

Imperial College London
Department of Bioengineering

Engineering Design Principles of Neural Fibres

Mohammad Ali Neishabouri

Submitted in part fulfilment of the requirements for the degree of
Doctor of Philosophy in Bioengineering of Imperial College London
and the Diploma of Imperial College London

Declaration

I herewith certify that all material in this dissertation which is not my own work has been properly acknowledged.

The copyright of this thesis rests with the author and is made available under a Creative Commons Attribution Non-Commercial No Derivatives licence. Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work

Mohammad Ali Neishabouri

Abstract

Our nervous system, like most information processing systems, faces 4 fundamental physical constraints. It has to transmit information quickly (1. *time*) and reliably (2. *noise*) while keeping its energy consumption (3. *energy*) and size/weight (4. *spatial scale*) at a minimum, to support the behaviourally determined fitness of the organism. These constraints are likely to enforce trade-offs to be made in the evolution of the nervous system. Taking this view, I investigated myelinated and unmyelinated axons across systems and species. I developed user interfaces and simulation methods for the Modigliani stochastic simulation software, and made a number of findings highlighted in the following.

Myelinated axons make up the majority of long-range connections in CNS and PNS. I derived size-dependent relationships for metabolic costs of action potentials in myelinated axons. The high density of sodium channels at Nodes of Ranvier set lower limits on myelinated axons' outer diameter ($0.3\mu\text{m}$), which is 3-fold that for unmyelinated axons. In contrast, thin, unmyelinated axons make up most of the local (cortical) connectivity. Using a variety of axon models and detailed models of synaptic calcium dynamics and vesicle release, I showed that the waveform of action potentials undergoes random changes whilst traveling along thin unmyelinated axons. These fluctuations translate into synaptic response variability. The diameter of unmyelinated axons sets energetic limits to signalling, and I derived diameter-dependent relationships for the maximum rate of burst and sustained firing. The latter depends on the density of pumps and metabolic cost of action potentials, but is counter intuitively independent of axon diameter.

My findings provide insights and scaling-relationships that relate the 4 fundamental design constraints for wiring brains. They allow us to quantitatively predict structure-function relationships, and form a basis for principled treatments of nerve disorders.

Acknowledgements

I remember, fondly, when I would read about “scientists” and their discoveries as a child, and wonder what it would take to become one. After three years of research, I still find it difficult to believe that I may soon become what I would have considered a real-life scientist back then. But I also realise how much I owe to those who accompanied me on this path.

I am especially grateful to my supervisor, Aldo Faisal, for believing in my ability and guiding me through my PhD. He never hesitated to answer my questions and discuss my ideas, and his contribution to this work can not be overestimated.

I would also like to thank other members of my lab and of the Department of Bioengineering: Alessandro, Anastasia, Andreas, Ariadne, Chin-Hsuan, Constantinos, Ekaterina, Feryal, Luke, Olga, Raj, Scott, William, Yesna and all the wonderful people in that department made my PhD an unforgettable experience. I am also grateful to the EPSRC and the department of Computing for their financial support.

I am thankful to my parents always valued their children’s education above all else, and never spared anything for us, and my sister Yassaman Neishabouri, whose belief in me helped me endure hard times. I am also thankful to my extended family who have always been present for me and supported me in my endeavours. They are too many names to cite them all, but I would like to thank Davoud and Leyla for supporting me in my choices.

I have lived separated from my extended family for the last 14 years. But I was fortunate enough to find incredible friends. Although the academic life made me unable to see them as much as I would like, Alexandra, Alexis, Anne-Caroline, Audrey, Aurélie, Aurélien, Bertrand, Charles, Chrystèle, Dolinda, Guillemette, Jérôme, Josquin, Julie, Laetitia, Louis, Marie, Nancy, Pablo, Pierre-Aimé, Pierre-Yves and Samuel are always present in my heart.

I have also been fortunate enough to be instructed by remarkable teachers who instilled in me the thirst for knowledge. For this, I am eternally grateful.

Finally, I want to thank Delphine Mercier and Pishi, who accompanied me on this journey. I am also grateful to the Mercier family who have always shown me the utmost kindness.

I could not have done this without you.

Contents

1	Introduction	15
1.1	Neurons	16
1.2	Action potentials and ion channels	17
1.3	Basic constraints on nervous systems	20
1.4	Basic constraints shape neural fibres	21
1.5	Structure of this dissertation	22
2	Modigliani II	23
2.1	Stochastic modelling of axons	23
2.2	Axon simulator	26
2.2.1	Cylindrical compartment types	27
2.2.2	Membrane currents	27
2.2.3	Anatomy and current injection	29
2.3	Ion channel kinetics	30
2.3.1	JSON format	31
2.3.2	Lua format	32
2.4	Other programs	33
2.5	Toward whole cell simulations	34

3	Trade-offs in the design of myelinated axons	36
3.1	Introduction	36
3.1.1	The metabolic cost of myelinated axons	38
3.2	Methods	39
3.2.1	Axon model	40
3.2.2	Anatomical and biophysical parameters	41
3.2.3	Diameter-generic model of myelinated axons	45
3.3	Results	48
3.3.1	Our model reproduces AP features and velocity	48
3.3.2	Metabolic cost of myelinated vs unmyelinated axons	51
3.3.3	Noise in myelinated vs unmyelinated axons	54
3.3.4	Robustness of results to parameter variation	58
3.3.5	Deriving the lower limit on axon diameter	60
3.4	Discussion	62
4	Axonal variability as a source of synaptic variability	69
4.1	Introduction	69
4.2	Methods	71
4.2.1	Axon models	71
4.2.2	Stimulus protocols	72
4.2.3	Modelling synaptic transmission	73
4.3	Results	75
4.3.1	Action potential waveform variability	75
4.3.2	Underlying ion channel fluctuations	79
4.3.3	Deriving biophysical scaling relationships	83
4.3.4	Waveform variability in spike trains	83
4.3.5	Variability in synaptic transmission	86

4.4	Discussion	91
4.4.1	Action potential waveform variability in thin axons	91
4.4.2	Synaptic variability from noisy action potentials	92
5	Does clustering of Na^+ channels on lipid rafts allow micro-saltatory conduction in C-fibres?	96
5.1	Introduction	96
5.2	Methods	98
5.3	Results	101
5.3.1	Microsaltatory conduction along Na^+ channel clusters	101
5.3.2	Change in shapes of APs results in lower metabolic costs	104
5.3.3	Propagation velocity over clustered Na^+ channels	107
5.3.4	Ionic mechanisms behind the reduction of metabolic cost	109
5.3.5	AP width in thin axons depends on axonal diameter	111
5.3.6	The observed effects in clusters are caused by lower Na^+ channel density .	112
5.3.7	AP efficiency depends on Na^+ density	113
5.4	Discussion	114
6	Axon structure-function relationships from homeostatic constraints	119
6.1	Introduction	119
6.2	Methods	123
6.3	Results	127
6.3.1	Simple theoretical calculations	127
6.3.2	Reversal potentials deteriorate after each AP	131
6.3.3	A simplified model for the homeostatic dynamics of reversal potentials . .	133
6.3.4	A model involving pump dynamics	136
6.3.5	The simplified model accurately predict maximum feasible firing rates . .	138
6.3.6	The effect of diameter dependent diffusion limits of ATP to the pumps . .	142

6.4	Discussion	145
6.4.1	Limits on firing rate of axons	146
6.4.2	Diffusion of ATP constrains the structure of thin axons	147
7	Conclusion	149
7.1	Summary of findings	150
7.2	Future research directions	153
7.2.1	Channel kinetics and noise	153
7.2.2	Mechanisms of energy supply	153
7.2.3	Effects of the four biophysical constraints on the synapse	154
7.2.4	Architecture of the brain	154
8	References	156

List of Tables

1.1	Abbreviations used in this dissertation	22
3.1	Anatomical data for myelinated axons	42
3.2	Electrophysiological data for myelinated axons	42
3.3	Electrophysiological data for unmyelinated axons	43
3.4	Ionic current properties for myelinated axons	43
3.5	Ionic current properties for unmyelinated axon	44
3.6	Q10 values used for ion channels kinetics	44
4.1	Simulation parameters	72
4.2	Variability in synaptic transmission	90
5.1	Simulation parameters	99
5.2	Summary of similarities and differences between myelinated axons and unmyelinated axons with lipid rafts	118
6.1	Simulation parameters	124

List of Figures

1.1	Anatomy of a neuron	17
1.2	The action potential	19
1.3	Basic constraints on the design of neural circuits	20
2.2	A model of a NaV1.6 channel	24
2.1	Class hierarchy of Modigliani	25
2.3	Voltage step simulations	34
3.1	Basic axo-geometric characteristics of myelinated axons	40
3.2	Saltatory conduction in the generic myelinated axon model	41
3.3	Extrapolation of anatomical data for myelinated axons	46
3.4	Simulated action potentials' widths and amplitudes	49
3.5	Action potential propagation velocity in myelinated and unmyelinated axons . . .	50
3.6	Metabolic cost of action potentials in myelinated and unmyelinated axons	51
3.7	Model for metabolic cost of signalling in myelinated axons	53
3.8	Action potential efficiency in myelinated and unmyelinated axons	54
3.9	Action potential and Na ⁺ current in unmyelinated and myelinated axons	55
3.10	Stochastic simulations of myelinated axons	56
3.11	Stochastic simulations of unmyelinated axons	57
3.12	Spontaneous firing rate in myelinated and unmyelinated axons	58

List of Figures

3.13	Influence of parameters on spontaneous action potential firing	59
3.14	Minimum internal diameter for myelinated axons	62
4.1	Allosteric synaptic vesicle release model	75
4.2	Definitions and methods	76
4.3	Random variability of AP waveform in thin axons of four different types	78
4.4	Random variability of AP waveform in thin SGA type axons of 3 diameters	80
4.5	Ion channel and current fluctuations underlie waveform variability	81
4.6	Travelling APs' waveform fluctuations scale with axonal diameter with a power law	84
4.7	Variability of the AP width and height scales with axon diameter	85
4.8	Waveform variability and resulting Ca^{++} influx variability in a spike train	88
4.9	Calculated distribution of synaptic response variability	89
5.1	Schematic overview of Na^+ channel clusters	100
5.2	Action potential propagation in a thin C-fibre axon	102
5.3	Microsaltatory propagation in a thin C-fibre axon	103
5.4	Action potential height and width as functions of channel distributions	105
5.5	Metabolic cost of action potential as functions of cluster configuration	106
5.6	Metabolic cost of action potentials for different channel distributions	107
5.7	Action potential velocity for different channel distributions	108
5.8	Action potential and sodium current waveform in Na^+ channel clusters	110
5.9	Action potential width in C-fibre axons as a function of axonal diameter	111
5.10	Metabolic cost and action potential velocity as a function of Na^+ channel density	112
5.11	Action potential efficiency for different channel distributions	114
5.12	Action potential and sodium current waveform in somas and axons	115
5.13	Deactivation kinetics of Nav1.2 and Nav1.8 channels	116
6.1	Action potentials depolarise ionic reversal potentials	121
6.2	Potential energy stored in ionic concentration gradients	129

List of Figures

6.3	Theoretical maximum sustainable firing rates	130
6.4	Influence of axonal diameter on the effect of each action potential	132
6.5	Maximum sustainable firing rate (analytical)	138
6.6	Maximum sustainable firing rate and pump densities	139
6.7	Higher pumping rates can lead to lower maximum firing rates	140
6.8	Maximum sustainable firing rate and firing durations	142
6.9	Maximum feasible burst firing rate	143
7.1	Summary of findings	151

1 Introduction

The need for rapid and accurate transmission of information, which is a requirement for fast, coordinated movement, is believed to be the driving factor for the evolution of nervous systems in animals. Despite all the subsequent sophistication, e.g. short and long term memory, abstract representations, or control of the organism's internal state (Kandel et al. 2000), the nervous system at its heart is still a system to receive and process (sensory) input, and (few exceptions aside) initiate and control movement.

Nervous systems are also extraordinarily complex. They are constituted of staggering numbers of building block, that are each connected to tens of thousands of other building blocks. There has been some success in a comprehensive mapping of these connections in the case of one of the simplest nervous system known, *Caenorhabditis elegans* (White et al. 1986; Hall and Russell 1991). But the sheer numbers involved in the case of e.g. a human brain and its 86×10^9 neurons (Azevedo et al. 2009) put such a mapping out of reach. This complexity calls for a higher level approach, based on basic biophysical theory. One such approach is to take advantage of the tools developed to deal with man-made information processing systems, or computers, in order to understand design principles of the brain.

There are, generally speaking, three constraints faced by any system dealing with information. Reduction of delays, whether between the reception of an input and emission of the appropriate output (latency), or the time necessary to process a given amount of input information, has

been a primary goal of all information transmission systems. Reliability is the second constraint faced by those systems. All messages need to be sent accurately, and unintended messages shall not be transmitted.

Rapid and reliable transmission requires the expenditure of energy. Nervous systems have to be built and maintained at great metabolic cost, and it also require energy in order to process and transmit information. The energetic requirements are the third constraint limiting the speed and reliability of information systems. Although the building blocks of nervous systems are very different from man-made computers, they necessarily face these same constraints, in addition to other potential constraints due to their specificity.

1.1 Neurons

The nervous system is made up of individual cells, called neurons (Ramon y Cajal 1897). Functionally speaking, the neuron can be divided into 3 distinct components: the soma – which contains the nucleus – dendrites and the axon (Fig. 1.1). From an extremely simplified point of view, the dendrites receive information, which is processed in the soma. As a results, a signal might be triggered in the soma or the axon initial segment and travel along the axon to the next processing stage.

From a physiological point of view, there is no discontinuity in the cytoplasm between the soma and its dendrites (Kandel et al. 2000). The axon, on the other hand, is cytoplasmically distinct from the rest of the neuron. Axons are typically longer than dendrites, and a complex network of neural filaments enables the transport of proteins and other essential molecules from the soma to all parts of the axon (Kandel et al. 2000) to supply the output of neurons, the synapses. Axons are generally classified into two broad categories, myelinated and unmyelinated axons. Myelinated axons are characterised by the sheaths of myelin wrapping around them. Each sheath is formed by compacted layers of bi-lipid membrane, joined through gap junctions (Hartline 2008). Myelin is produced by Schwann cells in the PNS, and oligodendrocytes in the CNS.

Myelinated axons are a hallmark of nervous system in vertebrates and a few invertebrates (Zalc 2006), and we will study them in Chapter 3. In contrast, very thin ($<0.2\text{ }\mu\text{m}$ diameter) axons in vertebrate CNS are always unmyelinated (Waxman and Bennett 1972), i.e. they do not possess a myelin sheath.

1.2 Action potentials and ion channels

The fundamental signal in the nervous system is the action potential (AP). Each AP is a transient change in the electrical potential across the membrane of the cell (Du Bois-Reymond 1848), which propagates from the soma along the axon and is transmitted from a neuron to another cell through synapses.

In its resting state, the electrical potential inside the cell is lower than the potential outside. The difference between the two potentials is called the membrane potential, and denoted V_m . Typical value for V_m are between -80 mV to -40 mV (Johnston et al. 1995). This resting potential is stable, i.e. if V_m is slightly changed, e.g. by injecting current through an electrode, it will return to its resting value. However, if V_m crosses a certain threshold (called the firing threshold), V_m will very rapidly increase by approx. 100 mV , before redescending back to its resting value. This sudden change of V_m is propagated to nearby membrane segments by active membrane processes, and can be observed at any point on the soma or along the axon.

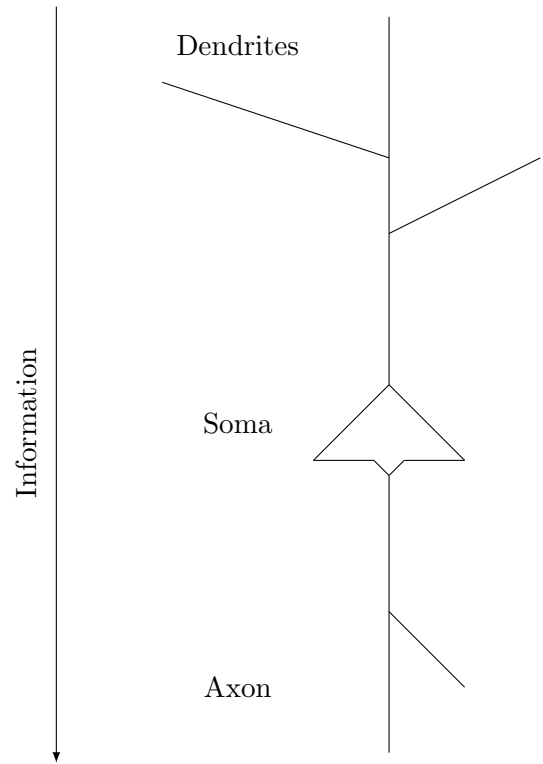


Figure 1.1: A schematic view of a cortical pyramidal neuron. Information is received at dendrites, processed in the soma and the axon's initial segment, and transmitted along the axon to the next stage.

The remarkable work of Hodgkin and Huxley (1952) revealed that the propagation of APs is an active process, in which the signal is preserved as it travels along the axon thanks to transmembrane voltage sensitive proteins, commonly called ion channels. Propagation of action potentials requires two distinct ionic currents (see Section 1.2). Na^+ channels are involved in the beginning phase, and amplify the signal. The existence of these channels, or the functionally equivalent Ca^{++} channels in earlier species (Hille 1992), is essential for active propagation of APs. K^+ channels, on the other hand, become active in the later phase of the AP, and mediate the return of the membrane potential to its resting value.

In their resting state, the inside of neurons contains less positive charges than the outside. The membrane potential is consequently negative. This resting value is typically between -80 mV to -40 mV. In the vicinity of the resting membrane potential, both K^+ and Na^+ channels are typically closed. The fraction of open K^+ channels is higher than that of Na^+ channels. As a result, the resting membrane potential is mainly determined by the current flowing through the few open K^+ channels. Depolarisation (In the context of neurons, depolarisation refers to an increase in the membrane potential from negative to positive values. Hyperpolarisation, on the other hand, refers to the decrease of the membrane potential) of V_m causes Na^+ channels to open. The concentration of Na^+ inside the cell is typically much lower than the outside concentration. Consequently, Na^+ ions enter the cell through Na^+ channels. This depolarises the membrane further. Thus, Na^+ channels provide a positive feedback loop and are responsible for the sharp rising phase of the action potential.

Near the peak of the action potential, Na^+ channels start inactivating. Inactivating Na^+ channels can, in addition to the *open* and *closed* states, be either *activated* or *inactivated*. Inactivated Na^+ channels are closed. In order to become reactivated (and potentially open again), the local membrane potential around the channel needs to become polarised. The inactivation of Na^+ channels near the peak of action potential ensures that they will stay closed during the repolarising phase, which prevents action potentials from propagating backward. Inactivation of Na^+ channels is essential because it limits the total amount of Na^+ current crossing the

membrane (the implications of this current for the cell are discussed in Chapter 6).

Slightly before the inactivation of Na^+ channels, the majority of K^+ channels open. The K^+ concentration is higher inside the cell than outside, meaning K^+ ions flow out of the cell through K^+ channels. This flow of ions repolarises the cell. The membrane potential regains its resting value after a brief period of hyperpolarisation. The spike in the membrane potential causes a flow of current into the adjacent membrane segments, where it provokes a depolarisation of the membrane potential. Thus, the action potential propagates along the membrane.

The action potential is commonly thought to be a unitary (all or none) event, i.e. information is encoded not in the shape of the action potential, but in the number of action potentials and their frequency, or equivalently the interspike interval (Adrian and Zotterman 1926), but recent experiments suggest the information could be encoded in the shape of the action potential (Polavieja et al. 2005; Shu et al. 2006; Kole et al. 2007). We investigate this possibility in Chapter 4.

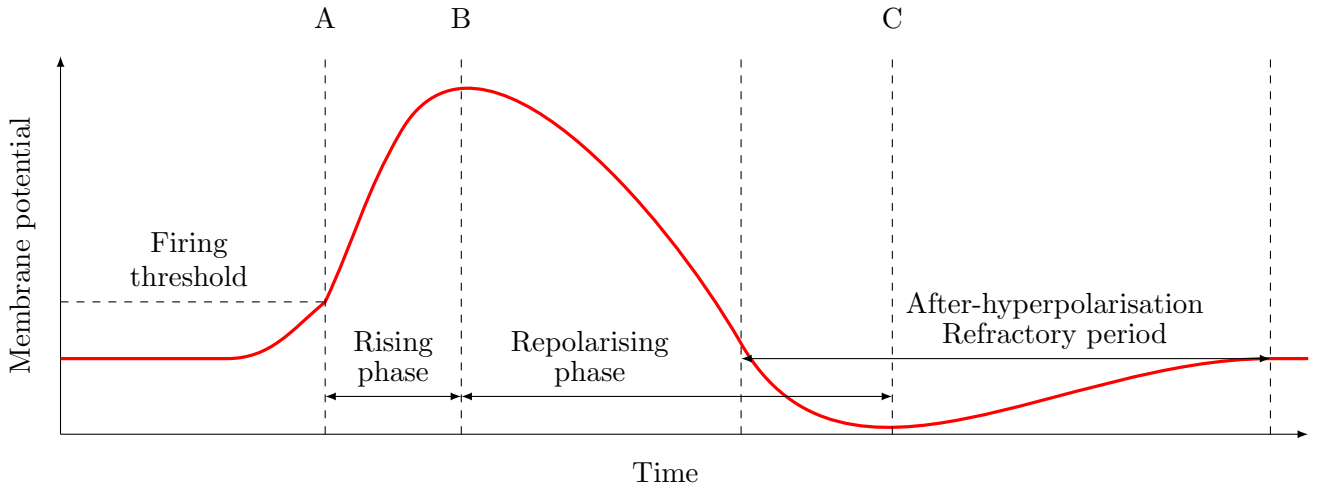


Figure 1.2: A schematic view of the action potential and the underlying mechanisms. **(A)** When the membrane potential is depolarised to the firing threshold, e.g. because of a depolarisation of an adjacent segments, Na^+ channels open. Na^+ ions flow into the cell and depolarise the membrane further. **(B)** Near the peak of the action potential, Na^+ channels deactivate and K^+ channels open. K^+ ions flow into the cell and **(C)** repolarise the membrane back to its resting potential after a brief overshoot.

1.3 Basic constraints on nervous systems

We can now discuss the basic constraints faced by nervous systems (Fig. 1.3) in more precise terms. Processing and transmission of information in neurons is accomplished by altering the membrane potential. The cell membrane is largely impermeable to ions and acts as a capacitance with a finite response speed, determined by the membrane time constant. The finite response range of neurons - signals range over 100 mV in amplitude and less than 1 kHz in action potential frequency - imposes limits on the total information throughput (Stemmler and Koch 1999). Rates of synthesis, release diffusion and uptake of chemical transmitters also limit the performance of neural fibres.

Random fluctuations are present at all levels of nervous systems (reviewed by Faisal et al. 2008). Ion channels are subject to thermodynamic noise that may cause spontaneous opening or closing (White et al. 1998; Faisal et al. 2005), called channel noise. Synaptic vesicle release, diffusion and molecular interactions (Laughlin 1989) are all stochastic processes. The existence of these sources of variability undermines reliable processing and transmission of information in neurons. Creation and maintenance of a nervous system is very metabolically expensive: this includes

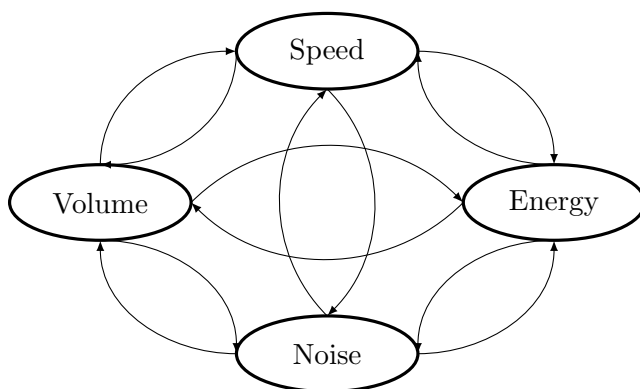


Figure 1.3: Basic constraints on the design of neural circuits. Energy, noise, speed and volume are linked to each other by basic biophysical principles. For example, reducing the diameter of an axon will decrease its volume and its metabolic cost. However, smaller axons are noisier, and conduct action potentials more slowly.

the cost of producing and maintaining neurons, their connections and support cells (astrocytes, oligodendrocytes and Schwann cells), to which we must add the cost of generating and propagating neural signals (Laughlin et al. 1998; Attwell and Laughlin 2001; Harris and Attwell 2012). The metabolic cost of action potentials in the CNS alone accounts for 22% of the resting

metabolic consumption (Laughlin 2001; Alle et al. 2009; Sengupta et al. 2010).

Finally, in the case of very dense circuits such as the brain, or in very small organisms, neural fibres are constrained by volume (see Niven and Farris 2012, for a review on miniaturisation of nervous systems). There is evidence that the wiring of the brain optimises the volume occupied by axons to reduce metabolic cost and conduction delays (Wang et al. 2008). The size of axons directly interacts with all 4 physical constraints: bigger axons increase the overall volume of nervous system and have a higher associated metabolic cost, while smaller axons conduct APs slowly. Moreover, noise imposes a lower limit on the diameter of axons (Faisal et al. 2005).

1.4 Basic constraints shape neural fibres

Volume, time, energy and noise constraints have contradicting effects on the function and structure of neural fibres. For example, the simplest way to reduce noise and thus increase the rate of information transmission, is to increase the number of channels carrying the signals, thus providing redundancy (Shadlen and Newsome 1994). However, more channels (e.g. more neurons encoding the same information) result in increased metabolic cost and volume (Laughlin 2001). Another example can be found in the adaptations necessary for high speed transmission in the squid giant axon. AP propagation velocity is proportional to the square root of the diameter in unmyelinated axons (Rosenblueth et al. 1948; Hodgkin 1954). The squid, for instance, has specifically evolved its giant (up to 1 mm diameter) axon in order to achieve the high velocity required for coordinating its muscles during the escape reflex (Eaton 1984), which is essential for the animal's survival. This has the disadvantage of increasing both volume and metabolic cost, as the energy necessary for transmitting signals in unmyelinated axons is proportional to the diameter.

My hypothesis is that the design of neural fibres is a manifestation of the trade-offs made between the four constraints (energy, reliability, space and time) they face. We aim to understand and quantify the impact of these four constraints on the anatomy and function of axons and the

interplay between them. With that knowledge, our goal is to uncover the engineering principles governing the design of neural fibres and nervous systems, explain their evolution and understand the physical basis of many neurodegenerative diseases and the nature of neural activity measures.

1.5 Structure of this dissertation

This dissertation is organised as follow. Chapter 2 presents work done on the Modigliani stochastic simulator (Faisal et al. 2002). Subsequent chapters are structured independently from each other. In Chapter 3 I investigate factors determining the choice between myelinated and unmyelinated axons. Chapter 4 presents work on the effects of axonally induced noise on AP waveforms and their impact at the level of the synapse. In Chapter 5, I look into the possibility and effects of micro-saltatory conduction along Na^+ channels clusters on lipid rafts in very thin C-fibre axons. Finally, Chapter 6 deals with structure-function relationships induced by metabolic constraints on thin axons.

Action potential	AP
Adenosine triphosphate	ATP
Central nervous system	CNS
Coefficient of variability	CV
Peripheral nervous system	PNS
Rat hippocampal interneuron	RHI
Squid giant axon	SGA
Standard deviation	SD

Table 1.1: Abbreviations used in this dissertation

2 Modigliani II

2.1 Stochastic modelling of axons

Ion channels are transmembrane proteins which selectively allow ions to cross the lipid bilayer. As is the case for almost any molecular process in biology, ion channels opening and closing are stochastic processes (reviewed in White et al. 2000; Faisal et al. 2008). When modelling neurons, it is often implicitly assumed that averaging large numbers of ion channels allows the elimination of the effects of individual channels' randomness at higher levels (such as spike timing). However, the coefficient of variability of the activity of a set of signalling molecules is inversely proportional to the square root of the number of molecules involved. In large cells, such as the squid giant axon which has played a key role in understanding neural mechanisms, the variability averages out quickly. In comparison, neuronal connections in our brains can have 10000 times smaller diameters. This implies that even a small number of channels randomly opening can have a systematic effect on the cell's behaviour (Faisal et al. 2005; Faisal and Laughlin 2007; Faisal et al. 2008). Moreover, neurons perform highly non-linear operations involving high gain amplification and positive feedback. Therefore, small random fluctuations can significantly change whole cell responses.

In order to capture the effects of the variability of ion channels, we need to use the stochastic partial differential equations describing the evolution of the membrane potential in space and time inside neurons. This requirement was the main motivation for the creation of Modigliani (Faisal

et al. 2002). Modigliani is a fast, accurate stochastic partial differential equation solver (Faisal et al. 2005). It is completely object oriented, and its classes are mapped onto relevant entities in neuroscience (Fig. 2.1). Using Modigliani, we can build complex neurons from the ground up by plugging objects, such as compartments or ion channels together and change them dynamically, i.e. at runtime.

In stochastic mode, Modigliani simulates each individual ion channel as a Markov process switching between finite states. A typical NaV1.6 channel, for instance, is simulated as an 8-state Markov process where transition probabilities depend on the membrane potential (see Fig. 2.2), according to well known kinetic rate functions that govern gating particles in the deterministic Hodgkin-Huxley type model (Hodgkin and Huxley 1952).

The choice of integration algorithm can have a crucial effect on the behaviour of the cell. We use the binomial algorithm (Faisal 2010), where the channels of each compartment are treated as a binomial population. At each step, binomial random numbers are generated to estimate the number of channels switching states based on the individual transition probabilities of each ion channel. This algorithm is chosen because of its accuracy as compared to the Langevin algorithm, and speed when compared to the Gillespie or Single channel algorithms. (For details about algorithms and their performance, see Faisal 2010).

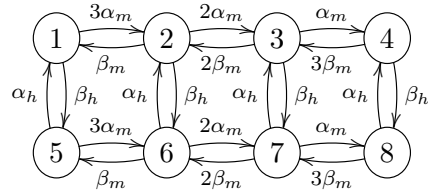


Figure 2.2: A model of a NaV1.6 channel. Each circle represents a possible state for the channel. State 4 corresponds to the open channel. States 5 to 8 are inactivated states. Transition probabilities (α_m , β_m , α_h and β_h) depend on the local membrane potential.

The original design of Modigliani aimed toward the creation of a library of classes. New simulations were then written as C++ programs that used said libraries. Although many of the programs written included some support for configuration files (allowing parameter variation without recompilation), many essential features (ion channel kinetics, axonal anatomy) had to be hard-coded. This approach had the obvious advantage of shortening execution times, but

this acceleration came with several disadvantages:

- Running simulations required knowledge of the C++ programming language.
- Each new simulation program had to be separately tested and validated.
- Bugs corrected in one program had to be manually corrected in all other simulation programs.

It soon became clear that, given the tremendous improvements in processing power since Modigliani was first written, it would be advantageous to add a set of standard programs to Modigliani. Each program would leverage the existing libraries to perform one set of simulations based on data supplied at runtime by the user. The scope of each of these programs would be much larger than that of programs using the previous architecture. Thus, we would also need a more powerful runtime configuration format, i.e. one that allows description of arbitrary axonal anatomy and ion channels.

I have implemented both the new set of programs and the required configuration format. This chapter presents one such program, and the configuration format in detail. Part of this work was presented as a lightning talk at the *Twentieth Annual Computational Neuroscience Meeting* (Stockholm, Sweden 2011).

2.2 Axon simulator

The most prominent of such programs is `axon_simulator`. `axon_simulator` leverages Modigliani libraries to simulate membrane currents in isopotential compartments of an axon, and propagates changes along the axon using the fast algorithm implemented by Faisal et al. (2002). `axon_simulator` is highly configurable. It is capable of simulating unmyelinated and myelinated axons, or generally any succession of cylindrical compartments. The configuration file for `axon_simulator` uses a standard data storage format, JSON (created by Crockford 2006), and is inspired by the format used by Neuron (Hines and Carnevale 1997). Neuron is one of the most

commonly used simulators in neuroscience, and the similarity of the configuration file formats makes axon_simulator easy to use by those already familiar with Neuron.

2.2.1 Cylindrical compartment types

The first step in defining an axonal segment is to define the types of compartments that are to be used. A compartment is a very short (as compared to the axonal length constant) segment of axon, which can be approximated to an isopotential membrane patch. The program expects to find a JSON array in the configuration file. This array contains all compartments types that are to be used to construct the axon. For an unmyelinated axon, the array will typically contain a single compartment type.

Listing 2.1: Properties of a compartment (axonal segment)

```
1 "compartments_parameters": [  
2   {  
3     "length" : 20,  
4     "Ra" : 70,  
5     "Cm" : 1,  
6     "currents" : [...]  
7   },
```

Each compartment type is defined by 4 parameters:

- length: The length of the compartment in μm . This refers to the length of the isopotential membrane, and not the total length of the axon we wish to simulate.
- Ra: The axoplasmic resistance in Ωcm .
- Cm: The membrane capacitance in $\mu\text{F cm}^{-2}$.
- currents: This JSON array contains the list of ionic membrane currents.

2.2.2 Membrane currents

Each compartment can contain an arbitrary number of ionic currents. Ionic currents are to be listed in the currents parameter of each compartment. Modigliani supports two types of

currents:

Passive (leak) currents are only defined by a constant conductance, and the amount of current will be calculated as a function of leak reversal potential, the membrane potential, and the conductance. In order to add a passive current to a compartment, a JSON structure containing two parameters must be added to the currents array. The parameters are:

- type: This string contains the type of current. It has to be set to "leak" for leak currents.
- GLeak: Leak conductance, in mS cm^{-2} .

Voltage gated ion channels are defined by 6 parameters:

- type: This parameter distinguishes voltage dependent conductances from passive conductance. For voltage dependent conductances, it should either be set to "json" or "lua". See Section 2.3 for more information.
- chModel: Short for channel model, this parameter defines the model to be used for the voltage gated ion channel. It must point to a .lua (if type=="lua") or .json (if type=="json") file.
- chAlg: Short for channel algorithm. This parameter determines the algorithm to be used to simulate the ion channel (Faisal 2010). Possible values depend on the value chosen for "type":
 - 1: Only for type=="lua", selects the deterministic algorithm.
 - 2: For both types, selects the single channel algorithm.
 - 3: For both types, selects the Gillespie algorithm.
 - 4: For both types, selects the binomial population algorithm.
- chDen: The density of channels per μm^2 .
- chCond: Short for channel conductance, the conductance of a single channel in pS.

- `chRevPot`: Short for channel reversal potential, the reversal potential for the ionic current in mV.

An example of a complete compartment parameter structure is reproduced below:

Listing 2.2: Currents attached to a compartment

```

1  "compartments_parameters":[
2      {
3          "length" : 20,
4          "Ra" : 70,
5          "Cm" : 1,
6          "currents" : [
7              {
8                  "type" : "leak",
9                  "GLeak" : 0.1
10             },
11             {
12                 "type" : "lua",
13                 "chModel" : "./RHI_sodium.lua",
14                 "chAlg" : 4,
15                 "chDen" : 23,
16                 "chCond" : 15,
17                 "chRevPot" : 55
18             },
19             {
20                 "type" : "lua",
21                 "chModel" : "./RHI_potassium.lua",
22                 "chAlg" : 4,
23                 "chDen" : 6,
24                 "chCond" : 14,
25                 "chRevPot" : -90
26             }
27         ]
28     },

```

2.2.3 Anatomy and current injection

Once compartment types are defined, we need to define the anatomy of the axon by stacking the compartments in the desired order. This task is accomplished by defining a table. Each entry in the table points to a (0 indexed) member of the compartments parameters table. A myelinated axon, for instance, uses three compartments types: one for the nodes of Ranvier (0), one for the paranode (1), and one for the internodes (2). The anatomy table would then contain: `[2, 2, 2, 2, 2, 1, 0, 1, 2, 2, 2, 2, ...]`.

In order to avoid having to write the huge table that would be necessary for a long myelinated axon, we instead define a lua script which is executed by the program. This lua script must then define a lua table containing the types of compartments to be used. In this way we have access to loops, which make this task trivial. An example of such a script is reproduced below.

Listing 2.3: A short lua script defines the anatomy of axons.

```
1 "anatomy_lua" : "compartments={};for i=0,499 do table.insert(compartments,i);
    end;";
```

Finally, a lua script defines the amount of current to be injected at the proximal end of the axon.

Listing 2.4: A short lua script determines the amount of current to be injected into the axon.

```
1 "inject_current_lua" : "function current(t) if (t>30 and t<30.1) then return
    0.3 else return 0; end; end;";
```

2.3 Ion channel kinetics

Ion channels kinetics were originally hard-coded in Modigliani libraries as classes. Each ion channel class implemented the required stochastic simulation algorithms independently. New ion channels had to be coded and compiled with the rest of the library, which made the process of adding new types of channels cumbersome, induced a significant amount of code replication, and increased the number of potential bugs simply because of the bigger code base. In addition, distributing Modigliani would have been impractical.

I reimplemented all stochastic simulation algorithms in a new base class, which does not rely on any prior knowledge of the underlying dynamics. In other terms, I implemented a generic Markov chain simulator which can be configured to have arbitrary number of states, and does not make any assumptions on the transition probabilities.

One of my aims was to be able to extract ion channel kinetics from experiments and simulate them in Modigliani in as automated a fashion as possible. Although most channel kinetics are in practice modelled using a rather small set of basic functions, my goal was to be able to model

arbitrary dynamics. Therefore, I designed and implemented two generic format for storing and retrieving ion channels.

2.3.1 JSON format

The first storage format for channel kinetics is also based on JSON. Ion channel kinetics for all (discretized) possible values of the membrane potential are independently stored in one file. Put simply, we store, for each value of the membrane potential, all transition probabilities. In order to simplify parsing this file, we chose a standard format, JSON. The JSON format is simple, with parsing libraries available in almost any language. A simple channel configuration file is reproduced below.

Listing 2.5: Sample Na⁺ ion channel defined in a JSON file

```
1 {  
2   "base_temperature": 20 ,  
3   "number_of_states": 8 ,  
4   "minV": -100 ,  
5   "maxV": 300 ,  
6   "step": 10 ,  
7   "open_states": [4] ,  
8   "transitions": [  
9     {"voltage": -100.0, "start": 1, "stop": 2, "probability": 0.16514202191351607, "q10":  
10      2.2 },  
11    {"voltage": -100.0, "start": 2, "stop": 3, "probability": 0.11009468127567737, "q10":  
12      2.2 },  
13    {"voltage": -100.0, "start": 3, "stop": 4, "probability": 0.055047340637838685, "q10":  
14      2.2 },  
15    {"voltage": -100.0, "start": 5, "stop": 6, "probability": 0.16514202191351607, "q10":  
16      2.2 },  
17    {"voltage": -100.0, "start": 6, "stop": 7, "probability": 0.11009468127567737, "q10":  
18      2.2 },  
19    ... ] }
```

These files contain all data needed to characterise an ion channel, and are typically generated using simple scripts written in another programming language e.g. Python. They can also be generated from virtually any source e.g. a database, a web link, or a pipe. At the beginning of each simulation, Modigliani loads each such file once, regardless of how many times the channels are used. This format allows to simulate arbitrary channel models. However, the generated JSON files are usually very large, and they can not be used for deterministic (as in the original

Hodgkin and Huxley 1952, models).

2.3.2 Lua format

The deficiencies of the JSON format led me to implement a second means of storing ion channel kinetics. In the new approach, ion channel kinetics are typically implemented in a simple programming language, Lua (Ierusalimschy et al. 1996). Lua scripts are then interpreted by Modigliani libraries.

Concretely, Modigliani now embeds a Lua interpreter which can execute Lua functions contained in a script. In order to populate the table of transitions probabilities, repeated calls are made to a Lua function. The implementation of this function is not constrained. Transition probabilities can come from a mathematical function, data files, databases, etc. A sample lua script is reproduced below.

Listing 2.6: Sample sodium ion channel defined in a lua script

```
1 base_temperature = 6.3
2 q10m = 3
3 q10h = 3
4
5
6 function AlphaM(vM)
7     if vM== -40 then return 1 end;
8     return 0.1*(vM+40)/(1-exponential(-0.1*(vM+40)))
9 end
10
11 function BetaM(vM)
12     return 4*exponential(-0.0556*(vM+65))
13 end
14
15 function AlphaH(vM)
16     return 0.07*exponential(-0.05*(vM+65))
17 end
18
19 function BetaH(vM)
20     return 1/(1+exponential(-0.1*(vM+35)))
21 end
22
23 local channel_functions=require('8_state_sodium')
```

2.4 Other programs

The implemented formats are flexible enough to be used for a whole family of programs, now implemented as a standard part of Modigliani. Notable programs in this list are described below.

- **Voltage step**

Thanks to the unified ion channel description, a single executable can now conduct voltage step simulations on arbitrary channels (Fig. 2.3). This program is particularly useful to reproduce patch-clamp experiments which aim to determine ion channel kinetics. The voltage step simulator serves to quickly validate models.

The choice of the simulation algorithm (deterministic or any flavour of stochastic simulations) is done using a command line switch. Thanks to the single input file we can guarantee that the same kinetics are used for all simulations.

- **Single compartment simulator**

A simpler version of `axon_simulator` is also implemented. This program only simulates a single spherical compartment, typically a soma. The only notable algorithmic difference between this program and the `axon_simulator` is the absence of any wave propagation in the single compartment case.

- **Neuronal networks**

Simulations of neural networks typically rely on fast simulation of a large number of neurons, most often modelled as a single compartment, connected to each other using model synapses. The neurons are typically modelled using the leaky integrate and fire model, sometimes with added white noise in order to take into account the stochasticity in the activity of neurons.

Modigliani implements a variety of fast and accurate algorithms for stochastic simulations of neurons. Network simulator is a new addition to Modigliani. This program simulates

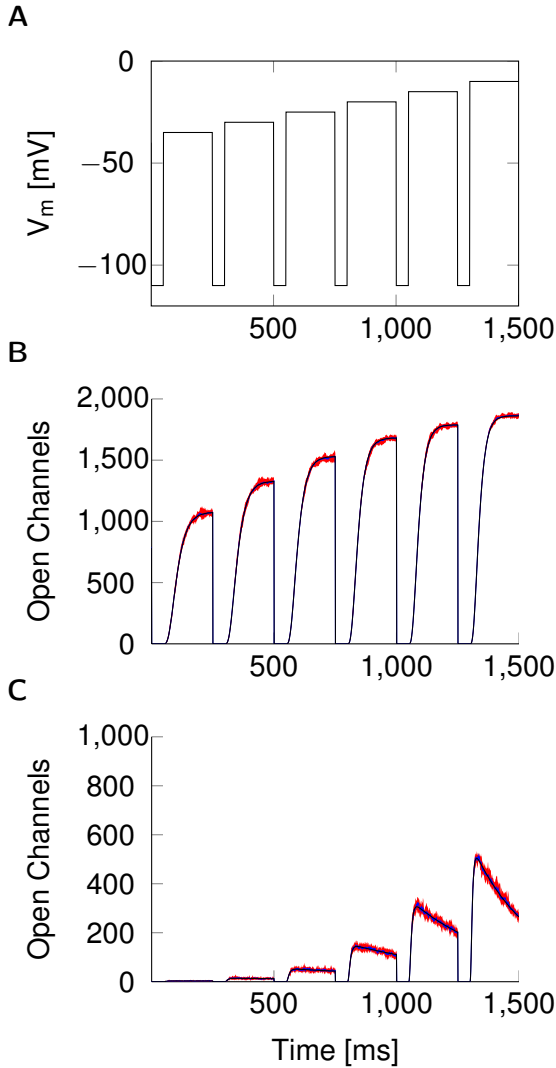


Figure 2.3: Voltage step simulations in Modigliani. (A) The membrane potential is held at a hyperpolarised value (-110 mV) for 50 ms, then brought to a varying target value. (B) Number of open Na^+ channels in stochastic simulations using the binomial population algorithm (Mean value in blue, range in red) and in deterministic simulations (black). The average of stochastic simulations is almost entirely hidden by the value given in deterministic simulations. (C) Number of open K^+ channels in stochastic simulations using the binomial population algorithm (Mean value in blue, range in red) and in deterministic simulations (black).

a set of spherical compartments which are linked together with simple synapse models. It can potentially be used for more biophysically plausible simulations of neural networks.

2.5 Toward whole cell simulations

Modigliani is at a mature stage, where it can be used for a variety of simulations. The new additions make it possible to conduct various simulations without any prior programming knowledge. However, some limitations subsist. One of the hallmarks of Modigliani is its original propagation

algorithm (Faisal 2004). This algorithm is considerably faster than those used in other simulation software, but can only be used for one dimensional cables. While this is fine for simulating axons and dendrite, we can not use it for whole cell simulations because of the presence of branching points in axonal and dendritic trees. The outlook is therefore to implement a generic propagation algorithm, and use it in the presence of branches in the model.

3 Trade-offs in the design of myelinated axons

3.1 Introduction

Most of the axons in the mammalian PNS, and a large portion of those in the CNS are myelinated (Zhang and Sejnowski 2000). Myelinated axons are composed of long segments (length= L) covered by a multilamellar myelin sheath (Fig. 3.1). The myelin sheath is composed of layers of fat that wrap around the axon. Two consecutive myelinated segments are separated by an unmyelinated segment called a node of Ranvier. The nodes of Ranvier are short (length= l , typically $1\text{ }\mu\text{m}$) and regularly spaced along the axon. Each node is surrounded by two paranodal regions, where layers of myelin (produced by Schwann cells in the PNS and by oligodendrocytes in the CNS) are attached to the axon. The presence of the myelin sheath changes the electrical properties of the internodal region, greatly reducing the membrane capacitance. E.g. for a $10\text{ }\mu\text{m}$ wide myelinated fibre, the internodal membrane capacitance is 1000 times smaller than the capacitance at a node of Ranvier. The leak conductance is reduced by a factor of 200 by the myelin wraps. This makes the propagation of electrical signals between two nodes very fast. The action potential (AP) jumps between the nodes of Ranvier, hence the term “saltatory conduction”, (Fig. 3.2). This mode of conduction is the main reason for rapid conduction in myelinated axons (Rushton 1951).

Myelination has arisen independently several times in evolution, as a means of dramatically increasing propagation velocity without requiring large axons (Hartline and Colman 2007). AP

propagation velocity in myelinated axon is known to be proportional to the axon's diameter (Rushton 1951; Smith and Koles 1970; Hartline 2008), whereas in unmyelinated axons, the velocity is proportional to the square root of the axon's diameter. The higher propagation velocity of myelinated axons is hypothesised to enable larger body sizes in animals, because it allows maintenance of coordination between distant body parts (Zalc 2006). But the increased velocity of myelinated axons comes at the expense of volume, since a myelinated axon is 1.4 to 2 times larger than an unmyelinated one of comparable nerve diameter (Hildebrand and Hahn 1978). In case of the densely packed cortex, which contains 4 km mm^{-3} of axons (Braitenberg and Schütz 1998), replacing the wiring length of unmyelinated axons with myelinated axons would cause an increase in volume of 2 to 4 times. The increased volume will push neurons further apart from each other and the increased distance between neurons would cause higher conduction delays, partially nullifying the velocity gained thanks to myelinated axons.

From the point of view of basic biophysical constraints, the choice between myelinated and unmyelinated axon is therefore linked (at least) to a trade-off between volume and velocity. A complete understanding of the influence of myelination on these basic constraints (*speed*, *volume*, *noise* and *energy*) is necessary in order to answer the question of what factors determine the myelination, or lack thereof, of an axon. But although transmission delay (Hodgkin 1954; Rushton 1951), metabolic cost (Aiello and Bach-y-Rita 2000; Attwell and Laughlin 2001; Alle et al. 2009) and noise (Faisal et al. 2005; Faisal and Laughlin 2007; Faisal 2010; Faisal et al. 2008) in unmyelinated axons are well understood, there is comparatively less quantitative biophysical data available on these parameters in myelinated axons. Notably, Harris and Attwell (2012) present a comprehensive energy budget for the white matter of the brain, although their treatment of the metabolic cost of APs is based on theoretical estimates for a single diameter and the results extrapolated to the whole white matter. Variability in AP threshold (Hales et al. 2004) and its effect on propagation reliability (Li et al. 2010; Ochab-Marcinek et al. 2009) have been investigated using stochastic simulations of very simplified models.

3.1.1 The metabolic cost of myelinated axons

The metabolic cost of signal transmission through neurons is of utmost importance in the context of evolutionary selection (Laughlin et al. 1998; Laughlin 2001). Understanding the impact of metabolic conditions and anoxia/hypoxia on the CNS also requires quantitative data on the metabolic cost of neuronal activity (Nicholls and Budd 2000; Ritchie 1985; Zandt et al. 2011). Finally, an accurate interpretation of data obtained by functional magnetic resonance imaging (fMRI) requires knowledge of the effect of neuronal activity on the energetic needs of neurons, since the change in blood flow detected in fMRI is indicative of the change in energetical needs, and not directly the change in activity.

The metabolic cost of an AP is commonly defined as the Na^+ charge crossing the membrane during the AP (Attwell and Laughlin 2001). This is because although the propagation of AP does not require the consumption of ATP, reversing the ionic charges after each AP is accomplished by ionic pumps. These pumps consume ATP in order to expel Na^+ out of the cell, and bring K^+ back into the cell. The amount of Na^+ ions crossing the membrane during each AP thus determines the cost (in ATP) of APs, as the cost of reversing Na^+ and K^+ currents are roughly equivalent (Karbowski 2009), and much greater than that of reversing other ionic currents.

Previous studies used electrical properties of APs to estimate the metabolic cost (Attwell and Laughlin 2001). The minimum metabolic cost of an AP is given by the amount of ions necessary to charge the membrane capacity by the AP amplitude. This is a lower limit on metabolic cost. In practice, during an AP, there is some overlap between the depolarising Na^+ current, and the hyperpolarising K^+ current. This overlap results in a transfer of ionic charges without any effect on the membrane potential. As a result, the total amount of ionic charges crossing the membrane is higher than the theoretical minimum required (Hodgkin 1975; Attwell and Laughlin 2001; Hasenstaub et al. 2010). The impact of the overlap and other inefficiencies in axons is estimated to quadruple the metabolic cost of APs in the squid giant axon (SGA) (Hodgkin 1975).

Recent studies (Alle et al. 2009; Sengupta et al. 2010) have suggested that metabolic efficiency is very sensitive to the underlying ionic currents, which determine the overlap between opposing Na^+ and K^+ currents activation periods. Rapid decay of the Na^+ current, combined with delayed onset of K^+ current can thus enable some neurons (Alle et al. 2009; Sengupta et al. 2010) to fire APs in a much more metabolically efficient manner, bringing the true cost much closer to the theoretical minimum cost. In this study, we directly calculate the Na^+ charge crossing the membrane by summing the Na^+ current during the course of each AP.

