Mathematical modeling of neurovascular coupling

Sebastian Sten

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Hjärnan kräver, för att bevara sin normala funktion, en kontinuerlig tillströmning av metaboliter så som syre och glukos, som bärs och levereras av blodomloppet. När ett hjärnområde aktiveras ökar förbrukningen av dessa metaboliter kvickt. Detta kompenseras snabbt för igenom att blodtillförseln till hjärnområdet ökar, vilket temporärt ökar syresättningen av blodet i det aktiverade hjärnområdet under flera sekunder, långt efter att aktiviteten avtagit. Detta fenomen utgör grunden för flera av de icke-invasiva tekniker som idag används för att kartlägga hjärnans funktion i både människor och djur. Ett exempel är funktionell magnetresonanstomografi (fMRI) som mäter lokala förändringar av syrehalten i hjärnan och använder detta som en markör för att lokalisera aktiverade hjärnområden. Användningen av fMRI har revolutionerat hjärnforskningen sedan den introducerades för 30 år sedan, men då tekniken indirekt mäter hjärnaktivitet genom syrehalten i blodet är det viktigt att förstå den serie av händelser som sker mellan ökad hjärnaktivitet och ökad blodtillförsel till hjärnområdet: den neurovaskulära kopplingen.

Den neurovaskulära kopplingen förbinder den elektriska aktiviteten i nervceller med lokala förändringar i blodflöde, blodvolym och metabolism av syre, genom ett komplext biokemiskt system av olika typer av hjärnceller som utsöndrar substanser som påverkar blodkärlen. För att uppnå en ökad förståelse för hur sådana komplexa biologiska system fungerar kan man använda sig av matematisk modellering och skapa en datormodell över systemet, som är en huvudgren inom forskningsområdet Systembiologi. I denna avhandling har vi utvecklat en serie av matematiska modeller som beskriver och undersöker de intracellulära biokemiska signalvägar som den neurovaskulära kopplingen består av, genom att använda oss av olika typer av experimentell data insamlad i flera olika arter: möss, apor och människor.

Artikel 1 undersöker två av de vanligast förekommande hypoteserna som beskriver den neurovaskulära kopplingen. Vi visar med hjälp av modellerna att varje hypotes var för sig inte kan förklara fMRI-data insamlad i människa, men en kombination av de två hypoteserna kan. Denna kombinerade modell kan även korrekt förutsäga hur mätdata bör se ut för olika fall den aldrig tidigare fått se.

Artikel 2 utökar denna modell till att även beskriva scenarion där syrehalten i blodet minskar på grund av att aktiviteten i hjärnområdet hämmas. Denna hämning fyller en viktig funktion då den reglerar aktiviteten i olika hjärnområden så att andra hjärnområden inte hindras från att utföra olika uppgifter.

Artikel 3 beskriver en ny typ av matematisk modell för den neurovaskulära kopplingen, som kan särskilja olika nervcellers bidrag till regleringen av blodkärlen. Detta är möjligt igenom experimentell data som genererats med hjälp av optogenetik, där en ljuskänslig jonkanal uttrycks i specifika typer av nervceller i möss. Med hjälp av en ljuspuls kan man då aktivera olika typer av nervceller var för sig. Modellen kan även beskriva hur narkosmedel förändrar den funktionella kärnregleringen, samt förutsäga effekten av olika typer av biokemiska hämmare.

I Artikel 4 utökas denna modell till att kunna beskriva och förutsäga experimentell data från de allra flesta tillgängliga mätmetoder som används för att undersöka den neurovaskulära kopplingen. Denna modell bidrar med insikter om hur olika typer av observerade fenomen uppstår på olika nivåer av signaleringskedjan.

Denna nya modellbaserade förståelse kring den neurovaskulära kopplingen ger möjlighet till en djupare analys av experimentell data relaterad till hjärnans funktion, med både kliniska och forskningsrelaterade tillämpningar.

ABSTRACT

The brain is critically dependent on the continuous supply of oxygen and glucose, which is carried and delivered by blood. When a brain region is activated, metabolism of these substrates increases rapidly, but is quickly offset by a substantially higher increase in blood flow to that region, resulting in a brief oversupply of these substrates. This phenomenon is referred to as functional hyperemia, and forms the foundation of functional neuroimaging techniques such as functional Magnetic Resonance Imaging (fMRI), which captures a Blood Oxygen Level-Dependent (BOLD) signal. fMRI exploits these BOLD signals to infer brain activity, an approach that has revolutionized the research of brain function over the last 30 years. Due to the indirect nature of this measure, a deeper understanding of the connection between brain activity and hemodynamic changes — a neurovascular coupling (NVC) — is essential in order to fully interpret such functional imaging data. NVC connects the synaptic activity of neurons with local changes in cerebral blood flow, cerebral blood volume, and cerebral metabolism of oxygen, through a complex signaling network, consisting of multiple different brain cells which release a myriad of distinct vasoactive messengers with specific vascular targets. To aid with this complexity, mathematical modeling can provide vital help using methods and tools from the field of Systems Biology. Previous models of the NVC exist, conventionally describing quasi-phenomenological steps translating neuronal activity into hemodynamic changes. However, no mechanistic mathematical model that describe the known intracellular mechanisms or hypotheses underlying the NVC, and which can account for a wide variety of NVC related measurements, currently exists. Therefore, in this thesis, we apply a Systems Biology approach to develop such intracellular mechanisms based models using *in vivo* experimental data consisting of different NVC related measures in rodents, primates, and humans.

Paper I investigates two widely discussed hypotheses describing the NVC: the *metabolic feed-back* hypothesis, and the vasoactive *feed-forward* hypothesis. We illustrate through multiple model rejections that only a model describing a combination of the two hypotheses can capture the qualitative features of the BOLD signal, as measured in humans. This combined model can describe data used for training, as well as predict independent validation data not previously seen by the model before.

Paper II extends this model to describe the negative BOLD response, where the blood oxygenation drops below basal levels, which is commonly observed in clinical and cognitive studies. The model explains the negative BOLD response as the result of neuronal inhibition, describing and adequately predicting experimental data from two different experiments.

In Paper III, we develop a first model including the cell-specific contributions of GABAergic interneurons and pyramidal neurons to functional hyperemia, using data of optogenetic and sensory stimuli in rodents for both awake and anesthesia conditions. The model captures the effect of the anesthetic as purely acting on the neuronal level if a Michaelis-Menten expression is included, and it also correctly predicts data from experiments with different pharmacological inhibitors.

Finally, in Paper IV, we extend the model in Paper III to describe and predict a majority of the relevant hemodynamic NVC measures using data from rodents, primates, and humans. The model suggests an explanation for observed bi-modal behaviors, and can be used to generate new insights regarding the underpinnings of other complicated observed behaviors. This model constitutes the most complete mechanistic model of the NVC to date.

This new model-based understanding opens the door for a more integrative approach to the analysis of neuroimaging data, with potential applications in both basic science and in the clinic.

List of Included Publications and Manuscripts

This thesis is based on the following papers, referred to in the text by their Roman numerals (I-IV).

- I Karin Lundengård, Gunnar Cedersund, **Sebastian Sten**, Felix Leong, Alexander Smedberg, Fredrik Elinder, Maria Engström. *Mechanistic Mathematical Modeling Tests Hypotheses of the Neurovascular Coupling in fMRI*. PLoS Comput Biol, 2016; 12: e1004971. <https://doi.org/10.1371/journal.pcbi.1004971>
- II **Sebastian Sten**, Karin Lundengård, Suzanne Tyson Witt, Gunnar Cedersund, Fredrik Elinder, Maria Engström. *Neural inhibition can explain negative BOLD responses: A mechanistic modelling and fMRI study*. Neuroimage, 2017; 158: 219–231. <https://doi.org/10.1016/j.neuroimage.2017.07.002>
- III **Sebastian Sten**, Fredrik Elinder, Gunnar Cedersund*, Maria Engström*. *A quantitative analysis of cell-specific contributions and the role of anesthetics to the neurovascular coupling*. Neuroimage, 2020; 215:116827. <https://doi.org/10.1016/j.neuroimage.2020.116827>
- IV **Sebastian Sten**, Henrik Podéus, Nicolas Sundqvist, Fredrik Elinder, Maria Engström*, Gunnar Cedersund*. *A multi-data based quantitative model for the neurovascular coupling in the brain*. In manuscript.

* Authors contributed equally

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Sebastian Sten
Linköping, July 2020

Abbreviations

20-HETE	20-hydroxyeicosatetraenoic acid.
AA	Arachidonic Acid.
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid.
ATP	Adenosine Triphosphate.
BK _{Ca}	Large-conductance calcium activated K ⁺ Channels.
BOLD	Blood Oxygen Level-Dependent.
cAMP	Cyclic Adenosine Monophosphate.
CBF	Cerebral Blood Flow.
CBV	Cerebral Blood Volume.
cGMP	Cyclic Guanosine Monophosphate.
ChR2	ChannelRhodopsin-2.
CMRO ₂	Cerebral Metabolic Rate of Oxygen.
COX	Cyclooxygenase.
CYP	Cytochrome P450.
DAEs	Differential Algebraic Equations.
EETs	Epoxyeicosatrienoic acids.
FIM	Fisher Information Matrix.
fMRI	functional Magnetic Resonance Imaging.
GABA	γ -aminobutyric Acid.
GLM	General Linear Model.
HRF	Hemodynamic Response Function.
IP ₃ R	Inositol-1,4,5-trisphosphate Receptor.
K _{IR}	Inward-rectifying K ⁺ channel.
LFP	Local Field Potential.
MCMC	Markov Chain Monte Carlo.
mGluR	metabotropic Glutamate Receptors.
MRI	Magnetic Resonance Imaging.
NIRS	Near-Infrared Spectroscopy.
NMDA	N-methyl-D-aspartate.
nNOS	neuronal Nitric Oxide Synthase.
NO	Nitric Oxide.
NPY	Neuropeptide Y.
NPY ₁ R	NPY receptor Y ₁ .
NVC	Neurovascular Coupling.
ODEs	Ordinary Differential Equations.
OG	Optogenetic.
PGE ₂	Prostaglandin E ₂ .
PL	Profile Likelihood.
PPL	Prediction Profile Likelihood.
RF	Radio Frequency.
RK4	Runge-Kutta 4th order.
SA	Simulated Annealing.
SEM	Standard error of the mean.
SOM	Somatostatin.
SSR	Sum of Squares Residual.
SSTR	Somatostatin receptors.
V _M	Membrane potential.
VASO	Vascular-Space-Occupancy.
VIP	Vasointestinal Peptide.
VPAC	VIP/PACAP receptor.
VSM	Vascular Smooth Muscle.

Mathematical Notations

α	Significance level.
$\alpha(p', p)$	Acceptance probability for an MCMC parameter candidate p' .
χ^2	Chi-square.
$CI(p)$	Confidence interval for the parameter p .
D	Profile likelihood confidence interval.
df	Degrees of freedom.
F_f	Probability distribution for f .
$F_f^{cdf-inv}$	Inverse cumulative density distribution for f .
h	Step size.
H	Hessian Matrix.
H_0	Null hypothesis.
$J(p)$	Negative log-likelihood function.
$J_{ssr}(p)$	Weighted sum of squares residual function.
$L(y p)$	Likelihood function.
λ, ϵ	Numerical weight.
M	Model structure.
n_d, n_o, n_e	Number of data points (d), observables (o), and experiments (e).
n_p, n_i	Number of parameters (p) and identifiable parameters (i).
p	A parameter or parameter vector.
p^0	Parameter starting point in optimization.
$\widehat{p}$	Parameter estimate of a global minimum.
p'	Candidate parameter point.
$P(p)$	Prior belief of parameter p .
$P(p y)$	Posterior parameter distribution.
$P(y)$	Unknown scaling factor ('evidence').
$PL_k(p_k)$	Profile likelihood trace for a parameter p_k .
$q(p)$	Proposal function.
R_1	Longitudinal relaxation rate.
R_2	Transverse relaxation rate.
R_2^*	Transverse relaxation rate subjected to inhomogeneities in the magnetic field.
σ	Measurement uncertainty.
s^x	Sensitivity of the state x .
$s^{\widehat{y}}$	Sensitivity of the observable y .
t	Time.
T	Test statistic of a given probability distribution.
$T_{\chi^2}^{cut-off}$	Test statistic threshold for rejection of H_0 .
u	Model input.
x	Model states.
$\dot{x}$	Derivatives of the states.
y	Experimental data.
$\widehat{y}$	Model output/observable.
z	Model states where the derivative is unknown (used in DAE models).
$Z(p)$	A quantifiable well-determined model property (core prediction).

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Introduction

“Men ought to know that from the brain, and from the brain only, arise our pleasures, joys, laughter and jests, as well as our sorrows, pains, griefs and tears. Through it, in particular, we think, see, hear, and distinguish the ugly from the beautiful, the bad from the good, the pleasant from the unpleasant . . . It is the same thing which makes us mad or delirious, inspires us with dread and fear, whether by night or by day, brings sleeplessness, inopportune mistakes, aimless anxieties, absent-mindedness, and acts that are contrary to habit.”

— Hippocrates, Fifth Century B.C.E

Our brain — a slimy wrinkled mound of grayish tissue — is the vessel through which our awareness of the external (*i.e.*, physical surroundings) and internal existence emerge: consciousness. The brain integrates a non-stop stream of information stemming from our sensory organs and presents this information in a coherent and unified manner, through which one can interpret and manipulate the physical environment. The human brain has evolved to possess higher-order cognitive functions such as abstract thinking and problem solving, which have aided the rapid evolution of mankind. However, *how* the higher-order cognitive functions of the brain emerge from the brain’s cellular, molecular, and anatomical structure is still not well understood. In search of answers to this question, scientists have during the last century, step by step, been able to probe and generate large amounts of experimental data from the brain, due to a series of major breakthroughs in biomedical imaging and other experimental techniques. In parallel to these experimental breakthroughs, the rise of computational power during the last decades has laid the foundation of artificial intelligence, which is heavily influenced by the naturally occurring design of the brain. This design is made up of an intangible weave of connections between neurons in the form of electrical synapses, which continuously change over time. Due to this complexity,

the brain relies on a constant supply of biological necessities such as glucose and oxygen, which are carried and delivered by blood. This doctoral thesis revolves around modeling how the brain manages to balance the supply and demand for blood by actively regulating the vasculature as a function of neuronal activity.

1.1 Thesis outline

Beyond this introductory chapter, this thesis is divided into five additional chapters.

Chapter 2 gives a brief historical background and introduction to neuroscience and Systems Biology, which leads up to the research questions which are studied in this thesis.

Chapter 3 provides background information regarding the current state-of-the-art understanding of the NVC and some of its pertaining aspects.

Chapter 4 introduces the reader to the core methodological concepts of Systems Biology and mathematical modeling, which is extensively applied throughout the thesis.

Chapter 5 summarizes the main findings and how they fit into the overall picture.

Finally, **Chapter 6** provides some concluding remarks and thoughts on the future outlook and potentials of the findings.

Background

The following chapter aims to introduce the necessary background information to the reader. This information entails a basic overview of neuroscience with an emphasis on the basic mechanism of balancing the supply and demand of blood in the brain, and a brief introduction to the interdisciplinary field of Systems Biology. With these concepts in place, the chapter ends with introducing the research questions that this thesis revolves around.

2.1 Anatomy and physiology of the mammal brain

This section provides a brief introduction to the anatomy and physiology of the mammal brain. The section begins with the basic building block of information in the brain — action potentials — and then continues upwards in scale, through neurons, the cortex, and finally to the whole-brain perspective. This is a short introduction to these concepts, and for a more comprehensive introduction to neuroscience in general, I refer the reader to [1].

The language of information in the brain — the action potential

The language of information in the brain is based on the transmission of rapid electrical impulses, commonly known as *action potentials*. The first recorded intracellular action potential (see Fig. 2.1, left) was captured in squid giant axons in 1939 by the Nobel prize recipients A.L Hodgkin and A.F Huxley [2]. The archetypal action potential is depicted in Figure 2.1 (right), and it exhibits three distinct phases: 1) depolarization, where the potential rapidly rise; 2) repolarization, where the potential abruptly shoots back downwards, and finally, 3) hyper-polarization, where the potential remains below the resting potential before slowly re-establishing the resting potential. The electrical

Figure 2.1: **The first intracellular recording of an action potential (left), captured in the squid giant axon.** The vertical scale shows the potential of the internal electrode in millivolts, using the extracellular seawater as zero potential. The sinusoidal wave peaks in the lower part of the figure correspond to a 500 Hz timing signal. Figure taken from [2] with permission. **Right:** The three phases of an action potential: rapid depolarization, repolarization, and finally, a period of hyper-polarization before returning to baseline. The different baseline potential values between left and right are due to different extracellular medium.

stimulation needs to be strong enough to initiate the response, meaning that the action potential can be viewed as an 'all-or-nothing' response.

Neurons

Action potentials originate from excitable cells, which express voltage-gated ion channels that are embedded in the lipid plasma membrane of the cell. The excitable cells are *neurons* which generate, receive, process, and transmit action potentials throughout the brain (Fig. 2.2A). The notion that the brain is comprised of individual neurons was first proven in the late 1800s, long after the idea that living tissue consists of individual cells was accepted. Initially, the brain was thought to be an exception; that the brain instead was made up of a continuous protoplasmic network, as hypothesized by the anatomist Joseph von Gerlach (1820–1896) in 1871 [3, 4]. This hypothesis was, in part, a result of methodological limitations, as neurons exhibit highly irregular shapes and long thin processes extending from the nucleus (Fig. 2.2). This made neurons hard to clearly distinguish using the only technique available at the time: conventional light microscopy. This experimental limitation was overcome by Camillo Golgi (1843–1926), who developed a new cellular staining technique in 1873: *la reazione nera* or Golgi's method [5, 6]. Golgi's method allowed for these small details to be clearly seen in the preparation. This technique was extensively used by both Golgi and Santiago Ramón y Cajal (1852–1934) to produce detailed drawings of neurons in many brain regions or different species (Fig. 2.2B) [7]. Golgi and Cajal were later awarded the Nobel prize in 1906 for these discoveries. These seminal

drawings laid the foundation for the 'neuron doctrine', which states that the brain is made up of discrete individual cells ('neurons') which are not structurally connected, but communicate through *synapses* (see [7]). A synapse is a site of information relay, which allows for an electrical signal to be relayed between two neurons through the use of chemical neurotransmitters (discussed below).

