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Hanne Stensola

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Thesis for the Degree of
Doctor Philosophiae
Faculty of Medicine
Kavli Institute for Systems Neuroscience /
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Hanne Stensola

Organizational Principles of Entorhinal Grid Maps

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Thesis for the Degree of Doctor Philosophiae

Trondheim, June 2016

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Norsk Sammendrag

Prinsipper for organisering av stedssansen

Nevrovitenskap er et relativt nytt felt med bidrag fra flere disipliner, som anatomi, fysiologi, psykologi og fysikk. Vår forståelse av hjernens funksjoner har hatt en enorm vekst de siste tiårene, både takket være teknologisk utvikling som har gjort nødvendige eksperiment mulig, og på grunn av stor konseptuell utvikling. Et underfelt i nevrovitenskap omfatter forståelsen av hvordan minner skapes og representeres i hjernen. Dette forskningsfeltet har hatt eksepsjonell fremgang. Med muligheten til å spille inn signaler fra populasjoner av enkle nevroner har et nevralt nettverk for intern stedssans blitt åpenbart, og dette har ført til innsikter i nevrale mekanismer bak hvordan minner skapes, konsolideres og hentes frem. Dette nevrale nettverket omfatter flere sammenknyttede hjernestrukturer og subområder med særegne kvaliteter som alle bidrar til hukommelsesprosessene. Innad i disse hjernestrukturene finnes det gradienter, både anatomiske og funksjonelle, som indikerer ulike nivå av prosessering av minner gjennom ulik grad av spatiell resolusjon. Ved det ene anatomiske endepunktet viser både stedsrepresentasjonen og episodisk hukommelse detaljerte attributter, mens i den andre enden av den anatomiske aksen er stedsrepresentasjonen bred og kontekstuell, og relasjonen til episodisk hukommelse er av en helhetlig og omfattende karakter. Disse variasjonene langs den anatomiske aksen kan være gradvise, eller de kan bestå av diskrét og parallell prosessering i en modulær organisering. Arbeidet i denne tesen demonstrerer at en viktig struktur oppstrøms for hukommelsens kjerne utviser en diskrét og modulær organisering av en kritisk komponent i stedssansen som er antatt å strukturere episodisk hukommelse via et internt generert koordinatsystem. Forekomsten av slike uavhengige og parallelle informasjonsstrømmer til hovedsetet for hukommelse har flere viktige implikasjoner for vår forståelse av stedssans og episodisk hukommelse. Videre har arbeidet i denne tesen demonstrert mekanismer bak forankring av det interne koordinatsystemet til geometrien i den eksterne verden. Dette knytter internt generert geometri til strukturen i miljøet.

Navn kandidat:	***Hanne Stensola***
Institutt:	***Kavli Institute for Systems Neuroscience*** ***Centre for Neural Computation***
Veiledere:	***Professor Edvard I. Moser & Professor May-Britt Moser***

Summary

Neuroscience is a fairly young field combining input from many disciplines such as anatomy, physiology, psychology, and physics. Our understanding of brain function has seen major growth in the last few decades, both due to technological developments that have aided experimental pursuits and in terms of conceptual and computational leaps. A subfield of neuroscience concerns how memories are formed and represented in the brain, and this area has shown exceptional progress. With the ability to record from populations of single neurons, a neural circuitry of internal representations of space has been described, revealing neural mechanisms behind memory encoding, consolidation, and retrieval. This circuitry spans several interconnected brain areas and subareas with heterogenous features which all have important roles in these memory processes. Gradients exist within these structures, both at the anatomical and functional level, indicating different levels of parallel encoding of memories at different spatial resolutions. At one anatomical pole spatial representations and episodic memory display detailed attributes, while at the other anatomical extreme spatial representations are broad and contextual, and the relation to episodic memory is comprehensive in character. These variations along an anatomical axis may be gradual or they may constitute discrete parallel processing in a modular organization. The work in this thesis has demonstrated that an important input structure to the seat of memory formation displays discrete modular organization of a critical spatial component believed to structure the formation of episodic memory via an internally generated coordinate system. The existence of multiple independent and parallel input streams from this structure has several important implications for our understanding of spatial cognition and episodic memory formation. Further, this work has demonstrated mechanisms for anchoring the internal coordinate system to the geometry of the external environment, linking internally generated geometry to external boundaries.

List of Papers

Paper I **The Entorhinal Grid Map is Discretized**
Stensola H*, Stensola T*, Solstad T, Frøland K,
Moser M.-B. & Moser E.I. (2012)
Nature, 492, 72-78

Paper II **Shearing-induced Asymmetry in Entorhinal Grid Cells**
Stensola T*, Stensola H*, Moser M.-B. & Moser E.I. (2015)
Nature, 518, 207-212

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load at the final stages of our projects during times of family increase. Thank you for holding the fort and keeping the projects moving while my brain was temporarily elsewhere.

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1. Introduction

1.1 Searching for the biological structure of memories

1.1.1 Memory systems to support survival

One of the most important features of the neocortex is to optimize adaptive behavior in order to maximize the chances of survival through evolutionary fitness. Current belief is that the brain can achieve this by generating schemas of knowledge about contingencies in the world through extraction of statistical regularities from multiple experiences, and use this knowledge to predict outcomes in current and future situations, predictions which then will guide decision making and ultimately behavior (Summerfield et al., 2014; Wang and Morris, 2010). This extraction of similarities from multiple experiences separated in time requires an ability to encode memories of episodes in a framework that is stable over time. A key aspect of such a framework is the spatial context in which an important episode occurred, such as finding food, water or shelter. In order to deliberately return to such locations from any given starting point when hunger, thirst or sleepiness motivates it, stable spatial representations must be available and retrieved. Further, every time an animal experiences that food was obtainable in a particular location, combined external inputs comprising the experiences will associate onto the same spatial framework, allowing all shared attributes to be strengthened while variable inputs are not, thereby continuously building and updating an evidence-based generalized representation. The brain thus needs to encode episodes in a spatio-contextual framework that can be repeatedly expressed in the same format. A second important requirement of such a system is the ability to avoid interference between similar, but distinct memories occurring under different contexts, such that extracted knowledge of contingencies can be context-dependent.

1.1.2 Where are memories located?

The search for this memory system has a long history. In 1948 Edward Tolman published a highly cited paper titled “Cognitive maps in rats and men”, in which he described several experiments culminating in a theory of a cognitive map. This theory stated that the brain develops and contains a comprehensive map of the external world, a map allowing animals to calculate shortcuts covering space not previously traversed (Tolman, 1948). The neural identity of this map remained unknown for several decades following Tolman’s paper, but an important discovery only a few years later put a candidate location in the searchlight.

This subsequent essential discovery came from a patient who was suffering from intractable epilepsy so devastating that the solution suggested by his doctor, William Scoville, was an experimental surgical procedure in an attempt to remove the source of epilepsy. This patient, Henry Molaison, had both his hippocampi and surrounding tissue removed in 1953, and after his surgery he was no longer able to form new episodic memories, as reported by Scoville and Milner (Scoville and Milner, 1957). His misfortune provided neuroscience with a location for episodic memory formation in the brain.

In the 60s and 70s experiments performed in Oslo would further the role of the hippocampus as a memory structure, through the discovery of long-term potentiation (LTP) in hippocampal slices (Bliss and Lomo, 1973). LTP is still regarded as the main cellular substrate for memory formation as experience (in the form of activation of synaptic inputs) can lead to long-lasting changes in connectivity (measured as responses to that input). This provided physiological substance to Donald Hebb’s postulate stating that memory is formed by selective strengthening of neuronal synaptic connections by repeated coactivation (Hebb, 1949).

1.1.3 Is the hippocampus a seat for memories or navigation?

At the same time as LTP was revealing the cellular mechanisms for memory in the hippocampus, John O'Keefe, and his student Jonathan Dostrovsky recorded from hippocampal neurons in awake rats. They reported that neurons in the hippocampus had selective responses to spatial location and suggested that the hippocampus contains a spatial reference map corresponding to the cognitive map suggested by Tolman (O'Keefe and Dostrovsky, 1971). This idea was further elaborated in detail in the subsequent book "The Hippocampus as a Cognitive Map" by O'Keefe and Nadel (O'Keefe and Nadel, 1978). The spatially selective neurons in hippocampus were named place units (O'Keefe, 1976), now referred to as place cells, and have since their discovery been studied extensively by numerous labs around the world. Neighbouring place cells have different peak firing locations so that even small local populations generate signature maps covering all parts of an environment, maps which are stable over multiple visits to that environment, providing the system with spatial specificity, stability, and redundancy, the latter potentially being particularly important for hippocampus due to its sensitivity to oxygen deprivation (Schmidt-Kastner, 2015).

Could the idea of hippocampus as a memory system be reconciled with the hippocampus as a spatial reference map? In 1987, Muller and Kubie published a paper describing how populations of place cells responded to changes to the recording environment (Muller and Kubie, 1987). They demonstrated that the hippocampal map would reorganize between two different enclosures, a circle and a square, a phenomenon known as remapping. Some place cells were only active in one of the environments, others would be active in both, but with uncorrelated locations of the fields. Now known as global remapping, this provides the hippocampus with orthogonal population codes for space in different environments (Leutgeb et al., 2005). In addition to global remapping, place cells can undergo rate remapping to subtle changes in an environment such as a change in the color of the walls (Leutgeb et al., 2005). Rate remapping is a cell-by-cell change in the firing rate within a spatially stable firing field, so that the spatial information is retained between the two contexts, but the population rate code changes with some place cells displaying increased peak firing rate and some place cells displaying decreased peak firing rate (Leutgeb et al., 2005). Rate remapping also occurs at the stem of a modified T-maze depending on whether the rat is turning right or left following the common stem, thereby orthogonalizing different behavioral contexts in the same spatial location (Wood et al., 2000). Global and rate remapping exemplify ways that the hippocampal map can reorganize between different situations and thus prevent interference with memory formation of similar episodes in different environments or under different contexts in the same environment, and the seemingly endless capacity to remap may allow the hippocampus to separate endless amounts of distinct memories. On the other hand, the stability of the map when a rat reenters the same environment may support the demand for a stable framework when extracting shared features of episodes separated in time to produce general knowledge of contingencies, or when using memory retrieval to produce context-dependent predictions of outcomes.

1.2 Entorhinal inputs to the hippocampus – an internal metric

1.2.1 Testing the self-sufficiency of hippocampus in map generation

The hippocampus is divided into distinct subfields with mainly unidirectional connectivity, known as the trisynaptic loop (Cappaert et al., 2015), shown in figure 1. Cortical input first reaches dentate gyrus (DG), an area of sparse activity due to large amounts of inhibition, and one of extremely few brain areas displaying adult neurogenesis (Aimone et al., 2011; Eriksson et al., 1998). DG then connects to CA3, an area with dense recurrent connections (Cappaert et al., 2015). The final synapse in this system is the projection from CA3 to the output area of the hippocampus, CA1, an area almost devoid of recurrent connectivity (Cappaert et al., 2015). Place cells are found in all these subfields of hippocampus, albeit with variations (Park et al., 2011), but for CA1 it is unlikely that they are generated in site due to the sparsity of recurrent connectivity. Therefore, CA1 may inherit the spatial information from CA3, which with its high level of internal connectivity may be able to generate such a spatial map through attractor or auto-association network properties (Rolls, 2007). However, the spatial input may also be provided from outside the hippocampus, such as from its main cortical input, the entorhinal cortex (Cappaert et al., 2015). Entorhinal cortex is a six-layered cortical structure that projects to the hippocampus in a layer-specific manner (Cappaert et al., 2015). Information from entorhinal cortex can reach the final hippocampal subfield, CA1, either indirectly from layer II (LII) via DG and CA3, or directly from entorhinal layer III (LIII) (Cappaert et al., 2015). To test the sufficiency and necessity of entorhinal inputs for generating place codes in CA1, two landmark studies isolated each of these inputs while recording from CA1 (Brun et al., 2008a, 2002). First the idea that place fields in CA1 may be solely inherited from CA3 was tested by surgical incisions at the end of CA3, thereby isolating CA1 from its hippocampal inputs (Brun et al., 2002). In these experiments CA1 still expressed place fields, albeit with reduced quality, demonstrating that extra-hippocampal input, dominated by entorhinal cortex, was sufficient to drive a spatial map in CA1 without the support of CA3 (Brun et al., 2002). The second study tested the necessity of entorhinal inputs to CA1 by selective lesioning entorhinal LIII, which constitutes the direct pathway to CA1 (Brun et al., 2008a). Without this direct entorhinal projection to CA1 place fields remained, but were compromised as shown by a significant reduction in spatial information (Brun et al., 2008a). Place fields in CA3 remained unaltered, demonstrating that the effect was not via the indirect route to CA1 via CA3 as could have been the case if damage to entorhinal LIII debilitated entorhinal LII output (Brun et al., 2008a). This latter study concluded that entorhinal inputs were necessary for normal spatial expression in CA1.

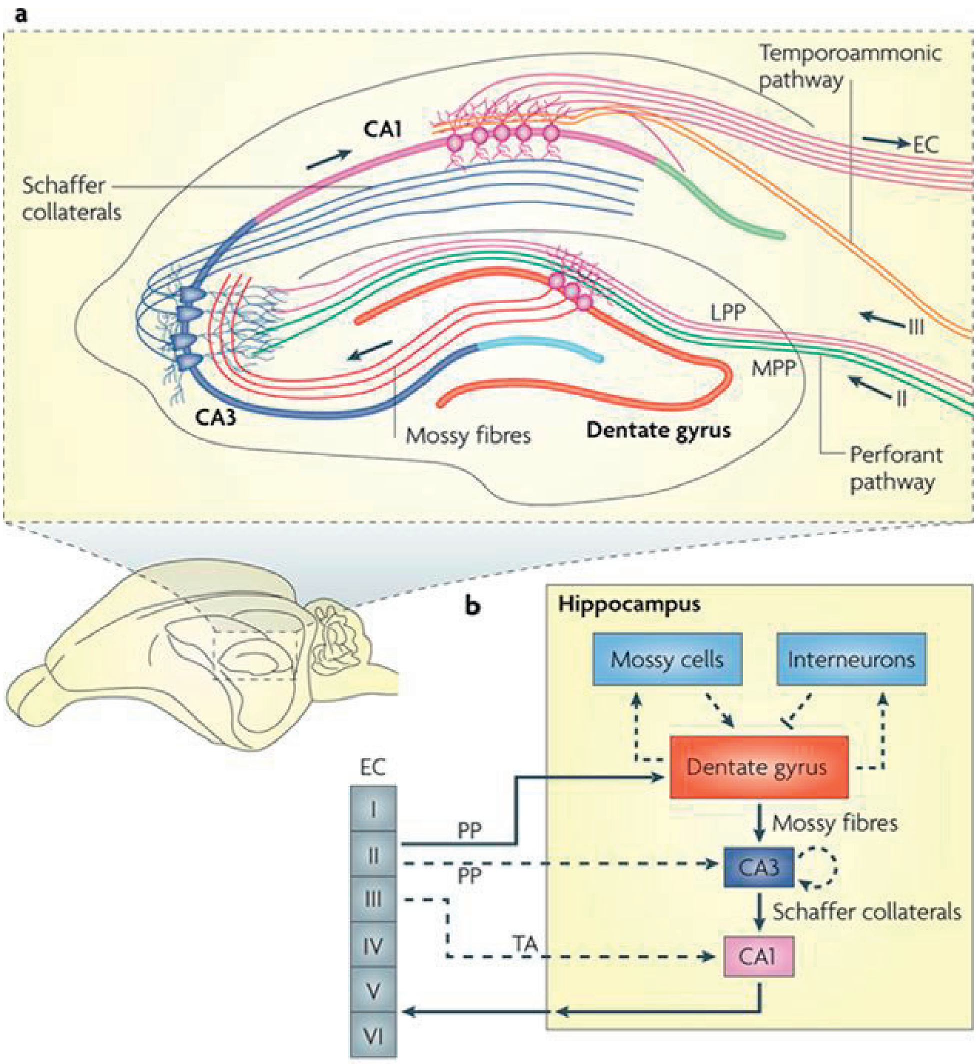
a
Temporoammonic pathway
CA1
EC
Schaffer collaterals
LPP
III
MPP
II
CA3
Mossy fibres
Dentate gyrus
Perforant pathway
b
Hippocampus
Mossy cells
Interneurons
Dentate gyrus
EC
I
II
III
IV
V
VI
PP
PP
TA
Mossy fibres
CA3
Schaffer collaterals
CA1

Figure 1: Overview of hippocampal anatomy and circuitry. The trisynaptic loop involves sequential input streams to DG (perforant path), CA3 (mossy fibres) and CA1 (schaffer collaterals) as outlined in a). These subfield all receive laminar-specific input from entorhinal cortex as shown in b). Entorhinal layer II project to DG and CA3, while layer III project to CA1. CA3 projects back on its own circuitry by heavy autoassociative connections. Hippocampal output reaches deep entorhinal layers. Figure adapted from Deng et al (Deng et al., 2010).

1.2.2 External input to hippocampus

What input does the entorhinal cortex provide to hippocampus? In order to reveal properties of neurons projecting to areas of current hippocampal place field recordings, electrodes were implanted to record neurons from dorsal entorhinal cortex (Fyhn et al., 2004). At the gross level, entorhinal cortex is separated into lateral and medial entorhinal cortex (Cappaert et al., 2015). To this day, extensive spatial correlates in the lateral entorhinal cortex have not been revealed despite much effort, whereas in the medial entorhinal cortex a substantial proportion of neurons have been found to be spatially selective (Deshmukh and Knierim, 2011; Fyhn et al., 2004; Hafting et al., 2005; Hargreaves et al., 2005). Here the focus will be on the spatially selective medial entorhinal cortex, thus unless specified otherwise all referencing to entorhinal cortex will regard medial entorhinal cortex.

