Regional and subpopulation rules for plasticity in the adult mouse hippocampus

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TABLE OF CONTENTS

Abbreviations iii
Acknowledgements iv
Preface 1
1. INTRODUCTION 4
1.1 EXPERIENCE-DEPENDENT PLASTICITY 4
1.1.1 Activity-dependent synaptic plasticity 4
1.1.2 Experience-dependent structural plasticity 5
1.2 THE HIPPOCAMPAL FORMATION 7
1.2.1 Hippocampal functions 7
1.2.2 Hippocampal anatomy and connectivity 11
1.2.3 Functional differentiation along the dorsoventral axis 18
1.2.4 Hippocampal formation: unitary structure or distinct domains? 21
1.2.5 Putative functions of the ventral hippocampus 22
1.3 MICROCIRCUITS AND SUBPOPULATIONS OF PRINCIPAL NEURONS 23
1.3.1 Selective connectivity and microcircuits 23
1.3.2 Defined subpopulations of principal neurons in the hippocampus 24
2. RESULTS 26
2.1 Local learning-related plasticity in ventral and dorsal hippocampus driven by reward-based behavioural requirements 26
2.1.1 Summary 27
2.1.2 Introduction 28
2.1.3 Results 30
2.1.4 Discussion 56
2.2 Experience- and learning-mediated structural plasticity in distinct subpopulations of principal hippocampal neurons 62
2.2.1 Summary 63

2.2.2 Introduction 64
2.2.3 Results 66
2.2.4 Discussion 83
3. GENERAL DISCUSSION 87
3.1 Behavioural dissociation along the longitudinal axis of the hippocampus 87
3.2 Significance of increased FFI connectivity upon learning 89
3.3 Hippocampal microcircuit: functional implications 89
4. EXPERIMENTAL PROCEDURES 91
4.1 Mice 91
4.2 Behavioural procedures 91
4.3 Tissue preparation 94
4.4 Microarray analysis 95
4.5 Imaging 95
4.6 Image analysis and data quantification 96
4.7 Drug delivery *in vivo* and stereotactic surgery 98
4.8 Statistical analysis 99
5. BIBLIOGRAPHY 100

Abbreviations

CA	Cornu Ammonis (hippocampal region)
cFC	contextual fear conditionoing
DH	dorsal hippocampus
DG	dentate gyrus (hippocampal region)
DIV	day in vitro
EC	entorhinal cortex
EE	enriched environment
FFE	feed-forward excitation
FFI	feed-forward inhibition
GABA	γ-amino butyric acid
GC	granule cell
IH	intermediate hippocampus
LEA	lateral entorhinal area
LMT	large mossy fibre terminal
LTD	long-term depression
LTP	long-term potentiation
MEA	medial entorhinal area
mGFP	membrane-targeted green fluorescent protein
mPFC	medial prefrontal cortex
NAcc	nucleus accumbens
oFC	olfactory fear conditioning
P	postnatal day
TA	terminal arborisation
VTA	ventral tegmental area

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If the human brain were so simple that we could understand it,
we would be so simple that we couldn't.
– Emerson M. Pugh

"Every man can, if he so desires,
become the sculptor of his own brain"
– Santiago Ramón y Cajal

Preface

One of the greatest discoveries of the past century in the field of neuroscience is that the adult brain can reshape itself. This extraordinary property to rewire its connections and "remodel" as a result of lifestyle, experience, learning or injury is called *neuroplasticity*. "Neuro" comes from "neuron", or nerve cell, and "plastic" means "malleable, changeable, modifiable". Only over the last decades, and mostly thanks to technological advancements, did the idea emerge that the brain is "plastic", which is in stark contrast to the common wisdom that the adult brain is hardwired, fixed and rigid.

Throughout life, we are faced with the necessity to efficiently categorise and compare incoming sensory information with previous experiences, in order to decide whether the new information should be retained and stored into memory. This is an adaptive process that is evolutionary advantageous in terms of survival, as it allows learning from previous mistakes and planning for the future. Just as the whole individual adjusts and changes his behaviour in response to external or internal events, so does our brain by remodelling its neuronal connections (a phenomenon known as "structural plasticity") in response to experience and learning. Therefore, altering our behaviour shapes the anatomy of the brain and vice versa, revealing an intimate relationship between structural rearrangements and a person's experience.

Storing, retaining and recalling memories from past experiences to modify behavioural responses are processes that strongly rely on the hippocampus (from *hippocampus* in latin, meaning sea horse (Figure 1)).

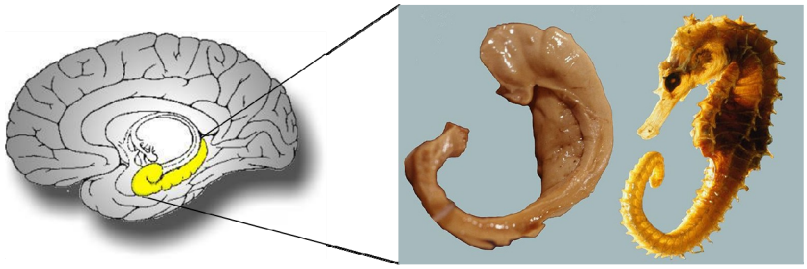


Figure 1: The human hippocampus
Left: schema of a human brain showing the location of the hippocampus (highlighted in yellow) in the medial temporal lobe. From http://www.ucl.ac.uk/cdb/research/okeefe/human_hippo.jpg
Right: Preparation of the human hippocampus and fornix by the Hungarian neuroscientist László Seress, 1980, and comparison with a sea horse. From http://en.wikipedia.org/wiki/File:Hippocampus_and_seahorse_cropped.JPG

The hippocampus plays a central role in various forms of episodic and relational memories (see section 1.2.1), and is a very well-suited model to study several aspects of learning and memory at the molecular, physiological, behavioural and psychological level. Its neuroanatomical organisation is unique: not only do distinct regional subdivisions (dorsal, intermediate and ventral hippocampus) and anatomical subregions (dentate gyrus, CA3, CA1) subserve different functions (Bannerman et al., 2004; Goodrich-Hunsaker et al., 2008; Kesner et al., 2004), but recent findings from our laboratory have demonstrated the existence of "microcircuits" in the hippocampus, consisting of subpopulations of genetically predefined principal neurons that are selectively interconnected across hippocampal subregions as a result of temporally matched schedules of neurogenesis and synaptogenesis (Deguchi et al., 2011). Moreover, our laboratory has shown that experience and learning lead to structural plasticity in the adult hippocampus (Bednarek and Caroni, 2011; Galimberti et al., 2006; Gogolla et al., 2007; Gogolla et al., 2009; Ruediger et al., 2011). However, whether such remodelling obeys region- and/or subpopulation-specific rules has not been explored.

Abstract

The aim of my thesis was to elucidate regional and subpopulation rules for structural plasticity in the adult mouse hippocampus, which can provide insights to information processing and memory formation within the hippocampal circuitry.

Previous studies have shown that dorsal, intermediate and ventral hippocampus have distinct coding and behavioural roles, consistent with the distinct afferent and efferent connectivities along the longitudinal (dorsoventral) axis of the hippocampus. In addition, evidence for distinct hippocampal regions has been provided in the form of discrete molecular domains of gene expression across the hippocampus. However, none of these studies has investigated the anatomy and connectivity at the level of individual identified neurons. Also, it still remains unknown whether structural plasticity upon experience and learning may differ along the dorsoventral axis of the hippocampus and across distinct mossy fibre subpopulations.

To address these questions, I mapped granule cell mossy fibre anatomy and connectivity throughout the hippocampus in three "sparse" Thy1 transgenic reporter mice (Lsi1, Lsi2 and Lsi3) that express membrane-targeted GFP in a subset of principal neurons. By combining behavioural and lesion experiments, high-resolution confocal microscopy and gene expression analysis, I provide evidence that distinct regions of the hippocampus (dorsal, intermediate and ventral) and distinct subpopulations of granule cells exhibit different anatomy and connectivity under baseline conditions and upon learning. Using the growth of filopodial synapses that mediate feed-forward inhibition to the network in CA3 as a specific readout for learning, I show that the dorsal hippocampus encodes spatial information and is specifically recruited for spatial learning, while the ventral hippocampus encodes goal-oriented information and is specifically recruited for goal-oriented learning. Moreover, the results reveal objective distinctions at the circuit level between hippocampal-dependent memory and hippocampal-dependent learning. In addition, I provide evidence that distinct granule cell subpopulations respond in unique ways to experience and learning, suggesting that principal neuron subpopulations may have distinct functional roles in hippocampal-dependent learning and memory.

1. INTRODUCTION

I will first introduce the concept of experience-dependent plasticity in the adult, with major emphasis on structural plasticity, and then extensively describe the hippocampal formation in terms of anatomical organisation, connectivity and function, highlighting the functional differentiation along its longitudinal axis.

1.1 EXPERIENCE-DEPENDENT PLASTICITY

One of the most fascinating features of the brain is its "plasticity", or the capability to remodel in response to changes occurring in the internal and external world. For a long time, it was believed that plasticity would be restricted to developmental and juvenile stages, but converging lines of evidence have clearly shown that this property is maintained throughout life.

There are mainly two forms of experience-dependent plasticity: functional plasticity (alteration in synaptic strength and neuronal excitability in response to an otherwise unchanged stimulus) and structural plasticity (physical rewiring of neuronal circuits by synapse formation, elimination or remodelling, axonal branching or addition of new neurons). Whether and how the different forms of plasticity are causally related to each other still remains to be clarified (Buonomano and Merzenich, 1998).

1.1.1 Activity-dependent synaptic plasticity

Synaptic plasticity, which can be short or long lasting, is the ability of excitatory and inhibitory synapses to respond to specific patterns of activation with long-lasting increases or decreases in synaptic efficacy. Long-lasting forms of activity-dependent synaptic plasticity are regarded as the most attractive cellular mechanism underlying the encoding and storage of memory traces into neuronal networks (Bennett, 2000; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999).

Long-term synaptic plasticity can affect both excitatory and inhibitory synapses in the central nervous system. Depending on whether long-term plasticity results in strengthening or weakening of synapses, this event is called long-term potentiation (LTP) or long-term depression (LTD), respectively. LTP was first described in the dentate gyrus at the perforant path – granule cell synapse (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973), whereas LTD was first reported in the CA1 area of the hippocampus (Lynch et al., 1977).

1.1.2 Experience-dependent structural plasticity

Besides changes in synaptic strength, structural modifications of neuronal connectivity provide another powerful mechanism to encode experience in the central nervous system. This phenomenon is called *structural plasticity*, whereby specific loss and gain of synapses or remodelling of pre-existing ones alter neuronal connectivity and modify the properties of neuronal networks and their functional output. As opposed to functional plasticity, which occurs within seconds to hours, structural plasticity follows a slower time scale (days to weeks) and allows sampling of wide synaptic territories (tens to hundreds of micrometres) through its large-scale rearrangements (Gogolla et al., 2007). Furthermore, structural plasticity can persistently modify local architecture of microcircuits quantitatively and qualitatively (Galimberti et al., 2006; Gogolla et al., 2007).

Direct visualisation of localised structural changes has become possible with the advancement of powerful technologies to allow *in vivo* imaging of single neurons deep into the brain (Brecht et al., 2004; Caroni, 1997; Feng et al., 2000; Svoboda and Yasuda, 2006). For example, it has been shown that new projections are generated after learning a challenging skill (Hofer et al., 2009; Xu et al., 2009; Yang et al., 2009) and novel connections are made following localised brain damage (Dancause et al., 2005).

Experience-dependent structural plasticity in adulthood

Experience-dependent modifications are widespread during development, for example during so-called "critical periods", which have been defined as a period of time during which neuronal connections are susceptible to experience-dependent modifications (Fu and Zuo, 2011; Hensch, 2004). For a long time it was believed that, after closure of critical periods of plasticity, neuronal circuits may become fixed in the adult. However, converging evidence over the last two decades has shown that experience-dependent plasticity occurs in the adult brain as well, and it is now widely accepted that neuronal circuits of the adult mammalian brain are dynamic and capable of undergoing extensive structural reorganisation in response to new experience throughout life (for review, see Fu and Zuo, 2011; Gogolla et al., 2007; Holtmaat and Svoboda, 2009). Importantly, such rearrangements not only occur in response to sensory deprivation or injury, but also under physiological conditions. In particular, persistent local circuit remodelling occurs in response to experience, learning, lifestyle and ageing.

a) Structural plasticity upon sensory manipulation and injury

In the adult, lesions and disease can lead to structural plasticity in the form of axon regeneration and local sprouting to promote repair (Dancause et al., 2005; Das and Gilbert, 1995; Florence et al., 1998; Yamahachi et al., 2009). In addition, studies in adult animals have shown that sensory manipulations alter dynamics of axonal arborisation (Marik et al., 2010) and dendritic spines (Knott et al., 2002; Trachtenberg et al., 2002).

b) Structural plasticity upon experience and learning

Most studies in the field have addressed structural plasticity in the neocortex, mostly at the level of dendritic spines, as a result of experience (Fu and Zuo, 2011; Hofer et al., 2006; Hofer et al., 2009; Holtmaat and Svoboda, 2009; Holtmaat et al., 2006; Trachtenberg et al., 2002; Wilbrecht et al., 2010) or learning (Komiyama et al., 2010; Roberts et al., 2010; Wang et al., 2010; Xu et al., 2009).

Complementary to structural plasticity occurring at postsynaptic spines, axonal presynaptic boutons and side-branches also undergo extensive structural plasticity in the adult brain as a result of experience and learning. As opposed to spine remodelling, one

feature of axonal plasticity is the capability to sample larger volumes of neuropil through large-scale rearrangements, thereby exerting a greater impact on circuit rearrangements (Gogolla et al., 2007). In fact, remodelling of axonal structures can range from local changes of synaptic contacts within microcircuits to assembly or dismantling of entire parts of local circuits (Gogolla et al., 2007). For example, housing mice in enriched conditions, a paradigm including a variety of visual and tactile stimuli, physical exercise and social interactions, has been shown to lead to massive presynaptic structural rearrangements in the hippocampus (Galimberti et al., 2006; Gogolla et al., 2009). In addition, structural remodelling of axonal structures upon learning has been reported in the cortex, hippocampus and cerebellum (Holahan et al., 2006; Kleim et al., 2002; Maguire et al., 2000; Ramirez-Amaya et al., 1999; Ruediger et al., 2011).

Compared to changes in synaptic strength alone (synaptic plasticity), new synapse formation resulting from structural plasticity could greatly increase the memory storage capacity of the brain (Chklovskii et al., 2004). In addition, stabilised new spines persist long after experience and may thus represent structural traces for earlier memories, thereby facilitating quicker adaptation of the brain to similar experiences occurring later in life (Hofer et al., 2009; Xu et al., 2009).

In conclusion, strong evidence supports the notion that experience-dependent structural plasticity occurs in the adult, both pre- and postsynaptically, and may play a role in supporting learning and memory.

1.2 THE HIPPOCAMPAL FORMATION

1.2.1 Hippocampal functions

Despite over 50 years of research and debate, there is still controversy over the basic general functions of the hippocampus (Fanselow and Dong, 2010). On one side, the hippocampus is viewed as a structure having a purely “cognitive” role in various aspects of declarative memory (see below). On the other side, it has been shown to be intimately linked to emotion, to regulate stress response and to be involved in affective aspects of

behaviour. Rather than being mutually exclusive, it is most likely that several functions co-exist. In this section, I will describe the main theories about hippocampal function that have been proposed from the second half of the 20th century.

1.2.1.1 Hippocampal functions: "cognitive" aspects

The role played by the hippocampal formation in learning and memory is widely accepted ever since the seminal works on the famous patient H.M., who underwent bilateral resection of large parts of the medial temporal lobe to cure epilepsy (Scoville and Milner, 1957). Damage to the hippocampus has been shown to cause anterograde amnesia, i.e. the inability to form and store new memories, as well as temporally graded retrograde amnesia, characterised by impaired retrieval of recent memories but often sparing of more remote ones. Procedural learning and memories, which are dependent on neostriatal structures (Squire, 2004), are not affected by bilateral hippocampal damage.

To a first approximation, memory can be subdivided into a short- and long-term form. In turn, long-term memory can be declarative (*explicit*) or non-declarative (*implicit*). Non-declarative memory is recalled unconsciously and refers to information about *how* to perform something. It is typically involved in training reflexive motor or perceptual skills, like riding a bike or playing the piano (Kandel et al., 2000). On the other hand, declarative memory is recalled by a deliberate, conscious effort and includes factual knowledge of people, places and events as well as their meaning (Kandel et al., 2000). The psychologist Endel Tulving proposed that declarative memory can be further subdivided into two classes, *episodic* and *semantic* memory (Tulving, 1972).

Episodic memory is the ability to remember personal past experiences and is based on temporal-spatial relations among them. It involves binding the "what", "where" and "when" of an event to create a relational representation that can be later recalled by partial input cues (Eichenbaum et al., 1999; Greene et al., 2001). For example, remembering that last night ("when") I was playing Chopin's *Fantaisie-Impromptu* ("what") in the living room ("where") is a form of episodic memory.

On the other hand, *semantic memory* underlies the ability to acquire general knowledge and remember facts about the world that are not necessarily related to specific personal events.

For instance, knowing that Chopin was a Polish composer and that Paris is the capital of France are forms of semantic memory.

There is no doubt that the hippocampus plays a prominent role in declarative memory, and several theories have been formulated in this respect.

a) Cognitive map theory: the hippocampus and spatial navigation

The "cognitive map theory" was originally proposed in 1978 by John O'Keefe and Lynn Nadel (O'Keefe and Nadel, 1978). According to this view, the role of the hippocampus is to mediate memory for spatial relations among objects in an environment (for review, see McNaughton et al., 2006; Moser et al., 2008). This idea is strongly supported by the existence of "place cells", which were discovered in the 1970's in freely behaving rats. These spatially selective cells fire whenever an animal is at a specific location in an environment (a cell's "place field") (O'Keefe and Conway, 1978; O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978), thereby allowing the formation of an internal representation of the outside world known as "cognitive map" (Kandel et al., 2000, 1264-1272; O'Keefe and Dostrovsky, 1971). Studies in humans also confirmed the central role of the hippocampus in spatial learning and navigation (Maguire et al., 1997; Maguire et al., 2000; Maguire et al., 2006).

b) Relational memory theory

In the 1990s, Howard Eichenbaum proposed a more general theory of hippocampal function, which incorporates spatial memory but also attempts to explain the role of the hippocampus in other aspects of declarative memory (Eichenbaum, 1999; Eichenbaum et al., 1999; Eichenbaum et al., 1992). According to this "relational memory theory", the function of the hippocampus is to rapidly generate relational representations of episodes, binding all kinds of stimuli into a unitary representation that can be later recalled from partial input cues (Eichenbaum et al., 1999; O'Reilly and Rudy, 2001). These rapidly learned relational representations are arranged according to familiar and novel features and allow for inferential relationships between episodes. Based on this theory, place cells do not encode space per se but rather relationships among subsets of cues. Indeed, place cell firing has been shown to be also affected by non-spatial variables, such as speed and

the presence of goals or reward in an environment (Eichenbaum, 1996; Hok et al., 2007; Lee et al., 2006; Royer et al., 2010). Further supporting this theory, several studies have reported the involvement of the hippocampus in non-spatial tasks based on relational learning, such as social transmission of food preference (Bunsey and Eichenbaum, 1995).

c) Episodic memory theory

In humans, declarative memory can be *episodic* or *semantic*. According to the "episodic memory theory" proposed by Endel Tulving and his colleagues, the hippocampus is critical for episodic, but not semantic, memory (Tulving, 1972; Tulving and Markowitsch, 1998). Several pieces of evidence coming from study on amnesic patients support this theory (Rosenbaum et al., 2000; Tulving, 2002). In contrast to the "episodic memory theory", the "relational memory theory" states that the hippocampus is also important for extracting common features across episodes and therefore plays a critical role in semantic memory as well (O'Reilly and Rudy, 2001). The role of the hippocampus in recollection (Eldridge et al., 2000; Prince et al., 2005; Yonelinas and Levy, 2002) is consistent with both the relational and the episodic memory theories, since recollection is a process that involves relational memory and is the prototypical form of episodic memory (Purves et al., 2008).

d) Declarative memory theory

Finally, according to the "declarative memory theory" proposed by Larry Squire and his collaborators, the hippocampus mediates all declarative memories, regardless of whether they are spatial or non-spatial, relational or non-relational, episodic or semantic (Bayley et al., 2003; Manns et al., 2003; Squire, 1992; Squire and Zola, 1998). In addition, this view also holds that the medial temporal lobe plays a time-limited role in the consolidation of declarative memory, such that memory for both episodic and semantic information encountered long before the onset of amnesia is unaffected (Suzuki, 2003).

1.2.1.2 Hippocampal functions: emotional aspects

In addition to its roles in cognitive functions, the hippocampus is also a regulator of stress and emotions (Dedovic et al., 2009; Jacobson and Sapolsky, 1991; McEwen, 1999). In support to this notion, several studies in rodents and humans have reported a close

correlation between hippocampal dysfunction and affective disorders (Bonne et al., 2008; Frey et al., 2007; Gray and McNaughton, 2000; McEwen, 1999). As described in section 1.2.3.2, the emotional and affective aspects of hippocampal function are mainly ascribed to the ventral (in rodents) or anterior (in primates) region of hippocampus. Overall, the role of the hippocampus in emotions can be as strong as its cognitive role in declarative memory.

1.2.1.3 A unifying theory of hippocampal function: division of labour?

Definitive consensus about hippocampal function is still missing, but according to the various theories described, there is strong evidence that it is involved in cognitive aspects of learning and memory such as spatial, relational, episodic and semantic memory, as well as in emotional and affective behaviour.

Are these views conflicting with each other or is it possible to formulate a "unified" theory of hippocampal function that includes all aspects discussed above? These theories can be reconciled if we consider the possibility that specific functions may be segregated within the hippocampal formation. In other words, several functions may co-exist, although being restricted to separate anatomical regions that are more or less, or even exclusively, involved in processing distinct aspects of behavioural functions (spatial memory, emotional and affective behaviour, recollection, etc.). This hypothesis may be supported by the notion that the hippocampus exhibits a complex three-dimensional organisation and is not homogeneous along its longitudinal axis in terms of connectivity (afferents, efferents and intrahippocampal), gene expression, neurochemistry, spatial resolution of space representation and behavioural functions (for review, see Fanselow and Dong, 2010). Before discussing in more detail the heterogeneity of the hippocampus and its implications (section 1.2.3), I will review its anatomical organisation and circuitry.

1.2.2 Hippocampal anatomy and connectivity

The rodent hippocampal formation is a C-shaped cortical structure situated in the caudal part of the brain (Figure 2). It is composed of three distinct subregions: the dentate gyrus (DG), the hippocampus proper (consisting of Cornu Ammonis regions CA3, CA2 and CA1)

and the subiculum (Amaral and Lavenex, 2007; van Strien et al., 2009). Its longitudinal axis is called the *dorsoventral* (or *septotemporal*) axis, as it runs from the *dorsal* pole (or *septal*, close to the septum) via the *intermediate* (or *splenial*) to the *ventral* pole (or *temporal*, close to the amygdala). Throughout this thesis, I will use the term *dorsoventral* to refer to the longitudinal axis of the hippocampus.

Understanding connectivity may be a first step to get insights into how hippocampal circuits process incoming information to form, store and retrieve memories. Therefore, in the following sections I will describe some the basic hippocampal circuitry with major emphasis on the perforant path and the mossy fibre pathway.

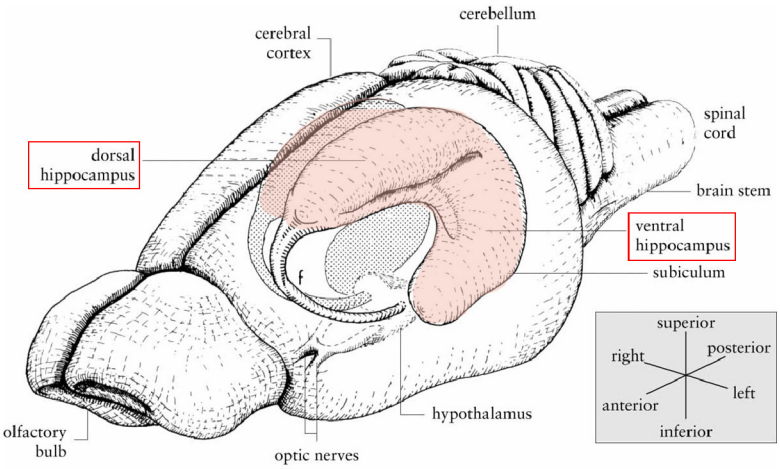


Figure 2: Three-dimensional representation of the rodent brain and the position of the hippocampal formation
Depiction of the rat brain and its main brain structures. Each hippocampus (highlighted in pale pink) is a C-shaped structure located in the caudal part of the brain. The top, anterior portion is the dorsal hippocampus, while the caudal and inferior portion is the ventral hippocampus (red boxes). Three orientation axes are shown in the bottom right panel. Modified from Amaral and Witter, 1995.

1.2.2.1 Trisynaptic circuitry

The basic trisynaptic circuitry of the hippocampus involves a mostly unidirectional flow of information (but see Scharfman, 2007) along excitatory synapses. Cortical inputs enter the DG from the entorhinal cortex (EC) and are sent via the mossy fibre pathway to CA3 pyramidal neurons. By means of their Schaffer collaterals, these neurons project to CA1,

which in turn project back to the EC (directly or via the subiculum), thereby giving rise to a closed loop (Figure 3).

The perforant path

The projection from the EC to the hippocampal formation is called the "perforant path" because it "perforates" the subiculum on its way to the DG and CA3. The *medial perforant path* arises from the medial division of the EC (MEA), whereas the *lateral perforant path* originates in the lateral division of the EC (LEA). In the mouse, neurons in layer II of the EC terminate in the molecular layer of the DG, whereas neurons in layer III project to CA3, CA1 and subiculum (van Groen et al., 2002; van Groen et al., 2003) (Figure 3).

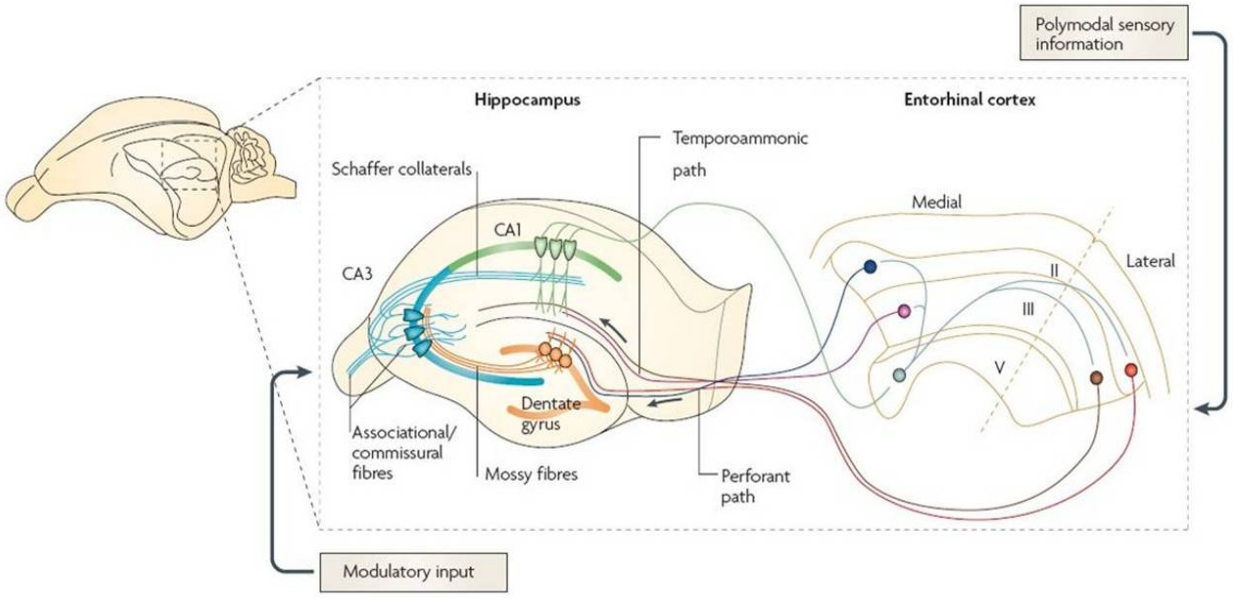


Figure 3: Schematic of the hippocampal circuitry
The main input to the hippocampal formation arises from the EC perforant path, which conveys polymodal sensory information from neurons in layer II to the DG and CA3 (not shown). Neurons located in the medial and lateral division of the EC give rise to the medial and lateral perforant path, respectively, which terminate onto the middle and the outer third of the DG molecular layer, respectively (blue and red lines). Granule cells, through their axons forming the mossy fibre pathway (orange), project to the proximal apical dendrites of CA3 pyramidal neurons, which in turn project to ipsilateral CA1 pyramidal cells via Schaffer collaterals and to contralateral CA3 and CA1 neurons through commissural connections. In addition, CA3 neuron collaterals give rise to a dense associative network interconnecting ipsilateral CA3 cells. From CA1, the information flows back to the EC (deeper layers) either directly or indirectly via the subiculum. Neurons from layer III of the EC project directly to CA1 (and subiculum, not shown), where they terminate onto stratum lacunosum-moleculare. The entorhinal projection from layer III is topographically organised: projections from the LEA terminate onto distal CA1 pyramidal cells and cells in proximal subiculum, while those originating from the MEA synapse onto cells located in proximal CA1 and distal subiculum (not shown). A small number of neurons from deeper layers of EC also contribute to this projection (not shown). From Neves et al., 2008.

From the DG, granule cell axons give rise to the mossy fibres that synapse onto proximal dendrites of CA3 pyramidal neurons in *stratum lucidum*. CA3 pyramidal cells, in turn, project to CA1 via so-called Schaffer collaterals, innervating both apical and basal dendrites of CA1 pyramidal neurons. Finally, CA1 sends its axons back to deep layers of the EC, either directly or indirectly via the subiculum (Amaral and Witter, 1989; Tamamaki and Nojyo, 1995). The deep layers of the EC then project back to the same cortical areas from where the information originated, thus giving rise to a closed hippocampal loop that processes cortical information.

Topographic organisation of the EC-to-hippocampus projection

In the rodent brain, the entorhinal cortex is located at the most caudal, ventral and lateral part of the brain (Figure 4a) and its position is particularly suited to serve as an interface between the neocortex and the hippocampal formation. As such, the EC is the main gateway to the hippocampus since it provides the main cortical source of input to the hippocampal formation and, together with the subiculum, it also serves as the major output area (van Groen, 2001). Importantly, the EC is the site of convergence of multimodal sensory and highly processed unimodal inputs, which in turn are conveyed to the hippocampal formation (Canto et al., 2008).

