From THE DEPARTMENT OF NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

CONDUCTANCE-BASED PRINCIPLES OF NEURONAL FIRING AND EXCITABILITY

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Front cover:
Single spike dynamics. A: noninjected pyramidal neuron. The first spike at 15 pA shows a long latency and a slowly developing afterhyperpolarization. B: same neuron as in A after the injection of a 1-μS slow K channel. The first spike occurs at 48 pA and exhibits a short latency and a pronounced afterhyperpolarization. Spikelets at subthreshold stimulus currents suggest the existence of an equilibrium point of the dynamics with complex eigenvalues, which makes a transition from stable (attracting) to unstable (repelling) as the threshold is exceeded. C: computational model reproducing the behavior in A. The first spike is at 40 pA. D: computational model reproducing the behavior in B after the addition of 1.5 μS. The first spike is at 82 pA. Higher levels of stimulation current and Kv conductance were needed in the model than in the experimental recording (most likely because this cell had a higher input resistance than average). *Zeberg H, Robinson HPC, Århem P. Density of voltage-gated potassium channels is a bifurcation parameter in pyramidal neurons. J Neurophysiol. 2015;113(2): 537-549*

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ABSTRACT

The brain is an electrical organ whose activity is determined by the flow of ions across cell membranes. As such, the conductances that control the ion flux is a key to our understanding of the nervous system.

Neurons process information by their ability to rapidly depolarize and fire action potentials. With the help of dynamical systems theory we have analysed the mechanisms behind this excitability. Specifically, we have used bifurcations theory to characterize the different dynamical pathways that lead to repetitive action potentials, or in terms of dynamical systems, to the emergence of limit cycles. We have demonstrated how down- and up-regulation of various conductances (e.g., potassium current) lead to altered firing patterns and altered threshold dynamics. Crudely, electrical activity in nerve cells can be classified in two main classes: resonators and integrators. It was previously believed that certain channels were needed for the different types, but we have shown that in many cases it is sufficient to change the native conductances to alter class. Using the dynamic clamp technique we have also tested this hypothesis in-vivo. By artificially increasing the potassium conductance in pyramidal cells in rat cortex we were able to demonstrate a shift in dynamical type.

Moreover, we have analysed synapse conductances between interneurons in neocortex. In particular, we have investigated fast-spiking interneurons, which recently were shown to be responsible for the generation of gamma oscillation (30-80 Hz). These neurons are interconnected by a combined chemical-electrical synapse. To understand how these synaptic interactions control synchronous firing, artificial synaptic conductances were injected into fast-spiking cells. Using standard techniques from synchronization theory, such as the phase response curve, we showed how this combined synapse is especially suited for entrainment over a wide gamma frequency band, whose upper and lower frequency limits are given by the electrical component and the inhibitory chemical component, respectively.

Conductances can also be studied at an ion channel level. We examined how local anesthetics block ion channel conductance by binding to the ion channel in an open state. This state-dependent block was analysing in terms of Markov-chains and mathematical formulae for the open probability under voltage-clamp were derived. This knowledge might be valuable for developing new principles for pharmacological treatment.

Theories must be tested against reality. The so-called dynamic-clamp technique allows mathematical models to be directly integrated using a real-time interface with living cells - a hybrid circuit is created between a computer and a nerve cell. The equations for the currents that one wishes to examine are simulated in a computer. The membrane potential is sampled and the current that would be passed through these artificial channels is injected into the cell, whereupon the voltage change and the current is calculated and injected again. The time scales are so short that this cycle must be performed within tens of microseconds. One problem with the dynamic clamp protocol has been the integration method used. We solved this issue by introducing a stable implicit Runge-Kutta method suited for stiff equations.

Understanding the inherent dynamics of different neuron types and their interplay with network activity is essential for understanding complex processes such as altered awareness levels caused by general anesthesia and psychopharmacological interventions. It is the hope that the findings in this thesis may add a small piece to the puzzle of understanding normal as well as pathological brain function.

LIST OF PUBLICATIONS

I. **Zeberg H**, Blomberg C, Århem P. Ion Channel Density Regulates Switches between Regular and Fast Spiking in Soma but Not in Axons. *PLoS Comput Biol*. 2010;6(4)

II. Gouwens N, **Zeberg H**, Tsumoto K, Tateno T, Aihara K, Robinson HPC. Synchronization of Firing in Cortical Fast-Spiking Interneurons at Gamma Frequencies: A Phase-Resetting Analysis. *PLoS Comput Biol*. 2010;6(9).

III. **Zeberg H**, Robinson HPC, Århem P. Density of voltage-gated potassium channels is a bifurcation parameter in pyramidal neurons. *J Neurophysiol*. 2015;113(2): 537-549

IV. **Zeberg H**, Wolk A, Århem P, Robinson HPC. A stable implicit integration method for injecting synthetic active conductances in excitable cells. *Manuscript*

V. **Zeberg H**, Nilsson J, Århem P. The Importanceof the Dissociation Rate in Ion Channel Blocking. *Manuscript*

CONTENTS

PREFACE

The introduction to this thesis has deliberately been written primarily as a prologue. This was done to put its work into a historical context. As the history of dynamical systems and their relationship to neuroscience is told, key concepts for the thesis are introduced. The choice of a historical focus also reflects the author's love for the history of science.

The theory of dynamical systems has played a central role in the work contained in this thesis. Therefore, this theory, especially when directed at biological systems, has been allocated a large portion of the introduction. The use of dynamical systems theory in close conjunction with experimental data is another distinctive character of this thesis.

The introduction uses some mathematics to present the type of systems studied and touches upon some of the relevant mathematical techniques. However, it has been my goal for the text to be accessible even to less mathematically oriented readers.

I received a great deal of assistance and guidance from several people to whom I must express my gratitude. First, I thank my main supervisor, Peter Århem (Karolinska Institutet), and my co-supervisors, Hugh Robinson (University of Cambridge) and Johanna Nilsson (Karolinska Institutet). For guidance on the more mathematical aspects of this thesis, I am deeply grateful to Clas Blomberg (Royal Institute of Technology).

I am also thankful to some academics with whom I corresponded while working on this thesis, namely, John Guckenheimer, Yuri A. Kuznetsov and Eugene Izhikevich.

Several other people assisted me in many ways during my doctoral studies. I list a few of them here, in no particular order: Kristoffer Sahlholm, Arvid Guterstam, Lotfi Khemiri, Abraham Wolk, Björn Meister, Fredrik Elinder, Ingemar Lindahl, Kaj Fried, Tomas Hökfelt, Gunnar Grant, Mårten Risling, Fredrik Ullén, Henrik Ehrsson, Stefan Plantman, Staffan Cullheim, Brun Ulfhake, Andreas Fahlström, Max Grönholdt Klein, Jonas Broman, Lars Winblad, Ulf Arborelius, Niclas Wisén, Tobias Karlsson, Arash Hellysaz, Liz Strandelin, Helén Asp, Karin Lagerman, Filip Lindholm, Anders Lindquist, Alessandro Motta, Richard Ågren, Christoffer Berger, Giovanni Gentile, Richard Andersson, André Fisahn and Sven-Ove Ögren.

“Everything changes and nothing remains still and you cannot step twice into the same stream”

Heraclitus, quoted in Plato's *Cratylus*

INTRODUCTION

Dynamical systems – *From Heraclitus to Poincaré*

The quote on the previous page is taken from the Greek philosopher Heraclitus of Ephesus (c. 535 – c. 475 BCE). It illustrates that the problem of change, especially its temporal aspect, has long fascinated people. Many pre-Socratic philosophers have their thoughts passed on as fragments quoted by later philosophers such as Plato (c. 428 – c. 348 BCE). Such quotations were often the basis for a critical review, and Plato argued against Heraclitus and his idea about the fundamental change of the object with the flow of time, as exemplfied by his question *"How can that be a real thing which is never in the same state?"* (Plato c. 375 BCE)

In Plato's world, one observes a state with the time set to "ever". By comparing observations, change can be inferred, but regardless of the number of observations, there is still a state between them, cf. Zeno's arrow paradox (Aristotle c. 350 BCE). As we will see, these problems and ideas, which thus have been with us for more than two millennia, serve as a foreground to a central theme in this thesis: *The theory of dynamical systems*. In addition, we will see that to some degree, the theory of dynamical systems will answer and resolve the problems with which Heraclitus and Plato were wrestling.

