FUNCTIONAL DIFFERENTIATION ALONG THE DORSO-VENTRAL AXIS OF THE HIPPOCAMPUS

Alessia Manganaro

Thesis submitted for the degree of
Doctor of Philosophy at the University of Oxford

Linacre College
MRC Unit of Anatomical Neuropharmacology
University of Oxford
Michaelmas 2013

Abstract

FUNCTIONAL DIFFERENTIATION ALONG THE DORSO-VENTRAL AXIS OF THE HIPPOCAMPUS

Alessia Manganaro

Thesis submitted for the degree of Doctor of Philosophy
Linacre College, University of Oxford
Michaelmas 2013

The hippocampus plays an important role in the processing of spatial memory. During exploration, theta oscillations (4-12 Hz) are prominent in the hippocampus, whereas during sleep and rest irregular sharp wave/ripple (SWR) events occur spontaneously in the hippocampus and may support memory consolidation. To date, the ventral sub-region of the rodents hippocampus, has received less attention relative to the more accessible dorsal part. It has been suggested that spatial information decreases along the septo-temporal axis in favour of coding salient features and coordinated oscillatory activity might enable the binding of spatial and nonspatial information. The first goal of my research was to investigate how the spatial representation by dorsal and ventral neurons is organised by theta oscillations in the hippocampal network. The second goal was to investigate the role of the ventral hippocampus in spatial learning. Finally, the third goal examined to what extent the firing relationships established during spatial learning are replayed during subsequent sleep in the ventral CA1.

I recorded the network activity of dorsal and ventral CA1 in rats performing a spatial memory task on the cheese board maze (Dupret et al., 2010). By using parallel multi-channel extracellular recordings in the dorsal and ventral portions of the hippocampus in behaving rats, I found that dorsal and ventral CA1 were theta coupled at particular times of the spatial learning. High coherence periods across the two regions were characterized by a strong speed-modulation of ventral theta oscillations, which was absent in other conditions. During sleep, it was found that SWR-related activity was presented in the ventral hippocampus as well, when the coordinated population activity established in spatial learning was reactivated within the two sub-regions. By contrast, reactivation across the two regions was observed outside the SWRs epochs. Overall, the data suggests that the ventral hippocampus might be involved in the processing of salient features of the environment such as rewards. On a temporal scale, this non-spatial information might be integrated to the spatial information provided by the dorsal hippocampus during theta oscillation. During sleep/rest periods, the coordinated communication of learned information might underlie the consolidation of memory traces.

Table of Contents

List of Figures and tables

Glossary

Ach	Acetyl Choline
BLA	Basolateral amygdala
CA	Cornu Ammonis or Ammons's Horn, comprising the CA1, CA2 and CA3 sub-regions of the hippocampus
CSD	Current source density
DG	Dentate Gyrus
EC	Entorhinal Cortex
EPM	Elevated plus maze
F	Fornix
eSWR	Sharp wave associated ripple patterns that occurs nested within, or near to theta oscillations during exploration
sSWR	Sharp wave associated ripple patterns that occurs during slow-wave sleep
GABA	Gamma-Amino-Butyric-Acid
GAD	Glutamate decarboxylase
HF	Hippocampal formation
HPA	Hypothalamic-pituitary-adrenal axis
Int	Interneuron
LEC	Lateral Entorhinal cortex
LFP	Local field potential
LM	Lacunosum Moleculare
LTD	Long term Depression
LTP	Long term Potentiation
MEC	Medial Entorhinal Cortex
mPFC	Medial prefrontal cortex
MS/DBB	Medial septum / dorsal band of Broca
MTL	Medial Temporal Lobes
NAs	Nucleus accumbens
NMDA	N-methyl-D-aspartate
PER	Perirhinal cortex
PFC	Prefrontal cortex
PHR	Parahippocampal region
POR	Postrhinal cortex
PP	Perforant path
Pyr	Pyramidal cell
SO	Stratum Oriens
SR	Stratum Radiatum
REM	Rapid Eye Movement Sleep
RN	Raphe nucleus

STDP	Spike Timing Dependent Plasticity
SUB	Subiculum
SWR	Sharp Wave / Ripple network event
VTA	Ventral Tegmental Area

Chapter I
General Introduction

Introduction

In humans, the hippocampus is a paired structure located within the medial temporal lobe system (MTL), corresponding to the medio-caudal part of the rodents brain. Traditionally listed as one of the major components of the limbic system, this complex archicortex is part of the large network connecting unimodal and polymodal associational areas including the parietal, temporal and frontal cortices. The hippocampus is highly reciprocally connected to the other mesolimbic structures, so it is not surprising it has been associated with a variety of high cognitive functions.

During the last century, two prominent theories have emerged about the main function of this brain structure. The first concerns the involvement of the hippocampus in the process of learning and memory. Memory can be defined as a complex cognitive function that allows the brain to acquire information about the internal as well as external environment, store it, and later, use it during appropriate conditions. The three steps are usually referred as encoding, consolidation, and recall of memory traces. Different types of memories can be defined based on the content and the process of acquiring. A first distinction is between explicit (declarative) and implicit (procedural or non-declarative) memories. Declarative memory can be divided into episodic memory and semantic memory, referring, respectively, to the storing of experienced events and to the knowledge of facts about things. These are distinct from implicit procedural memory for learned skills, in

that the former require the association between the spatio-temporal perception of context and the semantic contents of an event. The hippocampus has been suggested to be involved in this process of association. From an historic perspective, in the 1950s lesion studies in humans found that damage to the MTL lead to profound deficits in the ability to store new memories. Famous was the case of the patient H.M. (Henry Molaison, as revealed after his death), who lost the ability to form new memories after a bilateral surgical removal of a large portion of the MTL substantially damaged the hippocampus (Scoville and Milner, 1957). The description of the deficits Mr. Molaison reported comes to a hand to elucidate the complexity of the memory system and how this cognitive function is strictly related to learning processes. H.M. suffered from retrograde amnesia, essentially he lost his past memories dating back to 11 years before the surgery and he was also not able to retain any kind of new information for more than several minutes, showing impairment in what we would call short-memory processes. Remarkably, he was still able to learn new motor skills but the memory of its actions remained at a subconscious level. The definitive proof of the hippocampus' involvement came with the case of patient R.B. in the 1980s, who suffered from memory impairment following bilateral ischemic lesions circumscribed to the CA1 region of the hippocampus. This CA1 field can be considered the last stage of processing in this brain region, directing outputs to cortical regions as well as sub-cortical ones. The existence of a "multiple memory system" had been intuited by philosophers and

psychologists before, but the work with patients such as H.M. by Milner and colleagues settled the principles about the organization of memory and its classical definition formulated by Tulving and Squire.

In parallel, strong evidence has been collected in rodents to support the participation of the hippocampal network in memory processes related to spatial representation. Fundamental to this evidence, was the discovery that a subset of hippocampal pyramidal cells preferentially fire in a portion of the environment that an animal is exploring (O'Keefe and Dostrovsky, 1971). The existence of such "place cells" together with the spatial memory deficits reported by lesion studies (Morris et al., 1982) lead to the proposed theory of an internal "cognitive map" essential for memory-based navigation (Tolman, 1948; O'Keefe and Nadel, 1978). Therefore, information processed in the hippocampal memory system would enable animals to reach salient locations based on past experience. Consequently, it was proposed that spatial memory could be considered the equivalent of episodic memory in humans, comprising of a trace of the complex configuration of elements learnt and stored after experiencing the environment (see for theoretical overview Redish, 2000; Buzsaki and Moser, 2013).

Although those findings suggested the primary role of the hippocampus in the cognitive processing of spatial information, the hippocampus has also been implicated in the regulation of behavioural responses driven by emotional and motivational states. Specifically, different experimental approaches have since

confirmed its role in anxiety and reward-related processing. In relation to anxiety, the hippocampus is known to act on the hypothalamic-pituitary-adrenal (HPA) axis which controls the hormonal cascades related to the arousal-anxiety state and associated behaviours, indirectly contrasted by the amygdala (Bear et al., 2002). Indeed, the hippocampus contains numerous receptors that respond to glucocorticoids, such as cortisol, released by the adrenal glands in response to stress; so normally participates in a feedback loop, inhibiting eventually the release of additional hormone. In addition, the hippocampus is also strongly connected to the striatal and frontal cortical structures that constitute the reward circuitry. The presence of reward can modulate the firing of place cells (Breese et al., 1989; Hok et al., 2007), according to the idea that the hippocampus sub-serves the conjunction of non-spatial behavioural-relevant information with the spatio-temporal context (Young et al., 2004).

How the same brain circuits might operate in what appears to be the distinct processing of information is a compelling question in neuroscience. So far, a large number of the studies have focused on one of the two proposed functions of the hippocampus, revealing distinct key elements within the computations taking place in this brain area.

Recently, some attempts have been made to reconcile the data provided by anatomical and lesion studies, as well as physiological and behavioural ones. Intriguingly, it has been recently hypothesised that a functional differentiation exists

along the long axis of the hippocampus (dorso-ventral and antero-caudal axis, respectively, in rodents and humans). Indeed, certain heterogeneity of function has emerged at different levels of the hippocampus by looking at the expression profiles of genes and molecules and the anatomical connectivity patterns (Fanselow and Dong, 2010). In rodents, dorsal hippocampal but not ventral lesions impair spatial memory in spatial task such as the watermaze, T-maze and radial arm maze (Moser et al., 1995; Hock & Bunsey, 1998; Bannerman et al., 1999; Pothuizen et al., 2004). Critically, the analysis of the efferents indicates that the hippocampus can modulate emotional behavioural responses and reward circuitry mainly through projections from the ventral pole to the nucleus accumbens, amygdala, and the HPA axis (Sahay and Hen, 2007). Although the dorsal sub-region has been also associated with anxiety disorders (Padovan et al., 2000), the most up-to-date literature argues in favour of greater involvement of the ventral hippocampus. In agreement, selective lesions of the ventral pole result in a reduction of the anxiety related behaviours in rats and a close relationship between anxiety-related behaviours and the release of serotonin in the sub-region has also been observed (Bannerman et al., 2004; Rex et al., 2005).

As a result, many authors have proposed that distinct functions can be assigned to specific hippocampal sub-regions, with the dorsal hippocampal region preferentially involved in memory and spatial learning, and the ventral region

regulating emotional and motivated behaviours (Alves et al, 2004; Bannerman et al., 2004; Rex et al., 2005).

However, the ventral hippocampus might still play a role in spatial learning by providing emotion and reward context information. The question can be also formulated in terms of how the dorsal and ventral hippocampal neuronal populations are selected during learning to encode a long-term stable behavioural program when appropriate cues are presented. In this perspective, the organisation of communication between the two poles of the hippocampus may provide an insight into understanding the mechanisms underlying the acquisition, long-term storage, and reinstatement of neural memory programs for adapting behaviour in this brain region. In this thesis I aimed to investigate the representation of space in the dorsal and ventral CA1 sub-fields of the hippocampus, asking:

1. How is communication between the septal and temporal CA1 sub-regions organised by oscillatory mechanisms?

2. How is spatial information encoded in the hippocampal CA1 in the context of goal-directed behaviour?

3. Are waking patterns reactivated during sleep in the ventral hippocampus as well as in the dorsal hippocampus?

The present chapter will review neuroanatomical, physiological and behavioural evidence to support the existence of a dorso-ventral dissociation within the rat hippocampus. For the purpose of this thesis, I will focus on the spatial

correlates of hippocampal activity during encoding consolidation and retrieval of memory traces. The firing patterns of hippocampal principal cells will be reviewed, along with the network patterns orchestrating the activity of neuronal population within and across brain regions. Evidence on the involvement of the hippocampus in anxiety and the processing of reward-related information will be reported.

1.1 Anatomy of the hippocampal formation

In rodents, the hippocampal formation emerges at the posterior end of the septal nuclei and curves both ventrally and laterally over the thalamus and into the temporal lobe, the long axis being referred to as the septo-temporal axis. It comprises of the subiculum (SUB) and the hippocampus itself. Here the term 'hippocampus' is used to refer to Ammon's horn (Cornu Ammonis, including CA1, CA2 and CA3) and the dentate gyrus (DG). Together with the neighbouring entorhinal cortex (EC), the presubiculum, parasubiculum, and the perirhinal (PER) and postrhinal cortex (POR) constitutes the parahippocampal region (PHR) (Amaral and Witter 2004, van Strien et al. 2009).

1.1.1 The laminar structure of the hippocampus

The hippocampus is an archicortex structured in three layers. In the DG, the three layers comprise the molecular layer, where granule cell dendrites are located, the granule cell body layer or stratum granulosum, and the hilus, also termed the polymorphic layer, with excitatory mossy cells as well as a heterogeneous population of inhibitory interneurons. In the CA region, we recognize the stratum oriens, pyramidale and the radiatum/moleculare. The pyramidal layer contains most of the body of the pyramidal cells, which extend their basal dendrites to the inferior stratum oriens, while the apical dendrites are contained in the superficial strata radiatum/lacunosum moleculare. Below the stratum oriens, the hippocampus is

covered by the ‘alveus’ containing fibres projecting to retro-hippocampal structures, subcortical locations, and to the contralateral hippocampal formation.

1.1.2 Cortical and intrinsic connectivity of the hippocampal formation

The entorhinal cortex is the main source of cortical input to the hippocampus. It is usually divided into the medial EC (MEC) and lateral EC (LEC). Three bands can also be distinguished by transversal sectioning of the MEC/LEC in the dorsolateral, intermediate, and ventromedial bands. The perforant path (PP) begins in the layer II and III of the EC, the lateral perforant path from the LEC whilst the medial perforant path from the MEC (Fig. 1), and synapses amongst the granular cells of the DG (Bear et al., 2002), mostly ipsilaterally. Projections from EC layer II terminate differentially in dendritic regions of DG and CA3, overlapping throughout the entire extent of DG and CA3. Projections from EC layer III instead terminate in deep layers of CA1 and SUB and the pattern of their terminations are distinct compared to that seen in EC layer II inputs. The input from the medial PP terminates in the proximal part of CA1, the closest to CA3, and in the distal part of SUB, furthest from the border with CA1. The lateral PP input reaches, on the other hand, the distal part of CA1 and proximal SUB (Witter, 1993). The inputs from EC to DG, and then from DG to CA3, are homogenous (Witter, 1993). Each proximo-distal portion of CA3 receives input from the entire extent of DG, lateral and medial EC. The hippocampal formation output to EC originates from CA1 and SUB and mirrors the connectivity

received via the PP inputs, terminating in the deep layers V and VI (Tamamaki and Nojyo, 1995; Witter, 1993) (Fig. 1).

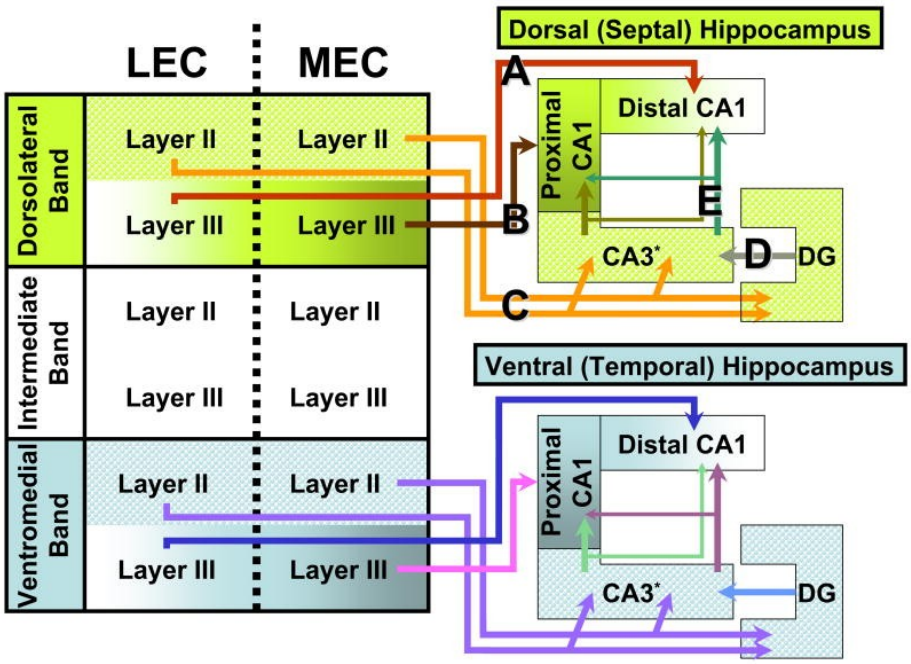


Figure 1. Distinct parts of the EC project to different level of the septo-temporal axis of the hippocampus

Schematic representation of the organization of the entorhinal-hippocampal connectivity. Indicated are the dorsoventral extent of the hippocampus, DG, CA1, CA3 areas and the projections from layer II III of LEC and MEC. The topographical arrangement of entorhinal-hippocampal reciprocal connections show that the dorsolateral band of entorhinal cortex (green) is preferentially connected to the dorsal hippocampus. Increasingly, more ventral and medial bands of entorhinal cortex (white and blus) are connected to increasingly more ventral levels of the hippocampus. Adapted from Ahmed and Mehta, 2009

Main EC inputs are complemented by intra-hippocampal connectivity specific for each hippocampal field. Generally, it is described by a unidirectional trisynaptic loop, in which the perforant path from the EC projects to the DG (and CA3), which afferents terminate in CA3, then CA3 pyramidal cells project to CA1 from where output is targeted back to the EC.

Prominent intrinsic connections include collateral fibres from granule cells to mossy cells in the hilus (Amaral and Witter, 1989). These excitatory cells (mossy cells) project to other granule cells contra and ipsilaterally for the whole extent of the septo-temporal axis, thus creating a loop of synaptic contact. One granule cell axon can innervate as much as 75% of the long axis of the DG, forming a polysynaptic associative network (Amaral and Witter, 1989). Mossy fibre axons also reach CA3, forming large synapses close to the somata of pyramidal cells (Henze et al., 2002). Additionally, axons of CA3 pyramidal cells directly innervate other CA3 cells both ipsilaterally and contra-laterally. This recurrent CA3 network resulted in a greater number of inputs from CA3 itself than from DG and EC projections combined. Approximately 12,000 CA3 synapses per pyramidal cell compared to 4,000 from the EC and 50 from mossy fibres were calculated, with each CA3 cell connected to around 2% of the others (Macvicar and Dudek, 1980; Miles and Wong, 1986).

In contrast, the CA1 region has few recurrent collaterals (Vangroen and Wyss, 1990). The Schaffer collaterals (axons) of CA3 represent numerically the largest input to CA1, more than the direct PP projection from layer III of the EC (Witter, 1993).

Evidence suggested that inputs from subcortical structures and network states could modulate the strength of these intrinsic inputs (see below).

1.1.3 The generation of spatial signal in the hippocampal system and the function of CA1

The formation of the hippocampal spatial map presumably occurs in the connections between DG and CA3, with the highest spatial specificity in the regions CA3. The connectivity between CA3 and CA1 shows topographical mapping along the transverse extent of the hippocampus: CA3 neurons closest to the DG hilus (proximal CA3) synapse with CA1 pyramids via the stratum radiatum, conversely distal CA3 closest to the CA1 region project to proximal CA1 via the stratum oriens (Ishizuka et al., 1990). Medial portions (in the transverse plane) of CA3 project to medial CA1. This separation of projections from the CA3 region maps to different populations of cells: CA3 neurons nearest the CA1 tend to project to the closest CA1 neurons, and CA3 neurons farthest from CA1 region tend to project to CA1 cells close to the subiculum, and neighbouring cells in the CA3 region project to neighbouring cells in the CA1 region (Brivanlou et al., 2004). It seems likely that each of these CA3–CA1 loops has access to a complete representation of space due to the overlapping inputs from DG and EC (see below) across the entire extent of CA3. Adjacent regions of CA1 and SUB are interconnected (distal CA1 and proximal SUB), as are the part of CA1 closest to CA3 (proximal CA1) and that part of SUB furthest from CA1 (distal SUB). The ‘middle’ third parts of CA1 and SUB are also interconnected. The

projections to EC from distal CA1–proximal SUB, proximal and CA1–distal SUB reflect the topology of the EC regions providing PP inputs (Tamamaki and Nojyo, 1995). Dorsal and ventral SUB as well as proximal and distal parts of the SUB target different areas (Witter, 2006).

Presumably, each of the described routes might convey information of a different nature or in different ways. CA1 and SUB are also innervated by the perirhinal and postrhinal cortices. The PER appears to be involved preferentially in object perception, so the related anatomical loop may serve in relaying this type of information through hippocampus and SUB; on the other hand, the POR seems to provide egocentric spatial or topographical information via strong connections with parietal cortex, as supported by lesion studies (Gaffan et al., 2004). The inputs from PER and POR do overlap at the level of DG/CA3 of the hippocampus, via LEC and MEC projections, respectively. However, the inputs to the downstream CA1 and SUB result segregated (Naber et al., 2000; Witter et al., 2000). The SUB projects subcortically to the anterior thalamus, nucleus accumbens and medial mammillary body in a rather laminar way. An extra feed-forward circuitry has been reported between EC, CA1 and SUB (Naber et al., 2001; Kloosterman et al., 2004), that appears to be a sort of feedback from proximal SUB to distal CA1 (Commins et al., 2002).

Different observations suggests that the CA1 input may be important for the formation of cell assemblies within SUB with perhaps the EC input acting to aid in

their selection. Distinct cell types might be involved in different functional loops. This raises a question regarding the function of CA1 (Gigg, 2006). A reasonably good spatial signal is provided to CA1 from EC and information regarding recall may be provided by CA3 (Brun et al., 2002; Nakazawa et al., 2002). Perhaps the function of CA1 is to integrate spatial, contextual (Hollup et al., 2001; Vazdarjanova and Guzowski, 2004) and mnemonic information and then pass this on to SUB, the principal hippocampal output, in a segregated manner for further processing and distribution. Differential inputs from PER and POR would then 'bias' these outputs to favour details regarding either the composition or spatial aspects of the environment, respectively. Recent evidence regarding the spatial signal from different parts of the EC supports this 'biasing'; medial EC provides a stronger spatial signal compared to that from lateral EC (Hargreaves et al., 2005). These data support the notion that the CA1-SUB loop that incorporates POR and medial EC is biased towards a more spatial representation of the environment when compared to that involving PER and lateral EC.

1.1.4 Subcortical input to the hippocampal system

The hippocampus receives non-cortical input from the amygdala, basal forebrain, basal ganglia, brainstem, thalamus and hypothalamus, most of which are likely to impose modulatory functional effects. For example, brainstem inputs include serotonergic projections from the raphe nucleus (to the hippocampus) and dopaminergic inputs from the ventral tegmental area (VTA) to the hippocampus and

EC. These monoamine neurotransmitters can modulate excitatory transmission between regions within the hippocampo-entorhinal system through pre- and post-synaptic mechanisms. In the CA1 region, these monoamines tend to reduce transmission from direct EC inputs (Otmakhova and Lisman, 1999; Otmakhova and Lisman, 2000; Otmakhova et al., 2005).

Basal forebrain structures, particularly the medial septum and the associated diagonal band of Broca (MS/DBB), project cholinergic and GABAergic fibres to the hippocampus and EC. This pathway is thought to be especially important in establishing the theta rhythmicity in behaving animals (see below). Both brainstem and basal forebrain inputs may fluctuate over the sleep waking cycle (Marrosu et al., 1995) and following exposure to novelty (Ihalainen et al., 1999), contributing to the flow of excitation through the hippocampal loop in different behavioural states (Hasselmo, 1999; Lisman, 2001; Lisman and Grace, 2005). Finally, these neuromodulators have been shown to influence synaptic plasticity within the hippocampus (Auerbach and Segal, 1994; Otmakhova and Lisman, 1998; Matsuyama et al., 2000; Lemon and Manahan-Vaughan, 2006).

1.1.5 Neuromodulatory inputs to the hippocampal formation along the dorso-ventral axis

The DG receives a prominent noradrenergic input arising from the locus coeruleus (LC). These fibers terminate mostly in the polymorphic layer of the DG as

well as in stratum lacunosum moleculare of CA3 and CA1 (Pickel et al., 1974; Swanson and Hartman, 1975; Loughlin et al., 1986). However, LC innervation is similar at all septotemporal extents (Loy et al., 1980). Like noradrenergic input, serotonergic input from the raphe nucleus (RN) terminates most heavily in the polymorphic layer of the DG and stratum lacunosum moleculare of CA1 (Conrad et al., 1974; Moore and Halaris 1975; Kohler and Steinbusch, 1982; Vertes et al., 1999). A large number of GABAergic interneurons appear to be preferentially innervated by serotonergic afferents (Halasy et al., 1992; McMahon and Kauer, 1997). Thus, the few serotonergic fibres innervating the hippocampus could have a crucial action by enhancing GABAergic inhibitory activity of hippocampal interneurons. Moreover, serotonergic innervation increases from septal to temporal levels (Oleskevich and Descarries, 1990). Lastly, histaminergic input from the tuberomamillary nucleus of the posterior hypothalamus (Vertes and Kocsis, 1997; Fano et al., 2011) increase from septal to temporal levels (Amaral and Kurz, 1985; Haring and Davis, 1985; Panula et al., 1989; Bjarkam et al., 2003).

1.1.6 A heterogeneous map of connections along the long axis of the hippocampus

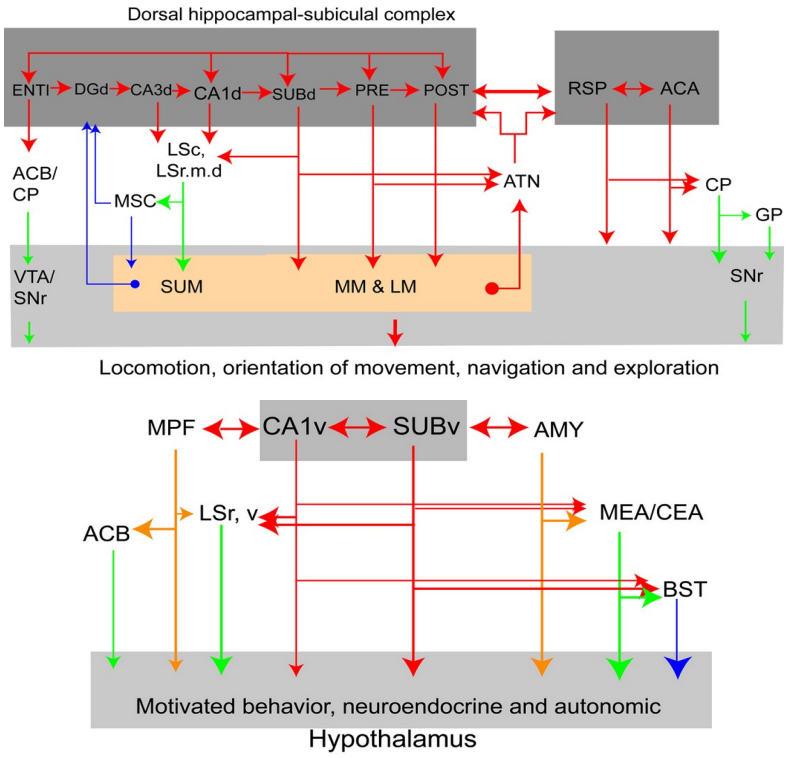
It has been a while since the first anatomical evidence demonstrated heterogeneity in the projection pattern originating at different septo-temporal levels of hippocampal CA1 (Lorente de Nó, 1934; Blackstad, 1956; Nauta, 1956; Raisman et al., 1966; Hjorth-Simonsen, 1973; Meibach and Siegel, 1977). For the purpose of the

thesis, I will focus on the differences in connectivity along the dorso-ventral axis of CA1 emerged by the use of anterograde and retrograde tracers, and on their broad functional implications. A recently published work by Agster and Burwell (2013) serves to characterize the connections within the HF and the parahippocampal regions, focusing on the proportions and strength of the projections along the dorso-ventral axis of the brain structures considered. After a general and traditional viewing of the principal connectivity, I will review the latest findings.

Generally, the CA1 field has quite widespread projections, to the contralateral cortex as well as the ipsilateral olfactory bulb, amygdala, and hypothalamus. When viewed on a flatmap, hippocampal CA1 association projections innervate an unexpectedly extensive ring of cerebral cortical areas. Topologically, the ring's caudal end consists of strong intrahippocampal formation connections arranged perpendicular (transverse) to the entire longitudinal axis of CA1 region and the hippocampal formation as a whole. Three longer projections extend rostrally. The first is a moderately strong dorsal projection to the retrosplenial area of the cingulate region (gyrus in humans) from the dorsal third of CA1 field. The second arises from the ventral two-thirds of CA1 and is an extensive ventral projection through the longitudinal association bundle to visual, auditory, somatosensory, gustatory, olfactory, and visceral cortical areas, as well as to the basolateral amygdalar complex and agranular insular and orbital areas. The third arises from the longitudinal extent of CA1. It is a strong cortical-subcortical-cortical projection

through the fornix system to the prefrontal, orbital, and olfactory areas that essentially lies rostral, at or near the rostral ends of the dorsal and ventral projections-in essence forming the rostral end of the peripheral cortical ring.

Swanson et al., 2006, documented for the first time axonal projections from the ventral end of hippocampal CA1 to widespread hypothalamic cell groups, as well as the existence of inputs directly to the thalamus. Intriguingly, hypothalamic cell clusters coordinated somato-motor and visceral-motor responses typical of



motivated or goal-oriented behaviour (Risold et al., 1997; Swanson, 2000). Only ventral regions of CA1 project directly to the amygdalar region, where the densest terminal fields are established in the postero-medial cortical and posterior baso-

medial nuclei. The anterior baso-medial and posterior basolateral amygdalar nuclei receive ventral CA1 axonal projections, project back to ventral CA1 (Petrovich et al., 2001), and integrate olfactory and gustatory information (Shi and Cassell, 1998). These results suggest a role in feeding behaviour. A projection from ventral CA1 to caudal regions of the piriform area (predominantly layer 2) and dorsal endopiriform nucleus was recently described (Chen et al., 2006). Inputs from the ventral CA1 are also reaching the prefrontal-orbital-agranular insular region and the somatosensory, gustatory, and visceral areas.

Figure 2. Schematic representation of the principal connectivity of the dorsal and ventral hippocampal networks.
Abbreviations ACA, anterior cingulated area; ACB, nucleus accumbens; ATN, anterior thalamic complex; CP, caudoputamen; DGd, dorsal domain of the dentate gyrus; ENTl, the caudolateral band of the entorhinal cortex; GP, globus pallidus; LM, lateral mammilarynucleus; LSc, the caudal part of the lateral septal nucleus; MM, medial mammilary nucleus;MSC, medial septal complex; PRE, presubiculum; POST, postsubiculum; RSP, retrosplenialcortex; SNr, reticular part of the substantial nigra; SUBd, dorsal subiculum; SUM,supramammillary nucleus; VTA, ventral tegmental area. AMY, cortical-like amygdalar areas (nuclei); BST,bed nuclei of the stria terminalis; CEA, central amygdalar nucleus; LSr, v, the rostral andventral parts of the lateral septal nucleus; MEA, medial amygdalar nucleus; MPF, medialprefrontal cortex; SUBv, the ventral subiculum
Adapted from Fanselow and Dong (2009).

Inputs to visual and auditory areas are predominantly from the dorsal CA1 sub-region, only light projections from the ventral part have been reported; whereas the strongest projection to temporal and parietal association areas, as PER, arises basically from the ventral two-third of the CA1 (Swanson and Cowan, 1977b; van Groen and Wyss, 1990; Cenquizca and Swanson, 2007).

The overall transverse organization of CA1-EC projections is well-known (Beckstead, 1978; Swanson et al., 1978; van Groen and Wyss, 1990a; Amaral and Witter, 1995). The dorsal end of CA1 projects most densely to lateral regions of the LEC and MEC, whereas progressively more ventral regions innervate progressively more medial regions of the LEC and MEC, with the medial entorhinal becoming progressively more dense from dorsal to ventral along the longitudinal axis. The transverse arrangement of the intra-hippocampal circuit is strengthened by recently reported projections from entorhinal area to CA1 and SUB fitting the pattern (Naber et al. 2001). As I previously said, the CA1-entorhinal projection mainly ends in layers III-V (Swanson et al., 1978; van Groen and Wyss, 1990a; Burwell et al., 1995). Additionally, dense termination in layers IV and V has also been reported, along with light projections to layers II and VI from dorsal levels of CA1, and moderate projections to layers II, III, and VI from more ventral levels of CA1.

Thalamo-cortical “feedback” projections of the medial hypothalamus controlling goal-oriented or motivated behaviours are confined to association projections from ventral hippocampal CA1 (Swanson 2000). The functional mechanisms sub-served by this cortico-hippocampal-thalamic connections are exceptionally complex although studies in humans point to its involvement in episodic memory associated with emotions that accompany spatial navigation for foraging or exploring specific goal objects (Steinvorth et al., 2005; Spiers and Maguire, 2006; Svoboda et al., 2006).

Overall, there is considerable experimental support in rats for differential functional localization along the longitudinal axis of field CA1 (Bannerman et al., 2004; Pentkowski et al., 2006; Fanselow and Dong, 2010) (Fig. 2).

1.1.7 Interneurons in the hippocampal system

In addition to excitatory cells, cortical circuitry is comprised of inhibitory interneurons. Named by their tendency to make local contacts (although long projecting ones between different structures are also known), they constitute approximately 20% of the cells in the cerebral cortex and hippocampus (Somogyi et al., 1998). There are many different types of interneuron, as many as 21 have been currently identified in the CA1/CA3 regions of the hippocampus alone, based on variations in morphology, biochemistry and electrophysiology (Somogyi and Klausberger, 2005; Klausberger and Somogyi, 2008; Somogyi et al., 2014) (Fig. 3). Indeed, different classes of interneurons express a number of different molecular markers, while they all are immunoreactive for GAD (glutamate decarboxylase) for the presence of the inhibitory neurotransmitter GABA. In addition, the dendritic and axonal arborisations, as well as the target neurons, are specific to each type (Freund and Buzsaki, 1996; Somogyi and Klausberger, 2005). Importantly, different interneuron types also show stereotypical firing and phase relationships with respect to different network oscillations (Klausberger et al., 2003; Klausberger et al., 2005;

Somogyi and Klausberger, 2005). Thus, interneuron types are thought to sub-serve separate network functions by controlling, synchronising, and integrating the flow of information through the hippocampal system. In this way, different interneuron types may orchestrate the activity of excitatory cells according to the behavioural state.

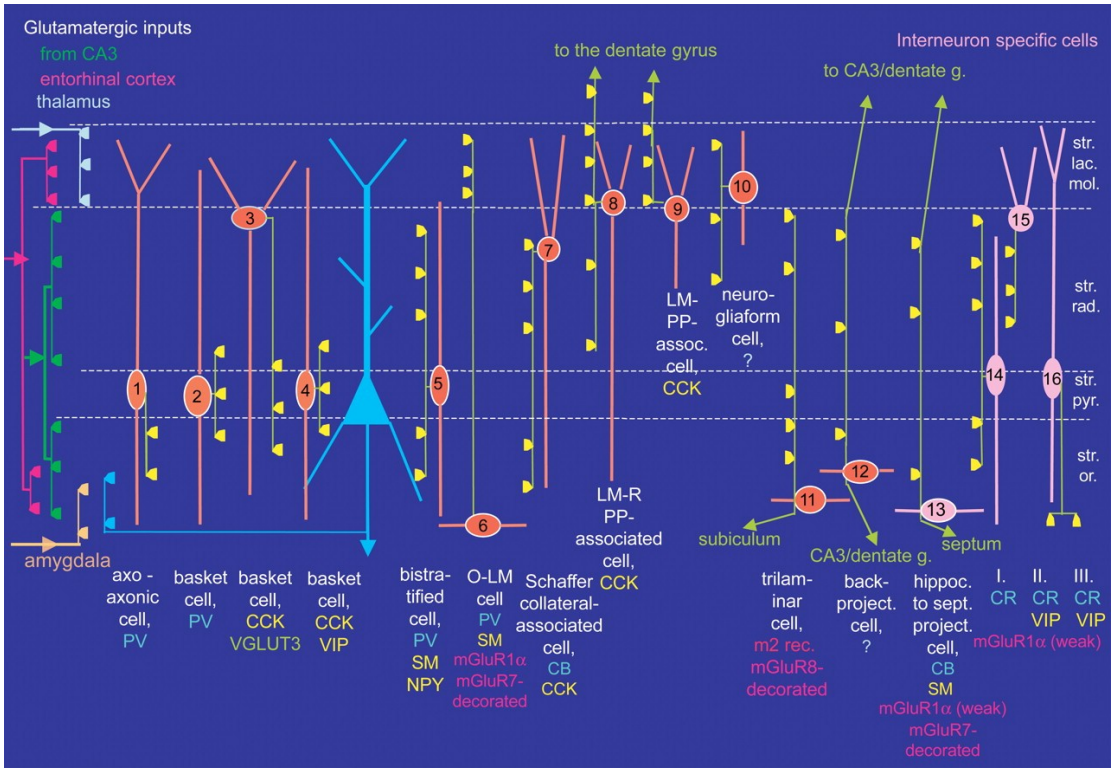


Figure 3. Innervation of pyramidal cells by GABAergic interneuron in the CA1 area of the hippocampus

The main lamina specific glutamatergic inputs are indicated on the left. The somata and dendrites of interneurons innervating pyramidal cells are shown in orange, those innervating mainly or exclusively other interneurons are shown in pink. Axons are shown in light green and the main termination zone of GABAergic synapses are shown by yellow symbols. The names of neurons, some of them abbreviated, are under each schematic cell and a minimal list of molecular cell markers is given, which in combination with the axonal patterns help the recognition and characterisation of each class. CB, calbindin; CR, calretinin; LM-PP, lacunosum-moleculare–perforant path; LM-R-PP; lacunosum-moleculare–radiatum–perforant path; m2, muscarinic receptor type 2; NPY, neuropeptide tyrosine; PV, parvalbumin; SM, somatostatin; VGLUT3, vesicular glutamate transporter 3.

Adapted from Klausberger and Somogyi (2008)

1.2 The spatial and behavioural correlates of hippocampal function

The firing of hippocampal cells shows striking functional correlates to spatial and non-spatial features of the environment the animal has experienced. The discovery of “place cells”, hippocampal neurons firing in relation to the animal’s location, led the investigators to propose a role in spatial navigation and maintaining of spatial maps for these brain structures (O’Keefe, 1979). Since then, an enormous amount of evidence has shown that excitatory cells in the CA3 and CA1 regions have place fields during exploration, so that the entire environment is represented in the collective firing of the cell population active in that context (O’Keefe, 1976; Wilson and McNaughton, 1993). The animal’s position is reliably inferred by the firing of such a population (Wilson and McNaughton, 1993; Brown et al., 1998).

From an evolutionary perspective, place cells were also found in birds, bats, primates, and humans (Rolls and O’Mara, 1995; Ekstrom et al., 2003; Siegel et al., 2005; Ulanovsky and Moss, 2007). In addition, cells in the DG SUB and EC show spatially selective firing patterns (Jung and McNaughton, 1993; Sharp and Green, 1994; Fyhn et al., 2004; Hafting et al., 2005; Barry and Burgess, 2007; Fyhn et al., 2007; Leutgeb et al., 2007). In particular, cells in the EC fire at multiple locations throughout the environment (Fyhn et al., 2004), showing a rigid and reliable structure in the spatial arrangement of their multiple fields: each field aligned with

the vertices of a tessellating hexagonal “grid” structure that covered the entire environment (Hafting et al., 2005). Similar to hippocampal place fields, the position (phase) of the grids in the environment seems to be widely distributed in a cortical volume, such that the whole environment can be represented in any recording location (Hafting et al., 2005). A detailed discussion of the spatial property of grid cells is beyond the purpose of the review. However, I need to mention that the change in place field dimension along the long axis of the hippocampus has been associated with the increase of the grid field size in relation to the topography of projections from the MEC bands to the long axis of the hippocampus (Brun et al., 2008b). Indeed, as long as we move towards the ventral extremity of the hippocampus, place cells have gradually broader fields, covering a larger portion of the environment (Jung et al., 1994; Maurer et al., 2005; Kjelstrup et al., 2008; Royer et al., 2010). As described in the anatomical section, the dorso-lateral band of the MEC projects to the dorsal half of the hippocampus, the intermediate band to the next quarter, and the ventro-medial band to the most ventral quarter of the hippocampus (Fig. 1). Thus, there exists a clear anatomical pathway for grid cell inputs to shape the output of hippocampal place cells. Besides one study attesting a topographically arrangement of place maps within CA1 and CA3 region (Hampson et al., 1996; Deadwyler and Hampson, 1999), the physical locations of the place fields and anatomical positioning of the cells do not correspond (Redish et al., 2001). Similarly, no relationship is evident along the transverse extent of the hippocampus,

although a certain degree of topographical connectivity between the two fields exists.

Other studies have revealed that different components of the context primarily influence hippocampal neuronal activity, such as discriminative cues and the behavioural state of the animal (Olton, 1978; Muller, 1987). Discrete events such as conditioned stimuli (Berger and Thompson, 1978), reward (Breese et al., 1989), and distinct odour cues (Wood et al., 1999), can modulate place cell activity, though whether this is independent of place remains a point of debate (O'Keefe, 1999). Therefore, place cells may be responsive to non-spatial information as well. In addition, CA1 pyramidal cell firing rates are boosted within novel environments (Nitz and McNaughton, 2004; Csicsvari et al., 2007), presumably following dopaminergic modulation via the VTA (Lisman and Grace, 2005). Similarly, the speed during locomotion has been shown to correlate somehow with place cell firing rates (McNaughton et al., 1983). These suggest that many factors, not just space, are modulating place cell activity, leading to the theory that hippocampal neuronal firing reflects the representation of conjunctions of stimuli with their behavioural significance and the context (space) in which they occur (Eichenbaum, 2004). In reference to the role in memory formation, it has been hypothesized that all those variables are part of the memory fragment acquired by the hippocampal network. It is generally accepted that such information is represented in addition to the spatial signal, rather than instead of it (Fenton et al., 1998; Olypher et al., 2002; Leutgeb et

al., 2005). Alternatively, different cell types in the hippocampus might have a distinct functional correlate, being specifically responsible for spatial or non-spatial component of the representation.

1.2.1 Global remapping and rate remapping

Hippocampal principle cell firing fields are not static in the sense that place cells fire in different locations from one environment to another, relative to the environment boundaries (Muller and Kubie, 1987; Bostock et al., 1991; Lever et al., 2002; Wills et al., 2005). This phenomenon is known as “remapping”, referring to the tendency of CA1 neurons to establish new firing fields in an environment that is novel and distinguishable from the familiar one, over a period of minutes (Wilson and McNaughton, 1993; Frank et al., 2004b; Leutgeb et al., 2004). Actually, the subset of cells active in the new environment is mostly composed of cells that were silent before and some remapping cells whose place field within one environment is not predictive of firing location in the second (Quirk et al., 1990; Bostock et al., 1991). The described “global remapping” is consistent with the idea of the existence of a hippocampal cognitive map based on place cells activity.

CA1 place map representations show also directional sensitivity when animals traverse a linear track (Markus et al., 1995) and are sensitive to changes in familiar environments. Movement of environmental cues can result in changes in place field location. Rotation of distal cues surrounding the maze leads to some of the place fields moving with the moved cue, while more complicated manipulations

of cues can lead to a heterogeneous mixture of responses (O'Keefe and Conway, 1978; Muller et al., 1987; Shapiro et al., 1997; Lee et al., 2004). This “partial remapping”: can also occur by changing the colour or odours in the environment (Anderson and Jeffery, 2003, Anderson et al., 2006). However, if the visual cues defining the environment are unstable then the cells appear to ignore external cues and follow the animal’s ideothetic cues, derived from vestibular, proprioceptive, and motor command (Jeffery and O'Keefe, 1999). Moreover, when external cues are all removed or the lights turned off, place fields remain stable as long as the animal has previously explored the environment in the light (Muller and Kubie, 1987; Muller et al., 1987; O'Keefe and Speakman, 1987; Quirk et al., 1990; Markus et al., 1994). Taken together, it has been proposed that place map representations may reflect the activity of a “path integration system” that calculates the position of the animal integrating the ideothetic information and anchored to the context defined by the spatial landmarks in the environment (O'Keefe, 1976; O'Keefe and Nadel, 1978; McNaughton et al., 1996; Etienne and Jeffery, 2004).

1.2.2 Functional differentiation along the longitudinal axis

Historically, significant emphasis has been placed on examining the functionality of distinct hippocampal sub-regions within the trisynaptic circuit (DG > CA3 > CA1) of septal hippocampal regions rather than functional differences across the areal or longitudinal expanse of the hippocampus (Anderson et al., 1971).

