Analysis of the Structure and Function of Dendrites and Dendritic Spines

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München, den 10. Mai 2023

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Contents

“Learn the rules like a pro, so you can break them like an artist.”

— Pablo Picasso

1 Summary

A single pyramidal cell carries about 30,000 synapses, and almost all of them can be found on dendritic spines. Spines are, therefore, an integral part of neuronal architectures. They are also essential for the mammalian nervous system. Despite their importance in memory formation and the integration of synaptic input, many aspects of the function of dendritic spines remain under debate.

Unfortunately, the small size of dendritic spines makes experimental investigation difficult, and a better understanding of their complex interactions with other parts of the nervous system requires the study of various aspects across different scales, including molecular organization, morphology, and the dendritic tree as a whole.

Quantitative theoretical models are needed to reveal the function of dendritic spines. The objective of this thesis is to explore different aspects of the structure and function of dendritic spines and dendrites, and to develop simplified but accurate descriptions of these systems. By building on the relationships between these elements, this work aims to develop a solid theoretical framework to improve our understanding of dendritic spines' role in synaptic integration and plasticity.

2 Introduction

Synapses facilitate communication and information exchange between neurons. A single hippocampal CA1 pyramidal cell (Fig. 1) receives synaptic input on more than 30,000 excitatory and 1,700 inhibitory synapses distributed across 12,000 μm of branches in the dendritic tree [39].

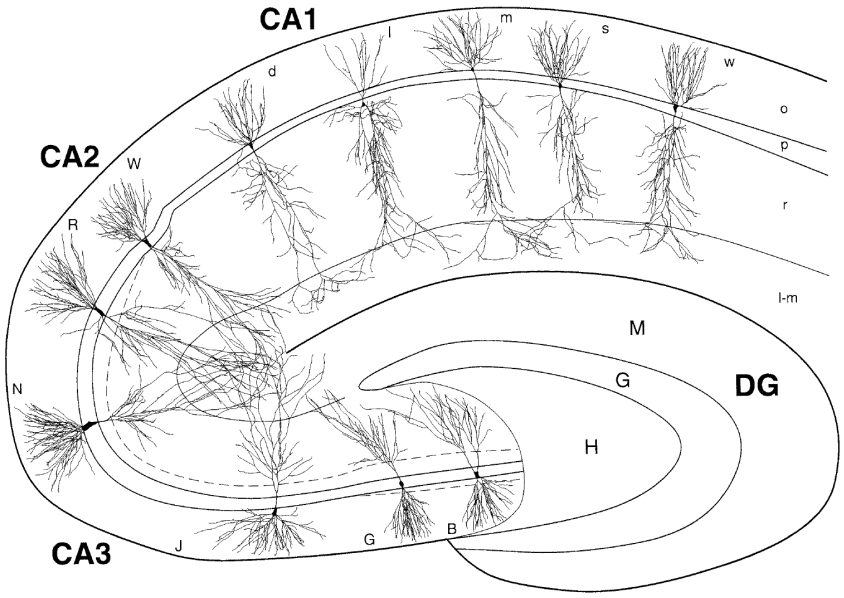

Figure 1: **Dendritic trees**
Pyramidal cells are a type of excitatory neuron commonly found in the cerebral cortex, the amygdala, and the hippocampus. The morphology of pyramidal cells can vary between different brain regions and even within different regions of the hippocampus, such as CA1, CA2, and CA3. In particular, the dendritic trees of pyramidal neurons in the CA1 field are typically smaller and more uniform in shape compared to those in the fields closer to the Dentate Gyrus (DG). On the other hand, pyramidal neurons in CA3 have the largest dendritic tree in the hippocampus. Although most pyramidal cells in CA2 are similar to those in CA3, they typically lack spines on proximal dendrites [30]. The cell body, or soma, of all pyramidal cells is located in the pyramidal cell layer (p) and has a roughly triangular or pyramid-like shape, which gives the cell its name. Typically, a single thick apical dendrite branches out from the top of the pyramid-shaped soma and grows into an elaborate dendritic tree that extends into the stratum radiatum (r) and stratum lacunosum-moleculare (l-m). The apical dendrite can be classified into three segments - proximal, medial, and distal - based on the density of spines along its length. The distal segment carries the highest spine density, and thinner dendrites with high spine density branch off from the distal section and constitute the majority of the apical dendrites. In addition to the apical tree, several dendrites grow out from the basal side of the soma, located in the stratum oriens (o). Along the basal dendrites, the spine density also increases with the distance from the soma. Figure reproduced from [30] (https://doi.org/10.1002/cne.903620103) with permission from John Wiley and Sons; permission conveyed through Copyright Clearance Center, Inc. (license number 5518760381561). Copyright © 1995 Wiley-Liss, Inc.