Figure 2.2: **Neurons underlie the electrical signaling in the brain.** **A:** A schematic drawing of a generic neuron. Note that the main parts of the neuron in this picture are the axon and the cell body (soma). Figure adapted from [8], with permission. **B:** Golgi-stained pyramidal neuron in the cerebral cortex of a cat. Figure adapted from [7], with permission. The drawing originates from the Cajal Institute in Madrid. **C:** Staining of neurons in the rat visual cortex using immunofluorescence techniques. Pyramidal neurons are shown as green cells, while GABA inhibitory interneurons are shown as red cells. The pyramidal neurons exhibit both apical (upwards) and basal (sideways) dendrites. The axons extend down from the soma in the lower portion of the image. This figure was adapted from [9], with permission.

Neurons are canonically comprised of four functionally unique regions (Fig. 2.2A): dendrites, the cell body (soma), the axon, and the axon terminals [1]. Dendrites are thin and spiny branches that extend outwards from the soma, and are the recipient of incoming signals from other neurons through synapses. On average, each neuron is connected through approximately 7000 synapses [10]. The soma houses the nucleus, which contains the genetic set of instructions that encode the synthesis of proteins in the neuron. Axons are long processes which conduct the action potential from the soma to a synapse. Some neurons sheath the axons in the protein myelin, which acts as an insu-

Figure 2.3: **Axial view of the human brain.** The characteristic ridges (gyri) and fissures (sulci) are seen on the surface of the cortex. The cortex contains a larger amount of neuronal cell bodies and therefore appears gray. The deeper white regions contain larger amounts of myelinated axons. Image reprinted from the Visible Human Project [11]).

lator to promote the electrical conduction of vast segments of the axon [1]. The axon terminal is found at the tip of the axon, which constitutes the pre-synaptic half of the synapse. The other half of the synapse is the post-synaptic dendrites of the receiving neuron. As no direct structural connection exists between the neurons, the electrical signal must be converted to an intermediary messenger, a neurotransmitter, which is released into the synaptic cleft. The neurotransmitter binds to specific receptors on the post-synaptic neuron, which when opened, induce an action potential, thereby relaying the 'message' further on.

Numerous different neurotransmitters are used in the central nervous system. Neurotransmitters are often classified as either excitatory or inhibitory, but the effect is not intrinsic to the neurotransmitter but instead depends on the post-synaptic expression of receptors. Nevertheless, glutamate and γ -aminobutyric acid (GABA) are the two most prevalent excitatory and inhibitory neurotransmitters used in the nervous system, respectively [1]. Excitatory neurotransmitters evoke small positive change in the post-synaptic membrane potential, whereas inhibitory neurotransmitters evoke a counter-acting effect. The release of neurotransmitters is dependent on the type of neuron activated. Excitatory neurons such as a pyramidal neuron (Fig. 2.2B–C, Fig. 2.6B) release glutamate when depolarized. Other types of neurons, such as GABAergic interneurons (Fig. 2.2C, Fig. 2.6B), are predominantly inhibitory and release GABA when stimulated.

Cortical arrangement of neurons

The large scale organization of the nervous system shares a similar structure in all mammals. The tissue which the brain consists of is commonly divided

into two parts: gray matter and white matter (Fig. 2.3). Gray matter contains a high density of neuronal cell bodies, while white matter contains mainly myelinated axons. As seen, the cortex (outer layer) of the brain is predominantly made up of gray matter, and has a characteristic folding pattern of grooves (denoted sulci) and ridges (denoted gyri). This clever structural pattern increases the surface area of the cortex (and thereby the number of neurons), which has allowed for higher-order cognitive abilities to develop. The

Figure 2.4: **The column-like structure of the cortex.** Each layer (see left side) possess a distinct selection of neuronal types and connections which spans both between and outside of the layers. The supply of blood is depicted with red (arteries and arterioles) and blue (veins and venules) blood vessels which penetrate the cortical column. White matter can be seen at the bottom of the column, with axons spanning both upwards and downwards directions. Image adapted from [12], with permission.

cortex has a column-like structure consisting of up to six (I–VI) distinct layers of neurons. These columns are aligned perpendicular to the surface (Fig. 2.4). The type of neurons and the intra-layer or extra-layer connections are specific to that layer and area, and different layers receive input from other cortical areas (see bottom of the figuring for traces of axons in white matter). Nevertheless, excitatory pyramidal neurons are commonly expressed in layer 5 (see *Z.L.P* in Fig. 2.4, dark pyramidal shapes), and inhibitory (GABAergic) interneurons in layer II/III (see irregularly shaped spiny neurons in Fig. 2.4). Note the vascular penetration of arteries and arterioles (red vessels),

2. Background

and correspondingly venules and veins (blue vessels), throughout the cortical column. The seminal work by Vernon Mountcastle (1918–2015) in the cat somatosensory cortex showed that neurons that respond to a specific stimuli often occupy the same layer in a cortical column. This insight spawned the idea that these cortical columns constitute the “elementary unit of organization” in the brain [13].

Finally, to transition from the structure of cortical columns to more large-scale cortical regions, the neurologist Korbinian Brodmann (1868–1918) used the cytoarchitectonic differences of the cortical columns to divide the brain into 52 distinct areas or regions [14]. Brodmann postulated that areas with different structural composition correspond to different functions of the brain [15], which holds true for some of the Brodmann areas today [15]. These areas are depicted in Figure 2.5.

Figure 2.5: **Brodmann’s map of cytoarchitectonics.** The numbers correspond to brain regions that possess a distinct cytoarchitecture. Image adapted from [16], with permission.

To summarize, we began this section with the function of action potentials; moving up through the morphology and function of neurons; their vertical alignment into cortical layers and columns, and finally, the large scale division of specialized cortical areas with the help of the Brodmann classification. Throughout this brief journey through the history and different spatial scales of neuroscience, we have acquired a small insight into the complexity of the mammal brain. A natural question arises, how does the brain maintain its normal function with all of this complexity?

Figure 2.6: Brief overview of functional hyperemia and the underlying biological system. **A:** The archetypal BOLD response exhibits three main characteristics to short stimuli: 1) a debated initial dip, 2) an over-compensatory response ('overshoot'), and finally, 3) a post-peak undershoot. **B:** The intracellular signaling basis of the NVC. Increased neuronal activity triggers increased cerebral metabolic rate of oxygen (CMRO₂), which can sometimes be observed as an initial dip (1). Simultaneously, neurons and astrocytes release vasoactive messengers upon stimulation, evoking changes in cerebral blood flow (CBF) and volume (CBV), which underlie the overshoot and post-peak undershoot phases of the response (2, 3). BOLD = Blood Oxygen Level-Dependent

2.2 A short introduction to the neurovascular coupling

The brain constitutes $\approx 2\%$ of the total body weight of an average adult human [17]. Despite this small weight proportion, the brain consumes $\approx 20\%$ of an adult's total energy [17]. To satisfy this large demand for energy, the brain requires a non-stop supply of metabolic substrates such as glucose and oxygen [18]. Such metabolic substrates are delivered through blood circulation, and can be transported over from blood to cerebral tissue in the capillaries through diffusion or receptor-mediated transport. This connection between brain activity and the supply of blood was first discovered by Mosso in 1881

[19], and later by Roy and Sherrington in 1890 [20]. These two studies unraveled a temporal and spatial connection between alterations in brain activity and local hemodynamic changes — a neurovascular coupling (NVC). We now know that the NVC describes how the communication between neural cells and the vasculature actively regulates cerebral blood flow (CBF), cerebral blood volume (CBV), and cerebral metabolic rate of oxygen (CMRO_2). More than a century later, a detailed view of the communication has evolved. The NVC is initiated by the synaptic activity of neurons, *brain activity*, which triggers increased metabolism of oxygen and glucose, as well as intracellular signaling cascades that culminate in the release of many different vasoactive molecules (see reviews in: [21–26]). These vasoactive messengers originate from different types of neurons, including pyramidal neurons, GABAergic interneurons, or other neural cells such as astrocytes (Fig. 2.6B).

The actions of these different cell types underlie a common response pattern that is often observed for local increases in brain activity. This response pattern consists of three main characteristics: 1) an initial rapid increase of CMRO_2 (Fig. 2.6A–B, see 1), followed by 2) large hemodynamic changes with a characteristic oversupply of blood (Fig. 2.6A–B, see 2). The oversupply of blood flow compensates fully for the increased metabolism, leading to an increased blood and tissue oxygenation, and is referred to as functional hyperemia. Finally, a post-peak undershoot can sometimes be observed, where the blood oxygenation and flow decrease below basal for a short time as it returns to baseline values (Fig. 2.6A–B, see 3). Functional hyperemia forms the basis of functional neuroimaging techniques, which exploit this increased blood oxygenation as a biomarker for brain activity [27]. These mostly non-invasive techniques include the widely used functional magnetic resonance imaging (fMRI) [28, 29] or near-infrared spectroscopy (NIRS) [30–32], which have been used to unravel a plethora of insights regarding brain function and cognition during the last 30 years [33]. In particular, fMRI captures a Blood Oxygen Level-Dependent (BOLD) signal, such as the response depicted in Figure 2.6A. Nevertheless, due to the indirect nature of this measure of brain activity, it is essential to have a complete understanding of the molecular and cellular chain of events underlying the NVC in order to fully interpret functional neuroimaging data. While this detailed chain of events still remains elusive, a broad picture of the essential mechanisms has slowly emerged. However, the analysis and interpretation of functional neuroimaging data is conventionally done in a statistical way that ignores the current understanding of the underlying biological mechanisms.

This statistical way is typically based on a correlational analysis between the observed BOLD-fMRI data (Fig. 2.7A) and a template of what the response should look like, using the general linear model (GLM) and its associated hemodynamic response function (HRF) (Fig. 2.7B) [34, 35]. This

Figure 2.7: **Analysis of fMRI: today and a vision for the future.** BOLD-fMRI data (A) are analyzed using a correlation analysis with a standard defined template, a hemodynamic response function (HRF) (B). This analysis produces statistical maps that depict areas with high or low correlations which are used to infer brain activity (C). In contrast, a model based on the underlying biochemical and physiological mechanisms could be utilized (D), which also could incorporate more data into the analysis. Such an approach would give model-based biomarkers (E–F), for instance, increased metabolism or increased activation of different subsets of neurons (F).

correlation analysis equates a high correlation between the measured BOLD-fMRI signal and the HRF as brain activity, and a low correlation as no brain activity, for the measured brain area (Fig. 2.7C). In contrast, the use of mathematical models which incorporate more information about the underlying system (Fig. 2.7D) can in principle include other measured observables in the analysis, such as CBF or CBV. Furthermore, such a model-based approach could also produce new model-based biomarkers (Fig. 2.7E). These biomarkers could potentially be more nuanced than the current correlation approach and identify things such as increased metabolism or increased activation of different subsets of neurons. (Fig. 2.7F). However, no useful mathematical model exists that could make this vision a reality. More specifically, models are needed that include biochemical mechanisms of the NVC. Such models, which are built upon biochemical or biological mechanisms, could be developed using tools and methods from the field of Systems Biology.

2.3 Systems Biology

An new interdisciplinary research field — *Systems Biology* — emerged in the early 2000s [36–39]. Systems Biology aims to provide a systems-level wide understanding of biological systems, and to capture how underlying system properties arise only when combining individual pieces from the same puzzle or system. This stands in contrast to the classical reductionist approach, where each component (or puzzle piece) is examined in isolation, a common approach in *in vitro* molecular biology [39]. Systems Biology rests on two core pillars: 1) acquisition of experimental data from the intact biological system, and 2) the use of mathematical models to analyze that data to generate new insights regarding the system.

The first concept, acquisition of experimental data from the system, is based on the fact that a systems-level understanding can only be obtained if enough diverse experimental data can be captured from the intact system. The second concept, mathematical modeling, is useful for analyzing complex data sets as it provides a framework for quantitatively testing hypotheses about the inner workings of the system, and in turn, how the captured experimental data was generated. As Systems Biology aims to capture all of the pieces of a given system, which are often complex and interconnected, the generated models and explanations are often also complex [36, 39]. This is typically the case for mechanistic modeling, where the components of the model, as well as the system properties that arise from the inter-connected components, can be understood in biological terms. Nevertheless, these types of mechanistic models have until recently rarely been applied to biological systems, due to previous experimental limitations of how much data that can be obtained from the system.

Models of the NVC

In the context of NVC, a variety of modeling approaches have been used for analysis of the NVC (see Table 1, first column). Early work by Friston *et al.* [41] and Buxton *et al.* [40, 52], and later by Havlichek *et al.* [42] introduced variants of the 'Balloon' model, a quasi-phenomenological model describing the interplay between CBF, CBV, and CMRO₂. While these models have been of great importance for the interpretation and development of fMRI, these models have three main limitations. First, these models are not based on the cellular and molecular mechanisms underpinning the NVC, and instead utilize phenomenological or bio-mechanical descriptions to connect neuronal activity to hemodynamic changes (see Table 2.1, second column). Second, these models cannot account for recent experimental data which show that arteriolar dilation is the main driver of the hemodynamic responses (see [53] and references therein), instead only accounting for a venous vas-

Table 2.1: Comparison of different published models describing the neurovascular coupling with regards to different aspects. The notations used in the table is, ‘Yes’ if the model features the stated aspect; ‘Partly’ if the model has a description that is not fully satisfying, and ‘No’ if there is no satisfying description of the aspect. As seen, no model currently exists that can describe all aspects. Table adapted from Paper IV, with permission.

Models	Intracellular mechanisms	All vascular compartments	Blood oxygenation dynamics	Full BOLD description
Buxton et al., 1998 [40, 52]; Friston et al., 2000 [41]; Havlicek et al., 2015 [42]	No	No	Partly	Partly
Barrett et al., 2012 [43–45]	No	Yes	Yes	No
Huppert et al., 2007 [46–48]	No	Yes	Yes	Yes
Yücel et al., 2009 [49]; Mathias et al., 2016 [50, 51]	Yes	No	Partly	Partly
Final work of this thesis	Yes	Yes	Yes	Yes

cular compartment (as these models were originally developed to describe the mismatch between CBF and CBV; see Table 2.1, third column). Third, these models do not explicitly include blood oxygenation and saturation dynamics (Table 2.1, fourth column), which in combination with the previous limitation, leads to only a partial description of the BOLD signal (Table 2.1, last column). Others research groups have developed more advanced electrical circuit equivalent vascular models, which remedy in particular the second and third limitation, such as Huppert *et al.* [46–48] or Barrett *et al.* [43–45]. Nevertheless, mechanistic models based on the current understanding of the molecular underpinnings of the NVC are lacking. Some models exist, such as models proposed by Yücel *et al.* [49] and Mathias *et al.* [50, 51]. However, these models have rarely been trained to time-resolved experimental data, and are typically connected to the ‘Balloon’ model in order to simulate the hemodynamic responses. Therefore, a model based on the core pillars of Systems Biology remains to be determined.

2.4 Aims and scope

The overall vision is to take the first steps towards obtaining a mathematical model built on the molecular mechanisms underpinning the NVC, which can be used to: 1) explain commonly observed NVC phenomena; 2) provide a more exhaustive interpretation of functional neuroimaging data with the potential of introducing new types of biomarkers, and 3) generate new insights into the functions of the NVC. More specifically, in this thesis, the main focus lies on applying the methods and tools used within Systems Biology to construct mathematical models built on the known mechanistic underpinnings of the NVC (Fig. 2.7D, orange box). These models are developed and investigated using *in vivo* experimental data from multiple different species and experimental modalities.

3

Neurovascular Coupling

Throughout this chapter, I will introduce the reader to the seminal studies behind the discovery of the NVC; the current state-of-the-art understanding of the molecular and cellular basis of the NVC; a selection of *in vivo* measurement modalities used to interrogate the NVC, and finally, how the NVC is affected by commonly used anesthetics within NVC research.

3.1 The discovery of a NVC

As briefly mentioned above, during the late 19th century, Angelo Mosso (1846–1910) and separately the duo of Charles Roy (1854–1897) and Charles Sherrington (1857–1952), provided the first evidence of a NVC by unraveling the coupling between brain activity and energy supply. Mosso observed brain pulsations occurring in the prefrontal cortex of a human subject with a post-traumatic skull defect, using a 'cerebral pulsometer' [19, 54] (Fig. 3.1). These pulsations held the same pace as the subject's heartbeat, but the amplitude of the brain pulsations increased independent of the heartbeat when the subject performed different cognitive tasks, such as reciting 'Ave Maria' (Fig. 3.1 top, the onset of the cognitive task is marked by the black arrow) [19]. These findings were followed up by the seminal work of Sherrington and Roy in 1890 [20], who employed a more direct approach which revealed that stimulation of sensory nerves in animals produced an increase in cerebral blood pressure and volume. Furthermore, the duo also observed that cerebral metabolites released during brain asphyxia (hypoxia/hypercapnia) dilated the cerebral vasculature in a compensatory manner [20]. With these key deductions and other findings, they arrived at the following conclusions:

"We conclude then, that the chemical products of cerebral metabolism contained in the lymph which bathes the walls of the arterioles of the brain can cause variations of the calibre of the cerebral vessels: that in this reaction

Figure 3.1: **Cerebral pulsations in the prefrontal cortex of a thin skulled human with simultaneously measured heartbeats at the forearm.** The subject was task with reciting 'Ave Maria', with the onset indicated by the black arrow. Image taken from [54] and [55], with permission.

the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations of functional activity” [20].

In other words, Roy and Sherrington proposed the mechanism of metabolic feedback, connecting cerebral metabolism with changes in localized CBF.

The proposal of the metabolic feedback hypothesis

Feedback mechanisms are a common and central component in the regulation of many physiological and biological systems. The metabolic feedback hypothesis of CBF regulation, proposed by Roy and Sherrington [20], states that signals which increase localized CBF are generated as a result of reduced supply of metabolic substrates and/or an accumulation of metabolic waste products (see review in [56]). An intuitive initiator for the neurovascular response could be for instance, a rapid reduction in the local concentration of oxygen and/or glucose, *i.e.* a negative feedback loop. Previous studies have shown that brain metabolism is more sensitive to reductions in oxygen concentration than glucose (see [57] and references therein). Indeed, earlier studies have shown that a decrease in cerebral tissue oxygen levels is strongly correlated with increased neuronal activity [58–60], and that changes in CBF

correlate with oxygen usage [61] *in vivo*. In addition, an *in vitro* study of rat brain slices demonstrated that oxygen levels in tissue determine the polarity of hemodynamic responses induced by activation of astrocytes [62]. However, correlation does not equal causality, and further available evidence suggests that the lack of oxygen or glucose does not directly increase the CBF ([63–65], also see [66] and references therein). In addition, the hemodynamic responses are not affected by hyperoxia for both normobaric [65] and hyperbaric [67] experimental conditions. These studies together suggest that neither oxygen or glucose directly evokes the transient CBF responses observed upon increased neural activity.

