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Hippocampal Microcircuits

A Computational Modeler's Resource Book

Second Edition

Springer

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Introduction

The hippocampus is amongst the most widely studied of mammalian brain regions hypothesized to play a role in the short-term storage of declarative memories. Recent years have witnessed a dramatic accumulation of knowledge about the morphological, physiological and molecular characteristics as well as the connectivity and synaptic properties of the various cell types found in the hippocampus. The microcircuits these cells form exhibit different rhythmic states such as theta (4–7 Hz) and gamma (30–100 Hz) oscillations in different behavioural conditions. This dynamic complexity presumably corresponds to specific functional processing of information. Much work has been devoted to trying to understand the cellular and network properties that generate these and other complex network patterns, but much is still to be done to decipher the function of the detailed microcircuits.

Microcircuits can be thought as functional modules that act as elementary processing units bridging the gap between single-cell activity, network activity and global brain function. Microcircuits can be found in many parts of mammalian nervous systems consisting of a complex architecture involving many different neuronal types connected in feedforward and feedback loops. Synaptic connections may be excitatory or inhibitory and target specific spatial compartments of a neuron. In addition to synaptic input, a neuron and the microcircuit it is a part of are subject to diffuse neuromodulatory signals. Neural synaptic transmission and neuromodulation combine to provide a complex dynamics of neural activity and presumed information processing in a neuronal microcircuit.

This book is the second edition of the 2010 *Hippocampal Microcircuits* and provides an updated snapshot and resumé of the current state of the art of the ongoing research avenues concerning the hippocampal microcircuits. The central aim of the volume is to provide a methodology to anyone interested in developing microcircuit-level models of the hippocampus. The book is divided into two thematic areas: (1) experimental background and (2) computational analysis. In the first thematic area, leading experimental neuroscientists discuss the morphological, physiological and molecular characteristics as well as the connectivity and synaptic

properties of the various cell types found in the hippocampus. Behaviour-related ensemble activity patterns of morphologically identified neurons in anaesthetized and freely moving animals provide insights on the function of the hippocampal areas. In the second thematic area, computational neuroscientists present models of hippocampal microcircuits at various levels of detail (e.g. single-cell level, network level). These models make use of the knowledge presented in the first thematic area to discuss the overall global function of hippocampal microcircuits (in areas CA1, CA3, dentate gyrus and entorhinal cortex). Synaptomics and connectomics models of hippocampal structures are initially discussed. Then, network models of memory, rhythm generation and spatial navigation are presented, followed by abstract and biophysical models of synaptic plasticity. Network models of hippocampal implicated disorders (epilepsy and schizophrenia) are then detailed and how their network topologies, connectivities and activities change in these diseases. Finally, two chapters are dedicated to describing simulator environments of single neurons and networks currently used by computational neuroscientists in developing their models and modelling tools to parametrically constrain them.

This engaging volume is invaluable to experimental and computational neuroscientists, electrical engineers, physicists, mathematicians and others interested in developing microcircuit models of the hippocampus. Graduate-level students and trainees in all of these fields will find this book a significant source of information. The following unique features make this volume distinct:

- It provides concise snapshots of experimental evidence rather than lengthy and detailed descriptions of the morphological, physiological and molecular characteristics as well as the connectivity and synaptic properties of the various cell types found in the hippocampus are presented. This evidence is often provided in either tabular and/or pictorial form.
- In contrast to previous editorial attempts in which main target audience was either the *entire hippocampus neuroscience disciplines*, this volume is targeting experimental and computational neuroscientists interested in *developing microcircuit models of the hippocampus*.
- Aside from presenting up-to-date experimental evidence on the hippocampal microcircuits, this second edition also suggests a didactic methodology approach of modelling microcircuits necessary to all computational neuroscientists interested in bridging the gap between the single-cell level, the network level and the behavioural level.

- All chapters not only discuss the current state of the art of experimental and computational research avenues regarding the hippocampal microcircuits but also provide a section on outstanding questions and areas in need of further clarification that will guide future research to be carried out by young and/or senior computational neuroscientists.

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Part I
Experimental Background

Stuart Cobb and Imre Vida

The hippocampus is one of the most intensely studied structures in the brain. It has been investigated at many different levels in an attempt to understand the neurobiology of cognitive functions, including learning and spatial coding. The accumulated knowledge of hippocampal anatomy, physiology, and function provides a rich repository of information that presents enormous opportunity to model different aspects of neuronal signaling and information processing within this structure. As a primary focus in neurobiology over many decades, studies of the hippocampus have also helped reveal elementary properties of neurons, their synapses, and the microcircuits they are embedded.

There are several reasons why the study of the hippocampus has been at the forefront of neurobiology research. These include the involvement of this brain structure in memory processes, spatial navigation, as well as major disease states. Another reason is the ability to readily recognize the hippocampus as well as target it in vivo and isolate it for in vitro investigations. Finally, a major impetus for focusing basic studies of the nervous system on the hippocampus owes to its apparently simple cytoarchitecture and circuitry and thus its tractability as a cortical "model" system.