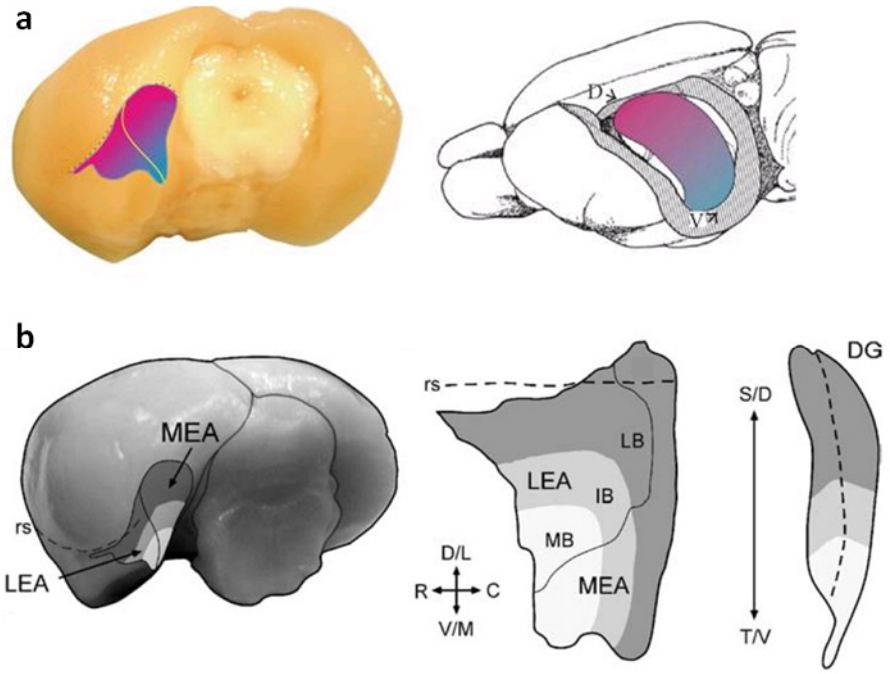


Figure 4: Representation of the topographical arrangement of entorhinal-hippocampal reciprocal connections. **(a)** Ventral posterior view of the rat brain with the cerebellum removed. A dorsolateral band of the entorhinal cortex (magenta) is preferentially connected to the dorsal hippocampus. Increasingly more ventral and medial bands of entorhinal cortex (purple to blue) are connected to increasingly more ventral levels of the hippocampus. The yellow line (left picture) indicates the border between the lateral subdivision of the EC (LEA) and the medial subdivision (MEA). **(b)** Middle and right: unfolded map of the pial surface of the EC and of the DG, respectively. The dashed lines represent the rhinal sulcus (middle panel, rs) and the crest of the DG (right panel). In each panel, the lateral band (LB) is shown in dark grey, the intermediate band (IB) in medium grey and the medial band (MB) in light grey. Other abbreviations: S/D = septal/dorsal; T/V = temporal/ventral; D/L = dorso/lateral; V/M = ventro/medial; C = caudal; R = rostral.
(a) from Canto et al., 2008, **(b)** from Kerr et al., 2007.

Classically, the EC has been subdivided into two main areas based on morphological grounds: the lateral entorhinal area (LEA), which occupies the rostrolateral portion of the EC, and the medial entorhinal area (MEA), which lies in the caudomedial part of the EC (Dolorfo and Amaral, 1998b; Insausti et al., 1997). Each of these subdivisions can been further subdivided into three discrete zones: the lateral, the intermediate and the medial band (Witter et al., 1989) (Figure 4b).

The projection from the EC to the hippocampus follows a topographic rule (Figure 4b), with distinct bands projecting to different and partly non-overlapping septotemporal levels

of the DG (Dolorfo and Amaral, 1998b; Ruth et al., 1982; Ruth et al., 1988). In particular, the most lateral band innervates the septal half of the DG (comprising the dorsal and part of the intermediate domain); the intermediate band projects to the third quarter of the DG; and the medial band terminates in the temporal part of the DG (ventral domain). The very sparse interconnectivity between the three bands and the different neuronal inputs they receive suggest that they represent functionally distinct units (Burwell, 2000; Dolorfo and Amaral, 1998a; Insausti et al., 1997). In general, the lateral band receives the most visuospatial information (mainly via adjacent perirhinal and postrhinal cortices), whereas the medial band receives prominent inputs from limbic and periamygdaloid cortices (Krettek and Price, 1974; Witter, 1993).

The mossy fibre pathway

The mossy fibre projection consists of unmyelinated axons of the glutamatergic granule cells running in *stratum lucidum*. The mossy fibre pathway is the only fibre system of the hippocampal formation to be organised in a lamellar fashion (Gaarskjaer, 1986; Henze et al., 2000). Indeed, bundles of mossy fibres run mostly parallel to the transverse hippocampal axis and they barely exhibit any divergence along the DV axis. The only exception occurs at the transition from CA3 (and CA2) to CA1, where mossy fibres make an abrupt turn caudally and travel parallel to the longitudinal axis (De No, 1934; Swanson et al., 1978b). The extent of the temporally directed component of the mossy fibre projection is strong at more dorsal levels (up to ~2 mm in the rat) and rather weak at more temporal levels (Amaral and Witter, 1989).

Mossy fibre axons exhibit three morphologically distinct presynaptic specialisations: large 'giant' boutons (large mossy fibre terminals, LMTs) that are thought to represent the main bodies of mossy fibres (Galimberti et al., 2006), small *en passant* varicosities and filopodial extensions emerging from the LMT core (Amaral and Dent, 1981) (Figure 5).

LMTs are large (> 2.5 μm in diameter) and potent presynaptic terminals that innervate complex clusters of dendritic spines called *thorny excrescences* (or *thorns*) on CA3 pyramidal neurons (Blackstad and Kjaerheim, 1961; Hamlyn, 1962; Rollenhagen et al., 2007). The mossy fibre synapses made by LMTs are very powerful and are also known as

"detonator synapses", due to their ability to generate large postsynaptic currents and potentials in CA3 pyramidal neurons under conditions of high activation (Henze et al., 2002; Lawrence et al., 2004; Maccaferri et al., 1998). LMTs can exhibit "satellites", or terminal appendices that are connected to the main core through 10 – 200 µm processes (Galimberti et al., 2006). Like core LMTs, satellites are larger than 2.5 µm in diameter, exhibit filopodia and establish excitatory contacts onto distinct postsynaptic pyramidal neurons, thereby mediating feed-forward excitation (FFE) (Figure 5). Furthermore, LMTs have been shown to exhibit structural plasticity as a consequence of age, experience and learning (De Paola et al., 2003; Galimberti et al., 2006; Ruediger et al., 2011).

As opposed to these powerful excitatory connections, mossy fibres establish synapses with inhibitory GABAergic interneurons in the hilus and *stratum lucidum* via *en passant* varicosities and LMT filopodial extensions (Acsady et al., 1998; Szabadics and Soltesz, 2009). In turn, these interneurons make inhibitory synapses on CA3 pyramidal neurons, thereby mediating feed-forward inhibition (FFI) (Figure 5). At low-frequency firing, FFI dominates over CA3 pyramidal neuron excitation (Acsady et al., 1998), providing powerful regulatory control over CA3 principal cell excitability and timing of action potential generation (Lawrence and McBain, 2003).

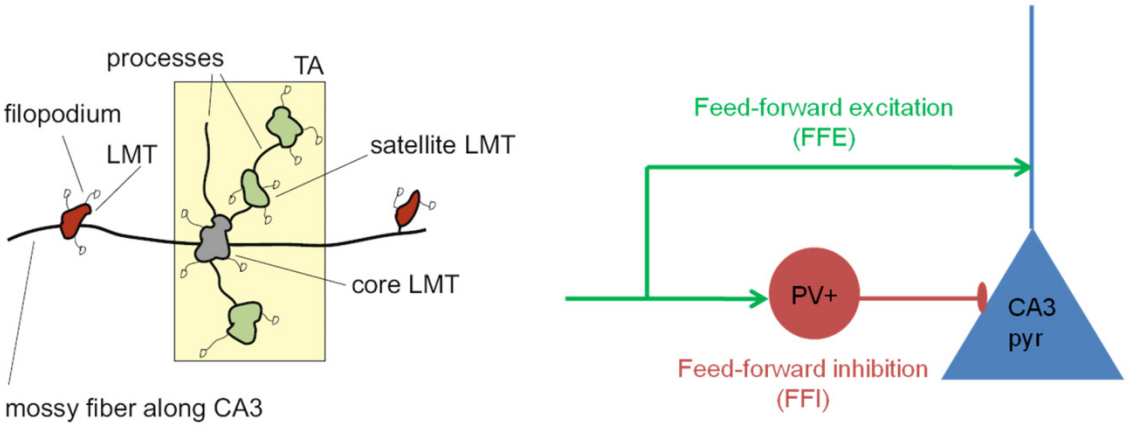


Figure 5: Feed-forward excitation and feed-forward inhibition arrangements in the mossy fibre projections
Left: schematic drawing of mossy fibre terminals (LMTs). LMTs can exhibit filopodia, which synapse onto inhibitory interneurons, and satellites (green) that are connected to the main core (grey) by processes. Terminal arborisations (TA's) consist of a core LMT, processes and satellites. From Galimberti et al., 2010. Right: schematic of feed-forward excitation (FFE) and feed-forward inhibition (FFI) circuit in the CA3 hippocampal region. PV+: parvalbumin-immunoreactive interneuron.

Taken together, the connectivity properties of the mossy fibre projections, its lamellarity and sparse connectivity, combined with the highly plastic properties of its LMTs (Galimberti et al., 2010; Galimberti et al., 2006; Ruediger et al., 2011), make the mossy fibre projection an ideal model system to study learning-related plasticity and its underlying rules.

1.2.3 Functional differentiation along the dorsoventral axis

Although the basic organisational principles within the hippocampus are repeated along the dorsoventral axis (Anderson et al., 1971), afferent and efferent connectivity changes from the dorsal to the ventral pole, suggesting a possible heterogeneity in the kind of information being processed by distinct regions of the hippocampus. In addition to differential anatomical connectivity, a large body of evidence from physiological, behavioural and gene expression studies supports the idea that the hippocampus is heterogeneous along its longitudinal axis and can be subdivided into three regions: dorsal, intermediate and ventral (DH, IH and VH, respectively). In the following sections, I will describe several aspects of hippocampal differentiation along the dorsoventral (DV) axis in support to the idea that DH and VH may be responsible for dissociable functions.

1.2.3.1 Differential connectivity along the dorsoventral axis

The topographic arrangement of entorhinal cortex inputs to the hippocampus (section 1.2.2.1) may give rise not only to an anatomical, but also to a functional differentiation along the DV axis of the hippocampus. The lateral band of the EC, which receives highly processed sensory and visuospatial information, projects to DH and IH, which are therefore more involved in the processing of exteroceptive sensory information, spatial learning and navigation. On the other hand, the medial band of the EC, which is preferentially innervated by limbic structures, projects to the ventral portion of the hippocampus, which may therefore be more implicated in processing interoceptive, visceral, emotional and affective information. Consistent with a functional segregation as a result of connectivity, lesions of the dorsolateral band of the EC reproduce the same behavioural impairments observed in

DH lesioned animals (impaired spatial memory), whereas lesions of the ventromedial band lead to reduced anxiety as observed after VH lesions (Steffenach et al., 2005).

Efferent projections originating from the hippocampal area CA1 show heterogeneity along the dorsoventral axis. Only dorsal, but not intermediate or ventral CA1, sends projections to the contralateral hippocampus (CA1, subiculum, postsubiculum, perirhinal and entorhinal cortices). On the other hand, only ventral, but not dorsal, hippocampus has unique connections to subcortical centres controlling reward, emotions, fear, internal state, and olfaction such as the ventral tegmental area (VTA) (Gasbarri et al., 1991; Gasbarri et al., 1994a; Gasbarri et al., 1997), the amygdala (Petrovich et al., 2001; Pitkanen et al., 2000), the hypothalamus (Kohler et al., 1985) and the anterior olfactory nucleus and olfactory bulb (van Groen and Wyss, 1990). In addition, only the temporal half of area CA1 projects to the medial prefrontal cortex, with connections from the intermediate CA1 being weaker and becoming stronger from the ventral CA1 (Jay and Witter, 1991).

Overall, the differential pattern of connectivity between DH and VH, which project to regions processing distinct inputs (e.g., visuo-spatial navigation versus emotional and affective information), can account for dissociations in behavioural functions along the DV axis.

1.2.3.2 Behavioural dissociation of the dorsal and the ventral hippocampus

In support to this view, an extensive number of studies have suggested a behavioural dissociation along the DV axis. Lesions or inactivation of the dorsal, but not ventral, hippocampus have been shown to impair performance on spatial learning tasks (Bannerman et al., 2002; Czerniawski et al., 2009; Fanselow, 2000; Lee and Kesner, 2004; Moser et al., 1993; Moser et al., 1995; Pothuizen et al., 2004). By contrast, lesions or inactivation of the ventral, but not dorsal, hippocampus reduce anxiety, hyponeophagia and fear expression (Bannerman et al., 2002; Bannerman et al., 2003; Kjelstrup et al., 2002).

1.2.3.3 Gradually increasing scale of spatial representation along the DV axis

Additional support for a preferential role of the dorsal hippocampus in spatial learning and memory is provided by physiological studies. Place cells have been reported throughout

the hippocampus, but the proportion is higher in DH (Jung et al., 1994; Poucet et al., 1994). Moreover, place fields in DH are small (0.5 – 1 meter) and sharp, whereas in VH they are much wider (about 10 meters) and less selective (Brun et al., 2008; Jung et al., 1994; Kjelstrup et al., 2008; Maurer et al., 2005). A recent study showed that place representation in the hippocampus occurs throughout the entire longitudinal axis of the hippocampus, with the scale of representation gradually increasing from less than 1 meter at the dorsal pole to ~10 meters at the ventral pole (Kjelstrup et al., 2008). Therefore, as for spatial resolution, DH is well suited in making precise representations of an environment, whereas VH only makes coarser representations.

1.2.3.4 Differential neurochemistry along the dorsoventral axis

Another aspect regarding heterogeneity along the DV axis concerns differential concentration of neurotransmitters and density of neuromodulatory innervation along this dimension. A marked concentration gradient, increasing from dorsal to ventral regions, has been reported for the following neurotransmitters: acetylcholine (Amaral and Kurz, 1985; Hoover et al., 1978; Hortnagl et al., 1991; Milner et al., 1983), noradrenaline (Hortnagl et al., 1991; Oleskevich et al., 1989; Young and Kuhar, 1980), serotonin (Hortnagl et al., 1991; Lombardi et al., 1987; Oleskevich and Descarries, 1990), dopamine (Hortnagl et al., 1991; Verney et al., 1985) and somatostatin (Hortnagl et al., 1991). Moreover, ventral CA1 and ventral subiculum receive the bulk of dopaminergic innervation (Verney et al., 1985). This concentration gradient of transmitters, neuromodulators, receptors and fibre termination density suggests that VH is associated with a greater density of neuroregulatory pathways than DH.

1.2.3.5 Gene expression studies

Recent studies based on genome-scale hippocampal gene expression have revealed that subfields of the hippocampal formation are parcelled into several discrete subdomains exhibiting unique and regionalised gene expression patterns (Dong et al., 2009; Lein et al., 2004; Thompson et al., 2008). Interestingly, especially in CA3, those patterns have been shown to delineate “molecular boundaries” along the dorsoventral and proximodistal axis

giving rise to nine distinct subregions. The sharpest boundaries involved the ventral quarter of the hippocampus, consisting itself of multiple discrete subdomains (Thompson et al., 2008).

Altogether, there is now convincing evidence at the behavioural, anatomical, physiological, neurochemical, genetic and molecular level that the hippocampus is heterogeneous along its longitudinal and proximodistal axes. However, whether the resulting domains represent functionally distinct units still remains to be confirmed.

1.2.4 Hippocampal formation: unitary structure or distinct domains?

Converging evidence for heterogeneity of the hippocampus along its DV axis could imply that different regions may perform different functions. However, the basic architecture of the intrinsic hippocampal circuitry is virtually equivalent throughout the entire longitudinal dimension (Anderson et al., 1971), raising the possibility that the hippocampal formation may operate as a unitary structure.

As mentioned in section 1.2.1, there are several types of hippocampus-dependent memories. Therefore, the question arises whether one common circuit subserves all of these functions or whether differential neuronal populations are involved. Also, it is not clear to what extent specific mnemonic functions are restricted to specific regions of the hippocampal formation. On the one hand, the multiple forms of hippocampus-dependent memory may be subtypes of a single, more general type of memory that involves the entire hippocampus. On the other hand, distinct hippocampus-dependent forms of memory may depend on separate intrahippocampal circuits, which may be segregated or overlaid (Moser and Moser, 1998).

Support to the first hypothesis comes from the intrinsic hippocampal circuitry, which revolves around the trisynaptic loop throughout the longitudinal axis and whose major characteristics are preserved in both dorsal and ventral domains (Anderson et al., 1971). In addition, place fields have been reported throughout the hippocampus (Jung et al., 1994), and it has been proposed that the dorsal and ventral hippocampus participate in similar processing of information (Rudy and Matus-Amat, 2005).

In support to the second view, recent evidence suggests that the hippocampus is functionally differentiated along its DV axis, and behavioural dissociation between the dorsal and the ventral hippocampus have been proposed (Bannerman et al., 2002; Bannerman et al., 2004; Bannerman et al., 1999; Moser et al., 1993; Moser et al., 1995). Accordingly, the dorsal and ventral hippocampus may perform dissimilar functions and be quite independent from each other. However, these two views (integrated unit versus separate domains) may not be completely incompatible. Owing to the similar basic architecture of hippocampal intrinsic circuitry along the DV axis, it is likely that the dorsal and ventral portions of the hippocampus use the same computational algorithm to process different kinds of information and perform different functions.

1.2.5 Putative functions of the ventral hippocampus

The ventral hippocampus is somewhat disconnected from the rest of the structure, both in terms of intrahippocampal and extrahippocampal connectivity (Moser and Moser, 1998) and section 1.2.3.1), and may therefore be executing distinct types of functions independently from the dorsal hippocampus.

Studies based on VH lesions have shown that it plays a role in fear and anxiety (Bannerman et al., 2003; Bannerman et al., 2004; Kjelstrup et al., 2002), but its role in learning and memory is less clear. Studies in animals and humans have reported a possible role of the ventral hippocampus (or anterior hippocampus in humans) in goal-directed behaviour (Burton et al., 2009; Viard et al., 2011) and novelty (Dolan and Fletcher, 1997; Strange et al., 2005; Strange et al., 1999; Tulving et al., 1996).

Altogether, although the precise role played by VH still remains to be determined, strong evidence suggests that it can be functionally different from and act independently of the dorsal hippocampus.

1.3 MICROCIRCUITS AND SUBPOPULATIONS OF PRINCIPAL NEURONS

Functionality of brain circuits often relies on specificity of connections, suggesting that connectivity patterns are not randomly established (Ko et al., 2011b; Song et al., 2005). In this last part of the introduction, I will mention examples of selective connectivity in the brain, with a major emphasis on subpopulations of principal neurons in the hippocampus, our model system to study connectivity and plasticity.

1.3.1 Selective connectivity and microcircuits

The neocortex exhibits sparse and highly specific synaptic connectivity (Song et al., 2005; Yoshimura and Callaway, 2005; Yoshimura et al., 2005), with functional columns being further organised into subcircuits (Kampa et al., 2006; Shepherd and Svoboda, 2005; Yoshimura and Callaway, 2005). Moreover, specific synapses form preferentially among sister excitatory neurons, suggesting that microcircuits develop preferentially within ontogenic radial clones of excitatory neurons in the developing neocortex (Yu et al., 2009). Therefore, investigating selective connectivity between defined subpopulations of neurons may shed light onto how information is processed in neural circuits.

Subpopulations of neurons in the nervous system often exhibit distinct connectivity which may relate to specific functions. In the basal amygdala, for example, at least two subpopulations of neurons have been identified based on their activity patterns exhibited during freezing behaviour (Herry et al., 2008): “fear neurons” and “extinction neurons”, which are active upon fear expression or extinction, respectively. These two subpopulations are integrated into discrete neuronal circuits that are differentially connected with the hippocampus and the medial prefrontal cortex. For example, “fear neurons”, but not “extinction neurons”, receive input from the ventral hippocampus (Herry et al., 2008), revealing how the importance of selective connectivity for reliable function of neuronal circuits.

1.3.2 Defined subpopulations of principal neurons in the hippocampus

Recently, a study from our laboratory demonstrated the existence of genetically defined subpopulations of principal neurons in all main subfields of the hippocampal formation (DG, CA3, CA1) (Deguchi et al., 2011). Taking advantage of sparse Thy1 mouse reporter lines (Lsi1 and Lsi2) based on a modified version of the *Thy1.2* promoter cassette (Caroni, 1997), it was shown that genetic sister neurons preferentially connect to each other across subfields, and that this selectivity results from temporally matched neurogenesis and synaptogenesis of neurons belonging to the same subpopulation. As a result, distinct microcircuits emerge in the hippocampus, but their functional significance still remains to be determined. In fact, it is plausible that not only different regions (dorsal versus ventral), but also distinct subcircuits within the hippocampus may be recruited for encoding and retrieving distinct episodic representations.

Abstract

In this work, I investigated region- and subpopulation-dependent rules for plasticity in the adult mouse hippocampus by adopting an approach combining transgenic mouse reporter lines, behavioural studies, high resolution imaging and microarray analysis.

The first part of the results reports a study carried out in collaboration with Sarah Ruediger (FMI, Basel) and aiming at functionally dissociating the roles of dorsal and ventral hippocampus in hippocampal-dependent learning. Taking advantage of "sparse" reporter mice (Thy1-Lsi1) expressing membrane-targeted GFP in a subset of neurons and using the growth of mossy fibre filopodial synapses that mediate feed-forward inhibition to the network in CA3 as a specific readout for learning, I show that the dorsal hippocampus encodes spatial information and is specifically recruited for spatial learning, while the ventral hippocampus encodes goal-oriented information and is specifically recruited for goal-oriented learning. Moreover, the results reveal objective distinctions at the circuit level between hippocampal-dependent memory and hippocampal-dependent learning.

The second part addresses the topic of microcircuits in the hippocampus and aims at identifying subpopulation-specific rules for structural plasticity. I show that LMT anatomy in three different subpopulations of granule cells (Lsi1, Lsi2 and Lsi3) differs under baseline conditions, and that distinct subpopulations respond in unique ways to experience and learning. These results suggest that distinct subtypes of granule cells are functionally different and that separate microcircuits are differentially recruited upon specific behavioural tasks.

2. RESULTS

2.1 Local learning-related plasticity in ventral and dorsal hippocampus driven by reward-based behavioural requirements

Dominique Spirig, Sarah Ruediger and Pico Caroni

Unpublished results

2.1.1 Summary

Converging evidence from connectivity, neurochemistry, gene expression and behavioural studies supports the notion that the hippocampus is heterogeneous along its dorsoventral (DV) axis (Fanselow and Dong, 2010; Moser and Moser, 1998; Moser et al., 1995; Thompson et al., 2008). Although the ventral hippocampus (VH) has been reported to play a role in anxiety-related and goal-oriented behaviours (Bannerman et al., 2002; Bannerman et al., 2003; Burton et al., 2009; Viard et al., 2011), little is known about its function in hippocampus-dependent learning. Here, we investigated the behavioural contribution of VH in the hippocampal-dependent Morris water maze (MWM) task and propose that it is involved in representing the nature of a goal-oriented learning task.

Using growth of mossy fibre filopodial synapses mediating feed-forward inhibition (FFI) connectivity onto CA3 pyramidal neurons as a readout for learning, as well as thorough behavioural analyses and rearrangement of CA3b pyramidal neuron ensembles upon MWM learning, we show that: 1) FFI connectivity in the dorsal hippocampus (DH) begins to increase after 5-6 days of MWM training and reflects spatial learning; 2) FFI connectivity growth in VH occurs within two days of a goal-oriented learning task; 3) VH is required for stability of performance throughout MWM training, but not for establishment of a reference memory; 4) FFI connectivity growth in DH upon a MWM task can occur independently from VH; 5) VH is recruited specifically during goal-oriented learning tasks, but not purely memory-based tasks (such as novel object recognition test).

Taken together, these results suggest that VH is critically involved in encoding goal-oriented information and is specifically recruited upon goal-oriented learning tasks. In addition, they provide further evidence for a functional dissociation between DH and VH, which are involved in two different aspects of learning paralleled by a selective local increase in FFI connectivity. While DH encodes spatial information and is recruited upon spatial learning, VH is likely involved in learning the nature of the goal of a task and is constantly engaged throughout learning.

2.1.2 Introduction

The hippocampal formation is a brain structure crucial for learning and memory. Its role is to produce relational representations of episodes, to arrange them according to familiar and novel features and to transfer some of that information into long-term storage sites (Eichenbaum, 1997; Squire, 2009). A growing body of evidence indicates that it plays a central role in encoding and retrieving information upon various spatial (Fanselow, 2000; Jung et al., 1994; Moser et al., 1995; O'Keefe and Conway, 1978) and non-spatial (Eichenbaum, 1996; Eichenbaum et al., 1992; Hock and Bunsey, 1998; Kennedy and Shapiro, 2004; Parsons and Otto, 2008) learning tasks.

Anatomical, connectivity, behavioural and physiological studies suggest the existence of a functional dissociation along the dorsoventral (DV) axis (Bannerman et al., 2004; Moser and Moser, 1998; Richmond et al., 1999). Furthermore, a recent study based on a genome-wide gene expression analysis identified molecular boundaries that revealed the existence of at least three distinct domains in the hippocampus: dorsal, intermediate and ventral, abbreviated here as DH, IH and VH, respectively (Thompson et al., 2008).

DH and IH are innervated by the lateral band of the entorhinal cortex (EC) and receive visual, auditory and somatosensory cortical information (Witter, 1986; Witter, 1993). Conversely, VH is reciprocally connected to the amygdala and hypothalamic nuclei (Petrovich et al., 2001; Pitkanen et al., 2000), which are involved in emotional, affective and motor output behaviours. As a result of this differential connectivity, DH predominantly processes visuo-spatial information and is thus implicated in encoding precise spatial representations of an environment (McHugh et al., 2008; Moser et al., 1993; Moser et al., 1995), whereas VH may have a prominent role in a variety of emotional or anxiety-related behaviours (Adhikari et al., 2010; Bannerman et al., 2004; Rogers et al., 2006; Yoon and Otto, 2007), goal-directed behaviours (Burton et al., 2009; Viard et al., 2011) and novelty (Dolan and Fletcher, 1997; Strange et al., 2005; Strange et al., 1999; Tulving et al., 1996).

The predominant role of DH in spatial learning is consistent with electrophysiological data indicating that, in comparison to VH, DH contains both a higher proportion of and more sharply tuned place cells (Jung et al., 1994). Moreover, place field size gradually increases

from the dorsal to the ventral pole (Kjelstrup et al., 2008), resulting in spatial representations that are sharp and precise in DH and more coarse in VH.

Although VH has been reported to be involved in emotion-related behaviours (Bannerman et al., 2002; Bannerman et al., 2003; Kjelstrup et al., 2002), very little is known about its possible roles in hippocampus-dependent learning and memory (Hunsaker et al., 2008). Addressing this question has remained particularly challenging owing to the lack of appropriate read-out strategies.

Studies from our laboratory have reported the existence of structural rearrangements of large mossy fibre terminals (LMTs) in the adult hippocampus in response to ageing, experience and learning in the dorsal hippocampus (De Paola et al., 2003; De Paola et al., 2006; Galimberti et al., 2006; Gogolla et al., 2009; Ruediger et al., 2011). Based on the notion that DH and VH are differentially involved in several behavioural tasks (Bannerman et al., 2002; Moser and Moser, 1998), we asked whether structural remodelling of LMTs would differ along the DV axis and, if so, whether this could give us insights into possible differential functional roles of DH and VH in hippocampus-dependent learning.

In order to address these questions, we took advantage of *Thy1-mGFP* (line Lsi1) reporter mice overexpressing membrane-targeted GFP in a subset of principal neurons (Caroni, 1997; De Paola et al., 2003; Galimberti et al., 2006). This "sparse" line allows high-resolution sampling of individual LMTs, whose morphology and structural plasticity can be efficiently analysed. We analysed LMT morphology in baseline conditions and upon various learning paradigms in the region CA3b along the entire DV axis. Our readout was mainly based on two parameters: LMT filopodial contents, which mediate feed-forward inhibition (FFI) onto CA3 pyramidal neurons (Acsady and Kali, 2007; Lawrence and McBain, 2003), and levels of c-Fos in CA3b pyramidal cells, an immediate-early gene whose upregulation has been correlated with activity-related plasticity in neurons (Kubik et al., 2007).

We found that contextual fear conditioning (cFC), which involves powerful hippocampus-dependent associative learning, elicited a comparable growth of FFI in DH and VH. By contrast, a hippocampus-dependent spatial task based on incremental learning (Morris water maze, MWM) involved different kinetics of FFI connectivity growth in DH, IH and VH.

As opposed to DH, where filopodial contents reached plateau levels after 9 days of training (Ruediger et al., 2011), levels in VH were readily increased after only two days of exposure to the "goal" (safety platform), even before criterion was reached (a reference memory only begins to form after 5 – 6 days (Ruediger et al., 2011)). In addition, we found that performance and learning strategy were affected in VH-lesioned mice not only during the initial phase of learning but throughout the entire training (9 days). A hippocampus-dependent task lacking a learning (or "goal") component (novel object recognition test), as well as a paradigm including only one single goal exposure, failed to increase filopodia numbers on granule cell LMTs in VH.

Taken together, our findings show that differential learning-mediated growth of feed-forward inhibition connectivity in DH and VH reflects their distinct role in hippocampal learning, namely spatial learning and goal-oriented learning, respectively. In addition, our results provide objective evidence at the circuit level for a dissociation between hippocampal memory and hippocampal learning.

2.1.3 Results

Region-specific characterisation of LMTs under baseline conditions

Given the widespread heterogeneity along the hippocampal longitudinal axis, we first asked whether LMTs from separate regional domains along the dorsoventral axis (DH, IH and VH) may exhibit different morphologies under baseline conditions. In order to reproducibly sample accurate dorsoventral locations and obtain a maximal number of LMTs per hippocampal region, we cut the hippocampi perpendicular to their longitudinal axis so as to obtain transverse lamellar sections from every level (see section 4.3 and Figure 1). This technique was not only required to identify DH, IH and VH in an unbiased manner, but also to preserve the course of mossy fibres, thereby facilitating the sampling and morphological analysis of LMTs in CA3b. LMT anatomy of naïve adult males aged between P55 and P80 was analysed in DH, IH and VH based on LMT size and complexity, mean number of filopodia per LMT and frequency of satellite LMTs (for a definition of satellites, see page 17).