Markedly, physiological findings in septal hippocampus have been attributed to the entirety of the septotemporal axis, suggesting that the hippocampus acts as a unitary structure for successful information processing. A variety of behavioral studies based largely on lesion data in rodents and neuroimaging data in humans support functional differentiation of hippocampal circuits along the long axis (Moser et al., 1995; Strange et al., 1999; see Bannerman et al., 2004 for review). As previously highlighted, dorsal hippocampal lesions in rodents are known to have the same effect of complete hippocampal lesions in severely disrupting spatial memory in tasks testing both reference and working memory such as the Morris water maze and the radial maze. Rats with ventral lesions normally perform well in those tasks. Those results were replicated in studies using local microinfusion of drugs for temporarily perturbate the ongoing activity of the target area (M.B Moser & E.I Moser, 1998; Pothuizen et al., 2004; Pentkowski et al., 2006). Having said that, some other studies raise the question whether the role for dorsal hippocampus in spatial learning and memory would be only preferential but not exclusive. Indeed, rats with a sufficient preoperative training can eventually acquire the Morris watermaze with a partial lesion either of the septal or temporal hippocampus, but not of the whole structure. Ventral hippocampus spared rats may even contribute to spatial learning at least in some cases (de Hoz et al., 2003; Ferbinteanu et al., 2003; Broadbent et al., 2004; Martin et al., 2005; Loureiro et al., 2011; de Hoz et al., 2014). For example, De Hoz and colleagues (2014) have recently shown that new spatial learning in a familiar

(reversal learning) but not in a novel context can be impaired by temporal hippocampal lesions made before acquisition. If the lesions were done after training, rats with dorsal hippocampal lesions were better than the ventral lesioned ones in new learning in a different environment. As the authors suggested prior experience might influence the ability of the animals to fulfil a spatial task and to acquire new information (see also Bast et al. 2009 on rapid place learning), so to use a strategy not only based on visuospatial information. This procedural correction might reveal a role for the ventral hippocampus in spatial learning for a different form of memory.

Additionally to the complex differentiation reported by lesion studies, there is considerable septotemporal variation in neuronal markers (Gusev et al., 2005), neuromodulation of plasticity (Maggio and Segal, 2007), and differences in the experiences that influence neurogenesis along the long axis (Snyder et al., 2009). While LFP data support functional differentiation across the longitudinal axis, the work of place cells provides convincing evidence that suggests variability in the spatial representation written across the surface of the septotemporal axis.

Principal cells in septal CA1 have long been demonstrated to have spatially restricted receptive fields, or “place fields” in a given environment (O’Keefe and Dostrovsky, 1971). These septal CA1 “place cells” are believed to provide a cognitive spatial representation of a given environment, with as few as 15-20 place cells predicting a rat’s location in space within a few centimetres (Wilson and McNaughton, 1993). The vast majority of place cell work has strictly focused on the

well-defined place fields of septal CA1, while the few studies at temporal regions have demonstrated that place field size systematically increases across the septotemporal axis (Jung et al., 1994; Kjelstrup et al., 2008; Royer et al., 2010). Place fields in septal hippocampus are small, concise, and refined, while in temporal hippocampus, place field sizes become much larger, reaching at least a meter in length (Jung et al., 1994; Maurer et al., 2005). Furthermore, recordings in CA3 pyramidal cells across the septo-temporal axis show place fields 5-6 meters in length when rats traverse an 18-meter long linear track (Kjelstrup et al., 2008). Royer et al., 2010 confirmed the increase in place field size in CA3 temporal hippocampus and suggested that non-spatial information was also coded by temporal CA3 principal cells. More specifically, CA3 principal cells dissociate between open versus closed arms on a radial maze as well as traversals toward and away from a reward positioned at the end of the arm (Royer et al., 2010). These data taken together suggest functional differentiation across the septotemporal axis of the hippocampus, further indicating that perhaps septal cells code for egocentric information, while temporal cells code allocentric information.

1.3 Network oscillatory patterns in the hippocampus: the oscillatory correlates of memory

Jung and Kornmüller reported regular electroencephalographic oscillatory (EEG) patterns in the hippocampus in the late 1930s. Some of the hippocampal local EEG oscillations were observed to change with behavioural state (Vanderwolf, 1969).

Oscillatory pattern in the local field potential (LFP) reflect the summation of extracellular currents generated by rhythmic spiking in a network of neurons. Because of the highly organized architecture of the hippocampus, the synaptic excitatory and inhibitory potentials impinging upon the laminar somato-dendritic organization of hippocampal neurons ultimately leads to the rhythmicity observed in the hippocampal field. LFP waveform characteristics, such as the frequency and amplitude of the signal, depend on the proportional contribution of multiple afferent sources as well as intrinsic properties of brain tissue (Buzsaki et al., 2012). Therefore, the LFP recorded throughout the hippocampal structure is drastically different in shape and amplitude depending on recording site (eg, CA1 vs DG).

This periodicity constitutes a temporal framework within which the activity of particular assemblies of cells can be grouped and segregated in order to serve distinct processing of information. The precise timing of neuronal cooperation is crucial for inducing change at the level of synapses, such as long-term plasticity (LTP), eventually regulating the mechanisms for cellular learning.

Prominent network oscillations reported in the rodent hippocampo-entorhinal system include the theta rhythm (5-12 Hz); the gamma oscillation (40-100

Hz); and ripple oscillations (~200 Hz) that occur during hippocampal sharp-wave/ripple (SWR) events. Each of these oscillatory patterns is thought to contribute to specific and distinct functional operations.

In the next paragraphs, I will review the main evidence to define the functional relevance of theta oscillation and SWR patterns in the hippocampal computations.

1.3.1. Theta Oscillations along the dorso-ventral axis

When a rat runs through an environment, large-amplitude theta oscillations (4–10 Hz) characterize consistently the hippocampal field potential. Those oscillatory patterns are a key element of spatial navigation and memory theories. Therefore, understanding the physiological function of theta oscillations might be crucial to uncover the neural basis of the computation taking place in the hippocampus. It has been shown that there is increased efficiency of memory encoding during periods of high theta amplitude (Seager et al., 2002).Theta may support memory by serving as a timing signal that causes the groups of neurons that are simultaneously active to spike at similar times and become linked via long-term plasticity (O'Keefe J and Recce, 1993; Buzsaki, 2005). During exploratory periods, synchrony is relatively low within the hippocampus (Csicsvari et al., 1998). As cells mainly fire within their place field, ensembles of cells that are jointly active represent a discrete region of the environment around the animal, or path along a trajectory. Thus during exploration, the behaviour of the animal drives different groups of cells with overlapping fields to

fire together. Since some cells fire in multiple environments, that activity of individual place cells provides limited information. However, assemblies of such cells may represent discrete places or trajectories.

Other theories stress the role of theta in computing location during spatial navigation. Specifically, theta may help compute an animal's spatial location during navigation by allowing grid cells in entorhinal cortex (Hafting et al., 2005) to represent location using computations based on theta phase (Burgess et al., 2007). Owing to the range of different functions that have been ascribed to theta, an emerging view is that theta oscillations are a fundamental signal that are essential for a range of behaviours (Buzsaki and Moser, 2013). Given the prominent role of theta oscillations in theories of mammalian brain function, it might be considered surprising that, in practice, there are many species where hippocampal theta oscillations are rarely observed. In particular, in humans evidence of human hippocampal rhythms at 4–8 Hz is weak (Halgren et al., 1978). Further, a recent study showed that 4–8-Hz theta oscillations are rare in bats (Yartsev et al., 2011). The apparent absence of theta rhythms issues theta-based theories of spatial navigation and memory (Giocomo and Moser, 2011). Certainly, theta oscillations cannot be critical for all mammalian memory and navigation if these oscillations are absent from many species.

Theta waves are observed in each sub-region of the hippocampus proper, as well as the EC (Mitchell and Ranck, 1980). Place is not just encoded by the firing rate

of cells during theta epochs but also by the timing of individual cells relative to the phase of theta oscillations (O'Keefe and Recce, 1993; Huxter et al., 2003), and inclusion of phase improves position prediction made by firing rate alone (Jensen and Lisman, 2000). Place- selectivity of CA1 pyramidal cells decays as a function of time distant to from the end of theta oscillations (O'Neill et al., 2006) and is absent when animals are restrained or immobile for long periods (Foster et al., 1989; O'Neill et al., 2006). Thus, place coding, in terms of both firing rate and spike timing, appears to be maximal during, or proximal to be linked with theta epochs. Theta activity is facilitated by enhanced levels of ACh during exploration, and theta amplitude positively correlates with hippocampal ACh levels (Marrosu et al., 1995; Monmaur et al., 1997). As noted above, increased ACh reduces synaptic transmission in the stratum radiatum, while showing a reduced effect on direct inputs from the EC, which are sufficient to drive place selectivity. Exploratory theta epochs, therefore, may be the time in which information from the EC is encoded within the hippocampus (Buzsaki, 1989; Hasselmo, 1999). In this scheme, the EC primarily communicates with the hippocampus, while output to the entorhinal cortex is limited. Indeed, perforant path stimulation during exploratory epochs gives rise to an attenuated response at each stage of the hippocampus, as compared to slow wave sleep and immobility epochs, where multi synaptic responses can be seen (Buzsaki, 1989).

1.3.1.1 Origin and mechanisms of theta generation

As noted above, the LFP signal represents synchronized synaptic input within the laminar organization of somato-dendritic field of hippocampal neurons.

Mechanistically researchers have defined the currents that generate the time varying LFP signal (current generation) as well as various sources that define the rhythmicity (rhythm generation). I will focus on rhythm and current generation in CA1.

Rhythm generation represents the mechanisms that are responsible for the coordination of the synaptic potentials involved in current generation into a frequency in the range of 6-12 Hz. It is important to note that few of the key elements involved in theta current generation (EC, CA3, CA1 pyramids), fire faithfully at roughly 1-5 Hz in phase with theta rhythm (Buzsaki, 2002; Patel et al., 2012), while many GABAergic interneurons discharge at 40-100 Hz (Ylinen et al., 1995; Hajos et al., 2004) on each theta cycle. In addition to intrinsic properties of basket cells, the medial septum provides a divergent input to all major cellular fields along the septotemporal axis, including the EC.

To provide “rhythm” to the theta oscillation, the MS serves as a pacemaker to regulate the frequency of the theta oscillation. Neurons important for this massive coordination of theta field potentials are MS cholinergic neurons (Brazhnik and Fox, 1999), MS GABAergic neurons that selectively target specific sub-populations of hippocampal GABAergic neurons (Freund and Antal, 1988; Borhegyi et al., 2004), MS

glutamatergic projections (Colom et al., 2005), and hippocampo-septal neurons that selectively target medial septal GABAergic neurons (Toth et al., 1993; Manseau et al., 2008). Since MS GABAergic cells spike in phase with theta (King et al., 1998; Brazhnik and Fox, 1999), this septo-hippocampal GABAergic projection provides theta rhythmic inhibition to hippocampal basket cells, which allows these cells to control the spike rate of hippocampal principal cells (Freund and Antal, 1988). In contrast, septo-hippocampal cholinergic projections terminate more diffusely and contact excitatory and inhibitory cells (Baisden et al., 1984; Amaral and Kurz, 1985; Wainer et al., 1985). These cholinergic cells provide an overall depolarizing effect to all hippocampal cells (Pitler and Alger, 1992). While cholinergic medial septal cells are thought to contribute to alternations in theta amplitude changes (Lee et al., 1994; Buzsaki, 2002), they lack the temporal resolution to contribute to rapid changes in theta power (Zhang et al., 2010). By contrast, cells that participate in theta current generation have the ability to produce theta amplitude changes on a finer temporal scale. While the MS appears to play the most important role in theta rhythm generation, subcortical inputs have the potential to participate in generating theta rhythmicity as well as modulating ongoing interactions among elements of the hippocampal circuit. Notably, serotonergic input originating from the locus coeruleus, noradrenergic projections from the raphe nucleus and histaminergic input from the mamillary nucleus of the posterior hypothalamus (Vertes and Kocsis, 1997; Fano et al., 2011) all impinge upon the hippocampus. The joint participation of the

highly topographic input of medial septal and subcortical afferents allows for the observance of the highly regular hippocampal theta rhythm.

While rhythm generation refers to the mechanisms that are responsible for the coordination of the synaptic potentials, current generation refers to the synaptic potentials that generate the extracellular currents that are recorded as the local field potential and lead to the rhythmic field oscillation. Several key players contribute to theta current generation in CA1 of the hippocampus. First, rhythmic afferent excitation from EC layer III neurons impinge upon the distal dendrites of CA1 cells (Alonso and Garcia-Austt, 1987; Chrobak and Buzsaki, 1998). Without this prime input from the EC, theta field oscillations are diminished (Bragin et al., 1995). Furthermore, intrinsic excitatory hippocampal neurons arising from CA3 pyramidal (principal) neurons contribute to theta generation (Kocsis and Buzsaki, 1999; Buzsaki, 2002). Moreover, local GABAergic interneurons soma and dendritic-targeting provide for the current generation of theta oscillations (Konopacki et al., 1992; Ylinen et al., 1995; Hajos et al., 2004).

1.3.1.2 The effect of locomotion on theta parameters

In addition to anatomical variation and the differences in place cell physiology, recent work supports variation in LFP signals across the septotemporal axis of the HPC (Maurer et al., 2005; Sabolek et al., 2009; Hinman et al., 2011; Penley et al., 2011; Patel et al., 2012). Early work investigating the behavioral correlates of the hippocampal theta signal observed its emergence during locomotion, specifically

running speed of the rodent (Vanderwolf, 1969; Teitelbaum and McFarland, 1971; Whishaw and Vanderwolf, 1973; McFarland et al., 1975). Furthermore, it has been shown that in septal HPC not only theta power, but theta frequency increases as a function of running speed (Rivas et al., 1996; Slawinska and Kasicki, 1998). The increase in theta power as a function of running speed has been established in more recent studies examining the septal pole of the HPC (Rivas et al., 1996; Bouwman et al., 2005) as well as functional differentiation of the theta signal across the longitudinal axis of the HPC (Hinman et al., 2011). Maurer and colleagues suggested that the positive linear association between theta amplitude and running speed decreases at mid-septotemporal levels, but lack of simultaneous recordings made it impossible to validate such a suggestion. Hinman et al., 2011, addressed the suggestion of Maurer et al. (2005) demonstrating that the positive linear association between speed and theta power systematically decreases with distance from the septal pole. This work further supports functional differentiation across the septotemporal axis.

1.3.2 Sharp waves, ripples: features and mechanisms of generation

During periods of slow movement, immobility and slow-wave sleep, theta oscillations in the hippocampal system are replaced by sporadic fast oscillatory events known as sharp-wave ripple (SWR) events. First described as irregular patterning dominating the LFP of CA1 during offline states, they are characterized by a large deflection of the field potential in the CA1 stratum radiatum, “sharp waves“ (SW), (Buzsaki et al., 1983), and concurrent high frequency (150-250 Hz) “ripple“ oscillations in the pyramidal layer (O'Keefe and Nadel, 1978; Buzsaki, 1986). SWR events tend to last for 40-120 ms at irregular intervals ranging from 200 ms to 50 s during off-line states (Buzsaki, 1986). SWRs were also observed during brief interruption of theta waves during exploration (eSWRs). Even though mechanistically similar, it has been argued that awake SWR events are functionally distinct from SWRs occuring during sleep and rest: eSWRs may reinforce the encoding of spatial associations in the hippocampus (O'Neill et al., 2006) and/or integrate recent events into memory (Foster and Wilson, 2006).

When SWRs occurred, the hippocampus can provide strong output to the EC, while entorhinal inputs to the hippocampus are relatively restrained. Given the high level of synchrony associated to SWRs, more than would be predicted by firing rates alone, it has been proposed that cell assemblies representing memory traces may be weakly established during exploration (Csicsvari et al., 2000). During SWR periods, organised firing patterns may serve to strengthen intra-hippocampal associations

through repeated activity (Buzsaki, 1989) as well as facilitate the transfer of stored information from the hippocampus to the cortex (McClelland et al., 1995).

SWRs are generated within the hippocampus. The elimination of the majority of the sub-cortical projections by fimbria-fornix lesion results in large amplitude SW and spikes in the EEG, suggesting that SWRs may be triggered by a reduction of those inputs during slow-wave sleep and immobility (Buzsaki, 1986; Buzsaki, 1989; Hasselmo, 1999). During SWR events, population synchrony increased within both the CA3 and CA1 regions, relative to theta epochs (Buzsaki, 1986; Ylinen et al., 1995; Csicsvari et al., 1998; Csicsvari et al., 1999b; Csicsvari et al., 2000). In addition, the activity in layer II/III of the EC is modest during SWR epochs, while layer V shows increased activity (Chrobak and Buzsaki, 1994).

After the depolarization of SWs, the CA1 and CA3 networks can resonate at either 110 or 170 Hz, in accordance to their resonant properties: while the CA3 region preferentially resonates at 110 Hz, the CA1 resonate at the higher frequency likely because of its stronger gain properties. Anatomical information in relation to the organization of axon collaterals also supports the differential level of excitation in the CA1 and CA3 regions (Ishizuka et al., 1990; Li et al., 1994; Wittner et al., 2007). Therefore, during a CA3 population burst, CA1 neurons are more strongly excited, because of the stronger convergence of CA3 afferents. Inhibition might also contribute for the change in gain. Previous work has demonstrated that with

increasing magnitudes of ripples, the recruitment of CA1 pyramidal cells is more effective than that of interneurons (Csicsvari et al., 1999b).

Ripples are most likely generated de novo in the CA1 region (Buzsáki et al., 1992, 2003; Ylinen et al., 1995; Csicsvari et al., 2003). Slow oscillations or sleep spindles (Sirota et al., 2003; Isomura et al., 2006; Mölle et al., 2006, 2009) can effectively trigger a SWR (Battaglia et al., 2004; Isomura et al., 2006). The hypothesized influence of EC during states of high synchronous cortical activity (UP state) can either advance or delay the next SWR event, depending both on the timing and the relative dominance of excitation versus inhibition. Interference between the hypothesized SW-pacing oscillator in the CA3 network and the EC-mediated inputs can result in irregular patterns. Finally, fluctuation of the strength of sub-cortical inputs may also contribute to the irregular nature of SWRs (Buzsáki et al., 1983; Buzsáki 1989).

So extra-hippocampal input related to the preceding behavioural experience, may trigger the onset of SWR coincides via a brief transient increase in CA3 firing activity (Buzsaki 1986, 1996). The population burst in CA1 will depolarize target cells and promote synaptic plasticity in these target regions. The experience-dependent changes in the cellular properties and synaptic connectivity within the network of may be reflected in the increase in ripple activity (King et al. 1999). In conclusion, SWs in stratum radiatum reflect the depolarization of the apical dendrites of CA1 and CA3 pyramidal cells, because of the synchronous bursting of CA3 pyramidal cells.

The spread of excitatory activity in the recurrent CA3 collateral system at times when the subcortical inputs are reduced would support the sustained burst activity (Buzsáki et al., 1983; Hasselmo and Bower, 1993). The excitatory drive of CA3 pyramidal cells during SWRs, can affect a large volume of the CA3 and CA1 region (Ylinen et al., 1995). The interaction between pyramidal cells and specific interneurons recruited by CA3 are also believed to trigger the ripple oscillation (Buzsáki et al., 1992; Ylinen et al., 1995; Csicsvari et al., 1999a; 2000; Brunel and Wang, 2003; Klausberger et al., 2003; Klausberger and Somogyi, 2008; Ellender et al., 2010; Mizuseki et al., 2011).

1.4 Reactivation of memories: sleep consolidation and retrieval.

Memory reconsolidation studies began in the 1960s when Donald Lewis first observed retrograde amnesia for a well-established memory in rats when activated by a reminder of the original learning experience (review, Lewis, 1979). These studies are the real origin of today's hypothesis of memory reconsolidation, even though neither Lewis, nor his colleagues, ever used the term. Lewis' studies clearly demonstrated that memory liability was not time-bound to acquisition, as the consolidation hypothesis holds. Experiments followed showing that the reactivation of well-consolidated spatial memory, acquired over many days, was dependent upon NMDA receptors to maintain stability reactivation (Przybyslawski and Sara, 1997; Torras-Garcia et al., 2005). However, drug-treated rats could relearn the task in only a few trials, suggesting only partial amnesia (Przybyslawski and Sara, 1997). Cue-dependent amnesia could also be obtained by blockade of noradrenergic beta receptors at reactivation of memories for both appetitive and fear-driven tasks (Roullet and Sara, 1998; Przybyslawski et al., 1999; see also Debiec and Ledoux, 2006).

The notion that off-line memory processing occurs during sleep has been around for a long time. Animal studies in the 1960s and 1970s were focused on the role of rapid eye movement (REM) stage. It was hypothesized that the high level of cortical activity associated with this sleep stage is associated with reactivation of

memory traces formed during wakefulness and that this stage of sleep serves to consolidate these memories (Hennevin and Leconte, 1971). The hypothesis was tested by selectively depriving rats of REM sleep or delaying its onset after learning (Hennevin and Leconte, 1971) although these early studies did not adequately control for the stressful effects of the REM deprivation. Another approach involved monitoring and quantifying the proportion of REM sleep after learning. Learning-dependent REM increases were found and were correlated with the rate of acquisition of a task over several sessions (Hennevin et al., 1974). These studies were carried out within a conceptual framework of Lewis, the idea being that a specific memory was activated by the cue, to become labile. The cortical activity associated with REM was thought to promote consolidation of the active network.

The discovery of spontaneous “replay” of neural ensembles in rats provided a strong impetus for renewed studies of the role of sleep in memory consolidation. Neurons that are entrained to fire together during awake behavior, tend to fire together again during sleep (Pavlides and Winson, 1989; Wilson and McNaughton, 1994). Replay of hippocampal neuronal ensembles active during previous behavioural episodes is another example of experience-dependent persistent neuronal activity thought to be involved in off-line memory consolidation (Pavlides and Winson 1989; Wilson and McNaughton 1994; Skaggs and McNaughton 1996; Nadasdy et al. 1999; Louie and Wilson 2001; Lee and Wilson 2002). These studies have focused exclusively on ensemble activity of place cells in the hippocampus

recorded in rats performing familiar tasks, requiring a high degree of locomotor activity, such as foraging for food or running on a track. The majority of these investigators, nevertheless, suggest that the ensemble replay represents a reactivation of a memory trace established during the previous behavioral episode, even though such behavioral activities do not necessarily involve acquisition of a new information and formation of new memories. It remains an open question whether reactivation occurs after acquisition of information unrelated to spatial encoding (Eschenko 2008).

Replay has been taken to represent a reactivation of circuits underlying the previously encoded information (the memory trace) and reinforces the notion that consolidation of memory occurs during sleep. The initial hypothesis, discussed above, focused on the REM stage, because of the high level of cortical activity. However most replay in rats seems to take place during non-REM sleep, although there are some exceptions (Louie and Wilson, 2001).

Replay occurs in strong temporal relation to high frequency oscillations in the hippocampus, called sharpwave/ripple complexes (Kudrimoti et al., 1999; Csicsvari et al., 2000; O'Neill et al., 2010, for review). Others have proposed that these sleep-associated high frequency oscillations provide the substrate to promote long-term plasticity and consolidation of the memory trace. Synaptic connections between cells in the reactivated network will be reinforced by the high frequency concerted firing (Buzsaki, 1989; Steriade et al., 2003). Recording hippocampal local field potentials in

the rat, we found a marked increase in the occurrence of ripples in the sleep session immediately following learning of an odour-reward association. Important from the reconsolidation perspective, there was a similar increase in ripples in well-trained rats that had been exposed to a reminder session, just before. Rats that did not learn the task had no increase in ripples (Eschenko et al., 2008). In a multi-session spatial discrimination task, an increase in ripples predicted an increase in memory performance in the following session (Ramadan et al., 2009). These increases in ripple activity, if they are indeed, accompanied by replay of learning related ensembles of neurons, will promote memory consolidation, or might even be viewed as a manifestation of a consolidation process.

Until recently, there have been no strong data linking the content of the replay with the subsequent expression of memory during wakefulness. Two studies have provided convincing new data relating the reactivation to subsequent memory performance. In one, using human subjects, the content of dreams was related to previous waking experience and subsequent memory performance (Wamsley et al., 2010). An analogous experiment in rats recorded ensembles of hippocampal neurons during learning, monitored their reactivation during hippocampal ripple activity, and then related this activity to subsequent performance on a memory test (Dupret et al., 2010). These very recent reports take us a step further in relating encoding of memory in ensemble activity, off-line reactivation of the memory trace and subsequent robust memory performance.

Since reactivation seems to be a key process to memory consolidation and reconsolidation in and out of sleep, it is essential to understand what governs this reactivation, the brain regions involved, and whether neuronal assemblies are actually reactivated at retrieval.

Many imaging studies in humans have confirmed the crucial role played by the frontal regions in executive control of effortful memory retrieval (review Wheeler and Buckner, 2004). Imaging approaches have been able to identify other brain regions that are activated during retrieval from different types of remote episodic memory, implicating among others, hippocampus and amygdala (Spiers and Maguire, 2007). In addition, the parietal cortex has now been identified as a major component of the retrieval network, especially when the retrieval is effortful and is initiated top down. Furthermore, a recent study demonstrates that co-activation of left medial temporal lobe regions and temporal–parietal cortices is greater for correct than incorrect retrieval of episodic memory. (Mendelsohn et al., 2010). Earlier, the amygdala and the noradrenergic nucleus locus coeruleus were also identified as key players in retrieval, specifically of emotional memories. At retrieval, these two structures were more tightly coupled during correct responses (Sterpenich et al., 2006).

A large number of pharmacological studies have established that the noradrenergic input to the amygdala is essential to memory consolidation after initial training (see McGaugh and Roozendaal, 2008 for review).

The slow oscillations serve to group faster rhythms such as cortical spindles and hippocampal ripples and therefore provide the background brain state for consolidation (Siapas and Wilson, 1998; Molle et al., 2006). As mentioned above, it is during this non-REM sleep-associated ripple activity that the ensemble replay occurs. If indeed, there is potentiation of synapses occurring during this high frequency concerted firing of activate memory networks, then release of norepinephrine at a critical period during the replay should reinforce the plasticity in those activated synapses (Harley, 2007). While this scenario is presently highly speculative, it merits further investigation.

Only a few investigators have attempted to integrate the sleep research with current ideas concerning reconsolidation (Sara and Hars, 2006; Stickgold and Walker, 2013). The noradrenergic system and perhaps other neuromodulatory systems may be a key to link off-line memory reactivation, retrieval, and memory reconsolidation processes at both synaptic and systems levels, in and out of sleep.

It has been proposed that memory is a dynamic property of the nervous system, being retrieved within current cognitive environments. Tulving and Thompson (1973) argued that remembering is an activity similar to perceiving, in the sense that it involves the apprehension and comprehension of contemporary stimuli in the light of past experience. Accordingly, new episodic memory, to be remembered in a meaningful way, must be consolidated within a pre-existing semantic memory. An updated version of Tulving's view can be found in a recent

extensive review of the literature on the neural mechanisms of source memory. “Both encoding and remembering are constructive and reconstructive; they are selective and influenced by a rememberer's knowledge, beliefs, biases, goals, agendas, and meta-memory assumptions active at the time (Mitchell and Johnson, 2009). In this view, there is no difference between consolidation and retrieval. Encoding is preceded by retrieval, so that subsequent off-line consolidation, in and out of sleep, is really reconsolidation.

1.5 The hippocampus as part of the reward circuitry

Due to its dense reciprocal connections (direct and indirect) with the ventral tegmental area (VTA) and nucleus accumbens (NAc), the ventral hippocampus is thought to take also part in the reward-related processing. The most characterized reward circuit in the brain comprises dopaminergic neurons in the VTA that project to the NAc, which is part of the ventral striatum. The principal neurons of the NAc are GABAergic medium spiny neurons. This VTA–NAc circuit is crucial for the recognition of rewards in the environment and for initiating their consumption, but these regions respond to aversive stimuli as well. VTA dopaminergic neurons also innervate several regions of the prefrontal cortex (PFC), central amygdala, BLA and hippocampus, as well as other areas. All of these so-called ‘brain reward regions’ are inter-connected in complex ways: for example, the NAc receives dense glutamatergic

innervation from the PFC, amygdala and hippocampus; and the PFC, amygdala and hippocampus form reciprocal glutamatergic connections with one another. The functional output of each of these regions is modulated by several types of GABAergic interneurons and, in the NAc, by cholinergic interneurons as well. Moreover, each of these regions receives serotonergic inputs from midbrain raphe nuclei and noradrenergic inputs from the pontine locus coeruleus, and several are innervated by hypothalamic peptide systems. Last, there is evidence that dopaminergic neurons in VTA also release glutamate or GABA, which may contribute to their functional effects (Hinasko et al., 2012; Tritsch et al., 2012).

Part of the literature posts for the valence of a stimulus strengthening memory encoding in the hippocampus. Together with other structures in the medial temporal lobe, the hippocampus might act as an interface between emotion/motivation and cognition.

The fact that ventral hippocampus is preferentially connected with these brain systems hints at the role that the region might play in translating hippocampal-dependent learning in behaviour. The information encoded by the hippocampus may eventually be released from its behavioural control when consolidated in the neocortex.

It has been suggested that people with mood or anxiety disorders have memory encoding errors that result in exaggerated or misinterpreted experiences of the event. Many studies have found reduced hippocampal volume in depression and

other stress-related disorders, although again this seems to be found mostly in studies of middle-aged or elderly subjects. Studies of hippocampal activity in depression are limited and often conflicting. To speculate, in individuals affected by mood and anxiety disorders, the hippocampus might not properly encode the reward value in memories.

1.6 The involvement of the hippocampus in anxiety

Besides the discussed established role in spatial learning and memory, the hippocampus may also regulate behaviours related to anxiety (Bannerman et al., 2004). Indeed, one of the major neuroanatomical theories of anxiety (Gray and McNaughton, 2000) claims a crucial role for the hippocampal formation in anxiety behaviour. This is supported by its dense reciprocal connections (direct and indirect) with the HPA. Volumetric studies in humans resulted in somehow opposite results, the expression of anxiety traits having been shown to be both positively and negatively related to hippocampal volume in normal controls as well as patients affected by similar depressive disorder (Rusch et al, 2001). Clearly, a broad range of symptoms and behaviours are related to anxiety, so the opposite findings might be reconciled to different aspects of the disorder. Interestingly, the hippocampal enlargement in anxious individuals reported by Rusch and colleagues could reflect an increased use related to stress hormone release (Rasmusson et al, 2003), in a similar

way to the increase observed by Maguire (2000) in taxi drivers in reference to navigation-related system.

The observation made by Rusch et al (2001) of larger right-sided hippocampi in anxious subjects can further support the Gray/McNaughton theory. Molecular data on anxiety are also available in rats and mice, confirming altered hippocampal morphology and function. The use of rodent models is considered fundamental to investigate the molecular mechanisms underlying anxiety. In this case, anxiety is often defined as the tendency to behavioural inhibition in a conflict circumstance. So, research has frequently focused on unconditioned avoidance behaviour test such as the elevated plus-maze (EPM). In this maze, anxiety is generated because the animal has a tendency to explore the open arms of the elevated plus maze, but the open arms are also potentially dangerous compared to the closed arms. Well-characterized rat models genetically selected for anxiety-related behaviour on the EPM has resulted in extreme anxiety phenotypes (Landgraf and Wigger, 2002, 2003). Furthermore, cellular (Salome et al, 2004) and molecular (Murgatroyd et al, 2004) data from this rat model are now emerging. Lesion studies strongly support the role of the hippocampus in anxiety (Gray, 1982; Gray and McNaughton, 1983; Gray and McNaughton, 2000; Deacon et al., 2002). Electrolytic or excitotoxic lesions of the hippocampus produce anxiolytic-like behaviours in the EPM and social interaction tests (Bannerman et al., 2002; Deacon et al., 2002).

The precise contribution of the dorsal and ventral regions on anxiety is still debated. Microinjection of the anxiolytic benzodiazepine midazolam into the dorsal hippocampus decreased the avoidance response (Menard et al., 2001). However, anxiolytic-like effects were reported after either excitotoxic or electrolytic lesions of the ventral hippocampus, but not the dorsal hippocampus (Kjelstrup et al. 2002). Ventral lesioned animals show behaviour consistent with a reduction in anxiety-related responses in the social interaction tests, the light/dark test, the EPM test (McHugh et al. 2004, Pentkowski et al., 2006). Those results were confirmed by more accurate studies in which the activity of the two regions of the hippocampus were temporarily perturbated by acute microinfusion of drugs such as muscimol or NMDA. On elevated plus maze, distinct behavioural results were obtained by muscimol infusion in the two regions of the hippocampus: reduced locomotion was possibly accompanied by anxiolytic effects after the ventral hippocampal muscimol. In contrast, dorsal and ventral hippocampal muscimol infusion caused distinct effects, consistent with anxiogenic effects and anxiolytic effects respectively (Zhang et al., 2014). Most recently, optogenetic tools in mice have been revealed crucial for Tye and colleagues to show that communication between the amygdala and the ventral hippocampus might control anxiety levels (Felix-Ortiz et al., 2013).

Interestingly, recent studies have also shown high theta coupling between the ventral hippocampus and the medial PFC during anxiety behaviour (Adhikari et al., 2010; Adhikari et al., 2011). By using cross-correlation spectral density of the LFP

recorded in dorsal and ventral subfields of the hippocampus as well in the medial PFC, they showed that the temporal region oscillates coherently with the cortical region in the theta range, more than with the septal portion of the hippocampus. Those results were confirmed by the population responses to theta oscillations detected in distal electrodes.

All together, the reported evidences confirm distinct role played by the dorsal and ventral hippocampi. Those will be taken into account when discussing my results.

Chapter 2
General aims

The rodents hippocampus has been shown not to be uniform along its dorso-ventral axis (also defined as the septo-temporal axis). Indeed, changes along the dorso-ventral axis have been described with respect to anatomical projections (Fig. 2, General introduction) and gene expression (Fanselow and Dong, 2009). Distinctly, the more dorsal parts of the hippocampus receive visuospatial input, mainly from the dorsal entorhinal cortex (Witter and Amaral, 2004). In contrast, the more ventral parts receive largely nonspatial, emotional reward-related afferents from the amygdala, the hypothalamus and the medial prefrontal cortex, projecting back to those structures (Rysold and Swanson, 1996). In agreement with this anatomical differentiation, lesion studies suggest that the two parts are engaged in spatial learning in a different way (Bast et al., 2009). Moreover these studies support the idea that the intermediate hippocampal region is also important as it would enable the integration of information from the dorsal and ventral regions.

So far, electrophysiological investigations have focused on the more accessible dorsal third of the hippocampus. In a recent study (Royer et al., 2010) directed to highlight the potential differences between the dorsal and the ventral parts of this structure: the spatial selectivity of hippocampal CA1 and CA3 place cells become coarser towards the more ventral pole (Royer et al., 2010), along with a gradual increase in the size of their firing fields, reaching several meters in large environments (Maurer et al., 2005; Kjelstrup et al., 2008). Moreover, reductions in the theta-band (4-10 Hz) local field potential (LFP) and theta-rhythmic neuron

activities are observed in the ventral area. The authors concluded that a shift from spatial to non-spatial representation exists along the dorso-temporal axis. Considering that the ventral hippocampus receives reward-related afferents, it can be further hypothesised that ventral hippocampal neurons might encode salient features of the environment.

Even though all of these findings converge on the hypothesis of a functional differentiation, it is still unclear whether this change is gradual or the dorsal, intermediate and ventral hippocampus might be functionally distinct. In this perspective, it might be crucial to analyse in detail single unit and network activities during the predominant hippocampal oscillations, that is, during theta oscillations (4-10 Hz) (Vanderwolf, 1969; Buzsaki, 2002) and fast 200 Hz events known as sharp wave/ripples (SWR) (Csicsvari et al., 1998; Csicsvari et al., 2000). Investigation of SWRs events is particularly important as these events are linked to memory consolidation. It is thought that hippocampus temporarily stores memory traces of waking experience, and during SWRs these memory traces are transferred to extrahippocampal location for their consolidation. As a support of this hypothesis it was shown that waking activity patterns are reactivated during sleep and these sleep patterns during SWRs can predict future memory performances.

The main aim of my project is to investigate communication between dorsal and ventral CA1 region in the rodent hippocampus, eventually providing evidence for

for the hypothesised role played by the ventral hippocampus in coding of behavioural-relevant places such as salient locations. This goal will be achieved by using parallel multi-channel extracellular recording in the dorsal and ventral portions of the hippocampus, during different behavioural states and context, including sleep free exploration and goal-directed navigation.

1. In chapter 4, I will characterise theta oscillatory network patterns in the ventral hippocampus and reveal how this activity is related to dorsal patterns. Given the behavioural dependency of theta oscillations, I will examine separately periods of exploration and sleep, looking at the temporal dynamics across the two CA1 sub-fields and the population response to local and distal events.
2. In chapter 5, I aim to investigate the nature of representations in the dorsal and ventral sub-regions of the hippocampus looking at the dorsal and ventral CA1 activity during spatial learning. In particular, I will examine the theta dynamics within and across the two sub-regions in relation to goal-directed behaviour. I will also search for the emergence of cells 'firing at goal locations in the ventral CA1, comparing the firing selectivity of ventral pyramidal cells and dorsal goal cells previously described.
3. In chapter 6, I will examine whether waking firing patterns of CA1 activity established during spatial learning are reactivating in sleep. Considering the relevance of hippocampal SWR patterns in the phenomenon, I will first

investigate the profiles and temporal relationship of those fast oscillatory events in the dorsal and ventral CA1, as well as the relative population responses.

Chapter 3
Materials and Methods

3.1 Surgical implantation of chronic recording electrodes

Under deep anaesthesia using isoflurane (0.5-4 %) and an initial dose of buprenorphine (0.1 mg/kg), 6 male Long Evans rats, aged 3-8 months and weight 250-420 g, were implanted with 16 wire tetrodes.

The tetrodes were built from twisting 4 individual tungsten wires 12 µm diameter (H-Formvar insulated with Butyral bond coat, California Fine Wire, Grover Beach CA), then heated to obtain a single bundle. The tips were gold plated to reduce the electrode impedance to 200-400 kΩ. Tetrodes were loaded in a custom-made microdrive that allows moving them independently (Fig. 1).

During surgery, a craniotomy was performed above the hippocampus (AP= -4.60/6; ML= 2/6) and the dura mater removed. The arrangement in the drive and the length of the tetrodes were designed to reach either the dorsal or the ventral pole of the hippocampus (7 tetrodes targeting dorsal CA1, 9 tetrodes targeting the ventral CA1; difference in length 6 mm between dorsal and ventral tetrodes). The electrodes were implanted with an outward angle of 8° into the cortex until their tips were about 1mm (dorsal) or 1.5 mm (ventral) above the CA1 region (Fig. 1). Paraffin wax

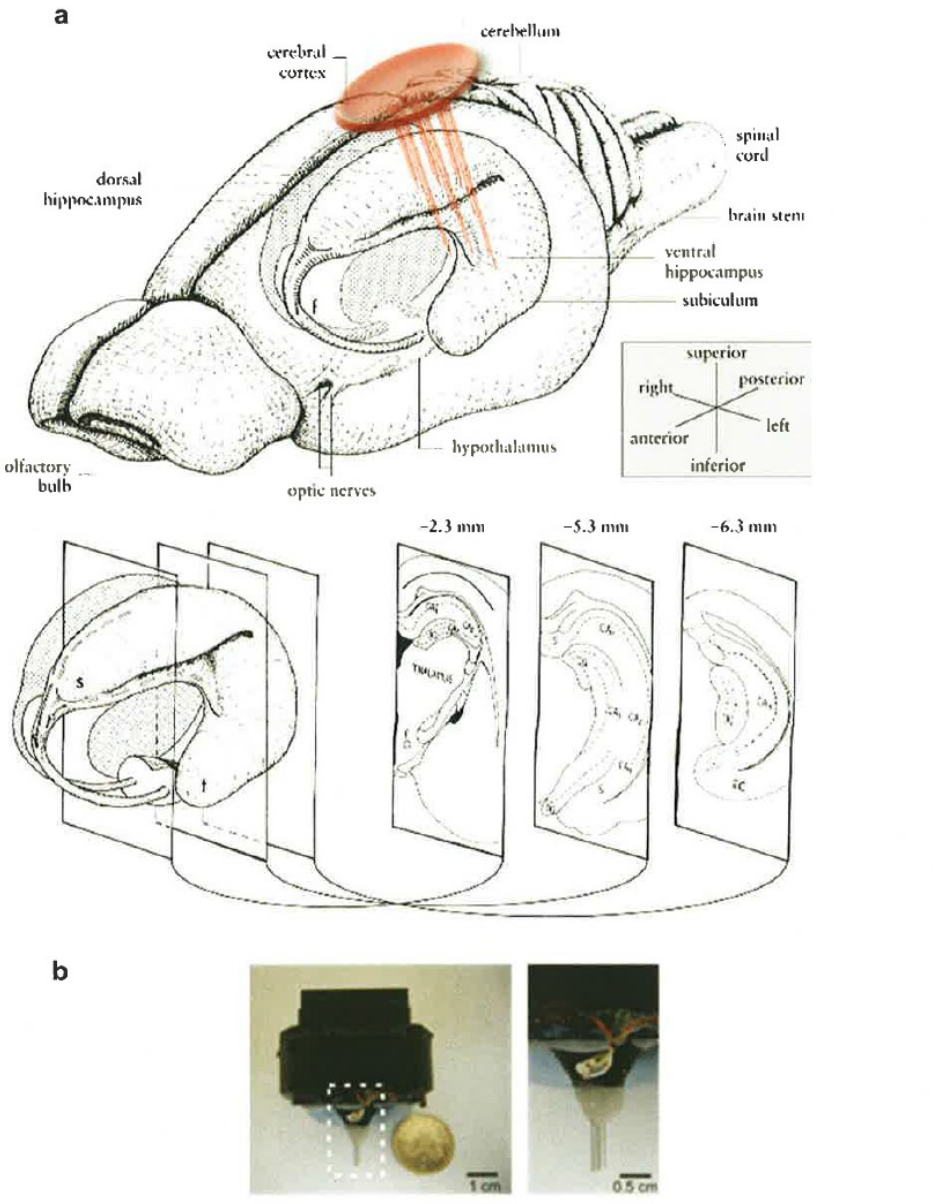


Figure 1. Experimental set-up. **(a)** The three dimensional organization of the rat hippocampus and related structures are illustrated. The microdrive (red) is schematically represented to show the position of the 16 tetrodes: 7 targeting the dorsal CA1, 9 tetrodes tge ventral CA1. At the bottom, CA1, CA2, CA3, DG, EC, fornix (f), subiculum (S), septal pole (s) and temporal pole (t) of the hippocampus are indicated in three coronal sections of the left hippocampus. Adapted from Cheung and Cardinal BMC Neuroscience 2005 (Amaral and Witter, 2004).
(b) A picture of the assembled microdrive. The right panel shows an enlargement of the electodes.

was used to seal the interface between the bottom of the drive and the craniotomy. Two stainless steel screws were inserted through the skull without touching the brain above the cerebellum, and used as ground and reference electrodes. 6 additional screws were used to anchor the microdrive to the skull. Dental acrylic was added to cover the screws and the middle–low part of the microdrive in order to create a durable case on the head of the animal. After 7 days of recovery, the tetrodes were lowered in small steps to reach their target (max ~250 µm in a day).

The electrodes were advanced until the CA1 pyramidal layer was reached. In both dorsal and ventral hippocampi, the CA1 pyramidal layer was identified by the presence of ripple oscillations (150-250 Hz) during immobility/sleep and high density of pyramidal cells and interneurons firing in correspondence to those events in the signal recorded from the electrode. The recordings started when at least one electrode for each targeted sub-region reached the pyramidal layer and the animal was able to perform the task (see paragraph 3.3).

At the end of the experiment, the rats were deeply anaesthetized with pentobarbital (1 ml/ 200 g), and electrical lesions were made (Intensity 20 µA, duration 8 s). The animals were then perfused through the heart with 0.9 % saline solution followed by 4% buffered paraformaldehyde solution for the post-mortem histological verification of the electrode tracks (Fig. 2).

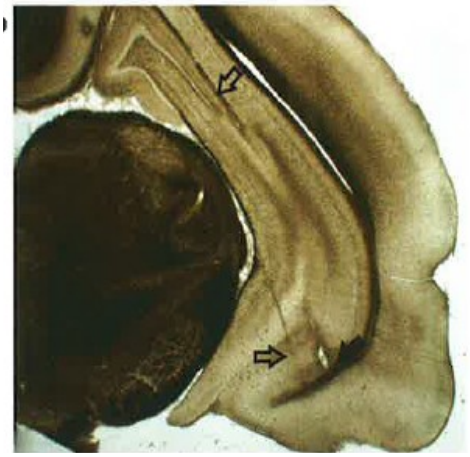


Figure 2. Nissl staining on a coronal section showing electrode tracks and electrolytic lesions marking the tips of electrodes (open arrows). Notice that the electrode tips in the CA1 region of the ventral hippocampus.

3.2 Recording and tracking

Wide band (0.1 Hz – 5 kHz) recordings were taken and then amplified local field potential (x1000, via a 64-channel Sensorium Amplifier, Charlotte, VT, USA) and multiple-unit activity were continuously digitalized at 20 kHz using a 64-channel analog-to-digital converter computer card (United Electronics Industries, Canton, MA, USA). Two 34-channel unity gain preamplifier panels (Axona Ltd, St Albans, Hertfordshire, UK) were used to reduce cable movement artefacts. An array of three LED clusters (blue, red, green) mounted on the preamplifier headstage was used to track the position of the animal (25 frames/s) through a digital video camera mounted at the top of the maze (Sony, Japan – 39,06 Hz sampling frequency). The animal's location was constantly monitored throughout the daily experiment and the data were analyzed offline. In parallel to the wide-band acquisition of the voltage data, a custom-made unit was generating square waves in a way to synchronise the tracking and the local field potential (LFP).

