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Comprendre la navigation spatiale à grande échelle à l'aide de la réalité virtuelle et des enregistrements électrophysiologiques multisites dans l'hippocamp

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    1. «Reward seeking behavior in Unpredictable Environments: Navigating without Cognitive Maps » (en cours de préparation)

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Résumé

Les animaux naviguent dans leur environnement en utilisant diverses stratégies. L'une d'elles repose sur la capacité de construire une carte cognitive interne de l'espace. Les cellules de lieu dans l'hippocampe sont considérées comme les éléments constitutifs de cette carte cognitive et participent à l'encodage des informations environnementales en formant des “champs de lieu”. Dans des labyrinthes de laboratoire à petite échelle, les cellules de lieu présentent généralement un seul champ de lieu, où ces cellules sont actives. Dans des études sur des environnements à grande échelle, il a été démontré que les cellules de lieu sous-tendent un codage multi-échelle de l’espace avec une couverture quasi-uniforme de l'espace. Cependant, peu de choses sont connues sur la manière dont les cellules de lieu encodent des environnements naturels à grande échelle qui sont, par nature,dynamiques, avec des distributions typiquement hétérogènes de repères et de localisations de récompenses. Étant donné que divers facteurs particient à la modulation de l'activité des cellules de lieu dans des environnements à petite échelle, il est possible que cette modulation puisse également se produire lors de l'encodage d'environnements dynamiques à grande échelle. Dans cette étude, nous avons cherché à étudier les facteurs qui pourraient moduler le codage des cellules de lieu dans un environnement à grande échelle en utilisant une configuration de réalité virtuelle semi-réaliste (VR) avec divers objets distribués de manière inhomogène et des récompenses distribuées de manière stochastique, similaire à des environnements naturels. Dans un tel cadre, nous n'avons pas observé de codage spatial de type cellules de lieu ; au lieu de cela, nous avons constaté que les souris pouvaient apprendre une règle générale sur l'environnement, l'abstraire de la configuration spatiale et naviguer dans l'environnement à grande échelle, probablement en utilisant une navigation basée sur des éléments visuels.

Mots clés : *cellules de lieu, hippocampe, modélisation informatique, enregistrements extracellulaires, réalité virtuelle pour rongeurs, navigation spatiale*

Abstract

Animals can navigate an environment using various strategies. One of these is the ability to build an internal cognitive map of the space. Place cells in the hippocampus are believed to be the building blocks of the cognitive map and participate in encoding environmental information. In small-scale laboratory mazes, place cells usually exhibit one place field. In studies of large-scale environments, it has been shown that place cells exhibit multiscale coding with quasi-homogeneous coverage of the space. However, little is known about how place cells encode large naturalistic environments that are highly dynamic, with typically inhomogeneous distributions of cues and reward locations. Considering that various factors have been shown to modulate place cell activity in small-scale environments, it is possible that this modulation can also occur during the encoding of dynamic large-scale environments. In this study, we aimed to investigate which factors could modulate place cell coding in a large-scale environment using a semi-naturalistic virtual reality (VR) setup with various inhomogeneously distributed objects and stochastically distributed rewards that are similar to natural settings. In such a setting, we did not observe place cell type spatial encoding; instead, we found that mice could learn a general rule about the environment, abstract it from the spatial layout, and navigate the large-scale environment, likely using schema-based navigation.

Key words: *place cells, spatial navigation, extracellular recordings, virtual reality for rodents, hippocampus, computational modelling*

Contents

Affidavit . . . 2
List of publications/patents and conference participations . . . 3
Résumé . . . 4
Abstract . . . 5
List of Figures . . . 8

I Introduction **9**

1 Introduction to Spatial Navigation **10**
1.1 What is spatial navigation? . . . 10
1.2 Path integration . . . 12
1.3 Cognitive map . . . 14

2 Hippocampal Formation **16**
2.1 The notion of hippocampal formation . . . 16
2.2 Anatomy and connectivity of the hippocampal formation . . . 18
2.2.1 Dentate gyrus . . . 18
2.2.2 Hippocampus . . . 19
2.2.3 Subiculum, pre- and parasubiculum . . . 25
2.2.4 Entorhinal cortex . . . 27

3 Neurophysiological Basis Of Spatial Navigation **30**
3.1 Spatial cells in hippocampal formation . . . 30
3.1.1 Place cells . . . 30
3.1.2 Head-direction cells . . . 32
3.1.3 Grid cells . . . 35
3.1.4 Other spatial modulated cells . . . 37
3.2 Rate coding and attractor dynamics in spatial navigation . . . 38
3.2.1 Rate Coding of Spatial Cells . . . 38
3.2.2 Continuous attractor network for HD and PC . . . 39
3.2.3 Continuous attractor network in entorhinal cortex . . . 41
3.3 Oscillations in hippocampal formation . . . 44
3.3.1 Theta-Oscillations and Phase Coding . . . 44
3.3.2 Sharp-wave ripples and their role in replay . . . 44

4 Spatial Resolution and its Role in Place Cell Coding **47**
4.1 Notion of spatial resolution in place cell coding . . . 47
4.2 Modulation of spatial resolution . . . 48
4.2.1 Modulation by sensory information . . . 48

4.2.2 Modulation by non-sensory information . . . 53
4.3 Spatial resolution in large-scale environments . . . 57

5 The Interplay Between the Rodent Visual System and Spatial Navigation 66
5.1 Understanding the Neural Circuitry of the Rodent Visual System . . . 67
5.1.1 Notion of the primary visual pathway in mice . . . 67
5.1.2 Neural circuits behind mouse visual system . . . 69
5.1.3 Visual streams to the hippocampal formation . . . 70
5.2 Influence of visual information on spatial navigation: bidirectional interactions . . . 71
5.2.1 Evidences of an influence of spatial navigation on visual information processing . . . 71
5.2.2 Vestibular and head-direction movement: visual control and signals in the mouse visual cortex . . . 73
5.3 Influence of vision on the object and boundaries representations . . . 75
5.3.1 Role of the visual cues . . . 75
5.4 Eye movements as an important component of spatial navigation . . . 78
5.4.1 Mice eye movements . . . 78
5.5 Changes in pupil diameter as an important behavioral characteristic . . 86
5.5.1 Pupil structure and neural circuits covering change in its diameter 87
5.5.2 Pupil dynamics across behavioral states and sensory stimulus modulation . . . 91

6 Hippocampus Role in Reinforcement Learning and Target-Finding Strategies 95
6.1 Role of dopamine in reward coding . . . 95
6.2 Reward coding in the hippocampus . . . 96
6.3 Modelling of the reward prediction during navigation . . . 99
6.3.1 Reinforcement learning . . . 99
6.3.2 Successor Representation in the hippocampal system . . . 101

7 Utilizing Virtual Reality to Study Spatial Navigation in Rodents 103
7.1 Virtual reality in scientific research . . . 103
7.2 Developing VR for rodent studies . . . 105
7.3 Limitations of VR for rodents . . . 107
7.4 Future perspectives in VR for rodents . . . 108

II Experimental Contribution 110

III Discussion 134
Conclusion . . . 140
Bibliography . . . 181

List of Figures

1.1 Animals could navigate using various external stimuli 12
1.2 Two primary concepts of spatial navigation strategies 13

2.1 Hippocampus and entorhinal cortex 18
2.2 Schema illustrating the different layers of the hippocampus in a transverse slice 20
2.3 Structure and connectivity of the hippocampal formation 22
2.4 Intrinsic connectivity of the hippocampal neurons in rodents 24
2.5 Entorhinal-hippocampal connectivity gradient in rodents 29

3.1 Schema illustrating the activity of different spatial navigation cells . . . 31
3.2 Examples of speed and time cells activity 38
3.3 Schemes illustrating theta-oscillations during navigation and replay during sharp wave-ripples 45

4.1 Effect of proximal objects on hippocampal cells spatial selectivity . . . 50
4.2 Effects of objects on place cells spatial resolution 52
4.3 Place cell coding in small vs large environment 58
4.4 Distribution of place fields in a large environment 59
4.5 Distribution of place fields in varied contexts in large environments . . 61
4.6 Distribution of place fields in bats in large environments 63
4.7 Place cell activity in four different large environments 64

5.1 The feedforward connections in the mouse visual system 68
5.2 Types of eye movements 79
5.3 Types of eye movements in mice 80
5.4 Pupil orienting circuit 88
5.5 Pupil correlation with ripple rate 93

7.1 Evolution of VR 104
7.2 VR systems for rodent studies 106

Part I

Introduction

Chapter 1

Introduction to Spatial Navigation

1.1 What is spatial navigation?

One of the key characteristics of living organisms is their ability to actively move. We can observe various types of movements in everything from bacteria and single-cell organisms to plants and animals all around the world. These movements serve various purposes, such as helping organisms navigate their environments in search of food, engaging in social interactions, finding shelter, and migrating to locations with better conditions. Thus, Gallistel, 1990 [1] proposes one of the definitions of navigation: “Navigation is the process of determining and maintaining a course or trajectory from one place to another”.

Growing up in Ukraine, one of the most fascinating natural phenomena to witness is the migration of birds. As a child, regardless of whether you were in the Donbas steppe, the forests of Volhynia, or the coast of Crimea, in every place during early spring, you would see white storks preparing their nests and settling in to start new families for the summer. As the autumn starts, the birds would begin their departure. Adults would tell you that they fly to the "вирій" (vyriy), a warm southern place where all the birds go. They would assure you that the storks would return next spring to the old homes if they were full of goods. As an adult, it remains impressive, despite learning that they migrate not to the "vyriy" but to southern Africa. This raises important questions: How do they know the locations of their winter and summer homes? How do they manage to cover distances spanning thousands of kilometers? How do they choose new nesting sites filled with resources, or decide to return to the same place? And how do they navigate in a rapidly changing world?

There are several mechanisms that help animals navigate. One common mechanism, found across a wide range of organisms from bacteria to mammals, is taxis. Taxis enables an organism to move towards (positive taxis) or away from (negative taxis) a stimulus. Examples of this include a plant orienting its leaves towards sunlight, bacteria moving along a chemical gradient to reach an area with a higher concentration of nutrients, or a dog following the scent of a treat. Taxis is an innate behavioral response that can be achieved through basic sensory processing, where the stimulus acts as a "beacon" guiding the animal’s movement. This form of navigation relies on a simple stimulus-response mechanism that requires the continuous perception of a goal or landmark [2]. While computationally simple—requiring only the association of a single landmark with a goal—this method lacks flexibility. If the beacon moves or disappears, the animal may become disoriented and unable to reach its destination.

Another mechanism, praxic navigation, involves reaching a target through a sequence of predefined movements. This strategy depends solely on the position and movement of the limbs, without reference to the initial starting point. However, praxic navigation is effective only if the starting point remains consistent. This type of navigation is observed in rats performing the Morris water maze task, a classic experiment for studying spatial navigation. When placed in a pool under complete darkness, always at the same location and orientation, rats can learn a stereotyped path to reach a hidden platform [3][4][5].

A further form of navigation is the ability of many animals to return to the starting point of a journey without relying on familiar visual cues. Instead, they continuously estimate their position relative to a reference point, such as their nest, using signals derived from self-motion. However, this method can result in computational errors over long distances. Animals may also navigate using various external cues, such as landmarks, objects in their environment, the Sun, or the Earth's magnetic field.

Migratory birds rely on the sun's position to determine their travel direction as was proven by German zoologist Gustav Kramer (1952) [6]. He observed migratory birds becoming restless, especially close to the time of migration when they were confined in cages. These birds exhibited erratic flapping behavior, often indicating the direction they needed to travel. He noted that on cloudy days, this restlessness decreased, and the birds did not display a clear sense of direction. He hypothesized that their behavior was influenced by the Sun. To test his theory, he strategically positioned mirrors on the cage windows to angle sunlight into the cage rather than directly. Kramer observed that the birds' directional orientation shifted in response to the altered angle of sunlight. Subsequent experiments have revealed that birds, bees (Figure 1.1A), and other animals can detect polarized light, enabling them to determine the sun's position even on cloudy days, as long as a small patch of blue sky is visible.

While the Sun serves as a valuable directional reference, many animals also possess the ability to detect the Earth's magnetic field, offering both directional and positional information. The initial suggestion that animals relied on Earth's magnetic field for determining geographic position dates back to Viguier in 1882 [7]. However, this observation lacked substantial support until more compelling evidence emerged, notably through observations of homing pigeons in the 1980s [8] and experiments conducted by Lohmann in the 1990s [9]. Lohmann's work demonstrated that loggerhead turtles could discern two magnetic parameters, inclination, and intensity, which likely played a role in their magnetic-driven navigation (Figure 1.1B). Similar findings began to emerge in various other species, including salamanders [10], birds [11], and other animals. Furthermore, other studies have been dedicated to the capacity of animals to inherit magnetic direction, a trait that may guide new generations toward warmer regions for wintering (for a detailed review see [12]).

Typically, environmental cues do not work in isolation. Animals often rely on a combination of sensory information, both internal and external, to determine their direction. An illustration of this multisensory navigation can be seen in something as simple as mosquitoes tracking humans on a warm summer night outdoors. Female mosquitoes employ a mix of sensory cues to effectively locate their host. Like many other insects, mosquitoes are naturally drawn to carbon dioxide exhaled by humans and other animals [13]. The detection of CO_2 triggers a strong attraction to visual objects, enabling mosquitoes to approach a host (Figure 1.1C). When in close proximity, they further confirm the host's presence using thermal cues [14]. This attraction

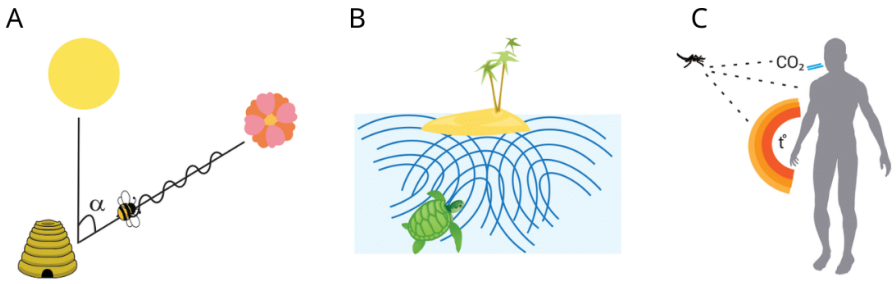


Figure 1.1: Animals could navigate using various external stimuli. (A) The duration of the waggle phase is proportional to the distance between the hive and the food source, and the angle between the axis of the waggle and the vertical direction provides the direction to the food source with respect to the Sun. **(B)** Loggerhead turtles could use magnetic fields for their navigation. **(C)**Female mosquitoes use multisensory cues to locate their host as carbon dioxide exhaled by humans, visual objects, thermal cues.

to visual objects in the presence of CO_2 represents an ingenious way for mosquitoes to be directed toward potential victims by utilizing multisensory information.

As observed in mosquitoes, various insects and animals, including homing pigeons, utilize visual cues either alone or in combination for both short and long-distance navigation and homing behavior [15]. For instance, Tim Guilford and his colleagues demonstrated that homing pigeons employ personalized routes to return home and appear to memorize several landmarks along the way [16]. This underscores the development of a route memory based on experience.

As we can see, animals could use a variety of cues, from self-generated to the external world ones, for different purposes: migration, searching for food, and homing. How do animals utilize these various types of information to navigate, recall their routes home, and explore new paths? To answer these questions, two primary concepts of spatial navigation strategies have emerged over the past decades: path integration and navigation using cognitive maps. In the upcoming chapters, I will delve into their principles and connections with each other.

1.2 Path integration

On a mountain hike, it's crucial to have a map detailing the various routes and landmarks. However, what should you do if you happen to lose your way while wandering around a forest? In such situations, you might rely on natural cues, such as a tree with broken branches, which could serve as a rough indicator pointing you in the direction of your campsite. But what about environments where such landmarks are scarce, like the open sea or deserts? Early sailors solved this problem when navigating the oceans. Their solution involved continuously tracking their position relative to their starting point. They achieved this by carefully measuring their speed and direction of travel, a technique known as "dead reckoning."

Charles Darwin (1873) [17] described a similar phenomenon within native Siberian tribes and suggested that animals might exhibit similar behavior. In response, John Murphy (1873) [18] stated that animals, similar to humans, could engage in "dead reckoning," or in modern terms, "path integration" (Mittelstaedt and Mittelstaedt, 1982) [19] (Figure 1.2A). He hypothesized that animals could keep track of their position by integrating vectors representing both the direction and magnitude of each

movement.

A compelling example of this navigational strategy can be observed in desert ants, Cataglyphis fortis, which can return directly to their nest even after covering extensive distances over featureless terrain during their outward foraging journeys [20]. Throughout their search for food, these ants continuously monitor the distance and direction of their travel. They then consolidate all these path segments into an estimated vector, pointing from their current location back to the nest. This vector guides them homeward as they carry their food haul. Path integration primarily relies on a distance estimate called odometry, often based on a step counter or pedometer. These self-motion-dependent cues are referred to as idiothetic cues and encompass vestibular input, optic flow, and proprioception. As it was shown before, different insects and animals could use compass information from various global cues like the position of the Sun, magnetic-field cues etc. However, it is still nor clear if they contribute to the path integration [21].

In mammals, the first exploration of path integration was done by Mittelstaedt and Mittelstaedt in 1982 [19]. Their study involved female gerbils tasked with retrieving pups that had been moved a considerable distance from the nest to a shallow cup. These gerbils had to locate their pups and navigate back home in darkness without relying on any external landmarks or references, relying solely on cues generated by their own movements. Consequently, it was essential for them to continually update their position relative to their starting point as they embarked on their journey, allowing them to return home at any point. The gerbils demonstrated the ability to locate the pups even in complete darkness and navigate back to the home nest in a straight path, even when the nest had been physically removed from their view. In further experiments involving the rotation of the cup, presumably below the gerbils' vestibular threshold, resulted in trajectory errors of a comparable magnitude. These findings, along with similar experiments conducted by Etienne in 1987, provided strong evidence that rodents employ a homing vector during their path integration strategy.

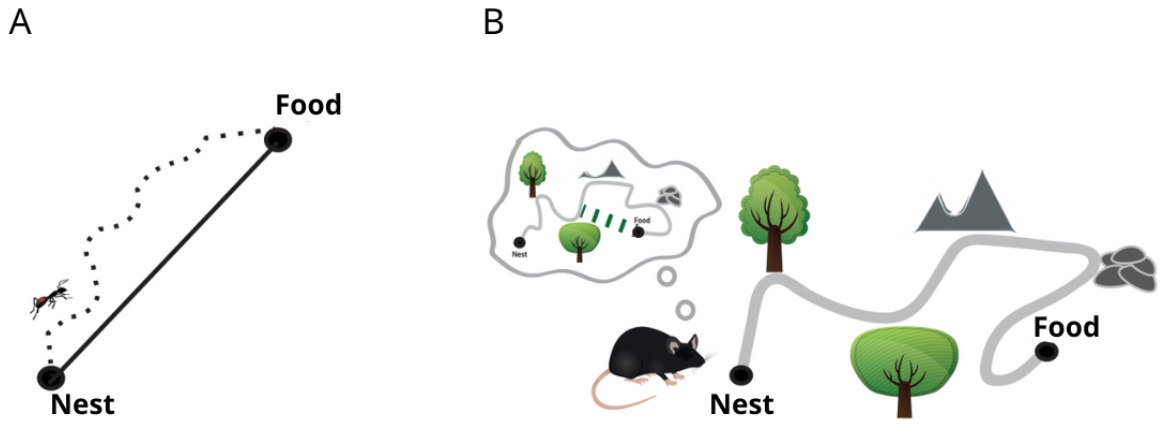


Figure 1.2: Two primary concepts of spatial navigation strategies. (A) Path Integration Strategy: Desert ants can return directly to their nest even after foraging (dashed line) by integrating the distance of their path. **(B)** Cognitive Map Strategy: Animals rely on external world cues to construct a map of their environment. The ability to create shortcuts (dashed line) is one of the characteristics of the cognitive map.

However, such a path integration strategy might work well for short trajectories,

but for longer ones, the integration errors at each step accumulate, leading to a significant error in position estimation. This can be corrected if the animal uses information from outside its body, such as environmental cues that provide details about the external surroundings. These cues help the animal determine its location, direction, or distance relative to objects or landmarks in the environment. Known as allothetic cues, they include visual, olfactory, tactile, and auditory signals, aiding navigation in complex environments rich in diverse sensory information.

Animals likely combine information from path integration with other spatial cues, weighing them according to their reliability. The integrity of the path integration signal can be disrupted by random noise at any point, making it less predictable for the animal. This noise may come from various sources, such as stochastic neural spikes or environmental disruptions that distort sensory cues. Navigation appears to rely on a mix of path integration and external cues, with path integration acting as a default strategy, especially when other options are limited. For navigating over long distances in environments rich in sensory cues, however, additional strategies are necessary.

1.3 Cognitive map

The examples of early navigation studies involving migrating birds, honeybees, and desert ants provide essential insights into how animals can use the sun or magnetic compass to orient themselves and how path integration enables them to return home using only self-motion signals. However, over long distances, this navigation can accumulate various errors, necessitating further recalibration with landmarks.

Any point in space can be defined by its location relative to an infinite number of other points. Consequently, animals face numerous choices when trying to remember the location of a goal. In a real-world environment, animals may hold multiple destination points, each with several routes leading to them and various landmarks that may or may not be usable for navigation. Furthermore, these goals, routes, and landmarks are dynamic and can change or become irrelevant over time. To address these complexities, a new spatial navigation concept has emerged.

In 1948, Tolman [22] conducted a series of behavioral experiments with rats navigating a "sunburst" maze to find a hidden reward. He demonstrated that their navigation could not be explained by a simple stimulus-response scheme because the animals, while searching for the reward, were capable of taking novel shortcuts, showing navigational flexibility instead of relying on known paths. This ability suggests that rats may use cognitive representations of the environment, which Tolman termed a "cognitive map." It's important to note that the term "cognitive map" is not exclusively used to describe navigation but also serves as a tool for organizing knowledge to enable rapid inference from sparse observations [23]. This concept can also be observed in the realm of social and logical cognition, akin to "schemas" in psychology [24][25], which has further evolved into schema-based cognitive therapy and is still widely used today [26].

Following Tolman's discovery, O'Keefe and Nadel (1978) [2] continued existing research on spatial navigation in various animals, supporting Tolman's idea that animals can form cognitive maps. They proposed that, in contrast to the idiothetic information used in path integration, animals rely on external world information (allothetic information) to construct a map of their environment. This map is described by the authors as not being a literal picture but an information structure that can be used to

generate map-like images and behaviors based on place information. This approach offers flexibility, resilience to landmark removal, and multiple paths to the goal. Another study by Morris in 1984 demonstrated rats' ability to navigate toward a hidden platform in a water maze using only visual cues located outside the swimming pool. The rats exhibited the capacity to find the hidden platform from different starting points.

However, most ethological studies were primarily conducted on insects and birds in large-scale environments, while experiments with mammals mostly occurred in small laboratory settings. In 2011, Tsoar et al. [27] used GPS trackers to record trajectories and conducted relocation experiments with cave-dwelling Egyptian fruit bats (Rousettus aegyptiacus) over consecutive nights. They demonstrated, for the first time, that mammals could navigate using cognitive maps over large-scale distances in their natural habitats, supporting O'Keefe and Nadel's concept (Figure 1.2B.

Another theory that complements the cognitive map theory rather than rejecting it is the theory proposed by Leonard and McNaughton in 1989 [28]. They combine path integration with the idea of a cognitive map, suggesting that location comprises various sensory and perceptual experiences that mix with an animal's movement within that location. They propose the existence of a preconfigured path integrator that serves as a general coordinate structure for the environment. To adjust and recalibrate this path integrator, animals (or humans) can use various cues or "local views" within the environment. Thus, from egocentric perceptions, an allocentric map of space is derived. The key difference between O'Keefe and Nadel's and Leonard and McNaughton's models lies in the computational description of their implementation in real-world environments. In O'Keefe and Nadel's model, the allocentric map stores relationships between landmark cues, which may take longer to form in a new environment. In Leonard and McNaughton's model, relations between landmarks are associated with a preconfigured coordinate system, requiring less space to store information about the environment.

Further investigations into mammalian navigation in large-scale environments, coupled with the discovery of neural correlates of space by O'Keefe and Dostrovsky in 1971 [29], have boosted updates to concepts related to cognitive maps, such as spatial schemas and others [30][31][32][33]. In the following sections, I will introduce the anatomy and connectivity of the hippocampal formation, a brain region known to play a crucial role in storing spatial information, and after, in Chapter 3, I will discuss studies on neurophysiological discoveries related to neural networks that support spatial navigation, complementing various spatial theories.