The hippocampal formation is a highly organized structure and has a striking appearance at the gross anatomical level. The complexity of the system can appear overwhelming at first. Nevertheless, there continues an evolution in our understanding of the constituent cells, their connectivity, their neurochemical and biophysical properties, and the emergent properties of these in terms of hippocampal-dependent behavior. However, many of the details remain to be established, and indeed significant gaps persist in our understanding of some key concepts.

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In this section, experimental neuroscientists discuss the salient structural and functional properties of the hippocampus. This includes morphological, physiological, and molecular characteristics as well as the connectivity and synaptic properties of the various cell types found in the hippocampus. We provide concise overviews of each aspect of hippocampal structure and function and, where possible, provide quantitative descriptions of the experimental findings. While we believe this will be a valuable summary for all readers interested in the biology of the hippocampus, by conveying often quantitative experimental data from different levels of complexity into a coherent picture, we hope this section will provide a valuable resource for researchers embarking on modeling different aspects of this system. In this second edition of *Hippocampal Microcircuits: A computational Modeler's Resource Book*, we have updated each chapter to reflect the most recent advances in understanding hippocampal cells and circuits.

In the first chapter "Connectivity of the Hippocampus," Menno Witter provides a comprehensive description of the major connectivity of the hippocampal formation. In this, he goes beyond the simplified classical models of anatomical organization to produce an updated and extended connectional scheme that incorporates important new as well as some older but hitherto overlooked details. In the chapter "Morphology of Hippocampal Neurons," Imre Vida and colleagues extend this overview to the microcircuit and single-cell levels. The chapter provides detailed quantitative descriptions of the morphology, major molecular markers, as well as connectivity of hippocampal neuron types. While the most detailed quantitative information is available for principal cells, this chapter provides a comprehensive summary of the major anatomically defined classes of interneurons, a rapidly developing area of hippocampal biology. In the chapter "Physiological Properties of Hippocampal Neurons," Marco Martina and Cheng-Chang Lien provide a detailed overview of the physiological properties of the different classes of hippocampal neurons from a single-cell biophysics perspective. Where possible, they provide detailed quantitative descriptions of the passive and active properties together with a discussion of the significance of these in shaping the electrical behavior of respective cell types. In the chapter "Glutamatergic Neurotransmission in the Hippocampus," Katalin Tóth moves from individual cells to consider excitatory synaptic communication between neurons in the hippocampus. In this, she provides a detailed yet accessible overview of glutamatergic transmission at different synapses in the hippocampus including key qualitative and quantitative differences in the physiology, biophysics, and pharmacology at different synapses and pathways. In the chapter "Fast and Slow GABAergic Transmission in Hippocampal Circuits," Marlene Bartos and colleagues provide an overview of GABAergic transmissions in hippocampal circuits. In this, they introduce a variety of different forms of GABAergic inhibition and discuss functional differences between ionotropic and metabotropic forms of GABAergic inhibition at different inhibitory synapses. In the chapter "Synaptic Plasticity at Hippocampal Synapses – Experimental Background," Jack Mellor reviews the divergent forms of synaptic plasticity that are characteristic of different hippocampal synapses. Ranging from short-term frequency facilitation to more enduring forms of synaptic plasticity, he provides a succinct summary of the experimental background

and highlights key literature in this area, as well as quantitative descriptions of plasticity at some major synapses. In the chapter "Neuromodulation of Hippocampal Cells and Circuits," Stuart Cobb and Josh Lawrence introduce the concept of neuromodulation and the many ways by which hippocampal cells and circuits can be regulated. Thereafter, they provide a detailed yet condensed summary of the main neuromodulator systems ranging from classical modulators (monoamines and acetylcholine) to neuropeptide modulators and paracrine/autocrine substances. In the chapter "Neuronal Activity Patterns During Hippocampal Network Oscillations In Vitro," Tengis Gloveli and colleagues describe the importance and relevance of neuronal activity patterns during hippocampal network oscillations in vitro. He provides a detailed account of the emergent electrical behavior of hippocampal networks including the importance of intrinsic cellular and synaptic properties in their genesis and modulation. In the chapter "Recording Identified Neurons in Awake and Anesthetized Rodents," John Tukker extends the concept of patterned neuronal activities by describing physiological patterns of neuronal activity that occur in vivo under anesthetic and conscious conditions. Under these circumstances, it is possible to observe highly stereotyped patterns of behavior within different morphologically identified principal and interneuronal cell types when viewed with respect to ongoing EEG states. This precise sculpting of neuronal activity in the temporal domain provides important insights into the spatial and temporal processing of synaptic signals during hippocampal activity in the intact network. In the final experimental chapter "Spatial and Behavioral Correlates of Hippocampal Neuronal Activity: A Primer for Computational Analysis," the late Howard Eichenbaum described spatial and behavioral correlates of hippocampal neuronal activity. By providing a succinct overview of the literature, this chapter offers a framework for considering the relationship between behavior, the activity of hippocampal neurons, and how these might be modeled.

