Nature neuroscience*, 7(5):446–451, **2004**.

Caeser, M., Brown, D. A., Gähwiler, B. H. and Knöpfel, T., Characterization of a calcium-dependent current generating a slow afterdepolarization of CA3 pyramidal cells in rat hippocampal slice cultures., *The European journal of neuroscience*, 5(6):560–569, **1993**.

Camerino, D. C. C., Desaphy, J.-F. F., Tricarico, D., Pierno, S. and Liantonio, A., Therapeutic approaches to ion channel diseases., *Advances in genetics*, 64:81–145, **2008**.

Cash, S. and Yuste, R., Linear summation of excitatory inputs by CA1 pyramidal neurons., *Neuron*, 22(2):383–394, **1999**.

Catterall, W. A., Voltage-gated sodium channels at 60: structure, function and pathophysiology., *The Journal of physiology*, 590(Pt 11):2577–2589, **2012**.

Catterall, W. A., Perez-Reyes, E., Snutch, T. P. and Striessnig, J., International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels., *Pharmacological reviews*, 57(4):411–425, **2005**.

Chapman, J. B., Johnson, E. A. and Kootsey, J. M., Electrical and biochemical properties of an enzyme model of the sodium pump., *The Journal of membrane biology*, 74(2):139–153, **1983**.

Charpak, S. and Gähwiler, B. H., Glutamate mediates a slow synaptic response in hippocampal slice cultures., *Proceedings of the Royal Society*, 243(1308):221–226, **1991**.

Choi, J.-S. S. and Waxman, S. G., Physiological interactions between Na(v)1.7 and Na(v)1.8 sodium channels: a computer simulation study., *Journal of neurophysiology*, 106(6):3173–3184, **2011**.

Chung, M.-K. K., Jung, S. J. J. and Oh, S. B. B., Role of TRP channels in pain sensation., *Advances in experimental medicine and biology*, 704:615–636, **2011**.

Clapham, D. E., Calcium signaling., *Cell*, 131(6):1047–1058, **2007**.

Clapham, D. E., Runnels, L. W. and Strübing, C., The TRP ion channel family., *Nature reviews. Neuroscience*, 2(6):387–396, **2001**.

Clem, R. L., Celikel, T. and Barth, A. L., Ongoing in vivo experience triggers synaptic metaplasticity in the neocortex., *Science*, 319(5859):101–104, **2008**.

Congar, P., Leinekugel, X., Ben-Ari, Y. and Crépel, V., A long-lasting calcium-activated nonselective cationic current is generated by synaptic stimulation or exogenous activation of group I metabotropic glutamate receptors in CA1 pyramidal neurons., *The Journal of neuroscience*, 17(14):5366–5379, **1997**.

Cosens, D. J. and Manning, A., Abnormal electroretinogram from a Drosophila mutant., *Nature*, 224(5216):285–287, **1969**.

Costigan, M., Scholz, J. and Woolf, C. J., Neuropathic pain: a maladaptive response of the nervous system to damage., *Annual review of neuroscience*, 32:1–32, **2009**.

Cox, J. J., Reimann, F., Nicholas, A. K., Thornton, G., Roberts, E., Springell, K., Karbani, G., Jafri, H., Mannan, J., Raashid, Y., Al-Gazali, L., Hamamy, H., Valente, E. M. M., Gorman, S., Williams, R., McHale, D. P., Wood, J. N., Gribble, F. M. and Woods, C. G., An SCN9A channelopathy causes congenital inability to experience pain., *Nature*, 444(7121):894–898, **2006**.

Crowder, E. A., Saha, M. S., Pace, R. W., Zhang, H., Prestwich, G. D. and Del Negro, C. A., Phosphatidylinositol 4,5-bisphosphate regulates inspiratory burst activity in the neonatal mouse preBötzinger complex., *The Journal of physiology*, 582(Pt 3):1047–1058, **2007**.

Cummins, T. R., Sheets, P. L. and Waxman, S. G., The roles of sodium channels in nociception: Implications for mechanisms of pain., *Pain*, 131(3):243–257, **2007**.

Damann, N., Voets, T. and Nilius, B., TRPs in our senses., *Current biology : CB*, 18(18), **2008**.

De Col, R., Messlinger, K. and Carr, R. W., Conduction velocity is regulated by sodium channel inactivation in unmyelinated axons innervating the rat cranial meninges., *The Journal of physiology*, 586(4):1089–1103, **2008**.

De Col, R., Messlinger, K. and Carr, R. W., Repetitive activity slows axonal conduction velocity and concomitantly increases mechanical activation threshold in single axons of the rat cranial dura., *The Journal of physiology*, 590(Pt 4):725–736, **2012**.

De Fusco, M., Marconi, R., Silvestri, L., Atorino, L., Rampoldi, L., Morgante, L., Ballabio, A., Aridon, P. and Casari, G., Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2., *Nature genetics*, 33(2):192–196,

2003.

Desai, B. N. and Clapham, D. E., TRP channels and mice deficient in TRP channels., *Pflügers Archiv : European journal of physiology*, 451(1):11–18, **2005**.

Destexhe, A., Contreras, D., Sejnowski, T. J. and Steriade, M., A model of spindle rhythmicity in the isolated thalamic reticular nucleus., *Journal of neurophysiology*, 72(2):803–818, **1994**a.

Destexhe, A., Mainen, Z. F. and Sejnowski, T. J., Synthesis of models for excitable membranes, synaptic transmission and neuromodulation using a common kinetic formalism., *Journal of computational neuroscience*, 1(3):195–230, **1994**b.

Destexhe, A. and Sejnowski, T. J., G protein activation kinetics and spillover of gamma-aminobutyric acid may account for differences between inhibitory responses in the hippocampus and thalamus., *Proceedings of the National Academy of Sciences of the United States of America*, 92(21):9515–9519, **1995**.

Dib-Hajj, S. D., Binshtok, A. M., Cummins, T. R., Jarvis, M. F., Samad, T. and Zimmermann, K., Voltage-gated sodium channels in pain states: role in pathophysiology and targets for treatment., *Brain research reviews*, 60(1):65–83, **2009**a.

Dib-Hajj, S. D., Black, J. A. and Waxman, S. G., Voltage-gated sodium channels: therapeutic targets for pain., *Pain medicine (Malden, Mass.)*, 10(7):1260–1269, **2009**b.

Dib-Hajj, S. D., Cummins, T. R., Black, J. A. and Waxman, S. G., From genes to pain: Na v 1.7 and human pain disorders., *Trends in neurosciences*, 30(11):555–563, **2007**.

Dib-Hajj, S. D., Cummins, T. R., Black, J. A. and Waxman, S. G., Sodium channels in normal and pathological pain., *Annual review of neuroscience*, 33:325–347, **2010**.

Dobretsov, M. and Stimers, J. R., Neuronal function and alpha3 isoform of the Na/K-ATPase., *Frontiers in bioscience : a journal and virtual library*, 10:2373–2396, **2005**.