Chapter 2

Hippocampal Formation

The role of the hippocampus in memory began to emerge through a series of scientific studies and observations in the mid-20th century. One of the key figures in this development were William Scoville and Brenda Milner. Their work with a patient known as H.M. played a pivotal role in our understanding of the hippocampus's role in memory [34]. H.M. underwent a bilateral medial temporal lobectomy to surgically remove the front two-thirds of his hippocampi, along with the parahippocampal cortices, entorhinal cortices, piriform cortices, and amygdalae, in an effort to treat his epilepsy. While the surgery successfully reduced his seizures, it had a profound impact on his memory. After the surgery, H.M. suffered from anterograde amnesia, which means he could no longer form new long-term memories. However, his short-term memory and some aspects of his cognitive function remained intact. Brenda Milner began studying H.M. in the 1950s and showed that the removal of his hippocampus had a specific impact on his ability to acquire and consolidate new factual information, such as names, places, and events. This led scientists to propose that the hippocampus played a crucial role in forming and storing explicit memories, including spatial memory. Subsequent research provided further evidence of the hippocampus's involvement in spatial navigation in humans. For example, a famous study of London taxi drivers revealed that qualified taxi drivers have a larger hippocampus (specifically the posterior part) compared to non-taxi drivers, and the size of the hippocampus was positively correlated with the duration of their taxi-driving careers [35]. Additional support for the role of the human hippocampus in spatial memory comes from studies involving patients, particularly those suffering from Alzheimer's disease [36].

In rodents, significant contributions to our understanding of the hippocampus and its role in spatial memory and navigation were made by John O'Keefe and Lynn Nadel in 1978 [2]. In their work, they elucidated the role of the rodent hippocampus in spatial navigation and the underlying physiological mechanisms. In this chapter, I will describe the structure and specific features of the hippocampal formation, which are crucial for a deeper understanding of the physiological basis of spatial navigation in this brain region, as discussed in Chapter 3.

2.1 The notion of hippocampal formation

The hippocampus, situated in the temporal lobe, is a crucial brain structure known for its fundamental role in memory and spatial cognition. Historically, Andreas Arantius, a prominent anatomist from the 16th century, coined the term "hippocampus" to

describe a unique human brain structure he observed on the floor of the temporal horn. This structure reminded him of the hippocampus creature from Greek mythology. Its name originates from the Greek words "hippos" (ιππς) meaning "horse," and "kampos" (καμπς) meaning "sea monster" or "sea creature." Thus, the term "hippocampus" persisted through the centuries.

Some scientists compared the curved formation of the hippocampus to a ram's horn. In 1742, De Garengeot named this structure "cornu ammonis (CA)" or "Ammon's horn," in reference to the mythological Egyptian god Amun Kneph, who was symbolized by a ram. This classical nomenclature is still used to describe the distinct subregions of the hippocampus, conventionally divided into CA1, CA2, and CA3. The functional counterparts of the hippocampus can be identified in the earliest vertebrates, notably those where the neocortex is minimal or absent. In teleosts like goldfish, this corresponds to the medial pallium, and in modern reptiles like turtles and lizards, it corresponds to the medial cortex [37].

Anatomically and functionally, the hippocampus forms a critical component of the larger hippocampal formation. The other parts of the hippocampal formation are the dentate gyrus, subiculum, presubiculum, and parasubiculum. It is also associated with the entorhinal, perirhinal, and parahippocampal cortices to form the medial temporal lobe (MTL) [38][39]. In humans, the hippocampus is less curved and has a more tube-like shape, whereas in rodents, it has a more pronounced "C" shape or "banana-like" curve. Its long, curved form is present across all mammalian orders and runs along a dorsal (septal) to the ventral (temporal) axis in rodents, corresponding to a posterior-to-anterior axis in humans (Figure 2.1).

Parts of the hippocampal formation have a non-homogeneous cortical structure. The hippocampus proper, dentate gyrus, and subiculum belong to the all cortical brain regions, which are evolutionarily considered to be more ancient and have fewer than 6 cortical layers. These three-layered cortical regions typically have a single neuronal cell layer with fiber-rich plexiform layers above and below the cell layer. The other parts of the hippocampal formation, presubiculum, parasubiculum, and entorhinal cortex (EC), exhibit a multilaminate structure and are grouped together under the term retrohippocampal region.

Comparing how the different parts of the hippocampal formation are connected to each other could help understand how these six areas with their unique structures are grouped together. In the neocortex, it's common for one region to send signals back and forth with another [40]. However, this is not the case for the connections among the parts of the hippocampal formation, as first described by Ramón y Cajal (1893) [41]. The entorhinal cortex is like the starting point of the inner hippocampal circuit. It gets a lot of information from the neocortex and passes it on to the granule cells in the dentate gyrus. This creates a one-way pathway called the perforant path. But the dentate gyrus doesn't send information back. Instead, the granule cells make connections with pyramid-shaped cells in the CA3 region of the hippocampus using mossy fibers. These CA3 cells then send signals to the CA1 area, which, in turn, sends signals to the subiculum.

From there, a part of the subiculum sends signals to the pre- and para-subiculum areas. Another part of the subiculum and the CA1 cells send signals deeper into the entorhinal cortex. This loop connects everything together, starting from the outer entorhinal cortex and ending in its deeper layers, showing that different parts work together, not separately. Early writings about the hippocampal formation focused on the first three connections in this circuit, which were grouped as the "trisynap-

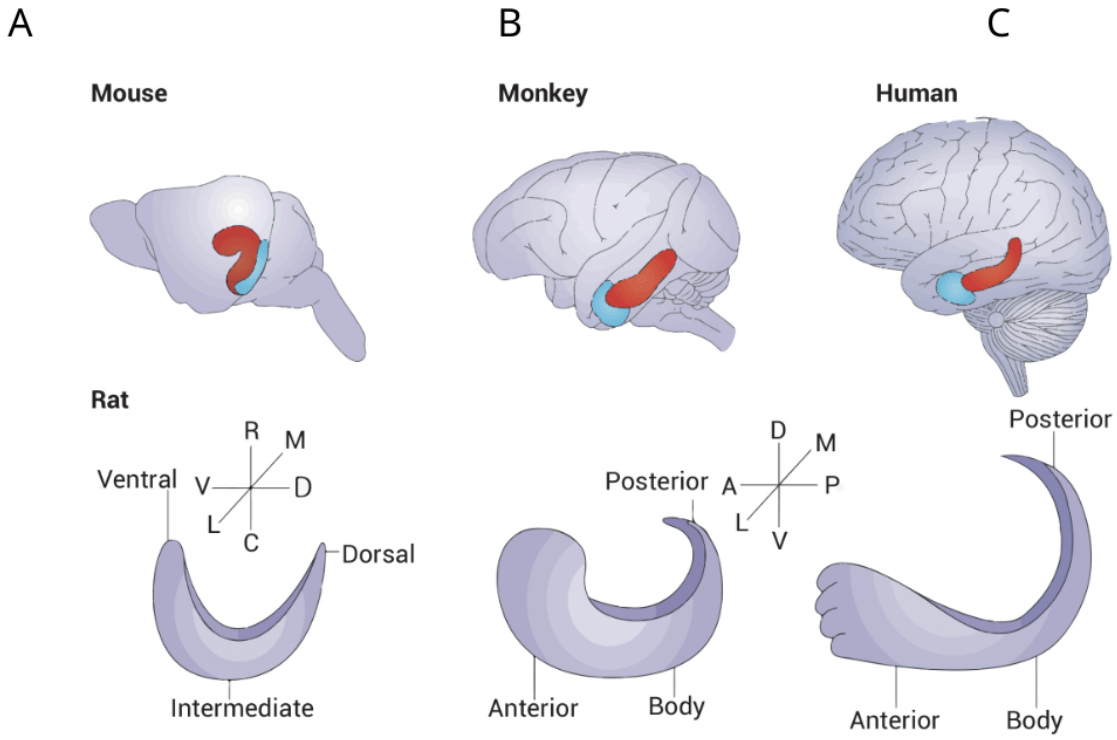


Figure 2.1: Hippocampus and entorhinal cortex. The full long axis of the hippocampus (red) in brains of mouse **(A)**, macaque monkeys **(B)** and humans **(C)**, with the entorhinal cortex in blue and schematic illustrations of the orientation of the hippocampal long axis respectively. The longitudinal axis is described as ventrodorsal in rodents and as anteroposterior in primates. A 90-degree rotation is required for the rat hippocampus to have the same orientation as that of primates. (Adapted from Strange et al., 2014 [42])

tic circuit" [43]. Moving ahead, we will look deeper into the structures within the hippocampal formation and its internal and external connections. These structural characteristics are crucial for supporting memory in the hippocampus, particularly spatial memory.

While the way the hippocampal formation is divided into regions remains similar in both rat and monkey brains, there are notable differences in certain subregions, especially CA1 and the entorhinal cortex. Later, we will discuss the main distinctions among human, primate, and rodent hippocampal formations.

2.2 Anatomy and connectivity of the hippocampal formation

2.2.1 Dentate gyrus

The dentate gyrus (DG) serves as the primary pathway for information entry into the hippocampus via the perforant path, discovered by Santiago Ramón y Cajal, in 1893 [41].

The dentate gyrus is a trilaminar cortical region with a distinct V or U shape. Its major cell type is the granule cell. In terms of cytoarchitectonic organization, it consists of three layers along the dorso-ventral axis: the superficial molecular layer

(*Stratum Moleculare*), which contains few cell bodies; the principal cell layer, containing densely packed granule cells (*Stratum Granulosum*); and a polymorphic cell layer, representing the third stratum of the dentate gyrus. This third layer is often referred to in the literature as the *hilus* [44]. The *Stratum Moleculare* is occupied primarily by dendrites of the granule, basket, and polymorphic cells as well as axons and terminal axonal arbors from the entorhinal cortex and other sources. Granule cells located in the *Stratum Granulosum* form a densely packed layer with small diameters (8-12 µm). Their dendritic tree is confined to the adjacent molecular layer. Between the *Stratum Granulosum* and the *hilus* lies the subgranular zone, a unique niche in the adult mammalian brain containing stem cells capable of differentiating into mature granule cells [45]. These granule cells integrate into the existing adult network. The axons of granule cells, known as mossy fibers, extend to the *hilus* and branch into multiple collaterals. In the hilus, these collaterals make contact with inhibitory interneurons and mossy cells [46].

The dentate gyrus serves as the initial component of the trisynaptic loop, projecting specifically to the CA3 region of the hippocampus via mossy fibers. These projections, which exclusively originate from granule cells, terminate in a confined zone just above the CA3 pyramidal cell layer [47][48][49][46]. Computational models of the hippocampus highlight the DG for its role in strong and flexible pattern separation, processing overlapping inputs from the entorhinal cortex into distinct neural representations. The separated signals are then projected onto the CA3 subfield, where CA3 receives three primary excitatory inputs: mossy fibers from DG granule cells, the direct perforant path input from Layer II of the EC, and recurrent collateral input from CA3 neurons themselves.

The mossy fiber pathway, a powerful unidirectional input from the DG, forms large synapses on the proximal apical dendrites of CA3 pyramidal cells, which robustly depolarize CA3 neurons. This suggests that the mossy fibers are crucial for introducing new pattern-separated representations to CA3, reducing interference, and supporting new learning [50].

2.2.2 Hippocampus

The hippocampus proper was largely described by Ramon y Cajal. He was the first who describe two major regions of it: a large-celled region that adjoins dentate gyrus and a smaller-celled region that follows from it. His student, Lorente de Nó, provided a more detailed characterization of this region and introduced modern nomenclature. Presently, the expansive cellular region is recognized as CA3 and CA2 segments, while the more compact cellular area is identified as CA1. The laminar arrangement within these two regions is commonly subdivided into five layers: *Stratum Oriens*, *Stratum Pyramidale*, *Stratum Lucidum* (in CA3), *Stratum Radiatum* and *Stratum Lacunosum Moleculare* (Figure 2.2).

The *Stratum Oriens*, is a relatively unobstructed cellular layer containing basal dendrites of pyramidal cells, inhibitory basket cells and horizontal trilaminar cells. The primary cellular layer *Stratum Pyramidale*, known as the pyramidal layer, is densely populated with pyramidal cells in CA1, and to a lesser extent in CA2 and CA3. The subsequent layer in CA3, the *Stratum Lucidum*, is positioned immediately above the pyramidal cell layer and is occupied by mossy fibers. Towards its septal extent, particularly within the hippocampus, the *Stratum Lucidum* slightly thickens, indicating the temporal bending and longitudinal course of the mossy fibers. This

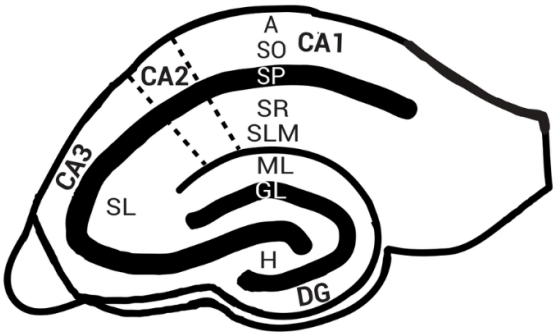


Figure 2.2: Schema illustrating the different layers of the hippocampus in a transverse slice. A: *Alveus*, SO: *Stratum Oriens*, SP: *Stratum Pyramidale*, SR: *Stratum Radiatum*, SLM: *Stratum Lacunosum Moleculare*, GL: *Stratum Granulosum*, ML: *Stratum Moleculare*, H: *Hilus*, SL: *Stratum Lucidum*.

thickening serves as a demarcation between the CA3 and CA2 regions of the hippocampus, however, part of mossy fibers can be found in CA2 region [51]. Found above the *Stratum Lucidum* in CA3 and directly over the pyramidal cell layer in CA2 and CA1, the *Stratum Radiatum* houses the suprapyramidal region. This is where the CA3 to CA3 associational connections and the CA3 to CA1 Schaffer collateral connections are situated. The outermost layer of the hippocampus, termed the *Stratum Lacunosum Moleculare*, receives terminations from fibers originating in the entorhinal cortex and the nucleus reuniens of the midline thalamus. Further elaboration on the detailed architecture and connectivity of the CA regions will be provided in the subsequent sections.

2.2.2.1 CA3

As previously discussed, the hippocampus features a prominent cell layer known as the pyramidal layer, comprised of pyramidal cells' soma that represent a major cell type within this brain region. These cells possess basal dendritic extensions that stretch into the *Stratum Oriens*, and apical dendritic extensions that reach toward the hippocampal fissure. Furthermore, in line with earlier explanations, the most proximal segment of CA3 pyramidal cells dendrites in the *Stratum Lucidum*, is targeted by mossy fibers originating from the dentate gyrus.

This Ammonic subfield can be subdivided into three additional subregions along its proximo-distal axis, as proposed by Lorente De Nó in 1934 [52]. The first subregion, CA3c, penetrates the hilus, followed by CA3b, and finally, CA3a, which corresponds to the curved portion adjacent to CA2. Notably, it has been demonstrated that the length and distribution of dendritic trees among CA3 pyramidal neurons are non-uniform and contingent upon the location of the cell body, a trend that gradually shifts along this proximo-distal axis. Cells situated in the *hilus* of the dentate gyrus exhibit fewer dendritic extensions within the *Stratum Lacunosum-Moleculare* layer and receive minimal input from the entorhinal cortex. Instead, they are contacted by a substantial number of mossy fiber terminals on thorny excrescences on both their apical and basal dendritic trees and are therefore heavily influenced by the granule

cells.

The CA3 region of the hippocampus exhibits several distinct intrinsic connectivities that set it apart (Figure 2.3). These include the CA3 to CA1 Schaffer collateral connections, CA3 to CA3 associational connections, and commissural projections extending to contralateral CA3, CA2, and CA1 regions. These CA3 projections have a broad distribution and are meticulously arranged throughout the hippocampal formation, showcasing a systematic gradient-like projection pattern. However, these projections are absent in subiculum, presubiculum, parasubiculum, and entorhinal cortex. The distribution of Schaffer collateral terminals is influenced by the location of their originating CA3 cells. Studies in rats have shown that individual CA3 pyramidal cells exhibit complex and distinct patterns of connectivity, with extensive axonal arborization, especially in the CA1 region, spanning about two-thirds of the hippocampus's longitudinal axis. Most recurrent collaterals from a given neuron remain within the same subfield (CA3a, b, or c) as their parent cell. In these subfields, CA3a cells near the CA2 region have more localized collaterals and primarily contact CA1 pyramidal cells near CA2 on their basal dendrites. Cells in the CA3b subfield target CA1 pyramidal cells located in the middle of the proximo-distal axis, while CA3c cells near the *hilus* project mainly onto the apical dendrites of CA1 and CA3 cells. CA3c neurons also have numerous collaterals in the hilus, with some projections reaching the inner third of the molecular layer and the subiculum. This distribution of axon collaterals into "patches" suggests that certain subpopulations of neurons receive denser innervation, whereas others receive sparser input [53].

The organization of the CA3 to CA3 associational connections is highly systematic. Proximal CA3 cells exclusively communicate with other nearby proximal CA3 cells. Conversely, associational projections originating from the middle and distal sections of CA3 span extensively across the transverse dimension of CA3, while also projecting extensively along the septotemporal axis. Notably, the density of these associational projections undergoes shifts along the septotemporal axis The strong recurrent connectivity between CA3 pyramidal neurons showed an ability to retrieve stored output patterns when presented with partial input patterns (pattern completion) [54][55][56][57][58]. These observations gave rise to a theory of hippocampal function where the CA3 recurrent circuit operates as a single attractor or auto-associative network. Which enables rapid association between the pattern of memories and provides the completion of the whole memory episode during its recall.

In rats, it has been observed that the same CA3 cells give rise to both ipsilateral and commissural projections to the CA3, CA2, and CA1 fields of the hippocampal formation in the opposite hemisphere [59][60]. While the commissural projections within the hippocampus generally exhibit a comparable topographical arrangement to the ipsilateral projections, often terminating in corresponding regions on both sides, there are slight variations in the distribution of terminals. These subtle disparities suggest that while the overall pattern is alike between the two hemispheres, nuanced differences exist in how the projections are dispersed on each side. However, these commissural connections are less prevalent in primates and are likely absent in humans.

For the extrinsic input to CA3 it is also worth mentioning entorhinal cortex projection. The collaterals of the perforant path fibers that project to the dentate gyrus also project to the hippocampus. They have a similar origin and laminar terminal distribution [61]. The projections from the entorhinal cortex to CA3/CA2 come from cells in layer II. Moreover, the same layer II cells also send collaterals that reach both

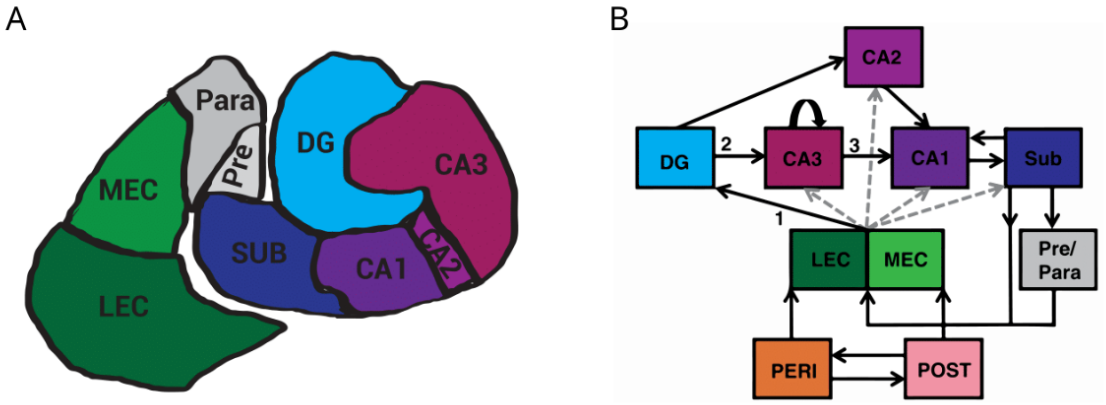


Figure 2.3: Structure and connectivity of the hippocampal formation. **(A)** The general structure of the hippocampal formation. **(B)** The general connectivity within the hippocampal formation. The direction of the trysinaptic loop is indicated by numbers. (Adapted from Poulter et al., 2018 [64])

the dentate gyrus and CA3/CA2 [62][63]. This suggests that similar information is conveyed to both structures, establishing a connection between them.

2.2.2.2 CA2

CA2, a section of the hippocampus proper, is typically excluded from the trisynaptic loop. Nevertheless, recent findings regarding its composition and connections highlight that CA2 holds a distinct role in processing information flow within the hippocampus [65][66][67][68].

In 1934, Lorente de Nó undertook the task of describing distinct cytoarchitectural and connectivity variations within the region known as regio inferior, as previously observed by Ramón y Cajal. He identified three distinct CA areas and highlighted significant differences among them. His findings revealed that pyramidal cells in CA2 boast comparable cell body sizes to those in CA3, as opposed to CA1. However, these CA2 pyramidal cells lack the specialized thorny excrescences characteristic of CA3 pyramidal cells, which receive input from mossy fibers stemming from the dentate gyrus. Furthermore, similar to CA1 cells, CA2 neurons also receive Schaffer collateral input from CA3 neurons.

Recent advancements in molecular, genetic, and connectivity studies centered around the CA2 region have illuminated its distinctive characteristics and prompted a reevaluation of its definition. Molecular markers specific to CA2 pyramidal neurons, including α-actinin 2 (ACTN2), Purkinje cell protein 4 (PCP4), striatum-enriched protein-tyrosine phosphatase (STEP), a regulator of G protein signaling 14 (RGS14), transmembrane AMPA receptor regulatory protein γ5 (TARP γ5), and others, exhibit noteworthy overlap, collectively identifying a substantial portion of CA2 pyramidal neurons [68][69][70][71]. Remarkably, a significant proportion of these neurons also align with an area housing axons from the dentate gyrus. Recent mouse-based research provides conclusive evidence that functional synapses form between DG axons and specific CA2 pyramidal neurons, recognizable through the expression of RGS14. This compellingly confirms that a significant molecularly defined segment of CA2 indeed receives input from the DG [51][72].

Additionally, recent studies challenge the traditional view of the mossy fiber pro-

jection from the DG to CA3, which was believed to run across lamellae of restricted width along the transverse axis of the hippocampus. Instead, these studies reveal that DG projections change direction upon reaching CA2, continuing along the long axis towards the ventral hippocampus. This indicates that information from the dorsal and intermediate DG can be transmitted caudally to the intermediate and ventral hippocampus, respectively [51].

The CA2 receives external inputs from vasopressinergic neurons in the paraventricular nucleus (PVN) and the median raphe. Furthermore, there exists reciprocal connectivity between CA2 and the supramammillary nucleus (SuM), the medial septum, and the diagonal bands of Broca [73]. The primary target of projections originating in area CA2, akin to those from area CA3, is the CA1 region within the hippocampus. However, there are three key distinctions within these projections.

Firstly, CA2 pyramidal neurons primarily project to the *Stratum Oriens*, a region occupied by the basal dendrites of CA1 pyramidal neurons. In contrast, CA3 pyramidal neurons mainly project to the *Stratum Radiatum*, encompassing the apical dendrites of CA1 pyramidal neurons. Secondly, in the *Stratum Oriens*, CA2 axons specifically target dendrites from a row of adjacent CA1 pyramidal neurons (Figure 2.4). Conversely, CA3 neurons target the row of CA1 pyramidal neurons positioned next to the *Stratum Radiatum*. Thirdly, the projection from CA2 to CA1 extends widely along the longitudinal axis of the rat hippocampus, spanning a considerable distance. However, the specifics of this longitudinal spread in comparison to CA3 projections remain unclear. These longitudinal projections from CA2 likely contribute to integrating information from the dorsal hippocampus into the more ventral regions. In addition to the projections directed towards CA1, the axons of CA2 pyramidal neurons, resembling those of CA3 neurons, extensively branch within both area CA2 and CA3, thereby establishing a high degree of local and recursive interconnectivity [74][72].

