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Pontificia Universidad Católica de Chile
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Tesis Doctoral

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Por

GONZALO VALDIVIA ULLOA

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Facultad de Medicina
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THE ROLE OF SLEEP IN THE ORGANIZATION OF SPATIAL REPRESENTATIONS DURING MEMORY FORMATION

Tesis presentada a la Pontificia Universidad Católica de Chile como parte de los requisitos para optar al grado de Doctor en Neurociencias

Por

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ANIMAL WELFARE

All experimental procedures related to animal experimentation were performed according to the protocol N° 180703002 approved by the Institutional Animal Ethics Committee of the Pontificia Universidad Católica de Chile and were carried out protecting animal welfare.

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LIST OF ABBREVIATIONS

CA1: CornuAmmonis area one

CA3: CornuAmmonis area three

GPS: Global Positioning System

Hz: Hertz

LEC: Lateral entorhinal cortex

LFP: Local field potential

LS: Long sleep

MEC: Mediall entorhinal cortex

ms: Millisecond

OPR: Object in place recognition

REM: Rapid eye movement

SWRs: Sharp-wave ripples

SWS: Slow-wave sleep

PC: Place cells

SD: Standard deviation

SS: Short sleep

µV: Microvolts

ABSTRACT

The study of place cells, discovered by O'Keefe (1971), has focused the spatial representation system in the processes and functions led by the hippocampus. Among these functions, the hippocampus plays a preponderant role in establishing spatial memory, in which sleep is fundamental, suggesting a plausible relationship between sleep and the spatial representations provided by place cells. In this line, there is intense research to establish whether sleep participates in the consolidation and configuration of spatial representations given by place cells and if this is related to spatial memory performance.

In this study, we evaluated the influence of sleep on the variations in the configuration of a spatial map given by changes in spatial context during a spatial memory task. Specifically, we first evaluated the influence of sleep on the performance of a spatial memory task, the object in place recognition (OPR), and performed single unit and local field potential recordings in the dorsal CA1 hippocampus in adult rats. Then, we evaluated the influence of sleep and its cardinal oscillations in the configuration of the spatial representations generated by place cells during OPR.

Our results show that sleep, specifically non-REM sleep, and its oscillatory patterns (SWRs, spindles, and delta waves), are directly correlated with performance in the OPR memory test. In addition, concerning single-unit recordings, we detected and isolated 612 units. In this line, the detection and classification of individual units were implemented successfully, which was crucial to evaluate the representation system by detecting place cells. We selected units according to their spatial activity, where 41 % were classified as place cells, and the remaining 59 % were classified as non-spatial cells. Also, during post-learning sleep, place cells were highly time-

correlated with non-REM oscillatory patterns, like SWRs and spindles. In addition, we evaluated mean firing rate and sparsity during the OPR task showing a general and sleep-independent increase in both parameters after sleep, which is possibly associated with novelty during test. Also, there was a sleep-dependent decrease in mean firing rate during post-learning sleep. Finally, we evaluated the stability of the spatial map on the task through a spatial correlation vector, showing that spatial stability in the novel, but not in the familiar arena zone, decreased associated with a longer duration of post-learning sleep.

In conclusion, our results suggest that Non-REM sleep and its oscillations affected spatial representation through the flexibility in the configuration of spatial maps due to changes in the environment. This is also associated with improved spatial memory since instability of place cell spatial configuration is relevant for memory updating during environment exploration.

CHAPTER 1

1. INTRODUCTION

SPATIAL REPRESENTATION SYSTEM

1.1.1 Spatial cells

One of the main functions of the hippocampus is to generate spatial representations that allow us to situate ourselves and navigate the environment (Burgess et al., 1994; John O'Keefe and Nadel, 1978). The idea that the hippocampus plays a crucial role in representing the environment arose when O'Keefe and Dostrovsky (1971) discovered specific hippocampal CA1 cells that fire when the animal is in a specific location. O'Keefe called these *place cells*. Ever since a large amount of research has been devoted to studying the spatial representation system of our brain, some of it will be presented on these lines.

O'Keefe and Nadel (1978) raised the idea that place cells are a central element in the generation of an allocentric cognitive map of the environment, giving a neural basis to the concept developed by Tolman(1948), which proposed the idea that navigation and exploration of space are generated and guided by an internal cognitive map that is produced by representations of the physical space, delivering the tools to create a spatial map and perform navigation (Eichenbaum, 2015)

This kind of cells are pyramidal cells present in hippocampal CA areas (Barnes et al., 1990) and are capable of encoding all local space (Moser et al., 2008; O'Keefe, 1976;

Wilson and McNaughton, 1993). In addition, place cells can represent different environments through changes in the configuration of their firing fields (Muller and Kubie, 1987). This implies that the position of the animal or the positions of particular objects concerning the environment are elements of the experience that can be stored in hippocampal networks (Eichenbaum et al., 1999; Leutgeb et al., 2005).

In addition to place cells, the spatial representation map is nourished by other cell types, among which we find the head direction cells, the boundary vector cells, and the grid cells **(figure 1).** Grid cells also have a significant body of data in the field of spatial function of the hippocampus. These cells were discovered by May-Britt and Edvard Moser (2005) and are characterized by firing in some regions of space; but, unlike place cells, they have several firing fields arranged in a grid pattern in what is called a tessellated pattern of the environment (Hafting et al. 2005; Doeller, Barry, and Burgess 2010).

The firing field of the grid cells is characterized by three components: scale (distance between adjacent firing fields), orientation (relative direction to an external reference), and spatial phase (x-y displacement relative to an external reference point) (Moser, Kropff, and Moser 2008). One of the possible functions of grid cells is their participation as elements of an internal metric system used during spatial navigation (Hafting et al., 2005; Moser, Kropff, and Moser 2008). In addition, these neurons would be involved in path integration system for space, participating in the generation of non-periodic spatial firing patterns (place fields) in the hippocampus (McNaughton et al. 2006).

Another spatial cell type is the head direction cells, found in the presubiculum and discovered by Taube, Muller, and Ranck (1990). These neurons fire only when the position of the animal's head points to a specific direction. Also, it has been seen that changes in the local environment can alter the head direction tuning, just as it does with place cells (Calton et al., 2003)

Finally, we find the boundary vector cells (BVC) that have extended firing fields parallel to the boundaries of the environment (maze or barrier edges). These cells

have been discovered in the subiculum (Lever et al., 2009) and medial entorhinal cortex (MEC) (Solstad et al., 2008). The discovery of these neurons shows that geometry is one of the critical spatial elements in developing the spatial cognitive map of the hippocampus (Hartley et al., 2014).

These cells together constitute the base of the cognitive map, generating a representation of the environment, places, and objects which is independent of orientation or specific position of the animal (Hartley et al. 2014; **figure 1**).

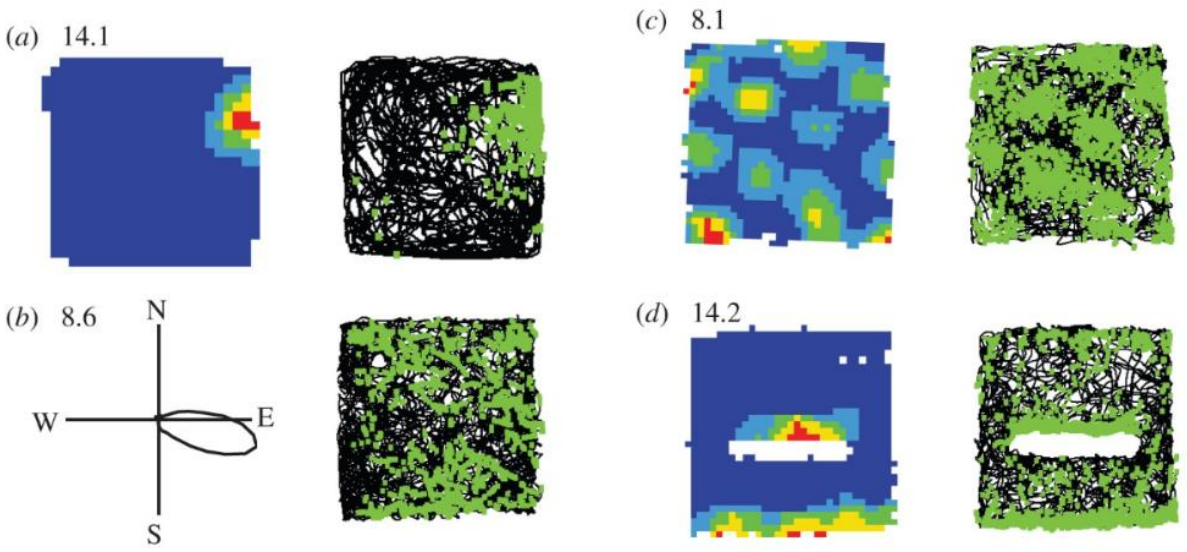


Figure 1. Spatial cells in the brain. The figure shows one example of each type of fundamental spatial cell: (a) place cell; (b) HD cell; (c) grid cell; (d) boundary cell. For each cell: left-hand column shows firing rate map (a, c, d) or directional firing polar plot (b), with peak firing rate in hertz shown top left of rate map/polar plot; right-hand column depicts path taken over whole trial (black line), on which are plotted the locations at which spikes were recorded (green squares). Adapted from Hartley et al. 2014

1.1.2 Changes in the environment and spatial map: global, partial, and rate remapping

One of the features of the spatial representation system given by place cells is that it can detect changes in the environment, generating variations in the space-dependent configuration of the firing fields of the same cell (Muller and Kubie 1987). This process is known as remapping and can occur when there are salient changes such as the color or geometry of the edges of a specific arena (Anderson and Jeffery 2003), even some cells can present remapping when the size of the maze changes without changing the external cues (Muller and Kubie 1987).

In this line, changes in the behavioral context can induce remapping in place cells even when the position or the spatial environment where the task is performed does not change (Markus et al. 1995; Moita et al. 2004; Wood et al. 2000). This evidence suggests a link between the activity of place cells and their firing field configuration with experience modifications.

There are different patterns of remapping. Specifically, two types of remapping that occur under different conditions have been described, rate remapping and global remapping (Colgin et al., 2008; **figure 2).** Global remapping is a phenomenon in which both the firing field and firing rate change. This type of remapping has been associated with local changes in the environment without changing the arena's location(Bostock, Muller, and Kubie 1991; Leutgeb, Leutgeb, Barnes, et al. 2005), as Muller and Kubie showed in 1987. In contrast, there are variations in the firing rate in rate remapping. This type of remapping has been observed in conditions where the location of the arena changes, but not the enclosure of it (Leutgeb, Leutgeb, Barnes, et al., 2005).

Some authors propose that changes in firing rate can encode non-spatial aspects of different experiences occurring in the same location, representing specific memory

episodes (Wood et al., 2000). Along this line, Kubie (2020) states that changes in the configuration of the spatial map are influenced not only by spatial variables but also by temporal variables, adding flexibility to the explanation of the changes observed in different contexts.

Changes in global or rate remapping will depend substantially on the degree of difference in experimental conditions. Neural populations are more likely to generate global remapping when environmental changes are more salient (Colgin, Moser, and Moser 2008).

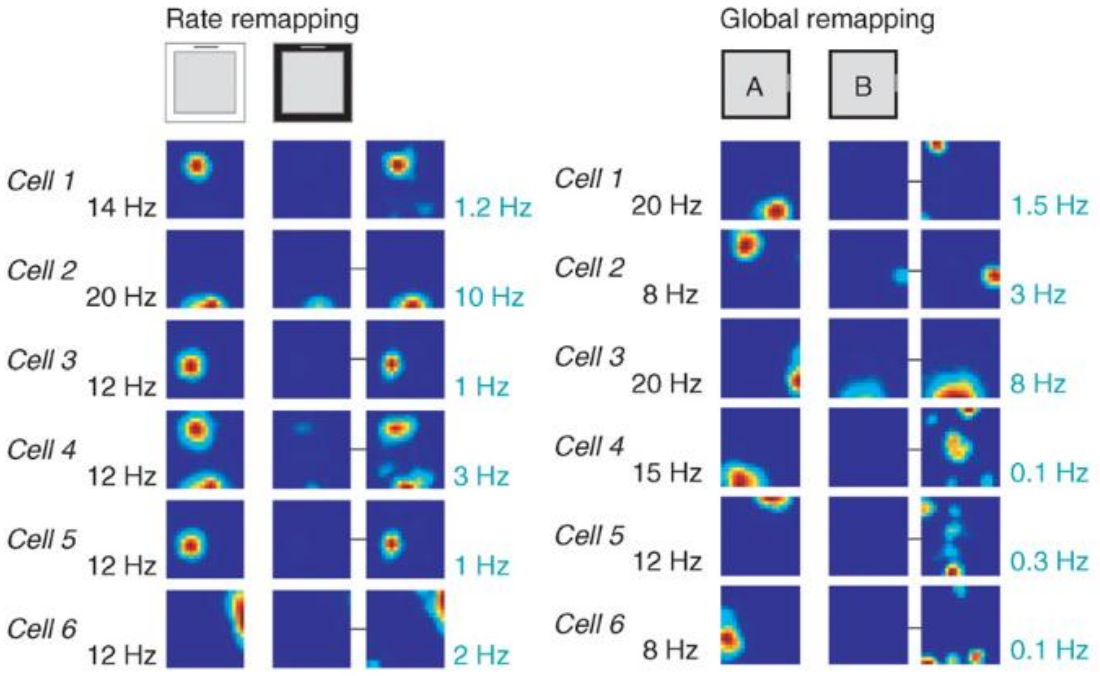


Figure 2. Global and rate remapping. Heat map of firing rate for 6 place cells. Animals were tested in boxes with a different color configuration in a constant location for rate remapping (white and black boxes) and identical boxes but in different places for global remapping (locations A and B). Peak firing rates are into the left. For the middle column, the scale is the same as for the left column. The right column contains the same data as the middle, but the

heat maps are scaled to their maximum values. Adapted from Colgin et al. 2008; originally from Leutgeb et al. 2005.

Most of the data is based on how the external environment configures the activity of place cells in the hippocampus within this field. However, certain studies have shown that landmarks present within the exploratory environment can generate changes in the configuration of these firing patterns (Cressant, Muller, and Poucet 1997). Scaplen et al. (2014) showed that rotations in the position of objects in the center of the maze could generate changes in the firing patterns. Such changes depend both on the salience of the object and the previous experiences in those conditions.

Although it has been established what kind of changes generate remapping in place cells, it has been observed that in environments where the changes are not so significant or salient generate a discordance in global remapping of the firing fields between different neurons of the same population (Tanila, Shapiro, and Eichenbaum 1997; Anderson and Jeffery 2003). These differences in remapping have been associated with the representation of different reference frames within the same neuronal population, as explained by Skaggs and McNaughton (1998). Under these conditions, remapping occurs in specific cells while others do not, depending on whether they encode an external (distal) or internal (proximal) reference framework.

This discordance in the configuration of the firing patterns was approached as partial remapping by Colguin et al. (2008), where they suggest that this phenomenon may reflect the formation of parallel maps within the same environment. Spatial remapping can occur when only part of the visual cues is changed, and the rest of the spatial map remains unaltered (Latuske et al., 2018). Along this line, the authors state that partial remapping results in heterogeneous changes in simultaneously recorded neurons, which means that while some neurons maintain stable firing fields, other neurons switch on or off.

Remapping thus acts as a neural mechanism that promotes the formation of different spatial representations in front of different contexts and experiences. On this line, place cells could organize overlapping items and events presented in different

contexts and avoid interference in the memory recall of similar situations, which could cause forgetfulness and learning disturbances (Colguin et al., 2008; Moser et al., 2015). Accordingly, some authors suggest that the function of place cells goes beyond allocentric navigation but instead organizes events that occur in a specific context, suggesting that the spatial representation system provides a spatial and temporal context as a mechanism for organizing memories (Eichenbaum and Cohen, 2014; Eichenbaum, 2017; Lisman et al., 2017).

1.1.3 Variation in spatial map configuration and its relationship with episodic memory

According to what we have presented previously, the hippocampal spatial representation system in which participate place cells is capable of encoding spatial and temporal context, allowing navigation and creating a cognitive map of the experienced space (O'Keefe and Nadel, 1978; Eichenbaum 1999; Leutgeb et al., 2005; Eichenbaum and Cohen, 2014; Lisman et al. 2017). In this sense, O'keefe and Nadel proposed at the end of the 70s that the hippocampus is the core of a neural memory system that provides an objective spatial framework within which the items and events of an organism's experience are located and interrelated (O'keefe and Nadel, 1978). This view was contrasted in those years by other groups that suggested that the hippocampus played a preponderant role in declarative memory system, through a relational processing mechanism that produces representations that bind in memory the elements of experiences and links memories via their common elements, composing a “memory space” (Cohen and Squire, 1980; Cohen and Eichenbaum, 1993; Eichenbaum, 1999; Eichenbaum and Cohen, 2014).

Currently, efforts have been made to reconcile both views, considering the hippocampal system and pace cells as an allocentric and relational representation system of experience that provides a spatial and temporal reference framework for the organization of memory (Smith and Mizumori, 2006; Eichenbaum and Cohen, 2014; Eichenbaum, 2017; Lisman 2017). However, the relationship between the

spatial representation system and declarative memory is still debated and researched.

In this context, the study of place cells features and function mechanisms related to spatial memory performance becomes relevant. Indeed, Deshmukh and Knierim (2013) have shown that the location of firing fields during the exploration of a square maze with the presence of objects inside it is associated with their position. When moving an object, some of these firing fields are maintained firing in the same place where the object was initially located. This feature is directly related to object location memory. In this line, it has been proposed that the reorganization and reactivation of assembly firing patterns in the hippocampus represent the formation and expression of new spatial memory traces, directly relating the activity of place cells with the formation of spatial memory (O'keefe and Speakman, 1987; Markus et al., 1995; Wood et al. 2000; Moser and Paulsen, 2001; Dupret et al. 2010). Other studies have shown that in the spatial memory task object-place recognition, the CA1 hippocampal cells represent the novel location of objects as a generalized novelty more than specific changes associated with the objects themselves (Larkin et al., 2014). Linked to the above, the selective activation of place cells through an optical strategy manages animals' performance during a spatial memory task, suggesting this neuronal group recruitment in memory acquisition processes (Robinson et al., 2020).

Several authors suggest that hippocampal circuits support the spatial component of episodic memory due to the ability to represent multiple contexts different activity patterns (Eichenbaum et al., 1999; Buzsaki and Moser, 2013; Alme et al., 2014; Moser et al., 2015; Sugar and Moser, 2019).

According to previously presented, diverse data about memory function and spatial representation, where the research that explains how the configuration of spatial representations system supports the spatial memory mechanisms is in the ongoing course and represents a pivotal action to understand how the hippocampus manages the memory function.

1.2 EPISODIC MEMORY

1.2.1 Hippocampus and memory

Memory is a fundamental cognitive function, and its storage is intimately tied to ongoing information processing in the brain. It is manifested in multiple ways by multiple functionally and anatomically distinct brain systems (Eichenbaum and Cohen 2001).

In this sense, memory can be classified, depending on its duration, into working memory, short term memory, and two types of long-term memory (LTM): declarative or explicit memory and non-declarative or implicit memory, each of them with different subclasses (Nadel and Hardt, 2011; Squire and Zola-Morgan 1991, **figure 3).**

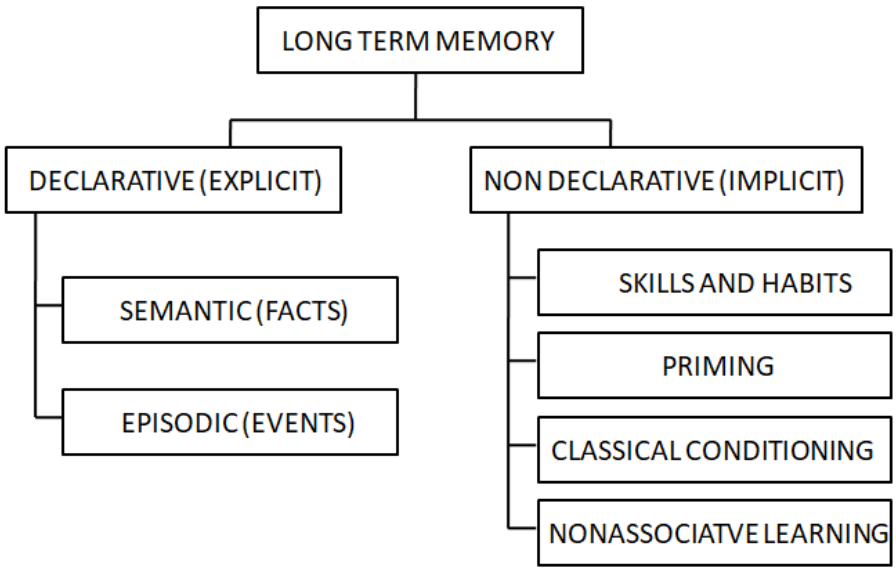


Figure 3. Classification of long term memory. Non-declarative memory refers to the capacity to acquire and recall information implicitly (non-conscious). Declarative memory refers to the ability to bring facts and experiences to the mind via conscious recollection (Adapted from Squire and Zola-Morgan, 1991).

Non-declarative memory comprises a heterogeneous collection of abilities, all of which comprise the capacity to acquire information implicitly (Squire and Zola-Morgan 1988), that is, a memory that allows us to execute a task evoking non-conscious information. This function depends on brain structures such as the striatum, the amygdala, the cerebellum, and the neocortex (Squire and Zola 1996).

On the other hand, declarative memory refers to the ability to bring facts and experiences to mind, that is, recall items in our memory via conscious recollection (Eichenbaum 1997). This declarative or explicit memory depends on the medial temporal lobe structures, specifically the hippocampus and associated cortical structures (Cohen and Squire 1980; Graf and Schacter 1985; Squire and Zola-Morgan 1991; Vargha-Khadem et al. 1997). The idea that the hippocampus could be involved in this type of memory arises from the study conducted more than 60 years ago in the patient Henry Molaison, better known as H.M., a man who suffered from amnesia after a surgery where part of the medial temporal lobe was removed bilaterally to treat epilepsy (Scoville and Milner 1957). H.M. was severely impaired in declarative memory, but his perceptual and cognitive abilities were intact, as well as his capacities for working memory and perceptual and motor skill learning (Corkin 1984; Scoville and Milner 1957).

Declarative memory can be classified as episodic or semantic memory (Tulving 1972). Semantic memory consists of stored information about the features and attributes that define concepts and processes that allow us to retrieve these concepts (Squire 1992; Martin and Chao 2001).

On the other hand, episodic memory is the ability to recall specific personal experiences in a unique spatial and temporal context (Tulving 1983; Eichenbaum 2017). Episodic memory has three ways that originate the spatiotemporal context. These ways are the "What" (as a reference to the memory on a specific item), the "Where" (as a reference to the place where the item occurred), and the "When" (associated to the moment in which it occurred said item) (Tulving 2002).

1.2.2 Pathways of episodic memory in the hippocampus

In episodic memory, the hippocampal memory system is composed of three main components: cerebral neocortical areas, the parahippocampal region, and the hippocampus itself (Eichenbaum 2000). In rats, the spatial memory system is similar to that of humans, with the hippocampus playing a primordial role (Eichenbaum et al., 2012).

Within the structure and organization of the network that supports this type of memory, two are the neural pathways involved in this type of memory. One of them is the "What" path, where the projections go from sensory areas of the cerebral cortex to the perirhinal cortex (PRC) and the lateral entorhinal cortex (LEC). The other one is the "Where" path that sends visuospatial information processed in retroespineal and parietal cortex to the parahippocampal cortex (PHC) and medial entorhinal cortex (MEC) (Suzuki and Amaral 1994; van Strien, Cappaert, and Witter 2009; Eichenbaum et al. 2012). After that, the lateral and medial entorhinal cortex sends projections to the hippocampus, where different representations of places and objects are mapped in a spatial context. From the hippocampus, the information can go upstream to higher cortical areas performing the inverse path, as shown in **figure 4** (Eichenbaum et al., 2012).

On the other hand, it has been established that the hippocampus mediates different cognitive functions depending on the specific zone of the dorsoventral axis. In this sense, it has been shown that the dorsal area of the hippocampus mainly mediates spatial memory while the ventral area mediates emotional responses (Bannerman et al., 2004; Strange et al., 2014). It has also been observed that these differences are expressed gradually along the dorsoventral axis, even indicating that the ventral hippocampus also participates in spatial processing (Kjelstrup et al., 2008). Similarly, Strange et al. (2014) state that there are multiple functional gradients along the hippocampal dorso-ventral axis, so this dichotomous model requires revision.

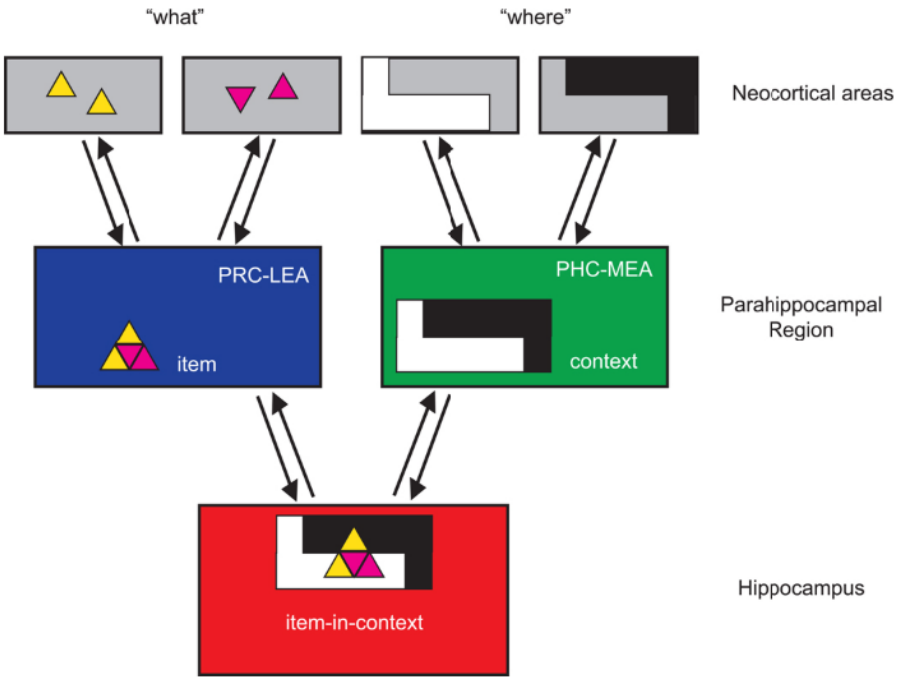


Figure 4. Schematic diagram of the medial temporal lobe memory system in mammals. The "what" stream of the neocortex projects differentially to the perirhinal cortex (PRC) and lateral entorhinal area (LEA), whereas the "where" stream of the neocortex projects differentially to the parahippocampal cortex (PHC) and medial entorhinal area (MEA). Those streams converge in the hippocampus, where items are represented in their experienced context. Outputs of the hippocampus are directed back to the parahippocampal areas and then the neocortical areas, which were the origins of the "what" and "where" stream inputs. Adapted from Eichenbaum et al., 2012.

1.2.3 Spatial memory

Within the episodic memory pathways of the hippocampus, the pathway of spatial memory (where) has been studied since its connection with another of the main functions of the hippocampus, the spatial representation system. In this sense, episodic memory processes need to code space dynamically and robustly.

As we presented above, the spatial memory component is supported by place cells. In this sense, it has been observed that in the dorsal hippocampus, place cells have smaller and more finely tuned place fields, associated with the processing of details in space, than in the ventral hippocampus, where there are more broadly tuned cells that represent global spatial markers (Kjelstrup et al., 2008; Evensmoen et al., 2013; Sugar and Moser, 2019). This variation along the hippocampal dorsomedial axis resembles the representation of grid cells in the entorhinal cortex, where there is an increase in the size of the firing fields from the dorsomedial area, which projects to the dorsal hippocampus, to the ventrolateral area of the medial entorhinal cortex, which projects to the ventral hippocampus (Hafting, 2005; Brun et al., 2008; Straneg et al. 2014; Evensmoen et al., 2015).

Related to this, Buzsaki and Moser (2013) present that the coding of spatial representation performed by place cells in the hippocampus must be capable, not only of generating a system of self-referenced navigation but also of having the ability to store different representations of the experience trough cell assembly sequences. The authors hypothesize that the mechanisms for representing a path through an environment are similar to those used to represent sequences in memory. In this line, the activity of place cells encodes not only spatial representations but also memory episodes (Leutgeb, Leutgeb, Moser, et al., 2005).To understand how the mechanisms of spatial memory use the different spatial representations, it is necessary to recognize which are the stages of memory formation where these spatial representations are encoded. Tulving (1972) already stated that memory had two necessary stages, the phases of encoding and retrieval. Eichenbaum et al. (2012) defined these stages for episodic memory, where the encoding phase involves the convergence of information about events and their context within the hippocampus, and retrieval allows driving the circuit to reactivate the convergent representation in the hippocampus, which reactivates the "where" and "what" stream to retrieve the context representation. They say that the ability to retrieve context from item information, that is, to remember where an event occurred (or vice versa), is a classic prototype of episodic memory.