Dubin, A. E. and Patapoutian, A., Nociceptors: the sensors of the pain pathway., *The Journal of clinical investigation*, 120(11):3760–3772, **2010**.

Dzubay, J. A. and Jahr, C. E., The concentration of synaptically released glutamate outside of the climbing fiber-Purkinje cell synaptic cleft., *The Journal of Neuroscience*, 19(13):5265–5274, **1999**.

Eder, P., Poteser, M., Romanin, C. and Groschner, K., Na(+) entry and modulation of Na(+)/Ca(2+) exchange as a key mechanism of TRPC signaling., *Pflügers Archiv : European journal of physiology*, 451(1):99–104, **2005**.

Egorov, A. V., Hamam, B. N., Fransén, E., Hasselmo, M. E. and Alonso, A. A., Graded persistent activity in entorhinal cortex neurons., *Nature*, 420(6912):173–178, **2002**.

El-Hassar, L., Hagenston, A. M., Bertetto, L. and Yeckel, M. F., Metabotropic glutamate receptors regulate hippocampal CA1 pyramidal neuron excitability via Ca2+ wave-dependent activation of SK and TRPC channels., *The Journal of physiology*, 589(Pt 13):3211–3229, **2011**.

Emery, E. C., Young, G. T. and McNaughton, P. A., HCN2 ion channels: an emerging role as the pacemakers of pain., *Trends in pharmacological sciences*, **2012**.

Endres, W., Grafe, P., Bostock, H. and ten Bruggencate, G., Changes in extracellular pH during electrical stimulation of isolated rat vagus nerve., *Neuroscience letters*, 64(2):201–205, **1986**.

Enyedi, P. and Czirják, G., Molecular background of leak K+ currents: two-pore domain potassium channels., *Physiological reviews*, 90(2):559–605, **2010**.

Esclassan, F., Coutureau, E., Di Scala, G. and Marchand, A. R., A cholinergic-dependent role for the entorhinal cortex in trace fear conditioning., *The Journal of Neuroscience*, 29(25):8087–8093, **2009**.

Faber, E. S., Sedlak, P., Vidovic, M. and Sah, P., Synaptic activation of transient receptor potential channels by metabotropic glutamate receptors in the lateral amygdala., *Neuroscience*, 137(3):781–794, **2006**.

Fairbanks, C. A., Kitto, K. F., Nguyen, H. O., Stone, L. S. and Wilcox, G. L., Clonidine and dexmedetomidine produce antinociceptive synergy in mouse spinal cord., *Anesthesiology*, 110(3):638–647, **2009**.

Fang, X., McMullan, S., Lawson, S. N. and Djouhri, L., Electrophysiological differences between nociceptive and non-nociceptive dorsal root ganglion neurones in the rat in vivo., *The Journal of physiology*, 565(Pt 3):927–943, **2005**.

Featherstone, D. E. and Shippy, S. A., Regulation of synaptic transmission by ambient extracellular glutamate., *Neuroscientist*, 14(2):171–181, **2008**.

Ferraguti, F., Crepaldi, L. and Nicoletti, F., Metabotropic glutamate 1 receptor: current concepts and perspectives., *Pharmacological reviews*, 60(4):536–581, **2008**.

Fertleman, C. R., Baker, M. D., Parker, K. A., Moffatt, S., Elmslie, F. V., Abrahamsen, B., Ostman, J., Klugbauer, N., Wood, J. N., Gardiner, R. M. and Rees, M., SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes., *Neuron*, 52(5):767–774, **2006**.

Fiala, J. C., Grossberg, S. and Bullock, D., Metabotropic glutamate receptor activation in cerebellar Purkinje cells as substrate for adaptive tim-

ing of the classically conditioned eye-blink response., *The Journal of Neuroscience*, 16(11):3760–3774, **1996**.

Fotuhi, M., Standaert, D. G., Testa, C. M., Penney, J. B. and Young, A. B., Differential expression of metabotropic glutamate receptors in the hippocampus and entorhinal cortex of the rat., *Brain research. Molecular brain research*, 21(3-4):283–292, **1994**.

Fowler, M. A., Sidiropoulou, K., Ozkan, E. D., Phillips, C. W. and Cooper, D. C., Corticolimbic expression of TRPC4 and TRPC5 channels in the rodent brain., *PLoS ONE*, 2(6), **2007**.

Frank, L. M., Brown, E. N. and Wilson, M., Trajectory encoding in the hippocampus and entorhinal cortex., *Neuron*, 27(1):169–178, **2000**.

Frankemolle, A. M., Wu, J., Noecker, A. M., Voelcker-Rehage, C., Ho, J. C., Vitek, J. L., McIntyre, C. C. and Alberts, J. L., Reversing cognitive-motor impairments in Parkinson's disease patients using a computational modelling approach to deep brain stimulation programming., *Brain*, 133(Pt 3):746–761, **2010**.

Fransén, E., Alonso, A. A. and Hasselmo, M. E., Simulations of the role of the muscarinic-activated calcium-sensitive nonspecific cation current INCM in entorhinal neuronal activity during delayed matching tasks., *The Journal of Neuroscience*, 22(3):1081–1097, **2002**.

Fransén, E., Tahvildari, B., Egorov, A. V., Hasselmo, M. E. and Alonso, A. A., Mechanism of graded persistent cellular activity of entorhinal cortex layer v neurons., *Neuron*, 49(5):735–746, **2006**.

Fraser, D. D., Doll, D. and MacVicar, B. A., Serine/threonine protein phosphatases and synaptic inhibition regulate the expression of cholinergic-dependent plateau potentials., *Journal of neurophysiology*, 85(3):1197–1205, **2001**.

Fraser, D. D. and MacVicar, B. A., Cholinergic-dependent plateau potential in hippocampal CA1 pyramidal neurons., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 16(13):4113–4128, **1996**.

Frick, A. and Johnston, D., Plasticity of dendritic excitability., *Journal of neurobiology*, 64(1):100–115, **2005**.

Frick, A., Magee, J. and Johnston, D., LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites., *Nature neuroscience*, 7(2):126–135, **2004**.

Fyhn, M., Molden, S., Witter, M. P., Moser, E. I. and Moser, M.-B. B., Spatial Representation in the Entorhinal Cortex, *Science*, 305(5688):1258–1264, **2004**.

Gadsby, D. C., Takeuchi, A., Artigas, P. and Reyes, N., Review. Peering into an ATPase ion pump with single-channel recordings., *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 364(1514):229–238, **2009**.

Gee, C. E., Benquet, P. and Gerber, U., Group I metabotropic glutamate receptors activate a calcium-sensitive transient receptor potential-like conductance in rat hippocampus., *The Journal of physiology*, 546(Pt 3):655–664, **2003**.