Dynamical systems are systems that have an aspect of time. From a broader perspective, they describe events, if events are defined as the changing of a state. More technically, dynamical systems consist of two components: a state x and a "rule" f of how this state changes with time (Strogatz 1994). Stated as a difference equation, we can write

$$x_{t+1} - x_t = f(x_t) - x_t$$

where x_{t+1} is the next (in terms of time) state after x_t and $f(x_t)$ is the "rule" for how the system develops. The difference equation is in some way a version of Plato's observed states and the change between them. More often, dynamical systems are expressed in terms of a differential equation

$$\frac{dx(t)}{dt} = f(x(t))$$

Although dynamical systems theory in the more modern sense were developed in the early 1900s, their fundamentals rest on Newtonian mechanics. Among other things,

Newton contributed the leap from the difference equation to the differential equation via the calculus of infinitesimals, which gave us the mathematical tools to solve Plato's (and Zeno's) problems of the everlasting state (Burton 2010) by mathematically treating an infinite number of observations. Although this technically solved the relationship between being in a state and being in constant change of state, the existence of infinites has been questioned by some of our greatest minds in mathematics, such as Leopold Kronecker (1823-1891).

The French mathematician Henri Poincaré (1854-1912) is often considered the father of the theory of dynamical systems (Holmes 1990). In his work on the problem of three bodies (Poincaré 1892, Poincaré 1905), Poincaré presents results about the behavior of solutions in terms of frequency, fixed points and stability, etc. These concepts are still the central properties of dynamical systems and are dealt with in terms of neuronal firing in the thesis. The Poincaré recurrence theorem is one of the many achievements of Poincaré. It states that that a dynamical system, if observed for long time enough, (almost certainly) will occupy a previously observed state. This theorem directly rebuts Heraclitus, who held that one never can descend into the same river twice!

That one can return to a previous state (often without having to wait an incredibly long time) has important consequences, namely, that a system will ultimately oscillate. Galileo Galilei had begun to study oscillations in the form of pendulum movements by the beginning of the 17th century (Drake 1978), but in the field of biological oscillations (addressed in this thesis), it was an engineer at Philips who came to have great influence.

The relaxation-oscillator and the phase plane – *A Dutch engineer and some vacuum tubes*

Telecommunications was an emerging technology in the early 1900s. At that time, the current in the electrical circuits (e.g., the receivers and transmitters) was controlled by vacuum tubes, and thus, there was an interest in studying the electrical properties of these types of circuits. The Dutchman Balthazar van der Pol (1889-1959), who had begun working at Philips in 1921 (Cartwright 1960), studied the oscillations that can occur in the vacuum-tube circuit (Van der Pol 1926, Van der Pol and Van der Mark 1927). He suggested the following model, which can be derived from the Rayleigh equation, to represent the oscillation phenomenon:

$$\frac{dx}{dt} = \mu\left(x - \frac{1}{3}x^3 - y\right)$$
$$\frac{dy}{dt} = \frac{1}{\mu}x$$

Here, that model is written in a two-dimensional form (van der Pol originally expressed the system as a single equation comprising both a first and a second derivative). Van der Pol called the oscillations phenomenon "relaxation oscillations" because the system had a non-sinusoidal repetitive signal. The equation system above is of great importance to the elaboration of computable biology . Some simple observations can be made just by looking at the system's equations:

i. The equations contain both positive and negative terms, meaning that the system has both positive and negative feedback;
ii. There is a higher-order term (x^3) that characterizes the system as non-linear; and
iii. In addition to the variables (x, y), the system contains a parameter (μ), thus allowing the system to potentially exhibit different behavior depending on the choice of parameter.

There are surely many more observations to make. These three, however, are characteristic of biological systems. Van der Pol himself understood the link to biological systems and built circuits to investigate the dynamics of the beating heart. In terms of neuroscience, the van der Pol equation could be shown to be a simplification of the standard model of neuronal firing (more on that issue below).

The two-dimensional nature of the van der Pol equation has an important consequence: it is easy to illustrate the system on a flat surface and therefore, its geometry and dynamics can be studied visually. The surface (or plane) is known as the phase plane of the system and is depicted in Figure 1b.

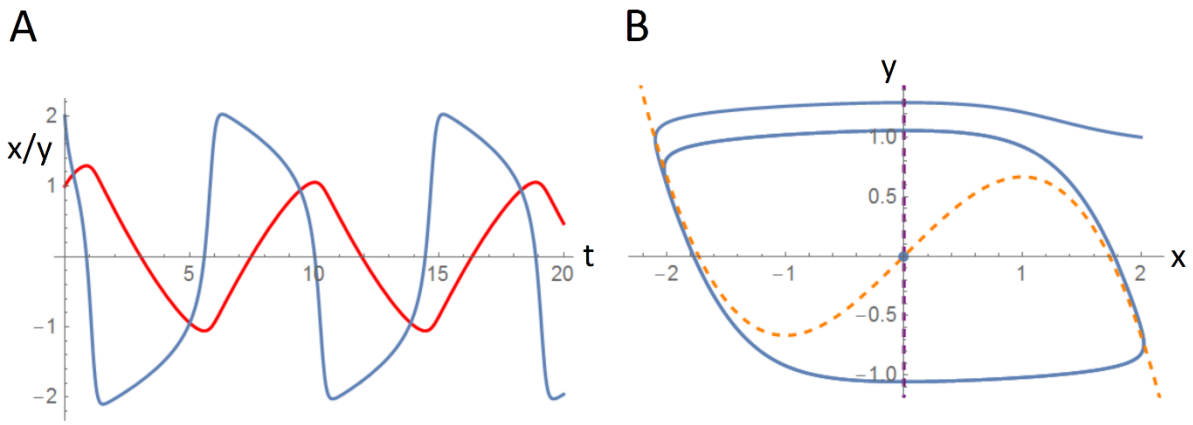

Figure 1. A) Oscillations for the van der Pol equations with $\mu = 3$. The blue line indicates the trajectory of x and the red line indicates the trajectory of y. Note the non-sinusoidal shape. B) Phase plane of the van der Pol equation. The blue line indicates the evolution of the system. The orange dashed line indicates the null-cline of x and the purple line indicates the null-cline of μ. The dot in the center (0,0) is the fixed point where the system is at rest.

The axes of the phase plane (Figure 1b) are the variables; therefore, in the case of the van der Pol equation, they represent x and y. Each point in the plane is associated with a vector (with a certain strength and direction) given by differential equations describing the system, so the phase plane is also called a vector plane. Figure 2 depicts the phase plane as a 'vector plot'. Moreover, each point can be observed as an initial value from which a trajectory can be drawn. There are numerous items that are important in the phase plane (Izhikevich 2010)

- Null-clines, i.e., the set of points for which one of the differential equations is zero. Thus, the van der Pol oscillator has two null-clines (dashed lines in figure 1b).
- Fixed points where the system is at rest. These are found in the intersection(s) of the null-clines, meaning that both differential equations are zero.
- Limit-cycles that describe the oscillations of the system.

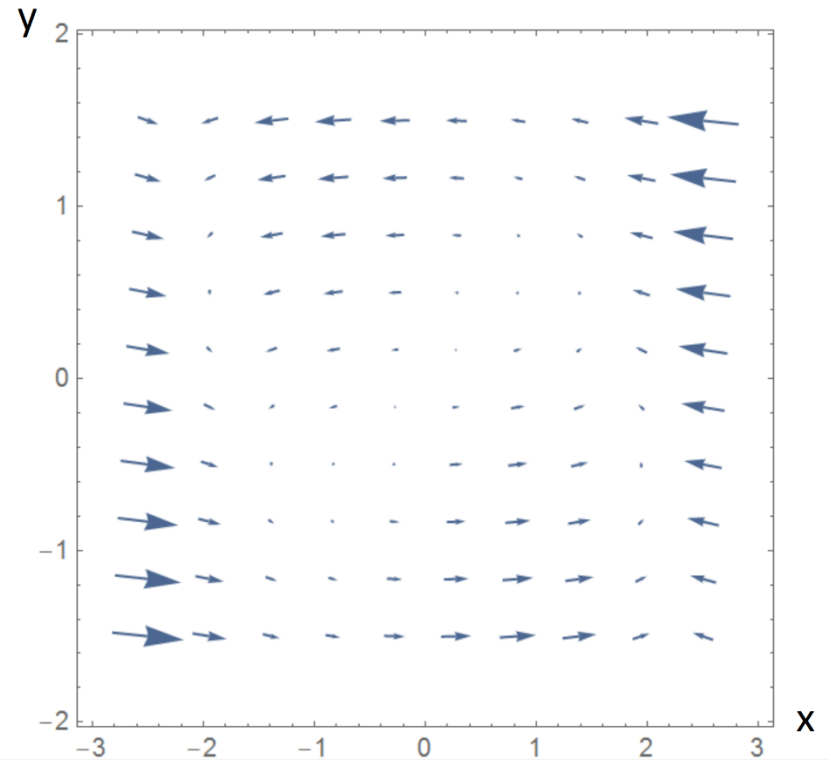

Figure 2. The phase plane of the van der Pol equation $\mu = 3$ with vectors showing the system behaviour for different initial values.