Using both deterministic and stochastic simulations, we set out to compare myelinated and unmyelinated axons from the perspective of basic biophysical constraints. Our goal is, starting from first biophysical principles, to derive the quantitative criteria which govern the evolutionary selection of the type of axons used in different parts of an organism and predict their functional/structural trade-offs. These are then compared with unmyelinated axons. An early version of this work was presented at the *Twentieth Annual Computational Neuroscience Meeting* (Stockholm, Sweden 2011).

3.2 Methods

We are interested in the very basic design principles of the myelinated axon. We deliberately chose to keep our model as simple as possible, while retaining the fundamental qualities of the fibre: the myelinated axon is, at the most basic level, a succession of passive cables, linked to each other by what engineers call buffers or relays.

The non-homogeneity of these axons makes their simulation more difficult than that of a soma or unmyelinated axon. Many parameters in myelinated axons such as myelin thickness, and internodal length vary with diameter (Waxman 2001). The myelinated axon cannot, for instance, be described by a single specific membrane capacitance value that is the same for all axonal diameters. McIntyre et al. (2002) developed a model of mammalian PNG myelinated fibres, which has since been widely used as for simulations of myelinated axons (McIntyre et al. 2004;

Miocinovic et al. 2006). We use this model as a base to elaborate a model of a generic myelinated axon. Data for diameters other than those explicitly provided by McIntyre et al. (2002) is extrapolated using known relationships, as explained in the following.

3.2.1 Axon model

In our model, we simulate a membrane made of 3 types of compartments: Nodes of Ranvier cluster a large number of fast transient Na^+ channels, persistent Na^+ channels, and slow K^+ channels. Myelin attachment point (paranodes) regions contain fast K^+ channels, and internodes do not contain any ion channels (Fig. 3.1). In this model, the only difference between paranodes and internodes is the presence of K^+ ion channel in paranodes.

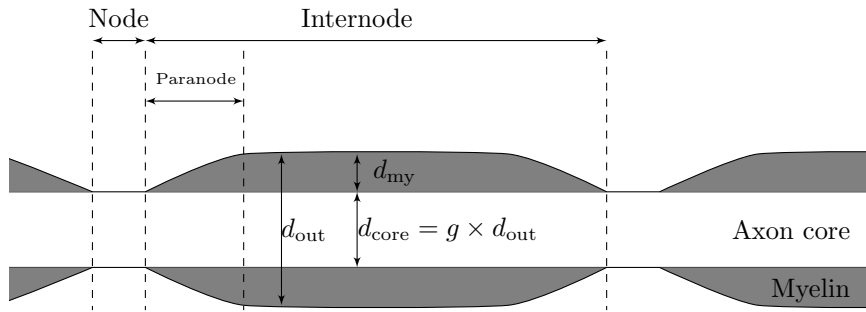


Figure 3.1: Basic axo-geometric characteristics of myelinated axons. Our model is made of 3 types of compartments. Nodal compartments are small and have a high density of Na^+ channels. Paranodal compartments are slightly longer, and contain fast K^+ channels. Internodes are passive.

Our goal is to provide a proof of concept biologically plausible axon, and to use it to estimate relevant biophysical quantities as well as to investigate the effects of a range of anatomical modifications.

Seven different fibre (outer) diameters were simulated: $0.3\ \mu\text{m}$, $0.5\ \mu\text{m}$, $1\ \mu\text{m}$, $2\ \mu\text{m}$, $5\ \mu\text{m}$, $10\ \mu\text{m}$ and $15\ \mu\text{m}$ (range of myelinated fibres in PNS). Although the diameter of the nodes of Ranvier is typically smaller than the diameter of the internodal region (Rydmark 1981; Rydmark and Berthold 1983), we chose to keep the same diameter over the length of the axon. There is also

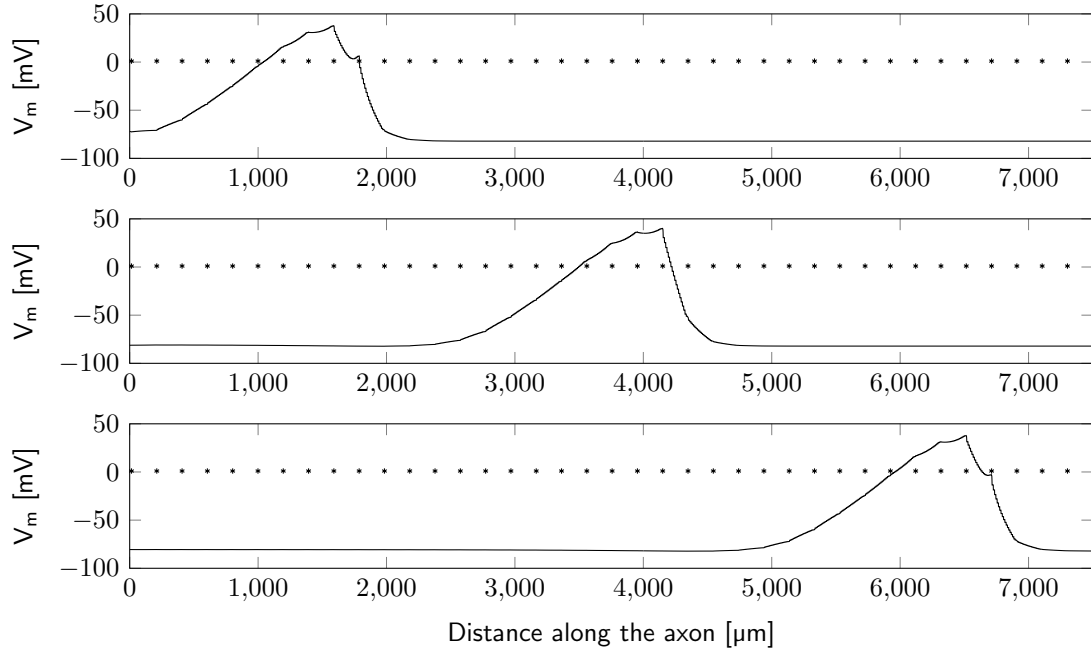


Figure 3.2: Saltatory conduction in our generic myelinated axon model ($d_{\text{out}}=1\mu\text{m}$). Each row represents a moment in time, $600\mu\text{s}$ apart. The AP propagates from left to right. Black dots represent the placement of node of Ranvier along the axon. The membrane potential has marked peaks on nodes of Ranvier. The bottom row shows a node of Ranvier depolarised in advance of the incoming membrane potential wave.

evidence that the difference in diameter is reduced (and eventually reversed, i.e the node of Ranvier become thicker than the internodal segment) for very thin myelinated axons (Tuisku and Hildebrand 1992).

In order to investigate the effects of myelination alone (as opposed to differences arising from different channel kinetics), we elaborated a model of an unmyelinated axon by using the nodal Na^+ and paranodal K^+ channels uniformly diffused along the axon. The density of channels was set to be the same as in the SGA model (Hodgkin and Huxley 1952; Faisal et al. 2005).

3.2.2 Anatomical and biophysical parameters

Our model of the generic myelinated axon is based on experimental data from mammalian peripheral nervous system (McIntyre et al. 2002) for which we derived diameter dependent

scaling relationships. This dataset contained data for axons of different diameters ranging from $5.7\text{ }\mu\text{m}$ to $16\text{ }\mu\text{m}$. As explained in the following, a number of adaptations were required, both to use the data in Modigliani, and to simulate smaller diameters. Basic parameters were kept the same as in the original model by McIntyre et al. (2002), and we cite the experimental data on which they are based for reference only.

Internodal length was based on experimental data from the cat ventral spinal root (Berthold et al. 1983), myelin lamellae count and core diameters were based on data from the same fibres (Berthold et al. 1983). The anatomical data is summarised in Table 3.1.

Outer diameter [μm]	5.7	7.3	8.7	10	11.5	12.8	14
Internodal length [μm]	500	750	1000	1150	1250	1350	1400
Myelin lamellae	80	100	110	120	130	135	140
Core diameter [μm]	3.4	4.6	5.8	6.9	8.1	9.2	10.4

Table 3.1: Anatomical data for myelinated axons. Data from McIntyre et al. (2002)

For the nodal capacitance, the authors used data from a frog (*Xenopus laevis*) myelinated fibre (Frankenhaeuser and Huxley 1964). Internodal capacitance was based on data from rats ventral root (Bostock and Sears 1978). Myelin capacitance and leak conductance per lamella were based on frog sciatic nerves (Huxley and Stämpfli 1949; Tasaki 1955). Electrophysiological data is summarised in Table 3.2. The parameters for our model of unmyelinated axon are summarised in Table 3.3.

Nodal Capacitance	C_m^{node}	$2\text{ }\mu\text{F cm}^{-2}$
Myelin capacitance	C_m^{myelin}	$0.1\text{ }\mu\text{F cm}^{-2}$
Axoplasmic resistivity	R_a	$70\text{ }\Omega\text{ cm}$
Myelin conductance	g_l^{myelin}	0.001 S cm^{-2}
Internodal axonal leak conductance	$g_l^{\text{internode}}$	0.0001 S cm^{-2}
Nodal leak conductance	g_l^{node}	0.007 S cm^{-2}

Table 3.2: Electrophysiological data for myelinated axons. Data from McIntyre et al. (2002)

Membrane Capacitance	C_m	$0.9 \mu\text{F cm}^{-2}$
Axoplasmic resistivity	R_a	$70 \Omega \text{ cm}$
Leak conductance	g_l	0.003 S cm^{-2}
Resting potential	E_{rest}	80 mV

Table 3.3: Electrophysiological data for our model of unmyelinated axon (McIntyre et al. 2002)

Fast Na^+ and slow K^+ conductance values are based on experimental data from human axons (Schwarz et al. 1995; Scholz et al. 1993). Persistent Na^+ conductance is based on data from human ulnar nerves (Bostock and Rothwell 1997). Resting potential is based on data from rat optic and tibial nerve myelinated axon (Stys et al. 1997). Ionic currents data are summarised in Table 3.4.

Maximum fast Na^+ conductance	$\bar{g}_{\text{Na}^+}$	3 S cm^{-2}
Maximum slow K^+ conductance	$\bar{g}_{\text{K}^+}^s$	0.08 S cm^{-2}
Maximum persistent Na^+ conductance	$\bar{g}_{\text{K}^+}^p$	0.01 S cm^{-2}
Na^+ Nernst potential	E_{Na^+}	50 mV
K^+ Nernst potential	E_{K^+}	-90 mV
Resting potential	E_{rest}	-80 mV

Table 3.4: Ionic current properties for myelinated axons. Data from McIntyre et al. (2002)

The model uses four voltage gated ion channel types. Persistent Na^+ channels are present with a density of $6.5 \mu\text{m}^{-2}$ in the node. Their conductance is 20 pS. They are modelled as a 4-state Markov chain, equivalent to the gating particle model of ($I_{\text{Nap}} = g_{\text{Nap}} \times p^3 \times (V - E_{\text{Na}^+})$), with $\alpha_p = \frac{0.0353 \times (V_m + 27)}{1 - e^{-(V_m + 27)/10.2}}$ and $\beta_p = \frac{0.000883 \times (-V_m - 34)}{1 - e^{(V_m + 34)/10}}$.

Fast Na^+ channels are present with a density of $2000 \mu\text{m}^{-2}$ in the node. Their conductance is 20 pS. They are modelled as an 8 state Markov chain equivalent to the gating particle model of ($I_{\text{Naf}} = g_{\text{Naf}} \times m^3 \times h \times (V - E_{\text{Na}^+})$), with $\alpha_m = \frac{6.57 \times (V_m + 20.4)}{1 - e^{-(V_m + 20.4)/10.3}}$, $\beta_m = \frac{0.304 \times (-V_m - 25.7)}{1 - e^{(V_m + 25.7)/9.16}}$, $\alpha_h = \frac{0.34 \times -(V_m + 114)}{1 - e^{(V_m + 114)/11}}$ and $\beta_h = \frac{12.6}{1 + e^{-(V_m + 31.8)/13.4}}$.

Slow K^+ channels are present with a density of $100 \mu\text{m}^{-2}$ in the node. Their conductance is 8 pS. They are modelled as a 2 state Markov chain equivalent to the gating particle model of ($I_{Ks} = g_{Ks} \times s \times (V - E_{K+})$), with $\alpha_s = \frac{0.3}{1+e^{(V_m+53)/-5}}$ and $\beta_s = \frac{0.03}{1+e^{-(V_m+90)}}$.

Finally, fast K^+ channels are present with a density of $12 \mu\text{m}^{-2}$ in the node. Their conductance is 17 pS. They are modelled as a 5 state Markov chain equivalent to the gating particle model of ($I_{Kf} = g_{Kf} \times n^4 \times (V - E_{K+})$), with $\alpha_n = \frac{0.0462 \times (V_m+83.2)}{1-e^{-(V_m+83.2)/1.1}}$ and $\beta_n = \frac{-0.0824(V_m+66)}{1-e^{(V_m+66)/10.5}}$. For our model of unmyelinated axon, ion channel related parameters are summarised in Table 3.5.

Na^+ channel density	80 channels / μm^2
K^+ channel density	20 channel / μm^2
Na^+ single channel conductance	20 pS
K^+ single channel conductance	8 pS
Na^+ Nernst potential (E_{Na^+})	50 mV
K^+ Nernst potential (E_{K^+})	-90 mV
Resting potential (E_{rest})	-80 mV

Table 3.5: Ionic current properties for unmyelinated axons.

Our simulations were run at 36°C , which is also the temperature for which McIntyre et al. (2002) developed their model and ran their simulations. Ion channel kinetics were scaled using Q_{10} values used in that paper¹. Q_{10} values and base temperatures are given in Table 3.6.

Parameter	Q_{10}	Base temperature [$^\circ\text{C}$]
Na^+ channel activation	2.2	20
Na^+ channel deactivation	2.9	20
K^+ channel	3.0	36

Table 3.6: Q_{10} values used for ion channel kinetics. Data from McIntyre et al. (2002).

1. The values used are not given in the paper, but can be found in the scripts available on ModelDB (Peterson et al. 1996).

3.2.3 Diameter-generic model of myelinated axons

The dataset contained data for the conductance and capacitance of one myelin layer, as well as those of the axonal membrane itself. In order to simulate the axon in Modigliani, we calculated the equivalent per surface values. The traditional way to calculate these values is to simply add capacitance and conductance in series, giving:

$$C_{sheath} = \frac{C_{my}}{\text{Number of lamellae}} \quad (3.1)$$

$$G_{sheath} = \frac{G_{my}}{\text{Number of lamellae}} \quad (3.2)$$

Where C_{my} is the per surface capacitance of one myelin lamella, and G_{my} the leak conductance of each lamella. However, these equations fail to account for the increase in surface as we get further from the axon. Outer myelin layers have a higher capacitance and leak conductance than inner layers. We define the capacitance of the i^{th} layer of the sheath as:

$$C_i = 2\pi L(d_{core} + id_{my})C_{my} \quad (3.3)$$

We capture this effect by multiplying each layer's per surface conductance by its own surface, and dividing the resulting value by the axonal membrane surface. The equations thus become:

$$C_{sheath} = \frac{1}{\sum_{i=1}^n \frac{d_{core}}{(d_{core} + id_{my})C_{my}}} \quad (3.4)$$

Similarly, we find the following equation for the leak conductance:

$$G_{sheath} = \frac{1}{\sum_{i=1}^n \frac{d_{core}}{(d_{core} + id_{my})G_{my}}} \quad (3.5)$$

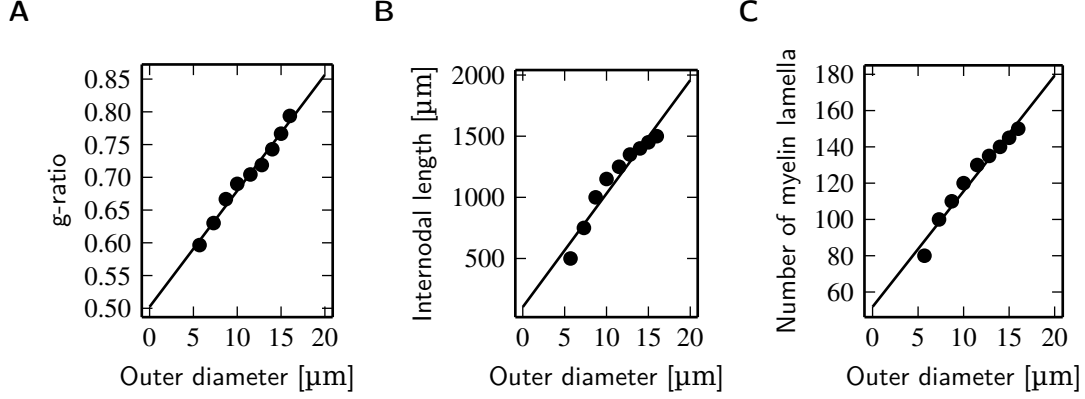


Figure 3.3: Extrapolation of anatomical data for myelinated axons. Black dots are given by McIntyre et al. (2002) based on experimental data (see text for details). We assumed that the g-ratio (**A**), internodal diameter (**B**) and number of myelin lamellae (**C**) were linear functions of the axonal diameter. Straight lines are given by fitting a line to the data, and are used in our model of generic myelinated axon.

We assumed that the distance L between two consecutive nodes changes proportionately to the diameter of the axon. Goldman and Albus (1968) suggest that the conduction velocity peaks on a broad maximum for $\frac{L}{d}$ between 90 and 200 (Brill et al. 1977). The linear dependence is also found in the experimental data set we used (McIntyre et al. 2002). The relevant values were extracted from available diameters (see Fig. 3.3), and assumed to hold for smaller axons (However, see Tuisku and Hildebrand 1992).

For myelinated axons, the g-ratio is defined as the ratio between inner and outer diameter:

$$g = \frac{d_{\text{core}}}{D_{\text{fibre}}} \quad (3.6)$$

Thus, $0 < g < 1$. The value of the g-ratio is commonly supposed to be constant, at 0.6 for PNS and between 0.77 and 0.8 for the CNS (Chomiak and Hu 2009). However, it has been suggested that the g-ratio might decrease for very thin axons (Tuisku and Hildebrand 1992). In our dataset, the g-ratio indeed varies linearly with diameter. We assumed this relationship to

hold for axons smaller than $5.7\mu\text{m}$ of diameter (see Fig. 3.3). Furthermore, we assumed that the number of myelin lamellae followed a linear relationship with the thickness of the myelin sheath, even though there is evidence that myelin lamellae become more compact the thinner the axon becomes (Simons and Trotter 2007).

We have thus constructed a model of a generic myelinated axon, which combines a significant number of findings on these axons. We validated this model using deterministic simulations. Our simulations reproduced the widths and amplitudes of AP waveforms as presented by McIntyre et al. (2002). Additional validation is provided by the AP propagation velocity, presented in Section 3.3.1.

For each diameter, we simulated a myelinated axon containing 40 nodes. The length of the axon studied is a crucial parameter for comparing the effects of noise in axons. Put simply, each section of the axon can independently generate a spontaneous AP. Therefore, the more segments there are (the longer the axon) the more spontaneous APs will be fired. However, in the case of myelinated axons, simply keeping the total axonal length constant would not provide a fair assessment of noise. This is because the internodal distance grows linearly with diameter. Thus, for the same total length, a thicker axon will have fewer nodes of Ranvier. Since channel noise is only generated in the nodal and paranodal regions, an axon having fewer nodes would exhibit fewer spontaneous APs, independent of its diameter.

Since we are interested in how noise scales with diameter in myelinated axons, we chose to keep the number of nodes constant. In order to have an accurate comparison between myelinated and unmyelinated axons, each unmyelinated axon was as long as the myelinated axon of the same outer diameter.

Simulations were carried out using the Modigliani stochastic simulation software available from <http://www.modigliani.co.uk> (Faisal et al. 2002). Axons were simulated for 300 ms. In each trial (10 total), an amount of current which was experimentally determined to reliably trigger APs was injected into the axon's extremity at 100 ms. The timestep and sampling frequency

were both 1 MHz for all simulations.

The propagation speed was calculated peak to peak. Metabolic cost was calculated by summing all Na^+ current during an AP, and subtracting the sum of Na^+ currents from a resting period of identical length. The sum was then divided by the total length to obtain the cost per length in cm.

3.3 Results

3.3.1 Our model reproduces AP features and velocity

We compare 3 features of APs generated by our model to experimental data. First, AP amplitude in our model was largely independent of diameter at approx. 120 mV (Fig. 3.4A). This is on par with the value reported by Schwarz et al. (1995) for a human myelinated axon at room temperature, or for a rat node of Ranvier both at 25 °C and 37 °C (Schwarz and Eikhof 1987). The amplitude of our APs is higher than those observed in lizard motor axons at room temperature (80 mV David et al. 1995).

Second, the width of APs generated by our model is in good agreement with APs recorded from nodes of Ranvier in rats at 37 °C by Schwarz and Eikhof (1987) (Fig. 3.4B) at approx. 0.2 ms. This value is lower than those observed at the same node (approx. 1.1 ms), or in human (Schwarz et al. 1995) and lizard motor axons (David et al. 1995) at 25 °C. This amount of variability is expected because of variation in ion channel kinetics due to temperature (Schwarz and Eikhof 1987).

The results from our simulations (Fig. 3.5, blue squares) show that the velocity in our simulated myelinated axons follows a linear relationship with the axonal diameter ($V[\text{ms}^{-1}] = 4.26d[\mu\text{m}]$, Fig. 3.5, blue line). It is worth noting that assuming a decrease in g-ratio as the axon becomes thinner, might lead us to underestimate the propagation velocity. For unmyelinated axons (Fig. 3.5, red squares), we can fit the velocity to the square root of the diameter ($V[\text{ms}^{-1}] =$

3 Trade-offs in the design of myelinated axons

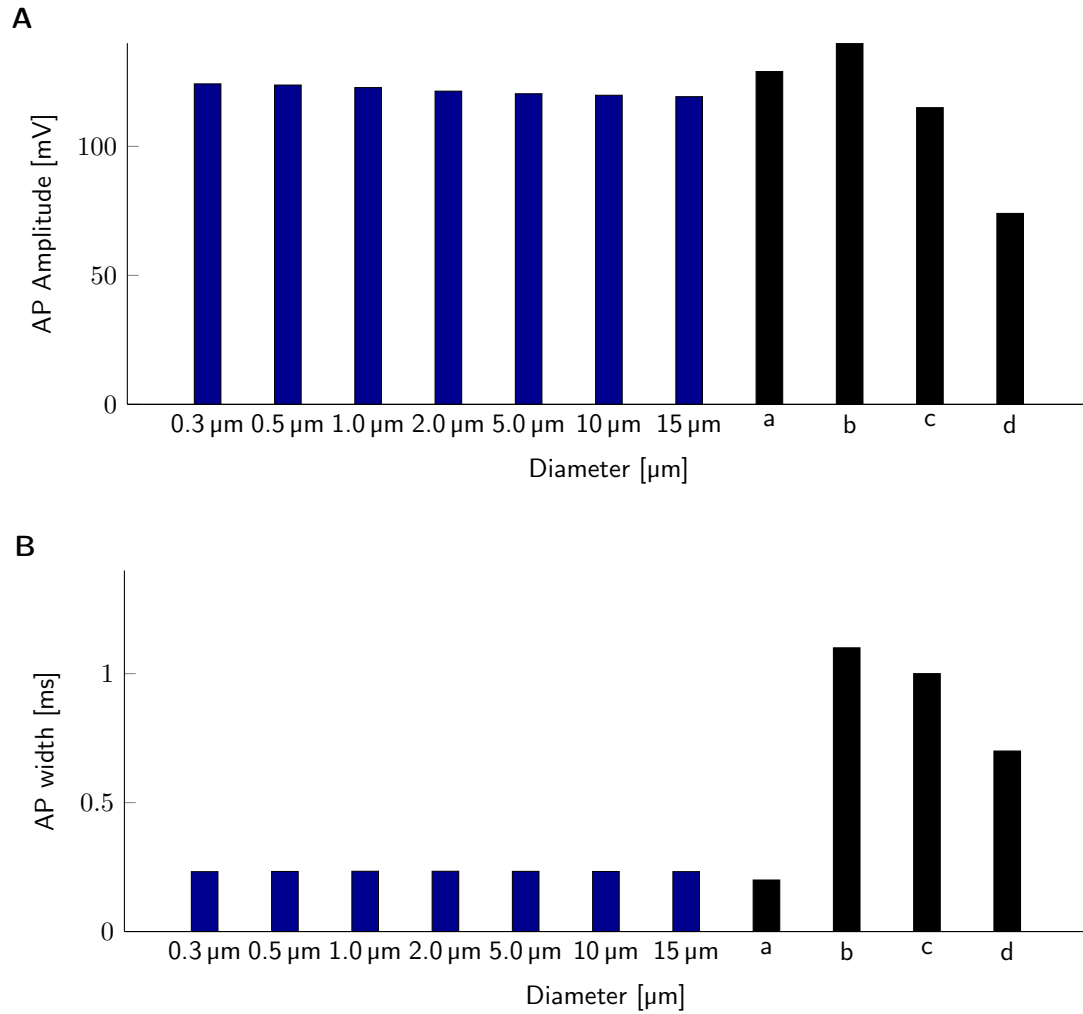


Figure 3.4: Simulated **(A)** AP amplitude and **(B)** AP width in nodes of Ranvier for different axonal diameter. Black bars represent experimental data from **(a)** rat myelinated nerve fibres at 37 °C (Schwarz and Eikhof 1987), **(b)** rat myelinated nerve fibres at 25 °C (Schwarz and Eikhof 1987), **(c)** human myelinated nerve fibres at 25 °C (Schwarz et al. 1995), and **(d)** lizard motor axons at 25 °C (David et al. 1995).

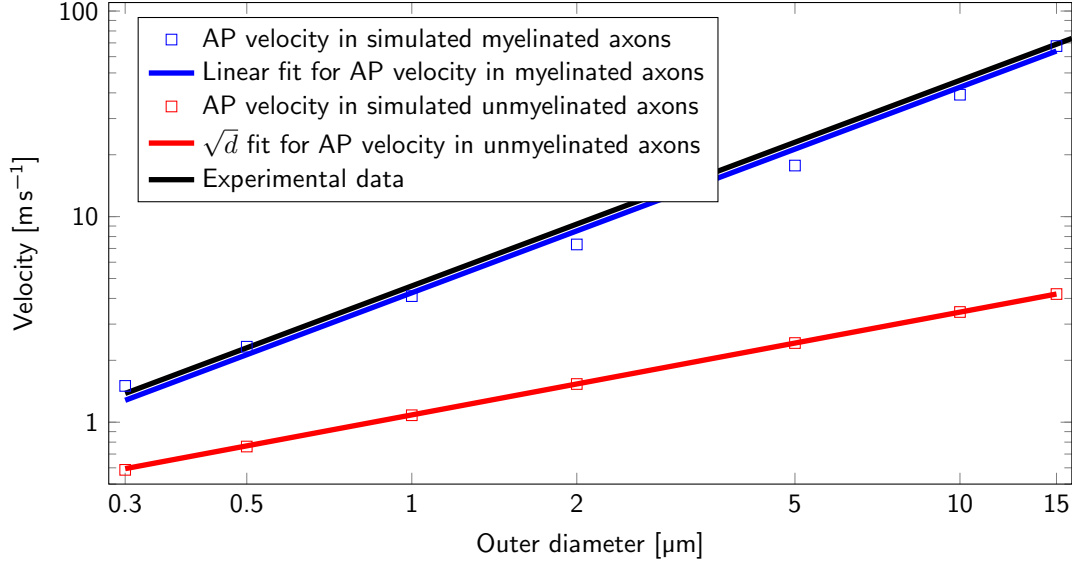


Figure 3.5: Action potential propagation velocity in myelinated and unmyelinated axons of same outer diameter. Thick lines are obtained by fitting data from simulation using a linear (blue) and a square-root (red) model. The black line represents experimental data from Boyd and Kalu (1979). Note the logarithmic scale.

$1.08\sqrt{d[\mu\text{m}]}$, Fig. 3.5, red line).

McIntyre et al. (2002) use data from cat hind limb (Boyd and Kalu 1979) in order to verify the velocity of APs in their model. Our somewhat simplified model reproduces the experimentally observed velocity for smaller diameter axons ($V = 4.6 \times d$) very faithfully (Fig. 3.5, the blue line compared to the black line).

We find that even for very small axons (0.3 μm outer diameter) the myelinated axon is faster than its unmyelinated counterpart for the same outer diameter (Fig. 3.5, leftmost squares). Our simulations suggest that an unmyelinated axon can be replaced with a myelinated axon of identical outer diameter that would conduct APs faster. Although this is not a new result (see discussion), the observed relationships between diameter and velocity validate our model, and allow us to turn our attention to other biophysical constraints.

3.3.2 Metabolic cost of myelinated vs unmyelinated axons

The cost per distance of APs in unmyelinated axons grows linearly with diameter (Fig. 3.6, red squares and line). The linearity is expected, as both the membrane capacitance and all ionic currents in unmyelinated axons are proportional to the membrane surface area, and therefore to the diameter (Attwell and Laughlin 2001; Alle et al. 2009). Note that we do not consider the cost of building and maintaining the axon, but only the cost directly associated with signalling. Interestingly, the relationship between the metabolic cost and the diameter of the axon is not

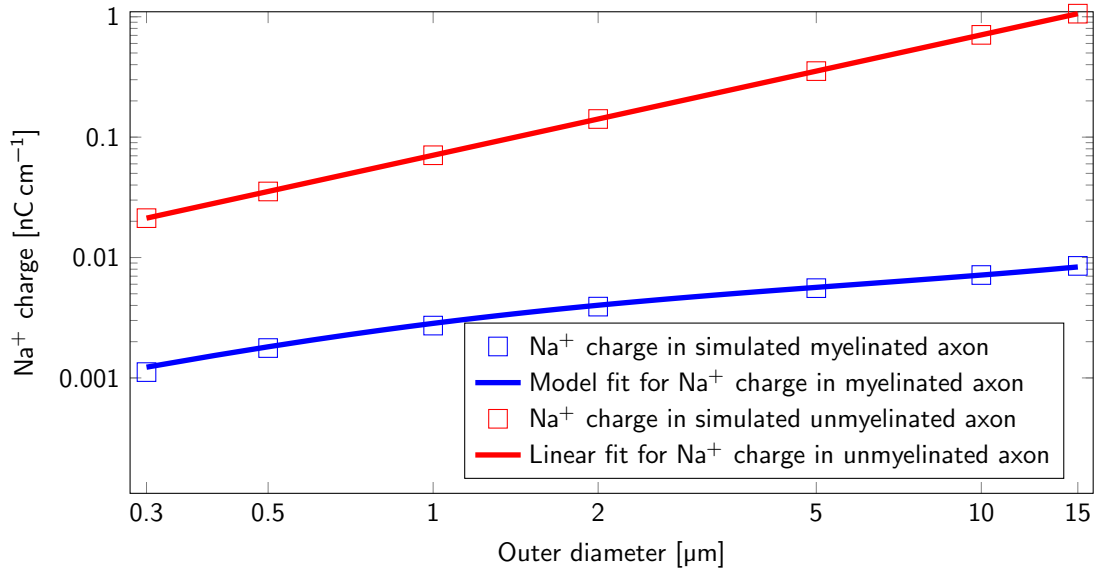


Figure 3.6: Metabolic cost of action potential in myelinated and unmyelinated axons of same outer diameter. The metabolic cost is defined as the Na^+ charge crossing the membrane during an action potential, from which we subtracted the Na^+ current during a period of no activity.

linear in myelinated axons (Fig. 3.6, blue squares and curve). In order to understand the non-linearity, we can use basic biophysical principles. We consider the myelinated axon as a sequence of unmyelinated axons, placed further and further apart as the diameter grows. The

total metabolic cost of transmitting an AP can be written as:

$$C_{\text{total}} = C_{\text{nodes}} + C_{\text{internodes}} \quad (3.7)$$

$$= C_{\text{nodes}} \quad (3.8)$$

$$= n_{\text{nodes}} \times C_{\text{node}} \quad (3.9)$$

Where C_{total} is the total cost of the AP, C_{nodes} the cost due to the nodes of Ranvier, $C_{\text{internodes}}$ the cost in the internodes (which is much smaller than that in the nodes, and nonexistent in our model), C_{node} the cost in a single node of Ranvier and n_{nodes} the number of nodes. C_{node} is proportional to the membrane surface area of each node S_{node} , which we can write as:

$$S_{\text{node}} = l\pi d_{\text{node}} \quad (3.10)$$

$$= l\pi d_{\text{out}}g \quad (3.11)$$

$$= l\pi d_{\text{out}}(g_0 + \alpha_g d_{\text{out}}) \quad (3.12)$$

Where S_{node} is the membrane surface area of a node of Ranvier, l the length of each node, d_{node} the nodal diameter, d_{out} the outer axonal diameter, and where we have replaced g with its diameter dependent expression $(g_0 + \alpha_g d_{\text{out}})$. By noting $n_{\text{nodes}} = \frac{\bar{n}_{\text{node}}}{l_0 + \alpha_n d}$ and substituting equation (3.12) in equation (3.9) we get:

$$C_{\text{total}} \sim \frac{\bar{n}_{\text{node}}}{l_0 + \alpha_n d} l\pi d_{\text{out}}(g_0 + \alpha_g d_{\text{out}})$$

Filling in with our anatomical data, we obtain the following expression:

$$C_{\text{total}} \sim \left(\frac{\alpha}{109 + 93d_{\text{out}}} \right) (0.5d_{\text{out}} + 0.017d_{\text{out}}^2) \quad (3.13)$$

The metabolic cost of propagating APs in myelinated axons (Fig. 3.6, blue squares). is not proportional to diameter, but can be much better fitted with our new model (Fig. 3.6, blue

line). This model has a single free parameter α . Its shape, for $\alpha = 1$, is plotted in Fig. 3.7. In the range of diameters of interest to us (between $0.1\ \mu\text{m}$ to $10\ \mu\text{m}$) the model has roughly the shape of a logarithm function.

The myelinated axon consumes less energy per AP, for all considered diameters. The difference is bigger the thicker the axon is. For the smallest diameters we simulated ($d_{\text{out}} = 0.3\ \mu\text{m}$), the unmyelinated axon is 20 times more expensive than the myelinated axon of equivalent outer diameter. For the largest axons considered ($d_{\text{out}} = 15\ \mu\text{m}$), the cost of the unmyelinated axon is almost two orders of magnitude higher than that of the myelinated axon. Overall, there is little doubt that although the difference is smaller in thinner axons, the metabolic cost of propagating signals in myelinated axons is much lower than that of unmyelinated axons of same outer diameter.

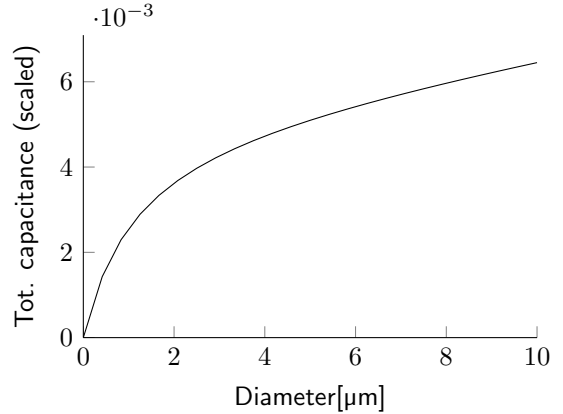


Figure 3.7: Shape of total capacitance model in myelinated axons. This curve can roughly be approximated with a logarithmic function.

We can estimate the efficiency of AP conduction in our model of myelinated axon by comparing the Na^+ charge transfer during one AP to the amount of charge necessary to depolarize the membrane up to the AP peak. This minimum cost is given by considering the total membrane capacitance over one node and the following internode. The results are plotted in Fig. 3.8.

The efficiency of our model unmyelinated axon is somewhere between that of the inefficient squid giant axon (25% Hodgkin 1975) and the efficient mossy fibres (77% Alle et al. 2009). The myelinated axon, on the other hand, is very inefficient (efficiency $\leq 10\%$). In order to understand this, we have plotted the AP waveform and Na^+ current in both type of axons (Fig. 3.9).

The shape of Na^+ current in the nodes reveals high reactivation of Na^+ channels in the repolarising phase of the AP in the nodes of Ranvier, compared to our model unmyelinated axon..

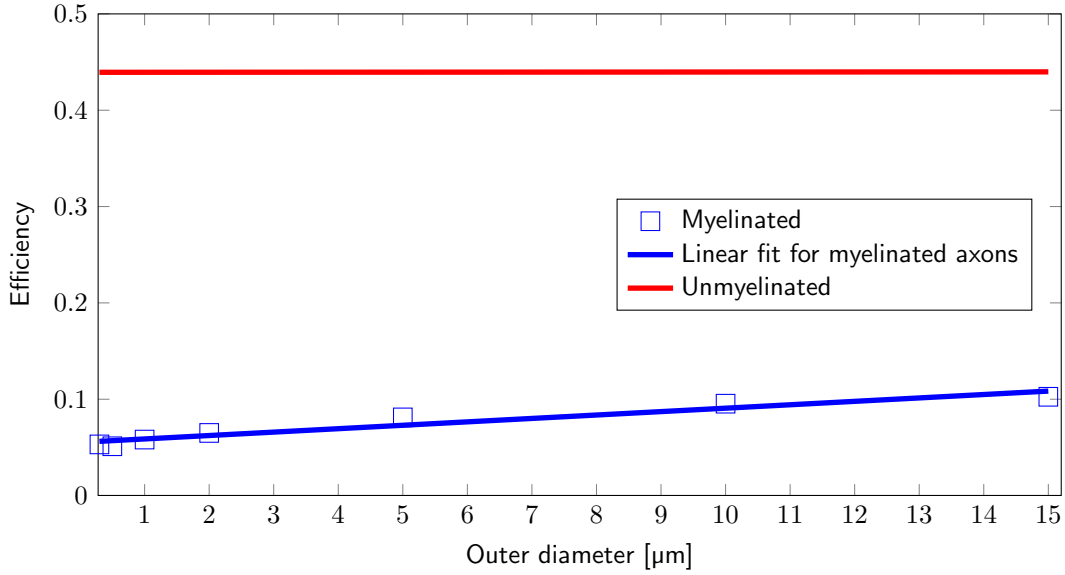


Figure 3.8: Action potential efficiency in myelinated axons of various diameter, compared to the efficiency in unmyelinated axons.

Reactivation of even a small number of Na^+ channels at this stage can result in a substantial current, because the membrane potential is far from the Na^+ reversal potential. The high density of Na^+ channels in the node of Ranvier, and the positive feedback provided by their opening is partially responsible for the reduced efficiency.

3.3.3 Noise in myelinated vs unmyelinated axons

Using stochastic simulations, we can assess the effects of channel noise in myelinated axons. Figure 3.10 shows the membrane potential in a single node of Ranvier of generic myelinated axons ($d_{\text{out}} = 0.5 \mu\text{m}$, $1 \mu\text{m}$, $1.5 \mu\text{m}$ and $2 \mu\text{m}$) over 10 trials (1 trial plotted in red).

For all axons, we inject a small amount of current (empirically determined to elicit one AP in the given axon) into the axon at the 80 ms mark in the figure. In the largest axon ($2 \mu\text{m}$ outer diameter, Fig. 3.10D), we observe a single AP triggered by the current injected. As we reduce the diameter, spontaneous APs are triggered. The amount of spontaneous APs increases as the diameter becomes thinner. For a $0.5 \mu\text{m}$ outer diameter axon (Fig. 3.10A), the rate of

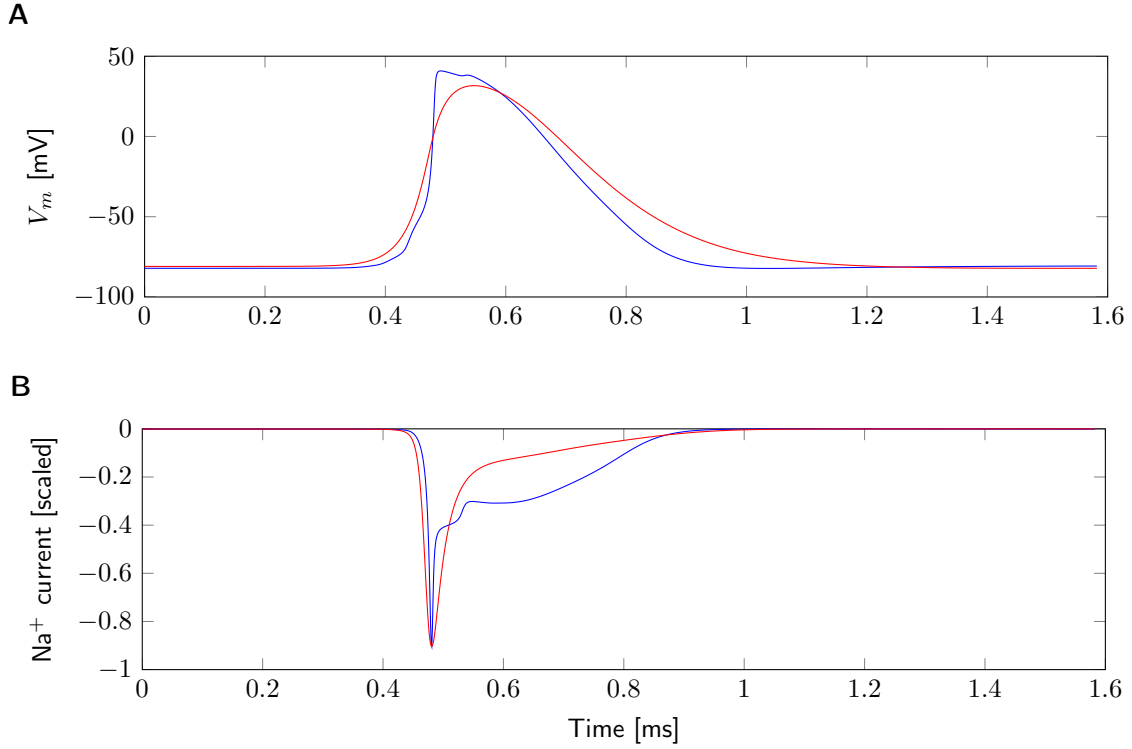


Figure 3.9: Na^+ current in myelinated axons has a more pronounced tail. **(A)** The AP waveform in an unmyelinated axon, compared to the AP waveform in a node of Ranvier. **(B)** Na^+ current in an unmyelinated axon compared to Na^+ current in a node of Ranvier. The currents have been scaled to have the same maximum amplitude.

spontaneous firing is approx. 100 Hz.

Surprisingly, our model of unmyelinated axon using the same ion channels is less affected by channel noise. Figure 3.11 shows the results of our stochastic simulations on unmyelinated axons. Even at $d=0.2\mu\text{m}$, our model of unmyelinated axon does not seem to suffer greatly from spontaneous firing (Fig. 3.11**B**). We verify that the ability of our unmyelinated model to conduct APs is intact by eliciting an AP at the 80 ms mark (in Fig. 3.11) which is reliably propagated.

The difference in the impact of noise in myelinated and unmyelinated axons is also visible in the spontaneous firing rate (Fig. 3.12). The spontaneous firing rate is much higher in very thin myelinated axons than in unmyelinated axons of same outer diameter.

3 Trade-offs in the design of myelinated axons

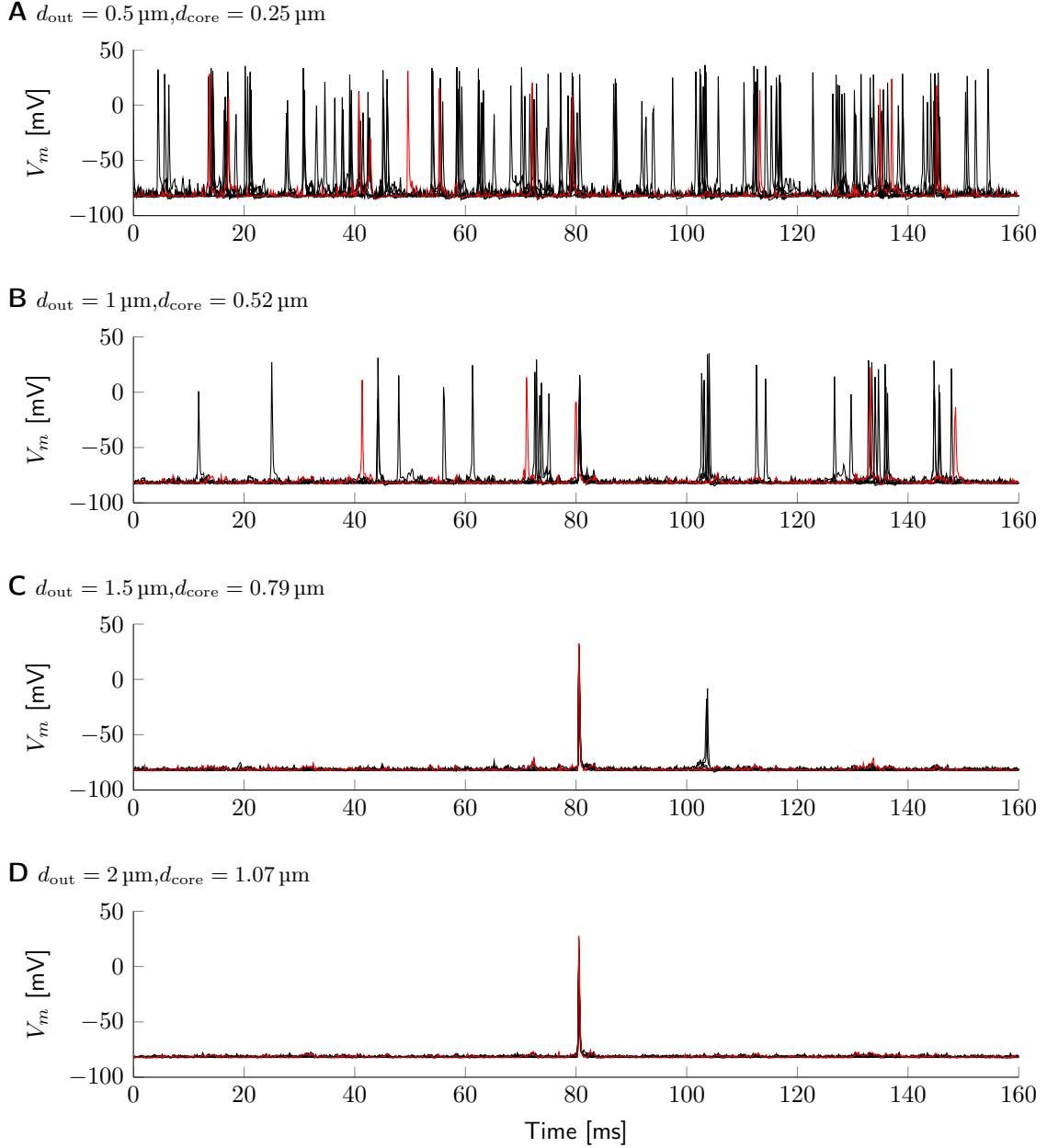


Figure 3.10: Spontaneous action potentials in small myelinated fibres. The results of 10 trials are superimposed (black). Results from the first trial are plotted in red. For all trials and all diameters, one AP was elicited at approx. 80 ms. All other APs are spontaneous. **(A)** Spontaneous APs in a $0.5 \mu\text{m}$ diameter myelinated axon **(B)** Spontaneous APs in a $1 \mu\text{m}$ diameter myelinated axon **(C)** Spontaneous APs in a $1.5 \mu\text{m}$ diameter myelinated axon **(D)** Spontaneous APs in a $2 \mu\text{m}$ diameter myelinated axon.

3 Trade-offs in the design of myelinated axons

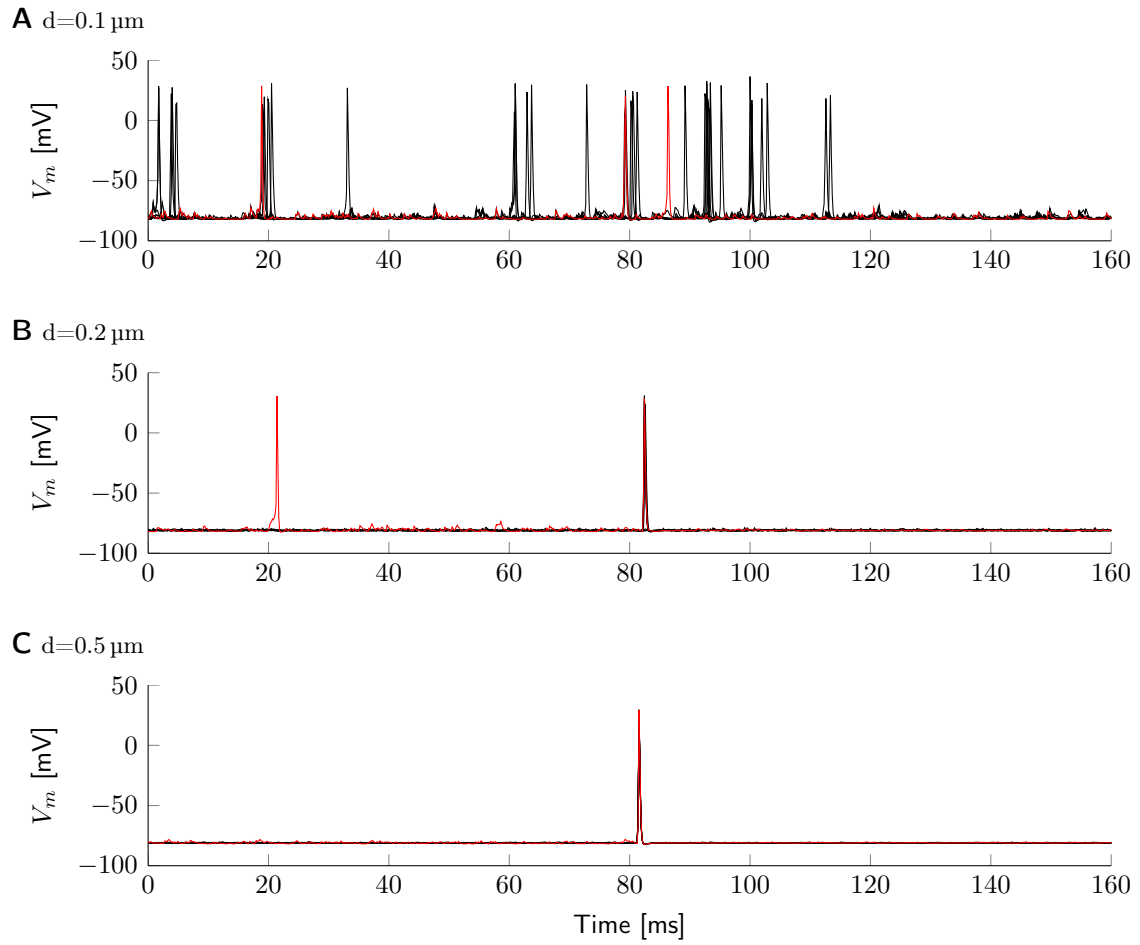


Figure 3.11: Spontaneous action potentials in small unmyelinated axons. The results of 10 trials are superimposed (black). Results from a single trial are plotted in red. For all trials and all diameters, one AP one elicited at approx. 80 ms. All other APs are spontaneous. **(A)** Spontaneous APs in a $0.1 \mu\text{m}$ diameter unmyelinated axon **(B)** Spontaneous APs in a $0.2 \mu\text{m}$ diameter unmyelinated axon **(C)** Spontaneous APs in a $0.5 \mu\text{m}$ diameter unmyelinated axon.