The vast majority of synaptic connections occur on dendritic spines (Fig. 2), which are small protrusions from the dendrites with a volume ranging from 0.01 to 0.30 μm^3 in the case of pyramidal cell spines [3]. Although highly heterogeneous, the spine morphology can typically be separated into a spine head and a spine neck [42]. The bulbous head contains the postsynaptic density (PSD) and is spatially segregated from the rest of the neuron, while

the thinner spine neck connects the spine head to the dendrite. The intracellular space of dendritic spines contains different organelles, such as the spine apparatus, and is filled with a dense actin cytoskeleton, that controls the size and shape of spines [7]. The organization of the cytoskeleton is distinctive for the spine's intracellular space and can be described as a dense mesh [32].

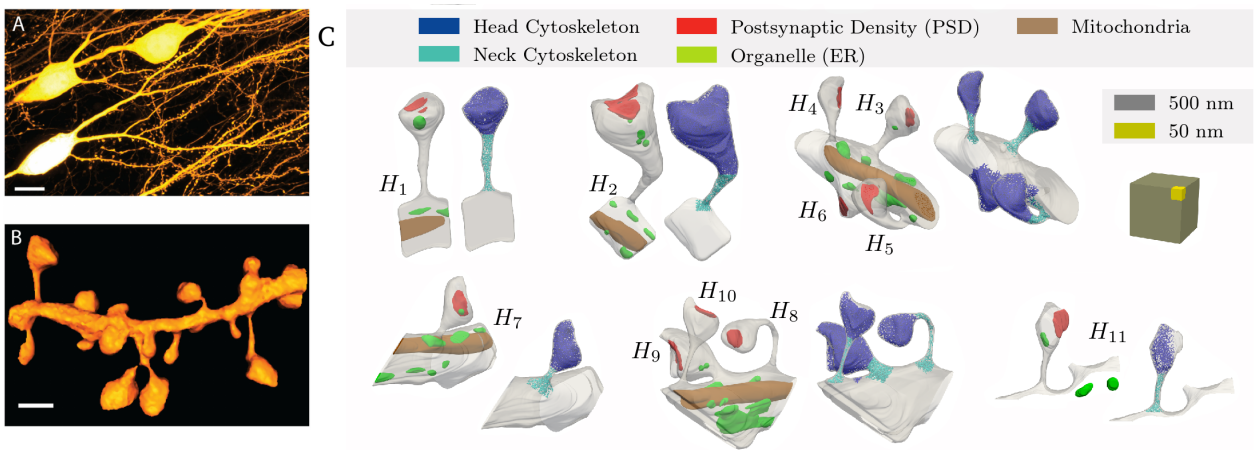

Figure 2: **Dendritic spines**
A) Basal dendrites grow out from the somata of three CA1 pyramidal cells in an organotypic hippocampal slice. A short section of the thicker apical dendrite can be seen on the left side of each soma. The basal dendrites are full of dendritic spines, whose density increases with the distance from the soma (scale bar: 10 µm). B) Dendritic spines on a section of a dendrite shown in A) with a high spine density (scale bar: 1 µm). C) A population of 11 spines from hippocampal pyramidal neurons. The spines were segmented from electron microscopy tomography data. The spine head contains the PSD, organelles such as the spine apparatus and vesicles, while the spine neck is considerably thinner than the spine head for most spines. The entire spine is filled with a dense mesh of cytoskeletal filaments and proteins. Figs. A) and B) reproduced from [53] (https://doi.org/10.3389/fpsyt.2016.00101). C) reproduced from [20] (https://doi.org/10.1523/ENEURO.0342-22.2022). A), B) and C) reproduced under the terms of the Creative Commons Attribution 4.0 International license (CC BY 4.0, https://creativecommons.org/licenses/by/4.0/)

Dendritic spines have been shown to play a role in learning [25, 40], and modifications to synaptic strength are thought to be a cellular mechanism for memory formation (Figure 3). The strength of a synapse is strongly correlated with the size and composition of the postsynaptic density (PSD), as well as the size and shape of the spine [5, 29]. A long-lasting increase in synaptic strength is called long-term potentiation (LTP), while a weakening is called long-term depression (LTD) [53, 55]. In addition to morphological changes of single spines, the growth of new spines or the removal of existing spines can also lead to structural changes in the connectivity between neurons (Fig. 4). In both cases, the changes related to synaptic plasticity are accompanied by a reorganization of the actin cytoskeleton [32, 43].