The spatial representation discovered in entorhinal cortex was different from hippocampal place maps in that individual entorhinal neurons expressed multiple spatial fields (Fyhn et al., 2004), and soon it was revealed that entorhinal spatial codes had a striking regularity where individual cells expressed fields in a repeating pattern of equilateral triangles tiling the extent of the environment (Hafting et al., 2005). Neighbouring cells expressed almost identical patterns, but with a spatial offset so that every part of the recording area was represented by the population (Hafting et al., 2005). The honeycomb-like mesh pattern expressed by these neurons gave them the name grid cells (Hafting et al., 2005). Grid cells from the same recording site expressed the same field size and interfield distance, known as grid spacing, as well as the same orientation of the basic geometric feature, the equilateral triangle (Hafting et al., 2005). In response to manipulations causing global remapping in hippocampus, local ensembles of grid cells expressed a concerted tilt, but manipulations leading to rate remapping in hippocampus did not change the grid cell expression (Fyhn et al., 2007). Contextual manipulations shown to cause intermediate levels of remapping in hippocampus have later been demonstrated to cause phase shifts in grid maps (Marozzi et al., 2015). These findings demonstrate that grid cells provide the hippocampal place map with a spatial component whose presence and local infrastructure is independent of context, but exact layout is environment-specific. Locally, the map behaves coherently and continuously provides spatial information due to the offset in neighbouring grid cells (Fyhn et al., 2007; Hafting et al., 2005). It was proposed that the grid pattern is locally generated by competitive interactions from surround inhibition between grid cells indirectly connected via interneurons, forming a self-sustainable attractor-network (Couey et al., 2013; Yoon et al., 2013), and that grid cells function as a path integrator that smoothly moves the hippocampal spatial representation by integration of self-motion cues such as speed and direction of movement (McNaughton et al., 2006). The grid map is likely the stable spatial reference frame that structures, updates, and anchors the place map on which external input can associate.

Later findings revealed other important aspects of a path integration network within entorhinal cortex; head direction cells (Sargolini et al., 2006), conjunctive head direction and grid cells (Sargolini et al., 2006), border cells (Solstad et al., 2008) and speed cells (Kropff et al., 2015) all work in concert with grid cells to provide the hippocampus with a complete spatial input system to run the internally generated spatial representation by self-motion, corroborating the idea of entorhinal cortex as an area for path integration. It has recently been demonstrated that environmental borders function as external landmarks that update the grid code to avoid accumulation of errors in the path integrator (Hardcastle et al., 2015). Border cells are obvious candidates for this mechanism, although the direct evidence for this is still missing.

1.2.3 Integrating spatial and nonspatial routes of information in memory formation

According to the attractor-map hypothesis of hippocampal function the internal place map is a continuous attractor landscape preconfigured within hippocampus in early development, and the entorhinal path integrator aids the movement within this attractor landscape through self-motion (Colgin et al., 2010; McNaughton et al., 2006; Samsonovich and McNaughton, 1997). By this theory, episodic memories are encoded in the associations of external input to the current coordinate-state of the internal map, as a ‘space-tag’, a process supported by the entorhinal path integrator system. The external inputs are likely provided by lateral entorhinal cortex, as nonspatial features such as odour and object responses have been found there (Deshmukh and Knierim, 2011; Tsao et al., 2013), and lateral entorhinal lesions affect rate remapping (Lu et al., 2013). Lateral and medial entorhinal inputs combine in DG and CA3, a likely location for where such associations are formed (Cappaert et al., 2015). Well established associations of external landmarks to internal coordinates may subsequently be used to anchor and update the path integrator to avoid accumulated errors and to correct when disoriented, stabilizing the internal map during environmental familiarization. In line with this, feedback from hippocampus has been reported to be vital to maintaining entorhinal grid patterns (Bonnevie et al., 2013).

It is interesting to note that in both the visual and auditory system there are distinctions between dorsal spatial streams and ventral nonspatial streams, so-called ‘what’- and ‘where’-pathways (Bizley and Cohen, 2013; Ungerleider and Mishkin, 1982). Mouse extrastriate visual areas lateromedial and anterolateral fields, areas specialized for processing spatial and nonspatial visual information, respectively, selectively target the spatial medial entorhinal cortex and the nonspatial lateral entorhinal cortex, connecting an early separation in the visual system with that in the memory system (Wang et al., 2011). It may be generally beneficial and widely adopted to process spatial and nonspatial information in separate, parallel streams. These are finally integrated at the level of hippocampus where nonspatial features are associated in a stable spatial framework in order to organize episodic memory (Knierim et al., 2006).

1.3 A long-axis dissociation of function

1.3.1 Topography in the brain

Neocortex displays a large amount of large-scale topographic mapping of the external world, both at the sensory cortices and at the motor cortex (Penfield and Boldrey, 1937). This topographic mapping has several advantages, perhaps most importantly it provides a stronger influence of neighbouring aspects of the sensory inputs and motor outputs, improving contrast by centre-surround organization of receptive fields and aiding smooth transitions of sensory representations and motor outputs in response to body movements, or supporting growth of local processing units by plasticity in response to training or during recovery after damage (Kaas, 1997). Primary sensory cortices project to secondary sensory cortices and further to higher-order cortices in a bottom-up hierarchical manner. The higher in this cortical hierarchy, the less prominent the topographic mapping becomes, and finally it vanishes completely (Kaas, 1997). A detailed anatomical study of this hierarchy in the visual system was performed by Felleman and Van Essen, and at the top of the hierarchy, they placed hippocampus (Felleman and Van Essen, 1991). Hippocampus would then be expected to have little or no topographic representation, and in terms of classical topographic mapping of the external world, this is true. The place fields expressed by hippocampal principal cells retain no apparent relationship to the structure of the outside world; neighbouring cells may have fields in vastly different parts of one environment and in overlapping parts of another environment through global remapping. The spatial representation appears to be completely generated by internal processes as no external features contain the information found in hippocampal neurons. This is also true for entorhinal grid cells, no hexagonal patterns exist in the physical environment, yet this is the way the brain represents space. Compared to topographic maps in early sensory cortices, which are shaped by bottom-up inputs, the internal maps expressed in entorhinal cortex and hippocampus can be viewed as top-down maps formed by internal processes at the top of the hierarchy.

1.3.2 Dorsoventral organization in the entorhinal-hippocampal circuit

Despite the absence of a topographic mapping to the external world, hippocampus and surrounding structures are far from homogenous in structure and function. The projections from entorhinal cortex to hippocampus preserve a long axis organization, despite quite extensive divergence and convergence (Cappaert et al., 2015; Dolorfo and Amaral, 1998a). This long axis runs in the dorsoventral plane in rodents, and in the anteroposterior plane in primates, but here the focus will be on rodents, hence the long axis will be referred to as the dorsoventral axis from now on. This preservation of a dorsoventral position between connected entorhinal and hippocampal neurons is accompanied by an important similarity in functional organization of the spatial representation; dorsal neurons of both areas express small spatial fields (place or grid fields) while ventral neurons of both areas express large spatial fields, with an apparent linear increase in field size with dorsoventral depth (Brun et al., 2008b; Hafting et al., 2005; Kjelstrup et al., 2008). In humans a similar long-axis gradient in resolution has been demonstrated for episodic memory representations, from small-scale detailed representations in the posterior end (corresponding to rodent dorsal hippocampus) to large-scale holistic representations in the anterior end (corresponding to rodent ventral hippocampus) (Collin et al., 2015), and also for spatial granularity (Evensmoen et al., 2015). This substantiates the idea that hippocampal involvement in spatial representation and episodic memory formation are interlinked, that the spatial codes seen in hippocampal neurons demonstrate a main organizational principle for episodic memory.

Lesion studies have uncovered functional differences between the ventral and dorsal poles of the hippocampus; whereas dorsal hippocampus was important for spatial memory (Moser et al., 1995), lesions of ventral hippocampus particularly affected unconditioned fear responses (Kjelstrup et al., 2002). These differences have led to theories of a hippocampal dorsoventral dissociation where the dorsal part is important for spatial representation while the ventral pole is essential for emotional processes. Studies in humans have added weight to this theory (Poppenk et al., 2013; Woollett and Maguire, 2011)

1.3.3 Anatomical differences between dorsal and ventral hippocampus

Could this dissociation be produced by differences in connectivity? Dorsoventral gradients in connectivity exists between the hippocampus and amygdala, a structure much studied for its role in fear processing, with lateral septum, a structure which further connects to the endocrine control centre, the hypothalamus, and with nucleus accumbens, a structure known for its role in reward processing (Strange et al., 2014). In addition to the graded connectivity differences of the hippocampus itself, entorhinal cortex also contains dorsoventral differences in connectivity that will affect the hippocampus via the preserved dorsoventral relationships of projection patterns between entorhinal cortex and hippocampus (Dolorfo and Amaral, 1998a). Dorsal entorhinal cortex receives inputs from areas more related to spatial processing such as retrosplenial cortex and postrhinal cortex, while ventral portions receive nonspatial input such as olfactory information from piriform cortex (Strange et al., 2014). These differences, with ventral hippocampus being more strongly connected to circuits involving emotions such as fear and stress responses, may be responsible for the functional distinctions reported in lesion studies. Other differences between dorsal and ventral portions of entorhinal cortex include gradients in gene expressions and inhibitory innervations by parvalbumin-positive interneurons (Beed et al., 2013; Ramsden et al., 2015). Hippocampus itself can be divided into several clearly demarcated dorsoventral domains based on gene-expression alone, the significance of which remains to be revealed (Dong et al., 2009; Thompson et al., 2008). Finally, the proportion of hippocampal subfields also vary in the dorsoventral axis; dorsally DG neurons outnumber CA3 neurons by more than tenfold, while ventrally CA3 neurons are in the majority of the two (Cappaert et al., 2015).

1.3.4 Spatial resolution and episodic memory formation along the dorsoventral axis

Despite much evidence distinguishing ventral and dorsal hippocampus, spatial representations in the form of place fields are still present in very ventral hippocampal neurons (Kjelstrup et al., 2008). A spatial code, albeit more diffuse, may thus have a role in emotional memory in addition to large-scale episodic memory. What purpose would large place fields serve in ventral hippocampus? And how would this differ from representations by small place fields in dorsal hippocampus? In dorsal hippocampus, the almost point-like place fields will cause the population code to change very swiftly during normal exploratory behavior. Individual place cells will make short appearances into the global place representation. This produces small temporal and spatial spread of overlapping activity between different cells, and fewer cells will have overlapping spiking activity at all. With a population code that changes quickly in time and space, external events associated with the place code will thus have to be very close in time or space to be associated together with the same population ‘space tag’. This further means that recall of associations in dorsal hippocampus can be very specific and will not necessarily lead to recall of close events except when indirectly associated and recalled as sequences. In ventral hippocampus place cells express large, undulating place fields generating a slowly changing population code, by which ventral cells have the opportunity to link external cues located further apart both in time and space. Associating the spatial population code with distributed cues in the ventral hippocampus leads to generalization of large spatial contexts and linking of episodes occurring further apart. The latter could be the mechanism behind large-scale comprehensive memory representations found in human anterior hippocampus, and is supported by the finding that ventral hippocampal neurons in rats slowly develop generalized representations about contexts while dorsal neurons rather develop location-specific rapid associations of events (Komorowski et al., 2013). An illustration of the idea is shown in figure 2.

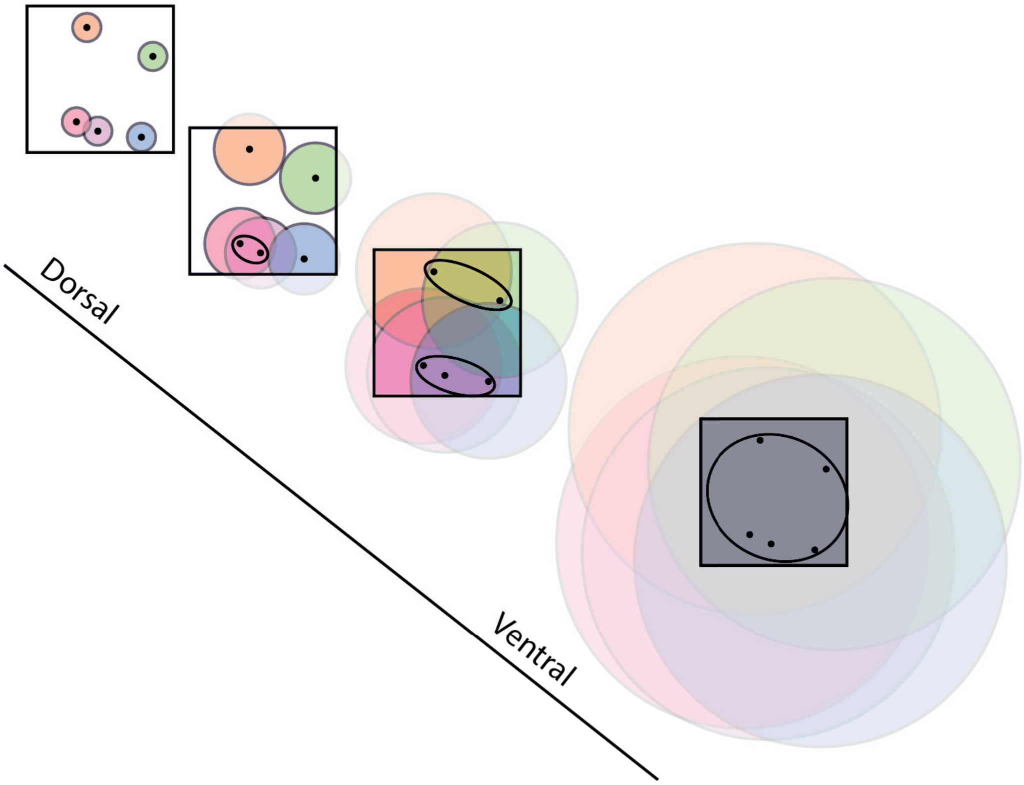

Figure 2: A schematic representation of spatial specificity and associations of events along the dorsoventral axis of hippocampus. Squares represent an environment, black dots represent events and the colored circles are example place fields centred at the location of the event. At the dorsal pole small place fields allow accurate location-specific associations of the events, but no associations are formed across events. Reentrance to particular locations may result in retrieval of specific events associated to a selective segment of a high resolution place map. At the ventral pole all the example cells are active during all events, and can integrate the association of context with multiple events. Reactivation of ventral place fields upon reentrance to any part of the environment may induce reactivation of all associations from events in the entire context. Intermediate levels would be expected at intermediate dorsoventral positions.

Via its connections with amygdala and nucleus accumbens (Cappaert et al., 2015), ventral hippocampal spatial generalization may associate salience, negative or positive, to entire environments. That emotional salience is more important for the type of spatial representations found in ventral hippocampus is not very controversial as emotional states are generally slowly changing and long-lasting once initiated, similar to the spatial population code in ventral hippocampus. A stronger olfactory input to ventral parts of the entorhinal-hippocampal circuit (Cappaert et al., 2015) agrees well with this dorsoventral distinction since olfaction stands out as the least spatially accurate sensory modality, with distribution of airborne chemicals greatly affected by air currents. In addition, olfaction serves an important role in detecting food or predators as early/far away as possible, a function well suited to large place fields and associations across great distances in ventral hippocampus.

1.3.5 Dorsoventral differences and variation in hippocampal subfield dominance

Extensive theoretical (Rolls, 2015) and experimental work (Gilbert et al., 2001; Knierim and Neunuebel, 2015; Leutgeb et al., 2007; Neunuebel and Knierim, 2014) support a distinction between DG and CA3 in two major processes, pattern separation, and pattern completion. Pattern separation, the process of representing similar contexts with distinct population codes, is important to avoid memory interference from similar environments or contexts and is thought to rely on the sparse coding in DG. Pattern completion, the process of retrieving an entire representation from incomplete input, is important to keep stable representations in the presence of noise or small alterations of familiar environments and is believed to rely on the heavily auto-associative network in CA3. These functional differences combined with the varying proportions of DG and CA3 along the dorsoventral axis, with DG neuron counts outnumbering CA3 in the dorsal pole and the reversed relationship in the ventral pole (Gaarskjaer, 1978), may indicate a stronger requirement for pattern separation in dorsal circuits, where detailed spatial representations are found, and more pattern completion in ventral circuits, where spatial representations are more comprehensive and contextual. Pattern completion may allow hippocampal ensembles to retrieve full representations to partial external cues, such as the smell of a familiar environment. This may be particularly important in ventral hippocampus since the large spatial extent of the maps cover many individual sources of external input associated to the environmental representation. At the dorsal pole pattern separation may be important to allow unique population codes at neighbouring locations, which may partly share external input from landmarks. This idea has also been suggested by Poppenk et al (Poppenk et al., 2013).

1.3.6. Hierarchical and parallel processing

A feature of sensory processing hierarchies which holds similarities to the dorsoventral axis of the entorhinal-hippocampal system is an increase in receptive field size with increased distance from sensory organs (Wilson and Wilkinson, 2015). Neurons in early sensory processing levels provide detailed and spatially accurate information while neurons later in the hierarchical processing stream display decreasing spatially restricted and increasing overarching information such as scene or face recognition, similar to hippocampal long-axis gradients of spatial accuracy. Confinements of genetic markers and connectivity described above support parallel processing in hippocampus. Yet, extensive longitudinal connections within DG, in mossy fibers connecting DG to CA3, and in Schaffer collaterals connecting CA3 to CA1 (Cappaert et al., 2015), may provide hierarchical processing in hippocampus. In sensory systems there are multiple levels of feedback and feedforward connections between areas, some even skipping hierarchical levels, indicating that there may be more parallel computations than traditionally appreciated (Felleman and Van Essen, 1991; Nakamura et al., 1993). Sensory and hippocampal systems may operate on similar mechanisms of combined parallel and hierarchical processing.