Another potential mechanism is that the waste products of cerebral metabolism such as CO_2 , H^+ , or lactate could evoke hemodynamic changes. Some studies have shown that astrocytes are capable of sensing experimental manipulations of CO_2 or H^+ , and respond to such manipulations with increased intracellular Ca^{2+} and release of adenosine triphosphate (ATP), which leads to the release of vasoactive signaling molecules [68, 69]. Nevertheless, no direct effect of CO_2 and H^+ can account for the transient changes in CBF evoked by stimulation [70, 71]. Finally, lactate is also released upon local increases in neural activity, and has been shown to positively modulate the hemodynamic response, most likely by up-regulating nitric oxide production (see review in [72] and references therein).

To summarize, these studies together suggest no direct effect of the cerebral metabolites on functional hyperemia, but instead, a subset of the metabolites can indirectly modulate and shape the response through effects on the production and release of intracellular vasoactive messengers. Therefore, the question arises: how are these vasoactive messengers released?

3.2 Feed-forward hypothesis governing the NVC

An alternative hypothesis to the metabolic feedback hypothesis is the idea of a feed-forward mechanism (see reviews in [22, 24, 26, 73]). A feed-forward mechanism describes how active neurons and astrocytes release vasoactive messengers or signaling molecules that evoke vasodilation or vasoconstriction, through direct or indirect action on the vasculature. The key difference between a feed-forward and a feedback regulated mechanism is that a feed-forward mechanism acts pro-actively, while a feedback mechanism acts reactively. Furthermore, in the context of the NVC, the signaling molecules which underpins the hemodynamic response are separate from those involved in cerebral metabolism. This hypothesis has gained traction with the discovery of many different feed-forward signaling pathways that influence the NVC (see Fig. 3.2). These discoveries have unraveled a basic chain of events, which includes multiple different cell types, signaling pathways, and

finally, secretion of different vasoactive messengers or molecules which act on their associated vascular targets.

This basic chain of events begins with the release of neurotransmitters from pre-synaptic neurons, which bind to specific ion channel coupled receptors expressed on the post-synaptic neurons. The main excitatory neurotransmitter, glutamate, bind to N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Activation of these receptors opens their ion-conducting pores, causing an influx of Na^+ and Ca^{2+} ions, and subsequently, depolarization of the neuron. Depolarization opens voltage-gated calcium channels, causing an additional influx of Ca^{2+} ions. In contrast, the primary inhibitory neurotransmitter GABA acts to prevent depolarization of neurons. GABA binds, for instance, to the ion channel-coupled GABA_α receptor, which opens Cl^- ion-conducting pores, causing an influx of Cl^- ions which counterbalance any depolarizing efforts. Excitatory neurons such as pyramidal neurons are glutamatergic, meaning that glutamate is released upon depolarization, and acts on astrocytes and other neurons. Inhibitory neurons, such as GABAergic interneurons release GABA upon depolarization and act on neurons or other neural cells. In total, these neuronal interactions form a canonical neuronal circuit, where pyramidal neurons excite the neighboring neural cells, and GABAergic interneurons regulate the activity of surrounding neurons. Furthermore, depolarization causes an influx Ca^{2+} ions, which in turn triggers cell-specific signaling pathways.

Pyramidal neurons

The rise in intracellular Ca^{2+} levels in pyramidal neurons (Fig. 3.2, gray triangle) activates phospholipases which cleave membrane phospholipids through a series of enzymatic steps into arachidonic acid (AA) [24, 26, 74]. AA is metabolized into prostaglandin E_2 (PGE_2) through a cyclooxygenase (COX)-2 rate-limiting conversion [75–78]. Finally, PGE_2 promotes vasodilation through activation of PGE_2 receptors EP2 and EP4 located on vascular smooth muscle (VSM) cells or pericytes, which up-regulates the synthesis of cyclic adenosine monophosphate (cAMP) (Fig. 3.2) [76, 79].

GABAergic interneurons

The rise in intracellular Ca^{2+} levels in GABAergic interneurons evoke the release of different vasoactive molecules or peptides (Fig. 3.2, yellow cell body). For instance, nitric oxide (NO) evokes vasodilation of arterioles, in both *in vitro* cortical slices and *in vivo* [80–83]. NO is released by specific interneurons which express the enzyme neuronal nitric oxide synthase (nNOS) [84], which is modulated by Ca^{2+} . A recent systematic review revealed that inhibition of nNOS had the largest effect on the hemodynamic response for *in vivo*

Figure 3.2: Simplified schematic illustration of the cellular pathways underlying the release of vasoactive messengers in the NVC. Neuronal signaling affects GABAergic interneurons (yellow oval) and pyramidal neurons (gray triangle), inducing fluctuations in membrane potential (V_M) through activation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA) or γ -aminobutyric acid ($GABA_A$) receptors. Depolarization induces an influx of Ca^{2+} ions. Ca^{2+} acts to facilitate various vasoactive signaling pathways in the different cells. In GABAergic interneurons, Ca^{2+} promotes the production and subsequent release of nitric oxide (NO) through up-regulation of neuronal nitric oxide synthase (nNOS), as well as facilitating the release of different neuropeptides such as neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), and somatostatin (SOM). In pyramidal neurons, Ca^{2+} promotes arachidonic acid (AA) synthesis via phospholipases (PL), which in turn is processed into prostaglandin E₂ (PGE₂) via cyclooxygenase-2 (COX)-2. Glutamatergic signaling also affects astrocytes (green rectangle), inducing a Ca^{2+} influx through the ATP-sensitive P2X receptor or by activation of the metabotropic glutamate receptor (mGluR). In astrocytes, AA is synthesized via phospholipase D2 (PLD2), and subsequently metabolized into three different vasoactive molecules: epoxyeicosatrienoic acid (EET) via cytochrome P450 (CYP) epoxygenase, 20-Hydroxyeicosatetraenoic acid (20-HETE) via CYP4A, and finally, PGE₂ via COX-1. These vasoactive messengers act indirectly by modulating the production of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) or directly on K^+ channels, through activation of messenger specific ion channels or receptors located on the cell membrane of vascular smooth muscle (VSM) cells or pericytes. Finally, the activation of astrocytes triggers the release of K^+ ions. K^+ act on inwards rectifying K-channels (K_{IR}) located on pericytes, inducing relaxation. Together, all of these different vasoactive messengers act on the vasculature, evoking

changes in cerebral blood flow (CBF). Neuronally-derived messengers act primarily on the arterioles, while astrocytic-derived messengers exert their effect on both arterioles and capillaries. Somatostatin receptor (SSTR); Neuropeptide receptor Y1 (NPY1R); VIP/PACAP receptor type 1 (VPAC1); large-conductance calcium-activated K⁺ channels (BK_{Ca}); Prostaglandin E2 receptor (EP).

studies (n=11) [85]. NO acts by increasing the synthesis of cyclic guanosine monophosphate (cGMP) in VSM cells [85].

GABAergic interneurons also express different neuropeptides which exert vasoactive effects both *in vitro* and *in vivo* [23, 77, 81, 86]. These neuropeptides can induce both vasodilation through vasointestinal peptide (VIP) [23, 81, 87], or vasoconstriction through both neuropeptide Y (NPY) [81, 88–90] or somatostatin (SOM) [81, 91, 92]. VIP acts on the VIP receptor type 1 which up-regulates the production of cyclic adenosine monophosphate (cAMP) in VSM cells [23]. NPY binds to the NPY receptor Y1 (NPY1R), a G_{αi}-protein coupled receptor which is expressed on the membrane of VSM cells [93, 94]. Activation of NPY1R inhibits the synthesis of cAMP, causing vessel constriction [95]. Finally, SOM acts possibly on somatostatin receptors (SSTR) to induce vasoconstriction [81].

To unravel the contributions of excitatory and GABAergic neurons to the evoked hemodynamic responses, optogenetic (see Section 3.3 for a brief overview) stimulation and/or different pharmacological manipulations can be used. One such study showed that the ratio between increased metabolic demand and the blood flow/volume change differs between selective activation of pyramidal neurons and GABAergic interneurons [96]. Pyramidal neurons have a much larger ratio, implying a larger metabolic cost associated with activation. In contrast, activation of GABAergic interneurons carries a low metabolic cost, and evokes a larger flow and volume increase as compared to pyramidal neurons [96].

Astrocytes

Astrocytes have end-feet ideally placed around smooth muscle cells on arterioles or pericytes, making them a potential mediator of the NVC (Fig. 3.2, green rectangle). Furthermore, astrocytes are known to regulate the basal tone of arteries [97–100]. The release of neurotransmitters such as glutamate and GABA can influence astrocytes directly. Glutamate binds to metabotropic glutamate receptors mGluR₁ and mGluR₅, resulting in an inositol-1,4,5-trisphosphate receptor (IP3R) mediated release of Ca²⁺ from internal storage pools (see [101] and references therein). The rise in intracellular Ca²⁺ levels in astrocytes activates phospholipase D2, which cleaves membrane phospholipids into AA [80, 102]. AA is metabolized into different vasoactive substances such as PGE₂ via COX-1, epoxyeicosatrienoic

acids (EETs) via cytochrome P450 (CYP), or 20-hydroxyeicosatetraenoic acid (20-HETE) via CYP4A (see [26] and references therein). EETs act on large-conductance calcium activated K^+ channels (BK_{Ca}), inducing relaxation through hyper-polarization. [24, 102–106]. In contrast, 20-HETE acts to contract VSM cells by inhibiting BK_{Ca} channels [107, 108]. Nevertheless, despite these signaling pathways, the astrocytes' role in evoking hemodynamic responses as a naturally occurring phenomenon is unclear, as adult human and mouse astrocytes lack expression of $mGluR_1$ and $mGluR_5$ [109]. In addition, mice that lack the expression of IP_3R through genetic knock-out still respond robustly to stimulation *in vivo*, indicating that astrocytes have little contribution to evoked hemodynamic changes through this pathway [110]. Nevertheless, a recently discovered mechanism describes how ATP that is released from post-synaptic neurons triggers an influx of Ca^{2+} ions into astrocytes through the ATP receptor $P2X_1$, provoking hemodynamic responses both *in vitro* and *in vivo* [80] (Fig. 3.2).

Finally, another vasoactive pathway involves inward-rectifier K_{IR} channels located on pericytes and endothelial cells which surround capillaries [111]. K_{IR} open upon extracellular increases of K^+ as a result of neural activity [111]. The opening of these channels induces hyper-polarization, promoting relaxation of pericytes and vasodilation of capillaries (Fig. 3.2).

In summary, many different vasoactive pathways exist that evoke both vasodilation or vasoconstriction *in vitro* and *in vivo*, and these pathways originate from multiple different neural cell types. The vasoactive messengers act primarily on the arterioles or arteries, but some have been shown to directly affect capillaries (such as in [80] or [111]). In contrast, venules and veins act strictly in a passive role. The currently available evidence points to that inhibitory neurons are the primary driver behind the observed hemodynamic responses of the NVC [53, 92, 96, 112, 113]. Nevertheless, to study the effect of these pathways in an intact physiological system, researchers have to rely on different *in vivo* measuring techniques.

3.3 *In vivo* measurement techniques for capturing NVC responses

The investigation of brain function and the NVC is reliant on the availability of experimental techniques capable of observing and measuring the ongoing biological and physiological processes in the brain. This section aims to provide a brief overview of some of the imaging modalities that have generated the experimental data used in this thesis. Nevertheless, this section is not a complete list of all the available experimental techniques.

The capture of hemodynamic responses using magnetic resonance imaging

This section provides a brief introduction to Magnetic Resonance Imaging (MRI) in the context of NVC research. For an extended description of the principles of MRI, I refer the reader to [114–116].

The basis of functional magnetic resonance imaging

MRI revolves around the phenomenon of nuclear magnetic resonance, where the use of a strong magnetic field captures information regarding the nuclear magnetic spin. The MRI signal arises from the nuclei of hydrogen atoms (H^+), which exhibit a magnetic moment (spin). If the hydrogen nuclei are affected by an external magnetic field (B_0), the spins will tend to be aligned with the direction of the field, attaining a lower energy conformation. The nuclei will precess around the direction of the applied magnetic field, resulting in a net magnetic vector in this direction. The frequency of this precession as well as the quota of nuclei in the lower energy conformation (*i.e.*, the size of the magnetic vector) is proportional to the strength of B_0 . Applying a radio frequency (RF) pulse at the same frequency as the precession frequency (the Larmor frequency), a proportion of the nuclei will shift to the high energy conformation, tilting the mean magnetic vector slightly such that it will start to precess around the applied magnetic field vector. The tilted magnetic vector will gradually return to its equilibrium, aligning once more with the direction of the applied field through the process of relaxation, emitting electromagnetic signals that generate current that can be measured in the receiver coils. This process of transmitting RF pulses and measuring the acquired current or signal in the coils is programmed in an MR pulse sequence (a series of RF pulses). Relaxation is measured as the time it takes to regain 63% of the equilibrium magnetization after an RF pulse. This is commonly referred to as the R_1 relaxation rate, which is a tissue-specific property. After excitation by an RF pulse, the nuclei will spin in phase coherence, which results in a maximal transverse magnetization. This transverse magnetization will decay due to spin-spin interactions or inhomogeneities in the magnetic field. The rate of the decay is referred to as the R_2 relaxation rate if no inhomogeneities are present, and R_2^* with inhomogeneities in the magnetic field.

A key revelation was found by Pauling and Coryell in 1936 [119]. They observed that hemoglobin exhibits different magnetic properties in the oxygenated state compared to the deoxygenated, with deoxyhemoglobin being *paramagnetic*, introducing local inhomogeneities in the magnetic field. Almost 50 years later, Thulborn *et al.* [120] showed that this oxygenation-dependent distortion of local magnetic susceptibility could be observed in blood using MRI (as it influences R_2^*). Finally, Ogawa *et al.* [28] extended this technique to be capable of producing functional images of the brain

Figure 3.3: **fMRI: from image to statistical maps.** **A:** R_2^* weighted images, which evolves over time. **B:** Plotting the signal from one cortical region over time yields a time series which might vary with a stimulation ('off' and 'on'). **C:** Statistically significant differences in the time series between 'off' and 'on' are conventionally shown overlaid on an anatomical image. R_2^* image adapted from [117], with permission. Middle time course adapted from [118], with permission.

in vivo, with the contrast of the image being dependent on blood oxygenation (Fig. 3.3, A). This study introduced the term BOLD-fMRI imaging, and spawned the research field of fMRI. Shortly thereafter, multiple research groups published findings illustrating the use of BOLD-fMRI imaging to map human brain function [29, 118, 121–124].

BOLD-fMRI is heavily dependent on the NVC, which enhances the internal contrast (blood oxygenation) upon increases in neuronal activity. Given a stimulus, such as a visual stimulation, spatially localized increases of CBF, CBV, and CMRO_2 occurs (with $\Delta\text{CBF} > \Delta\text{CMRO}_2$ as discussed in Section 2.2) in some cortical regions. These increases can be seen in the archetypal BOLD response to a brief stimulation (depicted in Fig. 2.6A), and it consists of three distinct characteristics: a debated initial dip (see [125, 126]), an overshoot of increased oxygenation, and finally, a post-peak undershoot. These characteristics constitute the qualitative behavior, which is separate from the quantitative (*i.e.*, measured values) nature of the response. This overshoot of blood oxygenation is reliably captured, increasing the contrast of the captured BOLD image between 'off' and 'on' conditions (Fig. 3.3, B). Correlating the captured experimental signals with the archetypal response patterns renders a statistical map of the cortical regions, which were selectively activated by the stimulus (Fig. 3.3, C).

Since those first reports, BOLD-fMRI has become one of the most widely used non-invasive techniques for investigating brain function in both research and clinical practice. This is due to BOLD-fMRI being non-invasive, its use of an internal contrast agent instead of an injected contrast agent, and

producing a relatively good spatial and temporal resolution. Despite the wide use, there are a number of important limitations to be considered with BOLD-fMRI. For instance, the BOLD signal is a weighted sum given by changes in CBF, CBV, and CMRO₂, which vary simultaneously in the cortical regions. Therefore, it is hard to propagate changes in the BOLD signal to the underlying neural physiology or the neuronal circuitry in the cortical regions. Nevertheless, different methods have been developed to quantify CMRO₂ changes from BOLD-fMRI measurements, often denoted ‘calibrated fMRI’ [61, 127]. However, these methods rely on theoretical frameworks based on a number of assumptions and limitations (see [128] and references therein).

Measuring CBF and CBV using fMRI

MRI can also be used to non-invasively measure CBF and CBV. For instance, CBF can be measured using arterial spin labeling techniques, which utilize blood water as a ‘tracer’. This is done in a series of steps. First, blood in the neck arteries is magnetically labeled. This labeled portion of blood is allowed to flow into the region the interest, generating a ‘tagged’ image of the region, as it includes signal from both the tagged blood and the static brain tissue in the region. Subsequently, a control image of the region is acquired without any magnetically labeled blood, so that the image only contains signal originating from the static brain tissue. The contribution from blood can now be calculated by subtracting the control image from the tagged image, producing a perfusion-weighted image. Nevertheless, the specific details of these steps vary with different implementations [129–131]. Data generated using this technique can be found in Paper IV.

Changes in CBV can be assessed using Vascular-Space-Occupancy (VASO) fMRI (see review in [132] and references therein). The VASO sequence builds on the fact that R_1 relaxation rate differs between tissue and blood. This can be exploited by applying an inversion pulse before the signal acquisition, which essentially nulls the contribution of blood to the acquired signal, but allows for a signal originating from tissue to be detected. The relative changes in the tissue-weighted signal are then associated with changes in CBV. To illustrate this, consider that the fractional composition of the voxel is 0.95 tissue and 0.05 blood. If CBV increases by 20%, the new fractions become 0.94 and 0.06 for tissue and blood, respectively, implying a $\approx 1\%$ decrease in the captured tissue signal. Thus, the signal is proportional to $1 - \text{CBV}$ [132, 133]. The conventional method is sensitive to the total CBV change, but subsequent improvements to the method can obtain volume changes on the level of arterial and venous compartments (see [134] for derivation). Data generated using this technique can be found in Paper IV.