Connectivity of the Hippocampus

Menno P. Witter

Abstract The aim of this chapter is to extend the standard simplified diagram of the connectional organization of the hippocampus found in many current textbooks, by adding details on the connectivity of area CA2 and on entorhinal intrinsic wiring. In the chapter, some of the 'traditional wisdoms' on hippocampal connectivity are discussed, emphasizing the need for a more inclusive framework to model the hippocampus. The chapter focusses on intrinsic connections, and many of the well-known extrinsic connections of the hippocampus will not be covered in this chapter, for two reasons. First, the information is already available at a summarized (meta) level, and a new summary would not assist those who need anatomical details to contribute to the explanation of the functional outcome of a study. Second, this chapter is meant to provide a framework of knowledge to support computational modelling of the region, and therefore only the most relevant and quantitative data on the connectivity of the hippocampus are covered.

Overview

In the first edition of this book, I pointed to the increasingly complex intrinsic wiring diagram of the hippocampus and that new data are being added at an increasing speed. With the emergence of cell-specific viral tracing techniques, the potential for a data explosion has become eminent, going hand in hand with an increase of the potential for false-positive or incomplete data. The relevance of interneurons in the local network, as well as the fact that interneurons contribute to long-range projections, has been integrated into current conceptualizations of the 'Connectivity of the Hippocampus'. Several comprehensive reviews have been published to which the reader is referred for many of the connections not covered in this chapter or for

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more details on the connections described here. An excellent, much more detailed resource can be found in a recent book chapter (Cappaert et al. 2015). Several online databases contribute to making this wealth of connectional data accessible as well (see further reading).

In contrast to this ever-expanding connectional knowledge base, many functional papers and reviews still use a simplified diagram of the connectional organization of the hippocampus as their reference, which we will here refer to as the standard view. The aim of this chapter is to extend this standard view, adding details that have been known for some time or have recently been provided, but apparently have not yet been incorporated in the commonly accepted connectional scheme for the region. For example, the increased insights on the connectivity of area CA2 are added in this second edition, as well as many new details on entorhinal intrinsic wiring.

I further aim to reinterpret some of the 'traditional wisdoms' on hippocampal connectivity, potentially pointing to the need of a changed functional framework for the hippocampus. The use will be made of a standardized scheme of connections which hopefully will facilitate easy dissemination of these adapted connectional concepts for the region. Many of the very well-known connections, such as all extrinsic connections of the HF and EC, will not be covered in this chapter, for two reasons. First, the information is already available at a summarized (meta) level, and a new summary would not assist those who need anatomical details to contribute to the explanation of the functional outcome of a study. Second, this chapter is meant to provide a framework of knowledge to support computational modelling of the region, and therefore I have selected what I consider the most relevant new data on the connectivity of the hippocampus, not of the brain.

Microscopical Anatomy and Nomenclature

Throughout the chapter, reference will be made to the hippocampal formation (HF) and the entorhinal cortex (EC) as the two main areas of interest. The HF in turn comprises three distinct subregions (Fig. 1): the dentate gyrus (DG), the hippocampus proper (consisting of CA3, CA2 and CA1) and the subiculum (Sub). The HF is a three-layered cortex that is easily differentiated from the EC, since the latter has more than three layers (see below). The deepest layer of the HF houses basal dendrites of principal cells and a mixture of afferent and efferent fibres and local circuitry – interneurons. Superficial to this polymorph layer is the cell layer, which is composed of principal cells and interneurons. On top, the most superficial layer or molecular layer contains the apical dendrites of the neurons and the large majority of axons that provide inputs. In the dentate gyrus, these layers are, respectively, referred to as the hilus, granular (cell) layer and molecular layer (*stratum moleculare*). In the CA-region, we find the deep polymorph layer (*stratum oriens*), followed by the pyramidal layer (*stratum pyramidale*), topped by the superficial or molecular layer. The latter is subdivided into a number of sub-layers. In CA3, three sub-layers are distinguished: *stratum lucidum*, representing

the mossy fibre input from DG; *stratum radiatum*, i.e. the apical dendrites of the neurons in stratum pyramidale; and, most superficially, the *stratum lacunosum-moleculare* comprising the apical tufts of the apical dendrites. The lamination in CA2 and CA1 is similar to that in CA3, with the exception that the *stratum lucidum* is missing in CA1 and absent or much less evident in CA2. In Sub, the superficial layer is generally referred to as molecular layer, sometimes divided into an outer and inner portion, and the remaining two layers are referred to as the pyramidal (cell) layer (*stratum pyramidale*) and *stratum oriens*. The latter is very thin and quite often not specifically differentiated from the underlying white matter of the brain. The EC, commonly subdivided into a medial (MEC) and a lateral (LEC) part,[1] is generally described as having six layers, a molecular layer (layer I), the superficial cell layer (layer II), the superficial pyramidal cell layer (layer III), a cell-sparse lamina dissecans (layer IV), the deep pyramidal cell layer (layer V) and a polymorph cell layer (layer VI).[2]

In order to understand the anatomical organization, it is relevant to describe the coordinate systems that define position within the HF and PHR (Fig. 1). For the HF, there are three relevant axes: the long axis, the transverse or proximodistal axis, which runs in parallel to the cell layer, starting at the DG; and the radial or superficial-to-deep axis, which is defined perpendicular to the transverse axis. In the EC, a similar superficial-to-deep axis is used in addition to mediolateral (proximodistal) and anteroposterior (rostrocaudal) axes.