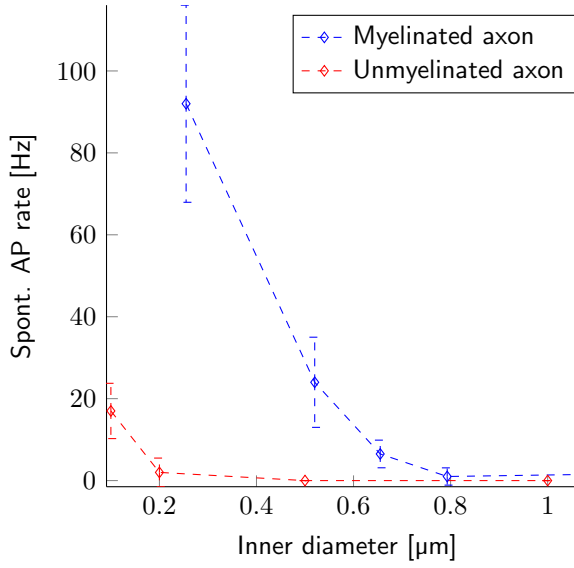


Figure 3.12: Spontaneous AP rate in myelinated and unmyelinated axons of same inner diameter. The rate is calculated from 10 trials, each trial lasting 200 ms (only the last 160 ms are plotted). For details on the length of axons see Section 3.2.3. Mean and SD plotted. The smallest unmyelinated axon ($0.1 \mu\text{m}$ diameter) has approximately the same spontaneous firing rate than a myelinated fibre of $0.5 \mu\text{m}$ outer diameter.

3.3.4 Robustness of results to parameter variation

In order to verify the robustness of this effect, we simulated a $0.5 \mu\text{m}$ diameter axon by varying some parameters. The results are plotted in Fig. 3.13.

Doubling the density of K^+ channels in the paranode had a very small effect on the rate of spontaneous firing, whereas a higher density of Na^+ channels (we only varied the density of transient Na^+ channels) dramatically increased the likelihood of spontaneous APs. Activation and deactivation of ion channels imply conformational changes of charged segments of proteins. The movement of charged segments consumes some energy which would otherwise have charged up the membrane. Therefore, channel gating acts as a capacitance. Its maximum value has been estimated at approx. $0.15 \mu\text{F cm}^{-2}$ for the squid giant axon with approx. 60 Na^+ channel μm^{-2} (Bezanilla et al. 1982), or 1 nF mS^{-1} (Crotty and Levy 2007; Sangrey et al. 2004; Sengupta et al. 2010), which in case of our model of the node of Ranvier is $1.5 \mu\text{F cm}^{-2}$. It is unlikely that such a huge change (250%) in membrane capacitance would have been overlooked in studies measuring the membrane capacitance of nodes. In order to investigate the effects of channel gating capacitance, we increased the nodal membrane capacitance by $0.45 \mu\text{F cm}^{-2}$, corresponding to 3 times the maximum voltage-dependent capacitance in the squid giant axon. This only had

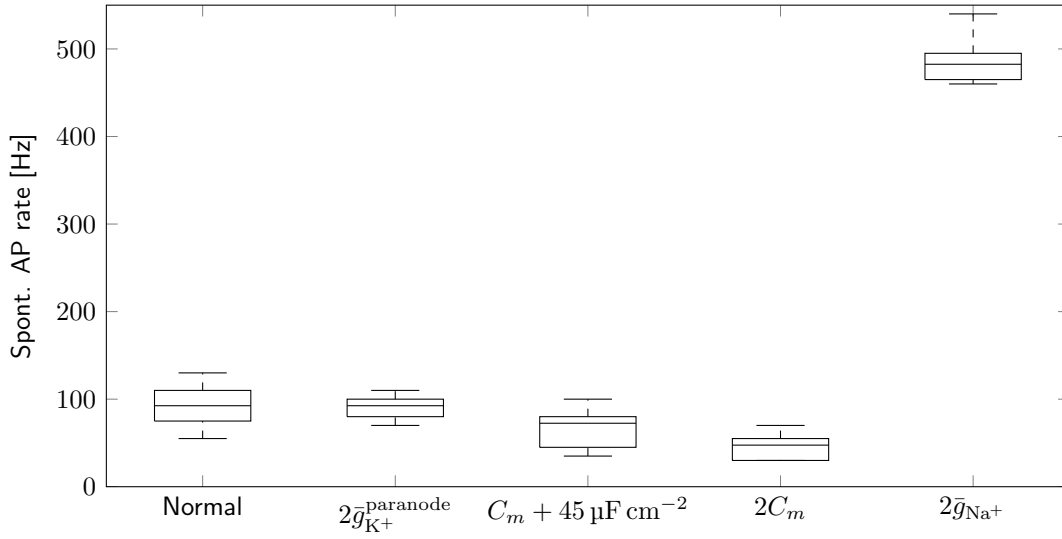


Figure 3.13: Influence of parameters on the spontaneous firing rate in a 0.5 μm outer diameter myelinated axon. Parameters varied are: density of K^+ channels in the paranode, nodal membrane capacitance (adjusted for the channel gating capacitance equal to 3 times the value in the squid giant axon, and also doubled) and the density of Na^+ channels in the node of Ranvier. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually (no outliers in this figure).

a moderate effect on the rate of spontaneous firing (approx. 70 Hz instead of approx. 100 Hz). But even with the nodal capacitance doubled ($2 \mu\text{F cm}^{-2}$), the spontaneous firing rate was still above 50 Hz. Note that such a huge change in membrane capacitance is not plausible (Faisal et al. 2005, supplementary material).

Finally, we investigated the impact of lower resting potentials by varying the leak reversal potential (E_{leak}). For $E_{\text{leak}} = -90 \text{ mV}$, the resting potential was -90 mV and no spontaneous APs were observed. However, APs triggered by the current impulse did not reach the fifth node. At $E_{\text{leak}} = -86 \text{ mV}$ the resting potential was approx. -86 mV . The triggered AP was not transmitted in 7 trials out of 10. In 8 trials, we observed spontaneous APs. Pooling data from all 10 trials results in a spontaneous AP rate of approx. 10 Hz. Increasing E_{leak} from its baseline value can only increase the spontaneous firing rate and was not considered in this analysis.

3.3.5 Deriving the lower limit on axon diameter

In order to quantify the higher sensitivity of myelinated axons to noise, we use to the original analysis regarding the limits set by noise on the diameter of unmyelinated axons in Faisal et al. (2005). The following expression gives the number of ion channels that need to open spontaneously in order to trigger a spontaneous AP in an unmyelinated axon.

$$n_0 = \frac{\pi}{4} d^{3/2} \frac{V_{AP}}{\gamma_{Na^+} E_{Na^+}} \frac{1}{\sqrt{R_m R_a}}$$

Where V_{AP} is the AP generation threshold, γ_{Na^+} is the conductance of a single Na^+ channel, E_{Na^+} is the Na^+ reversal potential, R_m the membrane resistance and R_a the axoplasmic resistance. The critical diameter is defined as the diameter where $n = 1$, i.e. the diameter for which the random opening of a single ion channel for indefinite time can trigger an AP.

$$d_{crit} = \left(\frac{4}{\pi} \gamma_{Na^+} \frac{E_{Na^+}}{V_{AP}} \sqrt{R_m R_a} \right)^{2/3} \quad (3.14)$$

Note that using this definition, the density of Na^+ channels does not play any role. Applying this equation directly to the myelinated axon, or more precisely, a node of Ranvier, will thus yield the same value for the critical diameter. But, higher densities of Na^+ channels necessarily lead to higher rates of spontaneous APs (Faisal et al. 2005). This density is relatively unchanged on unmyelinated axons, typically lower than 100 channels/ μm^2 (Hille 1992). This range of density is conserved across different taxa and the CNS-PNS divide, which allows equation (3.14) to retain its validity. In the case of the mammalian nodes of Ranvier, where the Na^+ channel density is typically 2000 channels/ μm^2 (Waxman and Ritchie 1985), thus 20 times higher or more, the obtained critical diameter is not necessarily accurate. Indeed, an earlier analysis by Chow and White (1996) yields a formula for the spontaneous firing rate which does depend on the density of Na^+ channels, although it is not obvious how to transform the firing rate into a critical diameter.

We can generalise equation (3.14) to nodes of Ranvier by considering the probability of spontaneous APs. For a cylindrical compartment of length l and diameter d , assuming there are N_{my} channels in a node, and n_{my} of those channels need to open spontaneously to trigger an AP, we can write:

$$p(\text{spontaneous AP}) = p(\text{at least } n \text{ channels open}) \quad (3.15)$$

$$= \sum_{i=n_{\text{my}}}^{N_{\text{my}}} p(i \text{ channels open}) \quad (3.16)$$

$$= \sum_{i=n_{\text{my}}}^{N_{\text{my}}} \binom{N_{\text{my}}}{i} p^i (1-p)^{N_{\text{my}}-i} \quad (3.17)$$

Where p is the probability for one channel to open spontaneously. For the node of Ranvier in thin myelinated axons, $20 \leq N_{\text{my}} \leq 100$, as given by multiplying the membrane surface area by the density of Na^+ channels. Approximating the binomial distribution to a Gaussian distribution, we can write:

$$p(\text{spontaneous AP}) \approx Q\left(\frac{n_{\text{my}} - N_{\text{my}}p}{\sqrt{N_{\text{my}}p(1-p)}}\right)$$

Where Q is the tail probability of the Gaussian distribution. We can now try to reflect changes in the density of ion channels, and hence in N , into changes in n . This will allow us to use equation (3.14) with the updated n value, which will take into account the increased density.

$$Q\left(\frac{n_{\text{my}} - N_{\text{my}}p}{\sqrt{N_{\text{my}}p(1-p)}}\right) = Q\left(\frac{n_{\text{unmy}} - N_{\text{unmy}}p}{\sqrt{N_{\text{unmy}}p(1-p)}}\right) \quad (3.18)$$

$$= Q\left(\frac{n_{\text{unmy}} - (N_{\text{my}}p/10)}{\sqrt{N_{\text{my}}p(1-p)/10}}\right) \quad (3.19)$$

$$= Q\left(\frac{n_{\text{unmy}} - N_{\text{my}}p}{\sqrt{10N_{\text{my}}p(1-p)}}\right) \quad (3.20)$$

Assuming $N_{\text{my}} \sim 10 \times N_{\text{unmy}}$. Q is a bijective function, which allows us to derive:

$$n_{\text{my}} = \frac{n_{\text{unmy}}}{\sqrt{10}} + N_{\text{my}}p\left(1 - \frac{1}{\sqrt{10}}\right) \quad (3.21)$$

Assuming $p \ll 1$, we can write $n_{\text{unmy}} \approx n_{\text{my}}\sqrt{10}$. In other words, in order to compensate for an increase in the density of Na^+ channels, we need to increase n in equation (3.14) by a factor of $\sqrt{\frac{\rho}{\rho_{\text{unmy}}}}$, where ρ is the actual density of Na^+ channels, and ρ_{unmy} the typical density in unmyelinated axons.

The change in n causes the critical diameter to change by a factor of $\frac{\rho}{\rho_{\text{unmy}}}^{\frac{1}{3}}$. Figure 3.14 shows the shape of this function for values of ρ between 1 and 20.

Replacing equation (3.21) in equation (3.14) yields a critical diameter of approx. $2.15 \mu\text{m}$ for the node, which translates into an outer diameter of approx. $0.42 \mu\text{m}$ assuming a g-ratio of 0.5. In Fig. 3.12, the $0.4 \mu\text{m}$ outer diameter myelinated axon has indeed roughly the same spontaneous firing rate than the $0.1 \mu\text{m}$ diameter unmyelinated axon.

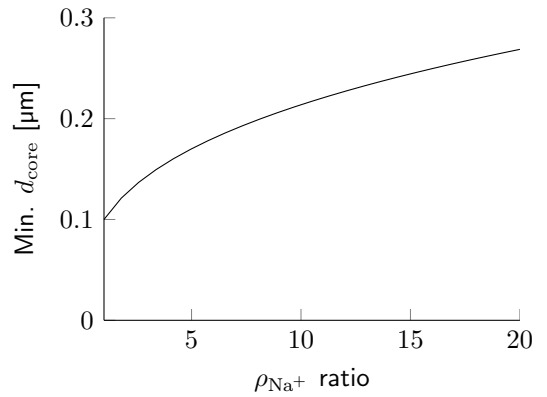


Figure 3.14: Minimum internal (d_{core}) diameter for myelinated axons as a function of $\frac{\rho_{\text{Na}^+}}{\rho_{\text{unmy}}}$.

3.4 Discussion

The central question in our work on myelinated axon is a simple one: what factors influence the choice of a myelinated axon over unmyelinated ones.

In other terms, can we exhibit design principles that would allow us to predict whether an axon should be myelinated, based on its location and function?

The answer to this question is known to depend on the diameter of axons. Larger axons tend to be myelinated, whereas smaller axons, tend to be unmyelinated (Waxman and Bennett 1972; Wang et al. 2008). We have developed a model of a generic myelinated axon, which takes into account a large body of literature on the influence of diameter on anatomical features of myelinated axons. Using this model, we can generate and simulate myelinated axons with a wide

range of diameters. Our simulations reproduce experimentally observed features of APs such as amplitude, width, and propagation velocity. This model allowed us to capture the influence of diameter on biophysical constraints, and to compare myelinated and unmyelinated axons in a principled way.

AP propagation velocity is the natural candidate. The fact that myelinated axons conduct APs faster has been known for a very long time (Rushton 1951; Smith and Koles 1970), and the mechanisms which allow this prowess are equally well characterised (Huxley and Stämpfli 1949; Fitzhugh 1962). Myelination is thought to have arisen specifically to solve the problem of accurate coordination of remote body parts without requiring huge axons like the squid giant axon (Hartline 2008).

This, however, brings us to another question. Why are all axons not myelinated? After all, there is no penalty for transmitting information too quickly. One possibility is that although myelinated axons propagate APs faster, this feature comes at the expense of volume. A myelinated axon is 1.4 to 2 times larger than an unmyelinated one of comparable nerve diameter. In case of the densely packed CNS (Abeles 1991), replacing unmyelinated axons with myelinated ones would cause a larger overall volume and the increased distance between neurons would cause higher conduction delays. The unmyelinated axons of the CNS are thus commonly believed to be the product of a trade-off between conduction delay and volume (Wen and Chklovskii 2005, 2010).

We can rephrase our initial question by eliminating the volume constraints, which cannot adequately be assessed from the study of a single axon. We want to know what factors influence the choice between a myelinated or an unmyelinated axon if the total volume they can occupy is fixed. In other terms, if we imagine that a new connection is to be made between two neurons placed at a certain distance, and that we can accommodate a given volume for it, how should we decide whether to use a myelinated or an unmyelinated axon?

As the results of our simulations show, for a constant outer diameter, the myelinated axon is

faster than the equivalent unmyelinated axon. This means that even by eliminating the volume constraint, the myelinated axon is preferable.

Another explanation, first given by Rushton (1951), is that there is a critical diameter underneath which unmyelinated axons actually conduct APs faster than myelinated axons. This limit was first thought to be $1\text{ }\mu\text{m}$, based on observations from vertebrate PNS, where the smallest myelinated axons have a diameter of $1\text{ }\mu\text{m}$. Observations from the CNS (Bishop and Smith 1964; Adinolfi and Pappas 1968; Waxman et al. 1972) led Waxman and Bennett (1972) to revise this diameter to $0.3\text{ }\mu\text{m}$ (Waxman and Bennett (1972) talk about the minimum diameter of axons, although it is not absolutely clear, from our reading of that paper, if this limit refers to that of the inner or the outer diameter. By digitising their figure, I established the smallest plotted fibre to have a diameter of $0.3\text{ }\mu\text{m}$ which, given the rather large g ratio of approx. 0.8, translates into an inner diameter of approx. $0.2\text{ }\mu\text{m}$). This is confirmed by the studies they have cited, although some of them are currently difficult to access. More recent data (Tolhurst and Lewis 1992) and our own simulations seem to contradict this hypothesis. Our results are in good agreement with McIntyre et al. (2002) and given the observed linear dependence of velocity on diameter, we consider our velocities to be valid. There is no reason to believe that myelination leads to a loss of velocity for any diameter.

It is therefore necessary to consider other constraints on the choice of axon type. Metabolic cost places severe limits on neuronal activity (Attwell and Laughlin 2001), which suggests that the brain may optimise energy efficiency in axons (Alle et al. 2009) as a design principle (Faisal et al. 2005). The cost of propagating APs is much lower in myelinated axons than their unmyelinated counter-parts. It is also substantially lower than that of APs in very energy efficient in-vivo unmyelinated fibres (Alle et al. 2009; Sengupta et al. 2010). We also find that the cost of APs in myelinated axons does not grow linearly with axonal diameter, because the internodal distance itself is dependent on diameter. Therefore, using the average diameter of myelinated axons in order to estimate metabolic cost of signalling in a population of axons (Harris and Attwell 2012) might lead to inaccuracies.

The estimates for the cost of APs in myelinated axons derived by Harris and Attwell (2012) are based on the capacitive minimum Na^+ current needed to charge the membrane to the AP amplitude. They use the average diameter myelinated axon in the nerve in order to derive the cost for all myelinated axons in the nerve. Their estimate for the minimum Na^+ current amounts to $0.0021 \text{ nC cm}^{-1}$. Our myelinated axon with $0.77 \mu\text{m}$ inner diameter has 60 myelin lamellae instead of 6. This is due to the lower g-ratio and increased myelin compaction in thinner diameter axons (see Methods for details). Our estimate of the minimum Na^+ charge, at approx. $0.0002 \text{ nC cm}^{-1}$, is an order of magnitude lower than that of Harris and Attwell (2012). Note that our model has a comparable internodal length ($237 \mu\text{m}$ as opposed to $240 \mu\text{m}$ used by Harris and Attwell 2012).

In order to estimate the actual Na^+ current, Harris and Attwell (2012) multiply the minimum Na^+ current by 1.3 (suggested by Alle et al. 2009, resulting in 77% efficiency). This results in an effective Na^+ charge of $0.0027 \text{ nC cm}^{-1}$, whereas our simulations yield $0.0035 \text{ nC cm}^{-1}$ for a myelinated axon of $1.45 \mu\text{m}$ outer diameter (efficiency $\sim 7\%$). Although these estimates for the total Na^+ current are remarkably similar, they result in very different efficiency estimates because of differences between the number of myelin lamellae between our model (based on data from the PNS) and that (based on data from the optic nerve) used by Harris and Attwell (2012). The inefficiency of our myelinated axons seems due to a late reactivation of Na^+ channels, which may play a role in high frequency firing (Hallermann et al. 2012). In fact, the higher reactivation is consistent with the findings of Hallermann et al. (2012, figure 6). The efficiency of our simulated unmyelinated axons was approx. 45%, lower than that observed by Alle et al. (2009). This can be explained by the fact that we use ion channel kinetics from myelinated axons, which are not necessarily the most efficient in unmyelinated axons. Although the unmyelinated axons were more efficient than unmyelinated axons, the minimum charge transfer necessary in the former is much greater than that of the latter. Overall, unmyelinated axons were much more metabolically expensive than myelinated axons. The ration between the two costs is much higher in our simulations than estimated by Harris and Attwell (2012). This is due to in part to the fact

that they compare axons with identical inner diameter, whereas we maintain the outer diameter constant.

The low metabolic cost of conducting APs in myelinated axons could however be countered by the metabolic cost of producing and maintaining the myelin sheath surrounding the axon (Harris and Attwell 2012). In that case, the choice of myelination would appear as a trade-off between maintenance cost, and activity cost. This however, does not explain why very thin axon ($<0.2\text{ }\mu\text{m}$ diameter) are never myelinated (Waxman and Bennett 1972). A thin axon collateral, for instance, fires at the same rate as the myelinated main axon because they are driven by the same axon hillock. In the brain, the collateral could theoretically even be myelinated by the oligodendrocyte that already myelinates a nearby section of the main branch. This would reduce both the initial and the ongoing cost of myelination even further.

Channel noise is the main source of variability in axons. The effects of random opening and closing of ion channels are known to scale with diameter in unmyelinated axons (Faisal et al. 2005; Faisal and Laughlin 2007). The smaller the axon, the larger an effect an individual ion channel exerts on the membrane potential. Channel noise can have a significant impact on the axon's performance for neural communication. The variability in APs' arrival times in axons can prevent the use of spike times to encode information (Faisal and Laughlin 2007; Wang et al. 2008). This would require information to be encoded e.g. using the number of APs arriving during a time window, which is much more costly because it requires more APs. In other terms, the presence of error changes the optimal encoding of information (Balasubramanian et al. 2001) and drives it to more metabolically costly codes (Polavieja 2002).

Channel noise constrains the miniaturisation of neural fibres by imposing a lower limit on the diameter of axons (Faisal et al. 2005). Myelinated axons are generally believed to be less noisy, but noise in this context usually refers to the variability of axonal propagation velocity (Kuriscak et al. 2002). Hales et al. (2004) show that stochasticity of Na^+ channels results in variability in AP threshold in myelinated axons. Similarly, Ochab-Marcinek et al. (2009) show that Na^+ channel noise in nodes of Ranvier can enhance AP propagation by firing APs even with subthreshold

signals, although very high densities of channels are likely to trigger spontaneous spikes which interfere with evoked APs (Li et al. 2010). The high Na^+ channel density in nodes of Ranvier leads to a high frequency of spontaneous APs, and thus be a factor constraining the anatomy of myelinated axons (Franciolini 1987). Our calculations show that the high density of Na^+ channels in the nodes of Ranvier increases the critical inner diameter of the axon to approx. $0.2\text{ }\mu\text{m}$. This is a new finding, and provides a novel basis for the distribution of white matter. This limitation is confirmed by our simulations.

Further evidence for the existence of such a limit is given by experimental data. Based on data from 6 mammals (shrew, mouse, rat, marmoset, cat and macaque), Wang et al. (2008) show that the lowest myelinated axon diameter is thicker than the lowest unmyelinated axon diameter, i.e. $0.1\text{ }\mu\text{m}$ (Faisal et al. 2005), by $0.4\text{ }\mu\text{m}$ to $0.5\text{ }\mu\text{m}$. This places the limit at approx. $0.5\text{ }\mu\text{m}$ outer diameter, in good agreement with our prediction. The data from Waxman and Bennett (1972) suggests a lower limit of approx. $0.2\text{ }\mu\text{m}$ for the inner diameter, very close to the derived value of $0.21\text{ }\mu\text{m}$. It is worth noting that unlike the axons in our model, very thin CNS myelinated axons ($d_{\text{out}} < 0.4\text{ }\mu\text{m}$) have a markedly high g ratio (approx. 0.8). This is expected, as the "normal" g ratio (about 0.5) for those thin axons would have required a nodal diameter underneath the limit we derived. We hypothesize that the observed discontinuity in g -ratios is precisely due to the fact that the nodal diameter has a lower limit imposed by noise. Moreover, in the CNS at least, there are inhibitory markers on some axons, which prevent oligodendrocytes from forming a myelin sheath (Aggarwal et al. 2011). This could be the biological mechanism by which thin axons are not myelinated.

The advantages of myelinated axons stem from the low capacity, less permeable internodal region of myelinated axons, which is the main proponent of saltatory conduction. Previous studies suggest that the length of this region has a crucial impact on propagation velocity, and the observed lengths correspond to the maximum velocity for a given diameter (Brill et al. 1977). Shorter internodal distances are sub-optimal with regards to conduction velocity. Longer internodal distances, on the other hand, lead to a very small current reaching consecutive nodes,

which increases the time needed to charge the nodal membrane, and can lead to propagation failure because of increased signal attenuation.

APs can only travel such long distances passively thanks to the high density of Na^+ channels at each node, which provides enough amplification for the signal to be regenerated. However, the high number of Na^+ channels at each node also increases the probability that enough of them open stochastically for a spontaneous AP to be fired. If the critical factor on the limit of axon diameter is the probability of spontaneous APs, we have shown that the high Na^+ channel density in nodes of Ranvier increases the lower internal diameter limit by approx. 2, meaning that the thinnest nodes of Ranvier need to be at least $0.2\text{ }\mu\text{m}$ thick. The smallest outer diameter for myelinated fibres is therefore between $0.3\text{ }\mu\text{m}$ to $0.4\text{ }\mu\text{m}$, depending on the g-ratio (0.5 to 0.8). This limitation arises from a fundamental property of myelinated axons, and is unlikely to be remediable.

4 Axonal variability as a source of synaptic variability

4.1 Introduction

The great majority of axons use action potentials (APs) to transmit information reliably to synapses. Once the AP arrives at the synapse, the characteristics of its waveform are fundamental in determining the strength and reliability of information transmission, as was extensively shown in the central and peripheral nervous system of both vertebrates and invertebrates (Klein and Kandel 1980; Llinas et al. 1981, 1992; Coates and Bulloch 1985; Augustine 1990; Delaney et al. 1991; Wheeler et al. 1996; Llinas et al. 1982; Gainer et al. 1986; Spencer et al. 1989; Sabatini and Regehr 1997; Borst and Sakmann 1999; Niven et al. 2003; Augustine 2001). Although the nervous system exhibits variability (noise) at all levels (reviewed by Faisal et al. 2008), it is generally assumed that little variability affects the AP waveform itself as it travels from the soma along the axon to the synapse, which would enable it to transmit information encoded e.g. in the width (Shu et al. 2006; Aldworth et al. 2012) of the AP.

The AP is mediated by voltage-gated ion channels which control the flow of ionic currents through the membrane. Thermodynamic fluctuations in voltage-gated ion channels result in probabilistic gating, producing random electrical currents called channel noise (reviewed by White et al. 2000). In thin axons, the behaviour of individual ion channels have significant

effects on the membrane potential dynamics due to the higher input resistance of those axons. Fewer channels sustain AP conduction and fluctuations in individual ion channels have a larger impact on the membrane potential in thinner axons. Faisal et al. (2005) have shown that channel noise sets a lower limit to reliable axonal communication at 0.08 μm to 0.1 μm diameter, a general limit matched by anatomical data across species. Above this limit, in axons of 0.1 μm to 0.5 μm diameter, channel noise causes variability in the rising phase of the AP and the axon's resting input resistance. Therefore, APs are jittered, shifted, added and deleted in an history-dependent way along the axon (Faisal and Laughlin 2007).

Attempts at investigating the impact of axonal noise at the synapse have so far been limited to rather large diameter axons (Aldworth et al. 2012; Sasaki et al. 2012). However, many unmyelinated axons are very thin (0.1 μm to 0.3 μm diameter, Wang et al. 2008). Examples include cerebellar parallel fibres (average diameter 0.2 μm , Sultan 2000), C-fibres implicated in sensory and pain transmission (diameter range 0.1 μm to 0.2 μm , Berthold and Rydmark 1978) and cortical pyramidal cell axon collaterals (average diameter 0.3 μm , Braitenberg and Schütz 1998). These thin unmyelinated axons make up most of the local cortical connectivity (Braitenberg and Schütz 1998) but the variability of the AP waveform in them is unknown. Basic biophysical considerations suggest that axonal noise sources are bound to introduce fluctuations in the shape of the travelling AP waveform in thin axons with immediate consequences for synaptic transmission (Faisal et al. 2005; Sasaki et al. 2011). Intracellular recordings from such thin axons are difficult to obtain. Extracellular stimulation offers only limited signal resolution and stimulus control, and tiny intracellular volumes limit the application of imaging methods to quantify AP waveforms accurately. This motivated the study presented here which uses biophysically detailed stochastic simulations of travelling APs in thin axons. We quantify waveform fluctuations of single propagating APs in terms of standard measures, namely AP width and height. We characterise how channel noise causes AP waveform fluctuations and show that these fluctuations scale with axon diameter according to a power law. We then study AP waveform fluctuations in propagating spike trains and estimate the measurable post-synaptic response variability it

would cause using synaptic models and published experimental data.

This work is based on Faisal and Laughlin (2004) which showed preliminary results for the SGA. Building up on the original idea, I built and simulated three additional axon models, including a model of cortical fibres which exhibit briefer APs. The model for synaptic Ca^{++} concentration and vesicle release rate is also novel. We presented part of this work at the *Ion Channels in Health and Disease Symposium* (Cambridge, UK 2013). A paper based on this chapter was accepted for publication in PLoS Computational Biology on 27/03/2014.

4.2 Methods

The variability of the AP waveform was estimated using detailed stochastic simulation of thin unmyelinated axons, where the stochastic kinetics of ion channels were individually modelled (Faisal 2012).

4.2.1 Axon models

Simulations were based on biophysical data and reproduced physiological data such as the amplitude and width of APs (but see Naundorf et al. 2006, for other models). Computations were carried out using the Modigliani stochastic simulator (Faisal et al. 2002, 2005), available from <http://www.modigliani.co.uk>, on a Linux PC using an Intel core i7 processor with the binomial algorithm (Faisal 2010). Simulations were carried out using Markov models of squid giant axon (SGA) channels (Na^+ channel gene GFLN1, K^+ channel gene SqKv1.1) as several independently constructed kinetic models exist for these channels. We used the original models given by Hodgkin 18 and Huxley (Hodgkin and Huxley 1952) as well as a more recent model with delayed opening (Patlak 1991) with little difference in results. These ion channel models captured the corresponding ion channel kinetics from patch-clamp experiments. To account for differences between the SGA and mammalian axon (reviewed by Bean 2007; Sengupta et al. 2010), we

confirmed the results using models of rat hippocampal interneurons (RHI) with Markov models of rodent ion channels (Wang and Buzsáki 1996) shown to have little overlap between Na^+ and K^+ currents (Sengupta et al. 2010), and a model of rodent C-fibre axons (Nav1.8) (Baker 2005). We chose to simulate all model axons at the temperature at which their channel kinetics were experimentally recorded. The RHI model was chosen for synaptic simulations because of its base temperature value. The parameters for each model are summarised in Table 4.1.

Parameter	SGA-HH	SGA-Patlak	RHI	C-fibre
Membrane capacitance [$\mu\text{F cm}^{-2}$]	1	1	1	0.81
Axial resistance [$\Omega \text{ cm}$]	35.4	35.4	70	70
Leak conductance [mS cm^{-2}]	0.3	0.3	0.1	0.14
Na^+ single channel conductance [pS]	20	20	15	20
Na^+ channel density per μm^2	60	60	23	62.5
K^+ single channel conductance [pS]	20	20	14	17
K^+ channel density per μm^2	18	18	6	10
Leak rev. pot. [mV]	-54.4	-54.4	-65	-61.14
Na^+ rev. pot. [mV]	50	50	55	79.6
K^+ rev. pot. [mV]	-77	-77	-90	-85
Temperature [$^{\circ}\text{C}$]	6.3	6.3	35	24

Table 4.1: Simulation parameters

4.2.2 Stimulus protocols

We used two sets of simulation protocol. In the first protocol, $0.1 \mu\text{m}$, $0.2 \mu\text{m}$, $0.3 \mu\text{m}$ and $0.5 \mu\text{m}$ (1 cm long) and $1 \mu\text{m}$ diameter axons (2 cm long) were stimulated in a single spike per trial framework ($N=250$ trials per diameter) to allow for fast parameter exploration. In the second protocol, we simulated long spike trains of 10 minutes duration. To this end, a $0.2 \mu\text{m}$ diameter (2 mm long) axon was stimulated with a zero-mean white noise current ($\text{SD}=0.01 \text{ nA}$, 1 kHz

corner frequency) injected at the proximal end. Membrane properties were set to $R_a=70\ \Omega\text{cm}$, $R_m=20\,000\ \Omega\text{cm}^2$, typical for cortical cells (Koch 1999). All axons had a resting potential of -65 mV . After visual inspection of the data, we used a threshold discriminator detecting AP height and aligned their waveforms at the rising half-peak potential crossing time. We measured voltage-traces of the AP waveforms at regular intervals (typical distance=10% of total axon length. We used shorter distances in the middle of the axon. See Fig. 4.2A) between 5% and 95% of the axon's length (0% being the axon's proximal end) to avoid measuring stimulus artifacts or boundary effects and to measure the evolution of the AP shape along the axon. The height and width of APs were defined according to Fig. 4.2C.

4.2.3 Modelling synaptic transmission

We estimated the impact of action potential waveform variability on synaptic transmission using two approaches. First, synaptic transmission was modelled using data and deterministic models from the Calyx of Held synapse (Schneggenburger and Forsythe 2006). This allowed us to compute incrementally how AP waveform variability affects the various stages of synaptic signal transduction. First, we computed how the incoming AP waveforms drive Ca^{++} channels.

We drive the Calyx- of-Held synapse with noisy spike train waveforms directly (voltage clamping the synapse) to circumvent any potential issues of impedance mismatch. We then compute Ca^{++} currents evoked by the AP waveform by integrating the dynamics of a Hodgkin-Huxley type conductance-based Ca^{++} channel model of Calyx synapses (Borst and Sakmann 1998b). We describe the Ca^{++} channel behaviour using conductance-based Hodgkin-Huxley type model with two identical gating particles (denoted m) with channel opening probability $p_{Ca} = m^2$ with corresponding rate functions $\alpha_m(V) = 1.78 \exp\left(\frac{V_m}{23.3}\right)$, $\beta_m(V) = 0.14 \exp\left(\frac{-V_m}{15}\right)$ and dynamics $\frac{\partial m}{\partial t} = \alpha_m(V)(1 - m) - \beta_m(V)m$ (Borst and Sakmann 1998a). The voltage-gated Ca^{++} channel was modelled here deterministically, and the waveform of the incoming Ca^{++} current was calculated using a reversal potential of $E_{\text{Ca}^{++}} = 64.3\text{ mV}$. This Ca^{++} current model simplifies the heterogeneity in both the biophysical and the pharmacological properties of calcium currents

found in Calyx-type synapses (Wu et al. 1998, 1999), but was shown to capture sufficient detail for quantitative modelling of synaptic transmission (Borst and Sakmann 1998b).

The transient $[Ca^{++}]_i$ encountered by vesicles in the proximity of Ca^{++} channels is shown to follow a time course similar to that of the Ca^{++} current (Schneggenburger and Neher 2000; Bollmann et al. 2000), i.e. the Ca^{++} concentration rapidly decline due to effects such as buffering (Meinrenken et al. 2003). We use the dynamics of Ca^{++} channels and modify them slightly so that the rise time is conserved, but the width at half-height becomes approx. 100 μ s longer (Schneggenburger and Neher 2000). The resulting waveform is then scaled to have a peak of approx. 12 μ M (Müller et al. 2010). The resting $[Ca^{++}]_i$ was 50 nM.

Finally, we modelled the impact of Ca^{++} concentration on transmitter release using an allosteric "5 state" model (Lou et al. 2005), which allows us to derive the instantaneous vesicle release rate (Fig. 4.1). According to this model, each vesicle can attach to up to 5 Ca^{++} . The number of ions attached determines the release probability, and the probability of attaching to a Ca^{++} ion depends on the Ca^{++} concentration. We translate the attachment and release probabilities into rates, and simulate these processes using a simple forward Euler algorithm. The rate of Ca^{++} attachment is equal to $N_{\text{freesites}}k_{\text{on}}[Ca^{++}]$, where $N_{\text{freesites}}$ is the number of Ca^{++} ions the vesicle can attach to ($5 - N_{\text{Ca}^{++}\text{ attached}}$), and k_{on} the base rate. The rate of Ca^{++} detachment is equal to $N_{\text{Ca}^{++}\text{ attached}}k_{\text{off}}[Ca^{++}]$, where k_{off} is the base rate. The vesicle release rate is proportional to $f^{N_{\text{Ca}^{++}\text{ attached}}}$. Note that vesicles can release with any number of Ca^{++} ions attached, although more Ca^{++} result in higher release rates.

Synaptic transmission dependence on the AP waveform was also modelled using experimental data for the much smaller rodent cerebellar Granule cell-to-Purkinje cell synapse. In this synapse the width of the pre-synaptic AP waveform, pre-synaptic Ca^{++} entry and the resulting post-synaptic currents were directly measured (Sabatini and Regehr 1997). To obtain an estimate of how AP waveform variability would affect this synapse, we passed the simulated APs' widths through the experimentally characterised relationship between postsynaptic response and AP width. We then computed the variability of the post-synaptic response over all APs.

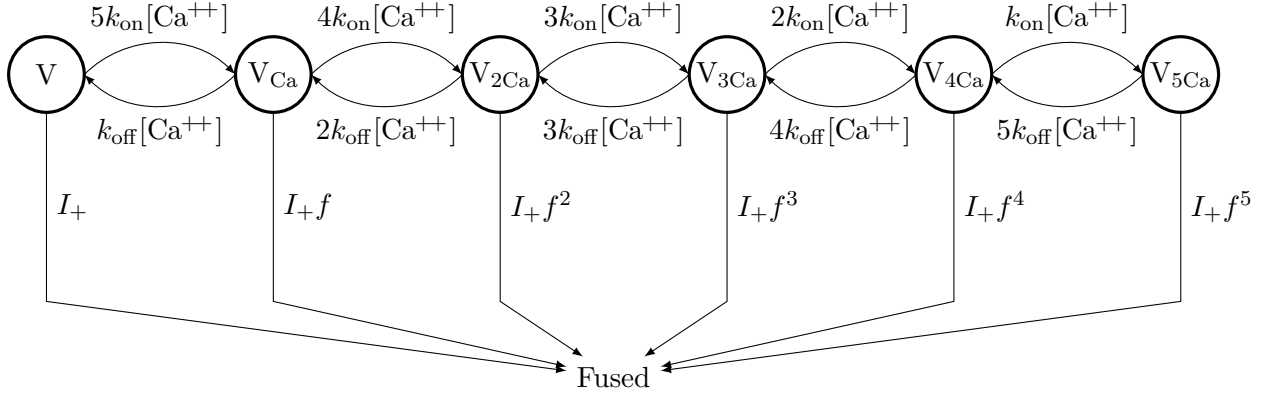


Figure 4.1: This model is used to estimate the vesicle release rate as a function of local transient Ca^{++} concentration. Transition rates between states depend on Ca^{++} local concentration in the vicinity of the vesicles. The release probability is higher the more Ca^{++} ions are bound to the vesicle, according to a factor $f = 31.3$. The base rate constant I^+ is set to $2 \times 10^{-4} \text{ s}^{-1}$.

4.3 Results

4.3.1 Action potential waveform variability

Stochastic simulations show that single APs propagating in axons of up to $1 \mu\text{m}$ diameter display large random variability in their membrane potential waveform as they propagate. We visualise this by measuring the AP waveform (membrane potential versus time) at various positions along the axon (Fig. 4.2A) and then align the waveforms at the instant of half-peak crossing. As a control, we simulated a deterministic axon, i.e. one that had the same set of biophysical parameters and stimuli but where we modelled the same ion channels as Hodgkin-Huxley-like deterministic conductances instead of the more biophysically realistic stochastic conductances. We observed no such wave form variability in the deterministic simulations. Since the stochastic kinetics of ion channels are the only source of variability given that all other parameters and stimuli are controlled, the variability of the travelling AP waveform observed here must be due to channel noise and thus entirely random in nature. Note, the AP wave form is not only variable across identical repeated trials with identical stimulus, but also varies as the same AP propagates along the axon.

4 Axonal variability as a source of synaptic variability

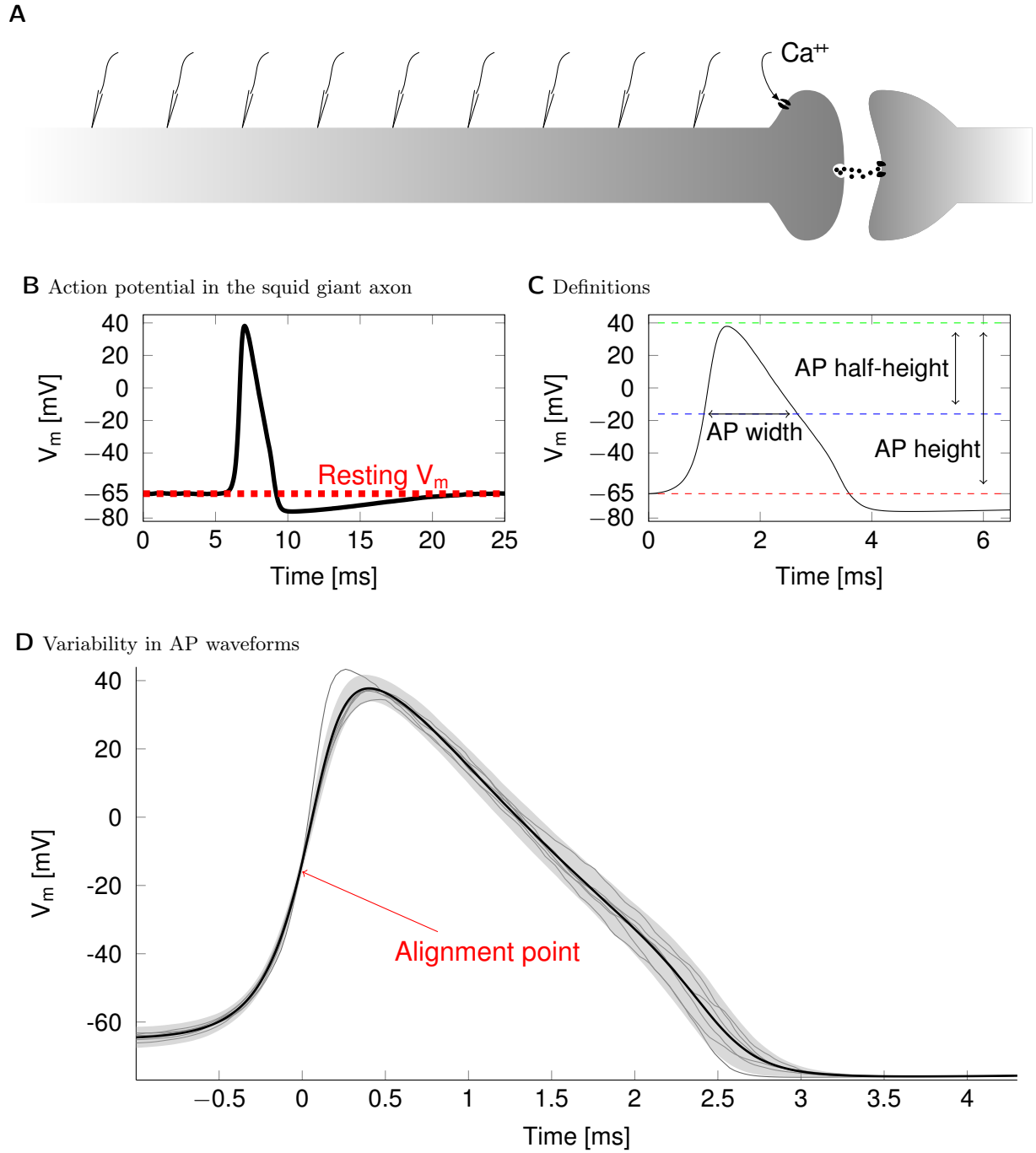


Figure 4.2: Definitions and methods **(A)** We record membrane potential and ionic currents at regularly placed points along the axon, and compare the **(B)** shape of the AP waveforms (black) and the resting membrane potential (dashed red). **(C)** Action potential features are determined individually for each AP. The amplitude of the AP is defined as the maximum membrane potential. The width is the delay between the crossings of the mid-height level. **(D)** AP shape fluctuations. Due to channel noise, APs triggered in an identical fashion will have different shapes across trials. Here APs in a $0.2\ \mu\text{m}$ diameter axon from 5 trials out of 250 are superimposed. The point at which the membrane potential crossed the half-height line is used to align APs.

Comparing the variability of the AP wave form in axons with identical biophysical parameters and ion channels, but varying axon diameter from 0.2 μm to 1 μm , shows that the wave form fluctuations become larger as the axon becomes thinner. The general structure of the variability profile remains preserved across diameters. The width of the propagating AP varies as it travels down a thin axon in the order of tenths of milliseconds (Figs. 4.3A, 4.3C, 4.3E and 4.3G). Similarly, AP height varies in the order of several millivolts (Figs. 4.3B, 4.3D, 4.3F and 4.3H). The variability is more pronounced the thinner the axon is (Fig. 4.4). The variations between proximal and distal AP shape are uncorrelated ($R^2 < 0.2$ across all diameters for both heights and widths). This implies that both, AP width and height become decorrelated with themselves (autocorrelation decreases) and between each other (cross-correlation decreases) the further the AP propagates.

Propagation of the AP alters the waveform due to two effects: a deterministic transformation and a stochastic variation mechanism. First, the waveform changes in a deterministic way after AP initiation (Swadlow et al. 1980) (Fig. 4.4D). The AP upstroke becomes steeper as the AP travels away from the AP initiation site and approaches its steady-state shape after several length constants¹ (after about 1 mm in a 0.2 μm diameter axon). The change in the average waveform matches the waveform change of the corresponding deterministically simulated axon and has been observed experimentally (Kole et al. 2007). This is because close to the proximal stimulation site, the AP shape is determined by the stimulus driving a fully resting axon, and the response speed is limited by the resting membrane's time constant. However, once the AP propagates the depolarisation is driven by the APs own axial current, which acts over the broad region (about a length constant) of the rising phase of the AP. Membrane resistance is reduced across this region due to the opening of voltage-gated channels in response to the rising of the AP. Therefore, the rise time of the travelling AP is faster than the rise time of the AP at the stimulus initiation site. The effect is more pronounced the bigger the axonal diameter is, because the larger cross-sectional area lowers the axial resistance. This deterministic transformation of

1. The length constant $\lambda = \sqrt{\frac{dR_m}{4R_i}}$ is a property of the passive membrane. It represents how well a subthreshold potential spreads along the axon.

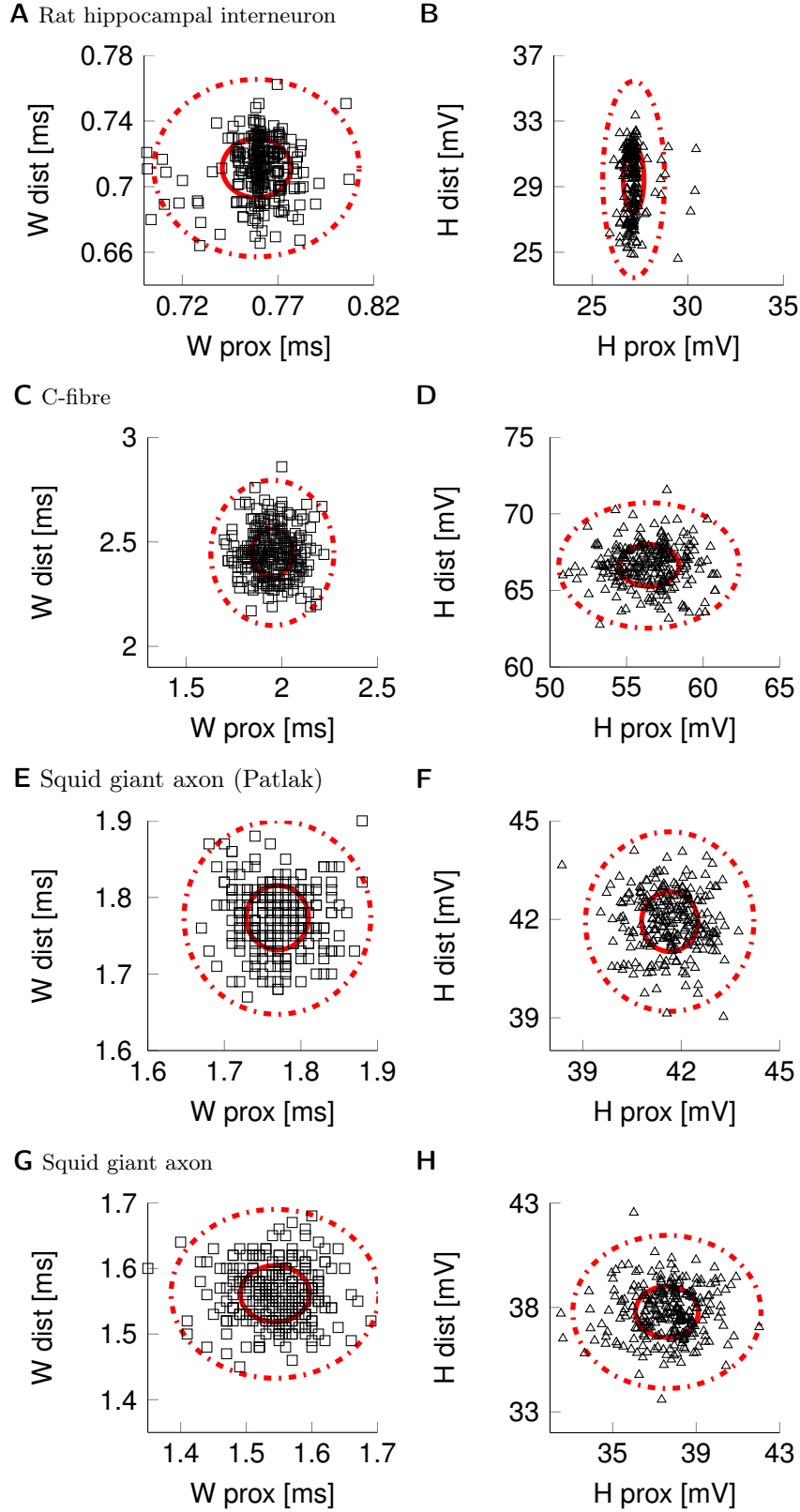


Figure 4.3: Subfigures display data for $N > 200$ single APs triggered by identical stimuli and initial conditions. (A) Distribution of AP width and (B) AP height (thick circle, $1 \times \text{SD}$; dotted circle $3 \times \text{SD}$) for rat hippocampal interneuron model axon. (C) Distribution of AP width and (D) AP height for C-fibre axons. (E) Distribution of AP width and (F) AP height for squid giant axons with Patlak channels. (G) Distribution of AP width and (H) AP height in squid giant axons.

the AP waveform is predictable, and could be compensated at the synapses without loss of information about the generating stimulus.