2.2.2.3 CA1

The pyramidal cells in the CA1 region are not only densely packed but are also smaller compared to other regions of the hippocampus, with a more regular morphology. In comparison to the CA3 layer, the cell bodies are thinner, emit a single dendrite that splits into the *Stratum Radiatum*, and typically end in a fine arborization in the *Stratum Lacunosum Moleculare*, often reaching the hippocampal fissure. Basal dendrites grow extensively in the *Stratum Oriens* and sometimes reach the alveus. The dendritic trees of CA1 pyramidal cells are more extensive but less compact, nearly three times longer than those of the granule cells in the dentate gyrus [75]. Despite their appearance, CA1 pyramidal cells, while not clearly segregated into distinct layers at first glance, are organized along a gradient of distinct morphological, molecular, and physiological features along the radial axis [76][77][78]. To describe this radial distribution, the terminology is borrowed from cortical anatomy, the deep and superficial parts of the layer are defined by the location of the neurons' basal versus apical dendrites. Superficial CA1 (sCA1), adjacent to the *Stratum Radiatum*, lies beneath deep CA1 (dCA1), which is closer to the *Stratum Oriens*. The *Stratum Pyramidale* consists of a stack of 5 to 8 cells spread over tens of micrometers in mice and a hundred in rats [79]. The sCA1 is more densely populated, with cells moderately smaller than those in the deeper layer. Another difference is that pyramidal cells in the superficial layer are less coupled by gap junctions, though the role of gap junctions in CA1 remains

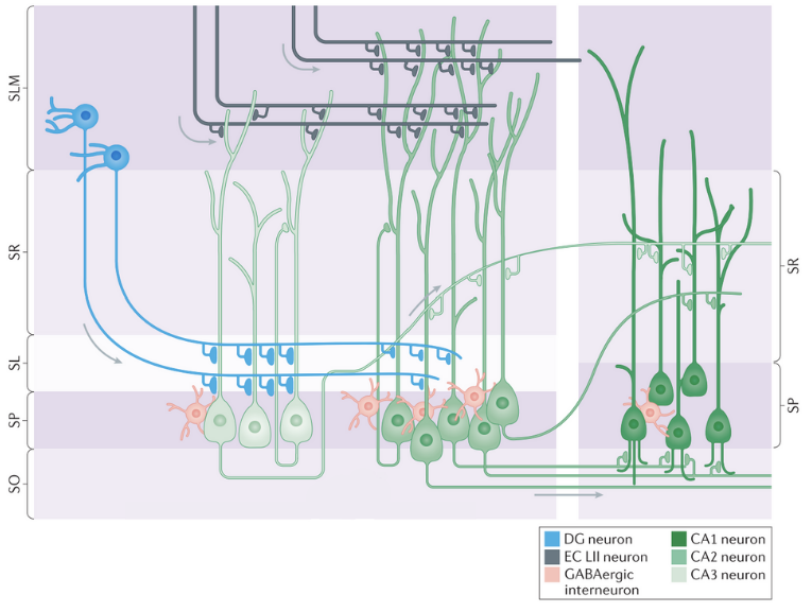


Figure 2.4: Intrinsic connectivity of the hippocampal neurons in rodents. Dentate Gyrus (DG) granule cells (blue) interact with CA3 (light green) in *Stratum Lucidum* and slightly with CA2 cells (medium green). Both CA3 and CA2 are connected to medial and lateral entorhinal cortex layer II (EC LII) neurons (grey) targeting the *Stratum Lacunosum-Moleculare* (SLM). CA2 neurons receive CA3 neuron projections targeting *Stratum Radiatum* (SR). CA1 neurons (dark green) receive projections from CA3 via Schaffer collaterals in *Stratum Radiatum* and to a smaller extent from CA2. CA2's main efferent projections aim at the basal dendrites of deep calbindin-negative CA1 neurons in *Stratum Oriens* (SO). CA2's *Stratum Pyramidale* has a higher density of reelin- and parvalbumin-positive interneurons (pink) compared to CA1 and CA3. (Adapted from Dudek et al. 2016 [68])

unclear [80][81]. This segregation along the radial axis may represent a general organizational principle in the hippocampus, potentially increasing its capacity for parallel task processing [82][76].

Regarding connectivity, CA1 is the final stage of the trisynaptic circuit and receives most of its input from CA3 axons via Schaffer collaterals (Figure 2.4). The distal part of CA3 projects to proximal CA1, while proximal CA3 projects to distal CA1 [83]. The third stage of the trisynaptic loop involves the projection from CA1 to the subiculum, where the proximal part of CA1 projects to the distal subiculum, and distal CA1 projects to its proximal part [84][85]. The backprojection from CA1 to CA3, as well as from the subiculum to CA1, has been found. The backprojection from CA1 to CA3 likely arises from inhibitory neurons in the *Stratum Radiatum* and *Stratum Oriens* of CA1, projecting to the same layers in CA3 [85]. The backprojection from the subiculum to CA1 originates from neurons in the *Stratum Pyramidale* of the subiculum, projecting to all layers of CA1 [86].

The projection from the entorhinal cortex to the CA1 region of the hippocampus displays significant differences compared to the projection to CA3/CA2. Firstly, the origin of the CA1 projection can be traced back to cells located in layer III of the entorhinal cortex, while the projection to CA3/CA2 originates from layer II. Secondly, the arrangement of terminals within the CA1 projection is characterized by a topographical organization, distinct from the laminar layout seen in the CA3/CA2 projection. Similar to CA3/CA2, the entorhinal fibers terminating in CA1 span the entire width of the *Stratum Lacunosum-Moleculare*. However, there is a notable divergence in their termination sites: fibers from the lateral entorhinal area target the

distal portion of CA1 (near the subiculum), whereas fibers from the medial entorhinal area target the proximal portion of CA1 (close to CA2). Consequently, depending on the position of a CA1 pyramidal cell along the transverse axis of the hippocampus, it will receive inputs from distinct sections of the entorhinal cortex. Despite having consistent dendritic lengths, CA1 cells receive unique inputs based on their location along the proximo-distal axis.

2.2.3 Subiculum, pre- and parasubiculum

The subicular complex comprises various cortical regions positioned between the hippocampus proper's area CA1 and the entorhinal cortex below, and CA1 and the retrosplenial cortex above [87]. This "subicular complex" is typically categorized into three subdivisions: the subiculum proper, the presubiculum, and the parasubiculum [88].

Despite their similarities, it is crucial to understand that they have unique neuroanatomical characteristics and should be viewed as separate cortical areas.

The line between CA1 and the subiculum is marked where the Schaffer collateral projection from the CA3 ends. In rodents, this point is where the dense layer of cells in CA1 changes into the thicker layer of the subiculum. Next to the subiculum is the presubiculum, which has more layers than the usual three found in other areas like the dentate gyrus, hippocampus, and subiculum. The presubiculum's outer layer is densely packed with small pyramidal cells that are close together. On the other hand, the parasubiculum has a wedge-shaped layer II with cells similar to those in the presubiculum, but they are a bit larger and not as tightly packed.

2.2.3.1 Subiculum

The subiculum is a component of the archicortical structures, characterized by its three distinct layers. These layers encompass: a molecular layer, seamlessly continuing into the *Stratum Lacunosum-Moleculare* and *Stratum Radiatum* of the CA1 field, an expanded pyramidal cell layer housing the cell bodies of principal neurons, and a polymorphic layer. Notably, the pyramidal cell layer in the subiculum exhibits a less compact arrangement compared to the CA1 area [89].

The main cell layer of the subiculum is dominated by large pyramidal neurons, constant in size and shape. These neurons extend their apical dendrites into the molecular layer while sending their basal dendrites deeper into the pyramidal cell layer. Alongside the pyramidal cells are numerous smaller neurons, known as subiculum interneurons [87][90]. The polymorphic layer sits deeper than the pyramidal cell layer and connects with the *Stratum Oriens* of the CA1 region. Recent research using immunohistochemistry [91] has provided more details about the subiculum's inner structure. They categorized it into two parts: proximal (near the CA1 region) and distal (further away from CA1), based on differences in cell architecture and how they react to calbindin [92].

The subiculum gets direct inputs from the hippocampal CA1 area and sends signals to various parts of the brain's cortex and subcortex. By spreading its connections far and wide, the subiculum appears to play a critical role in organizing the information coming out of the hippocampus. The subiculum gets inputs primarily from the CA1 area and entorhinal cortex (Figure 2.3). The way CA1 projects to the subiculum in the horizontal plane is similar to how CA3 projects to CA1. Neurons in the far end of CA1

(near the subiculum border) strongly connect with neurons in the nearby subiculum, while neurons near the CA2 area in CA1 connect with the distant subiculum near the presubiculum [85][93][94]. The subiculum also gets major inputs from layer III of the medial and lateral entorhinal cortices [95]. The entorhinal cortex's projection to the hippocampal formation is specific: layer III sends signals to CA1 and the subiculum, while layer II stellate cells send signals to the dentate gyrus, CA3, and CA2 [96][95][97][62][61]. Layer II pyramidal cells in the medial entorhinal cortex, also known as island cells, connect with CA1 and with the subiculum [98].

The ventral part of the anterior thalamic nuclei project to the temporal subiculum, while the dorsal part doesn't [99] (though see [100][101]). This suggests that the subiculum may receive signals related to head direction, modulated by theta rhythm from the anteroventral thalamic nucleus [102][100][103]. Additionally, the basal amygdala [104] and the nucleus reuniens of the thalamus [105] also send signals to the subiculum.

The dorsal subiculum sends connections to cortical and subcortical areas, including retrosplenial cortex, medial prefrontal cortex, entorhinal cortex, perirhinal cortex, and postrhinal cortex [106][107][108][49][109][94]. It also links to subcortical regions like the hypothalamic nuclei, nucleus accumbens, midline thalamic nuclei, and lateral septum [110][111][112]. The subiculum also communicates back to the CA1 area [113][114][86][115][116][117][118]. Some subiculum neurons also connect with newly formed granule cells in the dentate gyrus linked to memory and emotions [119][120][121].

2.2.3.2 Presubiculum and parasubiculum

Both the presubiculum and the parasubiculum, in contrast with the subiculum, are multilaminar structures. The presubiculum stands apart from the subiculum due to its closely arranged pyramidal cell layer located just above a cell-free zone (layers II and III), which seamlessly connects to the lamina dissecans in the entorhinal cortex (layer IV). Within these upper layers, small pyramidal cells are prevalent. Beneath layers II and III of the presubiculum lie its deep layers (below the lamina dissecans), which appear to extend continuously into the deep layers of the entorhinal cortex and the principal cell layer of the subiculum [87]. These deep layers comprise both pyramidal cells and a collection of polymorphic cells [52].

On the other hand, the parasubiculum distinguishes itself from both the presubiculum and the entorhinal cortex. Its layers II and III contain large, relatively densely packed pyramidal cells. Similarly to the presubiculum, its deeper layers seamlessly continue into those of the entorhinal cortex. However, unlike the presubiculum, there is no clear distinction between layers II and III.

While both the presubiculum and parasubiculum receive inputs from the subiculum, these inputs are not robust, and neither area appears to receive substantial input from the primary hippocampal fields (such as CA1 and CA3). This is unlike the subiculum itself, which receives a strong and extensive projection from CA1. However, similar to the EC, both the presubiculum and parasubiculum do receive significant direct input from the neocortex.

The presubicular projection is directed solely to the medial entorhinal cortex (Figure 2.3), where it primarily terminates in layer III and, to a lesser extent, in the deep part of layer I. This projection to the entorhinal cortex is topographically organized, with the location of the presubicular terminal field determined by both the

proximo-distal and dorsoventral positions of the presubicular cells of origin. The parasubiculum, in contrast, selectively innervates layer II of the entorhinal cortex. Unlike the presubiculum, the parasubiculum projects to both the medial and lateral entorhinal areas, although its projection to the lateral entorhinal area is less robust. Notably, Amaral and Witter (1995) emphasized the strong association of the presubiculum and parasubiculum with the anterior thalamic nucleus, suggesting a crucial pathway for thalamic influence on hippocampal formation. Lesions in the anterior thalamus have also been shown to be crucial for spatial firing activity in the subiculum, which is consistent with this assumption [122]. Thus, these areas may serve as an important channel through which the thalamus influences the hippocampal system.

2.2.4 Entorhinal cortex

When we talk about the entorhinal cortex, it is important to mention its crucial role in the flow of information through the hippocampal formation. It is the primary entry point for sensory data and the main channel for transmitting processed information back to the neocortex. Thus, an important loop of information within the hippocampal formation begins and ends in the entorhinal cortex. The entorhinal cortex exhibits a complex laminar structure. There are significant variations in the connectivity patterns among the layers of the entorhinal cortex. These patterns of connectivity are also consistent among rodents and primates, and have been broadly described using diverse molecular markers and genetic techniques [63][123][124].

The entorhinal cortex can be divided into two main regions: the lateral entorhinal cortex (LEC) and the medial entorhinal cortex (MEC). In rodents, the distinction between these two areas became particularly evident in their projections to the dentate gyrus. Axons from the LEC terminate in the outer third of the molecular layer, whereas axons from the MEC terminate in the middle third [125][126]. This medial versus lateral entorhinal cortex contrast was further supported by subsequent research, which revealed disparities in connectivity patterns and functional roles between these two regions.

In the entorhinal cortex studies, there are two main schemes of laminar organization that are commonly used. The first one suggested by Ramón y Cajal and modified later, defines six layers with four cellular layers (II, III, V, VI) and two acellular or plexiform layers (I, IV). The fourth layer is also called Lamina Dissecans. Lorente de Nó also proposed a six-layer scheme with five cellular layers (II, III, IV, V, VI) and a cell-free Lamina Dissecans (layer IIIb) between layers III and IV. Nowadays, this scheme is also adapted, with layer four being marked as a cell-free Lamina Dissecans.

Layer I of the entorhinal cortex is a cell-poor layer, containing mostly transversely oriented fibers. Layer II consists mainly of medium-sized to large stellate cells, along with small pyramidal cells grouped in clusters, especially in the lateral entorhinal area. Layer III contains cells of varying sizes and shapes, with a prevalence of pyramidal cells. The cellular-free layer IV is most prominent in parts of the entorhinal cortex adjacent to the rhinal fissure, particularly in the caudal regions of the cortex. In recent times, examinations of gene expression patterns in mice have uncovered a division within layer V consisting of two sublayers. The neurons in a Va express the transcription factor Etv1, while the lower sublayers marked by neurons expressing the transcription factor Ctip2 which helped in their further more detailed description. The principal neurons in each of these sublayers exhibit discernible differences in terms of their physical structure, projection pathways, and electrophysiological characteristics

[127][128]. Lastly, layer VI is composed of cells of varying sizes and shapes, forming a diverse population. The density of these cells decreases as it approaches the boundary with the white matter.

The MEC and LEC show notable differences in their laminar organization. In layer II of the MEC, there is a mix of excitatory, medium-sized pyramidal neurons and large multipolar neurons, known as stellate cells (SCs). On the anterolateral side, the laminar structure is similar but less uniform. In the LEC, layer II contains a combination of large multipolar neurons, fan cells, along with pyramidal and medium-sized multipolar neurons [63].

Not only structural distinctions but also variations in connectivity between MEC and LEC have contributed to their classification as two distinct entorhinal subregions. The idea that LEC and MEC serve as entorhinal areas operating within two parallel input-output pathways, responsible for encoding and storing "what" and "where" information, respectively [129][130].

The LEC receives stronger cortical projections than the MEC, with one-third of its input coming from cortical areas, including the piriform cortex and olfactory bulb, while the MEC receives about one-fifth [131][132]. The LEC is more connected to the frontal and insular cortices, whereas the MEC is more heavily innervated by the temporal, parietal, and occipital cortices. Both regions receive approximately one-third of their input from subcortical structures [133]. Consistent with its strong connection to the piriform cortex, the LEC is also innervated by the amygdala and other olfactory-related structures.

In contrast, the MEC receives substantial input from the dorsal thalamus, particularly the lateral posterior nucleus, which plays a role in visuospatial attention. The medial septum, crucial for generating the theta rhythm, also has stronger projections to the MEC than to the LEC [134][135][136]. The MEC primarily receives input from the hippocampal formation, including the hippocampus, pre- and parasubiculum, subiculum, and post- and perirhinal cortices, making up about 50% of its total afferent input, compared to one-third for the LEC [131][133]. The LEC's main hippocampal inputs come from the ventral hippocampus and perirhinal cortex, while the MEC is more strongly innervated by the pre- and parasubiculum, as well as the hippocampus [84][63][137].

As previously discussed, both the MEC and LEC have strong projections to the hippocampus, with the CA1 region contacted by both direct and indirect pathways. The indirect pathway involves layer II cells from the EC synapsing onto the dentate gyrus and CA3, eventually reaching CA1 via Schaffer collaterals. Specifically, stellate cells in the MEC and fan cells in the LEC from layer II project to the dentate gyrus and CA3. While stellate and fan cells are reelin-positive, another group of cells in MEC layer II, which are calbindin-positive, form an additional pathway where pyramidal cells project to CA1 as well as to the contralateral MEC [138][98][63]. It is also important to note that both the indirect and direct pathways from the MEC and LEC target different hippocampal subfields. In the indirect pathway, the projections converge on distinct populations of neurons in the dentate gyrus and CA3 at various levels of the dendritic trees [39].

In the direct pathway, MEC and LEC projections target different populations of neurons along the proximo-distal axis of CA1. Proximal CA1, near the subiculum, is targeted by the MEC, while distal CA1, closer to CA2, is targeted by LEC synapses. This distinction extends to subicular projections, where the MEC and LEC respectively target distal and proximal subiculum [39][139]. In rodents, these entorhinal

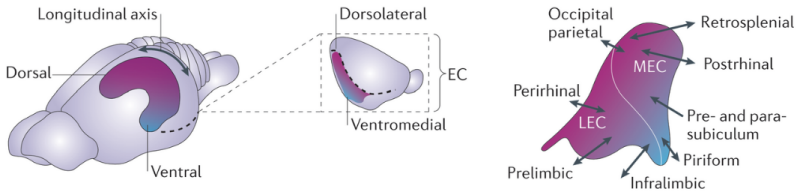


Figure 2.5: Entorhinal-hippocampal connectivity gradient in rodents. The figure shows the connectivity gradient between the hippocampus and entorhinal cortex (EC) in rodents. The dorsolateral EC band mainly connects to the dorsal hippocampus, while more ventral and medial EC bands (purple to blue) link to increasingly ventral hippocampus areas. A planar EC projection features a color gradient matching the hippocampal gradient, with arrows indicating major cortical projections to the EC. A white line marks the medial and lateral EC border. (Adapted from Strange et al. 2014 [42])

projections follow a dorsolateral-to-ventromedial arrangement (Figure 2.5), with the dorsolateral band of the entorhinal cortex primarily connected to the dorsal hippocampus. The ventral and medial bands of the entorhinal cortex are progressively linked to more ventral hippocampal levels [140][141].

The complex connectivity of the entorhinal cortex suggests it functions as a cortical hub, separating and pre-processing spatial and non-spatial information before distributing it to its hippocampal and cortical partners [142][63]. Also, a recent study found that, while MEC inputs to CA2 are not crucial for social memory formation, direct inputs from the LEC to CA2 are specifically activated during social exploration and are essential for forming social memories [143].

Chapter 3

Neurophysiological Basis Of Spatial Navigation

3.1 Spatial cells in hippocampal formation

Animals can use various strategies for self-location and navigation, two important ones being the cognitive map and path integration as it was described in Chapter 1.

Additionally, studies involving hippocampal lesions have not only demonstrated amnesia in patients with hippocampal removal [34] [144] but have also revealed various behavioral abnormalities, including disturbances in spatial navigation (see in-depth study of the early experiments in O'Keefe and Nadel, 1978 [2]). These findings have driven scientific investigations into the brain mechanisms underlying spatial navigation and memory within the hippocampal formation.

The first significant breakthrough occurred with the discovery of spatially modulated cells in the rat's hippocampus by O'Keefe and Dostrovsky in 1971 [29]. This discovery initiated decades of intensive research and debates regarding the neural basis of spatial navigation within the hippocampal formation. Subsequent studies have led to the discovery of other spatially modulated cells, advances in the theoretical framework and computer modelling of the networks involved in spatial navigation.

3.1.1 Place cells

In 1971, O'Keefe and Dostrovsky [29] conducted an experiment in which they recorded activity from several pyramidal neurons in the CA1 hippocampal region of rats navigating a square maze. Their study demonstrated pyramidal cells that fired action potentials when the rat traversed specific locations in the maze, with each cell having its own distinct spatial receptive field. They coined the term "place cell" for these neurons and referred to the locations where these cells preferentially fired as "place fields" (Figure 3.1A). It seemed that different place cells had different place fields, theoretically covering the entire environment, similar to pixels on a map. These discoveries, combined with Tolman's theory of a cognitive map in animals, led to the idea that place cells could serve as basic elements in the brain's construction of a cognitive map of the environment. Subsequently, place cells were also observed by Rank in 1973 [145], and O'Keefe and Nadel [2] further developed this concept in 1978 in their book "The Hippocampus as a Cognitive Map".

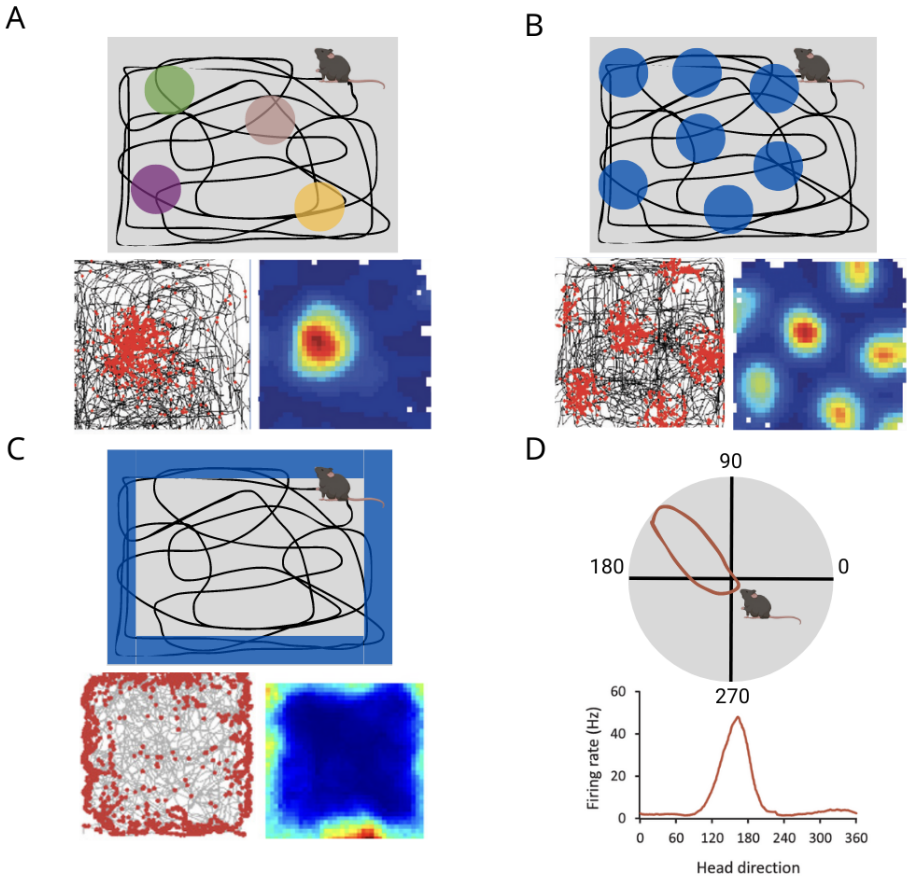


Figure 3.1: Schema illustrating the activity of different spatial navigation cells. The schema illustrates the activity of various spatial navigation cells (**A** - place cells, **B** - grid cells, **C** - border cells, **D** - head direction cells) while a rat is foraging in the environment. The path of the animal is shown by a black line. **(A)** At the top, colored circular areas show several place fields. Below, on the left, the rat's path in a maze is depicted by a black line, with neuronal spikes from a single cell marked as red dots. The area with the highest concentration of spikes represents a place field. On the right, a color-coded firing rate map of one hippocampal place cell is displayed (blue: low rate; red: high rate). (Adapted from Moser, Rowland, Moser, 2014 [146]) **(B)** At the top, blue circles illustrate the grid cell firing pattern in the maze. Below, on the left, similar to place cells, red dots represent individual spikes from one grid cell, with a rate map on the right displaying the firing pattern of the grid cell (blue: low rate; red: high rate). (Adapted from Moser, Rowland, Moser, 2014 [146]) **(C)** At the top, colored areas show an example of a border cell. Below, on the left, red dots represent individual spikes from one border cell, with a rate map on the right displaying the firing pattern of the border cell (blue: low rate; red: high rate). (Adapted from Long et al., 2024 [147]) **(D)** At the top, a polar plot depicts the activity of a head direction cell recorded as a rat forages for food in an enclosure. Below, a linear plot of the same cell's activity shows a peak at the preferred angle. (Adapted from Dudchenko et al., 2019 [148])

Since their discovery, various important observations have been made regarding the nature and characteristics of place cells. Place cells have been found not only in the dorsal CA1 region of the hippocampus but also in the dentate gyrus and CA3 region. However, the size and number of place fields vary among these regions. Also, the size of place fields systematically varies along the septotemporal axis of the hippocampus [149][150] and in relation to the size of the environment. In larger environments, a single cell may exhibit multiple place fields of varying sizes [151][152][153]. Notably, it was observed that place cells exhibit direction dependence when animals repeatedly traverse restricted paths [154]. In contrast, in open fields, place field firing rates

generally remain consistent regardless of the animal's orientation and travel direction [155]. Place fields could follow rotation of the visual cue indicating correct location, continue their firing after cue removal [156] and even be established in darkness [157].

