University of Groningen

From skin to brain

de Wiljes, Ot

DOI:
[10.33612/diss.123420732](https://doi.org/10.33612/diss.123420732)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
de Wiljes, O. (2020). *From skin to brain: modelling a whole-body coordination scenario of nervous system origin*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.
<https://doi.org/10.33612/diss.123420732>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

From Skin to Brain

Modelling a whole-body coordination scenario of nervous system origin

Oltman Ottes de Wiljes

The research described within this thesis was funded by NWO.

Copyright O. O. de Wiljes, except Chapters 3 and 4 and any reproduced illustrations.

License granted to Rijksuniversiteit Groningen according to the standard PhD-thesis license agreement.

ISBN 978-94-034-2612-9 (ebook)

Cover: detail of a visualization of a model as treated in this thesis, showing a wave front of activation travelling across the outside of the booklet, bottom to top. Darker red means a more recent action potential. 10% of cells possess elongations of length 4 (in terms of cells) in a random direction, allowing distant cells whose elongations intersect to connect. All cells affect their neighbours. This is the combination of parameters shown as case ‘B’ in the figures of Chapter 5.

This represents the equivalent of an excitable epithelium with a rudimentary nerve net.

university of
 groningen

From Skin to Brain

Modelling a whole-body coordination scenario of nervous system
origin

PhD thesis

to obtain the degree of PhD at the
University of Groningen
on the authority of the
Rector Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on

Monday 4 May 2020 at 12:45 hours

by

Oltman Ottes de Wiljes

born on 30 December 1982
in Utrecht

Supervisors

Dr. F.A. Keijzer
Prof. J.W. Romeijn

Co-supervisor

Dr. R.A.J. van Elburg

Assessment Committee

Prof. L.C. Verbrugge
Prof. G. Jékely
Prof. P. Godfrey-Smith

This thesis is dedicated to Tonny Albert Springer (1926–2011).

Contents

Introduction	1
1 Theorizing about nervous system evolution	5
1.1 Introduction	5
1.2 Nervous systems and their missing links	5
1.2.1 An explanatory gap	8
1.3 A lineage explanation for early nervous systems	10
1.4 An overview of theoretical proposals	13
1.4.1 Early theories	14
1.4.2 Modern theories	17
1.5 Two views on the production requirement	21
1.5.1 Neuron-focussed production: input-output	21
1.5.2 Nervous system-focussed production: internal coordination	22
1.5.3 Problems with the input-output approach	23
1.5.4 A simulation of the input-output view	24
1.6 Modelling nervous system origin	25
1.6.1 Formal models	25
1.6.2 A simulation of the internal coordination view	26
1.7 Conclusion	27
2 Constraints on a lineage explanation of nervous system origin	29
2.1 Introduction	29
2.2 Basal phyla	30
2.2.1 Eukaryote basis: Choanoflagellata	31
2.2.2 Porifera	31
2.2.3 Placozoa	33
2.2.4 Ctenophora	33
2.2.5 Cnidaria	34
2.2.6 Bilateria	35
2.3 Phylogenetics	37
2.3.1 Phylogenetic principles	37
2.3.2 Phylogenetics and phylogenomics	39
2.3.3 Tracking features through phylogeny	41
2.3.4 Crown group and stem group issues	41

2.3.5	Parallel nervous system evolution?	43
2.3.6	Lineage explanation constraints	45
2.4	Precambrian environment and ecology	47
2.4.1	Timing	48
2.4.2	Ecology	51
2.4.3	Ediacaran biota	52
2.4.4	Oxygen	53
2.4.5	Climate and temperature	54
2.4.6	Lineage explanation constraints	56
2.5	Behaviour	56
2.5.1	Behaviour in unicellular organisms	57
2.5.2	Feeding	57
2.5.3	Motility	59
2.5.4	Habitat	61
2.5.5	Lineage explanation constraints	62
2.6	Nervous system morphology and potential precursor tissues	62
2.6.1	Neuronal features	63
2.6.2	Cell & tissue specialization	67
2.6.3	Evo-devo and tissue development	68
2.6.4	Epithelia	69
2.6.5	Eyes	71
2.6.6	Lineage explanation constraints	71
2.7	Conclusion	72
3	Modelling spontaneous activity across an excitable epithelium: support for a coordination scenario of early neural evolution	75
3.1	Introduction	75
3.2	Methods	78
3.2.1	Model	79
3.2.2	Analysis	82
3.3	Results	83
3.4	Discussion	91
4	Modelling the effects of short and random proto-neural elongations	95
4.1	Introduction	95
4.2	Materials and Methods	99
4.2.1	Computational Model	99
4.2.2	Pattern Quantification	99
4.3	Results	101
4.4	Discussion	103
5	Modelling cell differentiation and axodendritic specialization	107
5.1	Introduction	107
5.1.1	Benefits of specialization	108

5.1.2	Modelling specialization	109
5.1.3	Lineage explanation constraints	110
5.1.4	Computational model implementation	111
5.2	Methods	113
5.2.1	Model	113
5.2.2	Analysis	115
5.3	Results	115
5.3.1	Individual model instances	115
5.3.2	Numerical analysis	117
5.4	Discussion	122
5.5	Conclusion	123
	Conclusion	125
	Appendix A: chapter 3 supplementary material	133
	Appendix B: chapter 4 supplementary material	139
	Glossary	145
	Bibliography	151
	Acknowledgements	167
	Curriculum Vitæ	169

Preface

Chapter 3 has been published under the following reference:

De Wiljes, O. O., Van Elburg, R. A. J., Biehl, M., and Keijzer, F. A. (2015). Modeling spontaneous activity across an excitable epithelium: Support for a coordination scenario of early neural evolution. *Frontiers in Computational Neuroscience*, 9.

Chapter 4 has been published under the following reference:

De Wiljes, O. O., Van Elburg, R. A. J., and Keijzer, F. A. (2017). Modelling the effects of short and random proto-neural elongations. *Journal of The Royal Society Interface*, 14(135).

Introduction

Understanding the human brain is one of today's primary scientific challenges. Solving this puzzle will likely allow us to optimize cognitive outcomes, potentially transforming human existence. Treatments and cures for mental illness are another likely result. Understanding human intelligence should allow the construction of true general artificial intelligence as well. The human brain is the most complex instance of a biological nervous system, a far broader category containing a wide variety of forms and functions: jellyfish, arthropod, and vertebrate nervous systems are differently structured and solve widely divergent behavioural problems for their possessors. In spite of these differences, these nervous systems share many characteristics: neurons have the same basic pattern throughout the animal kingdom. These nervous systems allowed the animal kingdom to become a major component of the current biosphere. Biological nervous systems in general are hardly better understood than the human brain. A better understanding of nervous systems in general will certainly help in understanding the human variety. Attempting to place the function and origin of nervous systems immediately leads to one question: how and why did the very first nervous systems evolve? Understanding how the first nervous systems evolved and operated should help to make sense of the basic operations of modern nervous systems.

Such help may be necessary as researchers are finding it hard even to understand how *Caenorhabditis elegans* manages to move about with its 302 neurons (Bargmann, 2012), let alone how it finds mates, avoids danger, and forages effectively. What we seem to miss is an understanding of the basic principles at work within nervous systems, despite having a lot of knowledge of the structure of neurons and their fundamental operation. Systematic research on the questions of how and why the first nervous systems evolved provides an interesting paradigm to investigate in a structured way how nervous systems may operate at a very basic level. Consequently, the central question is the evolutionary one: how and why did they evolve?

Theories of the origin of nervous systems go back to the late nineteenth century and new theories are being proposed up to today. Theorizing started soon after Darwin published his theory of evolution (Anctil, 2015). The initial history of the most prominent early proposals runs from Kleinenberg's ideas, published in 1872, to Mackie's (1970) important text and proposal (Lentz, 1968; Mackie, 1990). This early stage of theorizing used techniques that were limited to a tissue level of organization and it remained impossible to come to more definite claims concerning various options about the most plausible evolutionary trajectory. This changed with the advent of new molecular techniques and

genomics, bringing renewed interest in the question of nervous system origin (Lichtneckert and Reichert, 2007; Miller, 2009). The influx of molecular and genomic techniques allowed the detailed study of molecular differences and similarities between organisms that had few, if any, macroscopic similarities. These methods allow for analyses which are much more clear-cut and quantifiable than the cellular and tissue-level features previously used. In addition, expanding knowledge about the history of life on Earth based on geological evidence has provided a much clearer picture of the context in which nervous systems probably evolved. Pushed by this increase in techniques and knowledge, the study of the origins of the first nervous systems has recently become a major scientific enterprise (Miller, 2009; Moroz et al., 2014; Jékely et al., 2015a; Strausfeld and Hirth, 2015; Kristan Jr, 2016).

Frustratingly, while all these techniques have led to many new ideas and proposals concerning the evolution of the first nervous systems, the macroscopic multicellular organization of the animals involved remains enigmatic and proposals remain difficult to test. Most of the knowledge involved as well as the ways to test this knowledge applies at a molecular level, which does not translate in a self-evident way to the specifics of multicellular animal organization. Thus, while the presence or absence of molecular markers allows the inference of evolutionary relations between widely divergent lineages—including those currently with and without nervous systems—it remains very difficult to trace the morphology of the animal common ancestor at the stage before the origin of nervous systems as their extant descendants are very different. On the one hand, there are organisms without nervous systems like sponges. These are sessile and move only very slowly, if at all, while pumping water through their bodies to filter it for food. On the other hand, basic organisms with nervous systems are free-moving predators like jellyfish that hunt and digest their prey in an inner gut. These organisms are very different in their bodily organization and way of life, and given the lack of intermediate extant animal body plans it remains extremely difficult to get a clear picture of the first innervated (here and beyond used in the narrow meaning of ‘nervous system-having’) common ancestor. Likewise, the intermediate stages that connect the first animal common ancestor to this first innervated animal remain elusive (Arendt et al., 2015; Telford et al., 2015; Cavalier-Smith, 2017).

The result is an explanatory gap between innervated and non-innervated organisms. To provide an evolutionary explanation specifying how and why the first nervous systems evolved, an account is necessary that specifies how this gap was crossed in the deep past by a sequence of multicellular structures, each being ecologically and organizationally viable. Despite the many new insights in the biomolecular characteristics of early animals, difficulties remain in getting a clear grip on this evolutionary sequence (or sequences, if nervous systems arose more than once (Moroz, 2009)).

The present work aims to contribute to the explication how this explanatory gap has been crossed once, or possibly several times at some point in history. As we will discuss below, a variety of approaches and techniques provide handholds on understanding the crossing of this gap. Here, we add computational modelling as another technique. Computational modelling is well developed within computational neuroscience but so far the techniques described in this thesis have not been used to investigate the current problem.

Computational modelling provides a way to assess the characteristics and potential functionality of hypothetical neural configurations that may have constituted intermediate stages of nervous system evolution. Potentially relevant features can thus be tried out in a virtual way. Given the lack of living animal models, computational modelling is one of the few ways to investigate such intermediate bodily configurations.

The first chapter starts with an overview of the problem posed by the evolution of the first nervous system. This overview addresses the definition of neurons and nervous systems, the explanatory gap between animals with and without nervous systems and the explanatory ideal of a lineage explanation to cross this gap. A central point here is the differentiation between two conceptual frameworks for understanding how the very first nervous systems functioned, (a) as a connector between sensors and effectors (an Input-Output (IO) view), or (b) as mainly a muscle-coordination device (Internal Coordination (IC) view). Both interpretations have different repercussions for how the gap could have been crossed. While the characteristics of an IO view are relatively well-known, this is not the case for an IC view. In this chapter, computational modelling is subsequently introduced as a tool to investigate the potentiality of IC-based scenarios specifying how the explanatory gap could have been crossed.

Chapter 2 discusses the wealth of recent empirical findings and theorizing that bear on the question how the first nervous systems arose. Together these ‘data points’ provide the context as well as the constraints on any plausible lineage explanation for the evolution of early nervous systems. This chapter discusses the relevant animal phyla that provide information about the origins of nervous systems, the basic principles of phylogenetics, the state of the world during the relevant period, the kind of behaviour relevant for early nervous systems as well as the morphology of nervous systems and potential precursor tissues.

In Chapter 3 we present and discuss a first series of modelling studies that fit in with the various constraints sketched in the previous chapter. These modelling studies encompass an excitable epithelium rolled into a tube-like body. This epithelium consists of neuron-like excitable cells without elongations and only connected with their nearest neighbours. The aim of the models was to investigate whether, and if so to what extent, such a basic configuration would show coordinated activity across a tube-shaped body. We did find coordination, but in limited forms. In Chapter 4, we report a new series of modelling studies that add short and randomly oriented cell elongations, which have various enhancing effects on coordination across the body-tube. In a final set of studies, described in Chapter 5, further refinements and changes are added—most notably limiting the extensions to only a subset of cells—that further enhanced coordination options.

In the conclusion, we return to the main issue of the explanatory gap and whether and IC view on early nervous system functioning suggests possibilities for crossing it in a plausible evolutionary way. Our modelling studies strongly suggest a positive answer and we formulate two possible routes for bridging the gap, each with its own strengths and weaknesses.

Chapter 1

Theorizing about nervous system evolution

1.1 Introduction

This first chapter provides an overview of the problem posed by the evolution of the first nervous system, positions the problem within its current theoretical context, and introduces computational modelling as a tool to improve our understanding of how the explanatory gap has been crossed. In Section 1.2 we provide a definition of what we mean by ‘nervous systems’ compared to other tissues. This section also argues that the lack of extant intermediate stages constitutes an explanatory gap concerning the evolutionary transition from non-innervated to innervated animal organizations. Section 1.3 introduces Calcott’s (2009) notion of a lineage explanation as a way to articulate what would count as a satisfactory bridging of the explanatory gap introduced in Section 1.2. Section 1.4 provides an overview of early and more recent explanatory proposals for the evolution of the first nervous systems. Section 1.5 provides a more general perspective by differentiating between two general ways in which early nervous systems may have functioned: as input-output devices on one hand and as internal coordination systems on the other. Finally, Section 1.6 introduces the computational approach used and shows why it can be a valuable tool in dealing with the problem of early nervous system evolution.

1.2 Nervous systems and their missing links

To investigate the evolution of nervous systems, we should first specify what is meant by ‘nervous systems’. Nervous systems as discussed here are an exclusively animal feature, where ‘animal’ means ‘belonging to the biological kingdom of the Animalia’. Not all animals have nervous systems, but it is the presence of nervous systems that enabled the current position of the animal kingdom on Earth. We note that the word ‘nervous system’ is sometimes used in a wider sense that is not limited to animals (e.g. in discussions on artificial nervous systems and neural nets), but we will not take such cases into consideration here.

We understand nervous systems in the most general case to consist essentially of neurons, also called nerve cells. The presence of neurons will be used here as the key constitutive factor for a tissue to be considered a nervous system or being a part of a nervous system. Obviously, nervous systems are not only constituted by neurons. There are nervous system cells which are not neurons, such as human astrocytes and glial cells, which do not have synapses or clearly defined axons. Another example can be found in Cnidaria (a group of animals containing, among others, jellyfish, corals, and sea anemones): epithelial conductive cells (Anderson, 1980) present a borderline case of neuron-like cells. These have a neuron-like electrical transmission system as well as a clear information transmission role yet no axodendritic elongations, and their connectivity is based on gap junctions, not chemical synapses like canonical human neurons. See Section 1.2 below for the particulars of (chemical) synapses and gap junctions. Our focusing on neurons and their excitability, synapses, and axodendritic elongations is an explicit simplification, motivated by the importance and ubiquity throughout the animal kingdom of these cells and their features.

Neurons

Neurons are surprisingly consistent in general function and structure across animal groups. They are commonly characterized as cells that receive, transmit and pass on electric signals to other neurons or to effectors. While neurons can take many different shapes, at a general level these three separate aspects stand out as defining features:

- i First, the ability to transmit electrical signals—graded or spiking—across its electrically excitable cell membrane;
- ii Second, the ability to quickly, directly, and specifically interface with other cells through synapses;
- iii Third, the presence of cellular extensions (axons and dendrites) which allow signals to be sent across long distances to be delivered at specific destinations at specific times. We will refer to these extensions as axodendritic elongations.

Electrical excitability in neurons is achieved by actively maintaining differing concentrations of charged ions inside and outside the cell membrane, resulting in an electrical field across the membrane. The ability to manipulate ion concentrations across a cell membrane is not unique to neurons, since all living cells exhibit the ability to maintain differences in ion concentrations between the inside and outside of the cell. Here we describe the mechanisms found in neurons. The best known ionic mechanism involves balances of potassium (K^+) and sodium (Na^+) ions, but other mechanisms such as one using calcium (Ca^{2+}) also exist. Electrical transmission consists of a wave of ionic flux travelling across the cell membrane, allowing for a fast intracellular signal transmission system across the surface of the cell. The most common kind involves the action potential, a spike event. This process starts when a certain threshold in the electrical field is reached. Some cell types reach this threshold without external prompting; some types can reach this threshold via a sufficient level of outside nudging, for example by receptors

which affect ion flow or by direct ionic influx from another cell. When this threshold is reached, charged ions flow across the membrane in a travelling wave of threshold-crossing. This action potential results in depolarization, after which the concentrations need to re-set, a process called repolarization. During repolarization, the cell is less susceptible to threshold-crossing, a situation referred to as the refractory period. Some neurons use graded potentials instead of action potentials. In the case of graded potentials there is no spike event but only manipulation of the potential of the electrical field.

Synapses provide inter-neuron interfaces as well as interfaces between neurons and other tissues. There are two main types: chemical synapses and electrical synapses (also referred to as gap junctions). Chemical synapses are locations where, when triggered by electrical transmission across the membrane, exocytosis of neurotransmitters takes place. Generally, these neurotransmitters diffuse across a synaptic cleft to a postsynaptic receptor on another neuron or other cell. This way, activity can be transmitted between cells. When affecting another neuron, the postsynaptic receptors generally have some effect on the membrane potential, either facilitating or inhibiting a spike event or affecting the graded potential. There are many different neurotransmitters which can have differing effects on the postsynaptic cell, commonly either raising or lowering the probability of the postsynaptic cell crossing the threshold of an action potential. Synapses with potential-raising neurotransmitters are known as excitatory synapses; those with potential-lowering ones are known as inhibitory synapses. While our investigation focusses on chemical synapses (and when referring to ‘synapses’ we mean chemical synapses), we should mention gap junctions, also known as electrical synapses. These involve direct cytoplasmic connections between cells through membrane proteins, the eponymous gap junctions, which allow ions and small messenger molecules to flow directly from one cell into another. This way, a wave of electrical excitation can travel across cells. Chemical synapses are strongly associated with the nervous system. Gap junctions, however, occur in almost all tissues and animal groups (excepting sponges, which do not have them) and are not solely associated with the nervous system though they do occur in it as well.

Axons and dendrites are the spindly extensions of neurons also referred to as processes. These essentially bring the excitable cell membrane to more distant places, allowing waves of electrical excitation to travel across some distance to another location in the animal body. Axons send signals away from the cell body or soma and dendrites send signals towards the cell body (though exceptions to this rule exist). Both axons and dendrites show various degrees of branching, though axons generally exhibit a single main trunk.

Neurons may also exhibit additional features, such as susceptibility to neuromodulation (slow-acting, non-synaptic neurotransmitters diffusing through the nervous system); receptors of various stripes may be considered wholly or partly neuronal and neurons exhibit a large variety of shapes and sizes, but the three features enumerated here are both universal¹ and essential and thus form a good basis for the investigation of nervous

¹Though it must be noted that not all neurons possess all features; for example, some cnidarian nervous systems (for example in the cubozoan *rhopalum*, see Garm et al. (2006); Satterlie (2011)) possess cells that act like neurons but do not have clear elongations. One can also argue that some glandular or sensory tissues are nervous tissue and these may also lack these features. The point here is that all nervous systems have neurons with all three features.

system origin. Since this thesis concerns the transition from non-neural to neural, we often wish to refer to these features without implying the presence of fully-fledged neurons or nervous systems. Consequently, we use the phrase ‘neuroid features’ to refer to these, utilizing the term used by Mackie (1970).

Neural functions

Understanding the origin of nervous systems and neurons requires an account of their function. Interpreting the function of nervous systems and neurons is however more complex than is often acknowledged. The function most generally associated with nervous systems and neurons is behaviour modification: nervous systems enable animals to behave in intelligent ways that are sensitive to a broad variety of environmental features. While organizing behaviour is certainly an essential part of what nervous systems do and it will remain the crucial function targeted here, a broader picture of neural functions should be taken into account (Jékely et al., 2015a). Organizing behaviour itself involves both reacting to stimuli as well as various forms of intrinsic activity and muscle control and thus combines a variety of differentiated functions that will all play a role in an evolutionary account of nervous systems’ origin. In addition, modern nervous systems at least play a variety of roles, most notably the regulation of physiology and development (here ‘development’ is used in the strict sense meaning the progression from the zygote, the single-celled stage any unique individual animal goes through, to the adult stage of the life cycle), but also the regulation of gut activity (Furness and Costa, 1987), our immune system and stress responses (Trakhtenberg and Goldberg, 2011), and even host-microbiome interactions (Klimovich and Bosch, 2018).

Allowing an animal to respond quickly to environmental features is definitely not all that nervous systems do. Nevertheless, organizing behaviour remains plausibly its crucial feature since modern nervous systems are always connected to behavioural control. The modulation of development and physiology can be interpreted as supportive functions: developmental processes building the complex bodies capable of adaptive behaviour; physiological processes maintaining a complex balance between internal bodily requirements and external behavioural ones. Of course it may very well be that non-behavioural functions played a crucial role in the early evolution of nervous systems and the topic will surface whenever necessary below. However, in the present study the main focus will be on the organization of behaviour as the key function of early nervous systems.

1.2.1 An explanatory gap

The evolution of the first nervous systems consists of a series of events that happened a very long time ago and which played out when the most basic evolutionary lineages of the animal kingdom diverged (see Figure 1.1). First consider the five most basic phylogenetic groupings of animal life: Porifera (sponges), Ctenophora (comb jellies), Placozoa, Cnidaria, and Bilateria (a major clade or evolutionary group that contains all other and best known animals, for example flatworms, insects and mammals). Of these five, three (Ctenophora, Cnidaria and all Bilateria) have complete nervous systems and neurons that

Figure 1.1: A phylogenetic tree of the animal kingdom., including the closest non-animal relative, Choanoflagellata. This tree shows the most basal phyla (Porifera, Cnidaria, Placozoa, and Ctenophora) in contrast to all other animals, which together form the Bilateria. Triple forks lines indicate uncertainty about which group split off first.

fulfil the three criteria outlined above (electrical excitability, synapses, and axodendritic elongations) and together they are now referred to as Neuralia (Nielsen, 2008).² Although there are many differences between (and within) these three neuralian groups, at a basic level the organization of neurons and nervous systems is essentially similar: a neuron belonging to a jellyfish is not fundamentally different from a human neuron when it comes to the three main ingredients mentioned above (electrical excitability, synapses, and axodendritic elongations). In contrast, the two remaining phyla (Porifera and Placozoa) have no nervous systems or cells resembling neurons at all.³ The morphological and functional differences between these two non-neural phyla and Neuralia are huge and no currently known animal shows an intermediate condition that provides clear suggestions on how the gap between these two general groupings has been crossed and what the organisms that evolved nervous systems looked like.

The problem is acute for various reasons.

First, the relevant evolutionary events involve contrasts between three fundamentally different groups as exhibited by three different feeding strategies (Sperling and Vinther, 2010): Sponges have a water canal system that is open to the environment. They take up small food particles (microphagy) from the water passing through. The single placozoan species *Trichoplax adhaerens* feeds using external digestion with a ventral sole—the cavity between its ventral surface and the underlying substrate. All neuralians⁴ use some kind of internal gut to digest larger food particles (macrophagy) (Sperling and Vinther, 2010). Thus, the transition to animals with nervous systems also involved a major rehaul in feeding habits and the general functional organization of the animal body.

²Cnidaria and Bilateria together are often referred to as Eumetazoa as well, though this term is confusing as to whether or not it includes Ctenophora, which may be not directly related and may have evolved a nervous system in parallel—see Section 2.3.5.

³But see Leys (2015) and Dunn et al. (2015) for arguments on why we should not jump to conclusions regarding absence of neural mechanisms in sponges.

⁴Excepting secondary loss.

Second, inspection of genomic differences between these groups does not provide clarity regarding the signalling system of a group’s common ancestor. There is no ‘synapse’ gene; no ‘dendrite’ gene; no ‘turn single neuron into nervous system’ gene. The closest things found are genes which code for building blocks used to construct synapses but which are also present in Choanoflagellata (a single-celled sister group to animals, its closest relative) and Hox genes, which specify general body patterning but the role of which in specifying nervous system presence is impossible to tease out between Cnidaria and Bilateria.

Third, the relevant events lie deep in the animal phylogenetic tree and happened a very long time ago. This is additionally complicated by the uncertainty in the order and timing of the relevant events. What is clear is that animals with nervous systems were in existence at the start of the Cambrian, 541Ma (million years ago). Fossils of animals, recognizable ancestors to modern nervous system-possessing clades, with complex, active bodies (Trestman, 2013), compound eyes (Clarkson et al., 2006; Paterson et al., 2011), and even identifiable fossilized nervous tissue (Edgecombe et al., 2015) are a conclusive indication of nervous system presence. The origin of the first nervous systems must necessarily lie before this point in time, certainly after Animalia diverged from Choanoflagellata but before the divergence of Cnidaria and Bilateria. The order of intermediate divergences is in dispute and their timing is unclear on top of that. Whether specific researchers tend to earlier or later divergence depends on whether one takes the fossil evidence or calculations based on molecular phylogenetic data as leading. The differences are quite large: a conservative palaeontologist may estimate nervous system origin to be as recently as 560 Ma (see e.g. Budd, 2015) whereas the lower bound for phylogenomicists may be as long ago as 860 Ma (e.g. Dohrmann and Wörheide, 2017). This is a major influence on the plausibility of different options because an early origin implies rather different ecological circumstances and constraints impacting on the evolutionary transition compared to a late occurrence nearer the beginning of the Cambrian.

From our post-hoc vantage point, there seems to be a large discrepancy between innervated and uninnervated animals. No living representatives of intermediate groups have been found, although some features of animals which also possess fully-featured neurons may reflect structures ancestral to neurons, such as excitable epithelia (Anderson, 1980)—but these modern structures all coexist with full-featured nervous systems. Nevertheless, since evolution is a gradual process, there must have been intermediate stages. Species that embody partly developed neurons and nervous systems can be presumed to have existed at some stage and the explanatory gap that we currently witness just reflects that these intermediate forms are either all extinct or any survivors remain undiscovered. In this way, the organizational transition between uninnervated animals and Neuralia provides an explanatory gap that is waiting for a solution.

1.3 A lineage explanation for early nervous systems

The evolutionary transition from uninnervated to innervated animals involves changes at a range of levels, including changes in signalling molecules (Grimmelikhuijzen et al., 2004; Jékely, 2013), cell types (Arendt, 2008), and cell organization, such as synapses (Ryan

and Grant, 2009). These changes acquire their significance at the organismal level: what did they do for the animal in terms of functionality and resulting evolutionary fitness? We will focus on the level of organismal multicellular architecture: animal morphology. An account of how these changes came together and affected animal life ultimately consists of a specified sequence of evolutionary stages showing how the transition has taken place and how the changes affected the organism in its context.

This sequence is an evolutionary progression, and as such it is limited by specific constraints, dictated by the reality of the process of evolution through natural selection. A hypothesis of how these stages followed each other comprises an evolutionary model, and all evolutionary models should reflect the constraints imposed by evolution through natural selection. First, progression should be gradual: complex structures do not pop into existence all at once; differences between evolutionary stages are small. Second, snapshots of stages in time should contribute to the fitness of the organism in that ecological niche at that time. Evolution blindly follows fitness in the short term: broadly speaking, it cannot reach longer-term fitness maxima through local minima⁵.

To incorporate these constraints, we use the framework of a ‘lineage explanation’ (Calcott, 2009): a specific way to formalize evolutionary models and the two constraints outlined above. A lineage explanation is an evolutionarily valid narrative of how some biological feature came to be.

The sequence of states regarding a particular biological trait is divided into *stages*, the building blocks of a lineage explanation. Taking the eye as an example of a trait, Figure 1.2 illustrates the five distinct stages of eye evolution, from left to right (Nilsson and Pelger, 1994):

- i A triple layer of cells—consisting of transparent cells on top, light-sensitive cells in the middle, and pigmented cells below—functions as a light-sensitive spot, allowing the animal which possesses it to orient itself in the direction where light is coming from - a construction found in current-day sponge larvae;
- ii A depression forms in the middle of the light-sensitive spot, filled with transparent tissue, thus improving the directional resolution of incoming light;
- iii As more depression results in better directional resolution, the depression deepens, resembling a pinhole camera;
- iv Variations in the refractive index of the transparent body result in localized yet immutable lens-like functionality;
- v The aperture and the lens co-locate, improving visual resolution further still.

This lineage explanation works for the eyes of both vertebrates and molluscs, even though their derivation is subtly different. Both groups went through this process with very similar results, as evidenced by superficially similar eyes in vertebrates and cephalopods (a kind of mollusc) (Land and Nilsson, 2012). That this is a case of homoplasy (two

⁵In cases where population is small and selection pressure is limited, genetic drift may carry that population through a local minimum.

Figure 1.2: Stages of eye evolution, adapted from Calcott (2009), in turn adapted from Nilsson and Pelger (1994). (Figure reused with permission.)

functionally similar features with a different origin) instead of homology (two features with the same origin but not necessarily the same function) is proven by the fact that in cephalopods the light-sensitive cells are between the vitreous body and the neural layer of the retina, whereas in vertebrates the neural layer is between the vitreous body and the light-sensitive cells, thus necessitating a blind spot where the nerves need to pass through the light-sensitive cells. Cephalopods do not possess blind spots of this sort.

The trajectory of a biological feature or trait through evolutionary space is a continuous succession of stages. This procession is constrained by reality: change needs to be gradual, and each stage should be viable. Calcott (2009) calls these constraints the *continuity requirement* and *production requirement*, respectively.

- Continuity refers to the distance between stages. It is indicated in Figure 1.2 by the horizontal arrow. Evolutionary progression should be gradual, so in order to conform to the continuity requirement, the differences between stages in terms of morphology or genetic and developmental mechanisms required should be minimal. In the example of the eye, the morphological distance between stages is limited, so the lineage explanation of eyes holds in this regard.
- Production refers to the fact that all intermediate stages need to be functional: the feature needs to work. This is shown in Figure 1.2 by the vertical arrow, indicating that all morphologies must be ecologically viable in order to be accepted as part of the explanation. Evolution is not goal-directed and will not pass through less fit stages to reach a global optimum later on. Each separate stage needs to be viable in its own way. Still, the *kind* of useful, fitness-providing functionality need not be constant across stages: a trait may be useful for one thing in one stage and for something else in another stage. An example of a feature changing roles is feathers (Prum, 1999): the first stages of feather evolution did not aid or enable flight, but likely served as thermal regulation. As long as the function is evolutionarily adaptive in some way within a stage, the production requirement is satisfied. It is

important to stress that the production requirement is sensitive to environmental and ecological factors and involves two parts: first, the morphological mechanism needs to be able to perform a function; second, that function needs to be adaptive given the ecological reality.

Formulating a complete lineage explanation for the evolution of the first nervous systems constitutes a major scientific goal that requires dealing with many different kinds of difficulties. For present purposes it is important to differentiate two issues. First, there is the theoretical project of formulating a historical sequence of stages that constitutes a hypothetical lineage explanation, as well as providing an account how the various stages fulfil the production requirements. Second, whether any such proposal can be considered as a plausible option for a lineage explanation will depend on a broad variety of scientific work involving many different fields that will provide scientific claims that will either support or detract from that proposal. Relevant claims derive, for example, from work on phylogenetics—which itself is based on molecular and fossil studies but also on more theoretical work on molecular clocks—palaeoecology, morphology of the various phyla involved, developmental patterning, neural functioning and so forth. A lineage proposal must be consistent with the existing knowledge that can be brought to bear on the relevant events.

This thesis aims to contribute to the theoretical project of formulating a historically possible and plausible sequence of stages amounting to a lineage explanation proposal. The relevant scientific data regarding nervous system evolution will also be considered by discussing the most significant contributions and casting them as a series of constraints on potential lineage explanations (see chapter 2). Before we turn to constraints, we will turn to a discussion on earlier theoretical treatments of the origin of nervous systems.

1.4 An overview of theoretical proposals

The origin of nervous systems became a focus of evolutionary and physiological research soon after Darwin’s theory of evolution was published, leading to a series of proposals on how the evolutionary transition might have taken place (Anctil, 2015). The standard history of this early work starts with nineteenth-century proposals from Kleinenberg and the Hertwig brothers. The subsequent key figure in the twentieth century was Parker (1919) who provided a conceptually very convincing evolutionary sequence of steps leading to the first nervous systems. This proposal was later criticized by Pantin (1956) and Passano (1963), while, finally, Mackie (1970) provided an advanced account of Parker’s sequence, delivering the last major contribution of this early stage of theorizing (Lichtneckert and Reichert, 2007; Moroz, 2014; Anctil, 2015). All these early proposals relied exclusively on anatomical and physiological considerations. This paucity of reference points left a great deal of uncertainty, providing only limited constraints on theorizing. This made it difficult to test these proposals in a concrete way.

As Mackie predicted, the empirical situation changed from the nineteen-nineties onward (Mackie, 1990; Lichtneckert and Reichert, 2007). New molecular techniques enabled

more robust accounts concerning the structure of the deep branches of the animal phylogenetic tree, as well as a much clearer view on the molecular tools available at early stages of animal evolution (Dunn et al., 2015; Arendt et al., 2015; Dunn et al., 2018). Such data allowed theories of early nervous system evolution to be based on a much wider and more reliable set of relevant findings. For example: “A comparative genetic approach including cnidarians and ctenophores as well as different bilaterian groups may help to reconstruct different aspects of the first nervous system in evolution” (Lichtneckert and Reichert, 2007, p.310). With these new techniques, a new phase in the investigation of the evolution of early nervous systems came into being and a lot of new work has been done in recent years. Interestingly, some of the older theories remain influential, as they provide the context or starting point for later proposals.

The present section aims to provide an overview of both the older and more recent ideas and proposals for the stages involved in the evolution of the first nervous systems. The aim is to provide a historically ordered overview of these proposals, organized around the most relevant authors connected to these proposals. Some of the proposals stress the continuity requirement; others the production requirement.

1.4.1 Early theories

Parker

While Parker was not the first to write on the origin of nervous systems, he provided an account that became very influential (Parker, 1919). Parker’s evolutionary model is at heart still a compelling one for all its simplicity and parsimony given the data available at the time. The means available were limited: DNA was an unknown concept, electron microscopy would not be invented for another twelve years, so all Parker had at his disposal were comparative morphology, physiological and behavioural experiments, and optical microscopy. Parker’s evolutionary model proposes three crucial stages in the evolution of nervous systems:

- i In the first stage, the ancestor would have independent effectors, which is a term he uses to refer to cells which are both sensitive to the environment and adjacent to other cells, to which signals can be sent. He finds evidence of these cells in Porifera, which are uninnervated.
- ii In the second stage, the ancestor would exhibit receptor-effector pairs: a connected pair of cells, one with a sensory function on the outside of the animal, one with an effecting function on the inside of the animal.

He does not find explicit evidence of structures like these, but this is in and of itself not a problem for the theory: ‘missing links’ are ubiquitous in evolutionary biology, and eventually they tend to be found by scientific progress. This receptor-effector structure is Parker’s way of filling the explanatory gap. Presenting this structures would have made his argument much stronger, but he explicitly states that he has not identified it (Parker, 1919, p.200).

- iii Receptors connected to effectors by a third cell: a neuron forming a reflex arc, itself integrated with other neurons in a diffuse manner. This reflex arc would provide the benefits of such, allowing its possessor to exhibit reflexes. Such a system of neurons connecting sensors, effectors, and one another constitutes a nervous system, but not a centralized one.

Parker's account was heavily influenced by the notion of the reflex arc, as described in Sir Charles Sherrington's then recent book, 'The Integrative Action of the Nervous System' (1906). The notion of a reflex was a major organizing principle for understanding how nervous systems operated in order to produce behaviour and Parker's account was tailored to explain how the fundamental machinery for reflexes would have arisen during evolution.

In terms of the continuity and production requirements, Parker's proposal primarily stresses continuity: the steps between stages are clearly gradual and not too large. It is not a stretch to state that Parker added the middle stage as an evolutionary interpolation between the first undifferentiated stage and the third stage, with its reflex arc: there is no compelling functional reason for the second stage, and Parker explicitly lacked any evidence for it.

Pantin

The next important step in the formation of evolutionary theories of the origin of nervous systems came in the nineteen-fifties. Pantin provided two major insights: one pertaining to the production requirement and one regarding continuity.

First, we discuss Pantin's contribution to satisfying the production requirement: he investigates the functional affordances of intermediate systems, writing: "[a sensory-nervous network's] primary advantage is that it increased the area of the muscle sheet which is excited by a local stimulus." (Pantin, 1956, p.175) Just connecting a single point on the surface to a small number of muscle cells as Parker's intermediate stage implies does not confer this advantage: the stimulus remains local. By supposing connection to one another instead of local connection, a comparatively simple system of connected excitable and contractile cells would already surpass localisation without requiring any evolutionarily involved specialization. This way, Pantin puts the functional benefit of networked local interaction ahead of connecting over a distance with elongations. He satisfies the production requirement by showing the coordinative benefit of networked local connectedness.

Second, Pantin also contributes to continuity in nervous system evolution: he is the first to make the point that the electrical and chemical characteristics of nerve cells are not evolutionarily novel. While the specific properties of these electrical and chemical mechanisms in nervous systems will be explained further in chapter 2, the point Pantin makes is primarily important in a phylogenetic sense. He remarks:

[The chemical and electrical changes in nerve cells] are natural and incidental properties of cells which, as it were by accident, can be utilised for the construction of behaviour machines. (Pantin, 1956, p.172)

This means that a possible way for nervous systems to have originated is evolutionary pressure in the direction of better coordination latching on to these already existing phenomena and, over many generations, shaping them into something that improves control and coordination. This would have happened by changing, by way of minute variation, features which are already present and functional: changes in electrophysiology and chemical exocytosis. These features would have given cells the mechanisms needed to pass messages to their neighbours. These messages would allow the animal to behave without being restricted to localised responses. Pointing out that the building blocks were present saves the lineage explanation from having to explain their emergence, allowing less change between stages.

Passano

Passano, in an influential paper (Passano, 1963) further specifies Pantin's exploration of the functional benefit of intermediate stages of nervous system evolution, thus also contributing to satisfying the production requirement. Passano adds the concept of pace-makers to the discussion. He begins by agreeing with Pantin on the importance of an integrated muscle sheet before sensor cells:

Inputs of many receptors must merge on common "coordinators" before integration is achieved. Integration is as fundamental as conduction to any nervous system, a message also stressed by Sherrington. An organism at the phylogenetic stage showing isolated Parkerian triads, neither integrating nor conducting to more than a single effector, would have no advantage over a previous stage without such triads, and thus is evolutionarily implausible. (Passano, 1963, p.307)

He explains the formation of effector complexes by way of muscular cells specialising into pacemakers. After the evolution of the individual muscle cell, pace-makers and conducting myoid (muscle-like) cells arose, followed by a differentiation into cells mostly for moving and cells mostly for initiation and conduction, thereby allowing larger muscular structures to be coordinated. This would have happened locally at first, but organism-wide later on, adding different levels of pacemakers along the line, thereby providing the organism with ever wider ranges of response.

He supports this evolutionary model by referring to a number of rhythmic, recurring behaviour patterns in the species in which this specialisation first took place, the Cnidaria. The Cnidaria, at the time considered the most basal innervated phylum⁶ (a taxonomic rank below kingdom), have swimming movements regulated by different patterns of rhythmic pulses. Depending on the circumstances, these rhythmic patterns can overlap or supplant one another, resulting in a wide range of behavioural programs with only a small number of pace-maker systems. Passano's contribution clearly highlights the importance in terms of production of systemic behaviour regulation and endogenous activity.

⁶While Ctenophora may turn out to be more basal in the end, Cnidaria are certainly basal enough as well, so Passano's point still stands in that regard.

Mackie

Mackie postulates his evolutionary model in his 1970 paper, ‘Neuroid conduction and the evolution of conducting tissues.’, and after him, we use the word ‘neuroid’ to refer to features and structures like neurons which are not neurons. He based this account on his earlier discovery of epithelial conduction (Mackie, 1965). Such epithelia in cnidarians are capable of electrical conduction as well as contraction. Mackie’s theory proposes four distinct stages:

- i At first, a myoepithelial tissue sheet consisting of a single type of cell. These cells contract, sense, conduct and function as pacemakers;
- ii Followed by sinking in of contractile cells, retaining an upper layer of primarily sensing and conducting cells;
- iii A type of cell specialising in connecting the excitation of the external epithelium inside, to the myoid cells;
- v Specialisation of these connecting cells into sensory roles and connective roles, including secondary formation of networks of such cells.

While Mackie’s stages resemble those of Parker, the crucial difference is Mackie’s holistic approach. Mackie is specifically talking about tissues consisting of complexes of cells, whereas Parker is talking about single cells. Mackie’s model is thus better able to satisfy the production dimension by more clearly showing how the system would be useful to the whole animal. Nevertheless, with Mackie’s proposal attention did swing back to the continuity issue of specifying a gradual sequence of stages showing how nervous systems evolved as connecting devices between sensors and effectors. While acknowledged, the coordinative role of nervous systems became a part of the background again.

1.4.2 Modern theories

After Mackie’s proposal, major new developments had to wait for development of a multitude of new techniques, including but not limited to molecular and genomic studies as for example mentioned by Mackie (1990), Anderson (1989) and (Lichtneckert and Reichert, 2007). Our goal is not to provide a comprehensive overview but a representative sample relevant to the present discussion.