Second, axonal channel noise introduces non-deterministic, random variability in the propagating AP waveform. This stochastic component of waveform change has similar size as the deterministic component at the largest diameter but unlike the deterministic effect, its amplitude increases as diameter decreases (Fig. 4.4E). The stochastic effect is non-predictable and cannot be compensated for at the synapses. We find AP waveform fluctuations measured in standard deviations around the mean waveform are approx. 1-3 times larger in amplitude than resting membrane potential fluctuations caused by channel noise (Fig. 4.4E).

4.3.2 Underlying ion channel fluctuations

To measure how channel noise affects the propagating waveform one has to track the relevant quantities at corresponding points of the moving AP. To this end the time series recorded at closely spaced axonal positions (here, corresponding to a cylindrical membrane compartment of the axon model) are superimposed after having been aligned at the instant when the membrane potential crosses its half AP peak value. Thus, the individual quantities and their variability at corresponding points of the travelling AP are displayed at corresponding points (Fig. 4.5).

The variability of the waveform has a characteristic structure that is conserved across axon diameters: the first maximum in waveform variability is reached in the late rising phase of the AP (between half peak and peak depolarisation, see Figs. 4.6A and 4.6B). This is due to fluctuations in the number of opening Na^+ channels and Na^+ current (blue curves in Figs. 4.5B and 4.5C), as the first peak of Na^+ current variability is reached at half-peak depolarisation (Fig. 4.5C, shortly after 0 ms). This variability in depolarising Na^+ current accounts for the variations in AP height because the number of Na^+ open channels and their inactivation prior to reaching Na^+ reversal potential (the upper limit to AP height) determine how much the membrane potential is going to depolarise. K^+ channels begin to open later, and thus Na^+ channels carry

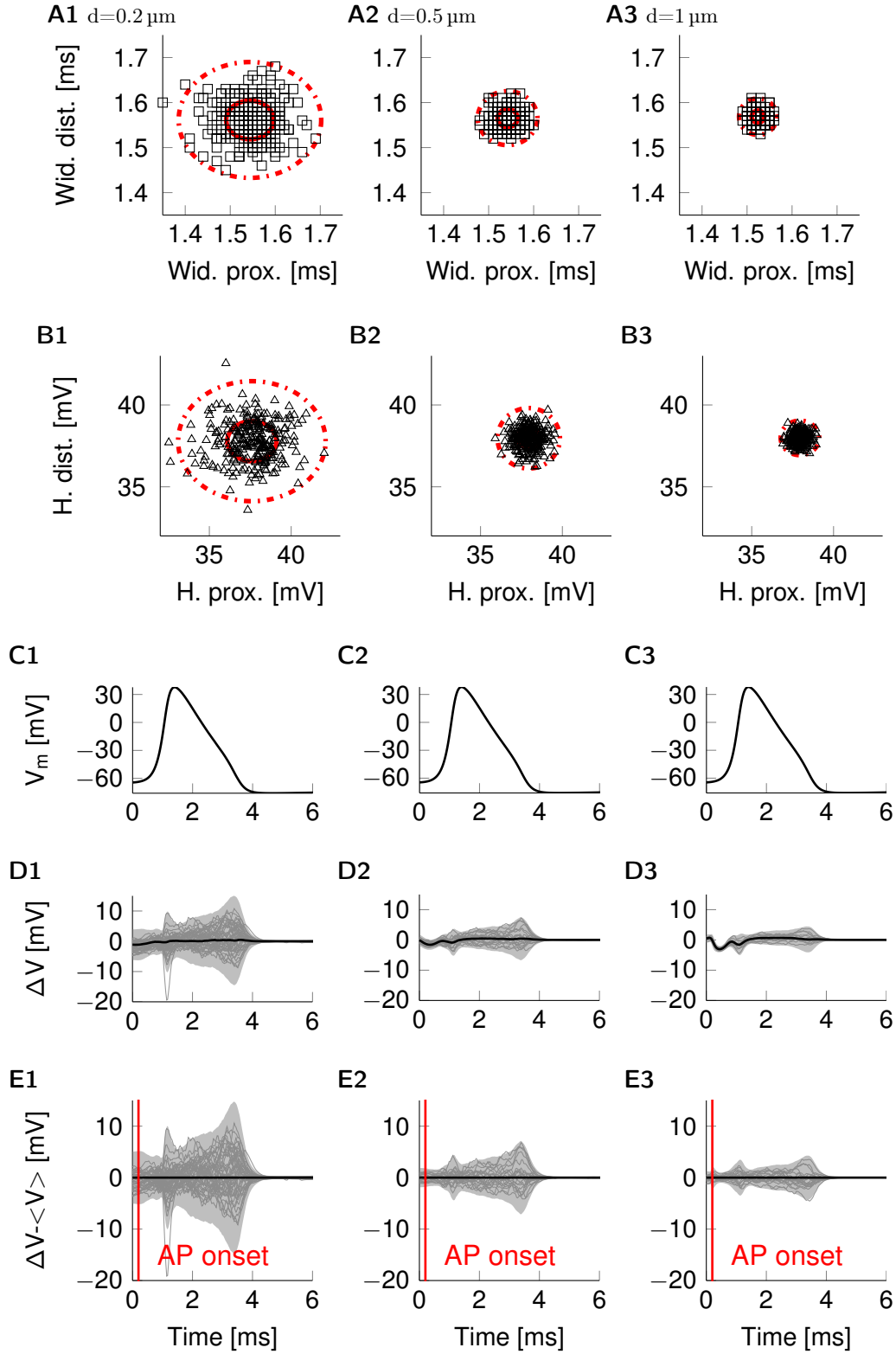


Figure 4.4: Random variability of AP waveform in thin SGA type axons of 3 diameters. The subfigures display data for $N > 200$ single APs triggered by identical stimuli and initial conditions in thin SGA model axons. **(A)** Distribution of AP width and **(B)** AP height (red circle, $1 \times \text{SD}$; dotted circle $3 \times \text{SD}$). **(C)** Mean waveform of the AP at the proximal site. **(D)** Pairwise difference between an AP's shape at the proximal and the distal location. The average difference is plotted in thick black, while the light grey shaded area represents the $3 \times \text{SD}$ range. Grey lines represent sample traces plotted individually. **(E)** Fluctuations around the mean pairwise difference. The average difference is plotted in thick black (0 by definition), while the light grey shaded area represents the $3 \times \text{SD}$ range. Grey lines represent sample traces plotted individually.

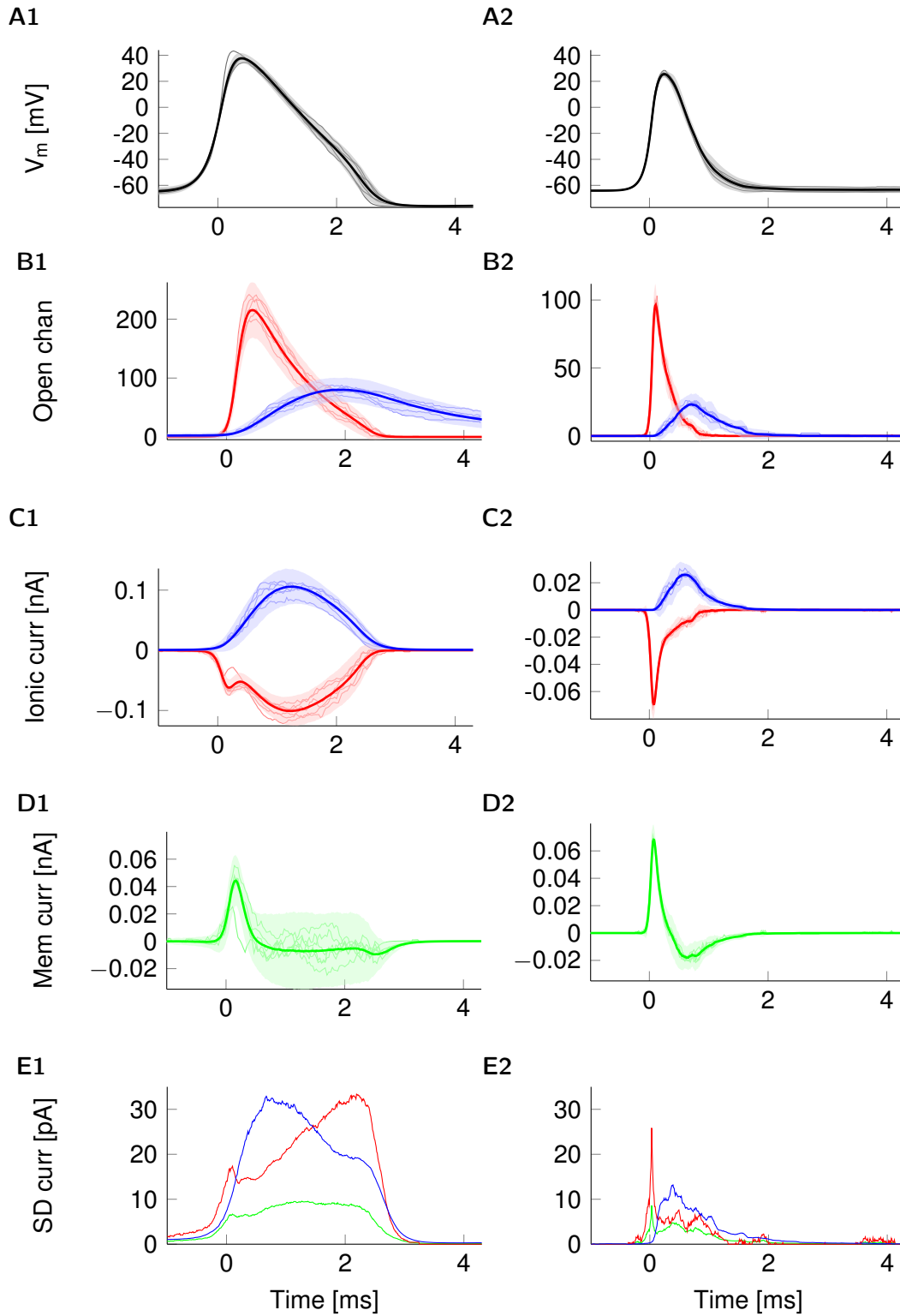


Figure 4.5: Ion channel and current fluctuations in an AP travelling along 0.2 μm diameter axons. All traces are aligned at the instant when the rising AP crosses half-peak. Data from the SGA model is on the left, while the right plots show data from the RHI model. Shaded areas in A-D are the 3 $\times$ SD envelope around the mean curve (dark curve). Light curves in A-D represent a sample of individual traces. (A) Membrane potential waveforms (B) Number of open Na^+ (blue) and K^+ (black) channels (C) Current flowing through Na^+ (blue) and K^+ (black) channels (D) Net membrane current (sum of Na^+ , K^+ and leak) (E) SD of Na^+ (blue), K^+ (black) and net membrane (green) currents. Red lines in A-D are the 1 $\times$ SD envelope around the mean curve (white lines in A-D).

most of the net membrane current in this phase of the AP (SD profile of Na^+ current, blue curve, and K^+ current, green curve, with net membrane current, black curve, in Fig. 4.5E).

The second, broad maximum in waveform variability is reached in the repolarising phase (Fig. 4.5A and Figs. 4.6A and 4.6B beginning at 1 ms and increasing up to 2.5 ms). As the rate of repolarisation (here, $<50 \text{ mV ms}^{-1}$) is much slower than that of depolarisation (here, $>200 \text{ mV ms}^{-1}$), variability in the height of the AP waveform translates into much larger changes of AP width. Note, AP width is measured between the up and down crossing of any given membrane potential level, here chosen to be half-peak depolarisation. Thus, AP width variability is mainly generated in the repolarising phase of the AP.

Variability in the repolarisation rate of the membrane is caused by a broad period of large fluctuations in net membrane current (Fig. 4.5D, between 0.75 and 2.25 ms). This broad period of net membrane current fluctuations is carried first by K^+ current noise and then by Na^+ current noise (Na^+ , blue, and K^+ , black, in Figs. 4.5B and 4.5C). After K^+ channels begin to open in the early repolarising phase, K^+ current fluctuations peak as K^+ channel opening probabilities increase and the binomially distributed variance of the number of open channels becomes larger. However, by the time maximum K^+ channel open probability is reached, fluctuations have decreased because electro-motive forces are larger close to the AP peak. An equally large and broad maximum in the fluctuations is due to noisy Na^+ channel inactivation in the late repolarising phase for analogous reasons, when Na^+ electro-motive forces are large and the probability of Na^+ channels inactivating has become large.

Thus, AP height variability is mainly caused by the fluctuating number of open and inactivating Na^+ channels during the upstroke of the AP. AP width variability is predominantly caused by the noisy repolarising phase of the AP, where both K^+ and Na^+ channels contribute to large fluctuations in the rate of repolarisation. Having described how channel noise affects a single AP's waveform, the question arises whether AP waveforms are more variable in spike trains, as APs may influence each other.

4.3.3 Deriving biophysical scaling relationships

This random variability of the AP waveform does not only result from AP initiation variability, but is also actively generated as the AP propagates along thin axons. The amplitude of waveform fluctuations (as defined by the standard deviation of the membrane potential at each point in time, see Fig. 4.6B) increases over-proportionally as axon diameter decreases below 1 μm (Figs. 4.6C to 4.6F). Plotting the relative variability as the CV (coefficient of variation, defined as the ratio of the standard deviation over the mean) of AP width or height, over axon diameter reveals a common power-law relationship of $d^{\frac{3}{4}}$ (Faisal and Laughlin 2007). The power law holds for the height of APs and their widths (Fig. 4.7), as well as the fluctuations in the overall shape of APs (Fig. 4.6). These relationships can be understood by analysing how the AP mechanism and the ion channel's stochastic nature produce the observed AP waveform variability and have been derived by Faisal et al. (2005). As they show, the ratio of membrane fluctuations over total number of channels is $\sqrt{(1-p)/(pd^{3/2})}$, where p is the open channel probability. Therefore the relative effects of channel fluctuations grows as $d^{-\frac{3}{4}}$.

4.3.4 Waveform variability in spike trains

Using a naturalistic white noise current stimulus protocol, we elicited spike trains for a period of 10 minutes in a 0.2 μm diameter axon (average cerebellar parallel fibre diameter) using the rat hippocampal interneuron model. Note that interspike intervals and AP triggering currents therefore varied as successive APs were triggered. The average firing rate was approx. 40 Hz. At the axon's distal end (measured at approx. 95% of the axon's total length to exclude boundary effects) waveforms showed considerable variation in the AP shape (Fig. 4.8A).

Plotting pairs of an AP's width measured at the mid and distal position revealed an uncorrelated structure (correlation coefficients 0.04 and 0.03 for AP width and height), as in the case of single APs. AP widths measured at half-peak had a coefficient of variation (CV=SD/average) of 6 % ($0.7 \text{ ms} \pm 0.04 \text{ ms}$) and AP amplitude (resting potential to peak) had a CV of 3 % ($93.7 \text{ mV} \pm$

4 Axonal variability as a source of synaptic variability

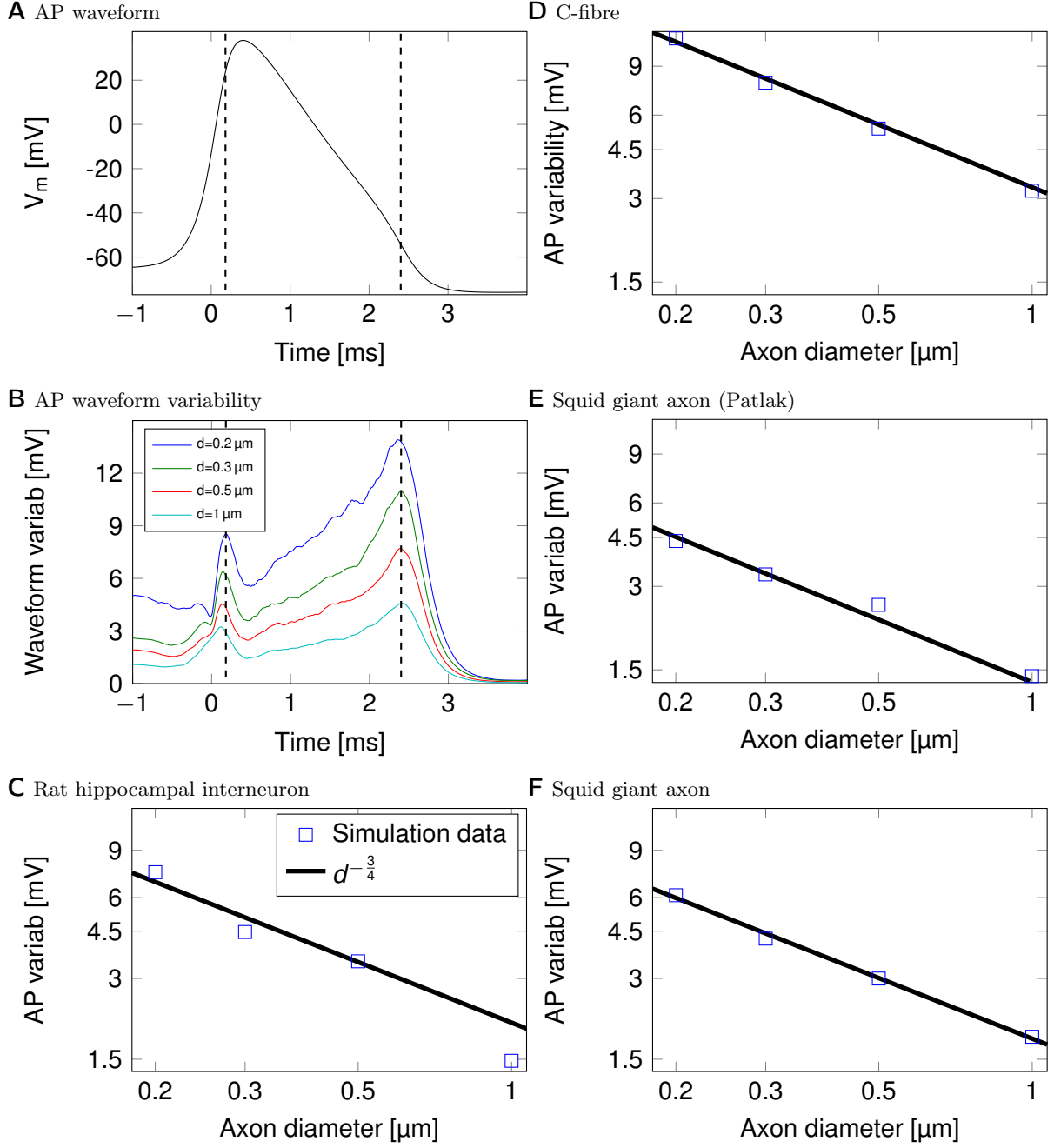


Figure 4.6: **(A)** Typical shape of an action potential in the squid giant axon. **(B)** The variability in the waveform at each moment in time ($N=250$). We define the variability as $3 \times \text{SD}$ of the membrane potential at each point in time. **(C)** Log-log plot of $3 \times \text{SD}$ of fluctuations in AP shape over diameter for a rat hippocampal interneuron. **(D)** Log-log plot of $3 \times \text{SD}$ of fluctuations in AP shape over diameter for a c-fibre axon. **(E)** Log-log plot of $3 \times \text{SD}$ of fluctuations in AP shape over diameter for a squid giant axon (Patlak channels). **(F)** Log-log plot of $3 \times \text{SD}$ of fluctuations in AP shape over diameter for a squid giant axon.

4 Axonal variability as a source of synaptic variability

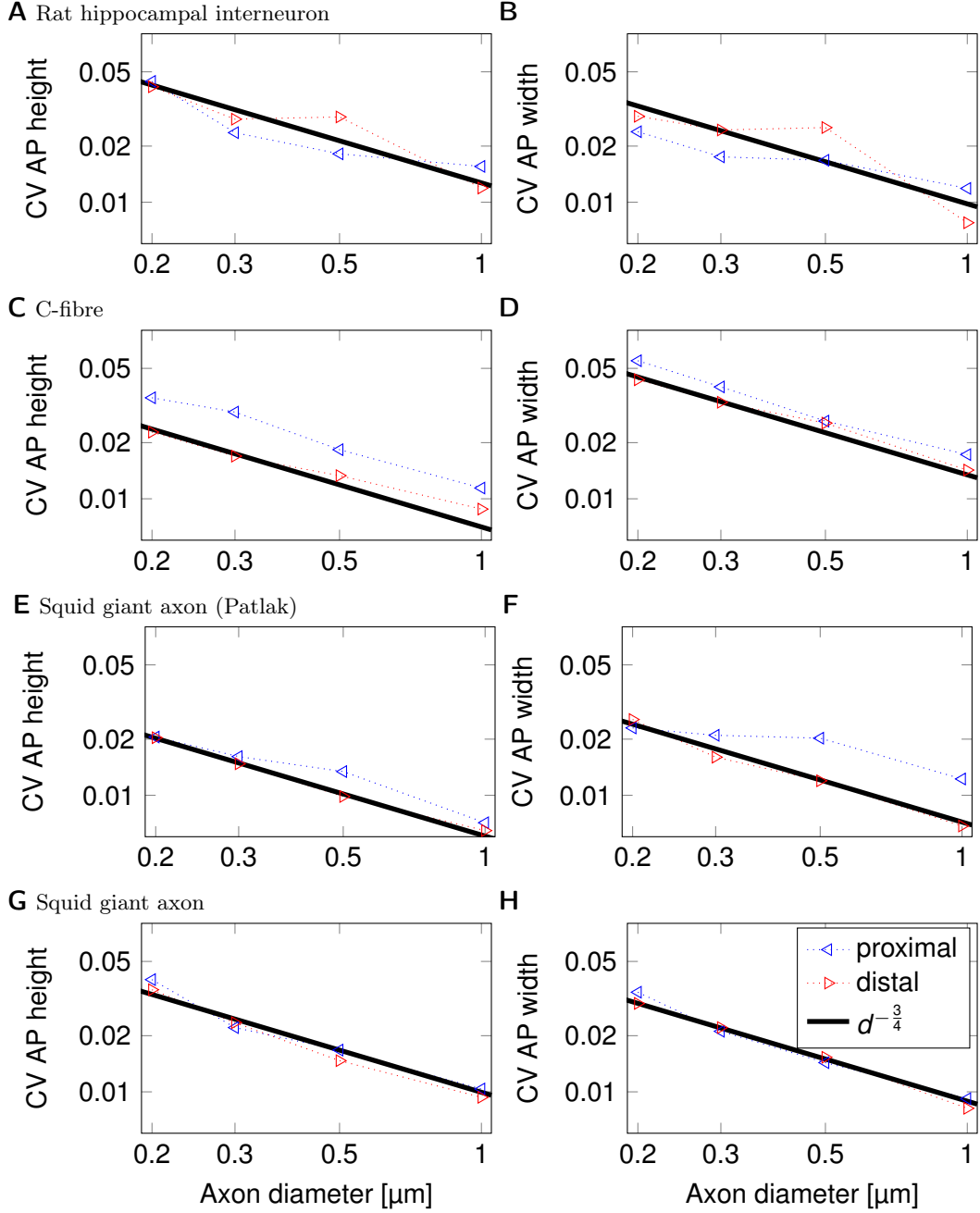


Figure 4.7: The power-law relationship is verified at both proximal (left-pointed triangle) and distal (right-pointed triangle) points in all four types of axons. (A) Log-log plot of CV of AP height over diameter for a rat hippocampal interneuron. (B) Log-log plot of CV of AP width over diameter for a rat hippocampal interneuron. (C) Log-log plot of CV of AP height over diameter for a c-fibre axon. (D) Log-log plot of CV of AP width over diameter for a c-fibre axon. (E) Log-log plot of CV of AP height over diameter for a squid giant axon with Patlak channels. (F) Log-log plot of CV of AP width over diameter for a squid giant axon with Patlak channels. (G) Log-log plot of CV of AP height over diameter for a squid giant axon. (H) Log-log plot of CV of AP width over diameter for a squid giant axon.

2.5 mV). AP waveform variability in spike trains was larger than in the case of individual spikes propagating. The standard deviation of change in AP height after propagating for 1 mm in the axon was 3.5 mV (0.05 ms for the width, $N=2000$), compared to 1.6 mV (0.04 ms for the width, $N=250$) for the single spike protocol. The profile of waveform variability (Fig. 4.8A) peaks close to AP threshold and, at a higher level, in the late repolarising phase. Random waveform variability has matching profiles in the spike train and the single AP protocol as it is caused in both cases by the AP mechanism itself.

This illustrates that AP waveform variability is a constantly acting random process, occurring independent of AP initiation or stimulus. Axonal variability is nonexistent in identical simulations where we replace stochastic ion channel models by the equivalent deterministic Hodgkin-Huxley type conductance models. Thus, all axonal variability observed here must result from the effects of the only source of noise modelled – channel noise in Na^+ and K^+ channels. It has previously been shown that the memory of voltage-gated ion channels causes an increased effect of membrane potential noise, affecting the speed of propagation (Faisal and Laughlin 2007). The same mechanism is also acting on the waveform.

We have quantified the impact of axonal channel noise on AP waveform variability and the biophysical mechanism behind it in thin axons. This previously neglected axonal noise effect will become relevant at the systems- and network level if it can influence synaptic transmission. We next modelled the synaptic transmission process from arrival to the AP to the post-synaptic response.

4.3.5 Variability in synaptic transmission

Synaptic transmission follows a general sequence of events leading to a post-synaptic response. An AP propagates down the axon and causes the opening of voltage-gated Ca^{++} channels resulting in the influx of Ca^{++} at the pre-synaptic terminal. Ca^{++} -sensitive proteins trigger the fusion of vesicles, which release neurotransmitters into the synaptic cleft. These transmitters diffuse

and trigger the opening of ion channels in the post-synaptic cell, producing a voltage response. Thus, AP waveform variability could perturb post-synaptic responses.

We estimate the impact of waveform variability for spike trains propagating down a 0.2 μm diameter axon using two distinct approaches: first, we model the individual stages of synaptic transmission in a synapse driven by our thin axons. Second, we use experimental data relating AP width and height to post-synaptic response to infer how the variability of the AP would transform into response variability.

Since there are very few quantitative models of the pre-synaptic mechanisms that link AP arrival to vesicle release, we use one of the most detailed available models, that of the Calyx-of-Held synapse (reviewed by Schneggenburger and Forsythe 2006). The Calyx is a very large synapse driven by a very reliable, thick axon terminal (1 μm average diameter), and care has to be taken when extrapolating results from this synapse to small synapses innervated by thin axons.

The simulations show that both calcium peak current and total Ca^{++} influx are subject to considerable variability with a CV of 3% and 9% respectively (Fig. 4.8B). The total inflow of Ca^{++} into the synapse and the associated increase in intracellular Ca^{++} concentration are subject to significant variability (Fig. 4.8C). The waveform variability in the repolarising AP phase - varying AP width - leads to a considerable spread in the total influx of Ca^{++} . This translates into considerable variability in vesicle release rate and the total duration of release (Fig. 4.8D) which mainly results from variability in the late AP waveform. Integrating the instant vesicle release rates yields the total amount of vesicles released in an AP (Fig. 4.9B). The variability of the total number of released vesicles is considerable as the CV is about 26% (mean 471 and SD of 121). EPSC variability can be directly estimated from vesicle release because experimental data has shown that under conditions of normal release probability, released quanta linearly summed to EPSCs (Meyer et al. 2001; Scheuss et al. 2002). This would suggest an EPSC variability of approx. 25% based on the Calyx-of-Held model for a synapse. We summarise the impact of AP waveform noise on successive stages of synaptic transmission for the Calyx-type synapse model in Table 4.2.

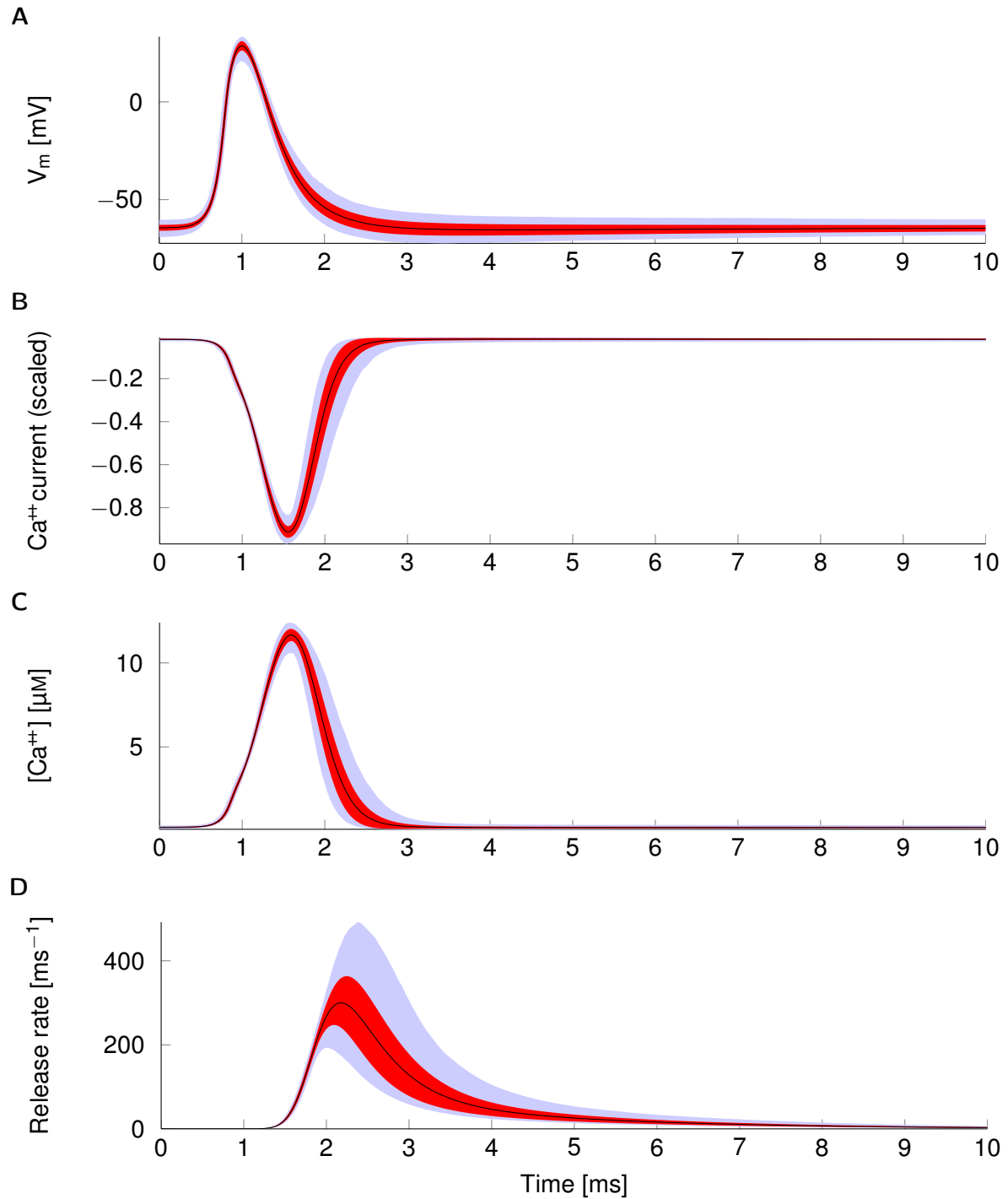


Figure 4.8: Variability in synaptic processes due to fluctuation in AP waveforms. For all subfigures, the mean waveform is plotted in black, SD in red, and 1%-99% quantiles in light blue. **(A)** Waveforms of 2000 consecutive APs arriving at the terminal end of an axon of $0.2 \mu\text{m}$ diameter axon with a model of the rat hippocampal interneuron. **(B)** Ca^{++} current resulting from the integration of the above AP waveforms into a model of a pre-synaptic Ca^{++} channel (see text for details). The current is scaled because we are only interested in its waveform. **(C)** Intracellular Ca^{++} concentration for a large CNS synapse obtained by scaling the Ca^{++} waveform, and lengthening it (Schneggenburger and Neher 2000). **(D)** Time course of vesicle release rates computed for a model of a Calyx-of-Held type synapse (see text for details).

4 Axonal variability as a source of synaptic variability

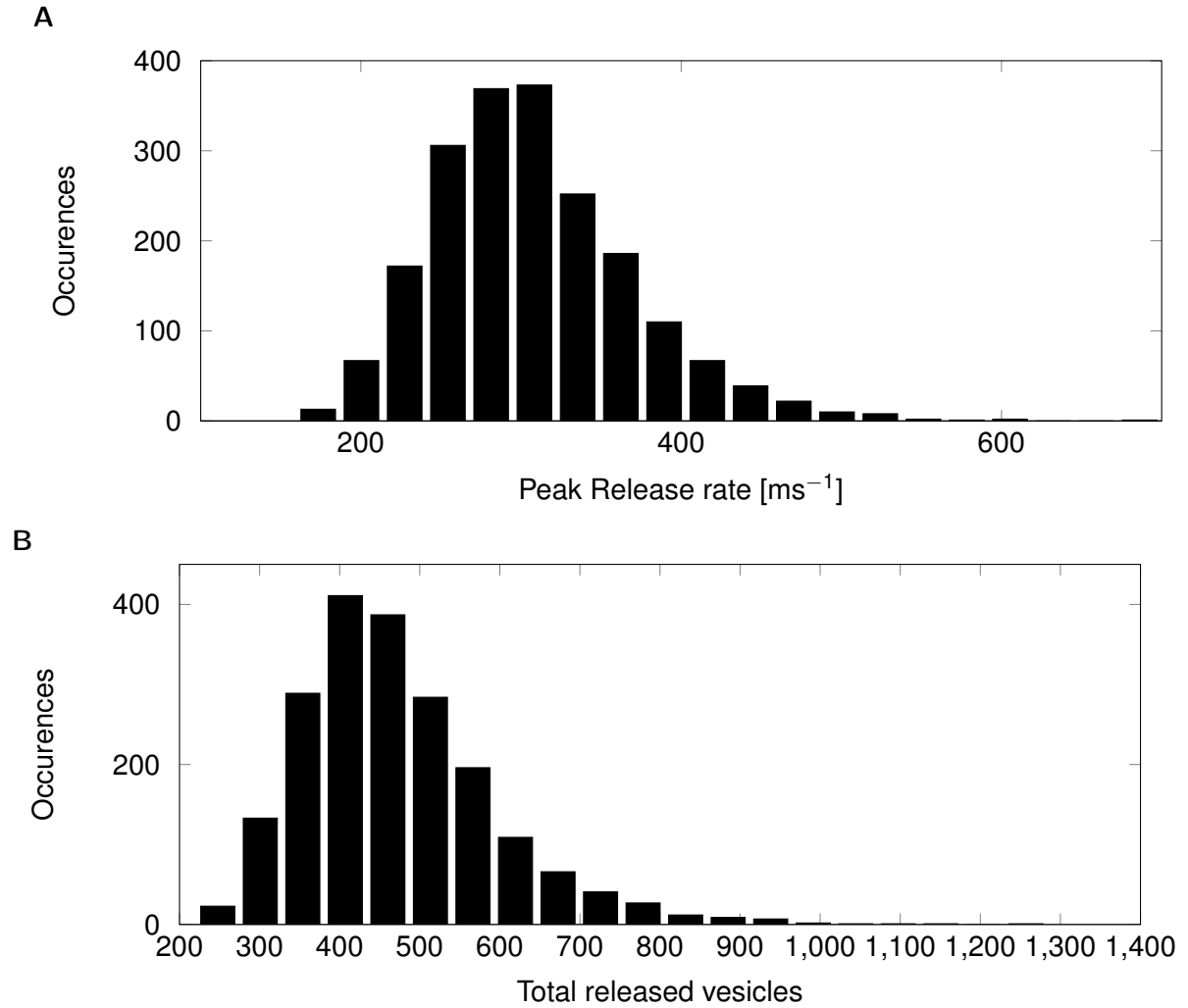


Figure 4.9: Calculated distribution of synaptic response variability. **(A)** Distribution of peak vesicle release rate in a large CNS synapse resulting from variability in ($N=2000$) AP waveforms (see text for details). **(B)** Distribution of the total number of released vesicles in a model of Calyx-of-Held type synapse for ($N=2000$) AP waveforms.

Stage	Signal	CV
Axon	AP width	6%
Pre-synaptic	Ca ⁺⁺ peak current	3%
Pre-synaptic	Ca ⁺⁺ influx	9%
Synapse	Peak [Ca ⁺⁺]	3%
Synapse	Vesicle release rate	10%
Synapse	Vesicles released	26%
Post-synaptic	EPSC amplitude	≈ 25%

Table 4.2: Computed signal variability in the successive stages of synaptic transmission for a Calyx-of-Held type synapse (Coefficient of Variation, CV=SD/av) driven by a 0.2 μ m axon.

To obtain a second, independent estimate of the impact of axonal waveform variability on synaptic variability we looked at experimental data from much smaller synapses. We used patch-clamp data that directly links AP width to EPSC amplitude in Granule-to-Purkinje Cell synapses. Sabatini and Regehr (1997) report a pronounced power-law relationship between AP width and EPSC amplitude for this synapse. We can use this relationship to translate waveform variability into synaptic response variability by combining our results on AP width variability (Fig. 4.9) and Figure 11 in Sabatini and Regehr (1997). Our thin axon APs showed a CV of 6% for AP width, which translates for the Granule-Purkinje Cell synapse to a CV of approx. 25% for the expected EPSC amplitude. Similarly, the computed total Ca⁺⁺ current CV would translate into an EPSC amplitude CV of approx. 25%. Thus, both synaptic signal transduction of the large Calyx-type synapse and the much smaller cerebellar synapse would translate AP waveform fluctuation in thin axons into a CV of 0.25-0.3 for their post-synaptic responses. Note, this amount of variability results from axonal noise alone, as we have not accounted for stochastic processes inside the synapse, such as stochastic Ca⁺⁺ channels, the vesicle release signalling cascade, neurotransmitter diffusion or post-synaptic receptors.

4.4 Discussion

4.4.1 Action potential waveform variability in thin axons

Axons are often thought of as faithful transmission channels for electrical impulses. But advances in experimental methods have allowed to reconsider these assumptions (Sasaki et al. 2011; Kole et al. 2007; Debanne et al. 1997), and sparked interest about the potential role of the axon as a computational unit in its own right (reviewed in Debanne 2004; Sasaki 2013). This is closely related to the question of how APs are translated at the synapse. Do synapses consider incoming APs as unitary events, or do they use information contained in the waveform to modulate the release of neurotransmitters? This has profound consequences for the information transmission rate and thus the efficiency of axonal conduction (Sengupta et al. 2013). We show here that the answer is likely to depend on the anatomy of the axon.

The AP that drives the synapse has to travel along an axon, yet the impact of axonal noise sources on the AP waveform in thin axons was, complete propagation failures set aside (Allen and Stevens 1994), not considered in previous studies. Thus, synaptic response reliability and variability have been in general attributed to mechanisms inside the synapse alone (Allen and Stevens 1994).

The results presented here show that in thin unmyelinated axons below 1 μm diameter, commonly found in the CNS and PNS, the travelling waveform of an AP undergoes considerable random variability. This random variability is caused by axonal Na^+ and K^+ channel noise which continuously acts during propagation and thus accumulates with distance. The variability of AP width and height, key parameters linked to synaptic efficacy, dramatically increased (the CV increasing by a factor of approx. 4, see Fig. 4.7) as diameter decreased from 1 μm to 0.1 μm . We predict this change by deriving a scaling relationship which is the direct result of the geometry and general biophysics of axons and thus independent of specific channel kinetics or other biophysical parameters (Faisal et al. 2005). Invariably, channel noise is bound to

increase as diameter decreases to the point that it affects the waveform of the AP. Therefore, we can observe the effects of this variability in CNS and PNS axons, in both vertebrates and invertebrates.

The range of the waveform fluctuations is about 4 to 6 times the SD, thus we found that AP widths vary by 0.1 ms to 1 ms in axons between diameter 0.2 μm and 1 μm . AP width fluctuations result mainly from K^+ channel noise and inactivating Na^+ channels during the repolarising phase of the AP. While Na^+ channel noise principally effects AP propagation speed and thus spike timing reliability (Faisal and Laughlin 2007), K^+ channel noise has more impact on waveform variability, although variability in Na^+ and K^+ channels partially compensate each other (Faisal et al. 2005). This fits well with genetic knock-out studies where one type of K^+ channels was removed from the central nervous system showed increased temporal response jitter (Kopp-Scheinpflug et al. 2003). The presence of axonal variability in thin axons suggests that information encoded in the shape of the AP waveform would be subject to corruption due to channel noise as it travels along the axon, making it beneficial to move any information processing directly involving the AP wave form downstream to the synapse itself. In fact, activity-dependent modulation mechanism specific to the pre-synaptic terminal are well-known and provide neurons with means for positive or negative feedback regulation of pre-synaptic Ca^{++} influx through regulation of the AP width at the synapse (Jackson et al. 1991; Cooper et al. 1998; Qian and Saggau 1999; Hu et al. 2001).

4.4.2 Synaptic variability from noisy action potentials

In general, the observable variability in synaptic responses could be due to two sources: (1) noise and/or (2) very complex mechanisms that appear random. We can distinguish to which extent these two sources of variability are present at the cellular level, by aggregating the effect of random variability generated by identified molecular stochastic processes, such as thermodynamic fluctuations in molecular conformations (Faisal et al. 2008).

Here, we considered axonal noise as a source of synaptic variability due to channel noise in axons. We modelled a Calyx-of-Held synapse and used data on the Cerebellar Granule-to-Purkinje synapse to estimate the effects of AP waveform noise on synaptic responses in the absence of detailed models for small synapses. Quantitative measurements and models of the mechanistic level of synaptic transmission are limited in small synapses by the technical difficulties to record from thin axon terminals ($<1\text{ }\mu\text{m}$ diameter, Sasaki et al. 2011) and the need to look at very short range connections ($<500\text{ }\mu\text{m}$). Therefore, we ignored pre- and post-synaptic activity dependent effects – which may reduce the effects of waveform variability – and used simplified synaptic transmission models.

Care has to be taken when extrapolating results from these synapses to small CNS synapses (Borst and Sakmann 1996; Harata et al. 2001), and extrapolating from any type of synapse to another – even synapses from the same parent axon – may be difficult when details are considered (Parker 2003). Bearing that in mind, individual active zones in the Calyx are known to be ultra-structurally similar to those found in small, bouton-like CNS synapses (Lenn and Reese 1966; Rowland et al. 2000; Sätzler et al. 2002) and the Calyx’s functional organisation corresponds to a parallel arrangement of several hundred conventional active zones in a single **bouton-like** terminal (Schneggenburger et al. 2002).

Mapping our AP waveform variability data for parallel-fibre like axons onto the empirical relationship between AP width and EPSC amplitude (Sabatini and Regehr 1997) showed a CV of approx. 25% for EPSC amplitude. Other synapses also display this common power-law relationship between AP width and synaptic response, suggesting that axonally induced random variability of the waveform would scale accordingly (Augustine 1990; Delaney et al. 1991; Sabatini and Regehr 1997). The detailed allosteric model of vesicle release rate for Calyx-type synaptic transmission produced comparable amplification of the AP waveform noise (CV increased from 6% to 25%).

Empirical synaptic response CV is typically between 20 and 60%. In all cases modelled here the extrapolated post-synaptic variability is considerable (CV of 25 to 30%) and suggests that the

observed synaptic variability could be partially explained by axonal noise. Axonal variability will show more impact in synapses 1 mm and more down the axon yet, due to the technical difficulties of finding cell pairs at these distances, their variability is little studied.

The Hodgkin-Huxley axon model and related deterministic axon models allow information about the stimulus to be retained in the AP waveform, e.g. stimulus strength is correlated with AP height (Jack et al. 1983). It has been shown in vitro that APs triggered and measured at the soma of the same cell can indeed encode information about the stimulus (Polavieja et al. 2005; Juusola et al. 2007), suggesting that this additional information may be decoded at synapses. Changes in the width of APs, whether due to a depolarised soma (Shu et al. 2006) or application of glutamate (Sasaki et al. 2011) have been shown to influence post-synaptic potentials (PSPs). Moradmand and Goldfinger (1995) studied the deterministic transformation of propagating AP waveforms in a paired-pulse framework and showed that the second AP waveform in the pair becomes increasingly stereotyped due to refractory interaction with the first AP. Thus, even in bursts, only the first spike would be the likely candidate to carry stimulus information in the waveform over long distances. Here, we show that for both single APs and spike trains, channel noise decorrelates the waveform (within the limits of the AP's regenerative dynamics) as the AP propagates even over short distances of less than 0.5 mm. Thus, any en-passant synapses along the path of an AP will be driven by randomly differing waveforms and produce different responses (even if the synapses were identical, Parker et al. 1998).

Synapses innervated by thin axons may have developed mechanisms to circumvent the problem of axonal variability. A simple solution would be to treat an incoming noisy AP waveform as a unitary event. Taking this view, small synapses on thin axons should treat APs as unitary signals and adjust their transduction mechanisms accordingly to be robust to axonal noise effects. This is, in addition to spontaneous APs, another way in which noise constraints affect neural coding (London et al. 2010).

Axons play an important active role for information processing that may be comparable to that of dendritic computation (Debanne 2004). However, axonal variability has traditionally not been

considered as a source of neuronal variability (Kandel et al. 2000), because the AP mechanism was considered highly reliable by extrapolating from classic studies large (3 orders of magnitude larger diameters) fibres such as squid giant axons . Yet, in densely connected central neural circuits the APs becomes sensitive to channel noise (Faisal and Laughlin 2007) . The effects of channel noise will inescapably increase non-linearly as diameter decreases due to the very nature of the AP mechanism (Faisal et al. 2005). Axonal channel noise will affect the reliability ($<0.1\text{ }\mu\text{m}$ diameter) and cause considerable variability to both timing ($<0.5\text{ }\mu\text{m}$ diameter) (Faisal and Laughlin 2007) and, as shown here, the shape of the AP in axons below $1\text{ }\mu\text{m}$ diameter. Thin unmyelinated axons typically innervate large numbers of small CNS synapses (Shepherd 2003) and are associated with, and required for the high level and density of circuit miniaturisation encountered in the cortex and the cerebellum (Chklovskii et al. 2002; Faisal et al. 2005).

The reliability with which information integrated at the soma can be decoded post-synaptically depends on distance and axonal diameter and is therefore linked to the anatomy of a neural network's wiring (Faisal and Laughlin 2007). In sensory and motor nervous systems, reliability is typically achieved by averaging over many release sites and high release rates. The corresponding large synapses are associated with large axons. However, in the cerebral cortex, hippocampus and cerebellum the dense connectivity within a restricted space limits the diameter of axons, the number of redundant axonal connections and the size of the synaptic contact areas. This makes synaptic transmission prone to the effects of axonal channel noise in thin axons innervating small synapses. The results presented here prompt careful experimental consideration, because paired-cell measurements and optical methods do not offer the control and resolution necessary to determine the source of PSP variability.

5 Does clustering of Na^+ channels on lipid rafts allow micro-saltatory conduction in C-fibres?

5.1 Introduction

Propagation of action potentials (AP) in axons relies on the concerted action of membrane-spanning selectively permeable ion channels. Myelinated axons feature a highly structured distribution of voltage-gated ion channels, with a characteristic clustering of Na^+ channels at the Nodes of Ranvier. Saltatory conduction in myelinated axons refers to the rapid propagation of the electrical waveform from each node to the next (the AP seems to jump between nodes). This mode of conduction allows faster, more reliable and, as we showed (Chapter 3), considerably more energy efficient propagation of signals than unmyelinated axons. In contrast, as we showed in Chapter 3, only unmyelinated axons, which are generally thought to feature uniformly distributed ion channels (Black et al. 1981), are found at diameters approaching the physical limits to axon diameter (Waxman and Bennett 1972) (d) at $0.1\text{ }\mu\text{m}$ (Faisal et al. 2005), thus making the high connection densities of mammalian cortex possible.

The number of ion channels on the surface of neurons' membrane is usually thought to be large enough to justify combining the individual channel conductances into a continuous measure

of overall conductivity (Dayan and Abbott 2001), as originally done by Hodgkin and Huxley (1952). However in the case of thin axons, the number of ion channels may be too small for these approximations to be valid. Faisal et al. (2005) showed that the behaviour of individual ion channels need to be taken into account, and that channel noise in very thin axons has a large effect, limiting the miniaturisation of fibres by imposing a lower diameter on axons at 0.1 μm . The conceptual transition from conductivity (per surface area) to density of channels, with each channel having only two possible conductance value corresponding to its open and closed states, involves investigating the effects of possible non-uniformities in the distribution of ion channels across the membrane.

Based on observations from the neonatal rat optic nerve, Waxman et al. (1989) hypothesised that action potentials could be propagated along thin ($d \approx 0.2 \mu\text{m}$) axons by “jumping” between individual Na⁺ channels placed a few microns apart. This postulated mode of propagation would be the analogue of saltatory conduction in myelinated axons (Huxley and Stämpfli 1949) and was termed micro-saltatory conduction. Faisal and Laughlin (2007) showed that probabilistic gating of ion channels due to thermodynamic fluctuations, or channel noise (reviewed by White et al. 2000) makes micro-saltatory conduction between individual Na⁺ channels impossible. This is because the very low diameters required for individual Na⁺ channels to have a measurable effect on the membrane potential make the axon overly sensitive to stochastic opening of Na⁺ channels, resulting in an excessive spontaneous AP rate.

In C-fibres, Pristerà et al. (2012) have recently discovered that NaV1.8, the voltage-gated Na channels of these 0.1 μm diameter unmyelinated axons (Baker 2005), are packed tightly together on lipid rafts. Lipid rafts are “dynamic, nanoscale, sterolsphingolipids enriched, ordered assemblies of proteins and lipids” (Pike 2006; Coskun and Simons 2010; Simons and Gerl 2010). They play a role in organising the cell membrane, and act as hubs for functional localisation of proteins (Pristerà et al. 2012). They also intervene in trafficking, clustering and electrophysiological properties of ion channels, and have an effect on cell excitability (reviewed by Pristerá and Okuse 2012). They are typically 0.1 μm to 0.3 μm long (Personal communication with Amber Finn and

Kenji Okuse, 2011), and placed approx. $5\mu\text{m}$ to $10\mu\text{m}$ apart (Pristerà et al. 2012). Disassociating lipid rafts and Nav1.8 channels in DRG neurons is correlated with impaired neuronal excitability (Pristerà et al. 2012).