However, further investigation into the influence of salient visual cues on place field formation revealed another intriguing characteristic. Manipulating salient cues, such as changing their color, size, or removing them, did not affect the location of the field from the center, but it could unexpectedly alter the angular coordinate of the place field [158]. Additionally, when visual cues were significantly altered, or the environmental shape changed, some place fields could shift their location or even disappear entirely. This phenomenon has been termed "remapping." Whether groups of place cells completely changed their maps or only partially did so depend on the conditions of the experiment. Scientists introduced terms like "global", "partial", and "local" remapping to describe whether only specific fields near particular cues were altered, or if there was a more widespread rearrangement of fields across the entire environment [159].

It was also demonstrated that remapping could occur not only in response to physical changes in the environment but also in response to contextual changes, such as aversive experiences [160]. Differences in place cells between CA1 and CA3 not only manifest in field sizes but also in their dynamic remapping during the learning of new environments. Thus, place fields in CA1 quickly appeared but tended to shift from one trial to another and remapped when the mice returned to the environment a day later. On the other hand, place fields in CA3 appeared more gradually but remained more stable from trial to trial and day to day [161].

Another interesting observation came from studying place cell activity in a 3D space, as many animals, including non-flying and flying mammals like bats, navigate in such environments. Single-neuron activity from hippocampal CA1 neurons was recorded in freely flying bats across two setups [162]. The first setup was a large cuboid-shaped flight room where the bats performed a natural foraging task, and the second was a smaller 3D cubic flight arena where they searched for food randomly. Most place cells (62%) had a single place field, and the fraction of cells with multiple fields was higher in the larger setup, similar to findings in rats where the number of 2D firing fields increased with the environment's size [151]. It was also shown, in both setups, place field locations were uniformly distributed along all axes and the radial dimension. When assessing whether place fields were the same size across dimensions, it was found that the sizes of individual fields along the three cardinal axes were quite similar. Only 9% of the fields showed significant elongation, mostly along the z-axis, while 91% were not significantly different from a sphere, showing no elongation in any cardinal direction. The nearly isotropic 3D coding observed in over 90% of bat hippocampal place cells may be influenced by the bats' natural 3D movement and echolocation, which provides volumetric sensory information, including depth. This isotropic coding likely evolved in bats to help encode and retrieve 3D spatial information, contrasting with land-dwelling mammals that rely mainly on horizontal movement and self-motion cues, and could apply to other animals navigating in 3D environments.

3.1.2 Head-direction cells

For succesful navigation, you need to know your direction or have a compass to indicate it, just as you need a map. Similarly, another important contribution to the cognitive

map in the hippocampus was made by discovering such a compass system by Ranck in 1984 [163]. He presented a video at the Society for Neuroscience Meeting of recorded head-direction (HD) cells in the dorsal presubiculum. The preference of these cells was not a location but the direction in the angular space, with each cell showing increased firing when the animal turned its head in a specific direction (Figure 3.1D) [164]. Later, cells with similar properties were also found in other brain areas, such as the entorhinal cortex [165], the anterior and lateral dorsal nuclei of the thalamus, as well as the retrosplenial cortex [166]. It has also been shown that by recording firing rates of a small subset of cells recorded concurrently, it is possible to reconstruct the animal's heading, providing valuable proof of the HD system being a so-called "compass system" in the brain [167][168].

There is also a strong connection between place field orientation and the HD system. In a cylindrical environment, rotations of a single salient cue that induce simultaneous rotation of HD cells tuning also induce rotations of place field locations by a similar amount. Lesion studies are consistent with this observation, whereby lesioning the HD system disrupts the ability of visual cues to control the orientation of place fields within the cylinder [169]. As discussed before, place cell firing in open environments is not dependent on the direction in which the rat is moving within a specific place or location. For example, they can fire whether the rat is moving east to west, west to east, or in any other direction. It has been found that this omnidirectionality of place cell firing is dependent on HD input since the lesioning of anterodorsal thalamic nuclei (ATN) or postsubiculum (PoS) makes place fields direction-dependent [169][170].

The HD system is influenced by a set of visual cues and idiothetic cues, but it is still not clear if one of these sensory information predominates over the others. Additionally, the HD signal has been be recorded from a rat pup in the dorsal presubiculum, MEC, and the anterodorsal thalamic nucleus as early as P11–12, an age when pups do not leave the nest autonomously, and their eyelids are still closed [171][172]. However, early HD signals show directional accuracy but remain unstable both within and in between trials. Once the eyes open, the HD system rapidly matures, with more cells firing directionally and a noticeable improvement in the quality and stability of the signal. Another experiment with cue-rotation also showed that the visual landmarks can influence HD responses within 24 hours of eye opening. These findings suggest that the directional network can form without visual spatial input, but patterned vision is key for accurate and reliable spatial orientation [172]. Early research suggests that self-motion cues control moment-to-moment firing, with periodic and rapid updating from distal cues at or beyond the boundaries of explorable space. Taube et al., 1995 [173] examined the impact of cue conflicts on HD responses, revealing that cue influence is not rigidly determined by cue type but is rather adaptable based on specific circumstances. Knight et al., 2013 [174] introduced conflicts between visual cues and idiothetic cues combined with background cues. Their findings showed that HD cells tended to follow the visual cues when conflicts were minor but exhibited compromise between the two cue sets when conflicts were substantial.

Recent development of virtual reality (VR) set-ups for rodents (see Chapter 5) helped to investigate interplay of sensory and idiothetic cues in PC and HD modulation. This is possible because VR allows for the control of visual stimuli, gains in an environmen, and the restriction of vestibular input through head-fixation, among other factors. In virtual environments that mimic linear spaces like treadmills, place cells show firing patterns influenced by both the distance the rodent moves and sta-

tionary visual cues on the virtual screen. In one of the studies [175] head-fixed mice were running on the spherical ball in a virtual linear track with visual cues. The ball-to-virtual movement gain was reduced by half, meaning the animals had to cover twice the distance on the ball with visual cues to get the same virtual translation as in normal gain trials. If place fields were based on self-motion cues like the number of steps since the start, they would shift backward, opposite to the movement direction in this setup. In the case of the visual cues and self-motion conflict in this experiment, the average place field locations shifted toward the start of the track. This indicates that both visual cues and distance travelled are used to control the majority of place cell firing [175].

In two-dimensional virtual environments with limited head movement, the specific firing patterns of place cells become less distinct. However, some influence from the distance the rodent travels is still observable [176]. But when the rodent has free body and head rotation in the virtual environment, place cells maintain stable position coding [177]. This suggests that vestibular signals, which are less active when the head is held still, play a vital role in integrating velocity and direction signals to create a coherent two-dimensional representation. This observation is consistent with earlier research showing that disrupting the vestibular system, either through inactivation or lesions, can disrupt place fields [178].

Navigating in real environments offer demands from animal orientation in 3D spaces. Recent studies on HD system in 3D environments showed two possible models: the rotational plane hypothesis and the dual-axis model. For the first model, when a rat moved from a horizontal surface to a vertical wall, it was found that HD cell activity remained consistent in the vertical plane, and the preferred firing direction (PFD) could be predicted based on its PFD in the horizontal plane [179][180]. The cell adjusted its reference frame by rotating the horizontal plane 90 degrees into the vertical plane, aligning with the rat's movement. These findings suggest that HD cells set their reference frame according to the animal's plane of locomotion. Studies where rats could explore all 360 degrees in the vertical plane along different walls also showed that the cell's PFD could follow its PFD in the horizontal plane, rotating its vector around any corner as the animal moved [181]. This demonstrates that the cells update their orientation by treating the vertical plane as an extension of the floor, shifting their reference frame to align with the animal's current plane of locomotion. This concept is known as the rotational plane hypothesis.

In the study by Page et al. [182] HD cell PFDs shifted between two opposing walls of a cuboidal surface, with orientations 180 degrees apart. This led to the proposal of two axes influencing cell firing in 3D space: the animal's yaw rotation around the dorsal-ventral axis and the orientation of the dorsal-ventral axis relative to gravity. These components accounted for a cell's PFD in any plane, forming the dual-axis model. The model predicts a 90° clockwise PFD rotation around right vertical corners and a 90° counter-clockwise rotation around left vertical corners. In contrast, the rotational plane hypothesis predicts no PFD shift, as the PFD follows the animal's plane of motion.

The studies on rats showed that when rats moved around horizontal corners, their HD cell responses matched the predictions of the rotational plane hypothesis. However, when they traversed vertical corners, the responses aligned better with the dual-axis model. So, HD cells align their directional representations to both local (current plane of locomotion) and global (gravity) cues across different behavioral tasks including navigation in darkness and passive rotation [183][181].

3.1.3 Grid cells

Having a map and a compass can make navigation easier during the day when visual and other sensory stimuli are accessible. However, what happens when the environment lacks them or when it's dark? In Chapter 1, I discussed the navigational strategy known as "path integration", which allows navigation in normal and such conditions. Another groundbreaking discovery in spatial navigation was made by the group led by May-Britt and Edvard Moser in 2005. They identified spatially modulated cells in the medial entorhinal cortex, which were termed "grid cells" [184]. Later, grid cells were also found in the pre- and parasubiculum as well [185]. Unlike place cells, grid cells exhibit multiple firing fields arranged in regular hexagonal or triangular lattice patterns, with varying peak firing rates (Figure 3.1B) [186]. Grid fields can be described by three key attributes: scale, which is defined by the spacing between neighboring peaks in firing rates; orientation, representing the alignment of grid axes in relation to a specified reference direction; and spatial phase, denoting the two-dimensional displacement of the grid relative to an external reference point [186]. The emergence of new data from the entorhinal cortex made it clear that grid cells might provide the brain's spatial map with a coordinate system that cannot be achieved through place cells in the hippocampus. This distinction arises from the seemingly random assignment of place fields to specific positions and the significant remapping of these fields when the environment changes.

Grid fields from cells recorded at the same location can exhibit significant variations in spatial phase. This means that even if they share the same scale and orientation, they may still be displaced relative to each other [186]. Similar to place cells, the scale tuning of grid cells varies along the dorsal-ventral axis, with smaller and closer-together fields in the dorsal medial entorhinal cortex and larger, more distant fields in the ventral part [184][187]. Grid cells can have fields with different scales, covering even large environments [188][189][190][191]. It has also been shown that stretching or resizing an already visited environment can expand the grid spacing in the elongated direction, along with scaling or remapping in hippocampal place cells [192][193]. This suggests that grid maps might be constructed through path integration but are influenced by external cues in a way that allows these cues to override path-integration dynamics.

The spatial periodicity of grid cells remains unchanged despite changes in the animal's running speed and direction. The persistent presence of grid fields and place fields in rats as they navigate in the dark aligns with the idea that self-motion information plays a crucial role in determining where these cells fire. This is supported by the rapid emergence of grid patterns in new environments and the consistent phase relationships between pairs of cells across all tested environments [184]. Considering all these observations, it is commonly accepted that grid cells could play a major role in path integration.

The coherence of the grid map is notably strong within groups or modules of grid cells that share similar scaling characteristics. This level of coherence is also observed among head direction cells [164]. Importantly, the coherence of both grid cells and head direction cells is not dependent on the animal's state and persists even during sleep [194][195]. These findings highlight a fundamental distinction between hippocampal and entorhinal spatial maps: hippocampal circuits are high-dimensional and capable of storing a vast number of patterns, while MEC maps are low-dimensional and inflexible, maintaining the same intrinsic structure across all behavioral contexts.

This consistency aligns with the characteristics expected from a path-integration-based map that keeps metric properties constant across different contexts and environments. In 2012, Stensola et al. [193] were able to record activity from up to 180 grid cells in the same animal, allowing them to conclusively determine whether grid cells clustered into groups with similar properties. They discovered that grid cells were indeed organized into at least four modules, each with its own scale, orientation, and asymmetric distortions. The scale change across these successive grid modules followed a geometric progression with a constant scale factor, validating prior predictions and theoretical analyses suggesting nested and modular organizations as the most efficient way to represent space at the highest resolution with the fewest cells [196][197].

The activity of grid cells within each module is well-organized and remains consistent across different environments and behavioral states. To understand how the activity patterns of these modules work together, simultaneous activity recordings were made of hundreds of grid cells in animals foraging in two conditions: one with light, where sensory cues help stabilize the representation of position, and another in complete darkness, where this stabilization is disrupted. The activity patterns of different modules were still tightly coordinated even in darkness, despite the internal representation of position within the MEC being significantly different from the actual position of the animal. These results suggest that the brain possesses internal mechanisms that dynamically coordinate how different modules represent position, ensuring they work together to encode a coherent and smooth trajectory [198].

Similar to HD cells, it was also unclear how grid cells would behave in a 3D environment. In a similar experiment where a rat traveled through volumetric space, it was demonstrated that grid cells maintained theta, head direction, speed coding, spike dynamics and spatial information and produced spatially stable firing fields. However, grid fields appeared sparser, larger, more variably sized, and irregularly arranged. Thus, as shown in 2D studies, grid self-organization is influenced by the environment's structure and/or movement affordances, suggesting that grid cells may not need to conform to regular patterns to support spatial computation [199].

A different perspective on the role of grid cells in spatial navigation and their role in defining the metric of space has recently been proposed by the Ulanovsky lab [200]. They argue that previous studies showed animal navigation in small, symmetric laboratory environments, whereas animals in the real world often navigate in geometrically complex, asymmetrical and large spaces. Changes in spatial geometry have been shown to disrupt grid cell activity patterns, raising questions about whether these patterns truly reflect the metrics of space in naturalistic environments. In addition, they argue that previous lesioning studies involving the entorhinal cortex have consistently failed to show significant impairment of animal performance in navigational tasks (however, see the study by Steffenach et al., [201] where lesioning in the dorsolateral band of the entorhinal cortex disrupted retention and retarded new learning in rats performing Morris water maze task; It has also been shown that medial septum inactivation, which abolishes the hexagonal firing patterns of grid cells induce impairment in rats performing spatial memory tasks [202][203]. This lack of critical effect contrasts with what might be expected if grid cells were solely responsible for an animal's global metric of space and perception of space. Instead, they propose that grid cells may provide a local metric for space, encoding a subjective, action-relevant spatial representation. Furthermore, grid cells may play a role in encoding important locations. The regular hexagonal pattern they form may serve as a basis for

representing distortions in space, and any deviations from this perfect order may signify significant points of interest. To address these questions and hypotheses, further studies involving animals navigating in more naturalistic and larger environments are needed.

3.1.4 Other spatial modulated cells

In addition to the spatially modulated cells mentioned above that are involved in navigation, other cells have been shown to play a role. Here I will briefly describe some of them.

Border cells

Border cells were discovered shortly after head-direction cells, as another type of spatially tuned cells in the medial entorhinal cortex and in the subiculum [204][205]. They fire along the geometric borders of the local environment, along one or sometimes several walls of the recording enclosure or along the edges of a platform (Figure 3.1C) [204]. Visual cues alone can activate border cell responses [177]. Border cells play a significant role in providing reliable landmark cues for the grid cell system due to their distinct geometrical nature. There is evidence supporting the idea that border cells are involved in error correction and enhancing the stability of place fields, especially in the absence of light [206]. For instance, when animals regularly encounter boundaries, it helps maintain stable place fields in the dark. In young rat pups, where border cells have developed but not grid cells, place cells show stronger stability and coherence near environmental boundaries [207]. Thus, neurons may represent the environmental geometry and anchor the spatial map to the surrounding borders, stabilizing it.

Speed cells

Speed cells are neurons whose firing rate is robustly correlated, typically positively and linearly, with the running speed of the animal (Figure 3.2A) and exhibits low levels of spatial and directional information. They constitute around 15% of MEC cells. The signal derives from proprioceptive or motor-efference information in the mesencephalon since the speed response is independent of visual input. Similar cells were also found in the hippocampus but make up a consistent small portion of cells (around 10%). Speed cells are thought to provide input to grid cells to support path integration. The presence of speed-responsive cells within the entorhinal-hippocampal network has important implications for the process of updating spatial maps as animals move through their surroundings [208].

Time cells

Time cells are hippocampal neurons that fire at specific moments of a temporal interval (Figure 3.2B) [209][210][211]. Time cells and place cells are essentially the same neurons, suggesting that they encode both spatial and temporal information, although these dimensions can be measured separately. This temporal organization within the hippocampus is present across various animal species, including humans. Time-cell firing patterns emerge during the learning process and serve to differentiate

specific memories while also predicting future memory performance. Probably, time-cell sequences are involved in more complex patterns than just sequential firing and are linked to organizing memories in temporal context within the hippocampus [212].

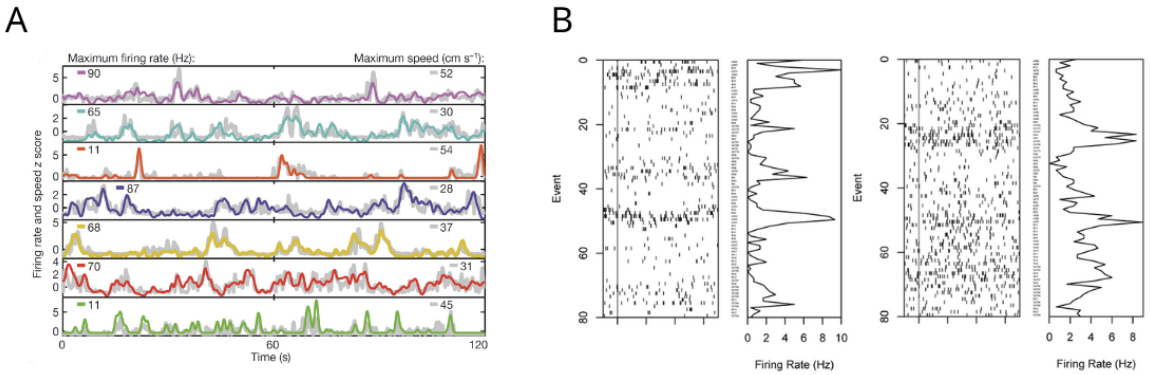


Figure 3.2: Examples of speed and time cells activity. (A) Traces showing z-scores for firing rate (color) and speed (grey) for seven representative entorhinal speed cells during 2 min of free foraging (different sessions). Maximum values of firing rate and speed are indicated (left and right, respectively). (Adapted from Kropff et al., 2015 [208])**(B)** Examples of time cells whose firing patterns correlate with time across multiple trials of a continuous recognition task. On the left, the event plot shows spikes fired during a recording session, with each row representing a different trial, aligned to the stimulus presentation (vertical line). On the right, the firing rate for each trial is displayed. The experiment shows robust autocorrelation in firing patterns, where the cell's firing rate changes consistently across trials. The first cell exhibits autocorrelation over a few trials (12 seconds, as each trial lasts about 4 seconds). The second cell shows autocorrelation over about 10 trials, corresponding to several dozen seconds. (Adapted from Howard and Eichenbaum, 2015 [213])

3.2 Rate coding and attractor dynamics in spatial navigation

3.2.1 Rate Coding of Spatial Cells

In the previous chapter, I discussed various spatial cells whose level of activity is modulated by several variables necessary for spatial navigation, such as location, distance, orientation in space, etc. These cells fire action potentials in preferential locations and remain silent in others, which represent their own "receptive field of the space." Additionally, it was shown that some cells, like place cells in CA1, have a flexible code of the environment with global or local remapping of their preferred fields when the environment changes [158]. Another important feature is rate remapping, which was described by Leutgeb et al. in 2005 [214]. In this study, they showed that when a CA1 cell was recorded in a rat in the same room but in two different shaped arenas (squared and circular shaped), the place fields did not disappear or change their location, but the firing rate of the cell substantially changed. These changes could be sufficient to make a field appear to be present in only one condition, unless the rate map graphs were rescaled. It seems that even if the firing location remains constant, alterations in external stimuli or internal factors like motivation, working memory, attention, or action plans can lead to significant variations in firing rate, [215]. In another study [216], it was shown that when the environmental shape was slowly transformed from a circle to a square, abrupt and widespread remapping occurred exclusively in cases

where rats had previously been permitted to move between these two shapes via a connecting tunnel. In contrast, when rats were pre-trained on both shapes in the same location, only changes in firing rate were observed. It suggests that path integrator is necessary to switch between rate and global remapping.

The discovery of rate remapping showed that there is not only a place code alone that encodes the location in the environment but also a non-spatial code on top of that which could modulate the spatial one. In further studies, it was shown that cells could respond to other stimuli like odors [139], sounds [217] or temporal stages of the experiments [218], and context [219]. This combination of spatial code with a non-spatial one serves as evidence of the hippocampal role in episodic memory, with spatial information being just one element within the encoded representation.

However, despite this evidence, studies using lesions to the MEC, the region thought to be responsible for path integration, found that place fields could still be detected in CA1. This raises questions about whether the hippocampal place map is truly dependent on path integration. It is crucial to highlight that even when the MEC is inactivated and doesn't affect hippocampal place selectivity, it still results in immediate remapping [220]. This suggests that the MEC plays a role in instantiating a specific map. Another possibility is that the spatial map is stored not in CA1 but in the CA3 recurrent network. In the absence of a strong MEC input, CA3 may retrieve previously constructed maps in a novel context. These discoveries, combined with the existence of multiple spatial cells, have prompted various theoretical studies aimed at explaining the organization of spatial maps and network interactions. In the following chapters, I will elaborate on neural network models for path integration and cognitive maps used for navigation.

3.2.2 Continuous attractor network for HD and PC

The first crucial step in further understanding the computational level of the hippocampal-entorhinal network and memory storage occurred in 1949 when Hebb proposed that activity could self-sustain in networks of recurrently connected neurons (Hebb, 1949) [221]. This self-sustaining neural activity is also known as attractor dynamics. Attractors refer to patterns of neural activity or states that a neural network tends to converge toward given certain input or initial conditions. These attractor states play a critical role in comprehending how information is stored and retrieved in the brain. They have been extensively studied in the context of memory and cognition, serving as foundational concepts for models of localized firing in early investigations of hippocampal function.

In 1977, Amari [222] introduced a mathematical model to describe the behavior of large populations of neurons in which each neuron possesses strong excitatory connections to its nearest neighbors. The strength of excitation decreases with distance along the network, unlike inhibition, which remains effective over longer distances. This network arrangement resembles a ring and is reminiscent of a Mexican hat in terms of connectivity [222]. This theory introduced the idea that information is not only stored as discrete items but can also be stored as continuous information distributed along a ring. This forms a continuum of discrete attractors forming a continuous attractor. This continuum can manifest in various dimensions: single-dimensional, such as a specific direction for head-direction cells; two-dimensional, such as a specific location in a 2D environment for place cells; or in multiple dimensions.

Later on, Skaggs and McNaughton with Redish, Elga, and Touretzky [223][224],

independently demonstrated how the concept of a ring attractor with local connectivity and global recurrent inhibition could be applied to model the firing behavior of head-direction cells. As previously described, HD cells exhibit selective firing patterns depending on the orientation of an animal's head in space. This selectivity primarily arises from the neural integration of signals related to the angular velocity of the head, as derived from the vestibular system. In the proposed model, cells are organized in a circle based on their preferred directions, with the strength of connections between two cells decreasing as the difference in their preferred directions increases. Consequently, this setup leads to a concentrated activity pattern or an "activity bump" centered around a specific direction, as described in Amari's Mexican-hat model. The activity bump naturally emerges because, within the bump, each neuron receives the maximum possible excitation from its neighboring neurons, making it the most stable state for the system. In the absence of input apart from random noise, the bump's location remains stable or may undergo slight random positional drift. To move the bump around the circular arrangement, additional inputs, such as those indicating head movements from the vestibular system and rotational visual cues, are required. These inputs can drive the bump clockwise or counterclockwise on the circle.