Even though the phase plane is merely the system shown on a two-dimensional surface (of course, this can be generalized to higher dimensions), a surprising number of mathematical details and theorems involve the phase plane. One important theorem is the Poincaré-Bendixons theorem (Poincaré 1892, Bendixson 1901), which states that the long-term behavior of a system that is bounded on a plane is either at rest at a fixed point or oscillates in a limit cycle. Intuitively, it is relatively easy to become familiar with this result. Start somewhere on a piece of paper and draw a random trajectory. Because one is not allowed to cross the trajectory (which would mean that the vector is not clearly defined), one must either stop at a certain point (the fixed point) or tie the trajectory together in a limit cycle.

Systems like the van der Pol equations can have different dynamics depending on the parameters. These changes in dynamics are called bifurcations. Although Poincaré studied these phenomena (Poincaré 1885), it was Russian mathematicians who formalized these shifts in dynamics.

Bifurcation theory – *The Moscow school of nonlinear dynamics*

During the 1920s, a strong research center in Moscow was created around mathematician/physicist Leonid Isaakovich Mandel (1879-1944) and one of his gifted students, Aleksandr Andronov (1901-1952). One of Andronov's earliest insights was the realization that there was a connection between Poincare's limit cycles and the number of oscillatory phenomena. Andronov predicted that the phase plane technique could explain oscillatory processes in chemistry, biology and technology (Bissell 1998) although, as discussed above, the theory was primarily developed for understanding electrical circuits. "Theory of Oscillators", which Andronov, A. A. Vitt and S.E. Khaiki wrote in 1937 (Andronov, Vitt et al. 1966), is a classic text.

As mentioned, a bifurcation is a qualitative change of a system's behavior – e.g., from rest to oscillations or from oscillation to chaos. A small change in one parameter can have a major impact on the system's behavior, which is sometimes referred to as the butterfly effect, a phrase coined by Edvard Lorenz (1917-2008). As will be discussed later in the thesis, this is also true for nerve excitation. A bifurcation can be said to be a mathematical description of threshold dynamics. There are numerous bifurcations, but it is the so-called Hopf bifurcation that is particularly important to the nervous system. Why, then—one might ask—did this paragraph not begin with information about Eberhard Hopf (1902-1983)? In the West, although the bifurcation is best known as the Hopf bifurcation, it is sometimes referred to as the Andronov-Hopf bifurcation. However, both the discovery and the more formal proof of the bifurcation belongs to Andronov (Gaiko 2013), who described it (without proof) at a 1931 conference (Mathematical Problems of Auto-oscillation); the classic book "Theory of Oscillators" contains a proof of the bifurcation. In the 1940s, Hopf generalized Andronov's results (Hopf 1942), which then bore Hopf's name.

Andronov et al. clarified the link between the characteristics of local points and the system's more global behavior, such as limit cycles and stationary movements. The local points of greatest importance are the so-called fixed points (sometimes called fix points, stationary points or equilibrium points). Fixed points are the points where the system of equations equals zero and thus, the system is stationary.

More formally, if we have the system of differential equations (written here with vector notation),

$$\frac{d\mathbf{x}}{dt} = f(\mathbf{x})$$

then a fixed point $\mathbf{x_0}$ is a point such that

$$f(\mathbf{x_0}) = \mathbf{0}$$

or, in other words, the root of the equation $f(\mathbf{x}) = 0$. Fixed points can be very useful for determining behavior. Crudely, a fixed point may have two natures:

- A stable equilibrium $\mathbf{x_0}$ that attracts nearby solutions, i.e., there exists $M > 0$ so that if $|\mathbf{x}(0) - \mathbf{x_0}| < M$, then $|\mathbf{x}(t) - \mathbf{x_0}| \to 0$ as $t \to \infty$.
- An unstable equilibrium $\mathbf{x_0}$ that repels nearby solutions, i.e., there exists $M > 0$ so that if $|\mathbf{x}(0) - \mathbf{x_0}| < M$, then $|\mathbf{x}(t) - \mathbf{x_0}|$ increases as $t \to \infty$.

This classification is based on the behavior of trajectories in the neighborhood of the fixed points. In the case of a stable equilibrium, this region (defined by M) is sometimes referred to as the attractor basin (Izhikevich 2010). A (local) bifurcation occurs when a fixed point changes its nature. Its stability properties can be fairly easily analyzed using linearization techniques. Consider the following coupled differential equations:

$$\frac{dx}{dt} = f(x, y)$$

$$\frac{dy}{dt} = g(x, y)$$

or in vector notation,

$$\frac{d}{dt}\mathbf{x} = f(\mathbf{x}).$$

We approximate the system with the two first terms in the Taylor expansion around (x_0, y_0):

$$f(x, y) \approx f(x_0, y_0) + \frac{df}{dx}(x_0, y_0)(x - x_0) + \frac{df}{dy}(x_0, y_0)(y - y_0)$$

$$g(x, y) \approx g(x_0, y_0) + \frac{dg}{dx}(x_0, y_0)(x - x_0) + \frac{dg}{dy}(x_0, y_0)(y - y_0)$$

Because (x_0, y_0) is a fixed point, the first term vanishes by definition and higher-order terms are negligible around the fixed point (under the assumption that the system is well behaved). Thus, we get

$$\frac{dx}{dt} = \frac{df}{dx}(x_0, y_0)(x - x_0) + \frac{df}{dy}(x_0, y_0)(y - y_0)$$

$$\frac{dy}{dt} = \frac{dg}{dx}(x_0, y_0)(x - x_0) + \frac{dg}{dy}(x_0, y_0)(y - y_0)$$

or in vector notation,

$$\frac{d\mathbf{x}}{dt} = \mathbf{J} \times (\mathbf{x} - \mathbf{x_0})$$

where **J** is the stability matrix or the Jacobian matrix (after Carl Gustav Jacob Jacobi, 1804-1851).

$$\mathbf{J} = \begin{pmatrix} \frac{df}{dx}(x_0, y_0) & \frac{df}{dy}(x_0, y_0) \\ \frac{dg}{dx}(x_0, y_0) & \frac{dg}{dy}(x_0, y_0) \end{pmatrix}$$

Let $\mathbf{u} = (\mathbf{x} - \mathbf{x_0})$ and the solution to the equation can be written as

$$\mathbf{u}(t) = \boldsymbol{A}_1 e^{\lambda_1 t} + \boldsymbol{A}_2 e^{\lambda_2 t}$$

where $\boldsymbol{A}_{1,2}$ are constants and $\lambda_{1,2}$ are eigenvalues of the Jacobian matrix. The eigenvalues can be used to determine the behavior of perturbations around the fixed point. Table 1 from paper I (Zeberg, Blomberg et al. 2010) presents the different natures that can be taken by a fixed point.

Character	Eigenvalues	Applies to
Stable spiral	$\lambda_{1,2} = -a \pm bi$	V_{s1} and V_{s3}
Andronov-Hopf bifurcation	$\lambda_{1,2} = 0 \pm bi$	V_{s1} and V_{s3}
Unstable spiral	$\lambda_{1,2} = a \pm bi$	V_{s1} and V_{s3}
Stable node	$\lambda_1 = -a_1$, $\lambda_2 = -a_2$	V_{s1} and V_{s3}
Saddle node	$\lambda_1 = a_1$, $\lambda_2 = -a_2$	V_{s2}
Saddle-node bifurcation	$\lambda_1 = 0$, $\lambda_2 = -a_2$	*Coalescence of V_{s1} and V_{s2}*
Bogdanov-Takens bifurcation	$\lambda_{1,2} = 0$	*Coalescence of V_{s1} and V_{s2}*

Two of the four eigenvalues are always real and negative and are not included in the table. *a* and *b* are real positive numbers.
doi:10.1371/journal.pcbi.1000753.t004

Table 1. Classification of fixed points. The character is defined by the eigenvalues. The rightmost column relates to the stationary potentials in scientific paper I of the thesis. Adopted from (Zeberg, Blomberg et al. 2010).

The equation above clearly shows why the eigenvalues are important. If the real part of $\lambda_{1,2}$ is positive, then the solution will "explode" away from (x_0, y_0), and thus (x_0, y_0) is unstable. If, however, the real part of $\lambda_{1,2}$ is negative, then the system will implode to (x_0, y_0), which is then stable. A typical case is a pair of complex eigenvalues, i.e.,

$$\lambda_{1,2} = a \pm bi$$

Near the fixed point, in this case, the system can be described as spirals (cf. Euler's formula $e^{ix} = \cos x + i \sin x$), either approaching the fixed point (i.e., stable, $a < 0$) or diverging from the fixed point (i.e., unstable $a > 0$). An Andronov-Hopf bifurcation occurs when a changes sign, or exactly at $a = 0$, or in other words, when a complex conjugate pair of eigenvalues of the linearized flow around a fixed point becomes purely imaginary. Subsequently, a Hopf bifurcation can only occur in systems of at least two dimensions. Figure 2 depicts such a bifurcation.