[1]The lateral and medial entorhinal cortex or Brodmann's areas 28a and 28b, respectively, have been further subdivided by a large number of authors (for a more detailed description and comparison of different nomenclatures used in the rat and in other species, the reader is referred to a number of reviews (cf. Witter et al., 1989)). In the rat, and likewise in the mouse, a further division into dorsolateral (DLE), dorsal-intermediate (DIE), ventral-intermediate (VIE), caudal (CE) and medial (ME) subdivisions have been proposed (Insausti et al., 1997, Hippocampus 7:146; van Groen et al., 2003, Hippocampus 13: 133–149). In monkeys, humans and in other species in which the entorhinal cortex was described, such as cat, dog, guinea pig and bat (Amaral et al., 1987 J Comp Neurol 264: 326–355; Witter et al., 1989, Progr Neurobiol 33:161–254; Buhl and Dann 1991, Hippocampus 1: 131–152; Insausti et al., 1995, J Comp Neurol 355: 171–198; Uva et al., 2004 J Comp Neurol 474: 289–303; Woznicka et al. 2006, Brain Res Rev. 52: 346–367), comparable partitioning schemes have been proposed. However, in case of most species, there is a tendency to consider the entorhinal cortex as composed of two primary components, the lateral and medial entorhinal cortex, most likely reflecting functional differences (see further Witter et al. 2017a, Front Syst Neurosci 11:46).

[2]Note that some authors have adopted a slightly different nomenclature in which the lamina dissecans is either without number or considered to be the deep part of layer III (layer IIIb), such that layer IV is used to designate the superficial part of layer V, characterized by the presence of rather large pyramidal cells that stain strongly for Nissl substance.

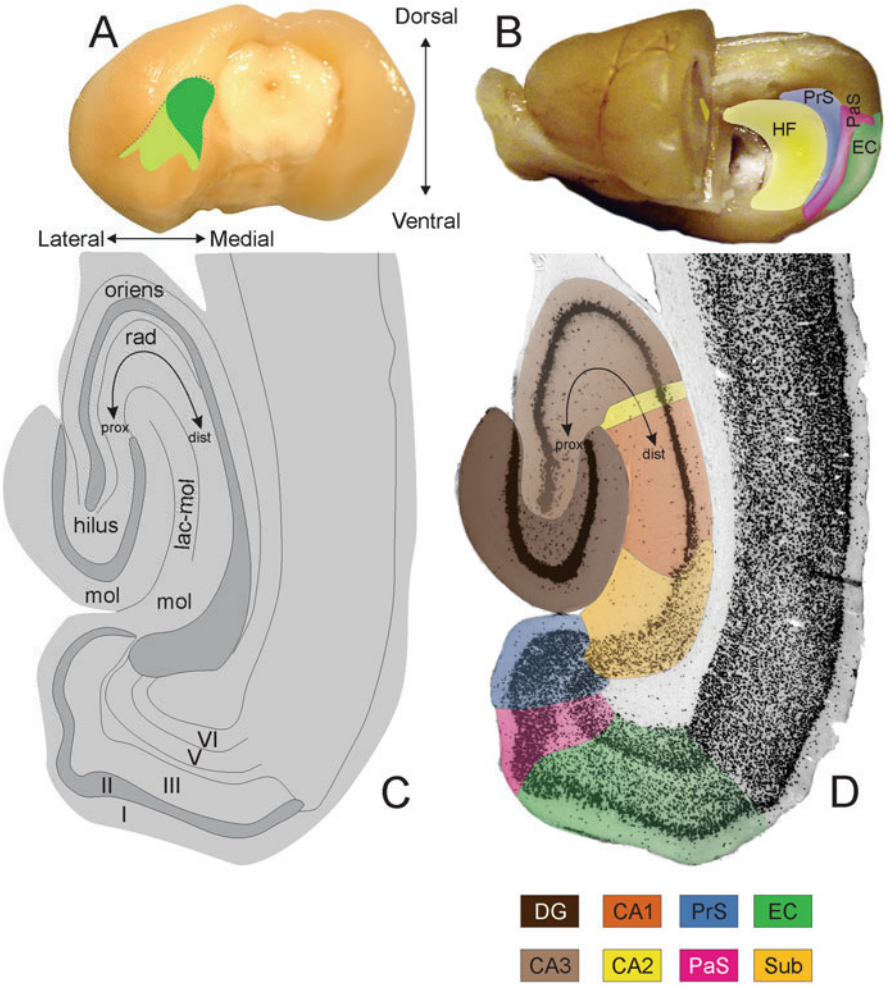

Fig. 1 Schematic representation of the position of the HF and the EC and main topological axes. (**a**) Posterior view of the rat brain showing the position of the LEC (light green) and MEC (dark green; modified with permission from Fyhn et al., 2004, Science 305: 1258–1264. (**b**) Lateral view of a partially dissected brain showing the shape and position of the HF and the longitudinal or dorsoventral axis, as well as the position and extent of the pre- and parasubiculum (PrS and PaS, respectively) and entorhinal cortex (EC) (Modified with permission from Boccara et al. 2010 Nat Neurosci.13:987. (**c**) Schematic drawing of a horizontal section illustrating the main nomenclature. (**d**) Horizontal section stained for the neuronal marker NeuN, illustrating the main subdivisions of HF and the EC