Jékely

One of those new developments is incorporated in a model proposed by Jékely (2011) (see also Jékely et al. (2008) and Jékely (2009)). This model is influenced by molecular and functional data on ciliated (possessing cilia, slender protuberances of a cell which can provide locomotion and sense movement) larvae of various animals groups. He begins by stressing that there are many multicellular organisms which achieve whole-body coordination without a nervous system, exemplified by particular ciliated larvae (such as sponge larvae). He then goes on to emphasize the metabolic costs of nervous systems:

while the potential coordinative payoff is large, so are the metabolic costs, which results in fast evolution, fast selection against inefficient features.

Jékely identifies that the one-on-few sensor and ciliary effector couplings found in uninnervated ciliated larvae are inefficient, since they require construction of many sensory cells where theoretically only a few would do. This is illustrated by a situation found in innervated ciliated larvae of the Spiralia, where the proportions of sensors and effectors reach one-on-a thousand: when sensory cells extend elongations to multiple ciliated cells, there is immediate metabolic benefit. This idea clearly resonates with Pantin’s idea. Besides added efficiency, this setup allows synapse-mediated signal amplification and a pass/fail filter for signals, which allows small changes in a sensory cell to result in large changes in target cells. These considerations lead him to conclude that nervous systems could have emerged as a way to economize on sensory costs and optimize signal gain, which clearly satisfies a production requirement: efficiency.

Jékely also mentions the integration of muscle in this scenario. In part he reiterates Mackie’s proposal, adding to that the idea that muscle cells and ciliary locomotion could be combined: he proposes muscle cells as a steering mechanism instead of propulsion in an animal where cilia are the primary effector.

Arendt

As an extension of the molecular techniques referenced by Lichtneckert and Reichert (2007), the field of evolutionary developmental biology (evo-devo) also resulted in theorizing about the origin of nervous systems.

Nervous systems from eye specialization Based on extensive experimental work on evolutionary development in invertebrates, Arendt et al. (2009) formulate a hypothesis on nervous system origin based on eye specialization. Broadly speaking, he states that from a multifunctional basis cell population with photosensitivity and some (intracellular) signal processing and transmission ability, cell populations specializing in photosensitivity on the one hand and information processing on the other would arise. The hypothesis supposes the following steps:

- i A multifunctional steering-eye (as present in Cnidaria and sponge larvae (Jékely et al., 2008; Jékely, 2009)), consisting of undifferentiated cells;
- ii Division of labour, splitting the undifferentiated cells of the multifunctional steering-eye into two types of cells, optimized for sensing and for generating movement;
- iii A stage wherein the sensing and movement-generating cell bodies move away from one another but keep connection, resulting in “simple axonal connection *and thus nervous system*” (emphasis added);
- iv As a final step, the sensor tissue specializing into receptor and pigment cell types, thus creating a structure representative of modern eyes.

Figure 1.3: Axons from eyes: Arendt et al. (2009). This progression illustrates how the functionality divided into locomotor ciliated cells (LCC), photoreceptor cells (PRC) and shading pigment cells (SPC) connected by axon-like structures could have arisen from pluripotent ancestor tissues combining their functionality. (Figure reused with permission.

Figure 1.3 illustrates this progression. This approach is specific regarding functional specialization in cell types, inferences supported by evidence, yet it still refers to single cells instead of groups of cells. In this it is reminiscent of Parker.

The chimeric brain Another theory originating from the Arendt lab involves the bilaterian brain as a chimera: consisting of two separate parts, fused together. The chimeric brain is an example of a hypothesis about nervous system evolution originating from evo-devo evidence. The authors observe that there are two distinguishable developmental origins of nervous tissue: the apical system and the blastoporal system. In all major bilaterian groups either system has its own set of patterning genes, which expresses in a comparable broad pattern. Where they overlap, the centralized brain sits. Interestingly, in Cnidaria these systems do not overlap and remain separate. The authors hypothesize that the overlap in these systems happened in the bilaterian common ancestor. (Tosches and Arendt, 2013; Arendt et al., 2016). While the bilaterian brain itself is not in the scope of the current investigation, the chimeric brain hypothesis has interesting repercussions for the ancestral state.

In this view, two separate systems evolved before coalescing into the bilaterian nervous

system. Whether these systems functioned as nervous systems is unclear, but certainly a collection of parts must have accumulated, making earlier stages of these systems both plausible intermediate stages on the road to a nervous system. What is more, these two systems now allow for two separate potential accounts of nervous system origin, possibly one of a Parkerian input–output system (the apical system) and one of a holistic, body coordination type (the blastoporal system).

Gastric pouches Evo-devo based theories also have a bearing on ancestral body shapes and how those bodies functioned, which in turn affects how nervous systems affect their possessors. Arendt et al. (2015) propose a sequence of body plans and feeding modes from gastrula to animal with gut. Here, they introduce the mucociliary sole as an intermediate stage where the underside of the common ancestor to innervated animals slowly gained a rostrocaudal axis. They posit this mucociliary sole-stage, with said sole as the bottom side of the animal and the focus of its feeding mechanism, as the staging point for neuronal specialization for whole-body coordination. Given that this system surrounds the blastopore, it can be inferred to mean the blastoporal system.

Keijzer

Not all evolutionary models are based on a single new finding. In a high-level conceptual proposal by Keijzer et al. (2013), attention is brought back to whole-body integration and the point made by Pantin: the ability to coordinate a whole body is functionally important. This links the evolutionary emergence of muscle tissue and nervous tissue to the accommodation and affordance of fast, macroscopic whole-body movement. According to the authors, this combination of nervous systems and muscle forms the core of an animal sensorimotor system. The authors also stress the importance of endogenous behaviour: an input-output system alone is far less productive than one allowing spontaneous behaviour. This work refers back to Pantin in terms of whole-body coordination and it also incorporates Mackie’s account of myoepithelia. It posits neural reflexes as a later refinement.

Overall, Keijzer’s work showcases the value of a bird’s-eye view of nervous system origin by identifying relevant findings from disparate disciplines through focus on what matters to the animal.

Moroz

It is commonly assumed in accounts of nervous system evolution that the Porifera, the major uninnervated animal group, are also the basal group. This means that the nervous system evolved once in a sister group to Porifera. The parsimony of this explanation diminishes significantly if the placement of Ctenophora relative to Porifera becomes unclear. In a broad selection of work (see e.g. Moroz, 2009; Moroz et al., 2014; Moroz and Kohn, 2016) Leonid Moroz argues on the basis of morphological, physiological, and phylogenomic data that Ctenophora, not Porifera, are the basal animal phylum and that nervous systems evolved twice. If one assumes that Ctenophora diverged first and that

sponges and Placozoa did not lose nervous systems secondarily, parallel evolution becomes the most likely explanation. While Moroz does not offer specific lineage explanations of nervous system origin, the simple fact that it is plausible that nervous systems evolved twice, or even more often, can affect evolutionary models of nervous system origin.

1.5 Two views on the production requirement

Having introduced lineage explanations as a way of making evolutionary models explicit and having set up the context by way of extant theories of early nervous system evolution, it is time to introduce the current approach. In this thesis, we intend to add to the understanding of how early nervous systems may have functioned; how the various, primitive ‘moving’ (i.e. dynamically interacting, not necessarily physically moving) parts of early nervous systems interacted, how the neurophysiological rubber might have hit the ecological road. This means we are primarily dealing with the production requirement: the main focus in terms of lineage explanation is on how various stages could have resulted in fitness-enhancing benefits. Two broad categories of theory regarding production can be isolated: input-output and internal coordination. These two views will be elaborated here.

1.5.1 Neuron-focussed production: input-output

Some theories are broadly neuron-focused in terms of their functional benefit. Examples of this include the theory proposed by Parker (1919) and the scenario of Arendt et al. (2009) introduced in Section 1.4.2. These theories assert that neurons arose in connecting sensors and effectors, either alongside each other or sequentially. These are also exemplified in the work of Braitenberg (1984), whose simplest ‘vehicles’ are based on sensors, effectors, and single connections between them. The idea is that these connections could have further expanded and specialized into nervous systems. This kind of theory is supported by the concept of the reflex arc (Sherrington, 1906), though we now know this may very well be a derived structure, and reflex-arc-like sensor-neuron-effector structures exist in marine zooplankton (Jékely et al., 2008). These structures are less likely to be derived than vertebrate reflex arcs, but still significantly likely so, given that these animals all possess centralized nervous systems and do not belong to basal phyla.

Attempting to understand the origin of nervous systems in terms of neurons, the micro-perspective, means considering the nervous system primarily as a collection of neurons. With the micro-perspective, the focus is more on the production of individual neurons than on the behaviour of the collective whole. This view of the origin of neurons will thus tend to structural questions: where did cells possessing the three features outlined in Section 1.2 (electrical excitability, synapses, and elongations) come from? How did they come to be shaped the way they are? What are their molecular building blocks, where did they come from, and how did they work within the neuron? As the functional repertoire of a single generalized neuron is limited, this micro-perspective tends to portray neurons primarily and initially as signal transmitters: sending a signal from one place to another;

taking input and giving output.

1.5.2 Nervous system-focussed production: internal coordination

The other category of theoretical work focuses primarily on large-scale activity and thus tends to stress nervous systems more than individual cells. Considering the origin of nervous systems primarily as the emergence of a system, the internal coordination-view, results in a high-level picture. These theories assert that nervous systems arose on the level of the multicellular body as a whole, as a way for multicellular animals to coordinate any movement at all. These are primarily exemplified by Pantin (1956) and Passano (1963), as well as by Arendt et al. (2009)’s second example. They hypothesize that nervous systems and their precursors arose as mechanisms to allow cells to perform any sort of coordinated behaviour, probably progressing from some kind of excitable epithelium to a nerve net. This is supported by examples of contractile, electrically signalling sheets such as those of *Sarsia*, outlined in Section 2.6.4, and the nerve nets commonly found in Cnidaria (such as those of *Hydra*) and Xenacoelomorpha.⁷

Focusing on the system leads to a view of nervous system origin regarding the nervous system’s role in animal bodies. Which beneficial options would this system have afforded its possessor? What can an animal with a nervous system do that one without cannot? Looking at it this way, it becomes clear that having a nervous system allows an entire animal body to be coordinated and to exhibit intrinsic behaviour patterns, both on a short time scale.

Input-output models tend to emphasize reacting to the environment. When discussing the origin of nervous systems, the assumption is often that the nervous system functions primarily as a reactive, information-processing feature, leading to an implicit emphasis on an input-output interpretation of nervous system evolution. This underrepresents the importance of internal coordination, without which an animal could not move at all.

Both the micro (input-output) and macro (internal coordination) views have merit and contain considerable explanatory value regarding the origin of nervous systems. Understanding the micro aspects is necessary for understanding how genetic features give rise to functionality at a cellular level, and those genetic features in turn provide clues about the phylogeny (evolutionary tree) of nervous systems. The macro view is essential for understanding how having a nervous system would benefit an entire animal body. In both views, the relation between function and structure is a key issue, while these different priorities suggest different evolutionary accounts.

⁷Like the reflex arcs referenced above, it generally cannot be ruled out that these structures are derived, though arguably particularly Xenacoelomorpha’s nerve net seems considerably less likely to be derived than any reflex arc, given that Xenacoelomorpha may well be the most basal eumetazoan (Hejnol, 2015; Telford et al., 2015).

1.5.3 Problems with the input-output approach

While a practical demonstration of efficacy of the input-output approach exists, there are reasons not to be satisfied with only a rigorous input-output approach for the production requirement in the lineage explanation of the early nervous system: it does have problems, as explained by Pantin and Passano (Section 1.4.1), and Keijzer (Section 1.4.2). In summary: it assumes the formation of sensors and effectors before coordination. What is the use of an effector for an organism which has no ability to coordinate its body? Specifically, how is it going to coordinate an effector without a nervous system? Unicellular effectors have only very limited utility for a multicellular animal. This violates the production requirement of a lineage explanation: the intermediate stage with unicellular sensors, effectors and connectors would not be better in terms of fitness. No structure combining unicellular effectors, sensors and connections between them has been documented in any extant organism.

Another complication for the input-output approach is that the basal nervous system appears to be a nerve net rather than a sensor-connector-effector structure. This will be explained in detail in the compilation of phylogenetic evidence in Section 2.2. The nerve net stage does not feature in an input-output narrative, or it is assumed to come afterwards.

Additionally, the input-output model does not specify how its structures manage a soft body. This becomes particularly problematic when considering the Braitenberg vehicles: they exist on a two-dimensional plane, and their effectors—of which there are only one or two—each have only one degree of freedom. Coordinating a soft body is an entirely different situation, with many more individual effectors (ciliated or contractile cells) and degrees of freedom, making it a high-dimensional problem.

Finally, the input-output approach requires strong diversification and targeting in terms of cellular tissue, a dependence upon cell specialization and developmental patterning. Given that these were early, newly multicellular animals, there is no reason to assume these abilities were present.

At first glance, the internal coordination approach provides a parsimonious account of nervous system origin which does deal with these issues. If the nervous system arose to allow multicellular animals to coordinate their bodies, turning multicellular tissue into a usable effector for a soft body, none of the problems affecting the input-output approach are an issue. However, unlike the input-output approach which has Braitenberg vehicles to demonstrate the plausibility of the paradigm, the internal coordination approach possesses no rigorous model. Thus we arrive at the central goal of the current research: to design and implement a simulation of the internal coordination approach to the production requirement of nervous system origin.

It is important to note that this does not necessarily disprove or discredit the input-output approach. Should this attempt at creating a rigorous implementation of the internal coordination approach succeed, thereby providing some validation, the input-output approach can remain relevant. It likely had a role in shaping nervous system evolution together with internal coordination, either concurrent with it or afterwards. A specific case of potential concurrency is outlined in Section 1.4.2, discussing the chimeric brain:

Figure 1.4: Braitenberg's vehicles exhibit different behaviour based on the connections between sensors and effectors. In these examples, all connections are positive: if a light sensor (represented by a cup) catches more light, it will send a stronger signal through its attached connector (the dotted red line) to the effector (the rectangles depicting wheels). Cross-connectivity, shown above, results in attraction to light whereas parallel connectivity, shown below, results in avoidance of light.

this proposal involves two loci of nervous system evolution, the apical and blastoporal systems, which later fused into one. One partial system could be mainly input-output focussed whereas the other could be internal coordination focussed.

While the input/output has Braitenberg vehicles, a more in-depth working model or simulation of the internal coordination view of the production requirement for the lineage explanation of nervous systems is lacking. How should one go about obtaining such a model?

1.5.4 A simulation of the input-output view

Both approaches assert functional benefits to animals with neurons, but they are of a different kind: input-output stresses the behaviour of single neurons while internal coordination stresses whole-body behaviour. What indication is there that intermediate stages did indeed confer functional benefits? How can we test whether the production requirement is actually met?

The increasingly complex progression of wheeled vehicles described by Braitenberg (1984) provides a practical example of why it works for the input-output approach. The initial vehicles are exceedingly simple. They possess sensors, wheels as effectors, and various connections between them. The simplest vehicles can be equated to stages of an input-output view. Equipped with only a minimum of sensors and effectors, these machines can exhibit targeted behaviours, such as moving toward or away from stimuli, depending on how the connections are laid. Figure 1.4 illustrates the principle of the Braitenberg vehicle.

Braitenberg vehicles are a conceptual model which can be straightforwardly implemented as a mechanical system with actual wheels, sensors and connectors as well as a computational simulation. They illustrate how little information processing is needed for sensors and effectors to result in goal-directed behaviour and thereby demonstrate that

the later stages of an input-output system are functional. We refer to later stages, since no Braitenberg vehicle lacks connections, though the very simplest one might as well not have any: it consists only of a single wheel, a single sensor, and a single connection.

1.6 Modelling nervous system origin

1.6.1 Formal models

Constructing models which provide testable hypotheses is the bedrock of science. There is a continuum in models, the rigorousness of which varies, from thought experiments to precise predictions from physical equations. This section is dedicated to the path toward obtaining a more rigorous model for satisfying the production requirement for a lineage explanation of nervous systems with the internal coordination approach.

Extensive thought experiments, like imagining what would happen to the behaviour of a metazoan common ancestor when equipped with synapses, or speculating about the usefulness of excitable epithelia without the presence of a nervous system, are attractive in a situation where there are many unknowns, such as the origin of nervous systems. Abstracting away the dimensions which are unknown yet utilizing the data points that we do have may still allow us to draw conclusions, albeit tentative ones, about the nature of the earliest nervous systems. Take axodendritic elongations: these are not useful nervous system components without synapses, provided one assumes the role of synapses is and was direct and targeted information transfer between cells. Without the ability to transmit any signals, being able to do so in another location does not add any benefit. However, simple thought experiments hardly prove anything. It is easy to imagine that adding synapses to an epithelium would result in some sort of connected sheet: this idea has been suggested as an intermediate stage already by Mackie (1970) and exists in reality, albeit with electrical synapses in excitable epithelia. Would chemical synapses also work? With just theories or thought experiments, it is not so easy to rule out that adding chemical synapses to an epithelium causes something functionally useful to an animal, thereby making it a poor fit in the Popperian sense: it does not deliver falsifiable, internally consistent predictions. Thought experiments alone simply lack sufficient rigour.

A next step would be to implement a more formal model version of the thought experiment, something with dynamically interacting, interdependent parts. Such a model may validate theories to some extent and provide the ability to make predictions about real systems. A model can take several forms. One of the most common scientific forms of theory with dynamically interacting parts that provides a usefully disprovable prediction is the mathematical function or formula, commonly used in physics to predict the behaviour of natural phenomena. Newtonian physics is a good example of this. The main limitation of this method is that mathematical tractability may be an issue: analytical mathematics deals poorly with multiple variables. When the number of variables is limited, when it is possible to limit the model to only a few moving parts, this method works very well. Common to all simulation and modelling approaches is that some part of the system is abstracted away, and some parts are implemented: Newtonian mechanics disregards

relativistic effects but work fine for human-scale physics.

More involved than a mathematical model, another kind of implementation strategy is modelling by simulation: recreating part of the proposed system in a virtual environment. In a sense, this is just a mathematical model with less extreme abstraction and without the possibility of mathematical analysis. However, these models may still provide predictions or suggest to which degree certain features or parameters are important. The least virtual form of simulation is some sort of mechanical device, such as the water flow systems used to model macro-economics (Bissell, 2007). Wind tunnels and wave tanks can also be considered the most practical example of this kind of simulation. Predating widespread adoption of computers, these efforts have been superseded by computational modelling. Examples of computer simulation include: models of bird flocking behaviour (Hildenbrandt et al., 2010); models of neuron structures (Prinz et al., 2004); weather forecasting, earth simulation, and protein folding. While computer simulations are dependent upon numerical methods for finding solutions, they allow models representing far more complex, multivalent interactions such as those needed in the areas mentioned above. This is the approach we take in this thesis, building on the techniques developed in computational neuroscience.

Monk

Computational models form yet another new frontier in theoretical options. In his PhD thesis and associated publications, Travis Monk (Monk, 2014; Monk and Paulin, 2014; Monk et al., 2015) uses a computational model to demonstrate that neurons could have arisen to allow predation, isolating high-density and low-frequency food sources (predation) as opposed to low-density and high-frequency food sources (filter-feeding). He does not explicitly claim that nervous systems arose to allow predation. To be precise, he claims that in a situation wherein high-density, low frequency nutrients are predominant, a foraging strategy involving an energetically costly mechanism which signals the presence of a food source is a superior strategy. Such a low-frequency, high-density situation could well have existed at the origin of animal multicellularity—but note that this may not resonate with modern-day interpretations of predation, which involves thorough specialization and biological arms races. The predation as envisaged by Monk is quite abstract and broad.

As this is primarily an ecological argument, the model remains abstract regarding the organismal implementation of this signalling mechanism. This rigorous indication that nervous systems are linked to predation *sensu lato* is an important element in a comprehensive understanding of early nervous systems: Monk provides evidence for the functional benefit of costly coordinative cells under specific ecological conditions, helping to satisfy the production requirement.

1.6.2 A simulation of the internal coordination view

Ideally, we would act like theoretical physicists and specify a mathematical model of the world which is internally consistent and possible to check with empirical data once it

becomes available. In the case of the internal coordination view on nervous system origin, there are too many uncertainties and variables to define a mathematical model. However, a computational model of early animal nervous system functionality may serve as a more rigorous thought experiment. The work of Monk and Paulin (2014) demonstrates proof of principle: computational modelling can provide additional information regarding early nervous system evolution.

Computational modelling will allow us to check the internal consistency of the internal coordination approach of early nervous system evolution. If, given the functional building blocks available to early nervous system possessors, a computational model built according to internal coordination principles cannot demonstrate some kind of coordination, that is an argument against the internal coordination approach. If the model demonstrates coordination, that supports internal coordination as a reason for emergence of early nervous systems.

1.7 Conclusion

As the previous sections have shown and chapter 2 will illustrate further, there are many uncertainties and hypotheticals regarding the origin of the first nervous systems. This makes consistent frameworks of thought about this origin particularly valuable, since any framework has important effects upon the way new evidence is interpreted. Ours being an evolutionary model, we introduced the concept of a lineage explanation to make explicit which rules should be followed by a narrative of nervous system origin. In establishing the theoretical context regarding nervous system origin two main frameworks of thought emerge: the input-output and internal coordination approaches.

While there are some problems with the input-output approach, it does have a simulation implemented, showing that it can be functionally beneficial, which helps to satisfy the production requirement. Although the internal coordination approach provides compelling explanations for things such as endogenous activity and multicellular coordination, it has remained under-represented in current discourse about nervous system origin. Demonstrating that the internal coordination approach holds up to the scrutiny of a rigorous simulation strengthens its case. No such simulation has been presented in the literature so far (the work of Monk and Paulin (2014) focusing on the ecological instead of functional) and this thesis is dedicated to the development of such a simulation and putting it in context of a lineage explanation of nervous systems.

Ultimately, we are attempting to further specify the lineage explanation of nervous systems by means of a simulation of the production requirement, using an internal coordination approach to modelling. To do so properly, it will be necessary to compile the empirical constraints on that lineage explanation, which we will do in chapter 2.

Chapter 2

Constraints on a lineage explanation of nervous system origin

2.1 Introduction

In the previous chapter we discussed the input-output and internal coordination views of how nervous systems could have arisen and that no working internal coordination-based model exists. We introduced the concept of a lineage explanation and how demonstrating the functional benefit of a nervous system for early animals would constitute satisfying the production requirement of that lineage explanation. The present challenge is to construct an internal coordination-based model of the production requirement of the lineage explanation of nervous system origin. In order to judge the validity and parsimony of any such model and advance in the direction of a better lineage explanation of nervous systems it is important to take as much relevant data as possible into account. This means tabulating relevant scientific findings and enumerating which constraints on a lineage explanation of nervous system origin emerge. That is the goal of this chapter.

‘Relevant data’ should be taken as a broad concept; it not only consists of straightforward empirical data. We also take it to refer to more abstract generalizations, concepts and hypotheses built on a broad variety of empirical approaches which fall short of high-level theories of nervous system evolution. For example, work on the phylogeny of the major animal groups provides insight about which groups split from other groups and in what order. This provides information about where in the animal tree nervous systems originate, and whether that happened only once or multiple times. As another example, a broad range of empirical methods provide information about the climate and oxygen levels on Earth during the period when nervous systems can be presumed to have first evolved; this is treated in Section 2.4. The ‘data’ on which the present investigation rests thus amounts to generalizing claims based upon empirical findings, originating from diverse scientific fields.

Being scientific findings, they are open to amendment, refutation, and reinterpretation when new evidence turns up. One important current discussion, for example, concerns whether Ctenophora (comb jellies, which possess nervous systems—see Section 2.2.4) split off from the main animal lineage before or after the Porifera (sponges without nervous sys-

tems); see Section 2.2.2 for details. If Ctenophora are basal (split off before the Porifera) this has fundamental repercussions for an account of the origins of nervous systems: Either they evolved at least twice in separate lineages, or the Porifera lost their nervous systems secondarily. Additionally, due to the compartmentalization between scientific disciplines, inconsistencies may occur when findings from different fields are combined. An example of this is the discrepancy in timing between the fossil record and molecular clock data (a technique using the mutation rate of genes to deduce when and in which order organisms diverged, see Section 2.4.1). More phylogenomic and developmental data should help to decide on the position of Ctenophora in the animal tree, but when it comes to questions relating to highly different sources of data—such as fossils and phylogenomics (evolutionary analysis of phylogenetics utilizing entire genomes)—it is often difficult to converge on a single account that accommodates most data (but see Cunningham et al. (2017) for a treatment of this problem).

Any acceptably parsimonious account of the origins of early nervous systems must take any scientific constraints into account. This means it ought to accommodate the data described in this chapter. Still, the data are not necessarily hard constraints but are open to interpretation. For example, if it can be shown that nervous systems and neurons may have evolved fairly easily, the claim that Ctenophora diverged first and developed nervous systems independently would be easier to accept and less controversial. Developing a lineage explanation of the origins of nervous systems is not a matter of passively accepting data; it means partaking in an ongoing weighting of the evidence with the goal of converging on a single account of how nervous systems first arose. A lineage explanation can itself act as a constraint: given some range of uncertainty in a scientific finding (of which this chapter contains many), parsimony within a lineage explanation may help reduce that uncertainty.

This chapter provides a systematic discussion of the relevant scientific findings concerning the evolution of the first nervous systems. First, in Section 2.2 the basal animal phyla are introduced. Section 2.3 then introduces phylogenetics and interprets the characteristics of the phyla of section one in light of their phylogeny. Section 2.4 investigates timing, the environment, and ecology relevant to the origin of nervous systems. Section 2.5 examines behaviour at the base of the animal tree. Finally, Section 2.6 delves into the morphology, development, and precursor tissues of early nervous systems.

All this information is relevant and the formulation of a lineage explanation should be consistent with it. Where applicable, the evidence is cast in such a light as to best outline the relevance to a lineage explanation of nervous system origin.

2.2 Basal phyla

Some major current-day animal phyla (a high-level clade or evolutionary grouping) possess nervous systems and some do not. Moreover, the structure and organization of nervous systems can vary significantly between the phyla which have them. Even though these are modern animals, comparing these groups can lead to insights about how nervous systems evolved, potentially revealing constraints on any lineage explanation. Inferring

characteristics of a common ancestor by those of its descendants is risky, as Section 2.3.4 will demonstrate. However, it can provide valuable clues: features common between two groups generally originate from their common ancestor. This is particularly likely if that feature exhibits the same derivation in development. Postponing in-depth conclusions to Section 2.3, the goal of this section is to provide an overview of all relevant animal phyla and the features salient to nervous system origin. These phyla form the basal (low in the phylogenetic tree) part of the phylogenetic tree of animal life, see Figure 2.3: Porifera (sponges), Ctenophora (comb jellies), Cnidaria (jellyfish, polyps, and corals), Placozoa (plaque animals), and Bilateria (all other animals).

2.2.1 Eukaryote basis: Choanoflagellata

First, we will take a look at the evolutionary neighbourhood of animals. Animals, also referred to as Metazoa, are eukaryotes: large-celled organisms with a nucleus and various organelles such as mitochondria and Golgi apparatuses, as opposed to Bacteria and Archaea, which do not have nuclei and organelles. Specifically, animals are multicellular eukaryotes with tissues but without rigid cell walls. Contrast this to plants and fungi, which do have cell walls, limiting their mobility; and to protists, which are unicellular or facultatively colonial. This broader eukaryote context is relevant since many of the building blocks, or at least the genes which were later coopted into those building blocks, are often also present in the closest eukaryote relatives. The group to which animals belong (together with for example fungi and slime molds) in said larger eukaryote context is called the opisthokonts, characterised by cells bearing a single posterior flagellum (whip tail) (Cavalier-Smith, 1987). The group most closely related to animals, and thus most interesting for our purposes, is the clade Choanoflagellata (King et al., 2008; Burkhardt et al., 2014), facultatively colonial (cluster-forming) bacterivorous eukaryotes with a prominent flagellum. The combined group of animals and choanoflagellates was dubbed ‘Apoikozoa’ by Budd and Jensen (2015), referring to the colonial nature of both animals and choanoflagellates, likely a basal feature of this group.

2.2.2 Porifera

Porifera are more commonly known as sponges. They are simple sessile filter-feeding animals, starting their life-cycle as a free-swimming larva equipped with cilia (small sweeping hairs, providing motility). These then settle down, filtering nutrients from the water by pumping water through an internal canal system using flagella. They differ from Choanoflagellata in the following ways:

- i Like many animals, they can grow to macroscopic size;
- ii They show tissue differentiation: different cells can have different *cell fates*: they permanently become a particular type of cell, performing a specific role within the animal.

Compared to other animals, this differentiation is very limited. Porifera do not clearly exhibit multiple germ layers. The concept ‘germ layer’ will come up repeatedly, so it

Figure 2.1: A strongly simplified example showing a cross-section of an animal with a through gut (a gastric cavity with two openings, a mouth and an anus) as organized into germ layers.

bears some introducing. Germ layers form the most fundamental tissue differentiation in development. Porifera are generally considered to possess only one germ layer; Cnidaria, Ctenophora, and Placozoa have two, distinguishable as ectoderm and endoderm, and Bilateria have a third layer, the mesoderm. The ectoderm is the layer covering the proper outside of the animal, and the endoderm is the layer covering the inside outside, i.e. the gut. Mesoderm is the layer responsible for structures in between such as organs and the endoskeleton. See Figure 2.1 for a schematic cross-section of a stylized organism with three germ layers.

Adult Porifera also lack strict bodily symmetry, a feature present in almost all other animal phyla. They do possess an epithelium (Dunn et al., 2015), a crucial animal morphogenetic feature, determining what is outside and inside an animal. They were originally classified as the most basal clade, but recent phylogenomic advances have moved Ctenophora to the fore as another contender for most basal phylum. However, other phylogenomic studies (Feuda et al., 2017) suggest that Porifera are indeed the most basal Metazoan phylum (see Section 2.3.5 for a more extensive treatment), so the point remains in contention. Given the considerable error margins in phylogenomic analysis, this is by no means definitive, but Porifera do make a plausible metazoan baseline in most respects.

Porifera do not possess nervous systems. Despite this, they do exhibit behaviour, even at the level of the entire animal, though their repertoire is limited. The ‘sneezing’ behaviour, in which the entire sponge contracts and expels water from its canal system in order to get rid of contaminants, is an example of this. Since they do not possess nervous systems, they use some other form of intercellular signalling. In terms of molecular building blocks used to engender said behaviour, there is overlap with animals which do have nervous systems: modern sponges use nitric oxide, glutamate and GABA, animal neurotransmitters, for behavioural control (Elliott and Leys, 2010). Porifera also possess a protein, PAM, used for activating neuropeptides, associated with nervous systems (Attenborough et al., 2012)—though it must be noted that the gene PAM is not specific to animals; it is also present in the green alga *Chlamydomonas* (Kumar et al., 2017). Sponges have senses, they have effectors, and they coordinate them with local, juxtacrine signalling using these neurotransmitters—but without neurons (Leys, 2015). Glass sponges (a subgroup of Porifera) even use calcium/potassium action potentials to quickly stop feeding (Dunn et al., 2015). On the genetic side, demosponges (the most diverse class of sponges, accounting for 90% of poriferan species) exhibit a slew of proteins associated with neural development: the neurogenic regulators NK, Six, Pax, and bHLH. However,

they lack the key body plan regulators Otx, Hox, and ParaHox which other phyla from Cnidaria (Galliot et al., 2009) and beyond use in nervous system development.

2.2.3 Placozoa

Placozoa are an enigmatic clade of very simple animals, shaped like plaques (for which they are named), of which until recently only one species was known (*Trichoplax adhaerens*)—only recently have other species been described (*Polyplacotoma mediterranea* (Osigus et al., 2019) and *Hoilungia hongkongensis* (Eitel et al., 2018)) They do not possess a digestive cavity but simply a top epithelium, a bottom epithelium, and a few cell types in between. They feed by ventral digestion: engulfing food, trapping it between their underside and the substrate, and digesting the contents of the resulting cavity (Schierwater, 2005). Limited in cell specialization overall, they lack a nervous system. They have no muscles *per sé*, but they do exhibit contraction, using it to change the shape of their body. They move using cilia.

The genome of *Trichoplax adhaerens*, the single placozoan species, has been sequenced, initially suggesting a position as a sister phylum to Bilateria (Srivastava et al., 2008) (meaning the trunk of the animal tree splits into Placozoa on one side and Bilateria on the other). They possess a number of genes associated with neurogenesis, but then again, so do Choanoflagellata and Porifera. Being a sister group to Bilateria and Cnidaria, having diverged after the Porifera, gives us little clarity with respect to its lack of nervous system being basal: since Ctenophora may be basal even to Porifera, the lack of a placozoan nervous system may be just as secondary as that of Porifera (Ryan and Chiodin, 2015). Recent work by Varoqueaux et al. (2018) shows that *Trichoplax* appears to coordinate by means of non-neural peptidergic signalling, suggesting that such juxtacrine signalling may predate synaptic connections.

Another problematic facet of Placozoa is that they are quite hard to place accurately in the metazoan tree. Since until recently only a single species was known, mutation rates were impossible to calibrate. This makes it impossible to tell whether differences from other reference species are due to being distantly related or due to high mutation rates or vice versa (a phenomenon called ‘long branch attraction’). This causes large margins of error when positioning the Placozoa, making it dangerous to draw any conclusions from this strange clade.

2.2.4 Ctenophora

Ctenophora, also known as comb jellies, are gelatinous, invertebrate animals superficially similar to jellyfish. Closer inspection reveals them to be profoundly different not just to ‘proper’ jellyfish (Cnidaria, see Section 2.2.5) but to all other animals: their bodies are neither bilaterally symmetrical like the eponymous Bilateria (see Section 2.2.6), nor radially symmetrical like Cnidaria, but *rotationally* symmetrical (Dunn et al., 2015). They are also characterized by rows or ‘combs’ of cilia which they use for movement. They actively hunt in the water column, using a mouth and a gut to capture and digest prey. They have muscles to deform their body and operate their mouth.

Ctenophora coordinate their body with a nervous system consisting of fully functional neurons, possessing all the features associated with them: electrical excitation, synapses, elongations, and various neurotransmitters. This nervous system is arranged in a net over the surface of the animal, with neurons more densely distributed around the mouth, the rows of cilia, and the statocyst, an organ which senses orientation.

Ctenophora were initially classed as a monophyletic group (an evolutionary branch with a single origin) titled coelenterates together with Cnidaria, owing to the fact that both groups possess two germ layers (ectoderm outside and endoderm inside), but phylogenetic data have shown that Ctenophora are at the very least a separate phylum and possibly even basal to Porifera (see Telford et al., 2015; Sperling and Stockey, 2018, for an overview). Intriguingly, while they exhibit clear developmental body plans, they do not appear to use the Hox or ParaHox genes which Cnidaria and Bilateria use to define their body plan (Ryan et al., 2010; Moroz et al., 2014)—this may be related to their distinctive radial symmetry.

Recent investigations of the genetic and developmental characteristics of the ctenophore nervous system imply that it consists of different molecular building blocks from cnidarians’ and bilaterians’ (Moroz and Kohn, 2016; Moroz et al., 2014). However, this is not without controversy. Given the long evolutionary time and likely simplicity of the ancestral nervous system, it is quite possible that both ctenophoran and ‘eumetazoan’ (bilaterian and cnidarian) nervous systems share a common ancestor and have changed to reflect the modern situation (Jékely et al., 2015b). Some see the presence of nervous system-related genes in Porifera as evidence of a secondary loss of that simple common ancestor nervous system in Porifera and Placozoa (Ryan and Chiodin, 2015).

2.2.5 Cnidaria

The phylum Cnidaria consists of jellyfish, sea anemones, polyps, and corals. These animals are characterized by the presence of two germ layers (endoderm and ectoderm), lack of a circulatory system, and the possession of unique nettle cells or cnidocytes, for which the phylum is named. Secondarily reduced Myxozoa¹ excluded, Cnidaria use a gut with a single opening to digest food, use muscles and a nervous system to coordinate their body. Life cycles of individual species vary greatly, with sessile and pelagic (in the water column) stages both being common, and often exhibiting both.

The cnidarian nervous system consists of fully featured neurons, organized in nerve nets distributed across its surface as well as in varying kinds and degrees of centralization. This centralization is generally in a number of rings around the gastric cavity (gut) opening, though species without centralization also exist (such as *Hydra*). Cnidarian neurons are multifunctional, exhibiting comparatively little specialization (Koizumi, 2016). The basal nature of Cnidaria combined with their developed nervous system results in them generally being considered an important clade to study in the context of nervous systems (Galliot et al., 2009; Kelava et al., 2015), and modern research methods are ren-

¹A class of Cnidaria, members of which are all obligate parasites, which have lost many advanced features.

dering the detailed functioning of nervous systems of Cnidaria increasingly tractable. This is particularly valuable since many bilaterian nervous system features are already present in Cnidaria (Bosch et al., 2016).

2.2.6 Bilateria

To non-biologists, the phyla described above may fail to strongly resemble canonical animals. All animals which do fit the description to the uninitiated, from molluscs and insects to fish and mammals, fall in the category of Bilateria: the bilaterally symmetrical animals. These animals possess nervous systems,² Hox genes, strong body patterning, and a dizzying array of body shapes and lifestyles, often exhibiting complex behaviour. This group has deep roots in the animal tree as well, with one early diverging group, the Xenacoelomorpha, being of particular interest regarding nervous system origin.

Xenacoelomorpha

Xenacoelomorpha constitute a recently established (Hejnol et al., 2009; Philippe et al., 2011a) group of very simple bilaterian worms, lacking a body cavity (coelom). Initially believed to be a simplified subgroup of platyhelminths (flatworms, an early diverging animal clade), in their own group, Xenacoelomorpha would be the most basal group of Bilateria (Hejnol, 2015; Telford et al., 2015). All Xenacoelomorpha possess nervous systems. The interesting part is that not all subgroups possess strongly centralized dorsal/ventral nervous systems the way other Bilateria do. The most basal subgroup of Xenacoelomorpha, the Xenoturbellida (consisting of 6 species of the genus *Xenoturbella*), possesses a nerve net and the later diverging clades exhibit progressively increasing centralization (Perea-Atienza et al., 2015).

Beyond Xenacoelomorpha

While animals beyond the Xenacoelomorpha are not directly relevant to nervous system origin as they all have advanced nervous systems, they do provide context, completing the phylogenetic frame of reference and serving to show the origin of any bilaterian bias to understanding the nervous system.

Currently extant Bilateria beyond the Xenacoelomorpha can be divided into two groups: Protostomia and Deuterostomia.

Deuterostomia include:

- i The chordates, the supergroup to which vertebrates belong, which in turn includes the lancelet fish *Amphioxus* and tunicates. Since vertebrates are a subgroup of chordates and thus Deuterostomia, this group includes humans;
- ii The echinoderms (sea stars, sea urchins and sea cucumbers);
- iii The hemichordates and acorn worms.

²Barring secondary loss events.

Deuterostomia are characterized by the primary developmental opening, the blastopore, developing into the anus. All these groups, even strange and otherwise relatively simple worms like the acorn worms, possess centralized nervous systems, though some with sessile life styles lose it at some point in their life cycle. The ascidians, a kind of tunicate (Cloney, 1982) provide a classic example of this. It is unclear whether the deuterostome common ancestor possessed a centralized nervous system—it might only have had a nerve net (Holland, 2015).

Protostomia, which include molluscs, arthropods, nematodes, platyhelminths (flatworms), chaetognaths (jaw worms), and annelids (ringworms) comprise a dizzying array of invertebrates. Do note that ‘invertebrate’ is a misleading term when applied to animal phylogeny: many species which may also be referred to as invertebrates fall outside Protostomia. Cnidaria and Ctenophora are considered invertebrate, and so are sea stars which are echinoderms, a deuterostome, and thus in a sister clade. The protostome clade is home to some very well-researched nervous system features: the giant squid axon described by Hodgkin and Huxley (1952) is from a mollusc and thus a protostome. The crustacean (arthropod, thus protostome) stomatogastric ganglion is a classic testbed for network effects in neuronal systems (Miller and Selverston, 1982) and for computational neuroscience as well (Prinz et al., 2004). The crustacean stomatogastric ganglion is an indicator of a particular characteristic of protostome nervous systems: these ganglia often exhibit identifiable neurons. This is a great boon to investigating the crustacean stomatogastric ganglion: it is roughly the same in all individuals, and the different cells have the same function in every individual, allowing for reproducible experiments. Another extreme example of identifiability is the model organism *Caenorhabditis elegans*: all its neurons (385 or 302, for males and hermaphrodites, respectively) are identifiable, making it an interesting case for analysis (e.g. Jarrell et al., 2012; Larson et al., 2018). Protostome nervous systems are practically always centralized in a ventral cord. A prominent example of evolutionary developmental investigation of early nervous systems, the study of phototaxis and chemical signalling in the nervous system of *Platynereis* larvae (Jékely et al., 2008), is also a protostome (*Platynereis* being an annelid). Protostomia are abundant in the Cambrian fossil deposits: quality fossils allow for definite identification of the protostome centralized nervous system by 518 Ma (Edgecombe et al., 2015).

As these examples show, there is extreme diversity within Bilateria. The centralized nervous system was a very successful adaptation, with many species currently exhibiting them. There is a bilaterian bias in looking at morphology, since we are bilaterians ourselves and more prone to looking at commonalities between Bilateria on one hand and Porifera, Ctenophora, and Cnidaria (basal phyla) on the other, than particularly basal phylum-specific adaptations and specialities. This results in large swathes of basal animal physiology and solutions to problems being overlooked: for example, ctenophoran rotational symmetry places it entirely outside ideas we have regarding body planning mechanisms of Bilateria and even Cnidaria, risking misrepresentation of ctenophoran body plan solutions. Porifera have a rich array of secretion molecules, the function of which is largely unknown at this time (Dunn et al., 2015), solving problems of which we may not be aware. This means that there is an observation bias in favour of finding a common

ancestor which exhibited the bilaterian features. While that is the face of modern animals and investigating the centralized nervous system is worthwhile in its own right, we should bear in mind that the origin of nervous systems likely lies in far simpler animals. We should therefore not let assumptions of bilaterian nervous system organization affect our judgement.