Optical imaging-based modalities

Optical imaging is conventionally employed to observe and measure a broad range of different modalities pertaining to the NVC in rodents *in vivo* [135]. Two-photon laser scanning microscopy is one prominent technique. This technique can be used to measure the velocity of red blood cells in blood vessels, or to directly measure changes in vessel diameter with excellent spatial, temporal, and depth resolution (see [88, 136, 137] for examples of application). Nevertheless, only a small number of vessels can be scanned at once. Experimental data consisting of vessel diameter changes are used in paper III & IV.

As with MRI, hemoglobin exhibits different properties with and without bound oxygen which can be detected using optical imaging, often denoted optical imaging of 'intrinsic signals' [135, 138]. More specifically, the absorption of light changes depending on if hemoglobin is bound to oxygen or not. This technique allows one to observe changes in oxy- and deoxygenated hemoglobin concentration. Furthermore, the sum of these changes reflects the change in total hemoglobin present in the measurement site, which can act as a proxy for changes in blood volume in the measured area. Experimental data capturing hemoglobin changes is used in paper IV.

One limitation with both of these optical techniques is that these techniques are affected by wavelength scattering in the tissue, limiting the experimental resolution (see reviews in [135, 139, 140]). Light in the visible spectrum is often used for data acquisition in both of techniques, which is strongly scattered by the bone of the skull. Therefore, these experiments are only performed in animals which have had a cranial window inserted into the skull, or with a genetically manipulated thin skull bone [135].

Non-invasive measurements of hemoglobin concentrations in humans can be performed using NIRS [141, 142]. This is due to weak absorption of near-infrared light by hemoglobin [135] and a small scattering in tissue and skull [143]. Nevertheless, NIRS suffers from low spatial resolution and low depth penetration, only capturing hemoglobin changes at the surface of the cortex. While fMRI has a higher spatial resolution and captures the whole brain at once, NIRS is a portable instrument with low noise during data acquisition. For all these reasons, NIRS is commonly used in a more clinical setting, for instance, in studies on infants or conversation [144–146].

Optogenetic stimulation

Optogenetics (OG) is an experimental technique that allows for a 'switch-like' control of neurons through the insertion of light-sensitive ion channels into the plasma membrane [147, 148]. The ion channel, *Channelrhodopsin*-

2 (ChR2) [149], opens when blue light illuminates the channel but does not respond to any other stimulation (*e.g.*, different wavelength, electrical stimulation). Thus, the neuron can be excited through the use of light pulses [150]. Typically, the channel is selectively expressed in a targeted subset of neurons, through the use of viral transduction methods (such as in [88, 92, 96]). This enables the selective activation of specific neurons, allowing for investigations of neuron-specific contributions to the evoked vascular response [112]. Data generated using OG perturbations are used in Paper III & IV.

Figure 3.4: **Simple introduction to optogenetics.** **A:** A conventional electrical stimulation will evoke a response from all neuronal cells within the cortical area. **B:** Illuminating a cortical area which lacks ChR2 expression with blue light will not evoke a response. **C:** Blue light opens the ChR2 channel, evoking a neuronal response in those neurons that express ChR2. Image taken from [150], with permission.

3.4 Pharmacological modulations of the NVC

To study the NVC and its pertaining aspects *in vivo*, scientists often employ the use of animal studies, and most of our current understanding regarding the inner workings of the brain stem from this practice. Nevertheless, one major issue (besides from translation from animal to human) is the *non-voluntary task conundrum* (see [151] and references therein). In contrast to voluntary humans, animals lack interest in participating in experimental studies. This issue can be circumvented through the use of head fixation, body restraint, habituation, behavioral training (especially to the noise of the MR gradients), and lastly, the use of anesthetics. The commonly used anesthetics within NVC research exert a broad range of effects on neurons and other neural cells, but can alleviate potential suffering due to the experimental setup. Nevertheless, these effects include: changes in neuronal excitability, vascular reactivity, cerebral metabolism, and other physiological processes (see reviews in [152, 153] and references therein). Anesthetics typically exert their mechanism of action directly on ion channels, for instance on the GABA $_{\alpha}$ receptor, the NMDA receptor, or on different types of K $^{+}$ channels (see review

in [154, 155]). However, how the action of different anesthetics propagate from altered ion-channel function to the conventionally measured NVC observables such as electrode measurements, CBF, CMRO₂, and blood oxygenation remain to a large extent unknown, due to the fact that few studies include both an awake and an anesthesia cohort.

Figure 3.5: Two examples illustrating the effects of anesthetics on observed hemodynamic responses. Comparison of dilation of arterioles/arteries (A, B) and veins (A) captured in the somatosensory cortex for awake and anesthetized mice from two studies: Drew *et al.*, (A) [137] and Uhlirova *et al.*, (B) [88]. **A:** Sensory stimulation consisting of air puffs aimed at the vibrissae with and without the administration of urethane. The administration of urethane decreases arterial dilation and removes the bi-modal response. The venous dilation is completely abolished. Image taken from [153], with permission. **B:** Brief optogenetic stimulation of GABAergic interneurons with and without administration of the anesthetic α -chloralose. A prolonged post-peak undershoot in the arteriolar diameter is observed during anesthesia. Image adapted from Paper III, with permission. Experimental data originally published in [88].

Nevertheless, a few studies exist [88, 137, 156–158]. The overall picture from these studies suggests that while anesthesia reduces neural activity, the hemodynamic measures (CBF, CBV, blood oxygenation) are suppressed to a greater extent relative to neural activity [153]. Furthermore, the quantitative relationship between neural response and hemodynamic response is highly dependent on the type and dosage of the anesthetic used [159–162]. For instance, Pisauro *et al.*, measured the spiking of neurons using multi-unit activity and CBV responses in awake and anesthetized mice [158]. For the awake condition, the spiking rates were ($\approx 20\%$) higher relative to anesthesia, but the CBV response was increased by $\approx 110\%$, relative to anesthesia. A similar disproportionate change in hemodynamic contra neural response has been observed when instead capturing local field potentials (LFP) in mice [156, 157]. Another study, Drew *et al.*, reports arteriolar and venular diameter responses during both awake and urethane conditions [137]. The use of urethane significantly reduces the arterial dilation, removing a bi-modal peak

response observed during awake condition and abolishes the venous dilation (Fig. 3.5A). In addition, Uhlirova *et al.*, reports a prolonged post-peak undershoot in arteriolar diameter during anesthesia, using an optogenetic stimulation of GABAergic interneurons [88] (Fig. 3.5B). Finally, anesthesia affects the timing of the hemodynamic responses across all species, causing a delay in the hemodynamic response as compared to awake [156, 158, 163].

The anesthetic used to capture the experimental data used in paper III (see [88] for original data publication), α -chloralose, is commonly used in rodent based research on the NVC as it preserves a robust hemodynamic response to sensory stimulation [164, 165]. It is known that α -chloralose potentiates the Cl^- ion conductance through the GABA_α channel by increasing the binding affinity of GABA [166]. Other studies on the physiological effects of α -chloralose suggests reduced baseline CBF values [152], increased blood pH, and decreased heart rate [167]. A recent study showed that VSM lacks the expression of the GABA_α receptor-channel complex [168], which suggests that α -chloralose does not directly act on the vasculature.

Together, these studies show that anesthetics significantly alter the hemodynamic responses commonly observed in the NVC, with the alterations being both dependent on the anesthetic used, the type of stimulation used, as well as the dosage of the anesthetic. For all these reasons, the use of anesthetics within the NVC research is an ongoing debate, and more studies of NVC related observables in rodents that are performed without anesthesia are emerging.

Mathematical Modeling in Systems Biology

Mathematical modeling is at the core of the research field Systems Biology. In Systems Biology, mathematical models are used to provide a quantitative understanding of complicated biological processes [36], and to unravel or provide insights of properties that cannot be assessed or measured directly using experimental techniques [169]. The use of mathematical models is widespread across many different organs and diseases. The size and scope of the models range from mechanistic models, describing the dynamics of one or two proteins, to genome-wide models within bioinformatics. A common application within mechanistic modeling is *hypothesis-based testing*, where hypotheses describing the mechanisms which govern the biological system are evaluated and compared [170, 171], which in some cases leads to the rejection of a hypothesis [171]. Models within bioinformatics are typically based on omics data, where the structural understanding is often fuzzier, making the approach and conclusions more data-driven. Through this broad spectrum of use, mathematical models have emerged as powerful tools for developing model-based treatment strategies for various diseases or as a platform for model-based decision making [172].

Different types of mathematical models in Systems Biology exist, such as deterministic models which feature no randomness [173], or stochastic models which are affected by random processes [174]. The choice of model type depends on the question at hand, as well on the biological system with its pertaining measurement observables under consideration. For studies that analyze the average behavior of a population over time, such as in this thesis, models based on deterministic differential-algebraic equations (DAEs) and ordinary differential equations (ODEs) are often employed. The main focus

of this chapter is to describe mathematical modeling in the context of differential equations. Nevertheless, some of the methods are relevant for other types of modeling frameworks, such as the iterative cycle of mathematical modeling.

4.1 Iterative cycle of mathematical modeling

As touched upon in Section 2.3, mathematical modeling of mechanisms rests on two core pillars: 1) model-based analysis of experimental data and 2) the use model-derived insights or predictions to validate the model by new experimental designs. These pillars form the basis of the iterative cycle of mathematical modeling, illustrated in Figure 4.1. This *modeling cycle* constitutes the practical framework for model construction, model-based hypothesis testing and data analysis, and finally, model predictions and model-derived insights or decisions. This framework is valid on two scales: on a small scale, concerning the individual modeler who performs the analyses, and on a larger group scale, suggesting how to organize an iterative collaboration between experimentalists who gather data and modelers who make the models [39].

Throughout this chapter, we will carefully examine some important aspects of the individual key steps in the modeling cycle (Fig. 4.1). I have chosen to focus on seven topics: model simulation, likelihood functions, parameter estimation, assessing goodness of fit, identifiability of parameters, and finally, the use of validation data for model selection.

4.2 Ordinary and algebraic differential equations

Throughout this thesis, all models are formulated using ODEs (Paper I-III) or DAEs (Paper IV). The general notation of an ODE model is as follows:

$$\dot{x} = f(x, p, u, t) \quad (4.1a)$$

$$x(t_0) = x_0(p) \quad (4.1b)$$

$$\hat{y}(t, p) = g(x(t, p, u), p) \quad (4.1c)$$

in which x are the state variables, describing the concentration or amount of an entity with respect to time; where $\dot{x}$ represent the derivative of the states with respect to time; where f and g are non-linear smooth functions; where p is the vector of unknown time-independent parameters; where u is the input signal associated with the experimental data, if applicable; where $x(t_0)$ are the initial values of the states at $t = t_0$, which are dependent on the parameters $x_0(p)$; and finally, where $\hat{y}$ are the model outputs corresponding to the measurement observables. Note that x , u , and y depend on time (t).

Figure 4.1: **The iterative modeling cycle used in this thesis.** (1) Combine experimental data captured from the biological system at hand with knowledge and hypotheses regarding the system, and construct a mathematical model. (2) Perform model-based hypothesis testing by fitting the model to the experimental data. This has two outcomes: a) if the model cannot describe the data, then reject the model; or b) if the model can describe data, it is passed on to the next step. If a), go back to the model construction and alter the model or hypothesis. If b), proceed to (3), where identifiable model predictions with low uncertainty are generated through model analysis. These predictions are favorably ones that can be experimentally validated or be used to design new experimental studies. (4) Validate the model against data of the model predictions or any other independent data that can be used for validation. If the model predictions do not overlap with validation data, start over at (1). Conversely, if the predictions match the validation data, the hypothesis is tentatively accepted. This loop can be iterated many times, each time refining the model further and expanding the mechanistic understanding of the system.

A DAE is the generalized case of an ODE, where the derivatives of some states x are unspecified, denoted z . z is instead solved for every time point through the use of algebraic relationships with x and t . These systems usually arise in *e.g.* electrical circuits. For further details of DAEs, I refer the reader to [175]. The general notation of a DAE is given by:

$$F(\dot{x}, x, z, p, u, t) = 0 \quad (4.2a)$$

$$F(\dot{x}(t_0, p), x(t_0, p), z(t_0, p), t_0) = 0 \quad (4.2b)$$

$$\hat{y} = g(x, z, p, u, t) \quad (4.2c)$$

where z represents the states for which the derivative function is unknown and F corresponds to the non-linear function of the system.

4.3 Model simulation

The lion's share of ODE models is solved ('simulated') numerically by the use of different step-based methods. These numerical methods are often divided

into two categories: one-step and multi-step solvers. One-step solvers, such as the well-known Euler step [176] (developed by the mathematician Leonhard Euler in the late 1760s), only require information about the system from the preceding step to calculate the next step. In contrast, multi-step solvers make use of multiple sequential points, which are weighted together to calculate the next step. Some methods are explicit, meaning that the value of the system at the next step is a function of the value at the present or previous steps. Other methods are implicit, such as backward differentiation formulas. An implicit solver calculates the solution for the next step by solving equations containing both the current and future steps of the system. These implicit methods are typically used if the ODE system is *stiff* and most conventional ODE solver packages, such as the SUNDIALS package [177], employ these implicit solvers. To practically illustrate a simulation, I will go through the classical explicit, multi-step method for ODE solving: the 4th order Runge-Kutta (RK4) approximation. RK4 was developed by the mathematician's Carl Runge and Martin Wilhelm Kutta during the late 1800s [178, 179]. First, consider the following initial value problem:

$$\dot{x} = f(t, x), \quad x(t_0) = x_0. \quad (4.3)$$

where $\dot{x}$ is the derivative of the variable x as given by the function f (which is dependent on time t , and x) and the initial value of x , $x(t_0)$, is given by x_0 . The variable x can be calculated using RK4 by:

$$x_{n+1} = x_n + \frac{h}{6} (k_1 + 2k_2 + 2k_3 + k_4) \quad (4.4a)$$

$$t_{n+1} = t_n + h \quad (4.4b)$$

where x_{n+1} is the RK4 approximation of $x(t_{n+1})$, which is determined by the present value (x_n) and an estimated slope, given by the weighted combined sum of k_i , $i \in 1, \dots, 4$, multiplied by the size of the temporal step (h). Explicitly, the slope k_i is given by:

$$k_1 = f(t_n, x_n) \quad (4.5a)$$

$$k_2 = f\left(t_n + \frac{h}{2}, x_n + h \frac{k_1}{2}\right) \quad (4.5b)$$

$$k_3 = f\left(t_n + \frac{h}{2}, x_n + h \frac{k_2}{2}\right) \quad (4.5c)$$

$$k_4 = f(t_n + h, x_n + h k_3) \quad (4.5d)$$

To simplify the meaning of k_i , a geometric representation is presented in Figure 4.2. Using this figure and Equation 4.5, one can observe that k_1 is based on the derivative of the starting point t_n , which is simply an Euler's step. k_2, k_3 are the derivatives at the mid-point of the step interval ($t_n + \frac{h}{2}$). Finally, k_4 is the derivative at the end of the step interval ($t_n + h$).

One important consideration with a fixed step method is that the choice of h is non-trivial: 1) selecting a too large h , the approximated derivatives

Figure 4.2: **Geometric representation of the 4th order Runge-Kutta formula.** k_1 represents the slope at (t_n, y_n) , calculated using Euler's step. k_2, k_3 are the derivatives at the mid-point of the step interval $(t_n + \frac{h}{2})$. Finally, k_4 is the derivative at the end of the step interval $(t_n + h)$.

are no longer a sufficient approximation of the true system; 2) if one instead selects a too small step size, numerical errors will accumulate, and as with 1), will result in an erroneous approximation of the true system. Modern ODE solvers negate this sensitivity to the choice of step size h by using an adaptive step size (see Fig. 4.3).

Figure 4.3: **Comparison between RK4 and a modern ODE solver within the SUNDIALS package.** Too large step size results in an erroneous approximation of the true system.

Following the typical modeling cycle outlined in Figure 4.1: once a model structure has been formulated, the parameters p which produce a model simulation that as closely as possible replicates observed experimental data, need to be determined. This is done by minimizing the disagreement between experimental data and model simulations. This disagreement is typically evaluated using the likelihood function.

4.4 Likelihood functions

Assuming independent, normally distributed additive measurement noise, the likelihood of observing data y given p is:

$$L(y|p) = \prod_{e=1}^{n_e} \prod_{o=1}^{n_o^e} \prod_{d=1}^{n_d^{e,o}} \frac{1}{\sqrt{2\pi}\sigma_d^{e,o}} \exp\left(-\frac{1}{2}\left(\frac{y_d^{e,o} - \hat{y}_d^{e,o}(p)}{\sigma_d^{e,o}}\right)^2\right) \quad (4.6)$$

where n_e is the number of experiments e ; where n_o^e is the number of observables o per e ; where $n_d^{e,o}$ is the number of data points d per o and e ; where $\sigma_d^{e,o}$ is the uncertainty of the data point, typically standard error of the mean (SEM); where $y_d^{e,o}$ is the measured data point; where $\hat{y}(p)$ is the corresponding simulated data point. By maximizing L , the maximum likelihood estimate of the unknown parameters p is found. In practice, it is more numerically efficient to minimize the equivalent negative log-likelihood function:

$$J(p) = -\log(L(y|p)) = \frac{1}{2} \sum_{e=1}^{n_e} \sum_{o=1}^{n_o^e} \sum_{d=1}^{n_d^{e,o}} \left[\log(2\pi(\sigma_d^{e,o})^2) + \left(\frac{y_d^{e,o} - \hat{y}_d^{e,o}(p)}{\sigma_d^{e,o}}\right)^2 \right] \quad (4.7)$$

If the measurement uncertainty $\sigma_d^{e,o}$ is known, $J(p)$ share the same optimal parameters with the measurement uncertainty weighted sum of squares residual (SSR) J_{ssr} :

$$J_{ssr}(p) = \sum_{e=1}^{n_e} \sum_{o=1}^{n_o^e} \sum_{d=1}^{n_d^{e,o}} \left(\frac{y_d^{e,o} - \hat{y}_d^{e,o}(p)}{\sigma_d^{e,o}} \right)^2 \quad (4.8)$$

The summation in Eq. 4.7 and Eq. 4.8 of the squared and normalized residuals corresponds to calculating the vertical distance between the simulated values $\hat{y}_d^{e,o}(p)$ and experimentally measured data $y_d^{e,o}$.