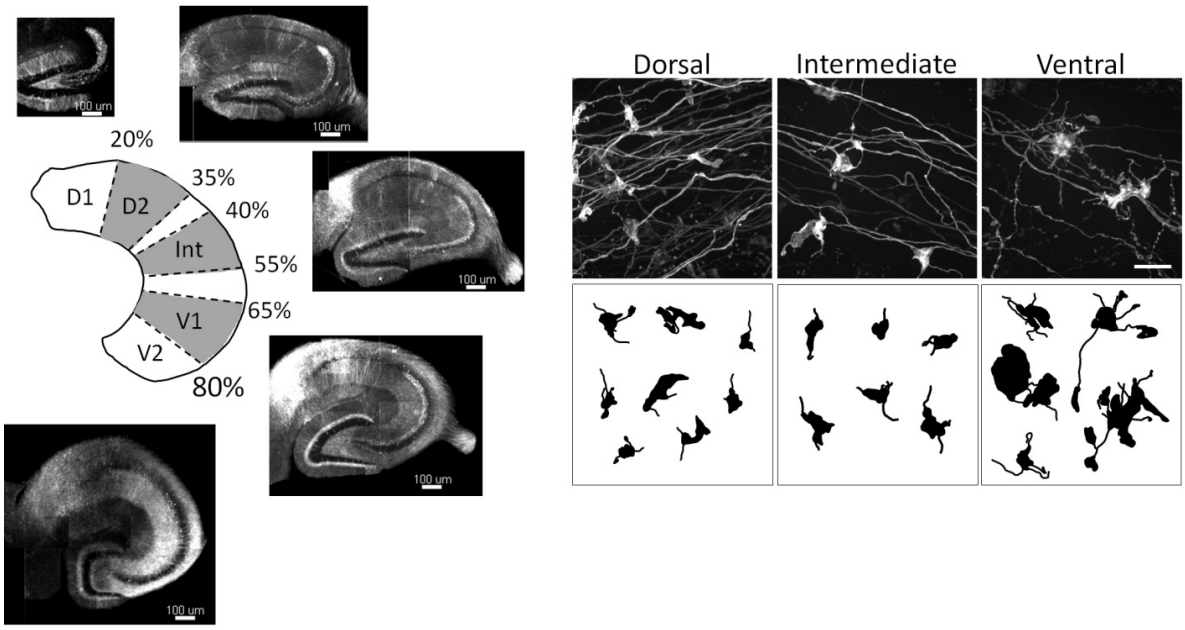


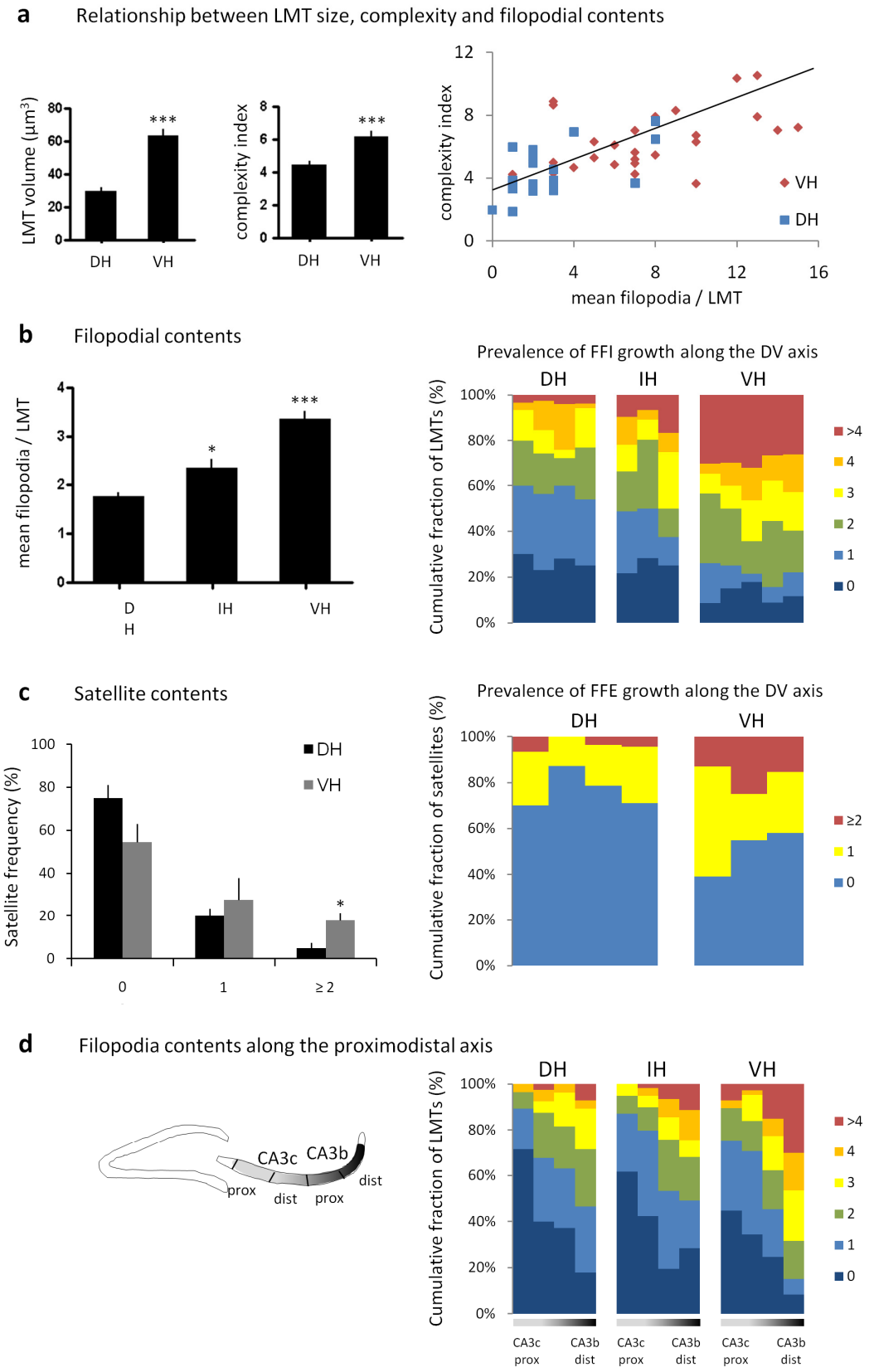
Figure 1: Characterisation of LMT morphology under baseline conditions along the dorsoventral axis.
(a) Schema showing the subdivision of the hippocampus into three domains: dorsal (D1+D2), intermediate (Int) and ventral (V1+V2). LMTs were analysed in the CA3b region of D2 (for DH), Int (for IH) and V1 (for VH) (highlighted in grey). A representative image of a hippocampal slice for each corresponding level is shown (lamellar sections). **(b)** Representative micrographs and camera lucida examples of LMT morphology from the dorsal (left), intermediate (middle) and ventral (right) hippocampus. Note the difference in size and complexity between dorsal and ventral LMTs. Scale bar: 10µm.

LMT complexity, defined as the convolution degree of LMTs, was expressed by the complexity index, corresponding to the ratio between measured surface areas and calculated surface area of a sphere of equal volume. On average, LMTs in VH were larger (2.13-fold, $p < 0.0001$) and more complex (1.39-fold, $p < 0.0001$) than in DH (Figure 2a), suggesting a higher synaptic transmission from core LMTs onto CA3 pyramidal neurons in VH (Galimberti et al., 2006). Interestingly, we found a positive correlation between LMT complexity and mean filopodial contents per LMT (Figure 2a), suggesting that higher synaptic transmission by larger LMTs may be counterbalanced by increased FFI (Lawrence and McBain, 2003). Compared to DH, filopodial contents in IH and VH were significantly increased (1.33-fold, $p < 0.05$ and almost 2-fold, $p < 0.0001$, respectively), revealing a growth in FFI connectivity along the longitudinal axis (Figure 2b). To estimate what fraction of LMTs was predominantly affected, we grouped LMTs according to their filopodial content (0, 1, 2, 3, 4 and >4 filopodia per LMT) in order to obtain a filopodia/LMT distribution (Figure 2b). Compared to DH, the fraction of LMTs without filopodia was not

changed in IH. However, a ~30% decrease in the portion of LMTs with one filopodium/LMT, combined with an approximately 3-fold increase in the fraction of LMTs with >4 filopodia/LMT revealed that, on average, at least 20% of the LMTs in IH established increased numbers of filopodia compared to DH. In VH, the difference was dramatic: here, we observed a 55% and a 65% decrease in the fraction of LMTs with zero and one filopodium/LMT, respectively. Concomitantly, a slight increase in the fraction of LMTs with 2 and 3 filopodia/LMT, as well as an 8.5-fold increase in the population with >4 filopodia/LMT, revealed that as many as 70% of the LMTs in VH exhibited increased filopodial contents compared to DH (Figure 2b). Taken together, these results reveal a steep, non-linear increase of FFI plasticity along the dorsoventral hippocampal axis under baseline conditions.

Since FFI connectivity was altered along the dorsoventral axis, we next asked whether FFE connectivity, mediated by "satellites", would differ as well. To this end, we quantified satellite frequency and found that it was higher in VH than in DH (Figure 2c). In DH, ~75% of LMTs did not exhibit any satellite, while this fraction dropped to 50% for LMTs in VH. Therefore, there were twice as many terminal arborisations in VH compared to DH. Moreover, LMTs with two or more satellites were rare (less than 5%) in DH but more frequent (~20%) in VH (Figure 2c), revealing increased FFE connectivity in the ventral hippocampus.

Since CA3 pyramidal neurons located at different positions along the proximodistal axis exhibit differential connectivity and may be functionally heterogeneous (Ishizuka et al., 1990; Witter, 2007), we investigated whether FFI connectivity in baseline conditions may also differ along the proximodistal axis of CA3. At all dorsoventral levels we found an increase in FFI connectivity along the proximodistal axis (from CA3c, closest to the hilus, to distal CA3b, closest to the fimbria). The fraction of LMTs with no filopodia decreased from 60-70% to 20-30% in DH and IH, while the fraction of LMTs with at least 3 filopodia per LMT increased up to 8-fold (Figure 2d). In VH, the increase in FFI connectivity along the proximodistal axis was steeper: the fraction of LMTs with zero and one filopodia decreased from 45% and 30% to 6% – 8%, respectively. At the same time, the fraction with 4 and >4 filopodia per LMT increased from 3% – 7% to 15% – 30 %, respectively (Figure 2d).


a
Relationship between LMT size, complexity and filopodial contents
LMT volume (µm³)
***
DH
VH
complexity index
***
DH
VH
complexity index
mean filopodia / LMT
VH
DH
b
Filopodial contents
mean filopodia / LMT
*
***
D
H
IH
VH
Prevalence of FFI growth along the DV axis
DH
IH
VH
Cumulative fraction of LMTs (%)
>4
4
3
2
1
0
c
Satellite contents
Satellite frequency (%)
DH
VH
*
0
1
≥ 2
Prevalence of FFE growth along the DV axis
DH
VH
Cumulative fraction of satellites (%)
≥2
1
0
d
Filopodia contents along the proximodistal axis
CA3c
CA3b
prox
dist
dist
prox
DH
IH
VH
Cumulative fraction of LMTs (%)
>4
4
3
2
1
0
CA3c prox
CA3b dist
CA3c prox
CA3b dist
CA3c prox
CA3b dist

Altogether, these results reveal a significant increase in FFI connectivity along the two main hippocampal axes (dorsoventral and proximodistal) under baseline conditions, which may differentially affect CA3 pyramidal neuron activity at different positions along the hippocampus.

To determine whether the observed increase in FFI connectivity along the DV axis is a property intrinsic to the hippocampus, we analysed filopodial contents in *ex-vivo* organotypic hippocampal slices (DIV 20) obtained from dorsal and ventral portions of the hippocampus (Figure 3a). We observed a 2-fold increase of filopodial contents between DH and VH, consistent with what we found in adult mice *in vivo* (Figure 3b and 3c). Interestingly, this factor of two was conserved in individual pups, regardless of their absolute mean number of filopodia (Figure 3d). These results suggest that the differential FFI connectivity between DH and VH is most likely a property intrinsic to the hippocampus.

Figure 2: Differential LMT morphology along the dorsoventral and proximodistal axes of the hippocampus (page 33)
(a) Relationship between LMT size, complexity and filopodial contents. Left: average volume and complexity of dorsal and ventral LMTs. The complexity index was defined as the ratio between measured surface area and calculated surface area of a corresponding sphere of equal volume. Right: positive correlation between filopodial contents and complexity index; linear regression, $r = 0.64$. N = 30 – 40 LMTs. **(b)** FFI connectivity growth along the dorsoventral axis. Left: mean filopodia numbers for LMTs in three hippocampal regions. LMTs in VH exhibit almost 2 times more filopodia per LMT than DH. Right: prevalence of FFI connectivity growth along the DV axis. LMTs were subdivided into five groups according to their mean number of filopodia (0, 1, 2, 3, 4 and >4). Each vertical row corresponds to one animal. N = 5 – 7 mice; 50 – 100 LMTs per region and per mouse. **(c)** Left: satellite frequency (% of total LMTs) in DH and VH. Right: prevalence of FFE growth in DH and VH. LMTs were subdivided into three groups according to their mean satellite content (0, 1, ≥2). Each vertical row corresponds to one mouse. N = 3 – 5 mice; 50-100 LMTs per region and per mouse. **(d)** Left: schema representing the CA3 region and its subdivisions into four domains along the proximodistal axis (from proximal CA3c, light grey, to distal CA3b, dark grey). Right: representative example of FFI connectivity growth along the proximodistal axis. Filopodia/LMT distribution (% cumulative fraction of LMTs) is represented for every dorsoventral level (DH, IH and VH, block of four rows) at different proximodistal positions along CA3 (each of the four vertical rows within a block represents, from left to right: proximal CA3c, distal CA3c, proximal CA3b and distal CA3b). Prox = proximal; dist = distal.
* $p < 0.05$; *** $p < 0.001$.

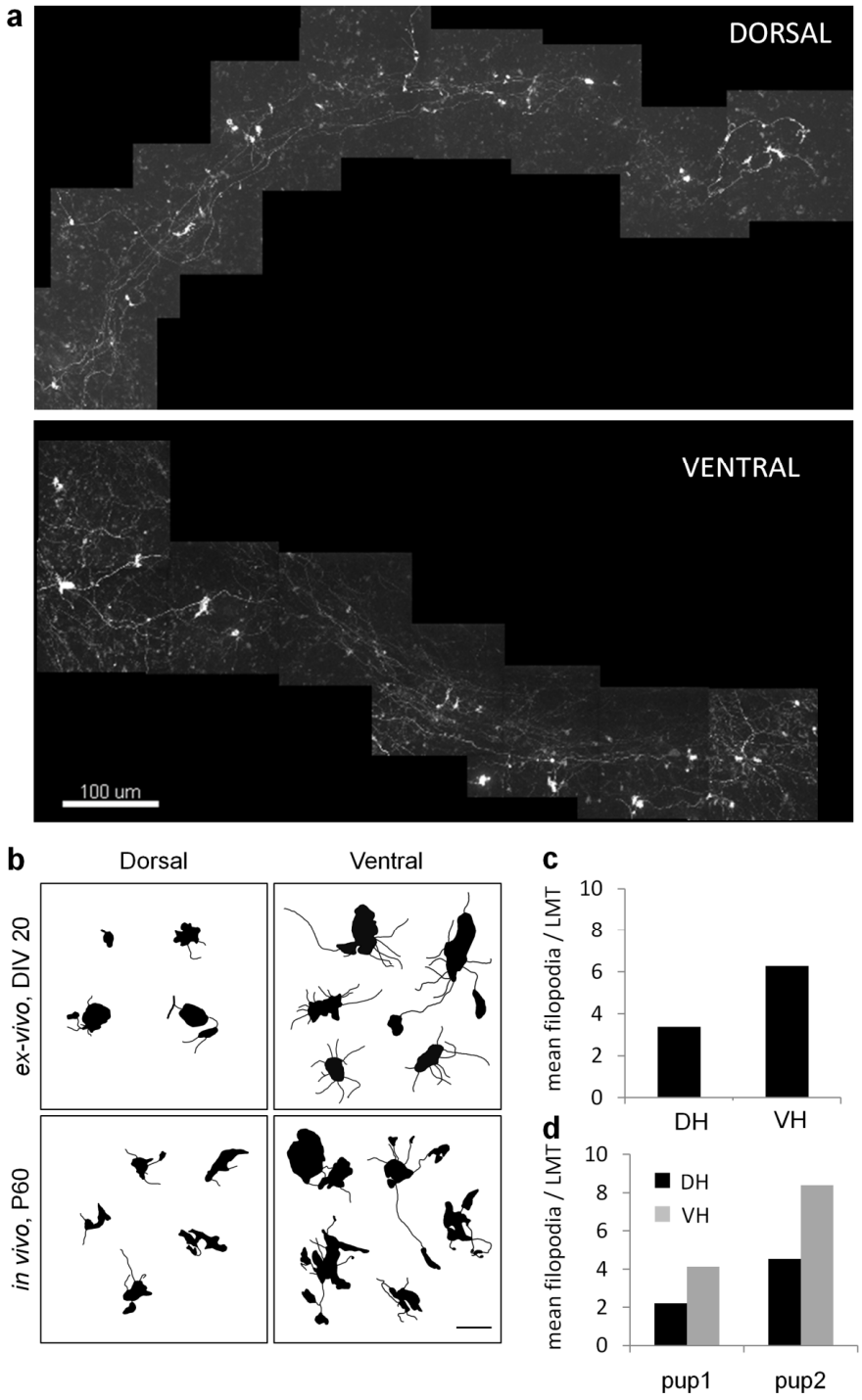


Figure 3: Increasing FFI connectivity along the DV axis is a property intrinsic to the hippocampus (page 36)
(a) Representative examples of a mossy fibre projection in organotypic hippocampal slice cultures from the dorsal (top) and the ventral (bottom) hippocampus at DIV 20. Slices were prepared from pups aged P7. **(b)** Ventral LMTs are more complex than dorsal LMTs both *ex-vivo* and *in vivo*. Representative camera lucida examples of LMTs in hippocampal slice cultures at DIV 20 (top) and *in vivo* from adult mice, P60 (bottom). **(c)** Mean number of filopodia per LMT in hippocampal slice cultures from DH and VH, DIV 20. N = 40 – 65 LMTs from 2 pups each. **(d)** Mean number of filopodia per LMT in hippocampal slice cultures from DH and VH for two individual pups (pup 1 and pup 2). Note that the ratio between dorsal and ventral filopodial contents is constant within one animal (2-fold).

Learning-induced plasticity along the DV axis

Since LMTs are known to remodel in response to ageing, experience and learning (Galimberti et al., 2006; Gogolla et al., 2009; Ruediger et al., 2011), we asked whether learning-induced plasticity would be differentially affected along the DV axis.

A recent study from our laboratory has shown that contextual fear conditioning (cFC) leads to a robust increase in FFI connectivity in the dorsal hippocampus (Ruediger et al., 2011). Since only the ventral, but not dorsal, hippocampus is reciprocally connected to the amygdala (Pitkanen et al., 2000), and since contextual fear conditioning is a behavioural paradigm that strongly depends on both brain structures (Bast et al., 2001; Bast et al., 2003; Biedenkapp and Rudy, 2009; Fanselow, 2000; Rudy and O'Reilly, 2001), we asked whether cFC may cause structural rearrangements in VH as well, and if so, to what extent.
We therefore subjected mice to a mild cFC protocol (see section 4.2) and tested them 24 h later for contextual fear memory (Figure 4b). We found that cFC elicited a rapid and robust growth of FFI connectivity not only in DH, but also in VH, as assessed by the massive increase in filopodial contents (Figure 4a). Remarkably, the kinetics of induction and the extent of FFI connectivity growth were very similar in DH and VH: filopodial contents were dramatically increased as early as 1 h after cFC (1.57-fold, $p < 0.01$ and 1.46-fold, $p < 0.001$, respectively) and reached maximal values after one day (1.83-fold change, $p < 0.001$ in both DH and VH) (Figure 4c). In order to estimate what fractions of LMTs were affected in VH, we plotted a filopodia/LMT distribution. The major shifts occurred in the LMT populations with 0 and 1 filopodia/LMT (2.7-fold decrease), 3 filopodia/LMT (6-fold decrease) and 4 and >4 filopodia/LMT (up to 2.4-fold increase) (Figure 4d).

In conclusion, we show that DH and VH exhibit the same kinetics of induction and magnitude of FFI connectivity growth upon cFC.

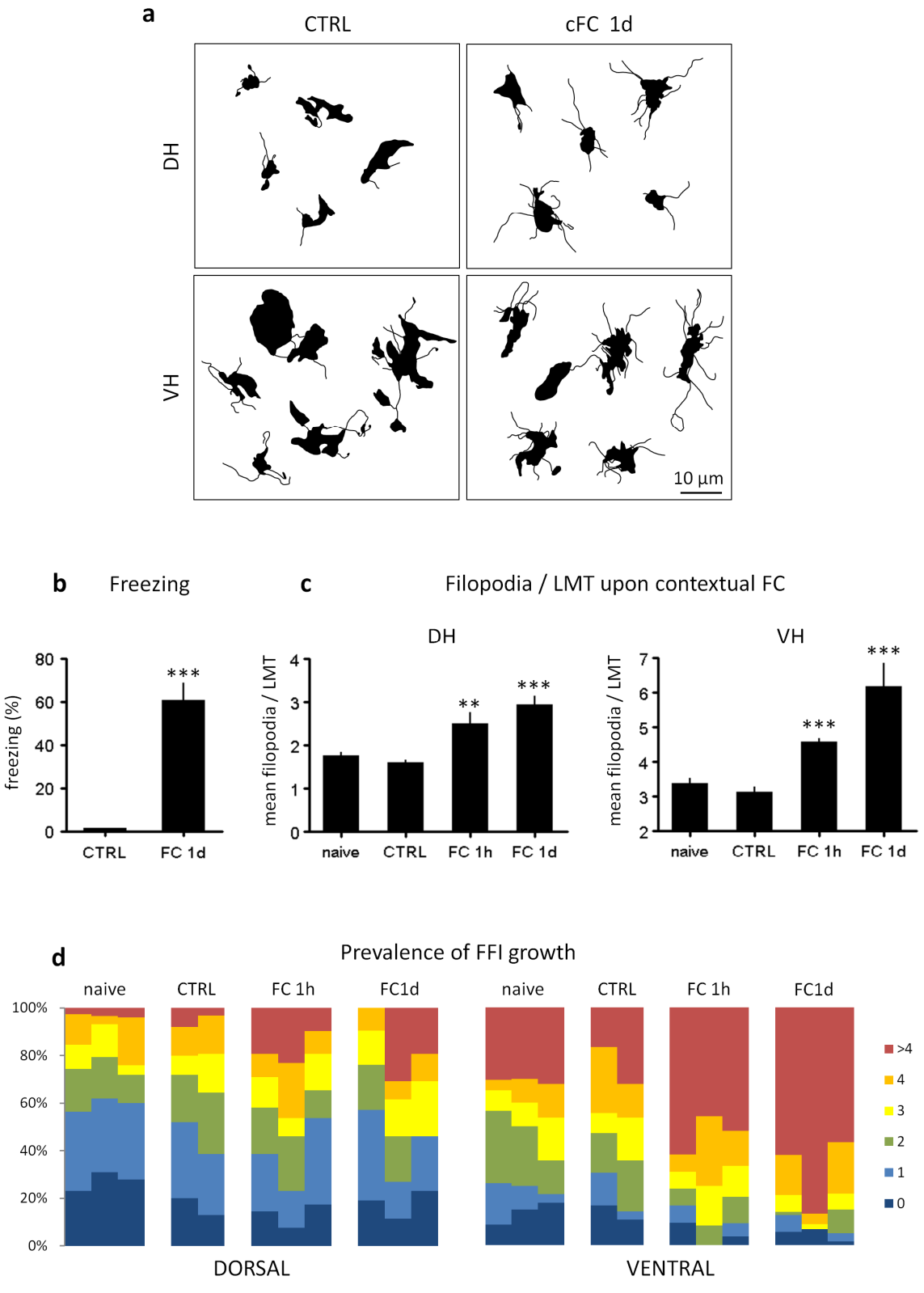

a
CTRL
cFC 1d
DH
VH
10 µm
b
Freezing
freezing (%)
CTRL
FC 1d
c
Filopodia / LMT upon contextual FC
DH
VH
mean filopodia / LMT
naive
CTRL
FC 1h
FC 1d
d
Prevalence of FFI growth
naive
CTRL
FC 1h
FC1d
100%
80%
60%
40%
20%
0%
DORSAL
VENTRAL
>4
4
3
2
1
0

Figure 4: Rapid and massive FFI connectivity growth in both DH and VH upon contextual fear conditioning (page 37)
(a) Dorsal and ventral LMTs exhibit increased filopodial contents upon contextual fear conditioning. Representative camera lucida examples of LMTs in dorsal (upper row) and ventral (lower row) CA3b in control (left) and 1d after contextual fear conditioning (cFC 1d, right). **(b)** Fear memory retention. Percentage of time spent freezing in the conditioning context 24h after training (n = 4 mice). **(c)** FFI connectivity growth 1 hour (1h) and 1 day (1d) after cFC in DH (top; modified from Ruediger et al., 2011, and VH (bottom). **(d)** Filopodia/LMT distribution for individual mice upon cFC in DH (left) and VH (right). Details as in Figure 2b. N = 50 – 100 LMTs from 3-5 mice each. ** $p < 0.01$; *** $p < 0.001$.

Olfactory fear conditioning does not lead to changes in FFI connectivity

Owing to its strong connectivity to the amygdala (Pitkanen et al., 2000), it could be argued that increased filopodial contents in VH upon cFC are specifically elicited by the emotional component of this aversive task. However, we have evidence that neither stress (e.g., acute swim stress, Figure 10) nor innate fear (S.R., unpublished results) cause any increase in filopodial contents in the hippocampus, therefore excluding these components as responsible for triggering FFI connectivity growth upon cFC.

To test whether fear learning in general was sufficient to induce FFI connectivity growth in VH, we designed another variant of the fear conditioning task lacking visuo-spatial cues, namely an olfactory fear conditioning (oFC) paradigm taking place in the dark. The chamber was abundantly wiped with 2% acetic acid, like in the standard version, but the light was turned off. Therefore, mice underwent fear conditioning to the olfactory component only (Figure 5a). In contrast to the cFC protocol, filopodial contents in DH or VH were not altered upon olfactory fear conditioning (Figure 5b and c), suggesting that fear learning per se is not sufficient to lead to FFI connectivity growth in VH.

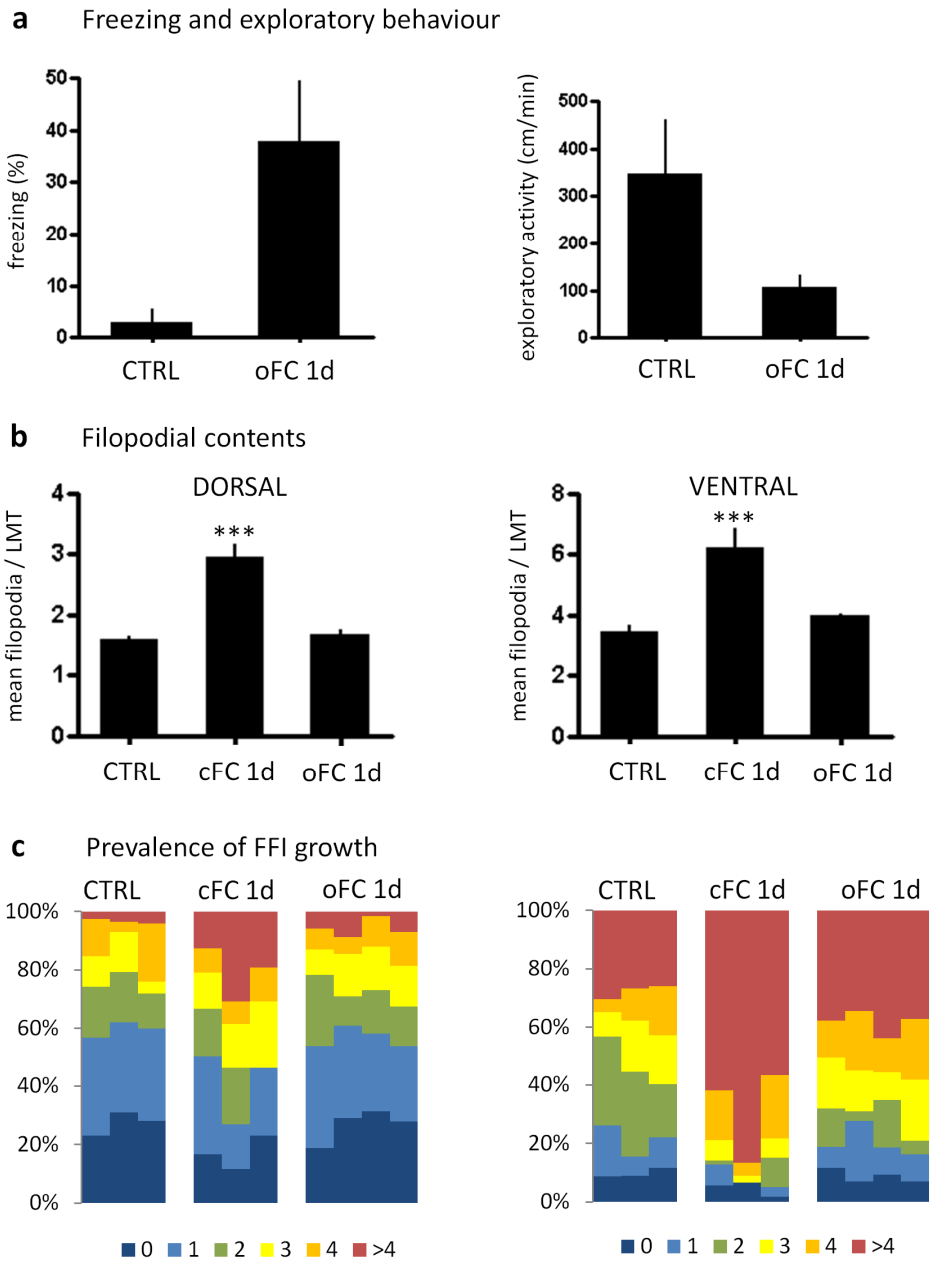


Figure 5: FFI connectivity is not changed upon olfactory fear conditioning
(a) Increased freezing and decreased exploration upon olfactory fear conditioning. Mice received 5 foot-shocks in a dark training chamber wiped with 2% acetic acid and were tested 24h later in a different context wiped with the same odour. Increased percentage of time spent freezing (left) and decreased exploratory activity (right) in the test context show that mice successfully formed an olfactory fear memory. **(b)** Filopodial contents one day after contextual (cFC) or olfactory fear conditioning (oFC) in dorsal (left) and ventral (right) hippocampus. CTRL mice (pooled from cFC and oFC experiments) underwent the same behavioural procedure but did not receive a foot-shock. **(c)** Distribution of filopodial contents in dorsal (left) and ventral hippocampus (right) upon contextual or olfactory fear conditioning, details as in Figure 2b. N = 50 – 100 LMTs from 3 – 5 mice each. ***$p < 0.001$.