Looking away from the prominent animal groups within protostomia and deuterostomia (like molluscs, vertebrates, and chordates) and instead observing their sister groups, we find that one body plan is present in all major bilaterian subgroups: the worm. Elongated and with a through gut, this body plan is absent from the most basal phyla.

2.3 Phylogenetics

The next step after having introduced the basal animal phyla is investigating how nervous systems emerged between them. For every instance of nervous systems evolving *de novo*, there is a common ancestor. Ultimately, given that our focus lies on expanding the lineage explanation of nervous systems, we want to trace back the intermediate stages of the nervous system through the phylogenetic tree: where did it originate, and in what kind of ancestors did the intermediate stages lie? It is possible to make inferences about these ancestors by looking at their descendants. However, this is not a trivial undertaking: the order of divergence of the basal clades is unclear and the inferences mentioned above can be ambiguous. We rely on the principles of phylogenetics, the study of evolutionary lineages, to investigate this. Phylogenetics is the science of dealing with the shape and branching-points of the tree of life and the purpose of this section is to introduce its methods, concepts, and terminology.

2.3.1 Phylogenetic principles

Phylogenetics works by comparing how extant phyla relate to each other in the phylogenetic tree. For a non-neural example illustrating phylogenetic principles close to home: are bonobos more closely related to chimpanzees or to humans? In other words, which of the following options is the case?

- A The group which would later diversify into humans and bonobos split from the group which would later be chimpanzees.
- B The humans split off from the group which would later diversify into the chimpanzees and bonobos.

When discussing phylogenetic trees, it is often very helpful to have a visualization of the groups at hand. This is referred to as a cladogram; Figure 2.2 shows two, outlining both scenarios. Scenario B is the real one, as the naming of the genera shows (i.e. the genus (a high-level clade) *Pan* (chimpanzees and bonobos) and the genus *Homo*): humans split off before chimpanzees and bonobos diverged from one another. This makes humans a so-called sister group to the genus *Pan*, which contains chimps and bonobos.

Figure 2.2: Two possible relationship scenarios regarding humans, bonobos, and chimpanzees. Sister groups outlined in dashed boxes. Situation B represents reality.

The study of phylogeny originally derived branchings like these using comparative morphology: ‘these two species of leafy shrubs have the same particular way of flowering and are similar in all other ways, so they are probably closely related’. While seemingly primitive, this approach nevertheless laid the groundwork for the modern study of phylogeny, and many broad strokes still hold. Life is still subdivided into kingdoms, though we have gained some; the levels of phylum, genus, and species are still adhered to, and comparative morphology of both currently extant species and fossil remains is still a valid and valuable avenue of research, particularly when used in conjunction with other, more modern methods (Hejnol and Lowe, 2015; Bosch et al., 2016). Those modern methods are molecular phylogenetics (phylogenomics) and evolutionary developmental biology (evo-devo).

2.3.2 Phylogenetics and phylogenomics

Molecular phylogenetics is the study of differences in genome between related species. For species which are currently alive, the entire genome can be sequenced; phylogenomics refers to molecular phylogenetics utilizing data from entire genomes. When two related species share a particular gene which was also present in a common ancestor, it becomes possible to estimate how long ago these species diverged, both in terms of time and relative to the divergence of other groups. This is done by considering the occurrence rate of mutations in those genes which are present in two different species, and tallying the number of differences between them. Doing this for a multitude of species then reveals which species are genetically more similar to one another and thus closer relatives. Computational methods then allow construction of phylogenetic trees based on these comparisons. Knowing the rate of mutation as well as the inferred shape of the tree also allows an estimate of how long ago groups diverged, which is called the molecular clock.

Better estimation of mutation rate and more genes to test improve results using this method, which is necessarily strongly statistical and computational in nature. The groups relevant to early nervous system evolution, the Porifera, Cnidaria, and Ctenophora diverged a very long time ago so the differences between their gene copies are large. Because of these long time spans, a good estimate of mutation speed is both critical and elusive. Consequently, results between studies can vary significantly, even when analyzing the same dataset (i.e., selection of sequenced genes shared between extant species) (Philippe et al., 2011b).

Critics of this method remain. Cavalier-Smith (2017), for example, is particularly vociferous in his opposition to molecular clocks, though he does not spare palaeontology either:

“A widespread explanatorily empty speculation that many groups originated long before their objective fossil dates is fuelled by deep uniformitarian prejudices about evolutionary rates that palaeontology long ago refuted, and three other prejudices/biases that synergistically led to the notion of a ‘slow burning fuse’—a journalistic slogan, not critical evolutionary thought, evaluation or synthesis. First is excessive confidence in the certainly false idea of a ‘molecular clock’ and in the reliability of current implementa-

Figure 2.3: Animal phylogeny as relevant to nervous system evolution.

tions of oxymoronic ‘relaxed clock’ computer programs (Eme et al., 2014). Second is uncritical acceptance of the dubious identity of some fossils used for calibration, driven by palaeontologists’ ‘my fossil is older than yours’ competition (Brasier, 2009).”

Practically speaking, when it comes to organizing the basal animal phyla, we need to accept uncertainty in the definition of the cladogram (King and Rokas, 2017). Even so, this is a valid way to learn something about deep biological history—a field in which any information is hard to come by. Molecular phylogenetics can give strong indications of what the genes of common ancestors of particular groups were like. Telford et al. (2015), for example, review research using this method to give a remarkably insightful overview of early animal phylogeny.

The basal animal cladogram

The phylogenetic relations between animal groups relevant to early nervous system evolution are both clues themselves as well as something upon which other clues have bearing. I use a visual representation of the basal animal phylogenetic tree with an emphasis upon nervous system evolution as a framework (Figure 2.3).

All these animal groups have been introduced in Section 2.2. There are uncertainties in this tree: the timings are unknown, it is unclear whether Ctenophora or Porifera are basal (i.e. split off from the trunk first), whether Cnidaria or Placozoa are basal (or whether they form a sister group together). What does it mean that the distribution of various levels of nervous system possession (no nervous system, a nerve net, or a centralized nervous system) does not seem to show a logical progression? What sort of conclusions do phylogenetic trees afford us, if any?

2.3.3 Tracking features through phylogeny

Establishing a phylogenetic tree allows following a feature through evolutionary time: if the tree is known and it is known which branches possess a feature, that feature originated in the common ancestor to the branches possessing the feature. Conversely, and this is how phylogenetics operated before gene sequencing and phylogenomics, groups sharing a feature indicates likelihood of relatedness.

This is often ambiguous, since parallel evolution (i.e. the same phenotypic/functional feature arising multiple times in necessarily slightly different ways, also referred to as homoplasy) does happen. However, novelty is rare, so most commonly a feature which exists in crown groups (the humans or the genus *Pan* from our previous example) arose in some common ancestor to both crown groups.

The possession of certain characteristics (such as opposable digits) by descendants of a particular common ancestor implies things about that common ancestor. It is reasonable to assume that the common ancestor of humans, bonobos, and chimpanzees possessed an opposable thumb. Conversely, if one member of a suspected group misses an expected characteristic (if bonobos lacked opposable thumbs, say), that implies that: a) they lost the characteristic, or b) they (in this case bonobos) are actually an outgroup: not closely related, not sharing a common ancestor with the rest of the group, or c) the characteristic evolved twice.

Option b can often be confirmed or excluded by developmental or genetic means: if vestiges of the characteristic remain, either genetic or in development, as tails do in humans, it is very likely lost secondarily (i.e. option a). It is also possible to exclude or confirm option c on grounds of developmental or genetic differences: the same functional adaptation is unlikely to arise in exactly the same way. Tracing nervous systems through the phylogenetic tree is not so easy, as reconstructing events so long ago poses particular difficulties.

2.3.4 Crown group and stem group issues

When looking at the intermediate stages of nervous system evolution, we want to know about the progression from the last uninnervated common ancestor: the node in the tree from which both innervated and uninnervated crown groups emerged. The collection of individuals between the common ancestor of a crown group, either innervated or uninner-vated, and the more ancient common ancestor of both innervated and uninnervated crown groups, is called the stem group. There is a stem group to the uninnervated crown group and there is a stem group to the innervated crown group. Any extinct branches from this stem are included in the stem group. The intermediate stages between uninnervated animals and innervated animals existed within the stem group to the innervated crown group.

Our main source of information about the stem group is the crown group: we infer things from their commonalities. There is little risk to this at the short distance between human and chimpanzee: we can freely assume common features between humans and chimpanzees existed in the common ancestor as well. These inferences tend to be

Figure 2.4: A phylogenetic tree illustrating the hypothesis that Cnidaria arose before Placozoa.

riskier when complex combinations of genes are in play and these genes have changed considerably over time. This is certainly true for nervous systems.

Consider Figure 2.4, a subset of Figure 2.3, with an embedded hypothesis: we suppose that Cnidaria are basal, and that Placozoa secondarily lost their nervous system. To support this claim, we seek to disprove that there is parallel evolution. Parallel evolution in this case means that Cnidaria developed a nervous system independent of the crown Bilateria (i.e. Xenoacoelomorpha and beyond, essentially all animals not poriferan, ctenophore, placozoan, or cnidarian). To do this, we need to look at the genes both groups in our comparison (Cnidaria and crown Bilateria) use. We find that most of the same genes that regulate nervous system development are used (Galliot et al., 2009). However, that does not give us conclusive evidence: where humans or chimpanzees could either use gene X or gene Y to achieve opponability, and humans used X and chimpanzees used Y, Bilateria and Cnidaria could have used a large selection of genes, and both did. They could very well have used those genes quite differently. Their nervous systems could also derive from a common ancestor, with some genes dropping out in one group, thereby giving the impression that different sets of genes were used in parallel. None of this is conclusive without exact details of *how* Cnidaria and Bilateria use their neural genes.

Another example of something that is a clue in some situations and inconclusive in others: my possessing a mutation of the ALDH2 gene means that acetaldehyde, a metabolic byproduct of ethanol metabolism, accumulates in my bloodstream when consuming ethanol, since my version of the ALDH2 gene metabolizes acetaldehyde at a reduced rate. This accumulation leads to specific physiological reactions, a particular phenotype, commonly termed the Alcohol Flush Reaction (increased blood flow in superficial capillaries when consuming ethanol, resulting in a red flush, hence the name). This mutation and associated phenotype is very common in East Asia, and nowhere else. This implies that I likely have some East Asian heritage (which happens to be the case, and the Alcohol Flush Reaction phenotype can be traced along that line in my family). I can confidently claim that my last Asian ancestor exhibited the Alcohol Flush Reaction. Indeed, it is highly probable that the common ancestor where the ALDH2 gene mutation

originated exhibited this phenotype, since it only involves a single gene and emerged relatively recently. Therefore, odds are very low that the role of the gene and the roles of other participants in the cascade have changed in the mean time.

However, when deep evolutionary time is concerned, pathways and pipelines of proteins may add up to different things and genes are coopted in rather different roles. Consequently, phenotype and gene cannot be correlated without reserve. For example, genes coding for a protein which is an essential part of the synapse are also present in Choanoflagellata, which are not even multicellular (Burkhardt and Sprecher, 2017). Genes which are neurogenic in other species are also present in sponges (Galliot et al., 2009). Genes can tell us things about structure of the phylogenetic tree, but *we cannot assume their functionality in the common ancestor and in descendant groups without modern representatives* (collectively called the stem group). This is also illustrated by Erwin (2015), who states that there are often macroevolutionary lags between genetic novelty and the corresponding functional novelty. Additionally, genetic novelties are often not immediately expressed in development (Erwin, 2015), further weakening the link between gene occurrence and phenotype. The fact that major eumetazoan phyla (Arthropoda, Cnidaria, Mollusca, Chordata) were already present before the Cambrian Explosion does not mean their representatives had the phenotypic characteristics typical of their current (or Cambrian!) crown group (Erwin, 2015). It is important to remember the long-standing admonition that the current representative of any phylum means little about its common ancestor. In other words, the groups which split off early on had their own however many millions of years of evolution. This means that it is unwise to consider a more primitive phylum to be representative of a common ancestor. This warning is worded elegantly by Pantin (1952) (on page 147):

At the outset let us remember to discard one notion that is apt to creep into discussion of the lower animals. They are not to be considered as poor and inefficient relics of our own evolutionary past. It may well be that the functional systems of our remote ancestors in part resembled those we find in the simpler animals today. But we are dealing with living animals and there is nothing inefficient about them.

Jellyfish, for example, are highly specialised predators with very sophisticated behaviours. It is very unlikely that the ur-cnidarian looked or behaved anything like this; in fact, it was probably excellently adapted to its ecological niche. The logical extension of this warning is not to consider the overlap of two modern phyla representative of the characteristics of its common ancestor (though it does, of course, ‘in part resemble’ it).

The only way to confidently say anything about phenotype in common ancestors is through palaeontological morphology (Budd, 2001), which has its own problems—animal fossils of this early era are sparse and hard to interpret.

2.3.5 Parallel nervous system evolution?

It is not certain whether Ctenophora or Porifera are the basal animal phylum. This is important since it may mean nervous systems evolved twice: once in Ctenophora and

once in the common ancestor to Cnidaria and Bilateria.

Since Ctenophora and Cnidaria possess fully featured nervous systems and Porifera do not, it would be counterintuitive if Ctenophora are indeed basal, because one would assume such a complex characteristic to have emerged only once. If, as was commonly accepted earlier (Lichtneckert and Reichert, 2007), Cnidaria and Ctenophora are sister groups, it is plausible that the nervous system arose once, some time before they diverged.

However, if Ctenophora and Cnidaria are not sister groups and Ctenophora are basal to Porifera, the Porifera sit between the Cnidaria and the Ctenophora. If the nervous system evolved once, the common ancestor to all three phyla must have possessed a nervous system, meaning Porifera lost it. Porifera sport such a convincing array of neural building blocks (messenger molecules, nervous system-related genes) that some researchers hypothesize that they did indeed lose neural cell types (Ryan and Chiodin, 2015), supporting the scenario in which nervous systems arose once and Ctenophora are basal.

If Ctenophora are basal and the common ancestor did not possess a nervous system, it must have arisen at least twice: once in Ctenophora and once in the group consisting of Cnidaria and Bilateria. While recent phylogenomic analysis (Feuda et al., 2017) suggests Porifera are indeed the basal clade, the existence of neuron-less Placozoa still allows the hypothesis that Ctenophora developed nervous systems independently. However, Placozoa only consist of a single species, decreasing the power of phylogenomic analysis regarding this phylum. This makes the case for Placozoa being primarily lacking in a nervous system less strong.

An additional reason why the case for parallel evolution of nervous systems is less likely with only the Placozoa as an intermediate is that a single species is a poor indication of primary absence instead of secondary loss, since a single species can quite easily lose features, and whether this is primary or secondary is impossible to check due to the lack of close relatives.

Another argument in favour of parallel origins lies in the bilaterian bias discussed in Section 2.2.6. Relying too much on Bilateria-focussed expectations risks discounting the divergent or parallel neuronal adaptations in Cnidaria and Ctenophora. Paying close attention to non-bilaterian solutions to behaviour problems should act as a counterweight to the assumption that the metazoan common ancestor was particularly bilaterian-like. We ought to let go of the exceptionalism regarding the complex bilaterian nervous system, which holds that nervous systems are so complex that they could never evolve more than once. If we accept that very basic nervous systems are a relatively simple step when provided with the building blocks available to the basal animal common ancestor (see sections 2.2.1 and 2.2.2), the case for parallel evolution of the nervous system becomes stronger.

Clues about whether or not features, in our case the nervous system, arose more than once can be found when investigating the developmental, genetic, and molecular constitution of the functional parts. If the ctenophore and cnidarian nervous systems use different genes and proteins, it is likely that their origins are independent. As such, the claims of Moroz and Kohn (2016) that precisely this is the case support Ctenophora being basal and nervous systems having evolved at least twice.

As a counterpoint to this, the warnings in Section 2.3.4 suggest that a lot may have happened in the interim. The simpler a common ancestor we suppose, the more different the phyla which could have emerged from it (Jékely et al., 2015b), which would argue for a common ancestor with a nervous system or some structure from which the nervous system evolved.

At some point the discussion becomes moot: if we posit that there was a single common ancestor with a nervous system, that system was likely so basic and unrecognizable that it becomes indistinguishable from a putative precursor system in a scenario where nervous systems arose twice. The question of how this process happened, once, twice, or even more times, remains, and a lineage explanation is valuable in all cases.

2.3.6 Lineage explanation constraints

As explained above, the intersection of features present in all descendent clades can be used to make informed guesses about the nature of the common ancestor. We want to know which neuroid features were present in animal common ancestors at various points predating demonstrably legitimate nervous systems—anything before the essentially certain arthropod compound eyes of *Anomalocaris*, approximately 515 Ma. (Paterson et al., 2011).

Even though there is fuzziness around the order of clade divergence and the timing thereof, if we accept this uncertainty in our suppositions we can make inferences about the nervous systems of common ancestors by comparing descendant clades. We will proceed in order from the most recent divergences to the most ancient one—for reference, see the cladogram, Figure 2.3.

Bilateria beyond Xenacoelomorpha

Starting at the bilaterian level—explicitly excluding the Xenacoelomorpha—where *Anomalocaris*’ certain, centralized nervous system is located, all clades have the following in common:

- i Complete neurons with synapses, electrical signalling, and elongations;
- ii Those neurons arrayed in a centralized nervous system arrayed along multiple body axes;
- iii Thanks to significant sophistication in developmental patterning genes.

These strong commonalities in nervous system structure between clades means they probably derived from a common ancestor featuring a well-defined (possibly elongated) body shape with a centralized nervous system (possibly in an anteroposterior cord).

Xenacoelomorpha

Going down one rung on the cladogram we find the Xenacoelomorpha. These animals, too, possess complete neurons and developmental patterning on par with the intersection

of protostomes and deuterostomes. The main difference here is that centralization appears in a less uniform manner, and a nerve net is present. Of particular interest is that the centralization appears to be less pronounced in the more basal clades within Xenacoelomorpha, which seems to indicate that centralization is secondary to nervous systems proper—possibly in the shape of a nerve net. Given the different kinds of centralization observed in the various subgroups of Xenacoelomorpha, this seems likely (Perea-Atienza et al., 2015). This would argue for a common ancestor to Bilateria which did have a diffuse nervous system but no centralization.

Cnidaria, Ctenophora

This supposition is strengthened when looking at the most basal innervated clades, the Cnidaria and Ctenophora, which we do not rank in order. They have sufficient overlap in nervous system characteristics to support the idea that a non-centralized nerve net is basal: there is a wide proliferation of centralization types between species in these phyla as well, in addition to the presence of nerve nets.

Porifera, Placozoa

The most important phylogenetic split is the one between those groups possessing nervous systems and those which do not, which is between Cnidaria and Ctenophora on one hand and Porifera and Placozoa on the other. Comparing these phyla in terms of nervous system components, we find the explanatory gap of Section 1.2.1 still quite present, with added confusion due to uncertainty about the order in which the groups diverged. The appearance of excitability, synapses, and elongations in a neuroid structure is still sudden: non-neural phyla appear to have no such structures at all while neural phyla have several: neurons and excitable epithelia. Even though no direct intermediate stages present themselves, we can identify constraints on a lineage explanation of nervous system origin.

First, many neural building blocks are present in Porifera. The extensive collection of nervous system-related genes in non-neural Porifera as well as neural phyla leads to the following sequence of conclusions:

- i Possession of a protein associated with the nervous system does not mean presence of a nervous system;
- ii Following i, possession of a gene coding for a characteristically neuronal protein does not imply presence of a nervous system;
- iii It is likely that other required parts for nervous systems were also already present in some common ancestor, performing some other function.

This means that a lineage explanation need not explain the presence of low-level building blocks.

Second, a nervous system is not a prerequisite for motile, free-swimming behaviour in a differentiated organism since ciliated sponge larvae do precisely that without a nervous system. Therefore, for small animals, the production constraints of a lineage explanation

should not be solely focussed on the ability to move at all. However, non-neural movement is either fast or in a large animal, not both: there is no indication that fast movement in larger animals is possible without a nervous system.

Comparing neural and non-neural basal phyla we also find that nervous systems correlate with fixed body plans: Porifera are relatively flexible regarding body plan, whereas Cnidaria and Ctenophora are not. This pattern continues higher up the tree: broadly, more complex developmental patterning allows for more complex nervous system structure and behaviour. Interestingly, Ctenophora do not use the same developmental patterning genes that Cnidaria, Placozoa, and Bilateria (together called ParaHoxozoa, precisely for this reason) do, yet both groups exhibit nerve nets. This means that *if* Ctenophora and Cnidaria shared a innervated common ancestor, it likely made do with very little developmental patterning as those patterning frameworks (i.e. Hox and ParaHox and their ctenophoran equivalents) appear to be a parallel (and thus later) developments. An alternative remains that nervous systems are parallel as well, which means that developmental patterning could have predated nervous system origin. Either way, developmental patterning available to animals evolving the very first nervous system was likely quite rudimentary: recently arisen, at the level of the ancestral sponge or the ur-eumetazoan—nothing as sophisticated as the patterning systems modern animals have. Consequently, any evolutionary model of nervous system development should include some basic developmental patterning but any model should not be reliant on extensive developmental patterning.

Eukaryote basis

Going even further down the tree, comparing non-neural animals with even more distant relatives may provide some information as well. Comparing Porifera and Choanoflagellata, we find that many genes present in the poriferan developmental toolkit predate split from the Choanoflagellata (e.g. Abedin and King, 2010; SebPedrs et al., 2013) and this includes components of the nervous system: the post-synaptic density protein Homer is present in choanoflagellates (Burkhardt et al., 2014). Obviously this single-celled (but colonial) organism does not use it for a nervous system since it does not have one. This further reinforces the first constraint of the previous section which states that a lineage explanation need not explain the basic building blocks.

2.4 Precambrian environment and ecology

The world was a radically different place during the long period between the origin of animal multicellularity and the Cambrian, the span in which nervous systems evolved. The purpose of this section is to place the origin of nervous systems in its time and in its environment in order to better understand the environmental pressures at play.

Timing is the first step: the period between the origin of animal multicellularity and the Cambrian is very long and this should be narrowed down. Having established a rough timetable, I will provide an overview of how environmental factors present at the relevant

time could have affected nervous system evolution. Three factors are covered:

- i Ecology, including Ediacaran (635–541 Ma) biota;
- ii Oxygen;
- iii Temperature and glaciation events.

The relevant period is so long ago that reliable information about it is hard to come by. There is geological evidence for the effect on ecology of environmental factors like temperature and atmosphere (e.g. Holland, 2006; Brocks and Butterfield, 2009; Sperling et al., 2010), but where life is concerned, the essential component of ecology, we are dependent upon fossils and phylogenetic inferences. These are necessarily subject to interpretation. By definition there were no recognizable animals at the time animal multicellularity arose. Photosynthesis was present and being performed by cyanobacteria (photosynthesizing bacteria) and algae. Colonization of dry land by plants happened after the Cambrian (Kenrick and Crane, 1997) and thus after the period relevant to nervous system evolution. At the start of the period considered here, the world was a place ruled by unicellular life both prokaryotic (i.e. bacteria, including cyanobacteria, and archaeobacteria) and eukaryotic (organisms with a nucleus and other organelles), agglomerating in deep, seafloor-covering biomats (Seilacher, 1999).

2.4.1 Timing

The interval between the emergence of animal multicellularity and the definite nervous systems of the Cambrian is certain to contain the origin of nervous systems, but at the cost of very inaccurate boundaries. It is possible to narrow down the interval using palaeontology and molecular clocks combined with phylogenetic inferences at the cost of confidence of containing nervous system origin.

Palaeontology: Budd

Palaeontology allows findings which are open to interpretation to be timed accurately relative to the time spans involved. Budd (2015) compiled palaeontological data to investigate the origin of nervous systems. He argues that trace fossils, remains of macroscopic indentations left on the benthos (sea bed) can be used to reason about the complexity of the nervous system of the animals which left them: earlier fossils leave simpler traces; later fossils leave more complex traces, implying the presence of a more sophisticated behaviour management system. Budd thus dates the emergence of nervous systems to the late Ediacaran and early Cambrian, 635–540 Ma.

Absence of palaeontological findings does not necessarily imply absence of innervated animals, but Budd claims that “animals undergoing an enormous radiation while leaving no trace in the fossil record is wildly implausible” (Budd, 2015, p.5), which means he considers earlier emergence of groups likely to have a innervated common ancestor (i.e. Bilateria, which underwent said enormous radiation) to be (‘wildly’) implausible. This would mean that the nervous system did not emerge before we find fossils of plausibly

innervated macroscopic animals, which would be during the late Ediacaran (treated in detail in Section 2.4.3).

Phylogenomics: Erwin

In contrast to palaeontology, phylogenomic analysis allows specific splits between animal phyla to be dated with relatively large margins of timing error. Findings in phylogenomics, which use molecular methods to derive the timing of splits in animal clades (see Section 2.3.2 for a detailed treatment) stand in opposition to Budd’s conclusions. Phylogenomic methods put the origin of the Metazoa at 800 Ma and indicate significant radiation in animal clades in the span between 800 and 550 Ma; see Figure 2.5 for details. This radiation encompasses the emergence of all innervated clades, which leads Erwin (2015) to propose two phases:

1. Up to 600 Ma, animals must have been small enough not to fossilize or leave traces. Phylogenomic comparison indicates that developmental and morphogenetic patterning must have been limited. Erwin supposes a last common ancestor to Bilateria and Cnidaria around 720 Ma which possessed a gut, a mouth, and multifunctional cells, as well as “well developed capacity for feeding, movement and sensation,” (Erwin, 2015, p.5) with neurons but not nervous systems.
2. From 600 Ma to the Cambrian explosion, characterized by increased ecological complexity, morphogenetic patterning increased and body size grew. From the neurons of the previous phase, nervous systems arose independently in Cnidaria, Ctenophora and Bilateria.

Erwin is vague regarding what exactly is meant by the neurons in phase 1 compared to the nervous systems in phase 2, nor does he go into any detail regarding how either system would have functioned. As our account focuses on neuronal features, the innervated common ancestor to Bilateria and Cnidaria suffices as an origin of nervous systems, timing it to around 720 Ma.

Reconciling palaeontology and phylogenomic timing

Given the large error margins of phylogenetic methods, a conservative informed estimate sets the emergence of nervous systems between an open-ended 800 (a phylogenomic lower bound) and a definite 540 (palaeontological upper bound) Ma. However, there is reason to be sceptical about palaeontological conclusions.

It is clear that by the Cambrian explosion, 530 Ma, crown bilaterian phyla are widely in evidence. Bilateria being a relatively advanced clade in this context, it is likely that some developmental scaffolding was present in an earlier age—necessarily the Ediacaran, being the directly preceding age (Valentine, 2002; Narbonne, 2005). Early metazoans need not have left fossil evidence at all: all or some of the Ediacaran biota, treated in detail in Section 2.4.3, may have been dead ends from an unseen stem which resulted in modern phyla. It is unclear to what extent the Ediacaran biota represent metazoan and eumetazoan ancestors. Some are probably representatives of some modern phyla;

Figure 2.5: Timing and phylum divergence overview from Erwin (2015). The blue horizontal bands indicate ice ages. (Figure reused with permission.)

some may not be. Apart from fossilized, macroscopic Ediacaran biota, there must have been smaller Metazoa. It is unlikely that early metazoans left fossils: they were newly multicellular and therefore small and likely soft-bodied, which makes for poor fossilization prospects (Cunningham et al., 2017). These kinds of animals, known as meiofauna (animals classified by their size; < 1 mm but $> 5 \mu\text{m}$), appear to have left no fossil evidence at all (Sperling, 2013). We should take care not to let the things that *did* fossilize bias our understanding.

Additionally, there is geological evidence of specific biomarkers suggesting the presence of a subgroup of Porifera, Demospongiae, at least 635 Ma (Brocks and Butterfield, 2009). This means the Porifera must have split from the animal trunk before that time and if Ctenophora are basal this pushes their split back even further. All in all, enough reason not to rely on Budd’s strict upper bound of recognizable fossils. As a result, whenever phylogenetic divergence timing estimates are mentioned in this thesis, we rely on molecular clock findings unless specified otherwise.

2.4.2 Ecology

Before animal multicellular life began its radiation into different clades some time before the Cambrian, unicellular organisms already went through radiations of their own, making it likely that a good number of their modern characteristics had been attained before the period in which nervous systems likely evolved. Information about the life of this period comes from two major sources: fossils or mineralized remains, and animal phylogeny. Phylogenomics allow us to determine at which point in the past clades diverged and molecular clocks allow us to time these splits, dating the origin of major groups: eukaryotes, for example, likely have an origin at around 2100 Ma, whereas Cyanobacteria originate 3500 Ma. Multicellularity, which likely originated multiple times (Parfrey and Lahr, 2013), in the broadest sense as agglomerations of organisms, originated as early as 3000 Ma (Grosberg and Strathmann, 2007; Knoll, 2003), yet the first undisputable multicellular animal fossils date to roughly 520 Ma (Valentine, 2002), though very probably various Ediacaran (635 Ma to 541 Ma) biota are animal as well—this is discussed in detail in Section 2.4.3. There are clues that interactions with bacteria strongly shaped the first animals and their common ancestor, up to and including wholesale adoption of genes by lateral transfer (Alegado and King, 2014).

It is hard to say anything about the point at which multicellular organisms with specialized cells arose and what kind of influence they had on the Precambrian ecology. What we do know is that microbial mats were common (Seilacher, 1999): agglomerations of micro-organisms which are also a feature of the modern world. These biomats can be up to centimetres thick. What exactly went on in these Precambrian microbial mats is hard to tell, but the absence of some things is clear: there were no large animals, no bioturbation, and no predation (Brasier, 2009). One can hypothesize based on the genes available to the organisms of the time when one assumes they coded for the same functional thing then that they do now. Based on such inferences Porter (2011) hypothesizes that unicellular eukaryotes (protists) evolved macrophagy, engulfing and digesting other

organisms, around 750 Ma, possibly even 1200 Ma. Size could have defended against being engulfed, and Porter hypothesizes that multicellularity could have been a response to this.

2.4.3 Ediacaran biota

Several groups of macroscopic specimens, believed to be animal in nature, have been identified as belonging in the Ediacaran biota: rotationally symmetric ones, believed to be ctenophores (Dunn et al., 2015); seemingly segmented, flattened ones, called *Dickinsonia* (Bobrovskiy et al., 2018); groupings of eukaryote cells believed to be animal in nature (the Doushanto formation) (Xiao et al., 2000), hypothesized to represent early stages of animal development, which might be bilaterian, might be cnidarian—but, according to Xiao et al. (2000), are definitely eumetazoan; a slug-like organism with a flat shell, called *Kimberella* (Fedonkin et al., 2007b); organisms with multi-sided symmetry (Droser et al., 2006); and even more enigmatic, branching shapes (the Lantian biota). How do these Ediacaran animals relate to metazoan crown groups? How did the Ediacaran biota live? Did they have nervous systems?

Many hypotheses exist; most conclusions about this era rely on abstraction and inference. Researchers generally claim to see modern phyla in the Ediacaran biota (Seilacher et al., 2003), but consensus is hard to find. The segmented nature of *Dickinsonia* suggests it is arthropoid or annelidoid in nature, but some (e.g. Valentine, 1992) hypothesize it was a polyp (i.e. a cnidarian); Sperling and Vinther (2010) suppose it was a placozoan. Recent reinterpretation of *Dickinsonia* and other iconic Ediacaran biota through an evo-devo lens indicates that they are likely metazoan in nature, at least (Dunn et al., 2018).

The resolution of fossils and its lack of clear resemblance to any modern species make it impossible definitively to relate *Dickinsonia* to any modern phylum, let alone to speculate about its physiology. *Kimberella* resembles a mollusc: it has a blob-shaped body and something resembling a shell. Some call it a mollusc (e.g. Fedonkin et al., 2007b; Fedonkin and Waggoner, 1997; Seilacher et al., 2003); some say it is not (Ivantsov, 2009). It most likely is some stem lophotrochozoan (Erwin, 2015) (a subgroup of the Protostomia introduced in Section 2.2.6 which includes molluscs). Budd (2015) also mentions that its classification is problematic and that clarifying its nature is important.

Considering the Ediacaran biota more generally, several authors provide useful higher-level hypotheses. Based on trace fossils, Jensen (2003) claims Ediacaran biota pushed themselves through sediment, that there was no burrowing. This would imply limited coordinative affordance. Contrary to this, Clark (1981) supposes that meiofaunal, burrowing animal ancestors with a coelom radiated into Turbellaria, Nemertea and Mollusca.

Given that all major eumetazoan phyla were likely already present in some proto-form (Erwin et al., 2011), what did the Ediacaran ecology look like? Life style appears to have been biomat-centric (Seilacher, 1999). Animal-on-animal predation appears absent; there is, in any case, no definitive evidence for it (Xiao and Laflamme, 2009). Sessile, benthic, and filter-feeding modes have been inferred (Narbonne, 2005). Either way, macroscopic organisms are in evidence of which we have no idea how they functioned, providing

very little information about potential early nervous systems.

2.4.4 Oxygen

The nervous system is an energy-hungry tissue (Laughlin, 2001), and aerobic metabolism is an efficient way of turning nutrients into ATP (adenine triphosphate, biologically usable energy).³ This makes the presence or absence of environmental oxygen a very important factor, especially regarding nervous systems.

We take an atmospheric oxygen percentage of about 20%, or .2 atm, for granted, but this was not the situation in the Precambrian. The state of oxygen in the environment over time can roughly be divided into five parts (Holland, 2006):

1. The first stage, until approximately 2500 Ma, is characterized by absence of atmospheric oxygen, with oceanic oxygen also largely absent with the possible exception of localized ‘oases’;
2. The second stage, approximately 2500 Ma to 1800 Ma, during which atmospheric oxygen levels rose to values estimated between 0.02 and 0.04 atm. Shallow oceans became mildly oxygenated and the deep oceans continued to be anoxic;
3. The third stage, approximately 1800 Ma to 800 Ma, saw oxygen levels remaining stable at the levels attained at the end of the second stage;
4. The fourth phase, approximately 800 Ma to 540 Ma and thus neatly coinciding with the period most important for the origin of nervous systems, saw a gradual increase in atmospheric oxygen approaching the .2 atm of today. During the extensive glaciations of this period, the deep oceans were anoxic.
5. The fifth phase, lasting until today, saw fluctuation in oxygen levels up to .3 atm and oxygenation of the oceans, albeit fluctuating as well.

The transition from phase 3 to phase 4 is likely to be very relevant for the development of animal life in general and the evolution of nervous systems in particular. Before then, oxygen levels were constant and low ($< 2\text{--}4\%$); afterward, oxygen was an increasingly salient environmental feature. For example, Planavsky et al. (2014) argue that the low oxygen conditions prevalent before 800 Ma probably prevented eumetazoans (i.e. any animal beyond sponges) from developing. Molecular clock studies agree with this, since they rarely date the origin of major eumetazoan clades before 800 Ma. Broadly speaking, it is likely that the rising oxygen levels allowed eumetazoan clades to evolve (Knoll, 2014).

Still, even after 800 Ma, oxygen levels were lower than today, and that might well have affected the suitability of the environment for animals with nervous systems, particularly larger ones due to problems transporting the oxygen to the tissue: larger animals require higher oxygen pressures to ensure sufficient access to oxygen away from the animal’s

³Interestingly, animals are far from obligate aerobic respirators; see Danovaro et al. (2010) for a known example of entirely anaerobic animals. While this example appears a secondary innovation, basal anaerobic metabolisms cannot be excluded (Mentel and Martin, 2010).

exterior, particularly with poorly developed circulation systems. Sponges, for example, lack any internal circulation system at all and utilize the water canal system which is also their feeding mechanism. Oxygen levels limiting the size of animals need not affect radiation of major animal groups; it could, however, have affected their size: there are no macroscopic metazoan fossils before 579 Ma. This is consistent with the hypothesis that oxygen limited body size, not the lack of evolutionary diversification (Erwin, 2015), making it more plausible that early radiation happened: we just do not see it because the lacking presence and internal transport of oxygen kept animals small.

The distribution of oxygen through the environment is also a relevant concern. The global glaciation periods rendered the oceans, the main locus of animal life at this point in time, anoxic—unsuitable for active animal life, certainly large animal life, potentially rendering extinct any aerobic metazoan-like clades which might have arisen before these glaciations.

Neurons are particularly sensitive to oxygenation levels, since they are energetically expensive (Laughlin, 2001; Lennie, 2003; Niven and Laughlin, 2008). With energy in short supply due to oxygen shortage, evolutionary pressure will quickly limit any neuron-like tissue. Even in high oxygen environments today, energy costs cause some animals to get rid of their nervous tissue. Ascidians (sea squirts, a tunicate, which is a chordate and thus bilaterian) significantly reduce their nervous systems after settling (Cloney, 1982). *Acropora millepora* (an anthozoan, which is a cnidarian) completely destroys its nervous system at some point in its life cycle (Ball et al., 2004)—the energy savings are worth sacrificing the coordinative benefits of possessing a nervous system.

Nervous systems are particularly useful in predation (Monk and Paulin, 2014) and avoiding it, which is one of the major reasons generally accepted why nervous systems became omnipresent in the Cambrian, an era of predatory arms races (Paterson et al., 2011). However, in modern ecology, low oxygen levels correlate with low carnivory (Sperling et al., 2014)—low oxygen areas do not support predatory metazoans. The only metazoans commonly found in such low-oxygen areas are polychaete worms.

2.4.5 Climate and temperature

Exact climatic details of this era are unknown. However, there is conclusive evidence indicating glaciation near the equator, especially in the era referred to as the Cryogenian, 720 to 635 Ma (Macdonald et al., 2010), right in the middle of our sensitive period. The majority of the Cryogenian is taken up by two glaciations, the Sturtian and Marinoan. These were so severe they may well have completely covered the globe in ice, resulting in a snowball Earth (Macdonald et al., 2010). The earliest estimates for the origin of Porifera place it before these glaciations (Brocks and Butterfield, 2009; Sperling et al., 2010) implying that Porifera at least survived these events, though they need not have resembled modern sponges.

Beyond these essentially cataclysmic periods of sustained low temperatures there is evidence of a significant glaciation later, during the Ediacaran, called the Gaskiers glaciation, circa 580 Ma. At this time there was sea-level glaciation near the equator, implying

Figure 2.6: Fossil record overview from Cunningham et al. (2017) (Figure reused with permission.)

environmental conditions not present in the past 580 million years. There are indications of rapid deglaciation afterward, with concurrent extreme environmental conditions, including chemical nutrients from weathering.

What precisely the meaning of these glaciations is in terms of animal evolution is hard to tell. Combined with deep ocean anoxia, conditions do not seem favourable for animal life.

One thing that is certain is that the end of a glaciation brings about deglaciation, which brings about extreme environmental conditions (disturbed ocean currents and the like) as well as a massive inflow of chemical nutrients, earlier locked in the ice, melting and flowing into the environment, available for life on which to thrive (Erwin, 2015). These events may have provided a fertile soil for the proliferation of animal clades. The Ediacaran fauna appear post-Gaskiers glaciation, for example. Cunningham et al. (2017) provide a current review of the timing of various Neoproterozoic (i.e. Precambrian) species of which there is fossil evidence in relation with glaciation. Figure 2.6 illustrates this—perhaps the most salient characteristic is the grey error bar in determining the exact age of the pre-Gaskiers clades.

2.4.6 Lineage explanation constraints

Nervous systems are energetically expensive (Laughlin, 2001). As indicated by the evolutionary model of Jékely (2011) and separate investigations on sensory tissue by Niven and Laughlin (2008), this expense acts as a counterweight to any functional production benefit. Modern low-oxygen environments exhibit low levels of predation (Sperling et al., 2014)—if animal-on-animal predation is the reason the early nervous system arose, it would have been after the rise of oxygen levels beyond .04%, 800 Ma.

Unless body shape was optimized for osmotrophy (diffusion feeding, see Section 2.5.2), as Laflamme et al. (2009) argue the Ediacaran Rangeomorpha and Erniettomorpha had, metazoa would have trouble transporting nutrients and oxygen throughout the body without a circulatory system. Consequently, many early non-rangeomorph or erniettomorph metazoans were probably small.

Modern low-oxygen environments do not necessarily exhibit low levels of nervous systems: the polychaete worms which inhabit these environments are innervated, unlike sessile animals, which get rid of their nervous systems because they do not need them. Clearly, there is some point to having a nervous system even in a low oxygen environment, so low oxygen levels in the time frame where nervous systems evolved are not a conclusive indication that no nervous systems were present.

In terms of constraints on the production requirement of lineage explanations, we can say that if nervous systems evolved before oxygen levels rose to modern levels, it would be more parsimonious if predation were not the reason since there is no neurally mediated modern predation under low oxygen circumstances. Many early metazoans likely being small implies that the nervous system emerged in small animals so a scenario of early nervous system origin which accounts for small animals is more parsimonious as well.

2.5 Behaviour

What do we know about the behaviour of early animals and what can that tell us about early nervous system evolution? As referred to in Section 2.3, drawing conclusions about animal phenotypes based on phylogenetic inferences is dangerous. Nielsen (2013) points out that there is no direct information about the ancestral eumetazoan life cycle based on the fossil record and genetics, which is the only evidence we have at this point. For all hypotheses in this section, it is true that they are based on exactly these kinds of dangerous inferences. For the very earliest animals, if they existed before 600 Ma, we only have indirect inferences based on molecular phylogenetics and geology. For later animals, those living in the Ediacaran, fossil evidence is also available.

This section seeks to give an overview of the possible conclusions that may be drawn regarding the behaviour of animals in the period during which nervous systems evolved, first putting behaviour in general in context, then progressing from feeding strategies to motility and habitats.

2.5.1 Behaviour in unicellular organisms

Neural circuits do not have a monopoly on the implementation of biological signalling algorithms (Baluška and Levin, 2016). Organisms without neurons are perfectly capable of exhibiting the behaviour necessary for their continued success in their particular niches. In the situation where nervous systems evolved, this non-neural signalling represented the baseline. Consequently, it is important to develop an understanding of behavioural options without possessing a nervous system, both to get a good idea of the behavioural ecology of the time when nervous systems evolved and to understand what extra benefits possessing a nervous system might have conferred.