1.4 Temporal codes in the spatial system

1.4.1 Theta oscillations may provide windows of opportunity for associations to form

A striking feature of neuronal activity in both hippocampus and entorhinal cortex is the timing of spikes related to an underlying oscillation in the local field potential (LFP). This oscillation occurs at a frequency of 4-12Hz during exploratory behavior (including immobile sniffing) and is known as theta oscillations. It is constantly ongoing while the animal is an active mode, sampling the external environment, and it is elevated by novelty and high running speed, perhaps reflecting a larger requirement for sampling in those situations (Hinman et al., 2011; Penley et al., 2013). Both theta power and frequency is increased at decision points, which may be due to similarly elevated sampling, but also increased efficiency through faster sampling (Belchior et al., 2014). Neurons typically express an intrinsic theta rhythm slightly faster than the LFP, causing neurons to shift the timing of preferred firing relative to the ongoing LFP theta (O’Keefe and Recce, 1993). This shift in timing is known as theta phase-precession, and it leads to sequences of spatial representations within single theta cycles; cells with place fields that the animal is about to exit fire earliest at the cycle, cells with place fields centred at the current location of the animal fire in the middle and cells with place fields that the animal is entering fire last (Skaggs et al., 1996). Individual theta cycles thus contain temporally compressed sequences of past, present and future location (Skaggs et al., 1996). Dorsally, a single theta cycle will hold shorter sequences, but with greater precision than ventrally due to the place field expansion along the dorsoventral axis and thereby the maximal field centre distances of overlapping fields. The slope of theta phase precession decreases with dorsoventral depth and very ventral CA3 cells have been found to display a single phase precession over a 10m distance (Kjelstrup et al., 2008). This difference means that dorsally a few or maybe even just a single theta phase will contain the same compressed sequence while ventrally the temporal code will remain the same for many theta cycles, providing repetitive windows of opportunities for associations of external inputs to a particular temporal sequence. This temporal compression and organization of sequences by theta phase precession may be essential to bind external features as strings of events through associations with common spatial representations, and may be another reason why human anterior hippocampus have been found to hold more comprehensive episodic memory, linking multiple events. Theta phase precession was first discovered in hippocampal place cells and later confirmed to also occur in entorhinal grid cells (Hafting et al., 2008; O'Keefe and Recce, 1993).

1.4.2 Theta oscillations and a theory of grid generation

In entorhinal cortex, the differences between intrinsic and LFP theta oscillations that cause theta phase precession was hypothesized to generate the grid pattern through oscillatory interference, and it was believed that the extent of this interference determined grid scale (Burgess, 2008; Burgess et al., 2007). The theory acquired some support from the finding of a gradient in intrinsic theta frequency in the dorsoventral axis (Giocomo et al., 2007), however, experiments with *in vivo* patch recordings of grid cells in awake animals later cast doubt on the feasibility of the theory (Domnisoru et al., 2013). For oscillatory interference to hold any plausible explanatory power regarding grid pattern formation and scale, increasing grid spacing along the dorsoventral axis will have to correlate with changes in oscillatory interference, that is, the difference between LFP and intrinsic theta rhythms of the grid cells.

1.5 Widespread sampling to reveal organizational principles

1.5.1 The hippocampal formation is a heterogenous structure

A single place cell or a single grid cell can do very little to support navigation or memory formation. It is the coordinated activity of entire populations of place and grid cells which generate the cognitive map. In order to understand the system that so clearly is fundamental to our capacity for episodic memory formation, we need to understand how populations of these spatially selective neurons act together. Developments in hippocampal research have made very clear that a place cell is not only a place cell, and the anatomical location of a place cell is an important piece of information to consider in any experiment. Place cells in DG, CA3 and CA1, and even along the transverse axis within these subfields, display quite different properties such as the number of fields in an environment, the tendency to remap to environmental changes and the ability to reorganize the population code in response to learning (Dupret et al., 2010; Lee et al., 2004; Leutgeb et al., 2004; Park et al., 2011). CA1 cells, but not CA3 cells, reorganize after learning new reward locations (Dupret et al., 2010). In this way, CA3 may keep a stable spatial reference within one environment while CA1 may provide support for context-dependent associations. In agreement with this, dorsal CA1, but not CA3, is important for post-training expression of contextual fear-memory (Hunsaker and Kesner, 2008). Even within subfields, there are distinct differences. CA1 is divided at the transverse axis and cells at the proximal part close to CA3 receive direct input from medial entorhinal cortex while cells at the distal part close to subiculum receive direct input from lateral entorhinal cortex (Cappaert et al., 2015). These differences in connectivity coexist with functional variations; compared to distal CA1, representations in proximal CA1 contain more spatial information and display a stronger modulation by LFP theta (Henriksen et al., 2010). Proximal CA1 also shows less overlap in immediate early gene expression upon exposure to two different environments, indicating stronger global remapping compared to distal CA1 (Hartzell et al., 2013). In addition, neurons in distal, but not proximal, CA1 respond to objects and odours, similarly to lateral entorhinal neurons (Igarashi et al., 2014; Ito and Schuman, 2012). These differences are important for functional roles in memory processing, and future studies on hippocampal processes should account for specific recording locations. In order to fully understand the hippocampal memory system, it will be important to know how these different areas function together. There is a requirement not only for population recordings but for multi-site population recordings.

1.5.2. Revealing global features of the grid map requires improved sampling

As previously mentioned, both hippocampus and entorhinal cortex express increasing spatial scales along the dorsoventral axis (Brun et al., 2008b; Hafting et al., 2005; Kjelstrup et al., 2008). In entorhinal cortex, it was found that this increase in spatial scale forms a linear relationship with anatomical distance from the dorsal end across animals (Brun et al., 2008b). Pooling of data from several animals provides little information into the specific organization of these different scales, however. Individual animals could potentially express only a few spacings, but unless different animals express the very same spacings, pooled data should look continuous. Whether scale increases gradually or abruptly along the dorsoventral axis was relatively unknown, although one report had shown an abrupt increase for the smallest scales of grid maps (Barry et al., 2007). The striking similarity of scaling within local recording sites also favors discontinuous changes over gradual ones (Hafting et al., 2005).

It was clear from the initial experiments that while grid orientation always displayed minimal variation in local grid populations, it did not necessarily appear similar across animals (Hafting et al., 2005), and it could be different or similar in grid cells with different spacings within animals (Barry et al., 2007; Hafting et al., 2005). Random internal settings of the path integrator at the time of first exposure to an environment could potentially determine the orientation selected for the grid pattern. Despite cross-animal differences in early reports, it remained relatively unexplored whether grid orientation would be restricted by certain features of the external environment. Whether a single or multiple orientations were expressed across the entorhinal cortex was unknown due to sampling methods, although it had been demonstrated that the two hemispheres could express different grid orientation in grids which also had different grid spacing (Hafting et al., 2005). There could be a similar change in orientation along the mediolateral axis as had been seen in spacing along the dorsoventral axis, providing animals with an anatomical coordinate system of both spacing and orientation, or orientation shifts could follow spacing. If grid orientations were to change with spacing or any anatomical feature, but with varying offsets across animals, different recording locations in different animals could account for why previous findings concluded that grid orientation was random.

At the anatomical level, one very distinct feature has inspired speculations about a modular organization in entorhinal cortex. In the human brain, entorhinal cortex has a ‘bumpy’ surface due to several local protuberances known as entorhinal verrucae (Simic et al., 2005). This patchy pattern co-occurs with differential expression of the enzyme cytochrome oxidase, which demarcates highly metabolically active areas (Burgalossi et al., 2011). Cytochrome oxidase stains reveal a patchy structure of entorhinal cortex in both primates and rodents, with islands of high neuron density separated by myelinated fibers (Burgalossi et al., 2011). In rat entorhinal cortex these patches are fairly small, about 100-150um in width and there may be more than a hundred of these patches in the entire rat medial entorhinal cortex, similar to human accounts of just over 100 patches (Burgalossi et al., 2011; Simic et al., 2005). Such patches could potentially contain islands of local grid cell populations with similar features such as spacing and orientation, as found in single recording sites.

1.5.3 Grid anchoring to external features

How is the internal geometric grid pattern related to the geometry of the external world? Experiments in rats have suggested that environmental borders are of particular importance. Rats tend to make rotational errors to geometrically equivalent locations when searching for food in rectangular environments, despite multiple contextual cues disambiguating these locations (Cheng, 1986), and place cells respond to environmental elongations or compressions with fields maintaining either fixed or relative positions to certain walls (O’Keefe and Burgess, 1996). Grid cells also display elastic properties when recording boxes are suddenly elongated or compressed, suggesting attachment to the borders of the environments (Barry et al., 2007; Solstad et al., 2008). Such anchoring may be provided by border cells found in entorhinal cortex (Solstad et al., 2008). Although direct evidence for this is still absent, it is in line with the observation that grid cells are reset upon wall contact, where border cells will be active (Hardcastle et al., 2015; Solstad et al., 2008). Other data suggest that cells in medial entorhinal cortex anchor to distant visual cues when local and global cues are rotated in opposite directions (Neunuebel et al., 2013). It is possible that grid anchoring differs between dorsal and ventral grid cells, and recordings from multiple locations are imperative to answer this question.

2. Objectives

Following the discovery of grid cells in entorhinal cortex, there was an initial wave of experiments that provided important insight into the properties of these neurons. These initial experiments had one major limitation; cell yields per animal were low and typically all neurons were recorded in one local area or one local site per hemisphere. Neurons were pooled across animals to study their attributes such as responses during hippocampal remapping, theta phase precession and spacing relative to dorsoventral depth (Brun et al., 2008b; Fyhn et al., 2007; Hafting et al., 2008, 2005). But pooling data across animals precludes insight into several aspects of how the grid map is organized on the large scale within animals, essential to understanding entorhinal effects on downstream targets, as well as on internal dynamics.

Theories of how grid patterns may combine to produce hippocampal place fields have typically depended heavily on the exact nature by which geometric features combine across the grid cell input population (Cheng and Frank, 2011; Solstad et al., 2006). Early reports indicated discrete organization of grid scale, but it was unknown whether this was the case for extended parts of the entorhinal cortex, or restricted to the most dorsal area (Barry et al., 2007; Hafting et al., 2005). Although single trajectories through extended entorhinal tissue had revealed discrete steps in spacing (Barry et al., 2007), different mediolateral positions could potentially contain other spacing values, providing the hippocampus with a relatively uniformly scaled spatial input. A primary goal of our experiments was to determine how grid spacing distributed across the grid map, and in particular, if grid spacing was confined to a select set of discrete steps. Systematic relationships between such potential steps may also provide clues about underlying processes that determine grid spacing.

As with grid spacing, the large-scale distribution of other geometric features of the grid had been difficult to establish. Initial findings suggested the existence of different grid orientations within animals, although recordings were from different hemispheres (Hafting et al., 2005) This difference could, however, potentially reflect a whole-hemisphere difference in grid features. Other work had demonstrated a tendency for grid cells with different grid spacing to display similar grid orientations within animals (Barry et al., 2007). Similar grid orientations could result from the limited mediolateral coverage of grid recordings in these animals, and different orientations across hemispheres may reflect distinct mediolateral positions in the two hemispheres. If the grid map has a capacity for several distinct orientations, shared boundaries between orientation and spacing distributions could point to geometrically coherent ensembles of grid cells.

Grid spacing and orientation represent two basic internal geometric dimensions by which the grid map may be organized. It is plausible that this organization spans several other geometric (and/or non-geometric) dimensions. For example, grid patterns may deviate from perfect hexagonal symmetry, and instead, display particular pattern distortions that appear consistent across local cell ensembles (Barry et al., 2007). Such distortions could represent a global error in the transformation of sensory or self-motion cues into spatial location, or they could be a consequence of non-trivial processes linked to spatial representation. If the grid map is organized according to several such features, the degree to which these cluster or overlap could indicate the extent of functional parcellation within the grid map. An overarching aim of our experiments was, therefore, to determine if various grid features were step-like or continuous across the map within animals and to quantify how much grid features clustered together.

The extent of grid map feature clustering would establish important bounds for theoretical considerations. However, feature clustering, or lack thereof, neither precludes nor guarantees functional separation. In particular, cells across the grid map may be capable of acting as a concerted unity despite grid feature clustering, or vice versa, as several independent ensembles that do not differ across such features. To establish the extent to which the grid map is functionally clustered, we sought to determine whether grid cells at distant locations would respond coherently to the same experimental manipulation and if any such difference would coincide with grid feature clustering.

Studying the organization of features in the grid map offers a window on organizing principles within this spatial neural circuit. However, little was known about how grid features interact with features of the external environment. Grid cells have been shown to be highly responsive to certain aspects of environment enclosure geometry (Barry et al., 2007; Derdikman et al., 2009). In order to maintain spatial invariance over time, grid patterns must somehow embed into the environment geometry, possibly mediated by anchoring the pattern to specific geometric features. If the grid pattern anchors to the surroundings through systematic interactions between geometric features of the grid pattern and the environment, there should be detectable feature trends across animals recorded in the same environment. It has been suggested that internally generated spatial maps anchor to boundaries in particular (Barry et al., 2007; O'Keefe and Burgess, 1996; Solstad et al., 2008). For the final set of experiments, we set out to resolve whether grid features were systematically related to environment geometry.

In order to address all these outstanding questions regarding fundamental aspects of entorhinal grid map organization, it was quintessential to perform large-scale recordings of grid cells from distant locations and with maximal anatomical continuity within animals, until then without precedence.

3. Synopsis of results

We successfully applied the use of Neuralynx hyperdrives which offered up to 12 independently moveable recording sites. These drives had been used for a long time in hippocampal recordings, but not yet with success in entorhinal cortex. In addition to these recordings that provided us with multi-site entorhinal recordings covering a range of mediolateral and dorsoventral positions within animals. We also implanted animals with traditional Axona Ltd. single-site recording equipment at an extreme angle, providing movement of tetrodes close to parallel to entorhinal layers, thereby extending the normal coverage from very local sites to long stretches of dorsoventral depth covered by slow tetrode advancements over long periods of time. These two methods were highly compatible; one provided us with extensive simultaneous recordings covering not only the dorsoventral but also the mediolateral axis, avoiding any potential slow changes in grid features over time in long-lasting experiments to be contributed to advancements in dorsoventral recording depth. The other method provided us with lengthy and continuous dorsoventral recordings, ensuring that discontinuity in grid features was not due to anatomical discontinuity in recording locations as with the multi-site approach. Features expressed by grid cells, such as grid spacing, grid orientation, pattern distortions and theta modulation were computed from recordings of implanted animals exploring familiar recording environments. In order to test the functional dependence of grid cells recorded at different locations in the multi-site approach, we manipulated the recording environment to induce the previously described phenomenon of grid elasticity (Barry et al., 2007). The two recording methods were employed by different experimenters, in different animals that were trained in different rooms and using different recording systems. Our results should thus not be attributed to particular training methods or subjective experimenter procedures.

Our multi-site method for grid cell recordings yielded amounts of grid cells from single animals that exceeded previous accounts by up to tenfold. We recorded grid cells at up to 10 distinct anatomical locations, providing us with the first simultaneous recordings so far of several different grid spacings within one hemisphere. With such numbers of neurons and anatomical spread of simultaneous recordings, we were able to uncover several novel and important principles of grid cell organization. We discovered that the grid map was indeed discretized into several modules with distinct grid spacing. Further, we revealed that these abrupt changes in grid spacings co-occurred with changes in several other features of the grid pattern, such as grid orientation, grid deformations expressed as ellipticity of the grid and theta frequency. We also described how these modules appear to be large, partly overlapping bands in the tissue, and that they act independently of each other in response to compression of the environment, thereby constituting large and independent parallel processing streams of spatial inputs to the hippocampus. We suggest that this functional independence of grid modules provide hippocampus with the enormous capacity to remap by reorganizing overlapping inputs from different modules onto hippocampal place cells.

Following from this we discovered that despite modules adopting distinct grid orientations within animals and distinct orientations appearing across different animals, there was a striking relationship between the geometry of the recording environment and grid orientation across modules and animals. We revealed that grid orientation aligned close to one of the cardinal axes of the recording environment, but with a slight offset which developed as animals familiarized to the environment. The absolute offset was on average 7.5°, an orientation which minimizes symmetry between the triangular grid pattern and the square recording environment, a potential mechanism to reduce disorientation in geometrically similar parts of an environment. We uncovered a process described by a simple mathematical rule that could in full reproduce both the orientation offset as well as the previously described distortion (ellipticity) in the grid pattern, providing a deep link between these phenomena. Shearing forces acting on simulated initial grid maps with perfect alignment to a cardinal axis and no ellipticity could reproduce the *in vivo* features we observed, and de-shearing grid maps we recorded reinstated non-elliptical and environmentally aligned grid patterns. In particularly large recording environments, we observed separate wall-alignment for different spatial sections of the grid pattern, suggesting that the grid map is composed of multiple linked sub-maps, each extending from different available landmarks.

4. Discussion

4.1 Modular organization of the grid map

4.1.1 Discontinuous grid spacing within animals

In paper 1 (Stensola et al., 2012) we describe how grid spacing is discontinuous within animals. We could, however, reproduce the linear and continuous increase in grid spacing with recording depth from previous reports (Brun et al., 2008b; Hafting et al., 2005) when pooling data across animals, since different animals expressed different specific grid spacing values. This is an important demonstration of the usefulness of high-yield sampling when revealing principles of functional organization in the brain.

Why individual animals expressed different grid spacings is still not known; even animals trained together in the exact same environment expressed different grid spacings. The relationships between different grid spacings were also not consistent across animals, neither ratios nor absolute increments, although on average the ratio was found to be close to 1.4, a value consistent between the different grid spacing increments and statistically significant. This value is close to the theoretically suggested optimal ratio for grid modules operating to decode position (Stemmler et al., 2015). It is possible that grid spacing is randomly selected from a distribution of possible spacings where the ratio between distribution peaks is 1.4, as illustrated in figure 3. Random selection could occur for each environment, only allowing small adjustments to an optimal set of useful grid spacings, e.g. a particular grid spacing could potentially be 30 cm in one environment and 35 cm in a different environment, but never 2 meters. This would aid hippocampal remapping by providing converging inputs specific to different environments, but this remains to be tested experimentally, although we have preliminary evidence for module-independent reorganization of grid spacing in different environments, as described below. It also agrees with the observation of quite variable increments across animals in the same environment.