The Standard Connectional View

According to the standard view (Fig. 2), neocortical projections eventually reach the EC, which in turn provides the main source of input to DG of the hippocampal formation. All subregions of the hippocampal formation are sequentially connected by a serial chain of connections. In short, the dentate granule cells give rise to the mossy fibre pathway which targets the CA3. Axons from CA3 neurons form the so-called Schaffer collateral projection, targeting CA1 and lastly, CA1 projects to Sub. Output from the hippocampal formation arises in CA1 and the Sub and is directed to the parahippocampal region, mainly, but not exclusively to the deep layers of the EC. This series of unidirectional connections has also been referred to as the extended trisynaptic circuit. In a more complex version, EC mediates two parallel projection streams by way of LEC and MEC, respectively, that each reflect major input/output differences. The EC is the source of the perforant pathway, which projects to all subregions of the hippocampal formation. Entorhinal layer II projects to the dentate gyrus, CA3 and CA2, whereas layer III projects to CA1 and Sub. CA2 has been added to the circuitry. Whether or not CA2 receives mossy fibre input is still debated, but recent data indicate that species differences might exist. For now, we assume that the mossy fibre projection is a characterizing feature of CA3. In turn, CA2 has strong projections to both CA3 and CA1. The projections to CA1 and subiculum show a complex topographical organization (Fig. 3). In the following sections, each of the connections of the more extended scheme will be reviewed, detailed and

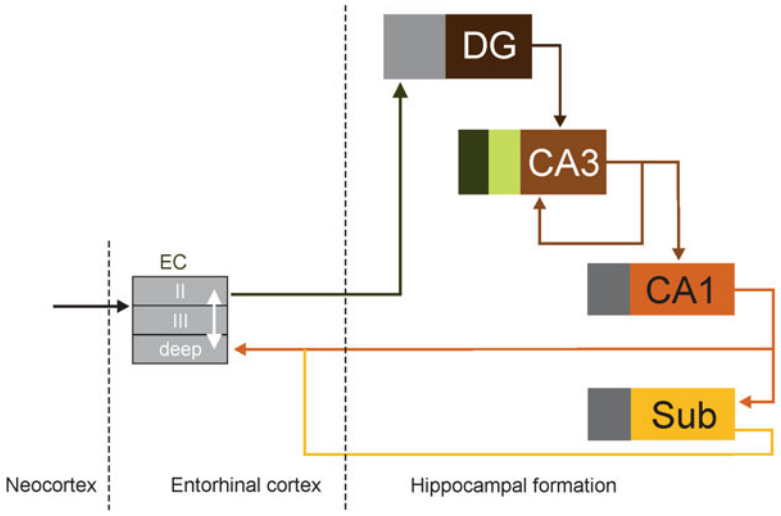

Fig. 2 The standard view of the entorhinal-hippocampal network. Layer II of EC originates the perforant pathway to DG. DG in turn sends the mossy fibre projection to CA3, where neurons originate the autoassociative projection as well as Schaffer collaterals to CA1. CA1 projects to Sub and both of them send return projections back to layer V of EC

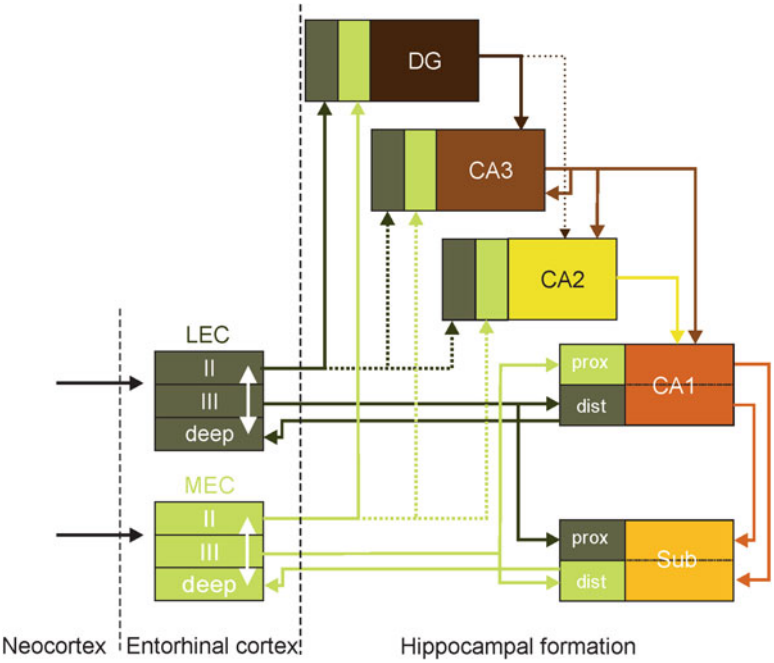

Fig. 3 The extended version of the standard view of the entorhinal-hippocampal network. CA2 was added to the network, as well as additional projections for Layer II of EC to CA3 and CA2 and the layer III projections to CA1 and Sub. The differential distribution of the projections from the LEC and the MEC along the transverse axis of the CA1 and the Sub has been included, as well as the organization of the projections from the CA1 to the Sub and from both back to LEC and MEC. The longitudinal topologies of neither connections are represented