Based on the above, it is necessary that the representation acquired during the encoding phase be stored and consolidated to be reactivated in the retrieval phase. This process of memory consolidation is the process by which new and transitory acquired information is integrated and stabilized into persisting long-term memory (McGaugh 2000; Inostroza and Born 2013). Due to the importance of the consolidation process for memory performance, the motivation to understand which are the factors or mechanisms that promote the consolidation of episodic memory in our brain and how these are performed by the hippocampus and the associated structures arises.

One factor that promotes and plays a key role in some forms of memory consolidation is sleep (Buzsaki 1998; Maquet 2001; Stickgold 2005; Born, Rasch, and Gais 2006). In this line, a research has shown that performance in a spatial memory test, object in place recognition (OPR), improves when animals sleep in the post-encoding phase (Inostroza, Binder, and Born 2013).

1.3 SLEEP AND MEMORY

1.3.1 Sleep and memory consolidation

One of the most interesting phenomena about our organism is sleep. Interestingly despite its importance and considering that we spend 1/3 of our lives in this state, the study of this process in the field of memory has taken the biggest stir just begun the XXI century, being a phenomenon that has not yet been fully explained, presenting a challenge for current neuroscience.

Sleep is defined as a natural, reversible, and global state of reduced responsiveness to external stimuli and relative inactivity, accompanied by loss of consciousness (Rasch and Born 2013).

It has been seen that sleep is involved in different brain processes promoting proper cognitive function (Tononi and Cirelli 2006; Diekelmann, 2014) and that sleep deprivation and sleep disruptions cause severe cognitive and emotional problems as decrements in attention, decision-making, and memory processes (Killgore 2010; Lim and Dinges 2010; Walker 2009).

In structural terms, sleep is divided into two main stages, Rapid Eye Movement or REM sleep and non-REM, also known as Slow Wave Sleep (SWS). In turn, non-REM is divided into 4 sub-stages in humans (Rechtschaffen and Kales 1968). In humans, it is essential to note that non-REM sleep is primarily present during early sleep, whereas REM is primarily present during the late state of sleep (Walker and Stickgold 2004; Rasch and Born 2013; **figure 5**). The influence of each one of these stages has been studied in different processes, being the hippocampus-dependent memory one of the most important (Marshall and Born 2007).

Several studies have tried to prove the influence of sleep on the processes involved in long-term memory; specifically, it has been found that sleep plays a fundamental role in memory consolidation process (Buzsaki 1998; Maquet 2001; Stickgold 2005; Born, Rasch, and Gais 2006). In this line, different effects of both REM and SWS

sleep have been found in this process. Some researchers have described REM sleep, characterized by the presence of theta oscillations, as a fundamental process of memory consolidation (Rauchs et al. 2004), while the vast majority of data points to the role of SWS on consolidation mechanisms (Peigneux et al. 2004; Marshall et al. 2006; Rasch et al. 2007; Diekelmann and Born 2010).

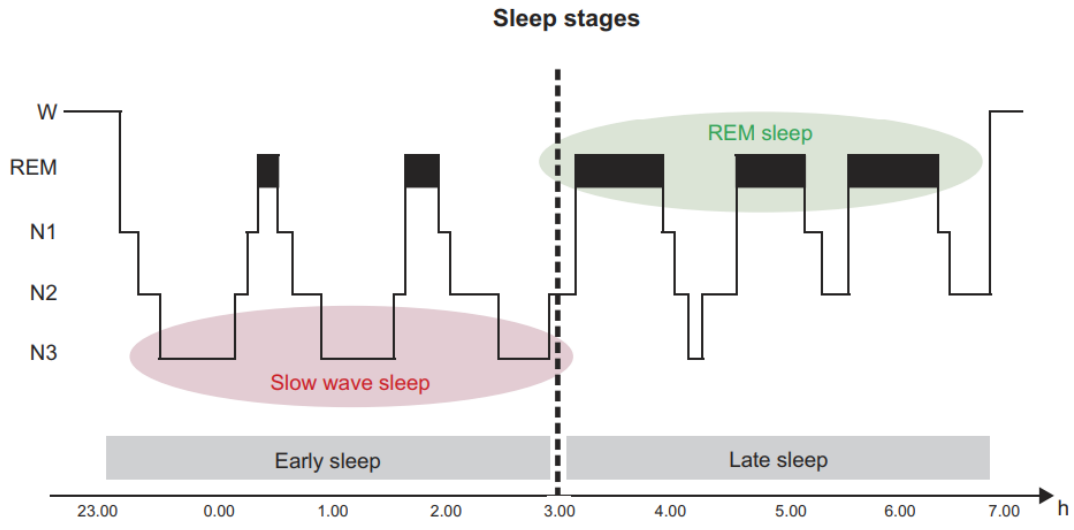


Figure 5. **Typical human sleep profile.** Sleep is characterized by the cyclic occurrence of rapid-eye-movement (REM) sleep and non-REM sleep. Non-REM sleep includes slow-wave sleep (SWS) corresponding to N3 and lighter sleep stages N1 and N2. According to an earlier classification system by Rechtschaffen and Kales, SWS was divided into stage 3 and stage 4 sleep. (Adapted from Rasch and Born, 2012)

Due to the specific role of SWS in episodic memory consolidation, it is essential to know the features of this stage and how they can be involved in that process. SWS is mainly associated with stages 3 and 4 of non-REM sleep (Marshall and Born 2007), characterized by the presence of slow cortical waves mainly in the delta range (1 to 4 Hz) (Steriade and Amzica 2003; Marshall et al. 2006), where they also present spindles oscillations (12 - 15 Hz) that have been proposed to represent an essential feature in the processing of memories during sleep (Schabus et al. 2004; Molle et al. 2002).

On the other hand, slow-wave sleep is characterized by sharp-wave ripples (SWRs) in hippocampal CA1 (Buzsaki, Leung, and Vanderwolf 1983; Molle et al. 2006; Eschenko et al. 2008). SWRs are short-lived fast oscillatory patterns of the LFP in the CA1 pyramidal layer (100-250 Hz) that are associated with large amplitude negative polarity deflections (40-100 ms) in CA1 stratum radiatum known as sharp waves (Buzsaki, Leung, and Vanderwolf 1983; Suzuki and Smith 1987; Buzsaki 2015). SWRs have been associated with Slow Wave Sleep and with resting periods in the awake state (Buzsaki 1986) and even during the exploratory behavior associated with a replay of place cells (O'Neill, Senior, and Csicsvari 2006; Karlsson and Frank 2009).

In this line, it has been found that SWR is directly involved in memory consolidation processes by transferring new hippocampal content to the neocortical circuits for long-term storage (Buzsaki 1989). One of the possible mechanisms is the coupling between SWR and the reactivation of neuronal assemblies that were activated during exploratory phases (i.e., encoding) of episodic memory (Pavlides and Wilson 1989; Buzsaki 1998; Jadhav et al. 2012; Ramadan, Eschenko, and Sara 2009; Girardeau et al. 2009). Interestingly, CA1 place cells are one of the neuronal populations that could be reactivated in these conditions (Pavlides and Wilson 1989; Wilson and McNaughton 1994; O'Neill, Senior, and Csicsvari 2006; Diba and Buzsaki 2007; Jadhav et al. 2012). In line with this, other studies have shown that spatial representations of place cells in a novel experience are processed during SWR in subsequent sleep (Hwaun and Lee Colgin, 2019).

In this sense, the reactivation of neurons that have spatial information occurred in hippocampal CA1 during slow-wave sleep coupled with SWR suggests that this mechanism is involved in the consolidation of spatial memories generated during spatial navigation.

1.3.2 Sleep and the reorganization of the hippocampal spatial map

Recently, some investigations have tried to elucidate the effect of the SWR on the hippocampal spatial map, but with different results. On the one hand, it has been seen that the optogenetic suppression of SWR during sleep periods in mice does not generate interferences in the stability of the spatial map when the animal visits a previously explored arena (Kovács et al. 2016).

Conversely, Roux et al. (2017) found that SWR is involved in the configuration of the spatial map. This idea is in line with what was proposed by van de Ven et al. (2016) that SWR is involved in the consolidation of hippocampal cell assembly patterns that represent novel environments. Roux et al. (2017) demonstrated that the optogenetic silencing of the SWR impairs the stability of the spatial map generated during the exploration of the cheeseboard maze in a learning task. Interestingly this research studies the configuration of the spatial map during active exploratory behavior in learning (exploration in a learning or memory task), which differentiates it from previous works where only the exploratory behavior (without cognitive tasks) was studied (Kovács et al. 2016, Van de Ven et al. 2016). Despite the above, no differences were found in learning in this investigation, even with changes in the spatial map stability.

Despite this contrast, there is a factor that has not been studied in detail in these investigations: the influence of sleep in the hippocampal spatial map configuration. On the other hand, as we have proposed here, the configuration of the spatial map during active exploration in spatial memory and learning tests has been poorly studied.

In this line, one of the critical factors to consider is that the studies that have been carried out in this area mainly aim to evaluate the stability of the spatial map in conditions where the spatial context of the explored maze does not change. In this sense, it is interesting and novel to see how sleep affects the reconfiguration of the spatial map when exploring novel environments as in a spatial memory task. This will give us an understanding of how spatial representation patterns are regulated in more

complex behavioral contexts. They can play an essential role in cognitive functions such as memory.

Although we know that changes in space and context generate changes in the spatial representation maps given by place cells (Muller and Kubie, 1987; Anderson and Jeffery 2003), we do not know how sleep affects these changes and how the variation in the reconfiguration of this spatial map could be related to spatial memory performance.

In this sense, while it has been established that sleep has a primordial function in spatial memory consolidation, where the reactivation of neurons encoding spatial information through specific activity patterns is involved, it is not clear how sleep promotes spatial memory consolidation and whether it plays a role in the configuration and reorganization of spatial representation patterns given by place cells during the exploration of environment.

For this and through the present work, we will evaluate the effect of sleep on the reorganization of hippocampal spatial and relate this effect with performance in spatial memory.

CHAPTER 2

2. HYPOTHESIS

The thesis committee approved the following general hypotheses on August 29th, 2018.

2.1 GENERAL HYPOTHESIS

Sleep promotes the stability of the hippocampal spatial map during changes in the environment, improving spatial memory performance.

CHAPTER 3

3. OBJECTIVES

The thesis committee approved the following general and specific objectives on August 29th, 2018.

3.1GENERAL OBJECTIVE

Evaluate the influence of sleep on the reorganization of hippocampal spatial maps and its relationship with spatial memory processing.

3.2SPECIFIC OBJECTIVES

The general objective presented is divided into two specific objectives.

3.2.1 Specific objective 1

Evaluate the influence of sleep on the performance of a spatial memory task and perform single-unit recordings in the dorsal CA1 hippocampus during the OPR task in rats

3.2.2 Specific objective 2

Evaluate the effect of sleep on changes in place cell activity patterns that occur in the spatial memory task and correlate them with behavioral performance.

CHAPTER 4

4. METHODS

4.1 EXPERIMENTAL DESIGN

4.1.1 Behavioral protocols

Efforts were performed to minimize the number of animals used and their suffering. All tests were conducted between 8.00 a.m. and 2.00 p.m. All experimental procedures related to animal experimentation were approved by the Institutional Animal Ethics Committee of the Pontificia Universidad Católica de Chile (protocol code: CEBA 180703002)

4.1.2 Animals

Adult male Long Evans rats (P61-P95, **table S1**) were obtained from the Animal House Facility of the Faculty of Medicine, Pontificia Universidad Catolica de Chile. Animals were kept in a room with a controlled temperature (22 ± 2° C), in light / dark cycles (12:12hr), with water and food delivered *ad libitum*. Eighteen animals were used for the experiments, where 13 of them performed only behavioral experiments (onwards *non implanted animals*), and the other 5 were implanted to perform hippocampal electrophysiological recordings (onwards *implanted animals*).

4.1.3 Spatial Memory Task, Object in place recognition (OPR) test.

4.1.3.1 Behavioral apparatus

The spatial memory task (OPR, **figure 6**) was performed in two arenas, a square (65x65 cm and 35 cm height) box and a circular (65 cm diameter and 35 cm height) open field maze made of PVC (gray for the floor maze, gray for the square maze walls, white for the circular maze walls). The maze was cleaned with 50% ethanol before and after each test trial, being careful not to leave traces of odors or biological material. For the rest phase **(figure 6)**, the retention box was a circular plastic box (30 cm in diameter) covered with a cotton blanket mounted on top, where the animal could move freely. The light was provided from the top of the recording room, allowing a uniform light in each sector of the maze. The noise inside the experimental room was relatively constant at all times (60 dB) and, in no case, exceeded 80 dB. The experimental room had 2D and 3D visual cues arranged outside the maze (distal), yet visible to the experimental animals from the maze. The objects used for the task were bottles made of glass and filled with corn shavings that avoid the reflection of the light. Their sizes were 20-25 cm in height and were cleaned with 50% ethanol before and after each trial allowing the elimination of all traces of odors and biological material. Finally, the recording area was enclosed by walls and a curtain that allowed isolation of the area from the rest of the room.

4.1.3.2 Behavioral Protocol

Handling: Handling was done for 10 minutes daily for 5 consecutive days. If the animals were used for surgery, handling contemplated manipulating the head, so the animal got used to that movement.

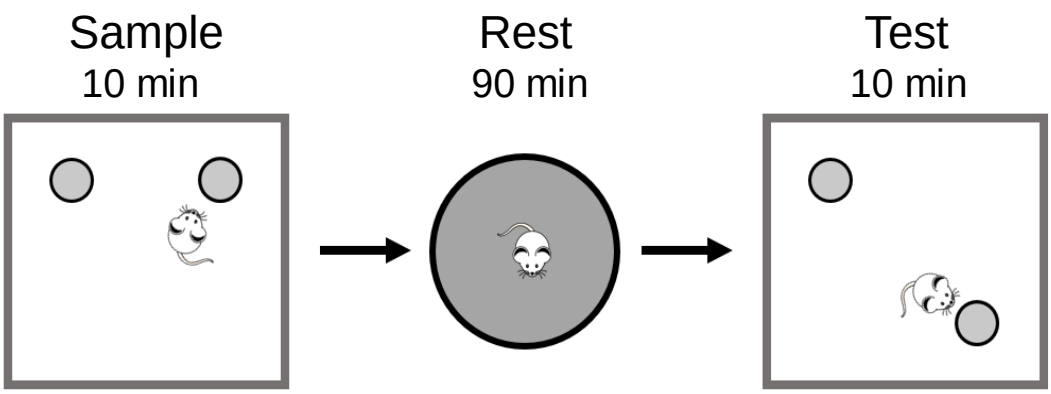


Figure 6. Test diagram for Object-Place recognition (OPR). The three phases are from left to right: sample, rest, and test. In the test phase, one object is moved to a different position than the one in the sample phase.

Habituation: Habituation was performed in the open field and retention box. For the Open field, habituation was performed in the absence of objects. The animal was placed inside the maze facing the wall (alternating the entrance wall) and allowed to explore for 10 minutes. Habituation to square or circular maze was made 3 times before the first OPR where that maze was used (two times the day before OPR, and 1 time 2 days before OPR). Habituation to the retention box was made for 3 hours, 3 times before the first OPR trial (two times the day before OPR, and 1 time two days before OPR).

Object-Place recognition (OPR) test: the protocol was performed as shown in figure S1. Two equal objects were placed at 15 cm from the corners for the sample phase. Then, animals were placed in the maze and explored for 10 minutes. The position of the objects and the entrances of the rat were randomized in every phase. After that, the rat was placed in the retention box for 90 minutes. Finally, for the test phase, one of the objects was moved to the opposite position to the sample phase, as shown in **figure 6** (the quadrant selected was diagonal to the quadrant of the non-displaced object). Again, the animal was entered looking towards the wall, through a

different wall to the one used in the sample. The animal explored for 10 minutes and then was returned to the housing box when the test ended. The video recording was performed in all experiments, and the animal's trajectory was analyzed using idTracker (http://www.idtracker.es).

The experiments were carried out during the first 6 hours of the light cycle, where slow wave sleep (SWS) is highest. For non-implanted animals, the experiments were repeated twice; the first time in the square maze, and the second time, 48 hours after, in the circular maze. For implanted animals, the number of trials and maze type is shown in **table S2**. The diagram of the experiment for all animals is shown in **figure7**. The protocol was adapted from Inostroza, Binder, and Born (2013)

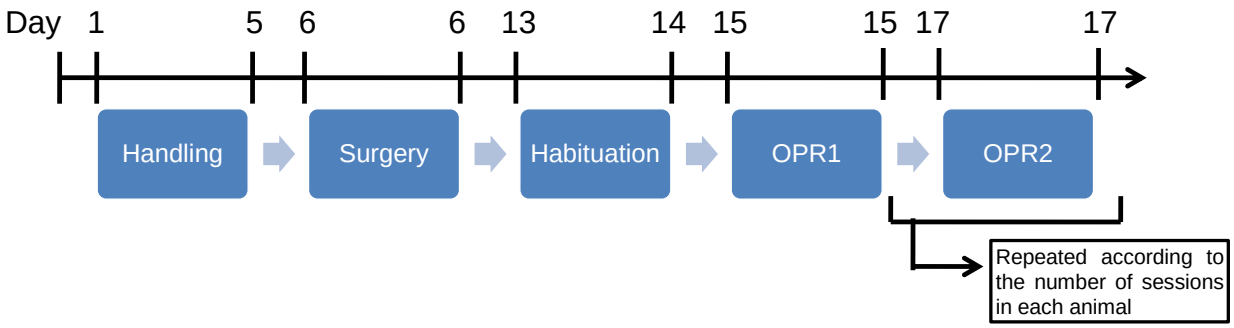


Figure 7. Experimental design for OPR task. The scheme represents the design for the experiments performed by implanted animals. The scheme is the same for behavior animals, but without surgery, so the animal goes directly from handling to habituation phase.

4.1.3.3 Scoring and discrimination index

Scoring was performed by quantifying the exploration time of each object using custom-developed software with MATLAB. The behavior was considered as exploration when the rat sniffed the object within a distance of 2 cm between them. The exploration index was calculated using the exploration time of each object according to the equation:

$$\textbf{Exploration index} = \frac{\text{Displaced object} - \text{NonDisplaced object}}{\text{Displaced object} + \text{NonDisplaced object}}$$

If the exploration index was close to 1, the rat explored more the displaced object (novelty condition); 0, the rat explored both objects equally, and -1, the rat explored more the non-displaced object (familiar condition). The protocol was adapted from Inostroza, Binder, and Born (2013)

4.2. RECORDING PROTOCOLS

4.2.1 Electrode preparation

For electrophysiological recordings, 8 Nickel Chrome tetrodes were chronically implanted in rats. They were mounted in a Harlan-8 hyperdrive (Neuralynx), and each tetrode strand was connected to an EIB-36 PCB card with a capacity for 32 channels. The stainless steel wires connected two grounds to stainless steel screws placed in the animal skull. One day before surgery, the impedance of each tetrode was lowered to 0.2 MOhm by coating each tip electrostatically with colloidal gold.

4.2.2 Surgery for Chronic Implantation

Rats were anesthetized with isoflurane (4% induction and 1.5 – 2% maintenance) and placed on a stereotaxic frame (David Kopf Instruments). The temperature was maintained at 37° throughout the procedure (3 – 4 hours) using a heating pad. The skin was incised to expose the skull, and a craniotomy (~1 mm in diameter) was made with a dental drill above the distal portion of CA1 subregion of the dorsal hippocampus in the right hemisphere (AP -0.36 mm and ML -0.22 mm from Bregma). The dura was gently removed to expose the cerebral cortex in this phase. To prevent desiccation, neural tissue was lubricated with a drop of mineral oil. All tetrodes were exposed 1 mm out of the metal tip of the Harlan-8 and inserted in the brain with a stereotaxic guide according to the calculated coordinates; after that, craniotomy was sealed with a silicone elastomer. Two screws were placed in the occipital bone and connected as short-circuit grounds; one screw was placed in the frontal bone and one in each lateral bone of each hemisphere to serve as mechanical support. Finally, the entire surface was sealed with dental acrylic cement. The animal was hydrated with saline solution (NaCl 0.9%) every hour during the surgery. After surgery, rats received a daily dose of enrofloxacin (10 mg/kg, Centrovet) for five days and supplementary analgesia with ketoprofen (5 mg/kg, Centrovet) for three days. Animals had at least a week of recovery before behavioral testing.

4.2.3 Recordings in Freely-moving animals

After the recovery period, tetrodes were be moved manually by no more than100 µm every 24 hours (2 turns of screws that support tetrodes). During this procedure, EIB-36 was connected to the amplifier (Amplipex KJE-1001) through a 32-channel headstage (HS3-Amplipex), and the signal of LFP (oscillatory patterns) and multiunit activity (rate and amplitude of neuronal spikes) was checked. Recording began when the characteristic signal of the pyramidal layer of the hippocampal CA1 sub-region was observed, such as a large-amplitude theta, sharp-wave ripples, and an abrupt increase in the density of units. One tetrode was used as a reference, and continuous recordings were performed at 20.000 samples per second. Each session was recorded with a video synchronized with the amplifier clock. After recordings, the obtained DAT files were transformed to Matlab format for further analyses using the LAN toolbox (http://lantoolbox.wikispaces.com/). Recordings were continuously performed during the entire OPR task and the rest phase.

4.2.4 Histology and recording site identification

After recordings, animals were anesthetized with isoflurane, electrolytically lesioned at each tetrode (5 µA of positive current for 10 s was applied to two channels of each tetrode), and allowed to recover for 48hr. After that, rats were terminally anesthetized and intracardially perfused with a saline solution followed by a 20 min fixation with 4% paraformaldehyde. Brains were extracted and postfixed in paraformaldehyde overnight before being transferred to PBS-azide and sectioned coronally (60-70 µm slice thickness). Sections were further stained for Nissl substance. Locations of shanks were performed under a light transmission microscope.

4.3 DATA ANALYSIS

4.3.1 Sleep analysis

Sleep scoring of non-implanted animals was performed visually according to the position of the animal **(figure 8).** If an animal spent 10 seconds or more immobile, that interval was considered sleep. This criterion was based on similar studies that visually evaluated sleep scoring (Sawangjit et al., 2018). Sleep scoring was performed using an electrode positioned in the hippocampal CA1 pyramidal layer for implanted animals. LFP signal of this electrode was downsampled to 1000 Hz, and time-frequency decomposition was performed with Fourier analysis using LAN toolbox to obtain the signal's power spectrum. The signal was analyzed in 10-second windows, where the raw LFP signal, the power spectrum, and the video recording were used to determine the stage of sleep. One of three different stages was selected for each window depending on the following criteria: for each window, if the power spectrum had a peak in delta oscillations (0.5-4HZ) and the animal was not moving, we identified it as non-rapid eye movement (non-REM) sleep; whereas if the power spectrum presented a peak in theta oscillations (4-10 Hz) and the animal was not moving, we classified it as Rapid eye movement (REM) sleep; finally, if the animal was moving, we select the period as Awake **(figure S1).** To select a window in any of these categories, the criteria had to be fulfilled at least in 50% of the window. It was cataloged as undetermined if there was no clarity in a specific window. This analysis was performed with a MATLAB routine (MathWorks, Natick, MA).

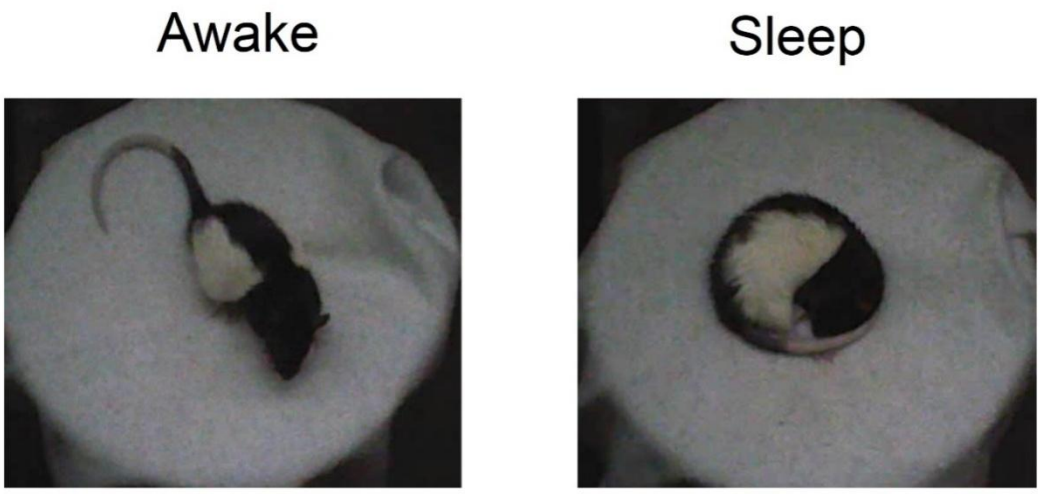


Figure 8. Visual inspection of sleep. Visual inspection was performed during the rest phase. On this, we detect two different behaviors. When the animal is exploring, it refers to awake, and when the animal is quiet or rolled upon it, it refers to the sleep stage.

4.3.2 Oscillation Analysis

4.3.2.1 Theta oscillation

Theta oscillations were detected by calculating the continuous ratio between the envelope of theta (4–8 Hz) and delta (2–3 Hz) frequency bands filtered from the hippocampus LFP and computed by the Hilbert transform. A ratio of 1.4 SD or higher during at least 2 s defined epochs of theta oscillations.

4.3.2.2 Delta oscillation

Delta oscillations were detected by calculating the envelope of the delta (1–3 Hz) frequency band filtered from the hippocampus LFP and computed by the Hilbert transform. Delta wave amplitude was Z-scored during all sleep periods and epochs with amplitude1.4 SD or higher, and at least 2 seconds were defined as delta oscillations.

4.3.2.3 Sharp wave ripples detection

Sharp wave ripples were recorded in dorsal CA1, as close to *stratum pyramidale***.** Hippocampal LFP was downsampled to 1 kHz and band-pass filtered (100-250 Hz) using a zero-phase shift non-causal finite impulse filter with 0.5 Hz roll-off. Next, the signal was rectified and low-pass filtered at 20 Hz with a fourth-order Butterworth filter. This procedure yields a smooth envelope of the filtered signal, then a z-score normalized using the whole signal's mean and standard deviation (SD). Epochs during which the normalized signal exceeded a 3 SD threshold and 50 ms of duration were considered events. The first point before the threshold that reached 1 SD was considered the onset, and the first one after the threshold to reach1 SD was considered the end of events. The difference between onset and end of events was used to estimate the ripple duration. We introduced a 50 ms-refractory window to prevent double detections. To precisely determine the mean frequency, amplitude, and duration of each event, a spectral analysis using Morlet complex wavelets of seven cycles was performed. The protocol was adapted from Logothetis et al. (2012), and the LAN toolbox (http://lantoolbox.wikispaces.com) was used for their implementation.

4.3.2.4 Spindles

Spindles were detected in the downsampled hippocampal signal (1 kHz), calculating the maximum normalized wavelet power in frequencies between 11 and 17 Hz. This signal was then *z*-score normalized, using the whole signal's mean and standard deviation (SD). Epochs during which the normalized signal exceeded a 1.4 SD threshold and 350 ms of duration were considered events. Only spindles detected on non-REM sleep were selected for analysis.

4.3.3 Spike sorting

Neuronal spikes were extracted from hippocampal recordings using semiautomatic clustering Klustakwik (https://github.com/kwikteam/klustakwik2/). This method was applied over the 32 channels (8 tetrodes), whose signal was filtered between 300-5000 Hz. Spike clusters were considered single units if less than 1% of their spikes had an interspike interval below 1 ms, and their auto-correlograms had a 2-ms refractory period. Timestamps of each spike cluster were obtained after this procedure to perform place cell analysis.

4.3.4 Place cell analysis.

4.3.4.1 Firing rate maps

Only data of epochs in which the animal ran faster than 5 cm/s were considered to identify place cells between hippocampal units. Each arena was divided into 5x5 cm bins, where the time that the animal spent in each bin was sorted to obtain an occupancy map. The animal position on the arena was determined by detecting a LED placed on the animal's head recorded at 30 fps. On the same line, spikes of each unit were sorted in map bins to obtain the fire map. After that, the firing rate was calculated using time and spikes in each bin to obtain a firing rate map. A Gaussian kernel (s.d. = 7 cm) was applied for firing rate maps to obtain smoothed maps used to compute spatial map parameters.

4.3.4.2 Spatial map parameters

Smoothed firing rate map was used to compute the parameters used to select place cells. The mean firing rate on the arena was calculated as the mean of all visited (bins where the animal spent more than 0.1 seconds. The Peak firing rate corresponds to

the highest firing rate on visited bins. Sparsity, which represents the proportion of the arena where a unit fire, was obtained using the formula:

$$\textbf{Sparsity} = \frac{\mathbf{Pi} * \boldsymbol{\lambda}\mathbf{i}^2}{\boldsymbol{\lambda}^2}$$

Where Pi is the probability for occupancy of a specific bin, λi is the firing rate of that bin, and λ is the mean firing rate. Finally, place fields were defined by a contiguous area starting from the cell's peak firing rate until it reached 2 × SD of the firing rates of all bins in the firing rate map (protocol adjusted from Høydal et al., 2019). Only contours that bounded an area of 6 or more bins (150 cm^2) were selected as place fields. After visual inspection of our putative place fields in the smoothed maps, this area was selected to avoid those derived from random firing in low traveled areas of the arena.