Time course of FFI connectivity growth in DH and VH in response to a hippocampus-dependent incremental learning task

To address the role played by VH in hippocampus-dependent learning, we investigated the time course of FFI connectivity growth upon a Morris water maze (MWM) task, a hippocampus-dependent task based on incremental learning. We trained mice on a MWM protocol consisting of 9 days of training during which they had to learn to locate a fixed hidden platform (Figure 6a). During this learning protocol, escape latencies decrease steeply over the first 3 – 4 days (transition phase), indicating rapid improvement, and later decreased further (refinement phase) to reach plateau levels of performance at 8-9 days (Figure 6b). On the day following the last day of training, mice underwent a probe trial consisting of a 60-second swim in the absence of platform, and reference memory was assessed by quantifying the percentage of time spent searching in the correct (target) quadrant. Values became higher than chance only after ~ 5 days of training and reached plateau levels at day 9 (Figure 6c).

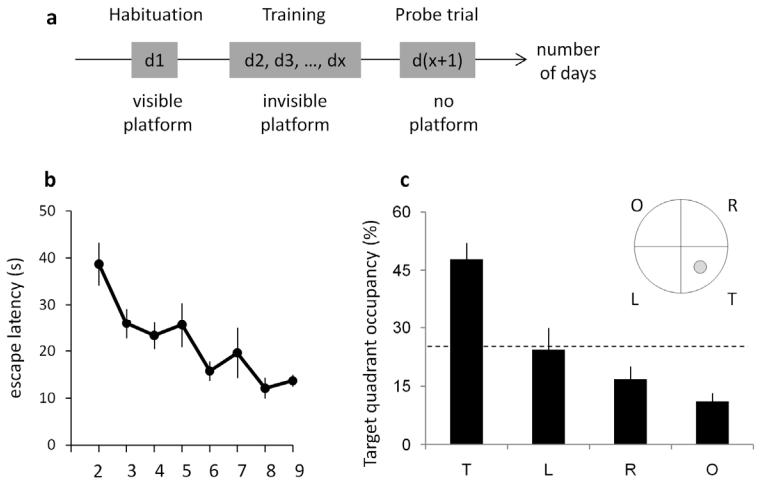
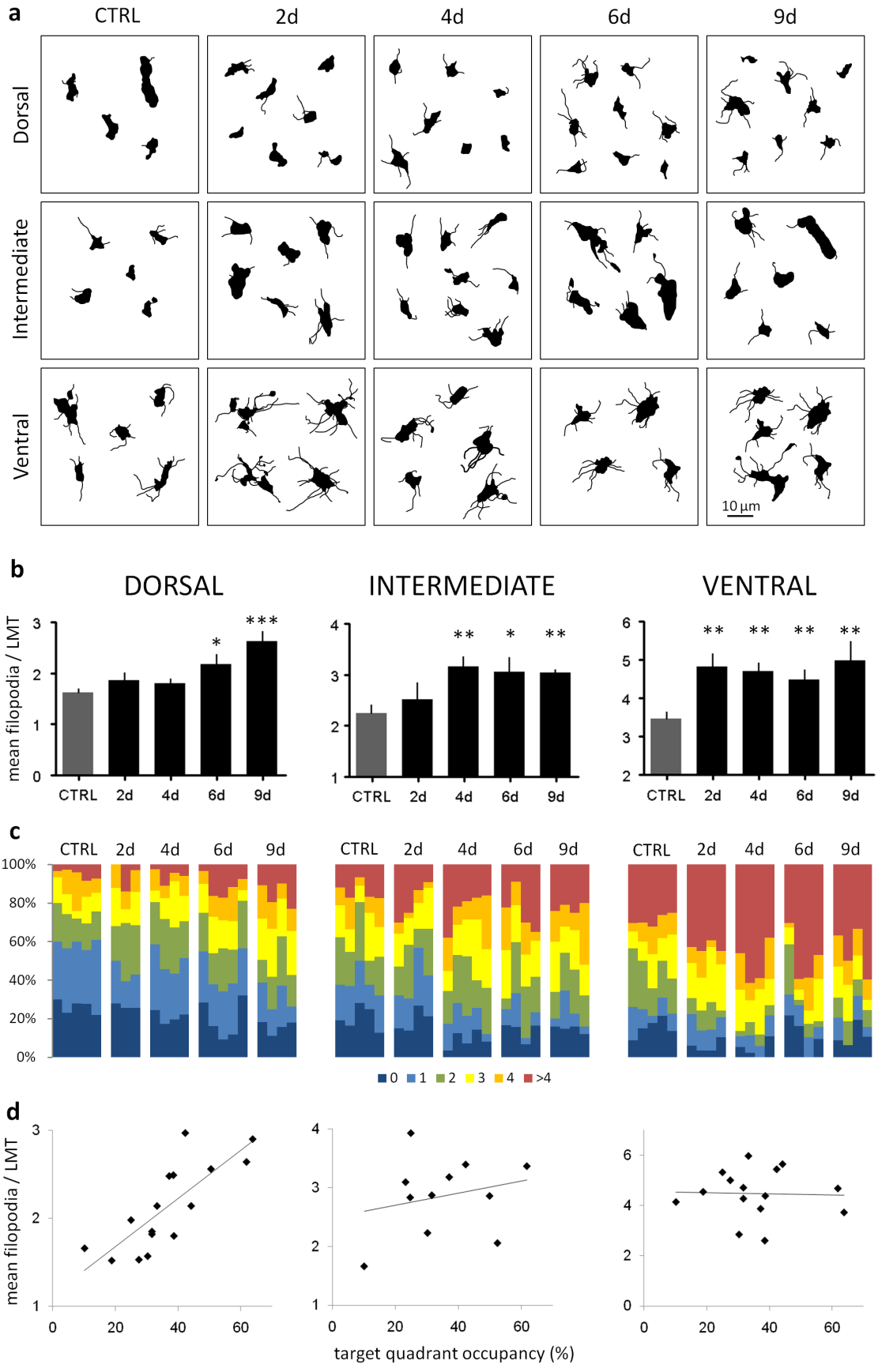


Figure 6: Morris water maze task
(a) Diagrammatic explanation of the (standard) training protocol. On day 1 (d1), naïve mice were habituated to the visible platform version of the task (4 swim trials, 60 s each, 5 min. inter-trial interval). Starting from the following day (d2), the platform was moved to the opposite quadrant and remained fixed (submerged) for the entire duration of the training (*x* days). On the day following the last training day (d(*x*+1)), mice underwent a probe trial (60-second swim) and were sacrificed within the following 10 min. **(b)** Mean escape latency to find the submerged platform. Values represent daily means (from four trials). **(c)** Performance during the probe trial. Time spent in each quadrant was recorded and expressed as a percentage of the total searching time. Levels around 50% (chance levels are at 25%, dashed line) indicate a clear preference for the target quadrant and hence successful reference memory formation. Top right: schematic drawing of the circular water maze, showing the location of the hidden platform (grey circle) in the target quadrant. L = left; O = opposite; R = right; T = target quadrant. N = 8 mice.

Our laboratory has recently shown that filopodial contents in the dorsal hippocampus increased only slightly over the first four days of training, whereas from the 5^{th} – 6^{th} day on they were markedly increased and culminated after 9 days (Ruediger et al., 2011) (Figure 7b). By contrast, we now found that filopodial contents in VH were significantly increased already by day 2 (1.39-fold, $p < 0.01$) and did not increase further at the following time points measured at 4, 6 and 9 days (1.36-fold, 1.29-fold and 1.44-fold, respectively; $p < 0.01$) (Figure 7b). The time course of filopodial induction in IH fell between that observed in DH and VH: mean numbers of filopodia per LMT reached plateau at day 4 (1.41-fold, $p < 0.01$), and at day 6 and 9 were 1.35-times higher than baseline ($p < 0.05$ for 6d and $p < 0.01$ for 9d)(Figure 7b). Interestingly, maximal fold change values were comparable across different hippocampal regions (1.6-fold in DH and 1.4-fold in IH and VH). These results reveal a differential onset of filopodia induction across hippocampal regions upon MWM (day 6 for DH, day 4 for IH and day 2 for IH), suggesting that distinct hippocampal domains may have different roles. Consistent with this notion, we found a regional dissociation in terms of correlation between filopodial contents and spatial memory precision. While this correlation was strong in DH (Ruediger et al., 2011), it was weaker in IH and absent in VH (Figure 7d), suggesting that separate regions of the hippocampus may be involved in processing distinct aspects of a learning task.

Figure 7: Differential time-course of FFI connectivity growth along the DV axis upon a MWM learning task and correlation with behavioural performance (page 42)
(a) Representative camera lucida examples of LMTs in dorsal, intermediate and ventral hippocampus as a function of the number of training days. Swim control mice (CTRL) underwent free swim trials in the absence of the platform. **(b)** Time course of FFI connectivity growth upon a MWM task. Note that plateau levels are reached after 9 days (left), 4 days (middle) and 2 days (right) of training in DH, IH and VH, respectively. **(c)** Time course of filopodia/LMT distribution for DH, IH and VH (details as in Figure 2b). **(d)** Correlation between reference memory precision and mean numbers of filopodia per LMT. Linear regression: $r = 0.79$ (left, DH); $r = 0.24$ (middle, IH) and $r = 0.04$ (right, VH). Each dot represents an individual mouse analysed between day 2 and day 9 of training procedure (60-100 LMTs each). Figure (**d**), left is modified from (Ruediger et al., 2011). N = 50 – 100 LMTs per condition and per mouse; n = 4 – 5 mice. * $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$


a
CTRL
2d
4d
6d
9d
Dorsal
Intermediate
Ventral
10 µm
b
DORSAL
INTERMEDIATE
VENTRAL
mean filopodia / LMT
CTRL
2d
4d
6d
9d
c
100%
80%
60%
40%
20%
0%
0
1
2
3
4
>4
d
mean filopodia / LMT
target quadrant occupancy (%)

How does an incremental learning task affect CA3 pyramidal cell ensembles along the dorsoventral axis?

Before exploring possible roles played by VH in a hippocampus-dependent learning task, we asked whether we would find a functional correlate of FFI connectivity growth at the level of CA3 pyramidal neuron ensembles. As a read-out for ensemble activity of pyramidal neurons in CA3b, we quantified the expression of the immediate early gene *c-Fos*, whose upregulation reflects activity-related plasticity (Kubik et al., 2007). For analysis, we considered c-Fos-immunoreactive (Fos+) cells that displayed an intensity value above a given threshold (see section 4.6).

In all three hippocampal regions, *c*-Fos expression increased across training days (Figure 8a). Compared to control mice, which underwent a single, 60-second long swim trial, c-Fos levels in DH were significantly increased after 4 days (2.51-fold, $p < 0.05$), 6 days (2.66-fold, $p < 0.01$) and 9 days of training (6.55-fold, $p < 0.01$). In IH, c-Fos levels increased across the training days and culminated at day 9 (4.44-fold, $p < 0.05$). Finally, in VH c-Fos levels were markedly increased as early as day 2 (almost three times higher than swim control mice) (Figure 8b). Interestingly, they were 2.08-fold (n.s) and 1.53-fold (n.s) higher than in DH and IH, respectively. Ventrally, c-Fos expression then slowly increased over the following days (4d: 3.53, $p < 0.05$; 6d: 4.16, $p < 0.001$) to reach highest levels at day 9 (5.81-fold, $p < 0.05$). At this time, 15% of all CA3b pyramidal neurons expressed c-Fos, or twice as many as after two days of training.

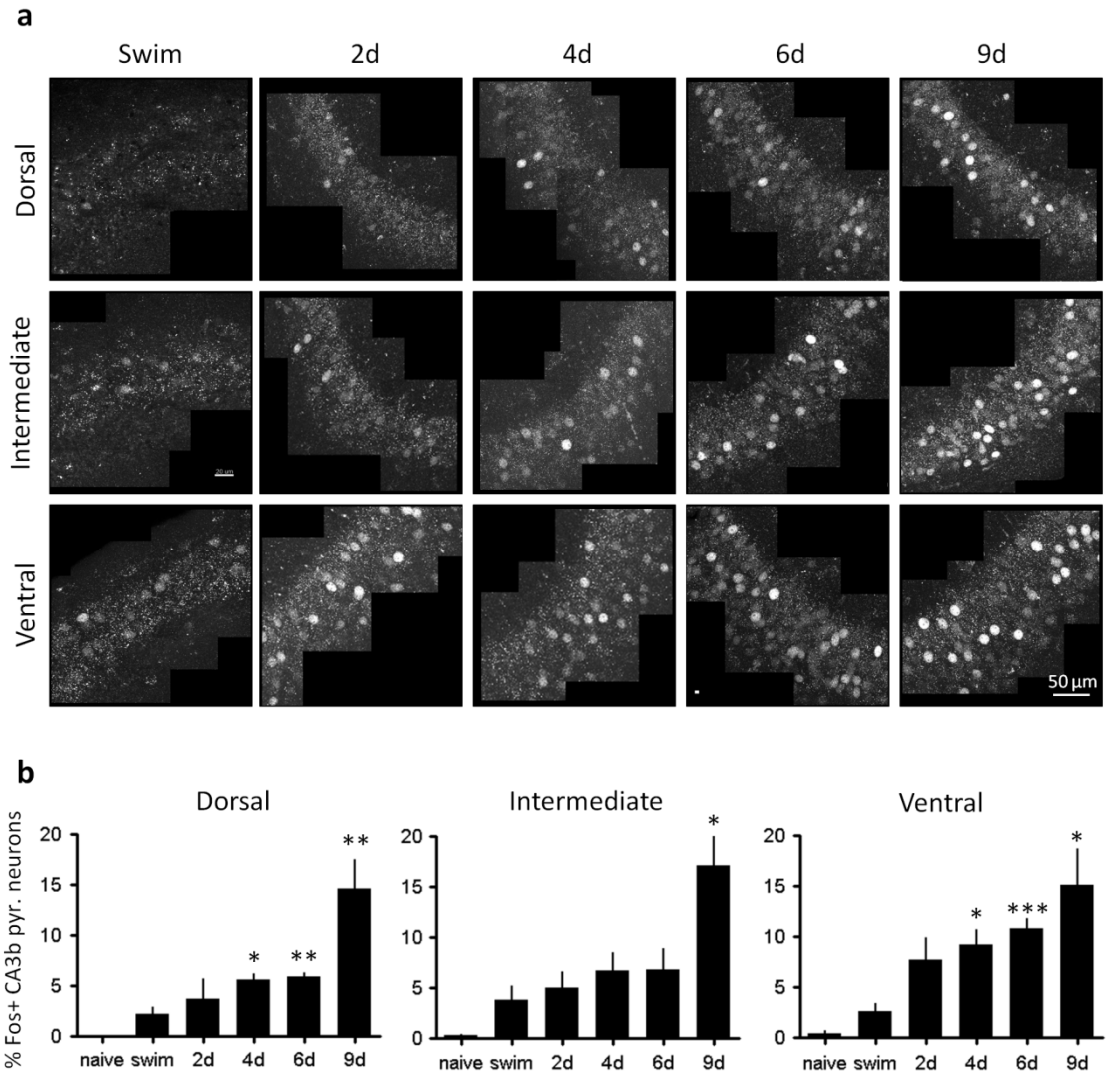


Figure 8: Region-dependent time course of c-Fos immunoreactivity during MWM training
(a) Representative micrographs of c-Fos immunoreactivity in dorsal, intermediate and ventral CA3b pyramidal cells across the training days (up to 9 days). **(b)** Quantification of Fos+ pyramidal neurons in CA3b in all three hippocampal regions over the training days (% of Fos+ over the total number of pyramidal neurons in CA3b). Values were compared to swim control mice which underwent a single 60-second swim trial in the absence of platform (swim). Note how values are already elevated on day 2 in the ventral hippocampus. For each region and animal, Fos+ cells were averaged from 3-4 sections. N = 3 – 4 mice.
* $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$

Three main findings emerge from these results: first, c-Fos levels increased across the training days in all three hippocampal regions; second, c-Fos levels and time course of FFI connectivity growth in DH and IH were comparable; third, levels after two days of training were more elevated in VH than DH or IH (2-fold and 1.5-fold higher, respectively).

Taken together, these results show that VH is the first hippocampal region to be recruited and undergo structural and functional changes upon an incremental learning task, both at the level of FFI plasticity and c-Fos expression.

FFI connectivity growth in VH may be induced by goal-oriented learning

What could be triggering, specifically in VH but not in DH, a growth of FFI connectivity and a higher recruitment of CA3 pyramidal neuron ensembles as early as two days after training on a MWM learning task? Based on the early time course of filopodia and Fos induction, as well as on the lack of correlation between filopodial contents and spatial memory precision in VH, we hypothesised that VH may be involved in learning the nature of the goal of a task. According to this view, we reasoned that early recruitment of VH would not be affected by modulating task difficulty or visibility of the platform, as long as the task involved learning a goal. To test this idea, we designed alternative MWM protocols and used FFI connectivity growth as read-out for VH recruitment.

Changing the size of the platform can be used to modulate task difficulty. For this purpose, we built two new platforms, which were twice (platform 2A) or half (platform 0.5A) the size of the standard platform (platform A). We found that when mice had to locate a larger platform (platform 2A), they exhibited reduced latencies throughout training (Figure 9a), reflecting the ease of the task. Remarkably, no spatial search strategy was recruited upon this less challenging task, and no reference memory was established (Figure 9b). Consistently, DH-mediated learning was abolished and no FFI connectivity growth was observed (Figure 9c). Conversely, DH was recruited upon a more difficult task (platform 0.5A), and accordingly reference memory was established and FFI connectivity in DH increased (1.77-fold, $p < 0.01$)(Figure 9a).

How would task difficulty affect FFI connectivity growth in VH? To answer this question, we trained mice for 2 or 6 days in a MWM task with a normal (A), a small (0.5A) and a large platform (2A). We found that, in all conditions, FFI plasticity in VH was markedly increased compared to baseline (CTRL) (on average, 1.4-fold change, $p < 0.001$) (Figure 9d), regardless of task difficulty. This result is consistent with the notion that understanding the goal of a task only requires goal exposure and is not dependent on its particular features (e.g., large or small platform).

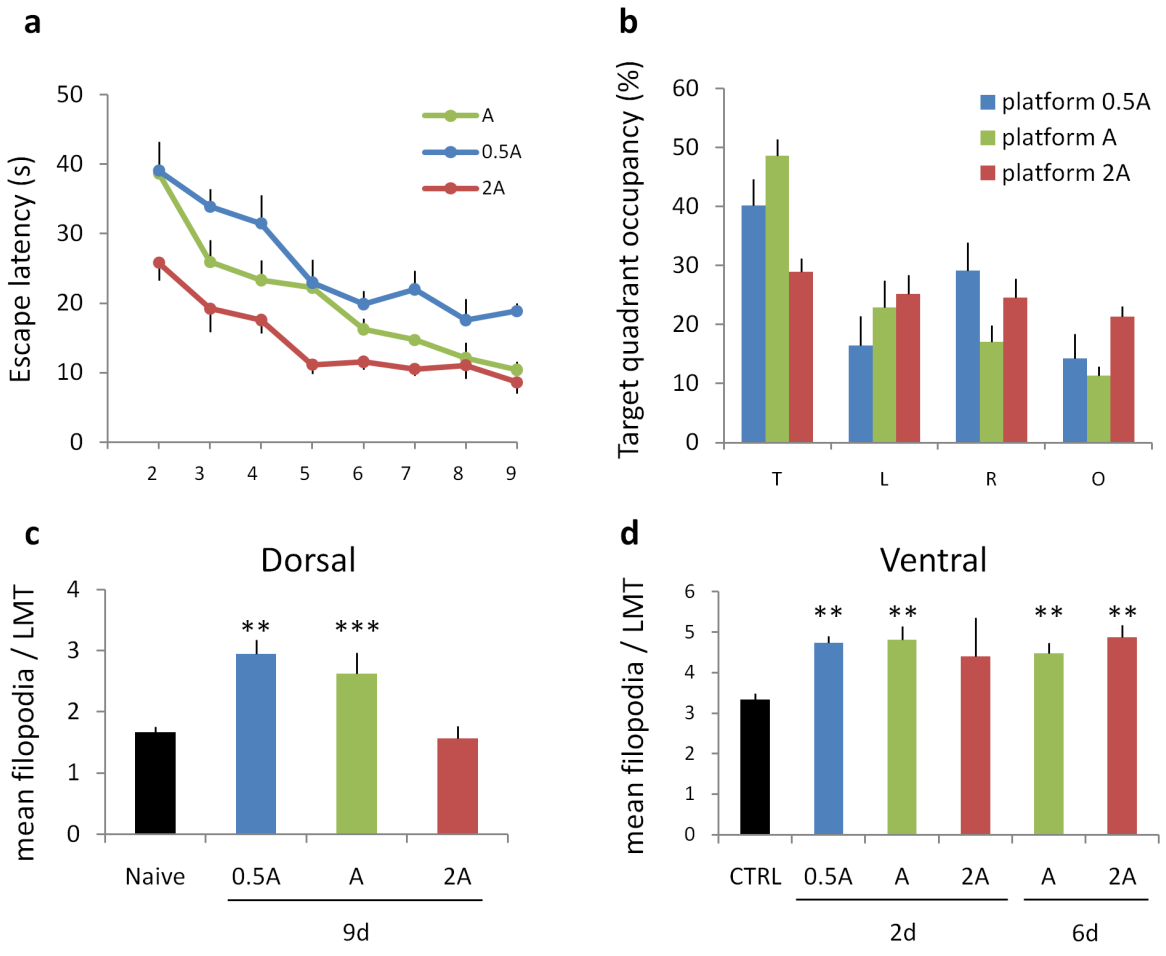


Figure 9: Effect of task difficulty on FFI connectivity growth in DH and VH.
(a) Effect of task difficulty on escape latency. Mice were trained for 9 days to locate a platform of variable size: standard (A), half (0.5A) or twice (2A) the size of the standard platform (10 cm diameter). **(b)** Failure to establish a reference memory upon a less challenging task (platform 2A). **(c)** FFI connectivity growth in DH is abolished upon a less challenging task (platform 2A) but present upon a standard (platform A) or more difficult (platform 0.5A) task. N = 4 – 8 mice per condition. **(d)** Increased FFI connectivity in VH regardless of platform size. Naïve and swim control mice were pooled into a single group (CTRL). N = 2 – 5 mice. ** $p < 0.01$; ***$p < 0.001$.

In support to the hypothesis that VH may be recruited upon a goal-oriented learning task, free swimming in the absence of a platform did not lead to increased FFI connectivity (Figure 10). Training the mice on a visible platform for two days triggered FFI connectivity growth in VH (1.59-fold, $p < 0.05$) (Figure 10), suggesting that platform presence but not its visibility plays a role in VH recruitment. Surprisingly, one single day of training on a visible platform did not increase LMT filopodial contents in VH (Figure 10), suggesting that at least two days of training in total are required to induce FFI connectivity growth in VH, regardless of the visibility of the platform.

Two options can be considered to explain this finding: on the one hand, merely the number of days after the initial exposure to the goal (platform) may be critical to increase filopodial contents, regardless of the number of trials; alternatively, it may be the number of training trials (in the presence of the platform) that is critical to induce FFI connectivity growth. In order to distinguish between these two possibilities, mice were subjected to two consecutive days of swim trials (4 trials per day). On the first day, the platform was visible, while on the second day it was absent (Figure 10). Mice were then sacrificed on the following day, so that in total they underwent two full days of "training" (one day with goal presence and one without). Interestingly, the mean number of filopodia per LMT was at baseline levels (Figure 10), suggesting that two days of performance in a goal-oriented task (i.e., two days of goal exposure) are required to engage VH. We next asked whether the first and the second goal exposure had to occur on consecutive days or whether they could also be temporally separated, for example by a seven-day gap (Figure 10). Upon this paradigm, filopodia contents were not increased compared to baseline (Figure 10), revealing that recruitment of VH upon the second exposure of a goal-oriented task only occurs if the delay between the first and the second exposure does not exceed a critical time window.

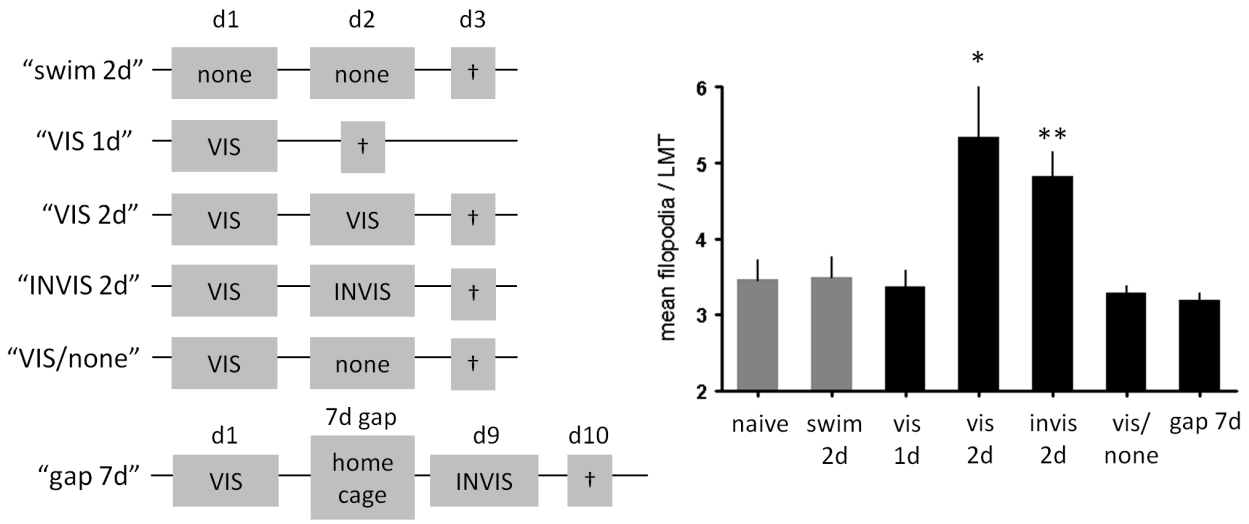


Figure 10: At least two days of goal-oriented learning are required for FFI connectivity growth in VH
Left: different paradigms to test FFI connectivity growth in VH during the early phase of training. The symbol † indicates the day on which mice were sacrificed. Right: Increased LMT filopodial contents after two days of training in the presence of the platform (regardless of its visibility), but not upon pure swimming (swim 2d) or only one day of goal presence (VIS 1d). N = 3 – 5 mice per condition. * $p < 0.05$; ** $p < 0.01$.

Altogether, these findings reveal an early engagement of the ventral, but not dorsal, hippocampus during an incremental learning task. More generally, we propose that VH is recruited upon a goal-oriented learning task after some "recognition" process takes place, which is time-sensitive.

To further investigate the relationship between FFI connectivity growth in VH and goal-oriented learning, we determined whether a hippocampus-dependent task lacking a learning component may fail to increase FFI connectivity in VH. For this purpose, we chose the well-established hippocampal-dependent novel object recognition test (NOR) (Broadbent et al., 2010; Broadbent et al., 2004; Clark et al., 2000). This paradigm involves recognition memory processes but not learning, and may thus be a good example to dissociate the contribution of learning versus memory in hippocampal FFI plasticity. As expected, we found that the NOR task did not alter filopodial contents in DH or VH (Figure 11), supporting the notion that FFI plasticity in the hippocampus may be learning-related.

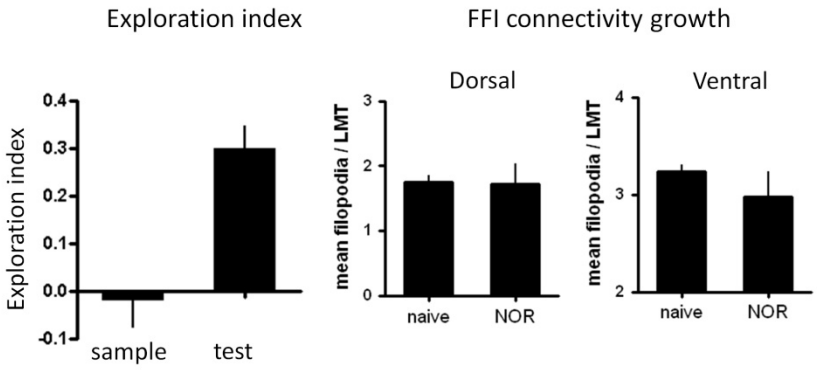


Figure 11: Novel object recognition test does not affect FFI connectivity in the hippocampus
Left: Recognition memory upon NOR test. On the habituation day (sample), mice were exposed to two identical objects. 24h later (test), one object was replaced and mice spent more time exploring the new object. Exploration index = (time exploring new object – time exploring familiar object) / (total time of exploration). N = 13. Right: no FFI connectivity growth in DH or VH upon NOR test. N = 3 mice.

VH is required for stability of performance throughout learning but not for establishment of a spatial reference memory

In order to causally define the role played by FFI plasticity in VH in the Morris water maze task, we performed pre-training bilateral ibotenate lesions of the ventral hippocampus (Figure 12a). After surgery, mice were given 7 days recovery before starting the water maze training. Following habituation (day 1, visible platform), animals underwent 8 or 10 days of training (invisible platform) and were tested for reference memory during the probe trial on day 10 or day 12 (no platform), respectively (Figure 12a). Lesions were histologically confirmed based on a Nissl staining (Figure 12b). Dorsal and intermediate portions of the hippocampus were spared in almost all cases (100% and 90%, respectively), while ventral hippocampal lesions were observed in 70% - 100% of the cases, with CA3 being the subregion most affected (Figure 12c).