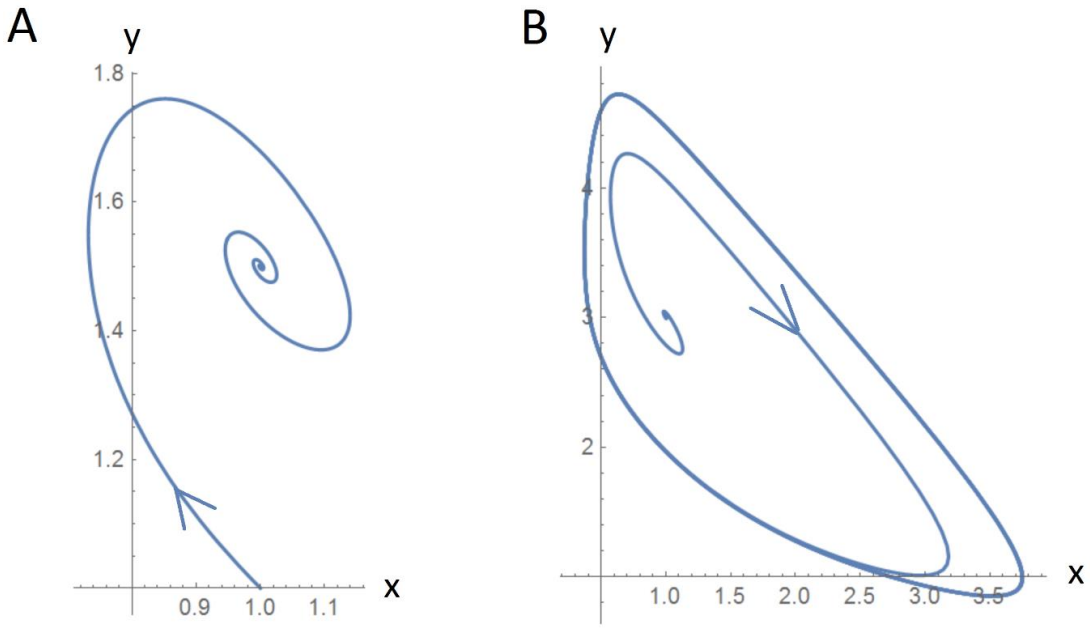

Figure 3. The Andronov-Hopf bifurcation illustrated in the Brusselator. A) The trajectory approaches the fixed point when $a = 1$ and $b = 1.5$. B) The trajectory leaves the fixed point and approaches a limit cycle if b is increased to $b = 3$. For $b < 1 + a^2$, the system is stable.

The Andronov-Hopf bifurcation is illustrated in figure 3 in differential-equation system called the Brusselator, which was proposed by Ilya Prigogine (1917-2003, Nobel laureate 1977) and his coworkers at the Université Libre de Bruxelles as a theoretical model for a type of autocatalytic reaction. The system is given by

$$\frac{dx}{dt} = a + x^2y - (b+1)x$$
$$\frac{dy}{dt} = bx - x^2y$$

This thesis investigates the Andronov-Hopf bifurcations in terms of neuronal impulses; it also involves more complex bifurcations (e.g., saddle-node bifurcations, double-limit cycle bifurcations, Bogdanov-Takens bifurcations and big saddle homoclinic bifurcations) that are intentionally omitted from the introduction.

Neuronal models and firing patterns – *A couple in Cambridge*

Having described the background of the development of the theory of dynamic systems and provided examples of changes of dynamics, we now turn to applying this theory to neuronal systems. In the summer of 1938, a researcher from Cambridge, Alan L. Hodgkin (1914-1998), went to the Marine Biological Laboratory in Plymouth to learn how to dissect the nerve fibers of the giant squid *Loligo forbesii* (Schwiening 2012). The fact that the giant squid could be used as an excellent model system was a breakthrough by John Zachary Young (1907-1997). The following summer, Hodgkin took the newly graduated Andrew F. Huxley (1917-2012) with him to the Marine Biological Laboratory. Initially, they worked with diffusion through nerve fiber (the axoplasm) using mercury droplets. The experiments were unsuccessful, but their work on the giant squid axon led to the idea to place an electrode on the inside of the nerve fiber. The same summer, the two men made the first intracellular measurement of a nerve impulse, or action potential, which was rapidly reported in *Nature* (Hodgkin and Huxley 1939). World War II then broke out, and the two had to set aside their studies on nerve impulses. Hodgkin worked to improve radar systems and pilots' oxygen masks while Huxley devoted himself to ballistics. These experiences proved valuable, giving Hodgkin experience in feedback systems and Huxley experience in manipulating equations (Schwiening 2012).

After the war, the two resumed their work on the giant squid axon and developed an ingenious apparatus for studying the electrical currents that flow across the axonal membrane. The apparatus they developed (or perhaps dramatically refined from Cole and Marmont's version) was based on using feedback systems to maintain a constant voltage across the cell membrane, thus allowing the study of the electric currents. This technique was named the voltage-clamp technique. This in itself was a great achievement, but the crowning glory of their work was Hodgkin and Huxley's model of the action potential that they could infer from voltage-clamp experiments (Hodgkin and Huxley 1952). In their model, the current is split into four parts (cf. Kirchhoff's current law): a capacitive current, a sodium current, a potassium current and a leakage current. See figure 4.

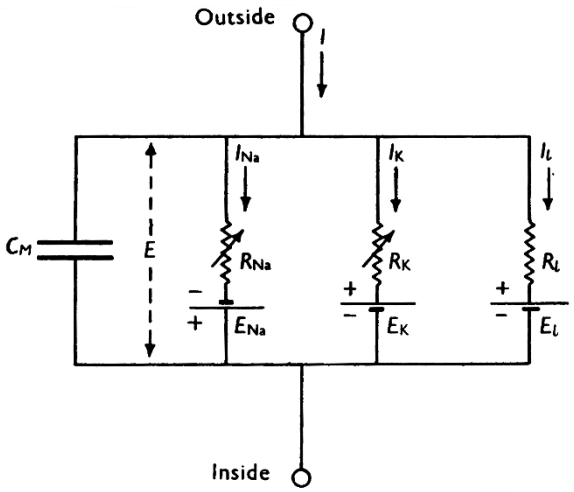

Figure 4. The circuit proposed by Hodgkin and Huxley to describe the currents across the giant squid axon. From (Hodgkin and Huxley 1952), reprinted with permission.

A capacitive current varies with potential. Using the voltage-clamp apparatus, they could also capture the sodium and potassium current's variance with potential. The Hodgkin-Huxley model is the basis for virtually all models of neurons to this day and is described as a system of coupled differential equations. One equation is given by the capacitive properties of the membrane and three equations describe how the sodium and potassium channels open and close:

$$C\frac{dV}{dt} = I_{stim} - \bar{g}_{Na}m^3h(V - E_{Na}) - \bar{g}_K n^4(V - E_K) - \bar{g}_{Leak}(V - E_{Leak})$$

$$\frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V)m$$

$$\frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V)h$$

$$\frac{dn}{dt} = \alpha_n(V)(1 - n) - \beta_n(V)n$$

In the system above, C is the membrane capacitance, V the membrane potential, E_i the reversal potential for the different currents, $\bar{g}_i$ the various conductances, $m/n/h$ gating variables that control the current and α/β the voltage-dependent functions. As discussed in terms of the van der Pol oscillator, the system is nonlinear, contains parameters that determine its behavior and receives both negative and positive feedback. The Hodgkin-Huxley equation enables the calculation of action potential (Figure 5 and 6).

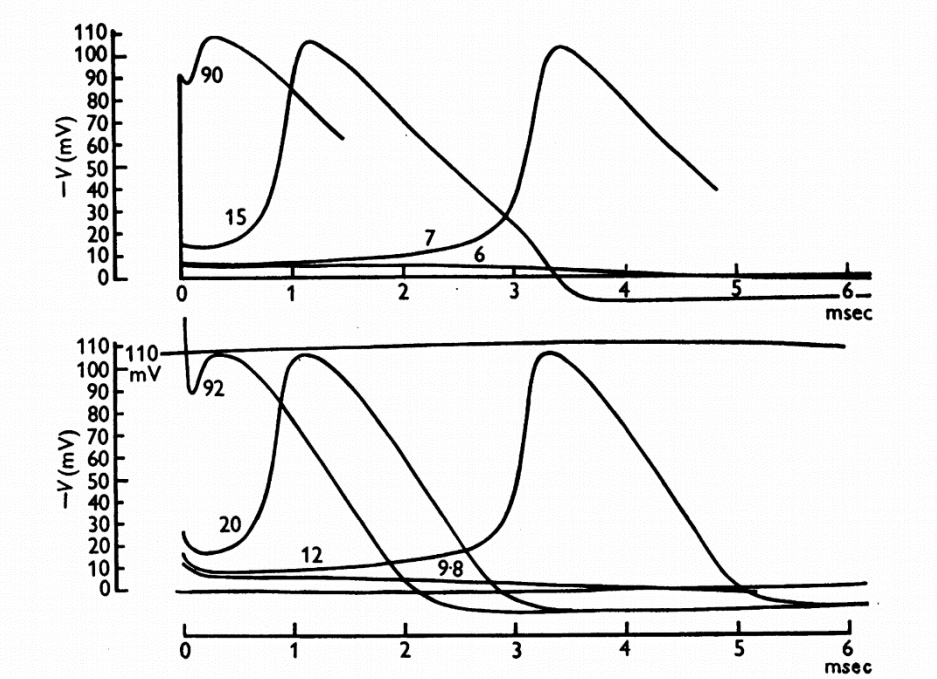

Figure 5. Calculated (upper panel) and recorded (lower panel) actions potentials from the original work of Hodgkin and Huxley (Hodgkin and Huxley 1952). Reprinted with permission.