4.3.4.3 Sigmoid fit

Sigmoid curve fitting was performed for all units during the sample phase. The mean firing rate (logarithm scale) v/s sparsity of all units were plotted and fitted to a sigmoid curve using the Matlab curve fitting toolbox (https://www.mathworks.com/help/curvefit/) from which the critical values were obtained. The equation resulting from this analysis was:

$$\textbf{Sparsity} = \frac{\mathbf{1}}{\mathbf{1} + \mathbf{exp}^{-\mathbf{0.72} * \mathbf{log\ firing\ rate}}}$$

We obtained the lower and upper inflection points from this fit, which delimit the linear zone of sigmoid fit, which represents the zone of the curve where pyramidal cells and precisely place cells are. This allowed to us discard cells with a low firing rate (less than 0.18) and high sparsity (sparsity more than 0.78) in our experiments.

4.3.4.4 Place cell selection

Units with firing rate over 0.18 Hz (lower critical point of sigmoid curve), sparsity less than 0.78 (Upper critical point of sigmoid curve), and the presence of at least 1 place field was classified as place cells. For sparsity, a shuffling with 1,000 iterations was carried out for the occurrence in time of each spike of a single unit, keeping the animal trajectory unchanged. Sparsity value for each iteration was used to compute the Z-score value, discarding those units that did not exceed -1.5 standard deviations. This threshold was selected after visual inspection of our putative place cells in the smoothed maps to avoid eliminating those cells that were place tuned.

All units that did not meet these criteria were cataloged as non-spatial cells. This classification was performed in both sample and test phases.

4.3.4.5 Cell group classification

Depending on the classification units as place cell or non-spatial cell both in sample and test, we sorted 4 different groups of units. Units classified as Place cells during both sample and test phases were sorted as *Keep place cells*. Those units classified as place cells during the sample phase but not during the test phase were sorted as *Down place cells*. Those units that were not classified as place cells during sample but were classified during test were sorted as *Up place cells.* These groups were considered as “place cells”. On the other hand, those cells classified as place cells neither during the sample nor during the test were sorted as *non-spatial cells.*

4.3.5 Cross-correlation analysis.

Hippocampal SWRs and neuronal spikes were cross-correlated by applying the "sliding-sweeps" algorithm (Abeles and Gerstein, 1988). A time window of ± 1 s was defined with the 0-point assigned to the start time of a ripple. The timestamps of spikes within the time window were considered as a template and were represented

by a vector of spikes relative to t = 0 s, with a time bin of 25ms and normalized to the total number of spikes. Thus, the central bin of the vector contained the ratio between the number of thalamic spikes elicited between ± 12.5ms and the total number of spikes within the template. Next, the window was shifted to successive SWRs throughout the recording session, and an array of recurrences of templates was obtained.

4.3.6 Population vector

In each trial, the spatial maps generated for each cell were assembled in a 3D matrix, where the z-axis corresponds to the number of place cells on that trial. Using this matrix, a population vector that contains the firing rate values of all the place cells in a given bin was created. This analysis was performed both for sample and test. After that, a spearman correlation between both sample and test population vectors was performed, obtaining a correlation coefficient (r) for each bin on the arena **(figure S2).**

4.3.7 Statistical analysis

All statistical analyzes were performed with Graphpad Prism 8 program (https://www.graphpad.com/scientific-software/prism/). The detail of the results, significance values, and type of test applied for each analysis are shown in **table S5.**

CHAPTER 5

5. RESULTS

5.1. SPECIFIC OBJECTIVE 1: EVALUATE THE INFLUENCE OF SLEEP ON THE PERFORMANCE OF A SPATIAL MEMORY TASK AND PERFORM SINGLE-UNIT RECORDINGS IN THE DORSAL CA1 HIPPOCAMPUS DURING OPRTASK IN RATS

5.1.1 Sleep correlates with better spatial memory performance during OPR task

In the present doctoral thesis, I have studied the influence of sleep on the performance of a spatial memory task. For this, first, I performed behavioral experiments in the OPR test **(figure 6)**. In this test, animals spent 90 minutes in a rest phase (between sample and test phases) where the time of sleep and wakefulness was quantified for each test trial in non-implanted (animals that performed the behavioral test only, without electrophysiological recordings) and implanted (animals that performed the task while their hippocampal activity was recorded) animals. In the rest phase animals were not disturbed (no sleep deprivation) so wakefulness and sleep were spontaneously induced.

The quantification of sleep duration was performed visually considering the posture of the animal in the rest cage **(figure 8)** and, also for implanted animals, the hippocampal LFP to determine the amount of REM and non-REM sleep time **(figure S1).** These results are shown in **figure 9A** for all animals (41 trials, 18 animals), in terms of the percentage of sleep duration. The distribution of sleep duration showed periods from 0 to 70 minutes **(table S3).**

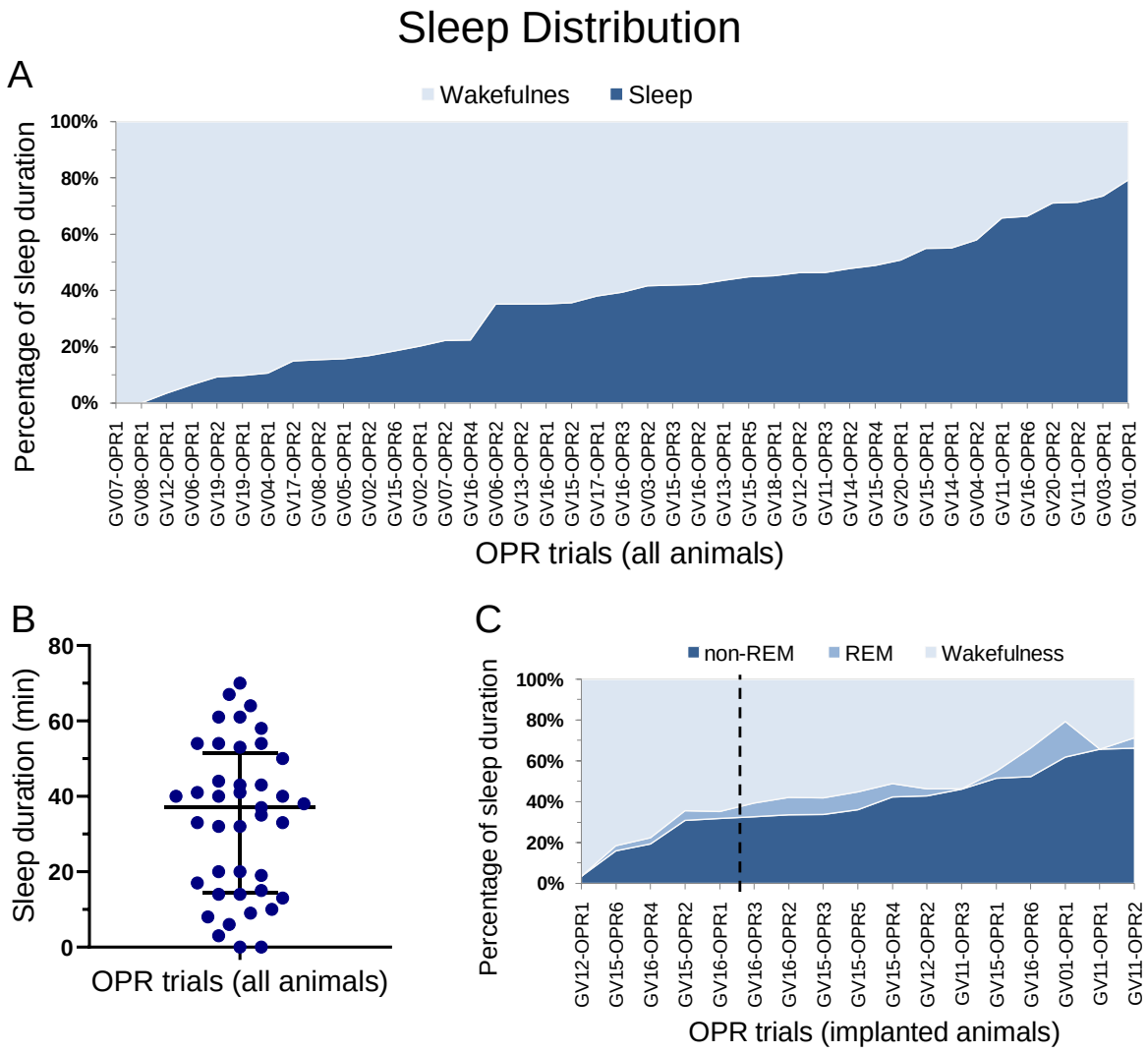


Figure 9. Distribution of sleep duration during the rest phase in the OPR task. **A)** Percentage of sleep during the 90 minutes of rest phase. The x-axis presents the number and test trials performed by each animal, from a total of 41 trials and 18 animals. **B)** Distribution of sleep duration in the rest phase per trial. The distribution is normal (Anderson-Darling test p=0.1227), with a median of 37 minutes and a mean of 34 minutes. **C)** Same as in **A** but for implanted animals. The dotted line marks the separation between the trials that are above the median (long sleep group, 11 trials, 5 animals) and below the median (short sleep group, 5 trials, 3 animals) of total sleep.

To characterize the distribution of sleep duration, the Anderson-Darling normality test was performed, showing that data followed a normal distribution (**figure 9B**, p=0.1227). The median sleep duration was 37 minutes, and the mean was 34 minutes. **Figure 9C** shows the distribution in implanted animals, ranging from 3 to 70 minutes of sleep duration. We separated the population in function of the median (i.e., median split) of the sleep duration, in this condition we defined a long sleep group (LS) composed of trials with more than 37 minutes of sleep and a short sleep group (SS) composed by trials with 37 or fewer minutes of sleep.

As we reported before, to maximize the number of trials performed by animals we used circular and square maze tests. On this line, and because a different experience and/or the repetition of a similar experience could induce different sleep patterns we evaluate differences between these two arenas and the influence of recurrence in OPR test on sleep duration.

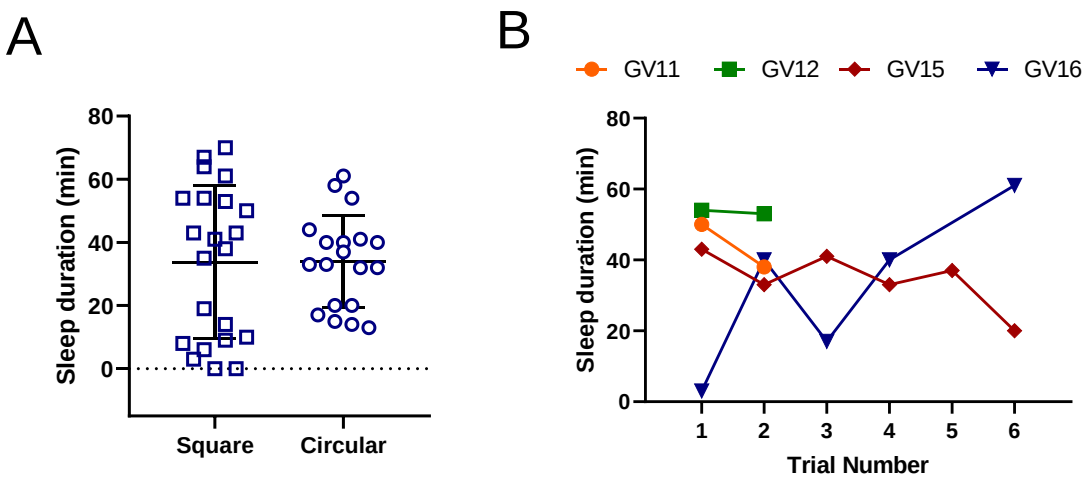


Figure 10. Influence of Maze shape on Sleep. **A)** Influence of maze shape on sleep duration in the rest phase of OPR. No significant difference was found between a square and circular maze. Unpaired T-test was performed, p=0.9791. **B)** Influence of trial number on sleep duration in the rest phase of OPR

Importantly, sleep duration was not different when the animal performed the OPR test in a square maze or a circular maze **(figure 10A),** also because of the variable change with no clear tendency sleep duration was not dependent on the number of trial performed **(figure 10B).**

After that, and to evaluate the influence of sleep on memory In OPR task as objective one claim, first, the preference index was calculated using the exploration time of each object in SS and LS groups according to the equation:

$$\textbf{Preference index} = \frac{\text{Displaced object} - \text{NonDisplaced object}}{\text{Displaced object} + \text{NonDisplaced object}}$$

If the exploration index is close to 1, the rat explored more the novel object (displaced); 0, the rat explored both objects equally and -1, the rat explored more the familiar object (not displaced). This index was computed every 1 minute to identify how the difference between groups varies during the test time (10 minutes).

To discard previous spontaneous object preference we evaluate the preference index during sample phase. Importantly the two objects were similarly explored **(figures 11B, C)** and the preference index was no different from zero **(figure 11A)**. This indicates that there is no prior preference for either of the two objects or positions in the arena, which is essential in the interpretation of the OPR results during the test phase.

On the other hand, now in test phase, **figure 12A** shows that there were differences in the preference index between the LS group and SS group from minute 3 onwards. These results were maintained for the non-implanted group **(figure 12B)** but not for the implanted group **(figure 12C).** However, for implanted animals, the SS group is not different from zero in all time points while the LS group is different in all time points (one-sample Wilcoxon-test. SS group p-value from 0.3125 to 0.9999, LS group p-value from 0.0010 to 0.0269, Table S5), so this suggests that groups were different, and raises the need for increase the number of trials to corroborate this hypothesis.

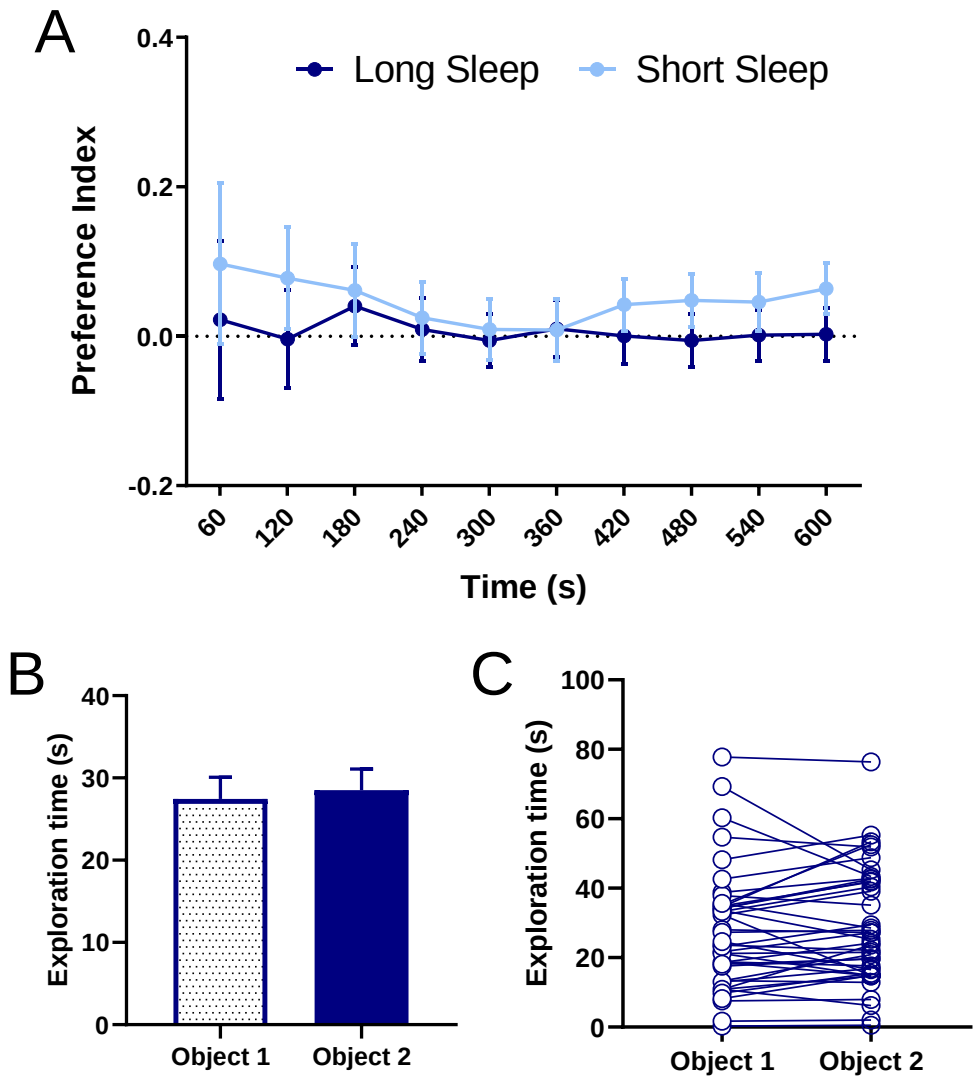


Figure 11. No object preference during sample in OPR test. **A)** Preference index during sample phase of OPR in LS and HS groups are not different between them (mixed-effect analysis test, table S5) and are not different from cero (One sample T-test, table S5) in all times. **B)** Exploration time of objects during sample is not different between object 1 and object 2 (paired T-test, p=0.4040). **C)** Same as in B but for each trial.

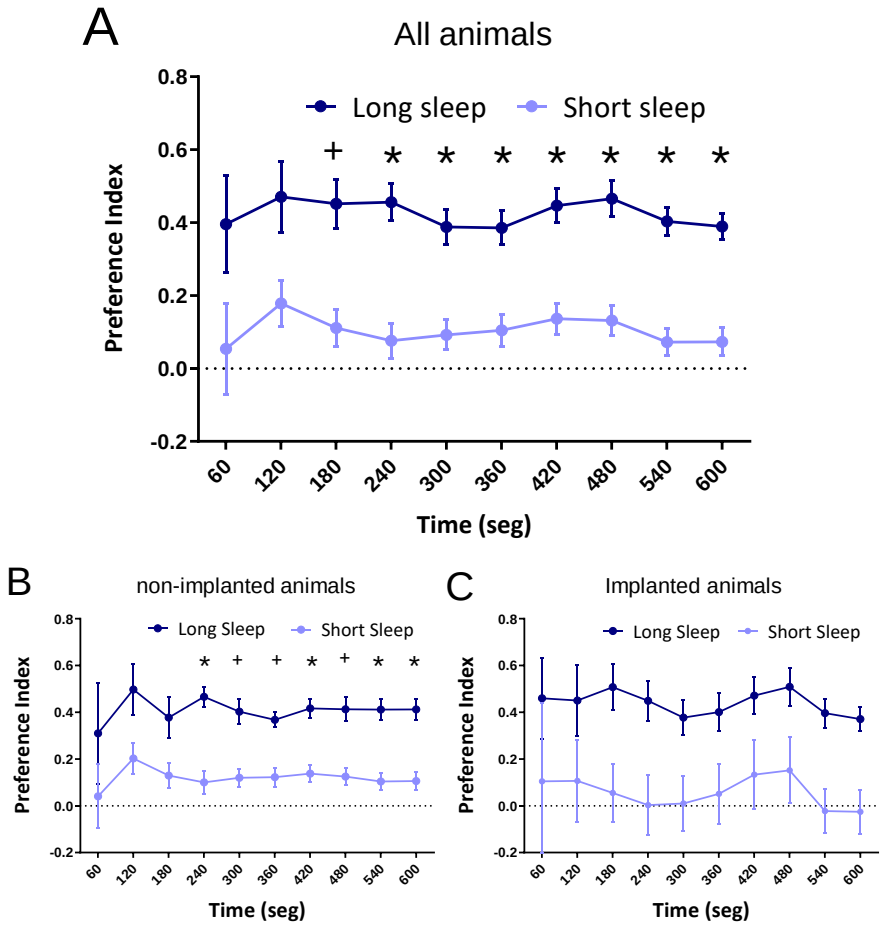


Figure 12. Sleep enhances the preference for the displaced object in the OPR test. Preference index during test time in spatial memory task OPR. **A)** Sleep correlates with a longer exploration of a displaced object during test phase. For each condition, the exploration time of the objects during the test was evaluated and the preference index was calculated. When the preference index is positive and close to 1 the animal explores more the novel object. For these experiments, 18 animals were used with a total of 21 trials in the long sleep (LS) group and 20 trials in the short sleep (SS) group. Significant differences were found between both groups from minute 3 and over. These results show that post-learning sleep enhances the performance of the animal's spatial memory. **B)** Same as in A, but just with non-implanted animals. The number of animals was 13, with a total of 15 trials in the SS group and 9 trials in the LS group. **C)** Same as in A but for implanted animals. The number of animals was 5 with a total of 5 trials in the SS group and 12 trials in the LS group. A mixed-effects analysis followed by Bonferroni's multiple comparison test was performed in each case (***** $p < 0.001$, **+** $p < 0.005$).

In addition, as for sleep, the preference index during the test was not different between the square maze and the circular maze **(figure 13A)**, also preference index was not dependent on the number of trials performed **(figure 13B)**. This indicates that preference index in not depend on the number of performed trials or maze shape, which is also essential in the interpretation of the OPR task.

Interestingly, the traveled distance was shorter during the test phase in comparison to the sample phase **(figure 14A),** but there was no difference between long sleep and short sleep groups **(figure 14B).** Also, there is no correlation between traveled distance in sample and test with memory performance **(figure 14C)** which indicates that traveled distance is not related to success or failure in memory performance in our conditions.

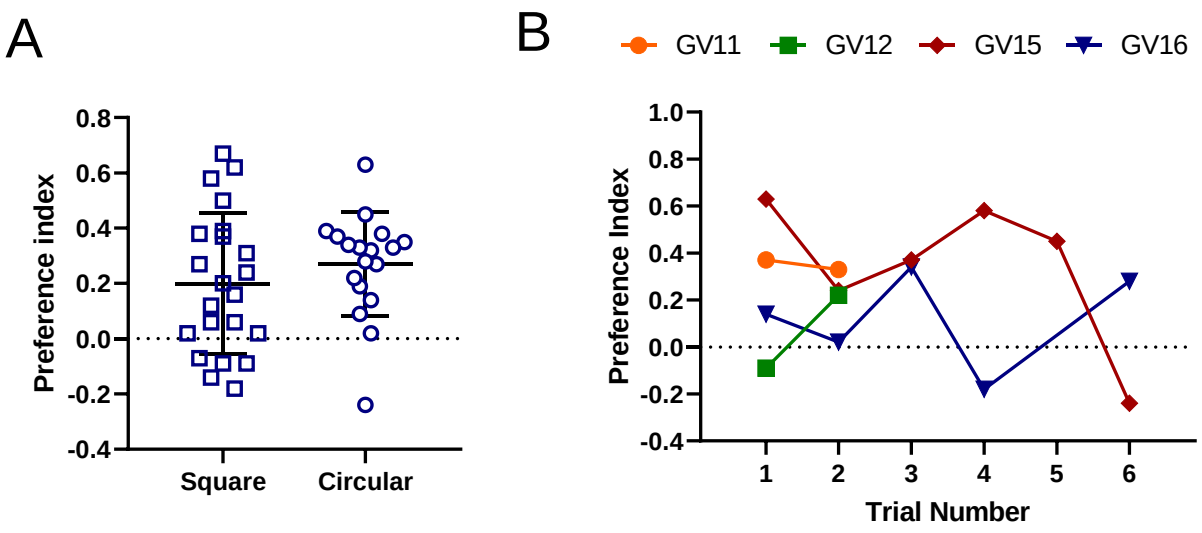


Figure 13. Influence of Maze shape on Behavior. **A)** Influence of maze shape on preference index in OPR test. No significant difference was found between square and circular mazes. Unpaired T-test was performed, p=0.3384.**B)** Influence of trial number on preference index on OPR test.

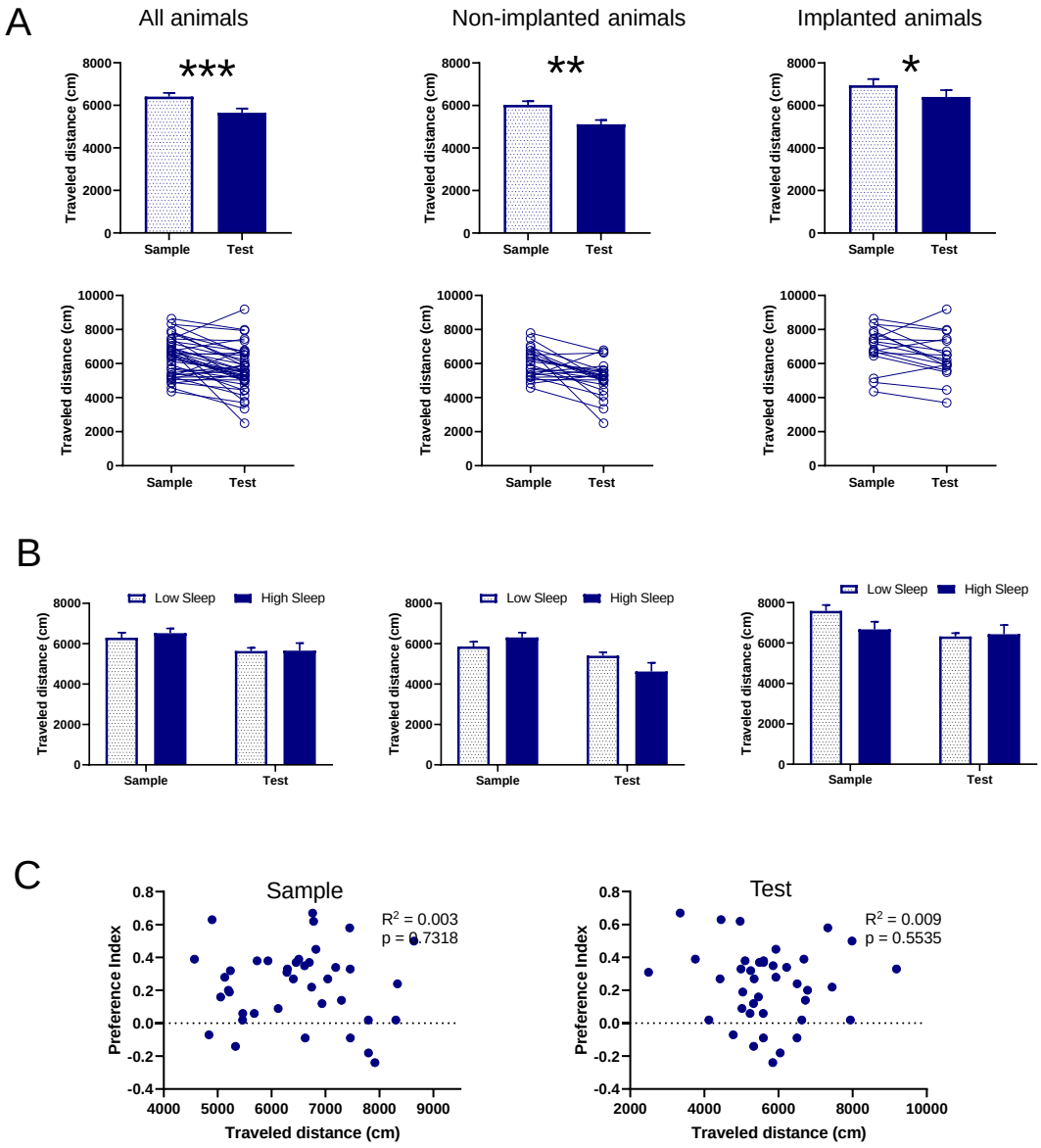


Figure 14. Traveled distance in the OPR task. A) Upper, distance traveled in sample and test phases of OPR test in all (left), behavior (middle), and implanted (right) animals. T-test was performed for each group (*** p <0.0001, ** p <0.001, *p<0.05). Lower, same as in upper but for each trial. **B)** Same as in A but separated by sleep groups. Two-way ANOVA was performed, and no difference was found between sleep groups (table S5). **C)** Correlation between preference index and distance traveled in sample (left) and test phases of OPR test (right). Each blue dot corresponds to one single trial. The correlation was performed using Pearson correlation coefficient test. p and R^2 values are shown in each plot.

These results indicate that sleep has a pivotal influence on the performance in OPR task performance, being consistent with previous studies (Inostroza et al., 2013) but also giving us a temporal dimension of the task that has not been previously evaluated. These experiments allowed us to establish that sleep has a robust effect on spatial episodic memory, an important requirement to be able to evaluate hippocampal activity through electrophysiological experiments in these conditions.
Interestingly, we found that in the analysis of all animals the SS group was not different from zero at most time points. This is probably because in our experiments sleep was never deprived, so it managed an improvement in spatial memory differently than what occurs when sleep duration is near to zero and memory consolidation is completely affected. This effect suggests the influence of sleep on memory not as an all-or-nothing phenomenon but rather as an effect relative to the amount of post-learning sleep. These conditions allowed us to evaluate the effect of sleep as a continuous, rather than a discrete parameter.

To assess the continuous nature of sleep in the rest phase, we correlated sleep duration in each trial with the preference index in the OPR test. **Figure 15A** shows a linear correlation between sleep duration and preference index at 10 minutes of the test phase. **Figures 15B and 15C** show that such correlation is present in both behaviors and implanted animals separately.