Acting in a way that fits with the environment is an essential trait of living organisms. We have a tendency to ascribe behaviour only to organisms which exhibit some obvious, visible movement. However, any kind of reaction to an organism's environment may be classified as behaviour. Plants growing toward the light, guard cells opening and closing the stomata in leaves in response to light intensity and humidity, fungi releasing spores, sponges pumping water—all clearly behaviour of organisms generally regarded as static. Behaviour is not limited to multicellular life, either. Unicellular organisms exhibit a wide array of responses to their environment: chemotaxis, procreation contingent upon nutrient levels, clustering, formation of biofilm—even active predation through phagocytosis as exhibited by amoebae (Cosson and Soldati, 2008). Unicellular organisms perform these behaviours by means of cellular machinery at the molecular level, such as membrane proteins, microtubuli, and intracellular signalling molecules. They move and react quickly, but are limited to certain sizes, though protists may grow relatively large, and it is theorized that they did in the period during which nervous systems could have evolved (Porter, 2011).

Either way, it is important to position the origin of nervous systems as a shade in a palette of organismal behaviours, not the origin of behaviour. It is plausible and likely that the origin of nervous systems broadened the behavioural toolkit of multicellular animals, and the context for that broadening is the behaviour of the organisms around them: relatively developed, potentially fast-moving unicellular organisms, and sessile or slowly moving multicellular organisms. Given the gradient between low oxygen levels and the concomitant sedate tempo of metabolism and the likely ubiquitousness of biomats, what sort of behavioural repertoire can we suppose in the preneural eumetazoan common ancestor, and in the animals with the very first nervous systems? Where did they live, how did they move in that habitat, if at all, and how did they feed?

2.5.2 Feeding

Given the ecological reality of life around the Ediacaran, what are the options for nutrient collection for a putative possessor of an early nervous system, and how could it help them? Feeding options are a useful starting location when investigating behaviour as there are phylogenetic clues providing additional context. Given the interesting transitions in feeding modes between animal phyla, with Porifera filter-feeding and innervated clades using a gut, feeding makes a useful framework.

Broadly speaking, animals have four possible feeding modes:

- i Macrophagy, engulfing relatively large food particles with a gut and digesting them internally. As these particles can be static, this does not necessarily qualify as predation, though predation is a form of macrophagy. Examples: vertebrates, Ctenophora.
- ii Microphagy, filtering relatively small food particles out of the water and digesting them locally, usually with a water canal system. Examples: Porifera.
- iii Ventral digestion, moving over relatively large food particles and digesting them externally, between a ventral sole and the substrate. Examples: putatively dickinsoniids, Placozoa (Sperling and Vinther, 2010).
- iv Osmotrophy, relying on osmosis and a large surface-to-volume ratio for molecular-sized nutrients to diffuse into the animal. Examples: putatively Erniettomorpha and Rangeomorpha, modern internal parasites (Laflamme et al., 2009). Osmotrophy in parasites is a secondary evolution; the Rangeomorpha and Erniettomorpha are long extinct. Additionally, no coordination of movement is required at all. Consequently, it is not relevant for this discussion and will not be treated further.

The relationships between microphagy, macrophagy, and ventral digestion are relevant: modern macrophagic clades possess nervous systems, while clades which rely solely on ventral digestion and microphagy (i.e. Porifera and Placozoa) do not possess nervous systems. Figure 2.7 shows the latest possible emergence of these feeding modes as discussed by Sperling and Vinther (2010) (they do not mention the Ctenophora). This picture is supported both from the top down and from the bottom up, and the interesting bit is in the middle.

From the top down, based on phylogenetic inference and morphology, Nielsen (2013) supposes that the eumetazoan ancestor was a holopelagic, planktotrophic gastraea: a moving predator, *sensu lato*, ergo macrophagous: this common ancestor would be located at the split between Cnidaria and Bilateria. As both phyla possess nervous systems, it is likely this common ancestor possessed a nervous system as well.

From the bottom up, microphagy is in evidence in modern-day Choanoflagellata (King et al., 2008; Cavalier-Smith, 2013) and in Porifera in combination with a water canal system. This makes microphagy the likely ancestral metazoan feeding mode. Whether or not the canal system is a purely Poriferan adaptation or one of the basal metazoan later lost by neural clades does not matter for this discussion, since feeding with a canal system does not require possession of a nervous system.

Within macrophagy we can distinguish between slow macrophagy of static particles and fast macrophagy of motile particles. Ventral digestion is also slow and on static particles. Judging by the fact that no modern predation (fast macrophagy on motile particles) exists in low oxygen situations, that only becomes a plausible mode of nutrient gathering with higher ($>.04\%$) oxygen levels. The computational modelling studies introduced in Section 1.6.1 (Monk and Paulin, 2014) show that having a nervous system allows for

Figure 2.7: Latest possible emergence of feeding modes adapted from Sperling and Vinther (2010)

this mode of feeding and that ecological conditions suitable for predation allow a costly nervous system to be supported. This is in line with fossil findings of at least the Cambrian and beyond. Nervous systems likely facilitated motile animal-on-animal predation, certainly so in the Cambrian (Paterson et al., 2011).

That is not to say that nervous systems arose solely to allow predation. The interesting development in feeding modes lies in ventral digestion. Since Placozoa are non-neural, ventral digestion does not require a nervous system. However, a common ancestor possessed of a ventral sole could be a step toward a gut. Arendt et al. (2015) hypothesize that developmental and evolutionary advancements of an initially non-neural mucociliary ventral sole, entailing more extracellular digestion and nutrients flowing into the gastric cavity, could have led to evolution of a nerve net through division of labour from mechanosensory-contractile cells in the body wall, enabling coordinated movement of mucociliary creeping and changes of body shape: note how whole-body motility (creeping) serves the feeding method.

If the origin of the nervous system lies deeper, as the mucociliary sole theory suggests, oxygen levels were likely lower as well (see Section 2.4.4). This meshes nicely with the finding that non-predatory modern polychaete worms thrive in low-oxygen environments (Sperling et al., 2014).

2.5.3 Motility

Following the proposal of the previous section that motility is associated with macrophagy which in turn is related to possession of a nervous system, it is valuable to investigate the coordinative needs for various kinds of motility plausibly available to early animals. Various options are apparent: cilia, muscles, and hydrostatic mechanisms. These should

not be considered mutually exclusive. What we are interested in is to what extent (combinations of) these motility methods require the coordinative affordance of a nervous system.

All of these carry certain conditions and presuppositions. Cilia are generally infeasible for larger animals due to physical limitations regarding viscosity (Purcell, 1977), though they are easy to coordinate, even without a nervous system (Elgeti and Gompper, 2013). Cilia are common in unicellular organisms, including the direct premetazoan ancestor. They are ubiquitous throughout the animal kingdom, and some species use cilia to achieve movement of the entire animal—for example, metazoan larvae from all major groups (Bilateria, Cnidaria, Porifera) and adult Ctenophora as well as planarian worms (a bilaterian clade) (Tyler and Rieger, 1999) do use it for whole body propulsion, albeit not exclusively: all phyla also exhibit contractile functionality alongside the cilia.

Unlike cilia, contractile tissue organized into muscles allow larger organisms to move their entire bodies, but doing so requires a lot of coordination when used in a multicellular organism. In order to usefully affect their possessor, muscles need to act in concert to achieve anything. The essential contraction and expansion mechanism itself is ancient: the basic cellular machinery needed for muscle movement, the actin-myosin module, predates the metazoan radiation (Steinmetz et al., 2012): it, too, was almost certainly available to the early animals. Marlow and Arendt (2014) state that in early metazoans, myosin light chain kinase was added to the acto-myosin module coupling the regulation of contractions with intracellular Ca^{2+} concentration—implying that in early Metazoa, the essentially unicellular acto-myosin module, allowing movement, was combined with some intercellular communication molecule, in this case Ca^{2+} , a likely candidate for early intracellular signalling, and still universally relevant today in nervous system physiology. In modern metazoa, organized muscle and the nervous system always coincide, strongly suggesting coevolution (Seipel and Schmid, 2005).

Findings regarding hydrostatic skeletons support this coevolution. Clark (1981) states that using muscles for propulsion, for example by means of retrograde peristaltic movement, which would allow burrowing, requires a coelom (internal body cavity) to provide a sufficiently deformable hydrostatic skeleton. The coelom is a relatively advanced developmental feature. The most basal neural phyla, Cnidaria and Ctenophora, do not possess such. Using it to burrow with any celerity likely also requires a nervous system. Kier (2012), on the other hand, states that any sort of constant internal volume is sufficient: no specialized coelom is required, and polyps' enclosed internal volume suffices to act as a hydrostatic skeleton. Muscular-hydrostats allow bodies to use muscle to move absent a rigid skeleton (Kier and Smith, 1985). This also requires muscle coordination and probably a nervous system. No animal using muscular-hydrostatic propulsion manages without a nervous system. Whether the primitive solution resembled any modern one is unclear.

Regardless of whether muscles propel in combination with cilia or hydrostats, they are always accompanied by nervous systems.

2.5.4 Habitat

The animals in which the nervous system originated must have lived in the ocean but there are still clear distinctions to be made how and where. There are four main options:

- i They could have been sessile, that is, static on the sea floor or some substrate like rock or bacteria-crusted soil, like modern sponges and many other animals with a sessile life stage;
- ii They could have been benthic, that is, motile in or on the sea floor;
- iii They could have been pelagic, living a drifting or actively navigated life in the water column;
- iv They could have been burrowing, making a habitat in the soil of the sea floor, either including mobile foraging or not.

Since the first definite evidence of any lifestyle is fossil evidence, it is logical to conclude that any animals of this time period were benthic or sessile. Animals moving around the ocean floor leave trace fossils and when static or mobile animals living on the sea floor get covered with sediment they tend to fossilize nicely in ways that allow them to be found easily. Conversely, it is hard to tell whether fossils of pelagic animals that died and fell to the sea floor were pelagic, benthic, or sessile. Nevertheless, Budd and Jensen (2015) hypothesize that the common ancestor to innervated animals was probably benthic. Whether the data points we have for this expose any conclusions to observation bias (since other modes fossilize more poorly) is impossible to tell; what we do know is that from circa 560 Ma we have definite trace fossils of something moving around (Jensen, 2003). These earliest trace fossils are straight and short; from 550 Ma more complex-looking patterns can be observed. What this means for the nervous system capabilities of the animals in question is hard to infer (Jensen, 2003), but it likely indicates muscular activity. Muscles would be used for steering cilia (as in modern planarian worms or ctenophores) or as a hydrostatic mechanism (like in modern Cnidaria), thereby implying presence of a nervous system.

Though a bilaterian fossil has been found together with its trace, dating to the late Ediacaran (Chen et al., 2019), it remains unclear what kind of animals produced the earliest traces—Seilacher et al. (2003), for instance, suppose they are worms, whereas Jensen (2003) refers to them simply as Ediacaran biota. When attempting to draw conclusions about the habitat of whichever fauna is mentioned, both benthic and burrowing lifestyles may be inferred: Jensen (2003) considers the traces to mean that the animals pushed themselves through sediment, and does not observe burrowing, whereas MacNaughton et al. (2000) claim to infer burrows of some unspecified bilaterian. Either way, they were macroscopic—whether or not they were fast is unclear.

Aside from trace fossils there are the enigmatic fossils of *Dickinsonia*, a prominent member of the Ediacaran biota referred to in Section 2.4.3. The conclusions drawn from their impressions on the sea floor vary: Cavalier-Smith (2017) and Sperling and Vinther (2010) use the same evidence to argue opposing points, Cavalier-Smith (2017) claiming

they were sessile and Sperling and Vinther (2010) claiming they moved around, foraging, making them benthic. It is entirely unclear whether any kind of nervous system was involved.

Some make more sweeping claims based on the available evidence: Narbonne (2005), for example, infers that both benthic lifestyles and sessile, filter-feeding lifestyles were present at this time, drawing an explicit parallel with later and modern animal groups.

Another option would be that the earliest animals, whatever their habitat, were too small to reliably fossilize. Based on phylogenetic inferences, Clark (1981) supposes the bilaterian (and thus innervated) common ancestor was meiofaunal, a burrowing animal—probably soft-bodied. In that case, the timing becomes impossible to establish with certainty.

2.5.5 Lineage explanation constraints

What does the information we have about basal metazoan behaviour tell us about the early nervous system? Section 2.5.2 shows that it does not appear necessary for a sessile, filter-feeding existence: modern sponges do not have one and sessile animals minimize it. Muscles, either in conjunction with a hydrostat or used for steering cilia, the fast macroscopic animal movement mechanism, always appear in conjunction with a nervous system, regardless of whether they are used for burrowing, grazing, swimming, or prey capture. All predation, macrophagy on motile particles, occurs in conjunction with a nervous system. However, the earlier emergence of a nervous system to aid in macrophagy on static particles cannot be excluded. Nervous systems appear to allow muscle-driven, whole-body motility and active predation.

There is added value to a lineage explanation of nervous systems which explains how it would allow soft-bodied animals to move, even without predation. The early origin of light chain kinases (Marlow and Arendt, 2014), linking muscles to Ca^{2+} concentrations and thus coordination is particularly salient in this regard, linking electrical conductivity with muscle movement, suggesting coincidence and coevolution of muscle use and nervous system-mediated coordination.

2.6 Nervous system morphology and potential precursor tissues

Given their phylogenetically basal origins, neural tissues were likely one of the earlier tissue specializations. However, considering the clues that nervous systems might have evolved more than once and that Porifera have tissue specialization but no nervous tissue, nervous tissue is unlikely to be the very first diverged tissue and probably arose from some other tissue. How and why that process took place may give hints as to the continuity and production of a lineage explanation of nervous system origin: the tissue from which neurons originated may have implications for the kind of functional roles intermediate tissues could have played or what kind of morphology it could have exhibited.

This section will first discuss the individual elements of neurons as outlined in Section 1.2: electrical signalling, synapses, and axodendritic elongations. Next, general considerations on specialization and development will be discussed. Finally, related or precursor tissue types will be treated: first epithelia, a precursor, then eyes, a related tissue.

2.6.1 Neuronal features

Beside considering nervous systems as a tissue, another way to gain insight into the origin of nervous systems is to investigate individual constituent parts. The salient characteristics of a neuron, those essential to nervous system functioning, without which we cannot talk about a neuron, are: electrical signalling, synaptic connections, and elongations (axons and dendrites). Investigating the origin of these specific features, paying attention to the order in which they appeared, may help us understand the functional progression of the early nervous system.

Electrical signalling

All living cells maintain ionic and osmotic balance with their environment by pumping ions in and out, which is done with ion channels and pumps. The mechanisms involved are ancient and ubiquitous. When discussing electrical signalling, I am referring to the ability to use these mechanisms to achieve a wave of electrical charge to move along a membrane, which is what an action potential is.

Using ionic currents to coordinate is not limited to the animal kingdom. Single-celled *Paramecium* uses waves of Ca^{2+} -ions to reverse its swimming direction if it hits an obstacle. Even plants use ionic waves to coordinate. The use of ionic mechanisms for coordination in animals is ubiquitous throughout the animal kingdom and was likely available to its common ancestor as well. Specific ion channels, such as the sodium channels found in nervous systems, may provide more information about particular lineages in animal electrical signalling in nervous systems. However, the findings are inconsistent. Liebeskind et al. (2011) claim that sodium channels predate the nervous system, whereas Barzilai et al. (2012) claim they arose independently in Cnidaria and Bilateria, precluding an ancestral version. Glass sponges use calcium/potassium action potentials to coordinate behaviour (Dunn et al., 2015), lending credence to the idea that these mechanisms are basal to Metazoa. It thus seems likely that the functional ability to signal electrically was available to animals at the point where first nervous systems arose, but which exact mechanism that was remains unclear.

More recent phylogenetic findings by Liebeskind et al. (2015) regarding ion channels used in nervous systems indicate rounds of gene loss as well as independent, rapid expansions. A net loss event occurred after the last bilaterian common ancestor, while other major loss events occurred in the stems of major deuterostome and protostome clades. Gains occurred independently in all major innervated clades as well as in the paraHoxozoan common ancestor. The cladogram in Figure 2.8 shows the locations on the phylogenetic tree of these gains and losses, following the branch ordering of Liebeskind

Figure 2.8: A cladogram following Liebeskind et al. (2015), showing loss and gain events of major ion channel families.

et al. (2015). Bearing in mind the injunction that genes being present or absent do not mean presence or absence of the thing they encode in modern animals (Section 2.3.6), this information is not conclusive regarding singular or multiple origins of nervous systems. It does, however, show a remarkable constancy in the major ion channel family genes from the choanoflagellates to the ParaHoxozoan common ancestor. The animal stem made do with very little expansion in ion channel families for a long time.

Liebeskind et al. (2015) opine:

The simplest explanation for this pattern is that nervous systems originated early, were very rudimentary for a long period, and then convergently evolved in complexity. (p.5)

This pattern also supports a situation where the basal animals had some non-neural coordination mechanism constituting most of the building blocks necessary to form a nervous system. Since the basis for expanding these ion channel families was already present in all groups, expansion would then independently provide additional benefit for separate clades which independently developed nervous systems.

In fact, the distinction between early origination or separate development into a nervous system from a non-neural coordination system lies in the definition of what constitutes a ‘nervous system’. Either way, here is evidence for some sort of ionically mediated intermediate system, either rudimentarily neural or not-quite-neural. How that in-between case functioned is precisely the sort of thing the production requirement of a lineage explanation should explain.

Synapses and gap junctions

Synapses are structures which allow identified connectivity between cells: only the receptors of the cell to which the synapse is attached are affected, and only from the presynaptic cell. Two major types of synapse exist: chemical synapses and gap junctions. Chemical synapses are the canonical kind associated with nervous systems which one finds in psychology textbooks: an arriving action potential causing exocytosis of vesicles, releasing neurotransmitters into the synaptic cleft and receptors on the postsynaptic side receiving the signal. Gap junctions, which are found in many tissues outside the nervous system, are direct cytoplasmic connections between cells. These allow small molecules to pass between cells, which lets the ion influx waves of an action potential directly traverse cell boundaries. Gap junctions also allow passage between cells of (developmental) signalling molecules. Unless specifying ‘gap junctions’, whenever we refer to synapses, we mean nervous system-associated chemical synapses.

While electrical signalling can be functional even in single-celled organisms, synapses—necessarily being a function of two cells—are a multicellular adaptation. There are a lot of data when it comes to the components, particularly of chemical synapses. Many of those components can be dated to before the emergence of metazoans. Emes and Grant (2012) write:

The major classes of synapse proteins and their respective functions in intercellular communication and adaptive responses evolved in prokaryotes and eukaryotes prior to the origins of neurons in Metazoa. (p.111)

Examples of the pattern include:

- i The post-synaptic density protein Homer, a synaptic component, which is present in choanoflagellates (Burkhardt et al., 2014)—an organism lacking a nervous system which is only facultatively multicellular;
- ii The structural components of the postsynaptic density, which are present in *Amphimedon*, a poriferan;
- iii Vesicle exocytosis, which is very broadly present as well, observed in unicellular holozoans. Homologs of the proteins involved in exocytosis have been identified in fungi and plants (Bosch et al., 2016). Kloepper et al. (2007) identify one such protein, SNARE, as a feature of the putative ur-eumetazoan.

Precursors to synaptic sub-structures likely served particular roles on their own, affording them a role outside the context of a nervous system. If synaptic parts can be shown to have served such non-neural roles, it becomes more plausible that synapses predate nervous systems as a whole. There are indeed indications that this is the case: Achim and Arendt (2014) find that the postsynaptic module initially acted as a general ‘chemosensory module’. For this role, the protein neuroligin is essential. Neuroligin is not present in *Trichoplax*, but can be found in the cnidarian *Nematostella* and in bilaterians. *Hydra*, another cnidarian, and *Mnemiopsis*, a ctenophore, do not have this protein,

but the presynaptic binding partner to neuroligin, neurexin, suggesting earlier presence of neuroligin. If all species mentioned show evidence of having possessed the neuroligin/neurexin pair, this suggests it existed in the last common ancestor of Ctenophora, Cnidaria and Bilateria. This would make at least all chemical synapses homologous.

Ryan and Grant (2009) and Emes and Grant (2012) date the origin of synapses proper to between Porifera and Cnidaria: a point which is not certain to have existed, given possible multiple origins of the nervous system in Cnidaria and Ctenophora. Based on Cnidaria, Galliot and Quiguand (2011) suppose that chemical synapses, at least, were a function of the last common ancestor of Cnidaria and Bilateria. The broad stroke of synaptic emergence at some point between uninnervated animals and ones having a nervous system is clear, but based on modern animals it is hard to tease out any role of synapses not involving the nervous system, leaving us with little idea of what any pre-neural role for synapses could have been. Recent work investigating paracrine signalling in *Trichoplax* (Varoqueaux et al., 2018) points in the direction of a possible transition: direct chemical signalling gradually becoming more local and more targeted.

As for electrical synapses (gap junctions), it is currently unclear whether chemical synapses or electrical synapses arose first. Molecular phylogenetic data (Abascal and Zardoya, 2013) suggest they arose roughly concurrently, potentially in mutual support (Ovsepian and Vesselkin, 2014). Abedin and King (2010), based on the occurrence of pannexin, also place the gap junctions between Porifera and eumetazoans, similar to chemical synapses.

Neurotransmitters, too, appear to predate nervous systems in general. The molecules commonly thought of as ‘neural’ predate neurons (Attenborough et al., 2012). More specifically, Achim and Arendt (2014), referring to Anctil (2009), state (p.105): “acetylcholine, GABA/glycine, neuropeptide and hormone signalling likewise predates the last common ancestor of cnidarians and bilaterians.” Sponges also possess many messenger molecules generally associated with nervous systems: nitric oxide, glutamate, and GABA, which they use for coordination. The mechanism sponges likely use is juxtacrine signalling: affecting close cells with excreted messenger molecules (Elliott and Leys, 2010; Leys, 2015).

A final complication lies in the directionality of synapses: bilaterian synapses are generally one-directional, going from the axonal end of the presynaptic cell to the dendritic end of the postsynaptic cell, giving synapses a clear polarity. This is not the case in basal phyla: Cnidaria, for example, feature bipolar neurons, in which the distinction between axon and dendrite is blurred. In these cells, synapses are often bidirectional (see e.g. Garm et al., 2006; Satterlie, 2011). Additionally, gap junctions or electrical synapses are always bidirectional.

Axodendritic elongations

Elongations are perhaps the most striking feature of neurons. In order to transmit electrical signals to a specific target in a short time over any distance, neurons require elongations. Generally, we identify receiving, postsynaptic dendrites and sending, presynaptic axons. Both these features also have computational properties: in dendrites, the branches

of dendritic trees allow for specific signal summation over sub-branches; in axons, the axon hillock determines whether somatic activation is sufficient to start sending an action potential along the axon. In more primitive animals, the binary distinction between axons and dendrites may be blurred. Cnidarian neurons, for example, are often bidirectional. That is, elongations are both afferent and efferent at the same time.

Axons and dendrites, the neural elongations, may be the only uniquely neuronal feature. Simple cellular elongations, however, are neither particularly neural nor animal, as elongated cellular fibres occur throughout all kingdoms of life. The uniqueness of axodendritic elongations comes from the combination of cellular elongation with synapses and electrical signalling. Since simple elongation is such a basic property, it is hard to distinguish which genes are responsible for specifically neural elongation, especially with regard to identifying its origin in deep evolutionary time (Hejnol and Lowe, 2015). Integration of those elongations into a body plan is another matter. This integration is governed by developmental mechanisms, such as the Hox genes. These mechanisms determine cell fates and where in the body tissues develop. The developmental properties of the different basal phyla are mentioned in their entries in Section 2.2. Inferences about nervous system origin based on between-clade comparisons of development are described in Section 2.6.3.

2.6.2 Cell & tissue specialization

Any kind of cell or tissue specialization, any kind of differentiated morphology is necessarily a function of multicellularity. Some facultatively colonial organisms exhibit some role attribution, for instance in slime molds (Bonner, 2015). The cell fates of animal tissues are generally determined in development. Our normal frame of reference is the human case, wherein development from zygote to adult individual is a one-way road of cell fate, except for gametes. This is not necessarily the case in other animal species: adult Porifera possess pluripotent cells; in animals with life cycles with multiple stages, cells change fates mid-existence. Any separate part of *Hydra* is capable of becoming an individual in its own right, indicating that it has the potential of generating pluripotent cells at growth sites. Either way, cell fates are a feature of multicellularity, and animals have evolved signalling systems to tell cells what their fates should be. The most well-known regulator of such signalling are the Hox genes. While Hox genes proper are generally considered bilaterian in nature, Cnidaria possess a similarly derived mechanism (Ball et al., 2004)—the whole family of body-patterning genes from Porifera on up is clearly related (Ryan and Baxevanis, 2007; Finnerty and Martindale, 1999) (this explicitly excludes Ctenophora, since they are quite possibly basal to Porifera).

The ancestral state in terms of cell specialization is a multifunctional cell (Arendt, 2008). Assuming that the ancestor to animals was a single species and not some agglomeration of multiple species, that first multicellular ancestor had to perform all possible functions with a single cell type. Once multicellularity is established, specialization becomes adaptive, as evidenced by the omnipresence of cell specialization in multicellular life: animals, plants and fungi all exhibit it. There is a large increase in complexity in terms of number of cell types between the eumetazoan ancestor and modern animals.

Comparing this cell type proliferation to the limited increase in the number of genes between the eumetazoan ancestor and modern animal, the number of proteins expressed per cell type must have decreased (repurposing pre-eumetazoan genes), meaning that cell diversity increase is a crucial mechanic in early eumetazoan evolution (Arendt et al., 2009). Where does the nervous system fit in when it comes to cell specialization? Several modern tissue types are associated with nervous systems, for various reasons. They, and the reasons why they are associated with nervous systems, will be discussed next.

2.6.3 Evo-devo and tissue development

The way genes result in various tissues is determined by embryonic development. Genes can switch on in specific cells, localized by signalling mechanisms. Those genes then determine the fate of the cell, the kind of tissue it becomes. Other genes may result in triggering other signalling mechanisms, and so on. Evolutionary developmental biology or evo-devo is the study of how development turns genes and epigenetic factors into phenotypes and how these processes evolved. The way specific features develop may contain clues about how they evolved. For example, at some point in embryonic development, humans possess a tail. In conjunction with the knowledge that our close phylogenetic relatives possess tails, this is evidence that human taillessness is a derived condition, not a primary one: our common ancestor possessed a tail at some point; we are not from a lineage which never developed one.

How does neural tissue fit into the narrative of early metazoan tissue specialization? Arendt et al. (2015) argue that the order of emergence of major morphological features between the eumetazoan common ancestor and the bilaterian common ancestor was blastaea (an animal consisting of a hollow, spherical clump of cells), gastraea (the same as blastaea, except invaginated, i.e. dented), mucociliary sole (i.e. ventral sole with mucus and cilia), nerve net, gastric pouches (a blind gut). Bearing in mind the possibility that Ctenophora are basal muddies the waters somewhat, since the concept of eumetazoan becomes fraught in terms of monophyly,⁴ but if we read Arendt to mean eumetazoan in the sense of common ancestor to all animals except Porifera and perhaps Ctenophora (depending on the outcome of that particular debate), the ordering becomes ctenophore-basality independent. On the same note, Kelava et al. (2015) state that there is conservation of the genetic network of nervous system development between Cnidaria and Bilateria. Whichever way the early nervous system was shaped, it appears to have been in place before the split between Cnidaria and Bilateria. The important message is that the nervous system appears very early on in the evolutionary narrative.

Regarding the animal ancestor in which this Bauplan arose, Erwin (2015), based primarily on phylogenomic analysis, writes: “the body size of early metazoans must have been small with limited developmental patterning of the embryo and a lack of complex morphogenetic patterning.” As Section 2.4.4 shows, oxygen concentrations were low and unstable at this time, probably placing primacy on opportunistic life strategies with easy

⁴After all, Eumetazoa are considered all animals except sponges, and if Ctenophora are basal to sponges yet included in the eumetazoans, that group is not monophyletic.

dispersal. The basis of foodwebs for clades other than Porifera is unclear. The cnidarian-bilaterian LCA (approx. 720 Ma) (Telford et al., 2015) was able to form primary and orthogonal body axes and regional organization of structures in relation to body axes.” More specifically regarding nervous system evolution, Seipel and Schmid (2005) write about this same basic metazoan: “we propose that striated muscle-based locomotion coevolved with the nervous and digestive systems in a basic metazoan Bauplan”. Not just the nervous system, but digestion, body axes: a head, an up and a down, were critical innovations of the early Metazoa. All species featured in the Cambrian explosion which are recognized as representatives of modern eumetazoan phyla possessed these innovations. Erwin et al. (2011) argue that the Cambrian explosion was the result of the developmental options resulting from the genetic toolbox of which this body axis innovation was the first indication.

Diving deeper into the lineage of the genes regulating this body patterning, the Hox gene family, we find that the intermediate steps are fuzzy. Sponges have only the rudiments of this toolbox (Srivastava et al., 2010), and some argue that there is reason to believe they were lost secondarily, the ‘ghost locus’ hypothesis (Ramos et al., 2012; Fortunato et al., 2014). Eumetazoa, even simple ones (Hejnol and Lowe, 2015), including Cnidaria (Watanabe et al., 2014) possess most. Ctenophora do not use Hox or ParaHox genes, but clearly do possess some kind of patterning mechanism. The molecular mechanisms behind ctenophore patterning are currently unknown (Lanna, 2015). Considering that an organism without any body plan or tissue difference would hardly be recognized as an animal at all, it is not surprising that these patterning genes are rooted so deeply in the animal tree. Though patterning is hard to quantify, the overall trend that more developed patterning correlates with more developed nervous systems seems to hold, which fits with the fact that developmental mechanisms are required to guide axodendritic elongations to specific places. The causation between more developed patterning and more developed nervous system is likely to be bidirectional, as better patterning leads to more specific application of neurons to behaviour problems while nervous systems help application of patterning to the body as well. Extrapolating to the earlier animals, we may infer that as patterning was likely very rudimentary, so was the nervous system.

2.6.4 Epithelia

The basic building material of the animal body, the first thing upon which developmental patterning impinges, is the epithelium. Epithelial tissue is one of the few tissues even more ubiquitous in the animal kingdom than nervous tissue. There is some discussion about whether Porifera truly possess epithelia. However, sponges possess genes associated with epithelia in other Metazoa and these tissues are similar in development, molecular machinery, and function (Leys et al., 2009). This means that sponge epithelia are certainly sufficiently homologous to other metazoan epithelia for the purposes of the discussion of nervous system origin (see Keijzer and Arnellos, 2017, for an overview and references).

Epithelia form a crucial part of animal architecture: in the simplest terms, defining what is outside and what is inside. Between each other, metazoan epithelial cells have

adherens junctions sticking them together and gap junctions allowing for intercellular signalling. On the inside, they have focal adhesions, integrin-based adhesion complexes connecting them to the underlying structure, the basal membrane. Developmental processes all over the animal kingdom use this machinery to define morphology and sub-specialization. In sponges, non-developmental coordination systems are primarily based on these epithelial mechanisms as well (Leys, 2015).

Epithelia forming the basis of developmental processes leads to an important generalization in comparative morphology: identification of different germ layers. Since there is progression in number of germ layers in basal clades from one to three germ layers, the common metazoan ancestor is generally considered to have possessed one germ layer, though some dispute this and hypothesize that the metazoan common ancestor had three germ layers (Cavalier-Smith, 2017; Seipel and Schmid, 2005).

Looking at how nervous systems are positioned in terms of germ layer between different phyla provides interesting clues: in vertebrates, the nervous system is entirely ectodermal (although some neural tissue developing from endoderm has been observed in sea urchins, which is also a deuterostome (Wei et al., 2011)). In Cnidaria, neurons arise both from the ectoderm and the endoderm. This means that either endodermal neurons arose independently in Cnidaria and sea urchins, or that neurons are basal to germ layer differentiation, putting the origin of neurons very deep indeed.

Epithelia also exhibit contractile behaviour, utilizing the cellular actomyosin cytoskeleton. Additionally, in the last common ancestor of Metazoa, epithelial cells possibly possessed cilia as well, adding more movement options (Nielsen, 2013). Between the features of coordination, movement, and structure, it is easy to imagine epithelia as the fundamental building material or ground pattern of early Metazoa (Nickel et al., 2011).

Excitable tissue

Excitable epithelia are sheets of epidermal tissue that have conductive properties (Mackie, 1965). While all epithelia are conductive to some extent, excitable epithelia providing behavioural responses on the level of the entire animal through extensive conductivity are common in Cnidaria. For example, in the hydrozoan species *Sarsia* and *Euphyssa*, the “subumbrellar ectoderm is a single layer of cells having striated fibres running circularly” forming “the swimming muscle” (Mackie and Passano, 1968). In this epithelial tissue, electrical impulses travel from cell to cell via gap junctions (electrical synapses) and are able to induce effector responses at points distant from the signals’ origin. Similar forms of excitable tissue are also present in muscle (Josephson, 1985; Mackie, 2004; Brink et al., 1996), sponge syncytial tissues (Leys et al., 1999) and myocardial tissues (Nash and Panfilov, 2004; Ten Tusscher and Panfilov, 2006; Ten Tusscher et al., 2007), all providing coordination without the need for a nervous system. There is a broad array of examples of non-neural coordination mechanisms inside and outside the animal kingdom, which evolved separately (Mackie, 1970). These are all modern tissues; to what extent ancestral tissues exhibited the same functionality is up for debate. However, given the very likely basal nature of epithelia and the reasoning that undifferentiated tissues are multifunctional, an excitable epithelium as a precursor tissue to both muscle and neuronal tissue

is plausible (Pantin, 1956).

2.6.5 Eyes

Photoreceptive organs are ubiquitous, not just in the animal kingdom. Animal eyes, though, are almost universally connected with a nervous system. Sponge larval photosensitivity (Leys and Degnan, 2001) is the primary exception. Photosensitivity being the more primitive feature, nervous features could have arisen from photoreception-based tissue specialization.

Arendt et al. (2009) directly use the evolution of eyes as a potential origin of the nervous system. This constitutes an entire lineage explanation: specialization in photosensitive tissue leading to elongated connections in turn leading to a nervous system. This hypothesis is treated in detail in Section 1.4.2.

Another important feature of eyes is that particularly multifaceted arthropod eyes are a distinguishable feature in Cambrian fossils: Clarkson et al. (2006), for example, describes trilobite eyes dated to 520Ma. Paterson et al. (2011) describes the eyes of the arthropod *Anomalocaris*, an active visual water column predator, dated to 515Ma. If we reasonably assume that these eyes are similar to modern arthropod eyes, which are deeply integrated with nervous systems and very hard to imagine without this integration, particularly in an active visual predator, we can confidently infer the presence of a fully functional nervous system by the mid-Cambrian.

2.6.6 Lineage explanation constraints

Individual neuronal features clearly predate nervous systems. The thing that makes them neural is how they came together. Molecular phylogenetics tells us little about how that happened, since its resolution cannot tell us at which point these characteristics changed from non-neural to neural. The only clue we have on the level of cellular machinery regarding an intermediate state of electrical excitability is the finding of Liebeskind et al. (2015), described in Section 2.6.1 which implies that a proto-nervous system arose early in the animal tree. The features already present must have coalesced into some proto-nervous or neuroid system at that point, but how and why is unclear.

The first animal tissues were likely little differentiated. Since nervous system tissue appears to predate the second germ layer (because it is expressed endodermally as well as ectodermally in Cnidaria whereas it is only ectodermal in Bilateria, suggesting neurons were already present before the establishment of the endoderm in Cnidaria) it likely differentiated very early. Add to this the correlation between stronger body patterning and nervous system complexity and it becomes clear that early nervous systems likely arose under circumstances of very limited specialization. Any lineage explanation of nervous systems should account for this, since assuming otherwise would violate the continuity requirement of lineage explanations.

A lineage explanation need not explain the emergence of individual neuronal features, since the molecular bits were present before. Having established that the building blocks

exist (see Section 2.3.6), continuity is satisfied with the caveat that developmental patterning is required to bring them together, which should have arisen simultaneously or earlier. The question remains how the features combined to provide functionality for the animal: the production requirement. What could synapses on their own add to coordination? Do synapses without elongations provide some coordinative benefit? How do synapses between adjoining cells compare to juxtacrine signalling or targeted secretory signalling (Varoqueaux et al., 2018)? In this case, the production requirement should be taken as a guide: whichever order of application produces something functional is plausible.

Nervous systems show a marked affiliation with (ectodermal) epithelia, both in terms of functional derivation, for instance in excitable epithelia, and developmental origin. The evolutionary models proposed by Pantin, Passano, and Mackie all incorporate this (see Section 1.4.1). These evolutionary models plausibly assume that neurons derive from epithelia.

Neurons likely predate the emergence of the endoderm. Having neurons might well have been a requirement for and coevolved with a gut-based lifestyle: no modern animal with a gut lacks neurons. This fits with the suggestion of Arendt et al. (2015) introduced in Section 2.5.2 which proposes neurons as a specialization from an initially non-neural mucociliary sole. Explaining the functional benefit of neurons to a gut-having animal would improve the parsimony of a production-explaining model.

2.7 Conclusion

Summarizing, in spite of uncertain timing and a general paucity of information, what are the constraints arising from scientific findings on a given lineage explanation of nervous system origin? Better yet, given the uncertainties present in the scientific background, which constraints are likely to improve the parsimony of a lineage explanation of nervous systems? Here, we coalesce all findings mentioned in this chapter.

- i As Section 2.3.6 shows, nervous system centralization appears to be a derived trait, so the first nervous systems were probably diffuse nerve nets. This makes a diffuse nerve net a good end point for a lineage explanation of the first nervous systems.
- ii Molecular building blocks of cellular neuronal components appear to predate nervous systems (sections 2.3.6 and 2.6.6). This means they need not be accounted for by the lineage explanation.
- iii Comparison between phyla in Section 2.3.6 shows that movement is possible without a nervous system, though macroscopic and fast movement is not. This means macroscopic and fast coordination should be a driver for a lineage explanation.
- ix Additionally, a lineage explanation ought to reflect that such timely multicellular coordination functions for soft-bodied animals (Section 2.5.5).

- iv That same comparison between phyla also shows that there is a correlation between developmental patterning and the resulting body plan and nervous system complexity. As the nervous system is an early innovation, patterning in the ancestor where it arose was likely rudimentary. A lineage explanation should work with the presence of limited developmental patterning and a rudimentary body plan, but still require some.
- v Investigation of the relevant environment in Section 2.4.6 shows through oxygen limitations and discrepancies between molecular clock findings and the fossil record that the animals in which the nervous system likely arose were probably small (meiofaunal). A lineage explanation should take small animals into account.
- vi Though macrophagous animal-on-animal predation never occurs without a nervous system, nervous systems occur in situations where that is not a feeding mode. The presence of nervous systems in low ($<.04\%$) oxygen situations and low oxygen in the relevant period implies that nervous systems need not have arisen to facilitate animal-on-animal predation, as further detailed in Sections 2.4.6 and 2.5.5.
- vii Section 2.4.6 indicates that neural tissue is energy-hungry. Reducing the energetic costs somehow would improve competitiveness, thus helping to satisfy the production requirement.
- viii Section 2.5.5 also shows that muscle and nervous systems coincide, making a lineage explanation using nervous systems to coordinate contractile tissue more parsimonious.
- x Ion channel derivations show that a proto-nervous or neuroid system probably arose early in the animal lineage (Section 2.6.6), which reinforces and supports constraints v, vi, and vii, which are in part dependent upon the early timing of nervous system origin.
- xi Basal phyla sport bipolar neurons with bidirectional synapses. Bidirectional synapses cannot be excluded as the ancestral form. Polarity, being dependent upon specialization, is a likely later refinement. The lineage explanation should account for bidirectional neurons.
- xii Section 2.6.6 concludes that epithelia, specifically ectodermal, make a very likely precursor tissue. This provides a lineage explanation with a starting point.
- xiii Nervous systems coincide with possession of a gut, as shown in sections 2.3.6, 2.5.5, and 2.6.6. A lineage explanation would be stronger if it can account for nervous systems allowing better use of a gut, or for non-sessile macroscopy which benefits from a gut (Section 2.5.2).

From these constraints we need to build a lineage explanation, a succession of stages with small changes between them which are individually functional. Such an account should have a starting position as a first stage and an end position as a final stage.

These should be derived from the constraints above. More directly, the constraints in this chapter also provide boundaries for the stages in the lineage explanation. We also find that some environmental factors could have spurred innovation. Summarizing, we can distill the thirteen points above into a starting position, drivers, limiting factors, and an end point:

- i The starting position: epithelia in small, soft-bodied animals with limited but present developmental patterning, with access to the molecular building blocks of neurons;
- ii Drivers: fast and macroscopic movement, muscle coordination, and the ability to utilize a gut, increasing developmental patterning, and not necessarily predation on active prey;
- iii Limiting factors: rudimentary developmental patterning and pressure to keep energetic demands low;
- iv The end point: a diffuse nerve net of bipolar neurons.

In the following chapters, we will use these four factors to investigate the functional value of potential intermediate stages.

From the starting location it is possible to speculate regarding the pre-neural role of synapses, especially when taking into account the point from Section 2.6.1 that an intermediate, proto-neural coordination system likely arose early. In the continuum of chemical sensors, paracrine and juxtacrine signalling, and synapses, there is increasing complexity and dependence upon multicellularity. Chemical sensors are useful to unicellular organisms. Paracrine signalling allows for multicellular coordination, but it is not specific at all—though recent findings show *Trichoplax* uses it to coordinate (Varoqueaux et al., 2018). Juxtacrine signalling a little more so, and synapses most. The more specific the communication, the more precise a collection of cells can be coordinated. Chapter 3 delves into the potential functionality of a coordination system based solely on specific close neighbour connectivity.

Strongly targeted axodendritic elongations require strong body patterning to guide developing elongations to specific targets. Given that such patterning only developed over longer animal evolutionary time, intermediate stages likely lacked such specific connections. Chapter 4 investigates the coordinative properties of such a non-specific mechanism.

Finally, Chapter 5 investigates how increasing developmental patterning could make the final transition toward the end point: a diffuse nerve net. Additionally, Chapter 5 suggests another potential order of events, starting with specialization rather than with an excitable epithelium.