when appropriate appended, starting with the entorhinal projections to the individual subdivisions of the HF.

Entorhinal-Hippocampal Projections

The elaborate Golgi studies of Ramon y Cajal and Lorente de Nó first demonstrated that EC is the origin of an immensely strong projection to HF. The latter became generally known as the perforant path(way). These observations were subsequently corroborated and extended in a seemingly continuous stream of tracing studies that drew attention to the many parallel entry routes for entorhinal inputs to HF, providing us with the contemporary image of EC projections to DG, the hippocampal fields CA1–CA3 and Sub. The total component of fibres was originally

named the perforant (temporo-ammonic) pathway by Cajal,[3] since the axons from EC perforated the pyramidal cell layer of the Sub. In the molecular layer of Sub, axons subsequently travel towards DG, crossing the hippocampal fissure, or course in stratum lacunosum-moleculare of CA1, CA2 and CA3, while making en passant synapses on the pyramidal neurons and interneurons in the CA fields, and continue into the tip of the molecular layer of the DG. There is an additional route for entorhinal fibres to reach targets in the hippocampus, referred to as the temporo-alvear tract. Axons in this pathway, which does not perforate the Sub, travel in the alveus and to some extent in stratum oriens below Sub and CA1–CA3 and will eventually traverse the pyramidal cells layer of the CA fields at specific points and continue to stratum lacunosum-moleculare where they terminate. Note that these axons target basal and apical dendrites of pyramidal cells as well as interneurons in strata oriens, pyramidale and radiatum.[4]

EC Projections to DG, CA3 and CA2

Cells in layer II of EC give rise to projections to DG, CA3 and CA2, and this observation has been made in most if not all species studied, including humans. It is likely that both the projections to DG and CA3 originate as collaterals from the same neuron and that the majority of neurons that project to DG and CA3 express marked levels of the protein Reelin, one of the two main cell markers for neurons in layer II. Details regarding the origin of CA2 projecting cells are unknown, but it is likely that these neurons also belong to the reelin-positive class of neurons. The other neuron class stains positive for the calcium-binding protein calbindin. These neurons give rise to widespread projections to the forebrain, but interestingly, about half of the population of EC layer II calbindin-positive neurons apparently issues local axon collaterals, contributing to an extensive, though yet now well-analysed intrinsic projection system. Only a small percentage of these neurons contribute to the projections to the hippocampus. Although the organization of the EC projection to DG has been described in much more detail than the EC to CA3 projection, the latter appears to follow organization principles like those that govern the projection from entorhinal layer II to DG. Generally, two components are differentiated which have their exclusive origin in LEC or MEC, respectively.

[3] See Witter et al. 2017 Brain Behav Evol 90:15–24 for details on the complex and sometimes confusing terminology used to describe EC-HF projections.

[4] Note that the term temporo-ammonic tract is often used to refer to all of the entorhinal projections to the CA fields but more commonly only to all fibres that reach CA1. In the temporal portion of the hippocampus, most of the entorhinal fibres reach CA1 after perforating the subiculum (classical perforant pathway). At more septal levels, however, the number of entorhinal fibres that take the alvear temporo-ammonic pathway increases. A third route taken by fibres from the entorhinal cortex involves the molecular layers of the entorhinal cortex, para- and presubiculum, continuing into the molecular layer of the subiculum. The latter route has not been given a specific name.

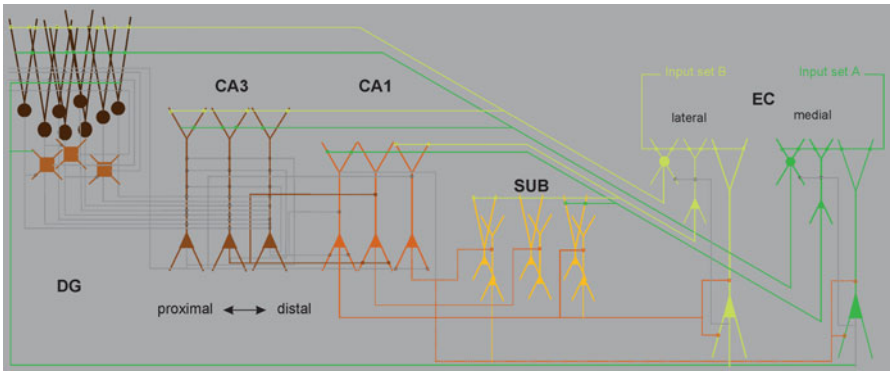

Fig. 4 Wiring diagram, illustrating the organization of the projections from layers II, III and V of the MEC and the LEC to the various subdivisions of the HF. Note the laminar terminal distribution of the layer II component to the DG and the CA3 and the restricted transverse terminal distribution of the layer III projection to the CA1 and the Sub