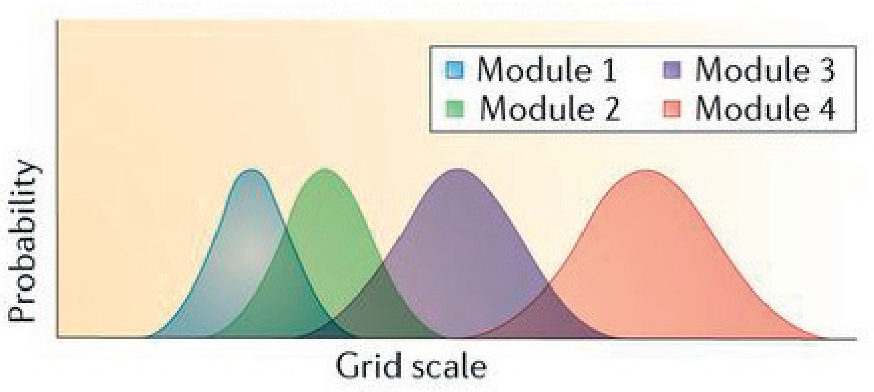

Figure 3: Grid scale may be selected from module-specific distributions where the ratios between the peak likelihoods for each module is approximately 1.4. Figure adapted from Moser et al (Moser et al., 2014).

4.1.2 Other grid features share the boundaries of grid spacing

We next revealed how a discontinuous expression of grid spacing correlated with abrupt changes in other features of the grid. One such feature was grid orientation. Grid orientation was known to differ across animals, but it was not known before whether one animal typically expressed more than one grid orientation within a hemisphere, or whether there was any gradient of grid orientations similar to the gradient demonstrated for grid spacing (Brun et al., 2008b; Hafting et al., 2005). Previous analysis in pooled datasets had not revealed any pattern of grid orientation with dorsoventral depth (Hafting et al., 2005). Given random grid orientation, as it was assumed to be, pooling data over animals would not be possible in the same way as for grid spacing, thus, the organization of grid orientation was relatively unexplored except for the initial discovery that local grid cells expressed the same grid orientation and two recording locations in different hemispheres could have grid cells with different grid orientation (Hafting et al., 2005). We verified that individual animals could express more than one grid orientation, but grid orientation did not follow a structured pattern of rotation in any anatomical axis. Grid orientation was always similar for all grid cells of the same spacing even when recorded at distant anatomical locations, so that whenever there was an abrupt difference in grid orientation, this was always consistent with an abrupt difference in grid spacing. Grid orientation was more similar for grid cells of the same spacing than for grid cells of different spacings, although several grid spacing steps within an animal often had very similar grid orientations as reported previously (Barry et al., 2007). This latter observation was the seed for paper 2 and will be described in detail below.

Another feature of the grid pattern that also shared the abrupt boundaries of grid spacing was a distortion of the grid pattern in terms of a warp along one direction, making the grid pattern hexagonality slightly elliptical. Previous grid reports had ignored this feature, perhaps since all local grid cells display the same distortion. It could easily be mistaken for an effect of different running speed in one direction, the amount of experience in parts of the environment, a difference in anchoring cues, or potentially a subjective experience of one environmental axis as longer or less safe, all of which may produce global anisotropies in the pattern. Our essential discovery was that simultaneously recorded grid cells with different grid spacing could express very different distortions while grid cells with the same spacing consistently expressed the same distortion of the grid pattern. This meant that the observed distortion was not due to behavior, the environment per se or subjective experience because then all grid cells should be distorted in the same way regardless of grid spacing. And the fact that it was common to grid cells of the same grid spacing entailed that it was not random imperfections or noise within the pattern either. The extent of distortion we observed was strikingly similar across animals, which also made us suspect that ellipticity in the grid pattern was a meaningful feature, one which remained a source of debate until paper 2, as will be described below.

4.1.3 Anatomical distribution of grid modules

These grid features described above were combined to objectively define grid modularity in our dataset. Grid modules consisted of grid cells with similar features of which spacing was the most striking. Grid modules appeared to span all layers as well as the mediolateral extent of entorhinal cortex, and no differences were seen between grid cells recorded in parasubiculum, another area expressing grid cells (Boccara et al., 2010), and those recorded from the same module in entorhinal cortex, suggesting modules are not area-specific variations. Strikingly, grid features across hemispheres also clustered tightly into global modules, indicating an important contribution of cross-hemisphere communication during grid pattern formation, which may be supported by contralateral excitation between neurons in LII (Fuchs et al., 2016).

The large anatomical extent occupied by single grid modules and our observation that several grid modules can overlap anatomically are somewhat difficult to reconcile with the description of hundreds of small patches in entorhinal cortex suggested to be an anatomical basis of modularity (Burgalossi et al., 2011). Still, it is possible that all cells in a single patch belong to a single module and connectivity across some, but not other patches can create grid modules spanning large anatomical areas. In this view, grid cells from different modules could also be close enough anatomically to be recorded on the same recording tetrode if the tetrode was placed in between two patches, but still not be connected to each other. This remains to be tested, but it is not an easy task. The function of the entorhinal patches remains elusive for now; if many interconnected patches make up a single module, why are they separated into these small islands? A different anatomical feature may be better suited for explaining our results; intrinsic connections of entorhinal cortex stretch for long distances within entorhinal bands, but not across them (Dolorfo and Amaral, 1998b). Such connectivity in the mediolateral direction may explain the large extent of single grid modules. The individual entorhinal bands correspond to projections to different dorsoventral levels in hippocampus (Dolorfo and Amaral, 1998a). Thus, areas of entorhinal cortex that project to the same dorsoventral level of hippocampus will be interconnected, but areas of entorhinal cortex projecting to different dorsoventral levels of hippocampus will not, with the possible exception of indirect connections via hippocampal longitudinal connections and subsequent feedback to entorhinal cortex (Cappaert et al., 2015). Entorhinal cortex is separable into three anatomically distinct bands (Dolorfo and Amaral, 1998b), a smaller number than the number of modules we observed in individual animals, implying that one band likely does not correspond to one module. Also, our data indicated extensive overlap of modules within the tissue, not complete separation. The separation of three entorhinal bands better fits the assumed division of three major dorsoventral parts of hippocampus; dorsal, intermediate and ventral (Dong et al., 2009), but further investigation of the specific anatomical correlates of grid modules and their impact on different hippocampal dorsoventral levels should be carried out.

4.1.4 Grid modules represent independent parallel input streams to hippocampus

The existence of grid modules suggests that spatial information to hippocampus enters in parallel streams with different spatial resolution, supporting theoretical predictions of optimal spatial representation by grid cells (Mathis et al., 2012). An important aspect of such parallel input streams is the extent of interaction between the separate modules. Given that different modules could express different direction of ellipticity, it seems unlikely that they are generated from the same external input just with different integration properties. Combined with ellipticity, the variable ratios in grid spacing within and between animals makes it unlikely that smaller grid spacings are generated from a harmonic of larger grid spacings or that larger grid spacings are generated from Moiré interference between smaller grid spacings. These observations hinted to independent processing streams of spatial information into hippocampus. To test whether grid modules could act independently of each other we performed an experiment where we manipulated the familiar recording environment by a compression. Varying effects on place fields have been described in response to similar manipulations of familiar environments; some place fields stretched along with environmental elongation, some fields shifted location and some fields split into two (O'Keefe and Burgess, 1996). In two separate studies, recorded grid cell responses to similar experimental manipulations demonstrated the presence of both elasticity and rigidity in the grid structure along the axis of manipulation (Barry et al., 2007; Solstad et al., 2008). Different degrees of elasticity in grid responses were reported in data from several animals and could potentially be explained by individual variations in anchoring of the grid or other internal processes (Barry et al., 2007; Solstad et al., 2008). Another possibility was that different responses could co-occur within animals, specific to distinct modules, as suggested by the similar responses across multiple cells with the same grid spacing that were recorded in the same animals (Solstad et al., 2008). The diversity in place field responses to manipulations of environment dimensions suggested that individual animals expressed more than one response in grid cells, but this required larger sampling within animals. We revealed the presence of both elastic and static responses in grid cells within individual animals in our environment compression experiment. Some grid modules retained internode relations, and fields outside the compressed environment were no longer present in the map. Other grid modules expressed grid pattern compression, matching the compression of the environment, so that all fields were still present in the compressed map. The geometry of the fields themselves remained unaltered, only their arrangement was affected by environmental compression, in line with the previous finding that individual grid fields are independent (Reifenstein et al., 2012). This finding has some important implications. It confirmed the coexistence of both static and elastic responses in grid patterns, which itself provides proof of principle of functional independence between grid modules. This further discredits the possibility that different grid spacings are generated by different integration of the same input, or by harmonics or Moiré patterns from inter-module interactions. Grid modules rather appear to represent separate and functionally independent spatial input streams to hippocampus.

Compression of the grid pattern in grid modules with large grid spacing should lead to increased field overlap of neighboring grid cells in these conditions. Given the theoretical importance of inhibition for grid pattern generation (Burak and Fiete, 2009; Couey et al., 2013), it is potentially a little surprising that closer packing of grids with different spatial phases does not affect the grid fields themselves as competitive interactions should escalate with such tension.

4.1.5 Functional differences between dorsal and ventral grid modules

Another observation from our compression experiment was that responses to this compression in the grid pattern were related to grid spacing. Modules with small grid spacing responded with static internode distances while modules with large grid spacing responded with a corresponding compression of distances between grid nodes. Response type did not depend on absolute spacing in the pre-compression grid pattern as we observed small-module responses in grid cells with larger spacing than grid cells with large-module responses in other animals. Still, the pattern of responses across animals indicated that static grid patterns are present in dorsal entorhinal cortex while dynamic grid patterns are located in ventral entorhinal cortex. Static grid patterns will not contain a complete contextual representation in the compressed environment as some fields of the original map are absent, but these cells will provide hippocampus with absolute distance independent of changes to the layout of the environment. Therefore, these neurons may provide proper metric input to hippocampus. In ventral entorhinal cortex, the dynamic representation of space indicates a map which has higher spatial relativity, such as designating the centre of the environment or in the North-West corner. These different responses could reflect a stricter convergence of self-motion cues or time, to movement of an attractor in dorsal modules compared to ventral modules. As entorhinal inputs converge in hippocampus, different amounts of input from separate modules onto individual place cells could potentially explain the diversity of place field responses in similar experiments, where individual place cells may keep both fixed and relative relationships to particular walls during environmental reshaping (O’Keefe and Burgess, 1996). In addition to grid cells, hippocampal place cells also receive input from entorhinal border cells and head direction cells (Zhang et al., 2013) as well as non-spatial input from lateral entorhinal cortex, all which could contribute to the final responses of place cells. Whether dorsal and ventral place cells respond differentially to environmental compression is another open question which should be tested.

Given that dorsal entorhinal would affect dorsal hippocampus more, it is likely that hippocampus as well will display such a dorsoventral distinction. The metric responses dorsally and the contextual responses ventrally do comply with the ideas of dorsoventral differences along hippocampus as discussed previously.

Grid recordings during environmental compression in the study of Barry et al. were of grid cells with small spacings (Barry et al., 2007). Why then did they find elastic responses when we consistently observed static grid patterns in dorsal entorhinal? These differences may be accounted to the size of the enclosure, which can affect grid anchoring as described below during the discussion of paper 2. While Barry et al. used environments of 70-100cm, our arena was compressed from 150cm to 100cm.

4.1.6 Is a modular organization unique to grid cells?

Should we expect modules in hippocampus as well? This question does require proper testing of place cells in experiments similar to ours, something which has not yet been done, despite the data having been collected (Patel et al., 2012). However, despite functional differences of ventral and dorsal hippocampus, there are some indications that this modular organization may be unique to the entorhinal spatial representation. Firstly, the convergence of entorhinal inputs to hippocampus suggests that individual place cells may actually receive input from more than one entorhinal grid module (Dolorfo and Amaral, 1998a). Secondly, intrahippocampal longitudinal projections connect both DG to CA3 and CA3 to CA1 across extended dorsoventral positions (Cappaert et al., 2015). Thirdly, the diversity of local responses in experiments similar to our compression experiment (O'Keefe and Burgess, 1996) as well as in remapping experiments (Jeffery and Anderson, 2003; Leutgeb et al., 2004), do not indicate the presence of local coherence as found in grid modules. Since hippocampal ensembles encoding an environment changes during global remapping, separate and coherent modules with high internal connectivity and low cross-connectivity seem unlikely. Fourthly, in our experience theta phase appeared synchronized across all entorhinal recording sites, similar to recordings in human entorhinal cortex (Mormann et al., 2008), only with the exception of a phase reversal when approximating LI, as has been described by others (Chrobak and Buzsáki, 1998). This stands in contrast to hippocampal theta which is known to propagate as a travelling wave along the dorsoventral axis (Lubenov and Siapas, 2009; Patel et al., 2012). While synchronized oscillations in entorhinal cortex may favour modular ensemble activity, a travelling wave would be more likely to favour continuous sequential activation. Finally, while grid cells thought to be generated in the entorhinal cortex express a modular arrangement, head direction cells in the same tissue do not bear the same modular organization (Giocomo et al., 2014). Since head direction cells exist in multiple brain areas and entorhinal head direction responses are most likely inherited from such inputs originating outside entorhinal cortex (Taube, 2007), similar convergence as grid to place cells in the head direction system may blur any potential modularity in earlier stages and create smooth gradients in resolution along the dorsoventral axis. The same mechanism may convert a modular grid organization to a continuous place cell map.

4.1.7 Independent grid modules and hippocampal remapping

What are the benefits of independent parallel spatial input streams? One potentially essential cause of hippocampal global remapping may be the ability of grid modules to independently reorganize between separate environments. As suggested by Fyhn et al (Fyhn et al., 2007), global remapping in hippocampal place cells may be attributed to one of two possible mechanisms outlined in figure 4. The first suggestion was that grid cells are organized in modules that can independently rearrange during global remapping, providing hippocampus with different overlapping inputs. Their second suggestion was that different sections of an infinite coherent map would be active in each environment. They noticed that during hippocampal global remapping grid maps rotate, a concerted response in local ensembles of grid cells from the same module (Fyhn et al., 2007). Global remapping occurs in hippocampal ensembles after partial entorhinal inactivation, demonstrating a causal role of changes in entorhinal input for global remapping (Miao et al., 2015).

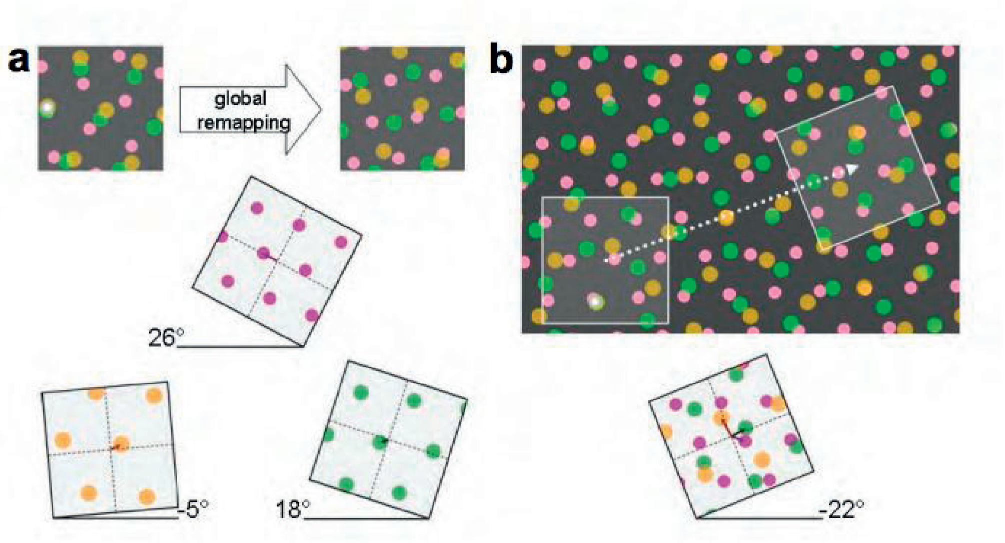

Figure 4: Two suggested mechanisms for how global remapping may be produced in hippocampus. Light squares at the bottom represent grid cells and their tilts during hippocampal global remapping. Dark squares at the top represent the combined overlap of grid inputs to a hippocampal place cell. The white dot displays a location where inputs overlap and the added excitation at this location produces a place field. In a) different grid maps display independent tilts of the grid pattern in response to relocations between two environments. The subsequent combined input to hippocampus will have different overlapping spatial locations, leading to a change in place field appearances. In b) relocations to different environments causes shifts in an infinite spatial map. Even concerted tilts between different grid modules would still be able to cause global remapping in hippocampus in this scenario. Figure adapted from Fyhn et al. (Fyhn et al., 2007)

Moving to a novel environment may produce temporarily increased grid spacing and reduced grid scores (Barry et al., 2012), two other features which may significantly alter input characteristics to hippocampus if independently set for different grid modules. From the independent responses across grid modules that we reported in our compression experiment, we provide support for the notion that grid modules may independently reorganize in different environments, and by this create distinct environment-specific patterns of spatial inputs to hippocampus leading to hippocampal global remapping.