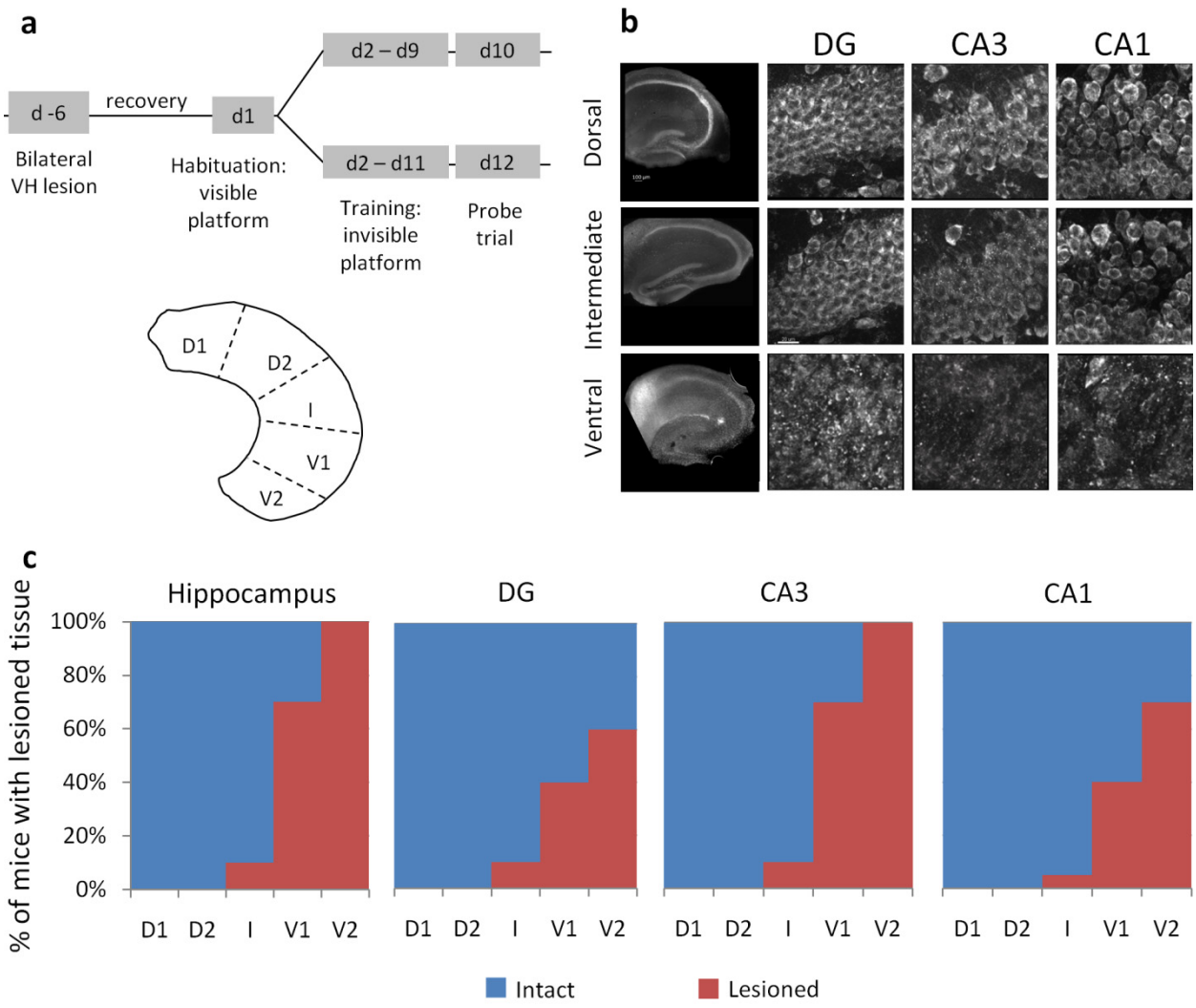
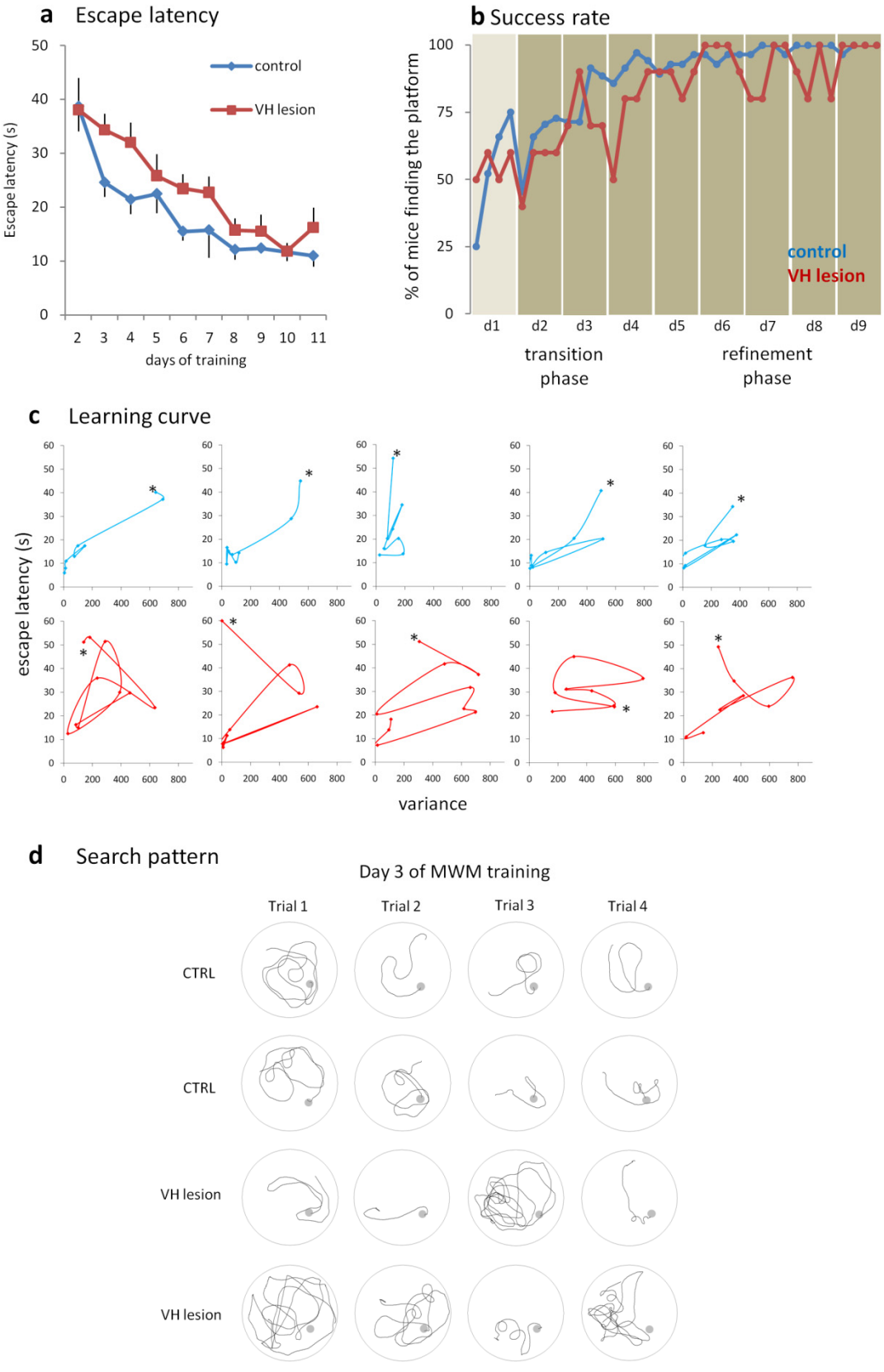


Figure 12: Histological confirmation of VH lesions
(a) Top: Experimental design. 10 mice underwent bilateral ibotenic acid lesions of VH and were allowed to recover for seven days before starting the standard MWM training (lasting for 9 or 11 days, N = 5 mice for each condition). Bottom: Schema showing the subdivision of the hippocampus into five regions along the dorsoventral axis. **(b)** Representative examples of hippocampal lesions in dorsal, intermediate and ventral hippocampus (Nissl staining). Left row: low-magnification micrographs of lamellar hippocampal sections of lesioned mice. Right: high-magnification micrographs of single subregions (DG, CA3 and CA1) in lesioned mice. Note how dorsal and intermediate regions of the hippocampus are intact, whereas ventral portions are lesioned. N = 10 mice. **(c)** Extent of hippocampal lesions. Left panel: percentage of mice with intact (blue) or lesioned (red) tissue in the hippocampus at different dorsoventral levels. Right panels: subregion analysis of hippocampal lesions (DG, CA3, CA1). N = 10 mice.

To investigate the role played by VH in the MWM task, we analysed several aspects of behaviour such as escape latency, learning curve, swimming pattern and learning strategy in control and lesioned animals.

The MWM learning process is bimodal and consists of two phases: a transition phase (day 2 – day 4) characterised by poor and highly variable performance, and a refinement phase (day 5 – day 9) characterised by good performance and lower variability (Figure 6b). We found that lesioned mice did not exhibit the characteristic rapid improvement in escape latency typical of the first phase of training, and performance was shifted by two days compared to control mice (Figure 13a). To further dissect behavioural performance and identify possible inconsistencies between control and lesioned mice, we compared, for each condition and for each trial and training day, the fraction of mice that succeeded in the task (e.g., the fraction of mice that found the platform, regardless of the time spent searching). This analysis across trials and days allows assessing after how many days of training the transition towards good performance occurs. This transition phase, defined by reaching and further maintaining at least a 70-75% success rate (i.e., 70-75% of all mice find the platform during a given trial), took place already at day 2 in control mice. In lesioned mice, however, the transition was shifted by two days and occurred only at day 4 (Figure 13b). These results show that involvement of VH, at least in the early phase of training, is required for behavioural performance.

Figure 13: Effect of VH lesions on behaviour and FFI connectivity growth in DH (page 52)
(a) VH-lesioned mice (red, n = 10) exhibit longer escape latency in the early phase of training compared to control mice (blue, n = 10). **(b)** Delayed improvement and instability of performance in VH-lesioned mice. Success rate across trials and days was expressed as the percentage of mice finding the platform, regardless of latency. In lesioned mice, shift to improved performance was delayed by two days in lesioned mice, and performance was very unstable across trials until day 9. **(c)** Performance in lesioned mice is highly unstable. Examples of learning curves in control (blue) and lesioned (red) mice showing escape latencies (y-axis) as a function of variance of daily trials (x-axis) over the training days. Every dot in the curve corresponds to the average value from four daily trials. High variance values reflect high variability (low stability) across trials within one training day. The first day of training (day 2, invisible platform) is labelled with a star. **(d)** Records of the search pattern during the four trials on day 3 for four representative animals: CTRL (top, 2 mice) and VH-lesioned (bottom, 2 mice).


a Escape latency
Escape latency (s)
control
VH lesion
days of training
b Success rate
% of mice finding the platform
control
VH lesion
d1
d2
d3
d4
d5
d6
d7
d8
d9
transition phase
refinement phase
c Learning curve
escape latency (s)
variance
d Search pattern
Day 3 of MWM training
Trial 1
Trial 2
Trial 3
Trial 4
CTRL
CTRL
VH lesion
VH lesion

Does VH also play a role during later phases of the MWM training? We found that at the end of the training (9 or 11 days), lesioned mice were able to form a reference memory (Figure 14a) and had comparable latency values as control mice (Figure 13a). These findings are consistent with a study by Moser et al. showing that rats are able to learn a MWM task with as little as 25% intact hippocampal tissue located in the dorsal hippocampus (Moser et al., 1995). However, we show here by means of a careful behavioural analysis that VH plays an active role throughout training (9 days). While performance in control mice consistently increased across trials to reach plateau after about 6 – 7 training days, daily success rates in lesioned mice were unstable across trials until day 9 (Figure 13b). To further assess VH involvement throughout training, we compared learning curves of individual control and lesioned mice. Learning curves resulted from plotting latency (mean of four daily trials) and its variance (from the four daily trials) over time (day 2 to day 9 or 11). In control mice, numbers consistently shifted towards lower latency and variance values within the first three days of training (Figure 13c). By contrast, latency values across trials in lesioned mice were much more unstable (i.e., high variance) and reached consistently low levels only at day 8 – 9 (Figure 13c). This instability was also evident when we analysed the search pattern across trials: while control mice tended to improve from the first to the fourth trial, lesioned mice exhibited unpredictable performance (Figure 13d).

Based on the notion that VH-lesioned mice were able to establish a correct reference memory, we asked whether FFI connectivity in DH would be comparable to control mice. After 9 days of training, DH filopodial contents in lesioned mice did not reach plateau values yet and were comparable to levels reached after 6 days of training in control mice (Figure 14b). Plateau levels corresponding to 9 days of training for control mice were reached after 11 days of training for lesioned mice, revealing a delay of 2 – 3 days for structural plasticity in the absence of VH.

Taken together, these results reveal a behavioural contribution of VH during the early phase of MWM task acquisition and performance stability throughout learning. In addition, they show that FFI connectivity growth in DH can occur in the absence of VH, suggesting a functional dissociation between DH and VH. These are engaged in two different learning processes that trigger selective FFI connectivity growth: while DH may be responsible for

establishing a reference memory of space upon initiation of a spatial searching learning strategy, VH is likely involved in learning the nature of the goal of a task and is constantly engaged throughout learning.

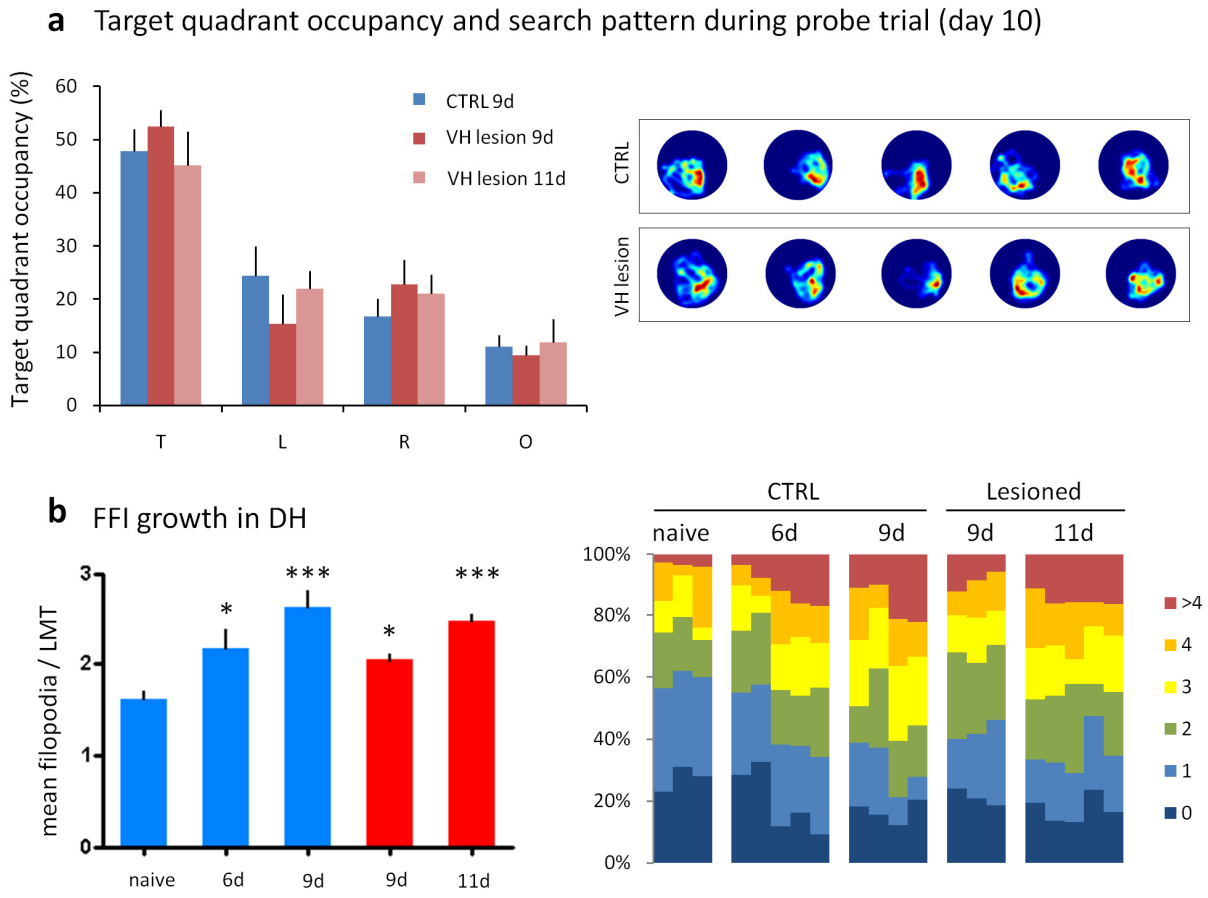


Figure 14: Reference memory and FFI connectivity growth in DH at the end of the training
(a) VH-lesioned mice are able to establish a reference memory. Left: all mice spent about 50% of the time searching in the target quadrant. Details as in Figure 6c. Right: examples of heat maps showing search strategy during the probe trial (day 10) in control (top row) and lesioned (bottom row) mice. **(b)** FFI growth in DH upon the MWM task is independent of VH. Left: mean number of filopodia per LMT in control (blue) and VH-lesioned (red) mice after 9 or 11 of training. (N = 10 mice). Right: comparison of filopodia/LMT distribution in control and lesioned mice after 6, 9 or 11 days of training. Lesion experiment: N = 10 mice (5 mice for 8 days and 5 mice for 10 days training). Control mice: N = 10. T = target quadrant; L = left; O = opposite; R = right. * $p < 0.05$; ***$p < 0.001$.

Neuromodulatory influence of FFI connectivity growth in VH

In a preliminary experiment, we investigated what neuromodulators may be involved in FFI connectivity growth in VH upon learning. It is widely accepted that dopamine is involved in learning and memory processes (for review, see El-Ghundi et al., 2007; Wise, 2004). For example, D_1 dopamine receptors activate a molecular cascade required for the late phase of LTP (Huang and Kandel, 1995) and are required for the persistence of hippocampus-dependent memory (Granado et al., 2008; O'Carroll et al., 2006; Rossato et al., 2009) and spatial learning and memory (El-Ghundi et al., 1999; Granado et al., 2008). To explore the role of dopaminergic neuromodulation on FFI plasticity in VH, we attempted to interfere with dopamine signalling. If our hypothesis holds true, namely that filopodial contents in VH are increased as a result of learning-related mechanisms that may involve LTP, interfering with the dopaminergic pathway mediating LTP should prevent filopodia induction upon MWM training. Therefore, we systemically injected mice with the drug SCH-23390, a specific D_1 receptor antagonist (Bourne, 2001), before subjecting them to the Morris water maze paradigm. The drug was administered 20 min. prior training, both on day 1 (habituation day, visible platform) and day 2 (invisible platform). Mice underwent a single probe trial (60-second swim without platform) on day 3 and were immediately sacrificed. Interestingly, SCH-23390 treatment abolished filopodial induction in VH upon MWM learning task (Figure 15), indicating that dopamine signalling is involved in the process of triggering FFI connectivity growth. Further investigations will be required to elucidate the role of dopamine in inducing filopodial content increase in VH upon a spatial learning task.

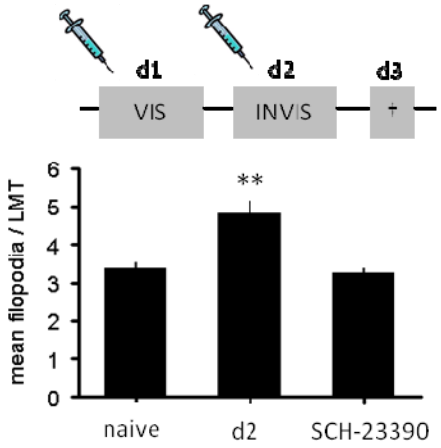


Figure 15: Dopamine modulation of FFI connectivity growth in VH
Systemic administration of the D_1 receptor antagonist SCH-23390 (0.005 mg/kg, i.p.) suppresses FFI connectivity growth in VH. The drug was administered on day 1 (habituation, visible platform) and day 2 (invisible platform) 20 min prior training; mice were sacrificed on day 3. N = 3 mice. ** $p < 0.01$.

2.1.4 Discussion

In this study we investigated how FFI connectivity at mossy fibres in CA3 relates to the dorsoventral axis of the hippocampus under baseline conditions and upon learning. We have shown that, under baseline conditions, LMTs exhibit region-dependent morphology and complexity, a property intrinsic to the hippocampus. In addition, we found that the time course and extent of filopodia induction was comparable upon contextual fear conditioning (cFC) in DH and VH. By contrast, FFI connectivity growth upon a spatial task based on incremental learning (MWM) occurred as early as two days after training in VH and only after 5 – 6 days in DH, indicating that DH and VH are involved in different aspects of a learning task. Importantly, LMT filopodial contents were not increased under conditions lacking a learning component, suggesting that learning is critical to induce FFI connectivity growth in VH. Interestingly, one day of exposure to the goal was not sufficient to induce FFI connectivity growth in VH. VH-lesioned mice were impaired in the initial behavioural performance and learning stability was affected up to day 9, suggesting the VH is required throughout the training. Taken together, our findings reveal different roles of DH and VH in a MWM task. We propose that DH is required to form a fine spatial map of the environment and to establish a precise reference memory through a searching learning strategy, while VH may be recruited upon a goal-oriented behaviour (Burton et al., 2009; Viard et al., 2011) and involved in learning the nature of the goal of the task (Gentile, 2000; Kumaran et al., 2009).

Different LMT morphology in DH and VH under baseline conditions

We found that, under baseline conditions, LMTs were larger and more complex ventrally than dorsally. Since LMT size and complexity correlate with higher synaptic densities and strength of excitatory responses in postsynaptic CA3 pyramidal neurons (Bednarek and Caroni, 2011; Galimberti et al., 2010), the prevalence of larger LMTs in VH may suggest that mossy fibre output is stronger on ventral CA3 pyramidal neurons. Interestingly, we found a positive correlation between LMT complexity and mean filopodia numbers per LMT, perhaps reflecting the need of increased FFI connectivity as a mean to counterbalance the stronger excitatory drive coming from larger core LMTs (Lawrence and McBain, 2003). The

ratio of granule cells to CA3 pyramidal neurons (GC:CA3) in DH is on the order of 5 – 7 : 1 , while in VH it may be around 0.8 – 0.5 : 1. Assuming that the total number of FFE release sites per pyramidal neuron may be the same throughout the hippocampus, individual CA3 pyramidal neurons would be contacted by more LMTs dorsally than ventrally. However, ventral LMTs are larger, more complex and thus more potent (Bednarek and Caroni, 2011; Galimberti et al., 2010), which may compensate for the lower convergence observed ventrally.

Overall, differential FFI connectivity along the DV axis in basal conditions may reflect the need to contain greater CA3 activation ventrally as a consequence of LMT morphology and intrinsic hippocampal connectivity. Analyses of active zone numbers in single LMTs from dorsal and ventral hippocampus as well as electrophysiological measurements will provide further information as to the possible functional implications of differential LMT size and filopodia contents in dorsal and ventral hippocampus.

Distinct roles of DH and VH in the MWM task

One of the surprising findings of this study was that induction kinetics of FFI plasticity upon an incremental learning task (Morris water maze) was not homogeneous along the DV axis. In DH, the time course of FFI connectivity growth reflected the time required to form a reference memory, and filopodial contents correlated with the extent of memory precision (Ruediger et al., 2011). Interestingly, this correlation existed also in IH but was much weaker, whereas it was completely absent in VH. Therefore, we conclude that both DH and IH are involved in spatial learning, with the degree of memory precision being higher at more dorsal levels. This is consistent with lesion studies showing that spatial learning can be supported by as little as 20-25% of residual hippocampal tissue located within the septal two-thirds of the longitudinal axis (Moser et al., 1995), and that the magnitude of the lesion within the dorsal-intermediate domains parallels the degree of spatial impairment (Moser et al., 1993). Moreover, the time course of c-Fos expression in CA3b pyramidal neurons upon MWM learning was virtually identical in DH and IH and very different from that in VH. As for connectivity, DH and IH receive inputs from the lateral and intermediate band of the EC, which convey visuo-spatial information from adjacent perirhinal and postrhinal cortices (Burwell and Amaral, 1998), whereas VH is connected to

the medial band of the EC and has unique connections to the mPFC, amygdala and hypothalamus, which process emotional, affective and interoceptive information (Petrovich et al., 2001; Pitkanen et al., 2000). Therefore, both in terms of connectivity and function, DH and IH are similar to each other, while the ventral hippocampus appears to be a somewhat separate entity. This dichotomy supports the idea DH and VH may represent independent units that process different aspects of a task.

Upon training on a less challenging version of the MWM task (platform 2A), we observed FFI connectivity growth in VH but not in DH. Under these conditions, mice did not develop any spatial search strategy and DH was hence not recruited. By contrast, mice trained on the standard (platform A) or on an even more difficult (platform 0.5A) task initiated a spatial searching learning strategy and established a reference memory as a result of DH recruitment. While DH may be involved in establishing a fine and precise spatial representation (Kjelstrup et al., 2008) upon initiation of a searching learning strategy, we propose that VH plays a central role in learning the nature of the goal of a task based on several pieces of evidence. 1) FFI connectivity in VH increased after two days of MWM training and was paralleled by a marked increase in c-Fos expression in CA3b pyramidal neurons within the same temporal frame. Combined with the impaired performance during the early phase of the MWM task, these data strongly suggest that VH is recruited early in the learning process and may encode "representing the goal of a task". 2) As opposed to DH, VH does get recruited upon a less challenging task (platform 2A), consistent with the hypothesis that goal exposure, but not task difficulty, matters. 3) A hippocampus-dependent memory task such as NOR does not alter FFI connectivity, consistent with the idea that goal learning is required to engage VH.

We showed that VH-lesioned mice were impaired in the early phase of the MWM task and that performance stability was affected throughout the learning process. However, we do not know whether the deficits observed at later stages reflect a constant engagement of VH or whether they may result from the lack of a functioning VH from the beginning, which may prevent subsequent stability of learning. Targeted inactivation of VH in the mid-late phase of training (e.g, from day 4 on) will permit to determine whether having an intact VH in the early phase is sufficient for performance stability at later stages, or whether VH

needs to be constantly engaged even after the early phase. Elevated Fos signals in VH all throughout the training days would rather support the latter hypothesis.

We have proposed that VH is engaged upon a goal-oriented learning task, but not upon a pure memory task. Learning can be defined as the process of acquiring new information for a behavioural purpose and memory as the ability to store and recall past experiences. Memory can thus be formed without learning, for example when making instant records of an experience. According to this definition, the novel object recognition test is a memory but not a learning task, since pure exploration in the absence of a motivational framework (no reward or punishment and hence no behavioural purpose) has no learning component. Consistent with the hypothesis that FFI connectivity growth in VH occurs upon learning but not pure memory hippocampus-dependent tasks, it has been reported that β-adducin knock-out mice, which have a defect in synapse stabilisation due to impaired linkage between the cell membrane cortex and the actin cytoskeleton (Bednarek and Caroni, 2011; Pielage et al., ; Rabenstein et al., 2005), are impaired in the goal-oriented MWM learning task (Ruediger et al., 2011), but not in the NOR test (Bednarek and Caroni, 2011), which does not involve learning. If VH undergoes FFI plasticity specifically upon learning but not pure recognition memory, we would predict that associating the NOR task with a goal (e.g. rewarding specifically one object over the other) would recruit VH-dependent learning and hence trigger FFI connectivity growth in VH. Accordingly, β-adducin$^{-/-}$ mice, which fail to establish increased filopodial contents upon learning (Ruediger et al., 2011), should be impaired in this associative version of the NOR task. Currently, we are re-expressing β-adducin$^{-/-}$ specifically in ventral granule cells to dissect its contributions to goal-oriented learning, and to test whether escape latencies in the early phase of training may be rescued. Moreover, if behavioural performance and learning stability on the MWM task are linked to connectivity growth in VH, we would predict that re-expressing β-adducin only in dorsal and intermediate granule cells would lead to the same behavioural impairments observed in the VH lesion experiment.

A novel finding of this work was that at least two days of goal exposure (four trials per day) were required to engage VH in terms of FFI plasticity. To test whether it is the total number of trials that is critical for VH recruitment, one could subject mice to a massive training (e.g,

8 trials on one single day) and assess filopodial contents one day later. Conversely, it may be that two days of goal exposure are decisive for filopodia induction. We found that, for FFI plasticity to occur, the delay between the first and the second exposure must not exceed a critical time window of seven days, but further experiments will be required to determine the shortest temporal separation and to dissect the processes underlying VH recruitment.

Neuromodulatory influence on FFI connectivity growth in VH

What neuromodulatory pathways could mediate filopodial induction in VH upon goal-oriented learning? Our preliminary experiment consisting of systemic blockage of dopamine D1 receptors suggests that dopamine signalling is required (directly or indirectly) to increase FFI connectivity in VH.

The hippocampus receives dopaminergic innervation from the ventral tegmental area (VTA) and the substantia nigra (Gasbarri et al., 1997; Gasbarri et al., 1994b), and indirectly from other brain structures such as the nucleus accumbens (NAcc). NAcc, like the VTA, is part of the mesolimbic dopamine system, which is central to reward processing in the brain (Kauer and Malenka, 2007). Dopamine release in VH from the VTA could be a signal for plasticity associated with approaching reward. Interestingly, DH and VH project to different target regions in NAcc. Dorsal subiculum preferentially innervates the core of NAcc, which is thought to mediate behavioural locomotor activation, while ventral CA1 and subicular areas densely innervate the shell of the NAcc, which is believed to contribute to reward and emotion (Brog et al., 1993; Degoulet et al., 2008; Groenewegen et al., 1987; Sellings and Clarke, 2003). Furthermore, the hippocampal formation (mostly CA1 and subiculum) projects to NAcc in a topographic manner: dorsal subiculum preferentially innervates the rostrolateral portion of NAcc, whereas ventral subiculum targets mainly the caudomedial portion (Groenewegen et al., 1999), which is more involved than the rostral part in reward processing (Ranaldi and Beninger, 1994).

Based on this knowledge, we speculate that repeated (at least twice) exposure to a goal-oriented learning task may be required to recruit VH, which in turn activates NAcc and VTA leading to dopamine release. This would promote plasticity in VH, possibly via structures downstream to NAcc that project back to hippocampus, like VTA (Lisman and Grace, 2005)

or medial septum (Swanson, 1977). Activation of NAcc and subsequent dopamine release may act as an associative reward signal coupled to the "goal-encoding" signal initiated by VH. However, further experiments will be necessary to elucidate the specific role of dopamine and its temporal involvement.

To summarise, our data provide further evidence in support of a functional dissociation between the dorsal and ventral hippocampus. Regional differences in LMT plasticity properties are present in baseline conditions and are most likely an intrinsic property of the hippocampus. Based on our findings resulting from a combination of behavioural, lesion and structural plasticity analyses, we propose that FFI connectivity growth in VH is induced upon a goal-oriented task as a result of learning the nature of the goal of the task. Future studies investigating the specific conditions and timing under which VH is recruited (based on increased FFI connectivity growth) will permit delineating more precisely the role played by the ventral hippocampus in learning.

2.2 Experience- and learning-mediated structural plasticity in distinct subpopulations of principal hippocampal neurons

Dominique Spirig and Pico Caroni

Unpublished results

2.2.1 Summary

In this study, we investigated structural plasticity of defined subpopulations of principal hippocampal neurons in the entire hippocampus by taking advantage of transgenic reporter lines expressing membrane-targeted GFP under the control of the neuron-specific *Thy1* promoter. Sparse labelling in lines Lsi1, Lsi2 and Lsi3 allowed visualisation of individual granule cell large mossy fibre terminals (LMTs).

We show that under baseline conditions Lsi1, Lsi2 and Lsi3 granule cells exhibit distinct degrees of LMT complexity as defined by the mean number of filopodia and "satellites" per LMT. Interestingly, we observed an increasing gradient of LMT complexity along the dorsoventral axis specific to Lsi1 and Lsi2. Structural plasticity upon environmental enrichment was different among the three lines: Lsi2 underwent the most dramatic changes (3.4-fold increase of LMT satellites), followed by Lsi1 (1.75-fold) and Lsi3 (no response). By contrast, contextual fear conditioning led to a massive increase of filopodial contents in Lsi1 and Lsi3 LMTs (1.8-fold and 2.5-fold, respectively), but to no detectable changes in Lsi2 LMTs. Upon an incremental learning task (Morris water maze), structural plasticity changes were comparable in dorsal Lsi1 and Lsi2 LMTs.