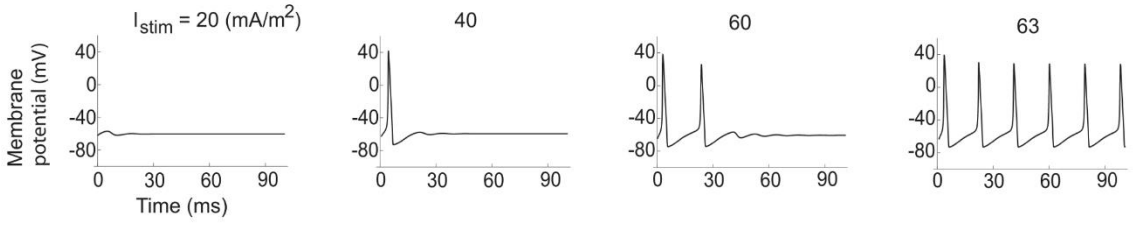

Figure 6. Threshold dynamics and action potentials as calculated by the Hodgkin-Huxley equations. As the stimulation current is increased, the models first elicit single spikes followed by damped oscillations and repetitive firing. Adapted from (Zeberg, Blomberg et al. 2010)

In a classic study, Alan Hodgkin also made an important classification of the firing patterns of neurons (in the crab *Carcinus maenas*) (Hodgkin 1948). In many respects, his division is still the basis for the analysis of the dynamic behavior of neurons. Using threshold dynamics and maximum frequency as characteristic features, he identified two major classes of repetitive firing axons (a third class of neurons that fired with difficulty was also identified): Class 1 axons start firing with low frequency, whereas class 2 axons begin with a relatively high frequency (typically 75 Hz) at threshold.

The class 1 axons posed a theoretical problem. How can a system composed of processes in the millisecond sphere be able to fire with hundreds of milliseconds between the action potentials? How can this slowness emerge? Figure 7 depicts these two classes.

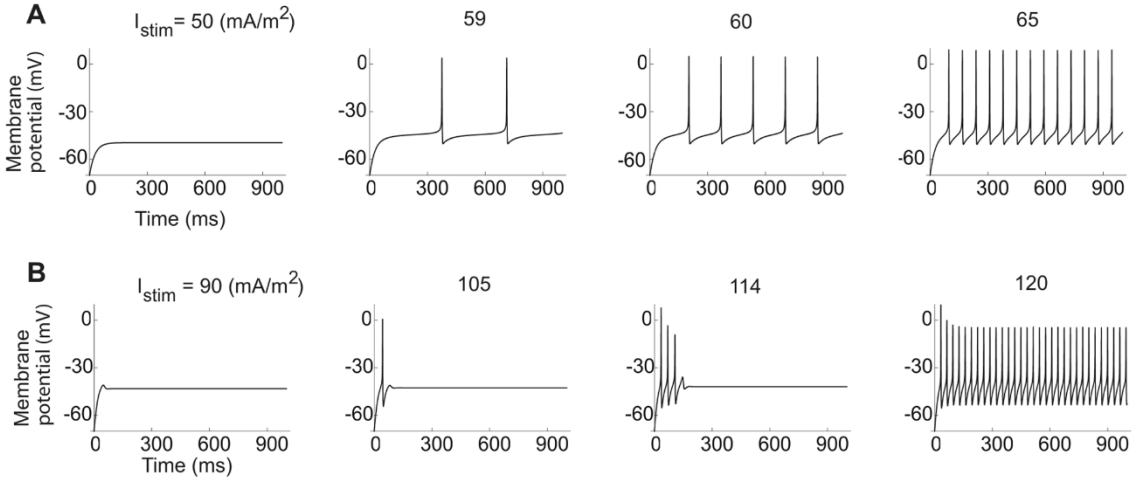

Figure 7. Different onset frequency at threshold. A) An example of a class 1 axon with low onset frequency as the stimulation current is increased. In these mathematical models, the neurons can be made to fire with infinitely low frequency. In practice, the threshold firing frequency for this class is often around a few Hertz. In terms of bifurcation theory, this onset is classified as a saddle-node on an invariant circle bifurcation (SNIC). B) An example of a class 2 axon with distinct onset frequency as the stimulation current is increased. The bifurcation that governs this shift is a subcritical Andronov-Hopf bifurcation. Figure adopted from (Zeberg, Blomberg et al. 2010).

Today, these two classes are more commonly referred to as regular and fast-spiking (Tateno and Robinson 2006, Prescott, Ratte et al. 2008, Zeberg, Blomberg et al. 2010), although the two phenotypes have more characteristics than just the threshold frequency. Dynamical systems theory can be used to explain the different threshold dynamics. The Andronov-Hopf bifurcation is one example of bifurcation in which the systems start with a distinct onset frequency. The slowness that was a problem for Hodgkin can be explained by a saddle-node on an invariant circle bifurcation (Ermentrout and Terman 2010, Izhikevich 2010, Zeberg, Blomberg et al. 2010).

It is difficult to overstate the importance of Hodgkin and Huxley's work. Despite the limited knowledge on how an action potential is formed, they managed to advance a theory that stands today. Their models provided hypotheses about how neurons obtain excitability. Even today, their work is an extraordinary example of how the phenomenological description of mathematical modeling can reveal mechanisms long before they can be observed directly. The two shared the 1963 Nobel Prize.

Low-dimension models and their solutions – *A maze of wires at the National Institutes of Health*

Not long after Hodgkin and Huxley presented their equations for the giant squid axon, a biophysicist named Richard FitzHugh (1922-2007) studied those equations' mathematical properties. FitzHugh worked at the Laboratory of the National Institutes of Health (NIH) in Bethesda, Maryland. With the help of analytical tools from nonlinear mechanics, developed under the leadership of Alexandr Andronov, he studied the system of equations (Izhikevich 2010). This was during a time when computers consisted of vacuum tubes. On an analogue computer consisting of a maze of wires and vacuum tubes that occupied half of a room, he plotted the time evolution of the variables in the Hodgkin-Huxley equations. The computer was constantly down and FitzHugh had to change several vacuum tubes each week. Moreover, it was problematic to address the complicated system of equations of the Hodgkin-Huxley model.

FitzHugh understood that the Hodgkin and Huxley equations were not only a biophysical description of the currents across a cell membrane but also a more generally described threshold phenomenon: how a system, like a nerve cell, may shift from rest to repetitive firing. To study this issue more closely, FitzHugh simplified the system to a two-dimensional model (Fitzhugh 1961). In response to a suggestion by his lab director, Kenneth S. Cole (1900-1984), Fitzhugh modified the van der Pol equations for a relaxation oscillator to fit the firing behavior of an axon. FitzHugh called the system the Bonhoeffer-van der Pol model. The systems FitzHugh proposed (Fitzhugh 1961) are given by the following coupled differential equations:

$$\frac{dV}{dt} = V - \frac{V^3}{3} - W + I$$
$$\frac{dW}{dt} = 0.08(V + 0.7 - 0.8W)$$

FitzHugh obtained this system from the following observations: (1) the kinetics of the variables n and h are slow relative to m; and (2) the sum of n and h is approximately constant (Keener and Sneyd 1998). The system had a resting state and could be made to oscillate if the stimulation current was increased. The transition takes place via the aforementioned Andronov-Hopf bifurcation. When FitzHugh published his results, the knowledge of nonlinear mechanisms and dynamical systems theory were limited among neuroscientists. FitzHugh's work ultimately laid the foundation for an entire field of research, which is often referred to as computational neuroscience.