Chapter 3

Modelling spontaneous activity across an excitable epithelium: support for a coordination scenario of early neural evolution

3.1 Introduction

Thinking about early nervous system evolution can be cast into two general sets of models (Jékely et al., 2015a):

1. Input-output (IO) models: nervous systems evolved initially as a way to connect sensory input devices to effector devices (Parker, 1919; Mackie, 1990);
2. Internal coordination (IC) models: nervous systems evolved initially as a device to coordinate internal activity, enabling multicellular effectors (Pantin, 1956; Passano, 1963; Keijzer et al., 2013).

Of these two broad scenario groups, input-output models are the most familiar and also closest to how present-day nervous systems are viewed, in particularly the human brain (Braitenberg, 1984). Internal coordination models have been less conspicuous. The central premise of internal coordination models is that multicellular coordination was a central constraint that heavily influenced the evolution of the first nervous systems (Pantin, 1956). Organizing a differentiated multicellular organism such that it can display coordinated movement involves specific challenges tied to the organism itself rather than to environmental features. While the main premise of coordination models is highly plausible (Monk and Paulin, 2014), so far little attention has been given to them and they are in a clear need for further elaboration and investigation. In this chapter we use a computational modelling approach to investigate the internal validity of some of the assumptions made by a recent case of an internal coordination model, the skin brain thesis (Keijzer et al., 2013).

The skin brain thesis stresses the close link between nervous systems and muscle-based motility: early animals evolved ways to coordinate contractile cells such that the animal body itself became a large and highly differentiated effector (Pantin, 1956). An abstract way to describe this contractile organization is in terms of a Pantin surface, defined as the total muscle surface that an animal has available for motility (Keijzer et al., 2013). A Pantin surface has a species-specific anatomy and provides a surface across which patterns of contraction-extension correspond with specific forms of animal motility. The skin brain thesis envisions the evolutionary context for the first (proto-) nervous systems as being intrinsically linked to a Pantin surface, where they are key to generating systematic patterns of activity across this contractile surface in ways that resulted in usable motility.

To test the internal coherence and plausibility of the skin brain thesis, we developed a computational model that simulates the hypothesized initial stage of early nervous system evolution. Here the Pantin surface and the nervous system are not yet differentiated but combined as an epithelium that is both excitable and contractile, a so-called myoepithelium. Leaving aside the contractile properties for now, the model’s purpose was to investigate to what extent the generic excitable properties of such an epithelium could actually provide a basic patterning device for inducing contraction-based motility at the organism’s scale.

Modern excitable myoepithelia exist for example in cnidarians, where electrical signaling takes place by direct electrical coupling through gap junctions and where they operate alongside a nervous system (Mackie, 1965; Josephson, 1985). In contrast, the skin brain thesis proposes a primitive epithelium that signals by local chemical transmission and action potentials. While hypothetical, such an organization is theoretically important as it provides the first step of a gradual evolutionary route towards basic neurons: protoneurons first evolved as cells within an excitable epithelium using directed chemical transmission of electrical signals to neighbouring epithelial cells, in this way enabling patterned contractions; axodendritic projections evolved subsequently to connect non-neighbouring cells, allowing more complex patterning compared to the initial set up (Keijzer et al., 2013).

The historical status of this particular configuration is hypothetical. However, it is consistent with findings from cnidarians (Satterlie, 2011), while it is also clear that such chemical signaling and in particular chemical synapses are an essential feature for the operation of modern nervous systems in clades that diverged early in evolution (Moroz, 2014). The evolutionary origin of chemical synapses is a subject of ongoing research (Sakarya et al., 2007; Ryan and Grant, 2009; Emes and Grant, 2012). At the moment, it is unclear whether electrical synapses (e.g. gap junctions) or chemical synapses arose first. An interesting proposal is that chemical synapses arose in conjunction with electrical synapses (Ovsepian and Vesselkin, 2014). Gap junctions have important roles in intercellular metabolic exchange and developmental patterning. Chemical synapses evolved from more general pre-existing mechanisms for paracrine signaling and for sensing: Presynaptic mechanisms share strongly conserved homologies with endocrine mechanisms (Klopper et al., 2007) and postsynaptic mechanisms have a sensory basis (Emes and Grant, 2012). Bringing these separate components together in the chemical synapse is envisioned by Ovsepian

and Vesselkin (2014) as an exaptation from these earlier functions, while gap junctions are cast in a developmental role. The intermediate evolutionary step our model seeks to address in this domain is a potential proto-mechanism for modern chemical synaptic transmission between individual cells: coordinating contraction across an epithelium by chemical signaling between neighbouring cells. Our model aims to investigate whether such a primitive organization could function as a basic coordinative device. If so, such an organization could provide a key stepping stone in the evolutionary transition from general, diffuse paracrine signaling to the closely targeted signaling enabled by synaptic transmission.

In addition, the skin brain proposal is relevant for the ongoing debate about the phylogenetic position of ctenophores (Moroz et al., 2014; Marlow and Arendt, 2014), which may imply that nervous systems evolved more than once. Not only does the skin brain thesis easily support a polyphyletic view on the origins of neurons and nervous systems as stressed by Moroz (2009, 2014), but by easing the adaptive transition from proto-nervous systems to full nervous systems it could also increase the general plausibility of a polyphyletic view.

Without claiming that this particular configuration actually appeared in this precise form during nervous system evolution, considering the explanatory potential of the proposal, investigating the characteristics of a chemically transmitting epithelium is highly relevant for the discussions on early nervous systems. The model provides a proof of concept of the patterning capacities of such a configuration and a first indication of the parameters that are important for controlling it. Taking this proposal as a tentative working hypothesis, the central question then becomes whether the generic properties of such a chemically signaling epithelium could provide a primitive but usable patterning device for a given Pantin surface. We limited ourselves to the question whether the modelled epithelia would show global scale patterns of activity, such as travelling waves that could in principle be used to drive primitive peristaltic contractions of the body.

The subject of waves in excitable media is well-founded in existing research, using an analytical approach supported by simulation. For an overview, see Boccaletti et al. (2006) and Bressloff (2014). This chapter considers locally-coupled 2D networks in finite topologies. Both locally coupled 2D networks and finite topologies are addressed separately in the literature: the stability of ring-shaped patterns on a torus is found in existing analytical work (e.g. Davydov et al., 2003; Kneer et al., 2014); simulations and experiments show wave annihilation through collision and edge effects (Copelli and Kinouchi, 2005; Zimmermann and Walz, 1997). Similar effects are observed in cardiac research (e.g. Li et al., 2009; Panfilov and Hogeweg, 1993). The effect of a refractory period appears hard to incorporate analytically: studies centering on refractory period (e.g. Li et al., 2009; Copelli and Kinouchi, 2005) rely on computational modelling. Our approach is similar to Copelli and Kinouchi (2005), which used n-state Greenberg-Hastings cellular automata implementing a refractory period in a triangular lattice. In contrast, we look at cylinders: topologies which are continuous in a single dimension and which may be asymmetrical; additionally, we use more realistic cell models.

3.2 Methods

Given that little is known about excitable epithelia as nervous system precursors, we need to decide how to deal with the large parameter space and the consequent model design decisions. For each parameter category, we need to decide what to do: abstract away, scan, or decide upon plausible values. We used the following rules of thumb: abstract away when possible, decide upon plausible values when there is little to no effect on system behaviour or a clear imperative to use a certain value, and scan when there is no such imperative and an effect on system behaviour. These are the parameter spaces and the decisions taken:

- *Cell model.* To represent a generic excitable epithelium in continuous time, an integrate-and-fire model with a refractory period was used as the simplest and most abstract model that allows us to investigate this system.
- *Cell model parameters.* Even the integrate-and-fire model has thresholds and time constants which need to be set or iterated over. A central functional requirement for systemic behaviour here is the generation of spontaneous activity. This can be accomplished by either using thresholds that result in spontaneous spiking or using a spiking threshold above the resting potential with some other added form of spontaneous activity. Either way, the level of activity has behavioural effects, so we need to scan this parameter. We have chosen to set thresholds at standard, roughly Hodgkin-Huxley-inspired values, which do not spike spontaneously. To initiate activity, we use the principle of random vesicle release.
- *Network macro-structure.* Epithelia are surfaces in space. The shape should approximate a very simple animal. The abstraction we have chosen is a tube with a skin thickness of one cell: geometrically simple and still representative of worm-like animals, a shape which is common in nature and a plausible shape for a precursor system. While we do not vary the shape itself, nor the thickness, we do perform parameter scans over various dimensions of tubes in terms of numbers of cells and various length-circumference proportions.
- *Network micro-structure.* Cells arranged on a one-cell-thick tube can still be connected and arranged in many ways. The simplest and most parsimonious way to arrange roughly spheroid cells on a surface is a triangular lattice, because of its isotropy, so that is the abstraction used. We are interested in local connectivity, so cells only connect with their neighbours in the grid. As for connection weights, we have chosen to set that at a value which is above spike threshold.

Implementation details resulting from these design decisions are detailed below.

To analyze the model outcomes we developed two indicators of whole-body coordination. These indicators can summarize simulation outcomes in a form suitable for visualizing the outcomes of parameter scans over the circumference and the length of the networks.

Table 1. Model Summary 1: Overview

Cell populations	Single excitatory
Topology	Triangular lattice on a cylinder
Connectivity	Nearest-neighbour
Cell	Integrate-and-fire
Chemical transmission	Delayed instantaneous membrane potential change
Noise	Independent fixed-rate Poisson processes of spontaneous vesicle release
Measurements	Wave-front orientation
Simulator	NEURON (www.neuron.yale.edu)
Code Availability	ModelDB, Accession number: 141132 (http://senselab.med.yale.edu/ModelDB)

3.2.1 Model

Our cell network model is built from single-compartmental cell models with integrate-and-fire dynamics. We reused a standard mechanism, IntFire1, from the NEURON simulation environment in which we introduced a delay-to-spike to reflect the time it takes chemical transmission to depolarize a cell and a refractory period, which basic integrate-and-fire lacks. Parameters for the model were inspired by the Hodgkin-Huxley model. The details of the integrate-and-fire model are provided in table 3.2. All model parameters are stated in the model summary tables (3.1 to 3.5). The model cells were arranged in a triangular lattice generated by a purpose-built weight generator, see Figure 3.1. This generator was designed for a network building library developed for the NEURON simulation environment (Carnevale and Hines, 2006). To facilitate parameter scanning, the multiple run control (van Elburg and van Ooyen, 2010) made available in ModelDB (<http://senselab.med.yale.edu/ModelDB>, accession number 114359) was used.

An important aspect of this model is spontaneous activity. We aim to develop a model system that is capable of self-generated activity patterns without explicit input from outside. Spontaneous activity can come from particular membrane potential dynamics, localized ion channel stochasticity, or from spontaneous vesicle release. Like the Hodgkin-Huxley model which inspired it, the integrate-and-fire parameters we use do not result in spontaneous spiking. We used spontaneous vesicle release to induce activity. Alternatively, non-noisy synapses and spiking membrane dynamics could be employed, but should yield the same results.

Data on spontaneous release frequencies of vesicles are still scarce and we found only one such study (Mackenzie et al., 2000). Experimentally, the maximum spontaneous vesicle release rate found was 0.25 Hz. In our model, which has integrated six chemical transmission sites into a single cell, this yields a total spontaneous vesicle release rate of 1.5 Hz per model cell. A reliable lower bound, other than zero, on the vesicle release rate is not available because many synapses failed to show spontaneous vesicle release during the experiment. From the biophysics of vesicle release we further know that vesicle release is calcium-concentration dependent (Augustine, 2001). As calcium concentration dynamics is known to vary with surface-to-volume ratio and with the concentration, mobility and

Figure 3.1: Model Network. A: Three dimensional organization of our model network, resembling a tube-shaped animal. The epithelium model consists of excitable cells arranged into a triangular lattice wrapped around a cylinder. B: Color coding used to refer to the three differently oriented wave fronts on this lattice. The size of a wave front is established by counting the number of neighbouring cell pairs on the corresponding wave front.

Table 2. Model Summary 2: Abstract Cell and Synapse Model

Name	intfire1ds
Type	Integrate-and-fire cell with fixed delay-to-spike and refractory period
Dynamics	<p>The model is derived from the standard IntFire1 mechanism of NEURON (Carnevale and Hines, 2006), but with a delay between threshold crossing and spiking which captures the delay due to the time it takes between the onset of chemical transmission and the effect on the membrane potential. As is common in these models $m = 0$ corresponds to resting potential and $m = 1$ corresponds to the firing threshold. When the cell is not firing and chemical transmission is absent, the membrane potential is decaying exponentially with membrane time constant $\tau_{membrane}$: $m = e^{-m/\tau_{membrane}}$ When chemical transmission commences m is increased by the weight w provided that the model is not in spiking or refractory state: $m \leftarrow m + w$. If m crosses threshold due to chemical transmission the model will indicate a spike at a time $\tau_{delaytospike}$ after threshold crossing. During this interval and the subsequent refractory period $\tau_{refractory}$ the model will simply ignore chemical transmission. On leaving the refractory period the membrane potential is reset to resting level: $m \leftarrow 0$.</p>
Parameters	<p>The parameters $\tau_{delaytospike}$ and $\tau_{refractory}$ were inspired by the Hodgkin-Huxley model. Transmission weights were chosen to be just above spiking threshold.</p> <p>$\tau_{membrane} = 15ms$ $\tau_{delaytospike} = 6\ ms$ $\tau_{refractory} = 20\ ms$ $w_{network} = 1.01$</p>

Table 3. Model Summary 3: Noise Model

Type	Description
Poisson process	Fixed rate $\nu_{spontaneous} = 10^{3-noiseparameter}$ Hz generator for each cell, the noise parameter is varied from 2 to 6.

Table 4. Model Summary 4: Network Structure

Triangular lattice on cylinder. The open ends are arbitrarily labeled East and West and both edges are aligned with the same primitive vector of the triangular lattice. Cell indices start at zero on a cell on the West edge. Indices are incremented by one for each lattice-constant-sized step on the edge in a direction arbitrarily marked as North. After labelling all the cells on this and subsequent rings, indexing continues stepping North from the first cell located North East of the last labeled cell until all cells are labeled. The number of cells on a single ring is specified by the circumference parameter, while the number of rings is specified by the length parameter.

Table 5. Model Summary 5: Analysis

Wave-front orientation preference is measured by counting how often two neighbouring cell pairs of a single orientation fire within 2 ms of each other. In Figure 3.2 we develop several visual representations which capture this information. In addition, spike activities are shown as snapshots capturing 6 ms of activity and as line graphs showing wave-front size as a function of time.

kinetics of the endogenous calcium binding proteins (Cornelisse et al., 2007), there is a large range of plausible vesicle release rates. We have therefore chosen to vary model vesicle release rates over 5 orders of magnitude around a value of 0.1 Hz. Thus we included the maximum directly observed vesicle release frequency in our range, but have a strong bias towards lower vesicle release rates. Generally speaking, we have tested our setup for a wide variety of noise levels.

We also investigated the possibility to introduce spontaneous network activity through spontaneous spiking resulting from stochastic ion channel gating. However, a short exploration using the model developed by Linaro et al. (2011) (available from ModelDB accession number: 127992) showed that this would lead to spike rates much lower than those induced with the vesicle release rates included in the model.

3.2.2 Analysis

As whole-body coordination is not a well-defined mathematical concept at present, it is crucial that we should choose good indicators of it. The triangular lattice favors three possible wave-front orientations; as indicators we have chosen the relative amounts with which these orientations appear in our simulations. Subpanel A1 of Figure 3.2 illustrates our analysis method and shows that pairs of neighbouring cells come in three different orientations: North–South, North East–South West, and South East–North West. For each of these orientations we count the number of neighbouring pairs that fire within 2 ms of each other. In subpanel A2 of the same figure we show how these raw counts (left) are translated into percentages (middle left), which are then used to set the diameter of the circles in the oriented circle pairs (middle left and right). This presentation, which we call **relative wave-front orientation prevalence**, is suited for the analysis of parameter scans, e.g. figures 3.5 and 3.7. We used this representation to present averages over all runs at a specific parameter setting.

Wave fronts propagate roughly perpendicularly to their own orientation. This idea leads to a second representation. Instead of showing the percentages directly, we add up the vectors normal to the wave fronts weighed by the same percentages used in our relative wave-front orientation prevalence representation. This results in bars effectively pointing the way the waves go. The longer the bar, the more prevalent the waves in that direction. For the North–South oriented wave-front, propagation is to the East or the West, i.e. parallel to the normal vector pointing West, similarly propagation is parallel to a South East-pointing vector for the North East–South West-oriented wave front, and parallel to North East pointing vector for the South East–North West-oriented

wave front. The choice of these normal vectors is not unique, we selected them in such a way that they point from the center to the oriented pair in the relative wave-front orientation prevalence representation. In Figure 3.2:A2 middle right we show the vector addition and the resulting vector. We call this representation **wave-front propagation orientation**. This representation is also suitable for the presentation of parameter scans and additionally allows us to show both the individual simulation runs and the average over simulation runs in a single figure. Supplementary Figure 3.8 uses this representation to show that we obtain similar results over twenty runs in which only the random number generator initialization is changed.

For the purpose of this study, visual inspection of preliminary simulations showed that both relative wave-front orientation prevalence and average wave-front propagation orientation are reasonably good indicators of the effects of body size and chemical transmission noise on whole-body coordination.

3.3 Results

In subpanel B of Figure 3.3 we see the temporal development of wave-front patterns on the short cylindrical network also shown three dimensionally in subpanel A of the same figure. After initial excitation the wave fronts grow in size uniformly in all directions until the wave fronts propagating longitudinally reach the edge of the cylinder and disappear. The remaining wave fronts propagate in both transverse directions. Provided no other noise-induced wave fronts interfere, these wave fronts eventually annihilate each other on the side opposite the wave front initiation point. In subpanel C of Figure 3.3, where the wave-front orientation counts are shown for the time interval depicted in subpanel B, we clearly see the phenomena we just described reflected. Initially all wave-front counts grow at the same rate, then the North–South oriented wave front, propagating longitudinally, dies out and the remaining wave fronts continue to grow in size. Wave-front counts are approximately stable during transverse propagation. The seemingly missing wave front at $t = 216ms$ is an artifact of the sampling rate: through delays and spike timings, wave fronts travel at a speed of roughly 1 cell per 6 ms, but not exactly. In this case, the speed was a bit slower, and this frame simply does not capture any cells firing.

In subpanel B of Figure 3.4 we see the temporal development of wave-front patterns on the elongated cylindrical network also shown in 3D in subpanel A of the same figure. After initial excitation, the wave front grows uniformly in all directions until the wave fronts propagating transversely annihilate each other opposite the wave front initiation point. What remains are two wave fronts propagating longitudinally. Provided no other noise induced wave fronts interfere with these wave fronts they will eventually reach the edge of the cylinder and disappear. In subpanel C of figure 3.4, where the wave-front orientation counts are shown for the time interval depicted in subpanel B, we clearly see the phenomena we just described reflected. Initially all wave-front counts grow at the same rate, then the South East–North West and South West–North East oriented wave fronts, that is, the wave fronts propagating transversely, annihilate each other. Subsequently, the North–South oriented wave fronts continue to grow in size until they become

Figure 3.2: Visual representations used. (A1) Yellow disks represent active cells at a given time; grey cells represent inactive cells. Neighbouring pairs of active cells come in three different orientations and are arbitrarily labeled with red, green and blue. To establish which orientation is dominant we simply count the occurrence of the orientation labels. (A2) The orientation label counts (left) are translated into relative wave-front orientation prevalences (graph left of middle) and represented by the diameters of the disks with the corresponding color labelling. In addition these counts are translated into an average wave-front propagation orientation by adding the normal vectors to these wave fronts with a weight proportional to their label count (diagram right of middle). Relative wave-front orientation prevalences and propagation direction are combined into a single representation for use in parameter scans (right). (B,C) Like A2 with different orientation label counts.

Figure 3.3: Wave patterns on a short tube (length: 8 cells, circumference: 32 cells, noise rate: 0.1 Hz). (A) Network geometry: the scissors indicate the line at which the tube is cut for presentation in B. (B) Snapshots of network activity during 4 ms intervals in an illustrative phase of the dynamics. The wave fronts propagating longitudinally die out at the network edges, causing the North–South oriented wave fronts to disappear. As a result, wave-fronts propagate predominantly transversely to the tube and the wave-front orientations are South East–North West and South West–North East. (C) Temporal development of different wave-front orientations, including all snapshot times shown in B.

Figure 3.4: Wave patterns on a long tube (length: 32 cells, circumference: 8 cells, noise rate: 0.1 Hz). (A) Network geometry: the scissors indicate the line at which the tube is cut for presentation in B. (B) Snapshots of network activity during 4 ms intervals in an illustrative phase of the dynamics. The wave fronts propagating transversely collide with each other, causing extinction due to the refractory period. Subsequently, the remaining wave-fronts propagating longitudinally dominate the dynamics and the North–South wave-front orientation dominates. (C) Temporal development of different wave-front orientations. Snapshot time markings are consistent with those in B.

approximately stable during longitudinal propagation.

To establish whether and under which conditions a surface of excitable cells generates coordinated patterns, we simulate our network at different network sizes and different noise rates. Our analysis methods allow us to scan a large parameter space for patterned activity. At an intermediate noise rate of 0.1 Hz, Figure 3.5 shows the relative wave-front orientation prevalences (represented by the diameters of the colored disks) and average propagation orientations (indicated by the orientation of the black bars) for various body lengths and circumferences of the excitable myoepithelium. In the corners of this figure we drew rectangles to illustrate the shape of the model network at the parameter settings used for the simulation in the corresponding corner. The ratio of circumference to length used in these drawings are understated with 1:1 (left bottom corner), 1:5 (left top corner), 5:5 (right top corner) and 5:1 (right bottom corner), while the actual sizes of the model network are 4:4, 4:256, 256:256 and 256:4, respectively. From the average propagation orientations in Figure 3.5 we see that there is a strong preference for longitudinal wave-front propagation if the model network axis is long compared to its circumference (upper left). In contrast, if the model network circumference is large compared to its axis (lower right), then we see a strong preference for transverse wave-front propagation. This is also visible from the relative wave-front orientation prevalences. Hence, we see that for these networks there is significant pattern formation. To extract how pattern formation scales with size we can study the change in pattern formation on the diagonals running parallel to the main diagonal (bottom-left corner to top-right corner). Along these diagonals body size varies while the ratio between axis length and circumference remains constant; the smallest body size is at the lower left side of these diagonals and the large body size at the upper right side. As we move along these diagonals to larger scale networks we observe that relative wave-front orientation prevalences equalize and average propagation orientation diminishes. This shows that pattern formation fails to reach network size when we move to large networks.

Noise drives activity in our network, but we also expect it to interfere with emerging patterns. The reduction of the noise rate might therefore rescue patterning in large-scale networks, and an increase in noise rate might destroy patterning in small-scale networks. Figure 3.6 illustrates the influence of the noise rate on whole-body coordination at a single fixed combination of length and circumference. In this figure each subpanel shows, for a specific noise rate, the temporal development of wave-front orientation prevalences. At the top of each subpanel we find the wave-front counts plotted versus time, followed by snapshots of the activity in the network. Subpanel A shows the low noise rate situation in which almost every excitation grows to network scale and induces coordinated activity. Subpanel B shows the intermediate noise rate situation in which many wave fronts grow to the short scale of the network but spreading on the long scale is often interrupted by collision with other wave fronts. Subpanel C shows the high noise-rate situation in which, in general, wave fronts are disrupted before reaching network scale and whole-body coordination at the network level is absent.

Figure 3.7 shows two parameter scans over network sizes, performed at the highest and the lowest noise rates used in this study. Both panels show relative wave-front orientation

Figure 3.5: Analysis of whole-body coordination for various body shapes. Relative wave-front orientation prevalences (represented by colored disk diameter) and average propagation orientations (indicated with an oriented black bar) are shown for various body lengths and circumferences of the excitable epithelium. In the corners, the corresponding body shape is indicated with a rectangle. Bottom left: small ‘square’ cylinder, top left: long cylinder with small circumference, top right: large ‘square’ cylinder, bottom right: short cylinder with large circumference. This parameter scan shows three effects: (i) more elongated networks show better developed longitudinal wave fronts, (ii) whereas shorter networks show better developed transverse wave fronts, (iii) however for fixed length-to-circumference ratios (visible on the diagonals running from bottom left to top right) we can see that with increasing size preference for transverse or longitudinally moving wave fronts is lost.

Figure 3.6: Development of wave-front orientation prevalences over time on a long tube (length: 32 cells, circumference: 8 cells) at three different noise rates. A. Low noise condition (0.01 Hz/cell). The graph at the top shows wave-front counts for all three orientations. It is clearly visible how activity first grows equally for all three orientations until the scale of activation pattern equals the tube's circumference, at which point in time the wave front moving longitudinally starts to dominate. B. At the intermediate noise rate (0.1 Hz/cell) we still observe growth of wave-front patterns to the scale of the animal, but occasionally several wave fronts are initiated in close succession leading to destructive interference. C. At a high noise rate (1 Hz/cell) wave fronts are initiated at a high rate and due to destructive interference with each other these wave fronts often fail to grow to the scale of the animal. As a result we no longer observe whole-body coordinated activity.

Figure 3.7: Analysis of whole-body coordination for the extreme noise rates used in this study. Relative wave-front orientation prevalences (represented by the diameters of the colored disks) and average propagation orientations (indicated with the oriented of the black bar) are shown for various body lengths and circumferences of the excitable epithelium. The two subpanels are organized as in Figure 3.5. (A) Low noise rate: 0.001 Hz, (B) High noise rate: 10 Hz. Compared to Figure 3.5 we find slightly stronger relative wave-front orientation prevalences and average propagation orientations at the low noise rate in panel (A). In contrast we clearly see the loss of whole-body coordination with increasing noise rates, as relative wave-front orientation prevalences become uniform and average propagation orientations are almost absent in panel (B).

prevalences (represented by the diameters of the colored disks) and average propagation orientations (indicated by the orientation of the black bars) for various body lengths and circumferences of the excitable epithelium. The two subpanels are organized as in Figure 3.5 but the simulations were performed at a low noise rate of 0.001 Hz (A) and a high noise rate of 10 Hz (B). Compared to Figure 3.5, we find slightly stronger relative wave-front orientation prevalences and average propagation orientations at the low noise rate in panel (A). In panel (B), in contrast, we see a loss of whole-body coordination with high noise rate, since relative wave-front orientation prevalences become uniform and average propagation orientations are almost absent at most parameter values in this scan. This shows that reducing noise can enhance the range of network size over which we find whole-body coordination, whereas increasing it will reduce this range.

3.4 Discussion

The simulations' results show that an excitable epithelial organization incorporating local chemical transmission and action potentials can lead to self-organized patterned activity at a whole-body scale under specific conditions. Three main features play a central role: noise, body dimensions and body size. Let us briefly consider each of these features.

Noise: Some form of spontaneous activity is required to initiate electrical activity. The cells included in our model epithelium showed spontaneous vesicle release that initiated action potentials which local chemical transmission subsequently spread out across the epithelium. This spontaneous activity could give rise to patterned activity at the body scale if the noise level was sufficiently low. At high noise levels, the subsequent spontaneous firing of action potentials disrupts already evolving large-scale patterns.

Body dimensions: At sufficiently low noise rates the relative epithelial dimensions, i.e. the length to width ratio, determines the type of whole-body coordination. Wave fronts travelling along the short dimension die out either through collision with the edge or through annihilation with a wave front travelling in the opposite direction from the same initiation site. Wave fronts travelling along the long dimension then travel on and finally die out through annihilation with an opposing wavefront or collision with the edge, respectively. At such vesicle release rates wave fronts travelling along the long dimension dominate the dynamics and lead to a primitive form of whole-body coordination. Thus body dimensions are a key feature for the emergence of body coordination under these circumstances.

Body size: In our model the scale of these patterns is determined by the rate of spontaneous vesicle release. We see whole-body coordination emerge only when the scale of these patterns matches roughly with one of the dimensions of the animal, i.e. matches with length or circumference. Consequently, we see a reduction of whole-body coordination with the increase of animal size.

We conclude that the generic properties of the modelled excitable epithelium enable a rudimentary form of coordinated patterning. This occurs without any sensory input, without any central pattern generators and without requiring any specific wiring or particular connections between the cells. Coordination can be cast as an ingrained self-organized

feature of such a multicellular organization.

The aim of our computational model was to investigate the potential functional validity of a chemically signaling excitable epithelium as hypothesized by the skin brain thesis for the evolution of early nervous systems (Keijzer et al., 2013). The model suggests that such an epithelial configuration could have acted as a primitive coordination device all on its own.

A note on robustness and validity of implications: the model is explicitly intended as a proof of concept, a particularly rigorous thought experiment, showing the principle of coordination through an excitable epithelium in a plausible configuration. We do not seek to claim that precisely this principle with precisely the mechanisms we outlined was at the basis of nervous system evolution; we merely claim that this is an example of how the coordination scenario could have worked. The computational experiment was also performed using a Hodgkin-Huxley cell model, and the results were the same, which serves as an indicator of the robustness of the principles. These results are detailed in the supplementary materials.

A theoretically important implication of the model is that it supports an improved lineage explanation (Calcott, 2009) for the independent and subsequent evolution of the two key features of modern neurons—synapses and axodendritic projections—by giving an account how targeted chemical signalling connections between adjacent cells could have functioned as part of a motility mechanism. Our results indicate that coordination in excitable epithelia can be cast as a potential evolutionary reason for the rise of the chemically induced transmission of electrical signals between neighbouring cells. These epithelial cells act as ‘protoneurons’, having only chemical transmission but no axodendritic elongations. From this point, adding short-range axodendritic projections to this organization can be hypothesized as a possible way to improve the coordinative properties of the epithelial organization modelled here. Short range axodendritic projections allow for faster spreading of activity thus allowing for spreading of activity to body scale in situations where otherwise this spread would be limited through interactions with other spontaneous activity. This account thus suggests a gradual evolutionary path towards nervous systems and thus bears on the current discussion concerning the monophyly or polyphyly of nervous systems evolution.

While such wider evolutionary implications must remain speculative at present, the model has also more immediate implications for the study of early nervous systems evolution. It is one of the first models targeting the now quickly expanding domain of early nervous system evolution. Though remaining very basic this model already shows how very specific features, such as body size, are likely to have been important features for early nervous systems in a way that would not be transparent without modelling. In addition, this model is clearly only a start. There are, potentially, many additional features which impact the function of primitive neural organizations. Therefore, a more complete understanding requires an evaluation of the impact of other features like ion channel composition, gap junctions and the development of neural processes on whole body coordination. This will also require development of measures of whole body coordination, as the measures employed in this study do not generalize to contexts in which the wave

fronts are more diffuse.

Figure 3.8: Analysis of propagation direction orientation for various body shapes and noise rates. Propagation direction orientation, averaged over a unique combination of length, circumference and noise rate is indicated by an oriented black bar. There are 19 individual runs per unique combination of length, circumference and noise rate. Propagation direction of a single run is indicated with an oriented grey bar. These grey bars usually largely overlap with the black bar, indicating that these experiments are highly reproducible. The orientation calculation is explained in Figure 3.2. (A) Noise rate: 0.001 Hz, (B) Noise rate: 0.01 Hz, (C) Noise rate: 0.1 Hz, (D) Noise rate: 1 Hz, (E) Noise rate: 10 Hz.

Chapter 4

Modelling the effects of short and random proto-neural elongations

4.1 Introduction

To understand how the very first neurons and nervous systems evolved, we need an account how each of neurons' most central characteristics came about: (a) their electrical signalling, (b) their synaptic connections and (c) their elongations (axons and dendrites). These three features are essential for making a 'full neuron'. All three features are central to nervous system functioning, and each has evolved into a wide variety of forms and modes of operation within the huge group of animals now known as the neuralia (Nielsen, 2008). From these three characteristics, graded and action potentials go back to unicellular organisms (Naitoh and Eckert, 1969; Greenspan, 2007; Liebeskind et al., 2011), and the same applies to macromolecular components of both the pre- and postsynaptic organization (Burkhardt, 2015; Ryan and Grant, 2009).

More difficulties remain with explaining how separate pre- and postsynaptic components came together to form synapses between separate cells, as well as how neural elongations first evolved. Both are tied to the multicellular morphology and functionality of proto- and early neuralia. To understand such early multicellular organizations, genomic and molecular evidence gives insufficient guidance (Smith et al., 2014; Nielsen, 2013; Hejnol, 2015), while other evidence remains inconclusive. Fossils of neuralia go back to the beginning of the Cambrian, 542 Ma (Million years ago) (Valentine, 2004) therefore synapses and neural elongations must have originated earlier. Body and trace fossils from the preceding Ediacaran (635 to 542 Ma) tend to be very different from modern animals and are difficult to interpret, although the presence of complex traces suggests the presence of some kind of nervous system at this time (Budd, 2015; Fedonkin et al., 2007a; Brasier, 2009). The first nervous systems may have evolved around this time, but molecular clock studies suggest the alternative option that the first neuralia evolved much earlier (Cunningham et al., 2017; Erwin et al., 2011). Leaving no fossils, such proto- and early neuralia could have lived as small meiofauna with sizes up to 1 mm (Wray, 2015; Erwin, 2015). It is also suggested that neurons and nervous systems evolved several times independently (Moroz, 2009; Moroz et al., 2014). To conclude, at present very little can

be said with certainty about the first neuralia, either their form, size or how and when they lived.

A systematic investigation and explication of potential evolutionary transitions from basic proto-neural configurations to neural ones will be beneficial here: this involves articulating possible trajectories that specify sequences of organismal organizations that span the transition from non-neural multicellular organizations to neural ones. Each step should consist of a functioning organism, while the consecutive steps from one organization to the next should be gradual, each one providing some improvement on the existing functionality (Calcott, 2009; Thornhill and Ussery, 2000; Budd, 2006). Investigating specific hypothetical transition trajectories will aid interpreting the limited empirical evidence and formulating more specific questions concerning this evidence.

A well-known example of such an idealized trajectory for nervous systems is provided by Braitenberg (1984). He formulated a sequence of configurations starting with a single neural connection between a sensor and an effector to which more connections could be added, eventually leading to increasingly complex neural circuits. Figure 4.1, for example, sketches a configuration with two connections. Braitenberg’s proposed trajectory is based on an Input-Output (IO) view on (early) neural evolution (Jékely et al., 2015a). IO views stress the functioning of neurons—and whole nervous systems—as connections between sensors and effectors. Initially these connections may have been simple and direct, but over evolutionary time they have become increasingly complex neural circuits governing behaviour. Neural elongations function here as specific and often long-distance connections between specific loci within an organism (sensors, effectors, or other neurons) (Jékely, 2011). While IO views seem well-suited for modern nervous systems, they start with organizationally and developmentally complex bodily organizations, which raises doubts about their suitability as a primitive condition. In addition, an IO view does not readily fit the surface-distributed nerve nets that are generally considered to represent the most primitive kind of nervous systems (Arendt et al., 2016), and which are found in cnidarians (Koizumi, 2016), ctenophores (Jager et al., 2011) and—possibly as a derived condition—in Acoelomorpha (Hejnol, 2015).

In contrast, we focus here on an alternative view, Internal Coordination (IC) (Jékely et al., 2015a), which stresses the need to acquire multicellular (bodily) coordination as an initial key task for early—and modern—nervous systems. Coordinating contractile (muscle) tissue for motility and reversible changes in body-shape is a central example here (Pantin, 1956; Keijzer et al., 2013; Keijzer and Arnellos, 2017) that imposes different functional demands on early nervous systems and neural elongations. Rather than focusing on neural elongations as a way to provide specifically targeted connections, an IC view opens up the possibility of acquiring neural elongations in a more gradual way.

We performed a modelling study to test the IC idea that simple—short and randomly connected—neural elongations can have played a significant behavioural role for proto-neuralia. Focusing on the option that the first neuralia evolved early, we targeted systems of limited (meiofaunal) size that consist of a few hundreds to thousands of cells. The starting point for this study consists of an excitable and contractile epithelium (a myoepithelium) that can provide primitive contractile motility (Mackie, 1970). Myoepithelia

Figure 4.1: A comparison between an input-output (IO) view as exemplified by a Braitenberg vehicle, top, and an internal coordination (IC) view, represented by an excitable (myo)epithelium with short and random connections, bottom. Behaviour of the respective systems is shown on the left; the wiring on the right.

with direct electrical connections by means of gap junctions between the epithelial cells exist in various extant animals, such as cnidarians (Mackie and Passano, 1968; Josephson, 1985). Here we build on the hypothesis that proto-neuralia could have possessed myoepithelia with similar electrical connections or with chemical signaling to conduct electrical activity from one cell to the next, either by juxtacrine signaling or basic chemical synapses (Keijzer et al., 2013; Keijzer, 2015; De Wiljes et al., 2015). Importantly, this configuration could have provided a scaffold to bring separate pre- and post-synaptic elements in adjacent cells together as a full synapse. However, here we only focus on the impact of neural elongations on an excitable epithelium where electrical activity is transmitted from cell to cell (see Figure 4.1).

The model consists of excitable cells connected to one another as a sheet to form an excitable epithelium where active cells activate their neighbours; electrical excitability generic enough to represent either a calcium-based mechanism or a sodium/potassium based one, and abstract excitatory synaptic transmission to represent either electrical or chemical transmission. The sheet of cells is rolled into a ‘body-tube’ to provide a general body surface on which activity takes place. This model does not include external senses, proprioception, or pacemakers. A Poisson process randomly initiates activity in single cells. Behavioural functionality is taken to consist of travelling rings of activation along the tube that drive a form of muscle-based peristalsis as sketched in Figure 4.1.

The model’s ‘body-tube’ does not mimic any specific animal, such as, for example,

Trichoplax or sponge larvae. Like Braitenberg’s vehicles, the model is made as abstract and general as possible to investigate in a generalizable and systematic way under what conditions an excitable epithelium produces patterned activity across its surface. The model is also very different from models of locomotion in modern animals (e.g. Stein et al., 1999) as it does not target motility itself but the evolutionary motivated question whether a very limited configuration of cells can potentially provide a basic controlling device for motility.

Earlier modelling showed that body-topology is a crucial factor here (De Wiljes et al., 2015): Body-tubes with a high length to width ratio enabled ring-shaped activity patterns travelling along the length of the tube, while a ring-shaped topology induced patterns travelling along the ring’s circumference. In the present study, we only used a tube-shaped topology as (a) this particular configuration is generally plausible as a basic animal shape; (b) a single example would be sufficient to make a case for the potential evolutionary relevance of short and random proto-neural elongations; and (c) by focusing on a single topology we could better focus on the relevance and impact of many different features of the elongations themselves, as will be discussed below.

For tube-shaped topologies, an excitable epithelium generates ring-shaped patterns of activation running along the long axis of a body-tube. These patterns are an emergent feature resulting from the topology of the system, the size measured in numbers of cells, random activations, and the refractory nature of the cells. However, the occurrence of such patterns at the whole body scale depends on the size of the body-tube: ring-shaped activity only occurs for smaller tubes and is lost in larger ones (De Wiljes et al., 2015). For larger body-tubes, the speed of the travelling waves of activation is too slow to entrain activity at the level of the tube itself, giving local activations the opportunity to destroy global patterning. Also, signalling to adjacent cells provided only travelling wave-fronts that remain thin and insignificant on the scale of the body-tube.

While other modifications of the model could also have changed the characteristics of the patterning observed, the specific evolutionary question we aimed to address was whether adding neural elongations could provide a mechanism to overcome the limitations of nearest neighbour signalling. We hypothesized that the patterns of activation across a body-tube would change when neural elongations were added. To keep the change as generic and basic as possible, we focused on the effects of elongations that are relatively short—that is, connecting cells that have only a few intermediate cells between them—while the connections are made randomly. Such short and random connectivity is undemanding in terms of body morphology, cell differentiation, and developmental patterning as it does not require preset destinations for these connections. Short and random elongations can therefore provide a small and plausible step in an evolutionary trajectory towards very primitive nervous systems.

To test the validity of this idea we investigated a number of variations of randomly connected configurations: we varied the fraction of cells with elongations, the length of the elongations, and the size of the body-tube. The presence of ring-shaped patterns of excitation travelling along the length of the tube was used as a biologically plausible form of coordinated activity that also could be measured both qualitatively and quantitatively

under various modelling conditions.

4.2 Materials and Methods

4.2.1 Computational Model

We reimplemented the model of De Wiljes et al. (2015) using the `brian2` package in Python (Goodman and Brette, 2008). All experiments use the following model:

- A 2D sheet of cells placed equidistantly in a triangular grid;
- The sheet is rolled into a cylinder to create a tube;
- Each cell is modelled by an integrate-and-fire model with a refractory period;
- Each cell has superthreshold connections to its direct neighbors;
- Each cell has a superthreshold per-cell Poisson process driver. In accord with De Wiljes et al. (2015) the rate of Poisson process is set at 0.1 Hz;
- We used a tube as body topology. The length to circumference ratio of the folded tube was fixed at 4:1. We varied overall size leading to length by circumference combinations, respectively, of 32 by 8, 64 by 16, 128 by 32, and 256 by 64;
- A fraction of the cells were given straight elongations in random directions, providing them with connectivity to each cell visited by the elongation; details can be found in Supplementary Material 1 in Appendix B. We performed a parameter scan over various elongation lengths: 1.5, 2, 2.5, 3, 3.5, 4, 6, and 10 grid spacings. Additionally, we included a condition without any elongations, but as in all cases above keeping nearest neighbour connectivity;
- Experiments have varying fractions of elongated cells. We performed a parameter scan over various elongated cell fractions. For fractions < 1 , cells to be elongated were picked randomly. The fractions used are .02, .05, .1, .2, .5, and 1;
- All connections have a 2ms synaptic delay. Transmission speed was not modelled explicitly as the used delay was assumed to incorporate a transmission delay that was plausible for the modelled system. For support for this assumption, see Supplementary Material 2 in Appendix B.