As a side note, one can easily re-arrange Eq. 4.7 to give the relationship between $J(p)$ and J_{ssr} as:

$$J_{ssr}(p) = 2J(p) - \sum_{e=1}^{n_e} \sum_{o=1}^{n_o^e} \sum_{d=1}^{n_d^{e,o}} \log(2\pi(\sigma_d^{e,o})^2) \quad (4.9)$$

Local optimization algorithms (see Section 4.5) utilize the gradient of the chosen likelihood function, with respect to the parameters $p_k, k = 1, \dots, n_p$

(n_p equals the number of parameters), in order to improve convergence to a local/global minima. Differentiation of $J(p)$ with respect to the parameters p gives:

$$\frac{dJ}{dp_k} = \sum_{e=1}^{n_e} \sum_{o=1}^{n_o^e} \sum_{s=1}^{n_d^{e,o}} \frac{1}{\sigma_d^{e,o}} \left(\frac{1}{2} - \left(\frac{y_d^{e,o} - \widehat{y}_d^{e,o}(p)}{\sigma_d^{e,o}} \right)^2 \right) \frac{d\sigma_d^{e,o}}{dp_k} \Big|_{(p)} - \frac{y_d^{e,o} - \widehat{y}_d^{e,o}(p)}{(\sigma_d^{e,o})^2} s_{o,k}^{y,e}(d) \quad (4.10)$$

Where $s_{o,k}^{y,e}(d)$ denotes the sensitivity of the output $\widehat{y}_d^{e,o}(p)$ at time point d with respect to parameter p_k . To note: if the experimental data uncertainty is parameter independent, i.e., $\frac{d\sigma_d^{e,o}}{dp_k} = 0$, the first term of the expression is eliminated. To calculate the likelihood gradient, several method exists, such as finite differences and forward or adjoint sensitivity analysis.

Finite differences

Finite differences gives an approximation of the objective function gradient with respect to p_k and is given by:

$$\frac{dJ}{dp_k} \approx \frac{J(p + ae_k) - J(p - be_k)}{a + b} \quad (4.11)$$

where $a, b \geq 0$ and e_k is the k th element in an unit vector of the parameters. In practice, three approaches to calculating this approximation are used: forward ($a = \epsilon, b = 0$), backward ($a = 0, b = \epsilon$) or central ($a = \epsilon, b = \epsilon$) differences, where ϵ is the parameter perturbation factor. Such a calculation is performed in three steps (see [180]):

- 1:** The model is numerically simulated, giving the state $x(t, p)$ and observable output $\widehat{y}(t, p)$ trajectories.
- 2:** The model is repeatedly re-simulated, each time perturbing one element of the parameter vector $p^{(k)} = p + \epsilon e_k$ for $k = 1, \dots, n_k$, resulting in new state and output trajectories for each perturbation ($x(t, p^{(k)})$ and $\widehat{y}(t, p^{(k)})$).
- 3:** The gradient $\frac{dJ}{dp_k}$ is calculated from the output $\widehat{y}(t, p)$ and the perturbed output $y(t, p^{(k)})$ using Eq. 4.11.

One can note that the method of forward and backward differences requires $n_k + 1$ function evaluations (simulations), while central differences requires $2n_k$ function evaluations. This makes the calculation of the gradient time consuming for large models.

Forward and adjoint sensitivity analysis

A method common within physics and classical engineering is to numerically solve for the sensitivity of the observables $s_{o,k}^{\widehat{y}}(d)$ during the simulation. This

can be done by differentiating Equation 4.1, giving the following expressions:

$$\begin{aligned} \dot{s}_k^x &= \frac{df}{dx} s_k^x + \frac{df}{dp_k}, & s_k^x(t_0) &= \frac{dx_0}{dp_k} \\ s_k^{\widehat{y}} &= \frac{dg}{dx} s_k^x + \frac{dg}{dp_k} \end{aligned} \quad (4.12)$$

where s_k^x is the sensitivity of state x with respect to p_k . This analysis is also performed in three steps (see [180]):

1: As previously, trajectories of the states $x(t, p)$ and observables $\widehat{y}(t, p)$ are calculated through simulation.

2: Using the state trajectory $x(t, p)$, the state sensitivities s_k^x and output sensitivities $s_k^{\widehat{y}}$ can be calculated for all parameters $k = 1, \dots, n_p$.

3: Finally, the objective function gradient $\frac{dJ}{dp_k}$ can then be calculated from $\widehat{y}(t, p)$ and $s_k^{\widehat{y}}$ using Equation 4.10.

One limitation with using forward sensitivity analysis is that calculating $\frac{dJ}{dp_k}$ using Eq. 4.10 requires the solution of Eq. 4.12. This is not an issue for small models with a few parameters or states, but it becomes computationally cumbersome for large models with thousands of parameters or states. However, it is possible to derive an expression of the gradient of the likelihood function that does not depend on the state sensitivities s_k^x , circumventing this issue. In short, one can introduce an adjoint state which is solved via a backward (meaning the state evolves backwards in time) differential equation (see [180] for detailed derivation). The key difference between adjoint and forward sensitivity analysis is that the state and output sensitivities (s_k^x , $s_k^{\widehat{y}}$) are not explicitly calculated. Instead, $\frac{dJ}{dp_k}$ is directly calculated by solving n_p one-dimensional integration problems over short time intervals [180]. In practice, this means that computational time required to calculate the likelihood function gradient using adjoint sensitivity analysis is only weakly dependent on the number of parameters in the model [180].

Figure 4.4 visualizes the comparison between these three methods: finite differences (blue line), forward sensitivity (orange line), and adjoint sensitivity (yellow line). As seen, for a small number of parameters (x-axis), the three different methods share a similar computational time (y-axis). However, for models with increasingly more parameters, forward and most prominently adjoint sensitivity analysis has a much shorter computational time, as compared to finite differences.

Forward and adjoint sensitivity analysis has been implemented in the 'Advanced Matlab Interface for CVODES and IDAS' toolbox [180, 181], which is

extensively employed throughout Paper III and IV for model simulations and gradient calculations.

Figure 4.4: **The computational time of likelihood gradient calculation with respect to the number of parameters using three different methods.** The computational time for finite differences and forward sensitivity analysis increases linearly with respect to number of parameters, while adjoint sensitivity analysis is only weakly linearly dependent on the number of parameters. Picture taken from [180], with permission.

4.5 Optimization — minimization of the likelihood function

With a suitable likelihood function chosen in the previous section, the next logical step is to locate the global minima in the typically multi-dimensional likelihood landscape. In other words, finding the parameters $\hat{p}$ which minimizes the negative log-likelihood function $J(p)$ (Eq. 4.7) or the SSR function $J_{ssr}(p)$ (Eq. 4.8). This is commonly denoted as:

$$\hat{p} = \arg \min J(p) \quad (4.13)$$

To search for the global minimum in the likelihood landscape (see Fig. 4.5 for a simple one parameter case), you typically employ one or several different available optimization algorithms or techniques. Generally, optimization techniques can be divided into two categories: local optimization and global optimization.

Local optimization

Multiple local optimization algorithms exist. A typical hallmark feature of local optimizers is that these typically are *deterministic*, i.e., given a specific

Figure 4.5: **The negative log-likelihood function landscape for a simple one parameter system.** In this case, a global minimum exists, as well as two local minima. Two starting points, p_1^0 and p_2^0 , are initiated in the landscape. Generally, local optimization strategies will not search in an upwards direction, missing the global minimum if starting from p_1^0 . In contrast, using p_2^0 as a starting point, the local optimization will converge to the true (global) optima. For a global optimization strategy, the algorithm can travel in an upwards direction, and will eventually converge to the global optima. Nevertheless, choosing a global strategy comes with the trade-off of higher computational costs and varying performance, as the global techniques typically include stochastic elements.

starting point p^0 , the individual optimizer will reach the same solution every time. Generally, a local optimization strategy locates the local minima in the neighborhood of the starting point p^0 , typically guided by the derivative of the negative log-likelihood function (see Section 4.4). As such, if we look at Figure 4.5 again, we see that given the starting point p_1^0 , a local optimization strategy will only find the left local minima, not the global minima. In contrast, initiating a local search at p_2^0 will result in locating the global minima. Therefore, local optimization techniques are heavily influenced by the start guess p^0 .

Below, I will go through two common local optimization methods: the simple *gradient descent* algorithm, and the more elaborate Newton method. In *gradient descent*, the algorithm iteratively steps in the negative direction

of the chosen likelihood function gradient until it fulfills the termination criteria, such as successful convergence or number of max iterations reached.

$$p^{n+1} = p^n - h \frac{dJ(p^n)}{dp} \quad (4.14)$$

where h is the step size and $\frac{dJ}{dp}$ is the gradient of the chosen likelihood function (Eq. 4.10). Note that this h is not the same as in Eq. 4.4, as there we step in the state space of x , while here we step in the parameter space of p . *Gradient descent* converges linearly, which is slower than other methods such as Newton or quasi-Newton methods. These methods incorporate the curvature of the likelihood function to quadratically converge to extreme points. Nevertheless, these methods require the calculation of both the gradient and the Hessian H , which are the second-order derivatives of the chosen likelihood function:

$$p^{n+1} = p^n - h \frac{dJ(p^n)}{dp} \frac{1}{H(p^n)} \quad (4.15a)$$

$$\text{where: } H(p_{i,j}^n) = \frac{d^2 J(p^n)}{dp_i dp_j} \quad (4.15b)$$

In Figure 4.6, a graphical comparison of the two local optimizers discussed above are shown (*gradient descent* as the purple line, and the Newton method as the gray line). Newton method takes a more direct path to the solution point $\hat{p}$ by utilizing the gradient and Hessian information. Nevertheless, the computation of the Hessian is cumbersome and non-trivial depending on the model complexity. Therefore, quasi-Newton methods utilize approximations of the Hessian, which are less precise but has a reduced computational cost. For more detail on approximating the Hessian, I refer the reader to Section 4.4 for a summarized view on available methods for calculating these gradients and to [182] for an in-depth view.

The main drawback of local optimization strategies is that the probability of getting stuck in local minima increases for highly complex or parameterized models. Nevertheless, multi-start optimization has been suggested to at least partly remedy this drawback (see [184] and references therein). Multi-start optimization works by dividing the parameter space into even grids, placing starting points in these grids (for instance using Latin Hypercube Sampling [185]). Next, a local optimization is launched for each point. This however becomes infeasible for highly parameterized systems, as you conventionally use m^n starting points, where m is the the number of grids, and n is the number of parameters. Nevertheless, this approach has been shown to perform well even for large models when coupled with precise calculations of the likelihood gradients [186].

Figure 4.6: **The iterative solution path taken for the two compared local optimizers:** gradient descent (purple line) and Newton method (gray line). As seen, the Newton method converges through a shorter path to the minima point ($\hat{p}$) from the starting point p^0 as it exploits the curvature of the likelihood (black contour lines) for a more direct path. In contrast, gradient descent converges by iteratively stepping orthogonally to the likelihood contours. Picture modified with permission from [183].

Global optimization

Global optimization methods are designed to circumvent the major drawback with local optimization: local methods can only search downhill in the likelihood landscape. This means that these methods are unable to continue searching if stuck in local minima (see Fig. 4.5). Therefore, global optimization algorithms include a way of escaping local minima, thus being able to climb uphill in the likelihood landscape and continue to the search for favorable parameter points. This is often achieved by including a stochastic process (randomness) in the optimization algorithm, meaning a different solution can be found every time the algorithm is executed, despite using identical starting points (assuming unsuccessful global convergence). These methods are typically referred to as *metaheuristic* in the numerical optimization field [187]. In the work presented in this thesis, two global optimization methods have been extensively used: Simulated Annealing [188, 189] in Paper I & II, and a Scatter Search [190, 191] based method (see [192, 193]) in Paper III & IV. Below, I will briefly go through the core aspects of these two methods.

Simulated annealing

Simulated Annealing (SA) is a global metaheuristic algorithm introduced by Kirkpatrick *et al.* in 1983 [189]. SA mimics the basic concept of the metallurgic process of annealing, where metal is repeatedly heated and cooled, which removes imperfections in the crystalline structure of the metal, and al-

allows the atoms to adopt their lowest energy conformations [173, 189]. In SA, this process is translated to the key variable *temperature*. The temperature T controls the probability P of the algorithm to a new candidate solution of the parameters p' , as compared to the current or optimal solution p , and this probability is in [189] expressed as:

$$P(p', p, T) \begin{cases} 1 & \text{if } J(p') - J(p) < 0 \\ e^{-\frac{J(p') - J(p)}{T}} & \text{otherwise} \end{cases} \quad (4.16)$$

where $P(p', p, T)$ is the probability of accepting the move with respect to p' , p , and T . As seen, the move is always accepted if $J(p') - J(p) < 0$. Typically, SA starts with a high T , meaning that the algorithm will accept large moves (uphill) in the likelihood landscape, allowing for a global search. The temperature is decreased iteratively, refining the found candidate solutions, with a decreased probability of accepting large uphill moves. This mechanism of accepting worse solutions is paired generation of a new candidate solution (based on the current solution), which essentially choose a neighboring point. This loop is completed when the temperature reaches zero, allowing no uphill moves to be made and thus acts as a local optimizer.

Figure 4.7: **Illustration of the Simulated Annealing optimization in the likelihood landscape, as a function of temperature.** The color scheme indicates the minimal temperature required for an accepted move/jump to that region of the parameter space during optimization. As seen, a high temperature (yellow) means that the optimization algorithm can climb far uphill, traversing large portions of the parameter space in the initial phase of the optimization, locating potential local minima. With decreasing temperature (green to blue color), the search is concentrated on favorable parameter regions (low $J(p)$). The initial parameter points p_1^0 and p_2^0 acts as potential starting points for the parameter search. Figure inspired by the work of [39].

Figure 4.7 depicts a simple illustration of the SA search pattern. The algorithm is initiated in a starting point (p_1^0 or p_2^0) and with a high T (yellow). The color depicts the minimal temperature required to accept the move to that portion of the parameter space. As seen, in the initial phase of the search, the algorithm can search the whole parameter space, making this the global search phase. As the temperature is iteratively reduced, the probability of accepting an uphill move is decreased [188, 189], concentrating the search in potential local/global minima. These search patterns are combined with the generation of new candidate parameter points through an user-defined function, for instance the Nelder-Mead simplex method [184, 194].

Scatter search

Scatter Search is a metaheuristic evolutionary algorithm that was first introduced by Glover in 1977 [190, 191]. Evolutionary algorithms are based on the process of evolution observed in biology, incorporating elements such as *mutations*, *cross-breeding*, and *generations* to evolve an initial seed of parameter candidates. Specifically, Scatter Search revolves around the use of a *reference set* of parameter candidates, which is iteratively updated throughout the optimization.

Figure 4.8 illustrates the basic steps underlying the Scatter Search algorithm. Scatter Search is initialized with a set of diverse initial starting points (p^0 , gray circles), which are subsequently further improved upon using a user-defined improvement method. This forms the basis of the reference set for the first iteration (blue circles, top right). This reference set is used to generate new candidate parameter points (subset) by for instance a linear combination of two or more reference solutions (dark gray circles, bottom right). These subset candidates are further improved upon and added back to the reference set. This forms an iterative loop, which stops when the termination criteria are fulfilled, typically a max number of iterations or a user specified duration. The improvement method is typically a local optimization algorithm, making Scatter Search a *hybrid* approach (combining both global and local search phases). This algorithm has been shown to perform well on both small and large kinetic models, especially in combination with the precise calculation of the likelihood gradient [184, 186].

4.6 Assessing goodness of fit using statistical tests

In the previous section, the topic of optimization and finding the parameters $\hat{p}$ which minimize the discrepancy between observed experimental data and model simulations was covered. We now want to test if the discrepancies between model and data are small enough, *i.e.*, assess the *goodness of fit* in order to determine whether to accept or reject the model [39]. This is

Figure 4.8: **General flowchart of the Scatter Search algorithm.** (1) Generate initial starting points (light gray dots), which are subsequently optimized using the chosen improvement method. These points constitute the initial reference set (light blue dots). (2) Create a subset of candidate parameter points by a linear combination of the reference set points (dark gray dots). (3) Improve the subset points through optimization. (4) Check termination criteria. If not fulfilled: update the reference set with some of the favorable subset points and repeat from (2).

formally done by evaluating the null hypothesis, H_0 , which tests if the given model structure $M(\hat{p})$, with its associated error model for the observables, has generated the experimental data.

Throughout the included works in this thesis, the χ^2 -test has been extensively used. The χ^2 -test is a statistical test for the size of the model residuals, introduced by Karl Pearson in 1900 [195, 196]. Given normally distributed noise, the sum of squares of N independent standard normally distributed variables (denoted y_i) follows a χ^2 -distribution with degrees of freedom equal to N :

$$\sum_{i=1}^N y_i^2 \sim F_{\chi^2} \quad (4.17)$$

One can note that in the case of normally distributed noise, the residuals calculated in Eq. 4.8 follow by definition a χ^2 -distribution [171, 197], and therefore the summarized cost value of Eq. 4.8 follow the χ^2 -distribution. In this case, the test statistic of the χ^2 -test, T_{χ^2} , is equivalent to the summarized cost value, $T_{\chi^2}^M$. As such, we can now determine whether to reject the model M or not by comparing the model-based test static value $T_{\chi^2}^M$ with a cut-off value $T_{\chi^2}^{cut-off}$. This cut-off value is given by the inverse of the χ^2 cumulative density function, with significance level α and df degrees of freedom ($F_{\chi^2}^{cdf-inv}$)

[39].

$$T_{\chi^2}^{cut-off} = F_{\chi^2}^{cdf-inv}(1 - \alpha, df) \quad (4.18a)$$

$$T_{\chi^2}^M = J_{ssr}(\hat{p}) \quad (4.18b)$$

$$if \ T_{\chi^2}^M > T_{\chi^2}^{cut-off}, \text{ reject } M(\hat{p}) \quad (4.18c)$$

where $M(\hat{p})$ is the model structure M evaluated for the optimal parameters $\hat{p}$. The significance level α is often chosen beforehand, but the degrees of freedom df needs to be determined. In the case of testing the model simulations on *independent* data (see Section 4.8), df are simply the total number of validation data points. However, one often tests the model on the same set of data that it was trained on, meaning that the residuals are not independent [198]. Nevertheless, this can be compensated for in multiple ways. The most theoretically sound way is to subtract the number of *identifiable* parameters from the total number of experimental data points (n_d) :

$$df = n_d - q \quad (4.19)$$

where q is the number of identifiable parameters. A parameter p is deemed *identifiable* if one can draw a bounded range in the parameter space, in which the parameter must lie (in order to pass a χ^2 -test, Eq. 4.18). If such a bounded range does not exist, the parameter is deemed *unidentifiable*. This topic is covered in detail in Section 4.7, along with other alternative ways of compensating for the lack of independence. Nevertheless, a common approach is to not compensate at all ($df = n_d$), making model validation a crucial and essential step (see Section 4.8).