Projections from LEC terminate in the outer half of the stratum moleculare of DG and the stratum lacunosum-moleculare of CA3 and CA2, whereas those from MEC terminate deep to the lateral fibres (Figs. 3 and 4). In DG, the entorhinal terminal zone occupies the outer two-thirds of the molecular layer, and in CA3/CA2, the entire radial dimension of stratum lacunosum-moleculare contains entorhinal fibres.[5]

There are conflicting papers on the transverse distribution of the layer II perforant path projection. Whereas in some studies no differences were reported, others reported that the lateral perforant pathway preferentially projects to the enclosed blade of the dentate gyrus and the medial component either does not show a preference or predominantly targets the exposed blade. In CA3 no indications have been found for a further transverse organization, although it should be mentioned that the distribution of apical dendrites makes it likely that neurons in the most proximal portion of CA3 are largely devoid of entorhinal input since their dendrites do not reach into the terminal zone in CA3. In the mouse and the monkey, no transverse organization has been described in either the DG or the CA3 projection.

[5]The laminar pattern has been extensively described in the rat and available data in mice, guinea pigs and cats indicate a similar laminar terminal differentiation between the lateral and medial components of the perforant path. In contrast, in the macaque monkey the situation is different in that irrespective of the origin in EC, at all levels of the dentate gyrus, projections have been reported to distribute throughout the extent of the outer two-thirds of the molecular layer and stratum lacunosum –moleculare in CA3. It is important though that in all species information from functionally different entorhinal domains converges onto a single population of dentate and CA3 cells.

EC Projections to CA1 and Sub

Layer III of the EC contributes a second component to the perforant path that selectively targets CA1 and the Sub (Fig. 3). Axons originating from the LEC and the MEC show strikingly different terminal patterns, but unlike the layer II projections, the difference is not along the radial axis but along the transverse axis. The projection that arises from the LEC selectively targets neurons in the distal part of CA1 (the part closest to the Sub) and in the adjacent proximal part of the Sub. In contrast, the projection from the MEC distributes selectively to the proximal CA1 and the distal Sub (Figs. 3, 4).[6] In their respective target domain, entorhinal fibres completely cover the radial extent of stratum lacunosum-moleculare of CA1 and the other portion of the molecular layer of the Sub.

In addition to the main innervations arising from layers II and III in the EC, a projection originating from deep layers has been described as well. In the DG, this deep layer component preferentially distributes to the inner portion of the molecular layer, the granule cell layer as well as the subgranular, hilar zone, where it establishes asymmetrical synapses onto granule cell dendrites as well as on their somata and onto spine-free dendrites in the subgranular zone. The latter most likely represent dendrites of interneurons (Fig. 4). In the other divisions of the HF, details on the distribution of this deep pathway are lacking.

Also, weak inputs from the PrS and PaS reach all hippocampal subfields, where they terminate throughout stratum moleculare/lacunosum-moleculare, overlapping with the inputs from the EC. The CA1 and Sub receive additional inputs from the perirhinal (PER) and postrhinal cortices (POR). The inputs from the PER and POR show a topology along the transverse axis comparable to that seen in case of the projections from the LEC and MEC, respectively. However, both projections have a strong preference for the extremes, such that the PER project to the most distal part of CA1 and the most proximal part of the SUB and the projections from the POR favour the opposite extremes.

Synaptic Organization

In the rat, a majority of the terminals of the perforant path fibres (around 90%) form asymmetric synapses and thus likely are excitatory, and no major differences have been reported between the lateral and medial components of the pathway. Fibres contact most frequently dendritic spines of dentate granule cells or of pyramidal cells in the CA fields and the Sub. A small proportion of the presumed excitatory

[6]In rodents, the layer II components from the LEC and the MEC apparently do not overlap with respect to their respective terminal zone in the molecular layer of the DG and likely the same holds true for CA3. It has not been established whether the same holds true for the respective layer III components, i.e. whether or not they have a zone of overlap in the centre part of CA1 or the Sub.

perforant path fibres terminate on non-spiny dendrites of presumed interneurons. In addition, a small proportion of the perforant path synapses is symmetrical, indicative of their inhibitory nature, and these likely target both interneurons and principal cells alike.