In this HD attractor model, there is also a second layer, often referred to as the "hidden layer." Neurons in this layer perform two essential functions: they receive information about the current head direction from the first layer and about how the rat's head is moving (e.g., whether it's turning left or right) from the vestibular system for example. Neurons sensing the rat's head-turning right send signals more to the right side of the head-direction cells they connect to, while those sensing the head turning left send signals more to the left. This action gives a push in the right direction, aiding in moving the "activity bump" in sync with the actual direction in which the rat's head is moving. This mechanism enables the system to track the rat's movement direction over time and is referred to as "angular path integration."

One year after this discovery, the continuous attractor network model for place cells was introduced. This model necessitated additional adaptations due to the two-dimensional encoding of place cells. In this model, cells are conceptually arranged on a two-dimensional sheet according to their firing locations in two-dimensional space. The strength of connections between neighboring excitatory cells decreases with the physical distance between their respective place fields. Global inhibition prevents activity from spreading, similar to the head direction system. Just like in the one-dimensional model, there's a distinctive concentrated activity region that spontaneously emerges. This activity region is supported by a two-dimensional hidden layer, which keeps track of the speed and direction of the animal's movement. This hidden layer preserves information about the rat's position from the continuous attractor layer and also considers the displacement vector formed by combining head direction and linear speed signals. Within this direction-specific layer, individual cells have the unique ability to encode both the rat's position and velocity vectors simultaneously. They achieve this by assimilating inputs related to head direction, running speed, and location information sourced from the attractor layer [225][226][227]. The connections originating from the continuous attractor layer to the hidden layer are established among cells that share the same positional preference. Conversely, the feedback connections that loop back from the hidden layer to the continuous attractor layer are fine-tuned in accordance with the directional preference of the source cell.

As the rat runs, the velocity-modulated cells in the hidden layer, which are selective for a particular direction, become active. They provide input that shifts the

activity bump in the chosen direction. The rate at which the firing rate of hidden layer cells increases with running speed determines the scale of the spatial representation. Stronger input from the direction-specific layer results in a faster movement of the activity bump, creating a rapidly changing, fine-grained spatial representation with small place fields. Conversely, reducing the speed dependence of hidden layer cells causes the activity bump to move more slowly, resulting in a coarser spatial representation with larger place fields [228]. Since this activity bump moves on a 2D sheet, the question arises of what happens when it reaches the edge of this plane. Samsonovich and McNaughton proposed an upgrade by conceptualizing this continuous attractor as a torus or donut shape. This shape has periodic boundaries, meaning that as the rat moves in a straight line, a particular cell should activate periodically. In a large two-dimensional environment, each cell would theoretically have multiple "place fields" arranged in a square grid. While it has been shown that place cells indeed have multiple place fields in large-scale environments, the absence of grid-like periodic place cells raised additional theoretical and experimental questions.

Furthermore, as discussed earlier, a subset of active hippocampal neurons remaps across different environments and contexts. To compute position in place cells, independent architectural adaptations for each environment would be necessary. Although computationally feasible, this raises the question of whether a single network matrix expressed in all environments could be more efficient [229].

3.2.3 Continuous attractor network in entorhinal cortex

The grid fields show features that were predicted by the toroidal model and are absent in the place cells. They have grid fields repeating at regular intervals over the entire environment [227], except that the unit cell of the grid is not a square but a rhombus with internal angles of 60 and 120 degrees [184]. The geometric arrangement and spacing of grid fields in layer II MEC neurons remain consistent regardless of the size or shape of the environment. The neighboring grid cells have similar grid spacing and orientation, but their grids are shifted relative to each other in a seemingly random manner, with all grid phases equally represented within a small region of the cortex. In the MEC, the relative offset (spatial phase) of grid fields between any two cells appears to be consistent across all environments. This property aligns with the concept of a universal chart proposed in theoretical models of path integration.

Grid cell activity patterns in layer II can be updated by input from afferent structures, but recent studies suggest that the integration of directional and positional information occurs within the grid network itself. This is achieved using neurons with conjunctive place and directional properties. Layers III, V, and VI of the MEC contain not only grid cells but also head direction cells and cells with both grid and head direction properties. All three cell types are positively influenced by an animal's running speed. Conjunctive cells are predominantly found in layers III and V and have extensive axonal projections to the grid cell population in layer II, suggesting that they could drive changes in the active grid cell population as an animal moves. These conjunctive cells likely receive input from grid cells in layer II, as predicted by the continuous attractor model.

Horizontal connections in MEC layers III and V suggest that attractor dynamics may be primarily accomplished in these deeper layers alone. In such a scenario, layer II might serve as an output layer, integrating activity from deep cells with different directional preferences to create a non-directional spatial representation .

As an animal moves through its environment, the grid cell network's location-specific activity is likely updated mainly through a path integration-based mechanism. This means that the firing of one set of cells depends on the previous network activity state, the distance and direction the animal has moved, rather than directly responding to environmental stimuli at a given location. This aligns with the fact that grid field relationships are invariant across different environments and remain unchanged when external landmarks are removed or displaced. Grids are also immediately expressed in a novel environment.

Additional evidence supporting the role of grid attractor networks comes from the observation that grid cells not only play a crucial role in self-motion-based navigation within an environment [230] but also exhibit alignment with specific landmarks as animals repeatedly explore the same surroundings [184]. This consistency in grid cell patterns in relation to external landmarks remains intact, regardless of the animal's starting point in its exploration. This observation implies that the hippocampus might be responsible for associating these path integration coordinates with distinct landmarks, thereby generating unique representations of different environments and the events occurring within them. Alternatively, this association could occur within the MEC itself, where grid cell activity integrates with sensory inputs from the postrhinal cortex [228].

An alternative model upgrade for grid networks demonstrates the potential for multiple representations of diverse environments to coexist, not only within the hippocampus but also within the grid code itself. This suggests that a population of grid cells has the capacity to encode various non-congruent metric relationships. This capability could enable a grid-like code to represent environments with varying geometries, as well as conceptual and cognitive spaces involving context-dependent metric relationships [231]. This proposal holds significance for advancing our understanding of grid network behavior in larger-scale environments.

Previous research primarily focused on grid cells recorded in small laboratory settings. In large-scale environments, observable distortions in the grid pattern raise the need to reconsider the continuous attractor model for grid cells. One possibility is that the experimentally observed distorted grid pattern may reflect distortions in the pattern of activity bumps on the neural sheet itself, particularly relevant for models involving grid cells with multiple activity bumps [232]. Such distortions could stem from alterations in the "Mexican hat" anatomical connectivity pattern, possibly due to competitive plasticity processes [233]. This local connectivity pattern could result in local spatial firing structures but not global ones, effectively creating a local metric for space. This scenario suggests that distortions may emerge slowly due to the gradual nature of plasticity mechanisms.

Another possibility is that while the pattern on the neural sheet remains perfectly periodic and undistorted, the velocity signal responsible for moving the bump of activity becomes distorted. This velocity signal could be influenced by subjective factors such as ease of movement, attention, or arousal, potentially leading to a grid pattern that correlates with subjective spatial perception. Alternatively, the velocity signal may experience distortion only in close proximity to strong reward signals, causing discrete distortions near critical locations while leaving the rest of the pattern intact. A third possibility is that inputs from other cell types, such as border cells, or sensory inputs entering the entorhinal cortex, could be distorting the grid pattern [200]. To gain insights into grid network behaviour, it is essential to conduct electrophysiological recordings in asymmetrical, naturalistic large environments. In such settings, various

visual inputs, along with the animal's arousal and attentional state, can be monitored to better understand the dynamics of grid cell activity.

3.3 Oscillations in hippocampal formation

3.3.1 Theta-Oscillations and Phase Coding

The study of brain oscillations in humans began with Hans Berger's recording of the initial spontaneous oscillation, known as the "Berger wave" or alpha wave. Subsequently, various other brain oscillations have been identified, with particular emphasis on theta oscillations. Grastyan's group was the first to demonstrate the significance of theta oscillations in the development of conditioned reflexes in animals [234] and in the regulation of motivation [235]. Additional studies have highlighted the cognitive and affective connections of hippocampal theta oscillations in various contexts (as reviewed by Buzsáki, 2002 [236]). Theta rhythm refers to a slow oscillatory activity occurring around 8 Hz, generally within the range of 5 – 12 Hz. This rhythm is most prominent in the LFP in the hippocampus when an individual is engaged in activities such as locomotion or active interaction with their surroundings. The power of theta rhythm increases during various behaviors, such as attention to predators [237] or a voluntary movement [238][239], like running on a track [240][241]. Notably, the frequency and amplitude of theta rhythm in rats increase with their running speed and jumping height [242], suggesting a role in encoding velocity and location information. In the hippocampus, theta rhythm is associated with learning and memory processes. Conversely, when an individual undergoes passive rotation, theta rhythm does not appear to intensify with cognitive demands (see the review by Michael E. Hasselmo and Chantal E. Stern, 2014 [243]).

O'Keefe and Recce described another feature of theta oscillation in spatial navigation. They observed that the place code not only followed the earlier described rate-code but also that place field spikes occurred at progressively earlier phases of the theta rhythm as the animal ran across the field. This phenomenon is known as "phase precision" (Figure 3.3A). The proposed mechanism could arise from the interference between two theta-band oscillations that are sensitive to velocity. The theta phase coding of distance within a spatial field is specific for each individual place cell. For example, if a rat successively enters the overlapping place fields of place cell 1, 2 and 3, when the rat is within the place field of place cell 2 this cell will fire during an intermediate phase of the theta cycle, while place cell 3 will fire during a later phase, and place cell 1 will fire during an earlier phase. Consequently, cells firing at late, intermediate, and early phases will represent positions ahead of, within, and behind the rat's position, respectively. Thus the temporal order of firing within each theta cycle corresponds to the spatial order of firing fields on the track. These sequential firing patterns on the theta cycle (termed theta sequences) can be observed on a linear track as well as when animals forage in open environments. This indicates that a significant amount of information is encoded in the ability of individual spatial cells to fire at different phases of the theta cycle (see review Céline Drieu and Michaël Zugaro, 2019 [244]).

3.3.2 Sharp-wave ripples and their role in replay

An important observation regarding the role of oscillations in hippocampal coding is that hippocampal neurons transiently increase their firing rate during sleep [211][245]. O'Keefe and Nadel noted that during periods of quiescence and quiet wakefulness, the LFP of CA1 exhibited short bursts (lasting 40–120 ms) of high-frequency oscillations

(140–250 Hz) superimposed on lower-frequency deflections. These high-frequency components were termed 'ripples,' and the combined signal is referred to as a sharp wave ripple [246]. It was during these sharp wave ripples that CA1 pyramidal cell activity was observed to dramatically increase [29]. Sharp wave ripple (SWR) events are associated with prominent multi-unit activity (MUA). Patterns of cell activation are observed when the animal is at rest or sleeping similar to the firing patterns of place cells during theta oscillations when the animal is in motion on a track. These patterns occur concurrently with sharp-wave ripples and are termed "replay" (Figure 3.3B) [240]. The replay can occur in the same or reverse order as the animal's movement on the track [247] and typically takes less time compared to when the animal is awake and navigating. In line with the concept that specific firing patterns of brain cells can enhance learning and memory, "replay events" in the brain are believed to recreate activity that occurred during recent experiences. These replay events appear to assist in learning and memory consolidation [248]. Offline rehearsal of routes may contribute to the development of valuable spatial maps or facilitate the transfer of rapidly acquired hippocampal spatial memories to brain regions outside the hippocampus [249].

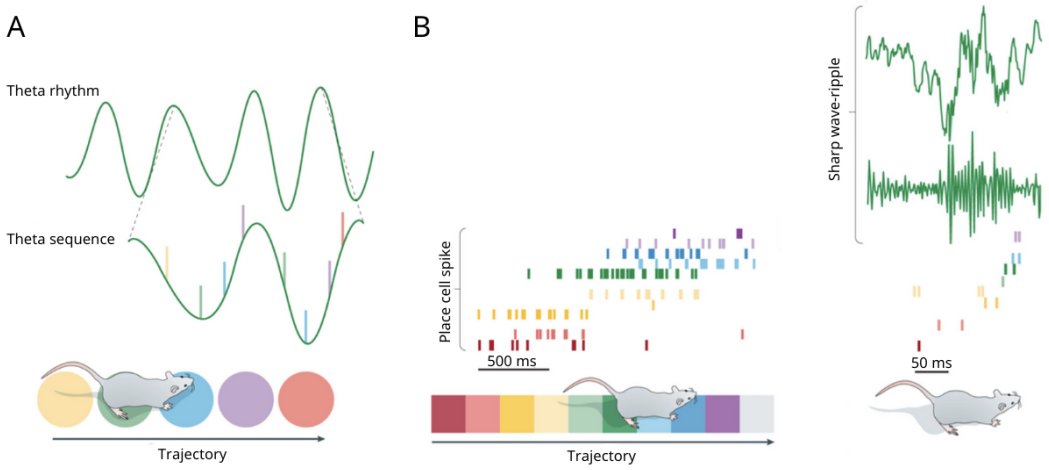


Figure 3.3: Schemes illustrating theta-oscillations during navigation and replay during sharp wave-ripples. (A) Theta sequences in the hippocampus during navigation. Successive locations in an animal's trajectory are represented by an ordered series of place cell spikes within individual theta cycles (theta sequence). Schematic representation of a path to be taken by a rodent (bottom), an ongoing theta rhythm (top), and the theta sequence of place cells for two magnified theta cycles (middle), whereby the place cells are colour coded according to the particular point in the path at which their place field is located. For individual place cells within a theta sequence, spikes occur at progressively earlier theta phases across successive theta cycles (theta phase precession). Because of this, spikes at early and late theta phases represent earlier and later locations, respectively, in the trajectory. **(B)**On the left, Presented spikes from successively activated place cells (top) as a rodent passes through the cells' place fields in a particular trajectory on a linear track (bottom). Each row of colored tick marks represents spikes from a different place cell. On the right, an example of a sharp wave-ripple (top), recorded during subsequent rest at the end of the linear track; a bandpass filtered (150-300 Hz) version of the sharp wave-ripple is shown immediately below the raw recording. Spikes from the place cell ensemble are shown to reactivate during the sharp wave-ripple in the same order as in exploration but on a faster time scale. (Adapted from Colgin, 2016 [250])

Another study has shown that in awake rats, sequences of sharp wave/ripple reactivation often foreshadow the rat's future path toward a specific goal [251]. In this context, Dragoi and Tonegawa, 2013 [252] discuss a related phenomenon known as

"preplay," where cell assemblies fire in a specific sequence before any relevant spatial experience occurs. They propose that preplay implies a level of pre-configuration within the hippocampal spatial network and that patterns of place cell activity during novel spatial experiences may involve selecting from a set of pre-existing cell assemblies. These intriguing findings present challenges to a simple explanation of replay solely as experience consolidation. However, they align with earlier observations that forward replay events tend to precede corresponding actions and support the idea that the hippocampus may play a role in both planning future behavior and representing present and past experiences.

Chapter 4

Spatial Resolution and its Role in Place Cell Coding

4.1 Notion of spatial resolution in place cell coding

In previous chapters, I have explored various navigation strategies that animals use to navigate their environments. One such strategy suggests navigating via a cognitive map of the environment. This map not only helps animals identify their location but also supports complex navigational tasks such as planning routes, discovering shortcuts, or spotting hidden targets [22][2][253]. For these functions to be possible, the cognitive map must accurately represent various elements of the external world, including environmental boundaries and landmarks. The identification of brain cells that encode spatial information has led to the understanding that this cognitive map is likely represented by spatially modulated cells, particularly place cells that encode specific environmental locations.

However, the idea of a cognitive map raises several questions when applied to real-world environments. Firstly, place cells do not always represent the same location consistently if environmental cues change [158] [254]. Moreover, the activity of place cells can vary based on the tasks an animal performs within a given space [255]. These findings highlight the difficulty of using a single place cell to accurately represent a dynamic and constantly changing real world, influenced by natural cycles (such as the seasonal change from fallen autumn leaves to a green spring forest) and the activities of other animals and humans. Secondly, assuming that each cell encodes a single place field, it would be computationally impossible to encode all potential routes and spaces that animals visit throughout their lives. Considering that even small mammals like rodents can travel up to 2 km from their nests, there simply are not enough neurons in the brain to cover such large areas. Hence, a model based on single-cell encoding appears insufficient for large naturalistic environments.

The 1993 study by Wilson and McNaughton marked a significant shift, moving from single-cell analysis to examining the activity of neural populations in spatial encoding. They showed that the population rate vectors of hippocampal cells robustly predicted an animal's spatial location [256]. By implanting tetrodes in the dorsal hippocampus of freely moving rats and simultaneously recording multiple cells, they found that comparing the similarity of population vectors over brief intervals with a mean vector calculated for the entire exploration period accurately reflected the animal's position. Notably, including both active and silent cells in the analysis

significantly improved the accuracy of reconstructing the animal's path from their activity. This demonstrates that it is not individual cells but the collective activity of all cells that encodes spatial information.

Observations from this study also revealed that the recorded place fields were variable — some were wider, others had a higher spatial firing rate, and some showed multiple firing fields, suggesting the presence of secondary place fields, while other cells did not form fields at all. These parameters—number, width, stability, among others—of place fields are indicative of spatial resolution. Similar to how image sharpness relies on pixel count, a greater number of smaller place fields suggests higher spatial resolution, whereas larger, sparser place fields suggest lower resolution.

This feature of place cell encoding, which distinguishes different areas with varying spatial resolutions, could be useful for large-scale areas by encoding some important areas with higher resolution, and vice versa. For example, let us look at a touristic city map. On it, some areas, like roads to museums and cafés, are highlighted with detailed markers on the street, city lights, or other significant cues to guide tourists to their destination. In contrast, unrelated areas, such as green bushes or building blocks, are marked with less precision, even though the actual structure of these places might be complex, having multiple buildings or hundreds of trees. This observation raises the question of whether the place cell map has similar properties, encoding different spaces with varying spatial resolutions. Exploring this question could provide deeper insight into the nature of the cognitive map in the mammalian brain. In this chapter, I discuss factors, both sensorial and non-sensorial, that could influence the spatial resolution of place cells and present what is currently known about spatial resolution coding in large-scale environments.

4.2 Modulation of spatial resolution

4.2.1 Modulation by sensory information

4.2.1.1 Self-motion cues

Animals use several types of internal signals, known as idiothetic cues, for navigation. These include proprioception, optic flow, vestibular feedback, and efference copies of motor commands. Such cues are crucial during path integration. In 1996, McNaughton [225] and colleagues developed an early model that suggested the scale of place fields within the dorsal hippocampus is modulated by these self-generated sensory cues. Several studies point to the role of idiothetic cues in the formation of place fields and support their role in scaling fields in the environment. For instance, studies like Quirk et al., 1990 [157] demonstrated that place fields could form in complete darkness, maintaining consistent scaling when light was later introduced. Markus et al., 1995 [255] further observed that although place fields maintained a similar spatial scale in dark versus lit conditions, there were notable reductions in firing rate stability and signal clarity. Further investigations have explored how altering animal movement affects spatial memory. A notable early study by Foster et al., 1989 [257] found that restricting limb movements in rats significantly decreased active place cells in the dorsal hippocampus. Later research, such as Terrazas et al., 2005 [258], showed that manipulating self-movement cues through assisted navigation on a mobile platform affected the spatial extent of place fields in the CA1 area of the dorsal hippocampus.

During assisted movements, there was a notable decrease in the number of active place cells and their peak firing rates, with place fields expanding up to three times compared to normal walking. Similar effects were observed in studies involving passive navigation, whether on motorized platforms or robots [259].

4.2.1.2 Distal cues

After the initial self-motion cues, a living being can access cues from the surrounding environment to help it orient and navigate in the external world. These are cues accessible to the sensory system and can be divided into distal and proximal cues. Distal cues are sources of information located in the external environment and can be distant from the observer. These cues help individuals perceive and understand objects and scenes around them, even from afar. Examples of distal cues include perspective, texture gradients, motion parallax (the difference in the speed of moving objects, which helps gauge distance), and atmospheric perspective (how the atmosphere affects the appearance of objects viewed from a distance). Proximal cues can refer to objects in the environment or any sensory stimuli that come into contact with an individual's sensory systems but in contrast to distal cues, proximal cues are closer to the observer. In laboratory experiments (see review by Knierim and Hamilton, 2011 [260]), distal cues often refer to objects on the walls of the laboratory room, while proximal cues are those located inside the experimental set-up.

In terms of animal navigation, particularly in structured tasks like dry mazes and the Morris water maze, which are commonly used in navigational studies, the role of cues has been extensively debated (see Knierim and Hamilton, 2011 [260] for a historical overview). It is widely accepted that rodents rely on distal sensory cues in water mazes to guide navigation and locate a hidden platform [261] [262]. Distal cues also influence the spatial configuration of place fields and the orientation of the hippocampal map (as detailed in Knierim and Hamilton, 2011 [260]). The impact of distal cues on place cell coding has been difficult to quantify and qualify since most studies contrast spatial coding in dark versus light conditions, where both distal cues and apparatus-bound proximal cues (like borders and internal parts) are affected, complicating conclusions about the specific role of distal cues. In a study by Aghajan et al., 2015 [176], dorsal CA1 cells were recorded while rats were randomly foraging in both a real-world environment and in 2D tasks within a virtual reality setup. It was shown that during the random-foraging task in VR, there was a strong reduction in the spatial selectivity of the cells. In another task, where reward-indicating pillars appeared at random locations in VR, the rats' excess path length between rewards was significantly shorter. However, no substantial difference in spatial selectivity was observed between these two VR tasks. In a third task, the reward-indicating pillars consistently appeared at fixed locations, alternating between two fixed positions. As the rats followed more predictable paths, locomotion cues—such as step counting from the last reward and the speed of visual flow—became spatially informative, as these cues repeatedly occurred at the same positions. Consequently, specific locomotion cues were consistently paired with distinct visual cues at each location. This systematic-pillar task led to spatially selective neural responses, with significantly greater spatial information and sparser rate maps compared to random foraging in VR (Figure 4.1). One possible reason for the increased spatial selectivity of cells in this task is the introduction of pillars as proximal cues. This likely helped the animal recognize the location of one or more nearby visual cues (such as floating pillars), which were linked

to rewards, stabilizing place cell coding at and between reward locations.

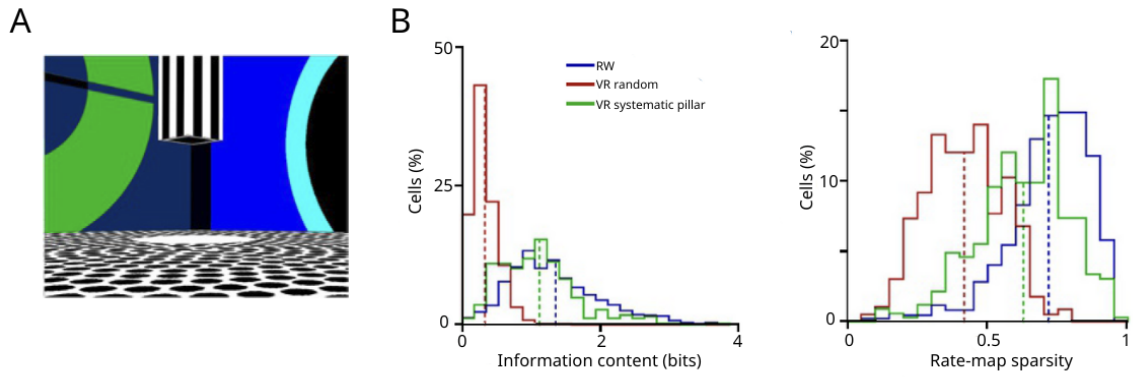


Figure 4.1: **(A)** Scheme of a maze where the reward location is marked by a pillar floating in virtual reality (VR random pillar). **(B)** On the left, the plot shows the spatial information content of cells during different task types: Real World (RW), VR random and VR systematic pillar tasks (where reward locations were marked by a proximal cue—a floating pillar). The spatial information content in the VR systematic pillar task was significantly higher than in VR random and only slightly lower than in RW. On the right, a similar comparison is made for spatial sparsity across the tasks. The spatial sparsity in the VR systematic pillar task was significantly greater than in VR random and nearly matched that of RW. (Adapted from Aghajan et al., 2015 [176])

4.2.1.3 Proximal cues

As mentioned earlier, proximal cues are those located close to the subject. Their perceived orientation and size may vary in relation to its movement. Potential proximal cues can include visual, olfactory, tactile, and auditory cues. While studies have demonstrated that auditory cues can trigger the formation of place cells [263] [264] similarly to tactile cues [265], these often occur in conjunction with visual [264] or olfactory cues [266]. However, there is no evidence that these cues independently affect the spatial resolution of place cells. Therefore, I will primarily focus on visual cues and then elaborate on olfactory cues.