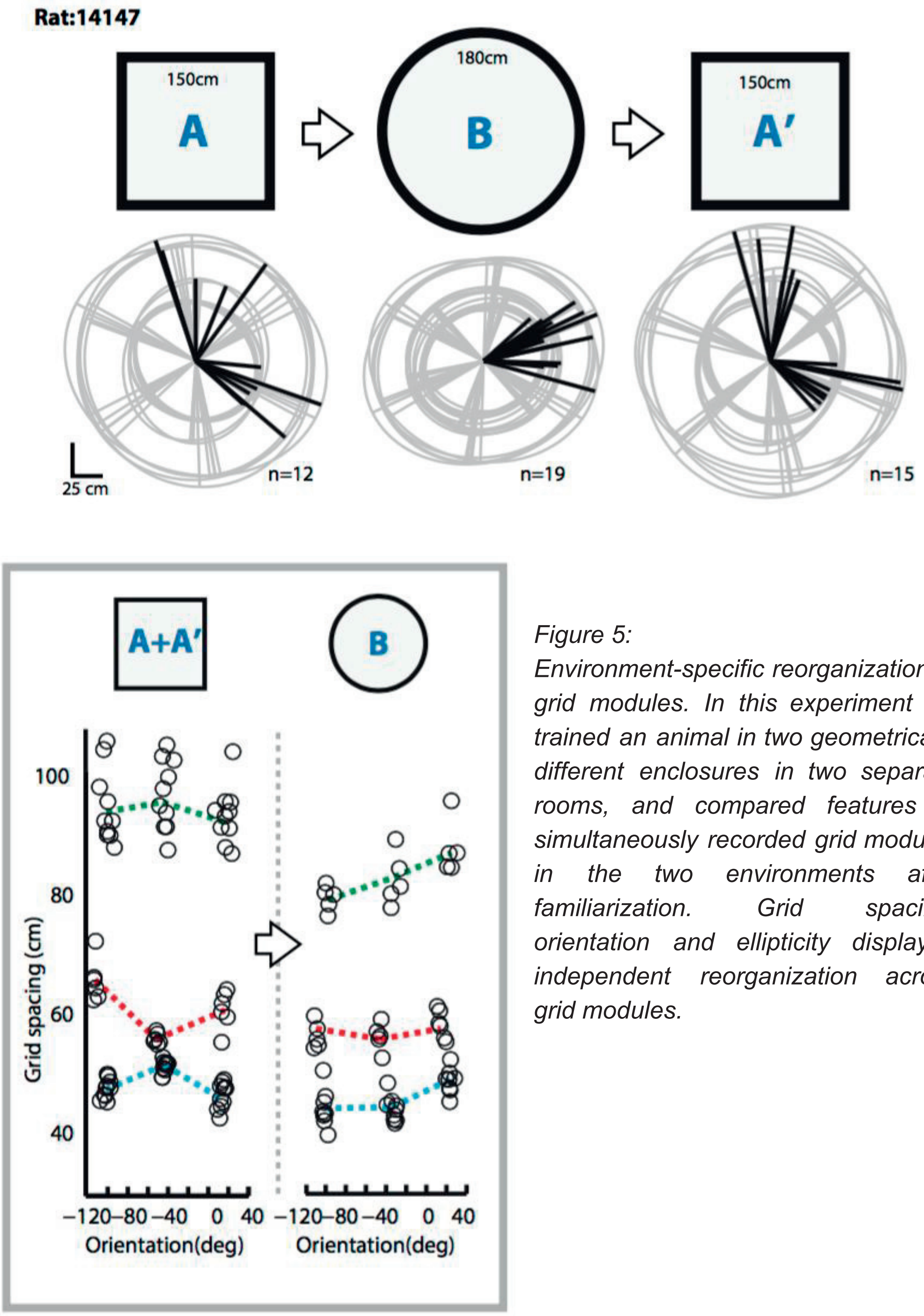

Figure 5:
Environment-specific reorganization of grid modules. In this experiment we trained an animal in two geometrically different enclosures in two separate rooms, and compared features of simultaneously recorded grid modules in the two environments after familiarization. Grid spacing, orientation and ellipticity displayed independent reorganization across grid modules.

Even small independent adjustments in converging inputs from as little as two grid modules can provide the hippocampus with an apparent endless capacity to form new maps in new environments (Monaco and Abbott, 2011). The changes in grid spacing, orientation, and distortion across modules require further testing in multi-environment experiments with simultaneous recordings from several grid modules and preferably hippocampal place cells, but as shown in figure 5, we have data to support these ideas.

4.1.8 Unresolved questions regarding grid modules

Our report of separate parallel spatial input streams from entorhinal cortex, with functional differences in a compression experiment, also raises a question regarding whether previously described features of grid cells best describe dorsal grid cells, which are the most common to record from given their higher frequency of fields per recording box area. Several studies should be carried out to evaluate properties of ventral grid maps in comparison to dorsal grid maps as well as their coactivity in simultaneous recordings. It is important to establish whether ventral grid cells respond similarly to dorsal grid cells during hippocampal remapping, particularly if ventral grid cells remain unchanged during hippocampal rate remapping (Fyhn et al., 2007). Another finding that was reported in dorsal grid cells is a fragmentation of the grid pattern in a hairpin-maze, where walls inserted from alternating opposing walls form a convoluted linear track in which the animal runs every other of multiple identical laps in opposite directions (Derdikman et al., 2009). It is unknown if ventral grid cells fragment in the same way or whether they instead represent the global environment independent of inserted walls.

Other outstanding questions regarding grid modules involve development and stabilization in novel environments. In novel environments grid cells display increased spacing which gradually decreases as the environment becomes familiar (Barry et al., 2012). The same holds for hippocampal place cells (Barry et al., 2012). The exact mechanism behind this phenomenon is unclear, but may involve gradually reduced anxiety levels during familiarization or increased spatial precision as landmarks become associated to the map and can be used for error correction, or a mixture of different underlying causes. It has been suggested that the expansion is a mechanism to reduce positional errors during periods of increased uncertainty (Towse et al., 2014). An important experiment that remains to be done is a systematic recording of multiple grid modules within animals during familiarization. This provides a good chance of revealing the temporal relationship of formation and stabilization of the grid pattern. Do small grids stabilize first, or do they follow after large grids have stabilized? Or do all grid modules stabilize simultaneously? All options are plausible. If the animal builds spatial control over its environment by first connecting small, detailed local information which becomes increasingly global as local maps become linked, one should expect small grids to stabilize first. Slow developing effects from one module to the next could be controlled by convergence in hippocampus and subsequent feedback to entorhinal cortex, a feedback demonstrated to be essential for maintaining a grid pattern (Bonnevie et al., 2013). Another option is that large grid modules form first as an approximate map of the environment, and smaller modules develop subsequently as the animal develops more accuracy. Grid modules may also display a similar familiarization development if the maps develop in parallel. Finally, grid modules may operate completely independently, even over time, with random temporal relationships between modules. Similar to grid formation and stabilization in new environments is the question of how grids form in development. Multi-site recordings in developing rats are potentially not feasible, but a temporal description of grid formation as has already been reported (Langston et al., 2010; Wills et al., 2010) could be systematically performed at different dorsoventral levels across animals.

4.1.9 Grid modules and intrinsic theta oscillations

The existence of grid modules also has impact on theories of grid cell formation, which have clustered into two main schools, oscillatory interference models and attractor-network models (Burak and Fiete, 2009; Burgess et al., 2007; Navratilova et al., 2012). The theory of oscillatory interference makes a particular prediction about an increasing offset between internal and external theta frequencies with increasing grid spacing (Burgess, 2008; Burgess et al., 2007). With grid modules being discrete, we should expect to find discrete steps in internal theta frequencies corresponding to grid spacing. Any differences in intrinsic theta modulation should also relate to actual grid spacing values, which vary quite a bit across animals. Data pooled from multiple animals had previously revealed a relationship between internal theta frequency and dorsoventral depth (Giocomo et al., 2007), also present in our pooled dataset. However, and again demonstrating the importance of high-yield data sampling from individual animals, we could not demonstrate the predicted relationship within animals. Grid modules did display discrete intrinsic theta frequencies, corroborating the discrete nature of grid modules also in the temporal domain, however, the association between grid spacing and internal theta frequency did not reveal a strictly monotonically increasing pattern across animals; grid modules with large spacings could have higher or lower theta frequencies than grid modules with smaller spacings in individual animals. The theory does not dictate that the internal frequency requires this tight correlation to grid spacing, but the interference created by differences between internal and external frequencies does (Burgess, 2008). The idea could still hold if grid cells of different modules were exposed to different external theta frequencies. This is, however, not feasible on account of the substantial overlap between modules in anatomical space; in a large proportion of recordings we identified more than one grid module in a single recording site and these would therefore share external theta. Our data do not confirm predictions from the oscillatory interference model of grid formation. It does, however, lend good support to the attractor-network model, which assumes networks of grid cells to share similar features and responses.

4.2 Rules for organization of grid orientation

4.2.1 Anchoring grid maps to external environments

How are grid cells anchored to the structure of the external environment? Anchoring is important as it prevents drift in the spatial representation. Such drift, if severe enough, could result in erroneous associations of events in hippocampus due to inaccurate coordinates and, as a consequence, incorrect hippocampal ensemble activity at the time of episodic-related input. Hippocampus may be somewhat tolerant to drift due to the recurrent character of CA3 networks, but it should optimally be restricted to a minimum and corrected whenever possible in order to maintain a stable spatial framework for experience and memory encoding. Minimizing drift should be particularly important for the high-resolution maps in dorsal populations as even small offsets here could lead to completely different population activity in hippocampus, while more tolerance should be expected ventrally.

Work from Cheng in 1986, in which rats were trained to obtain hidden food rewards in a rectangular environment, hinted at anchoring to geometric features of the environment as an important strategy (Cheng, 1986). Rats often made rotational errors in which they would search for the food reward in the corner at the diagonal of the correct location despite the presence of polarizing non-geometric cues (Cheng, 1986). These corners were in geometrically identical locations due to symmetries in the shape of the box. If geometrically equivalent locations cause confusion, one should assume that this would co-occur with a reorientation of the spatial map of the environment. Such errors should preferably be avoided in order to optimize adaptive behavior and avoid erroneous associations of external input. A square environment, as the one used in our experiments, will be even more geometrically confusing as four parts of the environment are geometrically equivalent. The need to disambiguate such geometrically equivalent locations may thus be even more pressing. A challenge when disambiguating geometrically similar locations is the geometry of the grid pattern, which itself may be ambiguous given its internal symmetries.

When superimposing a hexagonal pattern onto a square pattern, certain relative orientations of the two may result in more common symmetries than others. When the grid pattern is perfectly aligned to box coordinates, mirrored symmetry arises along two parallel walls perpendicular to the one the pattern aligns to. If the grid pattern aligns with a maximum offset to any box axes, mirrored symmetry arise along the diagonal. The further the grid pattern orients from a parallel symmetry, the closer it orients to a diagonal symmetry, and equal repulsion from both these solutions may put an optimal grid orientation in the middle between these. Parallel symmetries correspond to a grid orientation of 0°, and diagonal symmetries arise with a grid orientation of 15°. The midpoint of these would then be 7.5°.

4.2.2 Grid orientation aligns with axes of the external environment

In our dataset presented in paper 2 (Stensola et al., 2015) it was clear that grid orientation was not random. It could vary across animals and across modules within animals, but all orientation values clustered at values close to parallel to the cardinal axes of the environment, although with a conspicuous offset close to 7.5° in either direction, as suggested above may minimize symmetry. Grid orientation could fall on either side of environmental cardinal axes with no apparent pattern and similar likelihood, both across animals and across modules within animals. This indicates that grid orientation is set independently for each grid module, supporting our previous suggestion that grid modules are independent processing units. Despite possible differences in grid orientation across grid modules within animals, the similarity of grid orientation across modules and even across animals trained months apart suggests that internal and external geometry are somehow connected. The existence of different grid orientations within animals discredits causal impact from behavioral components in this connection. Grid orientation offset appeared to develop with environment exposure as grid cell recordings from novel environments had minimal offsets. This suggests that the offset develops as the grid map stabilizes in an environment, likely due to improved anchoring, which may arise from hippocampal feedback when the hippocampal map has established a rich representation of associated non-spatial features to the spatial coordinates. It would be interesting to know whether place maps in hippocampus display brief episodes of rotational symmetry errors when grid maps are parallel to geometric axes of the environment during novelty.

A potential benefit from the offset of grid orientation to environmental boundaries is that different segments along a single wall may become less ambiguous (figure 6).

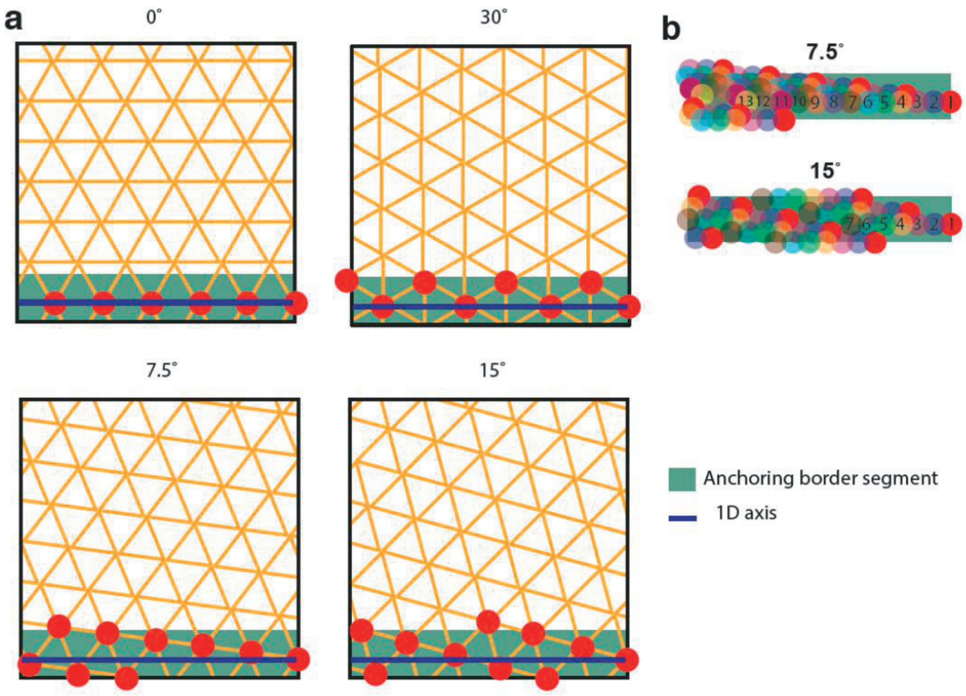

Figure 6: Wall alignment of grid orientation. A grid pattern oriented with 4 different solutions is shown in a). Perfect alignment of the grid to environmental axes produces ambiguous repetitions of the spatial code along the wall. Tilting the grid pattern within the environment reduces the frequency of this repetition to varying degrees. An offset of approximately 7.5° may be beneficial to reduce this ambiguous

repetition along the walls to a minimum. Plasticity mechanisms between grid cells with different spatial offsets and border cells may potentially generate this offset, as more grid phases will occupy border segments with an offset that reduces repetitions of single grid phases along the wall, as shown in b).

Due to the repetitive nature of grid patterns, grid input to the hippocampus will repeat during movement along a single wall. Input from border cells and head direction cells will be even more ambiguous in this situation since they will display similar firing along the entire path. The combination of ambiguous border and head direction input with repetitive grid input may pose challenging demands in downstream hippocampus during traversals along walls. Rats tend to move along walls of environments, particularly during novelty, and this may then be a source of positional uncertainty. Another issue with grid pattern alignment to border cell firing is that while maximizing co-activity with grid cells of some spatial phases, it minimizes the number of grid spatial phases that overlap at all with individual border cells (figure 6). If border cells provide direct excitation to grid cells, a continuous drive along borders may be present to all grid cells, but in competition with disynaptic inhibition from grid cells of different spatial phases (Couey et al., 2013). Equal distribution of grid cells with different spatial phases along walls, forcing grid orientation away from alignment, may be the solution that the pattern settles to (figure 6).

If grid cells are anchored to environmental walls by input from border cells in initial exposures, this may explain closer alignment in novel environments. Other anchoring mechanisms may develop over time, as suggested above, through hippocampal feedback and stabilize grid cells independently of border cell input. This hippocampal feedback may require binding of landmark and episodic features to the coordinate system in order to provide useful feedback, and this process that will depend on extended experience to evolve. The fact that grid patterns break down after hippocampal silencing despite the resistance of both border cells and head direction cells to this manipulation, demonstrates such a dependence on hippocampal feedback in familiar environments (Bonnevie et al., 2013).

In support of a role of border cells in providing anchoring of spatial representations during an early phase, it has been demonstrated that in developing rat pups, place maps first stabilize along environmental boundaries and only display full integrity when the grid system is developed (Muessig et al., 2015). Peripheral anchoring may potentially be provided by border cells in the absence of grid input, as these develop earlier (Bjerknes et al., 2014), suggesting that border cells may serve as a basic anchoring solution also for hippocampal maps, at least initially. This is further supported by the finding that septal inactivation, abolishing hippocampal theta, leads to loss of spatial specificity in hippocampal neurons in large novel recording environments, with the exception of some fields that retain specificity along walls (Wang et al., 2015). In the latter study, place fields were still present during septal inactivation, albeit less stable, on a novel, modified linear track where wall contact was continuously available (Wang et al., 2015). Particular aspects of grid and place cell establishment in novel environments are also not supportive of a role for grid cells in place field establishment (Yoon et al., 2013), which instead could be implemented by border cells.

4.2.3 Shearing relates grid orientation and pattern deformation

We detected that the offset in grid orientation was strongest for the grid axis closest to the cardinal axis that the grid aligned to, and the grid axis furthest away from this was only minimally different from 60° multiples of perfect alignment (0°). This supported our idea that the offset was developed over extended experience from a grid pattern originally parallel to one of the cardinal axes of the environment, an orientation still present in grid axes the farthest away from influences by assumed anchoring walls. It also connected the previously described grid ellipticity to a process of grid orientation dynamics, as the offset was not due to a full rotation of the grid, but to a deformation that fits a transformation known in continuum mechanics as shearing. By applying such shearing forces on our recorded grid cells, we could minimize ellipticity as well as orientational offset to the environmental cardinal axis, which we suggest is the reverse process of the original distortion. Shearing forces applied in one direction, perpendicular to the cardinal axis with the closest coupling to grid orientation, abolished the orientation offset, while shearing in the opposite direction did not change the orientation offset.