C-fibers are very thin unmyelinated peripheral axons responsible for transmitting nociceptive pain sensations (Lawson 2002). A variety of Na^+ channels are found on the membrane of C-fibres, including TTX-sensitive Nav1.6 (Black et al. 2002) and Nav1.7 (Black et al. 2012) channels. Voltage clamp experiments have shown that these TTX-sensitive channels are involved in amplifying subthreshold depolarizations, and are active during APs (Vasylyev and Waxman 2012). The slow-activating, slow-inactivating Nav1.8 channels play a crucial part in the generation and propagation of APs in these fibres (Akopian et al. 1999; Renganathan et al. 2001; Lai et al. 2003). As a result, these TTX-resistant channels are of particular interest for treating neuropathic pain symptoms (reviewed by Scholz and Woolf 2002). The clustering of Na^+ channels on lipid rafts resembles the structure of Nodes of Ranvier in myelinated fibers, and may permit micro-saltatory conduction in those thin axons. Here, we investigate whether this mode of propagation is indeed possible, and its potential benefits in terms of basic constraints faced by neural fibers. I have presented part of this work at the *14th World Congress on Pain* (Milan, Italy 2012), the *Sixty years of Hodgkin and Huxley Model* symposium (Cambridge, UK 2012), the *2nd Bernstein Conference* (Munich, Germany 2012) and the *Annual Meeting of the Society for Neuroscience* (New Orleans, USA 2012).

5.2 Methods

We investigated the effects of the lipid-raft clustering of Na^+ channels on the function of neural fibres, using both deterministic and stochastic simulations. In stochastic simulations, the changes of conformations of ion channels were individually modelled (Faisal 2012). Simulations were based on biophysical data from Baker (2005). Computations were carried out using the Modigliani stochastic simulator (Faisal et al. 2002, 2005, Chapter 2), on a Linux PC using an

Intel core i7 processor with the binomial algorithm, chosen because it allows accurate simulations that are less computationally intensive than the Gillespie algorithm (Faisal 2010). Our model of the C-fibre is the same than that used in Chapter 4. Parameters are presented in Table 5.1.

Parameter	Value
Membrane capacitance	$0.81 \mu\text{F cm}^{-2}$
Axial resistance	$70 \Omega \text{ cm}$
Leak conductance	0.14 mS cm^{-2}
Na^+ single channel conductance	20 pS
Na^+ channel density	$62.5 \mu\text{m}^{-2}$
K^+ single channel conductance	17 pS
K^+ channel density	$10 \mu\text{m}^{-2}$
Leak rev. pot.	-61.14 mV
Na^+ rev. pot.	79.6 mV
K^+ rev. pot.	-85 mV
Temperature $[\text{°C}]$	24

Table 5.1: Simulation parameters

Our C-fibre model axon contains only two types of voltage gated ion channels. We use a model of TTX-resistant Na^+ channels (NaV1.8) based on physiological data from (Baker 2005). The instantaneous Na^+ conductance in the model is given by $g_{\text{Na}^+} = \bar{g}_{\text{Na}^+} \times m^3 h$ where $\bar{g}_{\text{Na}^+} = 1.25 \text{ mS cm}^{-2}$. m and h follow the classical Hodgkin and Huxley (1952) dynamics, with rates $\alpha_m = 3.83/(1 + \exp((V_m + 2.58)/-11.47))$, $\beta_m = 6.894/(1 + \exp((V_m + 61.2)/19.8))$, $\alpha_h = 0.013536 \exp(-(V_m + 105)/46.33)$ and $\beta_h = 0.61714/(1 + \exp((V_m - 21.8)/-11.998))$. This transforms into the 8-state Na^+ channel model (Fig. 2.2) for stochastic simulations.

We also used the model for fast K^+ channels given in Baker (2005). The instantaneous K^+ conductance in the model is given by $g_{\text{K}^+} = \bar{g}_{\text{K}^+} \times n^4$ where $\bar{g}_{\text{K}^+} = 0.17 \text{ mS cm}^{-2}$. and the kinetic rates for n are given by $\alpha_n = 0.00798(V_m + 72.2)/(1 - \exp((-72.2 - V_m)/1.1))$ and

$\beta_n = 0.0142(-55 - V_m)/(1 - \exp((V_m + 55)/10.5))$. This transforms into a 5-state channel for stochastic simulation (for details, see Faisal et al. 2002).

We simulated both uniformly distributed and clusters of channels placed regularly along the $0.1\mu\text{m}$ diameter C-fibre axon (see Fig. 5.1). For the uniformly distributed Na^+ channels axon model, we use a single Na^+ channel conductance of 20pS , which translates into a density of $56.25\mu\text{m}^{-2}$, and a single K^+ channel conductance of 17pS , which translates into a density of $10\mu\text{m}^{-2}$. Single channel conductance values are putative (based on typical values for ion channels). For the clustered Na^+ channel model, we kept the density of K^+ channels constant in the region between lipid rafts. On the lipid rafts there are no K^+ channels. For all cluster configurations i.e all values of cluster length l and distance between clusters L , we simulated an axon long enough to contain 100 clusters. At each trial, we injected a small current step twice. The first evoked AP was only used to ensure the ion channels were properly initialised. We only used data from the second AP of each trial.

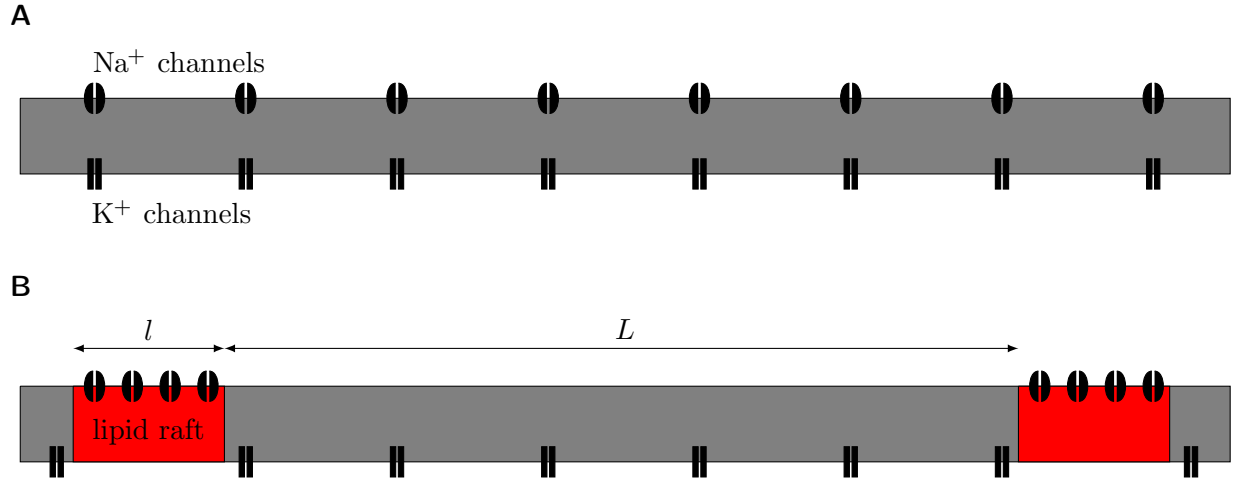


Figure 5.1: Schematic view of axonal models. **(A)** The null-hypothesis axon. Both Na^+ and K^+ channels are uniformly diffused along the axon. **(B)** Axon with Na^+ channels clustered together on lipid rafts. We model this axon by placing a compartment containing a high density of Na^+ channels at regular distances in between compartments containing only K^+ channels.

The width of each AP is measured between the half-width points (see also Fig. 4.2C). The metabolic cost of AP propagation is usually defined as the amount of ATP molecules necessary to

reverse the Na^+ current by Na^+ - K^+ -ATPase (Alle et al. 2009; Sengupta et al. 2010). However, we chose to keep this measure in terms of Na^+ charge and not convert it into a measure in terms of the amount of ATP molecules, because Na^+ charge is given directly by the amount of current crossing the membrane, and does not require additional assumptions on how the Na^+ charge is reversed. The processes involved in the reversal of ionic charges and more general metabolic constraints are discussed in Chapter 6.

5.3 Results

Figure 5.2 illustrates the propagation of an action potential in a C-fibre axon with uniformly distributed Na^+ channels, using both deterministic and stochastic simulations. In deterministic simulations, the AP waveform is kept constant while propagating through the axon. The activation profile of Na^+ channels, and hence the Na^+ current, is also the same at all points along the axon, as we expect.

In stochastic simulations using discrete, stochastic ion channels, there is considerable variability in the Na^+ current crossing the axon, as shown by the profile of Na^+ current Fig. 5.2D. In addition, the stochastic opening of each discrete channel has a minimum current flow determined by the single channel conductance, that is larger than the minimum conductance allowed in the deterministic model. This is visible in the absence of blue bands of low Na^+ current in Fig. 5.2D, although they are present at the beginning and end of the AP in Fig. 5.2C.

5.3.1 Microsaltatory conduction along Na^+ channel clusters

Clustering Na^+ channels on putative lipid rafts still allows AP conduction (Fig. 5.3). In both deterministic (Figs. 5.3A and 5.3C) and stochastic (Figs. 5.3B and 5.3D) simulations, the AP is sustained by the Na^+ current in clusters alone. Plotting the profile of the AP waveform (Fig. 5.3E) shows bumps in the waveform, corresponding to the placement of Na^+ channel

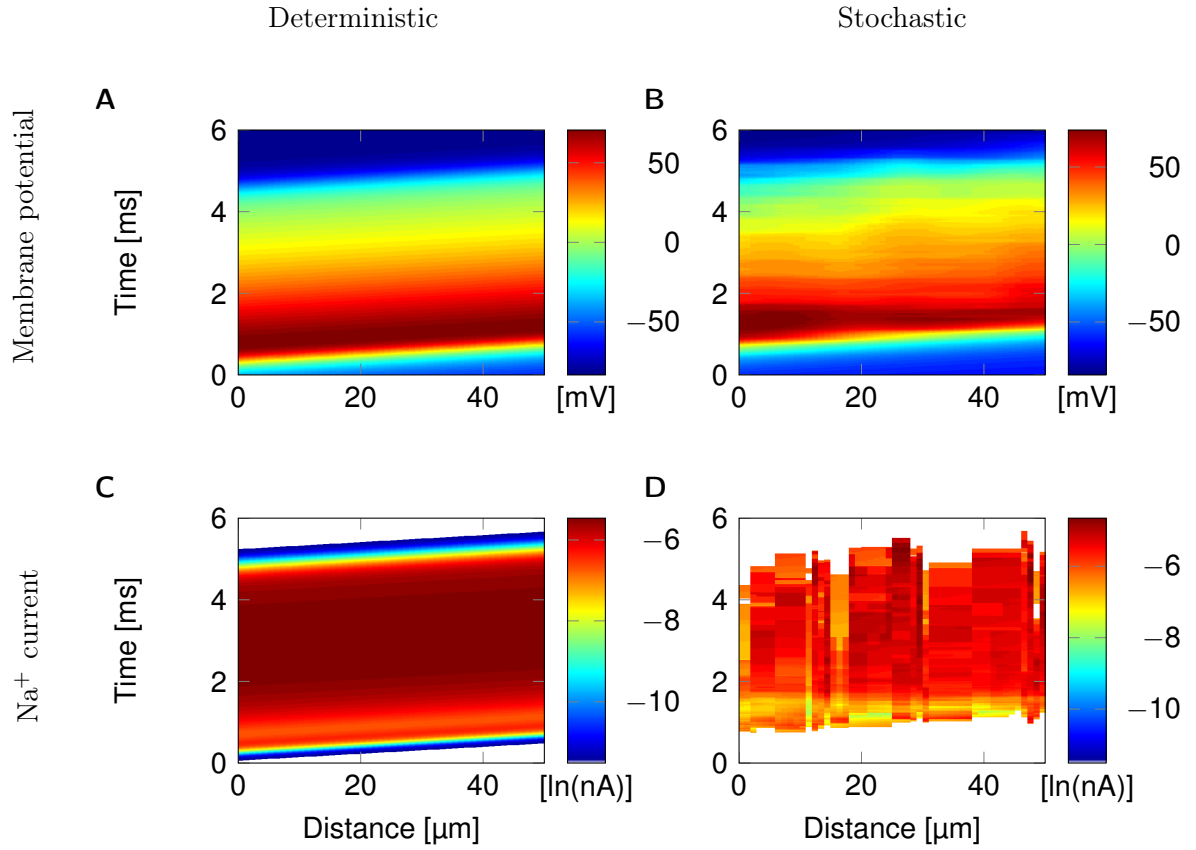


Figure 5.2: Action potential propagation in a $0.1\mu\text{m}$ diameter C-fibre axon with uniformly distributed Na^+ channels. (A) The membrane potential and (C) Na^+ current in a deterministic simulation. (B) The membrane potential and (D) Na^+ current in a single trial of stochastic simulations. White areas signify no current flow.

clusters.

The height of the AP is only slightly lower outside of Na^+ channel clusters. This is because L is much lower than the axon's length constant λ . Therefore the membrane potential over the intercluster region is roughly constant, and equal to that of clusters. Note that in myelinated axons, the amplitude of APs over the internodal regions is also not much lower than the amplitude in nodes of Ranvier (Bakiri et al. 2011). This is also confirmed by our simulations of a mammalian myelinated axon model (see Fig. 5.3F), based on data from McIntyre et al. (2002).

AP waveforms are slightly wider over clusters in both deterministic (Fig. 5.3A) and stochastic

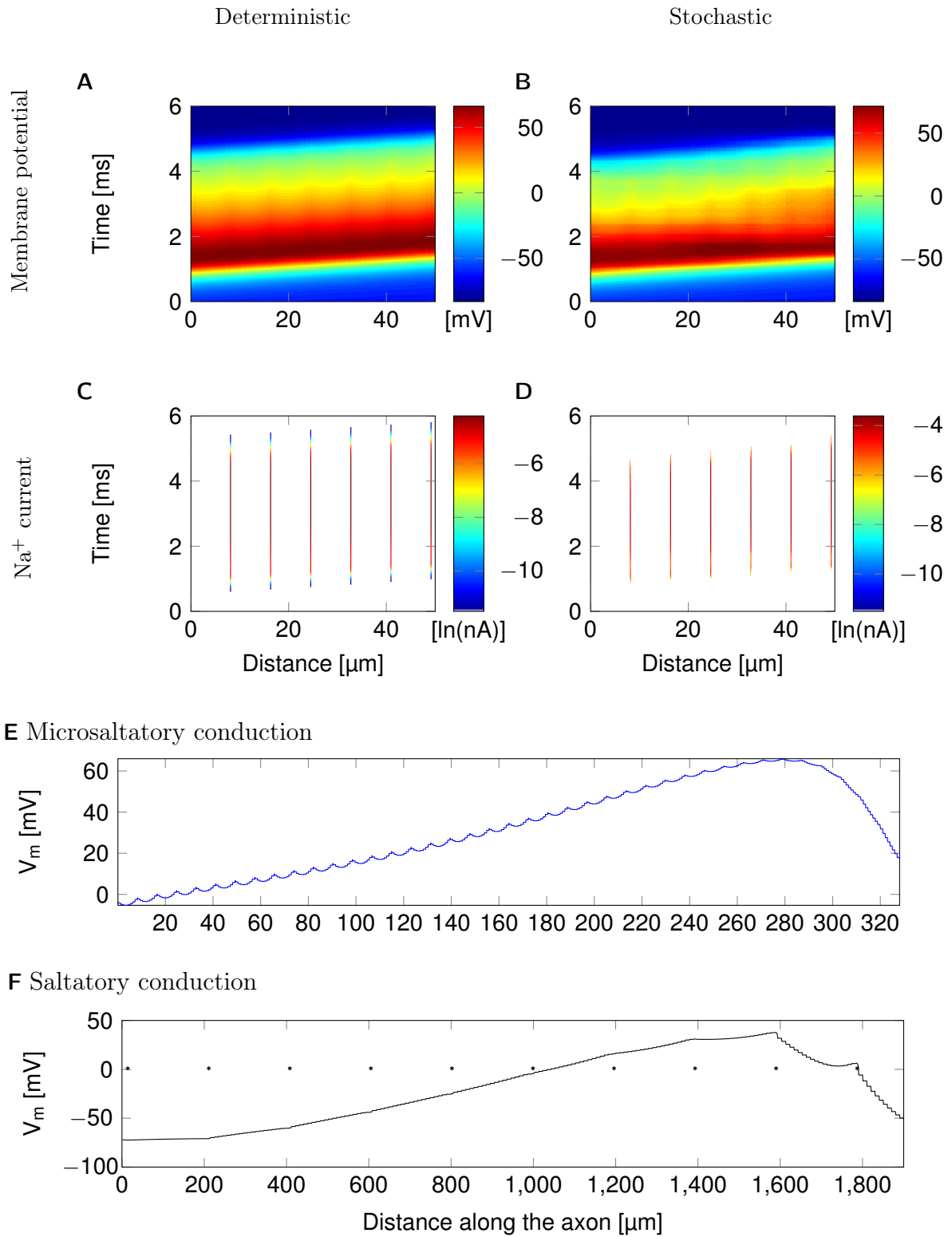


Figure 5.3: Microsaltatory conduction in a 0.1 μm diameter C-fibre axon with clustered Na^+ channels ($l = 0.2 \mu\text{m}, L = 8 \mu\text{m}$). (A) The membrane potential and (C) Na^+ current in a deterministic simulation. (B) The membrane potential and (D) Na^+ current in a single trial of stochastic simulations. In (C) and (D) the lines of Na^+ current are very fine due to the short length of clusters. (E) Profile of the AP waveform propagating in a C-fibre axon. The bumps in the waveform correspond to the placement of lipid rafts. (F) Profile of the AP waveform in a model of mammalian myelinated axon (using parameters from McIntyre et al. 2002).

(Fig. 5.3B) simulations. This effect is due to the reopening of Na^+ channels in the repolarising phase of the AP (Fig. 5.8). We investigated the influence of the length (l) of clusters, and the distance between them (L) on the shape of action potential. The results are plotted in Fig. 5.4.

Both AP width and height seem affected by the size and placement of Na^+ channel clusters. Longer clusters increase the width of APs almost linearly (Fig. 5.4A). The greater number of Na^+ channels in longer clusters also pushes the peak of APs toward the Na^+ reversal potential, increasing it from approx. 55 mV to 75 mV (Fig. 5.4B).

Increasing the distance between clusters, on the other hand, shortens the width of APs. With 0.2 μm long clusters 10 μm apart, the AP width is halved compared to placing the clusters only 1 μm apart (Fig. 5.4C). The height of APs is also reduced by furthering clusters, this time in an almost linear fashion (Fig. 5.4D) which is expected since the change is no longer limited by the Na^+ reversal potential.

5.3.2 Change in shapes of APs results in lower metabolic costs

The change in the shape of APs directly results into changes in their metabolic cost (Fig. 5.5). Shortening clusters lowers the amount of Na^+ charge crossing the membrane and thus the cost in ATP associated with pumping Na^+ ions back out of the cell (Fig. 5.5A). Increasing the distance between clusters also reduces the metabolic cost. The profile of the variation in metabolic cost closely follows that of change in AP width, suggesting that width, rather than height, determines the metabolic cost of firing APs (Figs. 5.4A, 5.4C and 5.5).

We can now compare the metabolic cost of APs in axons with clustered Na^+ channels to the cost in axons where Na^+ channels are uniformly distributed (Fig. 5.6). Experimental results (Personal communication with Amber Finn) suggest $l = 0.1 \mu\text{m}$ to $0.3 \mu\text{m}$ long clusters placed $L \approx 3 \mu\text{m}$ apart. The overall density of Na^+ channels is kept constant by assuming a density of $900 \mu\text{m}^{-2}$ in the clusters. In our simulation, this does not result in a significant change of metabolic cost. The metabolic cost for propagating APs over axons with both clustered and

5 Does clustering of Na^+ channels on lipid rafts allow micro-saltatory conduction in C-fibres?

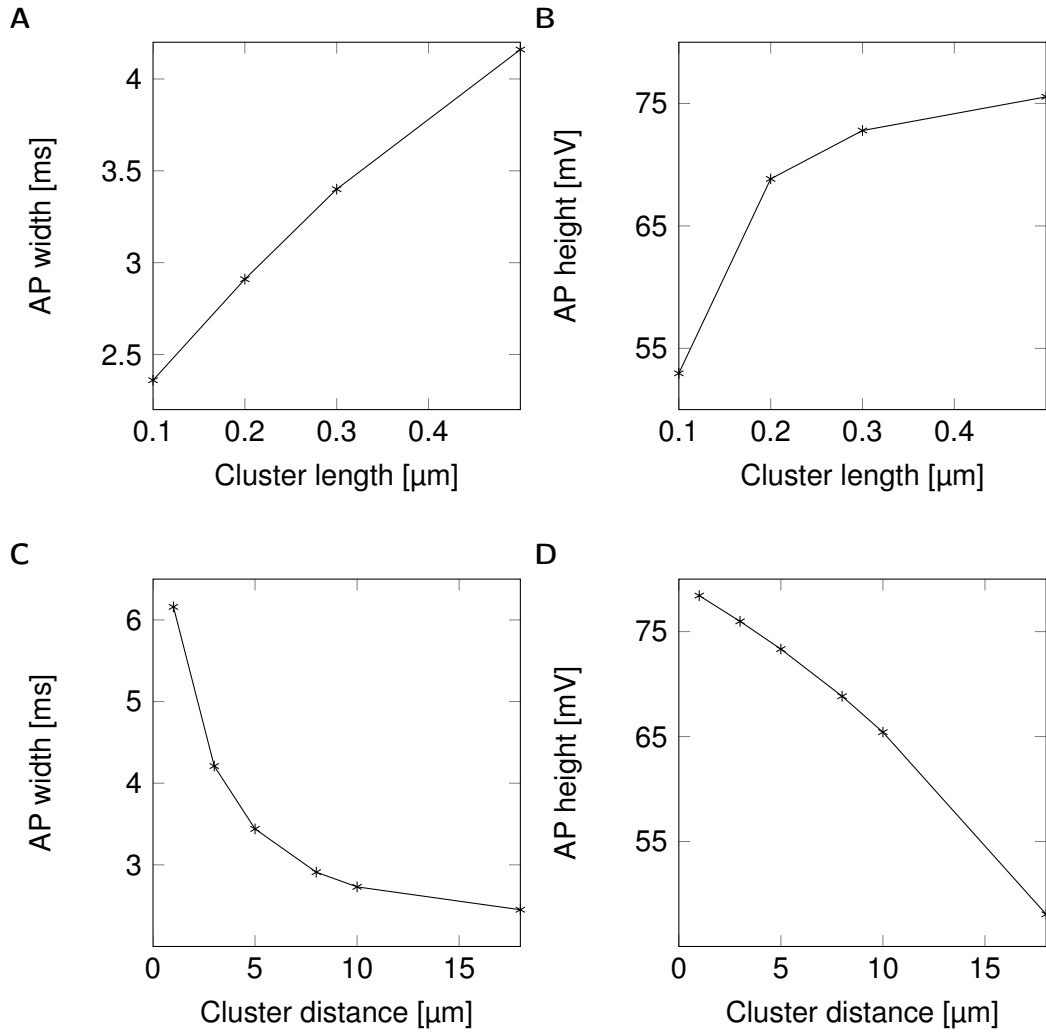


Figure 5.4: Action potential height and width as functions of channel distributions. When varying the length of clusters, the intercluster distance was set to 8 μm . While varying the intercluster distance, the cluster length was set to 0.2 μm . **(A)** Action potential width (measured at half-peak value) in a model axon with clustered Na^+ channels as a function of cluster length. **(B)** Action potential peak in a model axon with clustered Na^+ channels as a function of cluster length. **(C)** Action potential width (measured at half-peak value) in a model axon with clustered Na^+ channels as a function of distance between clusters. **(D)** Action potential peak in a model axon with clustered Na^+ channels as a function of distance between clusters.

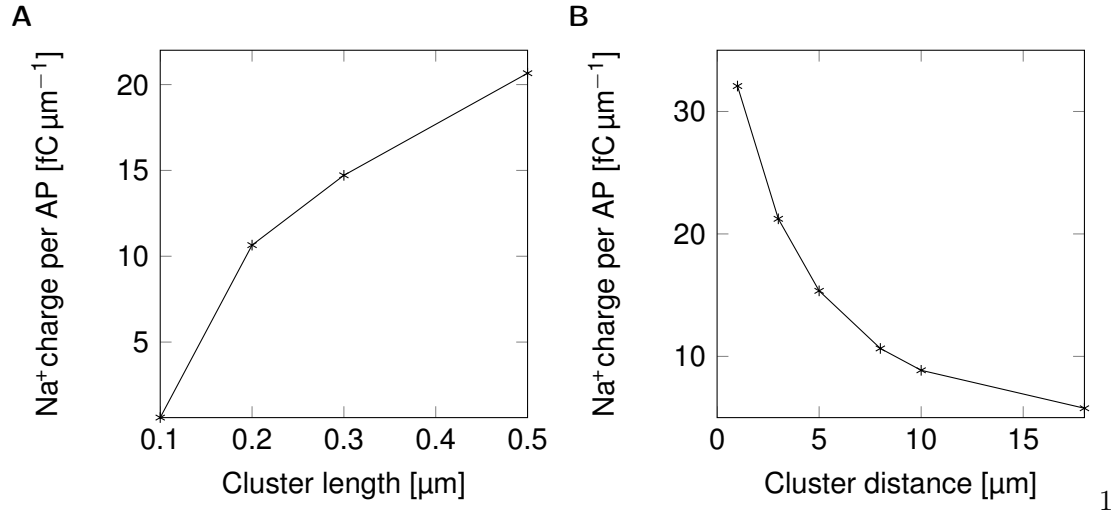


Figure 5.5: Metabolic cost of action potential as functions of cluster configuration in a 0.1 μm diameter C-fibre axon. **(A)** Metabolic cost of action potentials in a model axon with clustered Na^+ channels for varying cluster sized. Distance between clusters was set to 8 μm. **(B)** Metabolic cost of action potentials in a model axon with clustered Na^+ channels for varying intercluster distances. Cluster length was set to 0.2 μm.

uniformly distributed Na^+ channels is approx. 13 fC μm⁻¹ for the 0.1 μm diameter axon in deterministic simulations.

In stochastic simulations, the opening of a channel means that a conductance equal to that of the single channel is added to the membrane. This minimum current due to the discrete nature of ion channel conductance has an impact on the metabolic cost of APs. Stochastic simulations yield a median value of 16 fC μm⁻¹ per AP in axon with clustered or uniformly distributed channels.

Increasing L , and compensating by increasing the density of Na^+ channels in the clusters further lowers the Na^+ charge crossing the membrane. 0.2 μm long clusters placed 8 μm apart (Zeng and Tang 2009) are more metabolically efficient than uniformly placed Na^+ channels (approx. 11 fC cm⁻¹ in deterministic simulations). Shortening the clusters to 0.1 μm reduces the Na^+ charge even further, while maintaining the axon's capacity to propagate APs.

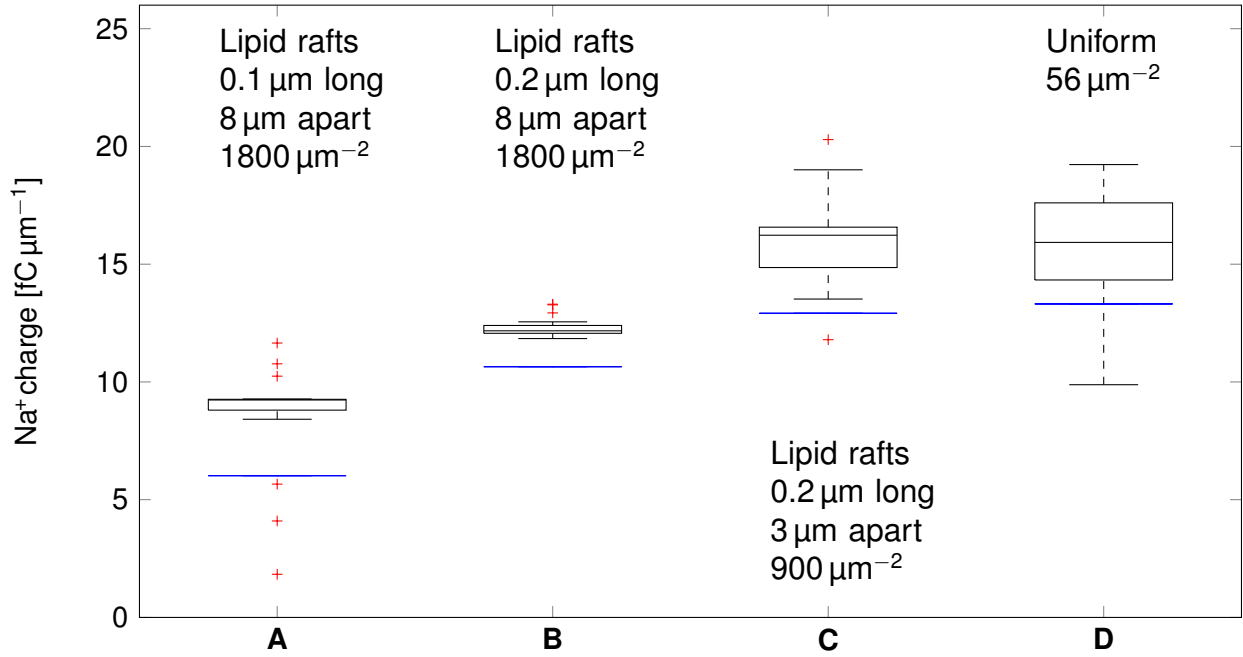


Figure 5.6: Metabolic cost of action potentials for different channel distributions in a 0.1 μm diameter C-fibre axon. Data was obtained using both deterministic (blue lines) and stochastic (boxes) simulations. (A) $l = 0.1 \mu\text{m}$, $L = 8 \mu\text{m}$, optimal values given by Zeng and Tang (2009), Na^+ channel density $1800 \mu\text{m}^{-2}$, (B) $l = 0.2 \mu\text{m}$, $L = 8 \mu\text{m}$, Na^+ channel density $1800 \mu\text{m}^{-2}$, (C) $l = 0.2 \mu\text{m}$, $L = 3 \mu\text{m}$, Na^+ channel density $900 \mu\text{m}^{-2}$, based on data on lipid raft placement given by Pristerà et al. (2012) and personal communication with Amber Finn, (D) Uniform distribution. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

5.3.3 Propagation velocity over clustered Na^+ channels

Due to their very small diameter, it is extremely difficult to obtain intracellular data from C-fibres, and therefore we can only estimate the propagation velocity in these fibres using extracellular recordings (Tigerholm et al. 2014). These estimations can not be reliably linked to axonal diameter. C-fibre axons are known for their very low conduction velocities. The conduction velocity is estimated to be 69 cm s^{-1} for a $0.25 \mu\text{m}$ diameter axon (Tigerholm et al. 2014).

Deterministic simulations yield a velocity of approx. 11 cm s^{-1} in both the axon with uniformly distributed Na^+ channels, and over clustered Na^+ channels (Fig. 5.7). In stochastic simulations,

we obtained a comparable median value. However, as was the case with the metabolic cost of APs, shortening lipid rafts or increasing the distance between them resulted in a reduction of the AP propagation velocity.

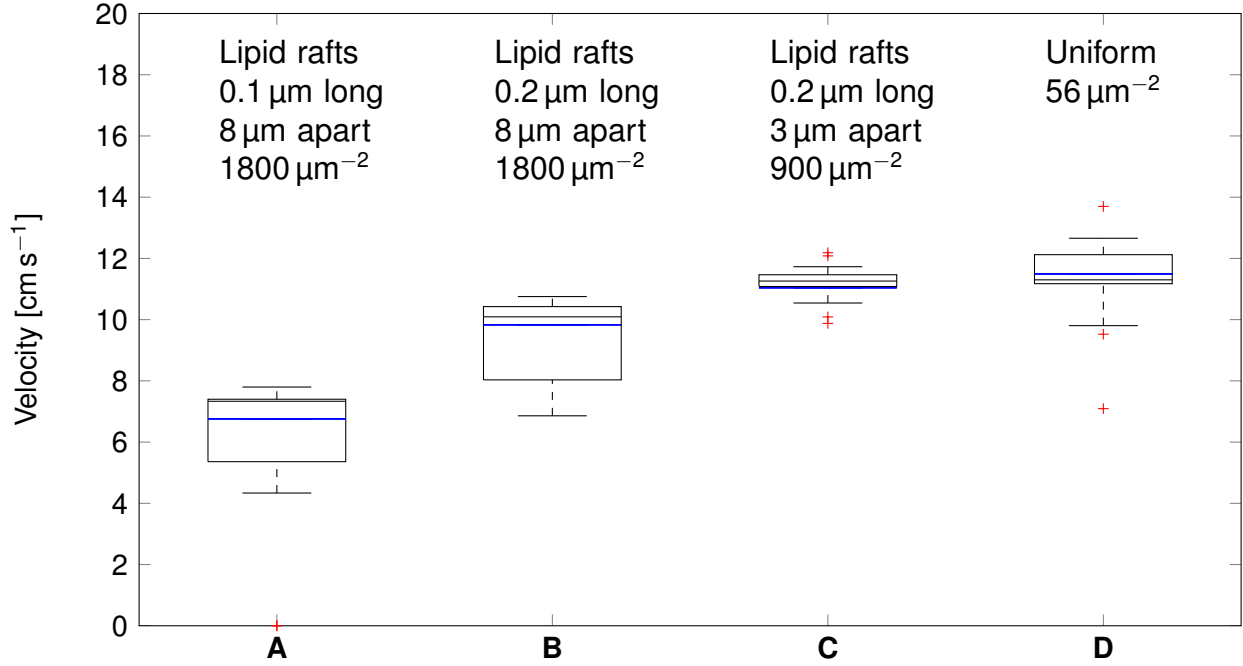


Figure 5.7: Propagation velocity of action potentials for different channel distributions in a 0.1 μm diameter C-fiber axon. Data was obtained using both deterministic (blue lines) and stochastic (boxes) simulations. (A) $l = 0.1 \mu\text{m}$, $L = 8 \mu\text{m}$, optimal values given by Zeng and Tang (2009), Na^+ channel density 1800 μm^{-2} , Note that in stochastic simulations some APs failed to propagate. (B) $l = 0.2 \mu\text{m}$, $L = 8 \mu\text{m}$, Na^+ channel density 1800 μm^{-2} , (C) $l = 0.2 \mu\text{m}$, $L = 3 \mu\text{m}$, Na^+ channel density 900 μm^{-2} , based on data on lipid raft placement given by Pristerà et al. (2012) and personal communication with Amber Finn. (D) Uniform distribution. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

This difference can be attributed to the lowered inward ionic current. In axons, membrane current not only depolarises the local membrane, but it also serves to drive the waveform of APs forward. A lower membrane current will result in slower depolarization of the membrane segment “ahead”, and thus in slower AP propagation. Increasing L , and partially compensating by increasing the density of Na^+ channels as done in Section 3.2 reduces the metabolic cost, and accordingly the propagation velocity of APs. In the most extreme case we considered,

with 0.2 μm long lipid rafts placed 8 μm apart, the median propagation velocity was approx. 7 cm s^{-1} . In this axon, stochastically simulated APs fail to propagate in 3 trials out of 20. Deterministic simulations yield a velocity of approx. 11 cm s^{-1} in both the axon with uniformly distributed Na⁺ channels, and over clustered Na⁺ channels (Fig. 5.7). In stochastic simulations, we obtained a comparable median value. However, as was the case with the metabolic cost of APs, shortening clusters or increasing the intercluster distance resulted in a reduction of the AP propagation velocity.

5.3.4 Ionic mechanisms behind the reduction of metabolic cost

In order to find the mechanism behind the reduction of metabolic cost, we plotted the instantaneous Na⁺ current and number of open Na⁺ channels over the course of an AP (Fig. 5.8). In the more metabolically efficient axon (green and red lines and shades), APs are shorter (Fig. 5.8A, red and green curves) than in the axon with uniformly distributed Na⁺ channels, or the axon with 0.2 μm long clusters placed 3 μm apart.

The shortening of APs is due to a shorter period of Na⁺ current activity (Fig. 5.8B) in metabolically efficient axons. The Na⁺ current seems to be relatively constant over the course of the AP. However, plotting the number of open Na⁺ channels (Fig. 5.8C) reveals that Na⁺ conductance reaches its peak near the peak of the AP. In the repolarising phase, the number of open Na⁺ channels decreases markedly due to inactivation. But approx. halfway through repolarisation, Na⁺ channels can open again. This late reopening causes the "bump" in the repolarising phase of the AP waveform. In the more metabolically efficient axons, the number of open Na⁺ channels decreases faster, the reopening is less pronounced, and the end of Na⁺ current is reached earlier. This explains the lower overall transfer of Na⁺ charge in these axons.

We can also explain the lower metabolic cost of APs in stochastic simulations compared to deterministic simulations of the same axon (Fig. 5.6). In stochastic simulations, there is more reactivation of Na⁺ channels in the repolarising phase as compared with deterministic simula-

5 Does clustering of Na^+ channels on lipid rafts allow micro-saltatory conduction in C-fibres?

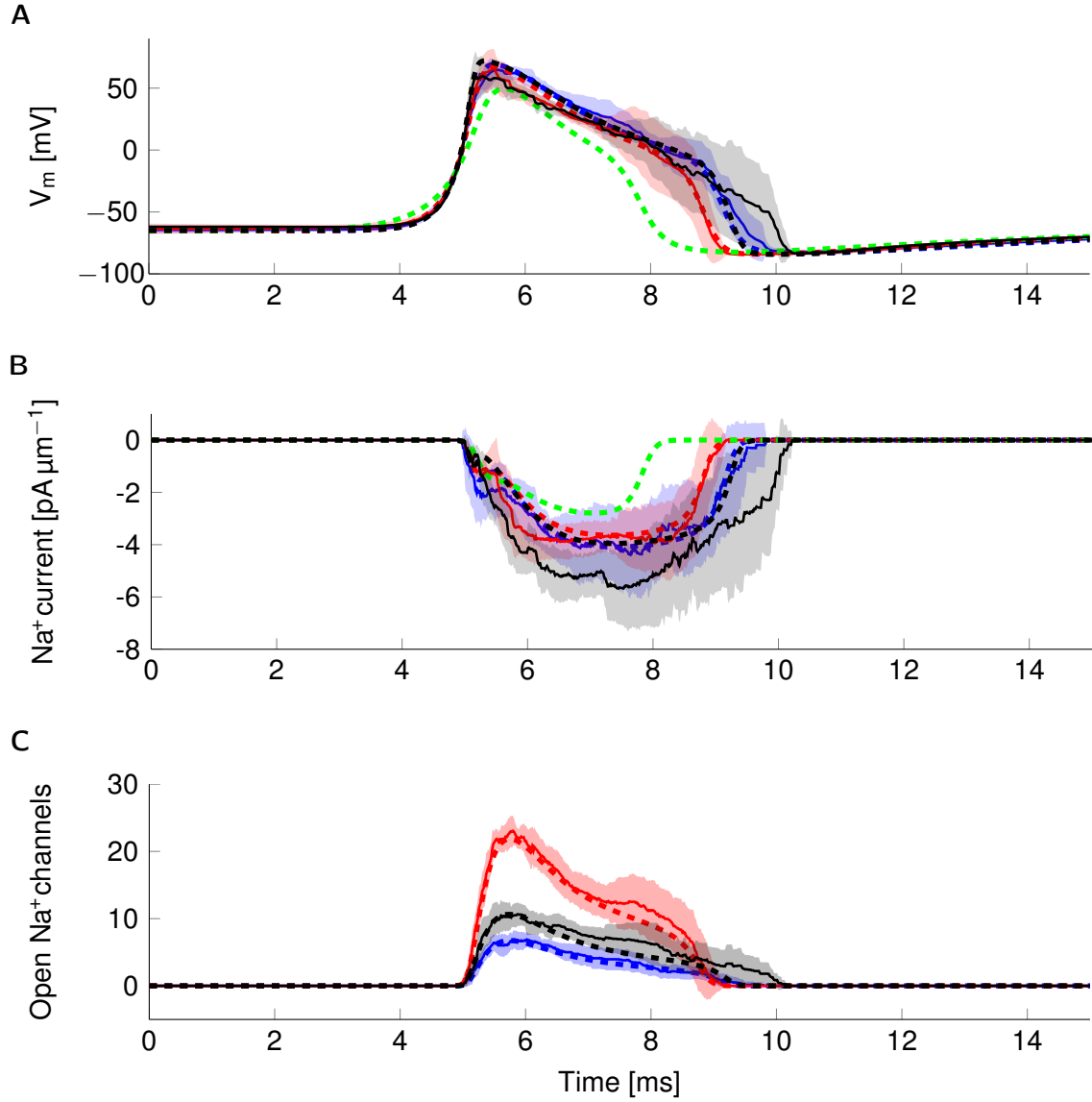


Figure 5.8: Action potential and sodium current waveform in uniformly channel density axons (Blue, STD shaded light blue, deterministic results in blue dotted line), in $0.2 \mu\text{m}$ long clusters placed $3 \mu\text{m}$ apart (Black, STD shaded grey, deterministic results in black dotted line), in $0.2 \mu\text{m}$ long clusters placed $8 \mu\text{m}$ apart (Red, STD shaded light red, deterministic results in red dotted line) and in $0.1 \mu\text{m}$ long clusters placed $8 \mu\text{m}$ apart (Dotted green line) in a $0.1 \mu\text{m}$ diameter C-fibre axon. **(A)** The action potential waveforms **(B)** Instantaneous Na^+ current and **(C)** open Na^+ channels in a single compartment. Although there are few Na^+ channels open in the repolarising phase of the AP, the larger difference between V_m and E_{Na^+} creates a Na^+ current comparable to that of the earlier stages.

tions (Fig. 5.8C). This effect is due to the "positive feedback" of Na^+ channels. The random opening of one Na^+ channel prolongs the repolarising phase, and makes the opening of other Na^+ channels more likely.

5.3.5 AP width in thin axons depends on axonal diameter

In larger diameter axons, each Na^+ channel opening during the repolarising phase has a smaller effect on the membrane potential. We thus predict that APs in larger diameters will be slightly shorter than those propagated in smaller axons. We check this hypothesis in stochastic simulations of long C-fibre axons (see Chapter 4 for information on methods).

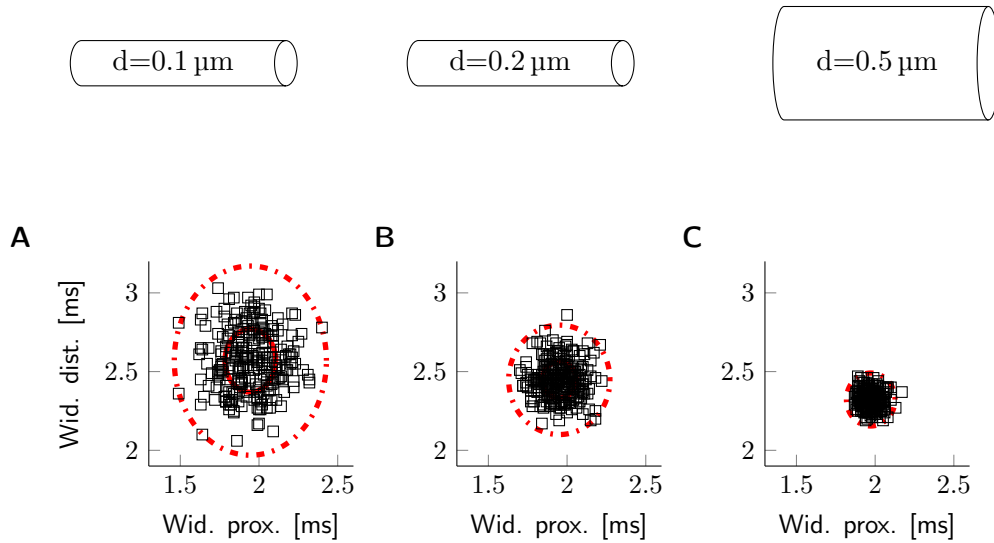


Figure 5.9: Action potential width (measured at half-peak) in C-fibre axons of **(A)** $0.1 \mu\text{m}$, **(B)** $0.2 \mu\text{m}$ and **(C)** $0.5 \mu\text{m}$ diameter. The data plotted here was obtained using the model described in Chapter 4.

Figure 5.9 shows the width of $N > 200$ APs in a measured at proximal and distance ends of a C-fibre model axon. At the distal end (y-axis), APs in the thinnest axon ($0.1 \mu\text{m}$ diameter, Fig. 5.9E) are wider than in the larger axon (approx. 2.55 ms vs. approx. 2.4 ms , Fig. 5.9C).

5.3.6 The observed effects in clusters are caused by lower Na^+ channel density

The effect of clustering Na^+ channels on the shape and metabolic cost of APs could simply be due to lower overall Na^+ channel densities. In order to verify if the clustering of Na^+ channels was in fact the responsible for the reduced metabolic cost, we simulated axon with uniformly distributed Na^+ channels by varying the density of said channels, and plotted the resulting metabolic cost (Fig. 5.10).

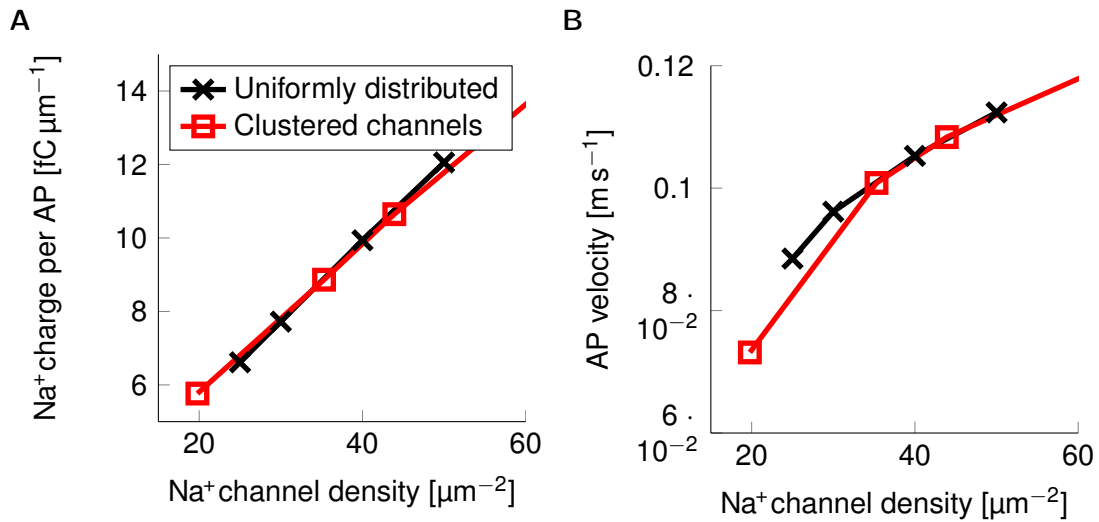


Figure 5.10: Metabolic cost and action potential velocity as a function of Na^+ channel density in a $0.1 \mu\text{m}$ diameter C-fibre axon. **(A)** Metabolic cost and **(B)** action potential velocity as a function of Na^+ channel density in both axons with uniformly distributed Na^+ channels (black) and axons with clustered Na^+ channels (red). Although the overall density of Na^+ channels clearly has an impact on both metabolic cost and velocity, there is no detectable effect from clustering Na^+ channels in lipid rafts.

There is no noticeable difference between the metabolic cost of APs in the axon with uniformly distributed Na^+ channels and the metabolic cost of APs propagating along an axon with clustered Na^+ channels if both axons have the same overall Na^+ channel density (Fig. 5.10A). Equivalently, the propagation velocity of APs is also the same in both types of axons, if the Na^+ channel density is kept constant (Fig. 5.10B). Our results show that the observed effect

on the metabolic cost is due solely to the reduced equivalent Na^+ channel density. That is, reducing the density of Na^+ channels in the uniformly distributed channels model produces the same short and metabolically efficient axons than in the clustered model.

5.3.7 AP efficiency depends on Na^+ density

Using our data, we can estimate the efficiency of AP propagation in C-fibre axons. Here we define the efficiency as Na^+ influx needed to charge membrane capacitance to AP peak/ Na^+ influx per AP. Na^+ influx to charge membrane capacitance to AP peak is given by $dC_m \Delta V$ where ΔV is the AP amplitude. The effective Na^+ influx per AP can be estimated using our simulation data. For the $0.1 \mu\text{m}$ diameter C-fibre axon, we have plotted the results in Fig. 5.11.

The C-fibre axon seems extremely inefficient. The axon with uniformly distributed ion channels is consuming almost 50 times the capacitive minimum current necessary to charge its membrane to AP peak. The least inefficient axon in this figure still is 20 times more expensive than the theoretical minimum. To check if this inefficiency is due to the fact that some of the current needs to be used to propagate the AP along the axon, we simulated a simple spherical membrane using the same ion channel densities and physiological data than the axon. We then compare the AP waveform in the spherical compartment and the axon in Fig. 5.12.

In the soma, the effective Na^+ current is approx. 20 times the capacitive minimum current. This inefficiency factor is much larger than to even notably inefficient axons such as the squid giant axon (Hodgkin 1975; Vetter et al. 2001). This inefficiency seems due to incomplete inactivation of Na^+ channels. Indeed, plotting the β_h function used for Na^+ channels in this chapter reveals a significant difference compared to that used for Na^+ channels in the squid giant axon, for instance (Fig. 5.13).