The analysing programs were written in C language and ran under Linux operating system (Fedora core 9-14, http://fedoraproject.org/). The statistics was performed using STAT 5.4 UNIX software package (Perlman, 1980) and mainly custom-made software.

3.3 Behavioural apparatus and training procedure

Rats were trained to perform the retrieval of the hidden rewards on a goal directed behavioural task referred to as cheeseboard maze task (apparatus: Kesner et al. 1991; Gilbert et al., 1998; task: Dupret et al., 2010). Briefly, the maze consisted of a circular arena of 120 cm in diameter made in plastic and painted black, standing 55 cm above the floor in the recording room (Fig. 3). 177 food wells (2.5 cm in diameter, 1.5 cm in depth) were evenly spaced into the surface (8 cm between the centers of each well). On the side of the board a wood-made black start-box (27 cm long, 19 cm wide and 59 cm high) was placed perpendicular to the rows of food wells, open at the top to allow tracking the animal inside and equipped with a frontal door (35 cm high).

Rats were trained to find three hidden food rewards placed in randomly selected wells. The locations were maintained fixed in a day, but changed from day to day. A black curtain surrounded the maze, avoiding the effect of any external cues. The training was performed after surgical implantation when the location of the electrodes was optimized to reach the CA1 region of the hippocampus. After the week of recovery period, rats were reduced to and maintained at 85% of their age-matched preoperative weight. Water was available ad libitum. Each animal was handled and familiarized with both the testing apparatus in the recording room and the general procedure before data acquisition. First, the rat was allowed to freely explore the whole apparatus for at least 30 min during 3 days. Then, the rat was

trained to chase for food-rewards and come back to the start-box. Three groups of visible food pellets (MLab rodent tablet 45mg, TestDiet) were spread out on the surface of the cheeseboard maze while the rat was inside the start box. For each trial, the door was temporarily opened, the animal was allowed to exit the box and retrieve all the rewards while another additional reward was placed in the glass-made cup situated within the start-box. Once all the rewards had been collected, the door was re-opened and the rat was gently directed back to the start box to find and consume the additional food reward within the start box. That procedure was repeated until the rat started to return back consistently on its own after having collected all the rewards within the board (~3-4 days). A similar procedure was applied over the following days, this time with three hidden rewards (i.e., one food pellet per baited location) within the cheeseboard maze (~2-3 days). To prevent the use of odour-cue during these experiments, food pellet dust was scattered across the maze before each experiment, the board was periodically wiped (using the towel used to handle the rat daily). In addition, the board was rotated relative to the start-box during the learning session and between rest and probe sessions. Regarding the learning session, every 3-4 trials the maze was rotated 90 degrees either clockwise or anticlockwise in a pseudo-random way. This initial phase of the experiment ended when the rat was familiar with the whole procedure.

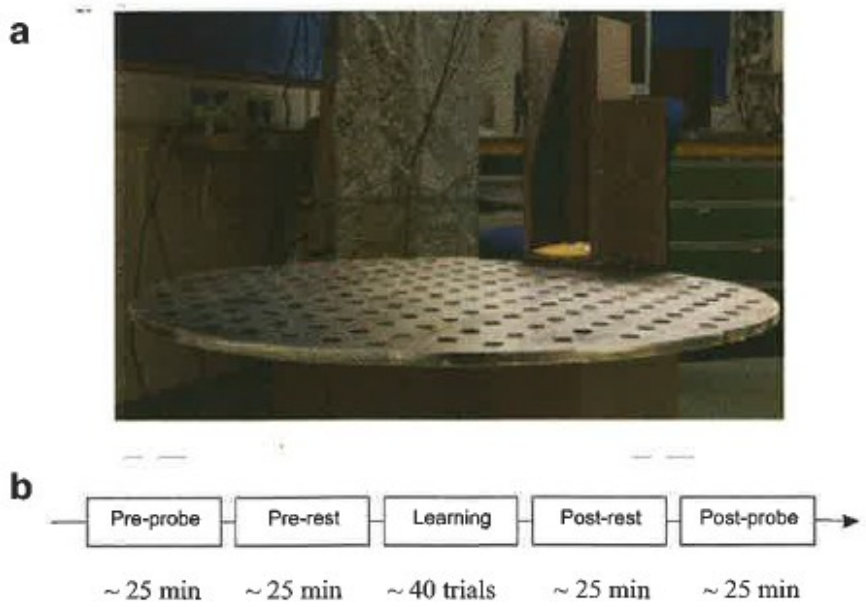


Figure 3. Behavioural apparatus and paradigm
(a) Behavioural apparatus. The photograph shows the cheeseboard maze and the connected start-box on the left side.
(b) Behavioural procedure comprising two no-rewarded probe sessions, one learning session and two immobility/sleep sessions. The sequence and the duration of each single session is indicated. Each recording day began with the "pre-probe" and ended with the "post-probe" which tested memory for old (from the previous day) and newly-learnt goal locations. Adapted from Dupret et al., 2010.

3.4 Behavioural testing

Each daily experiment consisted of a sequence of five recording sessions in the following order (bottom row in Fig. 3): a probe test ("pre-probe"), an immobility/ sleep rest session ("pre-rest"), a learning session, an immobility/sleep rest session ("post-rest") and a probe test ("post-probe"). The two probe tests (~25 min) were never rewarded. After both the pre-probe and the learning sessions, rats were allowed to settle down within the start box for the rest sessions (~25 min). During the learning session, rats were given successive trials (~40 trials) to find the 3 hidden rewards placed in randomly selected food-wells. As these baited locations changed from day-to-day but stayed fixed within a given day, this matching-to-multiple-places procedure required frequent updating of memory for goal locations in an otherwise unchanging environment.

In three animals for one or two consecutive days (see Table 1), the whole protocol was followed by one session (~25 min) in which the animals were left to explore a novel environment without food being provided. In the first day, the new environment was a grey cylindrical arena (with grey vinyl walls 40 cm high) placed on the stem of the cheeseboard maze, in the same area of the recording room. To maintain the novelty effect, the following day the novel apparatus consisted of a large heptagonal maze (1.6 diameter, with walls 25 cm high made by black painted card box) placed on the floor, in the same recording room. For both novel

environments, the floor consisted of Kraft paper (brown paperboard) unscented. After the novel exploratory session, the animals were left to sleep in the original start box (~25 min).

3.5 Unit isolation and clustering

In order to isolate and cluster hippocampal units, the continuously recorded wide-band signals were digitally band-pass filtered (0.8-5 kHz) as described before (Csicsvari et al., 1998). The power (root mean square) of the filtered signal was calculated in a sliding window of 0.2 ms for action potential detection. The standard deviation was calculated to estimate the variance of the baseline noise and to set a detection threshold. Spikes with a power of more than five times standard deviation from this threshold were selected. The spike features were then based on the principal component analyses. Automatic clustering software (http://klustakwik.sourceforge.net/; Harris et al., 2001) was used to segregate the detected action potentials in clusters belonging to putative individual units. The operation was manually refined by a graphical cluster cutting program (Csicsvari et al., 1998). Similar behavioural sessions were grouped to analyse changes in the firing patterns of cells over different states. Stability of the recorded units was verified by plotting spike features over time, by plotting the two-dimensional unit cluster plots

in different sessions and the constancy of waveform as well. Only units with clear refractory periods in the autocorrelation histogram were considered, meaning that cells with a certain amount of spikes in the ±2 ms bins were discarded. Clusters whose boundaries were not well defined (based on measuring the Mahalanobis distance between clusters; Harris et al., 2001) were also rejected.

3.6 Cell classification and spatial firing properties

Hippocampal units were classified as pyramidal cells or interneurons based on several extracellular features such as firing rate, waveform, spike waveform, spike width, asymmetry (Bartho et al., 2004;Csicsvari et al., 1998;Csicsvari et al., 1999b;Nitz and McNaughton, 2004). As previously described, typically interneurons were distinguished for having firing rate higher than 5 Hz and firing mode (the first moment of the autocorrelation function) of ~12 ms, whereas pyramidal cells were characterized for firing less than 10 Hz and having ~7 ms firing mode. Together, these two parameters allowed the strongest differentiation between putative interneuron and pyramidal cells in the dorsal pyramidal cell layer (Csicsvari et al., 1999b). No differences were noticed comparing the autocorrelation function of ventral units and well-studied dorsal units, therefore the same method of classification was applied. It is noteworthy to notice that, as we will see in the results, the firing of pyramidal cells in the ventral CA1 was generally higher than in the dorsal sub-region.

In 6 animals, totally 361 cells were identified and recorded in the dorsal CA1 pyramidal layer or stratum oriens. Of these, 310 were classified as pyramidal cells and 51 as interneurons. In the ventral CA1, 118 and 42 interneurons were identified. A further isolated 62 units were discarded from further analysis either because their anatomical location was estimated to be outside the hippocampus or in one case in ventral CA3, as suggested by histological verification and from the field analysis.

Spatial rate-maps were calculated as in prior work (for complete descriptions see Harris et al., 2001; Huxter et al., 2008; O'Neill et al., 2006). Briefly, I divided the camera view into a 2-dimensional array of bins, each representing ~10 cm2 within the recording environment. Both raw firing rates and occupancy were smoothed across the 2-dimensional array of bins by a kernel-based method using a Gaussian kernel smoothing function (SD=2 cm). Firing rates in each bin were taken as the number of spikes fired in the bin divided by the total time spent there (both smoothed).

3.7 Detection of LFP and oscillations

Definition

As described above, the LFP was generated by low pass filtering and then down-samping to 1252 Hz the wide band signal (Csicsvari et al., 1998). For each rat and for each day of recordings, I used one of the tetrodes in the dorsal CA1 pyramidal cell layer to detect periods of theta oscillations in the dorsal sub-region.

Each recording was separated offline into exploratory activity, immobility and sleep periods. For each of those sessions, the ratio of theta (5-12 Hz) to delta (2-4 Hz) power was calculated in 1600 ms windows, using Thomson's multi-taper method (Mitra and Pesaran, 1999; Thomson, 1982), as previously described (Csicsvari et al., 1999b). The behavioural state was then manually identified plotting this ratio in parallel to the running speed of the animal. The high threshold for the onset of theta periods was set to a ratio of 2 with a second low threshold of 1 for the end of these periods. Exploratory epochs included both locomotion and the presence of theta oscillations in the dorsal electrodes (seen in the theta-delta ratio) for 2.4 s interval. Waking immobility epochs were selected when speed was lower than ~2 cm/s and theta-delta ratio fell below the threshold of ~1 theta/delta ratio for at least 2.4 s time-windows. This off-line procedure allowed us to remove false detection of short segments containing movement artifacts. Sleep epochs labelled during the

experiment were confirmed offline by the presence of continuous immobility and occasionally REM-theta periods. Therefore periods classified as sleep included both real sleep and waking immobility. REM periods were removed from further analysis because they were too short in time.

The same procedures were repeated on a selected electrode located in the ventral CA1 pyramidal cell layer to detect periods of theta oscillations in the ventral sub-region.

Theta amplitude/ phase detection and spectral analysis

The procedures for detecting theta waves on each single electrode were performed using custom-made software as previously described (Csicsvari et al., 1998; Csicsvari et al., 1999b). Off–line, for each tetrode the EEG signal (LFP) of each channel was passed through a bidirectional digital Butterworth filter (2 Hz high–pass) and averaged with a 21 ms sliding window to remove high–frequency components. Time periods identified as waking exploration (see above) were band-pass filtered between 5-28 Hz using a Fast Fourier Transform (FFT) based filter. This band was chosen empirically to avoid phase delays in peak detection. So, power spectra were constructed by performing the fast Fourier transform, where the square modulus of each Fourier frequency coefficient represents the signal power at that frequency. The power spectrum was smoothed using a Gaussian kernel with an SD of 0.2 Hz A sinewave was fitted to the interval between pairs of positive–to–negative zero crossings in the filtered EEG signal, provided the interval fell within the theta

frequency range (5–12 Hz). The negative peaks within the selected theta epochs were used to build an amplitude histogram. The negative peaks of the theta waves were preferred to the positive peaks because more prominent in the hippocampal CA1 pyramidal layer (Csicsvari et al., 1998; Csicsvari et al., 1999b). A threshold for the identification of the oscillations was calculated by iteratively selected a range of threshold values starting with the lowest amplitude and progressing to the largest. For each value, the negative peaks were detected and counted. The threshold which resulted in the number of detected theta cycles closest to the expected 5-12 Hz theta activity was then considered the optimal value. This procedure enabled the identification of theta intervals and number of theta cycles in each session for each individual tetrode.

The mean theta frequency defined as the mean number of theta cycles per second, was calculated for each session as the inverse of the mean duration of a single wave ("Period") detected in the power spectrum.

The coherence analyses were conducted only based on power correlations. To calculate power correlations within and across sub-regions, the EEG signal from selected pairs of electrodes (one channel each) was band-pass filtered in the theta range (5-12 Hz) and power spectra obtained using the FFT and a sliding windows of 800 ms with a 50 % overlap. The coherence was calculated as the squared covariance at theta frequency between the two signals considered. In all figures in Chapter IV and V, the values plotted refer to the average over recording days and

rats, and error bars indicate the SE. Multiple Student's t-test were used for statistical comparison of measurements obtained (1) across and within the two CA1 sub-regions and (2) of sets of data corresponding to distinct recording sessions over days and animals. The independent samples t-test (unpaired t-test) was used when two separate sets of independent samples were compared (i.e. dorsal CA1 vs ventral CA1 electrodes). No correction was applied because the standard deviations were equal. When the analysis involved the same matched pairs of data units tested twice, Student's paired t-test was used (dependent t-test for repeated measures). Some concerns about the validity of the use of multiple t-test should be raised for two reasons. First, because the test assumes that each of the two samples belonging to a population normally distributed. Second, because the same data units are grouped repeatedly in different conditions and compared. In some cases through the results, the analysis of variance (ANOVA) might have been more appropriate to reveal hidden interactions between the groups, so to avoid misleading results due to dependent sampling (i.e. data were sampled in clusters) (Zar, 2003).

Analysis of the distribution of spikes from a single cell relative to the phase of detected theta oscillations was performed by Rayleigh statistical testing for circular uniformity (Zar, 2003). Cells with a p-value of < 0.05 were considered to be phase-locked. Watson-Williams circular test was used to assess the statistical significance of the population shift in the preferred phase of theta oscillations.

Detection of SWR events and ripple oscillation

For the detection of SWRs, recorded local field potentials were first band pass filtered (150-250 Hz), using a FFT based filter. The complete procedure was completely described before (Csicsvari et al., 1999a; O'Neill et al., 2006). Briefly, muscular noise and other high frequency artefacts were removed by differential subtraction of the filtered signal from a control electrode offline selected as checked to be placed outside the CA1 pyramidal layer. The power of the differential filtered signal was calculated for each electrode and summed across electrodes designed as being located in the CA1 pyramidal cell layer. The power was so calculated by summing the squared sample values within a sliding window (16 ms) and calculating the square root of this sum (root mean square). The mean and SD of the power signal were calculated to determine the detection threshold. The increase in power occurring during SWR events allows the detection of the beginning, peak and end of individual ripple episodes. The threshold for ripple detection was set to 7 SD above the background mean for dorsal CA1 electrodes, and 5 SD above the background mean for ventral CA1 electrodes. The beginning and the end of the oscillatory periods were identified as the points at which the power reduced to less than 1 SD. The positive and negative peaks of every wave identified on each CA1 tetrode were detected and included just in case the amplitude of the wave was above 1 SD

relative to the mean. SWRs epochs were finally separated into those occurring during theta periods (eSWRs), and those during sleep and rest (O'Neill et al., 2006).

3.8 Reactivation

Sleep reactivation of waking activity patterns was assessed by correlating the tendency of pairs of cells to fire together (cofiring) during learning with the cofiring measured in the rest session (before or after learning). Cofiring was so defined as the joint firing patterns of groups of cells during that session (Wilson & McNaughton, 2004). To measure the cofiring, I first established for each cell its instantaneous firing rate counts (IFRC) in 100-ms windows. When cofiring was calculated during slow-wave sleep associated SWRs periods, the time windows were centred on the peak of SWR. The Pearson correlation of the instantaneous firing rates of the paired cells in each window was established in pre-sleep, learning, and post-sleep sessions (O'Neill et al., 2006; O'Neill et al., 2008). Only those correlations in which both cells fired at least 20 times in the measurement time windows were considered. Cofiring reactivation was then assessed by comparing the correlation of the rest session with the learning cofiring, along the appropriate list of cell pairs. As the rat explored the maze before learning, reactivation during sleep after learning might reflect also activity from the pre-probe session. To control for those prior associations reflecting maze exploration before learning as well as baseline correlations I used a partial correlation to assess reactivation of learning waking patterns, correlating the joint

firing activity during learning with sleep cofiring after learning while controlling for sleep cofiring before learning (Pennartz et al., 2004; Tatsuno et al., 2006; O'Neill et al., 2008). The correlation was reversed to assess the degree to which baseline correlations existed in the pre-sleep session.

When the reactivation of waking firing patterns was investigated across CA1 regions, cell pairs were composed of one dorsal cell and one ventral cell simultaneously recorded.

Individual cell reactivation was also computed when at least 15 cells were simultaneously recorded from the other CA1 sub-region, with which to calculate cofiring. When single correlation values are shown in some figures the error bars indicated the standard error of the correlation coefficient (±SE). Regressions were compared using a Fisher's Z-test of the Pearson correlation coefficients (Zar, 2003).

Animal	Day	Dorsal CA1 pyramidal layer electrodes	*Pyr*	*Int*	Ventral CA1 pyramidal layer electrodes	*Pyr*	*Int*	Behaviour protocol
JC87	1	3	16	3	2	13	3	CB
	2	4	21	2	2	12	1	CB
	3	4	20	2	1	14	0	CB
JC88	1	3	22	0	3	20	2	CB
	2	5	33	3	2	13	2	CB
	3	5	30	3	2	14	3	CB
JC93	1	2	10	4	2	11	4	CB
	2	3	17	3	3	18	4	CB + N
	3	2	12	4	3	24	5	CB + N
	4	2	8	3	1	8	1	CB
JC98	1	6	48	3	Ventral CA3	16	4	CB
	2	5	41	4		12	3	CB + N
	3	6	44	1		14	0	CB + N
JC100	1	5	27	2	1	7	3	CB
	2	6	30	3	1	6	4	CB + N
	3	6	33	4	2	16	0	CB + N
	4	6	36	3	2	18	0	CB
JC102	1	7	44	3	2	4	5	EX
	2	7	38	3	3	18	5	EX
	3	8	55	4	4	33	3	EX
	4	6	36	2	4	22	6	EX
Jc104	1	4	21	3	2	26	3	CB
	2	6	34	3	3	24	2	CB + N
	3	7	32	4	3	23	3	CB + N
	4	7	36	3	1	16	0	CB

Table 1. Animal records. List of recorded units (Pyramidal cells and interneurons) and rec

Chapter IV
Oscillatory patterns along the dorso-ventral axis of the hippocampus: theta oscillation

Introduction

The network state of the hippocampus can be defined by oscillatory patterns. Oscillations are considered to constitute a spatio-temporal frame for the activity of neuronal ensembles within and across brain regions. Slower oscillations can coordinate the activity of large neuronal population, allowing communication across distal regions, whereas fast oscillations can enable local circuit processing, recruiting a smaller numbers of neurons. The most prominent frequency bands in ascending order are: theta oscillations (5-12 Hz), gamma oscillations (30-100 Hz) and sharp-waves associated to ripple oscillations (150-200 Hz). Each of these patterns is thought to serve a distinct and specific function in the hippocampal operations, and in general brain computations.

Theta oscillations dominate the hippocampal field potential during explorative behaviour (Vanderwolf, 1969) and REM sleep (Jouvet, 1969). The expression of theta oscillations has been strongly related to the process of learning (Lisman and Idiart, 1995; Buzsaki, 2005; Vertes, 2005) and representation of space (Foster et al., 1989; O'Neill et al., 2006) in which the hippocampus is involved. In association with sensory and motor inputs, other variables clearly affect the

amplitude and frequency of the theta signal along the septo-temporal axis of the hippocampus. It has been proposed that those changes would reflect a functional dissociation towards the temporal pole between theta oscillations and the processing of sensory information, which favours of attention to novelty or meaningful stimuli as reward (Bland 1986; Vinogradova 1995; Buzsáki 2002; Montgomery et al. 2008; Benchenane et al. 2010). The different nature by which information is brought by theta oscillation along the dorso-ventral axis of the hippocampus is supported by a systematic decrease in the relationship between theta power and locomotor speed towards the temporal pole (Hinman et al., 2012). Moreover, theta epochs in the temporal hippocampus seem to diminish over repeated behavioural experience (running episodes), while theta in the septal hippocampus does not. Therefore, septo-temporal variation in the theta signal can also be related to present and past experience. A recent model predicts that the mechanisms of hippocampal theta generation relative to spatial navigation and arousal-anxiety process underlie dissociable aspects of the theta frequency-speed relationship (the slope and intercept, respectively). In a recent paper, Wells and colleagues were able to isolate two components of theta generation operating in parallel in behaving animals and link them to anxiolytic drug action, novelty, and the metric for self-motion. Indeed, they showed that variation in slope predicted changes in spatial representation by CA1 place cells and novelty-responsive

behaviour, whereas variation in the intercept predicted anxiety-like behaviour (Wells et al., 2013).

In a study, Lubenov and Siapas (2009) have observed that the phase of theta waves advances systematically in the proximo-distal direction of the dorsal hippocampus (Lubenov and Siapas, 2009) and proposed a full cycle (i.e., 360°) phase shift between the septal and temporal poles of the hippocampus. The implication of their hypothesis is that outputs from the two poles of the hippocampus would affect their distinct targets in a temporally synchronous way. However, this prediction has been rejected by Patel et al. (2012), providing evidences only for a 180° shift in the theta phase between dorsal and ventral CA1 regions. They suggested that the hippocampus fulfils different function by the two sub-regions temporally desynchronised on occasions.

In order to shed light on the proposed hypothesis about the nature of theta oscillatory content along the dorso-ventral axis and the mechanisms of generation of those oscillatory patterns, in this chapter I will first investigate the physiological features of theta oscillations in the dorsal and ventral CA1 of the hippocampus during behavioural exploration and REM sleep. To study the spatial organization of theta oscillations, correlation in power, phase-shift, frequency and coherence of theta oscillatory events from the two poles of the hippocampal CA1 layers will be examined.

Results

Recordings were done during period of exploration and sleep. For the following analysis, unless stated, exploratory periods refer to probe sessions, in which the animals move on the cheese-board maze in absence of reward (see Materials and Methods). Sleep included pre and post sleep epochs, preceding and following the learning session.

4.1 Theta Oscillations along the dorso-ventral axis

Oscillations in the theta-band (5-12 Hz) were detected simultaneously in the pyramidal layers of the dorsal and ventral CA1 of the hippocampus, during exploration and REM sleep. Considering the behavioural dependence of theta oscillations, I first focused on the patterns occurring during exploration. As shown in the raw traces in figure 1A, in contrast to the continuous and rhythmic theta

oscillatory patterns observed in the dorsal CA1 pyramidal layer, theta oscillations were not easy to distinguish visually in the ventral CA1 region. Theta patterns were less prominent in the local field potential recorded at ventral sites and, when occurring in both sub-regions, the amplitude of ventral theta oscillations was smaller than the dorsal one. Consistently, the power spectra of simultaneously recorded local field potentials calculated on dorsal and ventral electrodes, exhibit a smaller peak at theta frequency for the ventral electrode (Fig. 1B). My observations are in agreement with earlier reports in the ventral CA3 sub-region (Royer et al., 2011; Patel et al., 2012).

It is known that both the amplitude and phase of theta oscillations vary as a function of depth within the hippocampus: in the dorsal CA1 region the amplitude is largest in the stratum lacunosum-moleculare. In addition, there is a 180° reversal of phase between lacunosum-moleculare theta and that in the CA1 pyramidal layer as a consequence of the postulated existence of two different theta current generators (Bland et al., 1975). To assess whether this is reliable along the dorso-ventral axis, I proceeded in calculating the average field profile of theta oscillations in dorsal and ventral CA1 subfield, relative to theta waves detected either at a dorsal or a ventral electrode located in the pyramidal layer (Fig. 1C). The averaged theta oscillation profiles showed again that the amplitude of the waves was larger in the dorsal sites. Still in both CA1 subfields, the amplitude was higher in the stratum radiatum layer. Looking at the phase of the theta waves, the ventral sites were out of phase

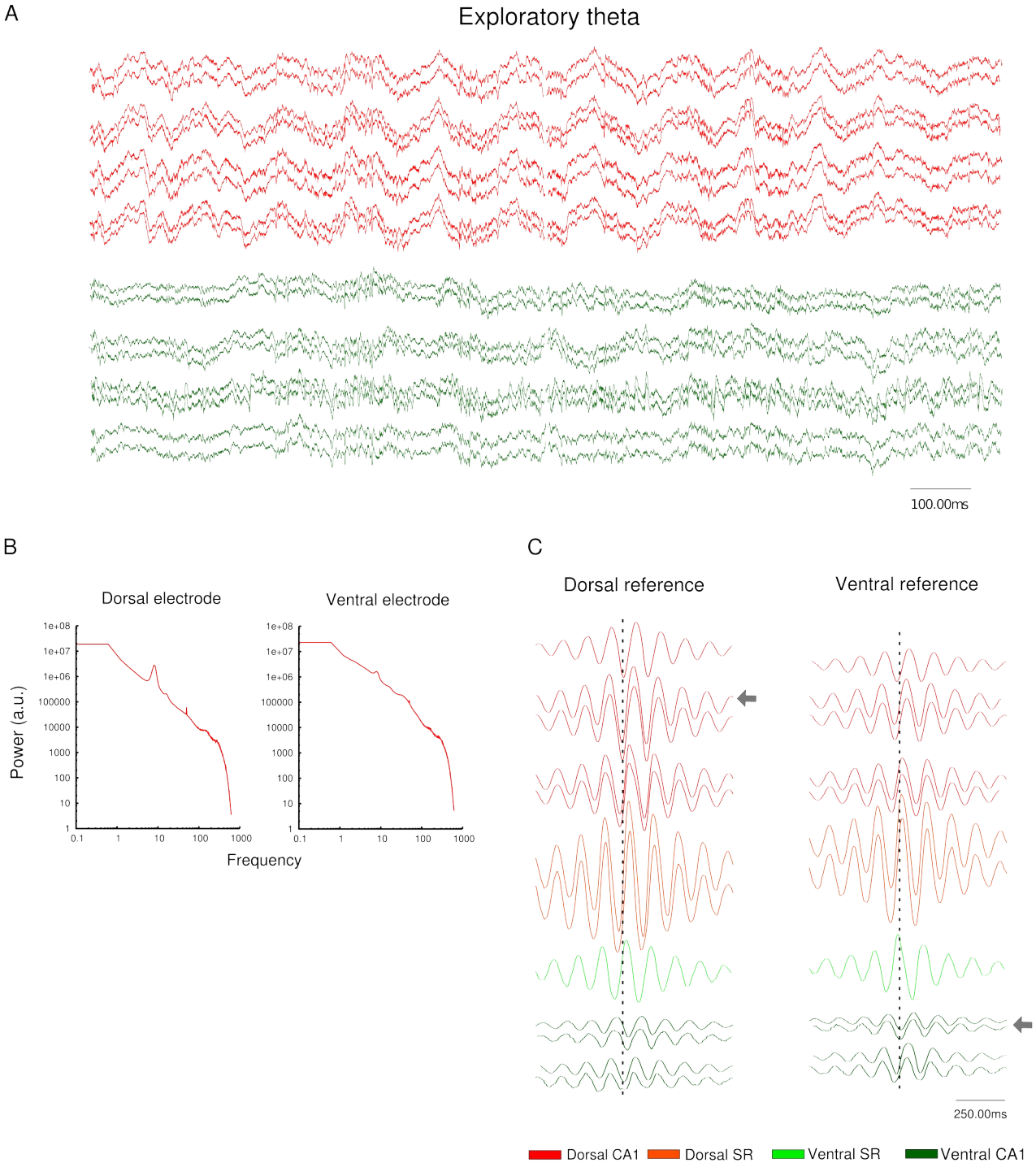

A
Exploratory theta
100.00ms
B
Dorsal electrode
Ventral electrode
Power (a.u.)
1e+08
1e+07
1e+06
100000
10000
1000
100
10
1
0.1
1
10
100
1000
Frequency
C
Dorsal reference
Ventral reference
250.00ms
Dorsal CA1
Dorsal SR
Ventral SR
Ventral CA1

Figure 1. Theta oscillations along the long axis of the hippocampus during exploration
(A) Example raw traces representing wide-band (1 Hz-5 kHz) potentials from tetrodes simultaneously recorded in dorsal (red) and ventral (green) CA1 pyramidal layers. Theta oscillations in the ventral recording sites are not easy distinguishable.

(B) Representative examples of the power spectra of the LFP recorded from the dorsal ventral CA1 pyramidal layer. Note the smaller peak at theta frequency (5-12 Hz).
(C) Average field potential profile of theta oscillations relative to theta waves detected at one dorsal electrode or one ventral electrode; reference electrodes are marked by arrows; the vertical dotted line marks the negative peak of the theta wave at the reference electrode. The displayed electrodes are positioned in the dorsal (red) and ventral (dark green) pyramidal layers, but one electrode in the dorsal stratum radiatum (SR) and one in ventral SR (orange and light green respectively). Note how the phase is gradually shifted from one layer to another.

relative to the dorsal pyramidal layer but, as expected, the degree of phase-shift varied amongst different ventral recording sites. The phase indeed was gradually shifted from one layer to another, with a phase reversal of almost 180° between the pyramidal and stratum radiatum layers in both sub-regions. As a result, theta waves in the dorsal and ventral pyramidal layers were in-phase. No difference emerged by changing the reference used.

4.2 Correlation of power and coherence of theta oscillations within and across hippocampal CA1 regions.

To investigate the relationship between the theta-band oscillatory events in the dorsal and ventral CA1 sub-regions, I examined the correlation of instantaneous theta-band power within region and across regions (Fig. 2A). For this analysis, I did not distinguish theta epochs according to behavioural state, so the power of the local field potential in exploration and REM sleep were analysed together. All the 6 animals recorded were included in those analysis. While the within sub-region correlation values were relatively high for dorsal CA1 (n = 52 electrode pairs$_{D/D}$, mean r-value = 0.621 ±0.025) and for ventral CA1 (n = 21 electrode pairs$_{V/V}$, mean r-value = 0.592 ±0.048) sites, the correlation dropped significantly across regions (n = 112 electrode pairs $_{D/V}$, mean r-value = 0.257 ±0.012) (df $_{DD/DV}$ = 162/ df $_{VV/DV}$ = 131 ,

p<0.01, unpaired t-test). This suggests that the oscillations in the two areas are not always related.

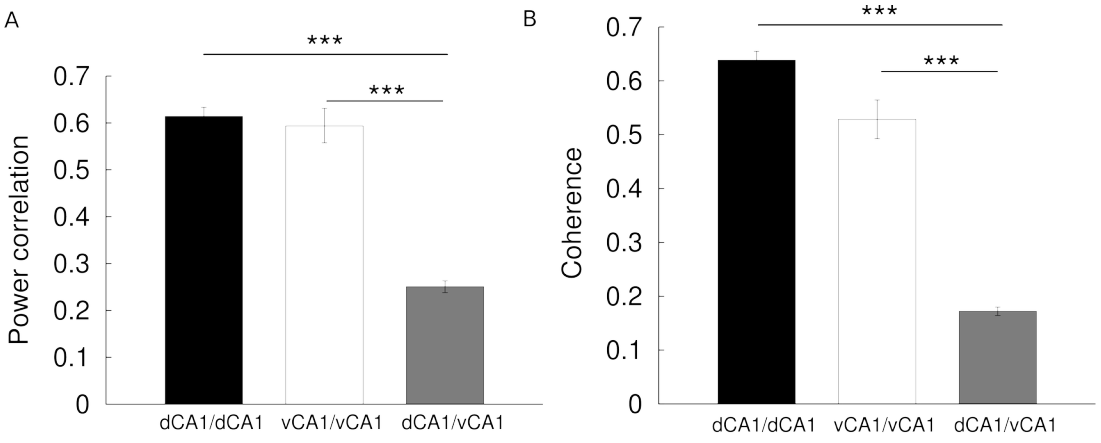


Figure 2. Correlation of instantaneous theta-band power and coherence within and across regions

(A) The power-power correlation in the theta band is significantly smaller between dorsal/ventral electrodes than between dorsal ones or ventral ones.

(B) Coherence in power decreases significantly across regions, but also between ventral electrodes than between dorsal ones. Theta oscillations during REM sleep sessions are not considered in the calculation.

Furthermore, similar results were obtained when I looked at the theta-band coherence between the dorsal and ventral CA1 subfields (Fig. 2B). The coherence in theta-band across regions (n= 112 electrode pairs $_{D/V}$, 0.177 ±0.003) was significantly lower than within either dorsal (n= 52 electrode pairs $_{D/D}$, 0.638 ±0.006) or ventral (n= 21 electrode pairs $_{V/V}$, 0.526 ±0.046) theta signal ($df_{DD/DV}$ = 162/ $df_{VV/DV}$ = 131, $p<0.01$, unpaired t-test).

4.3 Speed modulation of theta power along the septo-temporal axis

It is established that theta oscillations during exploration are affected by the speed of the animal. Indeed, it has been suggested that theta is part of the complex sensory-motor integration system animals use to navigate in an environment, keeping track of the actual location and distances to incorporate in the spatial map (Vanderwolf, 1969; O'Keefe and Recce, 1993). Both the power and frequency of theta oscillations increase linearly with running speed in the dorsal hippocampus. As discussed in the introduction, a functional dissociation towards the temporal pole between theta and the processing of spatial information can be seen in a weaker relationship between locomotor variables such as speed and theta parameters.

In order to test whether this dissociation is taking place, I examined how the running speed was related to the power of dorsal and ventral theta oscillations in all 6 animals. First, for each theta oscillatory event detected at electrodes located in the dorsal and ventral CA1 pyramidal layers, I calculated the instantaneous running

speed of the animal and correlated it to the theta-band power of that event. As shown in figure 3A, the power of theta waves detected at dorsal sites resulted positive correlated to speed (mean r value = 0.118 ±0.005, n = 473 theta events, $p<0.05$). In contrast, no speed modulation was observed for the power of theta in the ventral sites (mean r value = 0.015 ±0.006, n = 473 theta events, $p=0.23$).

Because of the decrease in the mean power coherence between the septal and temporal poles (see previous paragraph), I reasoned that the influence of speed might differ if I considered periods in which the local field potential of the two sub-regions oscillated coherently in the theta band. To test it, I selected periods in which the coherence between dorsal and ventral CA1 pyramidal layer was higher than the mean coherence plus twice the standard error (high coherence >=0.183, n = 242 theta events), and periods in which the coherence was lower than the mean coherence minus twice the standard error (low coherence <=0.171, n = 231 theta events). As shown in figure 3B, a positive correlation between the power of ventral theta and the speed emerged during high coherence epochs (mean r-value = 0.021 ±0.006, $p<0.05$), whereas the same variables were negative correlated when I considered low coherence epochs (mean r-value = -0.0168 ±0.003, $p<0.05$). The difference in the correlation values was statistically significant ($df = 470$, $p<0.0001$, paired t-test). Still, the correlation between theta power and speed (low coherence: 0.078 ±0.001; high coherence: 0.089 ±0.001) was significantly larger for dorsal sites than ventral sites in both conditions ($df_{lowcoh} = 460$, $df_{high\ coh} = 482$, $p<0.001$, unpaired

t-test). Finally, I examined whether strength of power-speed modulation in the dorsal regions predicted that in the ventral region. To this end, I performed the regression between theta power-speed correlation values measured at different dorsal and ventral sites across different animals and recordings sessions. The correlation coefficient was positive when epochs with high dorso-ventral coherence were considered (mean r-value across high coherence epochs = 0.398 ±0.080, n = 242 theta events, $p<0.05$) but they were not correlated at low coherence epochs (mean r-value across low coherence epochs = -0.014 ±0.077, n = 231 theta events, $p>0.5$).

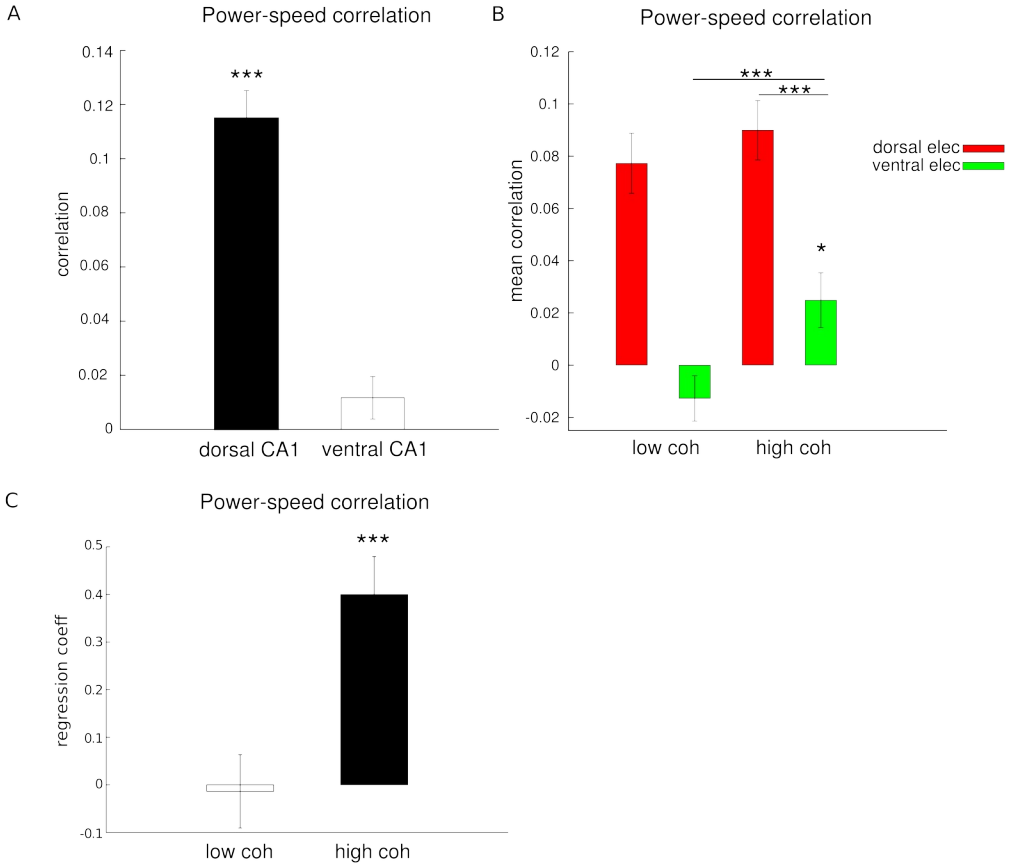


Figure 3. Speed modulation of theta power

(A) Theta power in the ventral CA1 is not significantly correlated to sleep, in contrast to the speed modulation observed for dorsal theta.

(B) The power-speed correlation at times when coherence between dorsal and ventral pyramidal layer theta was high and low. The mean correlation is calculated separately when theta power coherence between dorsal and ventral CA1 electrodes is higher or lower than the mean coherence value. Note the change from negative to positive correlation for ventral theta at high coherence, even though the speed modulation is still lower than for dorsal theta.

(C) Regression between theta power-speed correlation values calculated at dorsal and ventral sites for high and low theta coherence periods. The regression coefficient is not significantly different from 0 at low coherence and positive when the coherence is high.

4.4 Average frequency of theta in the dorsal and ventral CA1 during different behavioural states

I examined the mean frequency of theta oscillations during exploration and REM sleep as well. The mean frequency was obtained by averaging first the periods T of all cycles (distance in time domain between two consecutive negative peaks, see Materials and Methods) for each theta wave event detected at electrodes located either in the dorsal or the ventral CA1 pyramidal layers, and calculated the reciprocals of those values. I found that the mean frequency of theta oscillations changes between the dorsal and the ventral CA1 sites only during exploration, not during sleep (Fig. 4). Indeed, theta recorded at ventral CA1 sites had a significant lower frequency than dorsal CA1 theta during exploration (ventral CA1: 7.88 ±0.01Hz, n = 383 theta events $_V$; dorsal CA1: 8.2 ±0.01 Hz, n = 406 theta events $_D$; df = 787, $p<0.05$, unpaired t-test), instead during sleep the difference was not statistically significant (ventral CA1: 7.75 ±0.05, n = 90 theta events $_V$; dorsal CA1: 7.85 ±0.05, n = 86 theta events $_D$; df = 174, p=0.2, unpaired t-test). The comparison across behavioural states reveal that the difference noted in theta frequency between dorsal and ventral CA1 sites was a reflection of the increased frequency of dorsal theta during locomotion, whereas ventral theta oscillations maintained the same mean frequency. Presumably, the effect seen was a consequence of the theta frequency-speed relationship previously discussed.

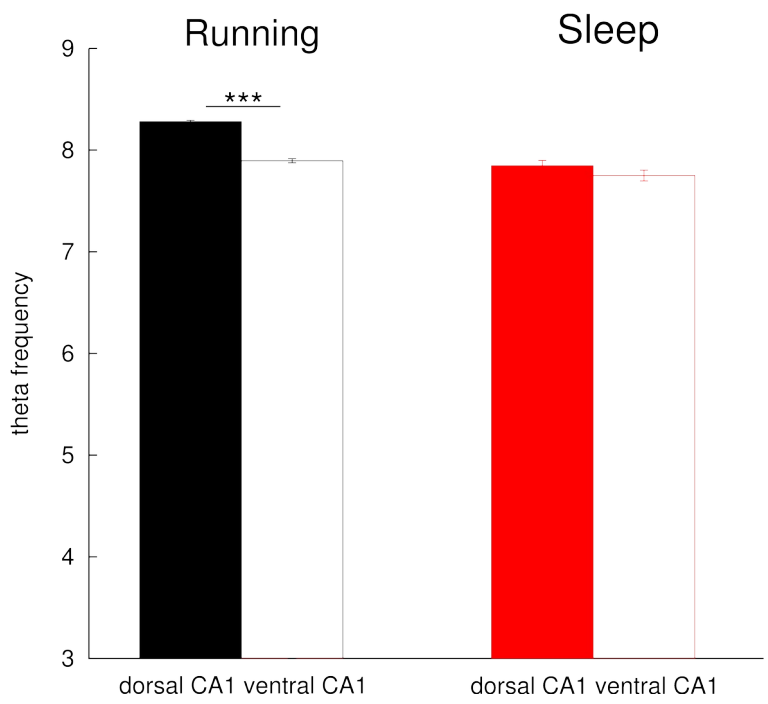

Running
Sleep
***
theta frequency
9
8
7
6
5
4
3
dorsal CA1 ventral CA1
dorsal CA1 ventral CA1

Figure 4. Average frequency of theta in the dorsal and ventral CA1

The average frequency of detected theta waves at dorsal and ventral electrodes is shown separately for exploratory periods and sleep. Note the significant slower frequency of ventral theta oscillations compared to dorsal theta oscillations, only in exploratory periods.

4.5 Theta phase shift between dorsal and ventral CA1 regions

The systematic phase-shift observed in the average field profiles (Fig. 1C), was further investigated looking at the distribution of theta phase difference between all pairs of dorsal and ventral electrodes. In the first place, I proceeded in calculating the phase difference between all the possible pairs of dorsal and ventral CA1 sites for each theta wave event detected simultaneously at dorsal and ventral CA1 regions. I plotted the distribution of the phase-difference between dorsal and ventral electrodes together with the distribution of phase-difference calculated between dorsal electrodes, so to use the latter as reference of the variability within a region (Fig. 5A). In contrast to the clear peak at 0° observed for the dorsal CA1 reference, a broader and multi-peak distribution appeared across the two sub-regions. This indicates variability in the temporal shifts of the local field in the theta-band along the septo-temporal axis.