Gladding, C. M., Fitzjohn, S. M. and Molnár, E., Metabotropic glutamate receptor-mediated long-term depression: molecular mechanisms., *Pharmacological reviews*, 61(4):395–412, **2009**.

Glitsch, H. G., Electrophysiology of the sodium-potassium-ATPase in cardiac cells., *Physiological reviews*, 81(4):1791–1826, **2001**.

Glynn, I. M., Annual review prize lecture. 'All hands to the sodium pump'., *The Journal of physiology*, 462:1–30, **1993**.

Gold, M. S. and Gebhart, G. F., Nociceptor sensitization in pain pathogenesis., *Nature medicine*, 16(11):1248–1257, **2010**.

Gracheva, E. O., Cordero-Morales, J. F., Gonzalez-Carcacia, J. A., Ingolia, N. T., Manno, C., Aranguren, C. I., Weissman, J. S. and Julius, D., Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats, *Nature*, 476(7358):88–91, **2011**.

Gracheva, E. O., Ingolia, N. T., Kelly, Y. M., Cordero-Morales, J. F., Hollopeter, G., Chesler, A. T., Sanchez, E. E., Perez, J. C., Weissman, J. S. and Julius, D., Molecular basis of infrared detection by snakes, *Nature*, 464(7291):1006–1011, **2010**.

Gregory, M. L. and Szumlinski, K. K., Impoverished rearing impairs working memory and metabotropic glutamate receptor 5 expression., *Neuroreport*, 19(2):239–243, **2008**.

Gross, S. A. A., Guzmán, G. A. A., Wissenbach, U., Philipp, S. E. E., Zhu, M. X. X., Bruns, D. and Cavalié, A., TRPC5 is a Ca2+-activated channel functionally coupled to Ca2+-selective ion channels., *The Journal of biological chemistry*, 284(49):34423–34432, **2009**.

Gulledge, A. T., Kampa, B. M. and Stuart, G. J., Synaptic integration in dendritic trees., *Journal of neurobiology*, 64(1):75–90, **2005**.

Gurkiewicz, M., Korngreen, A., Waxman, S. G. and Lampert, A., Kinetic modeling of Nav1.7 provides insight into erythromelalgia-associated F1449V mutation., *Journal of neurophysiology*, 105(4):1546–1557, **2011**.

von Bohlen und Halbach, O., Hinz, U., Unsicker, K. and Egorov, A. V., Distribution of TRPC1 and TRPC5 in medial temporal lobe structures of mice., *Cell and tissue research*, 322(2):201–206, **2005**.

Hallen, K., Huss, M., Kettunen, P., Elmanira, A. and Hellgrenkotaleski, J., mGluR-Mediated calcium oscillations in the lamprey: a computational model, *Neurocomputing*, 58-60:431–435, **2004**.

Hamada, K., Matsuura, H., Sanada, M., Toyoda, F., Omatsu-Kanbe, M., Kashiwagi, A. and Yasuda, H., Properties of the Na+/K+ pump

current in small neurons from adult rat dorsal root ganglia., *British journal of pharmacology*, 138(8):1517–1527, **2003**.

Handwerker, H. O., Classification of nociceptors - To what purpose?, *Pain*, 148(3):355–356, **2010**.

Hardie, R. C. and Minke, B., The trp gene is essential for a light-activated Ca2+ channel in Drosophila photoreceptors., *Neuron*, 8(4):643–651, **1992**.

Hargus, N. J. J. and Patel, M. K. K., Voltage-gated Na+ channels in neuropathic pain., *Expert opinion on investigational drugs*, 16(5):635–646, **2007**.

Harper, A. A. and Lawson, S. N., Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurones., *The Journal of physiology*, 359:31–46, **1985**.

Hartmann, J., Dragicevic, E., Adelsberger, H., Henning, H. A., Sumser, M., Abramowitz, J., Blum, R., Dietrich, A., Freichel, M., Flockerzi, V., Birnbaumer, L. and Konnerth, A., TRPC3 channels are required for synaptic transmission and motor coordination., *Neuron*, 59(3):392–398, **2008**.

Hartmann, J. and Konnerth, A., Mechanisms of metabotropic glutamate receptor-mediated synaptic signaling in cerebellar Purkinje cells., *Acta physiologica*, 195(1):79–90, **2008**.

Harvey, C. D., Collman, F., Dombeck, D. A. and Tank, D. W., Intracellular dynamics of hippocampal place cells during virtual navigation, *Nature*, 461(7266):941–946, **2009**.

Hasselmo, M. E., The role of acetylcholine in learning and memory., *Current opinion in neurobiology*, 16(6):710–715, **2006**.

Hasselmo, M. E. and Bower, J. M., Acetylcholine and memory., *Trends in neurosciences*, 16(6):218–222, **1993**.

Hasselmo, M. E. and Giocomo, L. M., Cholinergic modulation of cortical function., *Journal of molecular neuroscience : MN*, 30(1-2):133–135, **2006**.

Hasselmo, M. E. and Stern, C. E., Mechanisms underlying working memory for novel information., *Trends in cognitive sciences*, 10(11):487–493, **2006**.

Häusser, M. and Mel, B., Dendrites: bug or feature?, *Current opinion in neurobiology*, 13(3):372–383, **2003**.

Hayashi, K., Yoshihara, T. and Ichitani, Y., Involvement of hippocampal metabotropic glutamate receptors in radial maze performance., *Neuroreport*, 18(7):719–723, **2007**.

Herz, A. V., Gollisch, T., Machens, C. K. and Jaeger, D., Modeling Single-Neuron Dynamics and Computations: A Balance of Detail and Abstraction, *Science*, 314(5796):80–85, **2006**.

Herzog, R. I., Cummins, T. R. and Waxman, S. G., Persistent TTX-resistant Na+ current affects resting potential and response to depolarization

in simulated spinal sensory neurons., *Journal of neurophysiology*, 86(3):1351–1364, **2001**.

Heyward, P., Ennis, M., Keller, A. and Shipley, M. T., Membrane bistability in olfactory bulb mitral cells., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 21(14):5311–5320, **2001**.

Hines, M. L. and Carnevale, N. T., NEURON: a tool for neuroscientists., *The Neuroscientist*, 7(2):123–135, **2001**.

Hines, M. L., Davison, A. P. and Muller, E., NEURON and Python., *Frontiers in neuroinformatics*, 3, **2009**.

Hires, S. A. A., Zhu, Y. and Tsien, R. Y., Optical measurement of synaptic glutamate spillover and reuptake by linker optimized glutamate-sensitive fluorescent reporters., *Proceedings of the National Academy of Sciences of the United States of America*, 105(11):4411–4416, **2008**.

Hodgkin, A. L. and Huxley, A. F., A quantitative description of membrane current and its application to conduction and excitation in nerve, *The Journal of Physiology*, 117(4):500–544, **1952**.