These results show that sleep has a linear relation with spatial memory performance, rather than a discrete, all-or-none effect. While more time the animal spends sleeping in the rest phase, the better the performance in the OPR task. Also, the robust correlation in implanted animals suggests looking in more detail at different features in electrophysiological data both in the LFP signal (oscillations like SWRs, slow waves, and spindles) and at single-unit level that may be related continuously with memory performance. These results haven´t been previously reported in similar studies that evaluate the influence of sleep on memory performance (Inostroza et al., 2013; Ishikawa et al., 2014) and raise the possibility of not only evaluating the effect of sleep continuously on memory but also on other cognitive and physiological variables, including the spatial coding of place cells.

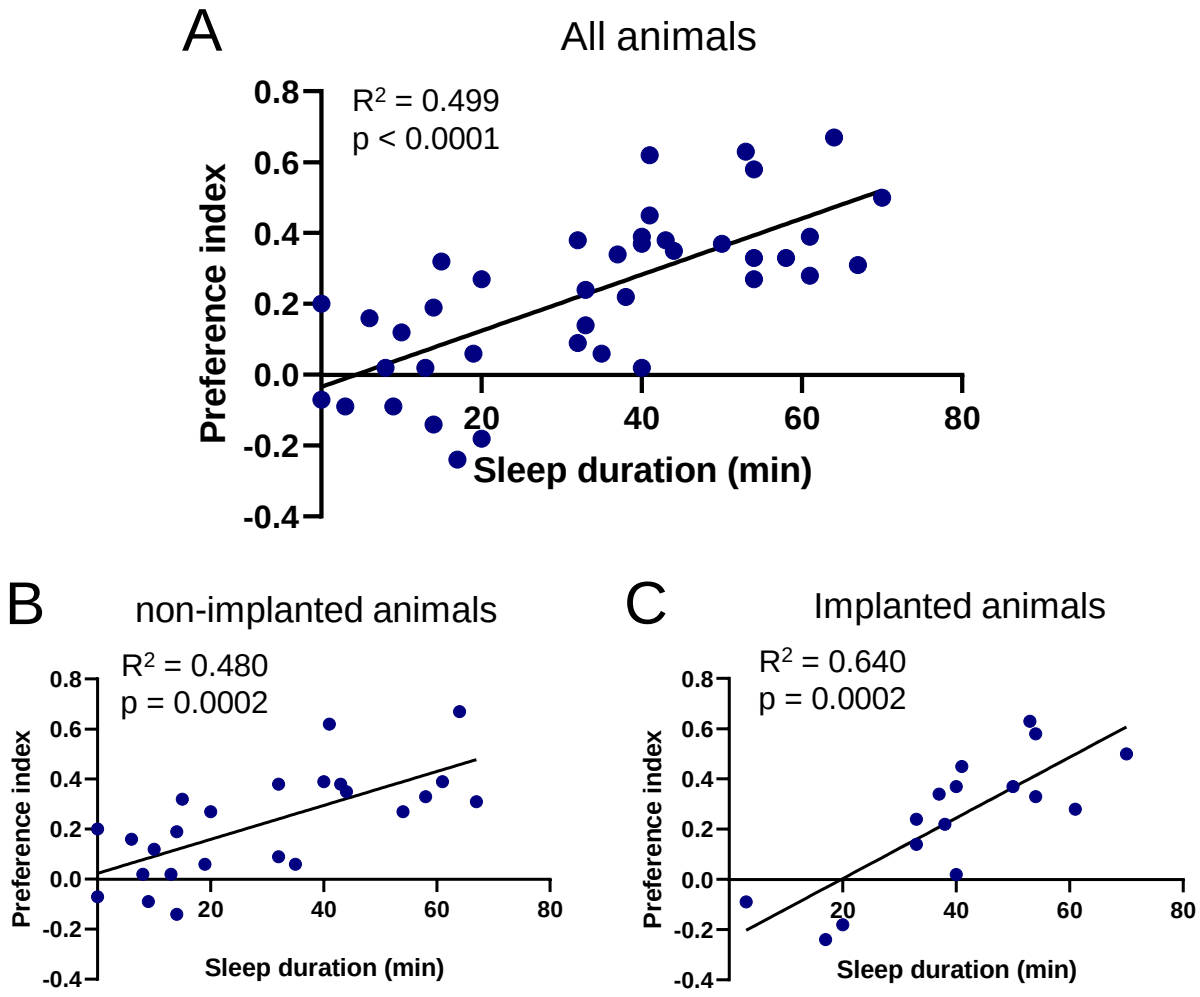


Figure 15. Correlation between sleep time and memory performance in the OPR task. A) Sleep time in the rest phase is directly correlated with the preference index in the task. Each blue dot represents a specific trial (41 trials and 18 animals); the preference index was calculated at the end of the test (10 minutes). **B and C** same as in A, but separately for animals that only perform behavior experiments **(B),** and implanted animals **(C)** respectively. The correlation was performed using Pearson correlation. p and R^2 values are shown in each plot.

5.1.2 Non-REM sleep duration and their oscillatory patterns correlate with a better spatial memory performance during OPR task.

Since we know that sleep is continuously correlated with spatial memory performance, it becomes interesting to see how the different stages of sleep, in particular REM and non-REM sleep, could be correlated with memory. For this, a correlation analysis was performed between the amount of non-REM sleep **(figure 16A)** and REM sleep **(figure 16B)** with OPR performance in implanted animals. These results show that only non-REM sleep was significantly correlated with performance in spatial memory, whereas REM sleep wasn't. Although it has been established that non-REM sleep plays a pivotal role in memory consolidation processes (Diekelmann and Born, 2010; Ackermann and Rasch, 2014), it had not been observed that there was a continuous positive correlation effect on task performance.

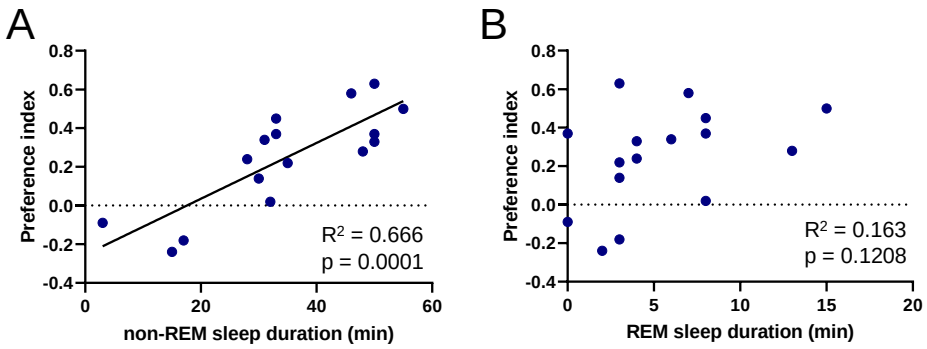


Figure 16. non-REM, but not REM sleep duration was correlated with memory performance in the OPR task. A) non-REM sleep duration in the rest phase was directly correlated with the preference index in the task. Each blue dot represents a specific trial (16 trials, 5 animals); the preference index was calculated at the end of the test (10 minutes). **B)** REM sleep duration was not correlated with the preference index. Animals and trials are the same as in A. Pearson correlation was performed. p and R^2 values are shown in each plot. Linear regression was plotted in graphs only when the correlation is significant.

These results confirm that sleep improves spatial memory performance early on the OPR task. Furthermore, in a novel way we find that sleep is continuously correlated with memory performance in this task and this correlation depends largely on the non-REM sleep.

Because we found a direct relationship between sleep, specifically non-REM sleep, and spatial memory, it was interesting to assess whether oscillatory activity during this phase of sleep was correlated with task performance. Based on the above, different oscillations that occur in sleep (SWRs, spindles, and delta oscillations, during non-REM sleep and theta oscillations, during REM sleep) were detected in our recordings **(figure 17)** and correlated with the preference index **(figure 18).**

Our results show that SWRs that occurred during sleep were positively correlated with task performance **(figure 18),** as were spindles **(figure 18B),** and the duration of delta oscillations **(figure 18D).** In turn, this correlation was not significant for SWRs during wakefulness **(figure 18C)** or the time of theta oscillation **(figure 18E).** These results demonstrate that oscillatory rhythms specific to non-REM sleep correlate with memory performance, whereas oscillatory patterns of REM sleep do not seem to contribute. This goes in line with the reported influence of non-REM sleep and complements that not only sleep but also their oscillatory events contribute proportionally to improving spatial memory.

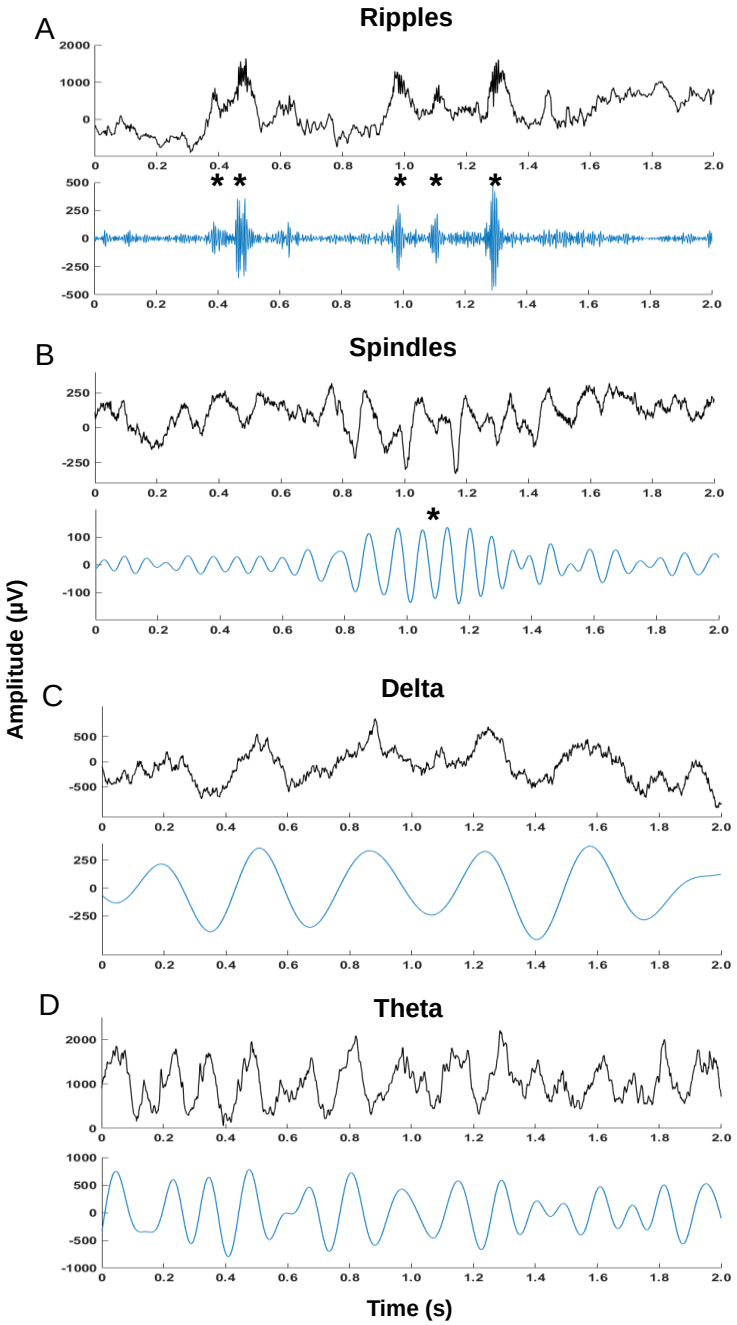


Figure 17. Brain oscillations are detected during sleep in the rest phase. **A)** Raw hippocampal LFP (upper) and filtered signal between 100 and 250 Hz to detect SWRs (lower). **B, C, and D** show the same as in A but with the filtered signal between 9 and 17 Hz for spindles, between 1 and 4 Hz for delta, and between 4 and 11 Hz for theta oscillations respectively. SWRs and spindles are marked with an asterisk.

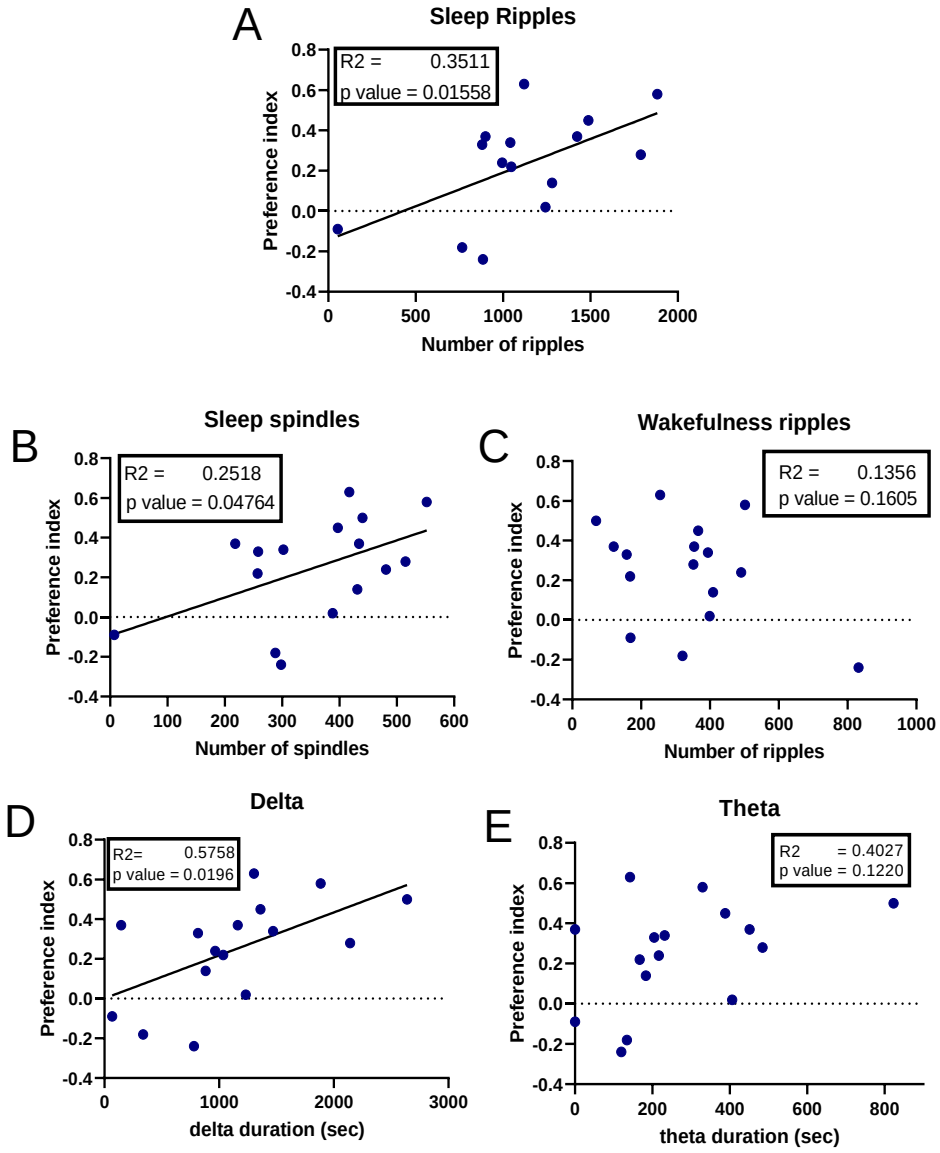


Figure 18. Oscillations of non-REM sleep are correlated with spatial memory performance. **A)** Number of SWRs during sleep in the rest phase is directly correlated with the preference index in the task. **B and D** show the same as in **A** but for spindles and time of delta oscillations respectively. **C)** Number of SWRs during awake in the rest phase is not correlated with the preference index in the task. **E)** Same as in C but for the time of theta oscillations. Each blue dot represents a specific trial (16 trials, 5 animals); the preference index was calculated at the end of the test (10 minutes). Spearman correlation was performed. Linear regression was plotted in graphs only when the correlation is significant.

5.1.3 Identification of Hippocampal cells in single unit recordings during OPR task.

Once we established the effect of sleep on a hippocampal-dependent cognitive ability such as spatial memory, and also established, through LFP recordings, how the oscillatory patterns in the hippocampus contribute to this effect, we evaluated the activity of hippocampal neurons associated with spatial coding during the spatial memory task and the effect of sleep on them.

For this, the first step was to record neuronal activity in the CA1 region of the hippocampus **(figures 19 and S2)** during OPR with stereotaxically implanted tetrodes. For the spike detection, we considered the last 4 recorded animals only, and units were selected through spike sorting using the klustakwik program (for the first animal we don't get well-isolated units). In 4 animals and 15 trials, 612 different units were detected (Table S4, GV11-OPR3 was not considered because the time of test phase in OPR did not reach 10 minutes).

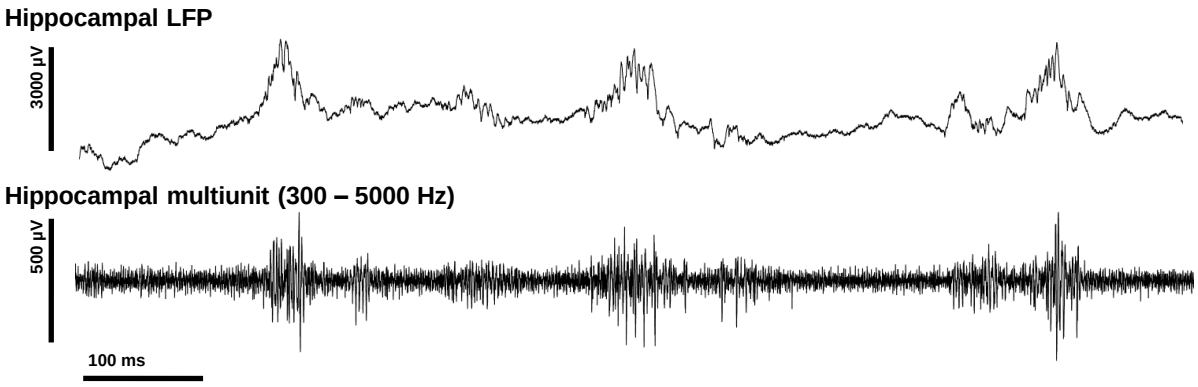


Figure 19. CA1 hippocampal LFP and multi-unit activity. Example recording of CA1 hippocampal LFP (upper) and multiunit spiking activity (lower).

To determine if hippocampal cells have activity in a specific place in the arena, heat maps of the firing rate in the maze for each neuron were evaluated for both the sample and the test phase. **Figure 20** shows the heat map generation process for the detected cells. Starting from a single unit **(figure 20A)** we obtain a raw map that represents the animal's trajectory and the position of each spike on the arena **(figure 20B)**. With that information, we constructed a heat map of firing **(figure 20C)** and occupancy **(figure 20D)**, and firing rate **(figure 20E)** in a binned map. After that, with the firing rate map, we generate a smoothed map **(figure 20F)** that was used to evaluate the physiological parameters of the neuronal response and the presence of place fields (see Methods). Finally, smoothed firing rate maps were used to evaluate whether these hippocampal units correspond to place cells or non-spatial cells.

Our results show that we were able to obtain cells that are active in a specific area of the environment, these cells called place cells and discovered by O'Keefe (1971) 50 years ago manage and establish the cognitive and spatial map in mammals (Tolman, 1949; Nadel and O'Keefe, 1978) and they allowed us, as we will show below, to be able to establish what is the role that the sleep has on the changes in spatial coding due to changes in the environment and turn if these changes have a correlate in the performance of spatial memory, thus suggesting a triangular relationship between sleep, spatial encoding, and memory.

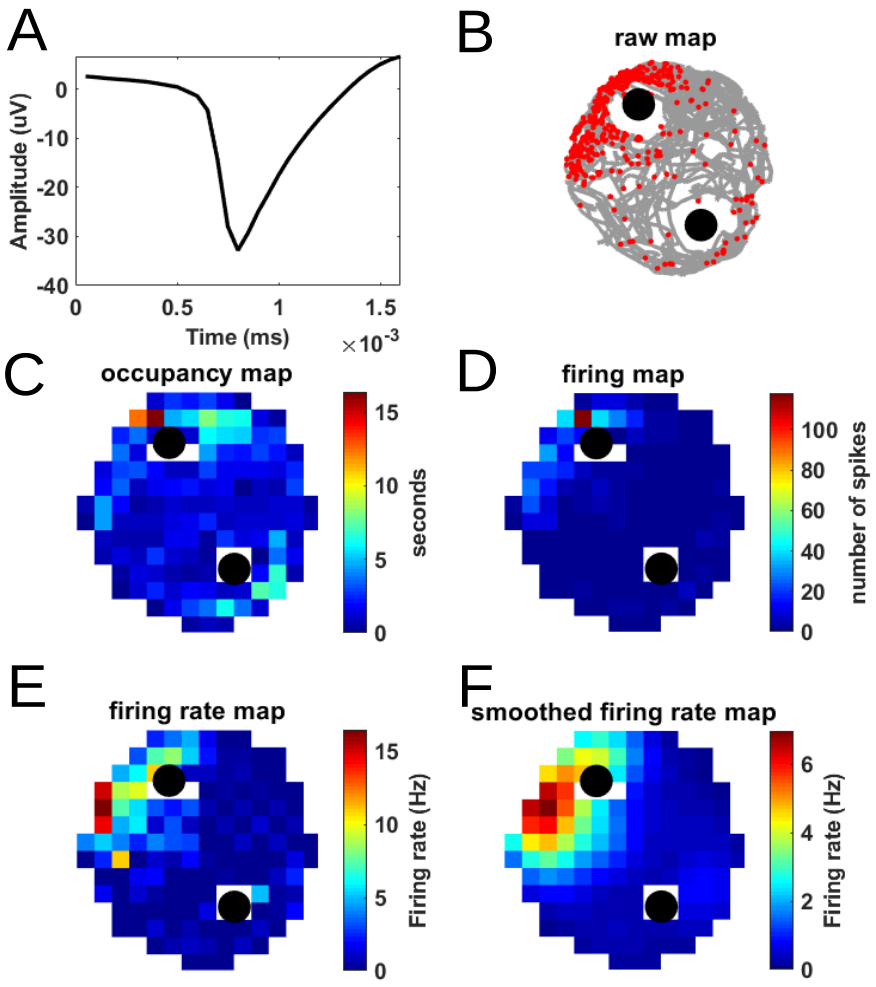


Figure 20. Heat map of firing rate in hippocampal CA1 cells. **A)** Unit detected by spike sorting. **B)** Raw plot represents the trajectory of the animal (grey lines) and the position at which each spike was discharged by the single unit presented in A (red blots). **C)** Occupancy map represents the time in seconds that the animal spent in the maze divided into 5x5 cm bins. **D)** Firing map shows the number of spikes in each bin of the maze. **E)** Firing rate map shows the firing rates in spikes per second (dividing the number of spikes by the occupancy in each bin). **F)** Smooth firing rate map represents the same firing rate map but with a smoothing process (gaussian (SD=7)). Bins that have less than 0.1 seconds of occupancy are shown in white. Black circles in each figure represent the position of the objects.

5.2. SPECIFIC OBJECTIVE 2: EVALUATE THE EFFECT OF SLEEP ON CHANGES IN PLACE CELL ACTIVITY PATTERNS THAT OCCUR IN THE SPATIAL MEMORY TASK AND CORRELATE IT WITH BEHAVIORAL PERFORMANCE.

5.2.1 Place cell classification on hippocampal units.

As we presented previously, we seek to establish the influence of sleep on spatial coding in place cells. For this, it is essential to be able to establish which of our hippocampal cells correspond to place cells and how the spatial representation of the changes according to variations in the environment during the OPR task.

Place cells are pyramidal cells that preferentially fire in a specific area of the explored environment (O'Keefe 1976). Based on this, and to characterize our hippocampal cells to find which one of them was a place cell, we evaluated the relation between the firing rate and sparsity, which corresponds to the proportion of the maze where the neuron was active (Skaggs et. al, 1996; **figure 21**). The measurement of sparsity allowed us to have a variable of spatial representation to be able to analyze our hippocampal cells. Other measurements such as information per spike (IPS) were not used because the presence of objects in the arena did not allow obtaining reliable data for this parameter. **Figure 21A** shows how the relation between firing rate (FR) and sparsity follows a sigmoid fitting, given by the equation "Sparsity = 1 / (1 + exp (-0.72 * Log Firing rate))**".**This equation was determined by the Matlab fitting tool cftool and its parameters were adjusted to obtain the smallest error in the fit to our data. This function has inflection points between which the relationship becomes almost linear **(figure 21B).** Under the lower critical point, we find units with mean firing rates below 0.2 Hz, so they have low dispersion (sparsity) because of their low activity levels. On the other hand, on the upper critical point, we have units with very large sparsity (over 78% of the maze), so they do not represent any specific area of the environment but rather tend to fire in most of the arena. The linear zone is

characterized by having units with firing rates from 0.2 to 10 Hz and has sparsity ranges that may indicate a preference for a specific area in the space. It is important to note that this adjustment was made considering the sample phase because it is the initial phase and reference point for the changes that did occur after, in the test phase.

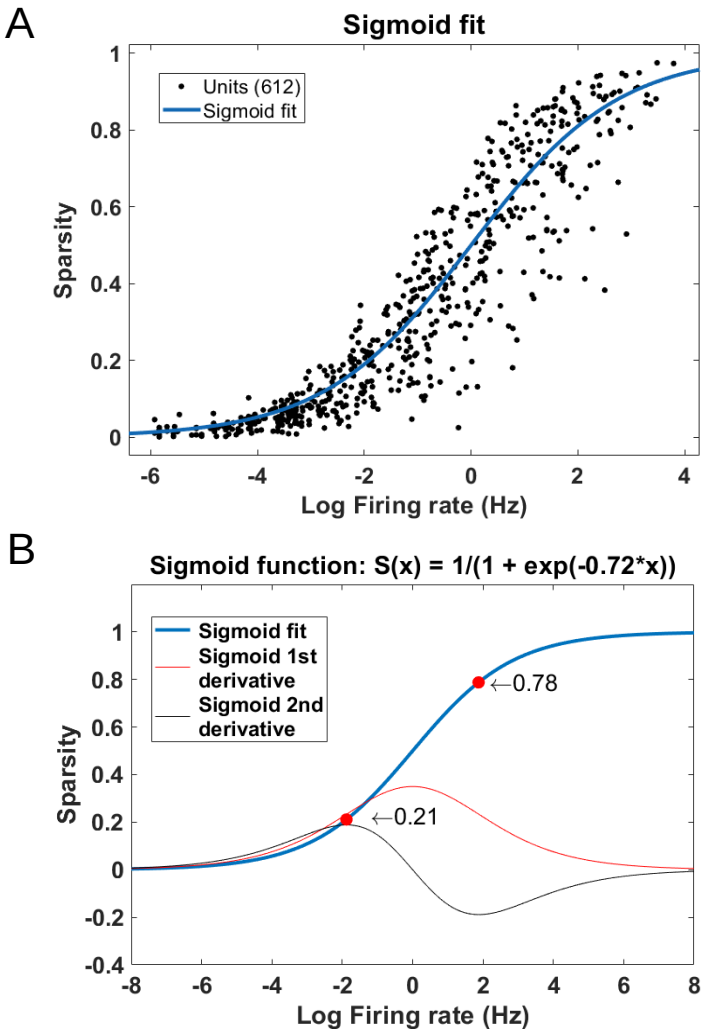


Figure 21. Sigmoid curve of the firing rate/sparsity relation. The relation between the log of firing rate and sparsity follows a sigmoid curve. **A)** Firing rate (log) and sparsity for each unit (black dots) during the sample phase in OPR task. This relationship follows a sigmoid curve (blue line) with the equation "Sparsity = 1/(1 + exp(-0.72*Log Firing rate))"**. B)** Critical

points of the sigmoid curve are presented in A (red blots). The values for each red blot correspond to sparsity values. R^2 for the sigmoid fit is 0.85

These results show us that we have a neuronal group that is active in a specific area of the environment. It is for this reason that we seek to characterize this response based on a central property in the place cells, which is the presence of place fields. The specific area in which a single unit fired when the animal was on it corresponds to the place field of that unit, which is characterized by having a firing pattern with a center that has a high firing rate, which is decreasing as the distance to this center becomes greater (O'Keefe 1976; Wilson and McNaughton 1993).

To detect place fields in our cells, an analysis was made considering a contoured area starting from the cell's peak firing rate until it reached 2 SD of the firing rates of the smoothed map **(figure 22).** Because contours can be generated with the smoothing of a few spikes, the minimum area that a place field must have is 6 bins or 150 cm2 (see methods). In **figure 22**, cell 3 presents a map that has a few random spikes in a specific place, but in bins with little occupancy, generating a significant firing rate in one single bin and a place field contour, however, if this contour doesn´t enclose a sufficient area of 6 bins was not considered as place field. This method allows us to detect place fields without considering random spikes on firing rate map.