4.2.2 Pattern Quantification

The system as described above produces spatiotemporal patterns. Previous work shows that given suitable noise rates, nearest-neighbour connectivity, and activation with a refractory period, travelling ring-shaped activity patterns appear. To quantify the degree and relevance of ring-shaped activity on the surface of the animal we developed a pattern detection method. We detect patternedness by comparing the activity in ring-shaped

Figure 4.2: This graphic shows how a condensed activity representation is calculated. Time steps t and $t+1$ show the process in detail. For each time-step, the body-tube—cut open in grey on the left—is divided into 16 ring-shaped segments (in this case, all 1 cell wide) for each of which the number of active cells—in red—is tallied. This numerical score is translated to greyscale values ranging from white (maximum) to black (minimum) providing a single vector for each time step.

segments with average activity over all segments. The activity in a segment is simply the number of cells active in a given ring-shaped segment in a given time frame.

We summarize this activity for a given run as follows:

- Divide the tube in n segments. A cell i is in segment s if $(s-1)/n \cdot l_{tube} \leq x_i < s/n$.
- A spike for a cell i in segment s takes place at a time t . The simulation is divided into time bins $[j\Delta t, (j+1)\Delta t)$.
- For each segment and time bin we can now count how many spikes take place: the matrix $C_{s,j}$ indicates the number of spikes in segment s during time bin j .

We set Δt to $3ms$, larger than the average synaptic delay between cells ($2ms$) but shorter than the refractory period ($20ms$). Within a time bin transmission between connected cells can occur but a single cell cannot fire twice. Empty and incomplete intervals at the start and the end of each run are discarded. The number of segments (n) is set to 16 for all systems in order to be able to compare measurements between the various body dimensions.

The ratio $T_{end}/\Delta t$ determines the number of intervals included in the analysis.

Visualizing $C_{s,j}$ as shown in figure 4.2 results in the condensed activity sequence plots found on the lower sides of panes A1-3 and B1-3 in Figure 4.3. Ring-shaped activity travelling down the tube shows up in this format as diagonal stripes, since a large amount of activation in a single ring-shaped segment travelling along the length of the animal and showing up in the next segment in a later time interval is a diagonal move.

To quantify the deviation in activity we performed the following steps. First, we define a per run normalized activity $\tilde{C}_{s,j}$:

$$\tilde{C}_{s,j} = \frac{C_{s,j}}{\frac{\Delta t}{nT_{end}} \sum_{s,j} C_{s,j}} \quad (4.1)$$

Using the normalized activities we can define a new patternedness measure P , which detects how activity deviates from homogeneity during each time steps and then calculates the root mean square:

$$P = \sqrt{\frac{\sum_j \sum_s (\tilde{C}_{s,j} - \bar{C}_j)^2}{(n \cdot (T_{end}/\Delta t) - 1)}}, \quad (4.2)$$

where $\bar{C}_j = \frac{1}{n} \sum_s \tilde{C}_{s,j}$

A high P implies low homogeneity over time and ring-shaped segments and thus strong patternedness over time and segments, allowing us to easily compare these values for various experimental conditions.

This measure compares the activity of a given segment not to the overall mean activity level but to the activity level in a particular time step. This way we disqualify situations where the whole body shows high and homogeneous activity in one time interval and low homogeneous activity in other time intervals ('flickering' behaviour).

4.3 Results

Central examples of our main results are presented in Figure 4.3. The panes on the left feature small systems measuring 32 cells in length and 8 in circumference. Those on the right represent large systems of length 128 and circumference 32. All systems have nearest neighbour (NN) signalling. Additionally, A2 and B2 have elongations of length 2, and A3 and B3 of length 4. These lengths refer to each cell's elongation length, implying that cells up to maximally 4 and 8 cells apart can become connected. Panes A1-3 and B1-3 each provide a detailed picture of the randomly initiated electrical activity across the cut open body-tube during six time steps of three milliseconds. Below is a condensed representation of this same activity across 100 time steps, providing a temporally extended overview of this activity by compressing all activity at a time-step into a line of 16 segments, scaled to the size of the body-tube (see Figure 4.2). As white reflects high levels of activity, travelling waves are shown as diagonal lines, unpatterned activity as smudges, and synchronous activation of all cells as a vertical line. Together, these panes give an indication of both the details and the more abstracted differences between the patterning resulting from the various conditions.

In addition to these qualitative results, panes A4 and B4 show graphs for the same small and large body-tubes, representing more extensive parameter scans, involving the NN condition and eight different elongation lengths (on the x-axis). The y-axis shows the measure of patternedness calculated as outlined in the Pattern Quantification section. The

Figure 4.3: Panes A1-3 and B1-3 each represent individual cases of sequences of activity across the body-tube for six different experiments (above), together with condensed representations spanning longer sequences (below). Individual time frames are identified by frame numbers, both above and below. Panes A1-3 show a small (32 by 8) body-tube, panes B1-3 a large one (128 by 32). Panes A1 and B1 represent nearest-neighbour connections only; A2 and B2 have in addition elongations of length 2; A3 and B3, have elongations of length 4. In all experiments shown, 50% of the cells have elongations. The graphs in panes A4 and B4 represent the average patternedness for the conditions shown above them (marked in the graph) together with the results for five more elongation lengths. The points scattered around the graph represent the 10 individual model iterations which were performed for each condition.

line represents the average patternedness for the given condition and individual markers represent individual model runs. The conditions represented in detail in A1-3 and B1-3 are marked as such within the two graphs.

As previously found (De Wiljes et al., 2015), small systems without added elongations exhibit ring-shaped patterns (A1), while these patterns are lost when the system is larger (B1). As hypothesized, when neural elongations are added to such large systems, ring-shaped patterns return (B2-3). However, such elongations are detrimental for smaller systems (A2-3), where they have a negative effect on patternedness (A4). This can be seen both by inspecting the examples and the quantitative results presented in A4 and B4.

The comparison between A4 and B4 shows the main effect of elongations on different topologies: For the small systems, elongations lower the patternedness (A4). For the large systems, elongations increase the patternedness, though returns do diminish for the longer elongations (B4).

The results discussed so far are based on systems where the probability of each cell having an elongation is .5 (i.e. the chance that any given cell has an elongation on top of nearest-neighbour connectivity is 50%) while we focused on two sizes of the body-tube. To investigate the effect of various elongation fractions (hereafter referred to as f) on patternedness, we also compared elongation fractions f across various body sizes and elongation lengths as described above. We also did these experiments for two extra body sizes: 64 by 16 and 256 by 64. The results are presented in Figure 4.4, which is similar to panes A4 and B4 of Figure 4.3 (included, respectively, as the lines marked with circles and diamonds in the pane marked ' $f = 0.5$ ') but with additional conditions regarding f as well as the additional body sizes.

Figure 4.4 shows the effect of f on patternedness. From top to bottom, f increases. For larger systems (darker red lines, marked with diamonds and triangles), having a higher fraction of cells with elongations improves patternedness. For the smallest system (yellow circles) elongations appear to be detrimental to patternedness. For the second-smallest system (orange squares), higher fractions (lower graphs) have a beneficial effect on patternedness for all but the longest two. However, low fractions (top graphs) show no such positive effect on patternedness—yet on the other hand, the low fractions also harm less for the longer elongation lengths.

Interestingly, for the larger body sizes, there appear to be diminishing returns to higher fractions. For the largest system (darkest line), patternedness for $f = 0.5$ is only marginally better than $f = 0.2$ for all elongation lengths.

4.4 Discussion

Our model represents an internal coordination (IC) approach to early nervous system evolution (see Figure 4.1). Our aim was to investigate IC scenarios that involve tissue configurations that are intermediate between non-neural and neural ones. In particular, we asked whether the presence of neural elongations providing random connections over very short distances could have functioned to enhance internal coordination for compara-

Figure 4.4: Comparison of the effect on patternedness of different elongation lengths and body sizes for six elongation fractions, $f = .02$ through $f = 1.0$. Line color and point shape represents different body sizes. Patternedness is shown on the each individual y-axis, the different elongation lengths are on the x-axis and individual plots represent the various values of f . The points scattered around the graph line indicate the 10 individual model iterations which were performed for each condition and the line itself represents the average.

tively small meiofaunal proto-neuralia. Our results show how such elongations can indeed enhance coordinated activity across very basic multicellular configurations. We discuss four specific implications of the modelling results.

First, short and randomly directed neural elongations allow patterned activity within larger multicellular organisms. Direct connections between adjacent cells within an excitable epithelium can provide a way to initiate and maintain coordinated patterns of excitability across a body surface. Such patterns could have enabled organized contraction. However, while this mechanism works for small body-sizes, such patterning deteriorates for larger bodies (De Wiljes et al., 2015). The model presented here shows that short and random neural elongations can support similar forms of patterning for increasingly larger multicellular organisms. Thus such very primitive forms of neural elongations provide a mechanism for adapting patterned activity to changes in body-size.

Second, neural elongations provide a way to scale the activity patterns themselves with respect to the size of the organism. An epithelial configuration only allows the spread of activation to adjacent cells, which limits the width of the patterns in the travelling direction to one or two cells (see Pane A1 and B1 of Figure 4.3). With elongations, the travelling patterns of activity can extend across more cells at every time-step. This allows the patterns themselves to become wider and scale up with larger body sizes (see Pane B2 and B3).

Third, for larger systems (See Pane B4 of Figure 4.3), patternedness tends to correlate positively with the length of the elongations, although even short elongations already provide an improvement in patterning. Thus, while even short elongations can be beneficial, lengthening them over evolutionary time provides a gradual path to further improve such patterning capabilities.

Fourth, while the influence of elongations on patterning tends to be more prominent when a larger fraction of cells have them, even a small fraction of cells with elongations can have significant effects depending on the size of the organism (see the top graph of Figure 4.4). Again, this provides an evolutionary path for a gradual improvement of the system.

Together these results provide a proof of concept that an organismal configuration relying on patterned activity across an excitable epithelium can use very basic neural elongations to maintain and improve patterning capacity for larger body-sizes. These changes can occur in small incremental steps allowing for a gradual evolutionary route towards increasingly complex neural elongations.

These findings have a broad conceptual relevance for understanding the very early evolution of both neurons and nervous systems. Rather than assuming that neurons must have come first and by aggregating together came to constitute the first nervous systems, here the sequence is reversed. With the IC view developed here, ‘nervous system functioning’ can be produced without full modern neurons—combining electrical signaling, synapses and elongations—by epithelia acting as a ‘proto-nervous system’ and relying on electrical signaling alone. Such epithelia could have provided a scaffold for gradually evolving full neurons and nervous systems. In this way, tissue configurations spanning the gap between non-neural and neural tissues become conceivable. The model presented

here shows that this speculative idea is indeed consistent and opens up new avenues for looking at the early evolution of nervous systems.

For example, the proposed outline of the piecemeal evolutionary assembly of neurons provides a new framework to make sense of the origins of nervous systems, which warrants further attention. Also, the focus on the intrinsic difficulties of evolving efficient muscle control provides a way to help explain the long cryptic evolutionary history of animals that is predicted by molecular clock studies (Cunningham et al., 2017; Erwin et al., 2011) while also providing a scaffold for the Cambrian evolution of new senses such as eyes (Parker, 2003) and predatory behaviour (Monk and Paulin, 2014). Finally, the IC approach developed here predicts an intrinsic connection between muscle control and the evolution of nervous systems. While it is widely acknowledged that muscle and neurons systematically co-occur in extant animals (Moroz and Kohn, 2016) the connection is potentially much more significant, and could constitute a fundamental feature of the animal senses as well (Keijzer, 2015; Keijzer and Arnellos, 2017).

To conclude, in comparison to the standard IO view that assumes the beneficial presence of long and specifically targeted neural connections, the results from this IC-based model provides new evolutionary scenarios that bring the neuron’s three main features—electrical signalling, synapses, and elongations—together in a gradual and plausible way.

Data Accessibility

The Python code of this model and its analysis are available on ModelDB (<https://senselab.med.yale.edu/modeldb/>) under accession number 231859.

Chapter 5

Modelling cell differentiation and axodendritic specialization

5.1 Introduction

As chapters 1 and 2 show, much is still unclear about the evolutionary origin of nervous systems. The nature and progression of intermediate evolutionary stages between the uninnervated animal common ancestor and animals with nerve nets is not defined. What is clear is that nervous systems are a very successful innovation, being present in the vast majority of animals and secondarily lost only in extreme cases (see Section 2.2 for details). It is also clearly a very early innovation: all hypotheses regarding its provenance agree that its origins lie deep in the animal tree. If one is interested in the specialization of neurons in particular, cell specialization in itself also becomes relevant. From the base of the animal tree, the number of specialized tissues tends to increase. In cell specialization, the ancestral state is pluripotent cells (Arendt, 2008). In non-specializing organisms like (colonial) protists, a single cell type has to perform all tasks, and specialization is logically only relevant to multicellular organisms. Indeed, cell type diversification and proper multicellularity are more or less synonymous (Maynard Smith and Szathmáry, 1997). Given the pluripotent starting point and the small steps of evolution, it is logical to assume that the very first metazoans only possessed very basic developmental patterning mechanisms.

Just as developmental patterning is only useful for multicellular organisms, nervous systems are only useful in multicellular organisms. While some degree of developmental patterning probably predates nervous systems, we can infer some things about the role of developmental patterning in nervous systems from modern animals. In currently extant animal clades, a more complex developmental patterning system tends to coincide with a more complex nervous system. This is partly because allowing axodendritic processes to target specific body-locations requires patterning to guide those processes. The non-neural common ancestor probably possessed only limited developmental machinery and it is likely nervous systems and developmental patterning systems co-evolved to some extent, considering the role of nervous systems play in developmental patterning: facilitating and guiding it. What did developmental patterning contribute to the early stages of nervous

system evolution? This chapter seeks to investigate how and why specialization of neurons may have occurred, given the limited developmental tools and the coordinative potential of the intermediate systems such a situation allowed.

What are plausible stages in this evolutionary model? Here, we summarize the information set out in chapters 1 and 2. The initial situation is likely a small, soft-bodied animal with a high degree of pluripotency and a low degree of body patterning. The likely end state is a nerve net, since that appears to be the most basal form of nervous system, a precursor to the more complex systems as well as a solution still present in modern animals (some Cnidaria, Ctenophora, some Xenacoelomorpha).

5.1.1 Benefits of specialization

There are clear benefits to specialization. An entity that focuses its efforts on a certain task will be better at that task than an unspecialized entity. While this may be to the detriment of other tasks, as long as there is some mechanism where tasks can be allotted individually but the benefit is accrued globally, a community of variously specialized entities will outperform a community of generalists. The most well-documented example of this is international trade, embodied in the Ricardoan principle of comparative advantage. This states that for one entity to specialize in one thing and to trade the output with another entity who specializes in another thing is beneficial as long as the exchange mechanism is fair. In animal cells the fitness benefits to individual cells via survival of the entire organism can be considered a fair exchange mechanism. Having neurons is good if it saves other cells the hassle of having to deal with coordinative activity, as long as the other cells take care of security, oxygen, movement, nutrients, procreation, and whatever else the community of cells needs to thrive.

Another clear benefit to specialization in the specific case of neuroid tissue is the fact that the metabolic costs associated with nervous system functionality are high. A clear indication of this lies in animals which lose their nervous system in particular life cycle stages, such as ascidians (Cloney, 1982), due to energetic cost. This does not tell us anything about *how* costly nervous systems are. Figures do exist for the human brain: it consumes 20% of the base metabolic production of the body while being only 2% of total mass (Sokoloff, 1960). While the human brain is far from an optimal proxy for early metazoan coordinative tissue, the dynamics at play may give us some idea of the most important pressures. Attwell and Laughlin (2001) calculate that the majority of energy consumed in the grey matter of the human brain is expended on action potentials (47%) and postsynaptic effects (34%)—both essential to the signalling role of neurons.

Harris et al. (2012) reinterpret the numbers from Attwell and Laughlin (2001) for mammalian metabolism and they infer that 22% of ATP is used in Na⁺ action potential reversal and 50% is used in reversal of ion fluxes through postsynaptic receptors. Another reinterpretation (Sengupta et al., 2010) infers 64% of energy going to synapses and 22% to action potentials. The reason for these reappraisals is that the models of Attwell and Laughlin (2001) are based on the squid giant axon: a large diameter cable optimized for fast transmission. Crotty et al. (2006) show that there is a trade-off at work in the case

of the squid giant axon: efficiency is sacrificed for transmission speed, resulting in large energy expenditure on action potentials.

Summarizing, about 80% of energy spent on nervous activity is spent on synaptic activity and action potentials, and depending on priority, between about 20 and 50 percentage points are being used for action potentials, the rest for synaptic activity. These are expensive activities. There are clear incentives to limit cells with neuroid activity and the level of that activity. It is not necessary for cells which have a non-neural function, such as effector cells and glandular cells, to perform these expensive activities. Non-neural cells may perform similar activities for different reasons, such as electrical excitability in muscle cells for intracellular coordination.

5.1.2 Modelling specialization

To investigate the role of specialization in early nervous system evolution we build on the results presented in chapters 3 and 4. These chapters lay out how intermediate neuroid systems can result in endogenous whole-body coordination. Summarizing, this is an internal coordination (IC) approach to behaviour in early nervous system evolution. The model framework demonstrates a plausible IC scenario for early nervous system evolution in which simple building blocks, plausibly available to early metazoa, progressively improve coordinative options. Chapter 3 shows that random activation and connection to nearest neighbours results in useful coordination given specific body dimensions. Chapter 4 expands on this by showing that adding long-distance elongations allows the system to scale to larger body sizes.

Adding specialization to the model presented in chapters 3 and 4 allows us to address the following issues:

- i The cells represented in the model as presented in chapters 3 and 4 do not utilize specialization (though other, non-modelled tissues in the animal probably exhibited at least minimal specialization). The constraints indicate that some limited specialization was likely present and more sophisticated developmental patterning correlates with neural complexity in modern animals. Presenting a possible role for specialization would improve parsimony of the satisfaction of the production requirement.
- ii The model as presented does not deal with energetic costs. This is a potential problem for the plausibility of the model as presented in chapters 3 and 4, as the mechanisms demonstrated are quite energetically expensive: all cells use action potentials and exocytosis. Using specialization allows us to investigate the relation between limiting the number of cells exhibiting neuroid features (and thus energetic costs) and the effect on coordination.
- iii The processes implemented in Chapter 4 consist only of a single elongation per cell. There is no branching or any attempt to cover a larger volume or surface of target cells. This does not reflect the processes found in modern cells, which exhibit both axons covering distance but also presynaptic and postsynaptic branching structures

which cover larger volumes. Specialization allows us to split these functional roles and investigate their effect on coordination separately. Different kinds of cell may exhibit different combinations of connectivity types.

We introduce ‘close neighbour connectivity’ as the term for volume connections. In previous chapters we used ‘nearest neighbour connectivity’. This is intentional: nearest neighbours stands for the adjacent six cells only. The connection to nearest neighbours is extended beyond directly adjacent neighbours (see Section 5.2.1) and now includes ‘close neighbours’ as well.

Also note that the term ‘elongation’ strictly refers to the singular processes as described in Chapter 4, which is separate from close neighbour connectivity. Elongations may be considered representative of axonal extensions while close neighbour connectivity provides dendrite-like connections. We use the term ‘axodendritic sophistication’ for the combination of elongations and close neighbour connectivity. Since we are examining the intermediate stages between non-neural and neural, we use the specific phrase ‘neuroid features’ to denote the core neuronal features enumerated in Section 1.2 (electrical excitability, synapses, and elongations—here implemented as axodendritic sophistication) without implying the presence of fully-fledged neurons.

Investigating specialization both in quality and quantity allows us to incorporate these points. Quantity, following point ii, means: ‘how many cells need to have neuroid functionality in order to result in coordinative functionality?’ Quality, following point iii, means: ‘how many minimal step improvements of neuroid functionality result in cells gaining significant coordinative functionality?’ Specifically, we re-envision the model developed in chapters 3 and 4, which consists of cells *all* exhibiting electrical excitability, nearest neighbour connections (the most basic form of close neighbour connectivity), and elongations. Instead of all cells possessing all features from the get-go, we investigate progressive addition of cells with basic axodendritic sophistication (quantity) and increasing the degree of axodendritic sophistication (quality).

In terms of investigating quality, increasing axodendritic sophistication is defined as follows: increasing elongation length, and close neighbour connectivity affecting increasing numbers of cells. We intend to observe the effect of varying degrees of these features: longer and shorter elongations, as well as close neighbour connectivity in a larger radius beyond only the nearest neighbour connectivity described in the previous two chapters.

Quantity is simply expressed by taking a varying proportion of all cells to express the features outlined above. We do not apply varying degrees of the same feature within a group, meaning that if some cells have elongations and others do not, all cells with elongations possess them to the same degree.

5.1.3 Lineage explanation constraints

Any evolutionary model should reflect the specific demands made by the evolutionary process, as outlined in Section 1.3. The potential stages of the hypothesis should have smooth transitions; the steps between stages should be as small as feasible. This is referred to as the continuity requirement. Additionally, as specified by the production

requirement, each individual stage should be of functional value to its possessor (Calcott, 2009).

If specialization is to be incorporated in the model, any lineage explanation constraints should be met. This means that the steps from an initial stage of undifferentiated non-neuroid tissue should be as small as possible. The smallest feasible step given the granularity of the model in this case means that a certain proportion of randomly picked cells in the total population starts performing some coordinative function while the remainder stays non-neuroid. The location of the randomly selected cells should be unbiased, since diversification in specific locations can be considered an extra evolutionary step. Another small step that can be imagined is increasing or decreasing the degree of a given trait, such as elongation.

Since our model outlined in chapters 3 and 4 did not include the effect of energetic efficiency on fitness, the production requirement was satisfied by the increase in coordination. With the addition of energetic efficiency, satisfying the production requirement also means finding a balance between expensive cells with neuroid functionality and their coordinative benefit. Consequently, the aim of this study is to explore the relationship between degrees of neuroid specialization and coordination: if the relationship is non-linear and a significant degree of coordination can be effected with a limited number of energetically expensive cells, specialization is adaptive.

The pressure on energy efficiency is particularly salient in light of the likely environmental situation at the time when nervous systems first emerged: oxygen levels were low (see Section 2.4.4). This period saw extremely cold climatic situations as well (see Section 2.4.5), putting a premium on energy conservation. While coordination and energy costs will remain impossible to quantifiably compare in evolutionary terms, a computational model allows us to investigate the relationship between progressive feature gain and coordinative performance in detail.

5.1.4 Computational model implementation

We use a computational model to investigate the progression from undifferentiated cells without neuroid functionality to a situation with specialized populations of neuroid cells and cells with a non-neuroid role. We use the model of coordination in early nervous systems detailed in chapters 3 and 4. This model represents a single-cell thick tissue layer of a worm-shaped animal. Chapter 3 shows that just two out of the three neuroid features (excitability and synaptic connectivity), when applied to all cells, results in quantifiable peristalsis-like spatiotemporal patterning without the need for any organized direction or specificity in connections. Chapter 4 shows that adding non-specific, random direction axodendritic elongations (the third basic characteristic) to all cells allows this system to be scalable to body size, with the coordination remaining quantifiable. The level of coordination depends on the length of the elongations. Now, we want to see how that quantifiable coordination responds to the addition of qualitative and quantitative cell specialization in the model.

Given the energetic cost of neuroid features, a situation wherein the number of cells

Table 1. Cell Types

Functionality \ Cell type	UC	CN	LD
Elongations received from	–	–	LD
Elongations sent to	–	–	LD
Close neighbours received from	LD,CN	LD,CN	LD,CN
Close neighbours sent to	–	LD,CN,UC	LD,CN,UC

with neuroid features is limited without significantly compromising coordination provides better fitness. The questions our model seeks to answer are:

- i On a sliding scale of the neuroid features present in the model, how much neuroid functionality is necessary to obtain useful coordination patterns?
- ii Given the various levels of neuroid feature presence of the previous question, how many cells in the body tube need to exhibit these features?

Summarizing, how much energetic expense required by neuroid features is needed to obtain a beneficial level of coordinative functionality?

To answer of these questions, we define three cell *types* and investigate various proportions between them. These cell types can fulfill two different *roles*: as background cells and as foreground cells, with the foreground cells being the type specialized the most in the direction of a nervous system. The three cell types are:

- i The unconnected cell (labelled UC for UnConnected), the simplest kind of cell in terms of coordinative functionality. These cells are receptive to neuroid activation but which do not establish connections themselves. These are envisioned as pure effectors, contractile cells. As such, they possess action potentials as sole neuroid feature. This type only serves as background cells.
- ii The close neighbour connectivity cell (labelled CN for Close Neighbour), connecting to close neighbours only. The amount of cells it connects to depends on the degree of close neighbour connectivity. In its most basic incarnation (only affecting direct neighbours), it is identical to the excitable epithelium cells featured in Chapter 3. These cells can function both as background cells and as foreground cells.
- iii The long-distance cell (labeled LD for Long Distance). These cells exhibit non-specific elongations as described in Chapter 4, which allows for connections over significant distances. In addition to these elongations, they also possess the close neighbour connectivity of CN-cells. As such, their neuroid functionality is a superset of the CN-cells. These cells only function as foreground cells.

The effect of the degree of various neuronal features is also being investigated. These are elongation length and close neighbour connectivity distance. Long-distance elongation length is simply the distance a single elongations travels from the midpoint of the originating cell. This parameter is described in detail in Chapter 4. We made no changes to the directions of the elongations: they remain random.

Close neighbour connection is a new addition, standing for an abstract, local structure which allows the cell to affect more than just directly adjacent cells. The exact implementation of this parameter is described in Section 5.2.1.

We want to use our model to investigate the scenario that energetic costs of neuroid activity were a driver of neuronal specialization. If it turns out that specialization does not improve energetic efficiency for similar levels of coordination, that scenario is disproved. If specialization does improve energetic efficiency without a loss in coordination, that confirms the plausibility of the scenario. Like our earlier work with this model, this is not intended as proof that events unfolded exactly this way; it is a rigorous thought experiment with dynamically interacting parts to demonstrate that things could very well have evolved this way, since it is internally consistent as well as able to accommodate the empirical findings in the area of nervous system origin.

5.2 Methods

5.2.1 Model

We extend the model described in Chapter 4 (in turn a reimplementaion of the model in Chapter 3) to include specialization. Summarizing chapters 3 and 4, our model system consists of an open-ended tube, a rolled-up sheet of cells one cell thick. The cells are arranged in a triangular lattice. For every cell, the dynamics are leaky integrate-and-fire, with an explicit refractory period of 20 ms. Synapses are super-threshold with a delay of 2 ms; this is taken to include transmission speed which is not modelled separately. While previous chapters varied the body sizes, in the experiments of the current chapter all systems have a circumference of 32 cells and a length of 128 cells. This size was chosen because previous results indicated that this size is not entirely uncoordinated without elongations, yet strongly benefits from them, even for short distances or a lower proportion of cells exhibiting elongations. Intercellular, synaptic connectivity is varied between experiments.

Contractile functionality is not modelled. Being a model of coordination, not biophysics of a soft body, we assume that contractile activity occurs when cells are electrically active, and we therefore follow chapters 3 and 4 in classifying travelling ring-shaped activation patterns as useful spatiotemporal patterning.

Cell types are defined by the kind of connectivity they possess (see table 5.1). Two types of potential connectivity are implemented. The first type is close neighbour connectivity, connecting to all cells in a radius around the presynaptic cell. The second type is connectivity mediated by elongations, as implemented in Chapter 4 and described in more detail in section 1 of appendix B. All connections, regardless of establishment method, are bidirectional.

Individual experiments each feature two cell types: one in a background role, and one in a coordinative foreground role.¹ Cells in the coordinative role possess greater neuroid

¹Though since the foreground cell proportions used include 0% and 100%, those instances only feature a single cell type.

Figure 5.1: Visual examples of connectivity types. Close neighbour connectivity is exhibited by CN-cells and LD-cells; connectivity by elongation only by LD-cells.

functionality than those in the background role. The following combinations were tested: CN-cells in an UC-cell background, LD-cells in an UC-cell background, and LD-cells in a CN-cells background. For each experiment, we vary the proportion of background cells to foreground cells. This allows us to investigate quantitative specialization. 7 different proportions of foreground cells are being tested: 0%, 2%, 5%, 10%, 20%, 50%, and 100%.

Qualitative specialization is implemented in several ways. First, in kind: gradually adding forms of neuroid features to entirely non-neuroid cell types. This is expressed in the progression of cell types, from non-neuroid UC-cells to limited connectivity in CN-cells to full connectivity in LD-cells. Second, qualitative specialization is varied in degree: we do this by varying elongation length and close neighbour connectivity. Long-distance elongation, a feature of LD-cells, is a variable known to strongly affect coordination in systems of the size tested here. We tested a number of LD-cell elongation lengths. With the distance between the middle of one cell and a neighbour in the grid defined as 1, the distances used are: 1.5, 2, 4, and 6. Close neighbour connectivity is the parameter governing the distance at which close neighbours connect. This parameter affects both CN-cells and LD-cells. The distances tested are (again in terms of the distance between the middle of one cell and its neighbour): 1 (meaning the 6 closest cells), 2 (the closest 18), and 3 (the closest 36). In effect, these numbers will end up slightly lower due to some cells being on or near the edge of the tube, meaning they will miss some neighbours and the connections to them.

5.2.2 Analysis

We are interested in the extent to which the patterns of spiking in the system are coordinated spatiotemporally under various conditions. For this, we use a measure of coordinatedness in terms of ring-shaped activity on the surface of the model as described in Chapter 4. This measure allows us to compare patternedness between experimental conditions. This method is similar to the one used in previous work with the exception that, since the current investigation only uses a single instance of bodily dimensions, normalization over total amount of activity per ring-shaped segment is unnecessary.

5.3 Results

5.3.1 Individual model instances

The results of the parameter scan performed are quantitative in nature, since patternedness is quantified. However, for a clearer understanding of what happens in individual experiments, we first present the behaviour of three separate instances. Figure 5.2 illustrates how spatiotemporal patterns emerge on the surface of an individual tube-shaped system. Ring-shaped patterns show up as horizontal lines travelling up or down.

Pane A shows the situation where all cells are CN-type, meaning that the only connectivity is every cell connecting to its closest neighbours. In this particular case, the close connectivity distance is set to 1, meaning only 6 direct neighbours are affected—this is in fact nearest neighbour connectivity. This case is representative of experiments in Chapter 3: synapses (nearest neighbour connectivity) and electrical excitability only.² Pane A illustrates the relative mis-scaling of the patterns and the interference of individual random events, though some coordination is in evidence, as can be judged by the presence of some horizontal bands.

Pane B shows a system with LD-cells in a background of CN-cells. It demonstrates how elongations allow the patterning of the system to scale much better: the horizontal bands are quite pronounced. In this specific case, 90% of cells are CN-cells and only 10% are LD-cells featuring elongations, meaning that this result occurs in spite of a limited number of elongation-possessing cells, representing specialization in terms of cell numbers, albeit only limited in terms of energy efficiency since the CN-cells still have neuroid functionality (they affect their neighbours in the same way they did in pane A).

Pane C shows a system similar to pane B, except that the CN-cells have been replaced with UC-cells, meaning that only 10% of cells, the LD-cells, have any neuroid functionality at all. Most cells are only receptive. This represents a situation with significant specialization both quantitatively and qualitatively. Tellingly, the patterns appear essentially unaffected.

The next step is checking to what extent the patterns hinted at in Figure 5.2 are confirmed by a quantitative analysis. The mutual interactions between the parameters

²In fact, the exact parameters used in pane A are also represented in Chapter 3, Figure 3.5: second row, fourth column, showing comparatively poor coordination.

Figure 5.2: Eight consecutive frames of three individual experiments. The tube is projected as a 2D surface; the axis of the tube runs top to bottom in each frame. Pane A shows noticeable but limited coordination in a system without LD-cells; only CN-cells with close neighbour range 1, so only connecting to the nearest 6. Pane B replaces 10% of cells with LD-cells possessing elongations of length 4, showing far more effective coordination even with a limited number of LD-cells. Pane C keeps the same 10% LD-cells, but all remaining CN-cells lose their close connectivity and are thus rendered into UC-cells: notice that the coordination pattern remains intact compared to pane B. Every frame shows 1 ms. The four different shades of red indicate how long ago a cell fired: more intense colouring indicates recent firing. Consequently, a single firing event shows up in four frames.

will be examined. This will allow us to establish whether specialization can help in providing coordination without resulting in a surfeit of neuroid (and thus energetically expensive) cells.

5.3.2 Numerical analysis

Numerical analyses allow us to show the patternedness of various experiments by reducing each one to a single patternedness value P as described in Section 5.2.2. The individual graphs in this section show the performance in terms of ring-shaped spatiotemporal patterning (via patternedness measure P , shown on the y-axis) for different parameter settings and different proportions of foreground-cells (on the x-axis). Individual markers represent single experiments; lines represent the average value of a given parameter setting. The experiments shown in the panes of Figure 5.2 are represented by the red letters in all figures in which they feature, putting them in context.

First, we present progressive addition of minimally neuroid CN-cells to a background of UC-cells lacking any neuroid functionality. These CN-cells have a close neighbour connectivity distance of 1, possessing only electrical excitability and synapses to their 6 neighbours. This makes them the least energetically costly cell type explored while still possessing some coordinative potential. Pane I of Figure 5.3 displays this progression, from a completely passive background without any coordinative ability on the left of the x-axis to the situation shown in pane A of Figure 5.2 on the right of the x-axis, a situation where all cells are connected to their 6 direct neighbours.

Pane II of Figure 5.3 explores progressive addition of LD-cells with various elongation lengths to backgrounds of UC-cells and CN-cells, representing specialization with a more advanced neuroid feature: elongations. Close neighbour connectivity distance is still limited to 1: CN-cells only affect their direct neighbours, and LD-cells only affect background cells which are their direct neighbours.

This progressions shown in Figure 5.3 allow the following observations:

- i Reconsidering the level of patternedness of the individual experiments shown in Figure 5.2, it becomes apparent that the measure of patternedness of case A is considerably lower than case B and that B and C are comparable in terms of patternedness;
- ii In the progression from a body consisting of UC-cells to one consisting of CN-cells (pane I), minimal patternedness is already visible at 2% and 5%, marginal patternedness at 10%, and definite patternedness at 20%, 50%, and 100%;
- iii At longer elongation lengths and higher proportions of LD-cells, a background of CN-cells instead of UC-cells appears to provide limited benefit in terms of patterning: the green asterisks and grey diamonds show very similar levels of patternedness in these conditions;
- iv Considering the grey diamonds in pane II, LD-cells in a background of UC-cells, we observe that connectivity is considerably better at low percentages of foreground cells when elongation lengths are greater;

Figure 5.3: Effects of specialization, elongation length, and background cell type on patternedness. Individual graphs in this figure display the proportion of foreground cells on the x-axis, patternedness P on the y-axis, and individual markers depict distinct experiments. The colour and shape of the markers indicate the type background cells in the system. Pane I displays increasing proportions of CN-cells with a background of UC-cells. In Pane II, individual graphs display different elongation lengths of LD-cells. This way, the effect of increasing LD-cell proportion on patternedness for various LD-cell elongation lengths can be compared between situations where the background cells are CN-cells and the background cells are UC-cells. The locations indicated by A, B, and C correspond with those panes in Figure 5.2. Close neighbour connectivity (LD and CN-cells only) is set to 1 in all experiments.

Figure 5.4: The interactions between close neighbour connectivity, elongation, background cell type and decreasing proportion of LD-cells and their effect on patternedness. In this figure, marker shape and colour indicate close coordination distance, while background cell type is shown in groups of graphs left and right. Pane I again shows CN-cells in a background of UC-cells. Pane II shows the case where LD-cells provide the foreground cells and CN-cells or UC-cells form the background. Various elongation lengths are displayed from top to bottom.

- v Comparing experimental conditions with similar levels of patternedness in pane II, we observe that elongation length can compensate for lower percentages of foreground cells. Achieving a patternedness level of 20 in a background of UC-cells with elongation length 2 requires a 50% proportion of LD-cells, while at elongation length 6 only 10% of LD-cells is needed.

Figure 5.4 displays the effect of greater neuroid functionality by adding distances beyond 1 in close neighbour connectivity to the parameter space. Like in Figure 5.3, pane I shows CN-cells with UC-cells in the background and pane II shows a foreground of LD-cells with either CN-cells or UC-cells in the background.

We observe the following in the results shown in Figure 5.4:

- i There is a clear tendency for situations with more connecting neighbours to have higher patternedness scores;

- ii This tendency is especially pronounced in pane I, where greater close neighbour connectivity allows CN-cells to affect more neighbours;
- iii The beneficial effect of affecting multiple postsynaptic cells is more marked in the case where UC-cells form the background and the proportion of LD-cells is low (the left side of graphs in the right column of pane II);
- iv In situations with many LD-cells in a background of UC-cells, the net added benefit of more close neighbour connectedness decreases as the percentage of LD-cells increases (the right side of the right half in pane II of Figure 5.4, where line with the orange squares (close neighbour distance 2) and the line with red pluses (distance 3) hardly deviate from each other): there is a large discrepancy in the number of connections but only a small effect on patternedness. This means that these kinds of systems are close to coordinating optimally without the necessity of connective cells affecting multiple postsynaptic target cells.

The number of synapses varies significantly between experimental conditions. This is relevant, since synapses carry metabolic costs. Consequently, in order to better judge energetic efficiency of the various conditions it is desirable to control for this number. The number of synapses resulting from close neighbour connectivity is fairly straightforward; the synapses resulting from elongations less so. Elongation length is relevant, but so is the proportion of cells possessing them. Especially in experiments with long elongations and high proportions, synapse counts run to the hundreds per cell. Fortunately, our experimental method allows exact and direct synapse counts. Figure 5.5 replicates Figure 5.4, only differing in that patternedness is directly divided by the number of synapses per cell.³

Figure 5.5 allows the following observations regarding energetic efficiency measured as patternedness per synapse:

- i A background of CN-cells is inefficient.

In the more efficient conditions with UC-cells as background, we find the following:

- ii Longer elongation conditions (4 and 6 in particular) allow greater efficiency at low LD-cell proportions;
- iii Independent of elongation length, at very low (2%, and to a lesser extent 5%) LD-cell proportions, close neighbour connectivity distance is more beneficial than at higher LD-cell proportions, where close neighbour distance 1 is more efficient.

³‘Number of cells’ refers to all cells, foreground and background, and since the number of cells between conditions is constant, the graph for ‘patternedness per synapse’ would look the same but for the scale of the y-axis. We chose ‘patternedness per synapse per cell’ ($P/\bar{s}$) because it is easier to relate to whole-body patternedness levels.

Figure 5.5: Effects of specialization, elongation length, and background cell type on patternedness, corrected for the number of bidirectional synapses per cell. The characteristics of this figure are identical to Figure 5.4 with the sole exception that patternedness is divided by the amount of synapses per cell (displayed as $P/\bar{s}$ on the sub-graph y-axes), thus accounting for metabolic cost and giving an indication of coordinative efficiency for the various experimental conditions.

5.4 Discussion

The model investigates two axes of specialization (or differentiation):

- Quantitative specialization: how many cells specialize in a coordinative role? This is implemented as the proportion of coordinative cells (LD-cells, on a background of either CN or UC-cells; or CN, on a background of UC-cells) in a given experiment.
- Qualitative specialization: how deep does the difference between cell types go? This is implemented by the per-experiment distinction of whether the background cells are CN-cells with close neighbour connectivity or UC-cells without any active connectivity. Additionally, qualitative specialization in degree is represented by elongation lengths and close neighbour connection distance.

What do the results in terms of these axes of specialization mean regarding the origin of nervous systems? Specifically, what do the results imply regarding the role of specialization facilitating improved coordination while limiting energetic costs?

Broadly speaking, coordination appears to improve as neuroid features are applied to more cells and as those features increase in degree. Looking at figures 5.3 and 5.4, the results show that patternedness correlates positively with the following:

- i Greater proportions of foreground cells;
- ii A background consisting of CN-cells instead of UC-cells;
- iii Longer elongations;
- iv Greater close neighbour connectivity.

The results also show situations where there is very little benefit to the addition of more neuroid functionality or cells: if elongations have length 4 or greater, for example, additional close neighbour connectivity adds only a small amount of extra patternedness, if any. Adding short elongations in a situation where the background cells have close neighbour connectivity greater than 1 has very little effect either—an obvious outcome, since the certain connectivity from close neighbour connections supersedes the potential connectivity from short elongations within similar distances.

More importantly, there are situations where only a small amount of extra cells with neuroid functionality can provide a remarkable improvement in patternedness. Adding only 5% of LD-cells with close neighbour connectivity of only 1 and elongations of length 4 to a background of UC-cells allows a similar level of patternedness (about 10) as the situation where *all* cells have close neighbour connections. Increasing that proportion to 10% roughly *doubles* patternedness (to about 20; the situation shown in pane C in Figure 5.2).

It is also important to note that even minimal addition of axodendritic sophistication in a limited proportion of cells (i.e. only 10% CN-cells in a background of UC-cells with close neighbour connectivity distance 1 as seen in pane I of Figure 5.3) results in some

improvement in coordination, resulting in a continuous gradient, allowing the continuity requirement of a lineage explanation to be satisfied.

None of this explicitly takes energy efficiency into account. In the end we are interested in the effect on fitness of the trade-off between coordination and energy efficiency. Neither the fitness effect of coordination nor that of energy efficiency can be measured, so we have to rely on plausible proxies. Patternedness, representing ring-shaped activity suitably scaled to body size, is our proxy for coordination. There are reasons to accept synapses as a suitable proxy for energy consumption. First, synaptic activity forms a significant part of the energy consumed by nervous systems and since any non-neuroid cells in this system could reasonably be expected to constitute some proto-myoid tissue, that would also possess action potentials, so there would be little relative energy difference. Second, we can reasonably assume synaptic costs to scale linearly: synaptic connections to 2 other cells should be roughly twice as expensive as connections to 1 other cell.

Lacking any indication of how to compare these two proxies, we simply divided patternedness by the amount of synapses per cell to get an indication which parameter combinations are most efficient, as shown in Figure 5.5. This shows that elongations and close-neighbour connectivity in combination with a low proportion of LD-cells in an UC-cell background is an efficient solution, providing significant patternedness while requiring relatively few synapses. Axodendritic sophistication allows more patternedness per synapse while limiting the overall investment in neuroid features.