Hogan, Q. H., Labat lecture: the primary sensory neuron: where it is, what it does, and why it matters., *Regional anesthesia and pain medicine*, 35(3):306–311, **2010**.

Hounsgaard, J. and Kiehn, O., Calcium spikes and calcium plateaux evoked by differential polarization in dendrites of turtle motoneurones in vitro., *The Journal of physiology*, 468:245–259, **1993**.

Hughes, S. W., Cope, D. W., Tóth, T. I., Williams, S. R. and Crunelli, V., All thalamocortical neurones possess a T-type Ca2+ 'window' current that enables the expression of bistability-mediated activities., *The Journal of physiology*, 517 (Pt 3):805–815, **1999**.

Huss, M., Lansner, A., Wallén, P., El Manira, A., Grillner, S. and Kotaleski, J. H., Roles of ionic currents in lamprey CpG neurons: a modeling study., *Journal of neurophysiology*, 97(4):2696–2711, **2007**.

Jacobs, J., Kahana, M. J., Ekstrom, A. D., Mollison, M. V. and Fried, I., A sense of direction in human entorhinal cortex., *Proceedings of the National Academy of Sciences of the United States of America*, 107(14):6487–6492, **2010**.

Jaeger, D. and Bower, J. M., Synaptic control of spiking in cerebellar Purkinje cells: dynamic current clamp based on model conductances., *The Journal of Neuroscience*, 19(14):6090–6101, **1999**.

Jaeger, D., De Schutter, E. and Bower, J. M., The role of synaptic and voltage-gated currents in the control of Purkinje cell spiking: a modeling study., *The Journal of Neuroscience*, 17(1):91–106, **1997**.

Jia, H., Rochefort, N. L., Chen, X. and Konnerth, A., Dendritic organization of sensory input to cortical neurons in vivo, *Nature*, 464(7293):1307–

1312, **2010**.

Johnston, D. and Narayanan, R., Active dendrites: colorful wings of the mysterious butterflies., *Trends in neurosciences*, 31(6):309–316, **2008**.

Johnston, D. and Wu, S. M., *Foundations of cellular neurophysiology*, MIT Press, **1995**, ISBN 0262100533.

Kanki, H., Kinoshita, M., Akaike, A., Satoh, M., Mori, Y. and Kaneko, S., Activation of inositol 1,4,5-trisphosphate receptor is essential for the opening of mouse TRP5 channels, *Molecular Pharmacology*, 60(5):989–998, **2001**.

Kath, W. L., Computational modeling of dendrites., *Journal of neurobiology*, 64(1):91–99, **2005**.

Katz, Y., Menon, V., Nicholson, D. A., Geinisman, Y., Kath, W. L. and Spruston, N., Synapse Distribution Suggests a Two-Stage Model of Dendritic Integration in CA1 Pyramidal Neurons, *Neuron*, 63(2):171–177, **2009**.

Kelly, T. and Rose, C. R., Sodium Signals and Their Significance for Axonal Function New Aspects of Axonal Structure and Function, in Feldmeyer, D. and Lübke, J. H. R. (editors), *New Aspects of Axonal Structure and Function*, chapter 3, pp. 35–53, Springer US, Boston, MA, **2010**, ISBN 978-1-4419-1675-4.

Kim, S. J. J., Kim, Y. S. S., Yuan, J. P., Petralia, R. S., Worley, P. F. and Linden, D. J., Activation of the TRPC1 cation channel by metabotropic glutamate receptor mGluR1., *Nature*, 426(6964):285–291, **2003**.

Knöpfel, T. and Uusisaari, M., Modulation of excitation by metabotropic glutamate receptors., *Results and problems in cell differentiation*, 44:163–175, **2008**.

Kouranova, E. V., Strassle, B. W., Ring, R. H., Bowlby, M. R. and Vasilyev, D. V., Hyperpolarization-activated cyclic nucleotide-gated channel mRNA and protein expression in large versus small diameter dorsal root ganglion neurons: correlation with hyperpolarization-activated current gating., *Neuroscience*, 153(4):1008–1019, **2008**.

Kovalchuk, Y., Eilers, J., Lisman, J. and Konnerth, A., NMDA receptor-mediated subthreshold Ca(2+) signals in spines of hippocampal neurons., *The Journal of neuroscience*, 20(5):1791–1799, **2000**.

Krishnan, A. V., Lin, C. S.-Y. S., Park, S. B. and Kiernan, M. C., Axonal ion channels from bench to bedside: a translational neuroscience perspective., *Progress in neurobiology*, 89(3):288–313, **2009**.

Kullmann, D. M., Neurological channelopathies., *Annual review of neuroscience*, 33:151–172, **2010**.

Lai, J., Hunter, J. C. and Porreca, F., The role of voltage-gated sodium channels in neuropathic pain., *Current opinion in neurobiology*, 13(3):291–

297, **2003**.

Lampert, A., O'Reilly, A. O., Reeh, P. and Leffler, A., Sodium channelopathies and pain., *Pflügers Archiv : European journal of physiology*, 460(2):249–263, **2010**.

Lansner, A., Associative memory models: from the cell-assembly theory to biophysically detailed cortex simulations., *Trends in neurosciences*, 32(3):178–186, **2009**.

Larkum, M. E. and Nevian, T., Synaptic clustering by dendritic signalling mechanisms., *Current opinion in neurobiology*, 18(3):321–331, **2008**.

Larkum, M. E., Nevian, T., Sandler, M., Polsky, A. and Schiller, J., Synaptic Integration in Tuft Dendrites of Layer 5 Pyramidal Neurons: A New Unifying Principle, *Science*, 325(5941):756–760, **2009**.

Li, S., Gosling, M. and Poll, C., Determining the functional role of TRPC channels in primary cells., *Pflügers Archiv : European journal of physiology*, 451(1):43–52, **2005**.

Lipscombe, D., Helton, T. D. and Xu, W., L-Type Calcium Channels: The Low Down, *Journal of Neurophysiology*, 92(5):2633–2641, **2004**.

Loewenstein, Y., Mahon, S., Chadderton, P., Kitamura, K., Sompolinsky, H., Yarom, Y. and Häusser, M., Bistability of cerebellar Purkinje cells modulated by sensory stimulation., *Nature neuroscience*, 8(2):202–211, **2005**.

London, M. and Häusser, M., Dendritic computation, *Annual Review of Neuroscience*, 28(1):503–532, **2005**.

Losonczy, A. and Magee, J. C., Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons., *Neuron*, 50(2):291–307, **2006**.

Losonczy, A., Makara, J. K. and Magee, J. C., Compartmentalized dendritic plasticity and input feature storage in neurons, *Nature*, 452(7186):436–441, **2008**.

Lu, B., Su, Y., Das, S., Liu, J., Xia, J. and Ren, D., The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm., *Cell*, 129(2):371–383, **2007**.