The underlying biochemical processes of synaptic changes in spines are triggered by calcium, which functions as a second messenger (Figure 4). The magnitude of changes in synaptic efficiency is correlated with the rise in calcium concentration. The direction of changes in synaptic strength, however, is determined by the temporal order of pre- and postsynaptic action potentials [41]. Presynaptic activity, i.e., the action potential, leads to the release of neurotransmitters from the presynaptic side, followed by the activation of postsynaptic ion channels. The influx of sodium through AMPA receptors causes a fast depolarization of the spine membrane. Subsequently, slower voltage-dependent NMDA-

type glutamate receptors (NMDARs) open, which are the main pathway for calcium influx from the extracellular space [11].

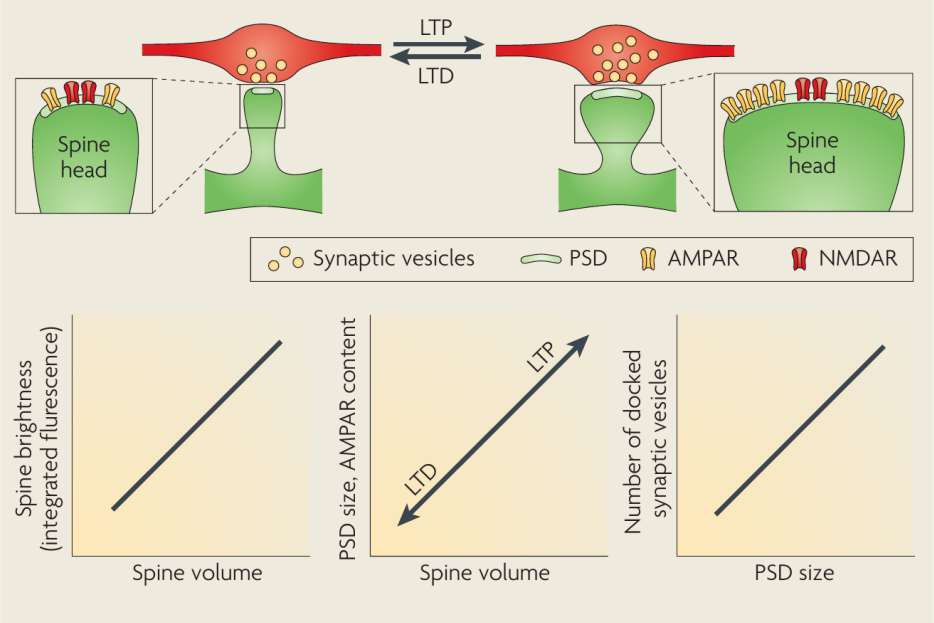

Figure 3: **Synaptic plasticity - Phenomena**
The presynaptic axon terminal contains neurotransmitters, such as glutamate, which are stored inside the presynaptic vesicles. Glutamate activates postsynaptic AMPAR channels. NMDAR channels are activated by glutamate and a coinciding depolarization of the postsynaptic membrane. The strength of a synapse is correlated with the spine volume, the size of the PSD, and the number of AMPAR channels present, as well as the number of presynaptic vesicles that are released into the synaptic cleft in response to a presynaptic action potential. Long-lasting modifications of synaptic strength, called LTP and LTD, are important for learning. Figure reproduced from [29] (https://doi.org/10.1038/nrn2699) with permission from SNCSC (Springer Nature); permission conveyed through Copyright Clearance Center, Inc. (license number 5518780435933). Copyright © 1969, Nature Publishing Group

Usually, action potentials are initiated at the axon hillock and travel down the axon. But in pyramidal cells (and also other cell types) the action potential can propagate back into the dendritic tree and into dendritic spines as a back-propagating action potential (bAP) [50]. This leads to a depolarization of the spine membrane caused by postsynaptic activity. Another type of postsynaptic event that can depolarize the spine head is the dendritic spike. A dendritic spike is similar to an action potential generated in the dendritic tree. This is usually a local event in a dendritic branch and occurs after strong localized input to a dendrite [48]. Different types of dendritic spikes exist; they can be short in duration in the case of dendritic sodium spikes or last tens of milliseconds in the case of calcium or NMDA spikes [36]. Calcium spikes can also also spread across larger regions of the apical dendrite [51].