Based on the above, the presence of place cells was established by assessing 3 variables: sparsity, firing rate, and the presence of place fields. First, those units that were in the linear zone of the sigmoid curve were selected; that is, units that had sparsity values lower than 0.78 and firing rates higher than 0.16 Hz, remaining 47% of all units for sample and 49% for test. This firing rate value was taken from the interpolation of minor critical point for sparsity with the x-axis of the sigmoid curve **(figure 22B).** In our group, the minimum value of firing rate was 0.18 Hz, which means that all neurons in this group discharged more than 100 spikes during the sample phase. At this point, a shuffling with 1,000 iterations was carried out for the occurrence in time of each spike of a single unit, keeping the animal trajectory unchanged. For each iteration, a spatial map was constructed and sparsity was calculated. With this we evaluated the Z-score value for sparsity, discarding those

units that did not exceed 1.5 standard deviations (see methods), remaining 35% of all units for sample and 39% for test. Finally, from this group, only units that had place fields were selected as place cells, remaining 32% of all units for sample and34 % for test **(Table S4).**

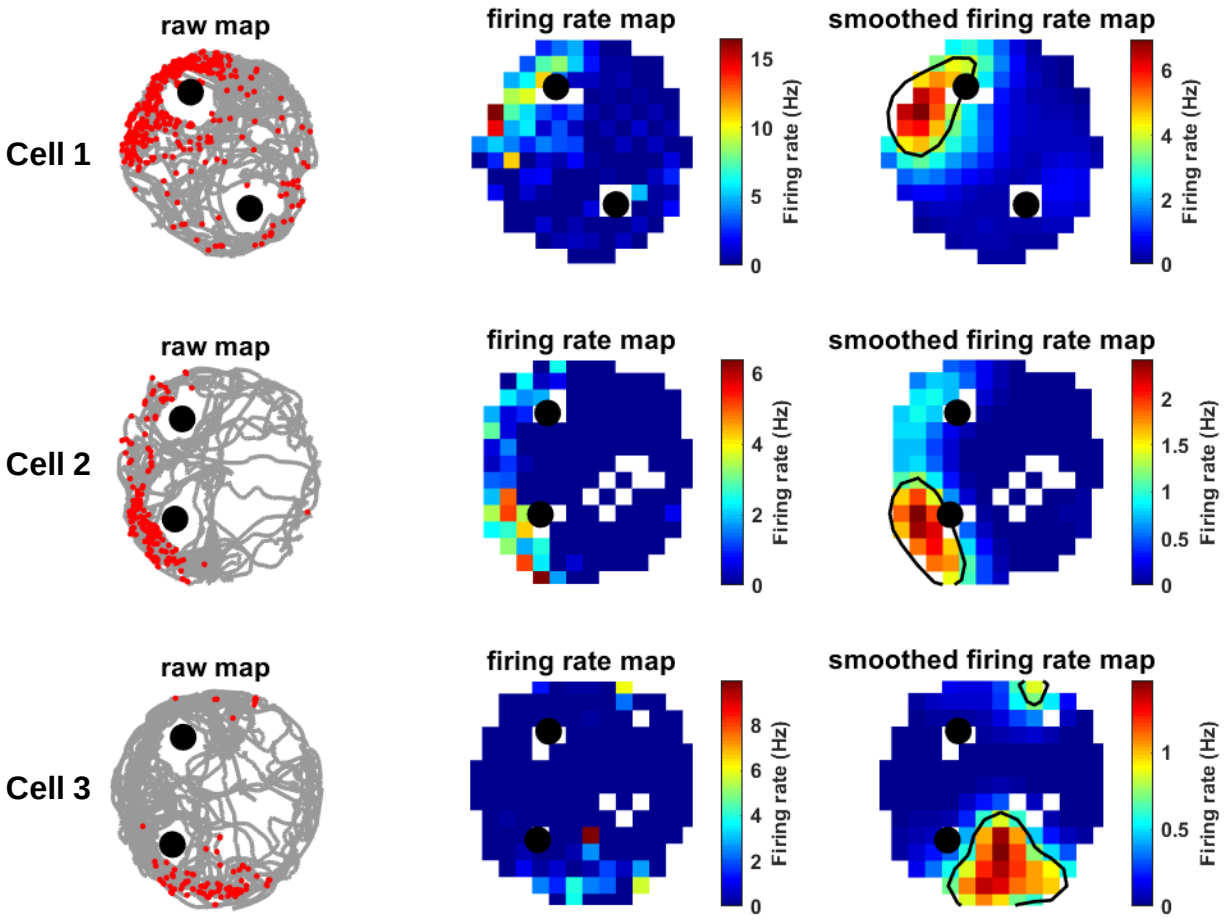


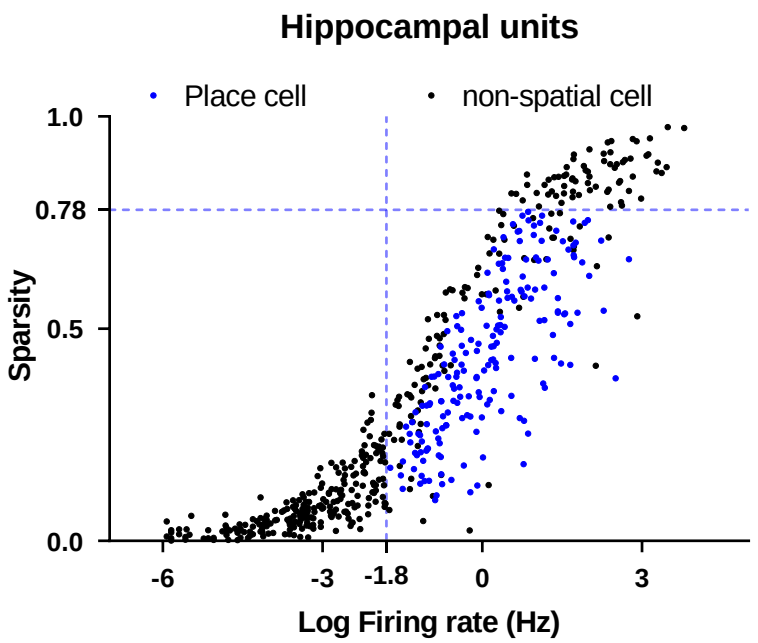
Figure 22. Identification of place fields in hippocampal cells. Left, raw plot represents the trajectory of the animal (grey lines) and the position of each spike in the single unit (red blots). **Middle,** firing rate maps. **Right,** smoothed firing rate maps with place fields. Black contour delimited the place field area. Only contours that enclose an area with more than 150 cm2 (6 bins) are considered as place fields. Cells 1, 2, and 3 have place fields (big contours), cell 3 have a small contour that is not considered a place field.

According to these criteria, **figure 23** shows the selection of units that we classified as place cells and their location in the plane defined by sparsity v/s firing rate (non-spatial cells, (**figure 23A).** This analysis was carried out in both the sample and test phases, so we have units that behave as place cells in one or both phases. This provides us with relevant information regarding the recruitment of each unit in the spatial representation during the OPR task, which could finally correlate with sleep and spatial memory performance.

On this line, of the total number of recorded neurons (n = 612) in the 4 animals and 15 trials **(figure 23B),** 4 groups of neurons were classified according to their place cell activity during the sample and test phases **(figures23C and 24).** Neurons that had place cell activity during both sample and test phases were classified as 'Keep place cells'. These were characterized by keeping a spatial map (presence of place fields) in both phases, although they might have changes in place field position or/and firing rate **(figure 24A).** Those cells that had place cell activity during the sample phase, but not during the test phase were classified as 'Down place cells', which were characterized by losing their place fields **(figure 24B).** Those cells that did not have place cell activity during sample phase, but did have it during the test phase were classified as "Up place cells" and were generally characterized by the appearance of place fields in the test phase **(figure 24C).** These three groups are considered "place cells".

On the other side, those cells that exhibited place cell activity neither during the sample nor during the test phase were classified as 'non-spatial cells'. In this last case, we could find cells that maintained very low firing rates both during sample and test, or those with high firing rates that discharged throughout the maze (**figure 24D)**. In total, we detected 255 place cells that corresponded to 41% of the total recorded population **(figure 23C)**.In our conditions, place cells had a mean firing rate between 0.2 and 15.7 Hz and a peak firing rate between 0.4 and 25.1 Hz. It is important to note that Down and Up place cells were characterized by remapping, since they change their firing rate, allowing the emergence of a place field in the case of Up place cells and the extinction of a place field in the case of Down place cells.

A



B

Animals	**4**
Trials	**15**
Units	**612**

C

Cell class	Number	%
Keep place cell	**134**	**22**
Down place cell	**57**	**9**
Up place cell	**64**	**10**
non-spatial cell	**357**	**59**
Total	**612**	**100**

Figure 23. Classification of place cells. **A)** Firing rate v/s sparsity for each unit during sample phase. Blue dots represent the cells that have a place cell activity and black dots represent units that don't have a place cell activity during sample. Blue dotted lines represent the cut points for sparsity and firing rate. **B)** Number of animals, trials, and units that we use in the analysis. **C)** The different cell classes on units that we report and their percentage over the total of units. Place cells are composed of Keep place cells that are units that have place cell activity during sample and test, Down place cells that have place cell activity just during the sample, and Up place cells that have place cell activity just during the test. Non-spatial cells don't have place cell activity either in sample or in test

Also, for Keep place cells, we identified global remapping (change of position and firing rate of place fields) or rate remapping (change just for place field firing rate; Muller and Kubie, 1987; Colgin et al., 2008; **figure 24A upper**), as well as cells that don't change their firing properties **(figure 24A, lower).** Defining our group of place cells allowed us to advance in the study of changes in the spatial maps during our spatial memory task to assess how sleep affects the activity of this particular group of cells. In line with this, some studies have been shown that oscillatory events that occur during sleep, specifically non-REM sleep, are temporally correlated with the reactivation of place cells (Buzsaki et al. 1992; Davidson et. al 2009).

These results show that there are indeed changes during the OPR task between sample and test phases, with a large number of cells that have remapping, among which those that stop representing the environment (Down place cells) or those where a spatial representation emerge (Up place cells). Interestingly those types of activity have not been extensively evaluated in other investigations.

At this point, it is interesting to be able to study whether these dynamic and differentiated changes for different groups of cells occur as a consequence correlated to different sleep patterns (in duration or stage) and finally if these differences can be correlated with the performance in spatial memory in our experiments.

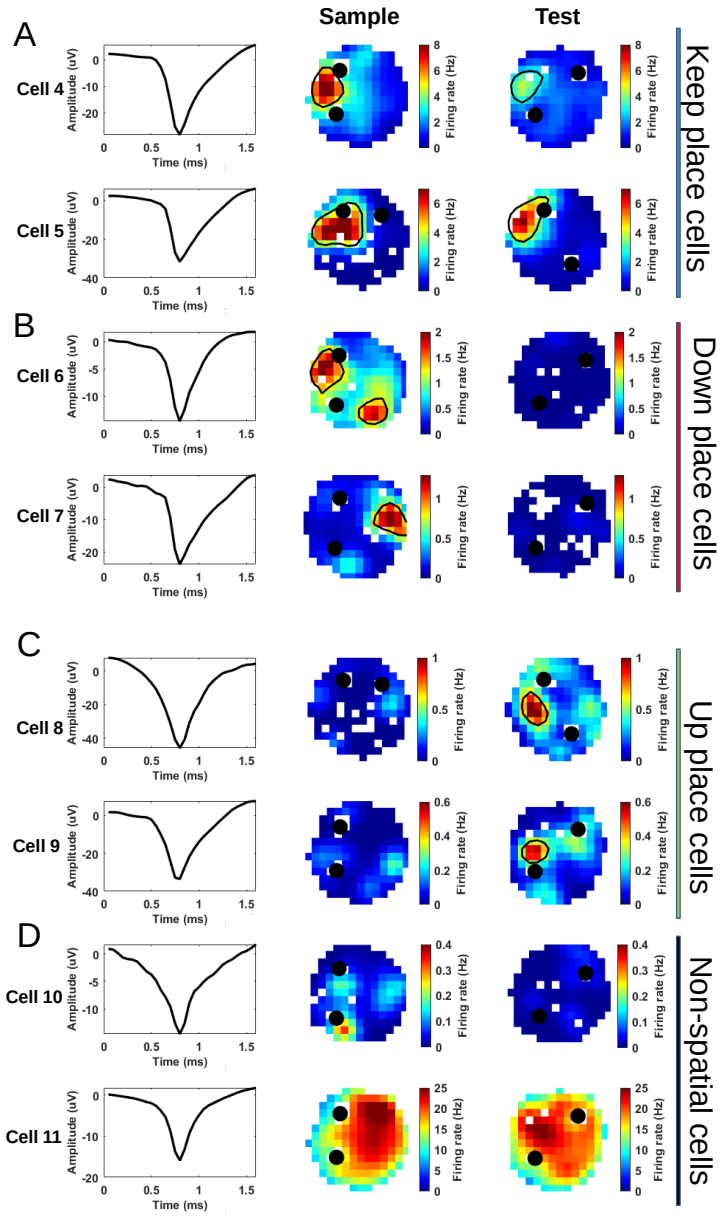


Figure 24. Spatial maps for the different 4 hippocampal cell groups. Left, Representative spike of each unit. Middle, smooth firing rate map in sample for each unit. Right, smoothed firing map in test. Place fields are contoured by black lines. **A)** Two representative examples of a keep place cell where place fields are present in sample and test**. B)** Two representative examples of a Down place cell, where the place fields disappear. **C)** Two representative examples of an Up place cell, where the place fields appear. **D)** Two representative examples of non-spatial cells where there are no place fields and firing rate is really low (upper), or where the firing rate is high (lower).

5.2.2 Influence of sleep on the reorganization of spatial representation

To what has been presented previously, it is interesting to see if sleep plays a role in the changes in place cell activity during OPR. As other research (Pavlides and Wilson 1989; Wilson and McNaughton 1994; O'Neill, Senior, and Csicsvari 2006; Diba and Buzsaki 2007; Jadhav et al. 2012) has shown, one of the phenomena that characterize the relationship between sleep and hippocampal cells is the reactivation of place cells during non-REM sleep stages. In this line, it was proposed that the reactivation of place cells associated with SWRs is essential for the processing of spatial information acquired during wakefulness and spatial memory (Diba and Buzsaki 2007).

In line with this idea, and to see if there was a reactivation of place cells during sleep in our recordings, we evaluate the cross-correlation between SWRs, spindles, and delta waves with hippocampal cells. **Figure 25**show that SWRs are significantly more correlated with place cells than with non-spatial cells, also for place cells this correlation is larger for SWRs that occur during sleep than for SWRs that occur during wakefulness, while for non-spatial cells there is no difference **(figure 25A)**. In addition, the density of SWRs during sleep is highly larger than during wakefulness **(figure 26)**, suggesting the predominant effect of sleep SWRs on place cells. Also, spindles **(figure 25C)** and delta waves **(figure 25E)** are more correlated with place cells than with non-spatial cells. Interestingly, Keep place cells are more correlated with sleep SWRs **(figure 25B),** spindles **(figure 25D),** and delta waves **(figure 25F)** than Down place cells. This implies that the reactivation of place cells during the occurrence of sleep oscillations could be related to the maintenance of spatial activity. In summary, these results show that Place cells, and specifically, cells that maintain a spatial map, are more correlated with oscillatory activity during non-REM sleep than cells that lost their spatial maps or non-spatial cells. This suggests the importance of non-REM sleep oscillations to keep the spatial activity in hippocampal cells.

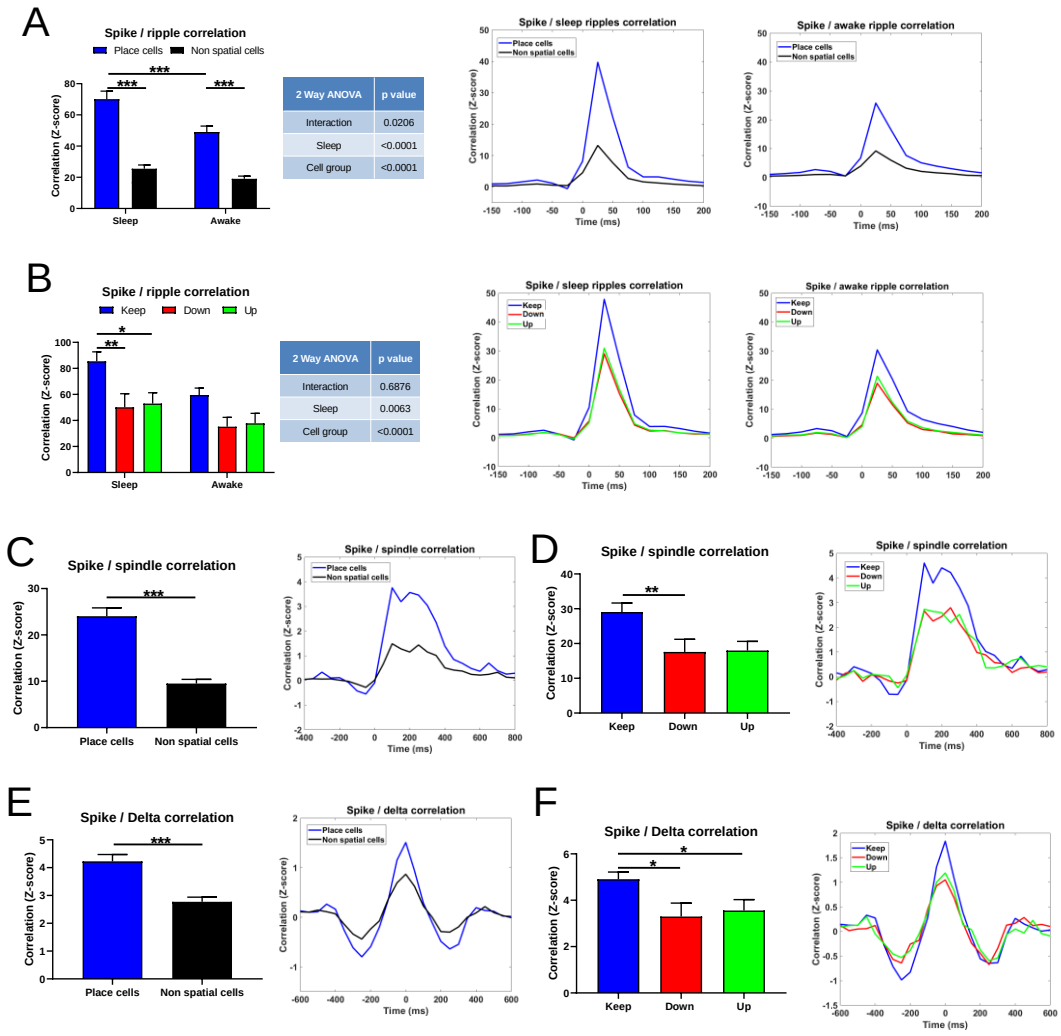


Figure 25. Correlation between ripple episodes and hippocampal neurons. **A)** Correlation (Z-score) between the onset of SWRs that occurred during sleep and wakefulness and spikes of place cells and non-spatial cells. **B)** same as in A but for three groups of place cells. **C)** Correlation (Z-score) between the onset of spindles and spikes of place cells and non-spatial cells. **D)** same as in C but for three groups of place cells. **E)** Correlation (Z-score) between the peak of delta oscillations and spikes of place cells and non-spatial cells**. F)** same as in E but for three groups of place cells. For A and B 2-way ANOVA followed by a Tukey test was performed. For C and E Mann-Whitney test was performed. For D and F Kruskal-Wallis followed by a Dunn's test was performed (*** p<0.0001,** p<0.001, *p<0.05, table S5). Bin used for correlations were 25 ms for SWRs and 50 ms for spindles and delta waves. The correlation was performed using the area under the curve in all cases.

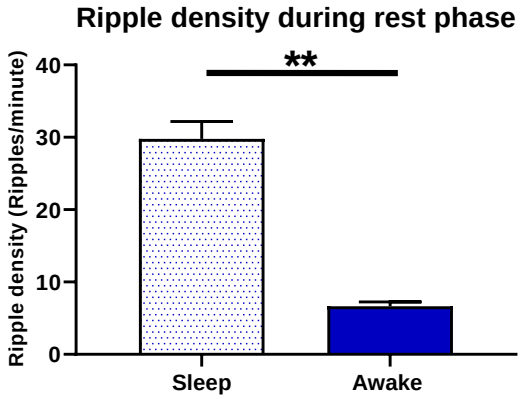


Figure 26. Ripple density is higher during sleep than during wakefulness. Ripple density during wakefulness and sleep are significantly different. Mann-Whitney was performed ($p<0.0001$).

To above, once we characterized the different cell groups, and because we saw that sleep oscillatory activity is more correlated with spatial cells that maintain spatial maps first we saw if sleep directly affects the variation in the proportion of place cells between sample and test in ORP task. **Figure 27A** shows that the time of sleep is not correlated with change in the percentage of place cells during test (proportion of cells that have a spatial map during test, that correspond to Keep place cells + Up place cells, respect to sample, that correspond to Keep place cells + Down place cells). Also, **figure 27B** shows that there is no correlation between sleep duration and the percentage of cells that keep the presence of a spatial map (Keep place cells).

Due to the observed changes both in the activity of the place cells as well as in their correlation with the oscillatory sleep patterns, but not in the proportion of spatial cells between both phases of the task, we saw if sleep affects any of the variables that define a place cell. As described previously, we define 3 variables to select place cells, which were, firing rate, sparsity, and presence of place fields. In line with this, we evaluate if these variables change over the task and if sleep is correlated with these changes.

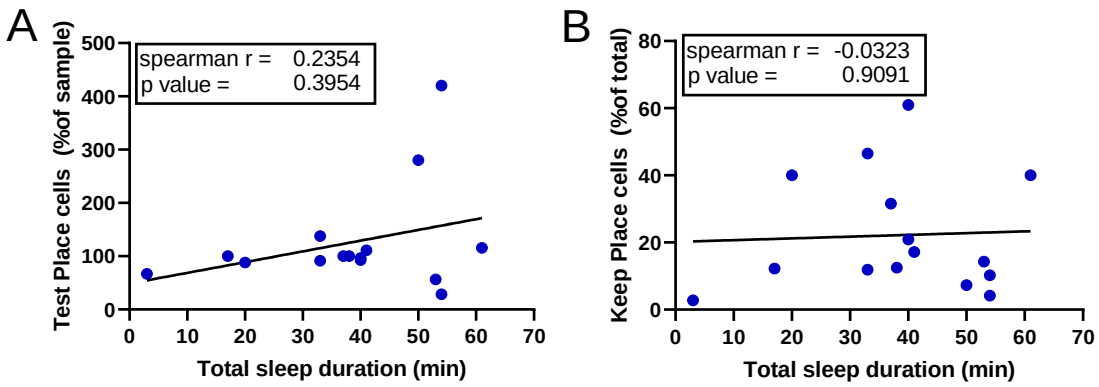


Figure 27. Change in the number of place cells doesn't correlate with the amount of sleep. **A)** Correlation between the percentages of total place cells during test (related to the number of place cells during sample). **B)** Correlation between the percentage of place cells that keep their classification during sample and test (related to the total number of units in each trial) and amount of sleep. Spearman test correlation was performed.

For that, we first established the general effect of the task phase on the firing rates of hippocampal units **(figure S3). Figure 28A** shows the firing rate in the three phases of OPR task in place cells and non-spatial cells. There is no variation between sample and test, also as was expected; the firing rate in sample and test is significantly lower in non-spatial cells than in place cells. Interestingly, firing rates during sleep in both groups is not different and, for place cells, is significantly lower than in sample and test. Also, **figure 28B** shows that this decrease in firing rate is present in the three groups of place cells, and that Keep place cells and Up place cells increase the firing rate in the test, while Down place cells keep a low firing rate shown during sleep. Interestingly, despite these differences in Down and Up place cells, there is no difference in sleep firing rate between them (**table S5**).

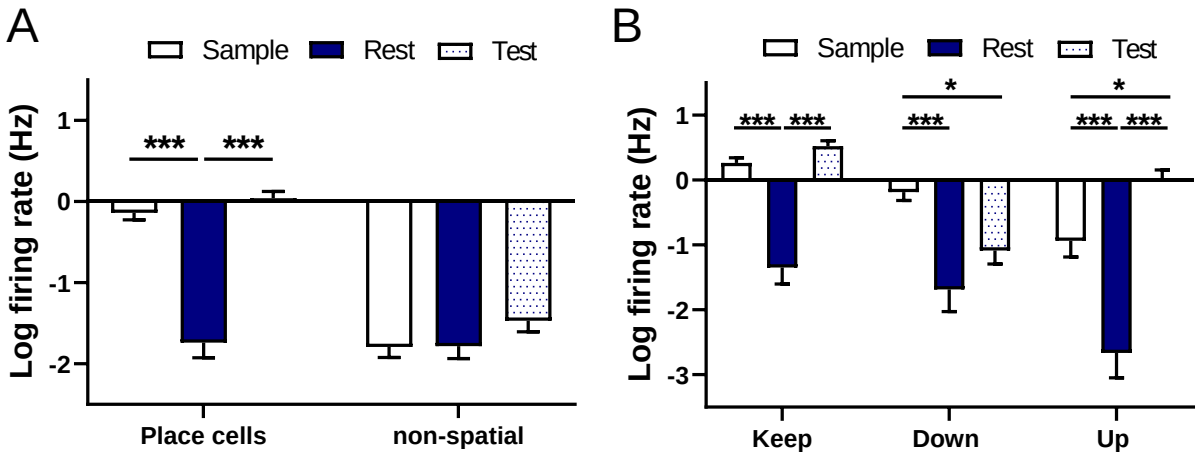


Figure 28. Firing rate during OPR task. **A)** Firing rate of place cells and non-spatial cells in sample, test, and sleep**. B)** Same as in A but for the three groups of place cells. Two-way ANOVA followed by a Tukey post-test was performed (***p<0.0001, *p<0.05, table S5).

To study further the relation between firing rate during sleep and OPR phases, we analyze the change in firing rate on these conditions for each cell. **Figure 21** shows the relation between firing rate during sleep and sample (left column), sleep and test (middle column), and sample and test (right column).

For place cells, about sleep firing rate, there are 2 different patterns, a group of cells that keep almost the same firing rate during sleep as in sample or test, and a group that decreases firing rate during sleep. Also, between sample and test, the whole group tends to maintain or increase slightly the firing rate during test **(figure 29A).** In specific, the three subgroups of place cells have a similar pattern to the whole group **(figure 29C, D, and E).** Unlike place cells, non-spatial cells have a more diverse pattern of change between phases and sleep, with units that fire more during sleep than in sample or test and vice-versa **(figure 29B).** On average place cells and non-spatial cells have the same sample-test firing rate relation (firing rate in test – firing rate in sample, **figure 29F**), but on the case of place cells, given by a different relation for each subgroup of cells, which is a slight increase in firing rate for Keep place cells, a decrease for Down place cells and an increase for Up place cells **(figure 29G)**.

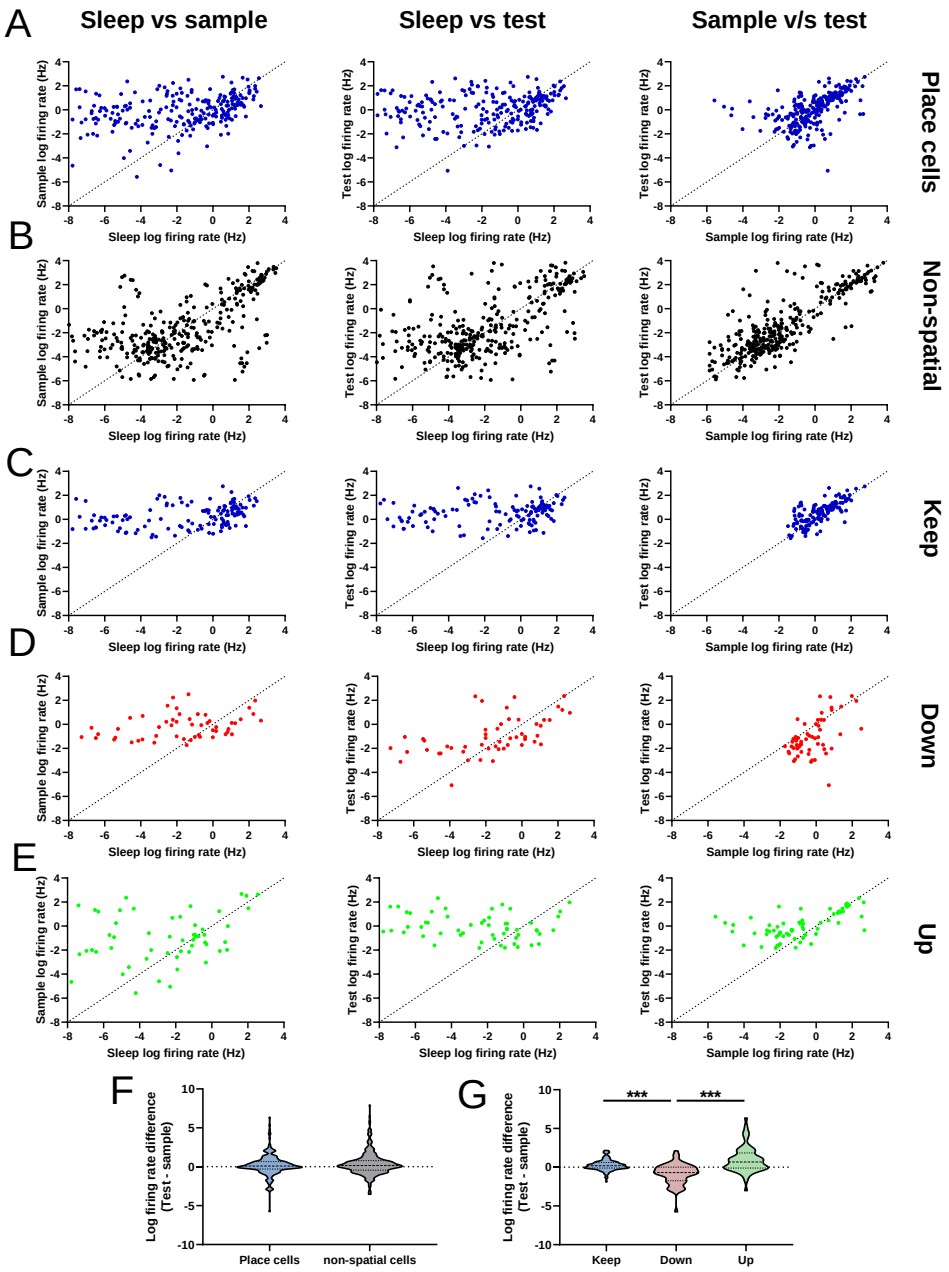


Figure 29. Place cell firing rate shift among OPR phases and sleep. Firing rate shift between sleep and sample (left), test (middle), and between sample and test (right) for **A)** place cells, **B)** non-spatial cell. **C)** Keep place cells, **D)** Down place cells, **E)** Up place cells. **F)**Log firing rate difference for place cells and non-spatial cells. **G)** Same as in C but for three groups of place cells. Mann-Whitney test was performed in F ($p = 0.5311$) and Kruskal-Wallis ($p < 0.0001$) followed by a Dunn's test was performed in G. (*** $p<0.0001$). Grid line represents identity.