Because previous results shown here supported the idea that high coherence periods between the dorsal and ventral CA1 sites might underlie flow of spatial information throughout the hippocampus, I repeated the analysis separately for of high and low coherence periods. As shown in figure 5B, the distribution of phase difference was indeed altered in high coherent periods compared to low coherent ones. The distribution was again smooth at low coherence, similar to the general result above, with a maximum probability at 288°. In contrast, a proper tuning was

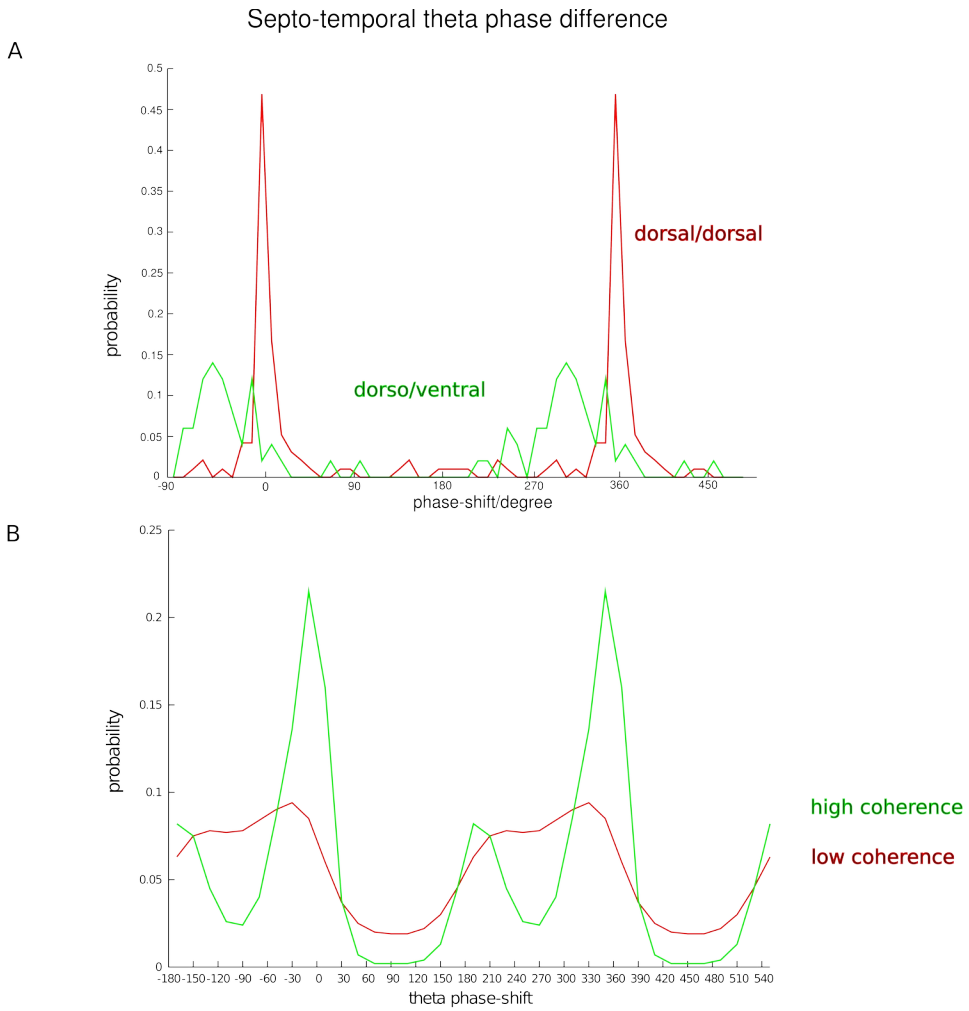


Figure 5. Theta phase shift between dorsal and ventral CA1 regions

(A) Distribution of phase difference of the theta oscillations between pairs of dorsal and ventral electrodes is shown in green. The shift is calculated as the mean difference of the instantaneous phase of detected waves in one electrode relative to the other electrode. For comparison, the phase shift is shown for pairs of dorsal electrodes (red) as well.

(B) The distribution of phase shift between dorsal and ventral electrodes are separately calculated for high (green) and low (red) coherent theta periods. High and low coherence periods are determined as in Figure 3. Note the two peaks at approximately 180° (half cycle) and 360° (one theta cycle) when the coherence

between dorsal and ventral theta is high, whereas at low coherence the phase shift is broadly tuned.

observed during high coherence epochs, revealing a bimodal distribution with a peak at 354° and a second smaller peak at 201°. Those results confirmed what previously obtained by the field average analysis (Fig. 1C); that is, almost a full cycle phase-shift between the septal and temporal poles of the hippocampus. Intriguingly, the temporal shift was present only when the coherence was higher. One explanation for the presence of a second peak might be the misleading positioning of some electrodes, most likely in the stratum radiatum as suggested by the phase-reverse observed (seen also in Fig. 1C).

4.6 Theta phase-locking of dorsal and ventral CA1 cells to local and distal oscillations during exploration

Next, I asked if the theta phase-shift observed between the two regions reflected on the preferred phase at which cells fire action potentials. Because the theta phase-preference of cells depends on the behavioural states, I first focused the attention on times in which the animals were exploring the maze. To address the question, I calculated the phase relationship between unit activity and theta oscillation relative to “local” theta events and relative to “distal” events, recorded from a reference tetrode located respectively in the CA1 pyramidal layer of the same sub-region and in the pyramidal layer of the other CA1 sub-region. For a given cell, each spike was assigned to a given phase (binning factor of 30°) of the normalized theta cycle, and the circular mean of action potential theta phases was summarized

in phase histograms showing the preferred phase only for cells significantly modulated ($p<0.01$, Rayleigh test of uniformity) (Fig. 6).

Generally, it was found that cells throughout the septo-temporal axis of the hippocampus were modulated by both local and distal theta oscillations. When I looked at the percentages of CA1 pyramidal cells significantly modulated (Fig. 6A), I found that less dorsal cells were modulated by distal theta oscillations than by local theta oscillations (local reference=80%, distal reference=48%, n=298). Surprisingly, the effect was reversed for ventral cells (local reference=61%, distal reference=85%, n=97). The phase histograms revealed a quite complex picture for the preferred phase. Dorsal pyramidal cells were, as expected, locked to the through of the local theta, whereas they were not exhibiting a common phase preference relative to ventral theta oscillations. In contrast, ventral pyramidal cells were phase-locked to local and distal theta oscillations, and interestingly they also showed a different preferred phase to the local theta oscillations than dorsal pyramidal cells. In particular, the firing of ventral cells was phase-locked nearly to the peak of the local theta oscillations, but to the descending phase of the dorsal theta waves. Polar plots of preferred phase and modulation depth of single cells (angle and length of the vectors, respectively) were built to show significantly modulated (red or green bars) and not-modulated cells (grey bars), separately for each group of cells and relative to dorsal and ventral theta (Fig. 6A, below phase histograms). To note, the length of the vectors was longer with dorsal reference than ventral reference for both groups of

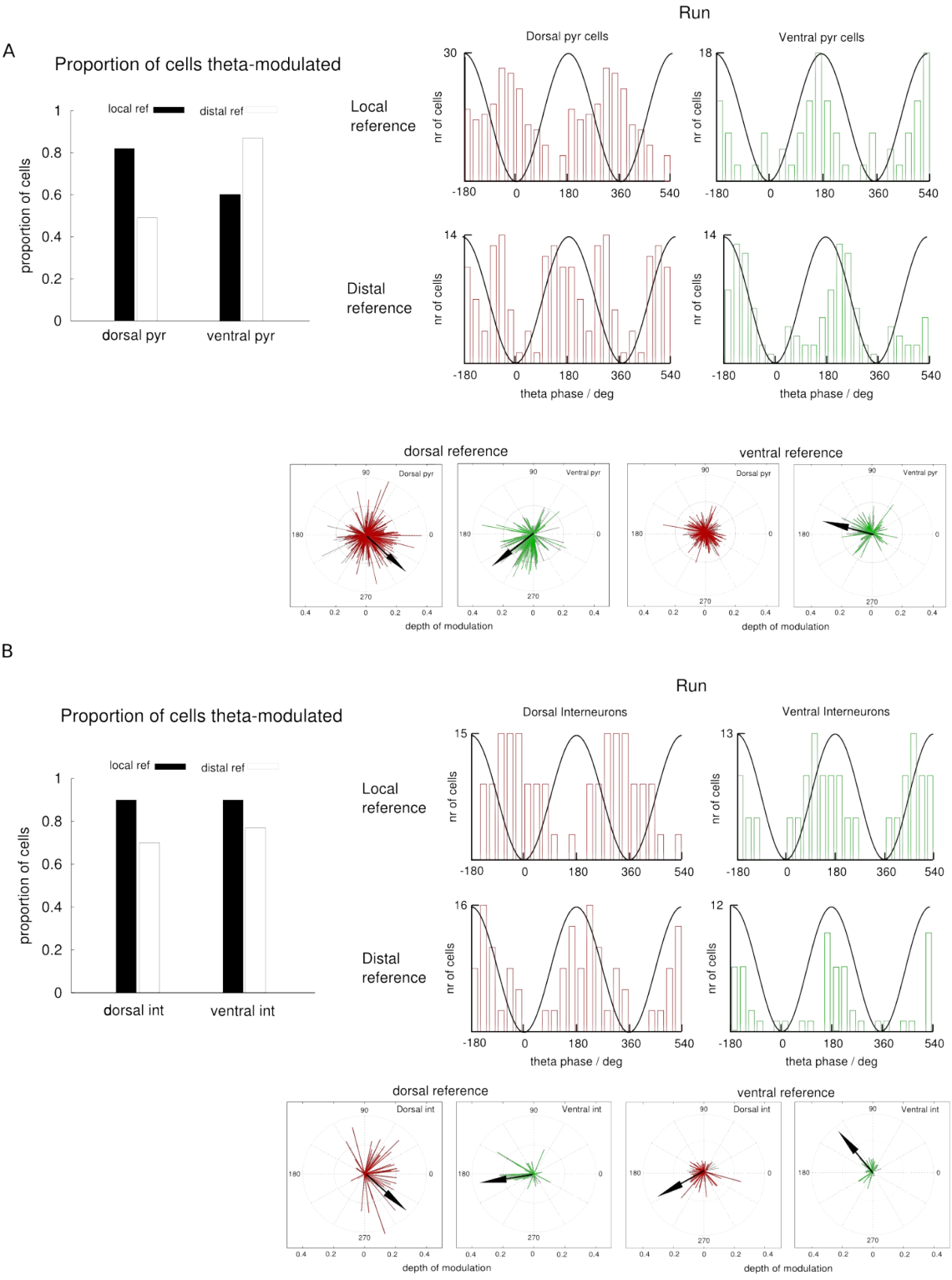

A
Proportion of cells theta-modulated
local ref
distal ref
proportion of cells
dorsal pyr
ventral pyr
Run
Dorsal pyr cells
Ventral pyr cells
Local reference
Distal reference
nr of cells
theta phase / deg
dorsal reference
ventral reference
depth of modulation
B
Proportion of cells theta-modulated
dorsal int
ventral int
Dorsal Interneurons
Ventral Interneurons

Figure 6. Phase-locking of dorsal and ventral CA1 cells to local and distal theta oscillations during exploration

(A) On the left, the histograms indicate the proportion of modulated cells (Rayleigh test for uniformity, $p<0.01$) for each group of cells. The frequency histograms summarize the preferred theta phase for the modulated dorsal and ventral CA1 pyramidal cells, relative to theta cycles detected in dorsal and ventral hippocampi during exploratory periods (peak of theta = 0°, 360°; trough = 180°). The polar plots at the bottom show the preferred phase for each modulated (red or green rays) and not-modulated (grey rays) cell separately with dorsal and ventral theta reference; the length of the ray represents the depth of modulation and the black arrows the preferred phase for the significantly modulated cells.

(B) The same as in A for dorsal and ventral interneurons.

cells, suggesting that not only dorsal pyramidal cells were modulated more by local theta oscillations but also the modulation was generally stronger. As indicated by the group mean vectors (black arrows), it also appeared that dorsal and ventral CA1 pyramidal cells exhibited a significant shift of ~90° ($p<0.0005$, Watson-Williams circular test) when comparing their theta phase-preference to dorsal theta ("dorsal pyr": mean angle=315°; "ventral pyr": mean angle=226°; $p < 0.01$, Rayleigh test for uniformity). The same comparison was not possible referred to ventral theta because the dorsal group of cells did not exhibit a consistent phase to the ventral oscillations, whereas ventral pyramidal cells tended to fire just before the peak of the local theta (mean angle=159°, mean vector length=0.38; $p < 0.01$, Rayleigh test for uniformity).

As shown in the figure 6B, the percentages of significantly theta-modulated CA1 interneurons ($p<0.01$, Rayleigh test for uniformity) were higher than the percentages of pyramidal cells. As expected almost all dorsal and ventral interneurons were modulated by local theta oscillations (dorsal interneurons=91%, n=43; ventral interneurons=90%, n=37). In comparison, in both populations a smaller number of cells were modulated by distal theta oscillations (dorsal interneurons=72%, n=43; ventral interneurons=78%, n=37). When I looked at the phase-preference distribution for each group of inhibitory cells, I noticed that both dorsal and ventral CA1 interneurons exhibited a consistent phase-locked phase to the local and distal theta oscillations. Shifts in the preferred-phase resulted

comparing local and distal reference for each sub-group, as better summarized in the polar plots (Fig. 6B, bottom). Similarly to what reported for pyramidal cells, the modulation was larger with dorsal reference as indicated by the length of the vectors. Focusing on the mean theta-phase of firing for each sub-group, while dorsal interneurons tended to fire before the through of dorsal theta oscillations, ventral interneurons showed a firing tendency after the peak of dorsal theta cycle ("dorsal int": mean angle=328°; "ventral int": mean angle=186°; $p<0.01$, Rayleigh test for uniformity), with a significant phase-shift of ~120° ($p<0.0005$, Watson-Williams circular test). During ventral theta oscillations, dorsal interneurons were phase-locked to the descending phase after the peak, whereas ventral interneurons tended to fire at the ascending phase of the oscillations ("dorsal int": mean angle=209°; "ventral int": mean angle=131°; $p<0.01$, Rayleigh test for uniformity), with a significant shift in the preferred phase of ~70° ($p<0.0005$, Watson-Williams circular test). Hence the changes in the preferred theta phase of pyramidal cells and interneurons were different when local and distal references are considered suggesting a different type of synchronisation difference.

4.7 Theta phase-locking of dorsal and ventral CA1 cells to local and distal oscillations during sleep

The same analysis described in the previous paragraph was repeated for theta oscillations recorded during REM sleep.

I found that cells 'firing was modulated predominantly by local theta oscillations but distal theta oscillations still influenced it. When I looked at the percentages of CA1 pyramidal cells significantly modulated ($p < 0.01$, Rayleigh test for uniformity) during sleep (Fig. 7A), I found that fewer dorsal cells were modulated by ventral theta oscillations than by local events (local reference=69%, distal reference=49%, n=298). Equally, ventral cells were more modulated by the local theta oscillatory patterns (local reference=48%, distal reference=31%, n=97). The phase histograms revealed that all groups of cells were phase-locked to dorsal and ventral theta: both dorsal and ventral pyramidal cells shifted their phase-preference slightly towards the descending phase of theta when comparing local and distal reference. As indicated by the group mean vectors (black arrows) in the polar plots (below in the panel 7B), indeed dorsal and ventral pyramidal cells were exhibiting a small but significant phase-shift of ~6° relative to dorsal theta ($p<0.001$, Watson-Williams circular test), less than previously reported for exploratory period ("dorsal pyr": mean angle=238°; "ventral pyr": mean angle=244°; $p < 0.01$, Rayleigh test for uniformity). When comparing their theta phase-preference relative to ventral theta, the phase-shift increased to 78° ($p<0.0005$, Watson-Williams circular test), ("dorsal pyr": mean angle=281°; "ventral pyr": mean angle=203°; $p < 0.01$, Rayleigh test for uniformity).

As shown in the figure 7B, the percentages of CA1 interneurons significantly theta-modulated ($p < 0.01$, Rayleigh test for uniformity) during sleep were lower

than during exploration, but comparable to the percentages of pyramidal cells. Dorsal and ventral interneurons were more modulated by local theta oscillations (dorsal interneurons=67%, n=43; ventral interneurons=59%, n=37), comparing to the modulation by distal theta

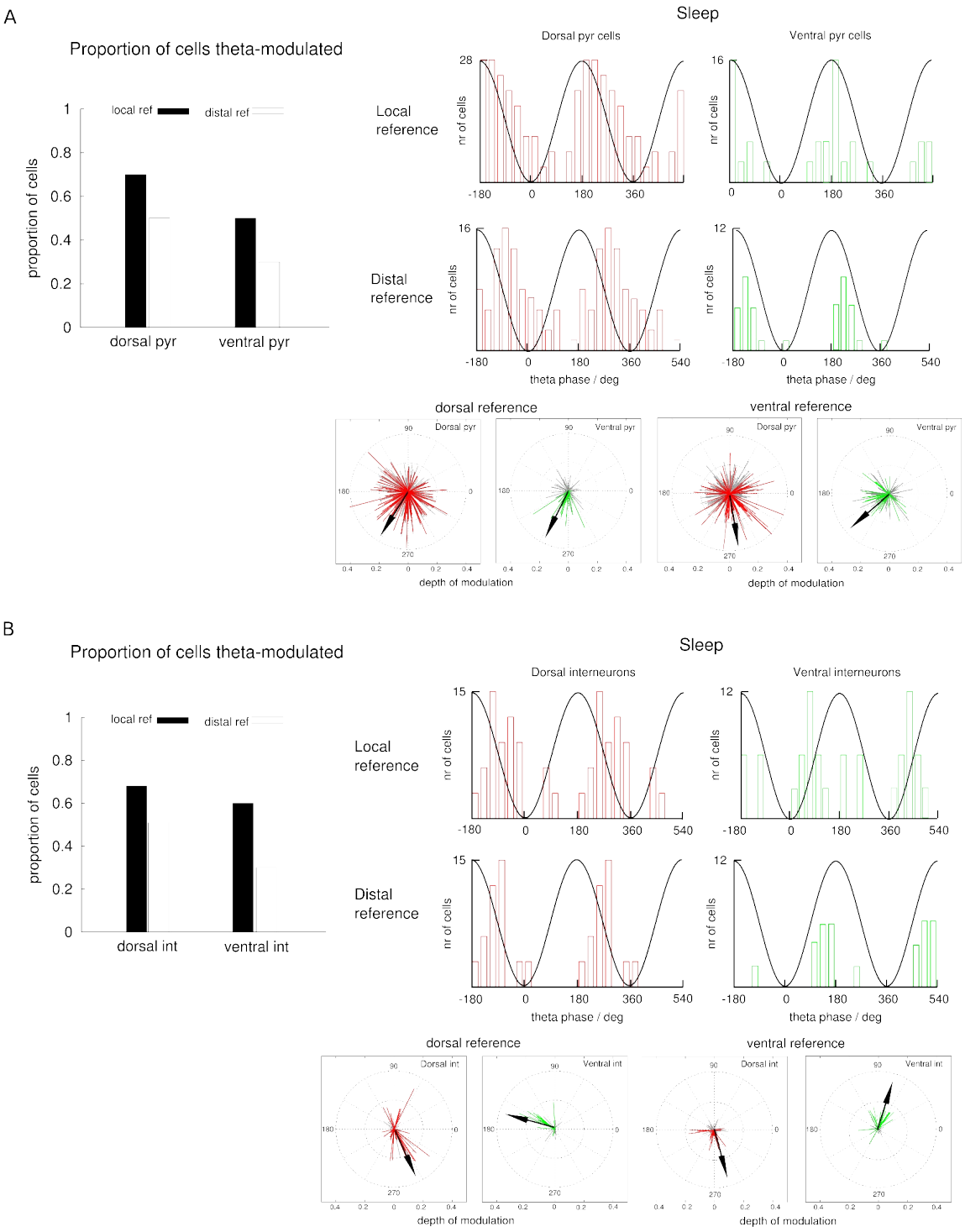

A
Proportion of cells theta-modulated
local ref
distal ref
proportion of cells
dorsal pyr
ventral pyr
Sleep
Dorsal pyr cells
Ventral pyr cells
Local reference
Distal reference
nr of cells
theta phase / deg
dorsal reference
ventral reference
depth of modulation
B
Proportion of cells theta-modulated
dorsal int
ventral int
Dorsal interneurons
Ventral interneurons

Figure 7. Phase-locking of dorsal and ventral CA1 cells to local and distal theta oscillations during sleep

(A) On the left, the histograms indicate the proportion of modulated cells ($p<0.01$) for each group. The frequency histograms summarize the preferred theta phase for dorsal and ventral CA1 pyramidal cells, relative to theta cycles detected in dorsal and ventral hippocampi during sleep (peak of theta = 0°, 360°; trough = 180°). The polar plots at the bottom show the preferred phase for each modulated (red or green rays) and not-modulated (grey rays) cells, separately for dorsal and ventral theta reference; the length of the ray represents the depth of modulation and the black arrows the preferred phase for the significantly modulated cells.

(B) The same as in A for interneurons.

oscillations for both populations (dorsal interneurons=50%, n=43; ventral interneurons=27%, n=37). When I looked at the phase-preference distribution for each group of cells, I noticed that dorsal CA1 interneurons were phase-locked to the local and distal theta oscillations. Also ventral interneurons showed phase-locking to both local and distal references, even though their numbers were relatively low. The group mean vector in the polar plots (black arrows, Fig. 7B, bottom) shows the preferred-phase for populations. Focusing on the mean theta-phase of firing for each sub-group, while dorsal interneurons tended to fire mostly at the descending phase of dorsal theta oscillations, ventral interneurons showed a firing tendency before the peak of the oscillation ("dorsal int": mean angle=288°; "ventral int": mean angle=167°; $p<0.01$, Rayleigh test for uniformity), with a significant phase-shift of ~120° ($p<0.0005$, Watson-Williams circular test). During ventral theta oscillations, dorsal interneurons were phase-locked to the descending phase, whereas ventral interneurons tended to fire at the ascending phase of the oscillations ("dorsal int": mean angle=278°; "ventral int": mean angle=85°; $p<0.01$, Rayleigh test), with a significant shift ($p<0.0005$, Watson-Williams circular test).

4.8 Depth of theta modulation

Although cells exhibited a phase-preference to fire action potentials relative to theta oscillations, phase variability exists when I looked at individual spikes (polar

plots, Fig.6 & Fig.7). The strength of modulation to theta oscillations reflects the fidelity of the spike timing of a cell relative to the oscillation. While in the dorsal CA1 layer the instantaneous phase relationship of many cells has been shown to change as a function of behaviour, the so-called theta phase precession (O'Keefe and Recce, 1993), spike-phase relationship is also crucial for synaptic plasticity, and in that case would require reliability of the cell firing in order to synchronize the activity of different neuronal populations.

The depth of modulation was calculated for all dorsal and ventral CA1 cells relative to a dorsal and a ventral theta reference, during exploration and sleep (Fig. 8) by calculating the length of the circular vector. Modulation depth was defined as the sum of the vectors representing the instantaneous phase of each spike fired by a cell. So, the length of the resulting vector reflects the tendency with which the cell fires at its preferred phase. During exploratory theta periods, dorsal CA1 pyramidal cells (n=310) showed a mean vector length of 0.120 ±0.006 relative to dorsal theta, weaker than that of CA1 interneurons (n=51) (0.160 ±0.014) , but in both cases larger than the values obtained relative to ventral theta (0.075 ±0.004; 0.082 ±0.009 respectively) (df_{pyr}= 618, df_{int} = 100, all $p<0.01$, paired t-test). Importantly, ventral CA1 cells showed markedly different effects. Ventral pyramidal cells (n=105) and interneurons (n=41) exhibited more modulated firing to distal theta than to the local, although the difference was not significant for ventral interneurons (“Ventral pyr”: 0.113 ±0.007;

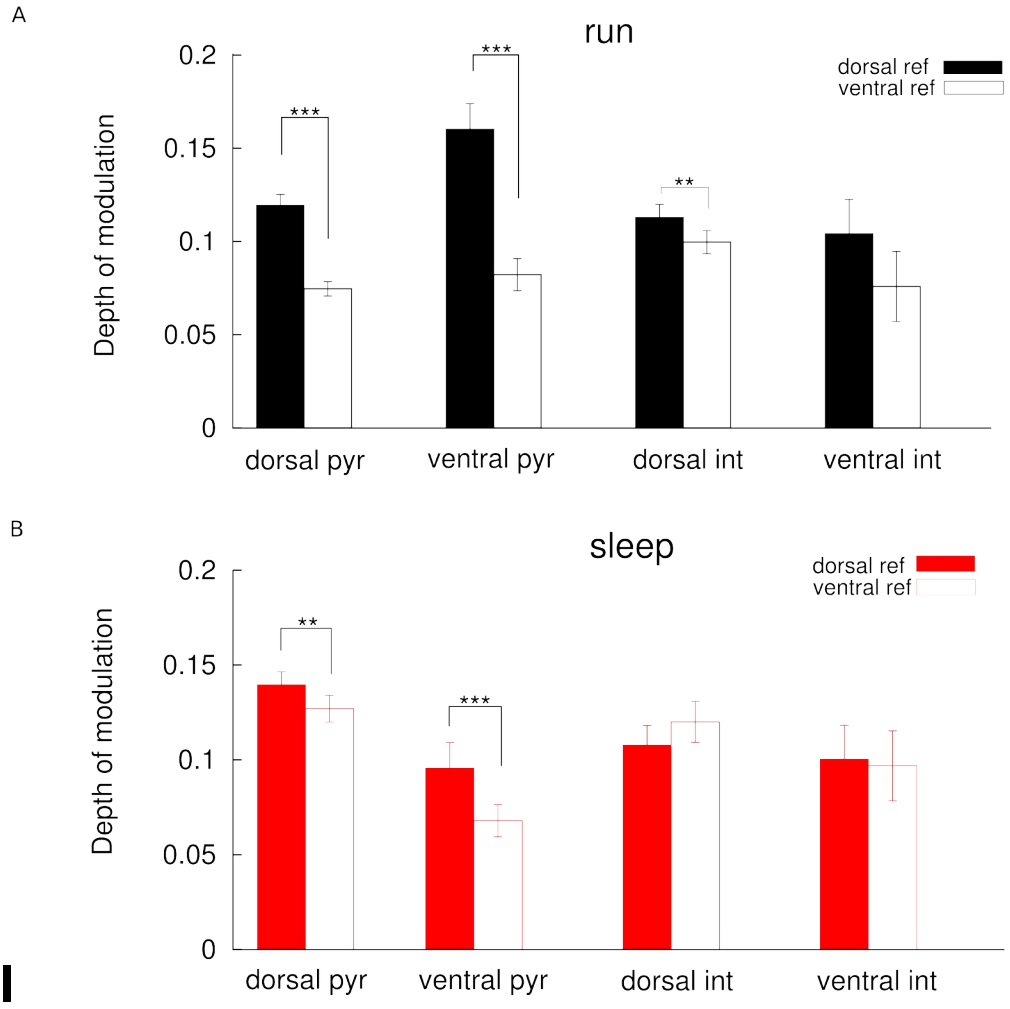


Figure 8. Depth of theta modulation

(A) Depth of modulation by theta oscillations (Rayleigh vector length) in dorsal and ventral CA1 during exploration. All groups of cells are significantly more modulated by dorsal theta (black boxes) than ventral theta (white boxes).

B) The same as in A during sleep periods. Here only dorsal cells are more modulated by dorsal theta (red boxes) than ventral theta (white boxes). Note that both dorsal and ventral pyramidal cells are more modulated by ventral theta during sleep than during exploratory periods, and more modulated than interneurons.

0.1 ±0.006 respectively; df=208, p<0.01, paired t-test; “Ventral Int”: 0.104 ±0.019; 0.076 ±0.019; p=0.214, df= 80, paired t-test).

By contrast, during sleep, only dorsal CA1 cells showed a stronger modulation to the local theta oscillations than to the ventral theta (“Dorsal pyr”: 0.139 ±0.007, 0.127 ±0.006 respectively; “Dorsal Int”: 0.096 ±0.013, 0.068 ±0.011 respectively; df_{pyr}= 618, df_{int} = 100, all p<0.05, paired t-test). Ventral CA1 pyramidal cells and interneurons did not show significant difference in the depth of modulation by local and distal theta oscillations (“Ventral pyr”: 0.108 ±0.010 0.120 ±0.011 respectively; df= 108, p=0.13, paired t-test; “Ventral Int”: 0.1 ±0.018, 0.097 ±0.001 respectively; df= 80, p=0.05, paired t-test).

Discussion

The results here have shown that periods of continuous theta oscillations occurred in the ventral CA1 regions during on-line states and sleep. However, those oscillatory patterns resulted either sporadic or characterized by lower amplitude when compared to theta oscillations observed simultaneously in the dorsal CA1 sub-region. However, the data indicate consistently a systematic distribution of the current sources of theta oscillations along the long axis of the hippocampus, reflected by a phase shift between pyramidal layer and stratum radiatum in both sub-regions. Importantly, the data presented here also support Lubenov and Siapas (2009) hypothesis of a full cycle phase-shift between theta oscillations recorded at the septal and temporal pyramidal layers. This is in contrast to what reported by Patel et al. (2012).

Consistent with the observations in the raw LFP and from the field average, I found a decrease in the correlation of instantaneous theta-band power across dorsal and ventral CA1 than within the same sub-field. Similar results were obtained measuring the power coherence in theta range between the signals recorded simultaneously at dorsal and ventral sites. My findings are in line with what

previously showed by Patel et al., where grouping recording sites in septal intermediate and ventral portions of CA1 region they found relatively high coherence between septal and intermediate sites, but less than 0.5 coherence between septal and ventral sites as well as intermediate and ventral sites. The result was consistent during run and REM sleep periods.

Different behavioural variables can modulate hippocampal theta oscillations. In particular, during online state the power of those patterns are known to be affected by the speed of the animal. No speed modulation was found in the ventral CA1, unless when the coherence between the dorsal and ventral sub-regions was relatively high. In contrast, the power of dorsal theta oscillations was positive correlated to the speed in all conditions, as expected. My results are partially in agreement to Patel et al., who reported how the ventral theta is not modulated by speed at all (2012). Remarkably, the highlighted positive speed modulation at high coherence between dorsal and ventral CA1 might indicate that the functional influence of speed and the power of dorsal theta oscillations are somehow temporary transferred to ventral theta oscillations. Accordingly, it can be hypothesized that some spatial information are transferred along the septo-temporal axis of the hippocampus during those temporal windows, or either communication is taking place.

The frequency of theta oscillatory patterns is also known to be dependent on the movement of the animal, so that decrease at low speed. I showed that the frequency of theta oscillations in the ventral CA1 was lower than in the dorsal CA1 during exploration, whereas the frequency did not differ during sleep. The last result is a further evidence of the independence of theta oscillations from the sensory variable represented by the speed of movement. The results here do not agree with published work where significant changes in theta frequency were found when comparing behavioural states (higher frequency during run than sleep), but not across CA1 sites at different levels of the long axis of the hippocampus during the same state (Patel et al., 2012). On the other hand, they are in line with the parallel reduction in frequency of intrinsic theta oscillations reported from the dorso-lateral to the ventromedial part of MEC (Giocomo et al., 2007; Giocomo and Hasselmo, 2008), mostly connected to the dorsal and ventral part of the hippocampus respectively (Cenquizca and Swanson, 2007).

While the coupling between dorsal and ventral CA1 in terms of power coherence (related to the amplitude of the oscillations) is not a clear sign of temporal relationship in theta frequency, the phase analysis confirmed undoubtedly synchronization between the two sub-regions at discrete times. I observed a more consistent phase relationship (narrower peaks) between theta oscillations at dorsal

and ventral sites when the coherence was high. In particular, I reported a shift of presumably almost one full cycle and not 6°, based on the assumption that the signal is travelling a distance of more than 8 mm. My data are not in agreement with the almost half-cycle theta shift reported by Patel et al. (2012) during exploration and even smaller during REM sleep, even though I might speculate that their results are similar to what I saw in the first place without splitting in high and low coherence periods. Indeed, the bimodal distribution (Fig. 5B) was not evident in the convoluted distribution saw in the first part of the analysis (Fig. 5A).

Despite the reduction of theta oscillations in the ventral hippocampus and the speed independence in the sub-region, my findings suggest that oscillations in the theta band may serve as a mechanism to temporally bind representations at different levels of the hippocampus. Such hypothesis of a temporal coordination was also supported by the phase modulation of dorsal and ventral CA1 cells by distal theta oscillations. Surprisingly, I found that during exploration more ventral pyramidal cells were modulated by dorsal theta waves than by ventral theta waves, whereas less dorsal cells and ventral interneurons showed modulation to distal events. The ventral population was also clearly phase-locked to dorsal theta oscillatory patterns, whereas only dorsal interneurons did relative to ventral oscillations. Moreover, the two populations were largely modulated by dorsal theta oscillations. During sleep, a small percentage of cells was modulated by the theta oscillatory patterns in both sub-fields, but all the sub-populations showed a phase

preference to local and distal events. Though the variegated distribution of preferred phases across the two CA1 sub-regions and in reference to dorsal and ventral theta oscillations, two things are worth to notice: first, the two populations of cells tended to fire at different phase relative to the local oscillations; second, the shift in the preferred phase between the two populations relative to the same oscillatory patterns was reduced from the awake state to the sleep state, probably reflecting the lower frequency of theta oscillations.

Given that the spike timing of a single cell relative to oscillations is considered a mechanism to induce synaptic plasticity, it is relevant that the firing modulation by distal theta waves for dorsal pyramidal CA1 cells changed during sleep, being larger than during on-line state. The same was not observed for ventral cells, whose firing was modulated in a similar way by local and distal theta oscillations in both states.

Overall, my findings demonstrate differences between the dorsal and the ventral-most parts of the hippocampus in the temporal dynamics of theta oscillations. I might hypothesize that, as theta oscillations change in the septo-temporal direction, spatial information become less precise and other types of non-spatial information become predominant. My study extends previous observation (Jung et al., 1994; Poucet et al., 1994; Maurer et al., 2005; Kjelstrup et al., 2008; Royer et al., 2010) of a septo-temporal shift of the neuronal representation toward the temporal end of the hippocampus.

The differences in the temporal firing properties of neurons in relation to LFPs in the dorsal and ventral regions might be linked to the spatial properties of hippocampal cells, strongly linked to hippocampal theta oscillations in the dorsal CA1 (Huxter et al., 2003; O'Keefe and Burgess, 2005). If so, the temporal shift in the population firing in relation to theta might be sufficient to separate the processing of non-spatial information in the hippocampus from incoming sensory inputs, otherwise of stored information of different nature during off-line states.

Chapter V
Network interactions between the dorsal and ventral hippocampi during spatial learning.

Introduction

The hippocampus is a key region in learning and memory processes. In humans, lesion and imaging studies have shown the involvement of this brain region in coding and retrieval of episodes (episodic memory). Since the discovery of hippocampal “place cells” in rodents (O’Keefe & Nadel, 1978), substantial evidence have suggested that the hippocampus is involved in spatial memory as well, and that might be inter-related to episodic memory. The parallelism can be crucial to reveal the mechanisms underlying how pieces of information are acquired, stored in hippocampal neuronal circuits, consolidated and eventually recalled. According to this view, when a rat is navigating in an environment, hippocampal neurons would act to decompose the spatial context in the same way they would do in humans experiencing an event. In both cases, the immediate output is to adapt the behavioural response in that situation, eventually creating a trace of that experience to store. Crucially, in rodents the activity of hippocampal cells correlate also to non-spatial features of the environments, developing specific firing responses during learning (Berger and Thompson, 1978; Breese et al., 1989; Wood et al., 1999).

Therefore, most studies were concerned with the role of the hippocampus on the acquisition and retention of spatial versus non-spatial information, as well as in contextual learning. As proposed first by Mishkin and colleagues in 1983, the hippocampus might be the perfect candidate to unify the “what” and “where” stream; in other words hippocampal cells could code for the conjunction of spatial context and non-spatial information and vice versa. The observation of single hippocampal neurons encoding moments in temporally structured experiences – so-called “time cells” – much as place cells encode locations in spatially structured experiences fill the gap on the role of the hippocampus in episodic memory and spatial mapping (MacDonald et al., 2011). As suggested by the authors, the fundamental function of the hippocampus might be to establish spatio-temporal frameworks for organizing memories.

The main aim of my project was to investigate the role of dorsal and ventral CA1 in spatial learning. The most accredited hypothesis is that the two sub-regions are functionally distinct, the dorsal hippocampus being involved in the processing of spatial information and the ventral hippocampus in the encoding of non-spatial information. Based on the anatomical connectivity, sensorial inputs of visuo-spatial nature indeed reach the septal pole of the hippocampus, whereas the temporal pole is receiving inputs mainly from brain structures crucial in emotional-reward processing. Moreover, neurons in the ventral hippocampus innervate directly to the mPFC and subcortical structures and therefore they are able to translate their

activity into behavioural responses. Accordingly, the spatial properties of cells decrease along the longitudinal axis (Jung et al., 1994; Maurer et al., 2005; Kjelstrup et al., 2008), and at least in CA3, dorsal and ventral principal cells develop different type of specificity during the same task (Royer et al., 2010; Komorowski et al., 2013). Following the logic of the existence of two parallel streams, the dorsal hippocampus may sub-serve the "where" function, whereas the ventral hippocampus might participate to the "what" pathway. It has been suggested that the intermediate region of the hippocampus might be the integrating point of these pathways (Bast et al., 2009).

To shed light on this apparent duality along the longitudinal axis of the hippocampus, I recorded simultaneously from dorsal and ventral CA1 sub-regions during a goal-directed spatial task, in which the animals had to collect rewards from multiple locations on a circular arena for more trials. So, the animals had to navigate in the maze, referred to as cheeseboard maze, in absence of intra-maze cues but having access to unchanged external cues. The position of the wells changed on a daily basis, although kept constant within trials in a day. The paradigm permits looking at the processing of the spatial information as well as the reward-related firing of cells during incremental learning in the dorsal and ventral CA1 regions. As reported previously by Dupret et al. (2010), principal cells in the dorsal CA1 region but not in the dorsal CA3 develop and mostly maintain a goal-related firing in the cheeseboard maze task. Here, I tested and compared the emergence of neuronal firing patterns in the septal and temporal CA1 thought to contribute to spatial

representation in such goal-directed task. The starting hypothesis was that the dorsal CA1 was preferentially involved in the acquisition and retention of the spatial information, but the motivational status influenced by the reward was supposedly affecting the ventral region as well, consistent with the inputs received by the reward-circuitry loop.

During online states, the spike timing of hippocampal pyramidal cells relative to theta oscillations are thought to constitute an important mechanism for coding information, and eventually, to communicate with other brain regions by synchronizing their firing activity. Evidence presented in the previous chapter supported that the dorsal and ventral hippocampi oscillate coherently in theta frequency in discrete periods. I suggested that flow of spatial information along the septo-temporal axis might take place in those temporal windows, as indicated by the observed speed modulation of the amplitude of ventral CA1, otherwise not correlated to the animals' exploratory speed during low coherence periods. Coherent theta rhythms as well as correlations between spike times of cortical neurons and hippocampal theta phase has also been reported by several studies recording simultaneously from the hippocampus and mPFC (Jones & Wilson, 2005; Young & McNaughton, 2009; Adhikari et al., 2010; Benchenane et al., 2010). The medial prefrontal cortex (mPFC) is thought to be critical for goal-directed action. This cortical region receives direct projections from ventral CA1 (Jay & Witter, 1991; Hoover & Vertes, 2007; see also Introduction), so the interaction between the two structures is likely reflected by ventral hippocampal activity. In line with the idea that

the hippocampus activates mPFC during goal-directed behaviours, the results of these studies support the conclusion that mPFC–hippocampal theta phase-locking is relevant for functions that are carried out by the hippocampus and mPFC. If memory trace formation (i.e. acquisition) required theta phase-locking between the regions, theta synchronisation should occur during the acquisition of learned behaviour. Remarkably, a recent study by Adhikari et al. (2010) shows a positive correlation between mPFC and ventral hippocampus in the theta band during anxiety-related behaviour in mice, but not with the dorsal hippocampus. In particular, the authors suggest that theta phase-locking between ventral hippocampal and mPFC would lead to suppression of anxiety-related response, mediating the animal behaviour.

So in this chapter, I will first analyse the change in theta synchronisation in the two CA1 sub-regions, separating exploratory periods according to session: pre-probe, post-probe and learning session.

In a second part, I will focus on the neuronal response at the level of single cell in the dorsal and ventral CA1, in searching of place and goal-related firing correlates. The analysis will be conducted comparing the results obtained in the learning with the probe sessions, looking at the dynamics of organization of neuronal firing in the dorsal and ventral CA1.

Results

The activity of dorsal and ventral CA1 networks was recorded during a hippocampal-dependent spatial memory task referred to as the cheeseboard maze task (see Materials and Methods; Dupret et al., 2010). Briefly, animals had to collect food from three different wells for almost 40 trials. The baited wells changed on a daily basis, so that each animal experienced different combination of goal-locations within the 3-5 recording days and every day had to update them. The learning session was preceded (pre-probe) and followed (post-probe) by ~25 minutes of exploratory sessions of the same maze when no reward was provided (alternating by rest/sleep times). Sequences of pre-probe/learning/post-probe were compared.

5.1 Behavioural performance on the cheeseboard maze task.

In five animals, a total of 14 recording sessions consisting of pre-probe/learning/post-probe sessions were included in the analysis. These were selected based on behavioural criteria, such as good exploration of the environment (spatial coverage) in both probe sessions (Fig. 1A, pre-probe and post-probe) and

~40 consecutive successful trials during the task. I measured the task performance of the animals in terms of distance travelled to collect all the rewards and coming back to the start box in each trial, which should decrease once the animal has learnt the locations of the three rewards. The tendency was already evident looking at the traces reconstructed from the position tracking system signal (Fig. 1A, blocks 1-4). To quantify and confirm, the learning curve was built by calculating for each of the 14 learning sessions the distance travelled per trial, then averaged across days and animals normalising the path length by the shortest distance for that day (Fig. 1B). The normalisation of the single learning curve was necessary in order to take into account the variability in the configuration of the three baited wells from one day to another. It emerged that the animals were able already after a couple of trials to reach the three rewarded wells and come back to the start box with shorter paths and maintaining the strategy, as suggested by the asymptotic trend of the curve.

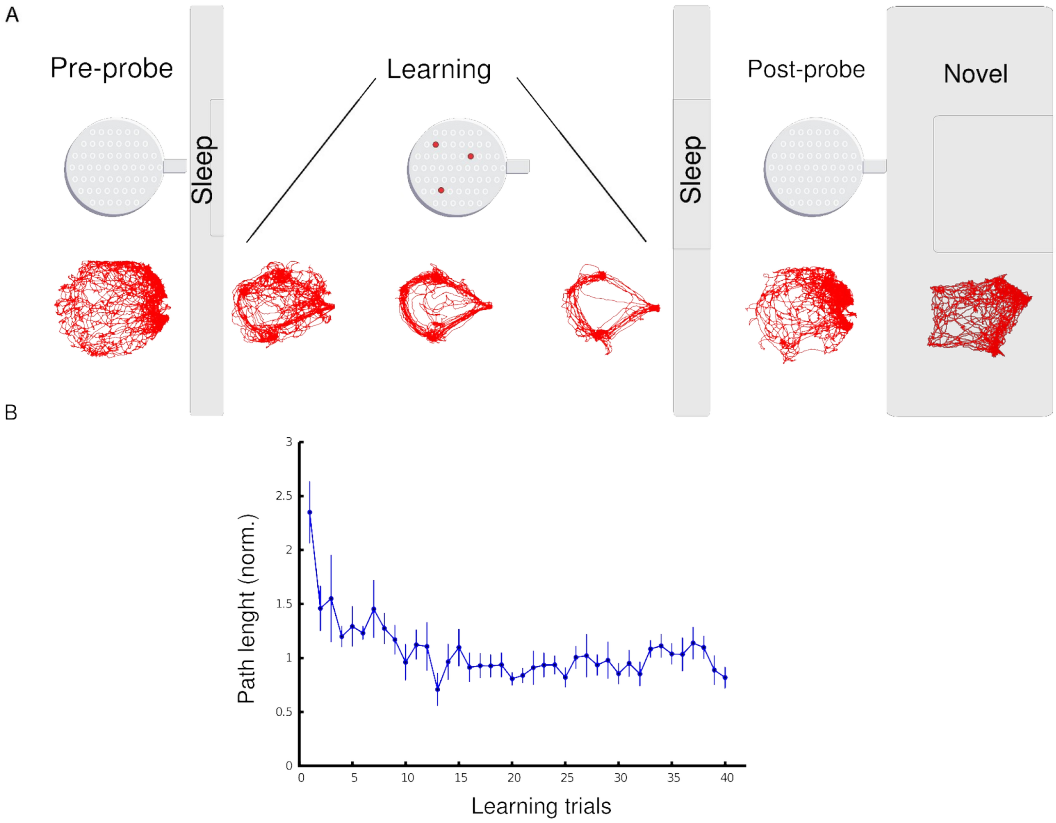


Figure 1. Behavioural performance on the cheeseboard maze task

(A) Representative example of an animal's path during pre-probe learning and post-probe sessions. For each probe session, only the first 10 min are shown; for the learning session, 4 blocks of consecutive 5 trials (black dots, baited wells). Note that in this case, the animal was exposed to a novel environment at the end of the paradigm. The spatial coverage is uniform for all the exploratory conditions, whereas in the learning session the path of the animal is refined consistently across blocks.

(B) Average learning curve for 5 animals and 14 learning sessions. The learning performance was estimated by the distance travelled to find all rewards per trial. Note the drop in the length of the path in the second trials and the asymptotic trend after the 4th trial.