Ion channels and their influence on the dynamics – *German precision and Markov chains*

If it took two British scholars to determine the mathematical model of the action potential, it was two German scholars who elevated the understanding of how nerve cells generate action potentials to new levels. In the early 1970s, the laboratories of London and Cambridge were still the center of electrophysiology. In 1971, a German physiologist named Bert Sakmann (1942-) left Munich to work as an assistant to the neurophysiologist Bernard Katz (1911-2003) at University College London (Sakmann 1991). In 1973, he returned to Germany and Göttingen to work with the physicist Erwin Neher (1944-). Sakmann had worked with the neuromuscular junction and Neher was engaged in a project on recording the current of individual channels in artificial membranes. They decided to try to measure the currents flux through single ion channels in cell membranes, publishing their first results in 1976 (Neher and Sakmann 1976). For years, until 1980, they optimized their technology—the so-called patch clamp technique. Suddenly there was a tool to observe the direct link between Hodgkin and Huxley's phenomenological description and the biological mechanism that governed the currents: the opening and closing of ion channels. For this achievement, Sakmann and Neher shared the 1991 Nobel Prize.

Generally, ion channels are assumed to be described by continuous-time discrete-state Markov chains (Colquhoun and Hawkes 1995). A key characteristic of a Markov chain system is that the system is considered to be memory-less, i.e., it possesses the Markov property. Although Andrei Markov (1856-1922) worked diligently with these systems, the more formal management of Markov chains was provided in 1922 by Norbert Wiener (1894-1964).

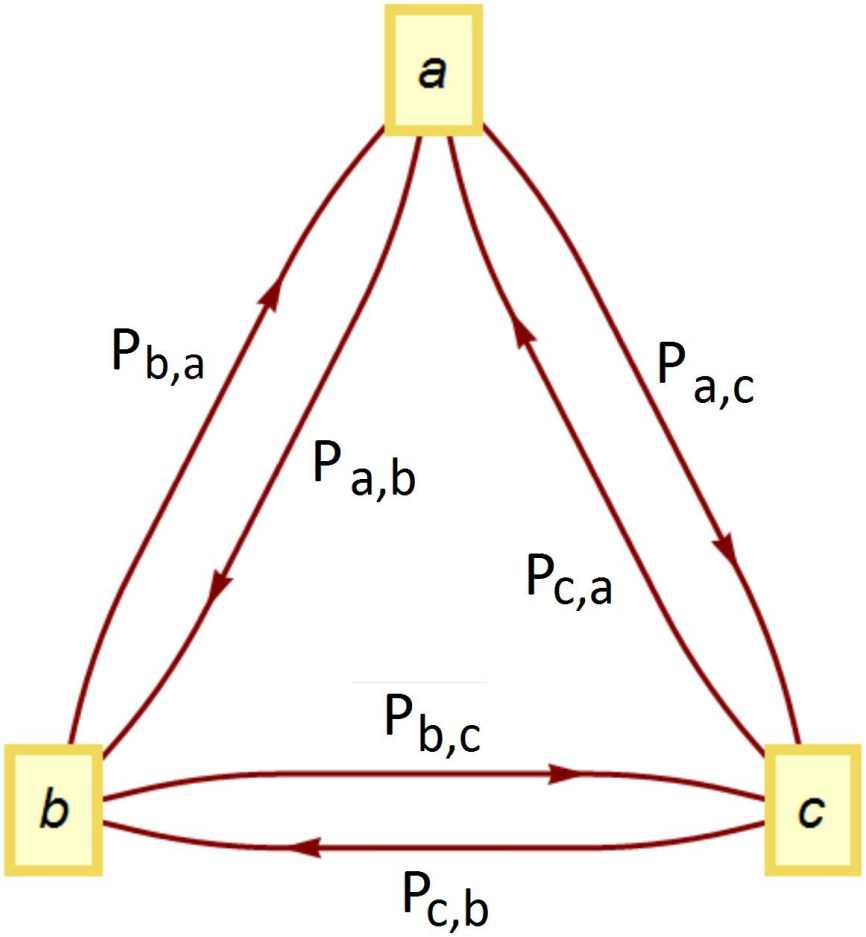

Figure 8. A Markov chain consisting of three states (a, b, c) with associated proabiliates $(P_{i,j})$.

A Markov chain consists of a number of states, $s_1, s_2, \ldots, s_n$ and a series of probabilities of going from state s_i to state s_j, which can be written $\Pr(s_j|s_i) = P_{i,j}$, where P is the transition matrix:

$$P = \begin{pmatrix} p_{1,1} & \cdots & p_{1,n} \\ \vdots & \ddots & \vdots \\ p_{n,1} & \cdots & p_{n,n} \end{pmatrix}$$

The transitions based on the above matrix have been compared to a frog jumping on a set of lily pads (Howard 2012). The frog jumps from lily pad to lily pad along with the transition probabilities in the matrix.

FitzHugh realized that the Hodgkin-Huxley formalism could be translated into Markov chains. In the case of the potassium current, the Hodgkin-Huxley expression is

$$I_k = \bar{g}_K \times n^4(V - E_K)$$

where $\bar{g}_K$ is the maximal conductance, V the potential across the membrane, E_K the Nernst potential for potassium and n the gating variable for the current – if $n = 0$, there is no current flux and if maximal current flux is observed then $n = 1$. The equation is equivalent to the following Markov scheme:

$$\begin{array}{ccccccccc} & 4\alpha & & 3\alpha & & 2\alpha & & \alpha & \\ c & \rightleftharpoons & c & \rightleftharpoons & c & \rightleftharpoons & c & \rightleftharpoons & o \\ & \beta & & 2\beta & & 3\beta & & 4\beta & \end{array}$$

With the identification of ion channels, and later, with the revolution in molecular biology, the kinetics of ion channels could be studied in detail. This thesis's scientific paper V explores analytical solutions to these systems of equations.

Brain oscillations – *Berger's wave and a windows to the mind*

So far, an introduction to dynamical systems has been presented, along with an explanation of the action potential and how this excitability is mediated by flow through single ion channels. Parallel to these findings, progress was made in understanding the macroscopic electrical operations of the brain. The German psychiatrist Hans Berger (1873-1941) made a crucial discovery in 1924, when he made the first electroencephalography recording. The story behind this discovery is exciting and somewhat unexpected. Sometime in 1883, Berger was involved in an accident on horseback (Berger had interrupted his mathematical studies and joined the cavalry). His sister felt that he was in danger and asked their father to send Berger a telegram to ensure that he was doing well. The event made a great impression on him and he became a believer in telepathy. His ambition to gain an understandng of the physiology of telepathy led him to develop electroencephalography (Berger 1940). Using this method, Berger discovered that if one closed his eyes, he acquired a rhythm in the electroencephalography recording with a frequency of 8-15 Hz, which was named Berger's wave (now better known as the alpha rhythm).

It was not until the late 1930s that his findings were generally accepted. By that time, Berger was suffering from severe clinical depression; he committed suicide in 1941.

Today, the following classification of brain oscillations is used (the exact limits vary in the literature)

- The delta rhythm (< 4 Hz)
- The theta rhythm (4-7 Hz)
- The alpha rhythm (8-15 Hz)
- The beta rhythm (16-31 Hz)
- The gamma rhythm (>32 Hz)

These different rhythms have been identified in various brain processes. A rhythm that has gained a great deal of attention during the last two decades is the gamma oscillation, probably because the Nobel laureate Francis Crick, together with Christof Koch, suggested that it is linked to the conscious experience (Crick and Koch 1990). Among other things, gamma oscillations are thought to be associated with sensory processing, motor control, and feature binding (Singer 1999, Buszaki and Draguhn 2004). An fascinating feature of gamma oscillations is that they can be generated locally in the brain cortex and turned on by different techniques such as electrical (Whittington, Stanford et al. 1997) or optogenetic (Cardin, Carlen et al. 2009) stimulation and through the use of pharmacological compounds (Whittington, Traub et al. 1995, Fisahn, Pike et al. 1998). In other words, the gamma oscillations are not only a feature of the brain as such but also a key feature of the tissue itself.

Fast-spiking interneurons are a type of neuron that is distributed throughout the cortex. There are certain connections among them composed of both an electrical and a chemical synapse (Galarreta and Hestrin 1999, Gibson, Beierlein et al. 1999, Tamas, Buhl et al. 2000). This combined synapse can both stimulate and inhibit the post-synaptic neurons. Additionally, the biophysical properties of fast-spiking neurons suggest a role in generating the gamma rhythm. As discussed above, fast-spiking cells have a distinct onset frequency of approximately 30 Hz (Tateno, Harsch et al. 2004), meaning that they can be entrained at this frequency.

This relates to the pair of complex eigenvalues that were discussed above in terms of the Andronov-Hopf bifurcation (eigenvalue $\lambda_{1,2} = 0 \pm bi$). Remember that using Euler's formula we can write

$$e^{bi \times t} = \cos(b \times t) + i \sin(b \times t)$$

with the imaginary part defining a characteristic frequency, i.e., $f = b/2\pi$. Richard Feynman (1918-1988) called this formula "our jewel" and "the most remarkable formula in mathematics" (Feynman 1977). This thesis's scientific paper II investigates the ability to synchronize firing between fast-spiking, connected interneurons and this combined chemical-electrical synapse.