By combining behavioural studies, high-resolution microscopy and microarray analysis, we show that the dorsoventral gradient of increasing LMT complexity may be specific to Lsi1 and Lsi2 granule cells, and that distinct subpopulations of principal hippocampal neurons respond in unique ways to experience and learning. One possible implication of these findings is that the hippocampal subpopulations have distinct functional roles in hippocampal learning and memory.

2.2.2 Introduction

Brain function often relies on specificity of neuronal connections (Ko et al., 2011a), suggesting that connectivity patterns are not randomly established. Indeed, subpopulations of neurons in the nervous system can exhibit distinct connectivity which may relate to specific functions. For example, functionally different subpopulations of neurons exhibiting differential connectivity have been described in the basal amygdala (Herry et al., 2008).

So far, little is known about functional subpopulations of principal neurons in the hippocampus, a brain structure critical for rapidly establishing relational representations of incoming stimuli and storing them into long-term forms of memory. The underlying circuitry has been extensively studied and, traditionally, has been characterised as trisynaptic and serially organised, with incoming signals from the entorhinal cortex flowing unidirectionally through the dentate gyrus, CA3 and CA1 region through excitatory connections and from there back to the entorhinal cortex, thus forming a loop.

Recent studies have identified substantial variability in gene expression patterns within CA1 and CA3 (Dong et al., 2009; Thompson et al., 2008), suggesting that hippocampal subregions are not homogeneous. Subfield heterogeneity is also supported by connectivity and physiological studies, revealing differential connectivity along the transverse (proximodistal) axis both in CA1 (Harvey et al., 2009; Naber et al., 2001) and CA3 (Ishizuka et al., 1990; Witter, 2007). In the case of CA1, electrophysiological recordings have shown that proximal and distal CA1 pyramidal cells are also functionally different, as revealed by their different degree of spatial modulation (Henriksen et al., 2010).

Recently, our laboratory has reported the existence of genetically identified subpopulations of principal neurons exhibiting preferential connectivity as a result of temporally matched neurogenesis and synaptogenesis (Deguchi et al., 2011). However, the functional significance of the resulting “microcircuits” has not been determined yet.

In this study, we tackle this question by first asking whether distinct subpopulations of principal neurons in the hippocampus exhibit different degrees of structural plasticity under baseline conditions and upon experience and learning. If so, this would imply that

there exist not only regional (section 2.1.3), but also subpopulation-dependent rules for plasticity in the adult hippocampus.

We took advantage of transgenic mouse reporter lines (Lsi1, Lsi2 and Lsi3) based on a modified mouse *Thy1.2* promoter cassette that stably expresses membrane-bound green fluorescent protein (mGFP) in a subset of principal neurons (De Paola et al., 2003; Galimberti et al., 2010; Gogolla et al., 2009). In particular, we exploited "sparse" *Thy1* lines (labelling few neurons) to investigate neuronal processes and structural plasticity of the granule cell (GC) large mossy fibre terminals (LMTs) by high-resolution confocal microscopy. Structural plasticity was assessed at the level of LMT filopodia and satellites. Filopodia synapse onto parvalbumin-positive interneurons (Ruediger et al., 2011), which in turn inhibit CA3 pyramidal neurons (Acsady et al., 1998); this feed-forward inhibition (FFI) is thought to mediate shunting of CA3 pyramidal cells at low-frequency mossy fibre activation (Henze et al., 2000; Lawrence and McBain, 2003). LMTs can exhibit "satellites" (terminal appendices that are connected to the main core through 10 – 200 µm processes) that establish excitatory contacts onto distinct postsynaptic pyramidal neurons (Galimberti et al., 2006), thereby mediating feed-forward excitation (FFE). Based on the regional differences in plasticity described in Lsi1 mice (section 2.1.3), we analysed structural plasticity in different subpopulations at the level of the entire hippocampus. To further gain insights into subpopulation-specific properties, we performed microarray analyses of dentate GCs from the dorsal and ventral hippocampus (DH and VH, respectively).

Our results reveal subpopulation-specific rules for LMT anatomy under baseline conditions. Random labelling of LMTs and microarray analysis of GCs further revealed that the steep gradient of increasing complexity along the dorsoventral (DV) axis appears to be a property specific to Lsi1 and Lsi2, as it was not detected to a comparable extent in Lsi3 or in a random population of GCs. In addition, we show that the degree of structural plasticity upon experience and learning is a subpopulation-specific property and depends on the behavioural paradigm. Based on these results we suggest that separate hippocampal subpopulations may have distinct functional roles in hippocampal learning and memory.

2.2.3 Results

Region- and subpopulation-specific LMT complexity under baseline conditions

Given the existence of different subpopulations of principal neurons in all main subfields of the hippocampus (Deguchi et al., 2011), we first asked whether they exhibited differential LMT morphology and structural plasticity in baseline conditions. In order to identify possible subpopulation-dependent rules for plasticity, we quantified the mean numbers of filopodia per LMT in the region CA3b of the dorsal and ventral hippocampus in Lsi1, Lsi2 and Lsi3 adult male mice.

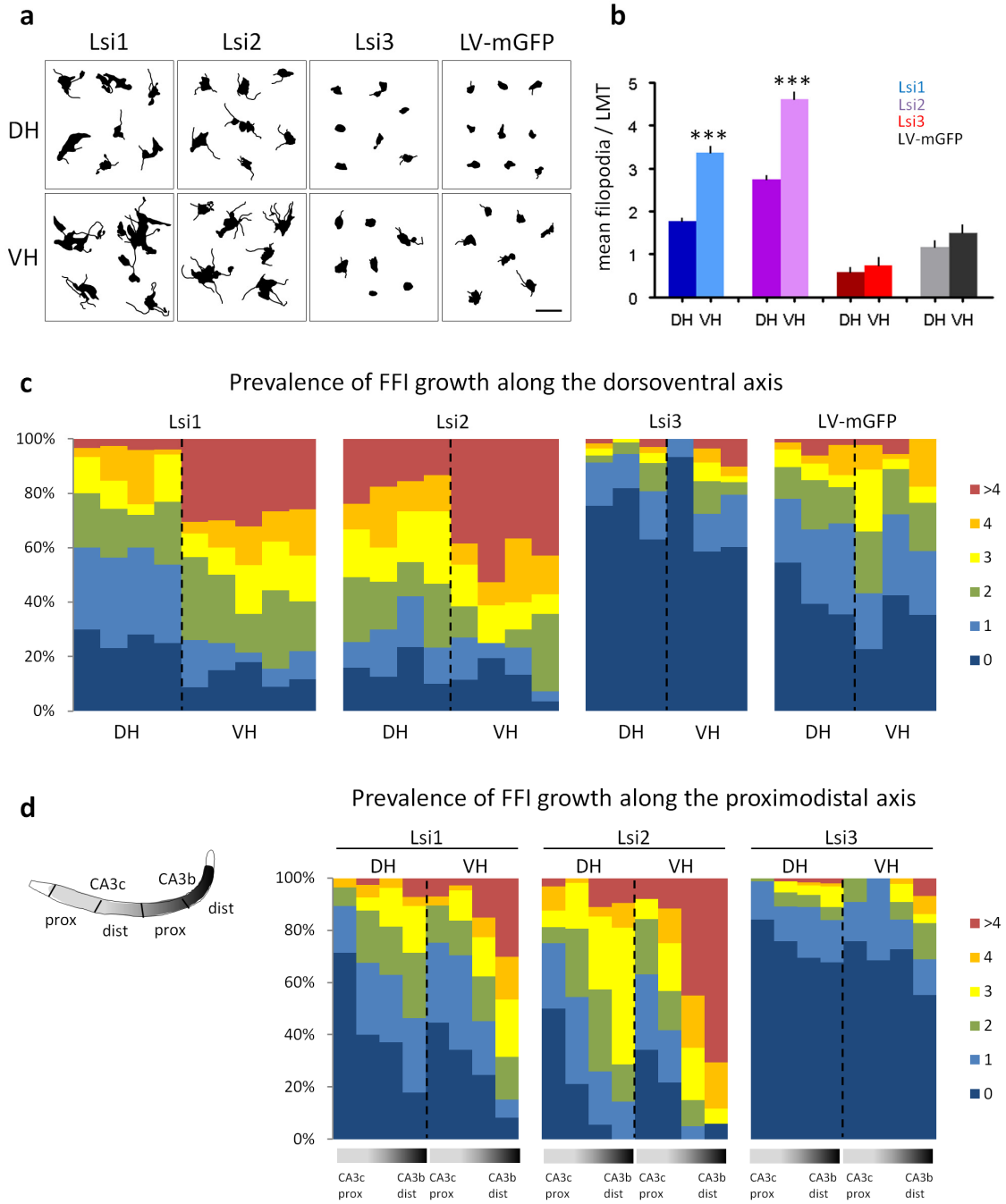
We found that LMT morphology in naïve mice greatly differed among subpopulations. Lsi1 LMTs were large, convoluted and heterogeneous in shape, in stark contrast to Lsi3 LMTs, which were smaller, globular and rather homogeneous. Lsi2 were more similar to Lsi1 LMTs, although they tended to be slightly smaller. In contrast to Lsi1 and Lsi2 LMTs, which exhibited filopodia and satellites under baseline conditions, these features were rare in Lsi3 LMTs (Figure 1a). Dorsally, the mean numbers of filopodia per LMT in Lsi1, Lsi2 and Lsi3 were 1.759 ± 0.076, 2.731 ± 0.097 and 0.575 ± 0.124, respectively. Ventrally, LMTs had on average 3.363 ± 0.155 (Lsi1), 4.605 ± 0.189 (Lsi2) and 0.738 ± 0.187 (Lsi3), respectively (values are mean ± S.E.M.) (Figure 1b). The mean fold changes of filopodial contents along the DV axis strongly differed across subpopulations of GCs. Lsi1 exhibited the most pronounced increase (1.91-fold, $p < 0.0001$), followed by Lsi2 (1.69-fold, $p < 0.0001$) and finally Lsi3 (1.28-fold, n.s.), where differences along the DV axis were negligible .) (Figure 1b).

To investigate whether Lsi1 and Lsi2 subpopulations of GCs may be “special” with regard to LMT morphology and increasing FFI connectivity along the DV axis, we transduced dentate gyrus GCs with a mEGFP-expressing lentivirus (LV-mGFP), thereby randomly labelling GCs in DH and VH (Galimberti et al., 2010). We found that the mean numbers of filopodia per LMT in randomly labelled GCs were 1.161 ± 0.166 in DH and 1.493 ± 0.195 in VH (Figure 1b). These values are consistent with the assumption that the fractions of Lsi1 and Lsi2 granule cells in DG are 20% and 15%, respectively (Deguchi et al., 2011), and that the remaining subpopulations are similar to Lsi3 in terms of filopodia (65%). Accordingly, in

LV-mGFP mice we detected only a slight but not significant increase of FFI connectivity along the DV axis (1.29-fold, n.s.) (Figure 1b), consistent with the notion that the majority of LMTs resemble the Lsi3 population.

To illustrate the different levels of FFI connectivity among the three subpopulations, we subdivided LMTs into groups according to their mean number of filopodia per LMT (from 0 to >4) and plotted a filopodia/LMT distribution (Figure 1c). We then calculated the fraction of LMTs with 0 and >4 filopodia per LMT, which in DH were on average 26.7% and 3.3% in Lsi1, 15.4% and 17.6% in Lsi2, and 71.6% and 1.8% in Lsi3, respectively. Ventrally, these values were on average 11.9% and 29% in Lsi1, 12% and 42.7% in Lsi2, and 62.7% and 6.83% in Lsi3, respectively (Figure 1c). To estimate what fractions of LMTs in CA3b were mostly affected along the DV axis in each subpopulation, we analysed filopodia/LMT distributions in more detail. In Lsi1 mossy fibres, the most prominent shifts from DH to VH were observed in the population of LMTs with no filopodia ("0" fraction), which underwent a 2.2-fold decrease, and in the population with more than four filopodia (">4" fraction), which increased by 8.6-fold. In Lsi2, the changes were less pronounced and the main difference in FFI connectivity between DH and VH was caused by a shift in the ">4" fraction of LMT, which increased by 2.4-fold. Finally, almost no difference between DH and VH was observed in terms of filopodial contents in Lsi3 LMTs.

Figure 1: Subpopulation-dependent LMT morphology and FFI connectivity under baseline conditions (page 68).
(a) Representative camera lucida examples of LMTs in Lsi1, Lsi2, Lsi3 and in lentiviral randomly labelled (LV-mGFP) GCs from the dorsal (DH) and ventral (VH) hippocampus. Scale bar: 10 µm. **(b)** Mean number of filopodia per LMT in DH and VH in Lsi1 (n = 7 mice), Lsi2 (n = 7 mice), Lsi3 (n = 5 mice) and lentiviral transduced mice (n = 3 mice). N = 50-100 LMTs per mouse and per region. **(c)** Prevalence of FFI connectivity growth along the dorsoventral axis in different GC subpopulations. LMTs were subdivided into five groups according to their mean number of filopodia (0, 1, 2, 3, 4 and >4). Each vertical row corresponds to one animal. **(d)** Increasing FFI connectivity along the proximodistal axis. Left: schema representing the CA3 region and its subdivisions into four domains along the proximodistal axis (from proximal CA3c, light grey, to distal CA3b, dark grey). Right: representative examples of FFI connectivity growth along the proximodistal axis for each subpopulation (Lsi1, Lsi2 and Lsi3). Filopodia/LMT distribution (% cumulative fraction of LMTs) is represented for every dorsoventral level (DH, IH and VH, block of four rows) at different proximodistal positions along CA3 (each of the four vertical rows represents, from left to right: proximal CA3c, distal CA3c, proximal CA3b and distal CA3b). Details as in (d).
Prox = proximal; dist = distal. ***$p < 0.001$.


a
Lsi1
Lsi2
Lsi3
LV-mGFP
DH
VH
b
mean filopodia / LMT
***
***
Lsi1
Lsi2
Lsi3
LV-mGFP
DH VH
DH VH
DH VH
DH VH
c
Prevalence of FFI growth along the dorsoventral axis
Lsi1
Lsi2
Lsi3
LV-mGFP
100%
80%
60%
40%
20%
0%
DH
VH
>4
4
3
2
1
0
d
Prevalence of FFI growth along the proximodistal axis
CA3c
CA3b
prox
dist
dist
prox
Lsi1
Lsi2
Lsi3
CA3c prox
CA3b dist

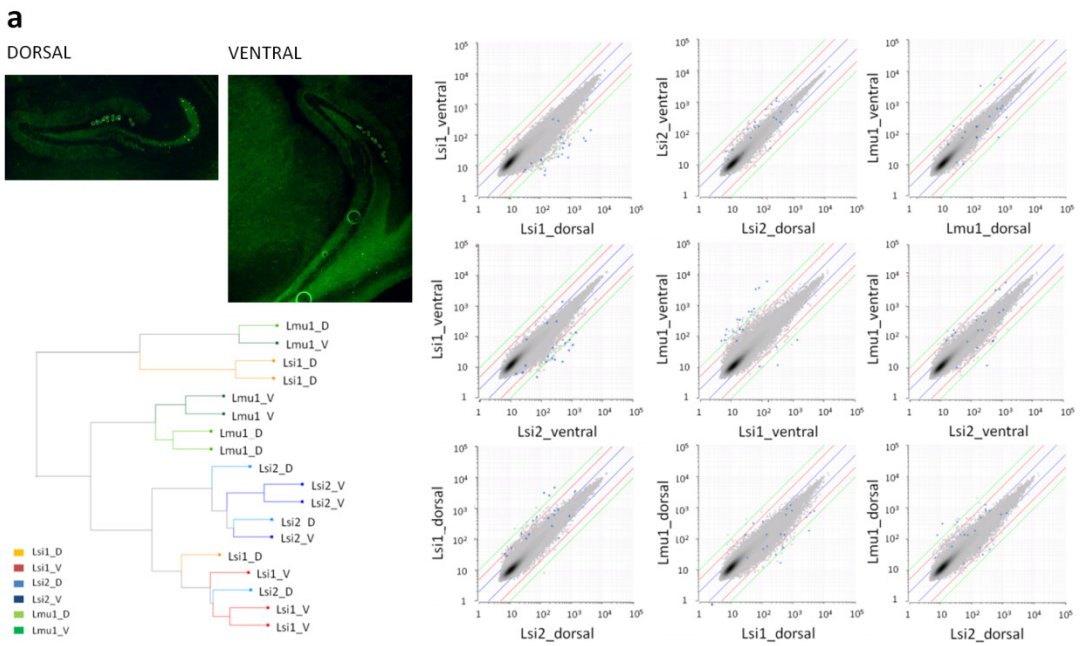
Altogether, these analyses show that the extent of feed-forward inhibition (FFI) connectivity under basal conditions and its increase along the DV axis is a subpopulation-specific property. Lsi1 exhibited the most marked differences between DH and VH, followed by Lsi2 and finally by Lsi3, where virtually no changes were observed. Based on these results, we conclude that Lsi1 and Lsi2 LMTs exhibit unique LMT features that are not shared by the majority of GCs.

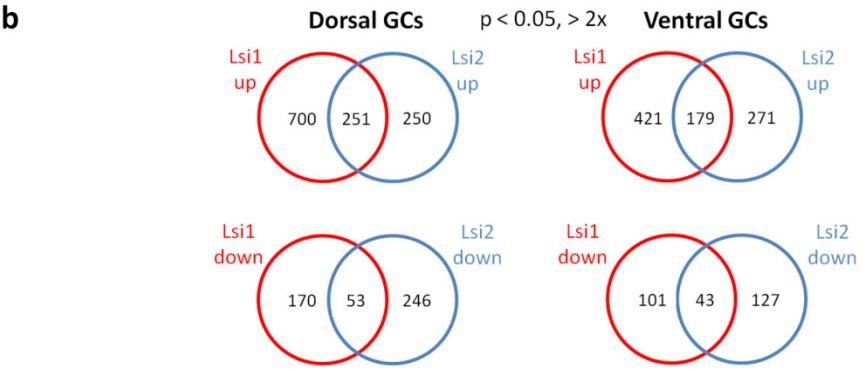
We next investigated LMT filopodial contents in CA3 along the transverse hippocampal axis (proximodistal dimension). In all three lines, we found a gradient of increasing FFI connectivity from the proximal CA3c (closer to the hilus) to distal CA3b region (closer to CA2), both in DH and in VH (Figure 1d). In order to express the steepness of the gradient in distinct subpopulations, we calculated the fold change for the increase in the ">4" fraction and for the decrease in the "0" fraction. Dorsally, the steepest proximodistal gradient was found in Lsi2 (3-fold increase and ~10-fold decrease, respectively), followed by Lsi1 (~3-fold increase and 4-fold decrease, respectively) and Lsi3 (1.7-fold increase and 1.2-fold decrease, respectively). The same trend was observed ventrally: Lsi2 exhibited the steepest gradient of increasing FFI connectivity along the proximodistal axis (9-fold increase and 5.8-fold decrease, respectively), followed by Lsi1 (4.2-fold increase and 5.4-fold decrease, respectively) and Lsi3 (increase from 0 to 7% and 1.4-fold decrease, respectively) (Figure 1d). Overall, we observed a gradient of increasing FFI connectivity in CA3 along the proximodistal axis in all three subpopulations, both dorsally and ventrally, suggesting that it may a general property of hippocampal circuitry.

Lsi1 and Lsi2 GCs differ strongly from average GCs: a microarray analysis

We next performed transcriptome analyses of Lsi1 and Lsi2 GCs from DH and VH in three adult male mice (8 weeks old). We individually collected 70 to 80 GCs from the distal half of the suprapyramidal blade of the DG by laser-dissection microscopy (Figure 2a), and then analysed them as pools on Affymetrix chips (Saxena et al., 2009). For comparison, similar sets of GCs were collected from three Lmu1 mice, whose broad mGFP expression in the DG (De Paola et al., 2003) make them well suited to represent a random population of GCs. We first asked how different Lsi1 and Lsi2 subtypes are compared to a random population of GCs (Lmu1) by expressing the number of genes that were at least two-fold up- or down-regulated ($p < 0.05$). In DH, Lsi1 contained 1174 differentially regulated genes (951 up- and 223 down-regulated), while in Lsi2 they were 800 (501 up- and 299 down-regulated). In VH, 744 genes were differentially regulated in Lsi1 (600 up- and 144 down-regulated) and 620 in Lsi2 (450 up- and 170 down-regulated) (Figure 2b). Since we found that dorsoventral differences in LMT morphology and plasticity were specific to Lsi1 and Lsi2 and negligible in a random population of GCs, we asked whether we could observe a more pronounced dorsoventral heterogeneity in Lsi1 and Lsi2 at the transcriptional level. In total, 1084 genes were differentially regulated in dorsal and ventral Lsi1 GCs (875 up- and 209 down-regulated in DH); 261 in Lsi2 (78 up- and 183 down-regulated); and only 150 in Lmu1 (76 up- and 74 down-regulated) (Figure 2c). Interestingly, the highest differential gene regulation was observed in Lsi1, the subpopulation of GCs exhibiting the strongest dorsoventral differences in terms of LMT complexity and filopodial contents. Likewise, fewer genes were differentially regulated in dorsal and ventral GCs of the average population, which at the morphological level also exhibited much less dorsoventral heterogeneity.

Altogether, these results confirm previous findings showing that Lsi1 and Lsi2 GCs represent subpopulations of principal neurons with unique properties (Deguchi et al., 2011; Galimberti et al., 2010) and suggest that the dorsoventral heterogeneity observed in Lsi1 and to a lesser extent in Lsi2 is a feature specific to these subtypes.


a
DORSAL
VENTRAL
Lmu1_D
Lmu1_V
Lsi1_D
Lsi1_D
Lmu1_V
Lmu1 V
Lmu1 D
Lmu1_D
Lsi2_D
Lsi2_V
Lsi2_V
Lsi2 D
Lsi2_V
Lsi1_D
Lsi1_V
Lsi2_D
Lsi1_V
Lsi1_V
Lsi1_D
Lsi1_V
Lsi2_D
Lsi2_V
Lmu1_D
Lmu1_V
Lsi1_ventral
Lsi2_ventral
Lmu1_ventral
Lsi1_dorsal
Lsi2_dorsal
Lmu1_dorsal


b
Dorsal GCs
p < 0.05, > 2x
Ventral GCs
Lsi1 up
Lsi2 up
700
251
250
Lsi1 up
Lsi2 up
421
179
271
Lsi1 down
Lsi2 down
170
53
246
Lsi1 down
Lsi2 down
101
43
127

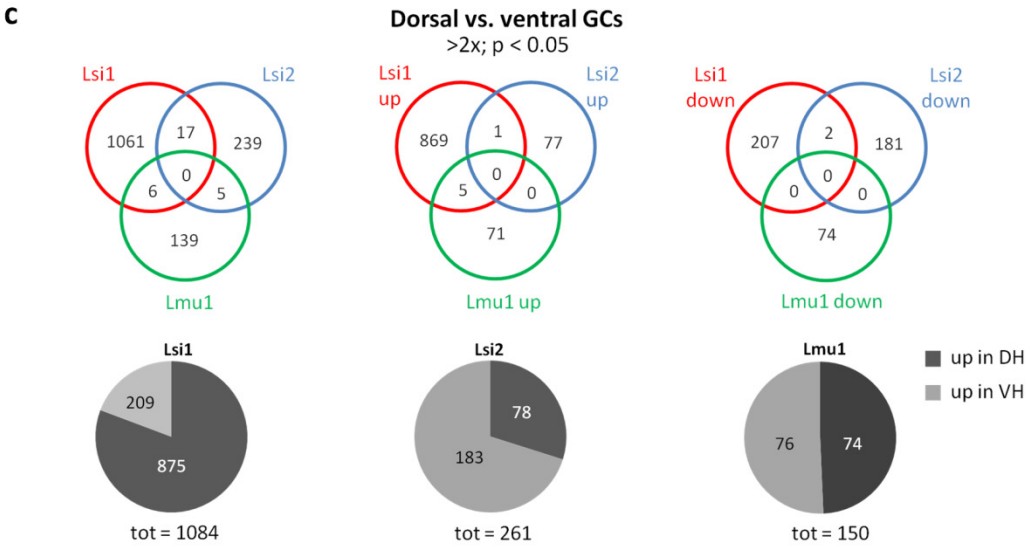

c
Dorsal vs. ventral GCs
>2x; p < 0.05
Lsi1
Lsi2
1061
17
239
0
6
5
139
Lmu1
Lsi1 up
Lsi2 up
869
1
77
0
5
0
71
Lmu1 up
Lsi1 down
Lsi2 down
207
2
181
0
0
0
74
Lmu1 down
Lsi1
209
875
tot = 1084
Lsi2
78
183
tot = 261
Lmu1
76
74
tot = 150
up in DH
up in VH

Figure 2: Distinct transcriptomes of dorsal and ventral Lsi1 and Lsi2 granule cells (page 71)
(a) Left, top: Laser-dissection microscopy. GCs were collected from the distal half of the DG upper blade from dorsal (Bregma -1.46 mm to -1.94 mm) and ventral (Bregma -3.28 mm to -3.44 mm) hippocampal cryosections. Left, bottom: *in silico* cell grouping. The unbiased hierarchical tree algorithm grouped granule cells according to subpopulation rules. Right: gene profiling quality. Region and subpopulation comparison of hybridisation data from adult male GCs (each dot represents one gene probe on the chips and is the average value from 3 mice, obtained using 70 – 80 GCs per mouse and per region). **(b)** Subpopulation-specific analysis. Number of genes up- or down-regulated in dorsal and ventral Lsi1 and Lsi2 GCs compared to average. **(c)** Region-specific analysis. Top, left: number of genes differentially regulated in dorsal and ventral hippocampus for each GC subpopulation. Middle and right: number of genes up- and down-regulated in each subpopulation, respectively. Bottom: for each subpopulation, fractions of the differentially regulated genes that are up-regulated in dorsal (dark grey) or ventral (light grey) hippocampus. Scale bar: 10 μm.

Subpopulation-specific structural plasticity upon environmental enrichment

We next asked whether experience-related plasticity may obey subpopulation-specific rules. To this end, we raised mice in an enriched environment (EE) for 20-30 days and kept age-matched control mice in single cages. Environmental enrichment provides animals with enhanced sensory, motor and social stimuli and causes molecular and plasticity changes that are beneficial for learning and memory (Baroncelli et al., 2010; Pizzorusso et al., 2007; Sale et al., 2009; van Praag et al., 2000). Data from our laboratory, mainly based on line Lsi2, have shown that EE leads to a massive increase in the number of satellites in DH (Galimberti et al., 2006; Gogolla et al., 2009). We thus asked whether this effect was specific to Lsi2 and whether it occurred throughout the longitudinal axis of the hippocampus (Figure 3a). In DH, we found that EE elicited the most dramatic FFE connectivity growth in Lsi2 LMTs (3.48-fold, $p < 0.05$). In Lsi1, satellite contents increased by 1.75-fold ($p > 0.05$). By contrast, EE completely failed to induce satellites in Lsi3 LMTs. In VH, we observed the same trend: the strongest increase in FFE connectivity was observed in Lsi2 (3.77-fold, $p < 0.01$), followed by Lsi1 (1.35-fold, n.s.) and no increase in Lsi3 (Figure 3c). Interestingly, the extent of EE-induced FFE plasticity was similar in DH and VH in each line, suggesting that both regions are equally recruited during this task but that the degree of the response is subpopulation specific (Figure 3c).

Does EE also affect FFI connectivity? If so, are there subpopulation differences? To answer this question, we expressed the mean number of filopodia per LMT in enriched versus control conditions in Lsi1, Lsi2 and Lsi3 LMTs. In all subpopulations, we found no significant changes in FFI connectivity, neither in DH nor in VH (Figure 3b).

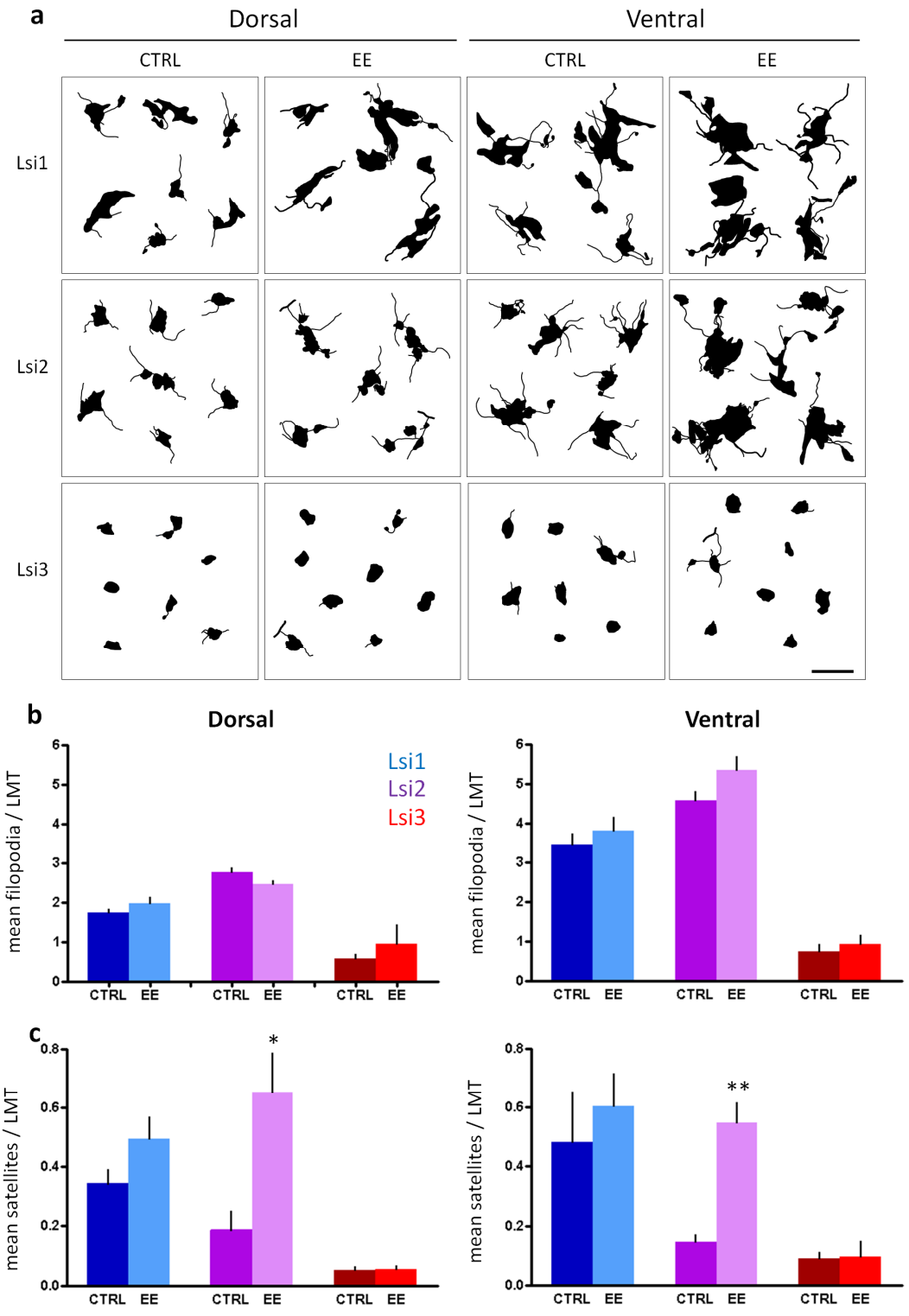

a
Dorsal
Ventral
CTRL
EE
CTRL
EE
Lsi1
Lsi2
Lsi3
b
Dorsal
Ventral
Lsi1
Lsi2
Lsi3
mean filopodia / LMT
0
1
2
3
4
5
6
CTRL EE CTRL EE CTRL EE
c
mean satellites / LMT
0.0
0.2
0.4
0.6
0.8
*
**
CTRL EE CTRL EE CTRL EE

Figure 3: Subpopulation-dependent structural plasticity upon enriched environment (page 73)
(a) Representative camera lucida examples of LMTs under control and enriched conditions in DH and VH in Lsi1, Lsi2 and Lsi3 mice. Scale bar: 10 µm. **(b** and **c)** Structural plasticity upon EE. FFI **(b)** and FFE **(c)** connectivity growth in Lsi1 (n = 5), Lsi2 (n = 4) and Lsi3 (n = 3) mice upon EE. N = 50 – 100 LMTs per region and per mouse. * $p < 0.05$; ** $p < 0.01$.