In order to confirm this, we simulated a spherical membrane compartment using physiological data from the C-fibre axon model, but with the same β_h function as the squid giant axon Na^+ channels. The shape of the AP waveform and Na^+ current in this model is plotted in

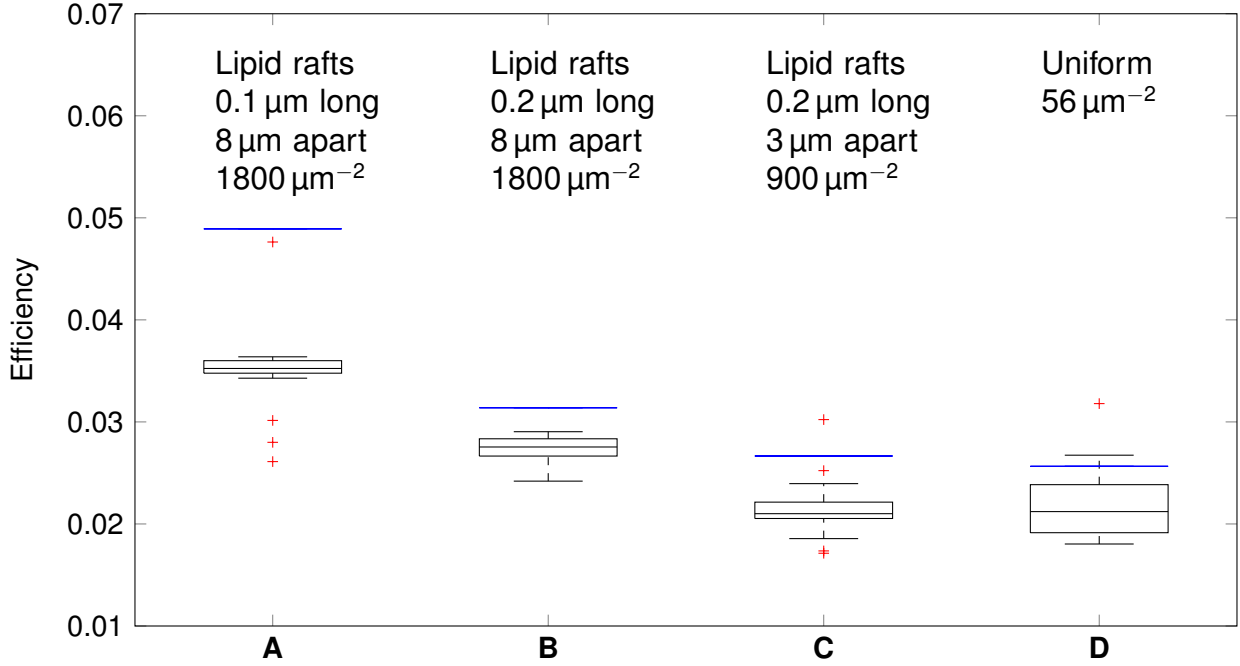


Figure 5.11: Efficiency of action potentials for different channel distributions in a 0.1 μm diameter C-fibre axon. Data was obtained using both deterministic (blue lines) and stochastic (boxes) simulations. **(A)** $l = 0.1 \mu\text{m}$, $L = 8 \mu\text{m}$, optimal values given by Zeng and Tang (2009), Na^+ channel density $1800 \mu\text{m}^{-2}$, **(B)** $l = 0.2 \mu\text{m}$, $L = 8 \mu\text{m}$, Na^+ channel density $1800 \mu\text{m}^{-2}$, **(C)** $l = 0.2 \mu\text{m}$, $L = 3 \mu\text{m}$, Na^+ channel density $900 \mu\text{m}^{-2}$, based on data on lipid raft placement given by Pristerà et al. (2012) and personal communication with Amber Finn. **(D)** Uniform distribution. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

Fig. 5.12. Action potentials in this model are much shorter than with the original kinetics for Nav1.8, and the total amount of Na^+ current crossing the membrane is correspondingly smaller. This results in a higher efficiency for the revised kinetics model, the inefficiency factor (Effective Na^+ current over minimum Na^+ current) being approx. 3, compared to approx. 17 for the soma with original Nav1.8 kinetics. These calculations take into account the difference in the amplitudes of APs between the two models.

5.4 Discussion

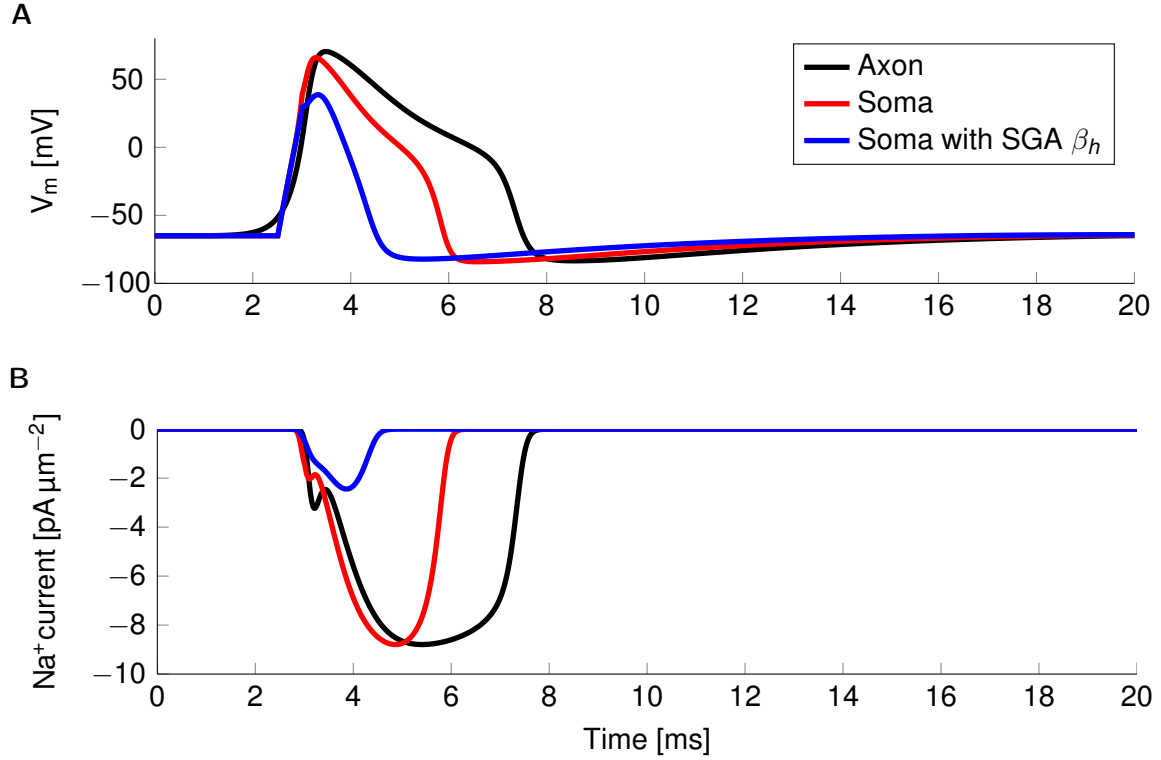


Figure 5.12: **(A)** Action potential and **(B)** scaled Na^+ current waveform in a uniform channel density axon (Black) and in a soma (Red), compared to the values obtained using a Na^+ channel model with β_h kinetics taken from the squid giant axon (Blue). The AP is wider and more metabolically expensive in the axon.

We find that microsaltatory conduction is possible in C-fibre axons with clustered Na^+ channels, i.e. action potentials (APs) can propagate from one cluster of Na^+ channels to the next in thin C-fibre axons. We also show how late reactivation of Na^+ channels affects the average shape of AP waveforms and increases the metabolic cost of APs in thin axons. Reducing the density of Na^+ channels in both axons with uniformly spaced Na^+ channels and axons with clustered Na^+ channels results in shorter and therefore more metabolically efficient APs.

Varying the length of clusters (l) and the distance between them (L) effects the shape of AP waveforms. Smaller clusters, as well as clusters placed further apart from each other, reduce the width of APs. This is due to reduced reactivation of Na^+ channels in the repolarising phase of the AP. Because in this phase the membrane potential is already far from Na^+ reversal potential (E_{Na^+}), a large current, comparable to the current at the peak of the AP, crosses the membrane

through any randomly reactivated Na^+ channel.

The reactivation of even a small number of channels maintains the membrane potential in a depolarised state longer. This in turn opposes the repolarisation of the membrane, leaving more time for the possible opening of other channels. This positive feedback effect makes APs slightly wider in stochastic simulations, where the possible stochastic opening of channels is taken into account. The discretisation of ion channel conductances amplifies this effect, by increasing the minimum conductance. Since the effect of the opening of each channel is bigger in smaller axons (Faisal and Laughlin 2007), reactivation of Na^+ channels results in slightly wider action potentials in thinner axons.

Our simulations lead to two new findings regarding the metabolic cost of propagating APs in C-fibres.

First, incomplete inactivation of Nav1.8 channels, the primary voltage gated Na^+ channels in C-fibres, leads to a long lasting Na^+ current. This in turn creates very wide APs, which are metabolically very expensive. The Na^+ charge transfer necessary for one AP in a spherical membrane using these Na^+ channels is 17 times more than the minimum charge transfer needed to depolarize the membrane to AP peak. This value is higher than 4, previously obtained for squid giant axon channels (Hodgkin 1975; Attwell and Laughlin 2001), and much higher than the very metabolically efficient channel kinetics (Alle et al. 2009; Sengupta et al. 2010). Although incomplete inactivation has been shown to allow fast spiking (Carter and Bean 2009), it is not clear why slow firing fibres such as C-fibres exhibit the same phenomena. Presumably, the very slow firing rates of these high-threshold fibres reduce the impact of metabolic cost of signalling

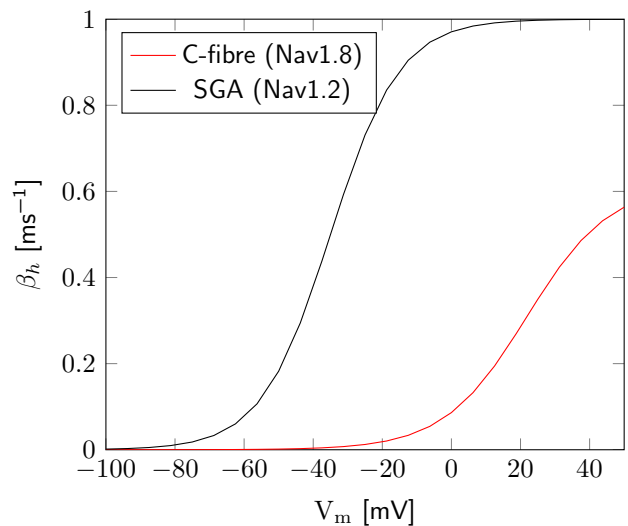


Figure 5.13: Difference of inactivation kinetics between Nav1.2 (SGA) and Nav1.8 (C-fibre) channels. Here we plot the β_h function, as defined in the m^3h , or 8-state Na^+ channel model. $\beta_h^{1.8}$ is much lower than $\beta_h^{1.2}$ over the whole biophysically relevant range.

in C-fibres. The very wide APs may have a functional role by ensuring a strong post-synaptic response (Klein and Kandel 1980; Augustine 2001), and thus prioritize APs carried by C-fibres. Another explanation may be that incomplete deactivation plays a role in ensuring transmission of APs in noise-prone thin fibres. Nav1.8 are not the only channels expressed on C-fibres (Black et al. 2002, 2012; Vasylyev and Waxman 2012) and there is evidence for other channel types to be present uniformly along these axons. It is possible that these channels allow for lower Nav1.8 densities. The role of the Nav1.8 channels could then be to ensure a wide action potential, and clustering them together would lower the overall metabolic cost. More detailed simulations are needed to test this hypothesis.

We also find that the cost of propagating APs in axons is significantly higher than that of an AP in a spherical membrane compartment. In our simulations, the cost of propagating action potentials in axons is roughly three times the cost estimated at the soma. The higher cost is associated with wider APs in the axon than in the soma. Our simulations use the same Na^+ channel kinetics in the soma model and in the axon, and the broadening effect can thus only be attributed to the spatial arrangement of the membrane, as opposed to channels kinetics (Hallermann et al. (2012) for instance, use different channel kinetics in their model which leads to narrower APs in axons).

The higher metabolic costs obtained in axons may suggest that estimates on the cost of APs derived from simulations conducted in somas (Sengupta et al. 2010) may underestimate the actual cost. This has a significant implication for the fraction of the brain's energy consumption devoted to the propagation of APs in axons (Attwell and Laughlin 2001; Harris and Attwell 2012).

Lipid rafts play a role in organising trafficking and localisation of proteins on the membrane and the clustering of Na^+ channels over lipid rafts may be beneficial in this context. Lipid rafts may allow colocalisation of ionic pumps and Na^+ sensitive channels with Nav1.8 channels, which may have some beneficial results on the cell's ionic homeostasis by placing ion pumps near the sources of current. A comparative summary between myelinated axons and unmyelinated axons

with clustered Na^+ channels is shown in Table 5.2.

Myelinated axon	Axon with lipid raft
Large (inner diameter $> 0.2 \mu\text{m}$)	Thin ($0.1 \mu\text{m}$ to $0.2 \mu\text{m}$ diameter)
Low membrane capacitance and leak conductance	Higher membrane capacitance
Low leak conductance	Higher leak conductance
Nodes of Ranvier placed at large distances	Clusters near each other ($< 20 \mu\text{m}$ apart)
Fast AP propagation	No change in propagation velocity
Low metabolic cost per AP	No change in metabolic cost

Table 5.2: Summary of similarities and differences between myelinated axons and unmyelinated axons with lipid rafts

Because there is no myelin sheath around C-fibre axons, the membrane capacitance and leak conductance are too high for Na^+ clusters to be placed at distances on the order of the axon's length constant ($\lambda \approx 200 \mu\text{m}$). In our simulations, the maximum distance L_{max} between clusters which allowed action potential propagation was approx. $20 \mu\text{m}$. The proximity of Na^+ channel clusters makes the waveform of the action potential virtually unchanged, compared to the waveform in an axon with uniformly distributed Na^+ channels. This is in stark contrast with myelinated axons, where the myelin sheath lowers the capacitance and leak conductance of the membrane. As a result, nodes of Ranvier can be placed much further apart. However, axons as thin as those studied here ($d = 0.1 \mu\text{m}$) can not be myelinated, as we showed in Chapter 3.

6 Axon structure-function relationships from homeostatic constraints

6.1 Introduction

Most of our classical understanding of axons comes from large, easy to study axons such as the squid giant axon. However, basic physical principles dictate that as axons become thinner, the effect of processes happening over their membrane becomes larger. This makes thin axons a prime candidate for studying the impact of biophysical constraints. Recently, advances in both theoretical (White et al. 2000; Faisal et al. 2005) and experimental (Sasaki et al. 2012) methods have permitted us to gain valuable insight into the world of small axons.

Action potentials (APs) are the fundamental signal of nervous systems. An AP is a short transient change of the membrane potential, which is propagated by an active process (Hodgkin and Huxley 1952). APs need to be short in order to enable high firing frequencies and thus high rates of information transfer. The rate of firing is on the other hand limited by metabolic constraints. 75% of the brains energy consumption is due to signalling costs (Attwell and Laughlin 2001) and energy considerations limit the timescale of information processing in neurons to the millisecond range (Attwell and Gibb 2005).

Propagation of APs requires the expenditure of a relatively large amount of energy in a very short

timespan. These requirements force the existence of a local pool of potential energy near the consumption site. In other words, the short duration of an action potential makes it impossible to "wait" for the energy to be converted from glucose, or even for any process involving ATP to take place. A neuron's energy store for immediate electrical signalling use is the potential energy stored in form of ionic concentration gradients over the membrane. The difference of ionic concentrations between the inside and outside of cells causes the ions to diffuse in the direction opposed to the concentration gradients. For example, the concentration of Na^+ inside mammalian nerves is 5 mM to 15 mM, as opposed to 145 mM outside (Johnston et al. 1995). This osmotic pressure pushes Na^+ inside the cell through voltage gated ion channels. The movement of Na^+ ions leaves negative charges behind them, and brings positive charges inside, creating the inward current responsible for the AP rising phase.

The presence of the bi-lipid membrane causes ions to accumulate on opposite sides of the membrane. This creates an electrical potential gradient, which opposes the diffusion of ions. Na^+ ions, for instance, are attracted to the negative charges they leave behind. The amplitude of this force depends on the electrical gradient, i.e. the membrane potential (V_m). The V_m value at which the electrical force cancels out the osmotic pressure is called the ionic reversal potential, or Nernst potential (Hille 1992). The Nernst potential depends on the concentration of ions inside and outside of the cell, and is given by the formula:

$$E_A = \frac{RT}{zF} \ln \left(\frac{[A]_o}{[A]_i} \right) \quad (6.1)$$

Where A is the ion of interest, R is the gas constant, T the temperature, z the ion's charge, and F the Faraday constant. Typical values are between 61 mV to 90 mV for Na^+ reversal potential (E_{Na^+}), and -90 mV for K^+ reversal potential (E_{K^+}) in mammalian neurons.

At rest, very few voltage gated sodium (Nav) channels are open. The resting V_m is therefore close to E_{K^+} . An AP, on the other hand, causes Nav channels to open. The influx of Na^+ drives the membrane potential toward E_{Na^+} , creating the raising phase of the AP. Once the Nav channels

close, Kv channels let K^+ out of the cell, pushing V_m back to a value near E_{K^+} . Ionic reversal potentials constrain the range of possible value for the V_m . Given the relative values of selective conductances, which are much higher for Na^+ and K^+ than for the other ions (Ca^{++} , Cl^-), the membrane potential can be assumed to be always between E_{Na^+} and E_{K^+} .

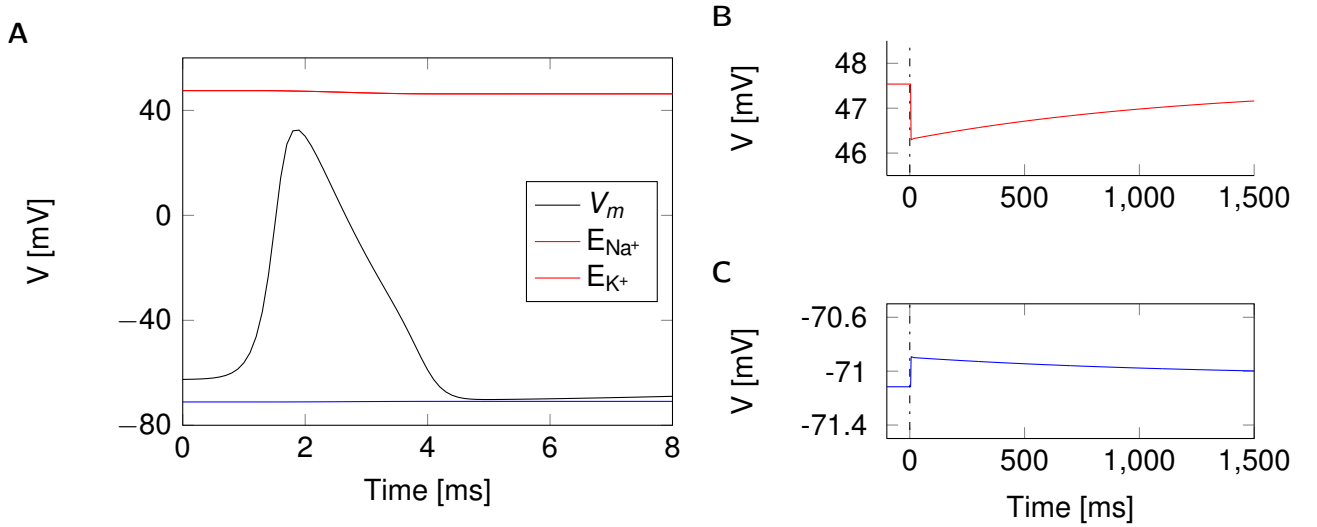


Figure 6.1: The effect of one action potential in a thin axon ($0.5\mu m$ diameter) using a model of the squid giant axon. **(A)** The waveform of an action potential (black), Na^+ reversal potential (red) and K^+ reversal potential (blue). The membrane potential is always between the reversal potentials. **(B)** Change in Na^+ reversal potential (red) after one AP (dashed line). **(C)** Change in K^+ reversal potential (blue) after one AP (dashed line).

With each AP, the difference of concentration is eroded due to the entry of Na^+ ions and exit of K^+ ions (Fig. 6.1). The total number of ions present is usually assumed to be high enough that this does not result in a measurable change in concentrations (Dayan and Abbott 2001). This assumption holds in case of large axons traditionally studied e.g. the squid giant axon, with a diameter of up to 1 mm (Hodgkin and Huxley 1952). However, in case of very thin axons e.g. C-fibres ($0.1\mu m$ to $0.3\mu m$ diameter Berthold and Rydmark 1978), the volumes involved are up to 10^8 times smaller per unit length than in the squid giant axon. Thus, in thin axons, the amount of ions displaced could result in a noticeable change in concentration, and reversal potentials which effect the cell activity (Forrest et al. 2012; Kager et al. 2000).

Concentration gradients are maintained by ionic pumps, which expel Na^+ out of the cell, and import K^+ into the cell. The energy necessary for the pumps is provided by mitochondria. There is experimental evidence that mitochondria are mobile inside the cell at rates of $\mu\text{m s}^{-1}$ (reviewed by Hollenbeck and Saxton 2005). There are mechanisms relocating mitochondria to areas with high energetic needs such as synapses or nodes of Ranvier (Zhang et al. 2010). Dysfunction in mitochondrial transport is known to negatively impact cell survivability (Sheng and Cai 2012; Edgar et al. 2008). It has also been shown that mitochondrial activity can be upregulated in response to sudden increases in cellular metabolic consumption (Hogan et al. 2009; Andrews et al. 2006). Because of their size ($0.5\ \mu\text{m}$ to $10\ \mu\text{m}$), mitochondria can not circulate inside axon of less than $0.5\ \mu\text{m}$ diameter. They must be located either at the branching point, or in a varicosity along the axon. ATP molecules, synthesised by the mitochondria, diffuse along the axon and provide the energy necessary to re-establish and maintain ionic concentration gradients. The finite diffusion rate of ATP along axons limits the supply of pumps, and may constrain the anatomy of thin axons.

In this chapter, we explore the impact of metabolic constraints (limited number of pumps and limited rate of ATP supply to pumps) in thin axons using detailed simulations. Our goal is to derive links between structure and function of axons using basic biophysical theory. In order to investigate the possible effects on the cell's ionic homeostasis, we keep track of changes in reversal potentials in real time, and model the activity of ionic pumps. A variety of computational models are available for the action of ionic pumps. At the lowest level the widely accepted Post-Albers model (Apell 1989) describes the pumping cycle in a manner similar to description of voltage-gated ion channel dynamics. In its basic form, the Post-Albers cycle is a consecutive chain of 15 possible conformational states for the pump. Transition rates between states are governed by ionic concentrations (Smith and Crampin 2004). Simplified models have also been proposed (Smith and Crampin 2004; Terkildsen et al. 2007; Gadsby et al. 2012) and aim to reduce the computational complexity. Higher level models have been proposed based on data from cardiac cells (Courtemanche et al. 1998). Based on data from canine brain (Astrup et al.

1981), Attwell and Laughlin (2001) suggest an equation of the form $\Delta i_{\text{pump}} \sim \Delta[\text{Na}^+]_i$ for the case where $[\text{Na}^+]_i$ varies by a small amount around the resting value. Based on data from cardiac cells and for the soma of Purkinje cells, Forrest et al. (2012) suggest a compatible relationship:

$$i_{\text{pump}} = d_{\text{pump}} \frac{V + 70}{(V + 80) \exp\left(\frac{K_{\text{Na}^+} - [\text{Na}^+]_i}{[\text{Na}^+]_{is}}\right)} \quad (6.2)$$

Where the cooperativity constant $[\text{Na}^+]_{is}$ equals 1 mM and the Na^+ affinity constant K_{Na^+} is 40 mM. Here we use a model of ionic pumps based on data from hippocampal pyramidal cells (Kager et al. 2000). We presented part of this work at the *Ion Channels in Health and Disease Symposium* (Cambridge, UK 2013), and the *Champalimaud Neuroscience Symposium* (Lisbon, Portugal 2012).

6.2 Methods

We develop a concentration based model of the AP, based on Hodgkin-Huxley (Hodgkin and Huxley 1952) and Nernst equations. Ionic concentrations are no longer assumed to be constant, but are constantly modified by taking into account current crossing the membrane through ion channels. Ionic reversal potentials were updated at each time step to reflect changes in concentrations.

Simulations were based on biophysical data. Computations were carried out using the Modigliani simulator (see Faisal et al. 2002, <http://www.modigliani.co.uk>), on a Linux PC using an Intel core i7 processor. In addition to the improvement detailed in Chapter 2, we implemented a new axon simulator program. At each time step, Na^+ and K^+ currents are used to calculate the number of ions crossing the membrane, and to update ionic concentrations inside and outside. Simulations were carried out using models of squid giant axon (squid giant axon) channels (detailed in Chapter 4. We also simulated a model of vertebrate C-fibre axons with Nav1.8 channels (Baker 2005), detailed in Chapter 5. The parameters for each model are summarised

in Table 6.1. We validated the model by verifying that the amplitude and width of APs were compatible with published data (Faisal et al. 2005; Baker 2005).

Parameter	Squid giant axon	C-fibre
Membrane capacitance [$\mu\text{F cm}^{-2}$]	1	0.81
Axial resistance [$\Omega\text{ cm}$]	35.4	70
Leak conductance [mS cm^{-2}]	0.3	0.14
Na^+ single channel conductance [pS]	20	20
Na^+ channel density per μm^2	60	62.5
K^+ single channel conductance [pS]	20	17
K^+ channel density per μm^2	18	10
Leak rev. pot. [mV]	-54.4	-61.14
$[\text{Na}^+]_i^{t=0}$ [mM]	20	6.4
$[\text{Na}^+]_o^{t=0}$ [mM]	144	144
$[\text{K}^+]_i^{t=0}$ [mM]	115	166
$[\text{K}^+]_o^{t=0}$ [mM]	6	6
K_{Na^+}	20	20
K_{K^+}	2.5	2.5
Temperature [$^\circ\text{C}$]	6.3	24

Table 6.1: Simulation parameters

We simulated thin axons (diameters between $0.2\mu\text{m}$ and $10\mu\text{m}$, the smallest axon being at least $0.1\mu\text{m}$ thick as shown by Faisal et al. 2005). The timestep was fixed at $10\mu\text{s}$. Each axon was divided into $20\mu\text{m}$ long compartments. Ionic concentrations in each compartment were calculated independently, and we neglected diffusion between compartments. Although diffusion is the primary mechanism for clearance of Na^+ from axon initial segments (AIS) and nodes of Ranvier (Fleidervish et al. 2010), our approach is justified for unmyelinated axons (excluding the AIS), because the APs, responsible for almost all changes in ionic concentrations, affect all compartments in the same way. The temporal difference in the effects of APs on concentrations

(determined by the propagation velocity of APs) is small compared to the diffusion time scale.

We use a commonly used model for the activity of ionic pumps (Bazhenov et al. 2004; Kager et al. 2000; Volman and Ng 2013). The pump kinetics were kept the same for both axons simulated:

$$i_{\text{pump}} = i_{\text{pump}}^{\text{max}} \left(\frac{[\text{K}^+]_o}{[\text{K}^+]_o + K_{\text{K}^+}} \right)^2 \left(\frac{[\text{Na}^+]_i}{[\text{Na}^+]_i + K_{\text{Na}^+}} \right)^3 \quad (6.3)$$

This model was chosen because it takes into account both the dependencies on extracellular potassium and on intracellular sodium concentrations. The parameter $i_{\text{pump}}^{\text{max}}$ is of utmost importance to this model, and a wide range of values have been reported in the literature. This is due in part to the different cells for which the value has been estimated, and in part because this parameter is typically used to fit the model to data, as opposed to being directly measured. In the case of hippocampal cells, values as high as $i_{\text{pump}}^{\text{max}} = 16 \mu\text{A cm}^{-2}$ are used (Volman and Ng 2013) to fit experimentally observed currents, an order of magnitude smaller than that used for nodes of Ranvier ($95 \mu\text{A cm}^{-2}$, Yu et al. 2012). We choose to express the pump current in more intuitive terms as a function of pump density d_p , maximum pump rate r_p and pump activity a_p :

$$i_{\text{pump}} = d_p r_p a_p = \left(\frac{[\text{K}^+]_o}{[\text{K}^+]_o + K_{\text{K}^+}} \right)^2 \left(\frac{[\text{Na}^+]_i}{[\text{Na}^+]_i + K_{\text{Na}^+}} \right)^3 \quad (6.4)$$

The maximum turn-over rate r_p seems to be relatively conserved across species, at approx. 200 cycles per second (Glitsch 2001). Calculating the pump density, however, proves more difficult. Simply converting the values given in the literature for the maximum pump current using the maximum pumping rate yields, for $i_{\text{pump}}^{\text{max}} = 16 \mu\text{A cm}^{-2}$, a pump density of 5000 pumps/ μm^2 at 200 Hz. This value can be put in perspective using an estimation of the size (projected on the membrane) of the single pump. Using figures from Shinoda et al. (2009), and approximating the size of the pump using that of the membrane, we can estimate the surface area of each pump at approx. 60 nm^2 , which sets the theoretical maximum pump density at approx. 16 000 pumps/ μm^2 (assuming that the entire membrane is covered in pumps.) However, i_{pump} is only a functional value, and typical values for pump current given physiologically plausible con-

centrations are much lower (Yu et al. 2012). In particular, at rest, $a_p \sim 0.1$, and the maximum value given the assumptions made here (constant outside K^+ concentration) is approx. 0.2.

For the sake of presenting a biophysically plausible pump density, we assume that the maximum effective pump current $i_{\text{pump}}^{\text{max}} \times a_p$ corresponds to all ionic pumps functioning at their maximum turn-over rate. More specifically, we replace equation (6.4) with

$$i_{\text{pump}} = d_p r_p \frac{a_p}{\max(a_p)} \quad (6.5)$$

$$= 5 \times d_p r_p a_p \quad (6.6)$$

This yields a more realistic pump density (Glitsch 2001), and will underestimate the actual number of ion pumps required. For the baseline level, we used a pump density of $d_p = 4000 \mu\text{m}^{-2}$, determined to be sufficient to counter leak K^+ current and restore reversal potentials after one AP. We also consider the case of a cell membrane with the maximum feasible pump density ($16000 \mu\text{m}^{-2}$). In doing so, we are considering two types of limits. The first is a biologically realistic set of limits bounded by the range of experimental values of specific pump currents. The second is theoretical – pumps packed with maximum density place an upper bound on neuronal performance.

Parameters for the initial Na^+ and K^+ concentrations were calculated using values from Kager et al. (2000) as initial estimates, and refining them to obtain desired reversal potential ($E_{\text{Na}^+} = 55 \text{ mV}$, $E_{\text{K}^+} = -77 \text{ mV}$ for the squid giant axon based on data from Hodgkin and Huxley 1952, and $E_{\text{Na}^+} = 79.6 \text{ mV}$, $E_{\text{K}^+} = -85 \text{ mV}$ for the C-fibre axon based on values from Baker 2005). Note that these values converge to resting values by the combined action of pumps and ion channels during simulations. Kinetic values for the Michaelis-Menten dynamics are taken from Volman and Ng (2013). Since each pump cycle extrudes 3 Na^+ and imports 2 K^+ , the changes in ionic concentrations due to pumps are given by

$$\frac{d[\text{Na}^+]_i}{dt} = -\frac{4 \times 3i_{\text{pump}}}{dF} \quad (6.7)$$

$$\frac{d[K^+]_i}{dt} = \frac{4 \times 2i_{\text{pump}}}{dF} \quad (6.8)$$

Here d is the axon's diameter, and the coefficient $4/d$ is due to the ratio between the surface area and the volume of a cylinder segment.

6.3 Results

The results of our simulations are presented as follows. We first present a simple analytical calculation of the changes in reversal potentials. We then show results from the extended Hodgkin-Huxley model. A model for the evolution of reversal potential and the limits on firing rates is then presented. Finally, we show the limits on the structure of axons due to the limited rate of diffusion of ATP.

6.3.1 Simple theoretical calculations

In order to obtain a lower bound of the impact of each AP, we assumed that during the depolarising phase, only Na^+ channels open, and no leak occurs. This gives a lower bound for the total number of Na^+ displaced with each AP :

$$Q = AC_m \Delta V \quad (6.9)$$

Here C_m is the specific membrane capacitance, A the membrane area and ΔV is the amplitude of the action potential. We assumed that external ionic concentrations were kept constant either due to a larger extracellular volume compared to the intracellular volume, or the action of glia

cells. Thus, after one action potential, the sodium reversal potential will become:

$$E_{rev}^{Na} = \frac{RT}{zF} \ln \left(\frac{[Na^+]_o}{([Na^+]_i)} \right) \quad (6.10)$$

$$= \frac{RT}{zF} \ln \left(\frac{[Na^+]_o}{[Na^+]_i^{t=0} + 4C_m \frac{\Delta V}{Fd}} \right) \quad (6.11)$$

An analogous formula can be obtained for the potassium reversal potential (E_{K+}). In order to calculate the change in concentrations, we would need to calculate the amplitude of each AP (ΔV). At rest, V_m is close to E_{K+} , while the peak of the AP is slightly lower than E_{Na+} (Dayan and Abbott 2001; Ermentrout and Terman 2010). A simple approximation of ΔV would be to set:

$$\Delta V = E_{Na+} - E_{K+} \quad (6.12)$$

Using this expression, we can write a simple simulation without taking into account the impact of ionic pumps in order to highlight the impact of an AP in this axons. This is a reasonable approximation for short time scales as the current produced by ionic pumps is much smaller than the current generated by each AP. We have plotted the evolution of E_{Na+} (Fig. 6.2A) and E_{K+} (Fig. 6.2B).

After each action potential, the influx of Na^+ hyperpolarises E_{Na+} (Fig. 6.2A). At the same time, the outflux of K^+ depolarises the potassium reversal potential (Fig. 6.2B). The amount of change in these potential depends on diameter. Large axons seem virtually unaffected, as the changes in concentration do not significantly affect the larger axons ($d=1 \mu m$ and $d=10 \mu m$) until after 100 APs. Thinner axons ($0.3 \mu m$ and low) on the other hand, seem much more sensitive. The smallest axon, at $d = 0.1 \mu m$, characteristic of C-fibres, and which is the lower limit imposed by noise on the diameter of axons (Faisal et al. 2005), sees a 15 mV shift in E_{Na+} after only 10 APs. For that axon, the E_{Na+} has collapsed to 20 mV after merely 20 APs.

More importantly from the point of view of survivability of the cell, E_{K+} is depolarised to approx. -60 mV. This places the resting membrane potential at over the firing threshold. The cell will

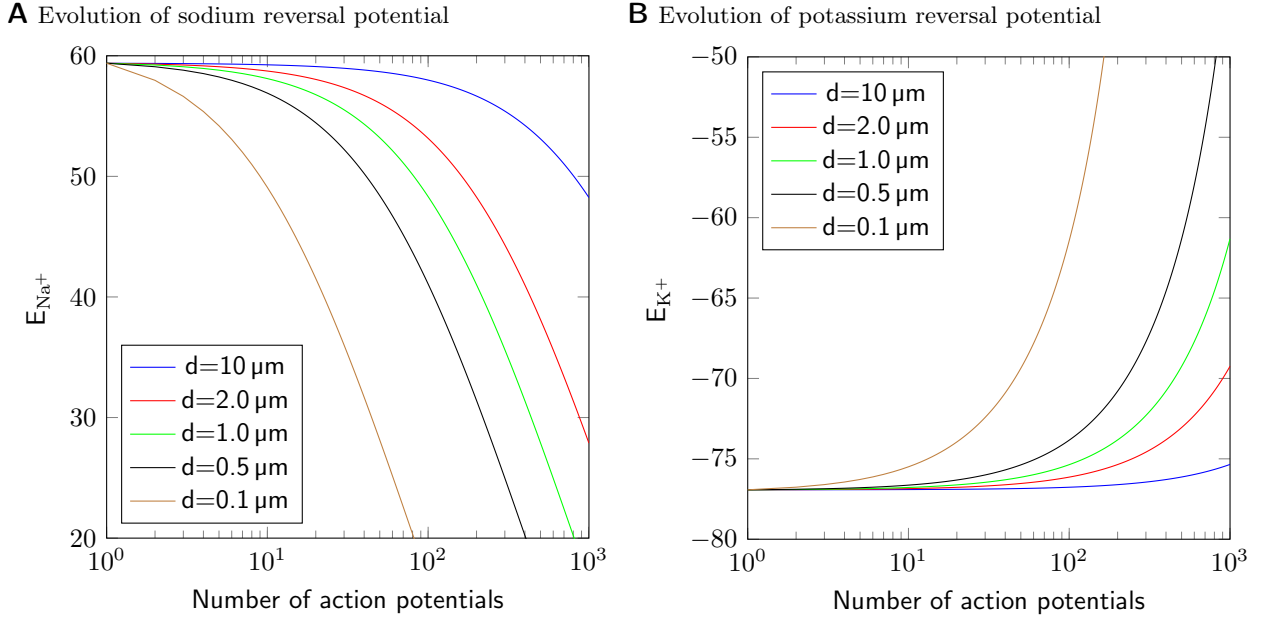


Figure 6.2: Evolution of ionic reversal potentials over the number of APs for 0.1 μm , 0.5 μm , 1 μm , 2 μm and 10 μm diameter axons. Here we simply ignore the influence of ion pumps, which operate on a much longer timescale. Note the logarithmic scale on the x axis. This plot gives us an upper limit on the number of action potentials a neuron can fire without consuming energy.

then enter a state of uncontrolled bursting. This can happen after 10-100 APs in thin axons, and lasts until the concentration gradients have been practically depleted. While our model does not allow us to find out if this state is reversible in vivo, it is likely that the depletion of ATP will lead to calcium pumps running backward and pumping calcium inside the cell, leading to cell death (Waxman 2006).

Making a few assumptions about the activity of pumps in the steady-state, we can already place an upper limit on the sustainable firing rate of axons as a function of pump density on the membrane surface. For the axons to be able to propagate APs at a constant firing rate for an infinitely long period of time, charge transfers due to each AP need to be reversed by pumps before the onset of the next AP. The charge crossing the membrane after each AP is given by equation (6.9). We consider the charge crossing the membrane per surface area $Q_m = \frac{Q}{A}$. This charge has to be reversed by the pump current before the next AP. Firing at f_{max} , and

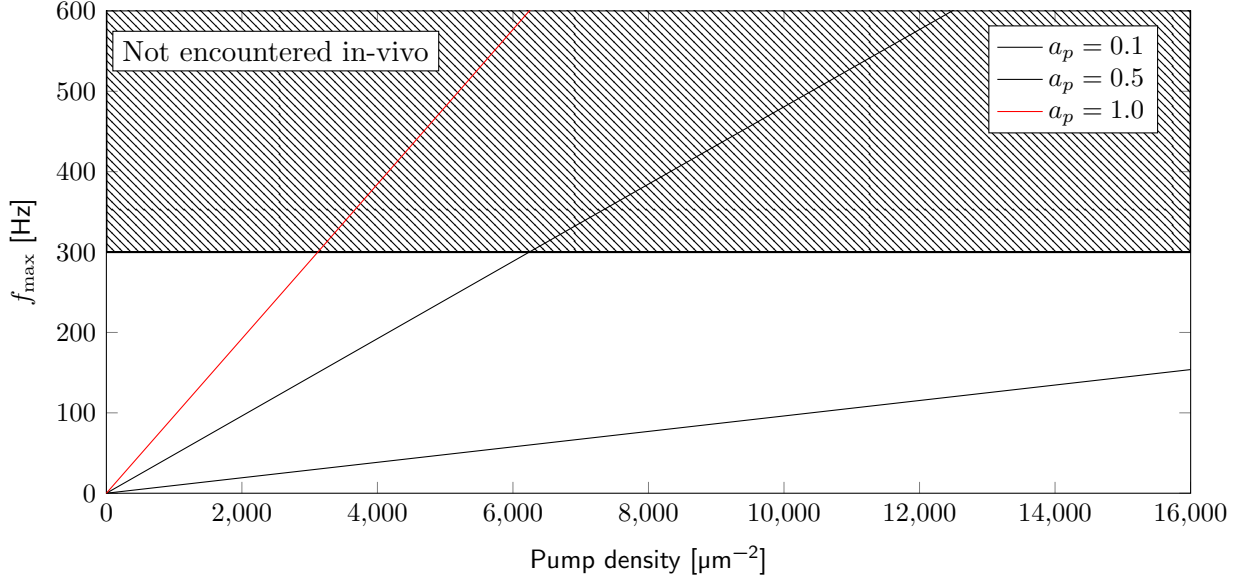


Figure 6.3: Theoretical maximum firing rates sustainable for perfectly efficient APs of amplitude 100 mV as a function of pump densities. Here we assume that the pumps have a constant activity rate, which can be understood as the average pump activity between two consecutive APs.

considering the limit case where the charges are reversed right before the next AP, we can write

$$Q_m = C_m \Delta V \quad (6.13)$$

$$= \frac{3i_{\text{pump}}^{\text{steady}}}{f_{\text{max}}} \quad (6.14)$$

$$= \frac{3d_{\text{pump}}a_{\text{pump}}^{\text{steady}}}{f_{\text{max}}} \quad (6.15)$$

Where $a_{\text{pump}}^{\text{steady}}$ is the steady state activity of the pumps (which can also be seen as the average activity over the interspike period) and $i_{\text{pump}}^{\text{steady}}$ the resulting steady state pump current. The factor 3 is due to the fact that each cycle of the pump expulses 3 Na^+ . We can plot the maximum sustainable firing rate as a function of pump density (Fig. 6.3).

The maximum sustainable firing rate is a linear function of the axon's pump density. In the case of e.g. Purkinje cells, which typically fire at 80 Hz (Cao et al. 2012), this translates into a minimum pump density of approx. $1700 \mu\text{m}^{-2}$ for $a_p = 0.5$ (i.e. assuming that the pumps are on average active at half their maximum rate), and $650 \mu\text{m}^{-2}$ for $a_p = 1$. These results assume

perfectly efficient APs and no limitation on ATP. Note that here we use a constant value for a_p , as opposed to using the Michaelis-Menten dynamics.

6.3.2 Reversal potentials deteriorate after each AP

The previous calculations rely on simplifying assumptions, and ignore the kinetics of ionic pumps. In order to obtain more accurate results, we simulated propagation of APs in axons using Hodgkin-Huxley type (Hodgkin and Huxley 1952) model of the squid giant axon, and a model of human C-fibres (Baker 2005). Each model axon was 2 mm long. APs are triggered by injecting a small amount of current at the proximal end of the axon. Our goal is to estimate the changes in ionic concentrations after each action potential, or in other words, the metabolic cost of APs. Figure 6.4 shows the changes in reversal potentials and the time required to reverse them for diameters between $0.2\text{ }\mu\text{m}$ to $10\text{ }\mu\text{m}$.

We find (Figs. 6.4A and 6.4B, red for E_{Na^+} , blue for E_{K^+}) an inverse relationship between the changes in ionic concentration gradients and the diameter of the axon for a given unit length. The change in concentrations due to a given number of ions crossing the membrane is inversely proportional to the volume which evolves as diameter squared. The number of ions crossing the membrane is itself proportional to the surface of the membrane which is proportional to the diameter. The changes in concentrations trigger a rise in the activity of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$.

We have plotted the half-restoration-times, defined as the time it takes for ionic pumps to reverse half of the AP induced change in E_{K^+} as a function of diameter (Figs. 6.4C and 6.4D). Again, we observe a linear relationship between the half-restoration-time of both E_{Na^+} and E_{K^+} . This relationship is also expected. The number of pumps, and thus the current due to their activity, is proportional to the area of the membrane. The changes in ionic concentrations, however, evolve proportionally to the volume. Thus, the scaling relationships we find in our simulations are dictated by basic biophysics and are independent of the specific membrane properties. We find the same overall relationship between changes in ionic concentrations and diameter in both

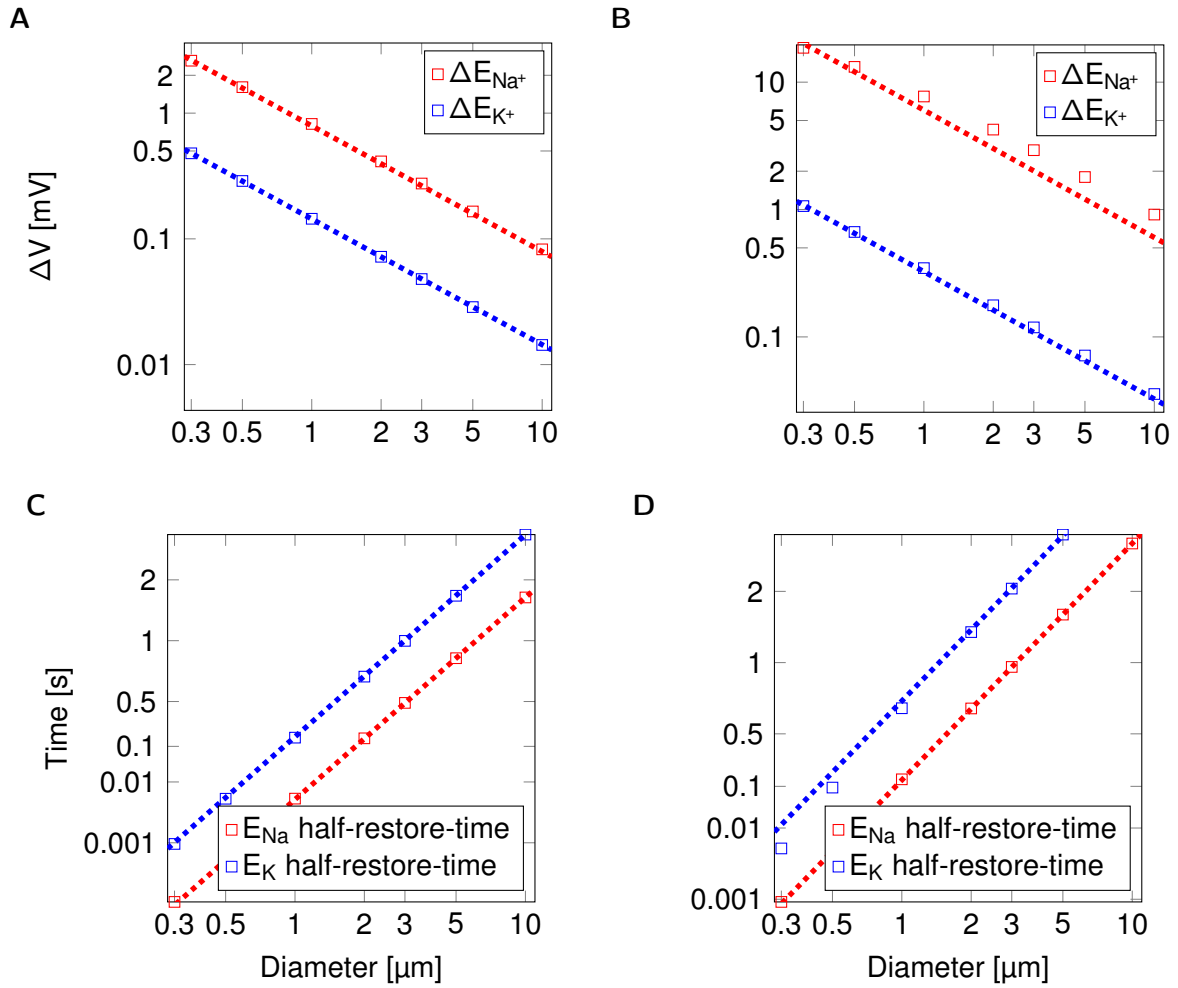


Figure 6.4: Influence of axonal diameter on the effect of each AP. **(A)** Change in ionic reversal potentials (Na^+ in red, K^+ in blue) after one AP in a model squid giant axon as a function of diameter. **(B)** Change in ionic reversal potentials (Na^+ in red, K^+ in blue) after one AP in a model C-fibre as a function of diameter. **(C)** half-restoration-time of ionic reversal potentials (Na^+ in red, K^+ in blue) after one AP in a model squid giant axon as a function of diameter. **(D)** half-restoration-time of ionic reversal potentials (Na^+ in red, K^+ in blue) after one AP in a model C-fibre as a function of diameter.

our models of squid giant axon and C-fibres.

The pumps restore the ionic concentrations and thus reversal potentials to their resting values. This however takes time, as the maximum current generated by the pumps (less than $100 \mu\text{A cm}^{-2}$) is much smaller than the typical values attained by ion channels during an action potential.

6.3.3 A simplified model for the homeostatic dynamics of reversal potentials

In order to investigate the constraints imposed by the deterioration of reversal potentials, we need a quantitative model of their evolution. For reasons stated above, E_{K^+} is of particular interest. We can approximate the evolution of reversal potentials when the axon is in a steady regime of firing. Two processes influence $[K^+]_i$. Each AP lowers the internal concentration, while pumps restore it. Since we aim to come up with an analytical solution, we will make several simplifications which we will discuss along the way. First, assuming the outer K^+ concentration is kept constant, E_{K^+} only depends on $[K^+]_i$. Second, we assume that each AP changes E_{K^+} by a fixed amount, so that the change in E_{K^+} in time due to APs is equal to $\bar{C}f$ where $\bar{C}$ is the change due to each action potential, and f the firing rate. Finally, we assume that the activity of pumps a_p is constant. We thus can write:

$$\frac{\partial[K^+]_i}{\partial t} = -\bar{C}f + \bar{p} \quad (6.16)$$

Here $\bar{p}$ is the rate of change due to the action of pumps. As we have shown in Section 6.3.2, $\bar{C}$ and $\bar{p}$ are both proportional to the diameter of the axon (d). We can thus rewrite them as:

$$\bar{C} = \frac{C}{d} \quad (6.17)$$

$$\bar{p} = \frac{p}{d} \quad (6.18)$$

The solution to equation (6.16) is simple:

$$[K^+]_i(t) = [K^+]_i^{t=0} - \frac{Cf - p}{d}t \quad (6.19)$$

Where $[K^+]_i^{t=0}$ the initial internal K^+ concentration. This equation is only valid for $Cf \geq p$, or in other words, the assumption of constant pumping activity is only valid to the point where they bring the concentration gradients to their resting level. We will introduce a slightly more complex model later in this section, but for now we can use this very simple model to gain some intuition about the processes involved.

If $Cf > p$, $[K^+]_i(t)$ will eventually reach a value which would put the resting potential above V_{th} . We call the concentration at which E_{K^+} is at its critical value $[K^+]_i^{crit}$. The condition $Cf > p$ is independent of diameter. However, the time it will take for $[K^+]_i(t)$ to reach $[K^+]_i^{crit}$ will depend on the diameter according to:

$$t = d \frac{[K^+]_i^{t=0} - [K^+]_i^{crit}}{Cf - p} \quad (6.20)$$

Equivalently, we can write the maximum firing rate the axon can sustain as a function of firing duration and diameter:

$$f = \frac{d ([K^+]_i^{t=0} - [K^+]_i^{crit})}{Ct} + \frac{p}{C} \quad (6.21)$$

The firing rate an axon can sustain scales linearly with diameter, and in inverse proportion to the duration of firing. For extremely long firing duration, this maximum sustainable firing rate approaches a limit independent of diameter at $\frac{p}{C}$. We can infer realistic values for the parameters in equation (6.21). We can assume $[K^+]_i^{t=0} - [K^+]_i^{crit} \sim 60 \text{ mM}$, which results in a change of approx. 20 mV in E_{K^+} at 36 °C. With $4000 \mu\text{m}^{-2}$ pumps functioning at 100 Hz ($a + p = 0.5$),

we can estimate:

$$\bar{p} = \frac{2d_p r_p a_p \times \text{area}}{N_A \times \text{volume}} \quad (6.22)$$

$$\sim \frac{5.3}{d} \text{mM s}^{-1} \quad (6.23)$$

Therefore, $p \sim 5.3 \text{ mM } \mu\text{m s}^{-1}$. Next, we need to estimate $\bar{C}$ as:

$$\bar{C} = \frac{Q \times \text{area}}{F \times \text{volume}} \quad (6.24)$$

$$\sim \frac{0.041}{d} \text{mM} \quad (6.25)$$

And therefore $C \sim 0.041 \text{ mM } \mu\text{m}$. This allows us to calculate the maximum feasible firing rate as a function of diameter and firing duration.

$$f[\text{Hz}] \sim \frac{60d}{0.041t} + \frac{5.4}{0.041} \quad (6.26)$$

$$\sim 130 + 1500 \frac{d[\mu\text{m}]}{t[\text{second}]} \quad (6.27)$$

These values do not take into account inefficiencies in the mechanism generating the AP, nor do they consider the ions crossing the membrane at resting state. However, they can serve as a rough estimate of sustainable firing rates. Assuming for instance an inefficiency factor of 70% for the AP, we get an estimated sustainable firing frequency of 90 Hz, very close to the observed firing frequency of Purkinje cells. We can give a more accurate estimate of the maximum firing rate in the case of the squid giant axon. Assuming an efficiency of 0.25 (Hodgkin 1975) in the AP generation mechanism is equivalent to multiplying Q and therefore $\bar{C}$ by four. We can estimate the resting state K^+ current by remarking that at -65 mV, $n_{\text{inf}}^4 \sim 0.01$, which translates into a change in K^+ concentration of approx. $2 \text{ mM } \mu\text{m s}^{-1}$ (the units are equivalent to that of p). The sustainable firing rate is therefore estimated at approx. 20 Hz, for $a_p = 0.5$.