In a dataset from a larger environment, similar, but more complex patterns were present. While all animals and all modules would display alignment to the same cardinal axis in the smaller environment, grid modules could align to either of the cardinal axes in the large environment, yet still with similar absolute offsets as seen in the small environment. Simple shearing transformations applied to minimize ellipticity did not reinstate a parallel grid as it did in the smaller environment, but a two axis corner-linked shearing did, suggesting the grid had multiple anchoring to the larger environment and that shearing forces acted independently from different parts of the box. This was further supported by local, but not global, 7.5° offsets from the environmental cardinal axes detectable in some grid patterns. Dividing the environment into segments revealed certain differences between the smaller and larger environments. While in the smaller environment grid patterns from box segments were similar, suggesting a unified map, in the larger environment the correlation of grid autocorrelations from the different segments were significantly lower, indicating conjoined sub-maps. In a further segmentation of the large environment, we found significant effects of position on grid score and ellipticity, with higher grid scores in the box centre and ellipticity more pronounced in the corners. Ellipse tilt displayed particularly low variance in the corner where the animal was released, suggesting that this may constitute an initial anchoring point.

The differences in anchoring between the two enclosure sizes hints to mechanisms of grid anchoring which depends on distances between essential landmarks, such as box walls. Initial experiments, which concluded with random grid orientation across animals, may have done so due to the use of even smaller enclosures (1m square enclosures), or circular recording environments (Hafting et al., 2005), the latter which we demonstrate contain a greater distribution of grid orientations.

4.2.4 Unresolved issues regarding grid anchoring to external environments

Based on our observations it is a plausible hypothesis that grid maps are anchored to environmental borders or corners. Corners are often the release point of animals in open field experiments, and at least in our experiments, the release points both during training and recording sessions, have been stereotyped, which may be the underlying reason for the abundance of cross-animal similarities. It remains to be tested whether systematic differences in initial environmental exposure, such as release from a start box in the centre of the environment, will alter grid anchoring and subsequent deformations by shearing. Another stereotyped behavioral feature during initial exposure to a novel environment is the tendency to sit in corners and move along walls as rats are agoraphobic. Such oversampling of these parts of the environment may be the underlying cause of anchoring to walls or corners. It has also been demonstrated that environmental borders serve to correct accumulated errors caused by inherent drift in path integration, and entorhinal border cells may be important for this as they display selective firing along the circumference of the environment (Hardcastle et al., 2015).

It is known that during familiarization with novel environments, both grid cells and place cells typically display a gradual compression of the map (Barry et al., 2012). We suspect that attachment of the grid pattern to particular anchoring points combined with this tendency of the grid pattern to compress during familiarization with an environment may be an underlying cause of grid shearing and deformation. Compression would always occur towards a centre point. If multiple anchoring points exist, as appears to be the case at least in the larger environment, there may be several such centre points, and forces may act simultaneously on these to compress the pattern while maintaining approximate overall grid structure. We did not have the required data to fully test the development of the shared grid patterns, but future experiments should explore this.

4.3 Are environmental borders the essential anchoring structure for grid maps?

4.3.1 Walls as anchoring structures for spatial maps in the brain

In hippocampal recordings, place cells were found to have particular strong attachments to environmental borders (O'Keefe and Burgess, 1996). When the recording environment geometry was altered by elongation or compression in either direction, place cells responded by maintaining either absolute or relative field distance to certain walls. Some place cells displayed field splitting, suggesting separate overlapping anchors in the training environment. Such response diversity could be due to different combinations of grid inputs from modules that either retain an absolute or a relative grid pattern in the modified environments, or that anchor to different walls. This should be tested in similar experiments with simultaneous entorhinal and hippocampal recordings.

In another study mentioned above, it was demonstrated that contact with walls in open field environments causes correction of accumulated errors in grid patterns (Hardcastle et al., 2015). The longer an animal has been away from the circumference of its environment, the larger the error.

4.3.2 Preliminary data supporting module-specific wall anchoring

Whether all walls have the same ability to reset the grid pattern in all modules remains to be determined, but we have preliminary evidence to suggest that there may be module-specific differences in anchoring within animals (Stensola et al, 2013, Abstr. Soc. Neurosci., abstract see figures 7-10). We performed a set of recordings where the animal explored a raised platform inserted into a familiar environment in order to test whether it would retain the map from the familiar environment or generate a separate map for the platform. The platform, a quarter of the size of the familiar environment, was positioned in one of four corners within the familiar recording box and moved to all quadrants with the rat still on the platform. The sequence of quadrant exposures was changed each day. We compared the combined maps from the four platform positions to the original map of the environment without the platform (figure 7).

We noticed that for the two grid modules with smallest grid spacing, the original map was adopted in two of the platform positions, and a new map was created for the two remaining positions (figures 8-9).

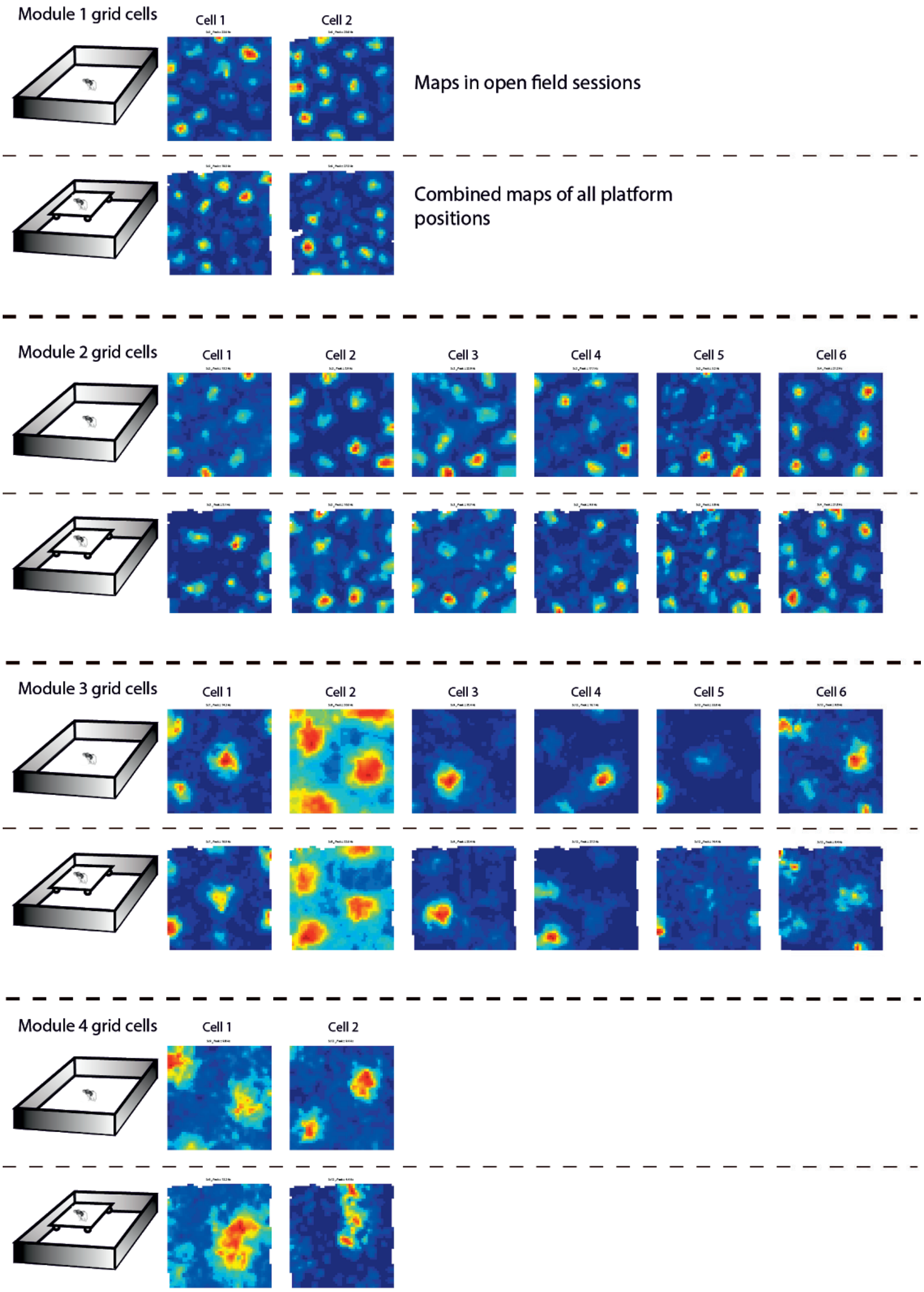
Module 1 grid cells
Cell 1
Cell 2
Maps in open field sessions
Combined maps of all platform positions
Module 2 grid cells
Cell 1
Cell 2
Cell 3
Cell 4
Cell 5
Cell 6
Module 3 grid cells
Cell 1
Cell 2
Cell 3
Cell 4
Cell 5
Cell 6
Module 4 grid cells
Cell 1
Cell 2

Figure 7: Platform experiment. We trained animals to run on a platform within a familiar enclosure. The platform was moved sequentially to different quadrants of the surrounding environment and the combined maps of the quadrant positions was compared to the map in the open field. Grid cells from 4 modules are shown. While module 3 grids appeared very similar in the two conditions, module 4 grids did not retain grid structure when the animal ran on a platform, and modules 1 and 2 both displayed grid-like structure, but with global pattern deviations.

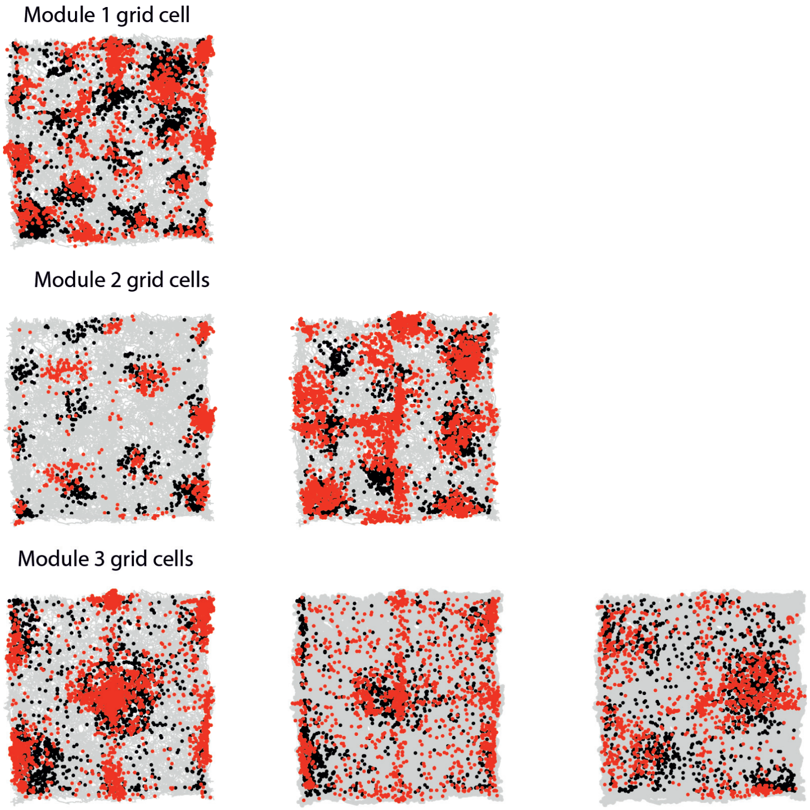

Figure 8: Comparison of grid maps in open field and on platform. Black dots indicate spikes from an open field session and red dots indicate spikes from the combined platform positions. A cell from module 1 is displayed in the top line. Along the West wall fields overlap fairly well in the two conditions, while in the North-East corner the offset is complete. The opposite pattern was true for grids in module 2, and for grids in module 3 the overlap was quite good for the entire environment.

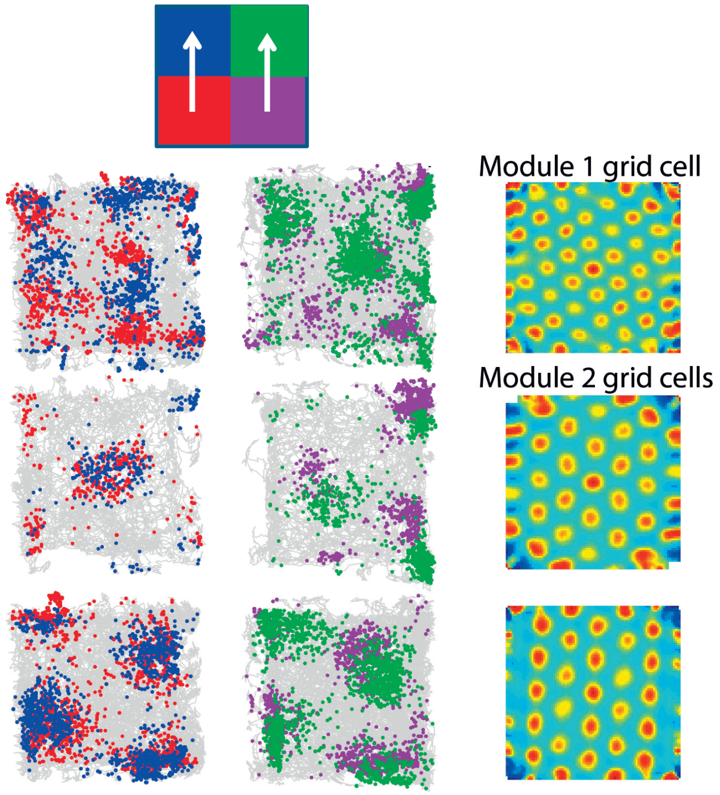

Figure 9: Comparison of grid maps at different platform positions. For the parts where there was little overlap between the open field and the platform maps, the animal could potentially have generated a map anchored to the platform, which then should not be affected by movement of the platform within the larger enclosure. For the cell from module 1, the top line, overlapping maps from two platform positions along the West wall, where there was good correspondence between open field and platform representations, did not reveal a platform-specific map, as expected if the map was retrieved from contact with anchoring walls in the surrounding environment. Along the East wall, where the map from the platform session displayed little overlap with the open field map, there was a good correspondence of field positions in the two platform quadrant positions, as expected if the grid anchored to the platform in these positions. For module 2 grids, the opposite pattern was present, suggesting module-specific wall anchoring. Different colored dots represent different quadrant positions of the platform.

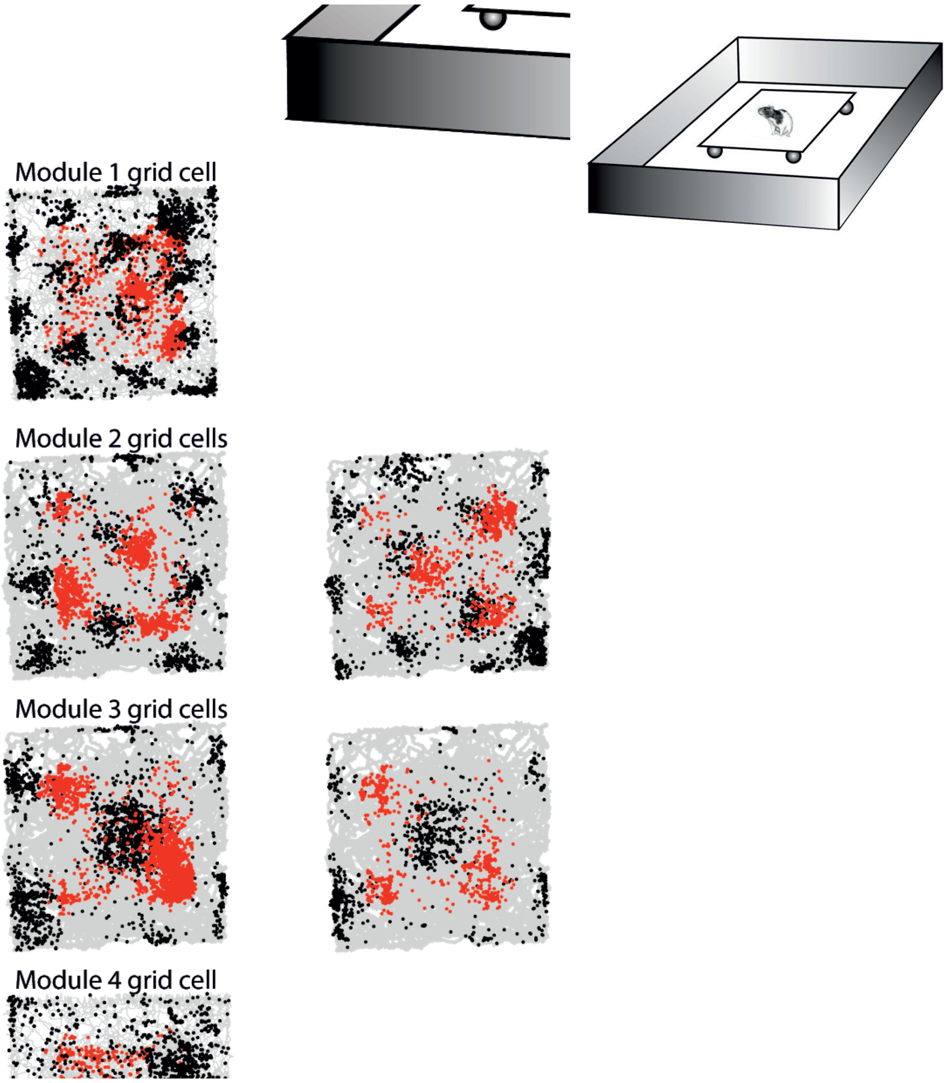

Figure 10: Comparison of grid maps in open field and on platform when the platform was positioned in the centre of the larger arena. In this condition none of the grid modules retained any aspect of the map from the open field, suggesting that direct wall contact is necessary to retrieve stored grid representations.

We hypothesize that direct contact with an anchoring wall is necessary to retrieve the original map. The two modules did not appear to anchor to the same wall, but to either of two parallel walls (figures 8-9). For a third module, we found complete retrieval of the original map on all four platform positions, indicating that this module with larger grid spacing anchored more globally to the environment (figures 7-8). A puzzling observation was that a fourth simultaneously recorded module appeared to respond in a complicated manner, not maintaining overall grid pattern structure (figure 7). The response of this module may indicate that grid maps with spacings irrelevant for the environmental size are used for other coding purposes in a dynamic manner. We subsequently positioned the platform to the centre of the larger enclosure, where the animal was not in direct contact with any of the walls of the larger environment, and in this condition, none of the recorded grid modules retained the original grid map (figure 10).