5.2 Theta power correlation and coherence between dorsal and ventral CA1 across sessions.

Because of the results of the previous chapter, I decided to examine the property of theta oscillations in the dorsal and ventral CA1 during exploration, separately for each session. Indeed, I reasoned that the variability in the power correlation and coherence of the theta signals between the two sub-fields might be related to the demand of the specific session, so that goal-directed exploration should be distinguished from unrewarded exploration of the same environment. Because learning is a dynamic process, I proceeded in splitting the learning in two parts. As shown in figure 2A, I first observed a significant increase in the power correlation of the theta oscillations between the two sub-fields during learning (n= time windows for calculating theta events, $p<0.0001$, paired t-test), comparing to the pre-probe and post-probe sessions (mean r- value pre-probe: 0.217 ±0.012, n = 62885 ; mean r-value 1st half learning: 0.339 ±0.014, n= 23992; mean r-value 2nd half learning: 0.314 ±0.012, n= 2392; post-probe: 0.168 ±0.011, n= 45584). The power correlation remained higher in both the first and the second half of the learning session compared to the probe sessions, but not significantly different between the two halves of learning ($p>0.5$, paired t-test). However, when I examined the theta power coherence across the dorsal and ventral hippocampus, a significant (n= time windows for calculating theta events, $p<0.0001$, paired t-test) increase in coherence between the dorsal and ventral theta patterns was observed mostly at the 1st half of learning (mean r- value pre-probe: 0.153 ±0.001, n = 62885; mean r- value 1st half

learning: 0.229 ±0.002, n= 23992; mean r- value 2nd half learning: 0.140 ±0.002, n= 2392; mean r- value post-probe: 0.164 ±0.002, n= 45584; all other p<0.001, paired t-test) (Figure 2B). I proceeded in segmenting the learning session in blocks of five trials and calculated the coherence between dorsal and ventral theta for each block, so to have a better temporal resolution of the increase observed. The other sessions were also divided in blocks of 5 minutes each. Interestingly, this revealed that, in contrast to the pre-probe session, in which there were no evident changes in the coherence values at the beginning of the block, the observed increase in theta coherence between the septal and temporal poles was almost confined to the start of the learning session (Fig. 2C). In the protocol, the maze was not changing across sessions. Nevertheless, the presence of the rewards might be considered as a sign of change in context, so to cause the increase in the theta signal in the ventral hippocampus (directly connected to the mPFC) and possibly flow of information between the two CA1 sub-regions, as I hypothesised. To exclude a possible effect of novelty for the change of context, I did the same analysis in 5 exploratory sessions of novel environments, recorded at the end of the whole protocol in three of the animals (out of the five previously selected). As shown in figure 2C, I did not find an abrupt change in the coherence at the beginning of the novel session such as in the learning session and at the start of the post-probe. What is more, the abrupt increase was also observed at the beginning of the following probe session, when the memory for the newly-learnt goal locations was tested.

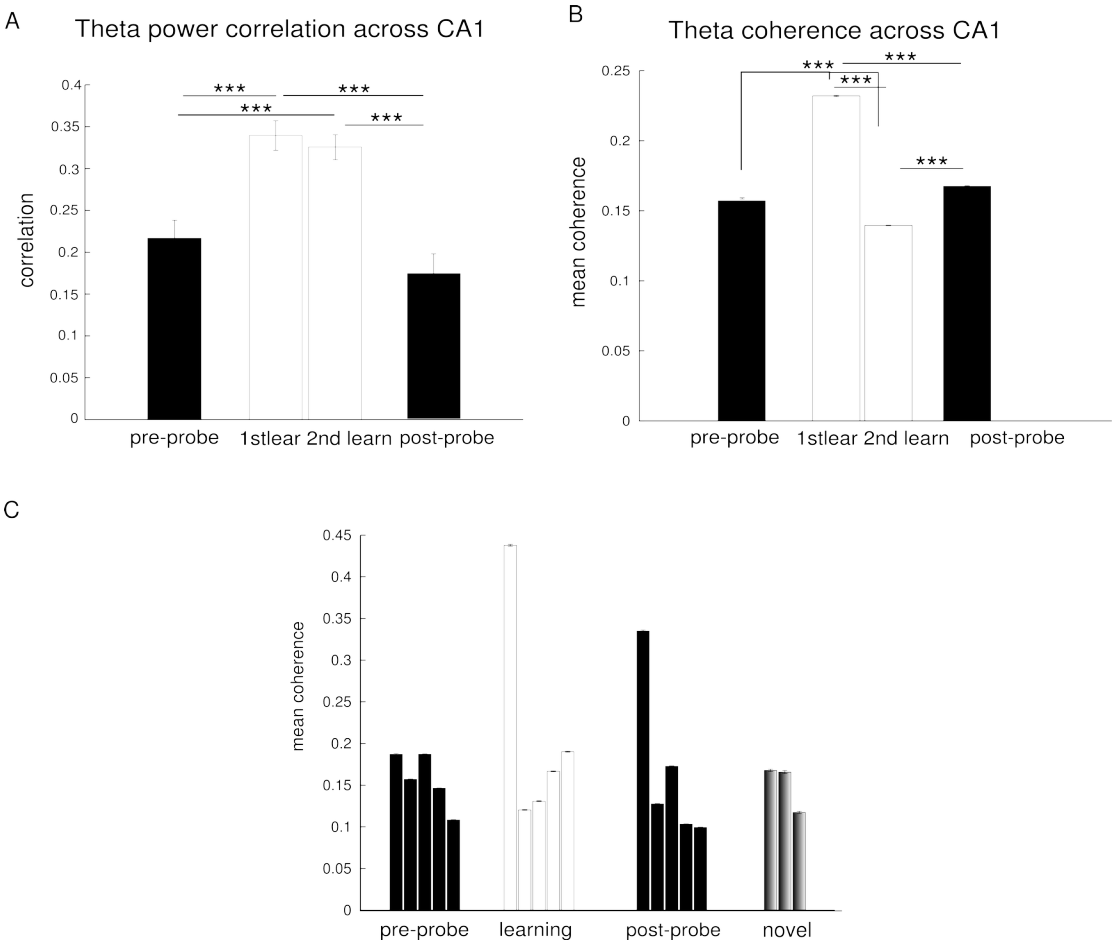


Figure 2. Correlation of instantaneous theta-band power and coherence across dorsal and ventral CA1 during pre-probe learning and post-probe

(A) The power-power correlation in the theta-band is significantly higher during the learning session.

(B) Increase of coherence in power across the CA1 subfields during spatial learning compared to probe sessions. Notice how the effect is relative to the first half of the session.

(C) The same as in B in only three animals (5 recording days) and splitting each session in small segments. Dorso-ventral theta power coherence increases significantly in the first block of 5 learning trials and in the first 5 minutes of the post-probe (1/5 of the session). The same initial peak is not observed in the pre-probe and during the exploration of a novel environment.

5.3 Speed-modulation of dorsal and ventral CA1 theta during learning.

I went on analysing the speed dependence of theta oscillations in the dorsal and ventral hippocampi for each session type. First, I calculated and compared the mean speed across sessions, to make sure that the analysis was not affected by different speed profile from exploratory sessions to the goal-directed one. As shown in figure 3a, the mean speed was not significantly changed among sessions (n= 14 recordings over 5 animals, df= 26, all $p>0.1$, paired t-test). Considering the different results obtained previously in examining times when theta coherence between dorsal and ventral CA1 sub-regions was either low or high, the speed modulation was tested separately for high and low coherence periods during pre-probe learning and post-probe. Here, it was evident that the emergence of a positive correlation between speed and ventral theta power observed previously at high coherence (see Chapter 4) was mainly restricted to the learning sessions. Indeed, theta oscillations in the ventral CA1 area were positively speed-modulated during high coherence only ("low coh": no sign; "high coh": mean r value 0.182 ±0.03), whereas either no significant ($p>0.2$, t-test) or negative correlation (all $p<0.01$, t-test) resulted during the probe sessions independently of the coherence between the two sub-regions (pre-probe: "low coh": no sign.; "high coh": -0.038 ±0.012; post-probe: :"low coh": -0.049 ±0.018; "high coh": no sign.). I also observed that only during learning, dorsal theta was significantly less modulated by speed when the coherence went down ("low coh": 0.021 ±0.020; "high coh": 0.122 ±0.031; n= mean r value for each high/low theta events, df = 205, $p<0.01$, paired t-test), otherwise no significative

differences were observed (pre-probe: “low coh”: 0.140 ±0.018; “high coh”: 0.128 ±0.017; post-probe: “low coh”: -0.141 ±0.020; “high coh”: 0.138 ±0.018; n=mean r value for each high/low theta event, $df_{pre\text{-}probe}$ =72, $df_{post\text{-}probe}$ = 83 ,all p>0.1, paired t-test).

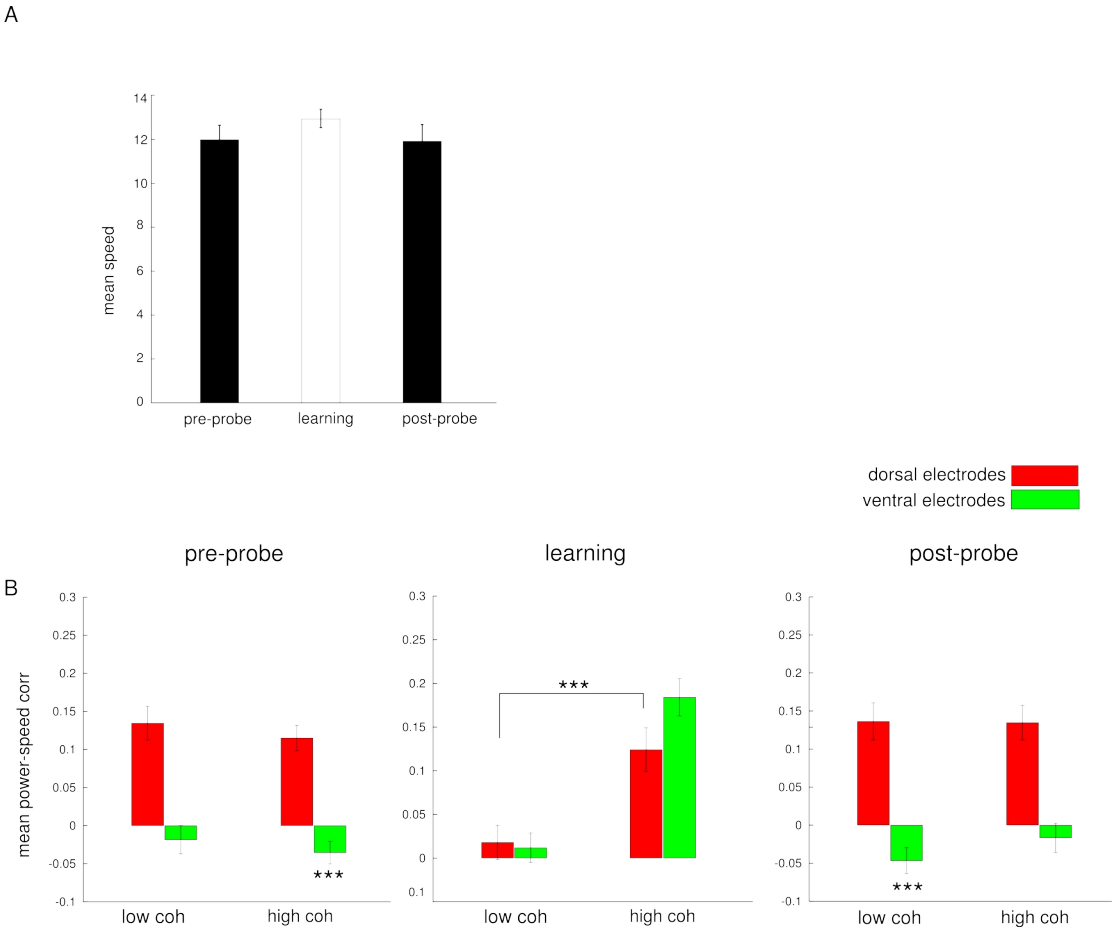


Figure 3. Speed modulation of theta power in relation to session type

(A) The histogram shows the mean speed calculated for the 5 animals in each session.

(B) Theta power-speed correlation for dorsal (red) and ventral (green) CA1 electrodes at times when power coherence between the two pyramidal layers is low or high. The correlation is shown separately for pre-probe learning and post-probe. During probe sessions, no significant differences are observed comparing low and

high coherence periods for both dorsal and ventral electrodes. Instead during learning, the correlation increases significantly in both sub-regions at high coherence, so that even the power of ventral theta results positive correlated to speed.

5.4 Frequency and phase relationship between dorsal and ventral CA1 theta during different sessions.

I further looked at the frequency of theta, that I previously reported being lower at the temporal level. Here, I examined the mean frequency of theta oscillations during the different sessions for dorsal and ventral oscillatory events. The mean frequency was obtained as described in the previous chapter. Briefly, I averaged the wavelength of each theta wave event detected at electrodes located either in the dorsal or in the ventral CA1 pyramidal layers for each session type, and calculated the reciprocals of those values. The mean frequency is shown in figure 4A for dorsal and ventral electrodes in the three behavioural sessions. I found that the mean frequency of dorsal theta oscillations (n= theta events detected at dorsal CA1 sites) was significantly higher than the one calculated at ventral sites (n= theta events at ventral CA1 sites) in all the three conditions (all $p<0.01$, unpaired t-test). The frequency of ventral theta oscillations did not change significantly across sessions (n= 14 recordings; pre-probe: 7.8± 0.01; learning: 7.9 ±0.01; post-probe: 7.8 ±0.02; $p>0.2$ in all conditions, df= 26, paired t-test), whereas at dorsal CA1 sites the difference between each probe session and learning was statistically significant (n= 14 recordings; pre-probe: 8.2± 0.01; learning: 8.5 ±0.01; post-probe: 8.2 ±0.01; $p<0.01$ in both conditions, df= 26, paired t-test) but not between pre and post-probe sessions ($p=0.233$, df= 26, paired t-test). Therefore, the comparison across sessions

revealed that the difference in theta frequency between dorsal and ventral CA1 sites was consistent in both learning and probe sessions, even though larger during the learning session. Presumably, the effect seen was a consequence of the theta frequency-speed relationship previously discussed. Considering the relation between speed and frequency of theta, I was also expecting to see an increase in ventral theta frequency correlated to the positive speed correlation found above. Instead, my data showed that theta frequency was constant at that level of the hippocampus, in contrast to the predictable increase in dorsal CA1.

Next, I compared the relative phase of the two oscillatory patterns in dorsal and ventral hippocampus during learning sessions. I proceeded in calculating the instantaneous phase difference between all the possible pairs of dorsal and ventral CA1 sites for each theta wave event detected simultaneously at dorsal and ventral CA1 regions. I plotted the distribution of the phase-difference between dorsal and ventral electrodes (Fig. 4B). Here, the analysis conducted across sessions revealed a change in the peak of the distribution that resulted tuned at 340 °during the learning session. This result was more similar to what was found at high coherence epochs in the previous chapter. During probes instead, the distributions were broadly tuned towards lower shift. Given that the skew of the distribution indicates somehow the consistency in the phase-shift, dorsal and ventral CA1 sub-regions were temporally coordinated by the phase of theta oscillations during learning. Overall, the evidence here supported the idea that the two poles of the hippocampus were more coordinated during the learning sessions.

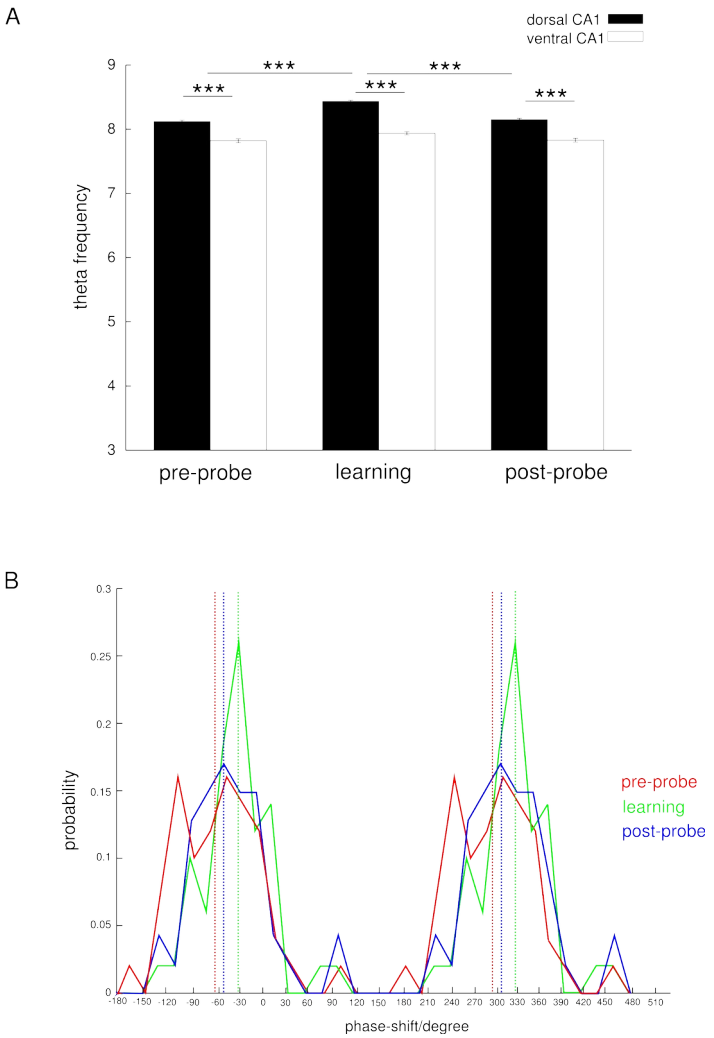


Figure 4. Average frequency and phase-shift of dorsal and ventral CA1 theta across sessions

(A) The average frequency of detected theta waves at dorsal and ventral electrodes is shown separately for each session type. Note the lower frequency of ventral theta oscillations comparing to dorsal ones in all the conditions, but also the higher frequency of dorsal theta oscillations during learning.

(B) Distribution of phase difference of the theta oscillations between pairs of dorsal and ventral electrodes are separately shown for pre-probe (red) learning (green) and post-probe (blue). The shift is calculated as the mean difference of the instantaneous phase of all the detected waves in one electrode relative to the other electrode during that session. To notice the probability distribution relative to the learning is more tuned than the pre and post probe ones (smaller skewing), showing an evident peak at approximately 330° (less than a theta cycle), with a shift of ~40° and ~30° respectively to pre-probe and post-probe sessions.

5.5 Spatial distribution and change in theta dynamics in the dorsal and ventral CA1.

As described above, dorsal and ventral CA1 sub-regions oscillate coherently in the theta domain predominantly when the animals were performing the cheeseboard maze task. Within these high coherent temporal windows, changes in the theta phase shift were observed between the two regions, and the speed modulatory effect seen in the dorsal site was not affecting the frequency of ventral theta. Additionally, the firing of the CA1 units in relation to the phase of the local and distal oscillations was altered, suggesting that the temporal binding along the septo-temporal axis of the hippocampus strengthened specifically during the learning processing. To investigate the nature of the processing beyond that, I moved to the spatial domain of the changes observed in the field analysis, looking for any correlation between high coherence between the two sub-regions and the behaviour of the animals.

Consecutive pre-probe, learning and post-probe sessions were analysed. The position of the animal in the maze was reconstructed for each time the coherence went up (green point, mean + 2SD) or down (red points, mean -2SD). In figure 5 examples from 6 recording days of the position of the animals at low and high coherence periods are plotted in the two dimensional space of the maze. The fewer green points corresponding at times of dorso-ventral high theta coherence tended often to spatially co-localised, specifically across trials. During probe sessions, as a

consequence of the assisted reduction of high coherence points, it was difficult to estimate any spatial modulation.

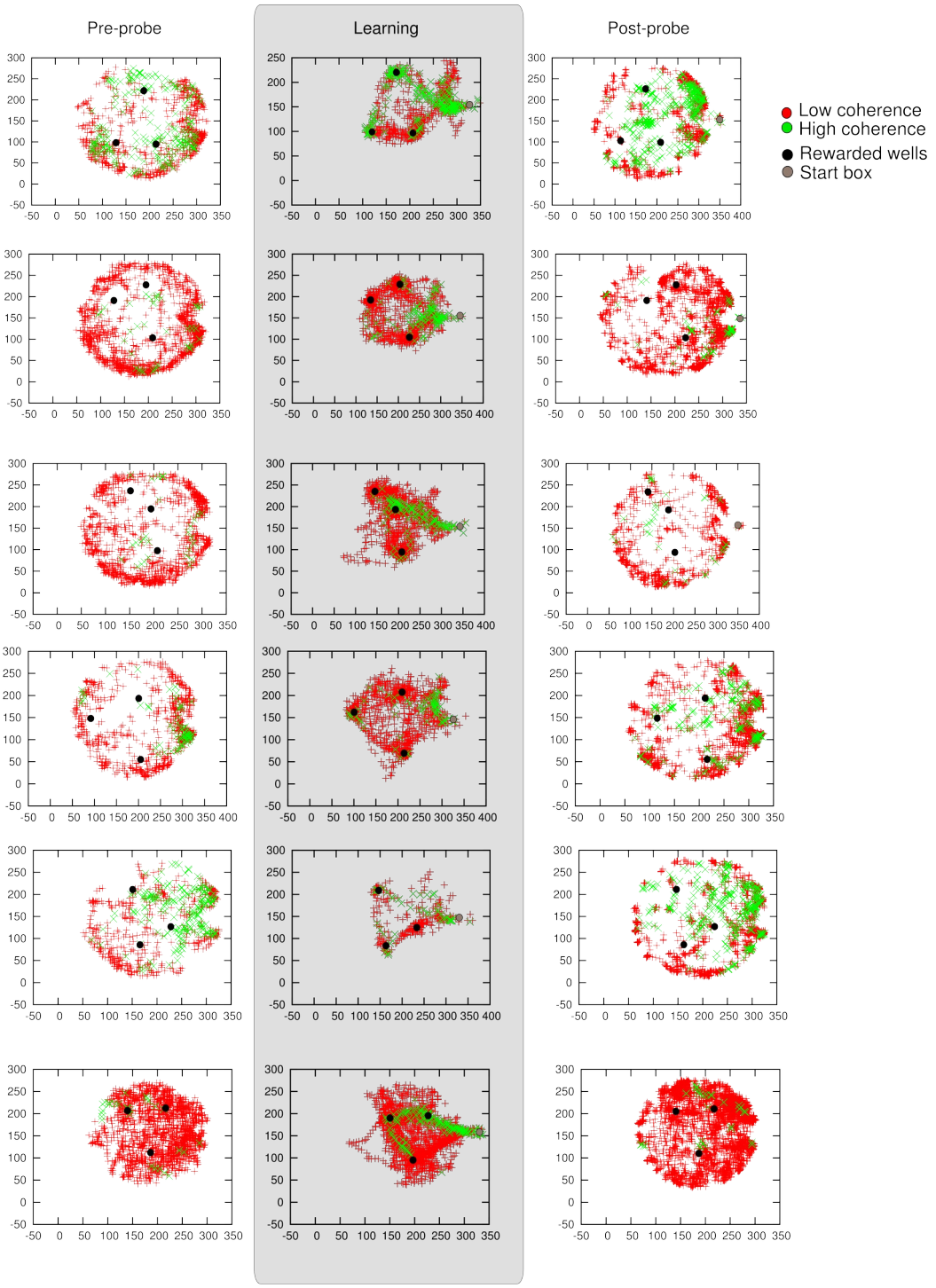


Figure 5. Relationship between the animals' position on the maze and theta power coherence between dorsal and ventral CA1

Example from 6 recording days of the position of the animals at low and high coherence periods plotted in the two dimensional space of the maze. Consecutive pre-probe learning and post-probe sessions are shown. The fewer green points corresponding at times of dorso-ventral high theta coherence tend often to be spatially clustered across trials.

To evaluate if the previous results were related to the increase in the power of the oscillations in the two regions, I looked at the spatial distribution of dorsal and ventral CA1 theta in relation to the power of the oscillatory events (Fig. 6). As shown in those examples taken from 2-3 consecutive recorded days in 3 different animals, I first observed a reduction of high ventral theta power epochs, as indicated by fewer points (green, right side in each panel) from one day to another in all the three animals. The position of the animals in the maze at times in which theta power was higher in the dorsal CA1 (grey, left side in each panel) was relatively constant from one day to another. Regarding the spatial distribution, I saw again a tendency to cluster at goal locations (cyan circles) and in correspondence of the start box (grey circles), as already noticed examining the peak in theta coherence.

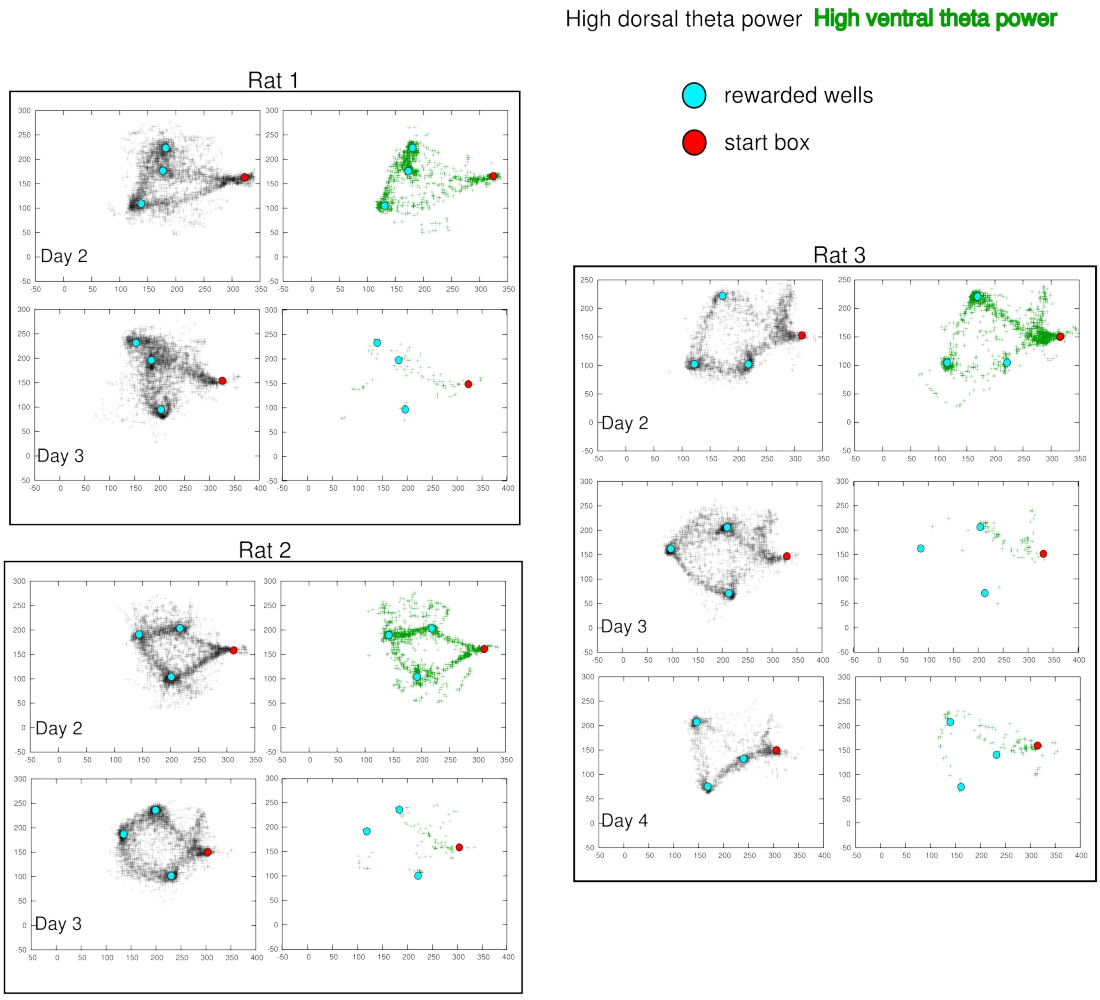


Figure 6. Spatial distribution of ventral and dorsal CA1 theta oscillatory events during learning in relation to power

Examples taken from 2-3 consecutive recorded days in 3 animals. The position of the animals in the maze at times in which theta power was high in the dorsal CA1 low (grey, left side in each panel) and high in the ventral CA1 (green, right side in each panel) during the learning session is plotted. A decrease in the number of points in correspondence to high ventral theta power is evident from one day to another in all the three animals. Note also the clustering at goal locations (cyan circles) and start box (grey circles).

5.6 Goal-related firing in dorsal and ventral CA1 regions.

To investigate how spatial memory is represented in both dorsal and ventral CA1 of the hippocampus during learning, I first looked at the activity of pyramidal cells, searching for any relation between their firing and the spatial location of the new goals. As described in the study by Dupret and colleagues, I first looked for place cells with a place field centred within one goal area by visual inspection of the spatial rate maps constructed by plotting the cell firing rate as a function of two-dimensional maze position (see Materials and Methods). Consistent with the above study and others previously (Hollup et. al, 2001; Markus et al., 2005; Hok et al., 2007), I found that 31 dorsal CA1 pyramidal cells out of 298 were firing in relation to goal locations during learning, 3 of them in correspondence of more than one rewarded well. Remarkably, a small number of ventral CA1 cells were also showing place fields around the goal locations, in particular 12 cells out of 97 were showing a place field at goal location, four of them firing at two goal locations. So, in my database the 10 % and 12 % of pyramidal cells in the dorsal and ventral CA1 sub-fields respectively showed to fire preferentially at goal locations (referred to as “goal-related cells”).

To illustrate the goal-related firing of pyramidal cells, I linearized the path to reduce the space to one-dimension. This computation was reasonable assuming the animals behaved in a stereotyped way after the first few trials, so the path could be decomposed into sequential linear segments from the start box to the first rewarded well and so on. The manipulation allowed us to compare properly the spatial but also

temporal tuning of the cells, looking at the firing peak of individual cells in relation to the three consecutive goal-locations. In figure 7 A, I see some examples of dorsal and ventral pyramidal cells classified as “goal-related cells”. Again, those results showed that in both sub-regions a subset of cells increase their firing at goal locations. Some pyramidal cells were showing a double peak in correspondence of two different goal-locations. In comparison, interneurons were occasionally broadly tuned to one goal location (see the couple of ventral interneurons at the bottom of Fig. 7 A). When I looked only at cells showing a single peak in correspondence of one goal-location, I noticed that in contrast to dorsal CA1 cells that quite distributed across the three goals, the ventral cells were showing a single peak just in correspondence of either second or the third goal location (Fig. 7 B). Even though I am referring to a few samples, still the tendency might reveal sub-region-specific differences in processing the value of the three goal-locations.

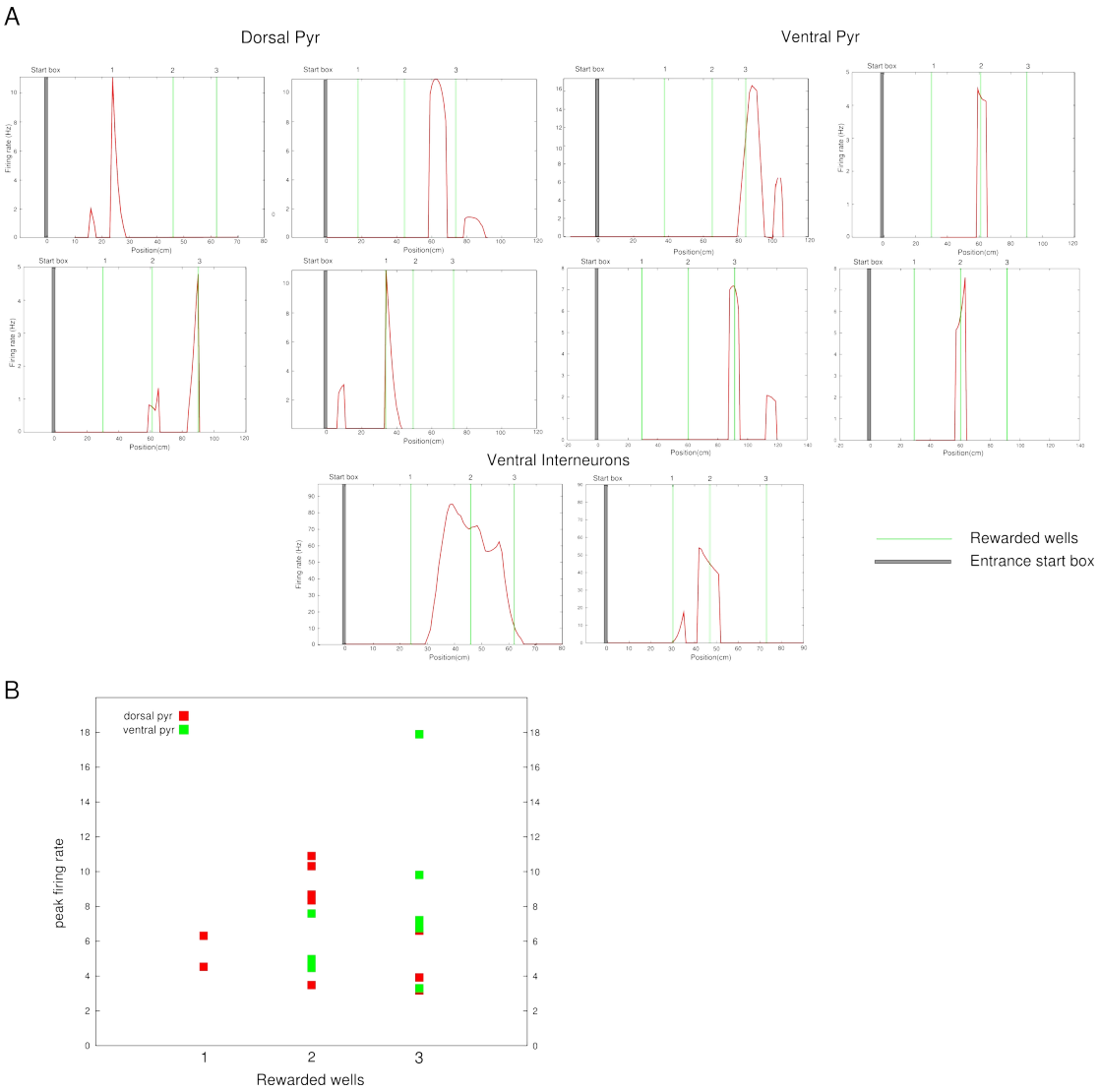

A
Dorsal Pyr
Ventral Pyr
Ventral Interneurons
Start box
Position(cm)
Firing rate (Hz)
Rewarded wells
Entrance start box
B
dorsal pyr
ventral pyr
peak firing rate
Rewarded wells

Figure 7. Goal-related responses of dorsal and ventral CA1 cells on the linearized path

(A) Linear spatial firing of individual dorsal and ventral pyramidal cells (4 in each case) during learning. Examples are selected from pyramidal cells recorded in distinct days from different animals, showing one or more linear place fields in correspondence to goal locations (green dotted lines) or start box (black dotted lines). The length of the linear path is different between days reflecting the change in the position of the three food rewards. A couple of ventral interneurons are also shown at the bottom. Note the broad spatial tuning of the latter.

(B) Distribution of goal-cells among the three rewarded locations. The wells are numbered according to the order in which animals are usually visiting them. On the y-axis, the cell firing peak at the goal locations is shown. Notice no ventral goal cells (green spots) in correspondence to the first well.

5.7 Two-dimensional analysis of the emergence of reward-related firing in dorsal and ventral CA1 regions.

Next, behavioural correlates were examined by using rate maps (Fig. 8, colour maps at the top row for each cell; see Materials and Methods), together with the position maps of cell firing obtained by plotting for each cell all the spikes as a function of the animal position along the path in the maze (Fig. 8, bottom row for each cell). Firing correlates of reward approaching were analysed for both dorsal and ventral CA1 pyramidal cells dividing the learning session in blocks of 5-7 consecutive trials. This approach was meant to reveal the temporal dynamics of the goal-related responses of dorsal and ventral CA1 pyramidal cells already observed when I linearized the path of the animals. In agreement to the results obtained previously, I observed the emergence of spatial firing preference in correspondence of one or more rewarded wells for a certain sub-population of dorsal and ventral pyramidal cells. The firing position was gradually shifted from the spatial preference shown in the probe session before (only first 5 minutes of the pre-probe were considered) to the newly-learnt goal-locations ("place-remapping"). As the examples in figure 8 show, those spatial representations developed from one block to another (cell 3-4-6). A variety of dynamic responses were still observed: while in some cases the cells which had already a place field at one goal location during the pre-probe session, increased the peak firing at learning (see dorsal cell 3 and ventral cell 7 in the fig. 8), other cells in both sub-fields showed a place field at the start box (see dorsal cell 2 and ventral cell 6). Regarding those "start box cells", it is worthy of note that during

pre-probe sessions the animals were rarely exploring this location, and the tuning of firing became sharp along the duration of learning and maintained during the post-probe.

To compare the spatial firing during probe sessions and learning, I restricted the analysis to only the first 5 minutes of exploration during probes as already mentioned. The reason was that the animals tended to cross the previous goal locations and to replicate the trajectory developed in the learning session, showing some memory of the learning task, as demonstrated by Dupret's study. When I tested whether the spatial firing established during learning was maintained in the following probe session, when no reward was present, I noticed that almost the 40 % of dorsal cells maintained the spatial preference acquired during learning. As for the ventral CA1 population, some pyramidal cells gradually developed a firing preference at a goal location too, while others were firing at one goal location only for one or more trial blocks; in both cases, clustering was seen in the spike-position maps during the first 5 minutes of the post-probe.

Collectively, my data show that "place-remapping" was taking place in both CA1 sub-regions and was driven by the goal location. In contrast to what I observed for dorsal CA1 goal cells, the reorganization of spatial firing along rewarded wells was maintained by only a small number of ventral CA1 goal cells during the post-probe session, even though the behaviour of the animal was showing remembrance of the newly-learnt goal locations. The results are in agreement with what reported by Dupret and colleagues for what concerns dorsal cells (2010). In their study, it was

also demonstrated that goal-oriented remapping was abolished in the cue-version of the task, when allocentric strategy was presumably used by the animals to locate the rewards (Dupret et al., 2010). Here, I showed that in contrast to dorsal CA1 goal-related activity, the ventral CA1 cells recognized as goal cells during learning, did not shown the same place preference for the whole learning. Overall, the dynamic of firing activity showed by the two sub-populations in the learning were distinct in two aspects: first, ventral cells seem to organize their firing at the goal locations since the beginning of learning but changed preference along the session, whereas goal-related firing was emerging slower in the dorsal CA1; nonetheless, the spatial firing of dorsal cells was more stable in the second part of the learning, and maintained in the following probe session. In light of the discussed paper, it can be argued the coding of ventral cells was anchored to the presence of rewards, not to spatial locations.

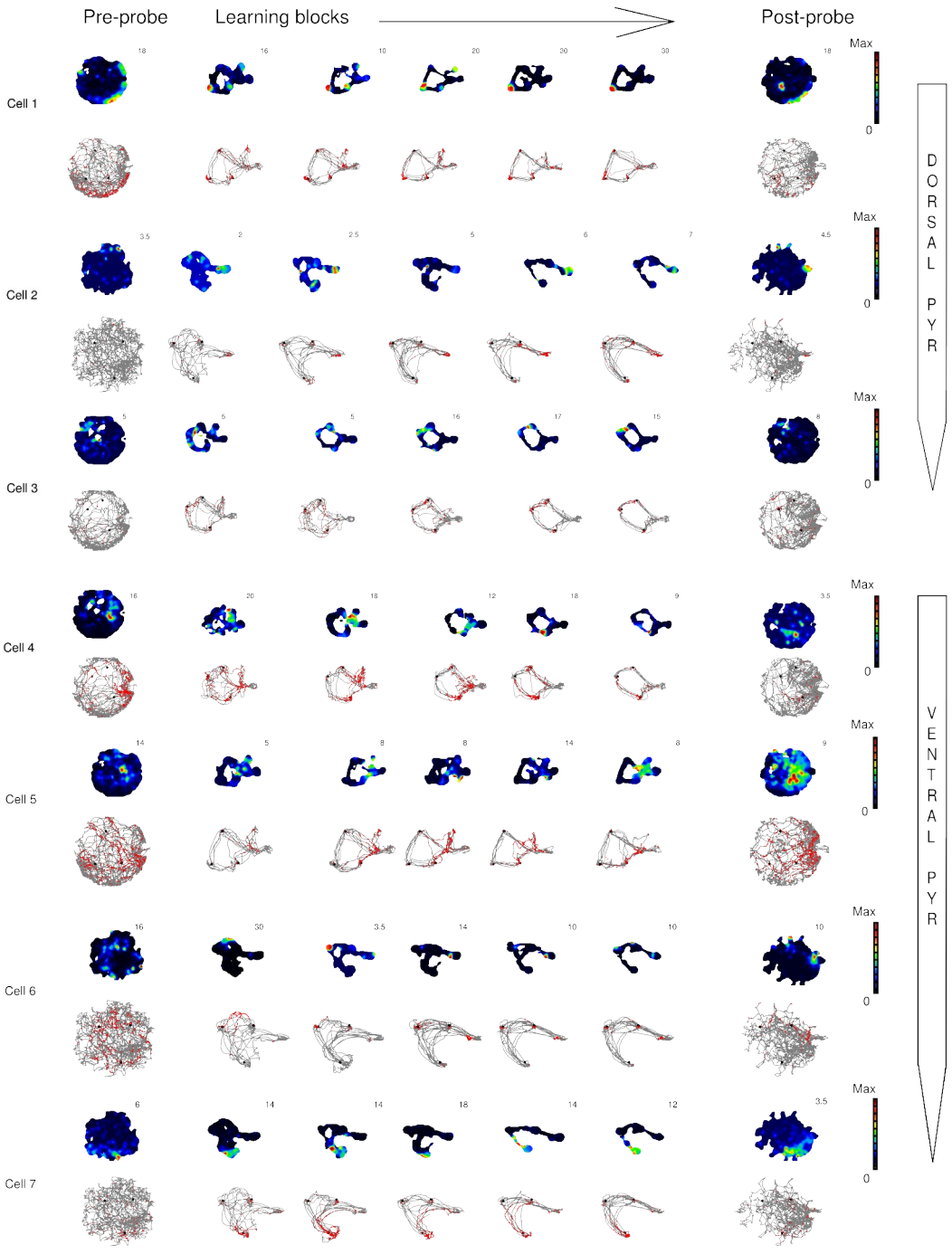

Pre-probe
Learning blocks
Post-probe
Cell 1
Cell 2
Cell 3
Cell 4
Cell 5
Cell 6
Cell 7
Max
0
DORSAL PYR
VENTRAL PYR

Figure 8. Examples of firing-rate responses and spatial firing distributions associated to goal-directed learning for dorsal and ventral CA1 pyramidal cells

Color-coded place-rate maps (top rows) and spatial distribution of individual spikes (bottom rows), for 3 dorsal and 4 ventral representative pyramidal cells recorded. Each row represents the firing patterns of a single cell in a recording day (from left to right: pre-probe, consecutive blocks of 5 learning trials, post-probe; goal locations indicated by dots). Note that the upper dorsal CA1 cell reorganizes its place field to a goal location whereas the middle cell firing at the start-box shows a stable place field across sessions, and the third one is showing a single place field not clearly related to one goal-locations. As for ventral CA1 pyramidal cells, cell 4 develops a place field at one goal location, whereas the last cell increases its firing at one goal location and the other two increase their spiking in correspondence of the entrance of the start box across blocks. Notice how the representative ventral pyramidal cells tend to reorganize their firing during learning (spatial remapping) but in a less stable way than dorsal CA1 cells, and at the end of the learning often do not maintain the place preference observed at the beginning of the session.

5.8 Place-remapping of dorsal and ventral CA1 pyramidal cells: correlation between the joint firing activity of cells during learning and pre/post-probe.

To further study the septo-temporal CA1 specificity of "place-remapping", I looked at the level of correlation between the spatial representation either at the beginning (first 5 trials) or at the end of the learning (last 5 trials) and the spatial representation during pre and post probe. Again, only the first 5 minutes of the probe sessions were taken in account. The analysis was conducted calculating first the joint firing tendency (cofiring) of dorsal CA1 cell pairs and ventral CA1 cell pairs during each of the sessions considered. Therefore, for cells showing spatial firing, the cofiring during online states reflects the degree of place field similarity between the paired cells (Wilson & McNaughton, 1994; O'Neill et al., 2008; Dupret et al., 2010). For each population of pyramidal cells, cells were paired (dorsal cells, n= 2395; ventral cells, n= 503) and the cofiring calculated as the correlation of their instantaneous firing rates (see Materials and Methods). The Pearson's correlation coefficients (R) obtained were averaged for each population and I proceeded in calculating the partial correlation between the cofiring during learning (beginning or end) and cofiring during one of the two probe sessions, controlled by the cofiring of the other probe session (histograms in Fig. 9). I found that in dorsal CA1 the joint firing activity patterns at the end of learning were more correlated to the cofiring measured after learning than during pre-probe (pre-probe, r = 0.267 ±0.006; post-probe, r = 0.410 ±0.005; n= dorsal CA1 cell pairs > 2395, $p<0.001$, Fisher t-test), whereas the beginning of the learning was equally correlated to the firing patterns of

the two probe sessions (pre-probe, r = 0.226 ±0.006; post-probe, r = 0.224 ±0.004; n= dorsal CA1 cell pairs > 2395, p = 0.671, Fisher Z-test). By contrast, the cofiring measured for ventral CA1 cell pairs during the last trials of the learning session was significantly less correlated to the post-probe than the pre-probe cofiring (r = 0.539 ±0.01; r = 0.389 ±0.014; n= ventral CA1 cell pairs > 503 , p<0.008, Fisher Z-test). In addition, the cofiring at beginning of learning was slighty more correlated to the post-probe cofiring than the pre-probe cofiring (pre-probe, r = 0.435 ±0.01; post-probe, r = 0.518 ±0.009; n= ventral CA1 cell pairs > 503, p<0.05, Fishers Z-test). Consequently, during the recall testing (post-probe), ventral cells tended to fire as at the beginning of the learning sessions, whereas dorsal cells showed firing activity presented at the end of learning. The higher values of cofiring reported for ventral CA1 cells might suggest that even though goal-related reorganization of spatial firing was shown during learning, pre-existing spatial representations continue to be present and compete during learning. Those results converged to Dupret's results and to what was found looking at the rate maps. Given that dorsal CA1 cells slowly developed a place field at goal locations that is maintained after learning, whereas ventral cells remapped faster, it is reasonable that the firing activity patterns at recall might reflect distinct information acquired during the learning session by the two sub-populations.