Since the discovery of dendritic spines by Ramón y Cajal more than a century ago [14, 22], electrical and chemical signals in spines have been intensely studied. The interaction between synaptic input and bAPs or dendritic spikes in spines has been found to be important for the induction of synaptic plasticity, as well as in shaping synaptic integration [35]. However, how exactly this works is still under debate, and the exact function of spines remains far from being understood.

It is well accepted that the spine neck chemically isolates the spine head from the den-

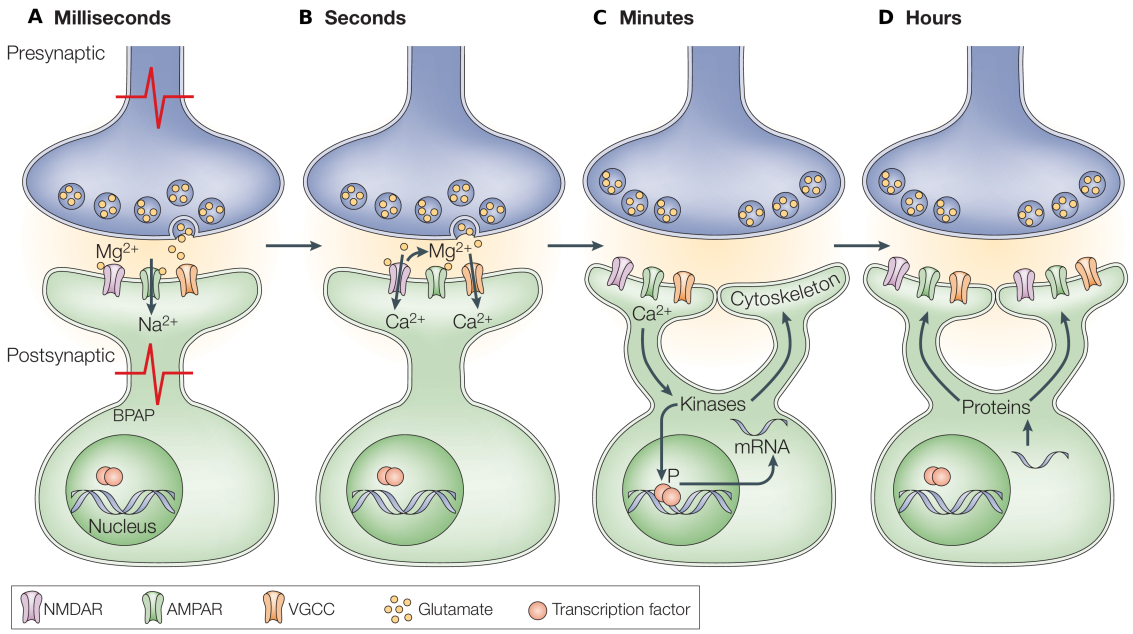

Figure 4: **Synaptic plasticity - Mechanisms**
A) Presynaptic action potentials lead to a release of neurotransmitters from the presynaptic side into the synaptic cleft. This activates postsynaptic AMPARs. The influx of sodium depolarizes the postsynaptic membrane of the spine. The spine head can also be depolarized by bAPs or dendritic spikes generated in the postsynaptic neuron. B) The depolarization lifts the magnesium block of NMDARs, which allows glutamate to activate NMDARs. Calcium will enter through NMDARs and voltage gated calcium channels (VGCCs). C) Calcium is a second messenger and activates several intracellular processes. In response to an elevated calcium concentration structural changes at the synapse occur. It affects the transcription of RNA, but also leads to morphological changes of the spine through cytoskeletal regulation. In turn this leads to a change of the size of the spine but can also induce the formation of new synapses. D) Newly synthesized proteins are transported to the spines and contribute to long lasting changes in the synapse. (Figure reproduced from [35] (https://doi.org/10.1038/nrn1301) with permission from SNCSC (Springer Nature); permission conveyed through Copyright Clearance Center, Inc. (lincense number 5519300417959). Copyright © 2004, Nature Publishing Group)