4.7 Identifiability of parameters and uncertainty analysis

Biological systems are inherently complex systems that pose difficult challenges in the measurement domain, especially *in vivo*, meaning that often few properties can be measured. Furthermore, most available observations are measured indirectly or under specific experimental conditions, limiting the applicability and quality of the observations. Together, these limitations mean that all parameters in a model might not be uniquely determined from the experimental data. Such parameters are referred to as *unidentifiable*, and can be detected by for instance using Lie algebra as presented by Sedoglavic [199]. In practice, the uncertainty of a parameter is given as an interval of values, which might be or not be limited. If the interval is not limited in any (positive or negative) direction, the parameter is commonly denoted as *structurally unidentifiable*. An intuitive example is if we would measure some quantity y^* which we postulate could be described by a relationship that is

dependent on two parameters p_1, p_2 and the variable x so that:

$$y^* = p_1 x - p_2 x \quad (4.20)$$

Using the algorithm presented in Sedoglavic [199], the parameters p_1 and p_2 would be identified as *structurally unidentifiable*. The reason for this is that the two decay terms in Eq. 4.20 are described in the exact same way, making it impossible to distinguish the two decay reactions from each other. In this case, the individual values of p_1 and p_2 are not uniquely determined, as an increase in one parameter can be fully compensated by a corresponding decrease of the other. Such a scenario can be remedied by simply introducing a new parameter $p_3 = p_1 + p_2$, which instead would be fully *identifiable*, meaning that we can identify a unique value for p_3 that satisfy the relationship. Nevertheless, given a common assumption that all parameters should be positive ($p > 0$), implying irreversible reactions, the parameters p_1, p_2 are now bounded in the negative direction, *i.e.*, a lower bound of the parameters exists. In this way, the parameters are unbounded only in the positive direction. Identifying unidentifiable and identifiable parameters (given the available data) can be important for assessing the predictive power of model predictions, or to draw model-based insights regarding the biological system at hand [171, 200]. Below, I will go through the three most common approaches for determining the identifiability of parameters.

Fisher information matrix

Historically, the oldest approach for estimating a lower and upper bound for a parameter is to use the Fisher Information Matrix (FIM) [173, 201], which is based on the Hessian or approximations thereof:

$$FIM_{a,b} = \sum_{i=1}^{n_d} \sigma_i \frac{d\hat{y}_i}{dp_a} \frac{d\hat{y}_i}{dp_b} \Big|_{\hat{p}} \quad (4.21)$$

where $a = b = 1, \dots, k$ and k is the number of parameters; n_d is the number of experimental time points; where σ_i is the variance of each experimental data point and where $\hat{y}_i$ is the model simulation of the observable at time point i using the optimal parameters $\hat{p}$. A confidence interval for each parameter can then be drawn as [202]:

$$CI(\hat{p}_i) = \hat{p}_i \mp T_t(\alpha, df) \hat{\sigma} \sqrt{FIM_{i,i}} \quad (4.22)$$

where $T_t(\alpha, df)$ is the student's t-test for confidence level α and df degrees of freedom, and where $\hat{\sigma}$ is the true variance of measurement data [173, 202].

The confidence intervals generated from the FIM rely on two key assumptions: 1) that the likelihood landscape in the neighborhood of the local/global

minima is described by an ellipse, implying that all parameters are identifiable (hence you need to assume identifiability to prove identifiability) [198, 202], and 2) that the observations depend linearly on the model parameters. For complex non-linear models, these assumptions might not be valid to make [202, 203]. Due to these limitations, other methods which do not rely on such assumptions have been developed.

Profile likelihood

Profile Likelihood (PL) determines profiles for each individual parameter separately. Using PL, the parameter p_k is traced along its numerical axis, for both positive and negative directions, allowing every other parameter pertaining to the model to change ($\tilde{p}_{j \neq k}$) for each value of p_k . This is typically expressed as:

$$\text{PL}_k(p_k) = \min_{\tilde{p}_{j \neq k}} J(\tilde{p} | \tilde{p}_k = p_k) \quad (4.23)$$

A PL analysis approximates the optimal path through the parameter space for each p_k , which describes the curvature of the likelihood function $J(p)$ on a global scale, as well as determining the feasible range of values for the parameter [200, 204]. The identifiability of the parameter p_k is determined by a threshold, specified as:

$$D := \text{PL}(p_k) - J(\hat{p}) \leq \chi_{\alpha, df}^2 \quad (4.24)$$

where $\chi_{\alpha, df}^2$ is the α quantile of the χ^2 distribution for df degrees of freedom. The choice of df is non-trivial, and is often set as: 1) $df = 1$, which is the theoretically correct value, yielding a narrow confidence interval, but does not account for the uncertainty present in experimental data; 2) $df = n_p - n_i$, where n_p is the number of parameters and n_i is the number of identifiable parameters (typically not known *a priori*), or 3) $df = n_p$, correcting for multiple-comparisons and giving simultaneous confidence intervals for all parameters. An additional common way of specifying the PL threshold is to use the $T_{\chi^2}^M$ as the absolute upper threshold, meaning that each point of the profile must remain in statistically significant agreement with the experimental data. In practice, the profile likelihoods are calculated using repeated-optimization [198, 205] or by using an integration-based approach as described in [206–208].

In Figure 4.9, three cases of identifiability are shown, calculated using a PL analysis. The top row (Fig. 4.9A, C, E) depicts the likelihood landscape contours as dependent on two parameters (p_1, p_2), while the bottom row (Fig. 4.9B, D, F) shows the trace of p_1 with the associated change in model agreement to data. The χ^2 -threshold for $df = 1$ is shown as thick solid lines in all graphs. In the left column, the trace of the profile is flat (Fig. 4.9B), thus failing to establish both an upper and lower bound of p_1 . This case for a PL

analysis of the same nature as Eq. 4.20, *i.e.*, *structural unidentifiability*. This particular issue cannot be resolved by increasing the quality of measurement data, but requires either reformulation of the model structure or new measurement observables. In the middle column (Fig. 4.9C, D), a lower bound of p_1 can be established, but not an upper bound (Fig. 4.9D). This case is commonly referred to as *practical unidentifiability*, where the trace is only limited in one direction. This case can sometimes be resolved given higher quality data, meaning less measurement noise, which would essentially move the χ^2 cut-off threshold in a downwards direction. However, the addition of higher quality data does not guarantee that the parameter will become identifiable. The final case is presented in the right column, where p_1 exhibit both an upper and lower bound (Fig. 4.9F), which means that the parameter is *identifiable* as there exist a limited range of values that the parameter must reside within for the model to agree with data.

Figure 4.9: **The three cases of parameter identifiability and the profile likelihood.** **Top row:** contour plots of the three different likelihood landscapes (solid lines) as dependent on two parameters (p_1 , p_2). The χ^2 -threshold is highlighted as thick black lines in both top and bottom row graphs. The dashed lines correspond to the profile likelihood trace. **Bottom row:** the profile likelihood trace of p_1 along with the associated change in the residual cost. **A, B** depicts structural unidentifiability, as the residual cost does not change along the trace of the profile. **C, D** depict practical unidentifiability, where only a lower boundary of p_1 can be established. Lastly, **E, F** depict the final case where the parameter is identifiable, as there exist a lower and upper bound. Originally published in [209]. Included with the permission of the authors.

Re-examining the example provided in Eq. 4.20, this case would for a PL analysis be considered *identifiable*. This is due to the fact that a lower bound (given the assumption $p > 0$) exists, and an upper bound can be calculated with respect to data, given by the fact that $p_3 = p_1 + p_2$ is identifiable (see example and discussion in [198]). For these reasons, the definition of *structural*

unidentifiability is dependent on the analysis method used, and the classical analysis of *structural unidentifiability* (such as in [199]) does not rule out that the parameter could be in practice identifiable (for instance using a PL analysis). This means that the classical way of denoting *structural unidentifiability* is of lesser importance in terms of identifiability of the model parameters.

Markov Chain Monte Carlo (MCMC) methods

An alternative to a PL analysis is to assess the posterior distributions of the parameters, which is typically performed using Markov Chain Monte Carlo (MCMC) sampling. The posterior distribution is defined in accordance to Bayes' theorem as:

$$P(p | y) = \frac{L(y|p) P(p)}{P(y)} \quad (4.25)$$

where $P(p | y)$ represents the probability of observing the parameters p given the measurement data y ; where $L(y|p)$ is the likelihood function maximizing the probability of observing data given p ; where $P(p)$ is the prior belief or knowledge about the parameter values, and finally, where $P(y)$ is a scaling factor, which is not known *a priori* but can for parameter inference purposes be ignored as it is independent of p . In analogy to the likelihood function, a maximum *a posteriori* estimate is gained by maximizing $P(p | y)$ which is equivalent to minimizing $J(p)$ or maximizing $L(y|p)$. If the prior belief ($P(p)$) is an uniform distribution on the interval given by the parameter boundaries, the posterior distribution corresponds to the likelihood as subjected to the prior. If the prior is not known, the prior distribution can be flat, meaning an equal probability for all possible parameter values. By repeated sampling using various MCMC algorithms, the posterior distribution will converge to the true target distribution asymptotically, given enough iterations.

The most widely known algorithm is the Metropolis-Hastings algorithm [210, 211]. It samples the posterior distribution of the parameters by performing random walks throughout parameter space. At every parameter point p , a candidate point p' is drawn from a proposal distribution $q(p)$. $q(p)$ is typically a normal distribution: $q(p) = N(p, \Sigma)$ with the covariance matrix Σ . The most common example of the algorithm is simply a normal distribution $N(p, 1)$. The candidate point is accepted with the probability $\alpha(p', p)$:

$$\alpha(p', p) = \min \left(\underbrace{\frac{L(y|p') P(p')}{L(y|p) P(p)}}_{\text{posterior ratio}} \cdot \underbrace{\frac{q(p|p')}{q(p'|p)}}_{\text{Hastings ratio}}, 1 \right) \quad (4.26)$$

This expression contains the likelihood ratio between the candidate and current parameter point, as well as the Hastings ratio, which ensures that the

sampler can reverse its previous move in space. This means that the sampler does not get stuck in one site. If the candidate point is more probable than the existing point, meaning that the posterior ratio is > 1 , the candidate point is accepted. However, if the candidate point is less probable, the point is sometimes rejected, with the likelihood of rejection increasing the less probable the candidate point is. Therefore, with many iterations (samples), the MCMC sampling algorithm will visit high probability regions many times, while rarely visiting low probability regions. This forms the posterior distribution of the parameters. MCMC methods have continued to evolve, and more advanced algorithms now exist. For instance, algorithms which include adaptation of the proposal distribution q such as the adaptive Metropolis algorithm [212]; incorporates the local geometry of the likelihood landscape such as in the manifold Metropolis adjusted Langevin algorithm, or multi Markov chain approaches such parallel tempering [213, 214].

Figure 4.10 show the similarity in parameter identifiability between an MCMC approach, the Region-Based Adaptive PARallel Tempering approach introduced by [214], and the conventional repeated-optimization PL analysis (Eq. 4.23). The test case consists of a pharmacokinetic model describing the hepatic uptake and degradation of an MR-compatible liver contrast agent, with MRI-based measurements of the amount of contrast in the liver and spleen compartments [215]. The model consists of five parameters. As seen, the PL traces for each parameter (Fig. 4.10, black lines) crosses the χ^2 -threshold ($\alpha = 0.05$, $df = 1$) at similar parameter values as the MCMC based posterior distributions of the parameters (Fig. 4.10, gray samples). The global optimization convergence points are shown as red dots, overlapping with the peaks of the posterior distributions. Nevertheless, two parameters: k_{hp} (top left) and k_{hb} (bottom left) are *practically unidentifiable* which both methods show: PL as the black line never crosses the χ^2 -threshold, and MCMC by having samples (albeit with low probability), in the non-limited direction. The graphs are truncated to -6 in $\log_{10}$ space for visual clarity.

While a PL analysis is performed on a per parameter basis, an MCMC analysis instead generates the posterior distributions for all parameters at once. As PL is computationally heavy to perform due to the conventional repeated-optimization approach, it becomes computational cumbersome for highly parameterized models. In such cases, MCMC might be a better choice but requires large amounts of samples in order to converge to the true posterior distribution. In this thesis, both PL and MCMC approaches has been extensively used. The simulation uncertainties in Paper III and IV are based on an MCMC approach, while key results in Paper II are based on the use of PL analysis.

Figure 4.10: **Comparison between MCMC and PL for a five parameter model of liver contrast agent uptake.** Normalized MCMC samples are shown as the gray histograms, while the profile likelihood trace is shown as the solid black lines. The χ^2 -threshold for $df = 1$ is shown by the horizontal red line. Three parameters are identifiable (k_{diff} , k_{ph} , k_y), while two are practically unidentifiable (k_{hb} , k_{hp}).

Generalizing parameter uncertainty to prediction uncertainty

The underlying methods behind both PL and MCMC can be extended from determining the uncertainty of parameters to any specified model property, $Z(p)$, that can be quantified from the model simulations [198, 200]. For PL, this is commonly denoted as the *prediction profile likelihood* (PPL), and can be estimated using the repeated-optimization method with a modified likelihood function:

$$J(p)_{PPL} = J(p) + \lambda |Z(p) - Z'| \quad (4.27)$$

where $J(p)$ is the residual cost function; where λ is the L1-weight, chosen to be sufficiently large to ensure that the added term is heavily favored to be minimized over $J(p)$; where $Z(p)$ is the model simulated property value for the given parameters p , and finally, where Z' is the fixated value to be reached for the model property, which is iteratively changed. This technique is used

in Paper II to look at the ratio between glutamate and GABA release, and to calculate the simulation uncertainty against estimation and validation data.

4.8 The necessity of validation data

We have now reached step 3 in the modeling cycle depicted in Figure 4.1, where model-based predictions are to generated and used for two purposes: 1) validating the model by comparing the model-based predictions to independent validation data, or 2) suggest new experimental designs for further investigation of both the biological system, and validation of the model structure. Using the methods described in Section 4.7, we now know how to generate model predictions with an upper and lower bound of uncertainty. However, why use validation data at all?

Model over-fitting and selection

Over-fitting a model occurs when the model contains too many parameters (*i.e.*, over-parameterized) as compared to both the quality and quantity of measurement data for which the parameters have been estimated on [171]. This is typically the case in Systems Biology, where available experimental data is usually limited and the model complexity is large due to the previous knowledge about the system. Over-fitting is often suspected if the model simulation fits the estimation data points *too well*, for instance fitting outlying data points or following the measurement noise too closely. If a model is over-fitted, the typical tell-tale sign is that it will not be able to predict independent validation data.

A paper by Sundqvist *et al.* [216] illustrates this common occurrence within Systems Biology. A polynomial example is presented in Figure 4.11. Here, simulated data (Fig. 4.11A–C, blue and red error-bars) have been generated using a 7th-degree polynomial as the ground truth, and corrupted using additive white noise of three different levels. For high measurement noise, a simple linear model is sufficient to describe the estimation data (Fig. 4.11A, blue symbols), but will fail to predict the validation data (Fig. 4.11A, red symbols). If the measurement noise is small, the polynomial degree must be high in order to fit the estimation data (Fig. 4.11C). Nevertheless, the added complexity to fit estimation data warps the ability to accurately predict the validation data (Fig. 4.11C, red symbols). Therefore, the correct model structure has to both be able to capture the dynamics in estimation data, but also correctly predict validation data, as quantified by having a low SSR (Eq. 4.8) against data used for validation (Fig. 4.11B).

To further illustrate this point, 14 different model structures, ranging from a first order to a 14th order polynomial expression, were fitted to the simu-

Figure 4.11: **Simple example using three polynomials.** For the three cases: synthetic data (red and blue symbols) generated by a 7th order polynomial (**B**, black line), plus some normal additive noise of three different levels. Using the χ^2 -test, high noise favors a simple model (**A**, black line), and low noise favors a complex model (**C**, black line). Nevertheless, these incorrect model structures have less predictive power of data used for validation (red symbols), unlike the true model structure (**B**). Image taken from [216] with permission from the authors.

lated noise corrupted data of 11 different levels. Model selection was carried out using four different methods:

1. Select the model structure with the lowest SSR (Eq. 4.8).
2. Select the model structure with the lowest χ^2 -test statistic, $T_{\chi^2}^M$, with the degrees of freedom corrected for number of parameter $df = n_d - n_p$ where n_d is the number of data points and n_p is the number of parameters.
3. Select the simplest model structure that pass the χ^2 -threshold, corrected for as in method 2.
4. Select the model with best prediction of the validation data (determined by the lowest SSR).

The model selection results are shown in Figure 4.12 with the left column depicting method 1) and 2), middle column depicting method 3), and finally, right column depicting method 4). The estimation SSR for the 11 different noise levels (blue, green, red lines) is shown for the different methods (Fig. 4.12A–C). The selected model is indicated by the colored dot for each noise level. The bottom row (Fig. 4.12D–F) depicts the model selection against the noise level. As seen, the first method of selecting the model with the lowest

Figure 4.12: **Polynomial example of model selection using four different methods.** For each graph: models with increasing complexity (1–14) on the x-axis, residual cost for estimation data (A–C) or the noise level of data (D–F) on the y-axis. A–C: Results for method 1 ('Lowest SSR') and 2 ('Simplest χ^2 ') (A); method 3 ('lowest χ^2 ') (B), and finally, method 4 ('lowest validation SSR'), (C). The different lines (blue, green, red) corresponds to different noise levels. Method 1 always chooses the most complex model (blue dots), while method 2 choose the simplest model that pass the χ^2 -threshold (yellow dots). The selection of method 3 is dependent on the noise level (green dots), while method 4 always selects the correct model structure (red dots). D–F: Same results as in A–C, but with measurement noise versus selected model structure depicted. Method 1 is biased towards the most complex model, model selection by method 2 & 3 is noise dependent, and method 4 always chooses the correct model structure, independent of the level of measurement noise. Image taken from [216] with permission from the authors.

SSR will always choose the most complex model (Fig. 4.12A, D). For the χ^2 -based model selections (method 2 & 3), the model selection depends on the measurement noise, and the correct model structure is rarely selected (Fig. 4.12A, D; B, E). For the last method (4), which uses validation data, the correct model structure is always chosen, independent of measurement noise (Fig. 4.12C, F).

Therefore, using independent validation data is crucial to validate models within Systems Biology. Furthermore, model selection using validation data is noise independent, favoring the correct model structure over too complex or too simple models.