5.5 Conclusion

The model results presented here clearly show that, given an internal coordination scenario for early nervous system evolution, a large proportion of neuroid cells is not required for high patternedness as long as sufficient axodendritic sophistication is present. Very basic specialization can solve the problem of the energetic costliness of neuroid tissue and provide the universal benefits of specialization. ‘Very basic’, since in the experiments shown here, there is no localization of any kind—elongations extend in random directions and different cell types are distributed randomly.

Interestingly, the configuration resulting from parameter combinations which appear to provide a reasonable balance between the number of cells with neuroid functionality and axodendritic sophistication (roughly 5% of cells, some elongation and a little close neighbour connectivity) strongly resemble a randomly connected diffuse nerve net. This lends credence to the idea that cell specialization provides a potential internal coordination (IC)-based path to nerve nets.

Long-distance elongations compensate for the numerical loss of neuroid cells as well as for greater body sizes. This means that in the internal coordination scenario for nervous system evolution sketched here there are three factors affecting coordination: first, size; second, limiting the overall investment in neuroid features, both of which affect it adversely; third, axodendritic sophistication, which affects it positively *but only up to a point*, since the results also show diminishing returns for more axodendritic sophistication. Specialization provides a crucial mechanism to balance the cost of axodendritic

sophistication and coordinative performance. While this abstract model and the proxies of synapse count and patternedness provide a useful first step, movement and metabolism remain unmodelled and no empirical check exists, so any conclusions should be taken as guidelines for thinking about nervous system origin rather than hard evidence.

All in all, these models provide a very robust hypothetical scenario for specialized neuroid cells with axodendritic sophistication arising from a non-neural epithelium, purely based on internal coordination. Given that ‘specialized neuroid cells with axodendritic sophistication’ substantially resemble nerve nets as found in modern basal animal phyla, this hypothetical represents a plausible path for the origin of the first nervous systems. The findings of this chapter in particular point out the central role of specialization (both qualitative, in axodendritic sophistication, and quantitative, in limiting the proportion of neuroid cells) in maintaining an energy-efficient, potentially evolutionarily fit coordination mechanism. A specific lineage explanation of early nervous system origin remains uncertain, since the model is highly idealized. However, since this model satisfies all constraints and the production and continuity requirements, an internal coordination scenario provides a good general outline of a nervous system origin lineage explanation.

Conclusion

In Chapter 1 we sketched the problem of the explanatory gap in nervous system evolution: innervated clades have fully featured neurons whereas uninnervated clades lack neurons altogether, and no intermediate stages are apparent in fossils or in extant animals. We opine that a lineage explanation (an evolutionary narrative in a progression of stages; see Calcott, 2009) of nervous system origin is wanting. Such a lineage explanation should satisfy two requirements: the continuity requirement, meaning that differences between stages should be small; and the production requirement, meaning that each individual stage should be functional for its possessor.

We follow Keijzer et al. (2013) and Keijzer (2015) by investigating the relationship between internal coordination and nervous system origin. Specifically, we investigate internal coordination as a way to satisfy the production requirement in the lineage explanation of nervous system origin. The internal coordination view hypothesizes that the first nervous systems allowed animals to endogenously coordinate their whole, multicellular bodies. We developed computational models with dynamically interacting parts to test the internal coherence and explanatory potential of this hypothesis. These models, described in chapters 3, 4, and 5 can be cast as suggestions for the stages in an internal coordination-based lineage explanation of nervous system origin.

The model fits the constraints

A good lineage explanation of nervous system origin should fit with all the relevant data available. In Chapter 2 we take stock of empirical data relevant to nervous system origin, including basal animal phyla, phylogeny, ecology, climate, evo-devo, and potential precursor tissues. From these data we have distilled a number of constraints:

- i The starting situation: epithelia in small, soft-bodied animals with limited but present developmental patterning, with access to the molecular building blocks of neurons;
- ii The drivers: fast and macroscopic movement, muscle coordination, and the ability to utilize a gut, increasing developmental patterning, and not necessarily predation on active prey;
- iii limiting factors: rudimentary developmental patterning and pressure to keep energetic demands low;

- iv The end point: a diffuse nerve net, comparable to the simplest extant nervous systems.

The constraints governing the starting situation fit specific parameter combinations of the excitable epithelia model as laid out in Chapter 3. The assumption that activity correlates with contraction makes the model laid out in Chapter 3 representative of soft-bodied animals. Small bodies are the part of the parameter space which exhibits coordination. Specifically, the parameter space showing the most useful, peristalsis-like coordination is the elongated, worm-like shape. Such a very simple body shape needs only basic developmental patterning: some shaping into a worm, being able to function as one, and the presence of an epithelium are all that is required, fitting that limiting factor. The model assumes an evolutionary step: the coalescence of previously present constituent synaptic building blocks into very basic synapses: bidirectional, without any kind of tuning, and noisy to boot. This is in line with the constraints regarding the starting position.

As laid out in Chapter 3, the model shows that small, worm-like body shapes with very basic synapses generate activity patterns which, when combined with contractile activity, could provide useful behaviour. This fits the driver of muscle coordination for fast macroscopic movement. Peristalsis also forms the basis of gut control, which fits that driver. Simple peristalsis is not specific enough to allow predation of fast prey, so the model meets that part of the constraints as well.

The usefulness of patterned contraction in tube-shaped bodies need not be limited to peristalsis in bilaterally symmetrical worms since the body shapes treated in the models are not primarily representative of a *bilaterally* symmetrical body plan: they also represent a radial body plan. Patterned contraction in a radial body plan can be functional as well, allowing for example rhythmic ‘reaching’ behaviour with tentacles as observed in modern anthozoans or the contraction of a swimming bell as in modern medusæ.

The extending scope of the model in chapters 4 and 5 remains within the constraints. First, in Chapter 4 we add extra neuronal features in the smallest possible steps: axodendritic elongations (we know that the molecular mechanisms for this were available beforehand) starting with only very short ones, in a random direction, without accounting for specialization in the model. Then, in Chapter 5, we introduce minimal kinds of specialization, thereby dealing with the limiting factor of energy consumption. Overall, the models presented in chapters 3, 4, and 5 fit with the constraints set out in Chapter 2.

The model demonstrates internal coordination

The main value of the models lies in the information they provide about how coordination relates to nervous system features. Our parameter scans demonstrate how coordination can react to different circumstances in a situation relevant for early nervous system evolution (i.e. small, soft, multicellular bodies with a very limited neural toolkit). Chapter 3 demonstrates that simply adding synaptic connectivity to a contractile sheet leads to fast, whole body coordination in a small enough, worm-shaped enough body. Chapter 4 demonstrates that even short and random elongations allow patterning to scale, including in larger bodies. Chapter 5 demonstrates that increased axodendritic sophistication

counterbalances a decrease in the number cells with a coordinative role and suggests an alternative lineage explanation that does not involve a full excitable epithelium.

Summarizing the results of chapters 3, 4, and 5, we find the following:

- i A synaptically coupled epithelium allows coordination in small bodies. Increased size decreases coordination in this system (Chapter 3);
- ii Adding axodendritic elongations allows coordination in larger bodies (Chapter 4);
- iii Having a synaptically coupled epithelium is an energetically inefficient way to coordinate (Chapter 5);
- iv Even low proportions of cells with more advanced axodendritic elongations in combination with non-excitable epithelial cells allows coordination, in a less expensive way (Chapter 5).

Having isolated these principles, they can help us take steps towards a lineage explanation of nervous system origin.

Towards a lineage explanation

From the findings of previous chapters, a lineage explanation can be constructed in several ways. We identify two options. These options center around the order in which the neuroid features (electrical excitability, synapses, and elongation), size increase, and specialization coalesced into a nervous system consisting of a diffuse nerve net. For both options, we presume the potential for electrical excitability and an epithelium to be present from the start. In what order do the other features arise, and how does that effect the potential fitness of their possessor?

Option 1: synaptically coupled epithelium first, specialization later

The progression of chapters 3, 4 and 5 carries an implicit ordering of feature incorporation, which can easily be elaborated into a lineage explanation. This lineage explanation option puts the incorporation of neuroid features—the innovations which we suspect were involved in early nervous system evolution—in the following order:

1. Synapses, the incorporation of which results in an excitable epithelium-stage (Chapter 3);
2. Body size, leading to a stage of slightly larger animals, coordinating less well;
3. Axodendritic sophistication, allowing better coordination in larger animals (Chapter 4), leading to a range of stages of various levels of body size and axodendritic sophistication;

4. Specialization, to ameliorate the energetic costs of neuroid functionality, leading to a diffuse nerve net (Chapter 5).

Each incorporation event plausibly affords potential improvement in fitness under suitable circumstances. The addition of synapses to an epithelium allows coordination resulting in a degree of fast, whole-body movement, potentially a great boon to its possessor under circumstances where no other multicellular organism was able to replicate that feat. Increased body size, even with a penalty to coordination, could be beneficial under circumstances where macrophagy was increasingly common: being bigger yet moving around means becoming harder to eat and more able to eat others. Axodendritic sophistication allows better coordination, even if tiny amounts of it are added: just short and random elongations will do, and this allows better coordination even in larger bodies. Each addition carries the potential for fitness improvement and each resulting stage is functional in its own right, thus satisfying the production requirement. The steps between stages are small, as are the steps of degrees within stages—such as elongation length—thus satisfying the continuity requirement and constituting a lineage explanation.

Option 2: specialization first

Another reading of the results of the previous chapters is possible. Instead of taking a synaptically coupled epithelium as a first stage, it is possible to consider chapters 3 and 4 more abstractly and take the implications on potential fitness from Chapter 5 as most important in terms of ordering of feature coalescence. Chapters 3 and 4 demonstrate the principles that underlie basic internal coordination with a minimal collection of building blocks. By incorporating energetic costs, Chapter 5 demonstrates that specialization is a crucial ingredient to keep energetic costs low, thereby improving the potential evolutionary suitability of this internal coordination-based scenario. Combining the likely presence of limited developmental patterning in the starting situation, the evolutionary importance of keeping energy costs low, and the finding in Chapter 5 that even limited axodendritic sophistication in a small number of cells results in some coordination means that there is a path from the starting situation to the end point. This path would look thus:

1. A small number of cells exhibits some minor axodendritic sophistication, resulting in very limited coordination;
2. Axodendritic sophistication increases to improve coordination, reaching an optimal balance between quality of coordination and energy expenditure;
3. With the possibility of more axodendritic sophistication to compensate for the loss of coordination resulting from larger body size (as demonstrated in chapters 3 and 4), it becomes possible to grow larger.

Steps 2 and 3 likely occurred intermittently: in fits and starts and at varying paces in different ecological niches. The important point is that there is a continuous path, with the constituent parts of axodendritic sophistication and size being able to increase gradually while providing increasingly better coordination for a given size. If at some

point the environment dictated that energy efficiency was the overriding pressure, sizes may well have remained small while axodendritic sophistication mainly balanced energetic costs. Conversely, if at another point predation was a significant driver, increased size may have been the factor for which axodendritic sophistication compensated. The exact degree to which specific variables changed at different points is not essential to a lineage explanation. The important point is that all ingredients were present beforehand, and that an internal coordination scenario perusing either lineage explanation option allows tiny evolutionary steps resulting in probable fitness improvement. In our view, the models presented in this thesis provide compelling support for an internal coordination-based lineage explanation of nervous system origin.

Lineage explanation consequences

These progressions of stages can have interesting implications when it comes to interpreting the empirical data of the relevant period as laid out in Chapter 2. The following conclusions are true for both options. For example, different body sizes suitable for different ecological niches explicitly suits speciation, with some species increasing axodendritic sophistication to offset increasing body size and others increasing axodendritic sophistication to become more efficient. This is consistent with the enigmatic early divergence of various innervated animal clades.

Several features very common to modern nervous systems were not included in this model, for example: unidirectional synapses, inhibitory synapses, any proliferation of neurotransmitter types, any regularly spiking cell membrane mechanism, neuromodulation, specifically targeted neurons, and centralization. All these features provide greatly increased coordinative options, yet none of these features are required for this internal coordination-based lineage explanation: it can work entirely without these features. This makes it more likely that they are later developments.

Additionally, the relative simplicity of axodendritic sophistication together with the significant potential benefits derived from it means that we should consider the threshold for nervous system evolution relatively low. This is especially true in the case where synaptic connectivity was already established. This means that nervous systems evolving separately in both Cnidaria + Bilateria (ParaHoxozoa) and Ctenophora should not be rejected lightly.

Philosophical context and implications

Our findings also affect the philosophical context. When comparing input-output (IO) and internal coordination (IC)-based views (Jékely et al., 2015a), our model suggests that IC-based scenarios allow a gradual evolutionary route towards a basic nervous system and neurons, which is less clearly available for IO-based scenarios. This support for an IC-based view bears on Godfrey-Smith’s influential environmental complexity thesis (1998; 2002), which states that the function of cognition is to enable the agent to deal with

environmental complexity. At least in its original version, the environmental complexity thesis stressed the importance of the *external* environment. IC models highlight the importance of dealing with internal bodily complexity as an additional condition for the environmental complexity thesis (Keijzer and Arnellos, 2017). The presented modelling work’s support for IC-based scenarios therefore bears on the evolution of cognition as well. The modelling studies also bear positively on recent ideas concerning the origins of animal sensing that stress self-initiated motion as playing an initial key role that enabled the multicellular integration of sensory feedback (Keijzer, 2015).

On a wider level, this modelling study fits in with a trend in current philosophy: turning to the evolutionary origins of nervous systems to develop accounts of mental properties such as subjectivity and consciousness. One example is the work of Feinberg and Mallatt (2016), who developed an account of consciousness that goes back to the Cambrian explosion and (presumably) the origins of complex brains and the structures enabling sensory experience. Godfrey-Smith’s bestselling “Other Minds: The Octopus and the Evolution of Intelligent Life” (2016) uses the deep evolutionary history of nervous systems to discuss issues related to the mind. Ginsburg and Jablonka (2019) recently provided an account that builds on early brains while stressing the key role of associative learning.

It is increasingly clear that the intelligent features that are characteristic for humans have a very long evolutionary history and philosophy should heed this history as a crucial source of ideas and background information when it comes to understanding animal minds, including the human variety.

Further research potential

With this model platform based on the internal coordination account of nervous system origin, a whole new area of research emerges. Any innovative research is likely to result in new subjects to investigate, but in our case, a particularly rich vista of further research options has opened up:

- i Other basic eumetazoan body plans should be investigated. Beside worm-shape another plausible basal eumetazoan body-shape is the gastraea (a gastrula, a clump of cells, with a hole): a tube has two openings, a gastraea only one, which is also the cnidarian body plan. The difference between one and two holes may strongly affect coordination (potentially beneficially).
- ii Adjusting noise levels in addition to adding pacemakers to the model is likely to improve consistency of activity waves. The peristaltic waves, while particularly striking when seen in motion, were not very consistent in terms of which direction of the worm-shaped body they were travelling in.
- iii The transition between completely non-synaptic transmission (e.g. some form of purely chemical paracrine or juxtacrine coordination) and synaptic transmission could be further investigated. This is particularly salient for lineage explanation

option 1, in which the transition between entirely non-neural and a synaptically coupled epithelium is quite abrupt.

- iv This transition between non-neural coordination and synapse-mediated coordination may benefit from closer integration of the model platform with extant animals. For instance, recreating (parts of) the behaviour of simple animals such as *Hydra*, sponge larvae, and *Trichoplax* may lead to better insights into animal coordination *sensu lato* and these insights could very well be integrated with the platform presented in this thesis.
- v The interaction between coordination and soft-bodied movement is an important factor in understanding modern animals as well as providing a clearer link between fitness and factors influencing coordination: coordinated movement is more directly adaptive than mere spatiotemporal patterns. Biophysics and the modelling of body movement in turn leads to possible inclusion of proprioception in the model. Body-body feedback loops may provide a wide array of behavioural options with very basic adjustments to the coordination system.
- vii So far we only used the most basic neuronal features conceivable; a wide array of neuronal features present in modern nervous systems is available for addition to the model framework: inhibition, neuronal polarity, neurotransmitter differentiation, and different cell membrane dynamics all have potentially far-reaching effects on the nerve net, particularly if biophysics are also modelled.

Appendix A: Chapter 3 supplementary material

Introduction

We illustrated our ideas about emergent whole body organization using an integrate-and-fire model. To show the robustness of our findings, we include here our simulations with the cell model replaced by a conductance based cell model.

Methods

Excitatory chemical transmission between nearest neighbors including the dynamics of messenger molecule exocytosis and receptor channel kinetics are modeled as double exponential conductance changes in receptor channel populations (Destexhe et al., 1994). Only a single type of excitatory chemical transmission is included.

Our methods are the same as in the main paper, but with the integrate-and-fire model with an absolute refractory period replaced by a Hodgkin-Huxley model. The details of the Hodgkin-Huxley model are provided in Tables 5.2 and 5.3, describing the cell model and the transmission model, respectively. With these parameters we can rerun our analysis code without a need for analysis parameter adjustments, so criteria for whether spikes in two neighboring cells are part of a wave front are unchanged.

Table 2. Model Summary 2.1 (Supplemental): Hodgkin-Huxley Cell Model

Name	HHCell
Type	Single compartmental Hodgkin-Huxley model
Dynamics	$C_m \frac{dV_m}{dt} = -A g_l (V_m - E_l) - A \bar{g}_K n^4 (V_m - E_K) - A \bar{g}_{Na} m^3 h (V_m - E_{Na}) + I_{syn}$ $\frac{dx}{dt} = -(x - x_\infty(V_m))/\tau_x \quad \text{with } x = m, h, n$ $\tau_x = 1/(\alpha_x + \beta_x)$ $x_\infty = \alpha_x/(\alpha_x + \beta_x)$
Parameters	$A = 400\pi \mu m^2 = 1257 \mu m^2$ $C_m = A c_m = 12.57 pF$ $\bar{g}_{Na} = 0.12 \text{ S cm}^{-2}, \quad E_{Na} = 50 \text{ mV}$ $\alpha_m(V_m) = \frac{-0.1(V_m+40)}{\exp(-(V_m+40)/10)-1}$ $\beta_m(V_m) = 4 \exp(-(V_m + 65)/18)$ $\alpha_h(V_m) = .07 \exp(-(V_m + 65)/20)$ $\beta_h(V_m) = 1/(\exp(-(V_m + 35)/10) + 1)$ $\bar{g}_K = 0.036 \text{ S cm}^{-2}, \quad E_K = -77 \text{ mV}$ $\alpha_n(V_m) = \frac{-0.01(V_m+55)}{\exp(-(V_m+55)/10)-1}$ $\beta_n(V_m) = .125 \exp(-(V_m + 65)/80)$ $g_l = 0.0003 \text{ S cm}^{-2}, \quad E_l = -54.3 \text{ mV}$

Table 3. Model Summary 2.2 (Supplemental): Chemical Transmission Model

Name	Exp2Syn
Type	Double exponential conductance based
Dynamics	$I_{syn} = -(V_m - E_{syn}) \sum_{pre} w_{pre} \sum_{t_{pre}+d_{pre} \leq t} G(t - t_{pre} - d_{pre})$ $G(t) = G_0 \frac{\exp(-t/\tau_{decay}) - \exp(-t/\tau_{rise})}{\exp(-t_{peak}/\tau_{decay}) - \exp(-t_{peak}/\tau_{rise})}$ $t_{peak} = \frac{\tau_{rise} \tau_{decay}}{\tau_{decay} - \tau_{rise}}$
Parameters	$G_0 = 1 \mu S$ $w_{pre} = 0.001 \text{ or } 0$ $d_{pre} = 0.75 \text{ ms}$ $\tau_{rise} = 0.05 \text{ ms}$ $\tau_{decay} = 2 \text{ ms}$ $E_{syn} = 0 \text{ mV}$

Supplementary Figure 6. Analysis of whole-body coordination for various body shapes. Relative wave-front orientation prevalences (represented by colored disk diameter) and average propagation orientations (indicated with an oriented black bar) are shown for various body lengths and circumferences of the excitable epithelium. In the corners, the corresponding body shape is indicated with a rectangle. Bottom left: small ‘square’ cylinder, top left: long cylinder with small circumference, top right: large ‘square’ cylinder, bottom right: short cylinder with large circumference. This parameter scan shows three effects: (i) more elongated networks show better developed longitudinal wave fronts, (ii) whereas shorter networks show better developed transverse wave fronts, (iii) however for fixed length-to-circumference ratios (visible on the diagonals running from bottom left to top right) we can see that with increasing size preference for transverse or longitudinally moving wave fronts is lost.

Supplementary Figure 7. Analysis of whole-body coordination for the extreme noise rates used in this study. Relative wave-front orientation prevalences (represented by the diameters of the colored disks) and average propagation orientations (indicated with the oriented of the black bar) are shown for various body lengths and circumferences of the excitable epithelium. The two subpanels are organized as in Figure 5.6. (A) Low noise rate: 0.001 Hz, (B) High noise rate: 10 Hz. Compared to Figure 5.6 we find slightly stronger relative wave-front orientation prevalences and average propagation orientations at the low noise rate in panel (A). In contrast we clearly see the loss of whole-body coordination with increasing noise rates, as relative wave-front orientation prevalences become uniform and average propagation orientations are almost absent in panel (B).

Supplementary Figure 8. Analysis of propagation direction orientation for various body shapes and noise rates. Propagation direction orientation, averaged over a unique combination of length, circumference and noise rate is indicated by an oriented black bar. There are 19 individual runs per unique combination of length, circumference and noise rate. Propagation direction of a single run is indicated with an oriented grey bar.

These grey bars usually largely overlap with the black bar, indicating that these experiments are highly reproducible. The orientation calculation is explained in Figure 2 of the main paper. (A) Noise rate: 0.001 Hz, (B) Noise rate: 0.01 Hz, (C) Noise rate: 0.1 Hz, (D) Noise rate: 1 Hz, (E) Noise rate: 10 Hz.

Appendix B: Chapter 4

supplementary material

Supplementary Material 1: Distant connection implementation details

Regarding implementation details, consider the case of cells arranged in a plane. Every elongated cell extends an axodendritic elongation p with length l_p in an arbitrary direction ϕ_p , relative to the x-axis. Where elongations cross each other they create bidirectional connections between the originating cells. Given any two cells i and j with coordinates (x_i, y_i) and (x_j, y_j) and elongation directions relative to the x-axis ϕ_i and ϕ_j respectively, a triangle emerges, with at one point cell i , another point cell j and the third point the crossing of their elongations. Two sides of this triangle consist of the elongations themselves. The third side, d , is the line between the cells, with an angle relative to the x-axis: ϕ_d . Assuming $\phi_i \neq \phi_j \neq \phi_d^4$, the sine-rule allows us to locate the crossing location, given the angles and the locations of the cells.

$$\frac{d}{\sin(\phi_j - \phi_i)} = \frac{\rho_i}{\sin(\pi - \phi_j + \phi_d)} = \frac{\rho_j}{\sin(\phi_i - \phi_d)} \quad (5.1)$$

Furthermore,

$$\sin(\pi - \phi_j + \phi_d) = -\sin(-\phi_j + \phi_d) = \sin(\phi_j - \phi_d) \quad (5.2)$$

Cell coordinates allow us to calculate d and ϕ_d :

$$d = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2} \quad (5.3)$$

$$\phi_d = \arctan\left(\frac{y_j - y_i}{x_j - x_i}\right) \quad (5.4)$$

Then we can solve for ρ_i and ρ_j :

$$\rho_i = d \cdot \frac{\sin(\phi_j - \phi_d)}{\sin(\phi_j - \phi_i)} \quad (5.5)$$

$$\rho_j = d \cdot \frac{\sin(\phi_i - \phi_d)}{\sin(\phi_j - \phi_i)} \quad (5.6)$$

⁴In practice, given the granularity of the random numbers used, this assumption always holds.

If, for a given pair of cells, ρ_i and ρ_j are shorter than l_p , so $0 < \rho_i < l_p$ and $0 < \rho_j < l_p$, the cells are connected. We loosened this constraint to $-\frac{1}{2} < \rho_i < l_p$ and $-\frac{1}{2} < \rho_j < l_p$ to account for crossings occurring on the cell body of the originating cell.

All the above, however, is predicated on an infinite plane, while our system is finite and tube-shaped. Although a tube is essentially a curved 2-dimensional plane, its contiguousness results in some particular cases: elongations which may wrap around the cylinder. If we assume the cylinder is formed by making the x-direction periodic, we should consider not only the crossing of elongations starting at (x_i, y_i) and (x_j, y_j) but also at $(x_i + k_i \cdot \odot, y_i)$ and $(x_j + k_j \cdot \odot, y_j)$, with k representing the number of windings and $\odot$ the circumference of the tube. We can limit the number of windings we need to consider, since the maximum elongation distance is limited: $|k| < (2 \cdot l_p / \odot) + 1$. The +1 is needed because the nearest copy might be on the other side of the tube's period. Another particular of the tube as opposed to a torus is that the crossing point may lie off the tube on the finite end, so we need to check whether the y-coordinate of the crossing, $y_{crossing} = \rho_i \cdot \sin \phi_i + y_i$ is on the tube: $-0.5 \leq y_{crossing} \leq (l_{tube} - 1) \cdot \frac{1}{2}\sqrt{3} + 0.5$, where l_{tube} is the number of rings on the tube and $\frac{1}{2}\sqrt{3}$ is the height of an equilateral triangle with sides of length 1.

Supplementary Material 2: The effect of transmission speed

Transmission speed of the signal affects the spatiotemporal pattern. Our main experiment does not incorporate transmission speed; instead, it uses a fixed synaptic delay, in essence assuming transmission speed to be fast enough not to matter on that scale. This supplement explores that assumption. How fast a transmission speed is fast enough, and how slow is too slow?

To investigate this issue, we extended the model to include transmission speed. The basic model only works in terms of abstract, size-less cells, so in order to arrive at a delay value using realistic transmission speeds we added a cell size parameter in addition to the transmission speed variable. The effect of transmission speed over a connection between two cells, i and j is modelled as a delay $t_{i,j}$ which is dependent on a) the transmission speed variable, v ; b) the distance between connected cells in terms of cell-breadths, $c_{i,j}$ (for distant connections this is $\rho_i + \rho_j$ —see the section on distant connection implementation above—and for nearest-neighbour connections this is 1); c) the cell size variable, s . These interact in the obvious way: $t_{i,j} = \frac{c_{i,j}s}{v}$. This transmission delay was added to the base synaptic delay of 2 *ms*.

The cell size parameter was set at 50 μm as eukaryote cells generally range between 10 and 100 μm and there is no good reason to assume anything else.

Regarding transmission speeds, for modern-day, human neurons the values vary between .5 *m/s* up to roughly 100 *m/s*. The giant squid axon achieves 27 *m/s*. These higher speeds are clearly the result of secondary adaptations to improve conduction speed: myelin for the human neurons and large diameter for the squid. Slower, unmyelinated human neurons are a good baseline to investigate how slow is too slow. We performed a parameter scan over transmission speeds between .01 and 10 *m/s*, increasing logarithmically. We also included an infinite speed condition, reflecting the parameters used in the main experiment, for ease of comparison. The proportion of cells with elongations was fixed at 50%. We scanned all body sizes and elongation lengths 0, 2, and 4.

Figure 5.9 shows the results of this experiment, arranged to accentuate the effect of transmission speed. Faster but realistic transmission speeds have results similar to having an infinite transmission time. The generally observed patterns break down entirely for the slowest transmission speed (0.01 *m/s*). Within any transmission speed category greater than 0.01 *m/s*, increasing elongation length still improves patternedness in cases where it otherwise would, indicating that given a sufficiently fast yet reasonable transmission speed the effect of elongations holds.

Figure 5.9: Illustration of how variation in transmission speed affects the patternedness measure (y-axis). Transmission speed (x-axis) decreases from left to right. Patternedness remains similar as transmission speed decreases to 1 m/s . Individual graphs represent elongation lengths: the top graph represents nearest-neighbour; the bottom graph a distance of 4 cells. Different body sizes are differentiated by color and point shape. There is no variation since these are single experiments.

Supplementary Material 3: Additional notes on pattern quantification

The constant n , denoting the number of segments into which the modelled body is divided, requires more scrutiny. The analysis yields different outcomes for different values of n . This supplement aims to provide an explanation of why we chose to set the number of segments to 16 instead of iterating over it or choosing another value.

First of all, we use a power of two when setting the length of our model. We force our segments to all have the same length and thus our possible choices for the number of segments are limited to powers of two as well. The shortest length used is 32, that is 2^5 and thus we have 2, 4, 8, 16 and 32 to pick from. The values 2 and 4 are theoretically allowable but do not fit our intuition on wave propagation. The value 32 is also allowed on theoretical grounds, but already nearest neighbor connections alone will spread out of the bin within a single time bin. This leaves us with two *prima facie* suitable candidates: $n = 8$ and $n = 16$, which we evaluated here.

Succinctly put, the qualitative results are similar when using different number of segments. Figure 5.10 shows that there is a difference in actual values but hardly a difference in overall shape. The effect of a lower number of segments is twofold: first, it causes patternedness to be higher for faster ring-shaped patterns. The optimum values will thus be biased towards longer elongation lengths, as those provide faster pattern propagation. Second, since more segments allows finer measurement the higher number of segments is able to show higher values overall. Even neat patterns within a large segment result in some lost signal. This also results in the effect visible in Figure 5.10, where 8 segments (top graph) for small systems (yellow circles) produces an improvement in patternedness between not having elongations at all and having elongations of length 2, since the broader patterns generated by elongations allow the segments to fill out whereas smaller segments get filled out even with patterns 1 cell wide.

Since the aim of our study is to establish qualitative changes in whole-body coordination, having a measure that is constant across multiple body sizes is important, and dividing the body-tube in a fixed number of segments accomplishes this. Whether that is 8 or 16 is of secondary importance for the purposes of this study.

Figure 5.10: Illustration of variation in segment number affecting the patternedness measure. Individual graphs represent segmentation options: the top graph represents 8 segments whereas the bottom graph represents 8 segments. Different body sizes are differentiated by color and point shape. Again, patternedness is shown on the y-axis, the different elongation lengths are on the x-axis. The points scattered around the graph indicate the 10 individual model iterations which were performed for each condition and the line represents the average.

Glossary

Animalia The taxonomic kingdom encompassing all animals.

benthic When referring to animals, indicating that it lives on the sea floor.

Bilateria An animal superphylum containing all animal phyla except Ctenophora, Porifera, Cnidaria, and Placozoa. Consequently, most commonly known animals reside in this group. This clade is treated in detail in Section 2.2.6.

Braitenberg vehicle Braitenberg vehicles (Braitenberg, 1984) are a progression of wheeled vehicles, starting very simple and becoming increasingly complex. They possess sensors, wheels as effectors, and various connections between them.

Cambrian A geological period lasting from the end of the Ediacaran, 541 Ma, to the Ordovician, 485.4 Ma, notable for the Cambrian Explosion. During the Cambrian Explosion, an event at the beginning of the period which lasted a few million years, modern-style animals emerged, leaving a wealth of fossil evidence.

Choanoflagellata A eukaryote sister group to animals, its closest relative. This clade is treated in detail in Section 2.2.1.

cilia Slender protuberances on a cell which can provide locomotion and sense movement.

clade An evolutionary grouping; a branch in the phylogenetic tree.

Cnidaria A basal animal phylum encompassing, among others, jellyfish, sea anemones, corals, and polyps. This clade is treated in detail in Section 2.2.5.

coelom A body cavity; the space inside the animal surrounding gut and organs.

crown group A collection of species consisting of the living representatives of the collection together with their ancestors back to their most recent common ancestor: all extant descendants of a given common ancestor.

Cryogenian A geological period lasting from the end of the Tonian, 720 Ma, to the Ediacaran, 635 Ma, remarkable for and named after its extensive periods of glaciation, the Sturtian and Marinoan glaciations, which were likely world-wide.

Ctenophora A basal animal phylum of animals also known as comb jellies, jellyfish-like organisms. This clade is treated in detail in Section 2.2.4.

Deuterostomia A major bilaterian subgroup featuring Chordata and Echinodermata, discussed in Section 2.2.6.

development When used in the limited biological sense, ‘development’ refers to the progression from zygote (the single-celled stage any unique individual animal goes through) to adult: the assignment of cell fates and the arrangement of tissues and morphology.

Ediacaran A geological period lasting from the end of the Cryogenian, 635 Ma, to the Cambrian, 541 Ma, remarkable for its enigmatic fossil finds, the Ediacaran biota, described in Section 2.4.3.

eukaryote Member of the domain Eukarya, large-celled organisms with a nucleus and various organelles such as mitochondria and Golgi apparatuses, as opposed to bacteria and archaea, which do not have nuclei and organelles.

Eumetazoa A grouping consisting of all animals excluding Porifera, characterized by true tissues and embryonic gastrulation. This term can be misleading as it is not necessarily monophyletic: it is unclear whether Ctenophora are basal to Porifera or not. Section 2.3.5 treats this in more detail.

evo-devo Short for evolutionary developmental biology, which compares the developmental processes of different organisms to infer the ancestral relationships between them and how developmental processes evolved.

excitatory When referring to a synapse, ‘excitatory’ means that the synapse will have an activating effect on the postsynaptic (i.e. target) cell, causing it to be more prone to fire.

gap junctions Molecular machinery which results in direct cytoplasmic connections between cells. This means molecules can diffuse between cells linked with gap junctions, including ions, which causes electrical excitability to transfer between cells as well: an electrical synapse.

germ layer The most fundamental tissue differentiation in animal development, introduced in detail in Section 2.2.2.

homology Homology is the phenomenon where features have the same evolutionary derivation, but not necessarily any functional similarity.

homoplasy Homoplasy is the phenomenon where functionally similar features have different evolutionary derivations.

inhibitory When referring to a synapse, ‘inhibitory’ means that the synapse will have a depressing effect on the postsynaptic (i.e. target) cell, causing it to be less prone to fire.

input-output A view on the origin of nervous systems which is neuron-focused in terms of functional benefit; see Section 1.5.1.

internal coordination A view on the origin of nervous systems which is systemic, considering the whole animal body rather than individual neurons; see Section 1.5.2.

juxtacrine signalling Like paracrine signalling, only in close proximity.

lineage explanation Introduced by Calcott (2009), a lineage explanation is an evolutionarily valid narrative of how some biological feature came to be: an evolutionary progression of stages, limited by specific constraints: the production requirement and the continuity requirement.

macrophagy An animal feeding mode involving engulfing relatively large food particles with a gut and digesting them internally. Examples: vertebrates, Ctenophora.

meiofauna A category of benthic animals defined by small size: < 1 mm but > 5 μ m.

Metazoa The kingdom of Animalia.

microphagy An animal feeding mode that involves filtering relatively small food particles out of the water and digesting them locally, usually with a water canal system as present in Porifera.

molecular clock A technique that uses the mutation rate of genes to deduce when organisms diverged, both in terms of the order in which splits took place and in terms of time.

monophyletic A clade with a single common ancestor.

myoepithelium An epithelium that is both excitable and contractile.

myoid Muscle-like.

Neuralia A term coined by Nielsen (2008), Neuralia is a potentially paraphyletic collection of phyla containing innervated animals: Cnidaria, Ctenophora, and Bilateria.

neuroid A neuron-like structure occurring in an animal, excluding actual neurons.

osmotroph Feeding via particle diffusion by differential osmotic balance, see Section 2.5.2.

Pantin surface The total muscle surface that an animal has available for motility (Keizer et al., 2013). A Pantin surface has a species-specific anatomy and provides a surface across which patterns of contraction-extension correspond with specific forms of animal motility.

phylogenetics Phylogenetics is the study of the branching of the tree of life. When did which clade split from its relatives? Which clades share a common ancestor, and which ones did not? How long ago did the lineages diverge? What does that tell us about their features? Phylogeny uses clues from various other branches of biology (evolutionary developmental biology, molecular biology, genetics, morphology) to answer these questions.

phylogenomics The intersection between evolution and genomics, phylogenomics utilizes the entire genome for phylogenetic ends: analyzing the evolutionary relationships between (groups of) organisms.

phylogeny An evolutionary tree containing the relationships between various (groups of) organisms.

phylum A phylum is a high-level taxonomic clade, an evolutionary grouping of animals which share a common ancestor. The level of phylum sits below ‘kingdom’ (e.g. Animalia). Examples of animal phyla include Panarthropoda (the grouping which includes insects, spiders, crustaceans, and their close relatives), Mollusca (which includes clams, slugs, squid, and octopodes), and our own, the Chordata (encompassing land and sea vertebrates as well as cartilaginous fishes and Tunicata, also known as sea squirts).

Placozoa A basal animal phylum consisting of a single described species, *Trichoplax adhaerens*. This clade is treated in detail in Section 2.2.3.

Porifera A basal animal phylum encompassing all sponges, filter-feeding sessile animals. This clade is treated in detail in Section 2.2.2.

production requirement In the context of a lineage explanation, the production requirement entails that individual stages of a given lineage explanation should be functional: they should provide some benefit to its possessor.

protists Eukaryotes which are not plants, fungi, or animals: for example, slime molds, amoebae, and choanoflagellates.

Protostomia A major bilaterian subgroup featuring Panarthropoda and Mollusca, discussed in Section 2.2.6.

refractory period An action potential results in depolarization, after which ionic concentrations inside and outside the cell need to reset, a process called repolarization. During repolarization, the cell is less susceptible to signals resulting in threshold-crossing. The time during which the cell is less receptive is called the refractory period.

stem group A stem group is a paraphyletic group composed of all descendants of a given common ancestor (a pan-group) minus the crown group (and therefore minus all living members of the pan-group).

Xenacoelomorpha A basal bilaterian clade, discussed in Section 2.2.6.

Bibliography

- Abascal, F. and Zardoya, R. (2013). Evolutionary analyses of gap junction protein families. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1828(1):4–14.
- Abedin, M. and King, N. (2010). Diverse evolutionary paths to cell adhesion. *Trends in cell biology*, 20(12):734–742.
- Achim, K. and Arendt, D. (2014). Structural evolution of cell types by step-wise assembly of cellular modules. *Current Opinion in Genetics & Development*, 27:102–108.
- Alegado, R. A. and King, N. (2014). Bacterial influences on animal origins. *Cold Spring Harbor Perspectives in Biology*, 6(11):a016162.
- Anctil, M. (2009). Chemical transmission in the sea anemone *Nematostella vectensis*: a genomic perspective. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 4(4):268–289.
- Anctil, M. (2015). *Dawn of the Neuron: The Early Struggles to Trace the Origin of Nervous Systems*. McGill-Queen’s University Press, Montreal & Kingston.
- Anderson, P. A. (1989). *Evolution of the first nervous systems*. Springer Science & Business Media.
- Anderson, P. A. V. (1980). Epithelial conduction: its properties and functions. *Progress in Neurobiology*, 15:161–203.
- Arendt, D. (2008). The evolution of cell types in animals: emerging principles from molecular studies. *Nature Reviews Genetics*, 9(11):868–882.
- Arendt, D., Benito-Gutierrez, E., Brunet, T., and Marlow, H. (2015). Gastric pouches and the mucociliary sole: setting the stage for nervous system evolution. *Philosophical Transactions of the Royal Society B*, 370(1684):20150286.
- Arendt, D., Hausen, H., and Purschke, G. (2009). The ‘division of labour’ model of eye evolution. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 364(1531):2809–2817.
- Arendt, D., Tosches, M. A., and Marlow, H. (2016). From nerve net to nerve ring, nerve cord and brain — evolution of the nervous system. *Nature Reviews Neuroscience*, 17(1):61–72.

- Attenborough, R. M. F., Hayward, D. C., Kitahara, M. V., Miller, D. J., and Ball, E. E. (2012). A “neural” enzyme in nonbilaterian animals and algae: preneural origins for peptidylglycine α -amidating monooxygenase. *Molecular Biology and Evolution*, 29(10):3095–3109.
- Attwell, D. and Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow & Metabolism*, 21(10):1133–1145.
- Augustine, G. J. (2001). How does calcium trigger neurotransmitter release? *Current Opinion in Neurobiology*, 11(3):320–326.
- Ball, E. E., Hayward, D. C., Saint, R., and Miller, D. J. (2004). A simple plan—cnidarians and the origins of developmental mechanisms. *Nature Reviews Genetics*, 5(8):567–577.
- Baluška, F. and Levin, M. (2016). On having no head: cognition throughout biological systems. *Frontiers in Psychology*, 7.
- Bargmann, C. I. (2012). Beyond the connectome: how neuromodulators shape neural circuits. *Bioessays*, 34(6):458–465.
- Barzilai, M. G., Reitzel, A. M., Kraus, J. E. M., Gordon, D., Technau, U., Gurevitz, M., and Moran, Y. (2012). Convergent evolution of sodium ion selectivity in metazoan neuronal signaling. *Cell Rep*, 2(2):242–248.
- Bissell, C. (2007). Historical perspectives—the MONIAC a hydromechanical analog computer of the 1950s. *IEEE Control Systems*, 27(1):69–74.
- Bobrovskiy, I., Hope, J. M., Ivantsov, A., Nettersheim, B. J., Hallmann, C., and Brocks, J. J. (2018). Ancient steroids establish the ediacaran fossil *dickinsonia* as one of the earliest animals. *Science*, 361(6408):1246–1249.
- Boccaletti, S., Latora, V., Moreno, Y., Chavez, M., and Hwang, D.-U. (2006). Complex networks: Structure and dynamics. *Physics reports*, 424(4):175–308.
- Bonner, J. T. (2015). *Cellular Slime Molds*. Princeton University Press.
- Bosch, T. C., Klimovich, A., Domazet-Lošo, T., Gründer, S., Holstein, T. W., Jékely, G., Miller, D. J., Murillo-Rincon, A. P., Rentzsch, F., Richards, G. S., et al. (2016). Back to the basics: cnidarians start to fire. *Trends in Neurosciences*, 40(2):92–105.
- Braitenberg, V. (1984). *Vehicles: Experiments in synthetic psychology*. MIT Press, Cambridge, MA.
- Brasier, M. (2009). *Darwin’s lost world: The hidden history of animal life*. Oxford University Press, Oxford.
- Bressloff, P. C. (2014). *Waves in neural media*. Lecture Notes on Mathematical Modelling in the Life Sciences, Springer, New York. Springer.