drite [53]. This allows for the control and maintenance of different calcium concentrations in the head compared to the parent dendrite. The formation of chemical compartments is, therefore, essential to regulate input-specific synaptic plasticity [23, 41]. Whether spines also form electrical compartments depends on the electrical resistance of the spine neck. If the spine neck resistance is high, the depolarization of the spine head and dendrite can differ by several millivolts [12, 19]. In this case, EPSPs are significantly attenuated when traveling from the spine head into the dendrite. Interestingly, this is not the case for electrical signals traveling in the other direction. BAPs and dendritic spikes fully invade the spine head without attenuation (see Fig. 5 and [33]).

But accurately measuring the electrical resistance of the spine neck is challenging. The isolation of the spine head from the dendrite and the small size makes voltage clamping of the spine impossible [8]. Consequently, the spine neck resistance can only be measured indirectly, based on a combination of theoretical models and different experimental methods [52]. Unfortunately, the experimental results remain inconsistent and measurements of the spine neck resistance of pyramidal neurons [1, 19] range from 27 [46] to 500 [24] megaohms and vary by an order of magnitude.

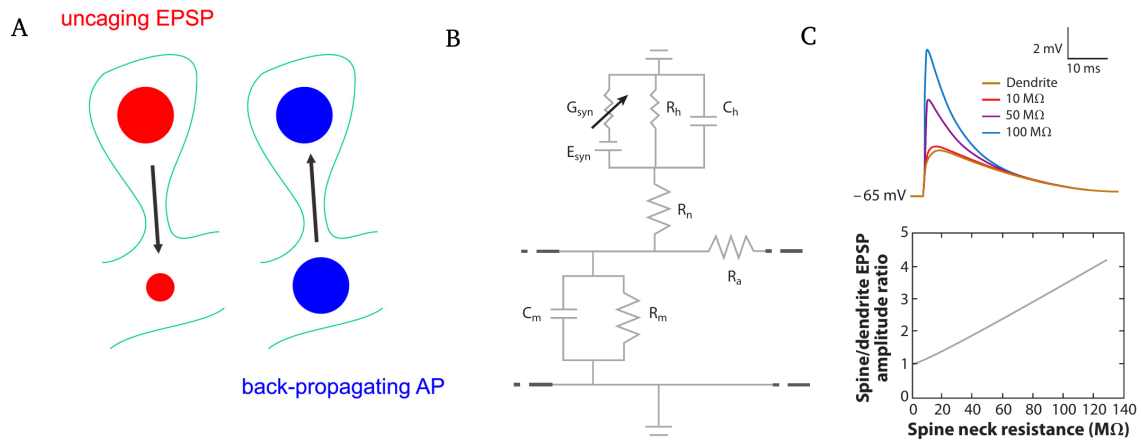

Figure 5: **Dendritic spines - Electrical properties**
A) Dendritic spines are assumed to form electrical compartments. EPSPs are attenuated in the dendrite, but a depolarization of the parent dendrite fully invades the dendritic spine. B) Simple electrical models of dendritic spines consider the electrical resistance of the spine neck to be the central parameter. C) However, measurements of the spine neck resistance are highly variable and differ from experiment to experiment. Depending on the spine neck resistance, the model in B) predicts different degrees of electrical compartmentalization of the spine. A) reproduced from [33] (https://doi.org/10.1016/j.celrep.2017.07.012) under the terms of the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY-NC-ND 4.0, https://creativecommons.org/licenses/by-nc-nd/4.0/). B) and C) used with permission of Annual Reviews, Inc., from [55] (https://doi.org/10.1146/annurev-neuro-062111-150455); permission conveyed through Copyright Clearance Center, Inc., Licence ID 1340305-1. Copyright © 2013 by Annual Reviews.

However, what could account for the high variability in measurements of the spine neck resistance?

Besides technical issues in the experiments, theoretical problems are also likely contributors. Since the small size of dendritic spines prevents accurate measurements of relevant parameters, model parameters are often unconstrained, which can impede a reliable interpretation of experimental results. Moreover, the conclusions based on numerical simulations are often uncertain since the outcomes highly depend on the model parameters [53].

In many cases, it is even unclear how to determine the relevant variables of the system under study [4]. For example, there are indications that ion concentrations could change in spine necks [34, 47], but this is usually ignored by most models [9, 19, 55].