These results show that throughout the task there are changes in the firing rate and that these changes are different between the groups, so it is interesting to evaluate if these variations are associated with sleep and specifically non-REM sleep.

To see this, first, we correlate the relationship between the amount of non-REM sleep and the firing rate of each unit during sleep in the rest phase **(figure 30, left).** For sleep duration, we use non-REM sleep, which represents 87% of total sleep (mean for all implanted animals, table S3), because non-REM sleep and their oscillatory patterns are highly correlated with spatial memory performance **(figures 16 and 18)** and with place cells **(figure 26).** These results show that the amount of non-REM sleep is correlated with the firing rate during sleep in non-spatial cells and place cells. Interestingly, for Keep place cells there is no correlation between these parameters, so the amount of sleep does not influence the firing rate during this period. Secondly, we correlate the amount of non-REM sleep with the variation of firing rate from sample to test **(figure 30, right),** these results show that, despite there being a different sample-test firing rate variation among cell groups, none of them correlate with these variations and the amount of sleep, so the effect of the change in firing rate is properly of the task, different for each group of cells and independent of sleep.

Another variable that characterizes a place cell and we evaluate in our data is sparsity. To study the effect of sleep on this variable first we evaluate variations in sparsity between sample and test. **Figure 31** shows these variations for place cells **(figure 31A, C)** and non-spatial cells **(figure 31B, C).** Similar to the firing rate, there are place cells that increase or decrease sparsity. In specific, Keep place cells maintain or slightly increase sparsity during test **(figure 31D),** Up place cells increase sparsity **(figure 31F),** mostly in cells that had a low sparsity during sample, and on average, there are no differences between them **(figure 31G).** On the other hand, Down place cells show a decrease in sparsity during test **(figure 31E, G).** Interestingly, among group cells, sparsity and firing rate has the same pattern of variation between sample and test, when firing rate increases or decreases, sparsity increases or decreases, respectively. These results suggest that there are variations

in spatial maps between sample and test putting the question if sleep affects these variations.

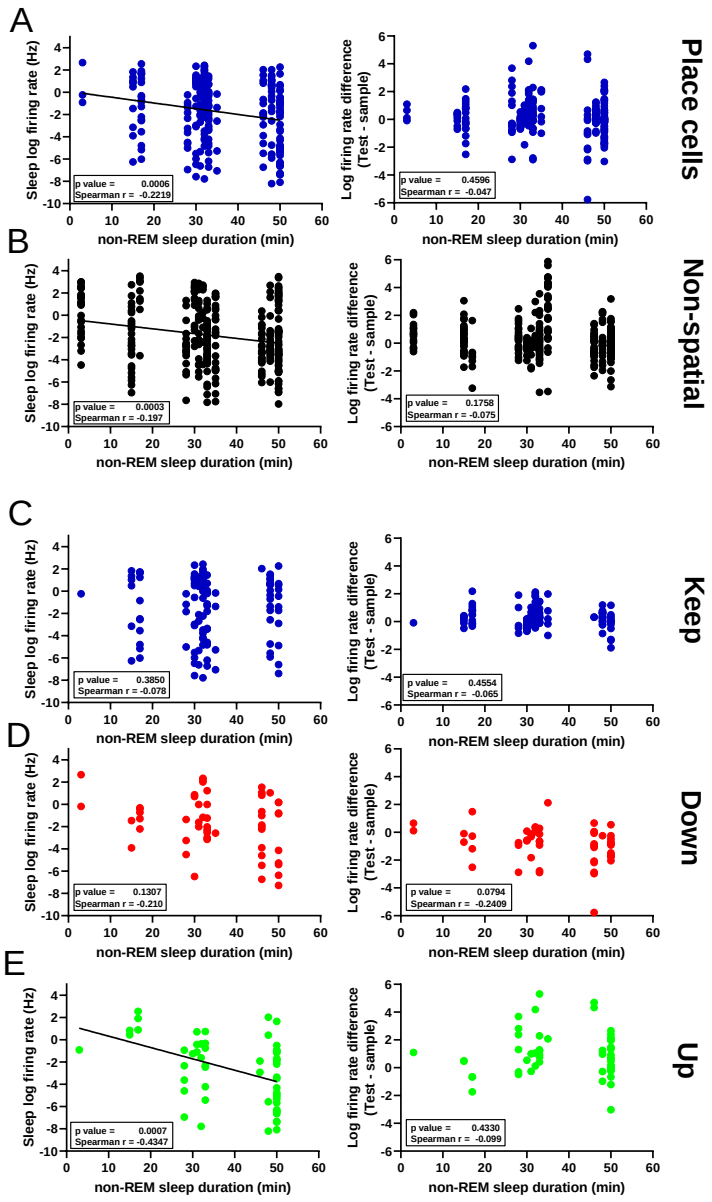


Figure 30. Correlation between non-REM sleep duration and firing rate during OPR task. Correlation between non-REM sleep time and firing rate during sleep in rest phase (left) and between non-REM sleep duration and firing rate difference in OPR phases (test – sample, right) for **A)** place cells, **B)** non-spatial cell, **C)** Keep place cells, **D)** Down place cells, **E)** Up place cells. Spearman correlation was performed. Linear regression was plotted in graphs only when the correlation is significant.

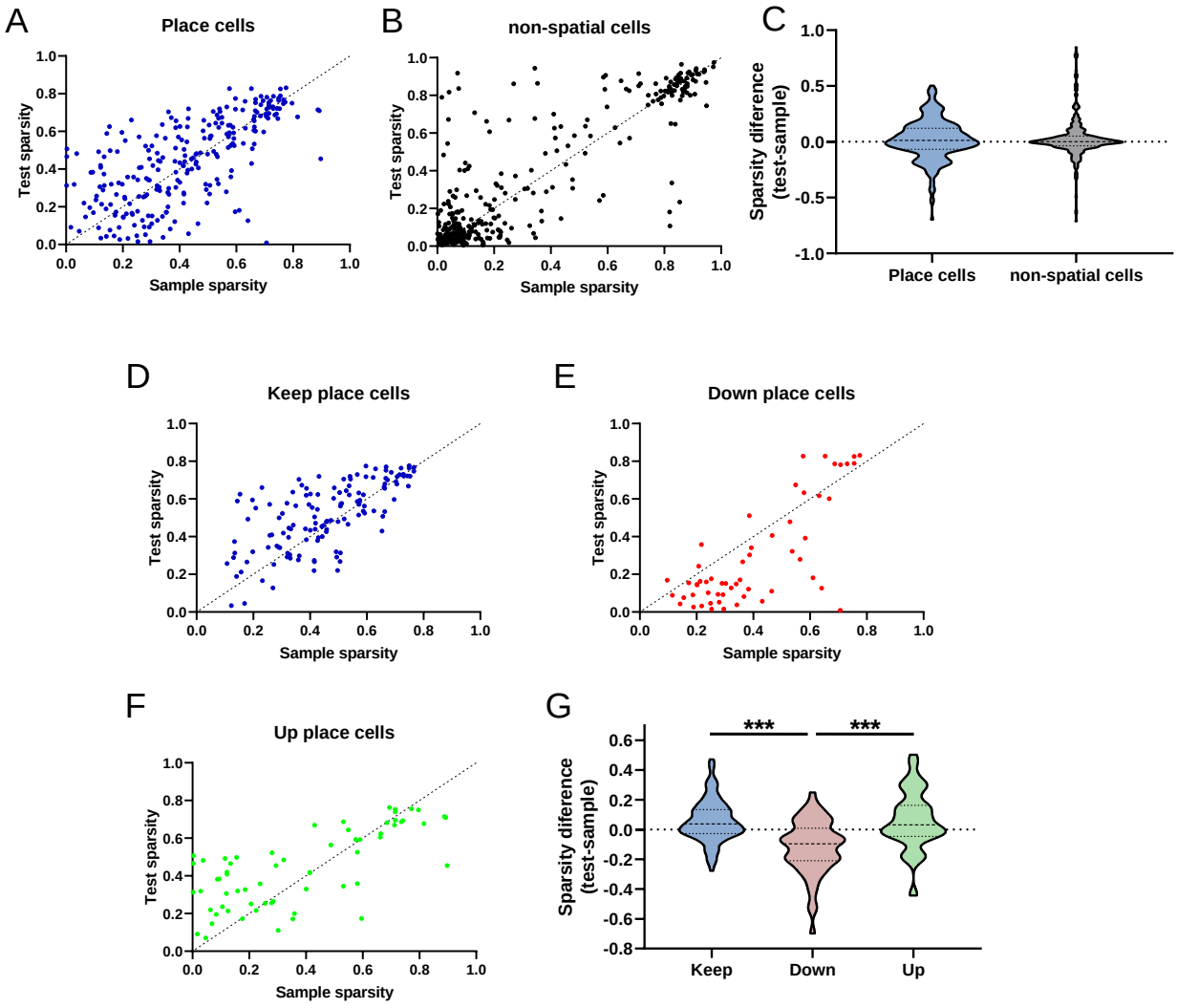


Figure 31. Place cell sparsity shift among OPR phases. Sparsity shift between sample and test for **A)** place cells, **B)** non-spatial cells, **D)** Keep place cells, **E)** Down place cells and F)Up place cells. C) Sparsity difference for place cells and non-spatial cells. **G)** Same as in C but for three groups of place cells. Mann-Whitney test was performed in C ($p = 0.666$) and Kruskal-Wallis ($p < 0.0001$) followed by a Dunn's test was performed in G. (*** $p<0.0001$). Grid line represents identity.

To above, we evaluate the effect of non-REM sleep over this sparsity changes among OPR tasks. For that, we correlate the amount of sleep (non-REM) with the variation of sparsity between the sample and test **(figure 32).** In the same way that for firing rate, non-REM sleep was not correlated with changes in sparsity for place cells and their different groups **(figure 32A, C, D, E)** nor the non-spatial cells **(figure 32B).**

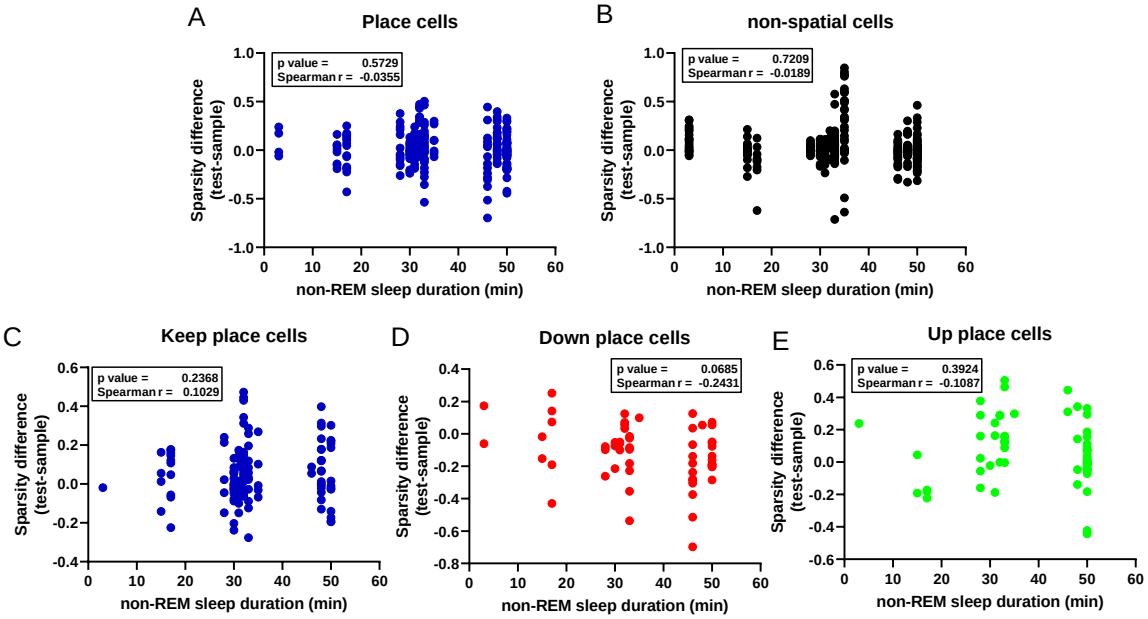


Figure 32. Correlation between non-REM sleep duration and sparsity in OPR task. Correlation between non-REM sleep duration and sparsity difference in OPR phases (test – sample) for **A)** place cells, **B)** non-spatial cells, **C)** Keep place cells, **D)** Down place cells, and **E)**Up place cells. Spearman correlation was performed.

This similarity with firing rate shows that these variations are related and OPR dependent. Also, these variables are general for the arena, so the changes in them represent more a main effect than a specific one. For those reasons it was interesting to see if more specific variables of spatial maps have sleep-dependent changes between sample and test.

To that, it was necessary to evaluate changes in the configuration of a spatial map over the OPR task. As we have commented previously, the configuration of spatial representation in place cells is given by the activity of that cell in a specific area of space. This specific area where the cell is active and adjacent to the peak firing rate (see methods) is what we here call place field. To this, we evaluated the number of place fields in Keep place cells during sample and test phases of OPR.

Figure 33A shows that, on average, the number of place fields is greater in test than in sample. However, this increase in the number of place fields is not correlated with total Sleep **(figure 33B)**, non-REM sleep **(figure 33C)**, or REM sleep **(figure 33D).** This result suggests that the increase in the number of place fields is a task-dependent change probably given by an increase in firing rate.

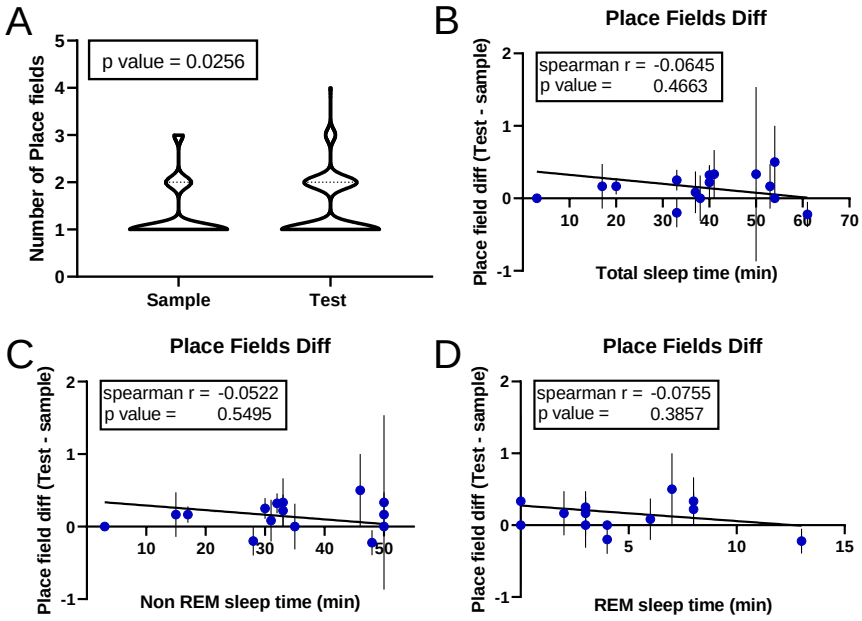


Figure 33. Number of place fields per cell changes throughout the task but is not sleep-dependent. **A)** Number of Place fields during sample and test for place cell group units (Mann-Whitney test was performed). **B)** Correlation between total sleep and change on place fields on place cell group. **C** and **D** are the same as in B but for non-REM sleep duration and REM sleep duration respectively. Spearman correlation was performed.

So far we have seen that although there are changes in the activity of the place cells, no changes have been seen in the general variables here analyzed that could be related to sleep. However, we know that changes in spatial representation may be associated with local changes, where the variables previously studied here are not necessarily affected. These local changes, such as variations in the location of the place fields or variations in certain specific areas of the arena (partial remapping), are not necessarily represented in the global variables (e.g., mean firing rate, sparsity), so it is necessary to be able to establish now whether the occurrence of these local changes may be related to the different sleep patterns in our experiments. For that, we first observe changes in the organization of spatial map along the task and we evaluate the correlation between sample and test spatial maps in each OPR trial. This was performed using a population vector analysis (Roux et al. 2017). For each spatial bin, a population vector that contains the firing rate values of all the place cells was created. After that, a spearman correlation between both sample and test population vectors was performed, obtaining a correlation coefficient (r) for each bin **(figure S4).**

This value represents the stability of the spatial map, being a measure of how much plastic the organization of spatial representations was. Altogether, **figure 34** presents the correlation maps, constructed with correlation coefficients of all bins in the spatial maps in each trial; also, the median population vector correlation was calculated **(figure 35).**

Here, spatial correlation was different depending on the specific zone of the maze. On this line, since in OPR there is a change in the position of objects, we identify a familiar zone, where there are no environmental changes between sample and test; and a novel zone, where the object that changes position is located. **Figure 35** shows that the median of the population vector correlation for each trial is not different between the familiar and the novel zone. However, the variability in the population vector correlation among trials suggests that other factors such as sleep could impact the stability of maps. On this line, we seek the role of sleep over this reorganization or map stability.

Figure 34. Population vector correlation maps. For all trials, the correlation maps represent the population vector correlation for all bins in the arena. This coefficient was obtained through Spearman correlation between the population vector in sample and test. Dark grey represents the position of objects during sample and light grey represents the position of objects during test. The grey line divides the novel zone of the arena, where the object in sample and test remained in the same place; and the novel zone, where the object during test was located in a different position than in sample.

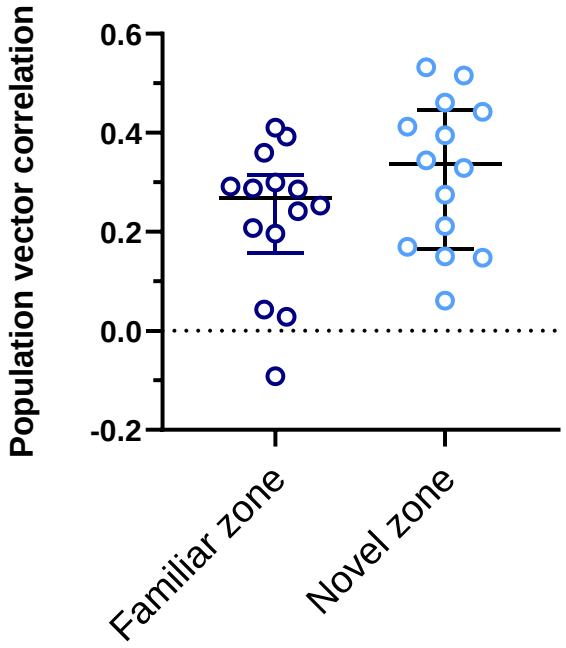


Figure 35. Median population vector correlation in OPR. The median correlation coefficient of each OPR trial for the familiar and novel zone of the maze. There was no significant difference between both maze areas. (Mann-Whitney test was performed, p-value = 0.163). Black bars represent the median and interquartile range.

For that, we correlate the time of non-REM sleep and their oscillatory patterns with the median population vector correlation **(figure 36).** These results show that considering the whole arena, non-REM sleep duration, delta duration, or SWRs do not influence the correlation between sample and test population vectors **(figure 36A, B, C,** respectively**).** Interestingly, spindles present an inverse correlation with map stability (correlation coefficient value, **figure 36D**). This result suggests that some sleep parameters could be related to spatial reorganization, and exhibit the importance of evaluating deeply this effect.

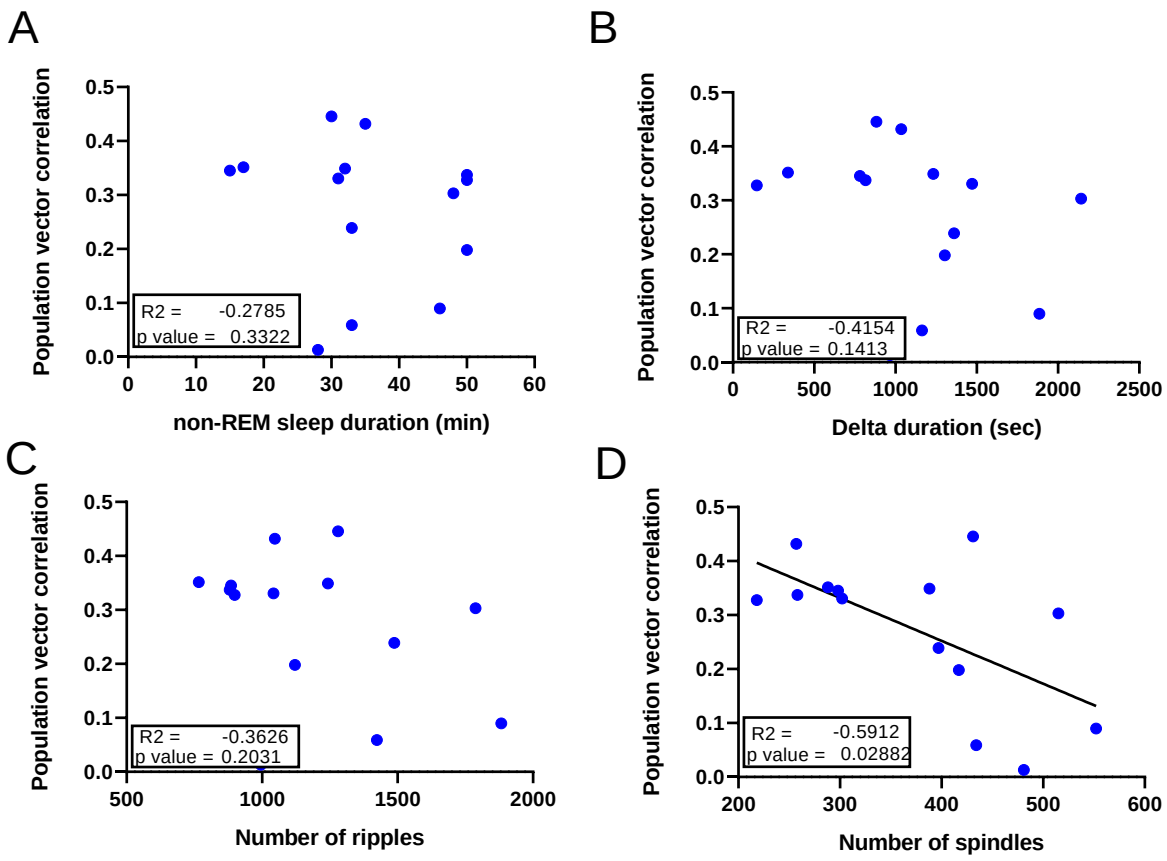


Figure 36. Non-REM sleep is not correlated with map stability in the OPR maze. A) Correlation between non-REM sleep duration and population vector correlation (median) between sample and test for each OPR trial. **B, C,** and **D** are same as in **A** but for delta duration, number of SWRs, and number of spindles respectively. In all cases, Spearman correlation was performed. Linear regression is plotted in graphs only when the correlation is significant.

Once we have observed the correlation map in a general way, it is interesting to be able to see if there are differences between the different areas. This is because on the map there is a familiar zone, that is, where there was no change in object positions, and a new zone, where there was a change in the position of objects (as mentioned above, in figure 26 both zones are delimited by a gray bar). In line with this, we evaluated the population vector correlation on the familiar and novel zone of

the OPR maze. **Figure 37** shows that non-REM sleep oscillations are inversely correlated with map stability in the novel zone, but does not correlate with the familiar zone. This same effect was present for REM sleep **(figure 38).** This suggests that in a complex environment; sleep promotes a remapping (reorganization of spatial configuration) in the zone where there were environmental changes but has no influence over the spatial map stability in the familiar zone. Furthermore, in a novel way, these results suggest that the effect that sleep exerts on the spatial representation of a complex environment may occur specifically for a specific area of it. This provides us with new information regarding how sleep can exert its influence on spatial representation in complex environments, that is, where there are three-dimensional objects and not just an empty arena.

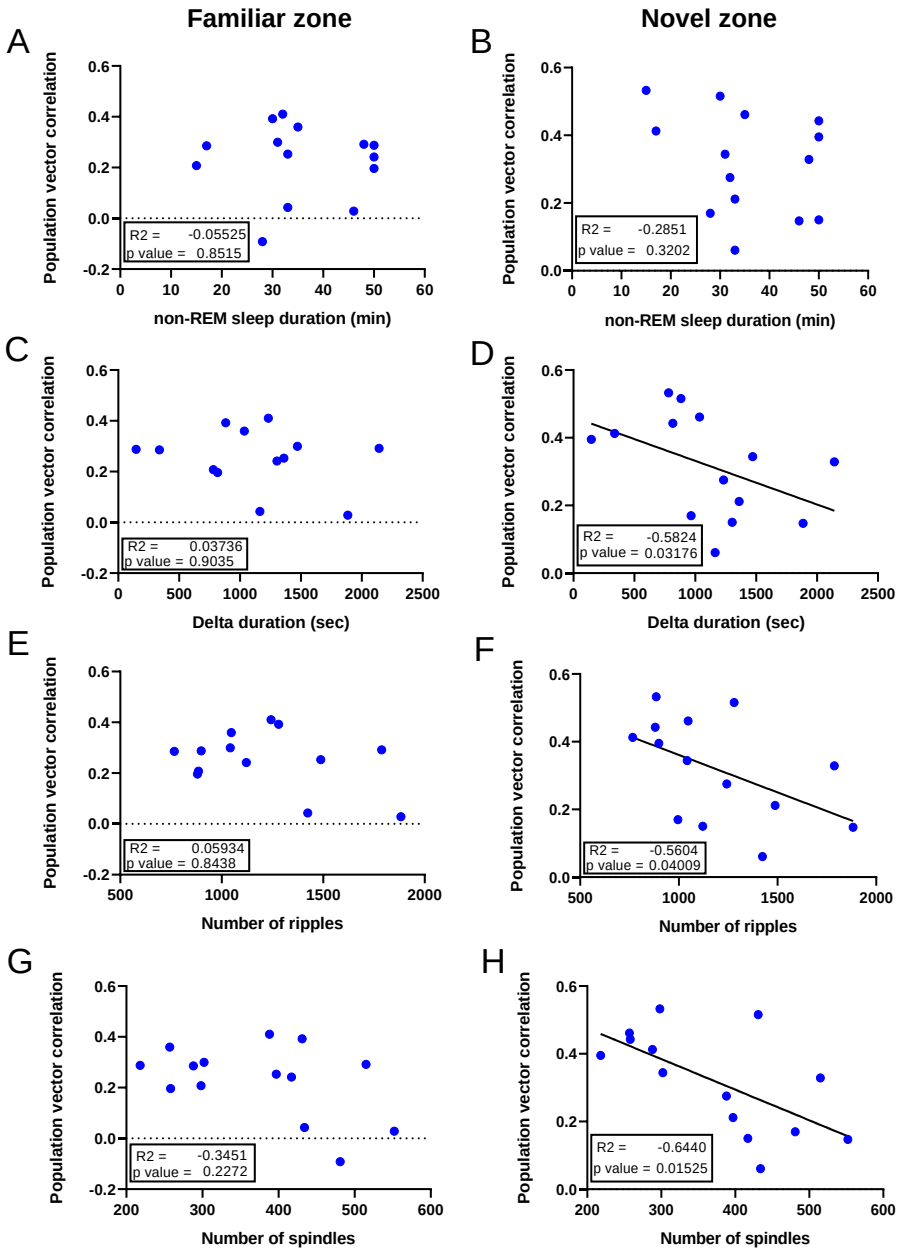


Figure 37. Non-REM sleep Oscillations are inversely correlated with map stability in the novel zone of the OPR maze. A) Correlation between non-REM sleep duration and population vector correlation (median) between sample and test for each OPR trial in the familiar zone of the OPR maze. **C, F, and G** same as in A but for delta duration, number of SWRs, and number of spindles respectively. **B, D, F,** and **H** same as in A, C, F, G respectively but for the novel zone of the OPR maze. In all cases, Spearman correlation was performed. Linear regression is plotted in graphs only when the correlation is significant.