6.3.4 A model involving pump dynamics

A slightly more complicated model can be obtained if we assume that the activity of pumps scales linearly as $[K^+]_i$ increases, so that the change in $[K^+]_i$ due to pumps can be written as $\bar{p}([K^+]_i - [K^+]_i^{t=0})$. We thus can write:

$$\frac{\partial [K^+]_i}{\partial t} = -\bar{C}f + \bar{p}([K^+]_i - [K^+]_i^{t=0}) \quad (6.28)$$

The solution to equation (6.28) is straightforward:

$$[K^+]_i(t) = [K^+]_i^{t=0} - \frac{Cf}{p} \left(1 - e^{-\frac{pt}{d}}\right) \quad (6.29)$$

This equation can give us more intuition about the processes involved. In absence of firing, the concentration is constant. In the limit where $t \gg 1$ s, the concentration will stabilise at:

$$[K^+]_i^{t=0} - \frac{C}{p}f \quad (6.30)$$

This means that assuming the asymptotic value is low enough for the resulting resting V_m to be below the firing threshold (V_{th}), this axon can continue firing at the corresponding firing rate indefinitely. In biophysical terms, between two consecutive APs, ionic pumps will restore a portion of ionic concentrations. If the delay between two consecutive APs is long enough, after the first AP the concentrations will have been restored to their resting level before the second one. The axon can thus fire indefinitely, as long as the firing rate is low enough. Interestingly, this limit on the firing rate is independent of diameter. It only depends on the cost of individual APs, and the performance of ionic pumps. This is consistent with the results of our theoretical calculations in Section 6.3.1.

But even if the firing rate is not infinitely sustainable, it can be maintained for a finite time before E_{K^+} becomes too depolarised. The concentration goes to its stable value with a time constant of $\frac{d}{p}$, in agreement with our results in Figs. 6.4C and 6.4D. The existing pool of potential energy is

large enough for at least a few APs to fire, meaning that any firing rate is theoretically possible, as long as it is not sustained for too long. Energy constraints do not directly limit the firing rate or the number of APs an axon can fire. Instead, there is an interplay between these two parameters. For a given diameter, the lower the firing rate, the longer the axon can sustain firing.

The fact that the indefinitely-sustainable-firing-rate is independent of diameter is due to the linear dependence of both ion channel current and pumping current on the diameter of the axon. Other results in this chapter, such as the expression for the maximum sustainable firing rate, depend on the simplified model.

Equation (6.29) allows us to calculate the maximum feasible firing ($F_{\max}$, in Hz) rate as a function of diameter and firing duration. Note that here we have made a number of simplifications. Notably, we keep the cost of successive APs constant, and do not impose an explicit limit on the pump current. Assuming that $F_{\max}$ is one at which E_{K^+} reaches the critical value at the end of the burst, we can write:

$$F_{\max}(d, t) = \frac{p ([K^+]_i^{\text{crit}} - [K^+]_i^{t=0})}{c \left(1 - e^{-\frac{pt}{d}}\right)} \quad (6.31)$$

This simplified model for the maximum sustainable firing rate has two parameters: c , which represents the change in the ionic concentration caused by one AP, and p the speed of restoration of the gradients.

The shape of $F_{\max}$ for two different pump densities is plotted in Fig. 6.5. Thicker axons ($2 \mu\text{m}$ diameter) can sustain the maximum firing rate allowed by ion channel kinetics for a short time (approx. 1 s). As the axon becomes thinner, and the firing duration becomes longer, the sustainable firing rate lowers. For very long firing duration, the maximum sustainable firing rate is less influenced by the diameter, and converges to approx. 2 Hz for $d_p = 4000 \mu\text{m}^{-2}$. Increasing the pump density increases the maximum sustainable firing rate. At the putative

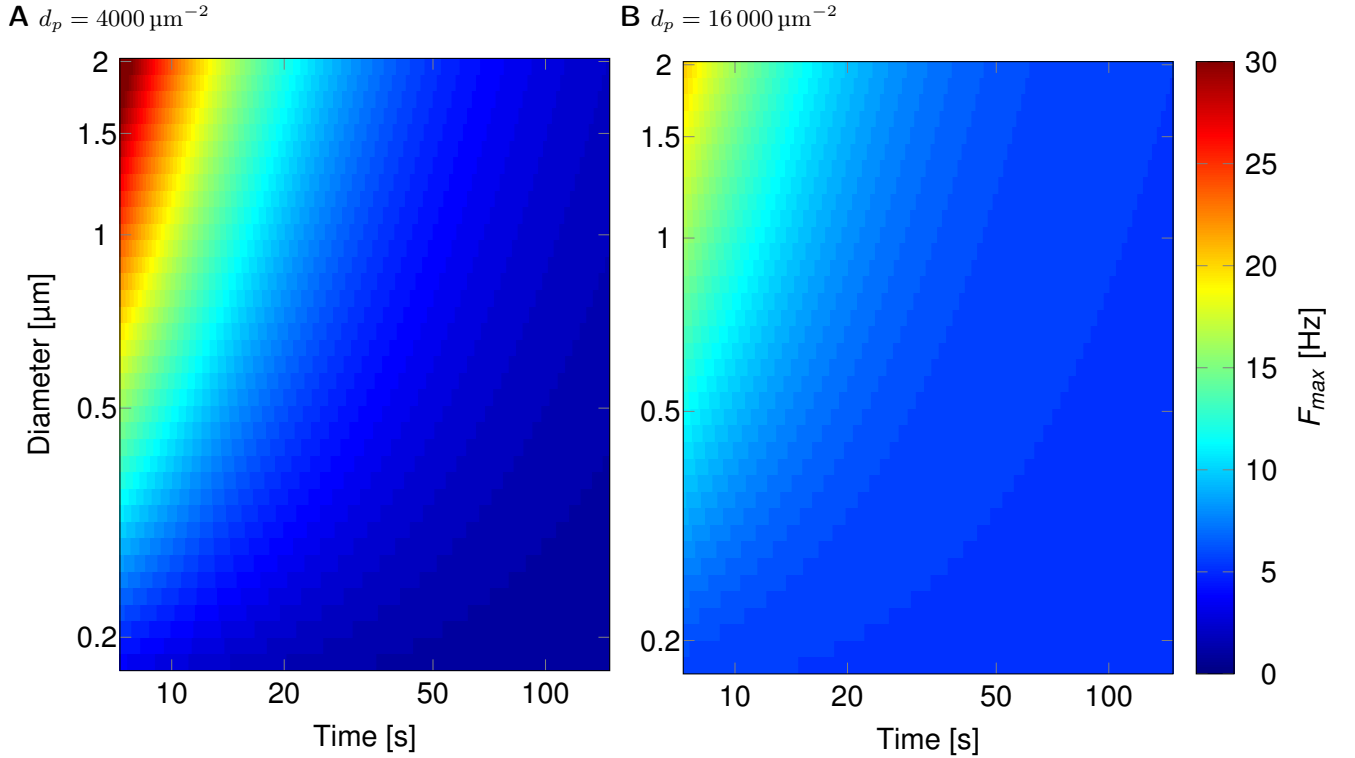


Figure 6.5: Color-map of maximum sustainable firing rates given by our simplified model using parameters from the squid giant axon. Data shown here is obtained by fitting the simplified model from equation (6.31) to the (A) blue curve and (B) red curve in Fig. 6.6. Over long time periods, the sustainable firing rate is independent of diameter (approx. 2 Hz for $d_p = 4000 \mu\text{m}^{-2}$ and approx. 6 Hz for $d_p = 16000 \mu\text{m}^{-2}$). For shorter time periods, larger axons can fire at higher rates. The refractory period prevents the axon from firing at more than approx. 40 Hz.

maximum pumping density $d_p = 16000 \mu\text{m}^{-2}$, $F_{\text{max}} \sim 6$ Hz. Next, we are going to check the validity of results given by the simplified model using biophysically principled simulations.

6.3.5 The simplified model accurately predict maximum feasible firing rates

Figure 6.6 shows the 10-second-sustainable firing rate for axons of different diameters. This data was obtained by injecting a small amount of current to induce APs at a given rate for 10 seconds. This is a plausible duration for a neuron to maintain a given firing rate. If the axon started firing at a higher rate before or did not stop firing after the 10 seconds mark, we deemed it unable to sustain the firing rate, since this would mean that the axon's resting potential has already

crossed V_{th} . Larger diameter axons ($d \geq 5 \mu\text{m}$) are able to sustain firing rates of up to 50 Hz for the whole duration of our simulations, and are limited by the actual channel kinetics rather than energy constraints. However, as the diameter decreases, lower firing rates start become unsustainable.

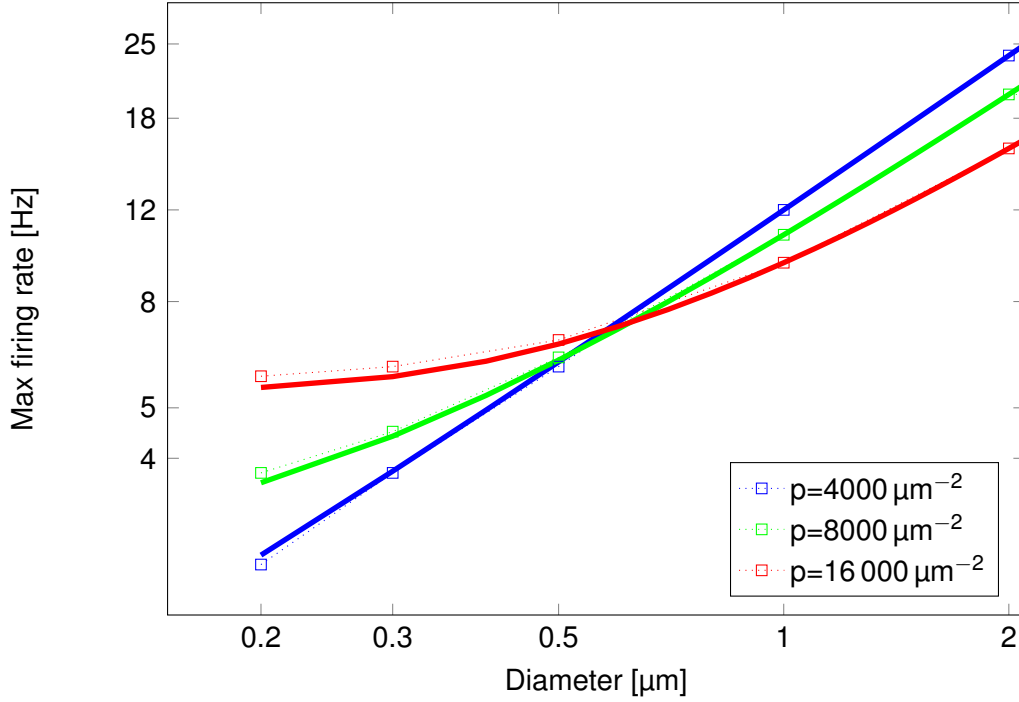


Figure 6.6: The maximum firing rate that can be sustained for at least 10 s as a function of diameter, for 3 different pump densities. Data was obtained by simulating each axon and determining if after the burst period the resting potential was restored. Lines are given by our simplified model fitted with data from the two largest axons for each pump density.

At the base-level pump density ($4000 \mu\text{m}^{-2}$), a $1 \mu\text{m}$ diameter axon fails to sustain firing rates above 12 Hz for 10 s. $0.2 \mu\text{m}$ diameter axons fail to go beyond 2.3 Hz (see Fig. 6.6). Increasing the pump density does have a beneficial effect on smaller axons. Using the maximum pump density allows firing rates of approx. 6 Hz and approx. 7 Hz for $0.2 \mu\text{m}$ and $0.3 \mu\text{m}$ diameter axons, respectively. Surprisingly, increasing the pumping density actually decreases the maximum sustainable firing rate in the larger studied axons. This is because after the first AP, successive APs have progressively smaller amplitudes (Fig. 6.7). The lower amplitudes are due to the depolarisation (toward 0) of both E_{Na^+} and E_{K^+} , which determine the maximum and minimum

values (respectively) for the membrane potential. As a result, the metabolic cost of each successive AP (which is proportional to its height) is lower. The reduction in AP cost allows the axon to fire for longer than if the AP amplitude was kept constant (Ritchie 1985). At each cycle, each pump displaces 3 Na⁺ but only 2 K⁺ (Jørgensen and Andersen 1988). Adding more pumps has a bigger effect on E_{Na+} than on E_{K+}, although it is the evolution of E_{K+} that determines the maximum firing rate as discussed in Section 6.3.1. By having too many pumps, E_{Na+} is kept high, which in turn increases the amplitude of the AP. This leads to more K⁺ current crossing the membrane after each AP in order to restore the resting potential. Since E_{K+} is not restored as fast as E_{Na+}, eventually the disproportion leads to a lower maximum sustainable firing rate.

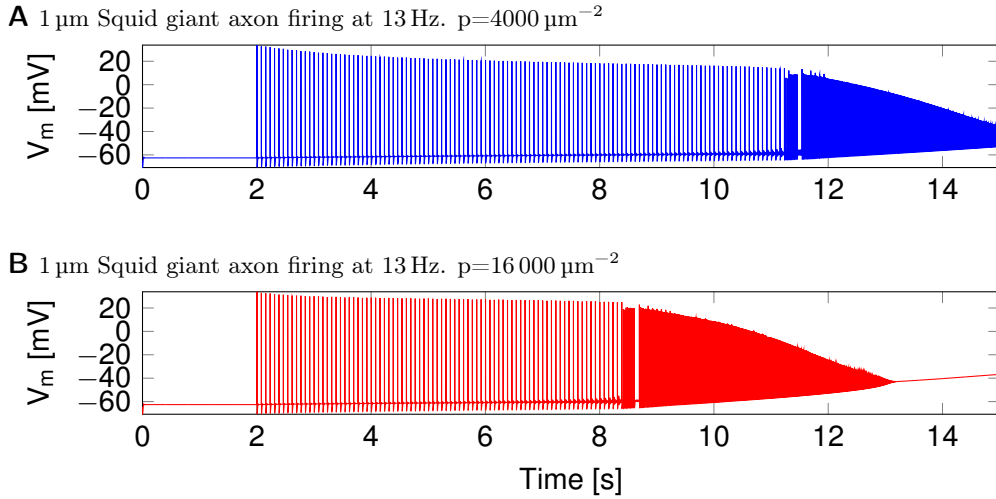


Figure 6.7: Higher pumping rates can lead to lower maximum firing rates in our model of the squid giant axon. **(A)** A 1 μm diameter axon with $4000 \mu\text{m}^{-2}$ pumps can fire at 13 Hz for more than 11 s. The height of action potentials quickly diminishes, leading to lower charge transfer per action potential. In contrast **(B)**, the same axon with $16000 \mu\text{m}^{-2}$ pumps starts bursting after less than 9 s. The height of action potentials is almost constant throughout this period.

Figure 6.6 also shows, in thick lines, our predictions for the smaller axons (0.2 μm , 0.3 μm and 0.5 μm diameter), fitted on the two larger axons (1 μm and 2 μm diameter) for all three pump densities. Our predictions, based on the simplified model in equation (6.28) are in good agreement with the results of our detailed simulations. In addition, our simplified model also predicts the nonintuitive influence of excessive pumping on the maximum sustainable firing rate.

This shows the validity of our simplified model with regards to the axonal diameter.

We can now verify this model with regards to the duration of firing. Our simplified model predicts that by restricting the duration of firing, the axon is able to sustain higher firing frequencies. This is equivalent to saying that the axon can consume energy at a higher rate (firing rate) if the duration for which it is consuming energy is shorter. We can test this hypothesis by simulating our model axon for a shorter period (e.g. 5 s instead of 10 s) and compare the maximum sustainable firing rates determined using our simulations to the ones predicted by the simplified model.

Figure 6.8 shows the results of our simulations for both 5 and 10 s firing durations at different firing rates. These duration were chosen because they allow us to see failure of conduction in the studied axons with firing frequencies that the squid giant axon can deliver. As expected, the sustainable firing rate is higher for the shorter firing durations. In accordance with our simplified model, the two curves representing maximum sustainable firing rates have a similar shape. Lowering the duration of the firing simply shifts the curves toward higher frequencies.

Assuming our simplified model retains its validity for different firing durations, we can look into shorter timespans. Many neurons exhibit bursting behaviour. A burst is defined as two or more spikes, followed by a period of inactivity (Izhikevich 2007). In bursting state, neurons can exhibit firing frequency as high as 300 Hz, which is a limit set by energy constraints (Attwell and Gibb 2005). Bursts can have a duration in the order of tens of milliseconds (McCormick and Pape 1990, thalamocortical relay neuron), all the way up to seconds (Del Negro et al. 1998, rodent trigeminal neurons) in healthy neurons.

Figure 6.9 shows the maximum feasible frequency for firing durations between 50 ms and 1 s. At our baseline pump density (Fig. 6.9A), even the thinnest axon (0.1 μm diameter) can fire at approx. 300 Hz for 50 ms. However, as the duration of burst increases, the maximum feasible firing rate decreases. The 0.1 μm diameter axon can only sustain 500 ms of firing at 60 Hz. The 0.3 μm diameter axon, average diameter of cortical pyramidal cell axon collaterals, which are

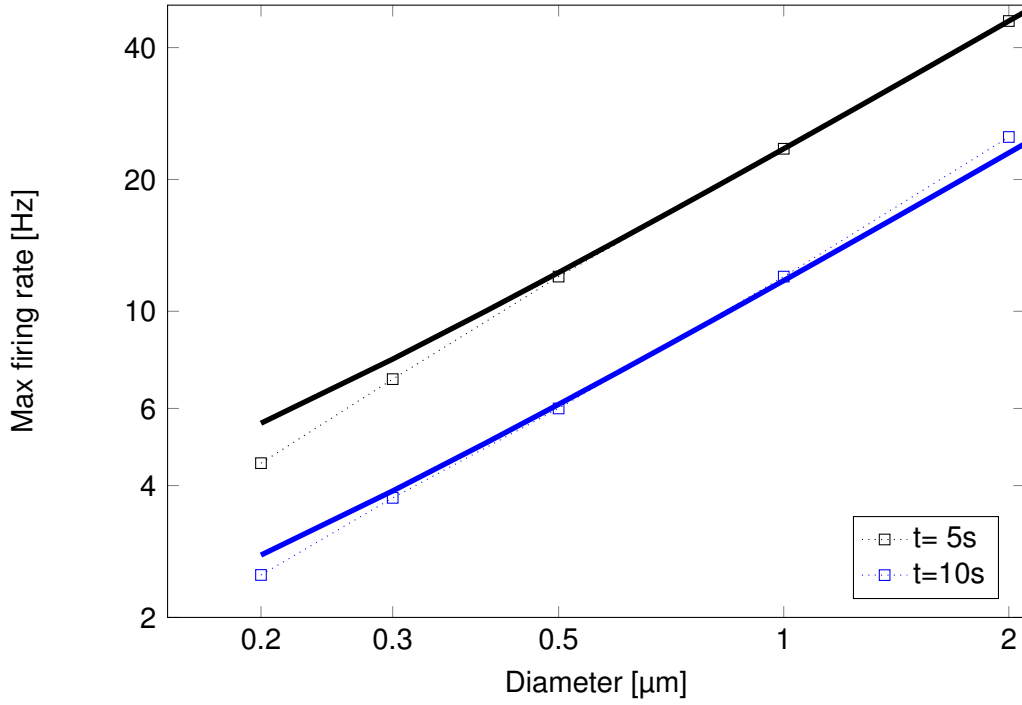


Figure 6.8: Maximum sustainable firing rates as a function of diameter for 5 s (black) and 10 s (blue) long firing periods ($d_p = 4000 \mu\text{m}^{-2}$). Square points are the results of extended Hodgkin-Huxley simulations. Thick lines are given by fitting the simplified model from equation (6.31) to the two larger diameters on the 5 s long simulation.

also known to exhibit bursting behaviour (see for example Egorov et al. 2002), can only fire at approx. 35 Hz for 1 s. Therefore, metabolic constraints also limit the duration of bursts in neurons. Increasing the pump density up to the maximum (Fig. 6.9B) does not offer a significant advantage. The timespan is so short that the added pumps do not have enough time to play a significant role.

6.3.6 The effect of diameter dependent diffusion limits of ATP to the pumps

The activity of Na-K pumps consumes significant amounts of energy. ATP must diffuse along the axon from the nearest mitochondria to the ionic pumps. Each segment of the axon consume some of the incoming ATP even at rest. Thus, the concentration of ATP obeys the following

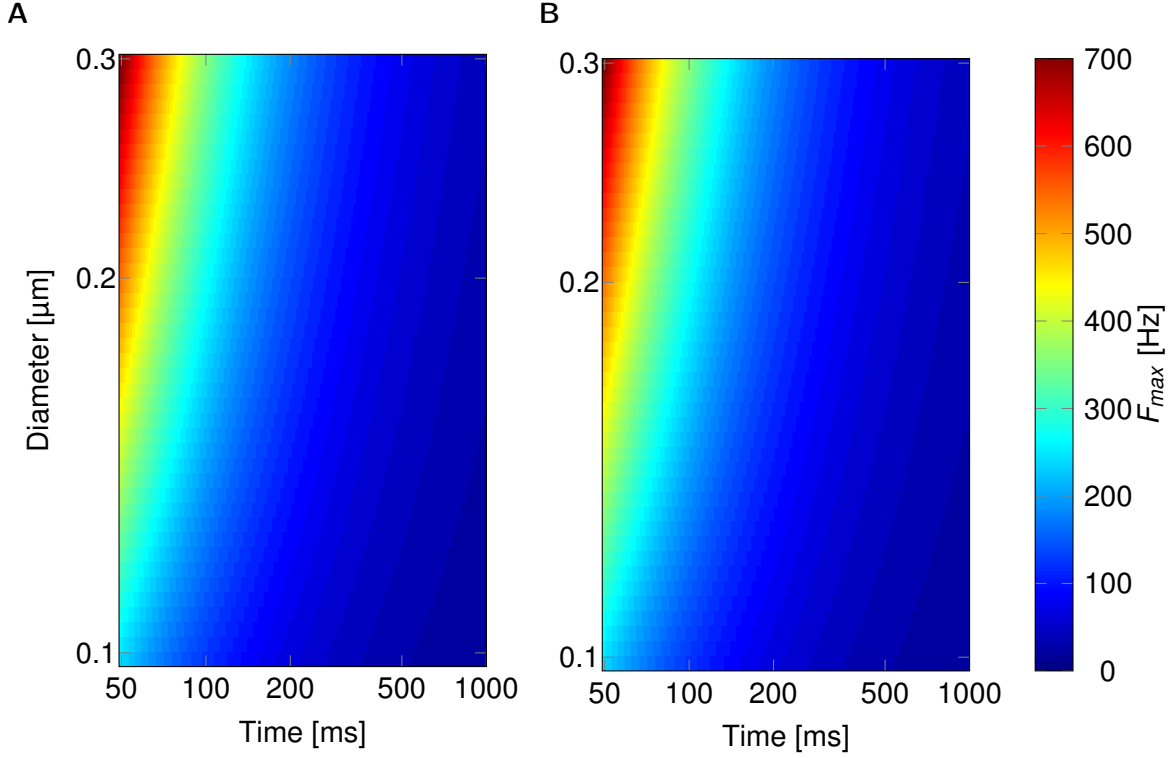


Figure 6.9: Color-map of maximum feasible firing rates given by our simplified model using parameters from the squid giant axon. Data shown here is obtained by fitting the simplified model from equation (6.31) to the (A) blue curve and (B) red curve in Fig. 6.6. Contrary to what we see in Fig. 6.5, the influence of pumps is limited on short time scales, even when the pump density is at its maximum.

equation:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - \bar{p} \quad (6.32)$$

Where $C(t, x)$ is the concentration of ATP, D is the diffusion rate, x the distance to the source of ATP (e.g. $x = 0$ being on a mitochondrion), and $\bar{p}$ is the amount of ATP consumed at rest, when no action potentials are being fired (the ionic pumps only need to reverse a small amount of membrane leak current). This equation is only valid for $C \geq 0$. Its static solution (for $t \gg 1$) is given by $C(x) = C_0 - \frac{\bar{p}}{2D}x^2$ (found by setting $\frac{\partial C}{\partial t} = 0$). The point x_0 at which C reaches zero represents the maximum tolerable distance between a point on the axon and its nearest source of ATP. Assuming a typical concentration of 10 mM (Beis and Newsholme 1975), and a diffusion rate of $100 \mu\text{m}^2 \text{s}^{-1}$ (Vendelin and Birkedal 2008), we can set out to estimate the rate

of consumption and thus the limit on distance:

$$l_{\max} = \sqrt{\frac{2C_0 D}{\bar{p}}} \quad (6.33)$$

$$= \sqrt{\frac{2C_0 D d}{p}} \quad (6.34)$$

The first way to estimate $l_{\max}$ is by using data from our simulations. At rest, the pumping rate in our squid giant axon models is approx. 4×10^{-5} nA in a 20 μm long compartment of a 1 μm diameter axon. This translates into a change of 0.55 mM s^{-1} in $[\text{K}^+]_i$ for a 1 μm diameter axon. Substituting these values into equation (6.34) gives $l_{\max} \approx 50 \mu\text{m}$ for the squid giant axon.

Another, independent, way of estimating this value is by requiring the pumping activity to reverse the resting state K^+ current. The resting state current can be estimated using Hodgkin-Huxley equations. The resting state K^+ conductance is given by $\bar{G}_{\text{K}^+} \times n_{\infty}^4$, where $\bar{G}_{\text{K}^+}$ is the maximum K^+ conductance, and n_{∞} the steady-state K^+ channel activation (Hodgkin and Huxley 1952). Typical values are $\bar{G}_{\text{K}^+} = 36 \text{ mS cm}^{-2}$, and $n_{\infty}(-65 \text{ mV}) = 0.32$, resulting in a resting ATP consumption of approx. 0.93 mM s^{-1} . This is a lower bound on ATP consumption, since it does not take any leak current into account. It translates into a maximum length of $l_{\max} = 46\sqrt{d}$ in μm , which is very similar to the value found using the actual pump current given by our simulations (50 μm).

The squid giant axon is never thin enough for the distribution of mitochondria to be an issue, as they can travel freely along the axon. The maximum length derived from diffusion of ATP is only of theoretical value in this case. But we can readily apply this calculation to the case of hippocampal mossy fibres. These fibres are known to be energy-efficient (Alle et al. 2009), which means that little current crosses their membrane during each AP. The membrane area of the mossy fibre is given by Alle et al. (2009) to be $9.864 \times 10^{-5} \text{ cm}^2$, and each second, 1.38×10^{-4} pmol ATP are consumed to maintain the resting membrane potential. The total surface and consumption are given for the whole mossy fibres, which comprises a large number of collaterals

and boutons of different diameters. Assuming that we can use these to obtain an accurate estimate of ATP consumption, we obtain 0.056 mM s^{-1} , requiring the maximum distance to mitochondria of the $0.4 \mu\text{m}$ diameter axon to be equal to $119 \mu\text{m}$. The longer maximum length shows the impact of more metabolically efficient fibres.

Finally, we can apply these calculations to the case of C-fibres. At rest, the pumping rate in our C-fibre models is approx. $4.6 \times 10^{-6} \text{ nA}$. This translates into a change of concentration of 0.095 mM s^{-1} for a $1 \mu\text{m}$ diameter axon. For a $0.2 \mu\text{m}$ diameter axon, we would expect the nearest mitochondria to be closer than $65 \mu\text{m}$. In anatomical terms, we would expect the presence of a mitochondria approx. every $130 \mu\text{m}$ along such axons. We will return to this result in the Discussion.

6.4 Discussion

The concentration gradients across the membrane act as “batteries”, fuelling the electrical currents necessary to trigger action potentials. The capacity of these “batteries” depends on the diameter of the axon. The lower the axon’s diameter, the less action potentials it can support relying only on its pool of potential energy. Ionic pumps constantly restore the ionic concentration gradients. We have shown, using basic biophysical theory that the finite number of those pumps, not to mention the necessarily finite supply of energy, limits the firing rate of action potential. We developed a simplified model for the evolution of internal K^+ concentration with time. Using this model, we show that the firing rate that can be maintained indefinitely is independent of diameter. However, for a given finite firing duration, the maximum firing rate the axon can maintain depends on the diameter according to a simple formula. We test this relationship by varying pumping density and firing duration, and show that our formula fits data from our simulations well. Finally, we show how the finite diffusion rate of ATP along axons limits the maximum distance between a point of the axon’s membrane and the nearest source of ATP.

6.4.1 Limits on firing rate of axons

The firing rate that an axon can sustain indefinitely is constrained by the cost of each action potential and the amount of ions that the pumps can displace. It is however independent from the diameter of the axon, since both the number of ions crossing through ion channels, and ions pumped, depend linearly on the membrane surface area. Conceptually, the maximum infinitely sustainable firing rate corresponds to an equilibrium between energy production, and energy consumption. If the impact of each AP can be reversed before the next, the axon will be able to fire indefinitely. Otherwise, each AP will diminish the potential energy of concentration gradients, which will eventually be depleted. For the squid giant axon, we estimate this limit to be in the region of 2 Hz to 6 Hz based on our simulation, although simply assuming a constant activity rate for pumps allows frequencies ≥ 20 Hz. More metabolically efficient neurons (Alle et al. 2009; Sengupta et al. 2010) will be able to sustain higher firing frequencies. Theoretical calculations are highly dependent on the density and activity of pumps. We do find, however, that simple estimates of pump densities in the case of high firing rate Purkinje cells are consistent with commonly observed values (Glitsch 2001) (although the values are not obtained from Purkinje cells).

Few neurons maintain a high firing rate indefinitely, as this would prove energetically unsustainable (Attwell and Laughlin 2001). By limiting the duration of fire, higher firing rates become sustainable. The maximum feasible firing rate for a given duration depends on the diameter of the axon. The larger the axon, the higher the firing rate it can sustain for a given amount of time. Our model of the squid giant axon can sustain firing rates of > 20 Hz at $2\text{ }\mu\text{m}$ diameter, as opposed to approx. 2 Hz (i.e. roughly the indefinitely sustainable firing rate) at $0.2\text{ }\mu\text{m}$ diameter. We can understand this by noting that larger axons have higher capacity “batteries” fuelling their action potentials. In other words, the larger the axon, the longer it can fire before the imbalance between production and consumption of energy depletes its reserves. Increasing the amount of pumps has a beneficial effect at lower diameters, and when the firing duration is long enough to allow pumps to have a significant effect.

The limits posed by this metabolic constraint are very strict. Firing at high rates for too long does not simply prevent the axon from firing APs. We find that the depolarisation of K^+ reversal potential from unsustainable firing rates induces a state of uncontrolled bursting by driving the resting potential over the firing threshold. The burst depolarises the membrane potential further and lasts until concentration gradients are depleted. This likely leads to cell death (Martínez-Sánchez et al. 2004; Zandt et al. 2011). Evidence for the impact of this constraint on the design on neural fibres can be found in the relationship between the average firing rate of a neuron, and the diameter of its axon. Mean firing rates are lower in smaller diameter axons than in larger ones (Perge et al. 2012).

Metabolic constraints are also likely to have provoked evolutionary adaptations in the function of neurons. Many neurons exhibit spike-rate adaptation, which limits the amount of time a neuron is firing at high firing rates (compared to the neuron's baseline firing rate). This is thought to enable them to be more energy-efficient (Pozzorini et al. 2013), and is known to be important for optimal information processing in neurons (Ermentrout 1998; Deneve 2008; Kilpatrick and Ermentrout 2011). We suggest that adaptation enables neurons to be smaller, and thus its evolution could play a role in miniaturisation and thus energy efficiency of nervous systems.

6.4.2 Diffusion of ATP constrains the structure of thin axons

The diffusion rate of ATP in axons limits the maximum distance from any point on the membrane surface to the nearest source of ATP. This source can be either mitochondria, a putative mechanism such as supply of ATP through gap junctions with glia cells, or an unknown mechanism. In the case of the squid giant axon, this is of little more than theoretical value, since the squid giant axon is always thick enough to allow the presence of mitochondria anywhere along the axon, and mechanisms for the transport of mitochondria in the squid giant axon are well characterised (Allen et al. 1982; Vale et al. 1985). The axon of mossy fibres is 3200 μm long ($d = 0.4 \mu\text{m}$), much longer than the length permitted by simple diffusion of ATP. However, the axon has a great number of varicosities (or boutons) spaced less than 15 μm apart (Viader et al.

2011), which are large enough to contain mitochondria. Many of these varicosities do contain mitochondria (Shepherd and Harris 1998). However, the existence of these varicosities is not necessarily due to metabolic needs of ionic pumps. The metabolic cost of synaptic transmission is estimated to be on average 3 times that of the APs (Attwell and Laughlin 2001; Sengupta et al. 2010). The presence of the mitochondria may be required by the high metabolic demands of synapses on the *boutons*, rather than the needs of the axon.

Our calculations do, however, serve as indirect evidence for other mechanisms when they seem to be in contradiction with observations, which is the case for C-fibres: C-fibres are very thin (down to $0.1\text{ }\mu\text{m}$) and also very long (up to approx. 1 m), as they can span from toes all the way up to the spinal cord. This is much longer than the maximum distance estimated in Section 6.3.6. We have not found any experimental evidence for varicosities along afferent C-fibres, which do not form synapses on the way to the spinal cord (Kandel et al. 2000). Therefore, we do not expect mitochondria to be present along C-fibre axons. This seems to contradict the hypothesis that the length of thin axons is limited because of the finite diffusion rate of ATP. However, and rather remarkably since they are not myelinated, C-fibres are bundled together by Schwann cells into Remak bundles (Nave and Trapp 2008). The Schwann cells have been shown to be electrochemically sensitive to the action potentials in axons they surround (Nave and Trapp 2008), and that they contribute energetically to the cells (Viader et al. 2011). We suggest that the Schwann cells supply C-fibre axons with ATP, allowing them to exceed the maximum length dictated by diffusion of ATP alone.

Metabolic constraints are relevant not only at the level of the nervous system, where they limit the size of the brain and its overall energy consumption (Karbowski 2009; Navarrete et al. 2011; Fonseca-Azevedo and Herculano-Houzel 2012; Kotrschal et al. 2013), but also at the level of a single neuron (Attwell and Laughlin 2001; Hasenstaub et al. 2010; Attwell and Gibb 2005; Harris and Attwell 2012). Metabolic constraints link the function and size of axons. Finally, the structure (placement of mitochondria or gap junctions, Remak bundles) of thin fibres is also constrained by the metabolic requirements of signalling.

7 Conclusion

Understanding the design and architecture of nervous systems, or reverse-engineering the brain, requires two fundamental understanding. The first one, perhaps the more immediate, is **how** do nervous systems receive, process, and transmit information. From the discovery of the action potential (Du Bois-Reymond 1848) to that of neurons (Ramon y Cajal 1897, Nobel prize in physiology or medicine, 1906), existence of functional regions in the brain (Broca 1878), neural codes (Adrian and Zotterman 1926, Nobel prize in physiology or medicine, 1932), mechanisms of action potential propagation (Hodgkin and Huxley 1952, Nobel prize in physiology or medicine, 1963), ion channels (Neher and Sakmann 1976, Nobel prize in physiology or medicine, 1991) and their structure (MacKinnon et al. 1998, Nobel prize in chemistry, 2003), study of neural networks etc., the primary approach has been about understanding how information is perceived, how decisions are made, and how they are transmitted to appropriate destinations.

The work of Hodgkin and Huxley (1952), however, made the investigation of the other, perhaps deeper, question possible. **Why** do nervous systems function the way they do? Having a perfect answer to this question would require, in addition to knowing the how of nervous system organisation and function, being able to provide a rational behind every choice made during the evolution of the nervous system.

It is unlikely such an answer will ever be found. The number of such choices is astronomical. Many of them may be random choices, or the consequence of the incremental nature of evolution

which might prevent it from finding the “right” solution to problems¹. Thus, any attempt to find meaning in all parts of the nervous system would necessarily be doomed. Finally, assuming that a choice made in the design of nervous systems is indeed optimal, we first need to understand what is being optimised. In other words, the first unknown is the actual cost-function guiding evolution.

Nonetheless, we can attempt to provide the rationale behind the design of at least parts of the nervous system using what we know from human made objects. Engineering principles suggest the cost-function to be based on the constraints faced by the nervous system: speed, noise, energy and space. These are all meaningful limitations, with direct consequences on the fitness of organisms. My goal was thus to find relationships between the design of a central part of nervous systems, axons, and the basic constraints for which they are likely to be optimised.

Most of this work focuses on thin axons. The processes governing the function of neural fibres are located on the membrane surfaces, but need to influence a 3D volume, their impact is not the same across all fibre diameters. In particular, thin axons are much more sensitive to the effects of processes happening on the membrane surface area. This makes them a prime target to investigate biophysical constraints.

7.1 Summary of findings

A quick summary of my findings is represented in Fig. 7.1. Myelinated axons are undoubtedly the solution to the problem of fast information transmission without taking up too much space (Zalc 2006). Saltatory conduction in myelinated axons is both faster, and as I showed using detailed models based on data from mammal PNS, metabolically more efficient than non-saltatory conduction in unmyelinated axons of same outer diameter. This remains true even as we approach the limits set to the diameter of unmyelinated axons by channel noise (Faisal et al.

1. A famous example of this is found in the giraffe recurrent laryngeal nerve. In fish, this nerves goes from the brain to the gills, passing behind the heart. The passage behind the heart is conserved even in animals with longer necks, resulting in a spectacular detour in giraffes.

2005).

However, experimental data show that there is a gap between the diameter of the thinnest unmyelinated axons, and the inner diameter of the thinnest myelinated axons. Unmyelinated axons can be as small as $0.1\mu\text{m}$ diameter, a limit set by stochastic openings of Na^+ channels (Faisal et al. 2005). In contrast, the thinnest known myelinated axons are at least $0.2\mu\text{m}$ thick (Waxman and Bennett 1972; Wang et al. 2008). Stochastic simulations revealed that myelinated axons seem more sensitive to the effects of channel noise, evidenced by the higher rate of spontaneous action potentials in thin myelinated axons. Theoretical calculations taking into account the high density of Na^+ channels in nodes of Ranvier set a lower limit on the inner diameter of myelinated axons (approx. $0.2\mu\text{m}$). This matches anatomical data on the diameter of thinnest myelinated axons (Waxman et al. 1972; Hildebrand et al. 1993; Wang et al. 2008).

Stochastic behaviour of ion channels also introduces noise into the signal. Stochasticity can trigger unwanted action potentials, suppress action potentials, jitter the timing of spikes, or split them into groups (Faisal and Laughlin 2007). Starting from work by Faisal and Laughlin (2004), I showed that in thin unmyelinated axons, the waveform of action potentials (and notably their height and width, which have a known effect on synaptic transmission) undergoes considerable

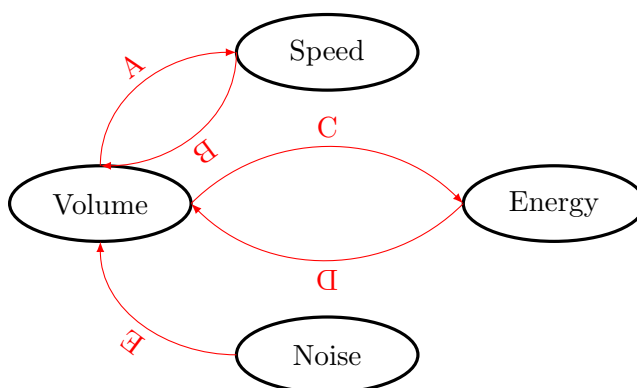


Figure 7.1: Summary of findings in this dissertation.

(A) For all diameters, myelinated axons conduct action potentials faster than unmyelinated axons. **(B)** The volume of axons limits their maximum sustainable firing rate, and hence their information throughput. **(C)** The volume of axons directly impacts the metabolic cost of signalling. The relationship between diameter and cost is linear in unmyelinated axons, but sublinear in myelinated axons. The volume also determines the amount of energy stored in concentration gradients across the membrane. **(D)** The limit rate of ATP propagation imposes a limit on the distance to nearest source of ATP in the case of very thin axons. **(E)** Noise imposes a lower limit on the diameter of myelinated axons. This limit is higher than the one imposed on unmyelinated axons.

variability due to random openings and closings of ion channels. The variability was present in all types of axons simulated, which included a model of cortical cells known for short action potentials and little overlap between Na^+ and K^+ currents. I showed that the strength of this effect varies with diameter according to a power law which is derived from basic probabilistic calculations. Using detailed models of synaptic Ca^{++} current and vesicle release, I showed how fluctuations in the action potentials waveform translate into synaptic variability, and can account for up to 30% of the observed variability in paired-cell recordings.

Metabolic constraints also have an exacerbated effect on thin axons. Active propagation of spikes requires an enormous amount of energy, which comes from the potential energy stored in ionic concentration gradients across cell membrane. Ionic pumps restore the concentration gradients by consuming ATP. I showed that, counter-intuitively, the rate of firing that an axon can sustain indefinitely is independent of diameter, but is set by the ratio between the cost of each action potential, and the rate and number of ionic pumps. For typical axons in the CNS this translates into peak sustainable firing rates of approx. 5 Hz. However, the diameter does have an effect on the burst firing rate. I propose a model relating axonal diameter, burst duration, and maximum sustainable firing rate. Lower durations of bursting make higher firing rates sustainable. Larger axon can also sustain higher firing rates than thinner axons.

The need for the ionic pumps to be supplied by ATP also constraints the anatomy of axons, as the diffusion rate of ATP to the pumps is dependent on the diameter. Using data from the resting state energy consumption several axon types, I show an upper limit on the spacing of sources of ATP (mitochondria, gap junction with glia cells, or other possible sources) set by the diameter of the axon, and suggest that glia cells are necessary to supply thin axons with ATP.

Advances in science, no matter how minor, often bring up more new questions that they solve. My work is certainly not exempt, and many questions remain unanswered.

7.2 Future research directions

7.2.1 Channel kinetics and noise

The rates of conformational changes in transmembrane proteins responsible for the active transport of action potentials, or ion channel kinetics, were first studied for their impact on the refractory periods of axons, and hence their maximum firing rate, which has a direct impact on the rate of transmission of information. More recently (Alle et al. 2009; Sengupta et al. 2010; Hasenstaub et al. 2010), their role in the metabolic efficiency of action potentials has been investigated.

The importance of channel kinetics on the sensitivity of channels to noise is yet unknown. In particular, the impact of mutations in genes coding ion channels (especially Na^+ channels, which provide positive feedback) on the stochastic properties of channel noise has not been studied. Work done on Modigliani during my PhD, and specifically the separation of the Markov chain simulator from the underlying transition probabilities, make it much easier to simulate a wide range of variations in channel kinetics. I plan to create a database of ion channels genes and associated mutations, and investigate their impact on higher (than 1) order statistics.

7.2.2 Mechanisms of energy supply

My calculation on the rate of diffusion of ATP along axons and the maximum distance to a source of ATP that can guarantee the presence of enough ATP molecule for ionic pumps to maintain the resting potential in spite of leak currents lead to rather surprising results, notably in the case of very thin C-fibres of the PNS. There exists a body of evidence pointing toward the role of support cells (Schwann cell, oligodendrocytes and astrocytes) in the metabolic cycle of neurons (Allaman et al. 2011; Saab et al. 2013). However, the mechanisms through which these cells can supply neurons with ATP are not discovered yet. Remak bundles are a prime candidate for experimental work on these mechanisms, which we believe are a necessity for the

very thin C-fibres.

7.2.3 Effects of the four biophysical constraints on the synapse

The processes taking place during synaptic transmission, both on the pre-synaptic and post-synaptic side, are stochastic in nature. The opening of Ca^{++} channels, diffusion of Ca^{++} , vesicle release, neurotransmitter diffusion, opening of ionotropic receptors, etc. are all stages which can introduce variability into the signal. But the impact of each stage on the overall synaptic variability is unknown. The interaction between axonally induced waveform variability and synaptically induced fluctuations in the EPSP is not fully investigated. The variability of the waveform is not homogeneous, and synaptic processes may be tuned to minimise its impact. Stochastic modelling of pre-synaptic processes will help us answer this question.

Synapses are also metabolically expensive (Attwell and Laughlin 2001; Sengupta et al. 2010). The impact of this cost, and possible how metabolic constraints effect the structure of neurons is of great interest, as it may allow us to answer another simple question about the architecture of nervous systems: what factors influence the choice between chemical synapses and gap junctions? When does the information processing capacity of chemical synapses compensate their cost? On the post-synaptic side, a large number of chemical and enzymatic reactions take place (Hayer and Bhalla 2005). By modelling these equations, we may be able to quantitatively measure the information capacity of synapses.

7.2.4 Architecture of the brain

The organisation of the brain is an area of active research (Chklovskii et al. 2002; Chen et al. 2006; Wen and Chklovskii 2010; Buzsáki et al. 2013; Pajevic and Bassar 2013). Mammal brains are organised in highly connected regions (using unmyelinated fibres), which are linked together by myelinated fibres. There have been many attempts to explain this organisation, and its variations in different species, as a result of minimising a cost function. Various candidates for

this cost function have been proposed, such as conduction delay (Chen et al. 2006), or costs related to coherent oscillations (Buzsáki et al. 2013). An alternative approach would be to postulate the existence of a cost function based on the 4 biophysical constraints, and fit its parameters to the experimentally observed architecture. This approach would allow us to infer the relative weight of different constraints over the evolution of nervous systems. The new data, concerning the metabolic cost and minimum size of myelinated axons will certainly prove useful.

The brain, with its 86 billion neurons, and 100 to 500 trillion synapses (Drachman 2005), with each synapse having at least the processing capability of a transistor, consumes a third as much power as a processor with merely 504 million transistors (intel Pentium G630). The brain is a remarkably efficient information processor, and we may someday be able not only to understand, but also predict using only what we know about basic biophysical constraints on the brain, how the architecture of the brain makes this prowess possible.

8 References

- Abeles, M. 1991. *Corticonics: Neural circuits of the cerebral cortex*. Cambridge: Cambridge University Press. (Cited on page 63).
- Adinolfi, A. M. and Pappas, G. D. 1968. “The fine structure of the caudate nucleus of the cat.” *J. Comp. Neurol.* 133 (2): 167–84. (Cited on page 64).
- Adrian, E. and Zotterman, Y. 1926. “The impulses produced by sensory nerve endings Part III.” *J. Physiol.* No. 1. (Cited on pages 19, 149).
- Aggarwal, S., Yurlova, L., and Simons, M. 2011. “Central nervous system myelin: structure, synthesis and assembly.” *Trends Cell Biol.* 21 (10): 585–593. (Cited on page 67).
- Aiello, G. and Bach-y-Rita, P. 2000. “The cost of an action potential.” *J. Neurosci. Methods* 103 (2): 145–149. (Cited on page 37).
- Akopian, A. N. et al. 1999. “The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways.” *Nat. Neurosci.* 2 (6): 541–8. (Cited on page 98).
- Aldworth, Z. N., Bender, J. A., and Miller, J. P. 2012. “Information Transmission in Cercal Giant Interneurons Is Unaffected by Axonal Conduction Noise.” *PLoS One* 7 (1): e30115. (Cited on pages 69, 70).
- Allaman, I., Bélanger, M., and Magistretti, P. J. 2011. “Astrocyte-neuron metabolic relationships: for better and for worse.” *Trends Neurosci.* 34 (2): 76–87. (Cited on page 153).
- Alle, H., Roth, A., and Geiger, J. R. P. 2009. “Energy-efficient action potentials in hippocampal mossy fibers.” *Science* 325 (5946): 1405–1408. (Cited on pages 21, 37, 39, 51, 53, 64, 65, 101, 116, 144, 146, 153).
- Allen, C. and Stevens, C. C. F. 1994. “An evaluation of causes for unreliability of synaptic transmission.” *Proc. Natl. Acad. Sci. U. S. A.* 91:10380–10383. (Cited on page 91).

- Allen, R. D., Metuzals, J., Tasaki, I., Brady, S. T., and Gilbert, S. P. 1982. “Fast axonal transport in squid giant axon.” *Science* 218 (4577): 1127–1129. (Cited on page 147).
- Andrews, H., White, K., Thomson, C. E., Edgar, J. M., Bates, D., Griffiths, I., Turnbull, D., and Nichols, P. 2006. “Increased axonal mitochondrial activity as an adaptation to myelin deficiency in the Shiverer mouse.” *J. Neurosci. Res.* 83 (8): 1533–9. (Cited on page 122).
- Apell, H. 1989. “Electrogenic properties of the Na,K pump.” *J. Membr. Biol.* 110:103–114. (Cited on page 122).
- Astrup, J., Sørensen, P. M., and Sørensen, H. R. 1981. “Oxygen and glucose consumption related to Na⁺-K⁺ transport in canine brain.” *Stroke* 12 (6): 726–730. (Cited on page 122).
- Attwell, D. and Gibb, A. 2005. “Neuroenergetics and the kinetic design of excitatory synapses.” *Nat. Rev. Neurosci.* 6 (11): 841–9. (Cited on pages 119, 141, 148).
- Attwell, D. and Laughlin, S. B. 2001. “An energy budget for signaling in the grey matter of the brain.” *J. Cereb. Blood Flow Metab.* 21 (10): 1133–1145. (Cited on pages 20, 37, 38, 51, 64, 116, 117, 119, 123, 146, 148, 154).
- Augustine, G. J. 1990. “Regulation of transmitter release at the squid giant synapse by presynaptic delayed rectifier potassium current.” *J. Physiol.* 431:343–364. (Cited on pages 69, 93).
- . 2001. “How does calcium trigger neurotransmitter release?” *Curr. Opin. Neurobiol.* 11 (3): 320–326. (Cited on pages 69, 117).
- Azevedo, F. A. C., Carvalho, L. R. B., Grinberg, L. T., Farfel, J. M., Ferretti, R. E. L., Leite, R. E. P., Jacob Filho, W., Lent, R., and Herculano-Houzel, S. 2009. “Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain.” *J. Comp. Neurol.* 513 (5): 532–41. (Cited on page 15).
- Baker, M. D. 2005. “Protein kinase C mediates up-regulation of tetrodotoxin-resistant, persistent Na⁺ current in rat and mouse sensory neurones.” *J. Physiol.* 567 (3): 851–67. (Cited on pages 72, 97–99, 123, 124, 126, 131).
- Bakiri, Y., Káradóttir, R., Cossell, L., and Attwell, D. 2011. “Morphological and electrical properties of oligodendrocytes in the white matter of the corpus callosum and cerebellum.” *J. Physiol.* 589 (3): 559–73. (Cited on page 102).
- Balasubramanian, V., Kimber, D., and Berry, M. J. 2001. “Metabolically efficient information processing.” *Neural Comput.* 13:799–815. (Cited on page 66).
- Bazhenov, M., Timofeev, I., Steriade, M., and Sejnowski, T. J. 2004. “Potassium model for slow (2–3 Hz) in vivo neocortical paroxysmal oscillations.” *J. Neurophysiol.* 92 (2): 1116–32. (Cited on page 125).