These interneurons are interesting from a biophysical and physiological perspective and are thought to be involved in numerous patophysiological processes. For instance, dysregulation of fast-spiking interneurons and gamma rhythm has been linked to both autism (Carlen, Meletis et al. 2012) and schizophrenia (Jadi, Margarita Behrens et al. 2015).

AIMS

A common thread throughout this thesis is how changes in conductances alter the dynamics of neurons. The thesis has the following four aims:

- To explore the hypothesis that ion channel density (i.e., conductance) is a bifurcation parameter of neuronal firing patterns (papers I and III)

- To identify how synapse conductances facilitate the synchronization of fast-spiking interneurons (paper II)

- To suggest an improved protocol for conductance injections (paper IV)

- To investigate blocking schemes of ion channel conductance (paper V)

METHODS

Linearization and bifurcation analysis techniques

For the full four-dimensional system of differential equation, the membrane potential associated with the fixed point was obtained by finding a root V_s to the equation

$$I\big(V, m_\infty(V), h_\infty(V), n_\infty(V)\big) = 0$$

where$I$ is the sum of the current across the membrane and $m_\infty(V)$ the steady-state value of the gating variable, i.e.,

$$m_\infty(V) = \frac{\alpha(V)}{\alpha(V) + \beta(V)}$$

and similarly for $h_\infty(V)$ and $n_\infty(V)$. The character of the fixed point, i.e., $(V_s, m_\infty(V_s), h_\infty(V_s), n_\infty(V_s))$ was then investigated by linearizing the differential equations and solving the equation $\det(\mathbf{J} - \lambda\mathbf{I}) = 0$, where **I** denotes the identity matrix and **J** the Jacobian matrix.

$$J = \begin{pmatrix} \frac{\partial \dot{V}}{dV} & \frac{\partial \dot{V}}{dm} & \frac{\partial \dot{V}}{dh} & \frac{\partial \dot{V}}{dn} \\ \frac{\partial \dot{m}}{dV} & -\alpha_m - \beta_m & 0 & 0 \\ \frac{\partial \dot{h}}{dV} & 0 & -\alpha_h - \beta_h & 0 \\ \frac{\partial \dot{n}}{dV} & 0 & 0 & -\alpha_n - \beta_n \end{pmatrix}$$

where $\dot{v}$, $\dot{m}$, $\dot{h}$ and $\dot{n}$ are the time derivatives. The four eigenvalues λ_i (i = 1, 2, 3 or 4) provide an approximate time evolution of the system in the sense that any perturbation **δr** around the fixed point **r*** can be written as

$$\delta\mathbf{r}(t) = c_1\mathbf{r}_1 e^{\lambda_1} + c_2\mathbf{r}_2 e^{\lambda_2} + c_3\mathbf{r}_3 e^{\lambda_3} + c_4\mathbf{r}_4 e^{\lambda_4}$$

where c_i (i = 1, 2, 3 or 4) depends on the initial conditions, and $\mathbf{r}_i$ (i = 1, 2, 3 or 4) is the associated eigenvector. Two of the eigenvalues in the present systems (here called λ_3 and λ_4) are always real and negative. Consequently, the remaining two eigenvalues determine the character of the fixed point. Equivalent techniques were used for two-dimensional systems.

Brain-slice preparation and electrophysiological recording

For the electrophysiological recordings, rat somatosensory cortex was used. Using a vibratome, slices with a thickness of 300 µm were prepared. The preparation was performed in a chilled solution composed of (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, and 25 glucose, oxygenated with 95% O_2, 5% CO_2 gas. Slices were then held at room temperature for at least 30 minutes before recording. The neurons were visualized with infrared differential-interference contrast videomicroscopy.

Slices were perfused during recording with oxygenated solution identical to the slicing solution with different temperatures depending on the experiment. Ten µM 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)-pyridaziniumbromide (gabazine), 10 µM D-2-amino-5-phosphonopentanoic acid (AP5), and 10 µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were used to block chemical synaptic transmission mediated by $GABA_A$, N-methyl-D-aspartic acid (NMDA), and α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptors, respectively. This was done to silence the slice.

The cell body of the neurons were the target for whole-cell recordings using patch pipettes with 3-5 MΩ resistance filled with an intracellular solution containing (in mM): 105 K-gluconate, 30 KCl, 10 HEPES, 10 phosphocreatine, 4 ATP, 4 $MgCl_2$, and 0.3 GTP, adjusted to pH 7.3 with KOH. Current-clamp recordings were conducted using an Axon Multiclamp 700B. Before seal formation, the liquid junction potential was corrected for. Signals were filtered and recorded with custom written software.

Dynamic clamp techniques

Artificial conductances were introduced in the recorded neurons using the dynamic clamp technique (Robinson and Kawai 1993, Sharp, O'Neil et al. 1993, Destexhe and Bal 2009). Briefly, conductances are introduced by injecting current according to Ohm's law:

$$I = g(V - E_{rev})$$

where g is the conductance, V is the membrane potential, and E_{rev} is the reversal potential for that specific conductance.

The membrane potential and the model for the conductance are constantly sampled and used to update the conductance. This loop must be performed within a timeframe on the order of tens of microseconds. The cell and the model thus can be viewed as a hybrid circuit between a living organism and a computer program. Since its birth in the early 1990s, the dynamic clamp technique has matured and is now a widely used tool in the field of neuroscience (Prinz, Abbott et al. 2004). The dynamic clamp technique is used in papers II-IV of this thesis. Paper IV explores how the protocol can be improved using Runge-Kutta integration.

Markov schemes of ion channel states

In a Markov chain, the outcome of a random event can affect the outcome of another event. Basically, in specifying a Markov chain, a set of states is defined, $S = s_1, s_2, \dots, s_n$. The sum of all of these states must equal one because the probability cannot exceed one. Next, probabilities are defined for how the system moves between these states, $\Pr(s_j|s_i) = P_{i,j}$, where P is the transition matrix

$$P = \begin{pmatrix} p_{1,1} & \cdots & p_{1,n} \\ \vdots & \ddots & \vdots \\ p_{n,1} & \cdots & p_{n,n} \end{pmatrix}$$

If the aim is to reproduce the situation of single-channel recording, this type of transition matrix can be used to describe how an ion channel shifts states, e.g., from a closed state to an open state or from an open state to a inactivated state. To reproduce the voltage-clamp experiment, the relative probabilities of being in a state are instead used. We can write this in terms of a differential equation:

$$\frac{d}{dt}\begin{pmatrix} s_1 \\ \vdots \\ s_n \end{pmatrix} = \begin{pmatrix} p_{1,1} & \cdots & p_{1,n} \\ \vdots & \ddots & \vdots \\ p_{n,1} & \cdots & p_{n,n} \end{pmatrix}\begin{pmatrix} s_1 \\ \vdots \\ s_n \end{pmatrix}$$

Then, the time evolutions of the states can be calculated. Often this is done numerically. However, for lower-order systems, it can be solved analytically, as in paper V.

RESULTS & DISCUSSION

Paper I

As discussed in the introduction, neurons can be classified based on their firing threshold frequency. This is noted by Hodgkin in a seminal paper (Hodgkin 1948). Two main types of dynamics have been identified in the brain's nerve cells (Tateno, Harsch et al. 2004, Tateno and Robinson 2006), which can be mathematically described in two ways. Type 1 dynamics show a continuous relationship between the frequency and the stimulation current and therefore, an arbitrarily low frequency at the threshold; Type 2 shows a discontinuous relationship and a minimum threshold frequency. The threshold frequency is a characteristic of the nerve cell's dynamic behavior, which in turn determines its role in the brain's oscillatory activity. Recently, neurons with a high threshold frequency, namely, parvalbumin containing fast-spiking interneurons, have been shown to be responsible for gamma oscillations (Cardin, Carlen et al. 2009) as one example of how network behavior is governed by the electrical properties of individual neurons.

The causal mechanisms of the firing pattern in nerve cells are still not fully understood (Bean 2007). It has been suggested that certain ion channels are responsible not only for different firing behaviors (Lien and Jonas 2003) but also for how these ion channels are combined (Prescott, De Koninck et al. 2008) and distributed. In this paper, we have pursued a different mechanism, namely, that ion-channel density can alter neurons' dynamical behavior based on previous studies of a hippocampal soma model (Arhem, Klement et al. 2006, Arhem and Blomberg 2007). This study investigated whether this shift could also occur in models of axons. Somewhat surprisingly, we found that this was not the case. Two standard models of axonal excitability—the Hodgkin-Huxley equations of the squid axon (Hodgkin and Huxley 1952) and the Frankenhaeuser-Huxley model of the myelinated nerve fiber (Frankenhaeuser and Huxley 1964)—displayed exclusively Type 2 dynamics (or no firing at all) for all possible combinations of ion-channel density. This might be an important feature of axons, limiting their flexibility and providing some type of homeostasis of axonal excitability. Taken together, these results suggest that the soma is electrically more flexible than the axon.