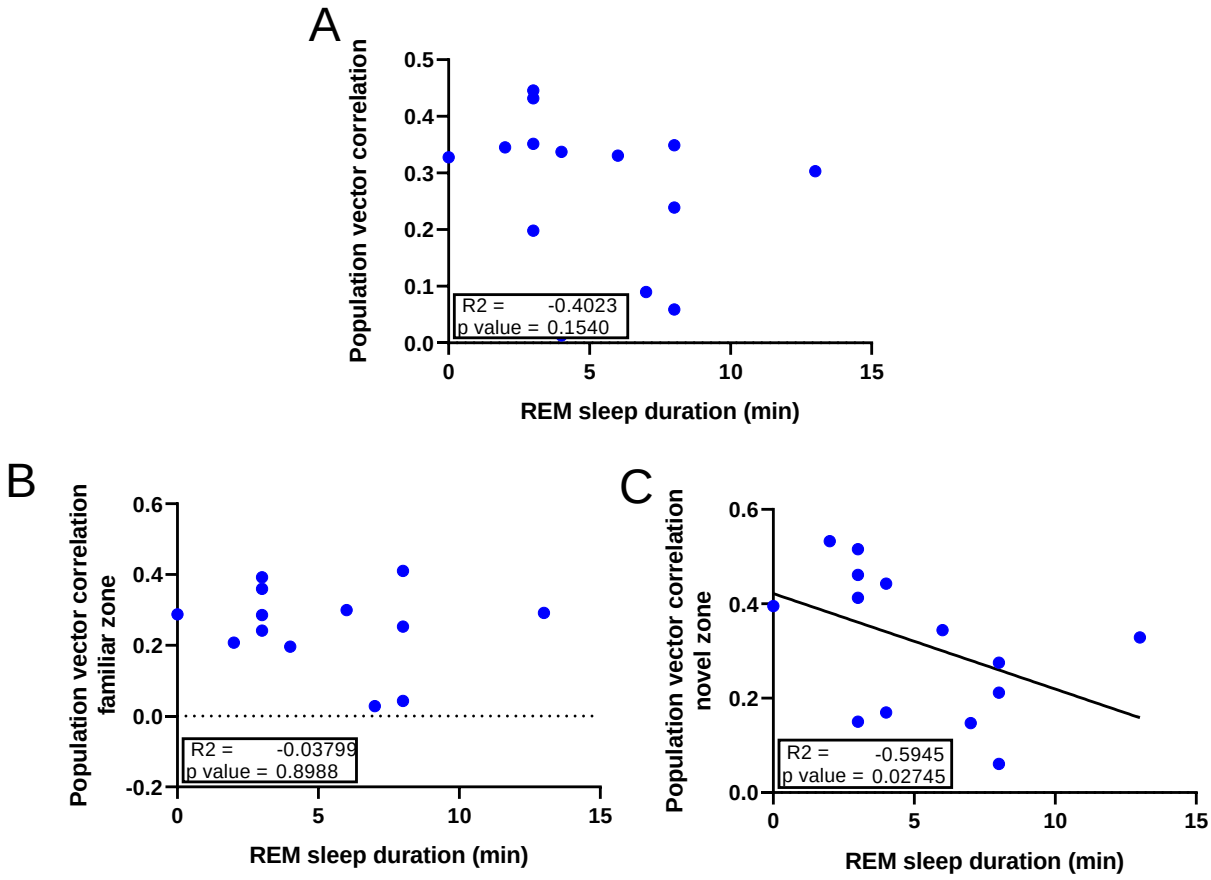


Figure 38. REM sleep is inversely correlated with map stability in the novel zone of the OPR maze. A) Correlation between REM sleep duration and population vector correlation (median) between sample and test for each OPR trial. **B** and **C** same as in A but for the familiar and the novel zone of the OPR maze respectively. In all cases, Spearman correlation was performed. Linear regression is plotted in graphs only when the correlation is significant.

5.2.3 Instability and reorganization of spatial representation improves spatial memory

Up to here, we have been able to elucidate that sleep and its oscillatory patterns were correlated with spatial memory performance **(figure 15, 18)** and reorganization of the spatial maps in the novel zone of the environment **(figure 37)**.

In line with this, one of the central points in our research was to be able to establish the relationship between sleep and spatial memory and also between sleep and spatial representation system. However, as we mentioned at the beginning of this work, both functions are carried out by the same cerebral structure and by the same neuronal group, in particular, the pyramidal cells of the hippocampus. It is because of the above, then, that it becomes relevant to be able to determine if the variables that we found in our experiments to be influenced by the sleep were correlated between them, so in particular, it is interesting to see if this reorganization of the spatial map is correlated with memory performance. **Figure 39A** shows that the decrease of stability in the spatial map given by the decrease in the population vector correlation was inversely correlated with memory performance in the OPR task. Also, and in the same way, as with sleep, this decrease in the stability of the spatial map occurs in the novel zone **(figure 39C)** and not in the family zone **(figure 39B)** of the arena.

This analysis suggests that the remapping of place cells in a novel area of the environment is necessary for an improvement in the performance of spatial memory and therefore puts the reorganization of spatial representation as a pivotal feature in spatial memory processes. In the same way, it includes sleep as a central element in this correlation, thus completing a triangular relationship between these three phenomena. This result represents a novel element both in the analysis of change of spatial representation in a complex environment as well as in the correlation between sleep, spatial memory, and the representation system given by the same recorded hippocampal cells.

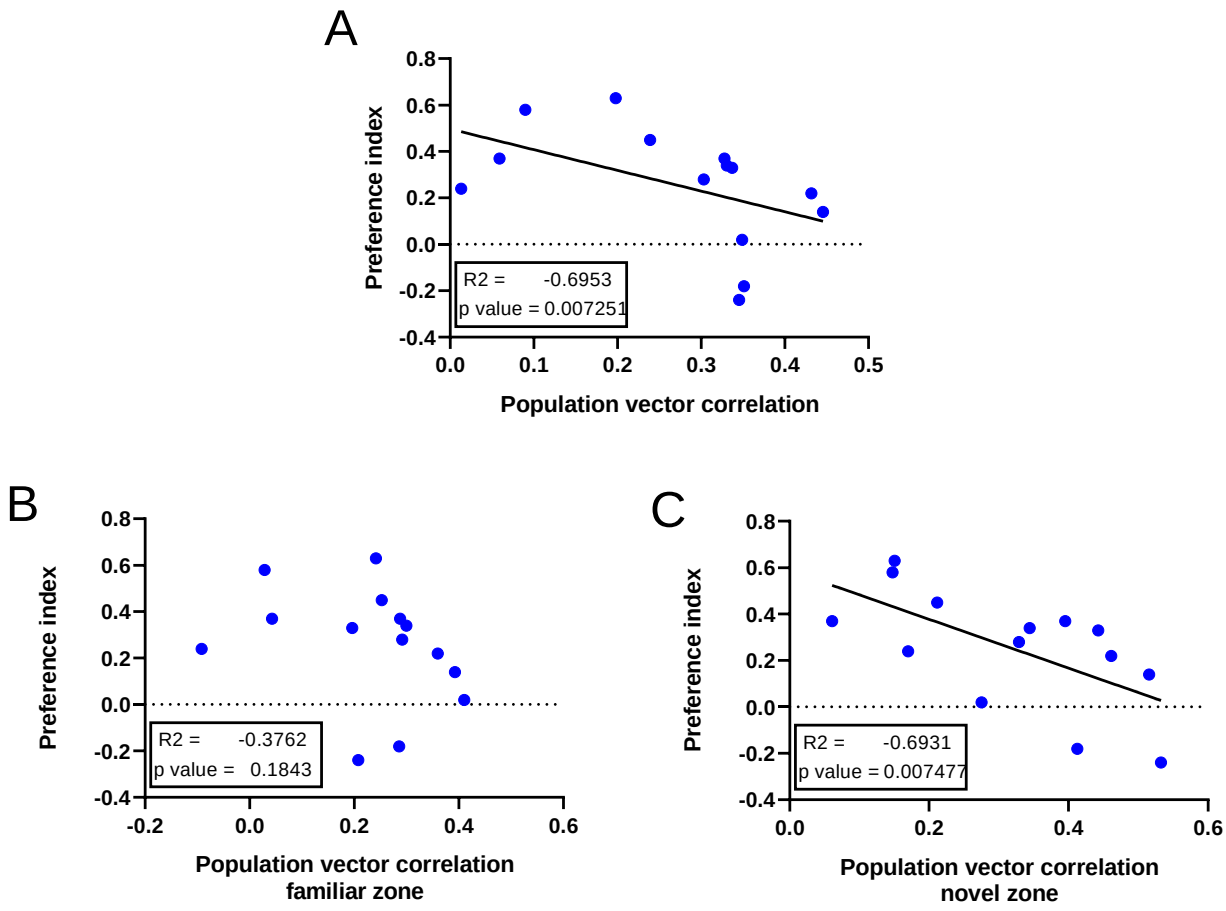


Figure 39. Memory performance is inversely correlated with map stability in the novel zone of the OPR maze. A) Correlation between REM sleep duration and population vector correlation (median) between sample and test for each OPR trial. **B** and **C** same as in A but for the familiar and the novel zone of the OPR maze respectively. In all cases, Spearman correlation was performed. Linear regression is plotted in graphs only when the correlation is significant.

CHAPTER 6

6. DISCUSSION

Our experiments in hippocampal cells during the OPR task allowed us to observe the effects of sleep on spatial memory and the spatial representation system. First, we replicated the findings associated with the role of sleep on the improvement of spatial memory, where we also established a continuous relation, finding that the longer the sleep time, the better the memory performance. Second, we observed that effects were specific for non-REM sleep and its respective oscillations, which, interestingly, have a significant temporal coupling with place cells present in hippocampal CA1. Third, we defined the three populations of place cells (up, down and keep PC) characterize their dynamics of activation, maintenance and silencing throughout the task, in addition to changes in firing rate and sparsity between sample and test phases for these three populations, finding that population that encodes both task phases vary partially, mainly associated with the novelty factor during the exploration of environment and where non-REM sleep exerts a general effect only on the decrease in firing rate of hippocampal neurons during rest phase.

Finally, our results suggest that non-REM sleep and its oscillations affect spatial representation by decreasing the stability of the spatial map where there are changes in environment, which also correlates with improvements in spatial memory performance, thus establishing a correlation between sleep, memory improvement and the flexibility of the spatial configuration given by the place cells.

The results obtained from the experiments performed in this work will be discussed in the following paragraphs.

6.1 SLEEP AND SPATIAL MEMORY

6.1.1 Sleep duration is crucial for spatial memory

In our experiments, rats had a spontaneous sleep-wake behavioral pattern during rest phase of OPR task, that is, without a sleep deprivation protocol. That promotes the animals to sleep for different amounts of time. This allowed us to have a continuous spectrum of sleep duration times, which has not been previously reported.

In general, sleep studies associated with memory tests are analyzed in a dichotomous way, where results are compared between the presence and absence of sleep (Diekelmann and Born, 2010; Inostroza et al., 2013; Nguyen et al., 2013). These studies are based on artificial sleep deprivation as a control condition to be compared with post-learning sleep. This could present a disadvantage since it can induce stress levels that could alter the behavior of animals and thus lead to erroneous interpretations of the observed phenomenon (Colavito et al., 2013). In line with this, it has been observed that sleep deprivation can produce alterations in hippocampal-dependent memory due to the increase in pro-inflammatory cytokines through the activation of microglia (Wadhwa, 2017).

On the other hand, although the study of sleep as a dichotomous variable allows evaluating the role that sleep has on memory consolidation processes, it does not allow a more in-depth analysis of how the variable condition of sleep duration and quality impact on their already known role in these processes. The above is interesting to evaluate because it is closer to the actual behavior of mammals, where both the quality and quantity of sleep vary between and within subjects (Campbell and Tobler, 1984; Nunn and Samson, 2017)

In humans, it has been seen that small amounts of sleep after an experience is sufficient to induce declarative memory consolidation processes, which are maintained even after the end of the sleep stage (Lahl et al., 2008). This is in line with our results, which show an improvement in spatial memory performance even in

animals that slept for short periods. This is consistent with the large body of literature that considers naps as critical events in memory consolidation, where short sleep events can produce an improvement in the consolidation of declarative memory (Mednick et al., 2003; Schabus et al., 2005; Rash and Born, 2013; Farhadian et al. 2021).

Tucker et al. (2006) showed that short periods of sleep improve declarative memory but not procedural memory due to the occurrence of non-REM sleep during these periods. In the same way, our results suggest that even in short periods of sleep, oscillatory patterns such as delta waves, SWRs and spindles improve spatial memory.

Due to this, it was interesting to evaluate whether the longer sleep duration improves memory capacity or behaves as an all or nothing phenomenon as historically studied. In Lahl et al. (2008), the authors also suggest that memory performance increases as sleep duration increases. That is in line with our results, which show a direct and robust correlation between the length of sleep with spatial memory performance, which suggests a direct causal relationship between both variables.

In humans, it has been seen that the duration of sleep is essential for declarative memory only in its first stage when Slow Wave Sleep occurs. After this period, no improvements are seen for this type of memory (Tucker and Fishbein, 2009). In addition, it has been observed that long periods of sleep (over 8 hours) can be counterproductive for memory and cognition due to alterations in the circadian cycle (Devore et al., 2014; Tsapanou et al., 2017). Our recordings were not made in long periods of sleep and sleep structure is different from rodents, so further experiments are required to see how this affects spatial memory in these animals.

The information presented here suggests that the development of research associated with sleep duration has been scarce in non-primate mammals. Our results, therefore, represent a novel approach to this analysis in rats, strongly suggesting a causal and continuous relationship between the duration of non-REM sleep and improvements in spatial memory, which generates a counterpoint on the

dichotomous view under which sleep has been studied. We propose that the duration of this physiological state is crucial for memory consolidation process.

6.1.2 Deep sleep and spatial memory consolidation

Some authors call deep sleep the increase in the time that animals spend sleeping during a specific time interval, which, as our results show, is associated with an increase in slow-wave sleep (Sawangjit et al., 2020). That study showed that deep sleep produced a persistent memory, even one week after a spatial memory task, which is associated with an increase in SWS and its oscillatory patterns. This suggests that more than the duration of total sleep, the duration of slow-wave sleep is essential in promoting episodic memory consolidation.

On this line, and reported by different groups (Plihal and Born, 1999; Peigneux et al., 2004; Inostroza and Born, 2013; Klinzing et al., 2019), our results suggest a key role of non-REM sleep on this type of memory, which is not the case for REM sleep. This was established by analyzing the different oscillatory patterns that underlie these sleep stages, particularly for non-REM sleep, which explain the observation of a continuous and direct relationship between sleep and memory raised in the previous section.

Several authors suggest that non-REM sleep features are fundamental to understand this phenomenon, where the presence and density of oscillations such as spindles, sharp wave ripples and slow waves are associated with improvement in spatial memory (Marshall et al. 2006; Girardeau et al. 2009; Maingret et al.; 2016; Latchoumane et al., 2017).

On this line, it has been seen that after learning tasks, spindles and SWRs are increased during slow wave sleep (Gais et al., 2002; Eschenko et al. 2008; Ramadan et al., 2009). Although we did not have a sleep control condition before the spatial memory task, we did find that the increase in these physiological events during sleep correlates with memory improvement. On these, the time of delta oscillations, the

number of spindles and the number of SWRs during sleep were positively correlated with spatial memory.

It is important to note that our results do not show a variation in this density but only an increase due to longer sleep duration. In line with the above, it has been seen that decline in memory processes associated with a decrease in the density of SWRs are present in mice with Alzheimer's; however, the longer they sleep, the adverse effects are diminished, behaving as a compensatory effect (Benthem et al., 2020). This allows suggesting the relevance of the number of events in memory consolidation processes.

Also, our results are in line with studies that report an improvement in declarative memory performance as the number of spindles increases (Tamminen et al., 2010). In animals, the same effect has been seen for recognition memory performance in the object in recognition task (Sawangjit et al., 2018). It has been suggested that thalamocortical spindles generate an influx of calcium into cortical pyramidal cells, leading to long-term potentiation (LTP) and strengthening of synaptic connections and plasticity that would be associated with improvement in memory consolidation and learning (Sejnowski and Destexhe, 2000; Rosanova and Ulrich; 2005; Marshal et al., 2006; Peyrache and Seibt, 2020).

In line with this, it is well established that during slow-wave sleep, SWRs allows the reactivation of hippocampal pyramidal neurons that were activated during the prior wakefulness (Pavlides and Wilson, 1989; Buzsaki 1998; Jadhav et al., 2012; Ramadan, Eschenko and Sara, 2009; Girardeau et al., 2009). This allows previous experiences to be represented through repetition of neuronal patterns of activity, strengthening synaptic connections and promoting plasticity in hippocampal cells (Joo and Frank, 2018; Ben-Yakov et al., 2015; Sadowsky et al., 2016; Pfeiffer, 2020).

Interestingly, it has been shown that hippocampal-dependent memory consolidation processes depend on co-activation between spindles, SWR, and slow waves (Diekelmann and Born, 2010; Dudai et al., 2015; Varela and Wilson, 2020) through a temporal and hierarchical phase locking between them (Siapas and Wilson; 1998;

Sirota et al., 2003; Clemens et al., 2007), promoting the hippocampal-cortical transmission of memory information (Buszáky, 1998). Particularly for spatial memory consolidation, Maingret et al. (2016) suggest that the reinforcement of hippocampal-cortical coordination between SWRs, cortical delta waves, and spindles through electrical stimulation results in the reorganization of cortical networks that promote memory consolidation during sleep. In line with this, spindles have recently been proposed to play a fundamental role in hippocampus-dependent memory consolidation only when coupled to the up-state phase of slow oscillations (Latchoumane et al., 2017).

In line with this, our results show that the increase in the quantity of these three oscillations during non-REM sleep is associated with a better performance in spatial memory, so it would be interesting to evaluate a possible temporal coupling between them and how this affects spatial memory in our conditions.

On the other hand, although it has been seen that during sleep, the reactivation of hippocampal pyramidal neurons such as place cells is coupled to SWRs (Diba and Buszáki, 2007), it is not clear if this neuronal replay is coupled to the triplet Slow waves-Spindles-SWRs. Interestingly, our results show that a temporal coupling occurs between Place cells and each of three non-REM sleep characteristic oscillations recorded in this work, more significant than in neurons that do not exhibit spatial coding. In addition, this effect is more significant for those neurons that were active during the encoding phase, before the sleep stage, and that encode the space after the sleep stage. This shows how neuronal replay, coupled to different non-REM sleep oscillations, occurs in neurons that remain active during all phases of the spatial memory task, suggesting the importance of this phenomenon in the reactivation of spatial representations during exploratory behavior.

Related to this, Dupret et al. (2010) showed that the better the synchrony between Place cells and SWRs, the better the performance in spatial memory. The authors suggest that this synchronization promotes the strengthening of spatial

representations by promoting neuronal plasticity, possibly through the activation of dendritic spikes (Losonczy et al., 2008), thus stabilizing the newly formed assemblies.

Finally, the information presented here raises the importance of the coupling and temporal synchrony between the oscillatory patterns that occur during non-REM sleep and the Place cells in their reactivation in the re-exploration of the environment, which suggests a correlation between this phenomenon and success in spatial memory consolidation processes. More analyses are necessary to establish whether, in our animals, the degree of synchrony between Place cells that remain active during the task correlates with improvements in behavioral performance.

6.2 SLEEP AND THE SPATIAL REPRESENTATION SYSTEM

6.2.1 Variations in place cell activity during the OPR task.

To analyze the changes in the activity of the place cells, it was first necessary to establish what happens with the dynamics of their activation during the exploration of novel contexts. Interestingly, our results show that there are place cells that emerge and others that are silenced during the task. In this sense, it has been shown that place cells, in the exploration of a novel environment, can develop place fields rapidly, progressively, that is, once the animal has passed through a particular place several times or just stayed silent (Sheffield and Dombeck, 2019; Dong et al. 2021).

In line with this, Frank et al. (2004) show that upon exploring a novel environment, place fields rapidly emerge from previously silent neurons. This emergence occurs through non-Hebbian synaptic plasticity mechanisms that occur through dendritic spikes of the hippocampal CA1 pyramidal cells during the exploration of the environment, generating behavioral timescale synaptic plasticity and thereby increasing the precision and stability of the place fields as the animal moves through the place field area (Sheffield and Dombeck, 2019). This shows that the emergence of new place cells during the exploration of the environment also depends on the duration of the exploration, so it is necessary to evaluate enough time for the development of spatial maps in our conditions since the variations observed in the spatial maps they can be influenced by their state of formation and not only by changes in the environment. On the other hand, our results show that the number of place cells that encode the spatial map varies in each trial, reaching around 30% of all cells.

The above suggests that the identity and quantity of cells that encode familiar environments do not predict the identity and quantity of cells that encode novel environments. These differences come not only from intrinsic spatial changes but also from the experience of exploration.

Interestingly, sleep does not influence the number of cells that remain active as place cells, suggesting that the possible changes in the configuration of the spatial map given by sleep could respond to specific changes in place fields rather than to patterns of activation or silencing of place cells.

Another relevant feature in the task performance is the change in place cell firing rate. As expected, both Up place cells and Down place cells vary their firing rate depending on their emergence or silencing, thus affecting sparsity since its value depends on the firing rate. On the other hand, neurons that keep their place cell activity show a slight increase in firing rate during the test phase. In this sense, similar results were described by Larkin et al. 2014, where firing rate increases in the exploration of modified environments for hippocampal CA1 pyramidal cells. In our results, the non-spatial cells also increase the firing rate; however, other analyses are necessary to know if this increase occurs in all cells or only in pyramidal cells since this group also includes interneurons.

As has been proposed, the increase in firing rate in CA1 pyramidal cells is induced by the novelty of the explored environment and promotes the re-exploration of a previously visited environment to update previously stored information (Karlsson and Frank, 2008; Van Elzakker et al., 2008; Larkin et al. 2014). In line with this and our results, exposure to complex novel environments increases sparsity (Bilkey et al., 2017). In addition, it should be remembered that the environment has been explored by the animal extensively since the habituation phase, suggesting that the increase in CA1 pyramidal cell activity allows spatial patterns previously stored in Lateral Enthorrinal Cortex (LEC) to be updated with recently collected information (Deshmukh and Knierim, 2011; Wilson et al. 2013; Tsao et al., 2013).

Interestingly, our results show that the increase in firing rate does not depend on sleep. However, it is essential to note that variations in mean firing rate could not represent local changes in spatial maps, so other analyses are necessary to see if sleep affects firing rate in a more specific area of the explored environment.

On the other hand, sleep does decrease the firing rate of hippocampal pyramidal cells during sleep. These results align with what is established in the synaptic homeostasis hypothesis, which proposes that synaptic strength is depressed during sleep (Tononi and Cirelli, 2014). This depression occurs to normalize the total synaptic strength that may have increased during wakefulness to restore cellular functions and selectivity. One of the key elements raised by this hypothesis is that those synapses that are reactivated during sleep are not significantly affected by this effect, unlike those that are not reactivated, improving the "signal to noise" relationship during replay in sleep consolidation processes (Tononi and Cirelli, 2014; Miyawaki and Diba, 2016).

In contrast, Hirase et al (2001) suggest that the increase in firing rate of recently activated neurons during sleep occurs together with a decrease in firing activity of the remaining population to maintain constant neuronal excitability. Interestingly, recent studies have suggested that in the hippocampus, non-REM sleep promotes the stabilization of firing rates in pyramidal cells and the inhibition of interneurons, while REM sleep exerts a selective decrease in the firing rate on those neurons that have low firing activity (Mizuseki and Miyawaki, 2017; Miyawaki et al., 2019), which is in line with our results since in those animals where the duration of sleep is more prolonged a more significant decrease in firing rate is observed.

In summary, the emergence of the spatial map during exploration is given by the activation, silencing, and maintenance of place cells activity, which is not necessarily predicted by the activity of these neurons during the previous exploration of the environment. Also, sleep does not directly affect the activation or silencing of place cells nor the changes in the mean firing rate during exploration of the novel environments; however it is not clear what effects it could have on the configuration of the spatial maps of those cells that keep their place cell activity.

The changes in the configuration of the spatial map are directly associated with the remapping that the place cells perform due to changes in the environment and focused this process as pivotal in active exploration during the performance of memory tasks.

6.2.2 Sleep promotes flexibility of spatial map due to environmental changes.

The activation and silencing of different groups of place cells during the exploration of the environment reveals the versatility of the spatial representation system. This versatility is also expressed through the remapping of place cells, where changes of representation are expected to occur in the exploration of novel environments and contexts, as well as stability in those experiences where a familiar environment is visited (Muller and Kubie; 1987; Thompson and Best, 1989; Colguin, Moser and Moser, 2008; Kubie et al. 2020). In the same way, when just some elements of the environment change, a partial remapping of the spatial map is expected (Anderson and Jeffery, 2003; Colguin, Moser and Moser, 2008, Latuske et al., 2018). In our experiments, based on the OPR test, changes in environment refer to the position of a proximal cue (i.e., object), while the general environment (i.e., distal cues) remains unchanged. As shown in this work, the re-exploration of this modified environment induces changes in the firing rate and position of place fields in some neurons. Similar results have been described by Vandrey et al. (2021), where they show that a change in position of a proximal cue was able to induce remapping in hippocampal neurons of CA1. Given the nature of our task in which an area of the arena changes while another remains unchanged (familiar vs novel area), in addition to the fact that population of neurons that encode the space between both phases of the task is different; a population vector analysis of the spatial representation was suitable.

In this type of test, in which there is a re-exploration of an environment already visited, other studies focus on analyzing the changes only in those neurons that remain as place cells in both stages, leaving aside a group of neurons that are activated in only one of both environments and that also participates in the coding of space. In contrast, the population vector allows considering all the coding neurons in a particular environment, which allows a more reliable representation of the spatial map.

Considering this, our results show low stability of spatial map during the test phase, which is expected due to changes in the object's location. Similar results have been

reported recently (Yuan et al., 2021). In addition, our results do not report differences between the correlation of the spatial map in the familiar zone and that in the novel zone of the arena, which shows some instability in the spatial maps even in familiar zones. This contrasts with different studies showing that spatial maps tend to be stable when environmental conditions remain constant (Thompson and Best, 1989; Ziv et al., 2013; Rubin et al., 2015; Roux et al., 2017; Kinsky et al., 2018).

This apparent contradiction could be explained because the distance between the objects can generate overlap between both zones and alter the result; being a small arena, the difference between zones may not be dichotomous and instead of a gradual transition that could depend on the frame of reference of each animal during the exploration. On the other hand, it has been seen that even in familiar environments, spatial maps are highly unstable when the environment is freely explored, without a goal-oriented task (Kentros et al. 2004; Jeantet et al., 2012; Kinsky et al. 2018; Sheintuch et al. 2020), as occurs in our experiments. This instability does not occur randomly but rather refers to different frames of reference that the animal can use during navigation and where attention to particular targets or cues plays a primary role (Knierim and Rao; 2003; Keinath et al., 2017; Muzzio, 2018; Kinsky et al. 2018).

On the other hand, it has been seen that different groups of place cells could represent different elements of environment, those that encode the location of objects and those that encode distal cues (Yuan et al., 2021). According to this, and given the features of OPR task, our results suggest that we recorded mainly cells that encode object location due to their greater instability associated with position change during the test. On the other hand, it has been shown that it can take several sessions for spatial map to be progressively developed until it reaches stability (Dupret et al., 2010; Kim et al. 2020), so a single 10-minute session could be insufficient to generate stable maps under our conditions and lead to variations seen in our results.

Interestingly, although our results show similar variations in spatial map stability between novel and familiar zone, we find a crucial difference in the correlation of

spatial map configuration with sleep. Our results show that in the novel zone and not in the familiar zone, the spatial map decreases its stability as the occurrence of non-REM sleep oscillations such as spindles, SWRs, and delta waves increase. This is in line with Kovacs et al. (2016) proposed, which states that in the re-exploration of familiar environments, SWRs are not related to the stability of spatial representations. Conversely, Roux et al., 2017 shows that the occurrence of SWRs promotes the stabilization of the spatial map; however, in the experiments carried out by these groups, there is no variation in either the explored environment or objects (animals walk through an empty arena) which marks a substantial difference from exploring a complex environment in which some proximal cues change its position.

Otherwise, recently Yuan et al. (2021) showed that the stability of spatial map in neurons that represent the location of objects is greater in those animals that have a sleep deprivation protocol. This is similar to our results and reinforces the idea that sleep could be playing a key role in making the spatial map more flexible in response to changes in the environment through place cell remapping. This had not been previously proposed and counterbalanced the idea of sleep as a stabilizer of the spatial map. In line with this, in humans, it has been proposed that flexibility in spatial representation systems during navigation is increased by sleep (Noack, Doeller and Born, 2021).