Visual cues

Initial studies on the impact of visual cues on place cell activity found it difficult to separate the effects of distal and proximal cues, with proximal cues often exhibiting greater variability and dependence on animal movement. However, in 1996, Gothard et al. [267] conducted a study that successfully separated the impacts of proximal and distal cues on place field development. They monitored place cells' activity in animals foraging in a cylindrical arena where food location was marked by two cylinders. As the animal explored, these landmarks were moved within the arena while their relative distances remained unchanged. The findings indicated that place cells could fire within a reference frame defined by proximal landmarks. Subsequent studies confirmed the influence of proximal visual cues on place cell activity. For instance, research by Deshmukh and Knierim in 2013 [268] demonstrated the development of multiple place fields equidistant from objects, while the study by Cressant et al. in 1997 [269] noted significant effects of peripheral objects on place cell map orientation, with a more pronounced impact than distal cues [270]. Contrary to initial assumptions, proximal objects were found to play a critical role in spatial navigation and in influencing place

cell firing. Further investigation revealed how proximal visual cues could affect map scaling and the spatial resolution of place coding. A 2004 study by Battaglia et al. [271] recorded hippocampal place cells in rats traveling through various conditions on a linear track, some enriched with local sensory cues and others deprived. The results showed that the place cell population vector decorrelated more quickly across adjacent positions in cue-rich environments compared to cue-poor ones, suggesting that local sensory cues can reduce the scale of spatial representation, although these observations were not replicated when comparing cue-rich and cue-poor sections of the same track. A subsequent study by Burke et al. in 2011 [272] involved rats navigating a circular track with several objects, recording intermediate hippocampal place cells. This study suggested that receiving projections from the LEC, known for processing object-related information, could enhance place cell coding in the presence of objects. Indeed, the presence of objects increased the number of place cells, reduced place field size, and enhanced the propensity to form place fields. A later study by Bourboulou et al. in 2019 [273] explored how visual proximal cues could influence spatial coding resolution. Mice navigated a 2-meter virtual linear maze under various conditions, including scenarios with and without 3D objects. The presence of local objects increased the proportion of spatially selective cells, improved spatial selectivity, and enhanced the stability of place fields, particularly near local cues (Figure 4.2). The study also demonstrated that these cues played a role in temporal place cell coding, such as theta phase precession and spike coordination on a theta timescale. Place fields were smaller and denser near objects, showing qualitative changes in place cell firing such as increased spatial selectivity, information content, and stability. These findings suggest that local cues can modulate spatial resolution in a virtual linear track, advancing our understanding of the hippocampal mapping system. Such local modulation by proximal cues could be beneficial to navigate complex naturalistic environments.

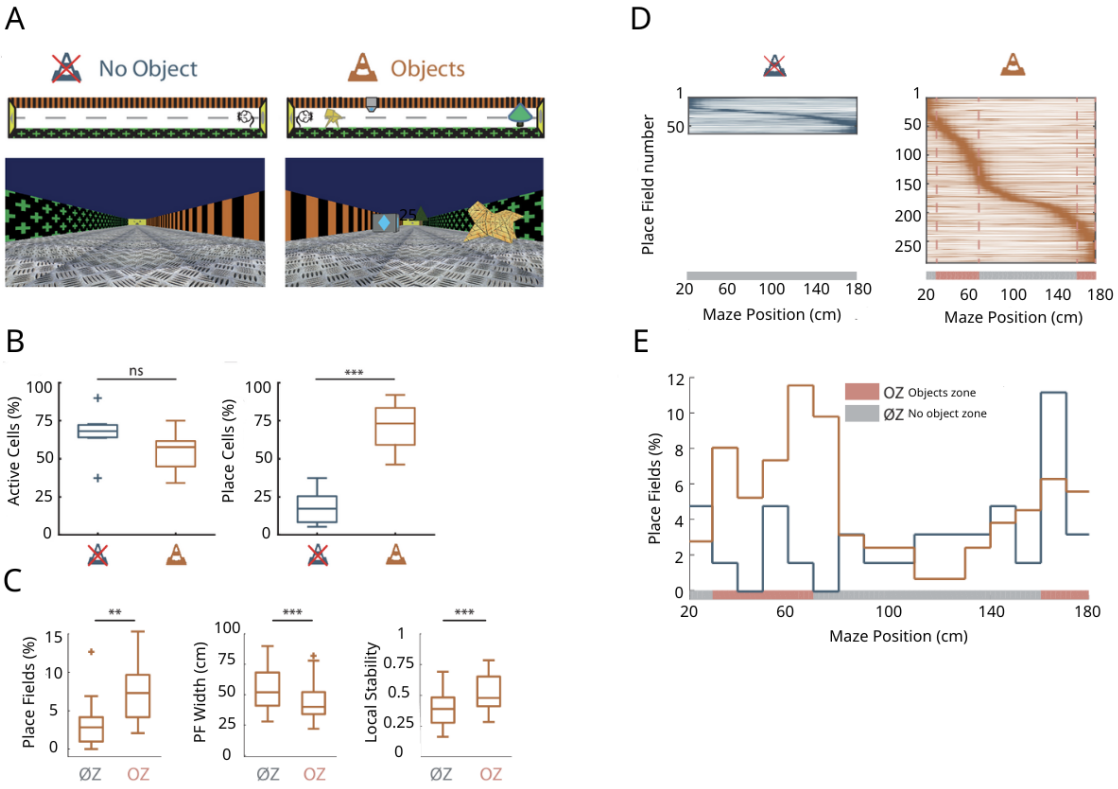


Figure 4.2: **(A)** Scheme of the virtual reality maze used in the experiments, showing a 1D virtual reality maze without objects and one with 3D objects inside. **(B)**The plot showing the percentage of place cells recruited among active cells in the maze with 3D objects. In the object maze, a greater number of place cells were recruited. **(C)** The width of place fields and the number of place fields were significantly higher in the Object Zones (OZ) compared to the No Object Zones (ØZ). Additionally, place cells showed increased stability in the OZ. **(D)** Color-coded mean firing rate maps of all place fields recorded in the maze without objects (left) and with objects (right). The color indicates the intensity of the bin's mean firing rate, normalized to the peak rate (maximum mean firing rate) during the recording session. Place cells are organized according to the location of their peak firing rate along the track. At the bottom, the tracks are divided into Object Zones (marked in red on the x-axis) and No Object Zones (marked in grey on the x-axis), with red dotted lines indicating the boundaries of the OZ in the track with objects. In the VR maze with objects, there is a greater number of place fields, along with a higher recruitment of these fields in the object areas. **(E)** Percentage of place fields at each spatial bin in the maze with objects (orange line) and without objects (blue line). Similar to the previous plot, there is an increased percentage of place fields in the object areas. (Adapted from Bourboulou et al., 2019 [273])

Olfactory cues

Not only visual cues but also other sensory inputs significantly impact the formation of place fields. For example, one study demonstrated that self-generated odors play a role in activating and stabilizing hippocampal place cells. In this experiment, hippocampal cell activity was recorded under various conditions, combining visual cues (light or dark) with olfactory cues (cleaned or uncleaned maze), as researchers observed cell behavior while animals searched for food in a circular polarized arena. The findings indicated that removing self-generated odors extended place fields, and place cell stability decreased under all conditions, particularly in the dark-cleaned scenario where some cells ceased firing, suggesting a reliance on multimodal cues for place field generation. Further exploration into the role of self-generated odors in navigation examined the effects of visual and odor cues when put in conflict, such as

by rotating objects or paperboard with odors [156]. Results showed that place fields rotated with visual cues and that olfactory cues alone were insufficient to anchor place fields without visual support. Another significant finding was that exploring a novel environment devoid of self-generated cues diminished place cell stability, indicating that odor cues could enhance exploration and contribute to place cell stability in new environments. Due to the volatile nature of odors on paperboard and their chemical changes over time, a subsequent study by Zhang and Manahan-Vaughan in 2015 [274] used four synthetic odors with stable concentrations. This study confirmed that odors can enhance place cell stability, and unlike earlier findings, showed that they can induce place field rotation following odor movement and that relocating odors can trigger remapping. In further experiments, mice navigated a virtual linear track using both odor and visual cues, followed by navigation in darkness relying solely on two odor gradients. It was observed using two-photon calcium imaging that animals effectively navigated using only olfactory cues, and place-modulated cells were active in the dark where only odors were present. When a uniform odor was introduced along the track, the active cell count decreased to 88%, highlighting the odor-dependent activity of the cells. Building on these insights, later research recorded hippocampal cell activity during olfactory-guided navigation tasks with localized odor cues [275]. Introducing an odor 1 meter from the track starts to reduce errors from the path integrator over longer distances, not only stabilizing place fields but also creating new spatial landmarks. This study illustrated that spatial representations are influenced not only by distance but also by a mix of path integration and olfactory inputs, showing how sensory cues refine spatial navigation by establishing precise and meaningful environmental landmarks. Moreover, enhanced place cell activity induced by odor cues at 1 meter increased the density of place fields further along the track. When the same odor cue was placed at both 1 meter and 3 meters from the start, the closer cue initially had a higher influence, resetting the path integrator and forming a distinct place cell representation. Over repeated training, place fields expanded to bridge the gap between these two identical cues, with the cue at 3 meters eventually promoting its own unique place cell activity and enhancing place field density at that location. This demonstrates how identical sensory features at different locations can be recognized as unique landmarks through a gradual, path integration-dependent process, enhancing navigational accuracy.

4.2.2 Modulation by non-sensory information

4.2.2.1 Modulation by goal

One of the hallmark characteristics of natural navigation is the existence of a goal, such as finding food, a mate, a nest, or escaping danger. This behavior is evident in classical spatial navigation tasks like the Morris water maze, where the animal's goal is to locate a platform to escape the water, or in various studies where the goal is to find food or water reward. If the hippocampus supports not only the spatial coding of an environment but also the "value" or "meaning" of the space, we would expect place cells to be influenced not only by stimuli that aid orientation, such as visual or tactile cues but also by the context of the environment and the internal state of the animal, such as the presence and location of a goal. Numerous studies have observed that place fields tend to cluster or activate more around the goal location, indicating an overrepresentation of these reward locations and suggesting a higher

spatial resolution at these points. For example, Dupret et al., 2010 found that during rewarded navigation, place cells in the CA1 region adjusted their positions towards the goal, this adjustment depended on NMDAR activation [276]. The adjustment was more pronounced in the deeper layers of CA1 than in the superficial layers. Other research has shown that local clustering of place fields at goal locations can be linked to place preference behavior [277] and to the memory of the goal location [278].

An earlier study by Kobayashi et al., 2003 demonstrated that a subset of place cells could adapt their activity patterns in response to rewards, indicating a form of neural plasticity or adaptation to new requirements. They used stimulation of the lateral hypothalamus as a reward mechanism in three different tasks and observed how place cells responded under various conditions, finding that 92% of place cells showed significant reward-related activity within their established fields, with consistent relationships between reward and place activity across different tasks [279].In another experiment, Lee et al., 2006 [280] studied how place cells responded during an automated T-maze alternation task. They found that 70% of these cells displayed varied activity, the "splitter effect", depending on the animal's past and future choices. As the animals gained more experience, these cells shifted their active zones toward the maze's goal. This adaptation was not observed if the maze's alternation element was removed, highlighting the necessity of this challenge for such neural changes. In a separate candelabra maze task [281] involving multiple goals, most place cells displayed multiple active fields linked to specific goals, yet no cells responded exclusively to all potential goals, indicating a more complex encoding of space than simple goal representation.

A study by Gauthier and Tank [282] identified a few cells that were activated exclusively at the goal, whether moved within the same virtual environment or transferred to a completely different one. These "reward-associated cells," which constituted 4.2% of active cells in both scenarios (or 0.8% of all cells studied), likely represent a minor subset of place cells that receive direct inputs from the reward system, potentially influencing other hippocampal cells regarding the location of rewards. Further studies have investigated whether the value of a goal can be represented in place cell firing. Tabuchi et al., 2003 found no evidence of hippocampal encoding of goal volume, perhaps due to the low spatial demands of the task [283]. Subsequent studies [284] [285] [286] explored whether place cell firing could represent changes in goal value. Lee et al., 2012 used a T maze with varying probabilities of reward in the two arms over blocks of trials, signaled by different tones [284]. They discovered that both CA1 (15%) and subicular (11%) cells reflected the animal's choice of goal and its outcome in both the current and previous trials. In a study by Duvelle et al., 2019 animals were required to navigate to an unmarked location in an open field and wait for a short duration (2 s) before a food pellet was released from an overhead dispenser, which the animal then had to locate [286]. This experiment separated the location of the goal from the act of obtaining the reward and facilitated the mapping of place fields since the animal explored the entire environment while searching for the reward. Previous findings indicated that CA1 place cells, whose fields were distant from the goal, emitted spikes when the animal paused in the goal zone [287], hinting at potential goal representation. In a specifically designed task featuring two goals offering varying amounts of food, researchers integrated an element of value-based decision-making into the spatial challenge. The study demonstrated that rats could distinguish between the two goals and displayed a preference for navigating towards the one with greater rewards, showcasing their behavioral adaptability to this variable. However,

there was no significant representation of place fields at the goals, nor a consistent encoding of goal value by place cells. The researchers concluded that place cells do not encode the value associated with spatial goals; instead, such information is likely processed in combination with other place information outside the hippocampal region.

A 2015 study by Aghajan et al. [176] showed that hippocampal neurons, which typically showed no spatial preference during random movement in a two-dimensional virtual environment, began to exhibit place-selective firing as the animals transitioned from random movements to running towards a reward within the same space. However, as noted earlier, spatial selectivity remained sparse, with improvement observed when reward locations were associated with introduced proximal cues (Figure 4.1). In another experiment rats were trained to approach reward ports in a two-dimensional open field based on visual cues. In this setting, hippocampal place cells managed both spatial and goal-directed information [288]. Their firing rates increased within their established place fields when the animal moved towards a specific goal, indicating place-selective firing that occurred irrespective of the animal's speed or direction. This supports the concept of a stable spatial map that functions independently of goal-directed actions. When allowed to roam freely in the field, these cells also demonstrated increased firing rates when the animal briefly moved towards remembered goals. Additionally, disrupting medial septal activity resulted in a reduction in goal-directed firing, although the spatial firing patterns remained consistent. These outcomes underscore the significant role of hippocampal circuits in integrating spatial and goal-directed information, enhancing the understanding of how these neural networks support complex navigational tasks.

4.2.2.2 Modulation by attention

As we discussed the goal modulation of place cell firing, we have observed that place cells are influenced not only by sensory information but also by behavior, including the internal states of an animal. A critical internal state in spatial navigation for both animals and humans is whether an individual is focusing attention on a particular space. Attention is a fundamental brain process essential for perception, cognition, and action [289]. It is described as "the process by which organisms select a subset of available information for enhanced processing and integration" [290]. Given the broad visual field of rodents, it might appear that they can perceive their entire visual environment at all times. However, while this might be feasible in simple scenarios with few, prominent stimuli, most real-world environments are too visually complex and cluttered for everything to be processed in detail across the entire visual field. Thus, sophisticated physical and mental attention mechanisms are necessary to direct processing resources toward significant and task-relevant information, helping to filter out irrelevant distractions [291].

As shown in a previous section, place fields can be influenced by an animal's goals and activities within its surroundings, which effectively dictate the focus or types of attention. Typically, these place fields gather around important environmental landmarks and behaviorally critical spots, indicating a preference for these types of cues. Moreover, place fields are customized based on prior experiences, allowing for the recall or initiation of distinct firing patterns even if there are no alterations in the environment or the animal's position, pointing to an attentional state driven by memory (see Soyer, 2023 [292] for more details). Indeed, several studies reported attentional

modulation of hippocampal representations in experimental studies. In a study by Kentros et al., 2004 [293], place cells were recorded from mice performing navigation under several behavioral conditions. In the first condition, there was no task, animals were simply fed and placed in the environment as a control group; a second condition involved foraging, where food-deprived mice chased randomly dropped food pellets; a third one featured a novel environment, similar to the foraging group but with sessions alternated with exposure to a new environment; and the last one involved a spatial task, where mice performed a demanding place preference task involving sudden noises and lights that they could stop only by locating an unmarked goal area using environmental cues. This last group required intense focus on environmental cues to succeed in the task [293]. After analyzing place cell stability, it was shown that long-term stability of place cell firing fields is linked to the level of attentional demands. This indicates that the management and recall of place cells are influenced by a cognitive process similar to attention. In another study, Muzzio et al., 2009 investigated whether the stabilization of the place field map was merely a result of increased general arousal or if it necessitated focused attention on specific visuo-spatial cues. They used two goal-directed tasks requiring the animals to concentrate on either visuo-spatial or olfactory cues to locate a reward.

In the visuo-spatial task, mice needed to learn a location based on visual cues, disregarding olfactory cues. Conversely, in the olfactory task, mice associated a particular scent with the reward, independent of location. Notably, both tasks utilized identical sensory cues (olfactory and visuo-spatial), with alterations including rotation or reorganization of these cues. If general arousal were the cause, place field stability would be similar across both tasks. However, if arousal selectively affected how the cues were processed, it could result in different levels of place field stability between the two tasks. The results showed that stable hippocampal spatial representation was only observed in the visuo-spatial task. This suggests that attention helps in selecting particular cues essential for successfully completing a task.

The difference might also relate to how the animals navigated to the goal; in the olfactory task, animals likely used a beaconing strategy, concentrating solely on the scent linked to the reward [294][295]. In a study by Fenton et al., 2010 [296], the researchers explored whether the hippocampal map statically reflects spatial information or dynamically adjusts based on changes in information that are the focus of immediate attention. This question comes from observations that place cell activity can be modulated on a one-second timescale by a behaviorally silent, attention-like process [297]. A key clue supporting this idea is that place cell discharge is often unreliable, firing either much faster or slower than expected based on the average location-specific rate [298] [299]. This unreliability, referred to as overdispersion, suggests that place cell firing may switch between two states as the rat's attention shifts between distal cues and local or self-motion cues [297].

In their research, Fenton et al., 2010 [296] investigated whether a greater need for navigation and attention to specific cues might cause hippocampal cells to align within a single reference frame, thereby reducing overdispersion. They observed that place cells recorded in rats navigating on a rotating arena to avoid an unmarked location associated with foot shocks exhibited less overdispersion compared to place cells recorded in control rats. Interestingly, they also found that overdispersion was lower when approaching a goal than when departing from one. They further demonstrated that a preference for using distal cues reduced overdispersion, suggesting that states akin to global attention can be distinguished by analyzing the overall firing patterns

and discharge rates of cell ensembles, each displaying unique characteristics. Detecting attention-like states could lead to more accurate reconstructions of a rat's path from the collective discharges of the ensemble. This indicates that attention can enhance the animal's focus on external sensory cues and directly affect the short-term variability in the firing of place cells. Similarly, Scaplen et al., 2014 demonstrated that the hippocampus processes visual cues differently depending on whether they serve as navigational landmarks or contextual elements [300]. This differentiation was influenced by factors such as the type of cue (for example, a distant landmark versus a small object on the ground), its prominence, and the animal's prior experiences. Results of these studies show that attentional effects can modulate place cell responses.

4.3 Spatial resolution in large-scale environments

In discussing spatial navigation, it is important to mention that the majority of studies, including those described previously, are conducted in laboratory environments. These environments do not adequately represent the complexity of real-world landscapes with their varied shapes, colors, and textures. Additionally, laboratory settings cannot replicate the true scale of natural environments. This limitation is significant, as navigating in such environments poses additional challenges that could benefit from a local modulation of spatial coding resolution. In large, complex environments, an animal needs to prioritize important locations over less interesting ones and dynamically update its cognitive map based on environmental changes.

To begin to address the question of how environments of a more realistic size can affect place cell coding, several studies have been carried out. For example, Fenton et al. [151] recorded hippocampal CA1 pyramidal cells from rats exploring two different environments. The first was a small, 68-cm-diameter gray cylinder with a polarizing card on the wall. The second was a modified monkey chamber [301] with a 150 x 140 cm footprint, equipped with stairs on three sides. In the chamber, rats could access two drinking spouts—one with plain water and the other with a sucrose solution—motivating them to traverse the stairs and forage for food for an hour. The chamber allowed the rats to see the surrounding room through its bars. For the experiments, the cylinder was placed within the chamber for the cylinder recordings and removed for the chamber recordings.

Using these two standard environments, one small and one enlarged, they showed that CA1 place cells with single place fields in the cylinder had multiple place fields in the six-times-larger chamber. In stable, familiar conditions, 89% of CA1 place cells discharged in multiple distinct locations. These randomly distributed multiple place fields appeared in all recorded animals in the large environment. However, the study did not indicate whether this distribution was overrepresented around reward areas or possible visual cues in the maze. They also showed that in a large environment, the scale and width of place fields were larger, though other characteristics, such as the overall firing rate, did not change. This study highlighted how place field sizes and distributions change with the enlargement of an environment (Figure 4.3) (Fenton, 2008).

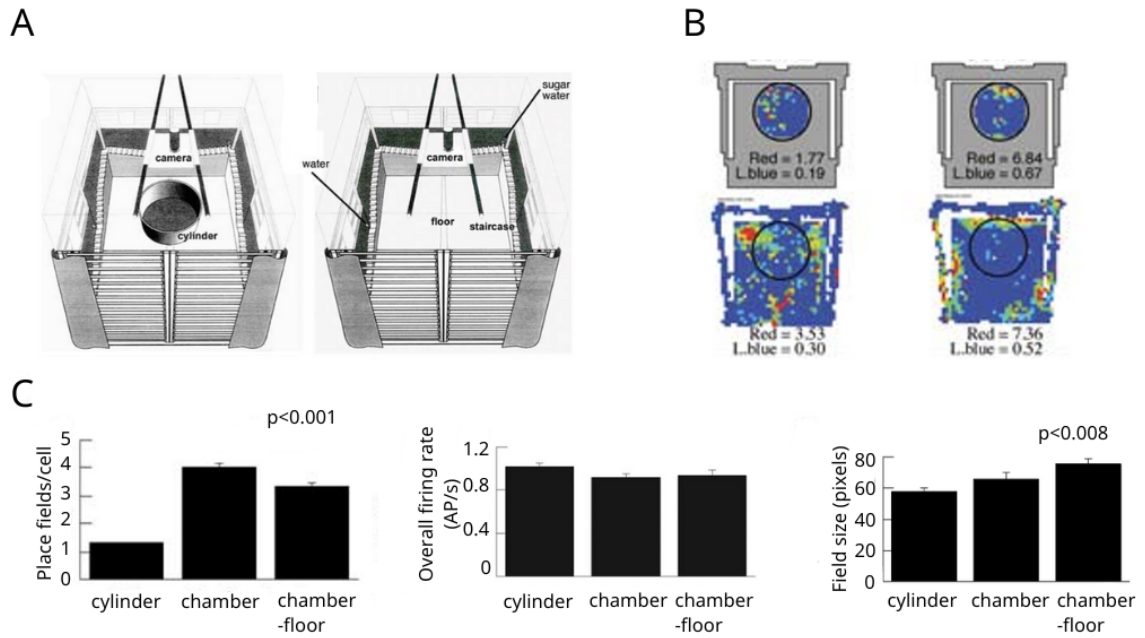


Figure 4.3: **(A)** Schematic of the 68-cm-diameter cylinder and the 150x140 cm chamber used in the experiments. **(B)** Examples of two cells' firing rate maps in the cylinder (top) and chamber (bottom). Blue indicates a low firing rate, while red represents a high firing rate. The firing patterns were independent between the two environments, with cells displaying a single place field in the cylinder but multiple place fields in the chamber. **(C)** On the far left, the boxplot shows the average distribution of the number of place fields per cell in the different conditions. In the larger environment (chamber), individual cells exhibited multiple place fields. In the center, there was no difference in the overall firing rate of cells across all environments. On the far right, place fields were slightly wider in the large environment compared to the smaller one. (Adapted from Fenton et al., 2008 [151])

A study by Rich et al., 2014 [152] shed light on the statistical structure of hippocampal place cells' activity during large-scale navigation, addressing two main questions: which cells fire in a large-scale environment and where they fire. They recorded 263 pyramidal cells from the dorsal CA1 region of 5 rats. The rats explored a previously unknown 48-meter long track, with both the room and track being novel to them. To explore whether there is a limit to the recruitment of place cells with increasing space, the researchers progressively extended the track across four epochs (3, 10, 22, and 48 meters). The analysis was restricted to periods when the animals explored the newly introduced sections in each epoch. The study found that with each new section, already existing place cells formed additional place fields, and more new cells were recruited to form place fields, consistent with previous observations (Figure 4.4A) [151]. These multiple place fields were irregularly spaced, which can be explained if the field locations are randomly chosen and follow a spatial Poisson process (Figure 4.4B, C).