Lujan, R., Nusser, Z., Roberts, J. D., Shigemoto, R. and Somogyi, P., Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus., *The European journal of neuroscience*, 8(7):1488–1500, **1996**.

Lütcke, H., Murayama, M., Hahn, T., Margolis, D. J., Astori, S., Zum Alten Borgloh, S. M. M., Göbel, W., Yang, Y., Tang, W., Kügler, S., Sprengel, R., Nagai, T., Miyawaki, A., Larkum, M. E., Helmchen, F. and Hasan, M. T., Optical recording of neuronal activity with a genetically-encoded calcium indicator in anesthetized and freely

moving mice., *Frontiers in neural circuits*, 4, **2010**.

Magee, J. C., Dendritic integration of excitatory synaptic input, *Nature Reviews Neuroscience*, 1(3):181–190, **2000**.

Magee, J. C. and Johnston, D., Plasticity of dendritic function., *Current opinion in neurobiology*, 15(3):334–342, **2005**.

Maingret, F., Coste, B., Padilla, F., Clerc, N., Crest, M., Korogod, S. M. and Delmas, P., Inflammatory mediators increase Nav1.9 current and excitability in nociceptors through a coincident detection mechanism., *The Journal of general physiology*, 131(3):211–225, **2008**.

Marcaggi, P. and Attwell, D., Endocannabinoid signaling depends on the spatial pattern of synapse activation., *Nature neuroscience*, 8(6):776–781, **2005**.

Marcaggi, P., Mutoh, H., Dimitrov, D., Beato, M. and Knöpfel, T., Optical measurement of mGluR1 conformational changes reveals fast activation, slow deactivation, and sensitization., *Proceedings of the National Academy of Sciences of the United States of America*, 106(27):11388–11393, **2009**.

Marder, E., Abbott, L. F., Turrigiano, G. G., Liu, Z. and Golowasch, J., Memory from the dynamics of intrinsic membrane currents., *Proceedings of the National Academy of Sciences of the United States of America*, 93(24):13481–13486, **1996**.

Marder, E. and Taylor, A. L., Multiple models to capture the variability in biological neurons and networks., *Nature neuroscience*, 14(2):133–138, **2011**.

Mateos, J. M., Benítez, R., Elezgarai, I., Azkue, J. J., Lázaro, E., Osorio, A., Bilbao, A., Doñate, F., Sarría, R., Conquet, F., Ferraguti, F., Kuhn, R., Knöpfel, T. and Grandes, P., Immunolocalization of the mGluR1b splice variant of the metabotropic glutamate receptor 1 at parallel fiber-Purkinje cell synapses in the rat cerebellar cortex., *Journal of neurochemistry*, 74(3):1301–1309, **2000**.

McIntyre, C. C., Miocinovic, S. and Butson, C. R., Computational analysis of deep brain stimulation., *Expert review of medical devices*, 4(5):615–622, **2007**.

Megías, M., Emri, Z., Freund, T. F. and Gulyás, A. I., Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells., *Neuroscience*, 102(3):527–540, **2001**.

Migliore, M. and Shepherd, G. M., Emerging rules for the distributions of active dendritic conductances., *Nature reviews. Neuroscience*, 3(5):362–370, **2002**.

Mikami, A., Masuoka, T., Yasuda, M., Yamamoto, Y. and Kamei, C., Participation of cholinergic system in memory deficits induced by blockade of hippocampal mGlu(1) receptors., *European journal of pharmacology*, 575(1-3):82–86, **2007**.

Minke, B., TRP channels and Ca2+ signaling., *Cell calcium*, 40(3):261–275, **2006**.

Minke, B., Wu, C. and Pak, W. L., Induction of photoreceptor voltage noise in the dark in Drosophila mutant., *Nature*, 258(5530):84–87, **1975**.

Miocinovic, S., Noecker, A. M., Maks, C. B., Butson, C. R. and McIntyre, C. C., Cicerone: stereotactic neurophysiological recording and deep brain stimulation electrode placement software system., *Acta neurochirurgica. Supplement*, 97(Pt 2):561–567, **2007**.

Miocinovic, S., Parent, M., Butson, C. R., Hahn, P. J., Russo, G. S., Vitek, J. L. and McIntyre, C. C., Computational analysis of subthalamic nucleus and lenticular fasciculus activation during therapeutic deep brain stimulation., *Journal of neurophysiology*, 96(3):1569–1580, **2006**.

Mogyoros, I., Kiernan, M. C. and Burke, D., Strength-duration properties of human peripheral nerve., *Brain : a journal of neurology*, 119 (Pt 2):439–447, **1996**.

Montell, C., The TRP superfamily of cation channels., *Science's STKE*, 2005(272), **2005**.

Montell, C., Birnbaumer, L. and Flockerzi, V., The TRP channels, a remarkably functional family., *Cell*, 108(5):595–598, **2002**.

Montell, C. and Rubin, G. M., Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction., *Neuron*, 2(4):1313–1323, **1989**.

Moran, M. M., Xu, H. and Clapham, D. E., TRP ion channels in the nervous system., *Current opinion in neurobiology*, 14(3):362–369, **2004**.

Mullins, L. J. and Brinley, F. J., Potassium fluxes in dialyzed squid axons., *The Journal of general physiology*, 53(6):704–740, **1969**.

Murayama, M. and Larkum, M. E., Enhanced dendritic activity in awake rats., *Proceedings of the National Academy of Sciences of the United States of America*, 106(48):20482–20486, **2009**.

Murayama, M., Pérez-Garci, E., Nevian, T., Bock, T., Senn, W. and Larkum, M. E., Dendritic encoding of sensory stimuli controlled by deep cortical interneurons., *Nature*, 457(7233):1137–1141, **2009**.

Naie, K. and Manahan-Vaughan, D., Regulation by Metabotropic Glutamate Receptor 5 of LTP in the Dentate Gyrus of Freely Moving Rats: Relevance for Learning and Memory Formation, *Cerebral cortex*, 14(2):189–198, **2004**.

Nilius, B., TRP channels in disease., *Biochimica et biophysica acta*, 1772(8):805–812, **2007**.

Nilius, B., Mahieu, F., Prenen, J., Janssens, A., Owsianik, G., Vennekens, R. and Voets, T., The Ca2+-activated cation channel TRPM4 is regulated by phosphatidylinositol 4,5-biphosphate., *The EMBO journal*,

25(3):467–478, **2006**.

Nilius, B., Owsianik, G., Voets, T. and Peters, J. A., Transient Receptor Potential Cation Channels in Disease, *Physiological Reviews*, 87(1):165–217, **2007**.

Niswender, C. M. and Conn, P. J., Metabotropic glutamate receptors: physiology, pharmacology, and disease., *Annual review of pharmacology and toxicology*, 50:295–322, **2010**.

Nusser, Z., Variability in the subcellular distribution of ion channels increases neuronal diversity., *Trends in neurosciences*, 32(5):267–274, **2009**.