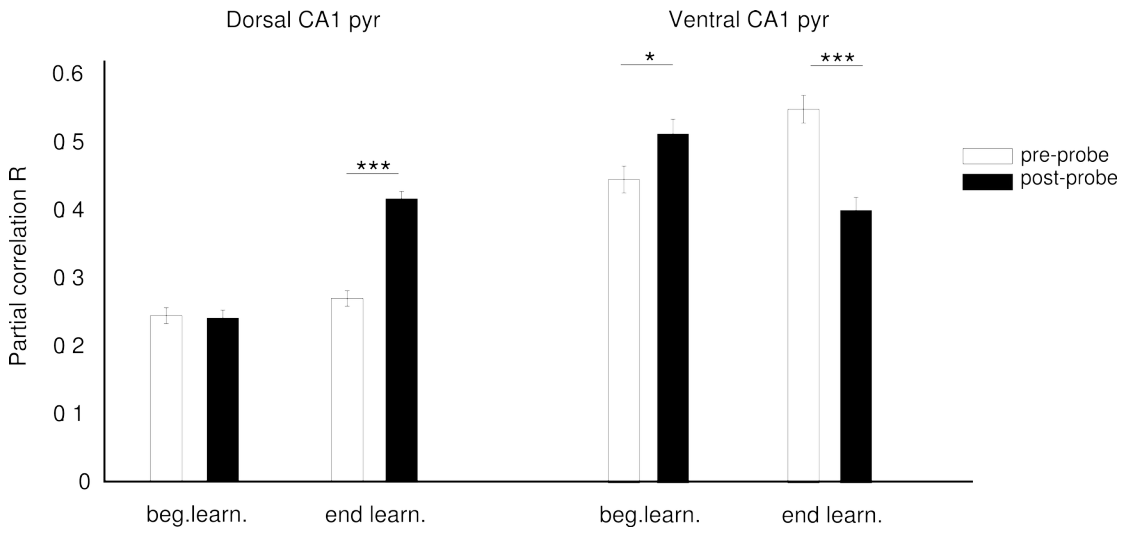


Figure 9. Place remapping of dorsal and ventral CA1 population on the cheeseboard maze

(A) For each population of pyramidal cells, correlation between joint firing activity during pre-probe/post-probe and joint firing activity calculated separately at the beginning (first 5 trials) and at the end (last 5 trials) of the learning session are shown. The correlation values represent the average of the Pearson coefficient for each learning session analysed, which was calculated as the partial correlation of the cofiring during learning and during one of the probe session, controlled by the cofiring of the other probe session.

5.9 Change in firing rate during learning for dorsal and ventral CA1 pyramidal populations.

In spite of the spike timing in relation to space and theta oscillation, information in the hippocampus might be represented also by the individual firing rate in the cell assemblies active in that particular environment (O'Keefe and Dostrovsky, 1971; Huxter et al., 2003; Leutgeb et al., 2005; O'Neill et al., 2006; Huxter et al., 2008). The hippocampal rate code is largely dependent on the set of excitatory and inhibitory inputs received. The temporal patterns of those shape the cellular response and eventually the CA1 outputs towards other brain regions. Therefore, the encoding of information during spatial learning will presumably arise in change (increasing or decreasing) of the mean firing rate of CA1 cells active in that specific environment.

To address the question whether reorganisation of firing rates ("rate remapping") was taking place on the cheeseboard maze besides "place remapping", I looked at the mean firing rate of dorsal and ventral CA1 pyramidal cells during the first and the second halves of the learning session. For each population, I considered only cells active (>0.5 Hz) for the whole duration of the learning sessions (dorsal CA1= 298 cells; ventral CA1= 97 cells). As shown in figure 10, the mean firing rate of ventral CA1 pyramidal cells tended to be higher in the first half, whereas no change was observed for dorsal pyramidal cells. Nevertheless, the comparison between the two CA1 sub-populations, revealed that the mean firing rate was significantly higher ($df= 393$, $p<0.01$ in both case, unpaired t-test) for ventral cells than dorsal cells in

both halves (dorsal pyr: first half= 1.52± 0.43, second half= 1.61±0.29; ventral pyr: first half= 3.43± 0.98, second half= 2.32± 0.51). This is in agreement to previous studies, showing a higher firing rate of ventral pyramidal cells than dorsal ones (Brun et al., 2009; Ahmed & Mehta, 2009 for a review). Since the pattern of projections along the longitudinal axis changes conspicuously, the CA1 mean firing rate reflects mainly the unique balance of the inhibitory and excitatory drives as well as the temporal convergence of the EC inputs gated by the CA3 sub-regions. Those resulted in the property of CA1 cells to be active in the environment, and how sparse and coherent the firing is at the level of single cell.

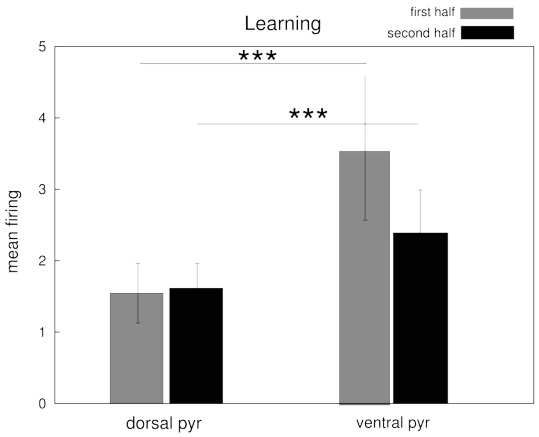


Figure 10. Firing rate of dorsal and ventral CA1 populations during learning

Average firing rate in the first half and second half of learning are compared for both populations. Notice that the mean firing of ventral CA1 pyramidal cells is in both conditions higher than the mean for dorsal cells, even though decreasing in the last part. Only cells that fired more than 0.5 HZ in both halves were considered.

Discussion

We saw in the previous chapter that dorsal and ventral CA1 oscillate coherently in theta frequency at discrete times. Higher degree of coupling between dorsal and ventral CA1 in theta frequency provides a mechanism to coordinate their functions. Periods of high coherence in the power of ongoing theta waves were characterized by a consistent phase-shift of almost one full cycle between the two sub-regions. Given that the independence of ventral theta oscillations from speed is indicative of the diminishing spatial inputs received, it was remarkable that the amplitude of ventral theta oscillations was modulated by this sensorial parameter at those times.

Here, I extend the previous results, showing that dorsal and ventral CA1 were oscillating coherently in the theta range preferentially during spatial learning. By using the cheeseboard maze task, I was able to examine separately periods of exploration and goal-directed spatial navigation performed in the same maze. Therefore, even maintaining the same familiar environment (the maze), the context was changed by introducing new components such as rewards. The location of the three rewarded wells was fixed for the whole duration of the learning session, and changed from one day to another. The spatial demand was also affecting the

context, the animals being required to collect the three rewards and come back to a starting point for more than 40 trials. All the animals were able to learn the task quite soon, as shown by the performance in terms of path length. I found that during spatial learning, the power of theta oscillations in the dorsal and ventral CA1 was correlated and highly coherent, particularly during the first trials of learning. It has been shown that mPFC and hippocampal networks oscillate coherently when the animal explores a novel environment and that novelty alters hippocampal theta parameters. Here, the increase was observed at the beginning of learning and recall (post-probe) but not when the animals were exploring a novel environment, ruling out the effect of novelty. Therefore, the change in theta dynamics previously observed seems to be related to some specific property of the context. The change was temporally enhanced at the beginning of the task and of the recall session. In addition, I found that the positive speed modulation of ventral theta oscillations previously reported was restricted to the learning session, at times in which coherence between the septo-temporal poles was relatively high. Importantly, the effect was not related to a change in the average speed of the animals across sessions, neither was it complemented by a change in the frequency of the oscillatory events. By contrast, dorsal theta oscillatory patterns had a higher frequency during spatial learning, as expected by other studies (Jeewajee et al., 2008b; Hinman et al., 2011; Richard et al., 2013). Athough, the coherence in the power of two distinct oscillatory events may already suggest coordination between the two patterns, the temporal binding of two oscillatory events is mostly supported

by the phase-relationship at each cycle. My analysis confirmed a constant phase shift of almost one cycle between dorsal and ventral theta oscillatory patterns during spatial learning. The difference in phase as calculated here was used as a raw measurement to infer the phase coherence. Appropriate analysis might be required to confirm my results.

I further showed a spatial discontinuity of theta coherence between the two sub-regions. Interestingly, high coherence tended to occur at salient places such as goal-locations and start box. The result might be the effect of the independent increase in theta power in the two sub-regions, so I looked at the spatial distribution of high power events for dorsal and ventral theta. Here, I found that the power of ventral theta oscillations decrease across days, whereas the same was not observed in the dorsal CA1 sub-region. The same effect of reduction was not observed in the coherence analysis (data not shown). Therefore, my results did not depend on the power of the oscillations in the two CA1 sub-regions.

In a recent study, Adhikari and colleagues (2011) found that the temporal portion of the hippocampus was more correlated to the medial prefrontal activity in the theta range than to the septal pole of the hippocampus during anxiety behaviour. They conclude that dorsal and ventral hippocampi might synchronize to process spatial information, whereas the ventral sub-regions would synchronize with mPFC in relation to anxiety. My results may confirm their prediction by showing that during a goal-directed navigational task, dorsal and ventral CA1 subregions were coupling in the theta range. The fact that the power of theta oscillations in the

ventral sub-region was exceptionally modulated during high coherence periods, might suggest that a flow of spatial information was indeed taking place.

In addition, I investigated the emergence of reward-related firing in the ventral sub-regions, looking at the presence of "goal cells" (Dupret et al., 2010). Those have been described as cells whose place field corresponds to rewarded locations during the task. In the above study, it was found that a percentage of pyramidal cells in the dorsal CA1 but not in the dorsal CA3 region developed a firing preference at one goal location during learning, and the place field remained stable after learning, when memory for those locations was tested in the post-probe. Here I found that "goal cells" were also present in the ventral CA1 regions, representing one or more than one goal location. Those cells were visually selected observing the linear place maps and the spatial rate maps. A first screening was done by looking at the position of the spikes for each cell. The data obtained by the dorsal sample was consistent to the Dupret's study, whereas the ventral sample showed some distinct features in relation to the temporal emergence of goal-related firing. First, I found that comparing to dorsal goal cells, ventral cells tended to fast remapping at goal locations during learning. Second, no ventral cells were found to have a single place field in correspondence of the first goal location, relative to the stereotyped path of the animal. It is important to notice that the sample of ventral cells was limited in number; therefore my results did not allow a real quantification of the phenomena observed. Still my evidence suggest that the firing of ventral cells was fastly driven by the change in context represented by the learning session. The presence of the

rewards was probably crucial as shown by the fact that the relocation of the firing during the post-probe observed for ventral cells, similarly to what shown by Dupret in dorsal CA3. Based on the absence of ventral CA1 goal cells at the first goal location, I might also speculate that cells in the ventral CA1 region distinguished the three three reward-locations and coding for the salience. Indeed, the three goals might differ in behavioural value to direct the animal choice (see General discussion). Both place remapping and rate remapping were observed in the ventral CA1. The place remapping suggested by the spatial rate maps was confirmed by the correlation between the firing activity patterns observed during the two probe sessions and the population firing activity either at the beginning or at the end of the learning. In agreement to the cited study, I found that the dorsal CA1 firing activity at the beginning of the recall session, when the animals actually were showing memory of the previous goal locations, was highly correlated to the firing activity patterns observed at the end of learning. In contrast to the above results, the firing activity patterns of ventral CA1 cells in the post-probe were more correlated to the beginning of the learning and less to the end of it, the last reflecting more the firing activity observed during the pre-probe. To interpret those results, two considerations are needed: first, I found that the mean firing of ventral pyramidal cells was higher than the mean firing of the dorsal sub-population; the firing of ventral cells was also quite sparse (spatially) during the probe sessions for ventral cells, comparing to the tuned spiking of dorsal pyramidal cells. Therefore, the probability that two active cells were firing together in the ventral CA1 region was

expected to be by chance higher than in dorsal CA1. This might have affected the cofiring measurement and explain the relatively high correlation values observed for ventral cells in all conditions. Beyond those effects, my data showed that the two populations differed in the temporal dynamics of changing their firing activity during learning and at recall, so that dorsal CA1 cells stabilized and maintained the spatial representations of the goals developed during learning whereas ventral cells did change the reprentation during learning. As I mentioned, rate remapping was also observed in the ventral CA1 during learning. I reported a decrease of the mean firing rate in the second half of the learning. The mean firing rate of CA1 cells active in that specific environment is largely dependent on the inputs received, which eventually determined the specific outputs towards other regions. Therefore, the change observed in the ventral CA1 presumably reflects the encoding of information during spatial learning.

Overall, my results support a model in which the dorsal and ventral hippocampus together provides contextual specificity and provides information to the goal-directed system for navigation. The term “context” as used here refer to any set of cues which situate the animal in place and time, a type of information thought to be encoded by the hippocampus (Nadel, 2008). In other words, the animal’s emotional state such as hungriness, anger, fear, can be included in the definition. The presence of reward surely influences the motivational state of an animal, thus its behavioural response in that context. The emotional system (comprising ventral striatum, amygdala, and ventromedial prefrontal cortex) might

provide those kind of informations to the ventral hippocampus, to be integrated with the spatial information processed in the dorsal hippocampus.

Chapter VI
Reactivation of learning-associated assembly patterns during sleep in the ventral hippocampus

Introduction

Memory theory postulates that after a memory trace has been formed in the brain, this needs to be consolidated and stored for future retrieval when circumstances (context) required. According to the "multiple-memory systems" theory, no brain structure would be dedicated specifically to memory storage on its own, but memories traces would be stored in multiple systems in relation to the nature of the processed information (White & McDonald, 2002). Because memory storages would work in parallel, either interference or convergence might occur with memory traces controlling the actual behaviour. Whether memory function is localized or more distributed in the brain circuits, much evidence support a crucial involvement of the hippocampus in memory processing. It has been shown that during offline (i.e. non-exploratory) states, cells in this brain region tend to maintain the temporal firing relationship previously acquired, so that the firing patterns observed in awake state are "reactivated" in successive sleep or immobility periods. In rodents, where this phenomenon was first demonstrated, the temporal structure of firing patterns seen in exploratory periods are strictly linked to the spatial firing of

cells, implicitly for place cells (Kudrimoti et al., 1999; O'Neill et al., 2008; Wilson and McNaughton, 1994). Indeed, it stands to reason that cells whose place fields are overlapping in an environment, have a higher probability to fire together in sleep and immobility. Moreover, it was shown that if animals repeatedly follow the same movement paths, the resulting spatio-temporal sequence of place cells is also reactivated (Skaggs and McNaughton, 1996; Lee and Wilson, 2002). Presumably, the tendency of place cells to fire in a previous established spatio-temporal order without any sensory inputs must reflect a change at the synaptic level. On a functional level, such replay of previous experience might underlie memory consolidation during sleep. A study from Dupret et al. (2010) has provided strong evidence that the reactivation of hippocampal cell assembly firing patterns represents the expression of new spatial memory traces, linking the newly learnt spatial information to sleep consolidation and subsequent retrieval. Specifically, the authors showed that during a hippocampal-dependent spatial task, the development of dorsal CA1 cell assemblies at salient locations such as rewards correlates with subsequent reactivation of the same assemblies in sleep. In addition, the reactivation of the new cell assemblies during sleep predicted the behavioural performance of the animal when immediately tested for memory retention.

In reference to the process of memory consolidation, network patterns dominating the offline states of the hippocampus are considered the substrates for newly acquired memories to be gradually transferred to neocortical stores. Specifically, large-amplitude local field potentials, known as sharp waves (SWs),

occur sporadically in the stratum radiatum of dorsal hippocampal CA1 during slow-wave sleep as well as immobility, consummatory behaviours, grooming (Buzsaki et al., 1983; Suzuki and Smith, 1987). In the correspondent CA1 pyramidal cell layer the so-called “ripple” fast-field oscillations (140 –200 Hz) are associated to SWs (SWRs) (O’Keefe and Nadel, 1978; Buzsaki et al., 1992; Ylinen et al., 1995; Csicsvari et al., 1999). During the duration of a SWR event (50-150 ms) 10–18% of all neurons discharge synchronously in the Cornu Ammonis/subicular complex (Chrobak and Buzsaki, 1996; Csicsvari et al., 1999a), a significant several-fold increase of population synchrony compared to theta oscillations (Csicsvari et al., 1999a). Past experiences of the animal influence the neuronal participation in the population discharge of SWRs (Buzsaki, 1989; Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996; Kudrimoti et al., 1999; Nadasdy et al., 1999; Lee and Wilson 2002; Foster and Wilson, 2006; O’Neill et al., 2006, 2008; Diba and Buzsaki, 2007; Karlsson and Frank, 2009; Singer and Frank, 2009; Dupret et al., 2010). The large network excitability and the behavioural-related spike content of individual cells associated with SWRs (Csicsvari et al., 1999a) could be crucial for both memory consolidation within the hippocampus and in transferring the formed memories to the neocortex (Buzsaki, 1989, 1996; Wilson and McNaughton, 1994; McClelland et al., 1995; Siapas and Wilson, 1998; Ji and Wilson, 2007; Axmacher et al., 2008; Eschenko et al., 2008; Molle et al., 2009; Ramadan et al., 2009). In support of this hypothesis, selective elimination or disruption of SWRs during post-learning sleep results in impairment of memory (Jadhav et al., 2012; Girardeau et al., 2009; Ego-

Stengel and Wilson, 2010; Nokia et al., 2010). More recently, it has been argued that awake and sleep SWRs might be functionally dissociable, the first being mostly involved in spatial working memory (Jadhav et al., 2012).

The occurrence of SWRs in the ventral CA1 region of the hippocampus was reported in a few papers, as a physiological mark to recognize the pyramidal layer when recording (Maurer et al., 2006; Patel et al., 2012). During the writing of this thesis, a study was published characterising the property and dynamics of SWRs along the dorso-ventral axis of CA1 region (Patel et al., 2013). With the purpose to extend the previous observations and hypothesis, in this chapter I will first examine the profile of SWRs and the temporal correlation of those events during slow-wave sleep in the two hippocampal CA1 sub-regions. Complimentary, the population response to local and distal events will be compared for both excitatory and inhibitory cells, during sleep as well as exploration.

Certainly, it has been shown that SWR events in the dorsal hippocampus are related to the reactivation of the cells' firing sequences established before. Specifically, the temporal order in which neurons fire in this burst of activity reflects in a compressed scale the sequences of cell spiking observed during waking exploration when animals follow repeatedly similar movement paths, while, in less constrained exploration of 2 dimensional environments, only places are reactivated (Wilson and McNaughton, 1994; Kudrimoti et al., 1999; Lee and Wilson, 2002; O'Neill et al., 2008; Dupret et al., 2010). Therefore, it has been hypothesised that this

preservation of organised pyramidal cell spiking during SWR reflects the replay of experience during sleep, such as might underlie memory consolidation. Further validation came from a study in which the selective elimination of SWR patterns after learning interfered with memory consolidation (Girardeau et al., 2009). Therefore, SWR-associated reactivation might underlie the consolidation of learned patterns in the hippocampus. Hippocampus-encoded memory traces would be consolidated for long-term storage and reinstating in cortical networks, by possibly reversing the flow of information from the hippocampal networks to the cortex during offline states (Marr, 1971; Buzsaki, 1989; McClelland et al., 1995).

So far, none of the studies has focused on the dynamics of reactivation at different levels of the long axis of the hippocampus. Starting from the hypothesis of a functional differentiation along the dorso-ventral axis, I might predict a scenario in which a cortical activity initiates a cascade of memory consolidation in the hippocampal network, through which replayed memories carrying different information would converge and sent back to the associational cortices.

To evaluate the theory of memory consolidation during sleep, I looked at the joint firing activity of hippocampal neurons along the longitudinal axis of the hippocampus. In this chapter, I aim to determine whether patterns of hippocampal activity observed during spatial learning are reactivated during sleep in the ventral CA1 as well as in the dorsal CA1. To examine the phenomenon after recently newly acquired memories, the analysis will be conducted within and across the two CA1 sub-regions of the hippocampus. The hypothesis predicts that simultaneous

reactivation at the two poles of the hippocampus should be coherent if the reactivation of the processed information needs to be coordinated throughout the brain structure before transferring to extra-hippocampal sites.

If replay events do take place also in the ventral pole, a critical question concerns whether those are also strongly related to SWRs, given that SWR-associated hippocampal-cortical interactions are presumably the substrate by which memory is consolidated during sleep. Therefore, I will first characterise the firing relationships between the dorsal and ventral CA1 to prove communication during offline periods of rest and sleep characterised by SWR patterns. Further, I will look at the relation between reactivation and the occurrence of dorsal and ventral SWRs. Eventually, this should give insights into the role and the nature of the content carried by those intrinsically generated oscillatory patterns.

Results

Recordings were done during sleep periods before (pre-sleep) and after (post-sleep) the learning sessions, in which the animals were left to rest in the familiar start box contiguous to the cheese-board maze (see Materials and Methods). Those ~30-minute sleep sessions included immobility/rest periods as well as real sleep. As previously described, epochs of long immobility and low theta/delta power ratio were offline selected and classified as slow wave sleep/rest (see Materials & Methods; O'Neill et al. 2008, Dupret et al., 2010). Sequences of sleep-learning-sleep were analysed.

6.1 Field potential during slow-wave sleep SWR patterns and awake SWRs.

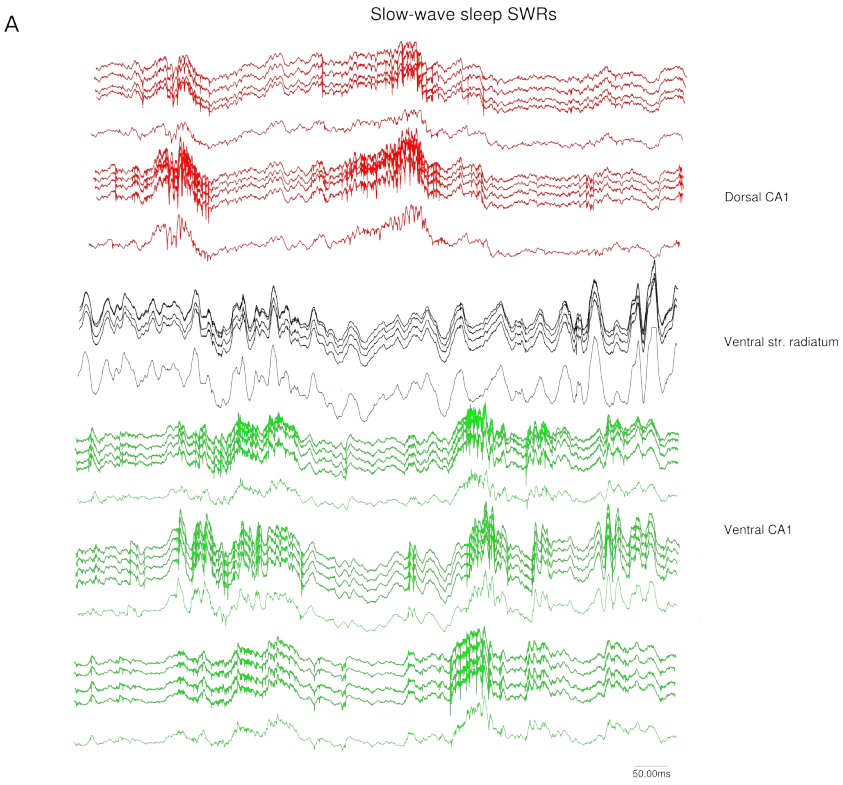
SWR activity was detected both in the dorsal and ventral CA1 sub-fields during sleep and exploration. In figure 1A, raw traces recorded from an animal resting in the start box show the occurrence of two subsequent SWRs in the pyramidal layer of dorsal CA1 (red traces), whereas only one is clearly evident in the ventral CA1 pyramidal layer (green traces). During sleep, such fast oscillatory events were less common in the temporal CA1 than in the septal sub-region, and not always

simultaneously occurring in the two sub-fields. The panel B depicts the occurrence of one SWR event during exploration (eSWRs) at ventral electrodes. A burst of spiking activity was observed in correspondence of the ripple oscillations. In this example, the LFP of dorsal CA1 appeared not affected by the simultaneous patterns in the temporal pole.

To investigate whether ventral SWRs preserved the same well-documented profile of the opposite pole of the CA1 region, I examined the field potential average calculated at each layer of CA1 during a single slow wave sleep period. For the analysis, I selected one reference electrode situated in the pyramidal layers in each sub-region, so the averaging was operating in time windows centred to the maximum ripple power recorded in those electrodes. As displayed in figure 2A (left side), the amplitude of the SW deflection was higher in the stratum radiatum for both sub-regions, and ripples were observed only in the pyramidal layers. Comparing the two reference systems, some differences emerged in relation to the amplitude and the occurrence of the patterns: first, the amplitude of the SW was larger in the dorsal CA1 region, second there was a less chance to see a deflection in the dorsal CA1 region when referring to events detected in the ventral CA1 than vice versa. The same profiles emerge when I considered waking SWRs (Fig.2A, right side). To notice, an envelope of theta waves was observed at dorsal electrodes but not in ventral ones as expected by the lower amplitude of ventral theta oscillations reported in the previous chapters.

Even though those first results did not globally explicate the characteristics of ripple fast oscillatory complexes and how they emerge, they surely suggest that SWRs were similar in the septal and temporal poles, starting from the larger SW in stratum radiatum to the associated ripple oscillation in the pyramidal layer. Moreover, SWR events in the two sub-regions of the hippocampus were not temporally synchronized.

Given that SWR patterns occurred in the ventral CA1 of the hippocampus as well as in the dorsal, I next investigated the temporal correlation between the fast oscillatory patterns in the two regions. I then calculated the cross-correlograms between the timing of dorsal and ventral SWRs by using a temporal window of 200 ms centred at the timing of dorsal SWRs and binning of 2 ms (those parameters were selected after a first calculation was made using larger windows of 500 ms and 5ms binning). As seen in figure 2B, when singular events were detected simultaneously in the pyramidal layers of the two sub-regions, there was high probability the ventral SWR was following the dorsal one with a delay of 2 ms (peak of probability=0.123). Considering the theorized mechanisms that generate SWRs, this might indicate a propagation of the events, presumably in relation to the upstream activity (CA3 or EC) that drive the synchronous discharge of the local CA1 population. A difference in the extension of those projections as well as the level of excitation/inhibition could lead the direction from the septal to the temporal CA1.


A
Slow-wave sleep SWRs
Dorsal CA1
Ventral str. radiatum
Ventral CA1
50.00ms

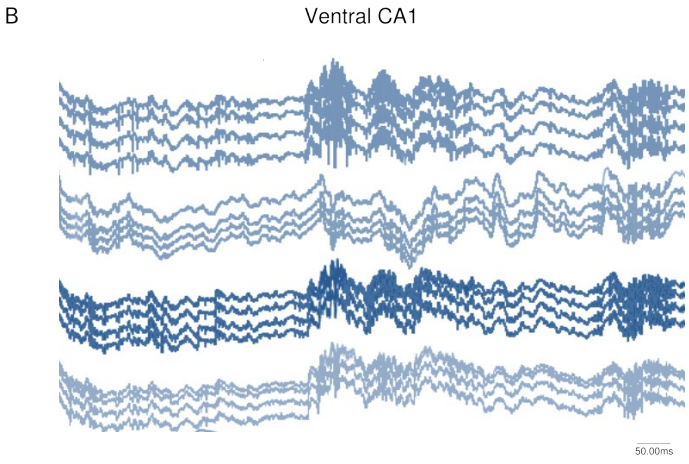

B
Ventral CA1
50.00ms

Figure 1. Field potential during slow-wave sleep SWR patterns and awake SWRs

(A) Wide-band traces (top, 1 Hz-5 kHz) from dorsal (red) and ventral tetrodes (green) located at the CA1 pyramidal layer. One tetrode in the ventral radiatum is also shown (black). The figure shows SWR events (see arrows). In the example, ventral SWRs follow dorsal SWRs patterns.

(B) As in A, but during exploration. Only 4 ventral tetrods are shown. Notice the absence of theta in LFP of ventral tetrodes around the eSWRs.

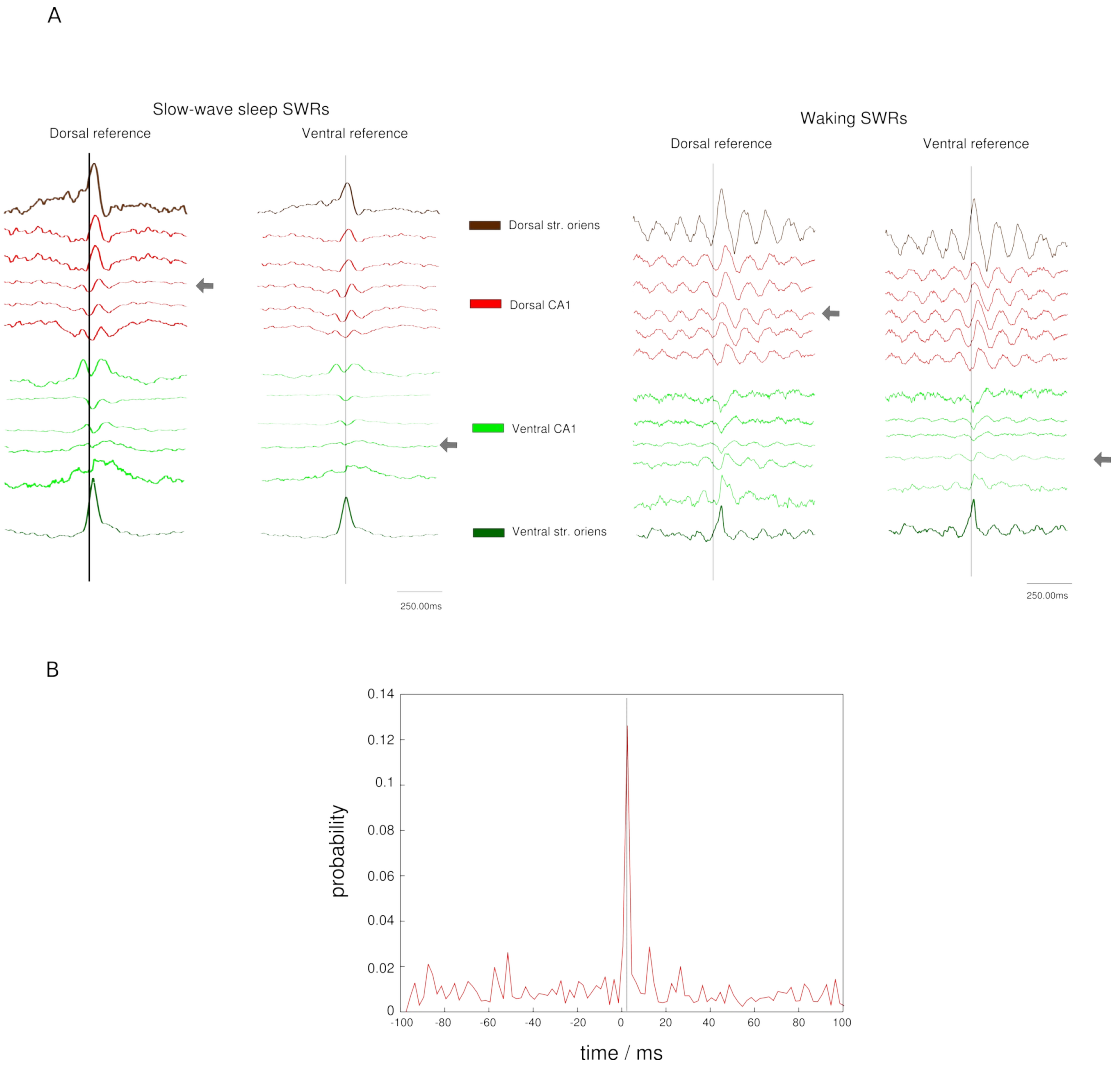


Figure 2. Slow-wave sleep ventral SWR patterns follow dorsal SWR events

(A) Average local field potential patterns relative to dorsal and ventral SWR patterns in a sleep session (left) and during exploration (right). In both cases, sharp waves are negative in the stratum radiatum (SR), and positive in the dorsal and ventral stratum oriens (orange and dark green respectively) in the two sub-regions. The arrows indicate the reference electrode.

(B) Average cross-correlograms of dorsal and ventral slow-wave sleep SWRs patterns. A temporal window of 200 ms centred to dorsal events and bins of 2 ms are used. Note the time-shifted peak at 2 ms, indicating that ventral SWRs occur after dorsal ones.

6.2 Both dorsal and ventral cells increase their firing at dorsal slow-wave sleep SWRs.

The field potential analysis showed similar profile for dorsal and ventral SWR patterns. It is known that dorsal CA1 cells tend to fire synchronously during SWRs (approximately 5-fold increase in the recruitment of pyramidal cells compared to theta epochs), reaching a several-fold increase in the firing rate of pyramidal cells (Csicsvari et al., 1999a; Csicsvari et al., 2000) and, with short delay, of sub-sets of interneurons relative to non-SWR periods (Csicsvari et al., 1999b; Klausberger et al., 2003; Klausberger et al., 2005). So, next I examined whether the firing rate of ventral CA1 cells was affected by those bursts of activity as well. I examined firing rate changes during SWR periods associated with slow-wave sleep, distinctly for pyramidal cells and interneurons. I also considered separately dorsal and ventral SWRs. As expected, both dorsal pyramidal cells (n= 298) and interneurons (n= 47) increased their firing rates during local slow wave sleep-associated SWRs (Fig. 3). Interestingly, even ventral cells increased their firing in correspondence of dorsal SWRs: indeed, ventral pyramidal cells (n= 92) showed a peak of activity temporally coupled to the peak of dorsal activity, whereas interneurons belonging to the ventral sub-region (n= 42) showed a delay in the peak relative to the peak of activity of dorsal interneurons. When I looked at ventral SWRs, I noticed that both excitatory and inhibitory ventral cells were increasing their firing in correspondence of those events. The baseline was constant, only the amplitude was smaller than the one

showed to local SWRs. This suggests a gain effect, more than a multiplicative one. Dorsal cells did not show modulation by events in the ventral sub-regions.

In summary, both neuronal populations firing was affected by local SWR complex, however moderate firing rate changes in correspondence to distal events were observed only for the ventral sub-populations: excitatory cells were increasing their firing coherently to the dorsal pyramidal cells, whereas the peak of firing for ventral interneurons was slightly delayed relative to the dorsal interneurons. By contrast, dorsal pyramidal and interneuron cells were not modulated by ventral SWRs. Generally, the increase in firing rate for ventral cells was modest, probably due to the already higher baseline (as seen when analysing the mean firing rate in the previous chapter), but still temporally aligned to the peak of the SWRs events.

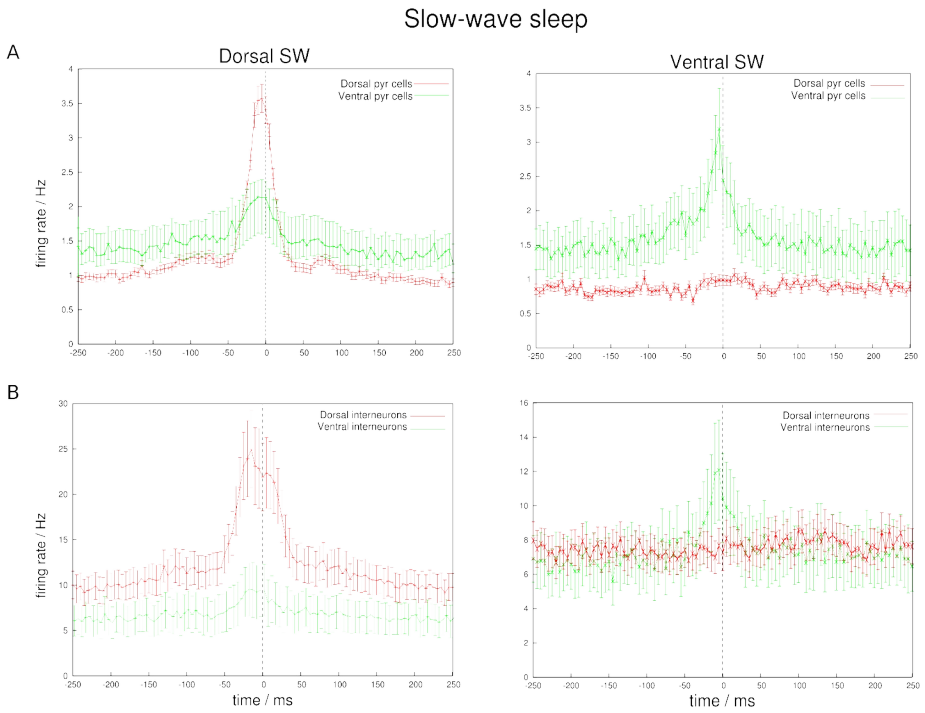


Figure 3. SWR responses of dorsal and ventral cells to local and distal slow-wave sleep associated patterns

(A) Mean firing rate histograms for dorsal CA1 pyramidal cells (n=298) and ventral CA1 pyramidal cells (n=92) relative to dorsal and ventral ripple events during sleep. The firing of individual cells was cross-correlated with the SWR peak times (i.e. the peak of maximum ripple power) and the cross-correlograms of different cells were averaged. Firing rates were measured in 5 ms bins in reference to SWR peaks. Error bars ± SEM.

(B) The same as in A for dorsal interneurons (n=47) and ventral interneurons (n=42).

6.3 Reactivation of learning-associated assembly patterns in the dorsal CA1 during SWRs.

Given that SWR events have been shown to be important for the reactivation of information in the dorsal hippocampal networks (O'Neill et al., 2008), these periods were first investigated for reactivation in the dorsal CA1. So, periods were selected during non-theta (offline) epochs when dorsal CA1 SWRs occurred.

As already described in the previous chapter, cofiring is an index of the tendency of two cells to fire at the same time. In the case of place cells, high cofiring during exploration will give a measure of overlapping in their place fields. During sleep, when no sensory inputs reach the hippocampus and other brain structures, cofiring might reflect the reactivation of learned associations developed during prior experience (see introduction to the chapter; Wilson and McNaughton, 1994; O'Neill et al., 2008; Dupret et al., 2010). The analysis of correlation between cofiring in exploration and cofiring in subsequent sleep was used to evaluate whether the activity present during exploration is reactivated during sleep/rest. The analysis was conducted in parallel selecting sleep periods in which either dorsal or ventral SWRs occurred. To take into account the activity present before exploration, that is the learning session, I calculated the partial correlation, using the pre-sleep cofiring as a control (McNaughton et al., 1994; Dupret et al., 2010). I proceeded in calculating the cofiring between a pair of cells as the correlation of their instantaneous firing rates, according to previous studies (Wilson and McNaughton, 1994; O'Neill et al., 2008;

Dupret et al., 2010). In figure 4a, I see that activity patterns associated with learning were reactivated in the subsequent sleep when SWRs occurred in the dorsal CA1 ($r = 0.345 \pm 0.014$; p-value <0.01). This correlation value was significantly higher than when I correlated the learning joint activity with the pre-sleep cofiring ($r = 0.263 \pm 0.012$; p-value <0.05) (all n= cell pairs > 2400, $p<0.0005$, Fishers Z-test). As expected from the field/unit responsiveness analysis showing that dorsal CA1 cells did not increase the activity during ventral SWRs, no reactivation was observed in those periods.

Because of the dynamic process of the learning, in which cell assemblies are thought to emerge across the trials and stabilize towards the end, I repeated the same analysis splitting the learning session in begin (first 5 trials) and end (last 5 trials) of learning. Indeed, data presented in two studies by Dupret and colleagues (2010; 2013) indicate that during the 40 trials in which the animal is spanning the maze and updating the position of the rewards from its past experience, cell assemblies associated to the new goal-locations are formed and compete with the one associated with the old goal-locations; in the second half of the session, the contingent cell assemblies emerged would become predominant and replayed in subsequent off-line states. My data were consistent with the previous results, showing that the end of the learning session was more reactivated than the first part during the post-sleep (respectively: $r = 0.408 \pm 0.006$; $r = 0.198 \pm 0.008$; all n= cell pairs > 2395, $p<0.0005$, Fishers Z-test), whereas no significative difference was

observed in the pre-sleep (respectively: r = 0.212 ±0.008; r = 0.206 ±0.007; all n= cell pairs > 2395, all p>0.05, Fishers Z-test).

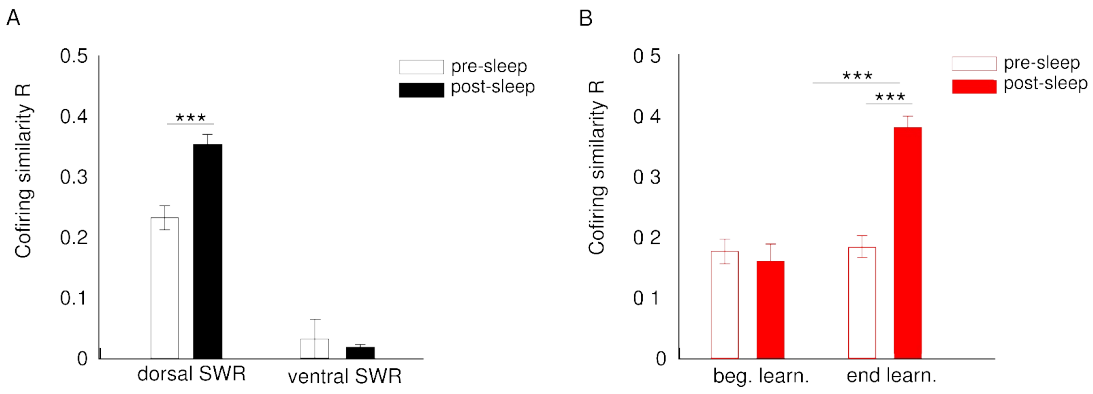


Figure 4. Reactivation of dorsal CA1 pyramidal cells during slow-wave sleep-associated SWRs

(A) Correlation between the joint activity of dorsal pyramidal cell pairs during learning and their sleep cofiring during local (dorsal) and distal (ventral) SWRs is shown. The correlation coefficients represent the partial correlation of joint firing activity patterns during learning and cofiring during sleep, controlled by the cofiring of the other sleep session.
(B) Reactivation during pre and post-sleep is shown separately for the beginning (first 5 trials) and the end (last 5 trials) of learning sessions. The correlations coefficients were calculated as in A.

6.4 Reactivation of learning-associated assembly patterns in the ventral CA1 during SWRs.

The reactivation analysis was repeated for ventral pyramidal cells. This time, reactivation in sleep was tested during dorsal SWRs and ventral SWRs periods. For each pair of ventral pyramidal cells, I proceeded in calculating the joint firing activity relative to the learning session and the cofiring probability during pre and post sleep. The partial correlation between the first values and the cofiring during post-sleep, controlled by the pre-sleep cofiring value, was calculated and averaged for all the pairs. The R-values obtained are displayed in the histograms of figure 5A: I noticed that post-sleep reactivation was stronger than pre-sleep reactivation when I considered ventral SWRs periods (respectively: r = 0.468 ±0.034; r = 0.249 ±0.042; n=cell pairs > 503, $p<0.001$, Fishers Z-test). Comparing to the results obtained with dorsal cells in the same condition (Fig.4A, dorsal SWRs), the r values were higher. Reactivation was observed during dorsal SWRs, even though no significantly different between sleep before and after learning (respectively: r = 0.149 ±0.042; r = 0.252 ±0.031; n=cell pairs > 503, p=0.23, Fishers Z-test).

As for dorsal pairs, I assessed whether the effect seen relative to the entire learning session was largely due to the reactivation of the last part of it, when new cell assemblies were established at least in the dorsal CA1 sub-field. A clear discrepancy in the results for the two population emerged. No significant difference was observed comparing the beginning (pre-sleep: r = 0.198 ±0.022; post-sleep =

0.315 ±0.044) and the end of learning (pre-sleep: r = 0.164 ±0.026; post-sleep = 0.337 ±0.042) (n=cell pairs > 503, all p>0.2, Fishers Z-test) (Fig. 5B). Therefore, my data seem to suggest that what was reactivated in the sleep subsequent to the learning was already present in the first half of the learning. I can hypothesise that cell assemblies associated to the new contingencies develop immediately in the ventral CA1 region, and are than reactivated after. The open question of course is whether the content of the replay events differs between the two CA1 sub-regions and so my results might be the mirror of distinct processes in act during the learning of the reward-place association.