At the population level, the researchers questioned if all cells had the same Poisson rate of field formation. If so, it would mean that as the environment increased in size, more silent cells would become active and form new place fields, with the observed number of fields per cell following a Poisson distribution. In this scenario, the distribution of the location of each cell's field closest to the start of the track would follow an exponential distribution, with the number of recruited cells increasing with the total length of the track. However, they found overdispersion in the number of fields per cell, indicating significant differences in the spatial rate of field

formation between cells. Many more cells than predicted had few or no fields, and only a small fraction had many fields as the environment size increased, with overall cell recruitment reaching 65% at 48 meters. Recording silent cells in a novel environment showed that despite being silent on one track, these cells could form place fields in another novel environment. These findings suggest that cells have different propensities to form place fields in a given environment, and this is not only dependent on the environment's scale. This difference in propensity can be explained by each cell having an independent Poisson process with its rate drawn from a gamma distribution (Figure 4.4D). As expected from such a distribution, the overall field density was uniform across the environment, uninfluenced by running speed or local track features. They also demonstrated memorylessness, invariant with respect to the starting point, a property of the Poisson process. Altogether the study shows that in large-scale environments different place cells have different propensities to form place fields and that the statistical description of place field distribution could be best explained by a gamma-Poisson model.

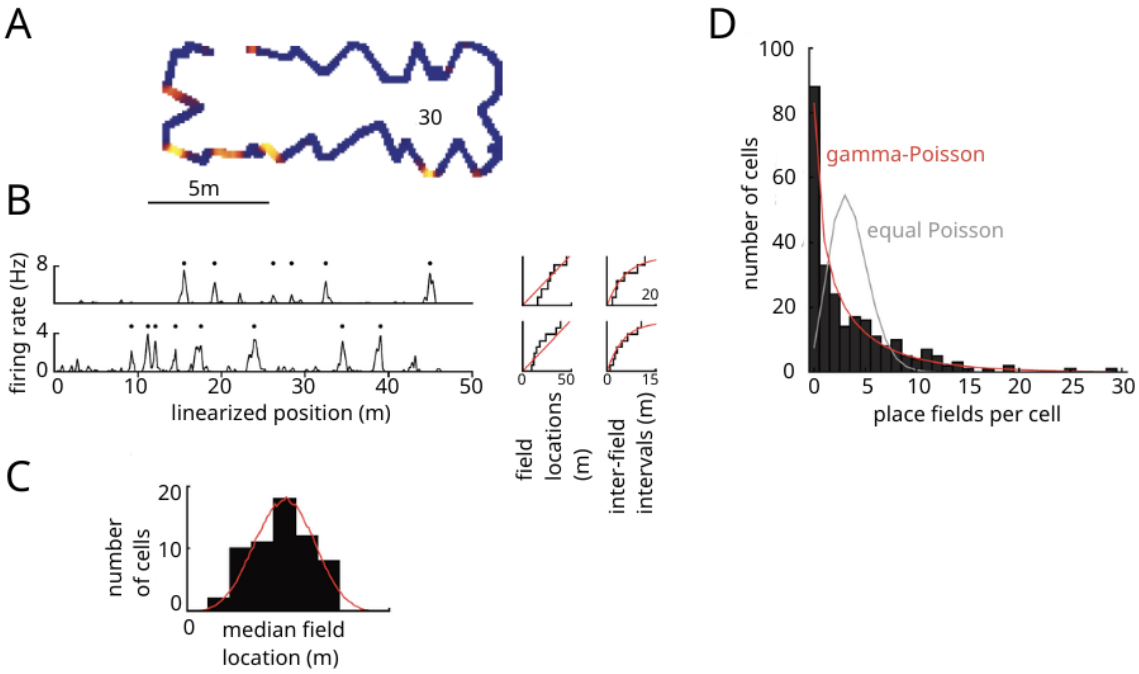


Figure 4.4: **(A)** Example of place cell activity along a 48-meter track, showing that place cell form multiple firing peaks in large environments. The firing rate is color-coded, with blue representing low activity and red indicating high activity. **(B)** An example of place fields from two cells in a single animal, with dots marking the centers of the detected fields. On the right, the cumulative distributions of field locations and interfield intervals are displayed in black, along with the uniform and exponential distributions predicted by a Poisson process in red. **(C)** The distribution of median field locations for all cells across animals, compared to the expected distribution for cells with uniformly distributed fields (represented by the red curve). **(D)** The number of place fields per cell is displayed, with the distribution assuming that each cell has an equal Poisson rate for forming place fields per unit length, based on the average number of fields per cell (gray). The red curve represents the fit of a negative binomial distribution, which results from gamma-distributed Poisson rates. (Adapted from Rich et al., 2014 [152])

One of the important questions raised by the study is what causes a cell to have a particular rate of expressing place fields. This could be due to preexisting differences between cells or a cumulative advantage mechanism during exploration. Preexisting

differences could result from variations in cellular excitability [302] [303] or synaptic inputs, making future place cells more excitable than future silent cells. However, it is possible that additional mechanisms contribute to the recruitment of cells to form place fields in a given environment. In this study, similar to Fenton et al. [151], the environment lacked cues or additional tasks that would require specific attention or motivation from the animal. However, other studies have shown that place field density can increase around goals [304] or visual cues [273]. Thus, it is possible that not only cell propensity but also the sensory content or valence associated with an environment could influence place field formation. Thus an important question remains: what would the population distribution look like under these varying conditions?

A partial answer to this question can be found in a follow-up study by Lee et al., 2020 [305]. In this study, mice ran on a spherical treadmill at the center of a half-cylindrical screen projecting monochromatic virtual environments (Figure 4.5A). The researchers used four different virtual reality environments with 40-meter-long tracks surrounded by unique objects. Each environment featured distinct odor cues, with rewards delivered randomly in the first set of experiments and fixed in the second set. By manipulating reward contingencies, they aimed to investigate the interaction between the value and propensity of cells to form place fields.

Using calcium imaging while mice performed the task, the study found that cell propensity was preserved across different environments, even in the dark, and over long periods, with some cells maintaining their propensity across two months. Place field density increased at reward locations (Figure 4.5B), with consistently rewarded areas showing 2-3 times higher CA1 field density. Cells with higher general field propensities also had higher reward field propensities, indicating these cells were more likely to form fields at rewarded locations. However, place cell activity at reward location was best explained by a conjunction of reward and place, rather than being specific to reward, as most cells contributing to increased field density at reward locations also had increased non-reward place fields propensity. Like a reward, novelty significantly modulated CA1 activity. CA1 pyramidal cells exhibit higher firing rates in the novel compared to familiar environments. Field numbers per cell were highly correlated between the novel and familiar environments, suggesting that novelty scales up each cell's propensity by a constant factor. This was confirmed by analyzing pairs of sessions where one was novel and the other familiar, showing lower calcium and field activity in familiar environments.

Following the results of this and previous studies, researchers propose that the "gamma × gain Poisson" model can statistically describe hippocampal representations across various conditions in large-scale environments. Each cell is assigned a propensity from a gamma distribution (Figure 4.5C), leading to the random expression of place fields in a spatially Poisson manner. Propensities are multiplied by common gain factors in rewarded and novel environments, and this propensity, predictive of a cell's activity, is preserved over time. However, the role of the gain factor and its modulation of place field formation in more complex environments still needs to be understood. Together with behavioral components, the use of such a model can facilitate the understanding of different navigational strategies, including the formation and navigation with a cognitive map, in large-scale environments.

In an effort to better understand navigational strategies in large-scale environments, Toledo et al., 2020 [306] conducted field studies with Egyptian fruit bats (*Rousettus aegyptiacus*, Pteropodidae). They tagged and tracked 172 bats over four years, monitoring their routes from their home cave to preferred fruit trees across a

19,000-ha area. Initial observations revealed that the bats rarely engaged in random searches. Instead, they consistently followed straight, goal-directed paths to a few favored locations. This behavior suggests that bats may be using a cognitive map as a navigational strategy to perform such behavior. To further explore this, it was tested whether the bats could take novel shortcuts between known locations far from each other—another key characteristic of cognitive map strategy. Remarkably, 41% of the bats were able to create new shortcuts. These new routes showed no differences from their regular paths in terms of straightness, turning angles, or headings. To confirm these results, an experiment with translocating bats to previously unvisited areas was performed. All translocated bats successfully took novel routes back to their regular foraging grounds, with no change in their flight behavior. Path integration, another possible navigational strategy, was not a possible explanation since the straightness of return flights did not correlate with the outbound flight paths or the total flight length. Additionally, on 13% of the nights, bats returned to a different cave than the one they had originally left from, sometimes more than 7 km away. In many cases, they only returned to their original cave after several nights, which would not be possible with path integration alone. These findings provide strong evidence that animals, like the Egyptian fruit bats, use cognitive maps for large-scale navigation in real-world environments.

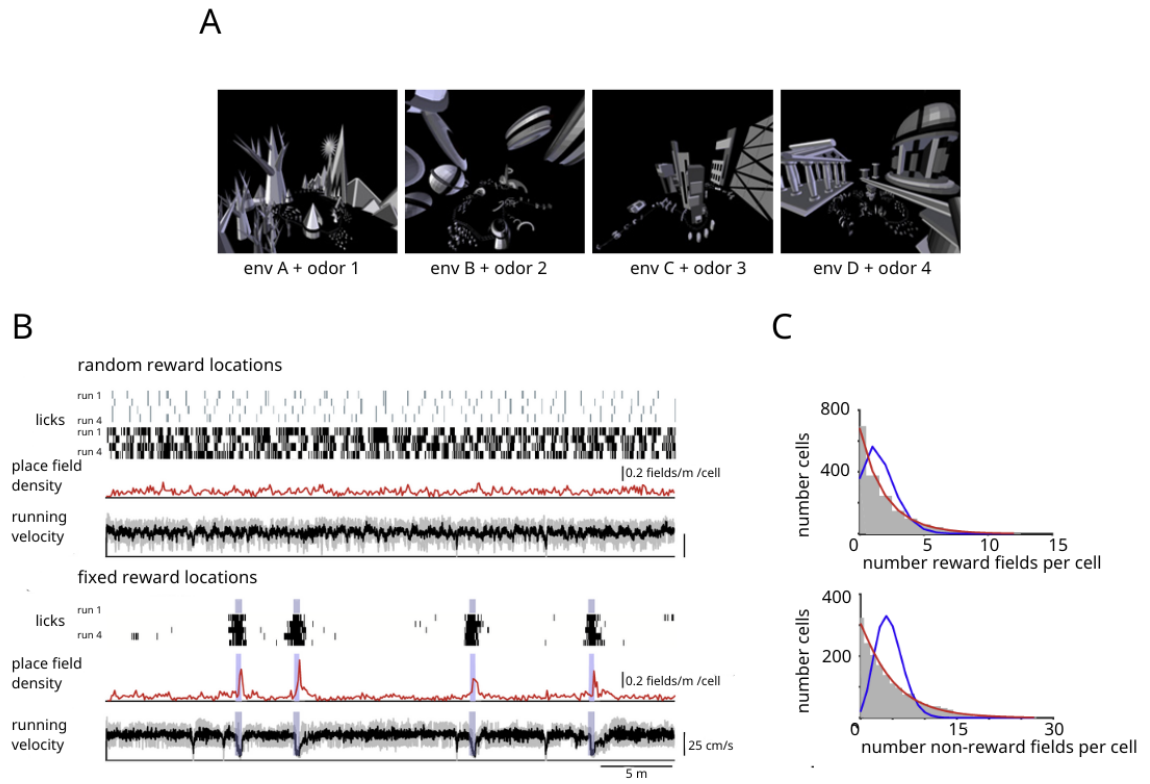


Figure 4.5: **(A)** Four different environmental contexts used in the study. **(B)** Water reward locations (blue), lick locations (black), place field density, and running speed (gray for each run, black for the average) during random reward sessions and sessions where rewards were given at four fixed locations per run. In the fixed reward sessions, place fields concentrated around the reward areas. **(C)** Distributions of reward and non-reward place field numbers per cell. Poisson (blue) and gamma-Poisson (red) fits are shown, with the distribution fitting a gamma-Poisson model, similar to the findings of Rich et al., 2014. (Adapted from Lee et al., 2020 [305])

Following this study, another set of experiments investigated the possible neuro-

physiological basis for large-scale environmental coding in the hippocampus of Egyptian fruit bats [307]. Pyramidal neurons were recorded from the dorsal CA1 while bats flew in a 200-meter-long tunnel, which included long and short arms with dispersed landmarks. In this large environment, place cells revealed multiple place fields at several different scales. On average, each direction had 4.9 place fields, with some neurons having more than 10 fields per flight direction (Figure 4.6A). Unlike smaller environments, where place fields can be as small as 30 cm, many neurons in this experiment exhibited much larger fields, ranging from 10 to 32 meters. However, some neurons also displayed small fields, less than 1 meter in size. Many place cells showed highly variable field sizes, with up to a 20-fold difference between the largest and smallest fields for the same neuron (Figure 4.6B). While most cells exhibited heterogeneous field sizes, some neurons had more uniform field sizes across their place fields, and a small minority of neurons had just a single place field, following the classical model of one place cell corresponding to one place field.

The majority of neurons had two key characteristics: multiple fields per neuron and a combination of both small and large fields even for individual cells. This raises the question of what drives this multiscale property. The influence of flight speed and differences between the long and short arms of the tunnel did not show any significant effects that could explain the multiscale representation. Another potential explanation involved the role of landmarks, with the idea that places with landmarks might be encoded with smaller, more numerous place fields. However, the cumulative distribution of field locations (Figure 4.6C) was linear as a function of position along the tunnel, with no overrepresentation near landmarks (Figure 4.6D). The place fields were uniformly distributed along the tunnel, and the entire range of field sizes appeared evenly spaced, with no notable clustering of small or large fields near landmarks.

The lack of overrepresentation and uniform distribution of place fields may stem from the limited importance or informativeness of the landmarks for the bats. Overrepresentation or encoding with higher spatial resolution might occur if the landmarks were more salient for navigation. Supporting this, the reward locations at both ends of the tunnel were overrepresented by place fields, while very large fields were found in regions with little spatial information over long distances. It could be suggested that the presence of landmarks alone does not play a role unless they provide information that is useful for the animal's navigation. In real-world environments, animals are surrounded by numerous visual and other cues, and for efficient navigation, they likely focus on the most informative cues to guide them to their destination. Future studies investigating the impact of informative versus non-informative cues in large-scale environments could provide further insight into how hippocampal place cells code for large spaces.

In a study by Harland et al., 2021 [308], place cell coding in a large-scale environment was tested using wireless recordings in a rat trained to follow a food-baited robot across an 18.6m^2 space. The results showed that the size of the environment influences the area covered by dorsal CA1 place cells, with the covered area increasing as the space expanded and each cell becoming active in several subfields of varying sizes. This suggests that place cells can form a multi-scale representation of space, allowing each cell to be active in multiple sections of the environment, potentially linking these areas spatially and enabling each location to be represented by a unique combination of subfields. The study proposed that ensembles of place cells with these abilities can generate complex multi-scale codes capable of supporting both coarse and fine-grained spatial representations. This upscaling of subfields could be an efficient

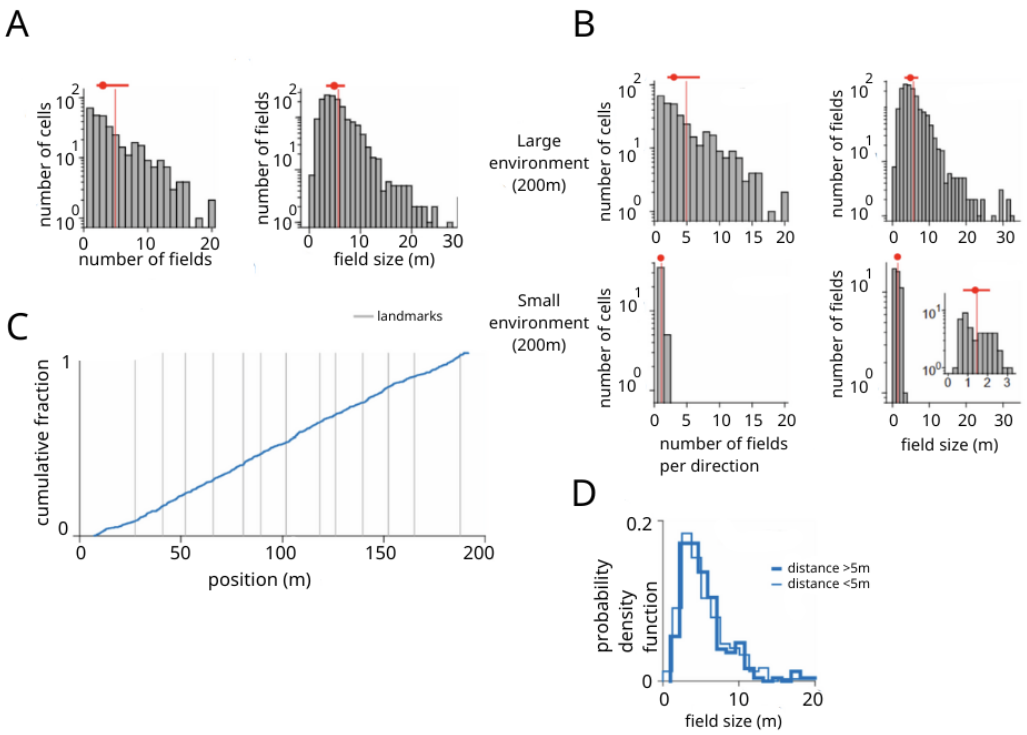


Figure 4.6: **(A)** On the left, the distribution shows the number of place fields per neuron for each flight direction, with the rightmost bar indicating cases where there are 20 or more fields per direction. The average number of fields per direction is 4.9. On the right, the distribution of place field sizes is displayed, with sizes ranging from under 1 meter (leftmost bar) to a maximum of 32 meters. **(B)** Distributions of the number of fields and field sizes are shown for the large-scale 200-meter environment (top row) and the small-scale 6-meter environment (bottom row). The red vertical lines represent the mean of each distribution, while the red horizontal lines and red dot indicate the interquartile range and median, respectively. **(C)** The cumulative fraction of peak firing-rate locations for all place fields along the tunnel is shown. Gray vertical lines mark the locations of landmarks. The place fields are evenly distributed throughout the flight path. **(D)** The distribution of field sizes is plotted separately for fields near landmarks (thin line, fields less than 5 meters from the nearest landmark) and fields farther away (thick line, fields 5 meters or more from the nearest landmark). No significant differences in field size were observed between fields located close to or far from landmarks. (Adapted from Eliav et al., 2021 [307])

way for a finite population of place cells to encode large environments. Supporting earlier findings, they observed that place cells exhibit multiple, irregularly arranged fields in large open spaces, but noted greater field enlargement than expected, with place subfields ranging widely in size in larger environments. Interestingly, despite this variability, the fields homogeneously covered space at the population level, even if individual cells had irregularly spaced subfields at the single cell level. In this experiment, there were no local cues, only distal cues on the walls of the recording chamber and room. The researchers highlighted the importance of testing in realistic environments, as natural settings are much larger and allow animals to move freely in 2D (or even 3D). However, real-world environments are also complex due to the presence of various visual and other cues which are important for spatial navigation. It would be interesting to explore how multi-scale subfields behave with additional sensory information, such as visual or olfactory cues or rewards since ensembles of place cells coding part of a space at multiple scales would likely require further differentiation for more informative locations.

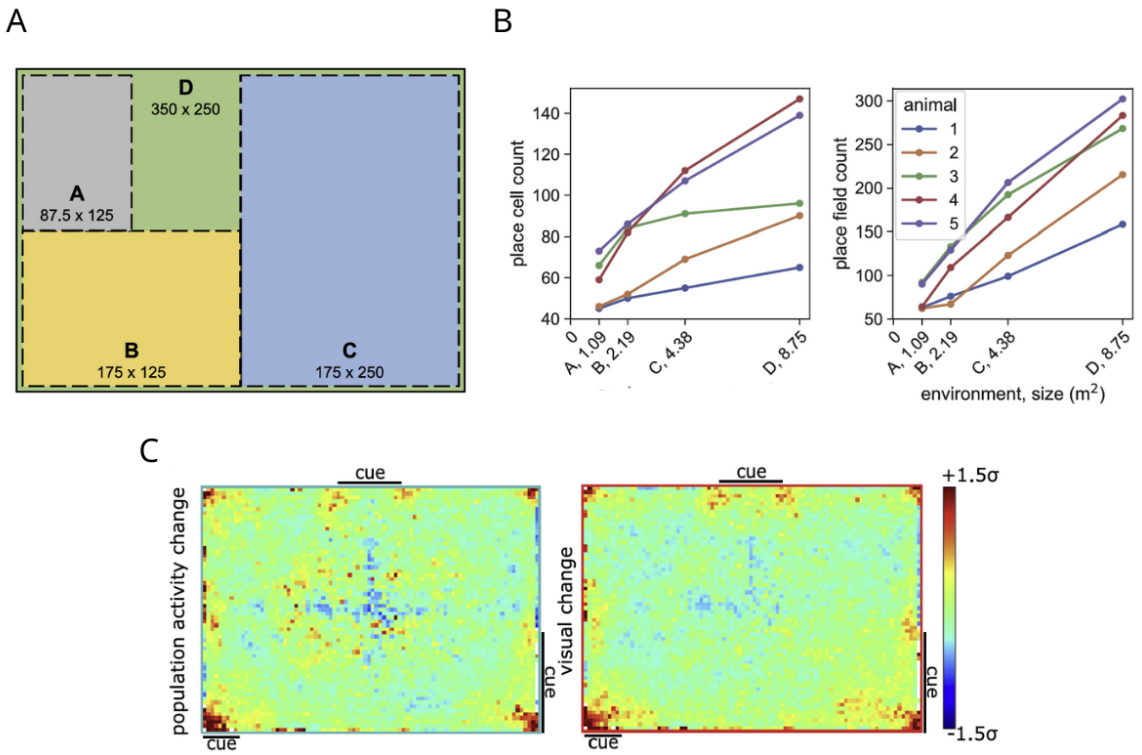


Figure 4.7: **(A)** Schematic representation of the four environments (A, B, C, and D), showing their relative dimensions (in cm) and locations within the experimental room. Each environment was characterized by a distinct set of cues. **(B)** The number of active place cells (left) and place fields (right) by environment area. **(C)** The spatially averaged rates of change for the place cell population and visual scenes are similar, with both showing increased activity near walls, corners, and cues. Notably, a local rise in change is observed at cue boundaries for both visual and population data. (Adapted from Tanni et al., 2022 [153])

Work by Tanni et al., 2022 [153] reveals the possible rules governing the statistics of the spatial code and its interactions with perceptual stimuli, particularly in large-scale environments. Place cell activity was recorded from rats foraging in environments of various sizes, up to 8.75m², with equal proportions (Figure 4.7A). Consistent with previous findings, the study showed that in larger environments, more place cells were activated, forming place fields, with more cells displaying multiple fields (Figure 4.7B). It was also observed that some cells had a higher propensity to form place fields. Cells that developed multiple fields in one environment were more likely to form fields in other environments as well. The number of fields per cell was best modeled by a gamma-Poisson distribution, which accounts for the varying propensities of cells to form place fields, as also noted in the study by Rich et al., 2014 [152].