Furthermore, models of dendritic spines could be based on invalid assumptions. Previous studies have proposed that the common neuroscientific models, such as cable theory, compartmental models, or Goldman–Hodgkin–Huxley–Katz models, might not be applicable to dendritic spines [15, 16, 17, 28].

As a result, it has not been possible to capture the collective behavior and the relationship between various components across different scales in dendritic spines so far.

Confronting these challenges, the main objective of this thesis is to establish a theoretical foundation to model dendritic spines. To accomplish this, different aspects of the structure and function of the dendritic tree and dendritic spines across various scales are studied and interconnected. The goal is to develop a simple but well-founded biophysical model of dendritic spines, step by step.

To establish a solid foundation for subsequent studies of dendritic spines, we investi-

gated the integration of synaptic input in the dendritic tree of a pyramidal cell model in the first peer-reviewed manuscript of this thesis [21]. The computer model incorporated dendritic spikes, backpropagating action potentials, various ion channels, and a realistic dendritic morphology [45]. We found that calcium spikes influenced the integration of synaptic inputs in individual dendritic branches, particularly in the tuft dendrites. However, the model did not include dendritic spines as the synapses were situated on the dendritic segments. This study provided essential insights into the computational properties of the dendritic tree and laid the groundwork for a better understanding of the role of dendritic spines in hippocampal pyramidal cells.

In the following, we shifted the focus to dendritic spines. The second peer-reviewed manuscript contains a study of the internal organization of dendritic spines. We statistically quantified the structure of the actin cytoskeleton based on data from electron microscopic tomography. We found that the entire spine is filled with a uniform and dense actin cytoskeleton and provide an accurate quantitative description of the internal organization in spines.

The third manuscript studies the effect of the spine's internal organization on biophysical parameters relevant to model ion concentrations and currents. We apply a method called homogenization to estimate the effective values of the biophysical parameters diffusion and permittivity based on the intracellular organization of dendritic spines. This results in effective Poisson-Nernst-Planck (PNP) equations.

In the fourth manuscript, we investigate the profiles of the ion concentrations and the electric potential in dendritic spines using PNP equations. We find contradictions to results from previous literature and quantitatively demonstrate that spines are well assumed as electro-neutral. Moreover, we show that important principles underlying cable theory are also valid in dendritic spines.

In the fifth and final manuscript, we present an extension of cable theory to accurately model dendritic spines. We confirm previous results that ionic concentrations in spines are transient during synaptic input and identify a new mechanism that will affect the spine membrane potential and calcium influx through NMDARs in dendritic spines.

We think that integrating the proposed spine model into compartmental neurons will help to better understand the role of dendritic spines in synaptic plasticity and synaptic integration.

3 Results

3.1 Manuscript 1

Tuft dendrites of pyramidal neurons operate as feedback-modulated functional subunits

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Author contributions:
F.E: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft
A.H.: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing
S.H.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

3.2 Manuscript 2

A Uniform and Isotropic Cytoskeletal Tiling Fills Dendritic Spines

Florian Eberhardt[1], Eric A. Bushong[2,3], Sébastien Phan[2,3], Steven Peltier[2,3], Pablo Monteagudo-Mesas[1], Tino Weinkauf[4], Andreas V. M. Herz[1], Martin Stemmler[1] and Mark Ellisman[2,3]

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Author contributions: F.E., S.Pe., A.V.M.H., M.S., and M.E. designed research; F.E., E.A.B., S.Ph., S.Pe., P.M.-M., T.W., M.S., and M.E. performed research; F.E., S.Pe., P.M.-M., M.S., and M.E. analyzed data; F.E., S.Ph., A.V. M.H., M.S., and M.E. wrote the paper

3.3 Manuscript 3

Quantifying the Influence of the Actin Cytoskeleton on Ion Transport in Dendritic Spines by Homogenization of the Poisson-Nernst-Planck Equations

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Author contributions: All work was performed by F.E.

3.4 Manuscript 4

Parameters of Cable Theory Are Mostly Unaffected by the Geometry of Dendritic Spines

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Author contributions: All work was performed by F.E.

3.5 Manuscript 5

Ion-Concentration Gradients During Synaptic Input Increase the Voltage Depolarization in Dendritic Spines

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Available at: https://www.biorxiv.org/content/10.1101/2023.08.03.551788v1

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Author contributions: All work was performed by F.E.