These results are consistent with our hypothesis that FFI connectivity is specifically increased upon learning but not upon mere behavioural experience (see section 2.1.4). Overall, our findings show that EE causes a net change in FFE both dorsally and ventrally in Lsi2. This effect is less pronounced in Lsi1 and absent in Lsi3, revealing that distinct subpopulations of GCs respond differently to behavioural experience.

Differential FFI connectivity growth upon a one-trial associative learning

Since we showed that structural plasticity responses to a novel experience are subpopulation specific, we asked whether structural remodelling induced by learning would also differ across subpopulations. For this purpose, we subjected Lsi1, Lsi2 and Lsi3 mice to a mild contextual fear conditioning (cFC) task (see section 4.2), a one-trial associative learning that elicits strong growth of FFI connectivity in the dorsal and ventral hippocampus in Lsi1 mice (Figure 4 on page 38). When tested for fear memory 24h later, all mice showed a dramatically increased freezing behaviour compared to control mice (Figure 4a). cFC led to a massive growth of FFI connectivity in DH and VH of Lsi1 (1.83-fold change, $p < 0.001$) and Lsi3 mice, where the fold change was even higher both dorsally and ventrally (2.69-fold, $p < 0.01$ and 2.04-fold, $p < 0.05$, respectively) (Figure 4c). Here, the fraction of LMTs with no filopodia shifted from an average of 75% (control) to 40% (cFC) (Figure 4b). Concomitantly, the fraction of LMTs with filopodia ranging from 2 to >4 increased considerably, revealing that, on average, at least 80% of the LMTs underwent FFI connectivity growth in response to cFC (Figure 4c). Surprisingly, filopodial contents were not altered in Lsi2 (Figure 4b and c), suggesting a subpopulation-dependent induction of FFI connectivity growth upon cFC. Similar to changes in FFE upon environmental enrichment, the extent of structural remodelling upon a one-trial associative learning varied across subpopulations but was homogeneous along the DV axis within one subpopulation.

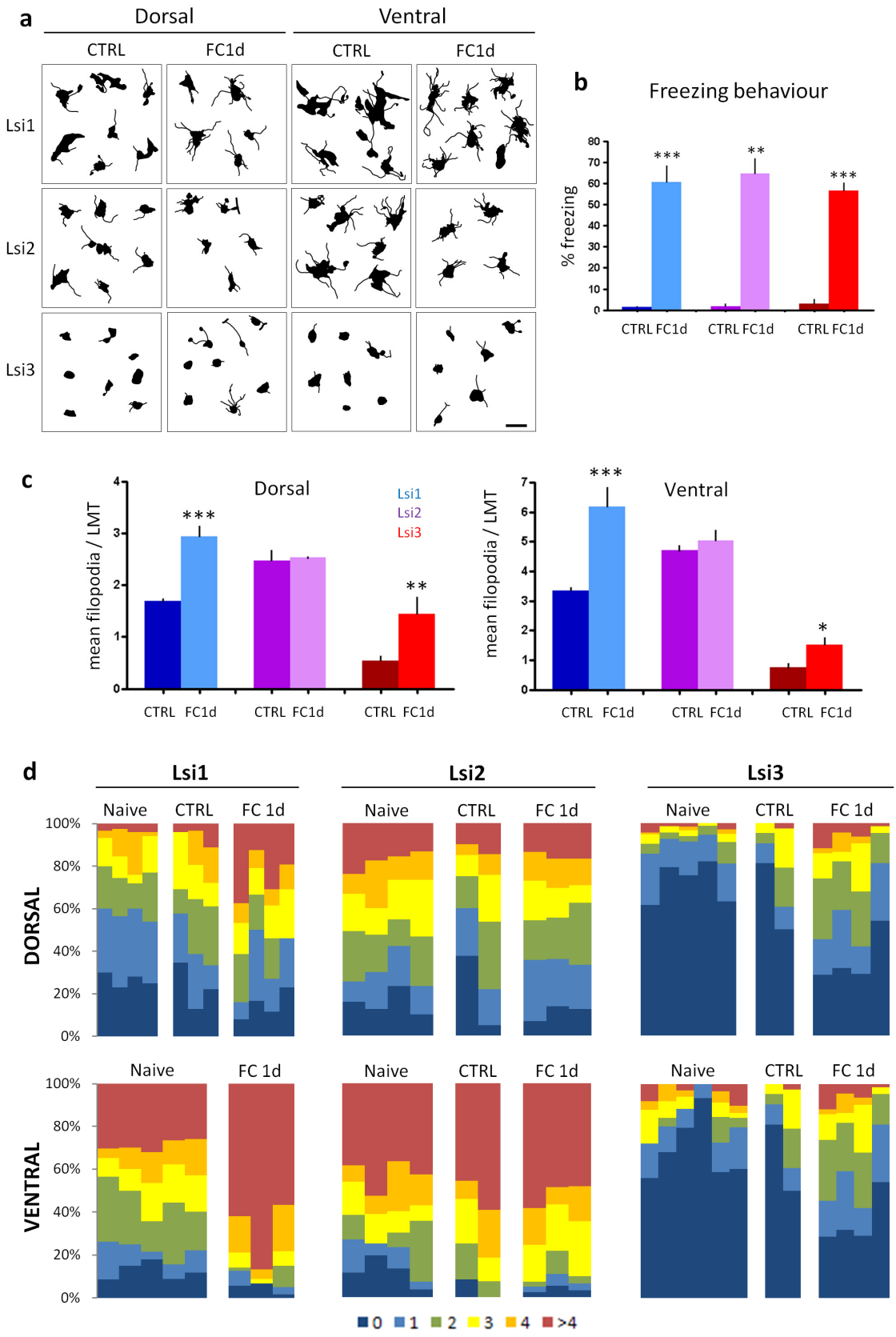
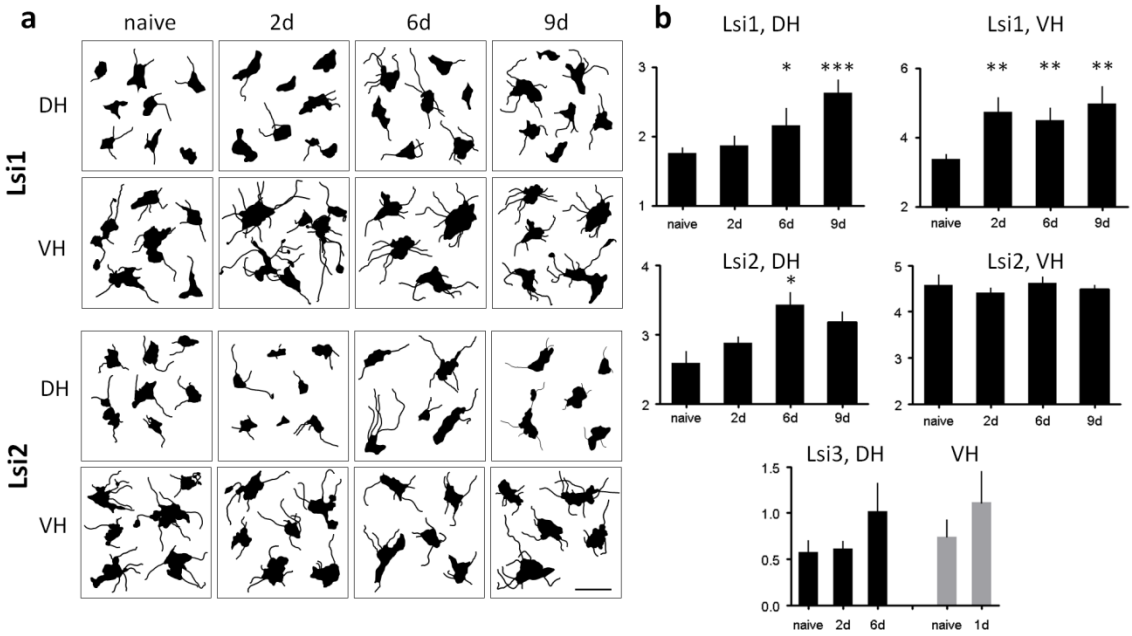

a
Dorsal
Ventral
CTRL
FC1d
CTRL
FC1d
Lsi1
Lsi2
Lsi3
b
Freezing behaviour
% freezing
***
**
***
CTRL FC1d
CTRL FC1d
CTRL FC1d
c
Dorsal
Ventral
Lsi1
Lsi2
Lsi3
mean filopodia / LMT
mean filopodia / LMT
***
**
***
*
CTRL FC1d
CTRL FC1d
CTRL FC1d
CTRL FC1d
CTRL FC1d
CTRL FC1d
d
Lsi1
Lsi2
Lsi3
Naive
CTRL
FC 1d
DORSAL
VENTRAL
100%
80%
60%
40%
20%
0%
0
1
2
3
4
>4

Figure 4: Subpopulation-dependent FFI connectivity growth upon contextual fear conditioning (page 75)
(a) Representative camera lucida examples of LMTs under control conditions and one day after contextual fear conditioning (FC 1d) in DH and VH in Lsi1, Lsi2 and Lsi3 mice. Scale bar: 10 µm. **(b)** Long-term fear memory 24h after cFC. Percentage of time spent freezing during the recall test. **(c)** Mean numbers of filopodia per LMT upon FC. Control (no US) and naïve mice were pooled into one group (CTRL). Lsi1 (n = 9 CTRL; n = 6 FC1d), Lsi2 (n = 8 CTRL; n = 3 FC1d) and Lsi3 (n = 7 CTRL; n = 4 FC1d). N = 50 – 100 LMTs per region and per mouse. **(d)** Prevalence of FFI connectivity growth upon FC in DH (top) and VH (bottom) in Lsi1, Lsi2 and Lsi3 mice. Details as in Figure 1c. * $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

FFI plasticity upon an incremental learning task in different subpopulations

Why did Lsi2 LMTs fail to exhibit FFI connectivity growth upon cFC, a protocol that triggers rapid and massive increase in filopodial contents in both Lsi1 and Lsi3 mice? To test whether Lsi2 GCs were not competent to establish increased filopodia numbers upon learning, we subjected Lsi2 mice to another hippocampal-dependent task based on incremental learning. Mice were trained on a Morris water maze (MWM) protocol for up to 9 days, during which they had to learn to locate a fixed hidden platform. Every day consisted of four swim trials lasting 60 seconds and separated by an interval of 5 min. On the day following the last day of training, mice underwent a probe trial consisting of a 60-seconds swim in the absence of platform. Reference memory was assessed by quantifying the percentage of time spent searching in the correct (target) quadrant. Values became higher than chance only after 3 days of training, and reached plateau levels at day 9.

In DH of Lsi2 mice we observed an increment in the number of filopodia per LMT after 6 days (1.33-fold, $p < 0.05$) and 9 days of training (1.23-fold, $p > 0.05$) (Figure 5b), ruling out the hypothesis that this subpopulation of GCs may not be competent to undergo FFI plasticity. In contrast to Lsi1 LMTs, where values increased to reach plateau levels at day 9 (Ruediger et al., 2011), highest values were already reached after 6d in Lsi2. Preliminary data from Lsi3 mice show an increasing FFI connectivity in DH (1.76-fold, $p > 0.05$) on day 6, suggesting that the observed increase in filopodial contents in DH may be a general effect of an incremental learning task, irrespective of subtype specificities. Upon the same task, filopodial contents in VH of Lsi1 mice were increased as early as day 2 and remained elevated for at least 9 days (Figure 7c in section 2.1.3). Preliminary results in Lsi3 mice also


a
naive
2d
6d
9d
Lsi1
DH
VH
Lsi2
DH
VH
b
Lsi1, DH
Lsi1, VH
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naive
2d
6d
9d
1d

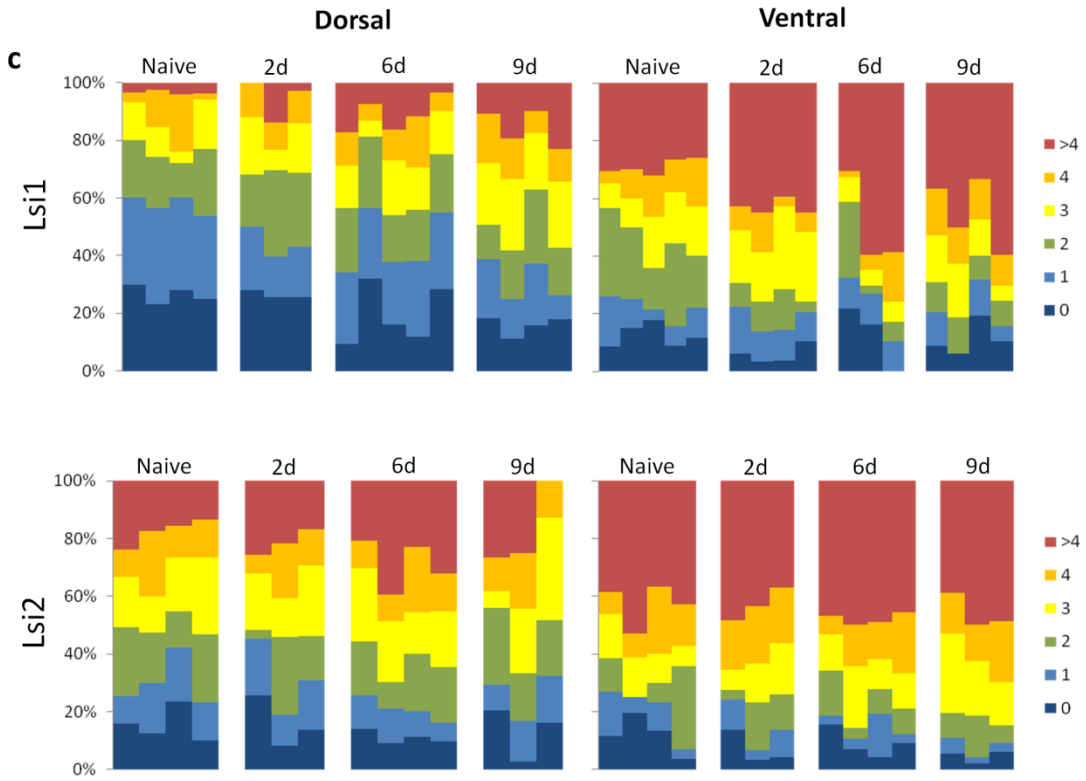

c
Dorsal
Ventral
Naive
2d
6d
9d
Lsi1
Lsi2
100%
80%
60%
40%
20%
0%
>4
4
3
2
1
0

Figure 5: Time course of FFI connectivity growth upon MWM training in different GC subpopulations (page 77)
(a) Representative camera lucida examples of Lsi1 and Lsi2 LMTs upon MWM training in DH and VH. Scale bar: 10 μm. **(b)** Time course of FFI connectivity growth upon MWM training in Lsi1 (n = 3 – 6 mice), Lsi2 (n = 3 – 4 mice) and Lsi3 (n = 2 mice) mice. (**c**) Prevalence of FFI connectivity growth upon MWM training in Lsi1 (top) and Lsi2 (bottom) mice. Details as in Figure 1c.

show increased FFI connectivity on day 2 (1.5-fold, $p > 0.05$) (Figure 5b). Surprisingly, however, FFI plasticity in VH of Lsi2 LMTs was not altered (Figure 5b), suggesting that the mechanisms of filopodial induction in VH may differ between distinct subpopulations of GC.

Taken together, these results show that dorsal Lsi2 GCs undergo FFI plasticity upon a MWM but not a cFC learning task, suggesting that individual GC subpopulations may be recruited upon distinct behavioural paradigms.

Thorny excrescences in distinct subpopulations of CA3 pyramidal neurons

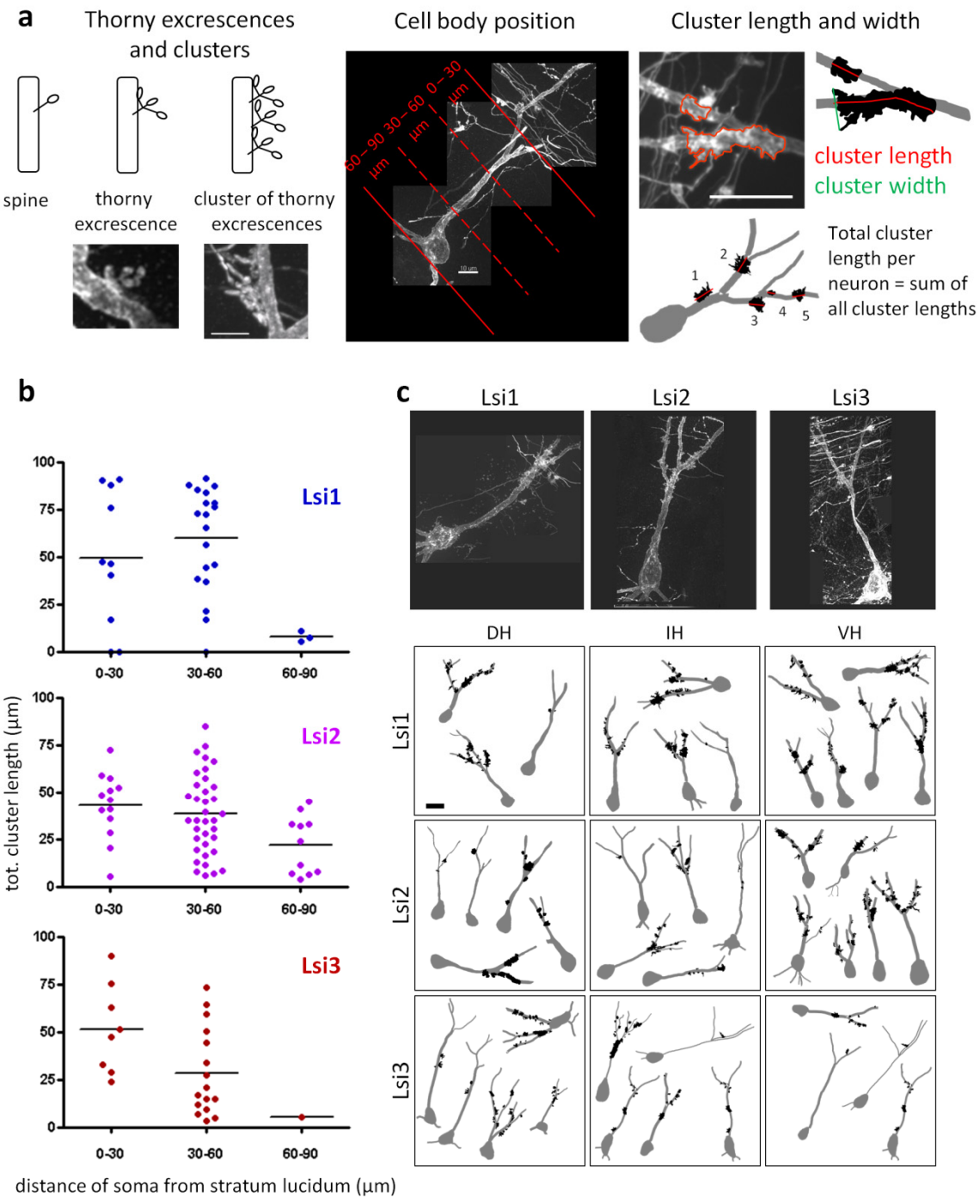
Mossy fibre terminals make synapses onto CA3 pyramidal neurons in *stratum lucidum*, which bear unique complex dendritic postsynaptic structures called thorny excrescences, a feature that they share only with hilar neurons (Chicurel and Harris, 1992; Fitch et al., 1989; Gonzales et al., 2001). As mentioned in the introduction, it has been shown that genetic sister principal hippocampal neurons are preferentially interconnected, so that Lsi1 (or Lsi2) GCs preferentially connect to Lsi1 (or Lsi2) CA3 pyramidal neurons (Deguchi et al., 2011). Because we observed different LMT morphology and plasticity across subpopulations, we asked whether the postsynaptic partners of LMTs (i.e., thorny excrescences on CA3 pyramidal neurons in *stratum lucidum*) may also exhibit subpopulation-specific features.

To analyse CA3 pyramidal neuron thorny excrescences (or thorns), we cut the hippocampi into 200-μm thick lamellar sections, so as to preserve most of dendritic tree extent in *stratum lucidum*. For analyses, we considered only pyramidal cells bearing thorns (Figure 6a), which we call thorny cells. CA3 pyramidal neurons are not homogeneous and fall into two major classes: 1) short-shaft pyramidal neurons, which exhibit short apical shafts, densely branched apical and basal dendritic trees and a large number of thorny

excrescences; cell bodies of these cells tend to be located higher in *stratum pyramidale* (closer to stratum lucidum); 2) long-shaft pyramidal neurons, characterised by a long apical shaft, relatively less dendritic branching in both the apical and basal trees and a small number of thorny excrescences; their somata tend to lie further away from *stratum lucidum* (Fitch et al., 1989). To verify whether the correlation between soma position and number of thorns may also apply to specific subpopulations of pyramidal cells, we subdivided labelled pyramidal neurons into three groups based on the distance of the soma from stratum lucidum (0-30, 30-60 and 60-90 μm).

Since single thorny excrescences cannot be resolved by light microscopy owing to their grouping into clusters, for each pyramidal neuron we measured the length of each thorn cluster and calculated the total cluster length along dendrites in *stratum lucidum* (Figure 6a). This measurement allows quantifying the extent of dendritic branch coverage by thorns. Consistent with the literature, we found that cells bearing more (or longer) thorns were located closer to stratum lucidum (0-30 μm group). Conversely, cells with less (or shorter) thorns were predominantly positioned further away from stratum lucidum (Figure 6b).

Figure 6: Regional and subpopulation analysis of CA3b pyramidal neurons thorny excrescences (page 80)
(a) Parameters to analyse thorny excrescences. Left: Schematic drawings illustrating postsynaptic structures: single spines (left), thorny excrescences (exhibiting a single neck and multiple heads) and cluster of thorny excrescences (uninterrupted stretches of excrescences along the shaft). Bottom: high-magnification micrographs examples of a thorny excrescence and a cluster of thorns. Scale bar: 5 μm. Middle: Cell body position expressed as the distance from *stratum lucidum*. Subdivision of *stratum pyramidale* into three portions: 0 – 30 μm, 30 – 60 μm and 60 – 90 μm from the beginning of *stratum lucidum*. Scale bar: 10 μm. Right: thorn cluster widths and lengths. Maximum intensity projections of 3D high-resolution stacks were generated and the outline of individual thorn clusters was drawn. Cluster length (red) was defined as the maximal length of the cluster along the dendritic stretch, while cluster width (green) was defined as the maximal length of the cluster perpendicular to the dendritic shaft. For every thorny CA3 pyramidal neuron we calculated the total cluster length, resulting from the sum of all individual cluster lengths. Scale bar: 10 μm. **(b)** Micrographs (top) and camera lucida examples of representative CA3b pyramidal neurons in Lsi1, Lsi2 and Lsi3 in dorsal, intermediate and ventral hippocampus. **(c)** Total cluster length of thorny cells (pooled from all regions and mice) as a function of cell body position (distance from *stratum lucidum*). Lsi1: N = 32 cells from 8 mice; Lsi2: N = 60 from 6 mice; Lsi3: N = 25 cells from 4 mice. Scale bar: 20 μm.


a
Thorny excrescences and clusters
spine
thorny excrescence
cluster of thorny excrescences
Cell body position
0–30 µm
30–60 µm
60–90 µm
Cluster length and width
cluster length
cluster width
Total cluster length per neuron = sum of all cluster lengths
b
Lsi1
Lsi2
Lsi3
tot. cluster length (µm)
0-30
30-60
60-90
distance of soma from stratum lucidum (µm)
c
Lsi1
Lsi2
Lsi3
DH
IH
VH

We found that the population of CA3 pyramidal neurons was very heterogeneous with respect to branch coverage by thorns (Figure 6c). At every dorsoventral level and in each subtype we could detect cells with only a few or several thorn clusters, with total cluster length per neuron ranging from a few to up to 100 microns. Interestingly, no differences along the DV axis were observed in Lsi1 in terms of total cluster length (Figure 7a). However, Lsi1 pyramidal neurons are very rare in dorsal CA3, which prevents an unbiased analysis. By contrast, branch coverage in Lsi2 expressed as total cluster length was higher in ventral pyramidal cells (1.78-fold, $p < 0.01$), while Lsi3 neurons exhibited the opposite trend (Figure 7a).

In order to identify possible subtype-dependent differences at the postsynaptic level, we carried out a region- and subpopulation-specific analysis of thorn clusters based on following parameters: total cluster length per CA3 pyramidal cell as well as length and width of individual thorn clusters. When we compared total cluster lengths per cell, we observed significant differences among subtypes only in the ventral hippocampus, where Lsi1 pyramidal neurons exhibited values almost two times higher than Lsi3 (1.9-fold, $p < 0.05$) (Figure 7b). As for individual thorn cluster widths, Lsi1 and Lsi2 exhibited comparable values at all dorsoventral levels (5-7μm). These were significantly higher than values measured in Lsi3 dendrites, both dorsally (1.28-fold, $p < 0.05$) and ventrally (1.63-fold, $p < 0.001$) (Figure 7c). Likewise, individual thorn cluster lengths were comparable in Lsi1 and Lsi2 in all regions and significantly higher than Lsi3 at all dorsoventral levels (1.33-fold, $p < 0.05$ in DH, 1.68, $p < 0.001$ in IH and 1.92-fold, $p < 0.001$ in VH) (Figure 7c).

These results show that thorn cluster properties are similar between Lsi1 and Lsi2 pyramidal neurons, but substantially different from Lsi3 cells, which tend to be smaller and exhibit in general shorter and less wide thorns. These findings are consistent with our results about LMT morphology and plasticity, as well as our microarray data, which together suggest that Lsi1 and Lsi2 represent subtypes of GCs with unique properties.

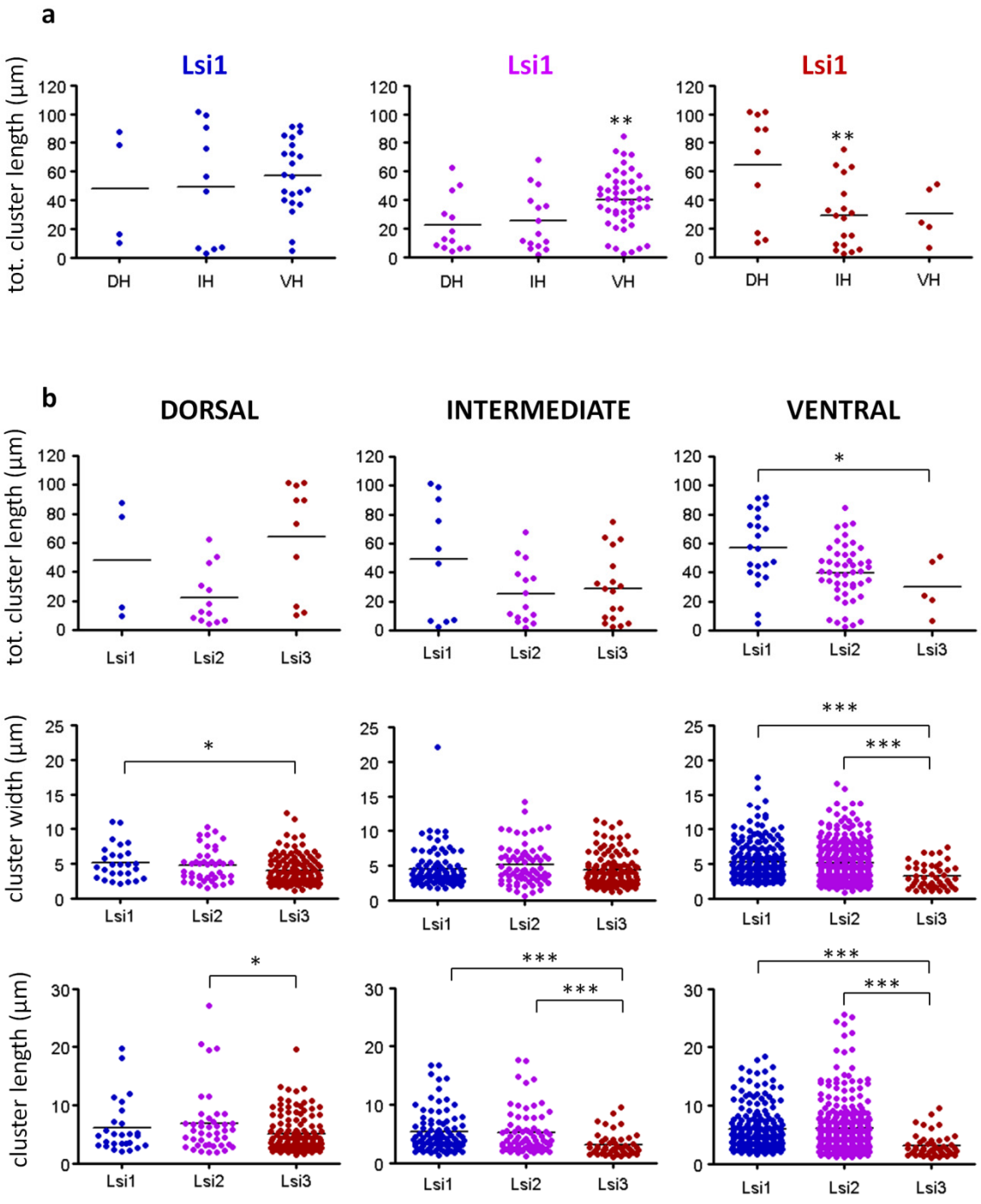


Figure 7: Regional- and subpopulation-specific characterisation of thorn clusters
(a) Regional characterisation of thorn clusters. Total cluster length per CA3b pyramidal neuron in different regional subdivisions of Lsi1, Lsi2 and Lsi3 mice. Lsi1: N = 36 cells; Lsi2: N = 76 cells; Lsi3: N = 33 cells from 4 – 6 mice. **(b)** Comparison of thorn clusters among subpopulations. Top row: Total cluster length per CA3b pyramidal neuron in DH, IH and VH (left, middle and right panel, respectively). Middle row: distribution of cluster widths. Bottom row: distribution of cluster lengths. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

2.2.4 Discussion

GC subpopulation-specific LMT morphologies along the longitudinal and transversal axes of the hippocampus

We have shown that distinct subpopulations of GCs exhibit morphological differences at the level of their LMTs. Lsi1 and Lsi2 LMTs were larger and contained filopodia and satellites under baseline conditions, whereas these features were rare in Lsi3 and in randomly labelled GCs. According to a previous study from our lab, 50%-55% of all GCs exhibit no LMTs with detectable satellites (i.e., no terminal arborisations, TAs) (Galimberti et al., 2010), and Lsi3 belongs to that group. Genetic factors likely underlie the specific properties of LMTs under baseline conditions, such as their morphology and propensity to form TAs and filopodia, possibly through differential expression of critical molecules supporting synaptic growth and/or process outgrowth during development.