4.9 Summary

In this chapter, an overview of the central aspects and methods within mathematical modeling has been presented. Mathematical modeling within biology has emerged as a powerful tool for studying many different diseases, organs, and biological systems, including the NVC (see Section 2.3 for an overview

of existing models of the NVC). This chapter illustrates the iterative cycle of mathematical modeling, as seen in Fig. 4.1), and covers many of the commonly used methods behind each of the steps involved. These methods and tools can now be applied to the question and aim outlined in Section 2.4: to develop a mechanistic model of the NVC.

5

Results

In this chapter, I present the main findings of my thesis. The results are presented in the natural order: *i.e.*, for Paper I–IV.

5.1 Paper I: evaluating two of the most widely discussed hypotheses underpinning the NVC

Figure 5.1: **The two studied hypotheses in Paper I.** **A:** The metabolic feedback hypothesis. Neuronal activity consumes energy in the form of glucose and oxygen, which triggers an increase in blood flow through a feedback loop. **B:** The feed-forward hypothesis. Neuronal activity triggers intracellular signaling cascades, culminating in the release of vasoactive messengers which act on the vasculature. Image adapted from Figure 1 in Paper 1.

In Paper I, we evaluated the two most widely discussed hypotheses describing NVC: the metabolic feedback hypothesis (see Section 3.1, Fig. 5.1A) and the feed-forward hypothesis (see Section 3.2, Fig. 5.1B), using BOLD-fMRI data consisting of two visual stimulation paradigms (see Paper 1, Experimental data). The two paradigms included an *intensity* aspect using different contrast levels of the visual cue, and a *frequency* aspect with a pair of

visual cues presented within a short time interval. The models were evaluated in regards to the ability to produce the qualitative features of the BOLD response: an initial dip, a main overshoot, and a post-peak undershoot (Fig. 2.6A), as well as the quantitative agreement with experimental data.

Neither hypothesis alone can explain all three characteristics individually

Figure 5.2: **The rejection of the metabolic feedback hypothesis.** Model simulation of the BOLD response (A, C) and glucose (B, D) for an unconstrained scenario (A,B), and a forced initial dip scenario (C,D). For each graph: model simulation (colored line), BOLD data (black symbols, with error bars depicting standard error of the mean (SEM)), and finally the length of stimulation (500 ms) is indicated by the black bar in the lower-left portion of A, C.

Following the modeling cycle outlined in Figure 4.1, multiple model structures capturing the main mechanisms of the metabolic feedback hypothesis was constructed. More specifically, for one of the model structures (see Fig. 5.1A; Fig. 5F in Paper I), glucose acts as the regulator in a negative feedback system. Thus, when glucose levels decrease upon stimulation, a corresponding increase in blood flow is evoked as a counteracting force. As can be seen, while the model can quantitatively fit the BOLD-fMRI data, it can not capture all of the qualitative features simultaneously. In Fig. 5.2A, the model does not produce an initial dip, and if an initial dip is forced as in Fig. 5.2C, the ability to produce a post-peak undershoot is diminished. Furthermore, in order to quantitatively describe the BOLD data, the model resorts to biologically implausible glucose behavior (Fig. 5.2B, D). Upon stimulation, the model glucose level is reduced to almost zero, ($< 1\%$ of the baseline value),

and remains there for several seconds. In a similar way, all other variants of a metabolic feedback system, including using oxygen level as the regulator, could not pass the hypothesis testing (see Paper I). In contrast, a model structure describing the feed-forward hypothesis could simulate all three BOLD characteristics and quantitatively agree with experimental data (see Figure 6 in Paper I). However, model analysis revealed that it could only do so in an implausible way, as the initial dip in the model was caused by rapid vasoconstriction before the overshoot phase of the response, which is not in line with previous insights and data (see discussion in [125, 126]). For this reason, the feed-forward hypothesis was rejected.

A combined model can quantitatively describe and qualitatively predict data

Figure 5.3: **A combined model can describe both data used for estimation and predict independent validation data.** A, D: Model estimation to data from the intensity (A) and frequency (D) experiment. B–C, E–F: Model predictions compared to validation data for the intensity (low (B) and medium (C) contrast) and frequency (two stimulations, 1 s (E) and 4 s (F) delay) experiments. For each graph: the x-axis depicts time in seconds, the y-axis is the normalized BOLD response, and experimental data is shown in black symbols with error bars representing SEM. For A, D, best simulation is shown as solid colored lines, with the corresponding model uncertainty as the black shaded area. For B–C, E–F, the model prediction uncertainty is shown as the red shaded area. The stimulation paradigm is indicated by the black rectangle in each graph, with each stimulation being 500 ms. Figure adapted from Paper 1.

Figure 5.4: **Archetypal characteristics of a positive and negative BOLD response to a brief stimulus.** The positive response depicts the three main characteristics: an initial dip, an overshoot, and a post-peak undershoot. The black rectangle in the lower-left corner of each graph indicates the stimulus length of 500 ms. **Right:** Zoomed-in view of the negative BOLD response shown in left. As seen, the response consists of only an undershoot phase. Image adapted from Paper II.

Based on these insights generated from the two hypothesis rejections, we constructed a new model that included key aspects from both the previous hypotheses. In this combined model, the initial dip is caused by a rapidly increased oxygen metabolism triggered by the stimulation, and the overshoot and the post-peak undershoot are the result of multiple feed-forward signaling arms which release vasoactive messengers. The combined model displayed all three qualitative characteristics with a good quantitative agreement to data (Fig. 5.3A). Model predictions (red shaded areas) of data from the *intensity* paradigm had adequate agreement with the corresponding validation data (Fig. 5.3B–C, black symbols). The model was also fitted to experimental data from the *frequency* paradigm, exhibiting corresponding good qualitative and quantitative agreement to training data (Fig. 5.3D). The corresponding model predictions of the BOLD response (red shaded areas) for a paired visual cue, with 1 s (Fig. 5.3E) or 4 s (Fig. 5.3F) delay between the two visual cues, agreed well with the corresponding validation data. For more details, see Paper I.

5.2 Paper II: negative BOLD responses — the role of inhibition

The final model structure presented in Paper I was developed for only positive BOLD responses. Nevertheless, negative BOLD responses are commonly observed for a wide range of cognitive or clinical studies [217–220]. An archetypal negative BOLD response for a brief stimulus is depicted against a positive response in Figure. 5.4. As seen, the negative response often exhibits a shallower deflection, as compared to its positive counterpart. Furthermore,

its qualitative features are less well defined except for the main bulk of the signal should decrease below baseline, but a brief post main response overshoot can sometimes be observed (see for instance experimental data in [221, 222]). Different hypotheses regarding the origin of the negative BOLD exists (reviewed in [223]) including hemodynamic steal [224, 225] or neuronal inhibition [221, 226, 227]. Hemodynamic steal describes how a positively activated area (in terms of BOLD) causes redistribution of blood, reducing the blood supply in the surrounding area. Neuronal inhibition instead describes how inhibitory activity of neurons reduces CBF and CMRO₂ in that local area.

Figure 5.5: **Model estimation to data consisting of brief visual stimuli evoked BOLD responses.** **A & B:** Experimental data (black symbols) depicting mean value and SEM for each time point compared to model simulation (black line) for both positive (**A**) and negative (**B**) BOLD responses. The 500 ms visual stimulation is indicated by the black rectangle in the lower-left portion of each graph. **C & D:** Area under curve (AUC) plots displaying the ratio between glutamate and GABA which have a positive or negative effect on Ca²⁺ inflow, respectively. The uncertainties (black error bar) depict a confidence interval ($p < 0.05$) for all possible model parameters calculated using a prediction profile likelihood (PPL) approach. The figure is adapted from Fig. 4 in Paper II.

As covered in Section 2.3, many models describing the various aspects of the NVC exist. However, none of these models describe any of the hypotheses underlying the negative BOLD response. Therefore, in Paper 2, we sought to extend the model developed in Paper I to incorporate the neuronal inhibition hypothesis. The neuronal inhibition hypothesis was chosen due to the availability of such experimental data: a visual-motor task and a working-memory task which BOLD-fMRI data did not contain adjacent areas exhibiting posi-

tive and negative BOLD responses, making the hemodynamic steal effect unlikely to be the underlying cause.

We trained this extended model using positive and negative BOLD responses acquired using the visual-motor task (see Paper II, Fig. 3). The estimated model agreed well with both the positive and negative BOLD-fMRI data, and the model simulation displayed the qualitative characteristics of both the positive (initial dip, main response, post-peak undershoot) and the negative (signal below baseline) response (Fig. 5.5A–B). Next, we quantified the ratio between the positive and negative contributions that glutamate and GABA exert on Ca^{2+} influx, respectively. This quantification was done using PPL analysis (see Section 4.7). The analysis revealed that the positive effect of glutamate is dominant for the positive BOLD response, as the ratio is > 1 (model confidence interval as black error bar) for all acceptable parameter sets (Fig. 5.5C), thus increasing the vasoactive signaling. In contrast, the negative effect of GABA is instead dominant for the negative BOLD response (Fig. 5.5D), with the ratio < 1 for all acceptable parameter sets. For more details, see Paper II.

5.3 Paper III: assessing cell-specific contributions to the NVC and the role of anesthesia

In Paper III, we assessed experimental data of arteriolar diameter responses captured in mice using OG stimulation of pyramidal neurons or GABAergic interneurons, as well as sensory stimuli, as presented in Uhlirova *et al.* [88]. These data were captured for both awake and anesthesia conditions, exhibiting qualitative and quantitative differences in-between the two conditions (Fig. 5.6A–B). Most prominently, a prolonged post-peak undershoot for the OG stimulation during anesthesia is observed (Fig. 5.6A). Currently, no existing model describes the vasoactive signaling pathways of the relevant neuronal sub-types (outlined in Fig. 5.6C) and their interplay, or include the effects of an anesthetic upon the evoked arteriolar response. We therefore sought to construct a first model describing the mechanisms and interplay between GABAergic interneurons and pyramidal neurons in the context of NVC, which include the effect of the used anesthetic. The constructed model is depicted in Figure. 5.6D, and consists of three layers: neuronal activity, intracellular signaling pathways which culminates in the release of vasoactive messengers, and finally, the vascular response.

The constructed model agrees well with data used for model estimation, which consisted of optogenetic and sensory stimuli in both awake and anesthetized mice (Fig. 5.7, compare black symbols with green lines). Furthermore, the model was used to generate predictions (Fig. 5.7, red shaded areas) of the effect of different pharmacological modulators on the evoked arteriolar

Figure 5.6: **A & B:** Anesthesia alters the evoked arteriolar diameter response for both optogenetic (OG) (**A**) and sensory (**B**) stimuli. The bars in the lower-left corner of each graph indicate the stimulus length of the OG and sensory stimuli, for both awake (red) and anesthesia (black) conditions. **C & D:** NVC representation of relevant signaling pathways and mechanisms (**C**), with the translated NVC model structure to explain these data (**D**). The model consists of a pyramidal neuron (gray triangle) and two GABAergic interneurons (yellow ovals), each with their respective vasoactive signaling pathways which influence the arterioles (red cylinder). See Fig. 2A–B in Paper III for further details. Figure adapted from Paper III.

response. These pharmacological modulations were: 1) the inhibition of the NPY receptor (NPY₁R) for both OG and sensory stimulations during anesthesia (Fig. 5.7,E–H), and 2) the blockade of glutamatergic signaling for OG stimulation during anesthesia (Fig. 5.7I–J). These model predictions agreed well with the corresponding independent validation data (Fig. 5.7, red symbols).

Figure 5.7: Model estimation and prediction of arteriolar diameter responses in awake (A–D) and anesthetized mice (E–J), captured using sensory (A–B, E–F) and optogenetic (OG) (C–D, G–H, I–J) stimuli. **A, C, E, G, I:** Simple illustrations of the experiment trial for each data set. Gray triangle: pyramidal neuron; yellow oval: GABAergic interneurons. Sensory stimulation and OG stimulation indicated by symbols, and the brown arrows are affected by anesthesia. **B, D, F, H, J:** The estimated model compared to experimental data. For each graph, the best model simulation (solid green line) with corresponding model uncertainty (black shaded areas), compared to experimental data (black symbols). Additionally, model predictions (red shaded areas) of different receptor inhibitors (see illustrations and legend) compared against corresponding independent validation data (red symbols). The length of the stimulation is shown by the black bar in the lower-left portion of each graph. The uncertainty of experimental data is presented as SEM. Figure adapted from Paper III.

To understand how the model explains the change in vascular dynamics induced by anesthesia, we analyzed how the model explains the key characteristic observed in the experimental data: the prolonged post-peak undershoot due to the anesthetic effect in the case of an OG stimulation of GABAergic interneurons (Fig. 5.6A, black symbols). The model simulations reveal that the prolonged post-peak undershoot can be explained by two parts. Firstly, the anesthetic agent acts on the neuronal activity (Fig. 5.8A–B), changing the excitability of the two GABAergic neurons, inducing amplitude changes in the response, which are propagated through the intracellular signaling pathways (Fig. 5.8C–F). Secondly, the vasoconstrictive effect of NPY follows Michaelis-Menten kinetics, converting the amplitude differences between awake and anesthesia at the neuronal level into duration changes at the level of the intracellular signaling pathways (Fig. 5.8C, E: compare awake with anesthesia). Finally, the two arms are summed together which forms the arteriolar diameter response (Fig. 5.8G). These amplitude and duration changes alter the arteriolar response from exhibiting a brief post-peak undershoot to a prolonged version, with minor changes in time to peak and peak amplitude (Fig. 5.8G: compare awake with anesthesia). For more details, see Paper III.

Figure 5.8: The model explanation of the anesthetic effect in the case of optogenetic stimulation of GABAergic interneurons. In the middle, a model schematic of the signaling pathways, and which reactions that are altered with anesthesia is depicted. The relevant states are shown: the neuronal dynamics of NPY (A) and NO (B) interneurons, the propagation to the vasoactive effect of respective neuron (C, E and D, F), and finally the vascular response (G). For each of these states, dynamics for awake (left graph) and anesthetized (right graph) model simulations are shown. Figure adapted from Paper III.

5.4 Paper IV: the final model can describe most relevant NVC measures

In Paper IV, we sought to extend the framework presented in Paper III to not only describe arteriolar diameter responses, but to incorporate most of the available hemodynamic NVC measures. This idea was driven by the fact that no currently existing model which describe the NVC can simultaneously account for a wide variety of data types (Fig. 5.9B), including: 1) compartmentalized CBV and CBF, 2) oxygen saturation measures (hemoglobin, BOLD-fMRI), 3) optogenetic stimulation of specific neuronal types, and finally, 4) pharmacological perturbations of intracellular signaling, including anesthetics. This new model is a combination of the model in Paper III, the hemodynamics model of Barrett *et al.* [43–45], and the BOLD signal derivation of Griffeth *et al.* [228] (Fig. 5.9A–B). This new model have a quantitatively good agreement to experimental data used for model training from multiple sources and species (Fig. 5.9A; Fig. 3, 5, 6, 7 in Paper IV), and can correctly predict independent data used for validation (Fig. 5 & 7 in Paper IV). Furthermore, this model can be used to examine complex behaviors observed in experimental data, such as in Figure 5.10.

Figure 5.9: **A:** Overview of the study conducted in Paper IV. Experimental data from different species (colored boxes) and modalities are used to develop a new combined NVC model. **B:** Comparison of existing NVC models, with regards to different aspects. The notations used are: ‘Yes’ if the model features a satisfying description of the aspect; ‘Partly’ if the model features a description that is not fully satisfying but nevertheless exists, and ‘No’ if the model lacks a satisfying description of that aspect. Figure adapted from Paper IV. See Table 2.1 for full references. HbX hemoglobin measures; CBV_i compartment specific CBV.

Here, the captured arteriolar responses to a sensory stimulation change from a one-peak (Fig. 5.10A) to a two-peak response (Fig. 5.10C), as the length of the stimulation increases. The model captures this transition between an uni-modal to a bi-modal response well (Fig. 5.10A–C, compare shaded areas and lines with symbols). Therefore, it is of interest to see how the model can replicate this behavior, by examining the last step before the arteriolar response: the summation of the vasoactive arms. In the model, the response to the shortest stimulation is produced by the fast release of NO from GABAergic interneurons, which produces the first peak in the arteriolar response (Fig. 5.10D–F, blue line) and which thereafter simply declines for the remainder of the stimulation. The other two signaling pathways exhibit slower kinetics, and are therefore negligible for the shortest stimulation. the dynamics of NO is also important for the two longer stimulations as the response declines quickly after the first peak. In the model, this is driven by the decline of NO, causing the decrease in arteriolar diameter observed after the first peak. For the two longer stimulations, a second peak is observed, which is driven by the slower PGE₂ signaling arm from the pyramidal neuron (Fig. 5.10E–F, green line). Finally, the NPY signaling arm only comes into play in order to explain the post-peak undershoot observed for the longest stimulation (Fig. 5.10F, red line). In summary, the first peak in the arteriolar response is due to rapid NO arm, the second peak is due to the PGE₂ arm, and finally, the post-peak undershoot (if observed) is due to the lingering effect of the NPY arm. This small example illustrates how these types of models can be used to investigate underlying mechanisms for observed complicated behaviors seen in various types of experimental data. For more details, see Paper IV.

Figure 5.10: Model estimation and explanation of experimental data consisting of arteriolar (A–C) diameter changes in awake mice for three different sensory stimulation lengths: 125 ms (A), 10 s (B), and 30 s (C). For each graph: experimental data (mean $\pm$ SEM) as red symbols; the best model simulation is seen as the red solid line, and the model uncertainty as the red shaded area. D–F: Model suggested explanation of the transition from a one-peak to a two-peak arteriolar response. Model simulations for the three stimuli lengths: 125 ms (D), 10 s (E), and 30 s (F) are shown. For each graph: model simulations (colored lines), and length of the stimulation (black bar). The x-axis depicts time, and the y-axis depicts the vasoactive effect on the arteriolar compartment (A.U). Figure adapted from Paper IV.

6

Discussion and concluding remarks

In this chapter, I will summarize the main findings from my papers, followed by a discussion of how these fit into the field of NVC research. I will also discuss some key limitations of my work, and provide some thoughts on the future outlook of my findings.

6.1 Summary of main findings and conclusions

Paper I

In Paper I, we show that neither of the metabolic feedback or vasoactive feed-forward hypotheses could on their own both quantitatively and qualitatively describe the experimental data. A combination of key aspects from both hypotheses could describe data used for estimation, as well as predict independent data used for validation.

Paper II

A model implementation of the neuronal inhibition hypothesis as underlying negative BOLD responses could describe data used for training, and successfully predict independent data used for validation. This model constitutes one of the first mathematical models investigating the underpinnings of the negative BOLD response.