Paper II

In this study, we aimed to describe how the network of fixed-spiking-type interneurons is able to have synchronous activity, which is important for brain activity in the gamma band. These types of neurons have been identified as Parvalumbin-containing (PV+), although in this study they were characterized as non-pyramidal in shape and with a fast-spiking phenotype; no other technique is used to mark them.

As noted above, these interneurons have combined chemical-electrical synapses (Galarreta and Hestrin 1999, Gibson, Beierlein et al. 1999, Kopell and Ermentrout 2004). Their synchronization ability was tested by recording a fast-spiking cell in a brain slice, which was connected to an artificial neuron in a computer utilizing the dynamic clamp technique. Next, by changing the strength of the conductance of the chemical and electrical connection, we could observe how these connections were necessary to create robust network activity.

Standard techniques from synchronization theory (Pikovsky, Rosenblum et al. 2001), such as stroboscopic observations and distributions of phases, were used to determine the degree of synchrony. We found that both parts of the combined synapse were important for the ability to obtain synchronous firing. The electrical component could cause a phase advance, whereas the inhibitory chemical component could cause an delay in phase, allowing for entrainment over a wide spectrum of frequency. Using mathematical simulations, we could then reproduce our findings. These findings have implications for how drugs such as benzodiazepines and alcohol affect the brain when adjusting the chemical synapses among these neurons.

Paper III

This paper is an experimental verification of some of the theoretical discoveries made in scientific paper I. In this study, we chose to study the most predominant neuron type in the cortex, i.e., the pyramidal cell. Utilizing the dynamic-clamp method, we introduced artificial channels in living pyramidal cells and therefore could study how the dynamic properties changed at various levels of conductance, which can be translated to ion-channel density. We found that increasing the potassium channels could change the pyramidal cells from Type 1 (having a very low threshold frequency) to Type 2 (with a distinct higher onset frequency). Other features of Type 2 firings were also observed, such as subthreshold oscillations and reduced latency to the first spike. Moreover, the action potential was resculptured with a more narrow spike width and was affirmed after hyperpolarization. We managed to reproduce the dynamics in a two-dimensional model that we developed. In that model, we found unexpectedly advanced mathematics that explained the changed bifurcation. The results from this study show that it is unnecessary to introduce specific channels to obtain different behavior from the neurons, but this can happen if the nerve cell changes its expression of existing channels. More generally, our results demonstrate that neurons' behavior is determined by the global interactions of their dynamical elements.

Paper IV

This study aims to improve the protocol for dynamic clamp. Dynamic clamp is a method which, with the aid of a computer and a real-time interface, can introduce currents that behave as channels or synapses in living cells (Robinson and Kawai 1993, Sharp, O'Neil et al. 1993, Destexhe and Bal 2009). The dynamic-clamp technique, or conductance injection, allows the experimenter to evaluate models directly in living cells. One problem with this technology is numerical instability because the differential equations must be solved numerically (Bettencourt, Lillis et al. 2008). Currently, Euler integration is used to solve the differential equations. In this work, we propose the use of a special method that is more suited for this type of equation. More specifically, the method used is a diagonally implicit Runge-Kutta method suited for a stiff equation system (Iserles and Nørsett 1990). Using both a mathematical model of the dynamic-clamp protocol and electrophysiological experiments, we show that this method is superior to the previous protocol. Given that the method is an implicit Runge-Kutta method, a root to an equation

needs to be obtained, which is done using a standard Newton-Raphson iteration (Press, Teukolsky et al. 2007). With modern processing units, the extra calculations required can be performed without a problematic long time-step. Therefore, the extra calculation seems to be a sensible trade off. The benefit of the proposed method is mainly resistance against numerical instability, but the scheme used also offers higher accuracy and real-time error control. This enhanced dynamic-clamp protocol presented in this thesis opens a path for future demanding experiments.

Paper V

In this paper, we aimed to explain how the dissociation rate of local anesthetics, i.e., how pharmacological compounds detach from an ion channel, affect the pharmacological profile and thus the conductance of the ion channel. We have previously shown that many local anesthetics bind to an open state (Nilsson, Madeja et al. 2008). This theoretical study accompanies an experimental paper in which potassium channels from the West African clawed frog *Xenopus laevis* were expressed in oocytes. The currents were then studied under voltage-clamp conditions and blocked using local anesthetics. An open ion channel can stop ion passage in different manners; a native closed state, an inactivated state and in the presence of a blocking agent, a blocked state. Here we investigate the interaction between states and provide principles of the dynamics of ion channels.

The blocking kinetics were treated mathematically in terms of Markov chains (the dynamics are generally considered to lack memory) and equations were derived that rule the behavior of the blocking agent, particularly whether the drug can reach a peak under voltage-clamp recording (this peak has been taken as proof of an open state blocking process). The mathematical formulae revealed that the dissociation rate played a crucial role in the exictence of a peak in the current waveform. Despite relatively simple models, unexpected complexity was observed. It is hoped that this knowledge will be used to develop new principles for pharmacological treatment.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

In this thesis study, we have investigated the temporal aspects of the nervous system from a electrophysiological and biophysical viewpoint. Despite the fact that it has been more than 120 years since the concept of biophysics was coined by Karl Pearson (1857-1936), biophysics has not been integrated with medicine as much as it deserves.

In our work on excitability, we applied dynamic systems theory (Izhikevich 2010) to understand the mechanisms that determine how a neuron fires impulses (Zeberg, Blomberg et al. 2010, Zeberg, Robinson et al. 2015). Specifically, we utilized bifurcations theory to characterize the various dynamical pathways that lead to action potentials. We have shown how down- and up-regulation of different conductances (such as the potassium current) lead to altered firing patterns and altered threshold dynamics. We wish to further develop these studies and place them into a medical context. Even changes of a more global nature, for example, epilepsy, are almost certainly rooted in the altered electrical properties of individual nerve cells. Epilepsy is usually characterized by highly synchronized activity (Engel, Pedley et al. 2007), which explains why certain neurons' ability to create resonance is of great significance (Ermentrout 1996, Gouwens, Zeberg et al. 2010, Zeberg, Gouwens et al. 2012). The majority of studies, by us and others, have identified two main classes of electrical activity in nerve cells: resonators and integrators (Izhikevich 2010). It was previously held that certain channels were needed for the different types, but we have demonstrated that in many cases, a change in the native conductances is sufficient to induce neurons to become resonators and thus prone to synchronic firing. In other words, we provide a biophysical model of epilepsy based on synchronization and bifurcation theory.

Hypothesis should be tested against reality. A powerful technique that we have used is the so-called dynamic-clamp technique, which allows mathematical models to be directly integrated using a real-time interface with living cells (Robinson and Kawai 1993, Sharp, O'Neil et al. 1993, Prinz, Abbott et al. 2004). The formulae for the currents that one wishes to examine, usually based on voltage-clamp experiments, are simulated in a computer. The membane potentials is sampled and the current that should be passed through these artificial channels is injected into the cell, whereupon the voltage change and the current

are calculated again. The time scales are so short that the current needs to be refreshed in intervals of the order of tens of microseconds. One problem with the technology has been the integration method used. We have solved this issue by using special mathematical techniques, applying a stable implicit Runge-Kutta method suited for stiff equations. In addition, we have developed the dynamic-clamp method to two new applications. First, we have constructed a setup that makes it possible to apply conductance injection on oocytes, thus enabling the study of the interaction between the firing patterns and the currents floating through the proteins expressed in the *Xenopus* oocyte. With this new approach, we have obtained preliminary results showing how so-called GIRK-currents, which are down-regulated pain conditions (Lyu, Mulder et al. 2015), among other diseases, affect the threshold for action potentials. The second development of the method that we have done is to apply it to the Node of Ranvier, which allows us to study how changing channels, or alternatively up- and down-regulated channels, may be involved in the pathogenesis of diseases such as multiple sclerosis (Waxman 2002) and amyotrophic lateral sclerosis (Bostock, Sharief et al. 1995).

Other mathematical aspects that we developed include a new way to assess the affinity between receptor and ligand using Markov chains. This makes it possible for the conventional two-electrode oocyte clamp to attack issues that previously could only be confronted through so-called binding studies.

Understanding the inherent dynamics of various neuron types and their interplay with network activity is essential for understanding complex processes such as altered awareness levels caused by general anesthesia and psychopharmacological interventions. It is the hope that the findings in this thesis may add a small piece of the puzzle for understanding normal as well as patological brain function.

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