A novel finding of this work was that the degree of LMT heterogeneity along the DV axis varied across subpopulations. It was most pronounced in Lsi1 LMTs and virtually absent in Lsi3 and randomly labelled LMTs, suggesting that it is an intrinsic property of specific subtypes of mossy fibres. Transcriptome analyses of dorsal and ventral GCs revealed that Lsi1 GCs exhibited the largest number of differentially regulated genes. Compared to the average GC population (Lmu1), we found about 7 times more differentially regulated genes in Lsi1 and almost 2 times more in Lsi2. These data suggest that the marked dorsoventral differentiation in Lsi1, and slightly less steep in Lsi2, is a genetically defined property of these subpopulations of GCs. By contrast, we found a gradient of increasing LMT complexity along the proximodistal axis in CA3 in all three GC subpopulations, both in dorsal and ventral regions, suggesting that it may be a universal property of hippocampal organisation. This feature may reflect differential connectivity of CA3 pyramidal neurons along the transverse axis. Proximal CA3c neurons receive strongest mossy fibre input from granule cells located in the infrapyramidal blade, the crest and the suprapyramidal blade of the DG (Claiborne et al., 1986), whereas distal CA3b pyramidal neurons are innervated most strongly by granule cells positioned in the suprapyramidal blade of the DG (Cutsuridis et al., 2010; Witter, 2007). Therefore, CA3 pyramidal neurons at different positions along the transverse axis are innervated by granule cells that in turn are different either in their

connectivity or in their functionality (Hara, 1990; Jaarsma, 1992; Scharfman 2002; Choi 2003; Witter 2007; Witter, 2010). In addition, as a result of the abrupt longitudinal turn taken by mossy fibres at the end of CA3 (De No, 1934; Swanson et al., 1978a), proximal and distal pyramidal neurons along CA3 may exhibit functional differences. In fact, at distal and dorsal portions of CA3, individual neurons receive mossy fibre inputs originating from widespread parts situated dorsally to their location, thus allowing these distal CA3 neurons to integrate the output of a substantial portion of the DG (Witter, 2007). Therefore, it is plausible to assume that proximal and distal CA3 portions may be functionally different. As a consequence, and since the CA3 to CA1 to subiculum connectivity is topographically organised (Amaral et al., 1991; Ishizuka et al., 1990), differential FFI connectivity along CA3 is likely to have a functional impact on the entire hippocampal circuitry.

Subpopulation-specific structural plasticity upon experience and learning

EE is a behavioural paradigm known to promote brain and hippocampal plasticity (van Praag et al., 2000). In particular, EE increases satellite frequencies in Lsi1 and Lsi2 LMTs (Galimberti et al., 2006) and may thus support subsequent hippocampal learning by locally rearranging CA3 pyramidal neuron ensembles. EE did not induce satellite formation in Lsi3 LMTs, suggesting that this subpopulation of GC mossy fibres is not competent to form TAs. This interpretation is consistent with a previous study showing that interference with EphA4 signalling in Lsi3 organotypic slices (a treatment that leads to TA formation in Lsi1 and Lsi2 mossy fibres) failed to induce TA formation in Lsi3 mossy fibres (Galimberti et al., 2010). New satellite formation upon EE was more pronounced in Lsi2 (3.5-fold increase on average) than in Lsi1 (on average 1.5-fold increase). Remarkably, Lsi2 GCs belong to the 10% - 15% fraction of cells that establish ≥ 2 TAs per mossy fibre, while Lsi1GCs are part of the 30% - 35% portion of cells that exhibit one TA per mossy fibre (Galimberti et al., 2010). Interestingly, the extent of satellite formation in DH and VH was comparable within one subpopulation, suggesting that both regions are equally recruited upon this behavioural paradigm.

Our results show that FFI connectivity in lines Lsi1 and Lsi3 was massively increased 24h after contextual fear conditioning (cFC). Moreover, the degree of filopodial induction

differed between subpopulations (1.8-fold in Lsi1 versus 2.3-fold in Lsi3), but was comparable in DH and VH within one subpopulation, suggesting that the extent of the response is a subpopulation-specific characteristic. Surprisingly, Lsi2 LMTs did not undergo FFI plasticity upon fear conditioning. However, they did exhibit increased filopodial contents after 6 days of training on an incremental learning task (MWM), arguing against the possibility that they may not be competent to undergo FFI plasticity.

Why is FFI connectivity growth upon cFC prevented in Lsi2 LMTs? One possibility may be that Lsi2 GCs constitute a subpopulation that is not recruited upon a fear conditioning task. In other words, the nature of a learning task (one-trial associative learning versus incremental learning) may determine the induction of FFI plasticity in combination with the specific genetic signature, such that a particular subpopulation of GCs may be responsive to some but not to other learning paradigms. Consistent with a current theory that different memories are stored in different subsets of neurons (Silva et al., 2009), it has been shown that a subpopulation of neurons in the lateral amygdala exhibiting elevated levels of cyclic adenosine monophosphate response element-binding protein (CREB) is preferentially activated during fear conditioning (Han et al., 2007), and that ablating or inactivating CREB neurons selectively erases fear memory (Han et al., 2009). However, it is still unclear whether distinct subpopulations in the hippocampus may also be preferentially recruited upon specific behavioural tasks. cFC is a task that strongly depends on the hippocampus and the amygdala (Fanselow, 2000; LeDoux, 2000). Interestingly, very few Lsi2 neurons are found in ventral CA1 and in the amygdala (Deguchi et al., 2011), two regions that are reciprocally connected (Pitkanen et al., 2000). Based on the speculation that microcircuits may extend beyond the hippocampal trisynaptic loop and include the amygdala, Lsi2 may label a subpopulation of neurons that are not recruited upon fear conditioning as a result of a putative low connectivity with the amygdala.

In the ventral hippocampus, MWM training caused FFI connectivity growth as early as two days after training in LMTs from Lsi1 (and Lsi3 mice; preliminary data). As we believe that such filopodial increase may reflect goal learning coupled to reward (see discussion 2.1.4), we were surprised by the lack of plasticity response in ventral Lsi2 LMTs. One possible interpretation could relate to the high number of filopodia/LMT present under baseline conditions in VH (4.6 ± 0.19), which may possibly preclude a further increase in FFI

plasticity upon learning. Theoretically, a "saturation" level for every LMT subpopulation may exist, whereby filopodial contents would not be able to grow beyond a given limit. Further experiments would be required to test this hypothesis, such as experimentally decreasing filopodial contents below baseline levels and subsequently subjecting mice to a learning task.

Taken together, these data show that LMT remodelling affecting FFE or FFI in response to experience or learning obeys as yet unidentified subpopulation-specific rules. Finding a means to experimentally alter filopodial numbers under baseline conditions and measuring the extent of plasticity changes may give us insights into possible mechanisms underlying those responses.

Subpopulation-specific properties of postsynaptic counterparts

Analysis of CA3 pyramidal neurons thorny excrescences, the postsynaptic counterpart of LMTs, revealed subpopulation-specific differences. The main finding was that Lsi1 and Lsi2 thorn clusters were quite similar to each other but significantly different from Lsi3 neurons in terms of total cluster length as well as individual thorn cluster width and length. Small and short thorn clusters in Lsi3 pyramidal neurons may be suited to accommodate small and simple LMTs, while more complex thorns in Lsi1 and Lsi2 may are consistent with larger and more convoluted presynaptic LMTs.

Given the pronounced heterogeneity of LMT morphology and complexity along the DV axis in Lsi1 and Lsi2 subpopulations of GCs, we would have expected to find a similar trend at the level of the postsynaptic counterpart. Surprisingly, regional differences were not detected in Lsi1 but only in Lsi2 pyramidal neurons, whose total cluster length was 1.8 times higher in VH than in DH. Owing to the extremely low fraction of Lsi1 pyramidal neurons in dorsal CA3 (Deguchi et al., 2011), the sampling of thorny Lsi1 pyramidal cells in DH was insufficient to address regional differences of pyramidal neuron thorn complexity in Lsi1 mice. Nevertheless, based on the notion that Lsi1 GCs preferentially connect to Lsi1 pyramidal neurons, we would expect to find larger and more complex thorns in ventral pyramidal neurons compared to dorsal cells to accommodate larger LMTs.

3. GENERAL DISCUSSION

In this thesis I have provided evidence that structural plasticity upon experience and learning in the adult mouse hippocampus is governed by regional and subpopulation rules. Here, I will discuss the significance and implications of these findings and suggest possible future directions to pursue this line of research.

3.1 Behavioural dissociation along the longitudinal axis of the hippocampus

Although a large body of evidence supports the notion that the hippocampus is heterogeneous along its dorsoventral axis (reviewed in (Fanselow and Dong, 2010; Moser and Moser, 1998), the specific contributions of the ventral hippocampus in learning have not been addressed. In the MWM task, for example, most studies have focussed on the end stage and the outcome of a task. Based on the findings that VH-lesioned animals are able to establish a spatial reference memory and that their performance at the end of the training is indistinguishable from control animals, it was concluded that the ventral hippocampus is dispensable for spatial learning (Bannerman et al., 2003; Moser et al., 1995). By contrast, the strength of our approach was the in-depth investigation of the behaviour at all stages of the learning process, not only considering escape latencies but carefully analysing search strategies and within-subject performance variability across trials. By this means, we were able to show that VH is required for initial performance and stability throughout the learning process. In addition, we took advantage of FFI connectivity growth as a parameter to assess hippocampal recruitment upon learning, and based on the different time course of filopodia induction we conclude that DH and VH are implicated in distinct phases and aspects of a learning task. Learning processes may be subdivided into an early and a late phase. According to a model proposed for skill acquisition (Gentile, 2000), the early phase of learning consists of acquiring the general concept of the nature of a task, a process involving understanding and attending to important features of the so-called “action goal” (Rao, 2006). In the case of the MWM task, the goal would be understanding that there is a submerged safety platform that has to be found. Late stages of learning are characterised by refinement of what has been learned in the early phase (Rao, 2006). In the MWM case,

performance improves by refining the searching strategy, leading to the formation of an increasingly more precise spatial map of the environment. The observed time course of FFI connectivity growth suggests that VH plays a critical role in the early phase of representing the nature of a task, while DH is crucial during the refinement phase of an incremental learning task. It is still unclear why two days of goal exposure are required to induce FFI plasticity in VH, but this could reflect the process of encoding the nature of the task (early stages of the learning process). The role for VH in goal-oriented behaviour (Burton et al., 2009; Viard et al., 2011) may be supported by its selective connectivity to limbic areas involved in novelty encoding, reward and expectation (Lisman and Grace, 2005), as well as to the mPFC (Jay and Witter, 1991), which plays a role in a number of "executive functions" such as attention, working memory, decision-making and goal encoding (Ragozzino et al., 1998; Ragozzino et al., 1999; Rich and Shapiro, 2009).

To determine whether our findings reflect a general principle that applies to all forms of hippocampal-dependent learning, one could monitor FFI connectivity growth upon other tasks that involve different kinetics of acquisition. Learning upon cFC is instantaneous, while establishing a reference memory in a MWM task requires at least five days (the time required to induce filopodia increase in DH). However, FFI growth in VH is induced already after two days of MWM training, which we interpret as representing the nature of the goal of the task. To test whether this hypothesis holds true, one could train mice on a radial-arm maze task, whose acquisition requires at least seven days, and assess whether FFI plasticity in VH may be shifted in time (assuming that learning the nature of the task occurs at later stages in this more challenging task).

To further explore the mechanisms underlying FFI plasticity in VH upon learning, one could investigate possible neuromodulatory contributions. Our preliminary results revealed a role of dopamine signalling, but more in-depth investigations about timing and site of action of dopamine will be required to precisely dissect the neuromodulatory influence on FFI plasticity upon learning. In addition, other brain structures may be involved in hippocampal FFI plasticity induction. Owing to the differential connectivity of DH and VH (section 1.2.3.1), it will be worth investigating separate systems and their specific influence on plasticity in different regions of the hippocampus.

3.2 Significance of increased FFI connectivity upon learning

It has been shown that *β*-adducin$^{-/-}$ mice, which do not undergo increased FFI connectivity upon learning, exhibit impaired performance in the early phase of the MWM training (Ruediger et al., 2011). Would performance be better, or would learning occur faster or more efficiently, if filopodial contents were elevated from the beginning? Would FFI connectivity induced by learning one task be advantageous to learn a second, unrelated task? It is still unclear whether it is a matter of network property or whether recruitment of specific ensembles of neurons is involved. According to the first view, a general chemical induction of filopodia (e.g. by acetylcholine) could in principle be sufficient to improve learning. Conversely, if separate ensembles are recruited for learning distinct tasks, filopodial induction brought about by a first task would not be beneficial for the second task.

3.3 Hippocampal microcircuit: functional implications

The functional significance of hippocampal microcircuits remains to be elucidated. Based on their different plasticity properties (Galimberti et al., 2010) and responses upon experience and behaviour, it will be important to investigate the role played by separate microcircuits in information processing in the hippocampus. Our finding that Lsi2 dorsal LMTs underwent FFI connectivity growth upon MWM but not fear conditioning may suggest that separate subpopulations are differentially recruited during the encoding and retrieval of episodic representations. One possibility to address this issue would be to use immediate-early genes, such as *c-fos*, to monitor recruitment of specific subtypes of GCs and pyramidal neurons upon different hippocampus-dependent behavioural paradigms.

In addition, it would be worth investigating whether microcircuits extend beyond those identified in the trisynaptic loop (granule cells and pyramidal neurons in CA3 and CA1). For example, genetic sister cells may be found in the layers of the entorhinal cortex projecting to the hippocampus, implying that information may be segregated into parallel routes already upstream of the hippocampus. In addition, this arrangement may exist at hippocampal output structures as well (e.g, amygdala). Similar to the presence of functional subpopulations of neurons in the basal amygdala ("fear neurons" and "extinction

neurons"), it is tempting to speculate that entire microcircuits may exist that are devoted to, or preferentially recruited upon distinct behavioural tasks ("fear microcircuits", "spatial learning microcircuits" and so on), but further investigations will be required to prove this hypothesis.

Another major finding was that the extent of structural plasticity differed across neuronal subpopulations. Upon enriched environment, increase in satellite numbers was massive in Lsi2, less pronounced in Lsi1 and completely absent in Lsi3 LMTs, suggesting that genetic factors may determine the propensity to establish satellites, possibly through differential expression of critical molecules supporting synaptic growth.

In conclusion, our results provide a solid ground to start dissecting differential functions of hippocampal regions and subpopulations.

4. EXPERIMENTAL PROCEDURES

4.1 Mice

Transgenic mice expressing membrane-targeted GFP in a small subset of neurons (*Thy1-mGFP^{Si1}* and *Thy1-mGFP^{Si2}*) were as described (De Paola et al., 2003; Galimberti et al., 2010). Line *Thy1-mGFP^{Si3}* was generated by Markus Sigrist in the laboratory of Silvia Arber (FMI, Basel).

All experiments were in accordance with institutional guidelines and were approved by the Cantonal Veterinary Office of Basel Stadt, Switzerland. Mice were kept in temperature controlled rooms on a constant 12h light/dark cycle, and experiments were conducted approximately at the same time during the light cycle.

4.2 Behavioural procedures

Unless stated otherwise, all experiments were performed with adult male mice aged between P55 and P65 at the onset of experiment. For all behavioural paradigms, mice were kept in single cages in a quiet room 3 – 4 days before training and for the entire experiment.

Enriched environment (EE)

At the age of P30, male mice (3 – 4 mice per cage) were housed for 20 to 30 days in a large (rat) enrichment cage containing several running wheels and several toys for exploration. Control mice were moved at the age of P30 to standard single cages for the same period of time (Galimberti et al., 2006; Gogolla et al., 2009).

Contextual fear conditioning

The contextual fear conditioning experiment was carried out as described (Ruediger et al., 2011). Briefly, the conditioning chamber was cleaned with 2% acetic acid before and after each session. Once placed inside the fear-conditioning chamber, mice were allowed to freely explore the apparatus for 2.5 min and then received five foot shocks (1 s and 0.8 mA each, inter-trial interval of 30s). Control mice were subjected to the same procedure except for omission of the foot shock. To test for contextual fear memory, mice were returned to the conditioning chamber during a test period of 2.5 min and sacrificed within 10 – 15 min.

In all the experiments, the acquisition and retention sessions were digitally recorded, and fear retention was measured as the percentage of time spent freezing. Freezing was defined as the complete absence of somatic mobility, except for respiratory movements. Exploratory activity was measured as body distance travelled over time.

Olfactory fear conditioning

Olfactory fear conditioning was identical to the paradigm described for contextual fear conditioning except that the whole procedure took place in the dark. To test for olfactory memory, mice were placed in a neutral context (cylindrical shape) wiped with the same odour as in the conditioning chamber (2% acetic acid). Freezing and exploratory behaviour were assessed as described above.

Morris water maze task

The Morris water maze consisted of a circular (diameter: 140 cm) pool filled with milky water in order to obscure the platform and allow efficient tracking of the animal's swim paths. Specialised software (Biobserve, Viewer II) sampled the position of the animal and provided measures such as latency, path length, swim speed and the amount of time spent in defined regions of the pool. The pool was homogeneously illuminated and surrounded by black curtains and spatial cues. In the visible version of the task, a circular escape platform (10 cm diameter) was labelled with a red tape and was placed in the NW quadrant at 1 cm above the water surface. In the invisible version of the task, the platform was submerged 0.5 cm below the water surface and placed in the SE quadrant. In the invisible version of the task, the platform was kept in a fixed position across the training days.

Mice were trained to find the platform during 4 trials a day for up to 11 days. For every trial within a day, mice were placed in the pool (facing the walls) from different starting locations which were pseudo-randomly assigned. Every training day consisted of four swim trials of 60 s each, separated by a 5-min. interval. At the end of each trial, mice were allowed to sit on the platform for 15 seconds (if they failed to find it by themselves, they were manually guided to it). Single probe trials to test reference memory were conducted one day after the last training session. Mice were released at a random start position and were allowed to swim for 60 seconds in the absence of the platform.

The "standard MWM protocol" was designed as follows: on the first day (day 1), naïve mice were habituated to the visible platform version of the task (4 swim trials, 60 s each, 5 min. inter-trial interval). Starting from the following day (day 2), the platform was moved to the SE quadrant and remained fixed (submerged) for the entire duration of the training (*x* days). On the day following the last training day (day *x*+1), mice underwent a probe trial and were sacrificed within 10 min. For experiments assessing c-Fos expression, mice were returned to their home cage for 90 min. prior perfusion. For the experiment modulating task difficulty, platforms of different sizes were used: the standard platform (A; 10 cm diameter), a small custom-designed platform (0.5A; 5 cm diameter) and a large custom-designed platform (2A; 20 cm diameter). Unless stated otherwise, the standard platform (A) was used in all experiments. Deviations from the standard protocol are described in the text.

Novel object recognition

Mice were handled for 5 min on two consecutive days and habituated to the testing arena for 15 min per day on the two following days. On the fourth day, each animal was allowed to explore for 10 min two identical objects placed in the arena. 24h later, one of the familiar objects was replaced with a novel object, and each animal was allowed to explore the arena and the objects for 5 min. The familiar and the novel object were different in shape and colour

Recognition memory was expressed by the exploration index (*I*), which was defined as follows:

$$I = \frac{T_{new} - T_{old}}{T_{new} + T_{old}},$$

where T_{new} and T_{old} represent the time spent exploring the novel and the familiar object, respectively.

4.3 Tissue preparation

Organotypic hippocampal slice cultures

Organotypic slice cultures from the dorsal or ventral hippocampus (see below for a definition) were prepared from P7 pups according to the Stoppini method as described (Gogolla et al., 2006). The culture medium was exchanged every third day. Slices were kept for 20 days *in vitro* before imaging (see below).

Lamellar hippocampal section preparation from the entire hippocampus

Mice were transcardially perfused with 50 ml ice-chilled 4% paraformaldehyde in PBS pH 7.4. Brains were collected and kept in fixation solution at least overnight at 4°C. Hippocampi were dissected, embedded in 4% agarose gel and sliced transversally on a tissue chopper (McIlwain) along the entire dorsoventral axis so as to obtain lamellar hippocampal sections of 100-125 µm thickness. The total longitudinal extent of the hippocampus was defined as 100% (0% corresponding to the dorsal and 100% the ventral pole). For analyses of the dorsal, intermediate and ventral hippocampus, sections were collected from the region D2 (20% – 35%), Int (40% – 55%) and V1 (65% – 80%) (see also Figure *1*A on page 31). For analysis of CA3 pyramidal neurons, 200- µm thick sections were produced.

Immunohistochemistry

The standard immunohistochemistry procedure was as follows: free-floating transverse sections were blocked for 1 h at room temperature in PBS-0.25% Triton-X-100 (PBS-T) containing 3% BSA, then incubated with the primary antibody solution (PBS-T, 3% BSA) over night at 4°C, washed three times 5-10 min. in PBS-T and subsequently incubated with the secondary antibody solution (PBS-T) for 2-3 hr at room temperature. After washing three times 5-10 min., sections were mounted in ProLong Gold antifade reagent (Molecular Probes), coverslipped (0.17 ± 0.01 mm, Assistant, Sondheim, Germany), sealed with transparent nail polish 24h after mounting and stored at 4°C until imaging.

Antibodies were from the following sources and were used as follows: primary antibodies rabbit anti-GFP (Molecular probes, Eugene, OR, USA), 1:1000; rabbit anti-c-Fos (Santa Cruz), 1:10’000; mouse anti-NeuN (Chemicon), 1:200. Secondary antibodies were

AlexaFluor 568 or 488 (Molecular probes) and use at the same dilution as the primary antibodies.

For evaluating the extent of lesions, neuronal cell bodies were counterstained with a Nissl fluorescent staining. Floating sections were rehydrated for at least 40 min. in 0.1 M PBS, pH 7.2 then washed for 10 min. in PBS-T and washed two times more for 5 min each in PBS. NeuroTrace 530/615 (Molecular Probes) was diluted in PBS at a dilution of 1:200 and sections were stained for 20 min at room temperature. Subsequently, they were washed 10 min. in PBS-T and additionally three more times, 30 min. each, in PBS to reduce the background. Finally, they were mounted in ProLong Gold antifade reagent (Molecular Probes), coverslipped (0.17 ± 0.01 mm, Assistant, Sondheim, Germany), sealed with transparent nail polish 24h after mounting and stored at 4°C until imaging.

For c-Fos immunochemistry, the standard immunohistochemistry procedure was the same described above, except that free-floating sections were blocked for 1h at room temperature in PBS-T with 10% BSA and washing steps after incubation with the primary antibody were 30 min each.

4.4 Microarray analysis

For transcriptome analysis, individual Lsi1, Lsi2 and Lmu granule cells were collected using laser-dissection microscopy from three independent mice each and analysed as pools on Affymetrix chips as described (Saxena et al., 2009). 70 to 80 GCs were collected from the distal half of the suprapyramidal blade of the dorsal (Bregma -1.46 mm to -1.94 mm) and ventral (Bregma -3.28 mm to -3.44 mm) DG. Average present calls were 46-48%. Microarray analysis was carried out using the data analysis software Expressionist (Genedata, Basel, Switzerland). The hierarchical tree was calculated based on the median values of each experiment by the clustering tool of Expressionist.

4.5 Imaging

For organotypic slice imaging, slices were placed in 2 ml of physiological Tyrode salt solution (Galimberti et al., 2006) at 37°C and imaged under controlled temperature

conditions on a spinning disk microscope consisting of a Yokogawa CSU22 confocal scanhead mounted on a Zeiss axioimager M1 and a Photometrics Cascadell:512B EMCCD camera using a 40x/0.75 water-immersion objective.

For high-resolution imaging of LMTs and thorny excrescences in fixed tissue, lamellar sections were imaged on an upright spinning disk microscope (see above) using an alpha Plan-Apochromat 100x/1.45 oil-immersion objective (Zeiss) and Metamorph 7.7.2 acquisition software (Molecular Devices, Sunnyvale, CA, USA). Standard settings were 85% laser power, 50 ms exposure time, gain 3000. Voxel size was 0.106 µm x 0.106 µm x 0.2 µm.

For c-Fos analysis, all samples belonging to the same experimental set were processed in parallel and acquired with the same settings on a LSM700 confocal microscope (Zeiss) using a EC Plan-Neofluar 40x/1.3 oil-immersion objective (Zeiss). Settings were defined so as to avoid saturation of signal and to still detect background levels outside cell clusters.

To evaluate the extent of hippocampal lesions, low-magnification overview images of Nissl staining were acquired on a wide field microscope based on a motorized Axioimager Z1 (Zeiss) and AxioCAm MRc/MRm cameras using a Plan-Apochromat 20x/0.8 objective (Zeiss) and Axiovision 4.8 software (Carl Zeiss, Toronto, ON, Canada).

4.6 Image analysis and data quantification

LMT analysis and filopodial and satellite counts

For Lsi3 and randomly labelled LMT analysis, sections were stained with an anti-GFP antibody (see above). For Lsi1 and Lsi2, the endogenous GFP signal was used. Transverse hippocampal sections from different dorsoventral levels (dorsal, intermediate, ventral hippocampus) were used for the analysis of LMT morphology and filopodial contents in CA3. Unless stated otherwise, LMTs from the CA3b region were analysed. In order to sample 50 – 100 LMTs per animal and per region (dorsal, intermediate and/or ventral), 3-4 confocal stacks per section along CA3b from at least 3 sections were acquired on average, depending on the expression level of the transgene. All objects that were completely

included in the 3D stack where analysed, as long as they were completely sufficiently isolated to be solved.

High-resolution 3D confocal stacks were analysed using Imaris 7.0.0 (Bitplane AG) software. We defined LMTs as mossy fibre terminal regions of >2.5 µm diameter in CA3a–c that were arranged either en passant or as side structures connected to the mossy fibre axon or another LMT by an axonal process (satellite, >2.5 µm) (Galimberti et al., 2006). Filopodia were defined as processes (≥ 2 µm long) emanating from the LMT core. For the quantification of LMT volumes and surface areas, confocal 3D stacks were analysed using Imaris 7.0.0 software by creating an isosurface object corresponding to each LMT. All identified objects were verified by eye inspection. Complexity was expressed by the complexity index, which was defined as the ratio between measured surface areas and calculated surface area of a sphere of equal volume.

Quantification of c-Fos immunoreactive neurons

For c-Fos analyses, three confocal stacks per section were acquired in CA3b from 3 – 5 sections per mouse and per region. 3D stacks were loaded and stitched with the XuvTool software (Emmenlauer et al., 2009)(FMI and LMB, Universität Freiburg). Fos+ cells were binned according to signal intensities using an automatic procedure (Imaris 7.0.0 spot detection tool), and the same threshold settings were used for quantification. For CA3b pyramidal neurons, threshold were defined as follows: high (> 650), medium (>400, < 650) and low (> 200, < 400). The fraction of Fos-immunoreactive cells was expressed as percentage of the total number of CA3 pyramidal neurons in the imaged neuronal layer.

Quantification of CA3b thorny excrescences

For analysis of CA3 pyramidal neurons, lamellar sections were stained with a GFP antibody (see above) in order to amplify the otherwise faint endogenous signal. 200-µm thick lamellar sections were produced in order to preserve as much of the dendritic tree extent as possible and up to 120 µm were imaged. For analyses, only pyramidal neurons bearing at least one thorny excrescence were considered. A thorny excrescence (or thorn) was defined as a complex spine with a single neck and multiple spine heads. A cluster of thorns was defined as a stretch of thorns that could be individually resolved. For analyses, 3D stacks were loaded and stitched with the XuvTool software (Emmenlauer et al., 2009)(FMI

and LMB, Universität Freiburg). MIPs were generated using the Imaris 7.0.0 software and the contour of every thorn was manually drawn. Cluster length was defined as the maximal length of the cluster along the dendrite, while cluster width corresponded to the maximal length of the cluster on the dimension running perpendicularly to the dendritic branch (see Figure 6 in section 2.2.3). Cluster lengths and widths were measured with the ImageJ open source software. The total cluster length per pyramidal neuron was defined as the sum of all individual cluster lengths on one neuron.

4.7 Drug delivery *in vivo* and stereotactic surgery

Lentiviral injections

Lentiviral constructs were a generous gift from Pavel Osten (Cold Spring Harbor Laboratories; (Dittgen et al., 2004)); cytosolic GFP was replaced in the expression cassette by the mGFP sequence (Bednarek and Caroni, 2011).

Coordinates for lentiviral injections into mouse DG were (in mm from Bregma): - 1.70 posterior, 1.10 lateral, -1.70 down (dorsal hippocampus); - 3.16 posterior, - 2.5 lateral, - 2.10 down (ventral hippocampus). Mice were sacrificed 11 days after injection.

Drug delivery *in vivo*

SCH-23390 (Tocris Bioscence) was dissolved in saline 0.9% and injected i.p. at a dosis of 0.05 mg/kg 20 min prior MWM training at day 1 (habituation) and day 2 (training with the invisible platform).

Ventral hippocampal lesions

A total of 10 Lsi1 mice received bilateral ibotenic acid-induced lesions of the ventral hippocampus. Ibotenic acid (Ascent scientific) was dissolved PBS to a final concentration of 10 mg/ml and injections of 50 nl were made at 3 sites. Injections coordinates were as follow (in mm from Bregma): - 3.08 posterior; 2.7 lateral; -3.2, -3.4 and -3.6 down (three sites). Mice were given 7 days recovery before training. Five mice were trained for 8 days and five for 10 days. After the probe trial, mice were sacrificed and lesions were evaluated by fluorescent Nissl staining.

4.8 Statistical analysis

Average values in the text and figures are expressed as mean ± S.E.M. Statistical differences were assessed by Student's *t*-test (GraphPad Prism4, GraphPad Software, La Jolla, CA, USA). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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Ich erkläre, dass ich die Dissertation „**Regional and subpopulation rules for plasticity in the adult mouse hippocampus”** nur mit der darin angegebenen Hilfe verfasst und bei keiner anderen Fakultät eingereicht habe.