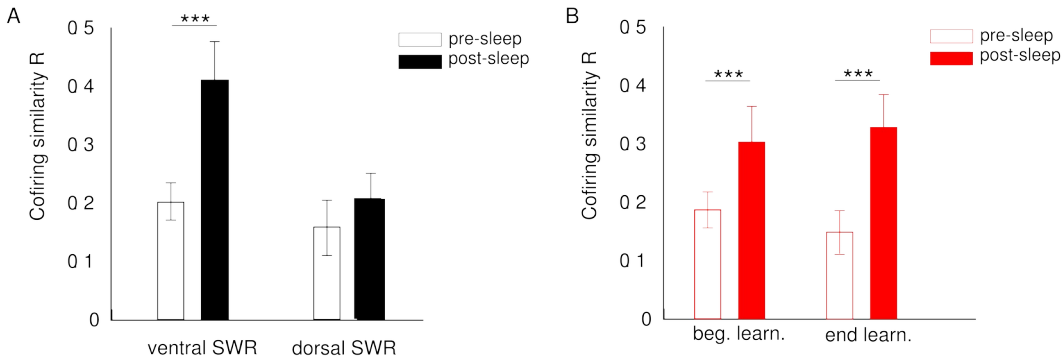


Figure 5. Reactivation of ventral CA1 pyramidal cells during slow-wave sleep-associated SWRs

(A) Correlation between the joint activity of ventral pyramidal cell pairs during learning and their sleep cofiring during local (ventral) and distal (local) SWRs is shown. The correlation coefficients represent the partial correlation of joint firing activity patterns during learning and cofiring during sleep, controlled by the cofiring of the other sleep session.

(B) Reactivation during pre and post-sleep is shown separately for the beginning (first 5 trials) and the end (last 5 trials) of learning sessions. The correlations coefficients were calculated as in A.

6.5 Reactivation of learning-associated assembly patterns across dorsal and ventral CA1 regions.

Next, reactivation between the two CA1 sub-regions was investigated. Across-region cell pairs were selected (composed of one dorsal and one ventral pyramidal cell), such that cofiring patterns reflected the activity of cell assemblies involving both structures. The analysis was conducted in parallel during non-SWRs and SWR periods, splitting as described above, the learning sessions in first 5 trials and last 5 trials (Fig. 6). In the first case, there was a significant increase in the correlation of learning cofiring with post-sleep relative to pre-sleep when considering the end of learning (pre-sleep: r = 0.145 ±0.019; r = 0.189 ±0.022; n=cell pairs > 798, $p<0.001$, Fishers Z-test). The across-region cofiring patterns measured at the beginning of the learning correlated less with both sleep sessions (pre-sleep: r = 0.232 ±0.019; r = 0.419 ±0.020; n=cell pairs > 798, $p<0.0001$, Fishers Z-test). It is worth noting, however, that the correlation between exploration and post-sleep cofiring patterns was greatest for no-SWRs periods and rather smaller when I considered dorsal SWRs periods especially when I looked at cofiring with pre-sleep.

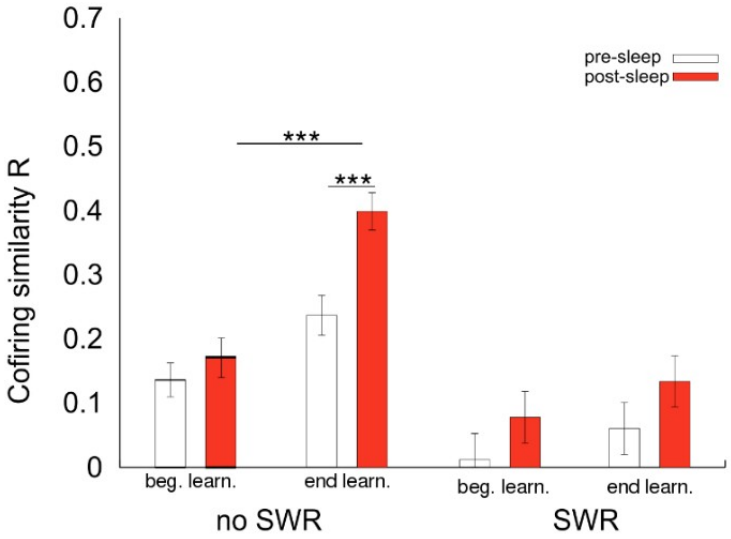


Figure 6. Across region reactivation of spatial learning activity patterns

Correlation between the joint activity during learning and the sleep cofiring of dorso-ventral cell pairs is shown. Reactivation during pre-sleep and post-sleep is separately compared during non-SWR and SWR periods.

Discussion

Hippocampal sharp waves along the dorso-ventral axis

Offline activity in the hippocampus is characterised by sporadic SWR events in which ripple oscillations and highly synchronous neuronal spiking are evident in CA1. So far, most of the studies regarding SWR patterns were referring to the septal part of the hippocampus. In this chapter, I looked at those fast oscillatory patterns both in the dorsal and ventral CA1, so to characterize the temporal profiles in the two sub-regions.

SWRs were observed in the dorsal and ventral CA1 LFP while the animal explored the environment (eSWR) as well as during sleep/rest periods (sSWRs). Generally, I found that SWRs in both sub-regions had similar profiles referring to the average field at each laminar level of CA1. This presumably suggests that dorsal and ventral SWRs are originated by the same mechanisms of inputs to the pyramidal layer of the CA1 region. Interestingly, I found that SWRs were more sporadic in the ventral CA1 and not synchronous to the dorsal events. When I looked at the temporal correlation between the two CA1 sub-fields, I saw that when SWR events co-existed in a temporal window of 200 ms in both regions, the ventral events were

following the dorsal ones. To date, a paper published by Patel and colleagues during the writing of this thesis, revealed in more detail the profiles and temporal relationship of SWR patterns along the whole long axis of the hippocampus. My results were consistent with what have been described in the above study. The authors reported that SWRs in the temporal CA1 were less prominent, in contrast to what they found in the intermediate hippocampus, where the probability of events simultaneous to the dorsal was higher. Despite the uncertainty in the mechanisms beyond, they concluded that ventral SWR patterns are less likely to propagate along the long axis because of the smaller amplitude, whereas SWRs in dorsal and intermediate hippocampus tend to co-occur or propagate in one way or another. Therefore, the results were reciprocally confirmed and they supported the idea that the content of those complex patterns in the two regions might differ. Still, sparse temporal windows might be available to coordinate them, as shown by the small lag between the two patterns in the cross-correlation analysis.

Firing rate of dorsal and ventral cells during sSWRs

During the 50-150 ms in which a SWR event occurs, the 10–18% of all neurons in the dorsal CA1 region discharge synchronously with a significant several-fold increase of population firing compared to theta oscillations ((Chrobak and Buzsaki, 1996; Csicsvari et al., 1999a). The burst of activity observed is thought to be

crucial for both memory consolidation within the hippocampus and in transferring the formed memories to the neocortex. The exceptionally excitatory state of the network might induce changes in the synaptic plasticity, replaying in a compressed scale the sequence of firing related to the just past experience. In this chapter, I was asking whether the ventral CA1 region was equally driven by the local SWR patterns, and whether distal events were also influencing CA1 assemblies at different levels of the long axis. I found that the firing of ventral neuronal population was largely affected by local SWR complexes. However, the unexpected result was the moderate firing rate changes in correspondence to dorsal slow-wave sleep associated events. The same was not observed for the dorsal sub-populations, which were not influenced by the incoming ventral sSWRs. Both pyramidal and interneuron cells were modulated by dorsal SWRs in the ventral CA1. The increase in firing rate for ventral cells was generally modest, probably due to the already higher baseline (as seen when analysing the mean firing rate in the previous chapter), but still temporally aligned to the peak of the local SWRs events. Regarding the distal events, the firing peak of ventral interneurons was following the increase of the dorsal sub-population and presumably, the burst of activity of pyramidal cells.

Reactivation of memory traces in the ventral hippocampus

It is hypothesised that offline activity, and particularly SWR events, might facilitate the consolidation of hippocampal memory traces, and/or their transfer to long-term storage in the cortical networks involved in the sensory representation of the original event (Marr, 1971; Buzsaki, 1989). Here I show that reactivation during sSWRs is taking place in the ventral CA1 region of the hippocampus after learning. I also analysed whether reactivation in the septal and temporal CA1 was taking place during the occurrence of SWR patterns in the other sub-region. As expected from the field/unit responsiveness analysis showing that dorsal CA1 cells did not increase the activity during ventral SWRs, no reactivation was observed for the dorsal population during ventral SWRs. By contrast, reactivation in the ventral CA1 sub-region was observed during dorsal SWRs (R-value > 0.140), even though no significative difference between the sleep before and after learning was found. The last result might be related more to the content of the SWRs in the ventral sub-region.

Data presented in two studies by Dupret and colleagues (2010; 2013) indicate that during the 40 trials in which the animal is spanning the maze and updating the position of the rewards from its past experience, cell assemblies associated with the new goal-locations are formed and compete with the one associated to the old goal-locations; in the second half of the session, the contingent cell assemblies would become predominant and replayed in subsequent off-line states. I replicate the analysis conducted by Dupret for goal cells, including the totality of the cells for each sub-region. A clear discrepancy in the results for the two populations emerged. The

results obtained for the dorsal population confirmed the previous studies, whereas no significant difference was observed comparing the beginning and the end of learning for the ventral CA1 population. The data seem to suggest that what was reactivated in the sleep subsequent to the learning was already present in the first half of the learning. It is important to connect those results to the analysis in chapter 5 regarding the place remapping. I saw that the beginning of the learning was more correlated to the recall session (following the post-probe) than the end of learning. Therefore, I might hypothesise that cell assemblies associated with the new contingencies developed immediately in the ventral CA1 region, and were the ones to be reactivated after. The open question of course is whether the content of the replay events differs between the two CA1 sub-regions and so my results might be the mirror of distinct processing in act during learning of the reward-place association with specific dynamics.

Reactivation across regions

It has been proposed that reactivation of CA1 firing patterns during sleep SWR facilitates the transfer of labile traces of episodic memories from the hippocampus to a site of longer term storage in the neocortex (Buzsaki, 1989; Wilson and McNaughton, 1994; McClelland et al., 1995; Kudrimoti et al., 1999; Hoffman and McNaughton, 2002; Lee and Wilson, 2002). So the hippocampus would coordinate the reactivation of prior experience during offline periods. This suggests that firing relationships established during the spatial learning are reinstated in the network

during sleep. In these perspective, I asked whether the reactivation along the dorso-ventral axis was coherent, that is whether any temporal relationship was established between distal activated cell assemblies. Based on the calculation of cofiring measured for pairs of dorsal and ventral CA1 cells, I found that the across-region cofiring patterns measured at the beginning of the learning correlated less with both sleep sessions. What is also apparent is that dorsal-ventral cells reactivation was not related to SWRs patterns. Indeed, the correlation between exploration and post-sleep cofiring patterns was greatest for no-SWR periods and rather smaller when I considered dorsal SWRs periods, especially when I looked at cofiring with pre-sleep.

Reactivation across brain regions has been observed, such that cofiring activity patterns between different brain regions during online states is also maintained during subsequent sleep. My data have demonstrated that, the information encoded during spatial learning were co-reactivated in the dorsal and ventral hippocampus, at times when SWRs do not occur in one of the two sub-regiosn. More recently, Peyrache and colleagues (2009) showed that reactivation in the prefrontal cortex preferentially occurs during hippocampal SWRs. In addition, reactivation of hippocampal-striatal ensembles seems to be stronger for reward-related neurons during sleep (Pennartz et al., 2004; Lansink et al., 2009). Such coordinated reactivation between the hippocampus and cortical or subcortical networks has been suggested to sub-serve a putative mechanism for the consolidation of memory traces in different areas of the brain. Intriguingly, it may

represent the association between modality-specific components of the original memory trace, such as place-reward in the cited study by Lansink and colleagues. The same phenomenon might take place first in the hippocampal network, to integrate the context related information acquired by the dorsal and ventral CA1 regions, eventually to be transferred to different cortical streams.

Chapter 7
General discussion

The results in this thesis present new data that highlight some of the mechanisms of communication between the dorsal and ventral CA1 sub-regions of the hippocampus. Although a functional differentiation has been hypothesized along the longitudinal axis of the hippocampus, they both might be implicated in the encoding, retrieval and storage of spatial memory processed by this structure. Therefore, understanding how they interact in the processing of spatial information is important to further our knowledge of mnemonic representation in the hippocampus.

This thesis provides support for the theory that communication between the septal and temporal level of the CA1 field in the hippocampus can be essential for the process in which information are encoded by the hippocampus during exploratory activity, and after consolidated during sleep and rest (Buzsaki, 1989). The CA1 region constitutes the final stage of hippocampal processing before the output to the EC and neocortical structures. Exploration is characterised by theta oscillations in the local field potential (LFP) of both the hippocampus and EC, whereas the "offline" state is characterised by irregular patterning of sharp wave/ripple (SWR) events in the hippocampus. It has been suggested that this state-dependent communication might facilitate the acquisition of memory traces during exploration, and the consolidation and long-term storage of memory during sleep and rest (Marr, 1971; Buzsaki, 1989).

7.1 Theta coupling between the septal and temporal pole of the hippocampus

The changes in the prominent theta rhythm along the septo-temporal axis of the hippocampus are considered crucial to understand the underlying cognitive operations associated to a sensorimotor experience. Alterations in the neuronal population activity are indeed reflected in the recorded LFP, so the changes in the theta-band power of the signal can monitor the relative activity of two areas. It has been shown that the power and frequency of theta oscillations become independent from locomotor indeces such as speed towards the ventral pole of the hippocampus, suggesting dissociation between the two poles of the hippocampus. Therefore, the temporal dynamics of theta oscillations in relation to ongoing sensory processing become relevant to investigate the role of the two sub-fields in processing spatial information. The present findings demonstrate that the amplitude of theta oscillations as well as the frequency is reduced at the level of the ventral CA1 sub-region relative to the theta oscillations simultaneously detected in the dorsal region. I also observed a general reduction in the theta coupling between the two sub-regions as measured by lower power correlation and coherence than the ones measured within sub-regions. Therefore, it might be concluded that these data strongly suggest the dissociation of the temporal region from the ongoing spatial information processing in the septal region. Nevertheless, I reported first that the power of ventral theta oscillations is modulated by the locomotive speed at times when coherence between the dorsal and ventral CA1 went up. Moreover, I showed

that a parallel change in the phase shift between the two oscillatory patterns resulted in approximately one theta cycle at times of high theta coherence. The results were also confirmed by the modulation of the neuronal firing activity relative to theta oscillations. Both pyramidal cells and interneurons in the two sub-fields were modulated by theta oscillations recorded in the same anatomical region. There was, however, evidence for modulation of the firing of neurons by distal oscillatory patterns. In particular, ventral CA1 cells were surprisingly more modulated by dorsal events than by ventral ones, showing that their activity is timed by the phase of dorsal theta oscillations. The depth of modulation of ventral pyramidal cells to dorsal oscillations was comparable with that of hippocampal dorsal cells and again slightly larger than the modulation by local theta oscillation.

Periods of high coherence in oscillatory events provide the temporal windows to synchronise the activity of distinct cell populations. Therefore, I went further in examining when it was the most likely to see coupling in the theta frequency band between the two poles of the hippocampus. By using a protocol in which a session of goal-directed navigation (learning session) was sided by sessions of free exploration of the same environment (probe sessions), I demonstrated that the demand of the task was crucial to synchronize theta oscillations at dorsal and ventral CA1: during the beginning of the spatial learning session an increase in power correlation and coherence was observed. As long as the coherence was high, ventral theta oscillations were positively correlated to speed during the learning task, whereas, during the probe sessions, this correlation was not present, irrespective of the theta

dynamics. This suggests a flow of spatial information along the longitudinal axis in the CA1 region in the context of goal-directed behaviour I used. Because the presence of rewards at goal locations is known to affect the activity of hippocampal assembly, I looked whether the theta coupling tended to occur at those salient places. Definitely, epochs of high coherence were clustered at goal locations and critically this was not a mere reflection of the increase of theta power in each single sub-field. If it was the case, the increase in power coherence would have been only a collateral effect in otherwise independent oscillatory patterns. Interestingly, the numbers of high theta power events in the ventral sub-regions decreased progressively in the same animal from one day to another. It is therefore possible that, communication between the two sub-regions is taking place preferentially during spatial learning, when distinct elements defining the context need to be integrated in the first place to guide the behavioural response.

These results corroborate the idea that both regions might be involved in the process of spatial learning and memory formation coming from recent studies in which either the dorsal or the ventral hippocampus was lesioned. Indeed, the relevance of the temporal region in solving spatial tasks was revealed at least in the context of rapid place learning and in animals receiving pretraining before lesioning the septal region (de Hoz et al., 2003; Ferbinteanu et al., 2003; Broadbent et al., 2004; Martin et al., 2005; Loureiro et al., 2011; de Hoz et al., 2014). Therefore, two aspects of my study become quite significant: first, the animals were pretrained, meaning that the maze and also the task were familiar and well-known before

starting the recordings; second, because the three baited locations were changed from one day to another, animals had to rapidly learn the novel places to efficiently collect the rewards, as shown also by the improvement in performance after few trials compare to naïve animals. So, the cheeseboard maze task is an example of rapid place learning, in which an update of the relevant spatial information is required in an otherwise familiar context to guide the behaviour. We might predict that if one of the two regions will be lesioned after pretraining animals would be impaired in the protocol I used. In reference to the cited lesion studies, it was also reported that septal hippocampal lesioned rats had a better performance across trials than temporal lesioned rats, due to a tendency of the ventral lesioned ones to go to the previous locations. It is tempting to suggest that the ventral hippocampus might provide the required flexibility to readapt the behavioural output, inhibiting pre-existing memory traces.

In another recent study, the activity of dorsal and ventral CA3 was investigated in rapid learning of the context-dependent object association (Komorowski et al., 2013). The authors provide evidence that the ventral hippocampus gradually develops the ability to generalize related events within a spatial context whereas the dorsal hippocampus events are rapidly associated to spatial context. In light of their results in CA3 and my in CA1, I might speculate that the ventral hippocampus might develop representations of the context by integrating spatial and non-spatial components, generalizing progressively with the

animal exposures to similar conditions. By contrast, the dorsal hippocampus might code for the specific spatial configuration of the context.

My conclusion is consistent with that of Adhikari et al. (2010), since it is proposed that ventral and dorsal CA1 would oscillate coherently in theta frequency to process spatial information during learning. Nevertheless, the independence of the theta signal in the two poles of the hippocampus, as reflected from the change in speed modulation, would allow processing of non-spatial information in the temporal pole. Therefore, communication with other regions such as medial PFC might occur at times of low coherence between the dorsal and ventral poles, where the broad phase shift suggests desynchronization in the outputs along the longitudinal axis of the hippocampus.

7.2 The role of ventral hippocampal CA1 in spatial learning: spatial firing in relation to reward

The role of specific brain structures implicated in reward-processing and reward-learning has been studied extensively in animals. Amongst these, the medial prefrontal cortex, amygdala, striatum and dopaminergic midbrain are showing strong neuronal correlates in relation to reward, even though the distinct contribution of each of these structures is in debate. It has been proposed that diverse cognitive processes are recruited to implement reward-related information perhaps reflecting the complexity of goal-oriented behaviour. The hippocampus is highly connected to what can be considered as an integrated network for reward

processing, thus it is not surprising that hippocampal cells have been also shown to fire in relation to it. The hippocampus likely conveys information about discrete spatial locations associated with valuable outcomes or saliency to enable structures such as the ventral striatum or prefrontal cortex to mediate behavioural responses toward rewards (Pennartz et al., 1994; Mulder et al., 1998; Lansink et al., 2012). Since the ventral hippocampus is directly connected to many of the above regions, it is reasonable to suggest that place-reward associations might depend on the coordinated activity of dorsal and ventral CA1 regions before conveying such spatial-contextual information to the downstream structures. In contrast to dorsal CA1 cells, ventral neurons showed low spatial selectivity (Kjelstrup et al., 2008; Royer et al., 2010). Interestingly, two recent studies showed that CA3 ventral cells were able to discriminate contexts as well as the moving towards and away from a reward at the end of a linear path (Royer et al., 2010; Komorowsky et al., 2013; see also previous paragraph). These results seem to confirm some of the conclusions of the previous paragraph regarding the integration of spatial and non-spatial information as reflected in theta dynamics. However, Dupret and colleagues have showed the emergence of reward-modulation of dorsal CA1 neuronal ensembles activity, in a goal-directed behavioural task. By using the same behavioural task (the cheeseboard task), here I showed that “goal cells” are present in the ventral CA1 as well as in the dorsal CA1, characterized by a tuned spatial firing field that is linked to at least one rewarded location. In addition, I observed cells in both sub-regions showing multiple firing fields but restricted to goals. I actually do not know whether those firing

patterns were present in the Dupret's study, because the criteria used in the paper sorted out multi-peak cells. Although I had a low sample of ventral CA1 cells in my experiments, I still found that in contrast to dorsal "goal cells", ventral CA1 "goal cells" did not show preference for first rewarded location in the sequence of the stereotyped path. I might speculate that a difference in the saliency/value of the three goal locations is transmitted to the ventral hippocampus, in relation to the spatial configuration confirmed only after the second goal was reached. In other words, the right context might be recognised only passing the second goal location and this will add value to the second and third reward station. The stereotyped consummatory behaviour of the animals can also be considered, since often they only collect the food at the first goal location and start chewing later on. I previously discussed the control role that the ventral hippocampus might have in inhibiting old memory traces when a rapid place learning is required (see 7.1). The first goal location would represent the spatial discriminant signalling the need to update the navigational path.

In the Dupret study, results from an intramaze-cued version of the task revealed that the reorganization of place maps take place only when those visual cues were not available, so the different strategies may correspond to different information being encoded in the dorsal CA1 region. Indeed not only less place cells were having their place field at reward locations, but also during the post-probe session cells were not firing anymore at those locations. The fact that in my study ventral cells firing at goal locations during learning but they were not maintaining

those firing preferences in the post-probe can be evidence for the analogy between the information processed across trials in this sub-region and when strong component of the context such as visual cues are present. As we said, lesion studies have also shown that animals in which only the ventral hippocampus is spared, are not impaired in new spatial learning in a familiar environment.

The hippocampal rate code mostly depends on the temporal patterns of excitatory and inhibitory inputs received. Rate remapping exerted a modulation effect on the active hippocampal neuronal assemblies, reflecting change (increasing or decreasing) of the mean firing rate of CA1 cells active in that specific environment (O'Keefe and Dostrovsky, 1971; Huxter et al., 2003; Leutgeb et al., 2005; O'Neill et al., 2006; Huxter et al., 2008). The encoding of information during spatial learning will presumably arise from rate as well as place remapping. I demonstrated that rate remapping was taking place in the ventral CA1 sub-regions but not in the dorsal CA1 when the mean firing rate of pyramidal cells at the beginning and end of learning was compared. The ventral CA1 sub-population was decreasing indeed the firing rate, even if it was still higher than the mean firing rate of dorsal CA1 pyramidal cells. Ventral CA1 showed remapping across learning suggesting that the network code not only changed in relation to behavioural states as defined by free exploration or goal-directed behaviour, but also in relation to the temporal dynamic implicated in the learning. Importantly, not only sensory changes in the environment can induce remapping. Switch from a foraging strategy to a goal-directed strategy in the same enclosure was found to induce remapping in the dorsal CA1 (Markus et al., 1995).

Recent studies have shown place cell remapping in response to changes in task in an otherwise stable environment (Ferbinteanu et al., 2011). As described, place remapping was taking place in both dorsal and ventral sub-regions of the CA1 during spatial learning, whereas rate remapping was observed only at temporal levels. Both phenomena were emerging during learning suggesting the shaping of the activity of the hippocampal networks for encoding information relative the context.

7.3 Oscillatory patterns in the dorso and ventral CA1 during sleep and rest

During offline periods including sleep and rest, the theta rhythm disappears from the hippocampal system, to be replaced by the sporadic occurrence of SWRs in the CA3-CA1 fields. It is thought that those fast oscillatory events are generated by mechanisms intrinsic to the hippocampus (Buzsaki et al., 1987; Draguhn et al., 1998). The spatial distribution of ripples along the longitudinal axis of CA1 has been extensively described in a paper by Patel and colleagues when this thesis was written (2013). In agreement with this study, I reported similar profile of SWRs in the dorsal and ventral CA1 sub-regions during sleep as well as online states. For the purpose of the thesis, I only focus attention on the temporal profile of the SWRs that co-occur in the two poles of the hippocampus, showing that the ventral SWRs tended to follow the dorsal ones. I further showed that both pyramidal and interneuron populations in ventral CA1 increased their firing in relation to local events, like dorsal CA1 cells.

Interestingly, an increase of the ventral population activity was also observed in relation to sleep-associated SWRs occurring in the dorsal region.

Given that sleep and rest-associated hippocampal ripples seem to participate in the consolidation of memory traces (Buzsaki, 1989; Wilson and McNaughton, 1994; Girardeau et al., 2009), the local emergence of ripples and the low levels of SWRs synchrony between the two poles of the hippocampus suggest that the information is segregated at each levels. This would provide support to the separation of the processed inputs received by different streams, such as the representations of emotional memory, and goal or reward-related information mainly at the ventral pole (Kjelstrup et al. 2002; Royer et al., 2010). Further support for this view comes from my results about the coherence between dorsal and ventral CA1 theta oscillations, showing that synchronous events in the septo-temporal axis can permit to integrate information conveyed by the two cortical streams at discrete times, while the synchronous ripple events may serve to integrate spatial and non-spatial information back to the neocortex (McClelland et al., 1995; Patel et al., 2012). Given that ventral CA1 cells showed a modest increase to dorsal SWRs, we might expect that spatial information is reactivated during some of the high amplitude dorsal SWRs coherently in the two sub-regions.

7.4 The role of ventral hippocampal CA1 in the consolidation of memory traces: reactivation of firing activity patterns emerged during spatial learning

It is hypothesised that off-line activity, and particularly SWR events, might facilitate the consolidation of hippocampal memory traces, and/or their transfer to long-term storage in the cortical networks involved in the sensory representation of the original event (Marr, 1971, Buzsaki, 1989). Evidence for this theory has come from the observation that the firing relationships established during exploration between CA1 pyramidal cells are reactivated during subsequent sleep, particularly SWR events (Wilson and McNaughton, 1994; Kudrimoti et al., 1999; O'Neill et al., 2008). Furthermore, this reactivation has been shown to correlate with subsequent memory performance (Dupret et al., 2010). Here I confirm the hypothesis that reactivation is associated to SWRs events, specifically when local patterns entrain the activity of cells separately in the dorsal and ventral CA1 sub-fields. So information learned during this goal-directed behaviour was later reactivated during sleep, coordinated by bursts of organised and self-generated hippocampal SWR activity. Therefore, the idea that SWR-associated cofiring does not only relate to the hippocampal spatial representation but to the behavioural experience of the animal in the recent explored environment is suggested by my data.

Furthermore, the work of Dupret et al., 2008 in dorsal CA1 has shown that the goal-related cell assemblies that dominate the end of the learning were the ones recruited strongly during SWRs; moreover, their relative reactivation predicted the behavioural performance of the animal on the recall memory test in the probe

session. Without selecting the goal cells because of the limitation of my data set, I assessed whether the SWR firing patterns in post-sleep reactivated more strongly with the end of learning patterns than the ones in the beginning. In regards to the reactivation observed in the dorsal CA1, my results show a stronger reactivation with end of learning study, in agreement with the Dupret et al. (2010) study. By contrast, no difference was found in comparing the reactivation of the activity at the beginning and end of the learning for ventral CA1 pyramidal cells. These data suggest that ventral cells seem to remap faster than dorsal CA1 pyramidal cells. Indeed, goal-related cell assemblies might emerge faster after few trials of the learning within the ventral hippocampus, in contrast to the suggested competitive dynamics for the establishment of new cell assembly in the dorsal CA1 area (Dupret et al., 2010 and 2013; Jezek et al., 2011). This would be in agreement to the role played by the ventral hippocampus in rapid spatial learning. In such circumstances, the initial performance might depend on the coordinated activity of this sub-region with the directly connected behavioural controlling brain regions (i.e. prefrontal cortex). Once spatial information would become stable during the learning, as reflected by the activity of dorsal hippocampal network, neocortical consolidation may be enabled and led by the dorsal hippocampal representations.

Nevertheless, the fact that firing activity of cells established during the learning were predominantly replayed during offline periods in both sub-regions is consistent with the idea that the reactivation of memory traces is involved in

mechanisms throughout the whole hippocampal networks where the experience is processed (Marr, 1971; McClelland et al., 1995).

7.5 The representation of conjunctive memory traces in the hippocampal system during sleep

An episodic-like memory trace is likely to require the simultaneous association of locations, objects and temporal information, and a representation of the familiarity of the context (Tulving, 1972; Howard et al., 2005; Eichenbaum and Lipton, 2008). The information processed in different structures converges on the hippocampus, so that spatial information reaches the dorsal hippocampus, while non-spatial information such as saliency or object-related information extends to the ventral hippocampus (see Eichenbaum and Lipton, 2008 for review). Here, the role of SWRs and theta oscillations were investigated in relation to the coherent reactivation in the dorsal and ventral CA1 areas of the hippocampus, which might support the consolidation of conjunctive memory traces.

In chapter 6 I showed that the joint reactivation of the activity patterns emerged during learning observed across the dorsal and ventral CA1 sub-regions was not associated to SWR patterns. Considering that dorsal and ventral pyramidal cells were highly modulated by both local and distal theta oscillations during off-line state and that they show a tendency to fire at particular phases in the theta cycle, I hypothesise that those periods can be significant for consolidation of the spatial information acquired during learning. If consolidation is linked to the firing timed by

theta oscillations, cell assemblies in each sub-region can be reactivated by the local occurrence of SWRs in a compressed time scale, whereas the synchronized activity of these assemblies is required for the more complex representations of the spatial context for the purposes of memory consolidation. Whether the theta-related firing interacts with the process of reactivation across regions remains to be an open question. Slow oscillations are a good candidate to sub-serve long-distance communication, for example at periods of high power coherence between the septal and temporal CA1 sub-regions as observed in my results. Because of the limitation in the number of goal-firing related cells, I was not able to assess whether those are preferentially involved in reactivation with dorsal CA1. I might expect ventral goal cells to play a role in the consolidation of spatial memory by participating in cell assemblies restricted to particular salient locations of the environment. As described above, one can speculate that the reactivation by collaborative cell assemblies might represent qualitatively different aspects of an experience. While dorsal CA1 activity reflect location-specific information, ventral CA1 representation can provide generalized information to connect different elements within a context; and the theta oscillations might provide the framework for the processing of different features at different phases, as well as unifying those multiple representations when the coupling between the distinct anatomical regions occur. The complete reactivation of a memory trace, eventually, would involve concurrent representation in structures that were not investigated here.

Future directions

Most of the analyses conducted were limited by the samples of cells simultaneously recorded. Future work will require collecting more data to compare the activity in the dorsal and ventral CA1 field. This will also enable the quantification of ventral cell firing during goal learning.

In addition, the use of non-spatial goal-learning task on the cheeseboard maze where local visual cues mark the location of the goals may attest whether allocentric learning is required for ventral firing or such firing is reward-related only.

The coordination of awake sharp-wave ripples between the two poles of the hippocampus needs also to be investigating during spatial learning, as it can reveal how salient spatial, contextual, and emotional components are merged in a memory trace.

Finally, co-reactivation of dorsal and ventral cell assembly patterns that occur outside the burst of activity represented by SWRs needs to be examined. My analysis did not go further to identify whether other oscillatory patterns such as gamma oscillations might be involved in the coordination across regions. Moreover, I might still ask whether reactivation of cell assemblies between the two sub-regions of CA1 is coherent and whether it is related to memory performances. By extending the analysis to the recall sessions following the sleep (probe), the last issue could be clarified.

Acknowledgements

I should start to say thank you to all the people that showed me how to play with science. In particular, to Jozsef and to all the members of the group that contributed to the work summarized in those pages. Alice, Barty, David, Joe (in a clear alphabetic order), you were precious. Thanks to all the MRC unit people that surrounded me in those four years, with their patient and friendly help. Thanks to Liz, Natalia, Jenny, Joe. I am really grateful to all of you. A particular mention to Mary, you did a great job for me, and give me the last support I needed. Of course, I am grateful to both my Hungarian supervisors who gave me the opportunity to stay in Oxford, and share their scientific view, sometimes going in the opposite directions, but equally sowing a great humanity.

And now a big thank you to all my friends in Oxford and spread around the world. Sorry I have been bothered you all those years, hope it was worthy. I would like to mention here Linda, Colin, Roberto, Fabrizio, Monica, Martina and “I fondaioli”. We shared a lot and I learnt a lot for you. Hope it will be the same in the future.

Finally, the last thought to all my family. My father, my mother, my sister and my sharing soul. You are in all the decisions and steps have been taken so far. Your contribution and influence are tremendous and you really believe in me more than I personally do. I love you unconditionally. I will be always linked to you.

Bibliography

Adhikari A, Topiwala MA, Gordon JA. Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. Neuron. 2010 Jan 28;65(2):257-69.

Adhikari A, Topiwala MA, Gordon JA. Single units in the medial prefrontal cortex with anxiety-related firing patterns are preferentially influenced by ventral hippocampal activity. Neuron. 2011 Sep 8;71(5):898-910.

Agster KL, Burwell RD. Hippocampal and subicular efferents and afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. Behav Brain Res. 2013 Oct 1;254:50-64.

Ahmed OJ, Mehta MR. The hippocampal rate code: anatomy, physiology and theory. Trends Neurosci. 2009 Jun;32(6):329-38.

Alonso A, García-Austt E. Neuronal sources of theta rhythm in the entorhinal cortex of the rat I Laminar distribution of theta field potentials. Exp Brain Res. 1987;67(3):493-501.

Alves SH, Pinheiro G, Motta V, Landeira-Fernandez J, Cruz AP. Anxiogenic effects in the rat elevated plus-maze of 5-HT (2C) agonists into ventral but not dorsal hippocampus. Behav Pharmacol. 2004 Feb;15(1):37-43.

Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience. 1989;31(3):571-91.

Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience. 2004;31(3):571-91.

Amaral DG, Kurz J. An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. J Comp Neurol. 1985 Oct 1;240(1):37-59.

Andersen P, Bliss TV, Skrede KK. Lamellar organization of hippocampal pathways. Exp Brain Res. 1971;13(2):222-38.

Anderson MI, Killing S, Morris C, O'Donoghue A, Onyiagha D, Stevenson R, Verriotis M, Jeffery KJ. Behavioral correlates of the distributed coding of spatial context. Hippocampus. 2006;16(9):730-42.

Anderson MI, Jeffery KJ. Heterogeneous modulation of place cell firing by changes in context. J Neurosci. 2003 Oct 1;23(26):8827-35.

Auerbach JM, Segal M. A novel cholinergic induction of long-term potentiation in rat hippocampus. J Neurophysiol. 1994 Oct;72(4):2034-40. PubMed PMID: 7823117.

Axmacher N, Schmitz DP, Wagner T, Elger CE, Fell J. Interactions between medial temporal lobe, prefrontal cortex, and inferior temporal regions during visual working memory: a

combined intracranial EEG and functional magnetic resonance imaging study. J Neurosci. 2008 Jul 16;28(29):7304-12. PubMed PMID: 18632934.

Baisden RH, Woodruff ML, Hoover DB. Cholinergic and non-cholinergic septo-hippocampal projections: a double-label horseradish peroxidase-acetylcholinesterase study in the rabbit. Brain Res. 1984 Jan 2;290(1):146-51.

Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, Zhang WN, Pothuizen HH, Feldon J. Regional dissociations within the hippocampus--memory and anxiety. Neurosci Biobehav Rev. 2004 May;28(3):273-83. Review.

Barthó P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsáki G. Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. J Neurophysiol. 2004 Jul;92(1):600-8.

Bast T, Wilson IA, Witter MP, Morris RG. From rapid place learning to behavioral performance: a key role for the intermediate hippocampus. PLoS Biol. 2009 Apr 21;7(4):e1000089.

Battaglia FP, Sutherland GR, McNaughton BL. Hippocampal sharp wave bursts coincide with neocortical "up-state" transitions. Learn Mem. 2004 Nov-Dec;11(6):697-704.

Benchenane K, Peyrache A, Khamassi M, Tierney PL, Gioanni Y, Battaglia FP, Wiener SI. Coherent theta oscillations and reorganization of spike timing in the hippocampal- prefrontal network upon learning. Neuron. 2010 Jun 24;66(6):921-36.

Berger TW, Thompson RF. Neuronal plasticity in the limbic system during classical conditioning of the rabbit nictitating membrane response I The hippocampus. Brain Res. 1978 Apr 28;145(2):323-46.

Bjarkam CR, Sørensen JC, Geneser FA. Distribution and morphology of serotonin-immunoreactive axons in the hippocampal region of the New Zealand white rabbit I Area dentata and hippocampus. Hippocampus. 2003;13(1):21-37.

Blackstad TW. Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. J Comp Neurol. 1956 Oct;105(3):417-537.

Bland BH, Anderson P, Ganes T. Two generators of hippocampal theta activity in rabbits. Brain Res. 1975 Aug 29;94(2):199-218.

Bland SK, Bland BH. Medial septal modulation of hippocampal theta cell discharges. Brain Res. 1986 Jun 4;375(1):102-16.

Borhegyi Z, Varga V, Szilágyi N, Fabo D, Freund TF. Phase segregation of medial septal GABAergic neurons during hippocampal theta activity. J Neurosci. 2004 Sep 29;24(39):8470-9. PubMed PMID: 15456820.

Bostock E, Muller RU, Kubie JL. Experience-dependent modifications of hippocampal place cell firing. Hippocampus. 1991 Apr;1(2):193-205.

Bouwman BM, van Lier H, Nitert HE, Drinkenburg WH, Coenen AM, van Rijn CM. The relationship between hippocampal EEG theta activity and locomotor behaviour in freely moving rats: effects of vigabatrin. Brain Res Bull. 2005 Jan 30;64(6):505-9.

Bragin A, Jandó G, Nádasdy Z, Hetke J, Wise K, Buzsáki G. Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat. J Neurosci. 1995 Jan;15(1 Pt 1):47-60.

Brazhnik ES, Fox SE. Action potentials and relations to the theta rhythm of medial septal neurons in vivo. Exp Brain Res. 1999 Aug;127(3):244-58.

Breese CR, Hampson RE, Deadwyler SA. Hippocampal place cells: stereotypy and plasticity. J Neurosci. 1989 Apr;9(4):1097-111.

Brivanlou IH, Dantzker JL, Stevens CF, Callaway EM. Topographic specificity of functional connections from hippocampal CA3 to CA1. Proc Natl Acad Sci U S A. 2004 Feb 24;101(8):2560-5.

Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A. 2004 Oct 5;101(40):14515-20.

Brown EN, Frank LM, Tang D, Quirk MC, Wilson MA. A statistical paradigm for neural spike train decoding applied to position prediction from ensemble firing patterns of rat hippocampal place cells. J Neurosci. 1998 Sep 15;18(18):7411-25.

Brun VH, Leutgeb S, Wu HQ, Schwarcz R, Witter MP, Moser EI, Moser MB. Impaired spatial representation in CA1 after lesion of direct input from entorhinal cortex. Neuron. 2008 Jan 24;57(2):290-302.

Brun VH, Otnass MK, Molden S, Steffenach HA, Witter MP, Moser MB, Moser EI. Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry. Science. 2002 Jun 21;296(5576):2243-6.

Brun VH, Solstad T, Kjelstrup KB, Fyhn M, Witter MP, Moser EI, Moser MB. Progressive increase in grid scale from dorsal to ventral medial entorhinal cortex. Hippocampus. 2008;18(12):1200-12.

Brunel N, Wang XJ. What determines the frequency of fast network oscillations with irregular neural discharges? I Synaptic dynamics and excitation-inhibition balance. J Neurophysiol. 2003 Jul;90(1):415-30.

Burgess N, Cacucci F, Lever C, O'Keefe J. Characterizing multiple independent behavioral correlates of cell firing in freely moving animals. Hippocampus. 2005;15(2):149-53. PubMed PMID: 15558542.

Burgess N, Barry C, O'Keefe J. An oscillatory interference model of grid cell firing. Hippocampus. 2007;17(9):801-12.

Burwell RD, Witter MP, Amaral DG. Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. Hippocampus. 1995;5(5):390-408.

Buzsàki G, Eidelberg E. Phase relations of hippocampal projection cells and interneurons to theta activity in the anesthetized rat. Brain Res. 1983 May 5;266(2):334-9.

Buzsáki G. Two-stage model of memory trace formation: a role for "noisy" brain states. Neuroscience. 1989;31(3):551-70.

Buzsáki G, Horváth Z, Urioste R, Hetke J, Wise K. High-frequency network oscillation in the hippocampus. Science. 1992 May 15;256(5059):1025-7.

Buzsáki G. Theta oscillations in the hippocampus. Neuron. 2002 Jan 31;33(3):325-40.

Buzsáki G. Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. Hippocampus. 2005;15(7):827-40.

Buzsàki G, Bickford RG, Ryan LJ, Young S, Prohaska O, Mandel RJ, Gage FH. Multisite recording of brain field potentials and unit activity in freely moving rats. J Neurosci Methods. 1989 Jun;28(3):209-17.

Buzsáki G, Moser EI. Memory, navigation and theta rhythm in the hippocampal-entorhinal system. Nat Neurosci. 2013 Feb;16(2):130-8.

Buzsáki G. How do neurons sense a spike burst?. Neuron. 2012 Mar 8;73(5):857-9.

Buzsáki G. Hippocampal sharp waves: their origin and significance. Brain Res. 1986 Nov 29;398(2):242-52.

Cenquizca LA, Swanson LW. Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Res Rev. 2007 Nov;56(1):1-26.

Chen S, Kobayashi M, Honda Y, Kakuta S, Sato F, Kishi K. Preferential neuron loss in the rat piriform cortex following pilocarpine-induced status epilepticus. Epilepsy Res. 2007 Apr;74(1):1-18.

Chrobak JJ, Buzsáki G. High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. J Neurosci. 1996 May 1;16(9):3056-66.

Chrobak JJ, Buzsáki G. Operational dynamics in the hippocampal-entorhinal axis. Neurosci Biobehav Rev. 1998 Mar;22(2):303-10.

Colom LV, Castaneda MT, Reyna T, Hernandez S, Garrido-Sanabria E. Characterization of medial septal glutamatergic neurons and their projection to the hippocampus. Synapse. 2005 Dec 1;58(3):151-64.

Commins S, Aggleton JP, O'Mara SM. Physiological evidence for a possible projection from dorsal subiculum to hippocampal area CA1. Exp Brain Res. 2002 Sep;146(2):155-60.

Conrad LC, Leonard CM, Pfaff DW. Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study. J Comp Neurol. 1974 Jul;156(2):179-205.

Csicsvari J, Hirase H, Mamiya A, Buzsáki G. Ensemble patterns of hippocampal CA3-CA1 neurons during sharp wave-associated population events. Neuron. 2000 Nov;28(2):585-94.

Csicsvari J, Hirase H, Czurko A, Buzsáki G. Reliability and state dependence of pyramidal cell-interneuron synapses in the hippocampus: an ensemble approach in the behaving rat. Neuron. 1998 Jul;21(1):179-89.

Csicsvari J, Hirase H, Czurkó A, Mamiya A, Buzsáki G. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. J Neurosci. 1999 Jan 1;19(1):274-87.

Csicsvari J, O'Neill J, Allen K, Senior T. Place-selective firing contributes to the reverse-order reactivation of CA1 pyramidal cells during sharp waves in open-field exploration. Eur J Neurosci. 2007 Aug;26(3):704-16.

de Hoz L, Knox J, Morris RG. Longitudinal axis of the hippocampus: both septal and temporal poles of the hippocampus support water maze spatial learning depending on the training protocol. Hippocampus. 2003;13(5):587-603.

de Hoz L, Martin SJ. Double dissociation between the contributions of the septal and temporal hippocampus to spatial learning: The role of prior experience. Hippocampus. 2014 Apr 17.

Deacon RM, Bannerman DM, Rawlins JN. Anxiolytic effects of cytotoxic hippocampal lesions in rats. Behav Neurosci. 2002 Jun;116(3):494-7.

Deadwyler SA, Hampson RE. Anatomic model of hippocampal encoding of spatial information. Hippocampus. 1999;9(4):397-412.

Diba K, Buzsáki G. Forward and reverse hippocampal place-cell sequences during ripples. Nat Neurosci. 2007 Oct;10(10):1241-2.

Dupret D, O'Neill J, Pleydell-Bouverie B, Csicsvari J. The reorganization and reactivation of hippocampal maps predict spatial memory performance. Nat Neurosci. 2010 Aug;13(8):995-1002.

Dupret D, O'Neill J, Csicsvari J. Dynamic reconfiguration of hippocampal interneuron circuits during spatial learning. Neuron. 2013 Apr 10;78(1):166-80.

Ego-Stengel V, Wilson MA. Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. Hippocampus. 2010 Jan;20(1):1-10.

Ekstrom AD, Kahana MJ, Caplan JB, Fields TA, Isham EA, Newman EL, Fried I. Cellular networks underlying human spatial navigation. Nature. 2003 Sep 11;425(6954):184-8.

Ellender TJ, Nissen W, Colgin LL, Mann EO, Paulsen O. Priming of hippocampal population bursts by individual perisomatic-targeting interneurons. J Neurosci. 2010 Apr 28;30(17):5979-91.

Eschenko O, Ramadan W, Mölle M, Born J, Sara SJ. Sustained increase in hippocampal sharp-wave ripple activity during slow-wave sleep after learning. Learn Mem. 2008 Apr;15(4):222-8.

Etienne AS, Jeffery KJ. Path integration in mammals. Hippocampus. 2004;14(2):180-92.

Fano S, Caliskan G, Behrens CJ, Heinemann U. Histaminergic modulation of acetylcholine-induced γ oscillations in rat hippocampus. Neuroreport. 2011 Aug 3;22(11):520-4.

Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron. 2010 Jan 14;65(1):7-19.

Felix-Ortiz AC, Beyeler A, Seo C, Leppla CA, Wildes CP, Tye KM. BLA to vHPC inputs modulate anxiety-related behaviors. Neuron. 2013 Aug 21;79(4):658-64.

Fenton AA, Wesierska M, Kaminsky Y, Bures J. Both here and there: simultaneous expression of autonomous spatial memories in rats. Proc Natl Acad Sci U S A. 1998 Sep 15;95(19):11493-8.

Ferbinteanu J, Ray C, McDonald RJ. Both dorsal and ventral hippocampus contribute to spatial learning in Long-Evans rats. Neurosci Lett. 2003 Jul 17;345(2):131-5.

Foster DJ, Wilson MA. Reverse replay of behavioural sequences in hippocampal place cells during the awake state. Nature. 2006 Mar 30;440(7084):680-3.

Foster TC, Castro CA, McNaughton BL. Spatial selectivity of rat hippocampal neurons: dependence on preparedness for movement. Science. 1989 Jun 30;244(4912):1580-2.

Frank LM, Stanley GB, Brown EN. Hippocampal plasticity across multiple days of exposure to novel environments. J Neurosci. 2004 Sep 1;24(35):7681-9.

Freund TF, Buzsáki G. Interneurons of the hippocampus. Hippocampus. 1996;6(4):347-470.

Freund TF, Antal M. GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature. 1988 Nov 10;336(6195):170-3.

Fyhn M, Molden S, Witter MP, Moser EI, Moser MB. Spatial representation in the entorhinal cortex. Science. 2004 Aug 27;305(5688):1258-64.

Fyhn M, Hafting T, Treves A, Moser MB, Moser EI. Hippocampal remapping and grid realignment in entorhinal cortex. Nature. 2007 Mar 8;446(7132):190-4.

Gaffan EA, Healey AN, Eacott MJ. Objects and positions in visual scenes: effects of perirhinal and postrhinal cortex lesions in the rat. Behav Neurosci. 2004 Oct;118(5):992-1010.

Gigg J. Constraints on hippocampal processing imposed by the connectivity between CA1, subiculum and subicular targets. Behav Brain Res. 2006 Nov 11;174(2):265-71.

Giocomo LM, Hasselmo ME. Time constants of h current in layer ii stellate cells differ along the dorsal to ventral axis of medial entorhinal cortex. J Neurosci. 2008 Sep 17;28(38):9414-25.

Giocomo LM, Zilli EA, Fransén E, Hasselmo ME. Temporal frequency of subthreshold oscillations scales with entorhinal grid cell field spacing. Science. 2007 Mar 23;315(5819):1719-22.

Giocomo LM, Moser EI. Spatial representation: maps in a temporal void. Curr Biol. 2011 Dec 6;21(23):R962-4.

Girardeau G, Benchenane K, Wiener SI, Buzsáki G, Zugaro MB. Selective suppression of hippocampal ripples impairs spatial memory. Nat Neurosci. 2009 Oct;12(10):1222-3.

Gray J.A. The neuropsychology of anxiety. (1st ed) Oxford University Press, Oxford (1982).

Gray J.A., McNaughton N. Comparison between the behavioural effects of septal and hippocampal lesions: a review. Neurosci Biobehav Rev. 1983 Summer;7(2):119-88. Review.

Gray J.A., McNaughton N.The neuropsychology of anxiety (2nd ed) Oxford University Press, Oxford (2000).

Gusev PA, Cui C, Alkon DL, Gubin AN. Topography of Arc/Arg31 mRNA expression in the dorsal and ventral hippocampus induced by recent and remote spatial memory recall: dissociation of CA3 and CA1 activation. J Neurosci. 2005 Oct 12;25(41):9384-97.

Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Microstructure of a spatial map in the entorhinal cortex. Nature. 2005 Aug 11;436(7052):801-6.

Hájos N, Pálhalmi J, Mann EO, Németh B, Paulsen O, Freund TF. Spike timing of distinct types of GABAergic interneuron during hippocampal gamma oscillations in vitro. J Neurosci. 2004 Oct 13;24(41):9127-37.

Halasy K, Miettinen R, Szabat E, Freund TF. GABAergic Interneurons are the Major Postsynaptic Targets of Median Raphe Afferents in the Rat Dentate Gyrus. Eur J Neurosci. 1992;4(2):144-153.

Halgren E, Babb TL, Crandall PH. Human hippocampal formation EEG desynchronizes during attentiveness and movement. Electroencephalogr Clin Neurophysiol. 1978 Jun;44(6):778-81.

Hampson RE, Deadwyler SA. Ensemble codes involving hippocampal neurons are at risk during delayed performance tests. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):13487-93.

Hargreaves WA, Gibson PJ. Effectiveness and cost of risperidone and olanzapine for schizophrenia: a systematic review. CNS Drugs. 2005;19(5):393-410.

Haring JH, Davis JN. Differential distribution of locus coeruleus projections to the hippocampal formation: anatomical and biochemical evidence. Brain Res. 1985 Jan 28;325(1-2):366-9.

Harley CW. Norepinephrine and the dentate gyrus. Prog Brain Res. 2007;163:299-318.

Harris KD, Hirase H, Leinekugel X, Henze DA, Buzsáki G. Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. Neuron. 2001 Oct 11;32(1):141-9.

Hasselmo ME. Neuromodulation and the hippocampus: memory function and dysfunction in a network simulation. Prog Brain Res. 1999;121:3-18.

Hasselmo ME, Bower JM. Acetylcholine and memory. Trends Neurosci. 1993 Jun;16(6):218-22.

Hennevin E, Leconte P. [The function of paradoxical sleep: facts and theories]. Annee Psychol. 1971;71(2):489-519.

Hennevin E, Leconte P, Bloch V. [Paradoxical sleep increase triggered by learning, extinction and relearning of a response based on a positive reinforcement]. Brain Res. 1974 Apr 12;70(1):43-54.

Henze DA, Wittner L, Buzsáki G. Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo. Nat Neurosci. 2002 Aug;5(8):790-5.

Hinman JR, Penley SC, Escabí MA, Chrobak JJ. Ketamine disrupts theta synchrony across the septotemporal axis of the CA1 region of hippocampus. J Neurophysiol. 2013 Jan;109(2):570-9.

Hinman JR, Penley SC, Long LL, Escabí MA, Chrobak JJ. Septotemporal variation in dynamics of theta: speed and habituation. J Neurophysiol. 2011 Jun;105(6):2675-86.

Hjorth-Simonsen A. Some intrinsic connections of the hippocampus in the rat: an experimental analysis. J Comp Neurol. 1973 Jan 15;147(2):145-61.

Hok V, Lenck-Santini PP, Save E, Gaussier P, Banquet JP, Poucet B. A test of the time estimation hypothesis of place cell goal-related activity. J Integr Neurosci. 2007 Sep;6(3):367-78.

Hollup SA, Molden S, Donnett JG, Moser MB, Moser EI. Place fields of rat hippocampal pyramidal cells and spatial learning in the watermaze. Eur J Neurosci. 2001 Mar;13(6):1197-208.

Hollup SA, Kjelstrup KG, Hoff J, Moser MB, Moser EI. Impaired recognition of the goal location during spatial navigation in rats with hippocampal lesions. J Neurosci. 2001 Jun 15;21(12):4505-13.

Hoover WB, Vertes RP. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct. 2007 Sep;212(2):149-79.

Huxter J, Burgess N, O'Keefe J. Independent rate and temporal coding in hippocampal pyramidal cells. Nature. 2003 Oct 23;425(6960):828-32.

Huxter JR, Senior TJ, Allen K, Csicsvari J. Theta phase-specific codes for two-dimensional position, trajectory and heading in the hippocampus. Nat Neurosci. 2008 May;11(5):587-94.

Ihalainen JA, Riekkinen P Jr, Feenstra MG. Comparison of dopamine and noradrenaline release in mouse prefrontal cortex, striatum and hippocampus using microdialysis. Neurosci Lett. 1999 Dec 24;277(2):71-4.

Ishizuka N, Weber J, Amaral DG. Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. J Comp Neurol. 1990 May 22;295(4):580-623.

Isomura Y, Sirota A, Ozen S, Montgomery S, Mizuseki K, Henze DA, Buzsáki G. Integration and segregation of activity in entorhinal-hippocampal subregions by neocortical slow oscillations. Neuron. 2006 Dec 7;52(5):871-82.

Jadhav SP, Kemere C, German PW, Frank LM. Awake hippocampal sharp-wave ripples support spatial memory. Science. 2012 Jun 15;336(6087):1454-8.

Jarrard LE, Luu LP, Davidson TL. A study of hippocampal structure-function relations along the septo-temporal axis. Hippocampus. 2012 Apr;22(4):680-92.

Jay TM, Witter MP. Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. J Comp Neurol. 1991 Nov 22;313(4):574-86.

Jeewajee A, Lever C, Burton S, O'Keefe J, Burgess N. Environmental novelty is signaled by reduction of the hippocampal theta frequency. Hippocampus.

Jeffery KJ, O'Keefe JM. Learned interaction of visual and idiothetic cues in the control of place field orientation. Exp Brain Res. 1999 Jul;127(2):151-61.

Jensen O, Lisman JE. Position reconstruction from an ensemble of hippocampal place cells: contribution of theta phase coding. J Neurophysiol. 2000 May;83(5):2602-9.

Jezek K, Henriksen EJ, Treves A, Moser EI, Moser MB. Theta-paced flickering between place-cell maps in the hippocampus. Nature. 2011 Sep 28;478(7368):246-9.

Ji D, Wilson MA. Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat Neurosci. 2007 Jan;10(1):100-7.

Jones MW, Wilson MA. Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol. 2005 Dec;3(12):e402.

Jouvet M. Biogenic amines and the states of sleep. Science. 1969 Jan 3;163(3862):32-41.

Jung MW, Wiener SI, McNaughton BL. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. J Neurosci. 1994 Dec;14(12):7347-56.

Jung MW, McNaughton BL. Spatial selectivity of unit activity in the hippocampal granular layer. Hippocampus. 1993 Apr;3(2):165-82.

Karlsson MP, Frank LM. Awake replay of remote experiences in the hippocampus. Nat Neurosci. 2009 Jul;12(7):913-8.

King AJ, Schnupp JW, Thompson ID. Signals from the superficial layers of the superior colliculus enable the development of the auditory space map in the deeper layers. J Neurosci. 1998 Nov 15;18(22):9394-408.

Kjelstrup KB, Solstad T, Brun VH, Hafting T, Leutgeb S, Witter MP, Moser EI, Moser MB. Finite scale of spatial representation in the hippocampus. Science. 2008 Jul 4;321(5885):140-3.

Klausberger T, Magill PJ, Márton LF, Roberts JD, Cobden PM, Buzsáki G, Somogyi P. Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. Nature. 2003 Feb 20;421(6925):844-8.

Klausberger T, Marton LF, O'Neill J, Huck JH, Dalezios Y, Fuentealba P, Suen WY, Papp E, Kaneko T, Watanabe M, Csicsvari J, Somogyi P. Complementary roles of cholecystokinin- and parvalbumin-expressing GABAergic neurons in hippocampal network oscillations. J Neurosci. 2005 Oct 19;25(42):9782-93.

Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science. 2008 Jul 4;321(5885):53-7.

Kloosterman F, van Haeften T, Lopes da Silva FH. Two reentrant pathways in the hippocampal-entorhinal system. Hippocampus. 2004;14(8):1026-39.

Kocsis B, Bragin A, Buzsáki G. Interdependence of multiple theta generators in the hippocampus: a partial coherence analysis. J Neurosci. 1999 Jul 15;19(14):6200-12.

Köhler C, Steinbusch H. Identification of serotonin and non-serotonin-containing neurons of the mid-brain raphe projecting to the entorhinal area and the hippocampal formation A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain. Neuroscience. 1982 Apr;7(4):951-75.

Komorowski RW, Garcia CG, Wilson A, Hattori S, Howard MW, Eichenbaum H. Ventral hippocampal neurons are shaped by experience to represent behaviorally relevant contexts. J Neurosci. 2013 May 1;33(18):8079-87.

Konopacki J, Bland BH, Colom LV, Oddie SD. In vivo intracellular correlates of hippocampal formation theta-on and theta-off cells. Brain Res. 1992 Jul 24;586(2):247-55.

Kudrimoti HS, Barnes CA, McNaughton BL. Reactivation of hippocampal cell assemblies: effects of behavioral state, experience, and EEG dynamics. J Neurosci. 1999 May 15;19(10):4090-101.

Landgraf R, Wigger A. High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behav Genet. 2002 Sep;32(5):301-14.

Landgraf R, Wigger A. Born to be anxious: neuroendocrine and genetic correlates of trait anxiety in HAB rats. Stress. 2003 Jun;6(2):111-9.

Lansink CS, Jackson JC, Lankelma JV, Ito R, Robbins TW, Everitt BJ, Pennartz CM. Reward cues in space: commonalities and differences in neural coding by hippocampal and ventral striatal ensembles. J Neurosci. 2012 Sep 5;32(36):12444-59.

Lee AK, Wilson MA. Memory of sequential experience in the hippocampus during slow wave sleep. Neuron. 2002 Dec 19;36(6):1183-94.

Lee I, Kesner RP. Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. Hippocampus. 2004;14(3):301-10.

Lemon N, Manahan-Vaughan D. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. J Neurosci. 2006 Jul 19;26(29):7723-9.

Leutgeb JK, Leutgeb S, Moser MB, Moser EI. Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science. 2007 Feb 16;315(5814):961-6.

Leutgeb S, Leutgeb JK, Moser MB, Moser EI. Place cells, spatial maps and the population code for memory. Curr Opin Neurobiol. 2005 Dec;15(6):738-46.

Lever C, Wills T, Cacucci F, Burgess N, O'Keefe J. Long-term plasticity in hippocampal place-cell representation of environmental geometry. Nature. 2002 Mar 7;416(6876):90-4.

Lewis DJ, Bregman NJ. Source of cues for cue-dependent amnesia in rats. J Comp Physiol Psychol. 1973 Nov;85(2):421-6.

Lewis DJ, Bregman NJ. The cholinergic system, amnesia and memory. Physiol Behav. 1972 Mar;8(3):511-4.

Lewis DJ. A cognitive approach to experimental amnesia. Am J Psychol. 1976 Mar;89(1):51-80.

Lisman JE, Idiart MA. Storage of 7 +/- 2 short-term memories in oscillatory subcycles. Science. 1995 Mar 10;267(5203):1512-5.

Lisman JE. Three Ca2+ levels affect plasticity differently: the LTP zone, the LTD zone and no man's land. J Physiol. 2001 Apr 15;532(Pt 2):285.

Lisman JE, Grace AA. The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron. 2005 Jun 2;46(5):703-13.

Loughlin SE, Foote SL, Grzanna R. Efferent projections of nucleus locus coeruleus: morphologic subpopulations have different efferent targets. Neuroscience. 1986 Jun;18(2):307-19.

Louie K, Wilson MA. Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. Neuron. 2001 Jan;29(1):145-56.

Loureiro M, Lecourtier L, Engeln M, Lopez J, Cosquer B, Geiger K, Kelche C, Cassel JC, Pereira de Vasconcelos A. The ventral hippocampus is necessary for expressing a spatial memory. Brain Struct Funct. 2012 Jan;217(1):93-106.

Loy R, Koziell DA, Lindsey JD, Moore RY. Noradrenergic innervation of the adult rat hippocampal formation. J Comp Neurol. 1980 Feb 15;189(4):699-710.

Lubenov EV, Siapas AG. Hippocampal theta oscillations are travelling waves. Nature. 2009 May 28;459(7246):534-9.

MacVicar BA, Dudek FE. Local synaptic circuits in rat hippocampus: interactions between pyramidal cells. Brain Res. 1980 Feb 17;184(1):220-3.

MacVicar BA, Dudek FE. Dye-coupling betwen CA3 pyramidal cells in slices of rat hippocampus. Brain Res. 1980 Sep 8;196(2):494-7.

Maggio N, Segal M. Striking variations in corticosteroid modulation of long-term potentiation along the septotemporal axis of the hippocampus. J Neurosci. 2007 May 23;27(21):5757-65.

Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RS, Frith CD. Navigation-related structural change in the hippocampi of taxi drivers. Proc Natl Acad Sci U S A. 2000 Apr 11;97(8):4398-403.

Manseau F, Goutagny R, Danik M, Williams S. The hippocamposeptal pathway generates rhythmic firing of GABAergic neurons in the medial septum and diagonal bands: an investigation using a complete septohippocampal preparation in vitro. J Neurosci. 2008 Apr 9;28(15):4096-107.

Markus EJ, Barnes CA, McNaughton BL, Gladden VL, Skaggs WE. Spatial information content and reliability of hippocampal CA1 neurons: effects of visual input. Hippocampus. 1994 Aug;4(4):410-21.

Markus EJ, Qin YL, Leonard B, Skaggs WE, McNaughton BL, Barnes CA. Interactions between location and task affect the spatial and directional firing of hippocampal neurons. J Neurosci. 1995 Nov;15(11):7079-94.

Marr D. Simple memory: a theory for archicortex. Philos Trans R Soc Lond B Biol Sci. 1971 Jul 1;262(841):23-81.

Marrosu F, Portas C, Mascia MS, Casu MA, Fà M, Giagheddu M, Imperato A, Gessa GL. Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. Brain Res. 1995 Feb 13;671(2):329-32.

Martin MV, Dong H, Bertchume A, Csernansky JG. Low dose quetiapine reverses deficits in contextual and cued fear conditioning in rats with excitotoxin-induced hippocampal neuropathy. Pharmacol Biochem Behav. 2005 Oct;82(2):263-9.

Matsuyama K, Drew T. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat I Walking on a level surface. J Neurophysiol. 2000 Nov;84(5):2237-56.

Matsuyama K, Drew T. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat II Walking on an inclined plane. J Neurophysiol. 2000 Nov;84(5):2257-76.

Maurer AP, Vanrhoads SR, Sutherland GR, Lipa P, McNaughton BL. Self-motion and the origin of differential spatial scaling along the septo-temporal axis of the hippocampus. Hippocampus. 2005;15(7):841-52.

McClelland JL, McNaughton BL, O'Reilly RC. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol Rev. 1995 Jul;102(3):419-57.

McFarland WL, Teitelbaum H, Hedges EK. Relationship between hippocampal theta activity and running speed in the rat. J Comp Physiol Psychol. 1975 Jan;88(1):324-8.

McGaugh JL, Roozendaal B. Drug enhancement of memory consolidation: historical perspective and neurobiological implications. Psychopharmacology (Berl). 2009 Jan;202(1-3):3-14.

McMahon LL, Kauer JA. Hippocampal interneurons are excited via serotonin-gated ion channels. J Neurophysiol. 1997 Nov;78(5):2493-502.

McNaughton BL, Barnes CA, O'Keefe J. The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. Exp Brain Res. 1983;52(1):41-9.

McNaughton BL, Barnes CA, Gerrard JL, Gothard K, Jung MW, Knierim JJ, Kudrimoti H, Qin Y, Skaggs WE, Suster M, Weaver KL. Deciphering the hippocampal polyglot: the hippocampus as a path integration system. J Exp Biol. 1996 Jan;199(Pt 1):173-85.

Meibach RC, Siegel A. Efferent connections of the septal area in the rat: an analysis utilizing retrograde and anterograde transport methods. Brain Res. 1977 Jan 1;119(1):1-20.

Menard J, Treit D. The anxiolytic effects of intra-hippocampal midazolam are antagonized by intra-septal L-glutamate. Brain Res. 2001 Jan 5;888(1):163-166.

Mendelsohn A, Furman O, Dudai Y. Signatures of Memory: Brain Coactivations during Retrieval Distinguish Correct from Incorrect Recollection. Front Behav Neurosci. 2010;4:18.

Miles R, Wong RK. Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. J Physiol. 1986 Apr;373:397-418.

Mitchell KJ, Johnson MK. Source monitoring 15 years later: what have we learned from fMRI about the neural mechanisms of source memory? Psychol Bull. 2009 Jul;135(4):638-77.

Mitchell SJ, Ranck JB Jr. Generation of theta rhythm in medial entorhinal cortex of freely moving rats. Brain Res. 1980 May 5;189(1):49-66.

Mitra PP, Pesaran B. Analysis of dynamic brain imaging data. Biophys J. 1999 Feb;76(2):691-708.

Mizuseki K, Diba K, Pastalkova E, Buzsáki G. Hippocampal CA1 pyramidal cells form functionally distinct sublayers. Nat Neurosci. 2011 Aug 7;14(9):1174-81. PubMed PMID: 21822270.

Mölle M, Born J. Hippocampus whispering in deep sleep to prefrontal cortex--for good memories?. Neuron. 2009 Feb 26;61(4):496-8.

Mölle M, Yeshenko O, Marshall L, Sara SJ, Born J. Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. J Neurophysiol. 2006 Jul;96(1):62-70.

Monmaur P, Collet A, Puma C, Frankel-Kohn L, Sharif A. Relations between acetylcholine release and electrophysiological characteristics of theta rhythm: a microdialysis study in the urethane-anesthetized rat hippocampus. Brain Res Bull. 1997;42(2):141-6.

Montgomery SM, Sirota A, Buzsáki G. Theta and gamma coordination of hippocampal networks during waking and rapid eye movement sleep. J Neurosci. 2008 Jun 25;28(26):6731-41. PubMed PMID: 18579747.

Moore RY, Halaris AE. Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat. J Comp Neurol. 1975 Nov 15;164(2):171-83.

Moser MB, Moser EI, Forrest E, Andersen P, Morris RG. Spatial learning with a minislab in the dorsal hippocampus. Proc Natl Acad Sci U S A. 1995 Oct 10;92(21):9697-701.

Moser EI. Learning-related changes in hippocampal field potentials. Behav Brain Res. 1995 Nov;71(1-2):11-8.

Moser MB, Moser EI. Distributed encoding and retrieval of spatial memory in the hippocampus. J Neurosci. 1998 Sep 15;18(18):7535-42.

Mulder AB, Hodenpijl MG, Lopes da Silva FH. Electrophysiology of the hippocampal and amygdaloid projections to the nucleus accumbens of the rat: convergence, segregation, and interaction of inputs. J Neurosci. 1998 Jul 1;18(13):5095-102.

Muller RU, Kubie JL. The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. J Neurosci. 1987 Jul;7(7):1951-68.

Muller RU, Kubie JL, Ranck JB Jr. Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. J Neurosci. 1987 Jul;7(7):1935-50.

Murgatroyd C, Wigger A, Frank E, Singewald N, Bunck M, Holsboer F, Landgraf R, Spengler D. Impaired repression at a vasopressin promoter polymorphism underlies overexpression of vasopressin in a rat model of trait anxiety. J Neurosci. 2004 Sep 1;24(35):7762-70.

Naber PA, Witter MP, Lopes da Silva FH. Differential distribution of barrel or visual cortex Evoked responses along the rostro-caudal axis of the peri- and postrhinal cortices. Brain Res. 2000 Sep 22;877(2):298-305.

Naber PA, Lopes da Silva FH, Witter MP. Reciprocal connections between the entorhinal cortex and hippocampal fields CA1 and the subiculum are in register with the projections from CA1 to the subiculum. Hippocampus. 2001;11(2):99-104.

Nádasdy Z, Hirase H, Czurkó A, Csicsvari J, Buzsáki G. Replay and time compression of recurring spike sequences in the hippocampus. J Neurosci. 1999 Nov 1;19(21):9497-507.

Nakazawa K, Quirk MC, Chitwood RA, Watanabe M, Yeckel MF, Sun LD, Kato A, Carr CA, Johnston D, Wilson MA, Tonegawa S. Requirement for hippocampal CA3 NMDA receptors in associative memory recall. Science. 2002 Jul 12;297(5579):211-8.

NAUTA WJ. An experimental study of the fornix system in the rat. J Comp Neurol. 1956 Apr;104(2):247-71.

Nitz D, McNaughton B. Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. J Neurophysiol. 2004 Feb;91(2):863-72.

Nokia MS, Penttonen M, Wikgren J. Hippocampal ripple-contingent training accelerates trace eyeblink conditioning and retards extinction in rabbits. J Neurosci. 2010 Aug 25;30(34):11486-92.

O'Keefe J, Burgess N. Dual phase and rate coding in hippocampal place cells: theoretical significance and relationship to entorhinal grid cells. Hippocampus. 2005;15(7):853-66.

O'Keefe J, Recce ML. Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus. 1993 Jul;3(3):317-30.

O'Keefe J, Dostrovsky J. The hippocampus as a spatial map Preliminary evidence from unit activity in the freely-moving rat. Brain Res. 1971 Nov;34(1):171-5.

O'Keefe J. Place units in the hippocampus of the freely moving rat. Exp Neurol. 1976 Apr;51(1):78-109.

O'Keefe J. Do hippocampal pyramidal cells signal non-spatial as well as spatial information?. Hippocampus. 1999;9(4):352-64.

O'Keefe J, Conway DH. Hippocampal place units in the freely moving rat: why they fire where they fire. Exp Brain Res. 1978 Apr 14;31(4):573-90.

O'Keefe J, Nadel L. The hippocampus as a cognitive map.. Oxford,Clarendon: -Oxford University Press; 1978.

O'Keefe J, Speakman A. Single unit activity in the rat hippocampus during a spatial memory task. Exp Brain Res. 1987;68(1):1-27.

O'Neill J, Senior T, Csicsvari J. Place-selective firing of CA1 pyramidal cells during sharp wave/ ripple network patterns in exploratory behavior. Neuron. 2006 Jan 5;49(1):143-55.

O'Neill J, Senior TJ, Allen K, Huxter JR, Csicsvari J. Reactivation of experience-dependent cell assembly patterns in the hippocampus. Nat Neurosci. 2008 Feb;11(2):209-15.

Oleskevich S, Descarries L. Quantified distribution of the serotonin innervation in adult rat hippocampus. Neuroscience. 1990;34(1):19-33.

Olton DS, Branch M, Best PJ. Spatial correlates of hippocampal unit activity. Exp Neurol. 1978 Feb;58(3):387-409.

Olypher AV, Lánský P, Fenton AA. Properties of the extra-positional signal in hippocampal place cell discharge derived from the overdispersion in location-specific firing. Neuroscience. 2002;111(3):553-66.

Otmakhova NA, Lewey J, Asrican B, Lisman JE. Inhibition of perforant path input to the CA1 region by serotonin and noradrenaline. J Neurophysiol. 2005 Aug;94(2):1413-22.

Otmakhova NA, Lisman JE. Dopamine selectively inhibits the direct cortical pathway to the CA1 hippocampal region. J Neurosci. 1999 Feb 15;19(4):1437-45.

Otmakhova NA, Lisman JE. Dopamine, serotonin, and noradrenaline strongly inhibit the direct perforant path-CA1 synaptic input, but have little effect on the Schaffer collateral input. Ann N Y Acad Sci. 2000 Jun;911:462-4.

Otmakhova NA, Lisman JE. D1/D5 dopamine receptors inhibit depotentiation at CA1 synapses via cAMP-dependent mechanism. J Neurosci. 1998 Feb 15;18(4):1270-9.

Padovan CM, Del Bel EA, Guimarães FS. Behavioral effects in the elevated plus maze of an NMDA antagonist injected into the dorsal hippocampus: influence of restraint stress. Pharmacol Biochem Behav. 2000 Oct;67(2):325-30.

Panula P, Pirvola U, Auvinen S, Airaksinen MS. Histamine-immunoreactive nerve fibers in the rat brain. Neuroscience. 1989;28(3):585-610.

Patel J, Fujisawa S, Berényi A, Royer S, Buzsáki G. Traveling theta waves along the entire septotemporal axis of the hippocampus. Neuron. 2012 Aug 9;75(3):410-7.

Patel J, Schomburg EW, Berényi A, Fujisawa S, Buzsáki G. Local generation and propagation of ripples along the septotemporal axis of the hippocampus. J Neurosci. 2013 Oct 23;33(43):17029-41.

Pavlides C, Winson J. Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. J Neurosci. 1989 Aug;9(8):2907-18.

Penley SC, Hinman JR, Sabolek HR, Escabí MA, Markus EJ, Chrobak JJ. Theta and gamma coherence across the septotemporal axis during distinct behavioral states. Hippocampus. 2012 May;22(5):1164-75.

Pennartz CM, Lee E, Verheul J, Lipa P, Barnes CA, McNaughton BL. The ventral striatum in off-line processing: ensemble reactivation during sleep and modulation by hippocampal ripples. J Neurosci. 2004 Jul 21;24(29):6446-56.

Pentkowski NS, Blanchard DC, Lever C, Litvin Y, Blanchard RJ. Effects of lesions to the dorsal and ventral hippocampus on defensive behaviors in rats. Eur J Neurosci. 2006 Apr;23(8):2185-96.

Petrovich GD, Canteras NS, Swanson LW. Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. Brain Res Brain Res Rev. 2001 Dec;38(1-2):247-89.

Pickel VM, Segal M, Bloom FE. A radioautographic study of the efferent pathways of the nucleus locus coeruleus. J Comp Neurol. 1974 May 1;155(1):15-42.

Pitler TA, Alger BE. Cholinergic excitation of GABAergic interneurons in the rat hippocampal slice. J Physiol. 1992 May;450:127-42.

Pothuizen HH, Zhang WN, Jongen-Rêlo AL, Feldon J, Yee BK. Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working spatial memory. Eur J Neurosci. 2004 Feb;19(3):705-12.

Poucet B, Thinus-Blanc C, Muller RU. Place cells in the ventral hippocampus of rats. Neuroreport. 1994 Oct 27;5(16):2045-8.

Przybyslawski J, Sara SJ. Reconsolidation of memory after its reactivation. Behav Brain Res. 1997 Mar;84(1-2):241-6.

Quirk GJ, Muller RU, Kubie JL. The firing of hippocampal place cells in the dark depends on the rat's recent experience. J Neurosci. 1990 Jun;10(6):2008-17.

Raisman G, Cowan WM, Powell TP. An experimental analysis of the efferent projection of the hippocampus. Brain. 1966 Mar;89(1):83-108.

Ramadan W, Eschenko O, Sara SJ. Hippocampal sharp wave/ripples during sleep for consolidation of associative memory. PLoS One. 2009 Aug 20;4(8):e6697.

Rasch B, Büchel C, Gais S, Born J. Odor cues during slow-wave sleep prompt declarative memory consolidation. Science. 2007 Mar 9;315(5817):1426-9.

Rasmusson AM, Vythilingam M, Morgan CA 3rd. The neuroendocrinology of posttraumatic stress disorder: new directions. CNS Spectr. 2003 Sep;8(9):651-6, 665-7.

Redish AD, Rosenzweig ES, Bohanick JD, McNaughton BL, Barnes CA. Dynamics of hippocampal ensemble activity realignment: time versus space. J Neurosci. 2000 Dec 15;20(24):9298-309.

Redish AD, Battaglia FP, Chawla MK, Ekstrom AD, Gerrard JL, Lipa P, Rosenzweig ES, Worley PF, Guzowski JF, McNaughton BL, Barnes CA. Independence of firing correlates of anatomically proximate hippocampal pyramidal cells. J Neurosci. 2001 Mar 1;21(5):RC134.

Rex A, Voigt JP, Fink H. Anxiety but not arousal increases 5-hydroxytryptamine release in the rat ventral hippocampus in vivo. Eur J Neurosci. 2005 Sep;22(5):1185-9.

Richard GR, Titiz A, Tyler A, Holmes GL, Scott RC, Lenck-Santini PP. Speed modulation of hippocampal theta frequency correlates with spatial memory performance. Hippocampus. 2013 Dec;23(12):1269-79.

Risold PY, Thompson RH, Swanson LW. The structural organization of connections between hypothalamus and cerebral cortex. Brain Res Brain Res Rev. 1997 Sep 19;24(2-3):197-254.

Rivas J, Gaztelu JM, García-Austt E. Changes in hippocampal cell discharge patterns and theta rhythm spectral properties as a function of walking velocity in the guinea pig. Exp Brain Res. 1996 Feb;108(1):113-8.

Rolls ET, O'Mara SM. View-responsive neurons in the primate hippocampal complex. Hippocampus. 1995;5(5):409-24.

Roullet P, Sara S. Consolidation of memory after its reactivation: involvement of beta noradrenergic receptors in the late phase. Neural Plast. 1998 Jul-Sep;6(3):63-8.

Royer S, Sirota A, Patel J, Buzsáki G. Distinct representations and theta dynamics in dorsal and ventral hippocampus. J Neurosci. 2010 Feb 3;30(5):1777-87.

Royer S, Zemelman BV, Losonczy A, Kim J, Chance F, Magee JC, Buzsáki G. Control of timing, rate and bursts of hippocampal place cells by dendritic and somatic inhibition. Nat Neurosci. 2012 Mar 25;15(5):769-75.

Rusch BD, Abercrombie HC, Oakes TR, Schaefer SM, Davidson RJ. Hippocampal morphometry in depressed patients and control subjects: relations to anxiety symptoms. Biol Psychiatry. 2001 Dec 15;50(12):960-4.

Sabolek HR, Penley SC, Hinman JR, Bunce JG, Markus EJ, Escabi M, Chrobak JJ. Theta and gamma coherence along the septotemporal axis of the hippocampus. J Neurophysiol. 2009 Mar;101(3):1192-200.

Sahay A, Hen R. Adult hippocampal neurogenesis in depression. Nat Neurosci. 2007 Sep;10(9):1110-5.

Salomé N, Salchner P, Viltart O, Sequeira H, Wigger A, Landgraf R, Singewald N. Neurobiological correlates of high (HAB) versus low anxiety-related behavior (LAB): differential Fos expression in HAB and LAB rats. Biol Psychiatry. 2004 Apr 1;55(7):715-23.

Sara SJ. Retrieval and reconsolidation: toward a neurobiology of remembering. Learn Mem. 2000 Mar-Apr;7(2):73-84.

Sara SJ. Strengthening the shaky trace through retrieval. Nat Rev Neurosci. 2000 Dec;1(3):212-3.

Sara SJ, Hars B. In memory of consolidation. Learn Mem. 2006 Sep-Oct;13(5):515-21.

SCOVILLE WB, MILNER B. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry. 1957 Feb;20(1):11-21.

Seager MA, Johnson LD, Chabot ES, Asaka Y, Berry SD. Oscillatory brain states and learning: Impact of hippocampal theta-contingent training. Proc Natl Acad Sci U S A. 2002 Feb 5;99(3):1616-20.

Shapiro ML, Tanila H, Eichenbaum H. Cues that hippocampal place cells encode: dynamic and hierarchical representation of local and distal stimuli. Hippocampus. 1997;7(6):624-42.

Sharp PE, Green C. Spatial correlates of firing patterns of single cells in the subiculum of the freely moving rat. J Neurosci. 1994 Apr;14(4):2339-56. PubMed PMID: 8158272.

Shi CJ, Cassell MD. Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. J Comp Neurol. 1998 Oct 5;399(4):469-91. PubMed PMID: 9741478.

Siapas AG, Wilson MA. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. Neuron. 1998 Nov;21(5):1123-8.

Siegel JJ, Nitz D, Bingman VP. Spatial-specificity of single-units in the hippocampal formation of freely moving homing pigeons. Hippocampus. 2005;15(1):26-40.

Singer AC, Frank LM. Rewarded outcomes enhance reactivation of experience in the hippocampus. Neuron. 2009 Dec 24;64(6):910-21.

Sirota A, Csicsvari J, Buhl D, Buzsáki G. Communication between neocortex and hippocampus during sleep in rodents. Proc Natl Acad Sci U S A. 2003 Feb 18;100(4):2065-9.

Skaggs WE, McNaughton BL, Wilson MA, Barnes CA. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. Hippocampus. 1996;6(2):149-72.

Sławińska U, Kasicki S. The frequency of rat's hippocampal theta rhythm is related to the speed of locomotion. Brain Res. 1998 Jun 15;796(1-2):327-31.

Snyder JS, Ramchand P, Rabbett S, Radik R, Wojtowicz JM, Cameron HA. Septo-temporal gradients of neurogenesis and activity in 13-month-old rats. Neurobiol Aging. 2011 Jun;32(6):1149-56.

Somogyi P, Tamás G, Lujan R, Buhl EH. Salient features of synaptic organisation in the cerebral cortex. Brain Res Brain Res Rev. 1998 May;26(2-3):113-35.

Somogyi P, Klausberger T. Defined types of cortical interneurone structure space and spike timing in the hippocampus. J Physiol. 2005 Jan 1;562(Pt 1):9-26.

Somogyi P, Katona L, Klausberger T, Lasztóczi B, Viney TJ. Temporal redistribution of inhibition over neuronal subcellular domains underlies state-dependent rhythmic change of excitability in the hippocampus. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 2014; 369(1635).

Spiers HJ, Maguire EA. The neuroscience of remote spatial memory: a tale of two cities. Neuroscience. 2007 Oct 12;149(1):7-27.

Spiers HJ, Maguire EA. Thoughts, behaviour, and brain dynamics during navigation in the real world. Neuroimage. 2006 Jul 15;31(4):1826-40.

Steinvorth S, Levine B, Corkin S. Medial temporal lobe structures are needed to re-experience remote autobiographical memories: evidence from HM and WR. Neuropsychologia. 2005;43(4):479-96.

Steriade M, Amzica F, Timofeev I. Cellular basis of transition between sleep and electrographic seizures. Med Sci (Paris). 2003 Oct;19(10):999-1002.

Sterpenich V, D'Argembeau A, Desseilles M, Balteau E, Albouy G, Vandewalle G, Degueldre C, Luxen A, Collette F, Maquet P. The locus ceruleus is involved in the successful retrieval of emotional memories in humans. J Neurosci. 2006 Jul 12;26(28):7416-23.

Stickgold R, Walker MP. Sleep-dependent memory triage: evolving generalization through selective processing. Nat Neurosci. 2013 Feb;16(2):139-45.

Strange BA, Fletcher PC, Henson RN, Friston KJ, Dolan RJ. Segregating the functions of human hippocampus. Proc Natl Acad Sci U S A. 1999 Mar 30;96(7):4034-9.

Suzuki SS, Smith GK. Spontaneous EEG spikes in the normal hippocampus I Behavioral correlates, laminar profiles and bilateral synchrony. Electroencephalogr Clin Neurophysiol. 1987 Oct;67(4):348-59.

Svoboda E, McKinnon MC, Levine B. The functional neuroanatomy of autobiographical memory: a meta-analysis. Neuropsychologia. 2006;44(12):2189-208.

Swanson LW, Hartman BK. The central adrenergic system An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. J Comp Neurol. 1975 Oct 15;163(4):467-505.

Swanson LW. Cerebral hemisphere regulation of motivated behavior. Brain Res. 2000 Dec 15;886(1-2):113-164.

Swanson LW, Cowan WM. An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J Comp Neurol. 1977 Mar 1;172(1):49-84.

Swanson LW, Wyss JM, Cowan WM. An autoradiographic study of the organization of intrahippocampal association pathways in the rat. J Comp Neurol. 1978 Oct 15;181(4):681-715.

Tamamaki N, Nojyo Y. Preservation of topography in the connections between the subiculum, field CA1, and the entorhinal cortex in rats. J Comp Neurol. 1995 Mar 13;353(3):379-90.

Tatsuno M, Lipa P, McNaughton BL. Methodological considerations on the use of template matching to study long-lasting memory trace replay. J Neurosci. 2006 Oct 18;26(42):10727-42.

Teitelbaum H, McFarland WL. Power spectral shifts in hippocampal EEG associated with conditioned locomotion in the rat. Physiol Behav. 1971 Oct;7(4):545-9.

Tolman EC. Cognitive maps in rats and men. Psychol Rev. 1948 Jul;55(4):189-208.

Torras-Garcia M, Lelong J, Tronel S, Sara SJ. Reconsolidation after remembering an odor-reward association requires NMDA receptors. Learn Mem. 2005 Jan-Feb;12(1):18-22.

Tóth K, Borhegyi Z, Freund TF. Postsynaptic targets of GABAergic hippocampal neurons in the medial septum-diagonal band of broca complex. J Neurosci. 1993 Sep;13(9):3712-24.

Ulanovsky N, Moss CF. Hippocampal cellular and network activity in freely moving echolocating bats. Nat Neurosci. 2007 Feb;10(2):224-33. PubMed PMID: 17220886.

Van Groen T, Wyss JM. Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. J Comp Neurol. 1990 Dec 15;302(3):515-28. PubMed PMID: 1702115.

Van Strien NM, Cappaert NL, Witter MP. The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. Nat Rev Neurosci. 2009 Apr;10(4):272-82. PubMed PMID: 19300446.

Vanderwolf CH. Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol. 1969 Apr;26(4):407-18. PubMed PMID: 4183562.

Vazdarjanova A, Guzowski JF. Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. J Neurosci. 2004 Jul 21;24(29):6489-96. PubMed PMID: 15269259.

Vertes RP. Hippocampal theta rhythm: a tag for short-term memory. Hippocampus. 2005;15(7):923-35. PubMed PMID: 16149083.

Vertes RP, Fortin WJ, Crane AM. Projections of the median raphe nucleus in the rat. J Comp Neurol. 1999 May 17;407(4):555-82.

Vertes RP, Kocsis B. Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. Neuroscience. 1997 Dec;81(4):893-926.

Vinogradova OS. Expression, control, and probable functional significance of the neuronal theta-rhythm. Prog Neurobiol. 1995 Apr;45(6):523-83.

Wainer BH, Levey AI, Rye DB, Mesulam MM, Mufson EJ. Cholinergic and non-cholinergic septohippocampal pathways. Neurosci Lett. 1985 Feb 28;54(1):45-52.

Wamsley EJ, Tucker MA, Payne JD, Stickgold R. A brief nap is beneficial for human route-learning: The role of navigation experience and EEG spectral power. Learn Mem. 2010 Jul;17(7):332-6.

Wells CE, Amos DP, Jeewajee A, Douchamps V, Rodgers J, O'Keefe J, Burgess N, Lever C. Novelty and anxiolytic drugs dissociate two components of hippocampal theta in behaving rats. J Neurosci. 2013 May 15;33(20):8650-67.

Wheeler ME, Buckner RL. Functional-anatomic correlates of remembering and knowing. Neuroimage. 2004 Apr;21(4):1337-49.

Whishaw IQ, Vanderwolf CH. Hippocampal EEG and behavior: changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. Behav Biol. 1973 Apr;8(4):461-84.

White NM, McDonald RJ. Multiple parallel memory systems in the brain of the rat. Neurobiol Learn Mem. 2002 Mar;77(2):125-84.

Wills TJ, Lever C, Cacucci F, Burgess N, O'Keefe J. Attractor dynamics in the hippocampal representation of the local environment. Science. 2005 May 6;308(5723):873-6.

Wilson MA, McNaughton BL. Reactivation of hippocampal ensemble memories during sleep. Science. 1994 Jul 29;265(5172):676-9.

Wilson MA, McNaughton BL. Dynamics of the hippocampal ensemble code for space. Science. 1993 Aug 20;261(5124):1055-8.

Witter MP. Organization of the entorhinal-hippocampal system: a review of current anatomical data. Hippocampus. 1993;3 Spec No:33-44.

Witter MP. Connections of the subiculum of the rat: topography in relation to columnar and laminar organization. Behav Brain Res. 2006 Nov 11;174(2):251-64.

Witter MP, Naber PA, van Haeften T, Machielsen WC, Rombouts SA, Barkhof F, Scheltens P, Lopes da Silva FH. Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways. Hippocampus. 2000;10(4):398-410.

Wittner L, Henze DA, Záborszky L, Buzsáki G. Three-dimensional reconstruction of the axon arbor of a CA3 pyramidal cell recorded and filled in vivo. Brain Struct Funct. 2007 Jul;212(1):75-83.

Wood ER, Dudchenko PA, Eichenbaum H. The global record of memory in hippocampal neuronal activity. Nature. 1999 Feb 18;397(6720):613-6.

Yartsev MM, Witter MP, Ulanovsky N. Grid cells without theta oscillations in the entorhinal cortex of bats. Nature. 2011 Nov 2;479(7371):103-7.

Ylinen A, Bragin A, Nádasdy Z, Jandó G, Szabó I, Sik A, Buzsáki G. Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. J Neurosci. 1995 Jan;15(1 Pt 1):30-46.

Young BJ, Fox GD, Eichenbaum H. Correlates of hippocampal complex-spike cell activity in rats performing a nonspatial radial maze task. J Neurosci. 1994 Nov;14(11 Pt 1):6553-63.

Young CK, McNaughton N. Coupling of theta oscillations between anterior and posterior midline cortex and with the hippocampus in freely behaving rats. Cereb Cortex. 2009 Jan;19(1):24-40.

Zar, J. Biostatistical Analysis. Prentice Hall International: Prentice Hall International; 1999.

Zhang QG, Han D, Hu SQ, Li C, Yu CZ, Wang R, Zhang GY. Positive modulation of AMPA receptors prevents downregulation of GluR2 expression and activates the Lyn-ERK1/2-CREB signaling in rat brain ischemia. Hippocampus. 2010 Jan;20(1):65-77.

Zhang WN, Bast T, Xu Y, Feldon J. Temporary inhibition of dorsal or ventral hippocampus by muscimol: distinct effects on measures of innate anxiety on the elevated plus maze, but similar disruption of contextual fear conditioning. Behav Brain Res. 2014 Apr 1;262:47-56.