Nuwer, M. O., Picchione, K. E. and Bhattacharjee, A., PKA-induced internalization of slack KNa channels produces dorsal root ganglion neuron hyperexcitability., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(42):14165–14172, **2010**.

Obreja, O., Hirth, M., Turnquist, B., Rukwied, R., Ringkamp, M. and Schmelz, M., The Differential Effects of Two Sodium Channel Modulators on the Conductive Properties of C-Fibers in Pig Skin In Vivo., *Anesthesia and analgesia*, **2012**.

Obreja, O., Ringkamp, M., Namer, B., Forsch, E., Klusch, A., Rukwied, R., Petersen, M. and Schmelz, M., Patterns of activity-dependent conduction velocity changes differentiate classes of unmyelinated mechano-insensitive afferents including cold nociceptors, in pig and in human., *Pain*, 148(1):59–69, **2010**.

Obreja, O., Ringkamp, M., Turnquist, B., Hirth, M., Forsch, E., Rukwied, R., Petersen, M. and Schmelz, M., Nerve growth factor selectively decreases activity-dependent conduction slowing in mechano-insensitive C-nociceptors., *Pain*, 152(9):2138–2146, **2011**.

Obukhov, A. G. and Nowycky, M. C., TRPC5 channels undergo changes in gating properties during the activation-deactivation cycle., *Journal of cellular physiology*, 216(1):162–171, **2008**.

Ochoa, J. L., Campero, M., Serra, J. and Bostock, H., Hyperexcitable polymodal and insensitive nociceptors in painful human neuropathy., *Muscle & nerve*, 32(4):459–472, **2005**.

Olive, M. F., Metabotropic glutamate receptor ligands as potential therapeutics for addiction., *Current drug abuse reviews*, 2(1):83–989, **2009**.

Ørstavik, K., Namer, B., Schmidt, R., Schmelz, M., Hilliges, M., Weidner, C., Carr, R. W., Handwerker, H., Jørum, E. and Torebjörk, H. E., Abnormal function of C-fibers in patients with diabetic neuropathy., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 26(44):11287–11294, **2006**.

Ørstavik, K., Weidner, C., Schmidt, R., Schmelz, M., Hilliges, M., Jørum, E., Handwerker, H. and Torebjörk, E., Pathological C-fibres in patients with a chronic painful condition., *Brain : a journal of neurology*,

126(Pt 3):567–578, **2003**.

Otsuguro, K.-i., Tang, J., Tang, Y., Xiao, R., Freichel, M., Tsvilovskyy, V., Ito, S., Flockerzi, V., Zhu, M. X. and Zholos, A. V., Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate, *Journal of biological chemistry*, 283(15):10026–10036, **2008**.

Otto, T. and Eichenbaum, H., Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task., *Behavioral neuroscience*, 106(5):762–775, **1992**.

Partridge, L. D. and Valenzuela, C. F., Ca2+ store-dependent potentiation of Ca2+-activated non-selective cation channels in rat hippocampal neurones in vitro., *The Journal of physiology*, 521 Pt 3:617–627, **1999**.

Pedersen, S. F. F., Owsianik, G. and Nilius, B., TRP channels: an overview., *Cell calcium*, 38(3-4):233–252, **2005**.

Pinto, V., Derkach, V. A. and Safronov, B. V., Role of TTX-sensitive and TTX-resistant sodium channels in Adelta- and C-fiber conduction and synaptic transmission., *Journal of neurophysiology*, 99(2):617–628, **2008**.

Pissadaki, E. K. K., Sidiropoulou, K., Reczko, M. and Poirazi, P., Encoding of spatio-temporal input characteristics by a CA1 pyramidal neuron model., *PLoS computational biology*, 6(12), **2010**.

Plant, T. D. and Schaefer, M., Receptor-operated cation channels formed by TRPC4 and TRPC5., *Naunyn-Schmiedeberg's archives of pharmacology*, 371(4):266–276, **2005**.

Poirazi, P., Brannon, T. and Mel, B. W., Arithmetic of subthreshold synaptic summation in a model CA1 pyramidal cell., *Neuron*, 37(6):977–987, **2003**.

Polsky, A., Mel, B. W. and Schiller, J., Computational subunits in thin dendrites of pyramidal cells., *Nature neuroscience*, 7(6):621–627, **2004**.

Purves, D., *Neuroscience*, Sinauer, 4th edition, **2008**, ISBN 9780878936977.

Raja, S. N., Meyer, R. A. and Campbell, J. N., Peripheral mechanisms of somatic pain., *Anesthesiology*, 68(4):571–590, **1988**.

Rakowski, R. F., Gadsby, D. C. and De Weer, P., Stoichiometry and voltage dependence of the sodium pump in voltage-clamped, internally dialyzed squid giant axon., *The Journal of general physiology*, 93(5):903–941, **1989**.

Rall, W., Branching dendritic trees and motoneuron membrane resistivity., *Experimental neurology*, 1:491–527, **1959**.

Rall, W. and Rinzel, J., Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model., *Biophysical journal*, 13(7):648–687, **1973**.

Ramsey, I. S., Delling, M. and Clapham, D. E., An introduction to TRP channels, *Annual Review of Physiology*, 68(1):619–647, **2006**.

Rang, H. P. and Ritchie, J. M., On the electrogenic sodium pump in mammalian non-myelinated nerve fibres and its activation by various external cations., *The Journal of physiology*, 196(1):183–221, **1968**.

Reichelt, W. and Knöpfel, T., Glutamate uptake controls expression of a slow postsynaptic current mediated by mGluRs in cerebellar Purkinje cells., *Journal of neurophysiology*, 87(4):1974–1980, **2002**.

Ren, D., Sodium leak channels in neuronal excitability and rhythmic behaviors., *Neuron*, 72(6):899–911, **2011**.

Reyes, A., Influence of dendritic conductances on the input-output properties of neurons., *Annual review of neuroscience*, 24:653–675, **2001**.

Riccio, A., Li, Y., Moon, J., Kim, K.-S. S., Smith, K. S., Rudolph, U., Gapon, S., Yao, G. L. L., Tsvetkov, E., Rodig, S. J., Van't Veer, A., Meloni, E. G., Carlezon, W. A., Bolshakov, V. Y. and Clapham, D. E., Essential role for TRPC5 in amygdala function and fear-related behavior., *Cell*, 137(4):761–772, **2009**.

Rose, C. R., Na+ signals at central synapses., *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, 8(6):532–539, **2002**.

Rudy, J. W. and Matus-Amat, P., DHPG activation of group 1 mGluRs in BLA enhances fear conditioning., *Learning & memory*, 16(7):421–425, **2009**.

Rusakov, D. A. and Kullmann, D. M., Extrasynaptic glutamate diffusion in the hippocampus: ultrastructural constraints, uptake, and receptor activation., *The Journal of neuroscience*, 18(9):3158–3170, **1998**.