Such a requirement for direct contact with environmental borders to retrieve and update an established grid map seen in our preliminary data, and in the study on error correction from boundary contact (Hardcastle et al., 2015), may be explained by a reliance on entorhinal border cells, which display selective firing along one or several environmental borders (Solstad et al., 2008). Border cells produce new fields in response to inserted walls positioned parallel to the cell’s initial fields (Solstad et al., 2008), and this extension of environmental boundary representations in combination with its potential influence on grid anchoring may also explain the fragmented and repetitive grid maps described in the hairpin maze (Derdikman et al., 2009). Similar extensions of geometrically equivalent representations may shed light on a recent finding in which rats in a recording environment consisting of two equal sub-compartments connected by a passageway, initially display local grid maps copied across the two geometrically identical environments (Carpenter et al., 2015). As the animals became more familiar with the multicompartment environment, a global map developed (Carpenter et al., 2015), suggesting that direct reliance on geometry may be more important in less familiar environments where fewer external associations have been made to anchor and stabilize the map via hippocampal feedback. Whether rats would similarly develop global maps in the hairpin maze over extended time is an intriguing possibility.

4.3.3 Do grid maps exclusively anchor to walls of the immediate environment?

In a study published simultaneously as our paper, similar pattern alignment to walls was described (Krupic et al., 2015). They also demonstrated that grid patterns were fixed to the recording box geometry during rotation of this relative to the environment (Krupic et al., 2015). This is in line with our own unpublished observations (see figure 11) across simultaneously recorded grid modules.

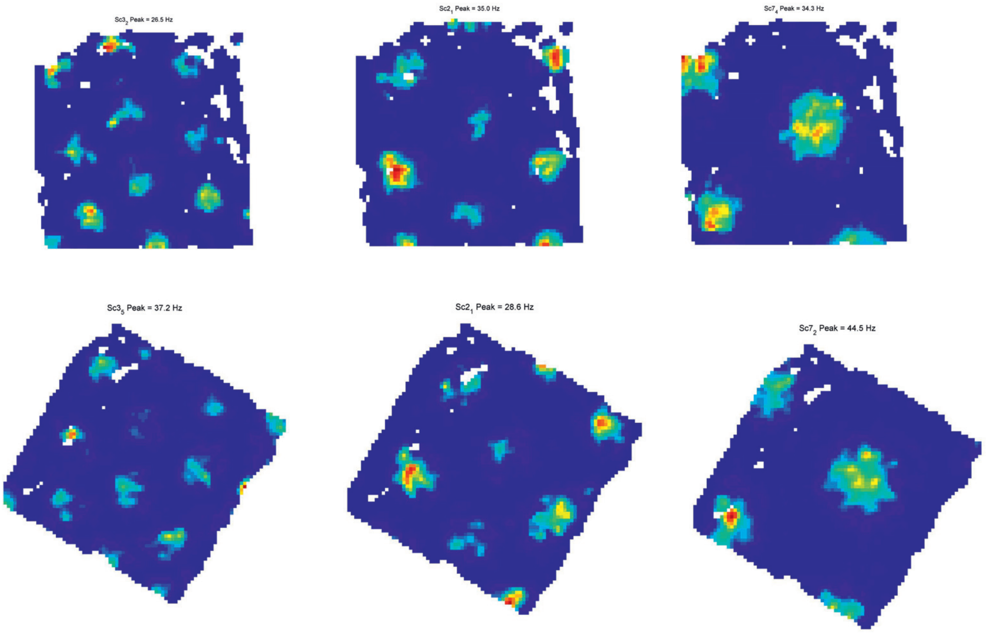

Figure 11: Rotation of the recording enclosure. Grid maps from 3 simultaneously recorded grid cells from 3 different modules all displayed rotation with the recording

enclosure, suggesting strong anchoring to the local cues over distal visual cues in the surrounding room.

Despite this demonstration of grid map attachment to recording boxes, we also have preliminary data demonstrating that this may be dependent on other factors, potentially the alignment of the recording box within the greater environment (figure 12). In a triangular recording box, we found that certain tilts of this box within the room maintained the same grid map anchored to the box while other tilts caused a sudden shift in the grid map (figure 12). The detailed mechanisms that anchor the grid to box geometry should be further studied.

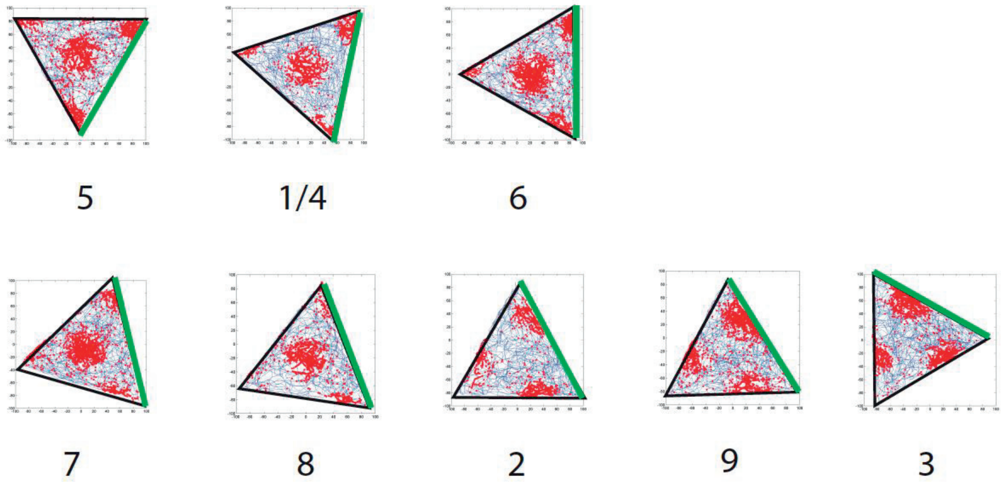

Figure 12: Abrupt shift in grid representation in response to rotation of the recording enclosure. This grid cell was recorded in a series of sessions in a large triangular environment. The green line indicates a wall which contained a white cue card. The number below each triangle indicates session number. Independent on session number, but dependent on tilt of the triangle in the surrounding environment, we detected an abrupt change in the grid map. This demonstrates that even if grid maps anchor to the immediate environment and follow this with some rotational tolerance, there is a limit to this adherence to the local enclosure. The abrupt difference seen here suggests strong attractor properties in the network.

Our findings are not in line with data from circular tracks where cells from medial entorhinal cortex anchored to global cues rather than local cues when these were rotated in opposite directions (Neunuebel et al., 2013). However, this can be explained by the geometry of the recording environments. In boxes rats may rely heavily on geometry to anchor grid maps, but in a circular maze, each point is geometrically equivalent. This may force anchoring to other features, such as distant visual cues. Anchoring to distant visual cues may be problematic, particularly in a nocturnal species like the rat, and may thus be less preferred as an anchoring solution if geometrical information is available. An important question which remains to be resolved is how grid cells anchor in the rich natural environments that rats normally reside in, as these rarely contain the same simple geometric features that standard recording environments in neuroscientific research labs have. Current development in wireless recording equipment and rapidly increasing data storage technology may make experiments in such naturalistic habitats feasible in the near future.

5. Concluding remarks

We have provided the first thorough description of grid cell organization across large anatomical extents. Our findings have revealed several important elements of grid organization, many of which had been predicted, in a modular arrangement of independent and parallel spatial streams with distinct spatial resolutions. We have also described mechanisms for grid anchoring that rely on the geometry of the external environment, and which display striking similarities across animals, suggesting universal anchoring principles.

There are large amounts of unanswered questions regarding the function of these parallel streams, some of which have already been mentioned. One of the most pressing may be the cross-module differences during hippocampal global remapping and the contributions of different grid modules to individual place cells. Such an experiment may provide essential insights into hippocampal remapping and the seemingly endless memory capacity of hippocampus.

Another important outstanding question is what determines grid spacing. Although we described an average ratio between grid modules that was consistent across module steps, the large variability both in specific spacing values and in ratios across animals exposed to the same environment suggests that grid spacing is determined by different mechanisms than grid orientation.

How grid cells anchor in naturalistic habitats with multiple nested environments will be essential to determine. We have already demonstrated partly segregated sub-maps in very large environments, suggesting that grid anchoring can only extend to a certain limit, potentially due to resetting of accumulated errors only when physically present at the anchoring location (Hardcastle et al., 2015) and that movement too far from this would cause drift in the code beyond what the hippocampus can tolerate without remapping. Based on our preliminary data from the platform experiments, a nested environment, we suspect that there will be a dorsoventral difference in the encoding of nested environments and that grid modules with small grid spacings may encode individual subsections separately while grid modules with larger grid spacings may provide more comprehensive representations. If error accumulation is relative to grid spacing, then grid modules with larger grid spacing could extend further from anchoring points before error accumulation becomes devastating for hippocampal encoding. This would agree with the findings of detailed and comprehensive episodic memory along the hippocampus longitudinal axis in humans and the ideas behind the different functional roles along the dorsoventral axis in both structures, detailed spatial representations dorsally and comprehensive contextual representations ventrally.

The existence of independent grid modules raises another important question: Is the presence of a certain number of grid modules fixed or does it depend on experience? Do animals that never experience a large environment need grid modules with very large spacings? Are the cells which potentially can be used for large context encoding ever active if large contexts are never experienced? Or do they adopt a different code than the grid pattern, as we see tendencies towards in the largest grid cells in our platform recordings where the local environment became too small to support meaningful grid structure in the largest module we recorded? It is a common experience that when people return to their childhood home after having lived away for a while, their hometown appears smaller than it used to. We also talk about seeing the world to expand our horizons. One can speculate that such experiences of larger environments lead to development of new, larger grid modules, which in turn can build better comprehensive representations via ventral hippocampus. Maybe it’s not the air, but the large distances and perspectives that make a hike in the mountains so therapeutical to many people, simply by activating ventral processing streams in the hippocampal formation and by that providing more comprehensive memory retrieval and opportunities for processing life events on a greater scale. A collaborative effort between neuroscience and social anthropology may test this by comparisons of abilities to link episodic memory at greater scales between nomadic groups of people and more spatially restricted farming communities.

Grid cells likely provide hippocampus with a continuous structure in space and time so that associations of nonspatial input can be correctly applied to existing representations in order to extract statistical relationships of the external world and create neocortical schemas that can generate predictions and guide decision-making and behavior. Such structure may serve as the underlying mechanism behind coherent experience which is qualitatively different from the fleeting experience during dreaming, conscious recollection of memories or imagination in which experience of time and space may defy physical laws. Determining the contribution of grid cells in such internal experience will be important in future research.

6. Ethical Issues

All experiments described in this thesis were conducted according to the Norwegian Animal Welfare Act (Lov om dyrevern, no 73 av 20. desember 1974) and the European Convention for the Protection of Vertebrate Animals used for Experimentation and other Scientific Purposes.

The research laboratory is licensed by the national authority for animal research and satisfies the requirements for rodent units as recommended by the European Convention. The experimenters involved were all certified via a compulsory course in laboratory animal science for researchers.

All experiments were specifically designed to minimize the number of experimental animals used and to maximize the animal's well-being through continual inspection by the researchers, qualified caretakers, and the laboratory vet.

7. References

Aimone, J.B., Deng, W., Gage, F.H., 2011. Resolving new memories: a critical look at the dentate gyrus, adult neurogenesis, and pattern separation. Neuron 70, 589–596.

Barry, C., Ginzberg, L.L., O'Keefe, J., Burgess, N., 2012. Grid cell firing patterns signal environmental novelty by expansion. Proc. Natl. Acad. Sci. U. S. A. 109, 17687–17692.

Barry, C., Hayman, R., Burgess, N., Jeffery, K.J., 2007. Experience-dependent rescaling of entorhinal grids. Nat. Neurosci. 10, 682–684.

Beed, P., Gundlfinger, A., Schneiderbauer, S., Song, J., Böhm, C., Burgalossi, A., Brecht, M., Vida, I., Schmitz, D., 2013. Inhibitory gradient along the dorsoventral axis in the medial entorhinal cortex. Neuron 79, 1197–1207.

Belchior, H., Lopes-Dos-Santos, V., Tort, A.B.L., Ribeiro, S., 2014. Increase in hippocampal theta oscillations during spatial decision making. Hippocampus 24, 693–702.

Bizley, J.K., Cohen, Y.E., 2013. The what, where and how of auditory-object perception. Nat. Rev. Neurosci. 14, 693–707.

Bjerknes, T.L., Moser, E.I., Moser, M.-B., 2014. Representation of geometric borders in the developing rat. Neuron 82, 71–78.

Bliss, T.V., Lomo, T., 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J. Physiol. 232, 331–356.

Boccara, C.N., Sargolini, F., Thoresen, V.H., Solstad, T., Witter, M.P., Moser, E.I., Moser, M.-B., 2010. Grid cells in pre- and parasubiculum. Nat. Neurosci. 13, 987–994.

Bonnevie, T., Dunn, B., Fyhn, M., Hafting, T., Derdikman, D., Kubie, J.L., Roudi, Y., Moser, E.I., Moser, M.-B., 2013. Grid cells require excitatory drive from the hippocampus. Nat. Neurosci. 16, 309–317.

Brun, V.H., Leutgeb, S., Wu, H.-Q., Schwarcz, R., Witter, M.P., Moser, E.I., Moser, M.-B., 2008a. Impaired spatial representation in CA1 after lesion of direct input from entorhinal cortex. Neuron 57, 290–302.

Brun, V.H., Otnass, M.K., Molden, S., Steffenach, H.-A., Witter, M.P., Moser, M.-B., Moser, E.I., 2002. Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry. Science 296, 2243–2246.

Brun, V.H., Solstad, T., Kjelstrup, K.B., Fyhn, M., Witter, M.P., Moser, E.I., Moser, M.-B., 2008b. Progressive increase in grid scale from dorsal to ventral medial entorhinal cortex. Hippocampus 18, 1200–1212.

Burak, Y., Fiete, I.R., 2009. Accurate path integration in continuous attractor network models of grid cells. PLoS Comput. Biol. 5, e1000291.

Burgalossi, A., Herfst, L., von Heimendahl, M., Förste, H., Haskic, K., Schmidt, M., Brecht, M., 2011. Microcircuits of functionally identified neurons in the rat medial entorhinal cortex. Neuron 70, 773–786.

Burgess, N., 2008. Grid cells and theta as oscillatory interference: theory and predictions. Hippocampus 18, 1157–1174.

Burgess, N., Barry, C., O'Keefe, J., 2007. An oscillatory interference model of grid cell firing. Hippocampus 17, 801–812.

Cappaert, N.L.M., Van Strien, N.M., Witter, M.P., 2015. Chapter 20 - Hippocampal Formation A2 - Paxinos, George, in: The Rat Nervous System (Fourth Edition). Academic Press, San Diego, pp. 511–573.

Carpenter, F., Manson, D., Jeffery, K., Burgess, N., Barry, C., 2015. Grid cells form a global representation of connected environments. Curr. Biol. 25, 1176–1182.

Cheng, K., 1986. A purely geometric module in the rat’s spatial representation. Cognition 23, 149–178.

Cheng, S., Frank, L.M., 2011. The structure of networks that produce the transformation from grid cells to place cells. Neuroscience 197, 293–306.

Chrobak, J.J., Buzsáki, G., 1998. Gamma oscillations in the entorhinal cortex of the freely behaving rat. J. Neurosci. 18, 388–398.

Colgin, L.L., Leutgeb, S., Jezek, K., Leutgeb, J.K., Moser, E.I., McNaughton, B.L., Moser, M.-B., 2010. Attractor-map versus autoassociation based attractor dynamics in the hippocampal network. J. Neurophysiol. 104, 35–50.

Collin, S.H.P., Milivojevic, B., Doeller, C.F., 2015. Memory hierarchies map onto the hippocampal long axis in humans. Nat. Neurosci. 18, 1562–1564.

Couey, J.J., Witoelar, A., Zhang, S.-J., Zheng, K., Ye, J., Dunn, B., Czajkowski, R., Moser, M.-B., Moser, E.I., Roudi, Y., Witter, M.P., 2013. Recurrent inhibitory circuitry as a mechanism for grid formation. Nat. Neurosci. 16, 318–324.

Deng, W., Aimone, J.B., Gage, F.H., 2010. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat. Rev. Neurosci. 11, 339–350.

Derdikman, D., Whitlock, J.R., Tsao, A., Fyhn, M., Hafting, T., Moser, M.-B., Moser, E.I., 2009. Fragmentation of grid cell maps in a multicompartment environment. Nat. Neurosci. 12, 1325–1332.

Deshmukh, S.S., Knierim, J.J., 2011. Representation of non-spatial and spatial information in the lateral entorhinal cortex. Front. Behav. Neurosci. 5, 69.

Dolorfo, C.L., Amaral, D.G., 1998a. Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. J. Comp. Neurol. 398, 25–48.

Dolorfo, C.L., Amaral, D.G., 1998b. Entorhinal cortex of the rat: organization of intrinsic connections. J. Comp. Neurol. 398, 49–82.

Domnisoru, C., Kinkhabwala, A.A., Tank, D.W., 2013. Membrane potential dynamics of grid cells. Nature 495, 199–204.

Dong, H.-W., Swanson, L.W., Chen, L., Fanselow, M.S., Toga, A.W., 2009. Genomic-anatomic evidence for distinct functional domains in hippocampal field CA1. Proc. Natl. Acad. Sci. U. S. A. 106, 11794–11799.

Dupret, D., O’Neill, J., Pleydell-Bouverie, B., Csicsvari, J., 2010. The reorganization and reactivation of hippocampal maps predict spatial memory performance. Nat. Neurosci. 13, 995–1002.