The spatial map flexibility, expressed as remapping of place cells, is a form of hippocampal synaptic plasticity since experience modifies neural circuits (Citri and Malenka, 2008). As we mentioned in this work, synaptic plasticity is crucial to memory consolidation processes in hippocampal neural circuits suggesting that remapping of spatial representations could be crucial for memory consolidation.

6.2.3 Spatial map flexibility correlates with improved spatial memory

Interestingly, the increase in spatial map flexibility, associated with sleep, correlates with a spatial memory improvement, where the lower the spatial stability in the novel zone, the better the performance in the task. Similar results are shown by Yuan et al. (2021), where it is indirectly shown that animals with good performance in memory have less stability of spatial map in those neurons that encode the location of objects in an OPR task and where the number of spindles was a good predictor of success in spatial memory. On the other hand, Dupret et al. (2010) showed that the reactivation and reorganization of the spatial map in a goal-oriented task were associated with improvements in memory performance. Concerning this, it has been seen in rodents that place cells that express c-Fos, a gene associated with memory formation in the hippocampus(Vann et al., 2001; He et al., 2002; Liu et al., 2012) spatial representations are less stable than those where the gene is not expressed (Tanaka et al. 2018). This suggests that plasticity of the spatial map is essential to sense and encode changes in the environment, which would be essential to update previously stored spatial maps and, therefore, a key function for spatial memory. This follows the idea originally proposed by O'keefe and Nadel (1978) that spatial map representation is a mechanism that brain has of organizing memory and, therefore, central to representation of context-specific experiences (Jeffery, 2018).

In line with this, in a novel experiment, Robinson et al. (2020) demonstrated that the selective activation of place cells allows managing the behavior of animals during a spatial memory task, providing evidence that these cells are not only a "GPS", rather, they actively influence exploration, mapping of the environment, and spatial memory performance. On the other hand, the flexibility of the spatial map associated with sleep due to variations in the environment, which is correlated with success in spatial memory tasks, suggests that the hippocampus drives flexibility in the representation of the environment during the achievement of a memory task, which highlights not only allocentric spatial navigation but also the context in which the task is developed. This is in line with the idea that the hippocampus also encodes information associated

with events, objects, and experiences, representing a mechanism for how memory is organized in the hippocampus (Eichenbaum and Cohen, 2014; Eichenbaum, 2017).

Finally, in a novel way, our results suggest that non-REM sleep and its oscillations affect spatial representation through the flexibility in the configuration of spatial maps due to changes in the environment. This is also associated with an improvement in spatial memory since instability of place cell spatial configuration is relevant for memory updating during the exploration of environment.

Since spatial representation system and memory formation are driven by hippocampal neural circuits, synaptic plasticity in memory formation process has an analogous effect over changes in spatial representation, which is promoted by sleep.

Future research is necessary to determine if different neuronal groups are responsible for encoding and processing spatial information associated with different frames of reference such as objects or distal cues and if sleep plays a specific role in any of these populations, taking part in memory formation processes in the hippocampus.

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES

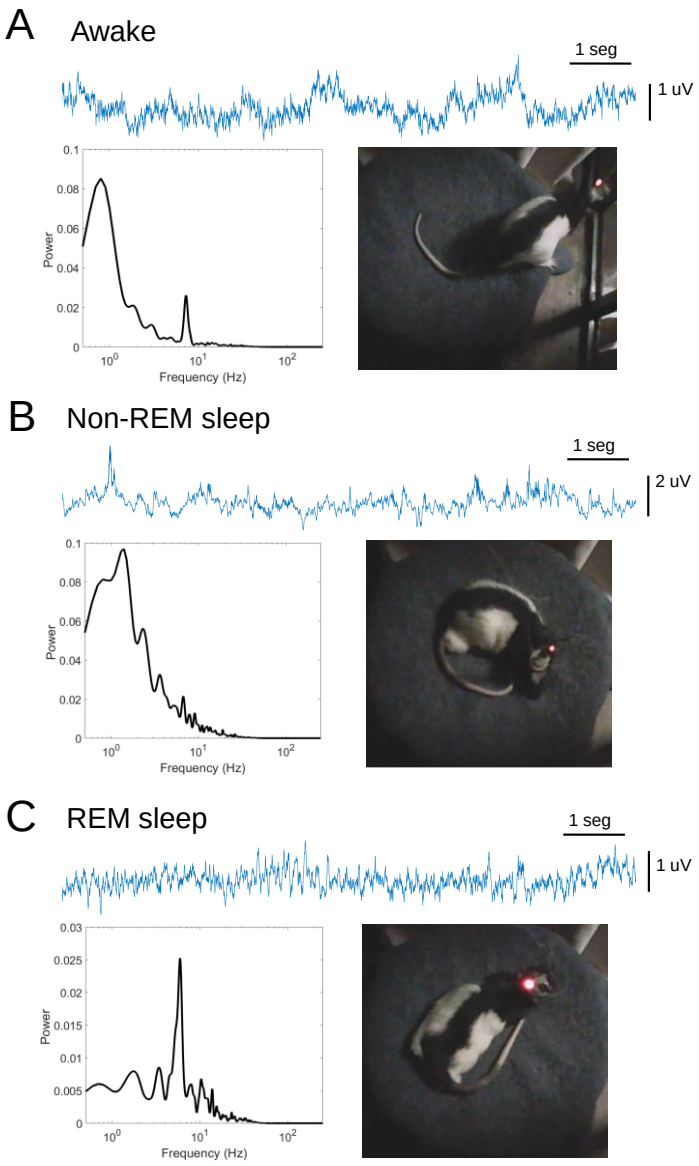

Figure S1. Sleep scoring using hippocampal LFP signal. Fourier analysis, Raw LFP data, and video recording were used to select each recording window (10 sec) as one of three different stages, in particular for A) awake, B) NREM and C) REM sleep. Animal: GV16-OPR3

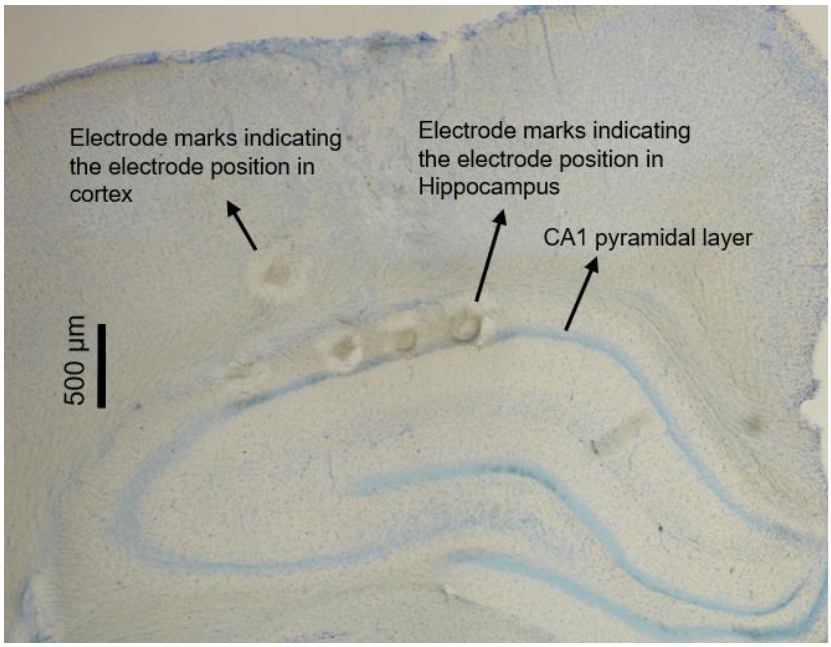

Figure S2. Hippocampal slice representing the position of electrodes in CA1 pyramidal layer. Nissl staining of hippocampal slice shows the position of the electrodes in CA1 and cortex. All the implanted animals were exposed to an electrolytic lesion through the tetrodes, then the brains were dissected, and hippocampal Nissl stained slices were obtained for the recognition of the final position of electrodes. Slice obtained from animal GV16

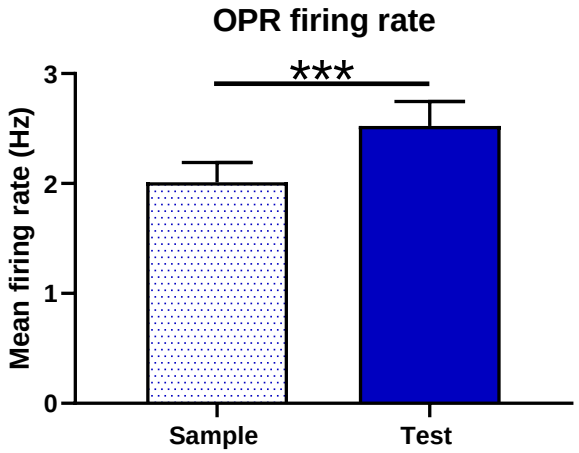

Figure S3. Firing rate in OPR task. Mean firing rate during test was higher than during sample phase in OPR task. Wilcoxon test was performed ($p<0.0001$).

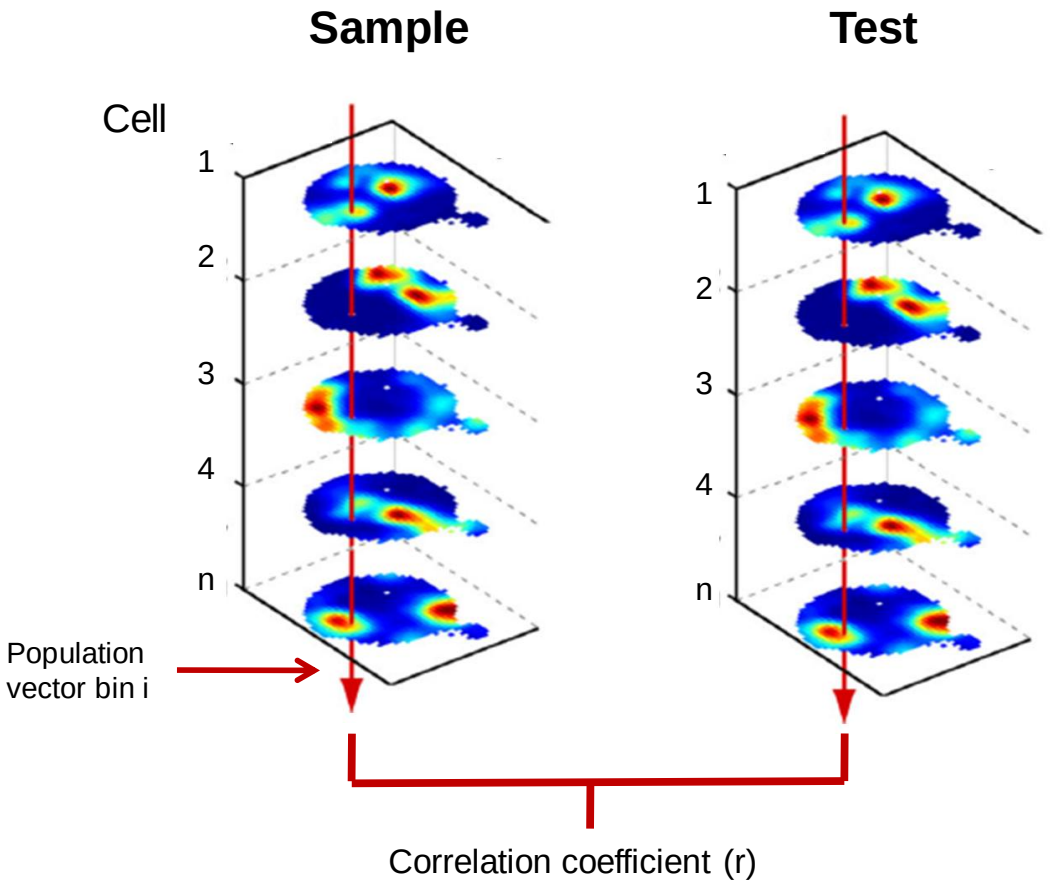

Figure S4. Population vector analysis. Population vector was created in sample and test phases. All units of one specific trial were ensembled in a 3D matrix, where the x-y axis represents the position of bins in the arena and the z-axis represents the different units. For each bin in the x-y axis, a vector that contains the firing rate values on that bin for all units (z-axis) was created. After that population vectors of sample and test were correlated to obtain a correlation coefficient (r) for each bin, generating a map of correlation for each trial. Spearman correlation was performed. Adapted from Roux et al., 2017.

Supplementary tables

Animal	Post-natal day at OPR1
GV01	95
GV02	87
GV03	87
GV04	87
GV05	85
GV06	91
GV07	91
GV08	93
GV11	83
GV12	83
GV13	61
GV14	61
GV15	96
GV16	96
GV17	70
GV18	70
GV19	78
GV20	78

Table S1. Animal age at OPR1. Table with the post-natal day of each animal when it performed the first OPR protocol.

Animal	Trial	Maze
GV01	OPR1	Square
GV11	OPR1	Square
GV11	OPR2	Circular
GV11	OPR3	Circular
GV12	OPR1	Square
GV12	OPR2	Circular
GV15	OPR1	Circular
GV15	OPR2	Square
GV15	OPR3	Circular
GV15	OPR4	Square
GV15	OPR5	Circular
GV15	OPR6	Circular
GV16	OPR1	Circular
GV16	OPR2	Square
GV16	OPR3	Circular
GV16	OPR4	Square
GV16	OPR6	Circular

Table S2. Trial number and Maze type in implanted animals. The number of each trial and the corresponding maze used for all implanted animals.

A All animals

Animal	Trial	Sleep	Awake
GV01	OPR1	70	18
GV02	OPR1	19	75
GV02	OPR2	15	74
GV03	OPR1	67	24
GV03	OPR2	40	56
GV04	OPR1	10	84
GV04	OPR2	58	42
GV05	OPR1	14	75
GV06	OPR1	6	86
GV06	OPR2	32	59
GV07	OPR1	0	90
GV07	OPR2	20	70
GV08	OPR1	0	99
GV08	OPR2	14	77
GV11	OPR1	50	26
GV11	OPR2	54	22
GV11	OPR3	43	50
GV12	OPR1	3	79
GV12	OPR2	38	44
GV13	OPR1	41	53
GV13	OPR2	32	59
GV14	OPR1	54	44
GV14	OPR2	44	48
GV15	OPR1	53	44
GV15	OPR2	33	59
GV15	OPR3	40	56
GV15	OPR5	41	50
GV15	OPR6	17	76
GV15	OPR8	54	56
GV16	OPR1	33	60
GV16	OPR2	40	54
GV16	OPR3	37	57
GV16	OPR4	20	68
GV16	OPR6	61	31
GV17	OPR1	35	57
GV17	OPR2	13	74
GV18	OPR1	43	52
GV19	OPR1	9	83
GV19	OPR2	8	78
GV20	OPR1	61	59
GV20	OPR2	64	26

B Implanted animals

Animal	Trial	NREM sleep	REM sleep	Awake	Undet
GV01	OPR1	55	15	18	3
GV11	OPR1	50	0	26	1
GV11	OPR2	50	4	22	1
GV11	OPR3	43	0	50	0
GV12	OPR1	3	0	79	0
GV12	OPR2	35	3	44	0
GV15	OPR1	50	3	44	3
GV15	OPR2	28	4	59	3
GV15	OPR3	33	8	56	2
GV15	OPR4	46	7	56	3
GV15	OPR5	33	8	50	1
GV15	OPR6	15	2	76	1
GV16	OPR1	30	3	60	0
GV16	OPR2	32	8	54	0
GV16	OPR3	17	3	68	1
GV16	OPR4	31	6	57	1
GV16	OPR6	48	13	31	1

Table S3. Sleep time of each trial in behavior and implanted animals. A) Sleep and awake time spent in rest phase per trial for behavior and implanted animals. **B)** Non-REM, REM, and awake time spent in rest phase for implanted animals (undet = undetermined time). All values are in minutes.

Animal	Trial	Units	Keep Place Cells	Down lace cells	Up place cells	Non-spatial cells
GV11	OPR1	41	3	2	11	25
GV11	OPR2	39	4	1	16	18
GV12	OPR1	36	1	2	1	32
GV12	OPR2	40	5	1	1	33
GV15	OPR1	42	6	10	3	23
GV15	OPR2	42	5	3	6	28
GV15	OPR3	43	9	5	4	25
GV15	OPR4	50	2	12	2	34
GV15	OPR5	35	6	3	4	22
GV15	OPR6	47	6	2	2	37
GV16	OPR1	43	20	3	1	19
GV16	OPR2	41	25	4	3	9
GV16	OPR3	38	12	3	3	20
GV16	OPR4	30	12	5	3	10
GV16	OPR6	45	18	1	4	22
Total number		612	134	57	64	357

Table S4. Number of units per trial in each hippocampal cell group. The number of units per trial for place cell, Up place cell, Down place cell, and non-spatial cell group.

STATISTICAL TABLE

Figure 9						
Figure 9B	Anderson-Darling Normality test (p-value)					
	0.1227					
Figure 10						
Figure 10A	Unpaired T-test					
	p-value					
	0.9791					
Figure 11						
	Mixed effect analysis		Bonferroni's multiple comparisons test		One Sample T-test	
			Short Sleep - Long Sleep		Short Sleep	Long Sleep
	Source of Variation	p-value	Minutes	adjusted p-value	p-value	p value
Figure 11A	Time	0,3815	60	0,6855	0,6693	0,0076
	Sleep	<0,0001	120	0,1703	0,0114	0,0001
	Time x Sleep	0,9945	180	0,0027	0,0399	<0,0001
			240	<0,0001	0,1291	<0,0001
			300	0,0004	0,0371	<0,0001
			360	0,0007	0,0245	<0,0001
			420	0,0002	0,0047	<0,0001
			480	<0,0001	0,0051	<0,0001
			540	<0,0001	0,0692	<0,0001
			600	<0,0001	0,0714	<0,0001
Figure 11B	Time	0,4234	60	0,9754	0,7722	0,1897
	Sleep	<0,0001	120	0,3156	0,0078	0,0019
	Time x Sleep	0,9982	180	0,2679	0,0338	0,0026
			240	0,0001	0,059	<0,0001
			300	0,0065	0,0078	<0,0001
			360	0,001	0,0094	<0,0001
			420	0,0007	0,0016	<0,0001
			480	0,003	0,003	<0,0001
			540	0,0007	0,0146	<0,0001
			600	0,0008	0,0162	<0,0001
Figure 11C	Time	0,3472	60	>0,9999	0,7739	0,0226
	Sleep	0,0268	120	>0,9999	0,5758	0,0135
	Time x Sleep	0,9881	180	0,1791	0,6783	0,0003
			240	0,2178	0,9819	0,0003
			300	0,3386	0,938	0,0004
			360	0,5332	0,7103	0,0006

			420	0,8487	0,4152	0,0001
			480	0,6575	0,3434	<0,0001
			540	0,0716	0,8272	<0,0001
			600	0,083	0,7956	<0,0001
				Anderson-Darling Normality test		
Figure 11A				0.5617	0.0313	
Figure 11B				0.6339	0.3982	
Figure 11C				0.4806	0.4144	
Figure 12						
Figure 12A	Mixed effect analysis		Bonferroni's multiple comparisons test		One Sample T-test	
			Short Sleep - Long Sleep		Short Sleep	Long Sleep
	Source of Variation	p-value	Minutes	adjusted p-value	p-value	p-value
	Time	0,6764	60	>0,9999	0,3791	0,8383
	Sleep	0,4552	120	0,9933	0,2687	0,9524
	Time x Sleep	0,9858	180	>0,9999	0,3349	0,4527
			240	>0,9999	0,6163	0,8315
			300	>0,9999	0,8283	0,8738
			360	>0,9999	0,8429	0,7954
			420	0,9957	0,2395	0,9901
			480	0,969	0,2017	0,8704
			540	0,9939	0,2545	0,9701
			600	0,9214	0,0812	0,9427
Figure 12B	Paired T-test					
	p-value					
	0,404					
Figure 13						
Figure 13A	Unpaired T-test					
	p-value					
	0.9791					
Figure 14						
	T-test					
	p-value					
Figure 14A Left	0,0001					
Figure 14A Middle	0,0015					
Figure 14A Right	0,0345					
	Two-Way ANOVA			Bonferroni's multiple comparisons test		
	Source of Variation		p-value			p-value
Figure 14B Left	Interaction		0,7073	Sample:Short Sleep vs.		>0,9999

			Sample:Long Sleep		
	Test	0,006	Sample: Short Sleep vs. Test:ShortSleep		0,5458
	Sleep	0,6452	Sample: Short Sleep vs. Test: Long Sleep		0,5914
			Sample: Long Sleep vs. Test: Short Sleep		0,1359
			Sample: Long Sleep vs. Test: Long Sleep		0,1472
			Test: Short Sleep vs. Test:Long Sleep		>0,9999
Figure 14B Middle	Interaction	0,7073	Sample: Short Sleep vs. Sample:Long Sleep		>0,9999
	Test	0,006	Sample: Short Sleep vs. Test: Short Sleep		>0,9999
	Sleep	0,6452	Sample: Short Sleep vs. Test:Long Sleep		0,0115
			Sample: Long Sleep vs. Test: Short Sleep		0,1216
			Sample: Long Sleep vs. Test: Long Sleep		0,0014
			Test: Short Sleep vs. Test:Long Sleep		0,2583
Figure 14B Right	Interaction	0,7073	Sample: Short Sleep vs. Sample:Long Sleep		>0,9999
	Test	0,006	Sample: Short Sleep vs. Test: Short Sleep		0,7605
	Sleep	0,6452	Sample: Short Sleep vs. Test:Long Sleep		0,5903
			Sample:Long Sleep vs. Test: Short Sleep		>0,9999
			Sample:Long Sleep vs. Test:Long Sleep		>0,9999
			Test: Short Sleep vs. Test:Long Sleep		>0,9999
	Pearson correlation test				
	R	p-value			
Figure 14C left	0.7318	0.003			
Figure 14C right	0.5535	0.009			
Figure 15					
	Pearson correlation test				
	R	p-value			
Figure 15A	0.499	<0.0001			
Figure 15B	0.480	0.0002			
Figure 15C	0.640	0.0002			
	Pearson correlation test				

	R	p-value				
Figure 16A	0.666	0.0001				
Figure 16B	0.163	0.1208				
Figure 18						
	Pearson correlation test					
	R	p-value				
Figure 18A	0.3511	0.01558				
Figure 18B	0.2518	0.04764				
Figure 18C	0.1356	0.1605				
Figure 18D	0.5758	0.0196				
Figure 18E	0.4027	0.1220				
Figure 25						
Figure 25A	2 way ANOVA		Tukey's test. Group comparison			p-value
	Source of variation	p-value	Sleep:Place cells vs. Sleep: Non-spatial cells			<0.0001
	Sleep	<0.0001	Sleep:Place cells vs. Awake:Place cells			0.0001
	Cell group	<0.0001	Sleep:Place cells vs. Awake: Non-spatial cells			<0.0001
	Sleep x Cell group	0.0206	Sleep: Non-spatial cells vs. Awake:Place cells			<0.0001
			Sleep:Non-spatial cells vs. Awake:Non-spatial cells			0.3539
			Awake: Place cells vs. Awake: Non-spatial cells			<0.0001
Figure 25B						
	2 way ANOVA		Bonferroni's test. Group comparison			p-value
	Source of variation	p-value	Sleep			
	Sleep	0.0063	Keep vs. Down			0.0043
	Cell group	<0.0001	Keep vs. Up			0.0115
	Sleep x Cell group	0.6876	Down vs. Up			>0.9999
			Awake			
			Keep vs. Down			0.0815
			Keep vs. Up			0.1452
			Down vs. Up			>0.9999
Figure 25 C	Mann-Whitney's test	p-value				
		<0.0001				
Figure 25 D	Kruskal-Wallis test	p-value	Dunn's multiple comparisons test		p-value	

		0.0078	Keep vs. Down	0.0034	
			Keep vs. Up	0.0647	
			Down vs. Up	0.4103	
Figure 25 E	Mann-Whitney's test	p-value			
		<0.0001			
Figure 25 F	Kruskal-Wallis test	p-value	Dunn's multiple comparisons test	p-value	
		0.0204	Keep vs. Down	0.0308	
			Keep vs. Up	0.0197	
			Down vs. Up	0.9381	
Figure 26	Mann-Whitney's test	p-value			
		<0.0001			
Figure 27					
	Spearman correlation test				
	R	p-value			
Figure 27A	0.2354	0.3954			
Figure 27B	-0.0323	0.9091			
Figure 28					
Figure 28 A	2 way ANOVA		Tukey's test. Group comparison	p-value	
	Source of variation	p-value	Place cells: Sample vs. Place cells: Rest	<0.0001	
	OPR phase	<0.0001	Place cells: Sample vs. Place cells: Test	0.9523	
	Cell group	<0.0001	Place cells:Sample vs. non-spatial:Sample	<0.0001	
	Sleep x Cell group	<0.0001	Place cells: Sample vs. non-spatial: Rest	<0.0001	
			Place cells: Sample vs. non-spatial: Test	<0,0001	
			Place cells: Rest vs. Place cells: Test	<0.0001	
			Place cells: Rest vs. non-spatial: Sample	0.9998	
			Place cells: Rest vs. non-spatial: Rest	>0.9999	
			Place cells: Rest vs. non-spatial: Test	0.7436	
			Place cells: Test vs. non-spatial: Sample	<0.0001	
			Place cells: Test vs. non-spatial: Rest	<0.0001	

			Place cells: Test vs. non-spatial: Test	<0.0001	
			non-spatial: Sample vs. non-spatial:Rest	>0.9999	
			non-spatial: Sample vs. non-spatial: Test	0.4609	
			non-spatial: Rest vs. non-spatial: Test	0.5074	
Figure 28 B	2 way ANOVA		Tukey's test. Group comparison		
	Source of variation	p-value	Keep		
	OPR phase	<0.0001	Sample vs. Rest	<0.0001	
	Cell group	<0.0001	Sample vs. Test	0.4888	
	Sleep x Cell group	0.0002	Rest vs. Test	<0.0001	
			Down		
			Sample vs. Rest	<0.0001	
			Sample vs. Test	0.0287	
			Rest vs. Test	0.2185	
			Up		
			Sample vs. Rest	<0.0001	
			Sample vs. Test	0.0108	
			Rest vs. Test	<0.0001	
Figure 29					
Figure 29 F	Mann-Whitney's test	p-value			
		0.5311			
Figure 29 G	Kruskal-Wallis test	p-value	Dunn's multiple comparisons test	p-value	
		0.0204	Keep vs. Down	<0.0001	
			Keep vs. Up	0.0595	
			Down vs. Up	<0.0001	
Figure 30	Spearman correlation test				
	R		p-value		
Left					
Figure 30A	-0.2219		0.0006		
Figure 30B	-0.1970		0.0003		
Figure 30C	-0.0780		0.3850		
Figure 30D	-0.2100		0.1307		
Figure 30E	-0.4347		0.0007		

Right					
Figure 30A	-0.0470		0.4596		
Figure 30B	-0.0750		0.1758		
Figure 30C	-0.0650		0.4554		
Figure 30D	-0.2409		0.0794		
Figure 30E	-0.0990		0.4330		
Figure 31					
Figure 31 C	Mann-Whitney's test	p-value			
		0.6656			
Figure 31 G	Kruskal-Wallis test	p-value	Dunn's multiple comparisons test	p-value	
		0.0204	Keep vs. Down	<0.0001	
			Keep vs. Up	0.9255	
			Down vs. Up	<0.0001	
Figure 32					
	Spearman correlation test				
	R		p-value		
Figure 32A	-0.0355		0.5729		
Figure 32B	-0.0189		0.7209		
Figure 32C	0.1029		0.2368		
Figure 32D	-0.2431		0.0685		
Figure 32E	-0.1087		0.3924		
Figure 33					
Figure 33A	Mann-Whitney's test		p-value		
			0.0256		
	Spearman correlation test				
	R		p-value		
Figure 33B	-0.0645		0.4663		
Figure 33C	-0.0522		0.5495		
Figure 33D	-0.0755		0.3857		
Figure 35					
Figure 35A	Mann-Whitney's test		p-value		
			0.163		
Figure 36					
	Spearman correlation test				
	R		p-value		
Figure 36A	-0.2785		0.3322		
Figure 36B	-0.4154		0.1413		

Figure 36C	-0.3626	0.2031		
Figure 36D	-0.5912	0.0288		
Figure 37	Spearman correlation test			
	R	p-value		
Figure 37A	-0.0552	0.8515		
Figure 37B	-0.2851	0.3202		
Figure 37C	-0.0374	0.9035		
Figure 37D	-0.5824	0.0318		
Figure 37E	0.0593	0.8438		
Figure 37F	-0.5604	0.0401		
Figure 37G	-0.3451	0.2272		
Figure 37 H	-0.6440	0.0152		
Figure 38	Spearman correlation test			
	R	p-value		
Figure 38A	-0.4023	0.1540		
Figure 38B	-0.0380	0.8988		
Figure 38C	-0.5945	0.0274		
Figure 39	Spearman correlation test			
	R	p-value		
Figure 39A	-0.6953	0.0073		
Figure 39B	-0.3762	0.1843		
Figure 39C	-0.6931	0.0075		
Figure S3	Wilcoxon test			
	p-value			
	<0.0001			

Table S5. Statistical data. All Statistical data used in the analysis.