4 Discussion

Dendritic spines are small structures located on the dendrites of neurons. They are an integral part of the connections between billions of neurons and are thus essential to the highly complex nervous system. Due to the interactions between the constituents of a complex system, the function of a system cannot be understood solely from the individual components [18]. Likewise, the function of dendritic spines cannot be fully understood without considering their interactions with other elements across different scales, including molecular organization, morphology, and the dendritic tree.

Despite their crucial role in the nervous system, many aspects of dendritic spines' function and role remain under debate. The objective of this thesis is to investigate various facets of the structure and function of dendritic spines and dendrites, with the aim of developing simplified yet accurate descriptions of the systems under study. By evaluating the relationship between the studied aspects, this work seeks to achieve a better understanding of dendritic spines. Ultimately, the goal is to improve our understanding of the role of spines in synaptic integration and plasticity.

In the first manuscript, we demonstrate that a simple two-layer model can effectively capture the integrative properties of pyramidal cells. Despite the elaborate morphology of the dendritic tree of a pyramidal cell, which includes various branches and ion channels [44], computational sub-units can be found in both proximal and basal dendrites [45]. We further identify calcium currents as a primary factor leading to the loss of independence of sub-units in the apical dendrites, which occur mainly in the tuft dendrites [38, 51]. However, the impact on computation can be captured by incorporating feedback sub-units into the two-layer model, again leading to a simplified description of the neuron. This caricature of a neuron is clearly different from a real cell, but it captures well the integration of synaptic input. The presented work demonstrates how simplified models can aid in better understanding specific aspects of complex systems by removing redundant variables and fixing interactions with other components of the system, such as synaptic input.

Finding a simplified description of the object of study and reducing dimensionality can also be achieved through statistical analysis. The crucial task in this regard is to identify meaningful statistical measures that can be applied to the studied object. If the statistical measures contain sufficient information, it should be possible to reconstruct a representative structure. Due to the permanent polymerization and depolymerization of the actin filaments, the exact position and length of individual filaments are not relevant when analyzing the actin cytoskeleton. Instead, properties of the mesh that are independent of the particular arrangement of each filament must be considered. In the second manuscript, this was achieved by computing distributions of distances, orientations, and lengths of nodes and branches of the mesh. The results provide information on the structure of the spine-cytoskeleton, the involved actin-related proteins [43], and the mechanical properties of the mesh [27]. This quantification also allows for comparison between different studies and can be related to functional differences [54] and morphological differences [5] between spines in future studies.

A question related to the electrical function of spines that arises after quantifying the spine's intracellular space is: how does the intracellular structure affect ionic fluxes in dendritic spines? One option to answer this question would be to set up a sophisticated biophysical simulation that includes the actin filaments at a nanometer resolution. However, this would require sophisticated software and high-performance computing clusters. An alternative approach is used in the third manuscript: replacing the complicated structure with a simpler one while keeping the relevant parameters unchanged. This can be accomplished by applying a method called homogenization to PNP-equations [49]. The result is a set of

partial differential equations in which the intracellular space is treated as a homogeneous material, but the impact of the nanoscopic organization is still considered. Effectively, the isotropic intracellular structure leads to a reduction of permittivity and diffusion inside the spine.

In the context of modelling neuroscientific systems, determining the essential and irrelevant variables to accurately describe the system under study is a crucial task. In the fourth manuscript, the validity of electrical parameters and underlying assumptions of cable theory for dendritic spines is investigated, to identify the minimal set of variables needed to accurately model spines. The study confirmed that regardless of spine geometry, the surface of the membrane and basic geometric measures such as volume or radius are sufficient to estimate the electrical parameters of the spine. Higher-order terms, such as the membrane curvature proposed in [17], can be ignored. Moreover, physiological ion concentrations are important to capture the electrical function of the membrane.

Once the minimal set of variables is identified to accurately describe a system, additional degrees of freedom do not necessarily improve the results, and removing essential variables can lead to contradictory results. This is particularly important in the case of spines.

Simplified electrical models predict a linear dependence of neck resistance and spine/dendrite EPSP amplitude ratio, as shown in Introduction Fig. 5C and in [55]. However, it has become evident that such simple electrical models do not include all relevant parameters to accurately capture the electrical behavior of the spine [28, 47].