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., Gage, F.H., 1998. Neurogenesis in the adult human hippocampus. Nat. Med. 4, 1313–1317.

Evensmoen, H.R., Ladstein, J., Hansen, T.I., Møller, J.A., Witter, M.P., Nadel, L., Håberg, A.K., 2015. From details to large scale: the representation of environmental positions follows a granularity gradient along the human hippocampal and entorhinal anterior-posterior axis. Hippocampus 25, 119–135.

Felleman, D.J., Van Essen, D.C., 1991. Distributed hierarchical processing in the primate cerebral cortex. Cereb. Cortex 1, 1–47.

Fuchs, E.C., Neitz, A., Pinna, R., Melzer, S., Caputi, A., Monyer, H., 2016. Local and Distant Input Controlling Excitation in Layer II of the Medial Entorhinal Cortex. Neuron 89, 194–208.

Fyhn, M., Hafting, T., Treves, A., Moser, M.-B., Moser, E.I., 2007. Hippocampal remapping and grid realignment in entorhinal cortex. Nature 446, 190–194.

Fyhn, M., Molden, S., Witter, M.P., Moser, E.I., Moser, M.-B., 2004. Spatial representation in the entorhinal cortex. Science 305, 1258–1264.

Gaarskjaer, F.B., 1978. Organization of the mossy fiber system of the rat studied in extended hippocampi. I. Terminal area related to number of granule and pyramidal cells. J. Comp. Neurol. 178, 49–72.

Gilbert, P.E., Kesner, R.P., Lee, I., 2001. Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1. Hippocampus 11, 626–636.

Giocomo, L.M., Stensola, T., Bonnevie, T., Van Cauter, T., Moser, M.-B., Moser, E.I., 2014. Topography of head direction cells in medial entorhinal cortex. Curr. Biol. 24, 252–262.

Giocomo, L.M., Zilli, E.A., Fransén, E., Hasselmo, M.E., 2007. Temporal frequency of subthreshold oscillations scales with entorhinal grid cell field spacing. Science 315, 1719–1722.

Hafting, T., Fyhn, M., Bonnevie, T., Moser, M.-B., Moser, E.I., 2008. Hippocampus-independent phase precession in entorhinal grid cells. Nature 453, 1248–1252.

Hafting, T., Fyhn, M., Molden, S., Moser, M.-B., Moser, E.I., 2005. Microstructure of a spatial map in the entorhinal cortex. Nature 436, 801–806.

Hardcastle, K., Ganguli, S., Giocomo, L.M., 2015. Environmental boundaries as an error correction mechanism for grid cells. Neuron 86, 827–839.

Hargreaves, E.L., Rao, G., Lee, I., Knierim, J.J., 2005. Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. Science 308, 1792–1794.

Hartzell, A.L., Burke, S.N., Hoang, L.T., Lister, J.P., Rodriguez, C.N., Barnes, C.A., 2013. Transcription of the immediate-early gene Arc in CA1 of the hippocampus reveals activity differences along the proximodistal axis that are attenuated by advanced age. J. Neurosci. 33, 3424–3433.

Hebb, D.O., 1949. The Organization of Behavior. Wiley & Sons, New York.

Henriksen, E.J., Colgin, L.L., Barnes, C.A., Witter, M.P., Moser, M.-B., Moser, E.I., 2010. Spatial representation along the proximodistal axis of CA1. Neuron 68, 127–137.

Hinman, J.R., Penley, S.C., Long, L.L., Escabí, M.A., Chrobak, J.J., 2011. Septotemporal variation in dynamics of theta: speed and habituation. J. Neurophysiol. 105, 2675–2686.

Hunsaker, M.R., Kesner, R.P., 2008. Dissociations across the dorsal-ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear. Neurobiol. Learn. Mem. 89, 61–69.

Igarashi, K.M., Lu, L., Colgin, L.L., Moser, M.-B., Moser, E.I., 2014. Coordination of entorhinal-hippocampal ensemble activity during associative learning. Nature 510, 143–147.

Ito, H.T., Schuman, E.M., 2012. Functional division of hippocampal area CA1 via modulatory gating of entorhinal cortical inputs. Hippocampus 22, 372–387.

Jeffery, K.J., Anderson, M.I., 2003. Dissociation of the geometric and contextual influences on place cells. Hippocampus 13, 868–872.

Kaas, J.H., 1997. Topographic maps are fundamental to sensory processing. Brain Res. Bull. 44, 107–112.

Kjelstrup, K.B., Solstad, T., Brun, V.H., Hafting, T., Leutgeb, S., Witter, M.P., Moser, E.I., Moser, M.-B., 2008. Finite scale of spatial representation in the hippocampus. Science 321, 140–143.

Kjelstrup, K.G., Tuvnes, F.A., Steffenach, H.-A., Murison, R., Moser, E.I., Moser, M.-B., 2002. Reduced fear expression after lesions of the ventral hippocampus. Proc. Natl. Acad. Sci. U. S. A. 99, 10825–10830.

Knierim, J.J., Lee, I., Hargreaves, E.L., 2006. Hippocampal place cells: parallel input streams, subregional processing, and implications for episodic memory. Hippocampus 16, 755–764.

Knierim, J.J., Neunuebel, J.P., 2015. Tracking the flow of hippocampal computation: Pattern separation, pattern completion, and attractor dynamics. Neurobiol. Learn. Mem. doi:10.1016/j.nlm.2015.10.008

Komorowski, R.W., Garcia, C.G., Wilson, A., Hattori, S., Howard, M.W., Eichenbaum, H., 2013. Ventral hippocampal neurons are shaped by experience to represent behaviorally relevant contexts. J. Neurosci. 33, 8079–8087.

Kropff, E., Carmichael, J.E., Moser, M.-B., Moser, E.I., 2015. Speed cells in the medial entorhinal cortex. Nature 523, 419–424.

Krupic, J., Bauza, M., Burton, S., Barry, C., O'Keefe, J., 2015. Grid cell symmetry is shaped by environmental geometry. Nature 518, 232–235.

Langston, R.F., Ainge, J.A., Couey, J.J., Canto, C.B., Bjerknes, T.L., Witter, M.P., Moser, E.I., Moser, M.-B., 2010. Development of the spatial representation system in the rat. Science 328, 1576–1580.

Lee, I., Yoganarasimha, D., Rao, G., Knierim, J.J., 2004. Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. Nature 430, 456–459.

Leutgeb, J.K., Leutgeb, S., Moser, M.-B., Moser, E.I., 2007. Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science 315, 961–966.

Leutgeb, S., Leutgeb, J.K., Barnes, C.A., Moser, E.I., McNaughton, B.L., Moser, M.-B., 2005. Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. Science 309, 619–623.

Leutgeb, S., Leutgeb, J.K., Treves, A., Moser, M.-B., Moser, E.I., 2004. Distinct ensemble codes in hippocampal areas CA3 and CA1. Science 305, 1295–1298.

Lubenov, E.V., Siapas, A.G., 2009. Hippocampal theta oscillations are travelling waves. Nature 459, 534–539.

Lu, L., Leutgeb, J.K., Tsao, A., Henriksen, E.J., Leutgeb, S., Barnes, C.A., Witter, M.P., Moser, M.-B., Moser, E.I., 2013. Impaired hippocampal rate coding after lesions of the lateral entorhinal cortex. Nat. Neurosci. 16, 1085–1093.

Marozzi, E., Ginzberg, L.L., Alenda, A., Jeffery, K.J., 2015. Purely Translational Realignment in Grid Cell Firing Patterns Following Nonmetric Context Change. Cereb. Cortex 25, 4619–4627.

Mathis, A., Herz, A.V.M., Stemmler, M., 2012. Optimal population codes for space: grid cells outperform place cells. Neural Comput. 24, 2280–2317.

McNaughton, B.L., Battaglia, F.P., Jensen, O., Moser, E.I., Moser, M.-B., 2006. Path integration and the neural basis of the “cognitive map.” Nat. Rev. Neurosci. 7, 663–678.

Miao, C., Cao, Q., Ito, H.T., Yamahachi, H., Witter, M.P., Moser, M.-B., Moser, E.I., 2015. Hippocampal Remapping after Partial Inactivation of the Medial Entorhinal Cortex. Neuron 88, 590–603.

Monaco, J., Abbott, L.F., 2011. Modular realignment of entorhinal grid cell activity as a basis for hippocampal remapping. J. Neurosci. 31, 9414–9425.

Mormann, F., Osterhage, H., Andrzejak, R.G., Weber, B., Fernández, G., Fell, J., Elger, C.E., Lehnertz, K., 2008. Independent delta/theta rhythms in the human hippocampus and entorhinal cortex. Front. Hum. Neurosci. 2, 3.

Moser, E.I., Roudi, Y., Witter, M.P., Kentros, C., Bonhoeffer, T., Moser, M.-B., 2014. Grid cells and cortical representation. Nat. Rev. Neurosci. 15, 466–481.

Moser, M.B., Moser, E.I., Forrest, E., Andersen, P., Morris, R.G., 1995. Spatial learning with a minislab in the dorsal hippocampus. Proc. Natl. Acad. Sci. U. S. A. 92, 9697–9701.

Muessig, L., Hauser, J., Wills, T.J., Cacucci, F., 2015. A Developmental Switch in Place Cell Accuracy Coincides with Grid Cell Maturation. Neuron 86, 1167–1173.

Muller, R.U., Kubie, J.L., 1987. The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J. Neurosci. 7, 1951–1968.

Nakamura, H., Gattass, R., Desimone, R., Ungerleider, L.G., 1993. The modular organization of projections from areas V1 and V2 to areas V4 and TEO in macaques. J. Neurosci. 13, 3681–3691.

Navratilova, Z., Giocomo, L.M., Fellous, J.-M., Hasselmo, M.E., McNaughton, B.L., 2012. Phase precession and variable spatial scaling in a periodic attractor map model of medial entorhinal grid cells with realistic after-spike dynamics. Hippocampus 22, 772–789.

Neunuebel, J.P., Knierim, J.J., 2014. CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. Neuron 81, 416–427.

Neunuebel, J.P., Yoganarasimha, D., Rao, G., Knierim, J.J., 2013. Conflicts between local and global spatial frameworks dissociate neural representations of the lateral and medial entorhinal cortex. J. Neurosci. 33, 9246–9258.

O’Keefe, J., 1976. Place units in the hippocampus of the freely moving rat. Exp. Neurol. 51, 78–109.

O’Keefe, J., Burgess, N., 1996. Geometric determinants of the place fields of hippocampal neurons. Nature 381, 425–428.

O’Keefe, J., Dostrovsky, J., 1971. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res. 34, 171–175.

O’Keefe, J., Nadel, L., 1978. The Hippocampus as a Cognitive Map. Oxford University Press.

O’Keefe, J., Recce, M.L., 1993. Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus 3, 317–330.

Park, E., Dvorak, D., Fenton, A.A., 2011. Ensemble place codes in hippocampus: CA1, CA3, and dentate gyrus place cells have multiple place fields in large environments. PLoS One 6, e22349.

Patel, J., Fujisawa, S., Berényi, A., Royer, S., Buzsáki, G., 2012. Traveling theta waves along the entire septotemporal axis of the hippocampus. Neuron 75, 410–417.

Penfield, W., Boldrey, E., 1937. SOMATIC MOTOR AND SENSORY REPRESENTATION IN THE CEREBRAL CORTEX OF MAN AS STUDIED BY ELECTRICAL STIMULATION. Brain 60, 389–443.

Penley, S.C., Hinman, J.R., Long, L.L., Markus, E.J., Escabí, M.A., Chrobak, J.J., 2013. Novel space alters theta and gamma synchrony across the longitudinal axis of the hippocampus. Front. Syst. Neurosci. 7, 20.

Poppenk, J., Evensmoen, H.R., Moscovitch, M., Nadel, L., 2013. Long-axis specialization of the human hippocampus. Trends Cogn. Sci. 17, 230–240.

Ramsden, H.L., Sürmeli, G., McDonagh, S.G., Nolan, M.F., 2015. Laminar and dorsoventral molecular organization of the medial entorhinal cortex revealed by large-scale anatomical analysis of gene expression. PLoS Comput. Biol. 11, e1004032.

Reifenstein, E.T., Kempter, R., Schreiber, S., Stemmler, M.B., Herz, A.V.M., 2012. Grid cells in rat entorhinal cortex encode physical space with independent firing fields and phase precession at the single-trial level. Proc. Natl. Acad. Sci. U. S. A. 109, 6301–6306.

Rolls, E.T., 2015. Pattern separation, completion, and categorisation in the hippocampus and neocortex. Neurobiol. Learn. Mem. doi:10.1016/j.nlm.2015.07.008

Rolls, E.T., 2007. An attractor network in the hippocampus: theory and neurophysiology. Learn. Mem. 14, 714–731.

Samsonovich, A., McNaughton, B.L., 1997. Path integration and cognitive mapping in a continuous attractor neural network model. J. Neurosci. 17, 5900–5920.

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B.L., Witter, M.P., Moser, M.-B., Moser, E.I., 2006. Conjunctive representation of position, direction, and velocity in entorhinal cortex. Science 312, 758–762.

Schmidt-Kastner, R., 2015. Genomic approach to selective vulnerability of the hippocampus in brain ischemia-hypoxia. Neuroscience 309, 259–279.

Scoville, W.B., Milner, B., 1957. Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry 20, 11–21.

Simic, G., Bexheti, S., Kelovic, Z., Kos, M., Grbic, K., Hof, P.R., Kostovic, I., 2005. Hemispheric asymmetry, modular variability and age-related changes in the human entorhinal cortex. Neuroscience 130, 911–925.

Skaggs, W.E., McNaughton, B.L., Wilson, M.A., Barnes, C.A., 1996. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. Hippocampus 6, 149–172.

Solstad, T., Boccara, C.N., Kropff, E., Moser, M.-B., Moser, E.I., 2008. Representation of geometric borders in the entorhinal cortex. Science 322, 1865–1868.

Solstad, T., Moser, E.I., Einevoll, G.T., 2006. From grid cells to place cells: a mathematical model. Hippocampus 16, 1026–1031.

Stemmler, M., Mathis, A., Herz, A.V.M., 2015. Connecting multiple spatial scales to decode the population activity of grid cells. Sci Adv 1, e1500816.

Stensola, H., Stensola, T., Solstad, T., Frøland, K., Moser, M.-B., Moser, E.I., 2012. The entorhinal grid map is discretized. Nature 492, 72–78.

Stensola, T., Stensola, H., Moser, M.-B., Moser, E.I., 2015. Shearing-induced asymmetry in entorhinal grid cells. Nature 518, 207–212.

Strange, B.A., Witter, M.P., Lein, E.S., Moser, E.I., 2014. Functional organization of the hippocampal longitudinal axis. Nat. Rev. Neurosci. 15, 655–669.

Summerfield, C., Christopher, S., de Lange, F.P., 2014. Expectation in perceptual decision making: neural and computational mechanisms. Nat. Rev. Neurosci. 15, 816–816.

Taube, J.S., 2007. The head direction signal: origins and sensory-motor integration. Annu. Rev. Neurosci. 30, 181–207.

Thompson, C.L., Pathak, S.D., Jeromin, A., Ng, L.L., MacPherson, C.R., Mortrud, M.T., Cusick, A., Riley, Z.L., Sunkin, S.M., Bernard, A., Puchalski, R.B., Gage, F.H., Jones, A.R., Bajic, V.B., Hawrylycz, M.J., Lein, E.S., 2008. Genomic anatomy of the hippocampus. Neuron 60, 1010–1021.

Tolman, E.C., 1948. Cognitive maps in rats and men. Psychol. Rev. 55, 189–208.

Towse, B.W., Barry, C., Bush, D., Burgess, N., 2014. Optimal configurations of spatial scale for grid cell firing under noise and uncertainty. Philos. Trans. R. Soc. Lond. B Biol. Sci. 369, 20130290.

Tsao, A., Moser, M.-B., Moser, E.I., 2013. Traces of experience in the lateral entorhinal cortex. Curr. Biol. 23, 399–405.

Ungerleider, L.G., Mishkin, M., 1982. Two cortical visual systems, in: Ingle, D.J., Goodale, M.A., Mansfield, R.J.W. (Eds.), Analysis of Visual Behavior. MIT Press, Cambridge, MA, pp. 549–586.

Wang, Q., Gao, E., Burkhalter, A., 2011. Gateways of ventral and dorsal streams in mouse visual cortex. J. Neurosci. 31, 1905–1918.

Wang, S.-H., Morris, R.G.M., 2010. Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. Annu. Rev. Psychol. 61, 49–79, C1–4.

Wang, Y., Romani, S., Lustig, B., Leonardo, A., Pastalkova, E., 2015. Theta sequences are essential for internally generated hippocampal firing fields. Nat. Neurosci. 18, 282–288.

Wills, T.J., Cacucci, F., Burgess, N., O'Keefe, J., 2010. Development of the hippocampal cognitive map in preweanling rats. Science 328, 1573–1576.

Wilson, H.R., Wilkinson, F., 2015. From orientations to objects: Configural processing in the ventral stream. J. Vis. 15, 4.

Wood, E.R., Dudchenko, P.A., Robitsek, R.J., Eichenbaum, H., 2000. Hippocampal neurons encode information about different types of memory episodes occurring in the same location. Neuron 27, 623–633.

Woollett, K., Maguire, E.A., 2011. Acquiring “the Knowledge” of London's layout drives structural brain changes. Curr. Biol. 21, 2109–2114.

Yoon, K., Buice, M.A., Barry, C., Hayman, R., Burgess, N., Fiete, I.R., 2013. Specific evidence of low-dimensional continuous attractor dynamics in grid cells. Nat. Neurosci. 16, 1077–1084.

Zhang, S.-J., Ye, J., Miao, C., Tsao, A., Cerniauskas, I., Ledergerber, D., Moser, M.-B., Moser, E.I., 2013. Optogenetic dissection of entorhinal-hippocampal functional connectivity. Science 340, 1232627.

8. Contributions (Papers I-II)

Paper I

Is not included due to copyright

Paper II

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