Paper III

In Paper III, we provide a first quantitative model of the cell-specific contributions to the NVC, including an explanation of the shift in dynamics that

occur with the use of an anesthetic agent. The analysis suggests that one of the GABAergic interneuron signaling pathways, NPY signaling, must include a saturating expression that translates differences on the rapid neuronal level to be converted to duration changes on the vascular level.

Paper IV

In Paper IV, we extend the model structure presented in Paper III to describe a majority of the existing relevant NVC measures via the addition of a vascular model consisting of: blood volumes and flows, oxygen transport and saturation, hemoglobin dynamics, and finally, the addition of a BOLD-fMRI signal. The model can describe and predict experimental data from rodents, primates, and humans, and suggest explanations for complex behaviors observed in experimental data. This model constitutes the most complete mechanistic model of the NVC to date.

6.2 The three phases of the BOLD response

As described already in Chapter 2, there are three phases to the archetypal BOLD response: the initial dip, the main peak or overshoot, and the post-peak undershoot (Fig. 2.6A). Before the thesis, no mechanistic model existed that explained plausible biological mechanisms that cause these three phases. With the results of the four papers in this thesis, we can now provide an updated mechanistic understanding of how these three phases are generated.

The initial dip

It is debated whether the initial dip even exists, because if it exists it is comparable in size to measurement noise (see [125, 126]). For this reason, only Paper I is concerned with the initial dip observed in BOLD-fMRI. In this paper, we find that the feed-forward signaling, caused by secretion of vasoactive substances, can in principle explain the initial dip. However, we reject that hypothesis, because it would imply that vasoconstrictive signaling happens directly within the first two seconds. Instead, we choose a model where the initial dip is caused by an initial rapid increase in oxygen metabolism.

The main overshoot

The increase in metabolism that causes the initial dip is in our models not causing the main overshoot component. This stands in contrast to the initially held belief of how the BOLD signal is generated. This initial belief was centered around a negative feedback: because the metabolism causes a drop in blood oxygenation, the blood flow increases thanks to a negative feedback that is a part of a control system (See Chapter 3, "The discovery of the NVC"). In

Paper 1, we reject this negative feedback hypothesis. This rejection was based on the fact that all implementations of the negative feedback hypothesis that could explain the main overshoot also had an unrealistic prediction: they predicted that the metabolite that was involved in the feedback went down to zero for several seconds (Fig. 5.2B, D). For this reason, we concluded that the main overshoot was caused by a feed-forward system involving intracellular signaling which leads to the secretion of vasoactive substances. This conclusion was supported by the fact that additional model analysis revealed no further apparent unrealistic model predictions, and that the model could explain both estimation data and predict independent validation data not used for model training (Fig. 5.3).

In Papers III & IV, we could return to these intracellular signaling pathways and unravel its more detailed composition by using pre-clinical data from mice. The most important component of those data is optogenetic perturbations, which allows us to more closely examine the role of different neuronal types. This analysis leads to a model, where the main response is caused by a combination of pyramidal neurons, which secrete PGE_2 , and interneurons which secrete NO and NPY. In Paper IV, we could refine this dual role further. For a short stimulation, the initial response regulates arterioles via the NO pathway. For longer stimuli, the NO pathway quickly subsides, and is overtaken by the PGE_2 pathway. In certain conditions, this leads to a bi-modal response, featuring two-peaks for the longer stimulations (Fig. 5.10).

The post-peak undershoot

In Paper I–II, the post-peak undershoot can be explained by the interplay between two vasoactive arms: one dilating and one constricting (see the minimized model in Paper 1, and the model in Paper II). These two arms exhibit highly similar dynamics, but the constricting arm has a prolonged time-scale, and is lower in magnitude. In total, the constricting arm briefly lingers on after the main response is over, causing the post-peak undershoot.

In Paper III & IV, we unravel the nature of these two arms in much more detail. Using the optogenetic perturbations, we can find conditions where the post-peak undershoot is observed or not, and also find conditions that impact the duration of the post-peak undershoot. Using these data, we are able to elucidate that the post-peak undershoot is caused by the NPY pathway. Furthermore, we can identify that this NPY pathway needs to have a saturation, since there are situations where the post-peak undershoot is prolonged but without an increased post-peak undershoot amplitude (Fig. 5.7). In Paper IV, this mechanistic unraveling is further refined by looking at data that specifically looks at the arteriolar diameter. For long stimuli, the arteriolar diameter has both a bi-modal main response, and a post-peak undershoot.

Our analysis based on this data again confirms that the post-peak undershoot is caused by the NPY pathway, which is the slowest of the three arms. In other words, compared to the situation in Paper I, we now know that the hypothesis of two vasoactive arms with different time scales is supported by detailed experimental data, and that the negative arm is made out of the slow NPY pathway.

Apart from our conclusions, there are also other explanations suggested in the literature that are worth mentioning. More specifically, the post-peak undershoot of the BOLD response can be caused by a slow return of venous blood volume for very long stimulations. In such cases, the post-peak undershoot is caused by a big blood volume in the venous compartment, which is filled with large amounts of deoxyhemoglobin. Because of this slow deoxyhemoglobin dynamic, the post-peak undershoot can appear in the fMRI-BOLD signal without it being present for CBF. For more details, see [229, 230]. Note however, the data studied here clearly shows that you only have a substantial venous volume for long stimuli (Paper IV, Fig. 3 & 7).

6.3 The three vascular compartments

There are three vascular compartments involved in the NVC: the arterioles, the capillaries, and the venules. These three compartments have different dynamics and regulations, and are all included in our final model (Paper IV). Let us therefore briefly consider them one by one.

Arterioles

Arteriole dynamics have been well characterized in rodent data used in Paper III and IV. These data include different optogenetic stimuli (pyramidal neurons, GABAergic interneurons), sensory stimuli, the addition of pharmacological inhibitors, and measurements for both awake and sedated conditions. In Paper III, we are able to explain all of these data, including independent validation data, and we therein provide a mechanistic explanation for how the different observed dynamics can be caused. In particular, we provide a first explanation for the difference between awake and sedated conditions. This is important since a large majority of data is available only for anesthesia conditions, and because anesthetics impact the mechanisms involved in the NVC. A part of this explanation concerns the different dynamics observed for optogenetic perturbations of GABAergic interneurons in awake and anesthesia conditions: a prolonged post-peak undershoot is observed for the sedated condition (Fig. 5.6A). Our analysis shows that this prolonged post-peak undershoot spanning more than 20 seconds is caused by a difference in amplitude during the short optogenetic stimulation through a sequence of three steps: 1) the difference in amplitude is propagated through the NPY pathway

and leads to a difference in the duration of the response; 2) the duration is preserved while the corresponding differences in amplitude is removed through a saturated step somewhere in the NPY pathway; 3) the entire response is caused by the sum of the NPY pathway and the NO pathway (Fig. 5.8).

In Paper IV, we further refine our understanding for the regulation of the arterioles. For short responses, there is a single peak which for increasing longer stimuli transforms into a bi-modal response of two peaks, and a post-peak undershoot. The explanation of these two phases is explained in Chapter 6.2, 'The main overshoot' and 'The post-peak undershoot', respectively. In Paper IV, we also considered arterial CBV data for humans. Unlike for arteriolar responses in mice, no bi-modal response is observed in humans, which instead exhibits a simple single peak response.

Capillaries

Not included in the thesis, we have a preliminary model of direct capillary regulation. However, no applicable *in vivo* data is available for this regulation, and therefore we choose not to include it in Paper IV. Instead, the capillary regulation in the model in Paper IV is exclusively caused by the vascular model propagating changes in blood pressure and flow from the arterioles to the capillaries. No plots are included in the paper, but the simulations display dynamics lying between the arteriolar and venular responses.

Venules

The venules are only studied in Paper IV. In Figure 3D–F in Paper IV, we see responses for short, intermediate, and long stimulations. For the shortest stimulation (125 ms), a hardly observable single peak response can be seen. For the longer stimulations, more dynamics can be observed. For the longest stimulation (30 s), a clearly defined single peak response is observed, which is explained well by the model. For the intermediate stimulation (10 s), the situation is more unclear: there may be a more complicated behavior seen in the data, but since the signal is almost below the detection limit (see Discussion in Paper IV), we choose to primarily present model simulations that anyway show a simple single peak response. Just as for the capillaries, the venules have no direct control, but their dynamics are simply caused by the vascular model propagating control from the arterioles. In Paper IV, we also study human data for venous responses (see Fig. 7 in Paper IV). For the positive BOLD, the venous response is highly similar to the arterial response with a single peak response. For the negative BOLD response, the behavior may be more complex, but the uncertainty of the data is high, and therefore model simulations show a simple response. Finally, our model stands in stark contrast to the widely studied Balloon model, which only describes the dynamics

of a venous compartment, even though the venous compartment has no direct control.

6.4 Negative BOLD

In Paper II, we examine the negative BOLD response for the first time. The model presented is a simple extension of the feed-forward model of Paper I. The primary extension consists of the addition of GABA, which inhibits instead of excites, the neural cells. This extension allows us to explain human data for both the positive and the negative BOLD response. The model structure in these two responses is the same, but we assume that some of the parameters for the intracellular signaling are allowed to change. This is an important weakness of this work. Nevertheless, the model is able to explain estimation data and independent validation data, in a way that is supported by statistical tests.

In Paper IV, we again return to the negative BOLD response by examining data from primates and humans. In data collected in primates (see [221]), it is seen that the negative BOLD response is accompanied by a corresponding negative deflection in the LFP. This supports our initial model, which also shows such a correlation between electrical activity and the BOLD response for the positive and negative BOLD response, respectively. Again our model is able to explain these data, and this time using the more advanced model structure that can explain all other data in Paper IV. Importantly, in contrast to the model in Paper II, no parameters are changed in the intracellular signaling between the positive and negative responses. Our initial simple explanation is further supported by the human data in Figure 7 in Paper IV. Here, the negative BOLD is captured with corresponding measures of CBV. While the CBV data for the two compartments, arteries and veins, are noise-polluted, the total CBV time series is well defined. It shows a similar decrease from baseline as the BOLD response. In summary, in all situations encountered in this thesis, the negative BOLD response is caused by a decrease already at the electrophysiological level, which is propagated to a similar drop in CBF and CBV. Note that the drop in blood flow is a prediction by the model, which is supported by independent validation data (Paper IV, Fig. 7E).

6.5 Limitations

As with all models, there exist a number of assumptions and limitations, which limit the conclusions that can be drawn. These limitations are now discussed on the general level for all of the presented models in this thesis.

Neuronal activity

First, our description of neuronal activity is quite simple throughout the different papers. For Paper I & II, we express the unspecified release of glutamate and GABA, and in Paper III & IV we employ a simple phenomenological relationship between GABAergic interneurons and pyramidal neurons. A more correct interpretation of neuronal activity would be to integrate our models with existing physical models of membrane potential fluctuations (see [231] and references therein). Furthermore, such a combination would open the door to including the structural and functional morphologies of specific subsets of interneurons (see [86]). This stands in contrast to the current lumped approach used in Paper III & IV, where we simply model an average NPY and NO interneuron.

Metabolism

Another highly simplified aspect of the presented models concerns cerebral metabolism. While metabolism of both oxygen (Paper I–II, IV) and glucose (Paper I–II) occurs in some of the models, this aspect could be expanded in two ways: 1) to include the commonly measured cerebral metabolites, often captured using magnetic resonance spectroscopy; 2) to include direct *in vivo* measures of tissue oxygen pressure. This would lead to a model capable of a detailed description of the ongoing metabolic processes during increased neuronal activity, and could further be extended to describe the energy consumption as a function of the opening and closing of ion channels during action potential firing.

The use of arbitrary units

Throughout the different models, the states are often specified in arbitrary units, meaning that the states represent biological values such as concentrations or amounts, but these are scaled by unknown constants. This limitation is often due to corresponding limitations of experimental data, as most of these intracellular molecules are hard to measure experimentally both *in vitro* and *in vivo*, and if measurable, this measurement often only captures relative changes.

Re-fitting of parameters between datasets

An important strength of this modeling is the use of independent validation data. In this usage, the parameters are trained on specific estimation data which determines the values of all the parameters. Then, without changing any parameter values, predictions are compared with new data, which are not a part of the estimation data. In contrast, there are important cases when

the parameters have been allowed to change between experiments, and these changes should be clearly specified.

In Paper I, two sets of estimation and validation data are used. In the first dataset (*intensity*), the model is trained on a single response and validated on other data where the contrast of the visual cue is lower. In the second dataset (*frequency*), the model is trained on a similar single response, but this time the validation data consists of a set of visual cues, with a 1 s or 4 s delay in-between the two cues. The parameters are held the same within these two datasets, but are allowed to be different between the two datasets.

In Paper II, there are also two different studies included. The first dataset (as published in [232]; Fig. 4 in Paper II) was used to develop and refine the model. The second dataset (as published in [233, 234]; Fig. 5 in Paper II) comes from another study, which means that the parameters were re-fitted when considering these data. Furthermore, the second dataset was subdivided into two parts: one part was used to re-fit the model to the new experimental setting, and the second part was used to validate the predictive ability of the model.

In Paper III, all data came from a single study ([88]). For this reason, we kept the parameters essentially the same for all data. The only difference in parameters allowed was for the electrophysiological part, which was allowed to be different between awake and anesthesia conditions. This difference is important because awake and anesthesia conditions do imply important differences in brain dynamics, which the paper sought to explore. However, the intracellular signaling was assumed to be the same for the two conditions. As usual, the complete set of data was subdivided into two parts: one part for estimation and one part for validation, and the parameters were kept the same when comparing simulations with the independent validation data.

In Paper IV, experimental data from many different sources were included. As usual, the general principle was that the parameters were allowed to change between experimental settings. This is reasonable, since different experimental settings do give rise to different observed dynamics, such as the presence or absence of a post-peak undershoot. Nevertheless, to utilize the fact that the same model structure could agree with this wide variety of data, we sought to make the model behave in a similar way when moving from one dataset to another.

Finally, we assume that most of the parameters are not known from before and thus should be estimated from data. This concerns all of the models in Papers I–III. For Paper IV, we re-used two models, one for the three vascular compartments, and one for the calculation of the BOLD signal. For both of

these re-used models, the parameters were retained from previous publications.

6.6 Future outlook

Connections to the electrophysiological and neuronal network models

Since the introduction of the Hodgkin–Huxley models, neuroscience has had a long tradition of applying mathematical models in order to understand and generate new insights regarding the inner workings of the brain. Since the original papers, these models have become increasingly realistic and complex. On the intracellular level, extensive experimental studies have unraveled the detailed mechanisms of ion channels. On the cell–cell communication level, models include gap-junction couplings, which describe communications between cells. Finally, on the whole-brain level, models describe physiological neural networks which perform central brain functions, such as object recognition and control of muscular actions [235–237]. One interesting such example is the object recognition models by Grossberg *et al.*, which describe the interplay between different brain regions in the complex task of recognizing objects when an eye is looking at different parts of an object (see [237] and references therein). Another fascinating example is the ‘BabyX’ project by Mark Sager *et al.* [238, 239]. This model describes many of the functions of a small child, such as interpretation and recognition of sounds, different emotional responses to different stimuli, and facial mimicking of the persons that the child is observing. All of these functions are simulated by the ‘BabyX’ model, in a way that involves the corresponding brain regions. However, none of these models include a realistic description of NVC. It is therefore an interesting challenge to connect our already existing NVC model with some of the above-mentioned models for electrophysiology and brain function.

Surpassing the capabilities of all other existing models for the NVC

There are some existing models for the NVC which are important to mention in this context, because these models are used for certain applications that we have not considered in this thesis. One of the most important such applications is displayed by the simple model by Davis *et al.* [127]. This model is important because it can combine data for the BOLD signal with CBF data in order to calculate an estimation of CMRO_2 . These three entities — BOLD, CBF, and CMRO_2 — are also included in our models, but we have not explored whether our models can be used for a similar purpose. In general, the combination of a variety of different data sources that describe different aspects

of NVC into the same model is one of the most important future potentials of our model. Using such data integration, it is likely that future versions of our model will be able to draw conclusions about mechanisms and the biomedical status of the brain, which have previously not been possible to obtain. This can open the door to many important applications within the clinic, basic research, and for the development of new treatments.

Clinical usage

Today, fMRI data is analyzed in a highly simplistic manner, which does not incorporate any mechanistic understanding of the biology involved. The current analysis is typically done by using the GLM approach, which looks at the correlation between observed fMRI-BOLD signal and the HRF (Fig. 2.7). This method equates brain activity with a high correlation: if the correlation is above a certain threshold, it is assumed that brain activity is present. This approach has a number of shortcomings. First of all, it does not make use of any biological understanding, even though much biological knowledge regarding the NVC is available. Second, because of the first shortcoming, this method can not incorporate other types of data into the same model. For this reason, the traditional GLM based approach can probably never go beyond a phenomenological assessment of brain activity to a more nuanced understanding of subtle differences between different brain areas or changes in the NVC. The model presented herein can, once sufficiently validated and analyzed, fundamentally improve this situation. In the future, we predict that it will be possible to use a more versatile MRI paradigm which is capable of collecting a wider array of data types and which can produce a battery of currently not available biomarkers. An important challenge for future research is to make this potential reality and to explore the power of such new biomarkers, both for direct clinical applications in diagnosis, prognosis, and patient stratification, and finally also for the identification of new treatments.

New treatments

We believe that our new model-based data analysis can be used to guide research and development of a wide variety of treatments. For instance, an improved mechanistic understanding of the neurological underpinnings of central neurological diseases can help guide pharmaceutical research. For this to become a reality, some important developments remain: the model *e.g.* needs to be adopted to different diseases, and a pharmacokinetic component describing the dynamics of potential drugs needs to be developed. Such model-based drug development is already a reality in most drug development companies, even though the most widely used application today is for pharmacokinetic models. Here, our research could open the door for more physiologically based modeling of the impact of the drug *i.e.*, pharmaco-

dynamic modeling. Finally, regarding pharmacological research, our model can in principle also become useful in the final approval stage, since the food and drug administration in the United States has now published guidelines for how and when models can be used in approval settings, *e.g.* to replace animal experiments or to reduce the size of clinical trials [240–242]. Apart from pharmacological treatments, our model could also be used to study other types of treatments commonly used to treat neurological diseases. Such alternative treatments could for instance include cognitive behavior therapy, psycho-dynamic treatments, and meditation. It is an interesting challenge for future research to use Systems Biology to provide a mechanistic underpinning for such alternative treatments.

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