- Brink, P. R., Cronin, K., and Ramanan, S. V. (1996). Gap junctions in excitable cells. *Journal of Applied Psychology Bioenergetics and Biomembranes*, 28(4):351–358.
- Brooks, J. J. and Butterfield, N. J. (2009). Biogeochemistry: Early animals out in the cold. *Nature*, 457(7230):672.
- Budd, G. (2001). Climbing life’s tree. *Nature*, 412(6846):487–487.
- Budd, G. E. (2006). On the origin and evolution of major morphological characters. *Biological Reviews*, 81(4):609–628.
- Budd, G. E. (2015). Early animal evolution and the origins of nervous systems. *Philosophical Transactions of the Royal Society B*, 370(1684):20150037.
- Budd, G. E. and Jensen, S. (2015). The origin of the animals and a ‘Savannah’ hypothesis for early bilaterian evolution. *Biological Reviews*.
- Burkhardt, P. (2015). The origin and evolution of synaptic proteins—choanoflagellates lead the way. *The Journal of Experimental Biology*, 218(4):506–514.
- Burkhardt, P., Grønborg, M., McDonald, K., Sulur, T., Wang, Q., and King, N. (2014). Evolutionary insights into premetazoan functions of the neuronal protein Homer. *Molecular Biology and Evolution*, 31(9):2342–2355.
- Burkhardt, P. and Sprecher, S. G. (2017). Evolutionary origin of synapses and neurons—bridging the gap. *BioEssays*, 39(10).
- Calcott, B. (2009). Lineage explanations: explaining how biological mechanisms change. *The British Journal for the Philosophy of Science*, 60(1):51–78.
- Carnevale, N. T. and Hines, M. L. (2006). *The NEURON Book*. Cambridge University Press, Cambridge, UK.
- Cavalier-Smith, T. (1987). The origin of fungi and pseudofungi. In *Evolutionary Biology of the Fungi, British Mycological Society Symposium*, volume 12, pages 339–353. Cambridge Univ. Press.
- Cavalier-Smith, T. (2013). Early evolution of eukaryote feeding modes, cell structural diversity, and classification of the protozoan phyla Loukozoa, Sulcozoa, and Choanozoa. *European Journal of Protistology*, 49(2):115–178.
- Cavalier-Smith, T. (2017). Origin of animal multicellularity: precursors, causes, consequences—the choanoflagellate/sponge transition, neurogenesis and the Cambrian explosion. *Philosophical Transactions of the Royal Society B*, 372(1713):20150476.
- Chen, Z., Zhou, C., Yuan, X., and Xiao, S. (2019). Death march of a segmented and trilobate bilaterian elucidates early animal evolution. *Nature*, 573(7774):412–415.
- Clark, R. (1981). Locomotion and the phylogeny of the Metazoa. *Italian Journal of Zoology*, 48(1):11–28.

- Clarkson, E., Levi-Setti, R., and Horváth, G. (2006). The eyes of trilobites: the oldest preserved visual system. *Arthropod Structure & Development*, 35(4):247–259.
- Cloney, R. A. (1982). Ascidian larvae and the events of metamorphosis. *American Zoologist*, 22(4):817–826.
- Copelli, M. and Kinouchi, O. (2005). Intensity coding in two-dimensional excitable neural networks. *Physica A: Statistical Mechanics and its Applications*, 349(3):431–442.
- Cornelisse, L. N., van Elburg, R. A. J., Meredith, R. M., Yuste, R., and Mansvelder, H. D. (2007). High speed two-photon imaging of calcium dynamics in dendritic spines: consequences for spine calcium kinetics and buffer capacity. *PLoS One*, 2(10):e1073.
- Cosson, P. and Soldati, T. (2008). Eat, kill or die: when amoeba meets bacteria. *Current Opinion in Microbiology*, 11(3):271–276.
- Crotty, P., Sangrey, T., and Levy, W. B. (2006). Metabolic energy cost of action potential velocity. *Journal of Neurophysiology*, 96(3):1237–1246.
- Cunningham, J. A., Liu, A. G., Bengtson, S., and Donoghue, P. C. (2017). The origin of animals: Can molecular clocks and the fossil record be reconciled? *Bioessays*, 39(1):1–12.
- Danovaro, R., Dell’Anno, A., Pusceddu, A., Gambi, C., Heiner, I., and Møbjerg Kristensen, R. (2010). The first metazoa living in permanently anoxic conditions. *BMC Biology*, 8(1):30.
- Davydov, V., Morozov, V., and Davydov, N. (2003). Critical properties of autowaves propagating on deformed cylindrical surfaces. *Physics Letters A*, 307(5):265–268.
- De Wiljes, O. O., Van Elburg, R. A. J., Biehl, M., and Keijzer, F. A. (2015). Modeling spontaneous activity across an excitable epithelium: Support for a coordination scenario of early neural evolution. *Frontiers in Computational Neuroscience*, 9.
- De Wiljes, O. O., Van Elburg, R. A. J., and Keijzer, F. A. (2017). Modelling the effects of short and random proto-neural elongations. *Journal of The Royal Society Interface*, 14(135).
- Destexhe, A., Mainen, Z. F., and Sejnowski, T. J. (1994). Synthesis of models for excitable membranes, synaptic transmission and neuromodulation using a common kinetic formalism. *Journal of Computational Neuroscience*, 1:195–230.
- Dohrmann, M. and Wörheide, G. (2017). Dating early animal evolution using phylogenomic data. *Scientific Reports*, 7(1):3599.
- Droser, M. L., Gehling, J. G., and Jensen, S. R. (2006). Assemblage palaeoecology of the Ediacara biota: The unabridged edition? *Palaeogeography, Palaeoclimatology, Palaeoecology*, 232(2):131–147.

- Dunn, C. W., Leys, S. P., and Haddock, S. H. (2015). The hidden biology of sponges and ctenophores. *Trends in Ecology & Evolution*, 30(5):282–291.
- Dunn, F. S., Liu, A. G., and Donoghue, P. C. (2018). Ediacaran developmental biology. *Biological Reviews*, 93(2):914–932.
- Edgecombe, G. D., Ma, X., and Strausfeld, N. J. (2015). Unlocking the early fossil record of the arthropod central nervous system. *Philosophical Transactions of the Royal Society B*, 370(1684):20150038.
- Eitel, M., Francis, W. R., Varoqueaux, F., Daraspe, J., Osigus, H.-J., Krebs, S., Vargas, S., Blum, H., Williams, G. A., Schierwater, B., and Wörheide, G. (2018). Comparative genomics and the nature of placozoan species. *PLOS Biology*, 16(7):1–36.
- Elgeti, J. and Gompfer, G. (2013). Emergence of metachronal waves in cilia arrays. *Proceedings of the National Academy of Sciences*, 110(12):4470–4475.
- Elliott, G. R. and Leys, S. P. (2010). Evidence for glutamate, GABA and NO in coordinating behaviour in the sponge *Ephydatia muelleri* (Demospongiae, Spongillidae). *Journal of Experimental Biology*, 213(13):2310–2321.
- Eme, L., Sharpe, S. C., Brown, M. W., and Roger, A. J. (2014). On the age of eukaryotes: evaluating evidence from fossils and molecular clocks. *Cold Spring Harbor Perspectives in Biology*, 6(8):a016139.
- Emes, R. D. and Grant, S. G. (2012). Evolution of synapse complexity and diversity. *Annual Review of Neuroscience*, 35:111–131.
- Erwin, D. H. (2015). Early metazoan life: divergence, environment and ecology. *Philosophical Transactions of the Royal Society B*, 370(1684):20150036.
- Erwin, D. H., Laflamme, M., Tweedt, S. M., Sperling, E. A., Pisani, D., and Peterson, K. J. (2011). The Cambrian conundrum: early divergence and later ecological success in the early history of animals. *Science*, 334(6059):1091–1097.
- Fedonkin, M. A., Gehling, J., Grey, K., Narbonne, G., and Vickers-Rich, P. (2007a). *The rise of animals: evolution and diversification of the kingdom Animalia*. JHU Press.
- Fedonkin, M. A., Simonetta, A., and Ivantsov, A. Y. (2007b). New data on *Kimberella*, the Vendian mollusc-like organism (White Sea region, Russia): palaeoecological and evolutionary implications. *Geological Society, London, Special Publications*, 286(1):157–179.
- Fedonkin, M. A. and Waggoner, B. M. (1997). The Late Precambrian fossil *Kimberella* is a mollusc-like bilaterian organism. *Nature*, 388(6645):868–871.
- Feinberg, T. E. and Mallatt, J. M. (2016). *The ancient origins of consciousness: how the brain created experience*. MIT Press.

- Feuda, R., Dohrmann, M., Pett, W., Philippe, H., Rota-Stabelli, O., Lartillot, N., Wörheide, G., and Pisani, D. (2017). Improved modeling of compositional heterogeneity supports sponges as sister to all other animals. *Current Biology*, 27(24):3864–3870.
- Finnerty, J. R. and Martindale, M. Q. (1999). Ancient origins of axial patterning genes: Hox genes and ParaHox genes in the Cnidaria. *Evolution & Development*, 1(1):16–23.
- Fortunato, S. A., Adamski, M., Ramos, O. M., Leininger, S., Liu, J., Ferrier, D. E., and Adamska, M. (2014). Calcisponges have a ParaHox gene and dynamic expression of dispersed NK homeobox genes. *Nature*, 514(7524):620.
- Furness, J. B. and Costa, M. (1987). *The enteric nervous system*. Churchill Livingstone.
- Galliot, B. and Quiquand, M. (2011). A two-step process in the emergence of neurogenesis. *European Journal of Neuroscience*, 34(6):847–862.
- Galliot, B., Quiquand, M., Ghila, L., De Rosa, R., Miljkovic-Licina, M., and Chera, S. (2009). Origins of neurogenesis, a cnidarian view. *Developmental Biology*, 332(1):2–24.
- Garm, A., Ekström, P., Boudes, M., and Nilsson, D.-E. (2006). Rhopalia are integrated parts of the central nervous system in box jellyfish. *Cell and Tissue Research*, 325(2):333–343.
- Ginsburg, S. and Jablonka, E. (2019). *The Evolution of the Sensitive Soul: Learning and the Origins of Consciousness*. MIT Press.
- Godfrey-Smith, P. (1998). *Complexity and the Function of Mind in Nature*. Cambridge University Press.
- Godfrey-Smith, P. (2002). *The evolution of intelligence*, chapter Environmental complexity and the evolution of cognition, pages 233–249. Psychology Press, Hove.
- Godfrey-Smith, P. (2016). *Other minds: The octopus, the sea, and the deep origins of consciousness*. Farrar, Straus and Giroux, New York.
- Goodman, D. and Brette, R. (2008). Brian: a simulator for spiking neural networks in python. *Frontiers in Neuroinformatics*, 2.
- Greenspan, R. J. (2007). *An introduction to nervous systems*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Grimmelikhuijzen, C. J., Williamson, M., and Hansen, G. N. (2004). Neuropeptides in cnidarians. In *Cell signalling in prokaryotes and lower Metazoa*, pages 115–139. Springer.
- Grosberg, R. K. and Strathmann, R. R. (2007). The evolution of multicellularity: a minor major transition? *Annu. Rev. Ecol. Evol. Syst.*, 38:621–654.
- Harris, J. J., Jolivet, R., and Attwell, D. (2012). Synaptic energy use and supply. *Neuron*, 75(5):762–777.

- Hejnol, A. (2015). *Acoelomorpha*, chapter 7, pages 56–61. Oxford University Press.
- Hejnol, A. and Lowe, C. J. (2015). Embracing the comparative approach: how robust phylogenies and broader developmental sampling impacts the understanding of nervous system evolution. *Philosophical Transactions of the Royal Society B*, 370(1684):20150045.
- Hejnol, A., Obst, M., Stamatakis, A., Ott, M., Rouse, G. W., Edgecombe, G. D., Martinez, P., Baguñà, J., Bailly, X., Jondelius, U., et al. (2009). Assessing the root of bilaterian animals with scalable phylogenomic methods. *Proceedings of the Royal Society of London B: Biological Sciences*, 276(1677):4261–4270.
- Hildenbrandt, H., Carere, C., and Hemelrijk, C. K. (2010). Self-organized aerial displays of thousands of starlings: a model. *Behavioral Ecology*, 21(6):1349–1359.
- Hodgkin, A. L. and Huxley, A. F. (1952). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *The Journal of Physiology*, 116(4):449.
- Holland, H. D. (2006). The oxygenation of the atmosphere and oceans. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 361(1470):903–915.
- Holland, L. Z. (2015). The origin and evolution of chordate nervous systems. *Philosophical Transactions of the Royal Society B*, 370(1684):20150048.
- Ivantsov, A. Y. (2009). New reconstruction of *kimberella*, problematic Vendian metazoan. *Paleontological Journal*, 43(6):601.
- Jager, M., Chiori, R., Alié, A., Dayraud, C., Quéinnec, E., and Manuel, M. (2011). New insights on ctenophore neural anatomy: immunofluorescence study in *Pleurobrachia pileus* (Müller, 1776). *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 316(3):171–187.
- Jarrell, T. A., Wang, Y., Bloniarz, A. E., Brittin, C. A., Xu, M., Thomson, J. N., Albertson, D. G., Hall, D. H., and Emmons, S. W. (2012). The connectome of a decision-making neural network. *Science*, 337(6093):437–444.
- Jékely, G. (2009). Evolution of phototaxis. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 364(1531):2795–2808.
- Jékely, G. (2011). Origin and early evolution of neural circuits for the control of ciliary locomotion. *Proceedings of the Royal Society B*, 278(1707):914–922.
- Jékely, G. (2013). Global view of the evolution and diversity of metazoan neuropeptide signaling. *Proceedings of the National Academy of Sciences*, 110(21):8702–8707.
- Jékely, G., Colombelli, J., Hausen, H., Guy, K., Stelzer, E., Nédélec, F., and Arendt, D. (2008). Mechanism of phototaxis in marine zooplankton. *Nature*, 456(7220):395–399.

- Jékely, G., Keijzer, F., and Godfrey-Smith, P. (2015a). An option space for early neural evolution. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 370(1684).
- Jékely, G., Paps, J., and Nielsen, C. (2015b). The phylogenetic position of ctenophores and the origin (s) of nervous systems. *EvoDevo*, 6(1):1.
- Jensen, S. (2003). The proterozoic and earliest Cambrian trace fossil record; patterns, problems and perspectives. *Integrative and Comparative Biology*, 43(1):219–228.
- Josephson, R. J. (1985). *Comparative Neurobiology: Modes of communication in the nervous system*, chapter Communication by conducting epithelia., pages 133–148. Wiley (New York).
- Keijzer, F. (2015). Moving and sensing without input and output: early nervous systems and the origins of the animal sensorimotor organization. *Biology & Philosophy*, 30(3):311–331.
- Keijzer, F. and Arnellos, A. (2017). The animal sensorimotor organization: a challenge for the environmental complexity thesis. *Biology & Philosophy*, 32(3):421–441.
- Keijzer, F., Van Duijn, M., and Lyon, P. (2013). What nervous systems do: Early evolution, input-output, and the skin brain thesis. *Adaptive Behavior*, 21(2):67–84.
- Kelava, I., Rentzsch, F., and Technau, U. (2015). Evolution of eumetazoan nervous systems: insights from cnidarians. *Philosophical Transactions of the Royal Society B*, 370(1684):20150065.
- Kenrick, P. and Crane, P. R. (1997). The origin and early evolution of plants on land. *Nature*, 389(6646):33.
- Kier, W. M. (2012). The diversity of hydrostatic skeletons. *Journal of Experimental Biology*, 215(8):1247–1257.
- Kier, W. M. and Smith, K. K. (1985). Tongues, tentacles and trunks: the biomechanics of movement in muscular-hydrostats. *Zoological Journal of the Linnean Society*, 83(4):307–324.
- King, N. and Rokas, A. (2017). Embracing uncertainty in reconstructing early animal evolution. *Current Biology*, 27(19):R1081–R1088.
- King, N., Westbrook, M. J., Young, S. L., Kuo, A., Abedin, M., Chapman, J., Fairclough, S., Hellsten, U., Isogai, Y., Letunic, I., et al. (2008). The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature*, 451(7180):783.
- Klimovich, A. V. and Bosch, T. C. (2018). Rethinking the role of the nervous system: Lessons from the *Hydra* holobiont. *BioEssays*, 40(9):1800060.

- Kloepper, T. H., Kienle, C. N., and Fasshauer, D. (2007). An elaborate classification of snare proteins sheds light on the conservation of the eukaryotic endomembrane system. *Molecular Biology of the Cell*, 18(9):3463–3471.
- Kneer, F., Schöll, E., and Dahlem, M. A. (2014). Nucleation of reaction-diffusion waves on curved surfaces. *New Journal of Physics*, 16(5):053010.
- Knoll, A. H. (2003). *Life on a Young Planet: The First Three Billion Years of Evolution on Earth*. Princeton University Press.
- Knoll, A. H. (2014). Paleobiological perspectives on early eukaryotic evolution. *Cold Spring Harbor Perspectives in Biology*, 6(1):a016121.
- Koizumi, O. (2016). Origin and evolution of the nervous system considered from the diffuse nervous system of cnidarians. In *The Cnidaria, Past, Present and Future*, pages 73–91. Springer.
- Kristan Jr, W. B. (2016). Early evolution of neurons. *Current Biology*, 26(20):R949–R954.
- Kumar, D., Strenkert, D., Patel-King, R. S., Leonard, M. T., Merchant, S. S., Mains, R. E., King, S. M., and Eipper, B. A. (2017). A bioactive peptide amidating enzyme is required for ciliogenesis. *eLife*, 6.
- Laflamme, M., Xiao, S., and Kowalewski, M. (2009). Osmotrophy in modular Ediacara organisms. *Proceedings of the National Academy of Sciences*, 106(34):14438–14443.
- Land, M. F. and Nilsson, D.-E. (2012). *Animal eyes*. Oxford University Press.
- Lanna, E. (2015). Evo-devo of non-bilaterian animals. *Genetics and molecular biology*, 38(3):284–300.
- Larson, S. D., Gleeson, P., and Brown, A. E. (2018). Connectome to behaviour: modelling *C. elegans* at cellular resolution [special issue]. *Philosophical Transactions of the Royal Society B*, 373(1762).
- Laughlin, S. B. (2001). Energy as a constraint on the coding and processing of sensory information. *Current Opinion in Neurobiology*, 11(4):475–480.
- Lennie, P. (2003). The cost of cortical computation. *Current Biology*, 13(6):493–497.
- Lentz, T. L. (1968). *Primitive nervous systems*. Yale University Press, New Haven.
- Leys, S. P. (2015). Elements of a ‘nervous system’ in sponges. *Journal of Experimental Biology*, 218(4):581–591.
- Leys, S. P. and Degnan, B. M. (2001). Cytological basis of photoresponsive behavior in a sponge larva. *The Biological Bulletin*, 201(3):323–338.
- Leys, S. P., Mackie, G. O., and Meech, R. W. (1999). Impulse conduction in a sponge. *Journal of Experimental Biology*, 202:1139–1150.

- Leys, S. P., Nichols, S. A., and Adams, E. D. (2009). Epithelia and integration in sponges. *Integrative and Comparative Biology*, 49(2):167–177.
- Li, J., Inada, S., Dobrzynski, H., Zhang, H., and Boyett, M. (2009). A modified FitzHugh-Nagumo model that allows control of action potential duration and refractory period. In *Computers in Cardiology, 2009*, pages 65–68. IEEE.
- Lichtneckert, R. and Reichert, H. (2007). *Evolution of nervous systems*, chapter Origin and evolution of the first nervous system, pages 289–315. Elsevier, Amsterdam.
- Liebeskind, B. J., Hillis, D. M., and Zakon, H. H. (2011). Evolution of sodium channels predates the origin of nervous systems in animals. *Proceedings of the National Academy of Sciences*, 108(22):9154–9159.
- Liebeskind, B. J., Hillis, D. M., and Zakon, H. H. (2015). Convergence of ion channel genome content in early animal evolution. *Proceedings of the National Academy of Sciences*, page 201501195.
- Linaro, D., Storace, M., and Giugliano, M. (2011). Accurate and fast simulation of channel noise in conductance-based model neurons by diffusion approximation. *PLoS Computational Biology*, 7(3):e1001102.
- Macdonald, F. A., Schmitz, M. D., Crowley, J. L., Roots, C. F., Jones, D. S., Maloof, A. C., Strauss, J. V., Cohen, P. A., Johnston, D. T., and Schrag, D. P. (2010). Calibrating the Cryogenian. *Science*, 327(5970):1241–1243.
- Mackenzie, P. J., Kenner, G. S., Prange, O., and Murphy, T. H. (2000). Vesicle number does not predict postsynaptic measures of miniature synaptic activity frequency in cultured cortical neurons. *Neuroscience*, 98(1):1–7.
- Mackie, G. O. (1965). Conduction in the nerve-free epithelia of siphonophores. *American Zoologist*, 5:439–453.
- Mackie, G. O. (1970). Neuroid conduction and the evolution of conducting tissues. *Quarterly Review of Biology*, pages 319–332.
- Mackie, G. O. (1990). The elementary nervous system revisited. *Integrative and Comparative Biology*, 30(4):907.
- Mackie, G. O. (2004). Epithelial conduction: recent findings, old questions, and where do we go from here? *Coelenterate Biology 2003*, pages 73–80.
- Mackie, G. O. and Passano, L. M. (1968). Epithelial conduction in hydromedusae. *Journal of General Physiology*, 52(4):600.
- MacNaughton, R. B., Narbonne, G. M., and Dalrymple, R. W. (2000). Neoproterozoic slope deposits, Mackenzie Mountains, northwestern Canada: implications for passive-margin development and Ediacaran faunal ecology. *Canadian Journal of Earth Sciences*, 37(7):997–1020.

- Marlow, H. and Arendt, D. (2014). Evolution: Ctenophore genomes and the origin of neurons. *Current Biology*, 24(16):R757–R761.
- Maynard Smith, J. and Szathmáry, E. (1997). *The major transitions in evolution*. Oxford University Press.
- Mentel, M. and Martin, W. (2010). Anaerobic animals from an ancient, anoxic ecological niche. *BMC Biology*, 8(1):32.
- Miller, G. (2009). On the origin of the nervous system. *Science*, 325:24–26.
- Miller, J. P. and Selverston, A. I. (1982). Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. IV. network properties of pyloric system. *Journal of Neurophysiology*, 48(6):1416–1432.
- Monk, T. (2014). *The Evolutionary Origin of Nervous Systems and Implications for Neural Computation*. PhD thesis, University of Otago.
- Monk, T. and Paulin, M. (2014). Predation and the origin of neurones. *Brain, Behavior and Evolution*, 84(4):246–261.
- Monk, T., Paulin, M. G., and Green, P. (2015). Ecological constraints on the origin of neurones. *Journal of Mathematical Biology*, 71(6):1299–1324.
- Moroz, L. L. (2009). On the independent origins of complex brains and neurons. *Brain, Behavior and Evolution*, 74(3):177–190.
- Moroz, L. L. (2014). The genealogy of genealogy of neurons. *Communicative & integrative biology*, 7(6):e993269.
- Moroz, L. L., Kocot, K. M., Citarella, M. R., Dosung, S., Norekian, T. P., Povolotskaya, I. S., Grigorenko, A. P., Dailey, C., Berezikov, E., Buckley, K. M., et al. (2014). The ctenophore genome and the evolutionary origins of neural systems. *Nature*, 510(7503):109–114.
- Moroz, L. L. and Kohn, A. B. (2016). Independent origins of neurons and synapses: insights from ctenophores. *Philosophical Transactions of the Royal Society B*, 371(1685):20150041.
- Naitoh, Y. and Eckert, R. (1969). Ionic mechanisms controlling behavioral responses of *Paramecium* to mechanical stimulation. *Science*, 164(3882):963–965.
- Narbonne, G. M. (2005). The ediacara biota: Neoproterozoic origin of animals and their ecosystems. *Annu. Rev. Earth Planet. Sci.*, 33:421–442.
- Nash, M. P. and Panfilov, A. V. (2004). Electromechanical model of excitable tissue to study reentrant cardiac arrhythmias. *Progress in Biophysics and Molecular Biology*, 85(2-3):501–522.

- Nickel, M., Scheer, C., Hammel, J. U., Herzen, J., and Beckmann, F. (2011). The contractile sponge epithelium sensu lato—body contraction of the demosponge *Tethya wilhelma* is mediated by the pinacoderm. *Journal of Experimental Biology*, 214(10):1692–1698.
- Nielsen, C. (2008). Six major steps in animal evolution: are we derived sponge larvae? *Evolution & Development*, 10(2):241–257.
- Nielsen, C. (2013). Life cycle evolution: was the eumetazoan ancestor a holopelagic, planktotrophic gastraea? *BMC Evolutionary Biology*, 13(1):171.
- Nilsson, D.-E. and Pelger, S. (1994). A pessimistic estimate of the time required for an eye to evolve. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 256(1345):53–58.
- Niven, J. E. and Laughlin, S. B. (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology*, 211(11):1792–1804.
- Osigus, H.-J., Rolfes, S., Herzog, R., Kamm, K., and Schierwater, B. (2019). *Polyplacotoma mediterranea* is a new ramified placozoan species. *Current Biology*, 29(5):R148–R149.
- Ovsepian, S. V. and Vesselkin, N. P. (2014). Wiring prior to firing: the evolutionary rise of electrical and chemical modes of synaptic transmission. *Reviews in the Neurosciences*, 25(6):821–832.
- Panfilov, A. and Hogeweg, P. (1993). Spiral breakup in a modified FitzHugh-Nagumo model. *Physics Letters A*, 176(5):295–299.
- Pantin, C. F. A. (1952). The elementary nervous system. *Proceedings of the Royal Society of London, Series B*, 140(899):147–168.
- Pantin, C. F. A. (1956). The origin of the nervous system. *Pubblicazioni della Stazione Zoologica di Napoli*, 28:171–181.
- Parfrey, L. W. and Lahr, D. J. (2013). Multicellularity arose several times in the evolution of eukaryotes (response to doi 10.1002/bies. 201100187). *Bioessays*, 35(4):339–347.
- Parker, A. (2003). *In the blink of an eye: how vision sparked the big bang of evolution*. Perseus Books, Cambridge, MA.
- Parker, G. H. (1919). *The elementary nervous system*. Lippincott, Philadelphia.
- Passano, L. (1963). Primitive nervous systems. *Proceedings of the National Academy of Sciences*, 50(2):306–313.
- Paterson, J. R., García-Bellido, D. C., Lee, M. S., Brock, G. A., Jago, J. B., and Edgecombe, G. D. (2011). Acute vision in the giant cambrian predator *Anomalocaris* and the origin of compound eyes. *Nature*, 480(7376):237–240.

- Perea-Atienza, E., Gavilán, B., Chiodin, M., Abril, J. F., Hoff, K. J., Poustka, A. J., and Martinez, P. (2015). The nervous system of xenacoelomorpha: a genomic perspective. *Journal of Experimental Biology*, 218(4):618–628.
- Philippe, H., Brinkmann, H., Copley, R. R., Moroz, L. L., Nakano, H., Poustka, A. J., Wallberg, A., Peterson, K. J., and Telford, M. J. (2011a). Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. *Nature*, 470(7333):255–258.
- Philippe, H., Brinkmann, H., Lavrov, D. V., Littlewood, D. T. J., Manuel, M., Wörheide, G., and Baurain, D. (2011b). Resolving difficult phylogenetic questions: why more sequences are not enough. *PLoS Biol*, 9(3):e1000602.
- Planavsky, N. J., Reinhard, C. T., Wang, X., Thomson, D., McGoldrick, P., Rainbird, R. H., Johnson, T., Fischer, W. W., and Lyons, T. W. (2014). Low Mid-Proterozoic atmospheric oxygen levels and the delayed rise of animals. *Science*, 346(6209):635–638.
- Porter, S. (2011). The rise of predators. *Geology*, 39(6):607–608.
- Prinz, A. A., Bucher, D., and Marder, E. (2004). Similar network activity from disparate circuit parameters. *Nature neuroscience*, 7(12):1345–1352.
- Prum, R. O. (1999). Development and evolutionary origin of feathers. *Journal of Experimental Zoology*, 285(4):291–306.
- Purcell, E. M. (1977). Life at low Reynolds number. *American Journal of Physics*, 45(1):3–11.
- Ramos, O. M., Barker, D., and Ferrier, D. E. (2012). Ghost loci imply Hox and ParaHox existence in the last common ancestor of animals. *Current Biology*, 22(20):1951–1956.
- Ryan, J. F. and Baxevanis, A. D. (2007). Hox, Wnt, and the evolution of the primary body axis: insights from the early-divergent phyla. *Biology Direct*, 2(1):37.
- Ryan, J. F. and Chiodin, M. (2015). Where is my mind? how sponges and placozoans may have lost neural cell types. *Philosophical Transactions of the Royal Society B*, 370(1684):20150059.
- Ryan, J. F., Pang, K., Mullikin, J. C., Martindale, M. Q., and Baxevanis, A. D. (2010). The homeodomain complement of the ctenophore *Mnemiopsis leidyi* suggests that Ctenophora and Porifera diverged prior to the ParaHoxozoa. *EvoDevo*, 1(1):9.
- Ryan, T. J. and Grant, S. G. N. (2009). The origin and evolution of synapses. *Nature Reviews Neuroscience*, 10(10):701–712.
- Sakarya, O., Armstrong, K. A., Adamska, M., Adamski, M., Wang, I.-F., Tidor, B., Degnan, B. M., Oakley, T. H., and Kosik, K. S. (2007). A post-synaptic scaffold at the origin of the animal kingdom. *PloS one*, 2(6):e506.

- Satterlie, R. A. (2011). Do jellyfish have central nervous systems? *Journal of Experimental Biology*, 214(Pt 8):1215–1223.
- Schierwater, B. (2005). My favorite animal, *Trichoplax adhaerens*. *Bioessays*, 27(12):1294–1302.
- Sebé-Pedrós, A., Burkhardt, P., Sánchez-Pons, N., Fairclough, S. R., Lang, B. F., King, N., and Ruiz-Trillo, I. (2013). Insights into the origin of metazoan filopodia and microvilli. *Molecular Biology and Evolution*, 30(9):2013–2023.
- Seilacher, A. (1999). Biomat-related lifestyles in the Precambrian. *Palaios*, 14(1):86–93.
- Seilacher, A., Grazhdankin, D., and Legouta, A. (2003). Ediacaran biota: The dawn of animal life in the shadow of giant protists. *Paleontological Research*, 7(1):43–54.
- Seipel, K. and Schmid, V. (2005). Evolution of striated muscle: jellyfish and the origin of triploblasty. *Developmental Biology*, 282(1):14–26.
- Sengupta, B., Stemmler, M., Laughlin, S. B., and Niven, J. E. (2010). Action potential energy efficiency varies among neuron types in vertebrates and invertebrates. *PLoS Computational Biology*, 6(7):e1000840.
- Sherrington, C. S. (1906). *The Integrative Action of the Nervous System*. Scribner’s Sons, New York.
- Smith, C. L., Varoqueaux, F., Kittelmann, M., Azzam, R. N., Cooper, B., Winters, C. A., Eitel, M., Fasshauer, D., and Reese, T. S. (2014). Novel cell types, neurosecretory cells, and body plan of the early-diverging metazoan *Trichoplax adhaerens*. *Current Biology*, 24(14):1565–1572.
- Sokoloff, L. (1960). The metabolism of the central nervous system in vivo. *Handbook of Physiology, Section I, Neurophysiology*, 3:1843–1864.
- Sperling, E., Frieder, C., Raman, A., Girguis, P., Levin, L., and Knoll, A. (2014). Oxygen, ecology, and the Cambrian radiation of animals. In *Integrative and Comparative Biology*, volume 54, pages E198–E198. Oxford University Press, Cary, NC, USA.
- Sperling, E., Robinson, J., Pisani, D., and Peterson, K. (2010). Where’s the glass? biomarkers, molecular clocks, and microRNAs suggest a 200-Myr missing Precambrian fossil record of siliceous sponge spicules. *Geobiology*, 8(1):24–36.
- Sperling, E. A. (2013). Tackling the 99%: Can we begin to understand the paleoecology of the small and soft-bodied animal majority? *The Paleontological Society Papers*, 19:77–86.
- Sperling, E. A. and Stockey, R. G. (2018). The temporal and enviromental context of early animal evolution: Considering all the ingredients of an “explosion”. *Integrative and Comparative Biology*, 58(4):605–622.

- Sperling, E. A. and Vinther, J. (2010). A placozoan affinity for *Dickinsonia* and the evolution of late proterozoic metazoan feeding modes. *Evolution & Development*, 12(2):201–209.
- Srivastava, M., Begovic, E., Chapman, J., Putnam, N. H., Hellsten, U., Kawashima, T., Kuo, A., Mitros, T., Salamov, A., Carpenter, M. L., et al. (2008). The *Trichoplax* genome and the nature of placozoans. *Nature*, 454(7207):955–960.
- Srivastava, M., Simakov, O., Chapman, J., Fahey, B., Gauthier, M. E., Mitros, T., Richards, G. S., Conaco, C., Dacre, M., Hellsten, U., et al. (2010). The *Amphimedon queenslandica* genome and the evolution of animal complexity. *Nature*, 466(7307):720.
- Stein, P. S., Grillner, S., and Selverston, A. I. (1999). *Neurons, networks, and motor behavior*. MIT press.
- Steinmetz, P. R., Kraus, J. E., Larroux, C., Hammel, J. U., Amon-Hassenzahl, A., Houliston, E., Wörheide, G., Nickel, M., Degnan, B. M., and Technau, U. (2012). Independent evolution of striated muscles in cnidarians and bilaterians. *Nature*, 487(7406):231–234.
- Strausfeld, N. J. and Hirth, F. (2015). Introduction to ‘origin and evolution of the nervous system’. *Philosophical Transactions of the Royal Society B*, 370.
- Telford, M. J., Budd, G. E., and Philippe, H. (2015). Phylogenomic insights into animal evolution. *Current Biology*, 25(19):R876–R887.
- Ten Tusscher, K. H. W. J., Hren, R., and Panfilov, A. V. (2007). Organization of ventricular fibrillation in the human heart. *Circulation Research*, 100(12):e87.
- Ten Tusscher, K. H. W. J. and Panfilov, A. V. (2006). Alternans and spiral breakup in a human ventricular tissue model. *American Journal of Physiology*, 291(3):H1088.
- Thornhill, R. H. and Ussery, D. W. (2000). A classification of possible routes of Darwinian evolution. *Journal of Theoretical Biology*, 203(2):111–116.
- Tosches, M. A. and Arendt, D. (2013). The bilaterian forebrain: an evolutionary chimaera. *Current Opinion in Neurobiology*, 23(6):1080–1089.
- Trakhtenberg, E. F. and Goldberg, J. L. (2011). Neuroimmune communication. *Science*, 334(6052):47–48.
- Trestman, M. (2013). The Cambrian explosion and the origins of embodied cognition. *Biological Theory*, 8(1):80–92.
- Tyler, S. and Rieger, R. (1999). Functional morphology of musculature in the acoelomate worm, *Convoluta pulchra* (Plathelminthes). *Zoomorphology*, 119(3):127–142.
- Valentine, J. W. (1992). *Dickinsonia* as a polypoid organism. *Paleobiology*, 18(04):378–382.

- Valentine, J. W. (2002). Prelude to the cambrian explosion. *Annual Review of Earth and Planetary Sciences*, 30(1):285–306.
- Valentine, J. W. (2004). *On the origin of phyla*. University of Chicago Press.
- van Elburg, R. A. J. and van Ooyen, A. (2010). Impact of dendritic size and dendritic topology on burst firing in pyramidal cells. *PLoS Computational Biology*, 6(5):e1000781.
- Varoqueaux, F., Williams, E. A., Grandemange, S., Truscello, L., Kamm, K., Schierwater, B., Jekely, G., and Fasshauer, D. (2018). High cell diversity and complex peptidergic signaling underlie placozoan behavior. *Current Biology*, 28(21):3495–3501.
- Watanabe, H., Schmidt, H. A., Kuhn, A., Höger, S. K., Kocagöz, Y., Laumann-Lipp, N., Özbek, S., and Holstein, T. W. (2014). Nodal signalling determines biradial asymmetry in *Hydra*. *Nature*, 515(7525):112–115.
- Wei, Z., Angerer, R. C., and Angerer, L. M. (2011). Direct development of neurons within foregut endoderm of sea urchin embryos. *Proceedings of the National Academy of Sciences*, 108(22):9143–9147.
- Wray, G. A. (2015). Molecular clocks and the early evolution of metazoan nervous systems. *Philosophical Transactions of the Royal Society B*, 370(1684):20150046.
- Xiao, S. and Laflamme, M. (2009). On the eve of animal radiation: phylogeny, ecology and evolution of the Ediacara biota. *Trends in Ecology & Evolution*, 24(1):31–40.
- Xiao, S., Yuan, X., and Knoll, A. H. (2000). Eumetazoan fossils in terminal proterozoic phosphorites? *Proceedings of the National Academy of Sciences*, 97(25):13684–13689.
- Zimmermann, B. and Walz, B. (1997). Serotonin-induced intercellular calcium waves in salivary glands of the blowfly *Calliphora erythrocephala*. *The Journal of Physiology*, 500(1):17–28.

Acknowledgements

First and foremost I owe gratitude to my supervisor, promotor, coauthor, and master thesis advisor dr. Fred Keijzer. The contents of this thesis can rightly be called a labour of love for the subject which Fred and I share and the majority of input has been Fred's. Our shared enthusiasm for the subject has resulted in many fruitful and thoroughly engaging discussions which have caused me great pleasure and fulfilment. I am thankful for his support, for letting me do things at my own pace and in my own way while supporting the process of producing both this thesis and the peer-reviewed publications which constitute Chapters 3 and 4. I am fortunate to count quite a few PhDs among my acquaintances and I have often heard them lamenting their relationships with their supervisors to various degrees. Without fail, any such remark caused me to be extremely grateful about the smooth and pleasant yet productive cooperation I have experienced with Fred over the course of this PhD.

I have not seen my copromotor, coauthor, and master thesis advisor Ronald van Elburg as much as I would have liked the last few years but his input has been of critical importance in producing Chapters 3, 4, and 5. I owe him particular gratitude for teaching me the ropes regarding the computational practicalities of nervous system modelling, useful principles in software development, and more than a few practical tips which I use on a daily basis. The technical side of the work presented in this thesis is quite different beast from the scientific and narrative side and it has been a great pleasure to me to discuss these matters at speed and in depth during the too sparse meetings we did have. It was interesting to experience Fred zoning out entirely during some my discussions with Ronald, a clear indication that this work really rests on the pillars of expertise provided by Fred and Ronald both.

My promotor prof. dr. Jan-Willem Romeijn has not been particularly involved in the content of this thesis yet I am particularly grateful for his unwavering support in the process, making it possible for Fred, Ronald, and myself to focus on the science and the details, with the result you find before you. I hope it speaks for itself.

Due to the specialized and somewhat left-field contents (for a philosophy department) of this thesis it has been a rather isolated experience, so I have had relatively little in the way of contact with faculty and fellow PhDs at the Philosophy department. Nevertheless, I would like to thank them for putting up with my mostly absenteeist behaviour and being good sports about my presentations of this work.

On the less professional front I would like to thank my boardgaming buddies for keeping me sane, particularly Boris Schuckink Kool for providing shelter along with the

boardgames and Xeryus Stokkel for agreeing to paranimf. I would also like to thank all of Botter for the *gezelligheid* in Groningen.

Retroactive thanks to Caroline and Barbara for showing me how it should be done and apologetic thanks to Doortje and Emma who I feel I have neglected somewhat through this process and thanks to my parents for getting me to this place.

Finally, thanks and love to Lisa, detailed expressions of which do not belong in public.

Curriculum Vitæ

Oltman Ottes de Wiljes (Ot)

Education

2014–2020 PhD, Behavioural and Cognitive Neurosciences and Graduate School of Philosophy Rijksuniversiteit Groningen. Defense April 6th, 2020.

2008–2010 Research Master Behavioural and Cognitive Neurosciences, C-Track: computational modeling of cognition Rijksuniversiteit Groningen. Attained MSc, cum laude, August 2010

2002–2005 Bachelor Biology Universiteit Leiden. BSc attained December 2005.

Bachelor project: Bio-informatics; Developing a GUI in APL.

1994–2000 VWO, Gymnasium Gemeentelijk Gymnasium Hilversum.

Work experience

2019–present Senior Quantitative Analyst, ABN AMRO N.V. (freelance)
Data modelling, technical data delivery, and technical consulting.

2017–present Founder, modelling expert, Scry.nl
Developing time series predictions applied to European energy markets.

2015–2017 (intermittently) BI Consultant, KPN (freelance)
Designing and creating data visualizations.

2011–2014 Data Scientist, Schuberg Philis B.V.
Computational data analysis, data visualization, and reporting.

2011 Consultant in Data Management, Capgemini Nederland B.V.

2006–2008 Consulting Business Intelligence Engineer, Sogeti Nederland B.V.
Reporting and ETL.

Publications

De Wiljes, O. O., Van Elburg, R.A.J., and Keijzer, F.A. (2017). “Modelling the effects of short and random proto-neural elongations”, *Journal of the Royal Society Interface* 15, 135.

De Wiljes, O. O., Van Elburg, R.A.J., Biehl, M., and Keijzer, F.A. (2015) “Modeling spontaneous activity across an excitable epithelium: support for a coordination scenario of early neural evolution”, *Frontiers in Computational Neuroscience* 9, 110.

De Wiljes, O. O., Van Elburg, R.A.J., Biehl, M., and Keijzer, F.A. (2010). “Early Nervous Systems: Theoretical background and a preliminary model of neuronal processes”, *ALife XII*

Notable Talks

De Wiljes, O. O., Van Elburg, R.A.J., and Keijzer, F.A. (2018). “Deciphering the evolutionary route to the first neurons”, *27th Annual Computational Neuroscience Meeting (CNS*2018)*