Increasing the complexity of the model, for instance with high-resolution finite element simulations based on PNP-equations does not necessarily solve the problems of reduced models, as seen in the case of neck resistance in [12]. The estimation of spine neck resistance in [12] almost perfectly coincides with the simple model presented in [55]. On the other hand, changing essential variables such as ion concentrations can lead to highly questionable speculations about electroneutrality, as presented in [28].

In the fifth and final manuscript, we set up a computer simulation of a dendritic spine. Based on our previous results, it contains the relevant variables to study electric currents in dendritic spines. The presented model can be considered an extension of cable theory, but compared to cable theory, this model contains the time-dependent concentrations of various ion species. Interestingly, a very similar approach was used by Lagache et al. [34]. In fact, the model presented there is based on the same equations but still arrives at contradicting results. For example, Lagache et al. predict a decrease in the neck resistance during an EPSP, whereas we rather expect an increase. The discrepancy is due to subtle differences in the models. While we allow for different diffusion constants of potassium, chloride, and sodium and a reduced concentration of free anions, [34] considers anions and cations of the same diffusivity and concentration. The comparison indicates that the diffusion constants and concentrations of the individual ion species are important variables to understand the electric function of spines. Moreover, it points out that even if relevant variables are known, the outcome of numerical simulations highly depends on the choice of the model parameters.

In experiments, the contradicting predictions provide an opportunity to test the models and identify incorrect assumptions. The fact that very similar models lead to contradicting conclusions could also help explain why measurements of spine neck resistance are still at odds [24, 52]. Due to the small volume of the spine, direct measurements of spine neck resistance are not possible, and the results are always based on indirect measurements in combination with theoretical models of the spine neck. It could well be that the different experimental setups are not the only reason for greatly diverging results but also that the underlying theoretical models are not compatible.

For example, fluorescence recovery after photobleaching (FRAP) experiments are often

used to estimate the neck resistance [1, 52]. However, the numbers are based on the diffusion times of dye and the intracellular resistivity in the neck. Both variables could depend on the individual intracellular organization of the spines. In addition, the resistivity of the cytosol could change during an EPSP as seen in the fifth manuscript and in [34]. Other measurements of the neck resistance are based on voltage-sensitive dyes [19] or calcium imaging [8]. In this case, the estimation of the neck resistance is usually based on a simple voltage divider equation, where the spine neck and dendrite are represented as ohmic resistors, and the additional assumption that synaptic current always equals the drift current through the neck. But due to the diffusion of ions, a simple application of Ohm's law might lead to faulty estimates (see manuscript 5).

We conclude that a good theoretical understanding is necessary for a better interpretation of experimental results in the spine. There should be a stronger focus on testing the underlying assumptions of measurements on the spine neck resistance but also other variables as synaptic currents, and calcium concentrations in future experiments and simulations. Without accurate biophysical models, the interpretations of the found results can be misleading.

The work presented in this thesis can be seen as a solid foundation for further development of more realistic models of dendritic spines. To extend the model in the future, additional variables need to be taken into account. One important question is the impact of calcium on the membrane potential, which is known to be a significant factor for the dendritic membrane potential [36, 51]. However, considering calcium in spines is complicated. Some spines contain a spine apparatus [31, 10], which acts as a calcium store. In addition, several calcium-binding proteins rapidly regulate the amount of free unbound calcium in the cytosol [26].

Another important factor in estimating electric currents is the high diversity of ion channels present in dendritic spines. Next to ionotropic glutamate receptors, voltage-gated calcium [24], or sodium [2] channels exist.

Finally, dendritic spines exhibit great morphological diversity. Significant differences exist not only between individual neuron types but also within a single neuron [20]. These differences in the spine morphology are known to influence the degree of functional compartmentalization [53, 55], leading to speculations, as for example stated in [37], that "these morphologically dependent degrees of compartmentalization lead to distinct states of metaplasticity at individual synapses".

Additionally, the presence of organelles (e.g., spine apparatus) in the spine neck can significantly change the electrical properties of a spine [13]. However, the distribution and location of these organelles are individually different [6], further supporting the idea that spines can be in different functional states.

We hope that the results presented here will help integrate models of spines with other models, better understand the role of spines in single neurons, and improve the interpretation of experimental results.

The following studies were cited in the Introduction and Discussion sections of this thesis.

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