- Bean, B. P. 2007. "The action potential in mammalian central neurons." *Nat. Rev. Neurosci.* 8 (6): 451–465. (Cited on page 71).
- Beis, I. and Newsholme, E. A. 1975. "The contents of adenine nucleotides, phosphagens and some glycolytic intermediates in resting muscles from vertebrates and invertebrates." *Biochem. J.* 152 (1): 23–32. (Cited on page 143).
- Berthold, C. H., Nilsson, I., and Rydmark, M. 1983. "Axon diameter and myelin sheath thickness in nerve fibres of the ventral spinal root of the seventh lumbar nerve of the adult and developing cat." *J. Anat.* 136 (Pt 3): 483–508. (Cited on page 42).
- Berthold, C. H. and Rydmark, M. 1978. "Morphology of normal peripheral axons." Chap. 2 in *Axon Struct. Funct. Pathophysiol.* Edited by Stephen G. Waxman, J. D. Kocsis, and P. K. Stys, 13–48. Oxford University Press. (Cited on pages 70, 121).
- Bezanilla, F., Taylor, R. E., and Fernández, J. M. 1982. "Distribution and kinetics of membrane dielectric polarization. 1. Long-term inactivation of gating currents." *J. Gen. Physiol.* 79 (1): 21–40. (Cited on page 58).
- Bishop, G. H. and Smith, J. M. 1964. "The sizes of nerve fibers supplying cerebral cortex." *Exp. Neurol.* 9 (6): 483–501. (Cited on page 64).
- Black, J. A., Foster, R. E., and Waxman, S. G. 1981. "Freeze-fracture ultrastructure of rat C.N.S. and P.N.S. nonmyelinated axolemma." *J. Neurocytol.* 10 (6): 981–993. (Cited on page 96).
- Black, J. A., Frézel, N., Dib-Hajj, S. D., and Waxman, S. G. 2012. "Expression of Nav1.7 in DRG neurons extends from peripheral terminals in the skin to central preterminal branches and terminals in the dorsal horn." *Mol. Pain* 8:82. (Cited on pages 98, 117).
- Black, J. A., Renganathan, M., and Waxman, S. G. 2002. "Sodium channel Nav1.6 is expressed along nonmyelinated axons and it contributes to conduction." *Mol. Brain Res.* 105:19–28. (Cited on pages 98, 117).
- Bollmann, J. H., Sakmann, B., and Borst, J. G. G. 2000. "Calcium sensitivity of glutamate release in a calyx-type terminal." *Science* 289 (5481): 953–957. (Cited on page 74).
- Borst, J. G. G. and Sakmann, B. 1996. "Calcium influx and transmitter release in a fast CNS synapse." *Nature* 383 (6599): 431–434. (Cited on page 93).
- . 1998a. "Calcium current during a single action potential in a large presynaptic terminal of the rat brainstem." *J. Physiol.* 506 (1): 143–157. (Cited on page 73).
- . 1998b. "Facilitation of presynaptic calcium currents in the rat brainstem." *J. Physiol.* 506:149–155. (Cited on pages 73, 74).

- Borst, J. G. G. and Sakmann, B. 1999. “Effect of changes in action potential shape on calcium currents and transmitter release in a calyx-type synapse of the rat auditory brainstem.” *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 354 (1381): 347–355. (Cited on page 69).
- Bostock, H. and Rothwell, J. C. 1997. “Latent addition in motor and sensory fibres of human peripheral nerve.” *J. Physiol.* 498 (1): 277–294. (Cited on page 43).
- Bostock, H. and Sears, T. A. 1978. “The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination.” *J. Physiol.* 280 (1): 273–301. (Cited on page 42).
- Boyd, I. A. and Kalu, K. U. 1979. “Scaling factor relating conduction velocity and diameter for myelinated afferent nerve fibres in the cat hind limb.” *J. Physiol.* 289:277–297. (Cited on page 50).
- Braitenberg, V. and Schütz, A. 1998. *Cortex: statistics and geometry of neuronal connectivity*. 2nd. Berlin: Springer. (Cited on pages 37, 70).
- Brill, M. H., Waxman, S. G., Moore, J. W., and Joyner, R. W. 1977. “Conduction velocity and spike configuration in myelinated fibres: computed dependence on internode distance.” *J. Neurol. Neurosurg. Psychiatry* 40 (8): 769–774. (Cited on pages 46, 67).
- Broca, P. 1878. “Anatomie comparée des circonvolutions cérébrales: Le grand lobe limbique et la scissure limbique dans la série des mammifères.” *Rev. Antropol.* 1:384–498. (Cited on page 149).
- Buzsáki, G., Logothetis, N., and Singer, W. 2013. “Scaling brain size, keeping timing: evolutionary preservation of brain rhythms.” *Neuron* 80 (3): 751–64. (Cited on pages 154, 155).
- Cao, Y., Maran, S. K., Dhamala, M., Jaeger, D., and Heck, D. H. 2012. “Behavior-related pauses in simple-spike activity of mouse Purkinje cells are linked to spike rate modulation.” *J. Neurosci.* 32 (25): 8678–85. (Cited on page 130).
- Carter, B. C. and Bean, B. P. 2009. “Sodium entry during action potentials of mammalian neurons: incomplete inactivation and reduced metabolic efficiency in fast-spiking neurons.” *Neuron* 64 (6): 898–909. (Cited on page 116).
- Chen, B. L., Hall, D. H., and Chklovskii, D. B. 2006. “Wiring optimization can relate neuronal structure and function.” *Proc. Natl. Acad. Sci. U. S. A.* 103 (12): 4723–4728. (Cited on pages 154, 155).
- Chklovskii, D. B., Schikorski, T., and Stevens, C. F. 2002. “Wiring optimization in cortical circuits.” *Neuron* 34 (3): 341–347. (Cited on pages 95, 154).
- Chomiak, T. and Hu, B. 2009. “What is the optimal value of the g-ratio for myelinated fibers in the rat CNS? A theoretical approach.” *PLoS One* 4 (11): e7754–e7754. (Cited on page 46).

- Chow, C. C. and White, J. A. 1996. “Spontaneous action potentials due to channel fluctuations.” *Biophys. J.* 71:3012–3021. (Cited on page 60).
- Coates, C. J. and Bulloch, A. G. M. 1985. “Synaptic plasticity in the molluscan peripheral nervous system: physiology and role for peptides.” *J. Neurosci.* 5 (10): 2677–2684. (Cited on page 69).
- Cooper, E. C., Milroy, A., Jan, Y. N., Jan, L. Y., and Lowenstein, D. H. 1998. “Presynaptic localization of Kv1.4-containing A-type potassium channels near excitatory synapses in the hippocampus.” *J. Neurosci.* 18 (3): 965–974. (Cited on page 92).
- Coskun, Ü. and Simons, K. 2010. “Membrane rafting: from apical sorting to phase segregation.” *FEBS Lett.* 584:1685–1693. (Cited on page 97).
- Courtemanche, M., Ramirez, R. J., and Nattel, S. 1998. “Ionic mechanisms underlying human atrial action potential properties: insights from a mathematical model.” *Am. J. Physiol. Heart Circ. Physiol.* 275 (1): 1522–1539. (Cited on page 122).
- Crockford, D. 2006. *The application/json Media Type for JavaScript Object Notation*. (Cited on page 26).
- Crotty, P. and Levy, W. B. 2007. “Effects of Na⁺ channel inactivation kinetics on metabolic energy costs of action potentials.” *Neurocomputing* 70:1652–1656. (Cited on page 58).
- David, G., Modney, B., Scappaticci, K. A., Barrett, J. N., and Barrett, E. F. 1995. “Electrical and morphological factors influencing the depolarizing after-potential in rat and lizard myelinated axons.” *J. Physiol.* 489:141–157. (Cited on pages 48, 49).
- Dayan, P. and Abbott, L. F. 2001. *Theoretical neuroscience : computational and mathematical modeling of neural systems*. Computational Neuroscience. Cambridge (MA): Massachusetts Institute of Technology Press. (Cited on pages 97, 121, 128).
- Debanne, D. 2004. “Information processing in the axon.” *Nat. Rev. Neurosci.* 5 (4): 304–316. (Cited on pages 91, 94).
- Debanne, D., Guérineau, N. C., Gähwiler, B. H., and Thompson, S. M. 1997. “Action-potential propagation gated by an axonal I(A)-like K⁺ conductance in hippocampus.” *Nature* 389 (6648): 286–9. (Cited on page 91).
- Del Negro, C. A., Hsiao, C.-F., Chandler, S. H., and Garfinkel, A. 1998. “Evidence for a novel bursting mechanism in rodent trigeminal neurons.” *Biophys. J.* 75 (1): 174–182. (Cited on page 141).
- Delaney, K., Tank, D. W., and Zucker, R. S. 1991. “Presynaptic calcium and serotonin-mediated enhancement of transmitter release at crayfish neuromuscular junction.” *J. Neurosci.* 11 (9): 2631–2643. (Cited on pages 69, 93).

- Deneve, S. 2008. “Bayesian spiking neurons I: inference.” *Neural Comput.* 20:91–117. (Cited on page 147).
- Drachman, D. A. 2005. “Do we have brain to spare?” *Neurology* 64:2004–2005. (Cited on page 155).
- Du Bois-Reymond, E. 1848. “Untersuchungen über thierische Elektrizität.” *Ann. Phys.* 151 (11): 463–464. (Cited on pages 17, 149).
- Eaton, R. C., ed. 1984. *Neural mechanisms of startle behavior*. 377. Springer. (Cited on page 21).
- Edgar, J. M., McCulloch, M. C., Thomson, C. E., and Griffith, I. R. 2008. “Distribution of mitochondria along small diameter myelinated central nervous system axons.” *J. Neurosci. Res.* 86 (10): 2250–2257. (Cited on page 122).
- Egorov, A. V., Hamam, B. N., Fransén, E., Hasselmo, M. E., and Alonso, A. A. 2002. “Graded persistent activity in entorhinal cortex neurons.” *Nature* 420 (November): 173–178. (Cited on page 142).
- Ermentrout, B. 1998. “Linearization of F-I curves by adaptation.” *Neural Comput.* 10:1721–1729. (Cited on page 147).
- Ermentrout, B. and Terman, D. 2010. *Foundations of mathematical neuroscience*. New York, New York, USA: Springer US. (Cited on page 128).
- Faisal, A. A. 2004. “Principles of information processing under biophysical constraints” [in English]. PhD diss., University of Cambridge. (Cited on page 35).
- . 2010. “Stochastic simulation of neurons, axons and action potentials.” In *Stoch. Methods Neurosci.* Edited by C. Laing and G. J. Lord, 297–343. Oxford: Oxford University Press. (Cited on pages 24, 28, 37, 71, 99).
- . 2012. “Noise in neurons and other constraints.” Chap. 8 in *Comput. Syst. Neurobiol.* Edited by N. Le Novère, 227–258. Cambridge, UK: Springer. (Cited on pages 71, 98).
- Faisal, A. A. and Laughlin, S. B. 2004. “Effect of channel noise on the propagating ap wave form and its potential impact on synaptic transmission.” *J. Physiol.* 555. (Cited on pages 71, 151).
- . 2007. “Stochastic simulations on the reliability of action potential propagation in thin axons.” *PLoS Comput. Biol.* 3 (5): e79. (Cited on pages 23, 37, 66, 70, 83, 86, 92, 95, 97, 116, 151).
- Faisal, A. A., Laughlin, S. B., and White, J. A. 2002. “How reliable is the connectivity in cortical neural networks?” Edited by D. Wunsch. *IJCNN '02. Proc. 2002 Int. Jt. Conf. neural networks*, 1–6. (Cited on pages 22, 23, 26, 47, 71, 98, 100, 123).

- Faisal, A. A., Selen, L., and Wolpert, D. M. 2008. “Noise in the nervous system.” *Nat. Rev. Neurosci.* 9 (4): 292–303. (Cited on pages 20, 23, 37, 69, 92).
- Faisal, A. A., White, J. A., and Laughlin, S. B. 2005. “Ion-channel noise places limits on the miniaturization of the brain’s wiring.” *Curr. Biol.* 15 (12): 1143–1149. (Cited on pages 20, 21, 23, 24, 37, 41, 59, 60, 64, 66, 67, 70, 71, 83, 91, 92, 95–98, 119, 124, 128, 150, 151).
- Fitzhugh, R. 1962. “Computation of impulse initiation and saltatory conduction in a myelinated nerve fiber.” *Biophys. J.* 2 (1): 11–21. (Cited on page 63).
- Fleidervish, I. A., Lasser-Ross, N., Gutnick, M. J., and Ross, W. N. 2010. “Na⁺ imaging reveals little difference in action potential-evoked Na⁺ influx between axon and soma.” *Nat. Neurosci.* 13 (7): 852–860. (Cited on page 124).
- Fonseca-Azevedo, K. and Herculano-Houzel, S. 2012. “Metabolic constraint imposes tradeoff between body size and number of brain neurons in human evolution.” *Proc. Natl. Acad. Sci. U. S. A.* 109 (45): 18571–6. (Cited on page 148).
- Forrest, M. D., Wall, M. J., Press, D. A., and Feng, J. 2012. “The sodium-potassium pump controls the intrinsic firing of the cerebellar Purkinje neuron.” *PLoS One* 7 (12): e51169. (Cited on pages 121, 123).
- Franciolini, F. 1987. “Spontaneous firing and myelination of very small axons.” *J. Theor. Biol.* 128 (2): 127–134. (Cited on page 67).
- Frankenhaeuser, B. and Huxley, A. F. 1964. “The action potential in the myelinated nerve of *Xenopus laevis* as computed on the basis of voltage clamp data.” *J. Physiol.* 171 (2): 302–315. (Cited on page 42).
- Gadsby, D. C., Bezanilla, F., Rakowski, R. F., De Weer, P., and Holmgren, M. 2012. “The dynamic relationships between the three events that release individual Na⁺ ions from the Na⁺/K⁺-ATPase.” *Nat. Commun.* 3:669. (Cited on page 122).
- Gainer, H., Wolfe, S. A., Obaid, A. L., and Salzberg, B. M. 1986. “Action potentials and frequency-dependent secretion in the mouse neurohypophysis.” *Neuroendocrinology* 43 (5): 557–563. (Cited on page 69).
- Glitsch, H. G. 2001. “Electrophysiology of the sodium-potassium-ATPase in cardiac cells.” *Physiol. Rev.* 81 (4): 1791–1826. (Cited on pages 125, 126, 146).
- Goldman, L. and Albus, J. S. 1968. “Computation of impulse conduction in myelinated fibers; theoretical basis of the velocity-diameter relation.” *Biophys. J.* 8 (5): 596–607. (Cited on page 46).
- Hales, J. P., Lin, C. S.-Y., and Bostock, H. 2004. “Variations in excitability of single human motor axons, related to stochastic properties of nodal sodium channels.” *J. Physiol.* 559 (3): 953–964. (Cited on pages 37, 66).

- Hall, D. H. and Russell, R. L. 1991. “The posterior nervous system of the nematode *Caenorhabditis elegans*: serial reconstruction of identified neurons and complete pattern of synaptic interactions.” *J. Neurosci.* 11 (1): 1–22. (Cited on page 15).
- Hallermann, S., Kock, C. P. J. de, Stuart, G. J., and Kole, M. H. P. 2012. “State and location dependence of action potential metabolic cost in cortical pyramidal neurons.” *Nat. Neurosci.* 15 (7): 1007–1014. (Cited on pages 65, 117).
- Harata, N., Pyle, J. L., Aravanis, A. M., Mozhayeva, M., Kavalali, E. T., and Tsien, R. W. 2001. “Limited numbers of recycling vesicles in small CNS nerve terminals: implications for neural signaling and vesicular cycling.” *Trends Neurosci.* 24 (11): 637–643. (Cited on page 93).
- Harris, J. J. and Attwell, D. 2012. “The energetics of CNS white matter.” *J. Neurosci.* 32 (1): 356–371. (Cited on pages 20, 37, 64–66, 117, 148).
- Hartline, D. K. 2008. “What is myelin?” *Neuron Glia Biol.* 4 (02): 153–163. (Cited on pages 16, 37, 63).
- Hartline, D. K. and Colman, D. R. 2007. “Rapid conduction and the evolution of giant axons and myelinated fibers.” *Curr. Biol.* 17:R29–R35. (Cited on page 36).
- Hasenstaub, A., Otte, S., Callaway, E., and Sejnowski, T. J. 2010. “Metabolic cost as a unifying principle governing neuronal biophysics.” *Proc. Natl. Acad. Sci. U. S. A.* 107 (27): 12329–12334. (Cited on pages 38, 148, 153).
- Hayer, A. and Bhalla, U. S. 2005. “Molecular switches at the synapse emerge from receptor and kinase traffic.” *PLoS Comput. Biol.* 1 (2): 137–154. (Cited on page 154).
- Hildebrand, C. and Hahn, R. 1978. “Relation between myelin sheath thickness and axon size in spinal cord white matter of some vertebrate species.” *J. Neurol. Sci.* 38 (3): 421–434. (Cited on page 37).
- Hildebrand, C., Remahl, S., Persson, H., and Bjartmar, C. 1993. “Myelinated nerve fibres in the CNS.” *Prog. Neurobiol.* 40:319–384. (Cited on page 151).
- Hille, B. 1992. *Ionic channels of excitable membranes*. 3rd. Sunderland, MA: Sinauer Assoc. (Cited on pages 18, 60, 120).
- Hines, M. L. and Carnevale, N. T. 1997. “The NEURON Simulation Environment.” *Neural Comput.* 9 (6): 1179–1209. (Cited on page 26).
- Hodgkin, A. L. 1954. “A note on conduction velocity.” *J. Physiol.* 125:221–224. (Cited on pages 21, 37).
- . 1975. “The optimum density of sodium channels in an unmyelinated nerve.” *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 270 (908): 297–300. (Cited on pages 38, 53, 113, 116, 135).

- Hodgkin, A. L. and Huxley, A. F. 1952. “A quantitative description of membrane current and its application to conduction and excitation in nerve.” *J. Physiol.* 117 (4): 500–544. (Cited on pages 18, 24, 31, 41, 71, 97, 99, 119, 121, 123, 126, 131, 144, 149).
- Hogan, V., White, K., Edgar, J. M., McGill, A., Karim, S., McLaughlin, M., Griffiths, I., Turnbull, D., and Nichols, P. 2009. “Increase in mitochondrial density within axons and supporting cells in response to demyelination in the Plp1 mouse model.” *J. Neurosci. Res.* 87 (2): 452–459. (Cited on page 122).
- Hollenbeck, P. J. and Saxton, W. M. 2005. “The axonal transport of mitochondria.” *Science* 118 (23): 5411–5419. (Cited on page 122).
- Hu, H. et al. 2001. “Presynaptic Ca^{2+} -activated K^{+} channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release.” *J. Neurosci.* 21 (24): 9585–9597. (Cited on page 92).
- Huxley, A. F. and Stämpfli, R. 1949. “Evidence for saltatory conduction in peripheral myelinated nerve fibres.” *J. Physiol.* 108 (3): 315–339. (Cited on pages 42, 63, 97).
- Ierusalimsky, R., Figueiredo, L. H. de, and Filho, W. C. 1996. “Lua - An Extensible Extension Language.” *Softw. Pract. Exp.* 26:635–652. (Cited on page 32).
- Izhikevich, E. M. 2007. *Dynamical systems in neuroscience*. Cambridge MA: Massachusetts Institute of Technology Press. (Cited on page 141).
- Jack, J. J. B., Noble, D., and Tsien, R. W. 1983. *Electric Current Flow in Excitable Cells*. 2nd editio. Oxford: Oxford University Press. (Cited on page 94).
- Jackson, M. B., Konnerth, A., and Augustine, G. J. 1991. “Action potential broadening and frequency-dependent facilitation of calcium signals in pituitary nerve terminals.” *Proc. Natl. Acad. Sci. U. S. A.* 88 (2): 380–384. (Cited on page 92).
- Johnston, Wu, Johnston, D., and Wu, S. M.-S. 1995. *Foundations of cellular neurophysiology*, 676. Cambridge, MA: MIT Press. (Cited on pages 17, 120).
- Jørgensen, P. L. and Andersen, J. P. 1988. “Structural basis for E1–E2 conformational transitions in Na, K-pump and Ca-pump proteins.” *J. Membr. Biol.* 103:95–120. (Cited on page 140).
- Juusola, M., Robinson, H. P. C., and Polavieja, G. G. de. 2007. “Coding with spike shapes and graded potentials in cortical networks” [in en]. *Bioessays* 29 (2): 178–187. (Cited on page 94).
- Kager, H., Wadman, W. J., and Somjen, G. G. 2000. “Simulated seizures and spreading depression in a neuron model incorporating interstitial space and ion concentrations.” *J. Neurophysiol.* 84 (1): 495–512. (Cited on pages 121, 123, 125, 126).

- Kandel, E., Schwartz, J., and Jessell, T. 2000. *Principles of Neural Science*. 1414. McGraw-Hill Medical. (Cited on pages 15, 16, 95, 148).
- Karbowski, J. 2009. “Thermodynamic constraints on neural dimensions, firing rates, brain temperature and size.” *J. Comput. Neurosci.* 27:415–36. (Cited on pages 38, 148).
- Kilpatrick, Z. P. and Ermentrout, B. 2011. “Sparse Gamma Rhythms Arising through Clustering in Adapting Neuronal Networks.” *PLoS Comput. Biol.* 7 (11). (Cited on page 147).
- Klein, M. and Kandel, E. R. 1980. “Mechanism of calcium current modulation underlying presynaptic facilitation and behavioral sensitization in Aplysia.” *Proc. Natl. Acad. Sci. U. S. A.* 77 (11): 6912–6916. (Cited on pages 69, 117).
- Koch, C. 1999. *Biophysics of computation*. Computational neuroscience. Oxford: Oxford University Press. (Cited on page 73).
- Kole, M. H. P., Letzkus, J. J., and Stuart, G. J. 2007. “Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy.” *Neuron* 55:633–647. (Cited on pages 19, 77, 91).
- Kopp-Scheinpflug, C., Lippe, W. R., Dörrscheidt, G. J., and Rübsamen, R. 2003. “The medial nucleus of the trapezoid body in the gerbil is more than a relay: comparison of pre-and postsynaptic activity” [in en]. *J. Assoc. Res. Otolaryngol.* 4:1–23. (Cited on page 92).
- Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., Immler, S., Maklakov, A. A., K, and Olm, N. 2013. “Artificial Selection on Relative Brain Size in the Guppy Reveals Costs and Benefits of Evolving a Larger Brain.” *Curr. Biol.* 23:168–171. (Cited on page 148).
- Kuriscak, E., Trojan, S., and Wunsch, Z. 2002. “Model of spike propagation reliability along the myelinated axon corrupted by axonal intrinsic noise sources.” *Physiol. Res.* 51 (2): 205–215. (Cited on page 66).
- Lai, J., Hunter, J. C., and Porreca, F. 2003. “The role of voltage-gated sodium channels in neuropathic pain.” *Curr. Opin. Neurobiol.* 13:291–297. (Cited on page 98).
- Laughlin, S. B. 1989. “The role of sensory adaptation in the retina.” *J. Exp. Biol.* 146:39–62. (Cited on page 20).
- . 2001. “Energy as a constraint on the coding and processing of sensory information.” *Curr. Opin. Neurobiol.* 11:475–480. (Cited on pages 21, 38).
- Laughlin, S. B., de Ruyter van Steveninck, R. R., and Anderson, J. C. 1998. “The Metabolic Cost of Neural Information.” *Nat. Neurosci.* 1 (1): 36–41. (Cited on pages 20, 38).
- Lawson, S. N. 2002. “Phenotype and function of somatic primary afferent nociceptive neurones with C-, Delta-or Aalpha/beta-fibres.” *Exp. Physiol.* 87:239–244. (Cited on page 98).

- Lenn, N. J. and Reese, T. S. 1966. “The fine structure of nerve endings in the nucleus of the trapezoid body and the ventral cochlear nucleus.” *Am. J. Anat.* 118:375–389. (Cited on page 93).
- Li, Y., Schmid, G., and Hänggi, P. 2010. “Noisy saltatory spike propagation: The breakdown of signal transmission due to channel noise.” *Eur. Phys. J. Spec. Top.* 1–6. arXiv: [arXiv:1008.5251v1](#). (Cited on pages 37, 67).
- Llinas, R., Steinberg, I. Z., and Walton, K. 1981. “Relationship between presynaptic calcium current and postsynaptic potential in squid giant synapse.” *Biophys. J.* 33:323–352. (Cited on page 69).
- Llinas, R., Sugimori, M., and Silver, R. B. 1992. “Microdomains of high calcium concentration in a presynaptic terminal.” *Science* 256 (5057): 677–679. (Cited on page 69).
- Llinas, R., Sugimori, M., and Simon, S. M. 1982. “Transmission by presynaptic spike-like depolarization in the squid giant synapse.” *Proc. Natl. Acad. Sci. U. S. A.* 79 (7): 2415–2419. (Cited on page 69).
- London, M., Roth, A., Beeren, L., Häusser, M., and Latham, P. E. 2010. “Sensitivity to perturbations in vivo implies high noise and suggests rate coding in cortex.” *Nature* 466 (7302): 123–127. (Cited on page 94).
- Lou, X., Scheuss, V., and Schneggenburger, R. 2005. “Allosteric modulation of the presynaptic Ca^{2+} sensor for vesicle fusion.” *Nature* 435 (7041): 497–501. (Cited on page 74).
- MacKinnon, R., Cohen, S. L., Kuo, A., Lee, A., and Chait, B. T. 1998. “Structural Conservation in Prokaryotic and Eukaryotic Potassium Channels.” *Science* 280 (5360): 106–109. (Cited on page 149).
- Martínez-Sánchez, M., Strigrow, F., Schröder, U. H., Kahlert, S., Reymann, K. G., and Reiser, G. 2004. “ Na^{+} and Ca^{2+} homeostasis pathways, cell death and protection after oxygen–glucose-deprivation in organotypic hippocampal slice cultures.” *Neuroscience* 128:729–740. (Cited on page 147).
- McCormick, D. A. and Pape, H.-C. 1990. “Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones.” *J. Physiol.* 431:291–318. (Cited on page 141).
- McIntyre, C. C., Grill, W. M., Sherman, D. L., and Thakor, N. V. 2004. “Cellular effects of deep brain stimulation: model-based analysis of activation and inhibition.” *J. Neurophysiol.* 91:1457–1469. (Cited on page 39).
- McIntyre, C. C., Richardson, A. G., and Grill, W. M. 2002. “Modeling the excitability of mammalian nerve fibers: influence of afterpotentials on the recovery cycle.” *J. Neurophysiol.* 87:995–1006. (Cited on pages 39–44, 46, 47, 50, 64, 102, 103).

- Meinrenken, C. J., Borst, J. G. G., and Sakmann, B. 2003. “Local routes revisited: the space and time dependence of the Ca^{2+} signal for phasic transmitter release at the rat calyx of Held.” *J. Physiol.* 547 (3): 665–689. (Cited on page 74).
- Meyer, A. C., Neher, E., and Schneggenburger, R. 2001. “Estimation of quantal size and number of functional active zones at the calyx of held synapse by nonstationary EPSC variance analysis.” *J. Neurosci.* 21 (20): 7889–7900. (Cited on page 87).
- Miocinovic, S., Parent, M., Butson, C. R., Hahn, P. J., Russo, G. S., Vitek, J. L., and McIntyre, C. C. 2006. “Computational analysis of subthalamic nucleus and lenticular fasciculus activation during therapeutic deep brain stimulation.” *J. Neurophysiol.* 96:1569–1580. (Cited on page 39).
- Moradmand, K. and Goldfinger, M. D. 1995. “Computation of long-distance propagation of impulses elicited by Poisson-process stimulation.” *J. Neurophysiol.* 74 (6): 2415–2426. (Cited on page 94).
- Müller, M., Goutman, J. D., Kochubey, O., and Schneggenburger, R. 2010. “Interaction between facilitation and depression at a large CNS synapse reveals mechanisms of short-term plasticity.” *J. Neurosci.* 30 (6): 2007–2016. (Cited on page 74).
- Naundorf, B. B., Wolf, F., and Volgushev, M. 2006. “Unique features of action potential initiation in cortical neurons.” *Nature* 440 (7087): 1060–1063. (Cited on page 71).
- Navarrete, A., Schaik, C. P. van, and Isler, K. 2011. “Energetics and the evolution of human brain size.” *Nature* 480 (7375): 91–93. (Cited on page 148).
- Nave, K.-A. and Trapp, B. D. 2008. “Axon-glial signaling and the glial support of axon function.” *Annu. Rev. Neurosci.* 31:535–561. (Cited on page 148).
- Neher, E. and Sakmann, B. 1976. “Single-channel currents recorded from membrane of denervated frog muscle fibres.” *Nature* 260:799–802. (Cited on page 149).
- Nicholls, D. G. and Budd, S. L. 2000. “Mitochondria and Neuronal Survival.” *Physiol. Rev.* 80 (1): 315–360. (Cited on page 38).
- Niven, J. E., Vahasoyrinki, M., Kauranen, M., Hardie, R. C., Juusola, M., and Weckstrom, M. 2003. “The contribution of Shaker K^{+} channels to the information capacity of *Drosophila* photoreceptors.” *Nature* 421:630–634. (Cited on page 69).
- Niven, J. E. and Farris, S. 2012. “Miniaturization of nervous systems and neurons.” *Curr. Biol.* 22:R323–R329. (Cited on page 21).
- Ochab-Marcinek, A., Schmid, G., Goychuk, I., and Hänggi, P. 2009. “Noise-assisted spike propagation in myelinated neurons.” *Phys. Rev. E. Stat. Phys. Plasmas. Fluids. Relat. Interdiscip. Topics* 79. (Cited on pages 37, 66).

- Pajevic, S. and Basser, P. J. 2013. “An optimum principle predicts the distribution of axon diameters in normal white matter.” *PLoS One* 8 (1): e54095. (Cited on page 154).
- Parker, D. 2003. “Variable properties in a single class of excitatory spinal synapse.” *J. Neurosci.* 23 (8): 3154–3163. (Cited on page 93).
- Parker, D., Söderberg, C., Zotova, E., Shupliakov, O., Langel, U., Bartfai, T., Larhammar, D., Brodin, L., and Grillner, S. 1998. “Co-localized neuropeptide Y and GABA have complementary presynaptic effects on sensory synaptic transmission.” *Eur. J. Neurosci.* 10 (9): 2856–2870. (Cited on page 94).
- Patlak, J. 1991. “Molecular kinetics of voltage-dependent Na⁺ channels” [in en]. *Physiol. Rev.* 71 (4): 1047–1080. (Cited on page 71).
- Perge, J. A., Niven, J. E., Mugnaini, E., Balasubramanian, V., and Sterling, P. 2012. “Why do axons differ in caliber?” *J. Neurosci.* 32 (2): 626–638. (Cited on page 147).
- Peterson, B. E., Healy, M. D., Nadkarni, P. M., Miller, P. L., and Shepherd, G. M. 1996. “ModelDB: an environment for running and storing computational models and their results applied to neuroscience.” *J. Am. Med. Informatics Assoc.* 3:389–398. (Cited on page 44).
- Pike, L. J. 2006. “Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function.” *J. Lipid Res.* 47:1597–1598. (Cited on page 97).
- Polavieja, G. G. de. 2002. “Errors drive the evolution of biological signalling to costly codes.” *J. Theor. Biol.* 214 (4): 657–664. (Cited on page 66).
- Polavieja, G. G. de, Harsch, A., Kleppe, I., Robinson, H. P. C., and Juusola, M. 2005. “Stimulus history reliably shapes action potential waveforms of cortical neurons.” *J. Neurosci.* 25 (23): 5657–5665. (Cited on pages 19, 94).
- Pozzorini, C., Naud, R., Mensi, S., and Gerstner, W. 2013. “Temporal whitening by power-law adaptation in neocortical neurons.” *Nat. Neurosci.* 16:942–8. (Cited on page 147).
- Pristerà, A., Baker, M. D., and Okuse, K. 2012. “Association between tetrodotoxin resistant channels and lipid rafts regulates sensory neuron excitability.” *PLoS One* 7 (8): e40079. (Cited on pages 97, 98, 107, 108, 114).
- Pristerá, A. and Okuse, K. 2012. “Building excitable membranes: lipid rafts and multiple controls on trafficking of electrogenic molecules.” *Neuroscientist* 18 (1): 70–81. (Cited on page 97).
- Qian, J. and Saggau, P. 1999. “Modulation of transmitter release by action potential duration at the hippocampal CA3-CA1 synapse.” *J. Neurophysiol.* 81 (1): 288–98. (Cited on page 92).
- Ramon y Cajal, S. 1897. “Leyes de la morfología y dinamismo de las células nerviosas.” *Rev. Trim. Micrográfica* 2:1–12. (Cited on pages 16, 149).

- Renganathan, M., Cummins, T. R., and Waxman, S. G. 2001. "Contribution of Nav1. 8 sodium channels to action potential electrogenesis in DRG neurons." *J. Neurophysiol.* 86:629–640. (Cited on page 98).
- Ritchie, J. M. 1985. "A note on the mechanism of resistance to anoxia and ischaemia in pathophysiological mammalian myelinated nerve." *J. Neurol. Neurosurg. Psychiatry* 48 (3): 274–277. (Cited on pages 38, 140).
- Rosenblueth, A., Wiener, N., Pitts, W., and Ramos, J. G. 1948. "An account of the spike potential of axons." *J. Cell. Comp. Physiol.* 32 (3): 275–317. (Cited on page 21).
- Rowland, K. C., Irby, N. K., and Spirou, G. A. 2000. "Specialized synapse-associated structures within the calyx of Held." *J. Neurosci.* 20 (24): 9135–9144. (Cited on page 93).
- Rushton, W. A. H. 1951. "A theory of the effects of fibre size in medullated nerve." *J. Physiol.* 115 (1): 101–122. (Cited on pages 36, 37, 63, 64).
- Rydmark, M. 1981. "Nodal axon diameter correlates linearly with internodal axon diameter in spinal roots of the cat." *Neurosci. Lett.* 24:247–250. (Cited on page 40).
- Rydmark, M. and Berthold, C. H. 1983. "Electron microscopic serial section analysis of nodes of Ranvier in lumbar spinal roots of the cat: a morphometric study of nodal compartments in fibres of different." *J. Neurocytol.* 12:537–565. (Cited on page 40).
- Saab, A. S., Tzvetanova, I. D., and Nave, K.-A. 2013. "The role of myelin and oligodendrocytes in axonal energy metabolism." *Curr. Opin. Neurobiol.* 23 (6): 1065–1072. (Cited on page 153).
- Sabatini, B. L. and Regehr, W. G. 1997. "Control of neurotransmitter release by presynaptic waveform at the granule cell to Purkinje cell synapse." *J. Neurosci.* 17 (10): 3425–3435. (Cited on pages 69, 74, 90, 93).
- Sangrey, T. D., Friesen, W. O., and Levy, W. B. 2004. "Analysis of the optimal channel density of the squid giant axon using a reparameterized Hodgkin-Huxley model." *J. Neurophysiol.* 96 (2): 2541–2550. (Cited on page 58).
- Sasaki, T. 2013. "The axon as a unique computational unit in neurons." *Neurosci. Res.* 75 (2): 83–88. (Cited on page 91).
- Sasaki, T., Matsuki, N., and Ikegaya, Y. 2011. "Action-potential modulation during axonal conduction." *Science* 331 (6017): 599–601. (Cited on pages 70, 91, 93, 94).
- . 2012. "Heterogeneity and independency of unitary synaptic outputs from hippocampal CA3 pyramidal cells." *J. Physiol.* 590 (19): 4869–4880. (Cited on pages 70, 119).

- Sätzler, K., Sohl, L. F., Bollmann, J. H., Borst, J. G. G., Frotscher, M., Sakmann, B., and Lubke, J. H. R. 2002. “Three-dimensional reconstruction of a calyx of Held and its postsynaptic principal neuron in the medial nucleus of the trapezoid body.” *J. Neurosci.* 22 (24): 10567–10579. (Cited on page 93).
- Scheuss, V., Schneggenburger, R., and Neher, E. 2002. “Separation of presynaptic and postsynaptic contributions to depression by covariance analysis of successive EPSCs at the calyx of held synapse.” *J. Neurosci.* 22 (3): 728–739. (Cited on page 87).
- Schneggenburger, R. and Forsythe, I. D. 2006. “The calyx of Held” [in en]. *Cell Tissue Res.* 326 (2): 311–337. (Cited on pages 73, 87).
- Schneggenburger, R. and Neher, E. 2000. “Intracellular calcium dependence of transmitter release rates at a fast central synapse.” *Nature* 406 (6798): 889–983. (Cited on pages 74, 88).
- Schneggenburger, R., Sakaba, T., and Neher, E. 2002. “Vesicle pools and short-term synaptic depression: lessons from a large synapse.” *Trends Neurosci.* 25 (4): 206–212. (Cited on page 93).
- Scholz, A., Reid, G., Vogel, W., and Bostock, H. 1993. “Ion channels in human axons.” *J. Neurophysiol.* 70 (3): 1274–1279. (Cited on page 43).
- Scholz, J. and Woolf, C. J. 2002. “Can we conquer pain?” *Nat. Neurosci.* 5:1062–1067. (Cited on page 98).
- Schwarz, J. R. and Eikhof, G. 1987. “Na currents and action potentials in rat myelinated nerve fibres at 20 and 37 C.” *Pflügers Arch.* 409:569–577. (Cited on pages 48, 49).
- Schwarz, J. R., Reid, G., and Bostock, H. 1995. “Action potentials and membrane currents in the human node of Ranvier.” *Pflügers Arch* 430 (2): 283–292. (Cited on pages 43, 48, 49).
- Sengupta, B., Faisal, A. A., Laughlin, S. B., and Niven, J. E. 2013. “The effect of cell size and channel density on neuronal information encoding and energy efficiency.” *J. Cereb. Blood Flow Metab.* 1–9. (Cited on page 91).
- Sengupta, B., Stemmler, M., Laughlin, S. B., and Niven, J. E. 2010. “Action potential energy efficiency varies among neuron types in vertebrates and invertebrates.” *PLoS Comput. Biol.* 6 (7). (Cited on pages 21, 39, 58, 64, 71, 72, 101, 116, 117, 146, 148, 153, 154).
- Shadlen, M. N. and Newsome, W. T. 1994. “Noise, neural codes and cortical organization.” *Curr. Opin. Neurobiol.* 4 (4): 569–79. (Cited on page 21).
- Sheng, Z.-H. and Cai, Q. 2012. “Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration.” *Nat. Rev. Neurosci.* 13 (2): 77–93. (Cited on page 122).
- Shepherd, G. M., ed. 2003. *The Synaptic Organization of the Brain*. 5th ed. 736. New York (NY): Oxford University Press. (Cited on page 95).

- Shepherd, G. M. and Harris, K. M. 1998. “Three-dimensional structure and composition of CA3→CA1 axons in rat hippocampal slices: implications for presynaptic connectivity and compartmentalization.” *J. Neurosci.* 18 (20): 8300–8310. (Cited on page 148).
- Shinoda, T., Ogawa, H., Cornelius, F., and Toyoshima, C. 2009. “Crystal structure of the sodium-potassium pump at 2.4 Å resolution.” *Nature* 459 (7245): 446–450. (Cited on page 125).
- Shu, Y., Hasenstaub, A., Duque, A., Yu, Y., and McCormick, D. A. 2006. “Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential.” *Nature* 441 (7094): 761–765. (Cited on pages 19, 69, 94).
- Simons, K. and Gerl, M. J. 2010. “Revitalizing membrane rafts: new tools and insights.” *Nat. Rev. Mol. Cell Biol.* 11:688–699. (Cited on page 97).
- Simons, M. and Trotter, J. 2007. “Wrapping it up: the cell biology of myelination.” *Curr. Opin. Neurobiol.* 17 (5): 533–540. (Cited on page 47).
- Smith, N. P. and Crampin, E. J. 2004. “Development of models of active ion transport for whole-cell modelling: cardiac sodium–potassium pump as a case study.” *Prog. Biophys. Mol. Biol.* 85:387–405. (Cited on page 122).
- Smith, R. S. and Koles, Z. J. 1970. “Myelinated nerve fibers: computed effect of myelin thickness on conduction velocity.” *Am. J. Physiol.* 219 (5): 1256–1258. (Cited on pages 37, 63).
- Spencer, A. N., Przysieznik, J., Acosta-Urquidí, J., and Basarsky, T. A. 1989. “Presynaptic spike broadening reduces junctional potential amplitude.” *Nature* 340 (6235): 636–638. (Cited on page 69).
- Stemmler, M. and Koch, C. 1999. “How voltage-dependent conductances can adapt to maximize the information encoded by neuronal firing rate.” *Nat. Neurosci.* 2 (6): 521–527. (Cited on page 20).
- Stys, P., Lehning, E., Saubermann, A. J., and LoPachin, R. M. 1997. “Intracellular Concentrations of Major Ions in Rat Myelinated Axons and Glia: Calculations Based on Electron Probe XRay Microanalyses.” *J. Neurochem.* 68 (5): 1920–1928. (Cited on page 43).
- Sultan, F. 2000. “Exploring a critical parameter of timing in the mouse cerebellar microcircuitry: the parallel fiber diameter.” *Neurosci. Lett.* 280 (1): 41–44. (Cited on page 70).
- Swadlow, H. A., Kocsis, J. D., and Waxman, S. G. 1980. “Modulation of impulse conduction along the axonal tree” [in en]. *Annu. Rev. Biophys. Bioeng.* 9:143–179. (Cited on page 77).
- Tasaki, I. 1955. “New Measurements of the Capacity and the Resistance of the Myelin Sheath and the Nodal Membrane of the Isolated Frog Nerve Fiber.” *J. Physiol.* 181 (3): 639–650. (Cited on page 42).

- Terkildsen, J. R., Crampin, E. J., and Smith, N. P. 2007. “The balance between inactivation and activation of the Na⁺-K⁺ pump underlies the triphasic accumulation of extracellular K⁺ during myocardial ischemia.” *Am. J. Physiol. Heart Circ. Physiol.* 293 (5): H3036–45. (Cited on page 122).
- Tigerholm, J., Petersson, M. E., Obreja, O., Lampert, A., Carr, R., Schmelz, M., and Fransén, E. 2014. “Modeling activity-dependent changes of axonal spike conduction in primary afferent C-nociceptors.” *J. Neurophysiol.* 111 (9): 1721–35. (Cited on page 107).
- Tolhurst, D. and Lewis, P. 1992. “Effect of myelination on the conduction velocity of optic nerve fibres.” *Ophthalmic Physiol. Opt.* 12 (2): 241–243. (Cited on page 64).
- Tuisku, F. and Hildebrand, C. 1992. “Nodes of Ranvier and myelin sheath dimensions along exceptionally thin myelinated vertebrate PNS axons.” *J. Neurocytol.* 21 (11): 796–806. (Cited on pages 41, 46).
- Vale, R. D., Schnapp, B. J., Reese, T. S., and Sheetz, M. P. 1985. “Movement of organelles along filaments dissociated from the axoplasm of the squid giant axon.” *Cell* 40 (2): 449–54. (Cited on page 147).
- Vasylyev, D. V. and Waxman, S. G. 2012. “Membrane properties and electrogenesis in the distal axons of small dorsal root ganglion neurons in vitro.” *J. Neurophysiol.* 108 (3): 729–40. (Cited on pages 98, 117).
- Vendelin, M. and Birkedal, R. 2008. “Anisotropic diffusion of fluorescently labeled ATP in rat cardiomyocytes determined by raster image correlation spectroscopy.” *Am. J. Physiol. Cell Physiol.* 295 (5): C1302–C1315. (Cited on page 143).
- Vetter, P., Roth, A., and Hausser, M. 2001. “Propagation of action potentials in dendrites depends on dendritic morphology.” *J. Neurophysiol.* 85 (2): 926–937. (Cited on page 113).
- Viader, A., Golden, J. P., Baloh, R. H., Schmidt, R. E., Hunter, D. A., and Milbrandt, J. 2011. “Schwann cell mitochondrial metabolism supports long-term axonal survival and peripheral nerve function.” *J. Neurosci.* 31 (28): 10128–10140. (Cited on pages 147, 148).
- Volman, V. and Ng, L. J. 2013. “Computer Modeling of Mild Axonal Injury.” *Neural Comput.* 25:1–36. (Cited on pages 125, 126).
- Wang, S. S.-H., Shultz, J. R., Burish, M. J., Harrison, K. H., Hof, P. R., Towns, L. C., Wagers, M. W., and Wyatt, K. D. 2008. “Functional trade-offs in white matter axonal scaling.” *J. Neurosci.* 28 (15): 4047–4056. (Cited on pages 21, 62, 66, 67, 70, 151).
- Wang, X.-J. and Buzsáki, G. 1996. “Gamma oscillation by synaptic inhibition in a hippocampal interneuronal network model.” *J. Neurosci.* 16 (20): 6402–6413. (Cited on page 72).
- Waxman, S. G. 2001. “Transcriptional channelopathies: an emerging class of disorders.” *Nat. Rev. Neurosci.* 2 (9): 652–659. (Cited on page 39).

- Waxman, S. G. 2006. "Axonal conduction and injury in multiple sclerosis: the role of sodium channels." *Nat. Rev. Neurosci.* 7 (12): 932–941. (Cited on page 129).
- Waxman, S. G. and Bennett, M. V. L. 1972. "Relative conduction velocities of small myelinated and non-myelinated fibres in the central nervous system." *Nature* 238 (85): 217–219. (Cited on pages 17, 62, 64, 66, 67, 96, 151).
- Waxman, S. G., Black, J. A., Kocsis, J. D., and Ritchie, J. M. 1989. "Low density of sodium channels supports action potential conduction in axons of neonatal rat optic nerve." *Proc. Natl. Acad. Sci. U. S. A.* 86 (4): 1406–1410. (Cited on page 97).
- Waxman, S. G., Pappas, G. D., and Bennett, M. V. 1972. "Morphological correlates of functional differentiation of nodes of Ranvier along single fibers in the neurogenic electric organ of the knife fish *Stern archus*." *J. Cell Biol.* 53 (1): 210–24. (Cited on pages 64, 151).
- Waxman, S. G. and Ritchie, J. M. 1985. "Organization of ion channels in the myelinated nerve fiber." *Science* 228 (4707): 1502–1507. (Cited on page 60).
- Wen, Q. and Chklovskii, D. B. 2005. "Segregation of the brain into gray and white matter: a design minimizing conduction delays." *PLoS Comput. Biol.* 1 (7): e78. (Cited on page 63).
- . 2010. "To Myelinate or Not to Myelinate?" Edited by D. Feldmeyer and J. H. Lübke. *New Asp. Axonal Struct. Funct.* (Boston, MA):103–113. (Cited on pages 63, 154).
- Wheeler, D. B., Randall, A., and Tsien, R. W. 1996. "Changes in action potential duration alter reliance of excitatory synaptic transmission on multiple types of Ca²⁺ channels in rat hippocampus." *J. Neurosci.* 16 (7): 2226–2237. (Cited on page 69).
- White, J., Southgate, E., Thomson, J., and Brenner, S. 1986. "The structure of the nervous system of the nematode *Caenorhabditis elegans*." *Phil. Trans. R. Soc. Lond.* 314 (B 1165): 1–340. (Cited on page 15).
- White, J. A., Klink, R., Alonso, A., and Kay, A. R. 1998. "Noise from voltage-gated ion channels may influence neuronal dynamics in the entorhinal cortex." *J. Neurophysiol.* 80:262–269. (Cited on page 20).
- White, J. A., Rubinstein, J. T., and Kay, A. R. 2000. "Channel noise in neurons." *Trends Neurosci.* 23 (3): 131–137. (Cited on pages 23, 69, 97, 119).
- Wu, L.-G., Borst, J. G. G., and Sakmann, B. 1998. "R-type Ca²⁺ currents evoke transmitter release at a rat central synapse." *Proc. Natl. Acad. Sci. U. S. A.* 95 (8): 4720–4725. (Cited on page 74).
- Wu, L.-G., Westenbroek, R. E., Borst, J. G. G., Catterall, W. A., and Sakmann, B. 1999. "Calcium channel types with distinct presynaptic localization couple differentially to transmitter release in single calyx-type synapses." *J. Neurosci.* 19 (2): 726–736. (Cited on page 74).

- Yu, N., Morris, C. E., Joós, B., and Longtin, A. 2012. “Spontaneous excitation patterns computed for axons with injury-like impairments of sodium channels and Na/K pumps.” *PLoS Comput. Biol.* 8 (9): e1002664. (Cited on pages 125, 126).
- Zalc, B. 2006. “The acquisition of myelin: a success story.” *Novartis Found. Symp.* 15–25. (Cited on pages 17, 37, 150).
- Zandt, B., Haken, B. ten, Dijk, J. G. van, and Putten, M. J. A. M. van. 2011. “Neural dynamics during anoxia and the “Wave of Death”.” *PLoS One* 6 (7): e22127. (Cited on pages 38, 147).
- Zeng, S. and Tang, Y. 2009. “Effect of clustered ion channels along an unmyelinated axon.” *Phys. Rev.* 80 (2): 021917. (Cited on pages 106–108, 114).
- Zhang, C. L., Ho, P. L., Kintner, D. B., Sun, D., and Chiu, S. Y. 2010. “Activity-dependent regulation of mitochondrial motility by calcium and Na/K-ATPase at nodes of Ranvier of myelinated nerves.” *J. Neurosci.* 30 (10): 3555–3566. (Cited on page 122).
- Zhang, K. and Sejnowski, T. J. 2000. “A universal scaling law between gray matter and white matter of cerebral cortex.” *Proc. Natl. Acad. Sci. U. S. A.* 97 (10): 5621–5626. (Cited on page 36).