Rush, A. M., Cummins, T. R. and Waxman, S. G., Multiple sodium channels and their roles in electrogenesis within dorsal root ganglion neurons., *The Journal of physiology*, 579(Pt 1):1–14, **2007**.

Safronov, B. V. and Vogel, W., Properties and functions of Na(+)-activated K+ channels in the soma of rat motoneurones., *The Journal of physiology*, 497 (Pt 3):727–734, **1996**.

Saltelli, A., *Sensitivity Analysis in Practice: A Guide to Assessing Scientific Models*, Wiley, 1 edition, **2004**, ISBN 0470870931.

Scanziani, M. and Hausser, M., Electrophysiology in the age of light, *Nature*, 461(7266):930–939, **2009**.

Schaefer, M., Homo- and heteromeric assembly of TRP channel subunits., *Pflügers Archiv : European journal of physiology*, 451(1):35–42, **2005**.

Schiller, J. and Schiller, Y., NMDA receptor-mediated dendritic spikes and coincident signal amplification., *Current opinion in neurobiology*, 11(3):343–348, **2001**.

Schmelz, M. and Schmidt, R., Microneurographic single-unit recordings to assess receptive properties of afferent human C-fibers., *Neuroscience letters*,

470(3):158–161, **2010**.

Schmelz, M., Schmidt, R., Bickel, A., Handwerker, H. O. and Torebjörk, H. E., Specific C-receptors for itch in human skin., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 17(20):8003–8008, **1997**.

Schmidt, R., Kleggetveit, I. P. P., Namer, B., Helås, T., Obreja, O., Schmelz, M. and Jørum, E., Double spikes to single electrical stimulation correlates to spontaneous activity of nociceptors in painful neuropathy patients., *Pain*, 153(2):391–398, **2012**.

Schmidt, R., Schmelz, M., Forster, C., Ringkamp, M., Torebjörk, E. and Handwerker, H., Novel classes of responsive and unresponsive C nociceptors in human skin., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 15(1 Pt 1):333–341, **1995**.

Schmidt, R., Schmelz, M., Torebjörk, H. E. and Handwerker, H. O., Mechano-insensitive nociceptors encode pain evoked by tonic pressure to human skin., *Neuroscience*, 98(4):793–800, **2000**.

Schulz, B., Fendt, M., Gasparini, F., Lingenhöhl, K., Kuhn, R. and Koch, M., The metabotropic glutamate receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) blocks fear conditioning in rats., *Neuropharmacology*, 41(1):1–7, **2001**.

Schwindt, P. C., Spain, W. J. and Crill, W. E., Long-lasting reduction of excitability by a sodium-dependent potassium current in cat neocortical neurons., *Journal of neurophysiology*, 61(2):233–244, **1989**.

Scimemi, A. and Beato, M., Determining the neurotransmitter concentration profile at active synapses., *Molecular neurobiology*, 40(3):289–306, **2009**.

Scriven, D. R., Modeling repetitive firing and bursting in a small unmyelinated nerve fiber., *Biophysical journal*, 35(3):715–730, **1981**.

Segev, I., Sound grounds for computing dendrites., *Nature*, 393(6682):207–208, **1998**.

Segev, I. and London, M., Untangling dendrites with quantitative models., *Science*, 290(5492):744–750, **2000**.

Segev, I. and Schneidman, E., Axons as computing devices: basic insights gained from models., *Journal of physiology, Paris*, 93(4):263–270, **1999**.

Selvaraj, S., Sun, Y. and Singh, B. B., TRPC channels and their implication in neurological diseases., *CNS & neurological disorders drug targets*, 9(1):94–104, **2010**.

Serra, J., Re-emerging microneurography., *The Journal of physiology*, 587(Pt 2):295–296, **2009**.

Serra, J., Bostock, H., Solà, R., Aleu, J., García, E., Cokic, B., Navarro, X. and Quiles, C., Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and

rats., *Pain*, 153(1):42–55, **2012**.

Serra, J., Campero, M., Ochoa, J. and Bostock, H., Activity-dependent slowing of conduction differentiates functional subtypes of C fibres innervating human skin., *The Journal of physiology*, 515 (Pt 3):799–811, **1999**.

Serra, J., Solà, R., Aleu, J., Quiles, C., Navarro, X. and Bostock, H., Double and triple spikes in C-nociceptors in neuropathic pain states: an additional peripheral mechanism of hyperalgesia., *Pain*, 152(2):343–353, **2011**.

Shalinsky, M. H., Magistretti, J., Ma, L. and Alonso, A. A., Muscarinic activation of a cation current and associated current noise in entorhinal-cortex layer-II neurons, *Journal of neurophysiology*, 88(3):1197–1211, **2002**.

Sheets, P. L., Jackson, J. O., Waxman, S. G., Dib-Hajj, S. D. and Cummins, T. R., A Nav1.7 channel mutation associated with hereditary erythromelalgia contributes to neuronal hyperexcitability and displays reduced lidocaine sensitivity., *The Journal of physiology*, 581(Pt 3):1019–1031, **2007**.

Shoham, S., O'Connor, D. H. and Segev, R., How silent is the brain: is there a "dark matter" problem in neuroscience?, *Journal of comparative physiology*, 192(8):777–784, **2006**.

Sidiropoulou, K., Lu, F.-M. M., Fowler, M. A., Xiao, R., Phillips, C., Ozkan, E. D., Zhu, M. X., White, F. J. and Cooper, D. C., Dopamine modulates an mGluR5-mediated depolarization underlying prefrontal persistent activity., *Nature neuroscience*, 12(2):190–199, **2009**.

Sidiropoulou, K., Pissadaki, E. K. K. and Poirazi, P., Inside the brain of a neuron., *EMBO reports*, 7(9):886–892, **2006**.

Sjöström, P. J., Rancz, E. A., Roth, A. and Häusser, M., Dendritic excitability and synaptic plasticity., *Physiological reviews*, 88(2):769–840, **2008**.

Snape, A., Pittaway, J. F. and Baker, M. D., Excitability parameters and sensitivity to anemone toxin ATX-II in rat small diameter primary sensory neurones discriminated by Griffonia simplicifolia isolectin IB4., *The Journal of physiology*, 588(Pt 1):125–137, **2010**.

Spruston, N., Pyramidal neurons: dendritic structure and synaptic integration, *Nature Reviews Neuroscience*, 9(3):206–221, **2008**.

Squire, L. R., *Encyclopedia of neuroscience*, Elsevier Academic Press, **2009**, ISBN 0080446175.

Steuber, V. and Willshaw, D., A biophysical model of synaptic delay learning and temporal pattern recognition in a cerebellar Purkinje cell., *Journal of computational neuroscience*, 17(2):149–164, **2004**.