In the DG, entorhinal synapses make up at least 85% of the total synaptic population, and they target mainly apical dendrites of granule cells. Interneurons that are innervated are those positive for parvalbumin, as well as those positive for somatostatin and NPY. No details have been reported for the CA3, but on the basis of quantitative analyses on reconstructed single neurons (Matsuda et al., 2004), one may assume that a large majority of the excitatory entorhinal fibres terminate on spines, i.e. indicating synapses with pyramidal cells, and only a minor percentage terminate on shafts, taken to indicate presumed contacts with interneurons. Although in the stratum lacunosum-moleculare of the CA3 inhibitory terminals make up approximately 10% of the total population, it has not been established whether these all belong to local interneurons or whether part of them have an entorhinal origin. No studies to date have looked into possible interneuron targets for perforant path fibres in the CA3. In stratum lacunosum-moleculare of the CA1, about 15% of the total population of synapses is inhibitory, and the other 85% are excitatory. Unlike the situation in the DG and CA3 where most if not all of the synapses in stratum moleculare/lacunosum-moleculare are of entorhinal origin, in the CA1 the total population of excitatory terminals likely have three different origins, the EC, thalamic midline nuclei such as nucleus reuniens and the amygdala.[7] Regarding entorhinal inputs, over 90% is asymmetrical, i.e. excitatory terminating on spines, and around 5% is excitatory terminating on shafts. Almost no symmetrical, i.e. inhibitory entorhinal fibres have been reported in CA1. The terminals on shafts likely indicate that interneurons are among the targets and recently interneurons that reside at the interface between strata lacunosum-moleculare and radiatum have been identified as recipients of entorhinal input.

In the Sub, the situation in the superficial half of the molecular layer is likely to be comparable to that in stratum lacunosum-moleculare of CA1 with the adding complexity of having even more inputs distributing here, including those from PrS and PER/POR. Of the entorhinal synapses, over 90% is excitatory and 80% terminates on spines and 10% on dendritic shafts, likely of interneurons, including those containing the calcium-binding protein parvalbumin, and the remaining are symmetrical terminals. The postsynaptic targets have not been identified anatomically, but electrophysiological data indicate that pyramidal cells that project back to EC are among the targets, an observation that has not been corroborated by anatomical findings (own unpublished data).

[7] Amygdala inputs reach only the ventral two-thirds of the CA1 and the Sub. The dorsal one-third of both fields is devoid of input from the amygdala.

Projections from CA1 and Subiculum to Entorhinal Cortex

Transverse and Laminar Organization

The dentate gyrus and CA3 field of the hippocampus do not project back to EC. Thus, the recipients of the layer II projection do not have any direct influence over the activities of EC. It is only after the layer II and layer III projection systems are combined in CA1 and Sub that return projections to EC are generated. The return projections mainly terminate in the deep layers (V and VI) although a component ascends into the superficial layers. The main targets of these output projections are in layer V, where likely two or three different subgroups of principal neurons reside. The cells in the deeper part, referred to as layer Vb, stain positive for the transcription factor Ctip 2, whereas those in the superficial layer Va stain for Etv1. Projections from CA1 mainly target neurons in layer Vb, whereas subicular output seems to target both layers. Whereas neurons in Va originate the main extrinsic projection system of EC, those in Vb project preferentially intrinsically, targeting layers Va, III and II (Fig. 5). In case of the projections from the Sub, up to 93% of fibres form asymmetrical, i.e. excitatory synapses onto dendritic spines (68%) and

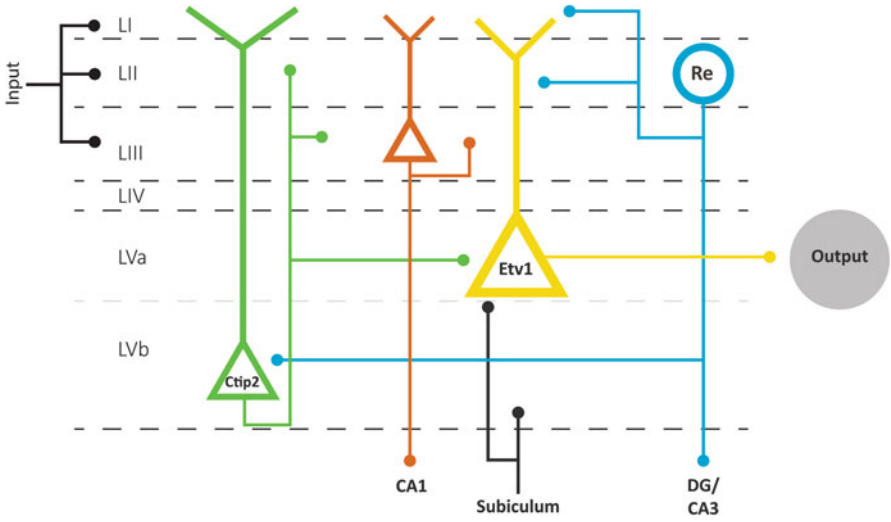

Fig. 5 Wiring diagram of some of the main intrinsic and extrinsic connections of EC. Reelin-positive layer II neurons project to DG, CA3 (and CA2), whereas neurons in layer III project to CA1 (and subiculum). Return projections from CA1 target mainly neurons in layer Vb, whereas those from the subiculum distribute to both Vb and Va. Neurons in layer Vb give rise to strong intrinsic connections to layers Va, III-I. Note that the intrinsic component originating from layer II calbindin-positive neurons is not indicated (Modified with permission from Witter et al. 2017a, Front Syst Neurosci. 11: 46)