Steuber, V., Willshaw, D. and Van Ooyen, A., Generation of time delays: simplified models of intracellular signalling in cerebellar Purkinje cells., *Network*, 17(2):173–191, **2006**.

Strübing, C., Krapivinsky, G., Krapivinsky, L. and Clapham, D. E., TRPC1 and TRPC5 form a novel cation channel in mammalian brain., *Neuron*, 29(3):645–655, **2001**.

Stuart, G., Spruston, N. and Häusser, M., *Dendrites*, Oxford University Press, **2007**, ISBN 0198566565.

Sun, W., Miao, B., Wang, X.-C. C., Duan, J.-H. H., Wang, W.-T. T., Kuang, F., Xie, R.-G. G., Xing, J.-L. L., Xu, H., Song, X.-J. J., Luo, C. and Hu, S.-J. J., Reduced conduction failure of the main axon of polymodal nociceptive C-fibres contributes to painful diabetic neuropathy in rats., *Brain : a journal of neurology*, 135(Pt 2):359–375, **2012**.

Suzuki, W. A., Miller, E. K. and Desimone, R., Object and place memory in the macaque entorhinal cortex., *Journal of neurophysiology*, 78(2):1062–1081, **1997**.

Talavera, K., Nilius, B. and Voets, T., Neuronal TRP channels: thermometers, pathfinders and life-savers., *Trends in neurosciences*, 31(6):287–295, **2008**.

Tamsett, T. J., Picchione, K. E. and Bhattacharjee, A., NAD+ activates KNa channels in dorsal root ganglion neurons., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(16):5127–5134, **2009**.

Taylor, A. L., Goaillard, J.-M. M. and Marder, E., How multiple conductances determine electrophysiological properties in a multicompartment model., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(17):5573–5586, **2009**.

Thomas, R. C., Electrogenic sodium pump in nerve and muscle cells., *Physiological reviews*, 52(3):563–594, **1972**.

Vasylyev, D. V. and Waxman, S. G., Membrane Properties and Electrogenesis in the Distal Axons of Small Dorsal Root Ganglion Neurons in vitro., *Journal of neurophysiology*, **2012**.

Vazquez, G., Wedel, B. J., Aziz, O., Trebak, M. and Putney, J. W., The mammalian TRPC cation channels., *Biochimica et biophysica acta*, 1742(1-3):21–36, **2004**.

Venkatachalam, K. and Montell, C., TRP Channels, *Annual Review of Biochemistry*, 76(1):387–417, **2007**.

Villereal, M. L., Mechanism and functional significance of TRPC channel multimerization., *Seminars in cell & developmental biology*, 17(6):618–629, **2006**.

Voets, T., Droogmans, G., Wissenbach, U., Janssens, A., Flockerzi, V. and Nilius, B., The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels., *Nature*, 430(7001):748–754, **2004**.

Wang, H., Westin, L., Nong, Y., Birnbaum, S., Bendor, J., Bris-

MAR, H., NESTLER, E., APERIA, A., FLAJOLET, M. AND GREENGARD, P., Norbin is an endogenous regulator of metabotropic glutamate receptor 5 signaling., *Science*, 326(5959):1554–1557, **2009**.

WANG, X.-J. J., LIU, Y., SANCHEZ-VIVES, M. V. AND MCCORMICK, D. A., Adaptation and temporal decorrelation by single neurons in the primary visual cortex., *Journal of neurophysiology*, 89(6):3279–3293, **2003**.

WEIDNER, C., SCHMELZ, M., SCHMIDT, R., HAMMARBERG, B., ORSTAVIK, K., HILLIGES, M., TOREBJÖRK, H. E. AND HANDWERKER, H. O., Neural signal processing: the underestimated contribution of peripheral human C-fibers., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(15):6704–6712, **2002**.

WEIDNER, C., SCHMELZ, M., SCHMIDT, R., HANSSON, B., HANDWERKER, H. O. AND TOREBJÖRK, H. E., Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin., *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 19(22):10184–10190, **1999**.

WEIDNER, C., SCHMIDT, R., SCHMELZ, M., HILLIGES, M., HANDWERKER, H. O. AND TOREBJÖRK, H. E., Time course of post-excitatory effects separates afferent human C fibre classes., *The Journal of physiology*, 527 Pt 1:185–191, **2000**.

WENG, X., SMITH, T., SATHISH, J. AND DJOUHRI, L., Chronic inflammatory pain is associated with increased excitability and hyperpolarization-activated current (Ih) in C- but not AÎŽ-nociceptors., *Pain*, 153(4):900–914, **2012**.

WILLIAMS, S. R. AND WOZNY, C., Neuroscience: The chain reaction of dendritic integration., *Current biology*, 19(20):R956–R957, **2009**.

WU, L.-J., SWEET, T.-B. AND CLAPHAM, D. E., International Union of Basic and Clinical Pharmacology. LXXVI. Current Progress in the Mammalian TRP Ion Channel Family, *Pharmacological Reviews*, 62(3):381–404, **2010**.

YAN, H.-D. D., VILLALOBOS, C. AND ANDRADE, R., TRPC channels mediate a muscarinic receptor-induced afterdepolarization in cerebral cortex., *The Journal of neuroscience*, 29(32):10038–10046, **2009**.

YANG, Y., WANG, Y., LI, S., XU, Z., LI, H., MA, L., FAN, J., BU, D., LIU, B., FAN, Z., WU, G., JIN, J., DING, B., ZHU, X. AND SHEN, Y., Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythermalgia., *Journal of medical genetics*, 41(3):171–174, **2004**.

YOSHIDA, M., FRANSÉN, E. AND HASSELMO, M. E., mGluR-dependent persistent firing in entorhinal cortex layer III neurons., *The European journal of neuroscience*, 28(6):1116–1126, **2008**.

YOSHIDA, M. AND HASSELMO, M. E., Persistent firing supported by an intrinsic cellular mechanism in a component of the head direction system., *The Journal of neuroscience*, 29(15):4945–4952, **2009**.

YU, F. H., YAROV-YAROVOY, V., GUTMAN, G. A. AND CATTERALL, W. A.,

Overview of Molecular Relationships in the Voltage-Gated Ion Channel Superfamily, *Pharmacological Reviews*, 57(4):387–395, **2005**.

Zhang, Z., Reboreda, A., Alonso, A., Barker, P. A. and Séguéla, P., TRPC channels underlie cholinergic plateau potentials and persistent activity in entorhinal cortex., *Hippocampus*, 21(4):386–397, **2011**.

Zhang, Z. and Séguéla, P., Metabotropic induction of persistent activity in layers II/III of anterior cingulate cortex., *Cerebral cortex*, 20(12):2948–2957, **2010**.

Zimmermann, K., Leffler, A., Babes, A., Cendan, C. M. M., Carr, R. W., Kobayashi, J.-i., Nau, C., Wood, J. N. and Reeh, P. W., Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures., *Nature*, 447(7146):855–858, **2007**.