When examining the distribution of place fields within environments, it was found that field size was influenced by proximity to boundaries. Smaller place fields were densely concentrated near the edges, while larger fields were more sparsely distributed in the center. This suggests that the total area covered by place fields across all active cells scales proportionally with the size of the environment—smaller environments compensate with a greater number of fields, while larger environments feature fewer but larger fields. Despite these differences, the proportion of co-active cells, the average firing rate, and the overall activity pattern remained consistent across all regions, indicating a robust mechanism that regulates place cell activity.

Interestingly, the size of the place fields and the density of their peak firing rates

varied systematically, implying that hippocampal activity changes at different rates depending on the animal's location and the direction of movement. When the animal moved toward or away from a wall, there was a greater shift in place cell activity, while movement parallel to the wall triggered less changes. A virtual reality version of the maze was used to test whether changes in visual information perceived by the animal during movement could affect place cell activity. The results showed that both the amount of visual change and the rate of change in place cell activity increased as the animal approached boundaries (walls, corners) (Figure 4.7C). Importantly, filtering the data based on the animal's movement direction revealed that the variation depended not only on the animal's position but also on its direction of movement. Walls and visual cues that were perpendicular to the animal's movement played a key role in driving visual changes, closely mirroring the direction-filtered changes in place cell activity.

These findings highlight a balance between homeostatic regulation at the population level and the rate of perceptual change, which naturally increases near boundaries, visual cues, and other areas where the visual scene shifts rapidly as the animal moves. Using information theory, this relationship between visual change and place cell accuracy can be understood more clearly. Fisher information quantifies how much a variable (such as the visual scene) informs about an unknown parameter (in this case, the animal's position). When visual changes occur more rapidly, like near walls, the brain receives more precise information about the animal's location and movement leading to higher Fisher information. The tuning of place cells—how precisely they respond to location—adjusts accordingly. Essentially, as the visual scene changes more rapidly, the response of place cells becomes more precise, with smaller place fields to capture more specific spatial information. Previous research has shown that place cell activity contains more spatial information in the presence of visual cues, with place fields near these cues being the most informative [297] [273]. Moreover, in simple neural models, a neuron's tuning width is inversely related to the Fisher information it carries [167]. Assuming the place code maximizes the transmission of information up to the limit imposed by vision, this would predict that place field width is inversely proportional to the rate of change in the visual scene.

Further investigation into the effect of visual scene changes on place cell activity in complex large environments filled with various cues could provide deeper insights. Comparing individual and population-level place cell activity in such settings, along with measuring the Fisher information of each location, could enhance our understanding of how large, complex environments are encoded by hippocampal CA1 cells.

Chapter 5

The Interplay Between the Rodent Visual System and Spatial Navigation

The majority of animals heavily rely on visual cues for their navigation. Consider squirrels in the forest as they skillfully navigate through the branches to locate food sources, such as nuts and seeds. They depend on visual cues to find their carefully hidden food stocks. Similarly, dolphins excel at hunting prey in environments with limited initial visual stimuli as seawater. From polar bears searching for seals on icy floes to kangaroos in the Australian outback, even your pet dog attempting to steal a sandwich from the table, or a lost student trying to find their way to a class auditorium on the first day of studies—all these examples highlight the significance of visual landmarks in navigation within their respective environments.

Typically, rodents, like many other animals, rely on external visual cues to orient themselves within their environment, along with self-motion signals such as eye, head, and body movements. A common task for investigating spatial navigation in rodents is the Morris water maze. In this task, rats must swim in a circular pool filled with opaque water to successfully locate a hidden submerged platform, using distal visual cues. When these visual cues are rotated, the animals usually swim to the wrong location indicated by the cues highlighting their prime role to guide navigation [262][309]. Similar visual cue rotation also induces corresponding rotations of the place fields of hippocampal place cells in circular arenas [310]. Proximal cues such as intramaze objects can also modulate the activity of cells in the hippocampus [268][273], LEC as well as in the MEC [311] [312]. These cells called object cells or landmark vector cells discharge when animals are always at the same distance and angle from an object irrespective of their head and body orientation. This is particularly surprising in the MEC where neural activity was previously associated preferentially with self-motion cues such as body translation and head rotation. In addition, recent studies using animals navigating in virtual reality environments reported that a subset of hippocampal place cells can be activated by pure visual stimuli, such as the presentation of a single moving bar of light [313] or passive exploration of the environment [175]. These and other studies provide strong evidence that visual cues not only modulate observed behavior during navigation but also influence the encoding of information in the corresponding spatial navigation neural circuits.

The recent development of experimental designs utilizing virtual reality [314][315] and recordings in both head-fixed and freely moving animals [316] has revealed that a significant portion of neuronal activity in primary sensory areas is modulated by self-motion signals. Therefore, in this chapter, I will discuss the rodent visual system and

its bidirectional connection with the brain's spatial navigation system, in addition to eye movement and pupil dilation as one of the crucial indicators of this bidirectional interplay.

5.1 Understanding the Neural Circuitry of the Rodent Visual System

To better understand the influence of visual information on spatial navigation signals in the brain, it is important to understand the anatomical and circuit organization of the visual system. The mouse visual system has been extensively studied through numerous anatomical, genetic and electrophysiological investigations, making it a prominent model system. In the subsequent discussion, I will delve into the mouse visual system, using it as an illustrative example. Initially, I will examine the anatomical structure of the mouse visual system, providing information on the fundamental principles of vision and the intricate composition of the eye.

5.1.1 Notion of the primary visual pathway in mice

Vision is a complex process involving the eye's ability to detect and focus light, and the brain's capacity to interpret these signals, allowing for the perception of color, depth, motion, and detail within the visible spectrum. Initially, wavelengths of light pass through the anterior segments of the eye: the cornea, the lens, and the vitreous body. Subsequently, they traverse the entire thickness of the retina, reaching the photoreceptors situated in the outermost layer of the neuronal retina, farthest from the vitreous body. This layer of the retina, where photoreceptors and their synaptic connections reside, is referred to as the "outer retina." In contrast, the "inner retina" is composed of diverse interneurons (bipolar, horizontal, amacrine cells) and ganglion cells along with their synapses.

Two primary types of photoreceptors exist: cones (responsible for color and daytime vision) and rods (for dim light and nighttime vision). Activation of photoreceptors by light leads to the modulation of glutamate release at the synapses between photoreceptors and inner retinal neurons, thereby activating bipolar cells [317]. In the cone pathway, bipolar cells directly transmit the signal to retinal ganglion cells (RGCs), whereas in the rod pathway, an additional relay station involving amacrine cells is required [318]. While this vertical retinal pathway serves luminosity detection, it is insufficient for discerning visual contours or patterns. Pattern vision is facilitated by lateral neuronal pathways within the retina, involving lateral inhibitory activity by horizontal cells and the ON-OFF organization of RGCs.

Once visual information is generated in the retina as a suitable physical stimulus, electrical signals travel to the brain through RGC axons, which form the optic nerve upon exiting the eye. The optic nerves primarily target distinct areas in the brain (Figure 5.1): the dorsal lateral geniculate nucleus (dLGN) in the thalamus, the superior colliculus (SC) in the midbrain, the suprachiasmatic nucleus (SCN) in the hypothalamus, and the pretectum between the thalamus and midbrain [320]. Briefly, the dLGN functions as a thalamic relay station, transmitting visual signals via optic tracts to the visual cortex. The SC coordinates head and eye movements with sensory targets. The SCN is involved in the circadian response to light, while the pretectum

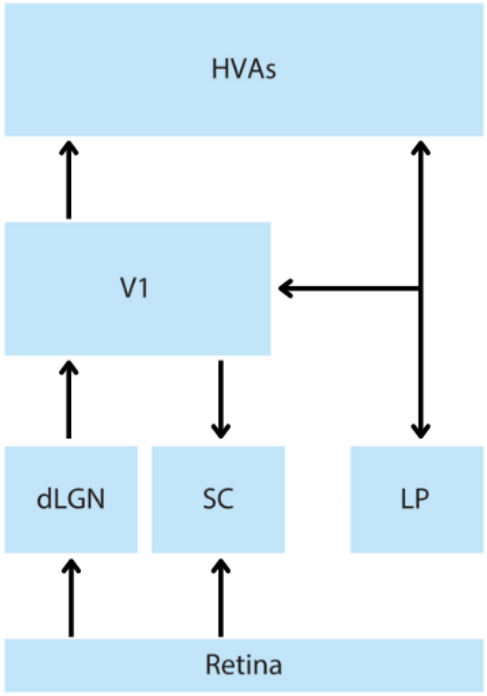


Figure 5.1: A schematic illustrating the feedforward connections in the mouse visual system: dLGN (dorsal lateral geniculate nucleus), HVAs (higher-order cortical visual areas), LP (lateral posterior nucleus of the thalamus), SC (superior colliculus), and V1 (primary visual cortex). (Adapted from Glickfeld and Olsen [319])

primarily governs the pupillary light reflex in response to changes in ambient luminance [321]. In the primary visual pathway, the visual code is ultimately interpreted in V1 and further in associative visual areas containing the "what" and "where" visual pathways [322][323]. Recently, another visual pathway connecting the pulvinar nucleus to the SC and to the perirhinal cortex has been described in parallel to the classical LGN to V1 pathway [324].

Mice possess a substantial number of rod cells in their retinas, which are specialized for low-light conditions. In contrast, they have a relatively limited number of cone photoreceptors, constituting approximately 3% of their photoreceptor population, as compared to rods [325]. It's worth noting that the human retina is similarly dominated by rod cells, with cones accounting for merely 5% of photoreceptors [326]. Unlike humans, where the central part of the fovea is primarily populated by rods, the mouse retina exhibits a minor peripheral-central cone gradient [327].

Rod photoreceptors in mice are most sensitive to wavelengths within the greenish light spectrum, around 500 nm [328][329]. The opsin pigments in mouse photoreceptors comprise two types: short-wavelength opsin (S-opsin) and medium/long-wavelength opsin (M-opsin). The absorption peaks for these opsins are approximately 360 nm and 508-510 nm, respectively [330]. Given that the conventional visible light spectrum ranges from around 390 nm to 700 nm, the absorption peak of the mice S-pigment falls within the UV range, indicating their adaptation for nocturnal vision. Notably, the absorption peak of M-opsin (508–510 nm) in laboratory rodents occurs at a relatively shorter wavelength range compared to human M-opsin, which peaks at 531 nm [331]). The absence of long-wavelength-sensitive opsins renders their vi-

sion less responsive to long-wavelength red light (above approximately 600 nm) under conditions of low luminosity.

While laboratory rodents possess only two cone opsins in their retinas, a significant proportion of their cones are not exclusively S- or M-cones; they are actually dual cones, capable of responding to both UV and the green color range, as previously mentioned. These mice also exhibit chromatic pathways at the RGC level [332], LGN [333], and V1 [172], indicating the presence of certain aspects of color vision. However, it is important to acknowledge the evident anatomical distinctions between rodent and primate eyes, resulting in substantial variations in visual capabilities between these species. Nevertheless, the fundamental structure and function of the retina share notable similarities between rodents and primates, particularly in the context of the retinal periphery [321].

As optic nerve fibers proceed toward the brain, a majority of them intersect at a specific juncture known as the optic chiasm. Consequently, signals originating from the right vision hemifield are directed to the left side of the brain, and vice versa. This phenomenon of signal crossover varies across different species and is more prominent in creatures with lateral eye placement. In the case of mice, approximately 97% of optic nerve fibers project to the opposite hemisphere of the brain [334]. This configuration results in a relatively limited binocular visual field for mice.

Species whose eyes are positioned laterally, such as mice, have large visual fields, a characteristic that could be of greater importance for animals that are prey. Being able to cover a large visual field enables them to detect the presence of predators. In the case of mice, their visual field covering an impressive 270 degrees along the horizontal axis and 120 degrees along the vertical axis would be particularly suitable for detecting aerial predators [335][336][337].

In conclusion, the unique visual system of mice, characterized by their abundance of rod cells and specialized cone photoreceptors, highlights their adaptation to low-light conditions and broadens their lateral field of view. The predominant optic nerve crossover enhances their peripheral awareness, while the prevalence of dual cones and chromatic pathways hints at potential color vision capabilities.

5.1.2 Neural circuits behind mouse visual system

In mice as in other mammals, specific visual pathways are devoted to the processing of visual information. The flow of neuronal activity along these brain regions influence how visual information is processed to make sense of and respond to what is seen. As explained further, the organization of the mouse brain's visual network suggests that it is divided into distinct pathways, each of which sends specific signals to the brain's perception, cognition, and action centers. Much like the visual systems of other mammals, the retinal cells in mice project to two specific brain regions: the midbrain's superior colliculus and the thalamus's dorsal lateral geniculate nucleus (LGN). The axons from these regions mainly terminate in layer 4 of the primary visual cortex (V1), with some sparse branching into other layers [334][338][339].

Inputs to V1 are organized in a retinotopic manner, meaning that specific regions of the visual field evoke responses in corresponding parts of V1. By observing retinotopic maps and analyzing V1's anatomical connections, researchers have identified 11 distinct higher visual areas in mice [340][341][342]. These areas surround V1 and are named according to their position relative to V1: lateromedial (LM), later intermediate (Li), anterolateral (AL), rostrolateral (RL), anterior (A), anteromedial (AM),

posteromedial (PM), posterior (P), and postrhinal (POR) areas. The projections from V1 to these higher visual areas originate in layers 2/3, 4, and 5 of V1 [343]. An anatomical study involving rats revealed that neurons in the superficial layers of V1 tend to connect with the corresponding superficial layers of the higher visual areas, whereas deeper layers in V1 establish connections with the deeper layers of the higher visual areas [344]. V1 establishes connections with the higher visual areas (HVAs) toward the end of the first week after birth (around P7) and continues to develop over the subsequent three to four weeks [345][346]. This developmental process largely occurs after the opening of the eyes, indicating that the formation of this pathway is greatly influenced by experiences during this period [346][347].

Following the identification of 11 distinct visual areas in the mouse cortex, there has been significant interest in understanding how these areas function. In primates, visual areas are traditionally categorized into two processing streams: the dorsal stream for processing movement information ("where" visual steam) and the ventral stream for object identification ("what" visual stream) [348] [349]. Similar attempts have been made in mice, with one influential approach based on anatomical connectivity by the Burkhalter group [340][350]. Their categorization places areas LM, Li, P, and POR along the ventral ("what") stream due to their connections with the entorhinal cortex and temporal association areas. The dorsal ("where") stream encompasses AL, RL, PM, AM, and A, linked to connectivity with retrosplenial, anterior cingulate, and motor cortices [350].

The functional specialization of these higher visual areas in mice has been studied based on preferences for specific spatial and temporal frequencies, or sensitivity to global motion (see review [319]). However, a full understanding of how these different areas support behavior has proven challenging. Extensive examination of responses in visual cortical neurons to various visual stimuli reveals that a substantial portion of neurons do not respond to classical visual cues like drifting gratings [351]. Nonetheless, vision supports a range of behaviours, including navigation, avoiding predators, and prey capture. Thus, investigating the mouse's visual system alongside natural behaviors could provide deeper insights into its functional organization [352].

Notably, there is evidence of spatial signals within the mouse visual cortex, along with changes in visual response due to spatial navigation [353]. Therefore, it is crucial to comprehend the pathway through which visual information travels from visual areas to the hippocampus.

5.1.3 Visual streams to the hippocampal formation

The primary pathways through which the hippocampus receives input from the cortex are the medial (MEC) and lateral (LEC) entorhinal cortices. In the preceding chapter, I detailed the anatomical distinctions between these two areas, particularly the types of spatial cells they host. Specifically, the MEC accommodates cells that encode aspects connected to self-motion, such as grid cells, speed cells, and head-direction cells. In contrast, the LEC contains neurons that encode information linked to objects, like object vector cells and trace cells.

It was suggested [354] that these regions, which receive inputs from various sensory and motor areas, represent the endpoints of the "what" (for LEC) and "where" (for MEC) streams of visual processing. These streams are then integrated within the hippocampus. In terms of function, self-motion-related data would benefit from signals tied to optic flow. On the other hand, representing landmarks and objects requires

information related to visual recognition, such as fine features and object identity. Consequently, visual information relevant to navigation can be broadly categorized into one stream for object and landmark features, and a second stream for optic flow signals.These two streams of visual information directed to the hippocampus could ultimately influence the activity of place cells and their representation of the environment.

The following section will delve into a detailed exploration of how behavioral factors influence visual signals, particularly the interplay between vision and spatial navigation.

5.2 Influence of visual information on spatial navigation: bidirectional interactions

5.2.1 Evidences of an influence of spatial navigation on visual information processing

Previously, when discussing vision research, the focus was on observing rodents as they passively explored visual objects or patterns. However, navigation involves more than just passive observation—it includes active exploration of the environment through movements and locomotion. In fact, studies involving neuronal recordings from head-fixed rodents that were freely running on a spherical treadmill showed that the activity of neurons in the primary visual cortex (V1) was influenced by the animals' locomotion. This change in neuronal activity wasn't only seen when there were visual stimuli present; it was also observed in the absence of light [355]. Other research has shown that as running speed increases, there is a corresponding increase in pupil dilation and changes in the power spectrum of the LFP [356][357]. Additionally, there's an uptick in the spiking activity of neurons in V1 and HVAs that respond to visual stimuli when the animals are running [314][358][359][360][361][362][363][364]. In addition, a part of V1 neurons was found to be tuned to speed or a weighted sum of speed and visual flow [314][359][360]. Taken together, these findings suggest that visual information processing is influenced by locomotion. This concept has led to the notion that visual processing might also be affected by the distance traveled and the spatial context, implying that visual stimuli could be influenced by spatial navigation.

As mentioned earlier, vision serves as an important sensory input that rodents can utilize for various activities such as social interactions, finding food, and protecting themselves from predators. All of these activities require effective navigation within their environment. Mammals, including rodents, rely on a combination of external sensory cues and their own movements (like eye, head, and body motions) to create an internal understanding of where they are in relation to their surroundings. The significance of vision in these behaviors is attributed to its superior ability to offer accurate information about distant spatial features. In rodents, for example, vision plays a crucial role in determining the position of objects that are beyond the range of their whiskers [365][366].

In the context of vision, researchers have extensively studied how visual cues influence the activity of neurons associated with navigation, such as place and grid cells in the hippocampus and medial entorhinal cortex [367][186][27][368][369]. However, the impact of navigational signals on the processing of visual information has not been

thoroughly explored. Only a limited number of studies have examined how the visual system functions during navigation [314][315].

Traditional perspectives on the visual system have emphasized that visual information processing initially occurs in a way that's centered around the eyes (retinotopic), and then progressively integrates with egocentric and allocentric information in higher brain areas for tasks like spatial navigation. Such a hierarchical model of visual information processing would suggest that areas before HVAs like the primary visual cortex are non-sensitive to spatial information, thus, the modulation of neuronal activity by spatial location could not be observed there. To test this idea, Ji and Wilson (2007) [370] were among the first to record activity from the visual cortex of the freely-moving rats. Surprisingly, they found that specific areas in the visual cortex fired in response to certain locations in the environment, resembling what is known as "place fields," even when the visual patterns presented in those locations were similar. However, their study and similar ones [371] had limitations, as they involved animals exploring real environments where controlling visual stimuli was difficult (e.g., lighting, the animal's viewpoint). Thus, the location specificity may be due to the difficulty of controlling visual stimulation in freely moving animals.

Fortunately, recent advances in technology, like virtual reality systems for rodents, have overcome these challenges and opened up new avenues for studying vision during navigation [357]. These systems allow a head-fixed animal to navigate through a virtual environment by running on a treadmill or ball. By combining this virtual reality setup with high-resolution imaging or recordings from multiple electrodes, researchers have been able to conduct more studies on how the mouse visual system works while the animals are moving [358][359][360] [361][362][363].

Saleem and colleagues utilized a virtual reality setup designed for rodents to be able to test activity in V1 during navigation with control of visual stimulus due to VR. It has a straight corridor with two visually identical sections. The expectation with visually identical segments is straightforward: if neuronal responses are purely driven by the visual scenes, the responses should be identical in both segments of the environment. However, the surprising finding was that many neurons in the primary visual cortex (V1) did not exhibit identical responses in these matching segments. This suggests that the responses were influenced by factors beyond visual input. The influence of these non-visual signals on neuronal activity was so pronounced that some neurons showed a single peak of activity in the environment. This spatial influence was much stronger than what would be expected by chance in neurons primarily responsive to visual stimuli. Furthermore, this effect could not be attributed to other non-visual factors like the animal's running speed, rewards, eye movements, or pupil size [372]. Interestingly, similar spatial modulation of visual responses was observed even when visual cues were absent, particularly in relation to the position of rewards [373][374]. This indicates that neurons in V1 exhibit a significant degree of response modulation based on the spatial context, implying that visual processing in these neurons is strongly influenced by the environment in which the animal is situated.

The next step was to verify if the response in V1 is related to the signals encoded in the areas involved in spatial navigation, particularly, the CA1 region of the hippocampal formation. This time, Saleem and colleagues simultaneously recorded activity from both the V1 and the CA1 regions using multi-electrode arrays while mice were navigating in the virtual corridor [372]. It was shown that neural populations in both areas represented the environment, and through a Bayesian decoder, they were able to accurately determine the animal's position based on the activity of these

two populations. Significantly, the decoded positions from V1 and CA1 were highly correlated, even sharing common errors. This correlation indicated that V1 and CA1 indeed carried the same spatial information.

In other experiments, researchers trained mice to perform a navigation task using visual cues along a virtual corridor to reach a rewarded location [374]. After training, V1 neurons in layer 2/3 became strongly responsive to the reward location, with the majority of these neurons firing when the reward location was reached. When these mice were tested in the same corridor without visual cues, relying only on their self-motion, some neurons continued to respond to the expected reward location. In this and similar study, it was shown that these neurons reacted based on the distance the mouse had traveled toward the reward, revealing the presence of spatial representations in V1 even without visual cues, which is much like how the hippocampus responds [374] [375]. Additionally, similar to hippocampal place cells, V1's spatial responses were also influenced by theta oscillations recorded in the hippocampus [375]. This suggests that V1 neurons might integrate visual data with movement-related signals, such as distance traveled, to estimate an animal's spatial position. Alternatively spatial position information could be broadcasted from the hippocampus to various cortical areas. Indeed recent lesion experiments showed that the representation of spatial information in the visual cortex is lost following hippocampal lesions [376].

These findings reveal that responses to visual stimuli are influenced by both movement and the surrounding spatial conditions. This means that the response to a particular visual stimulus under static conditions might differ from the response when that same visual stimulus is encountered during navigation. For instance, research demonstrated that the activity elicited by a drifting grating varied depending on whether it was passively viewed by a stationary animal or if it was linked to the animal's movement within a virtual environment [377]. Furthermore, a subset of V1 neurons was identified as encoding discrepancies between the animal's running speed and the visual motion. These neurons were discovered in experiments where the visual motion was disconnected from the animal's actual locomotion within a virtual reality setting [360][378] [379][380]. These outcomes provide strong evidence that signals related to navigation actively shape the activity of V1, whether or not visual stimuli are present.

5.2.2 Vestibular and head-direction movement: visual control and signals in the mouse visual cortex

5.2.2.1 Influence of visual stimuli on the head-direction cells

During navigation, visual landmarks can serve not only as markers for specific locations but also as beacons or compasses for directional orientation. In this regard, vestibular signals within the brain, combined with head movements, play a crucial role. As previously discussed, head-direction cells were initially identified in the postsubiculum and have since been observed in other regions such as the subiculum [164], lateral dorsal nucleus [381], anterior dorsal nucleus of the thalamus [173] [382], anteroventral nucleus of the thalamus [103], MEC [383], and RSP [384]. Although the representation of head direction is widely distributed across various brain regions, the neurons' activity in each of these regions encodes the same head direction. Head-direction cells activate when the animal's head is oriented in a specific direction, and collectively, these cells represent all possible directions.

Remarkably, the head-direction system can function without reliance on vision. HD cells continue to remain active even in darkness [164][385]. However, during extended periods without visual cues, their representation gradually drifts away from the world-centered reference frame, leading to the accumulation of errors [381][386] [32]. Experiments conducted in well-lit environments have revealed the presence of vision-controlled bidirectional HD cells [387][388]. Notably, strong visual cues in bright surroundings can serve as anchors for maintaining the representations of HD cells. Additionally, these visual cues have the ability to reset the HD cell representations to their original positions when lights are reintroduced after a period of darkness [381][389]. In a specific experiment involving animals moving between two connected chambers with similar geometries and distinct visual cues in each chamber, the alteration of a visual cue by 180° caused a subset of HD cells in the retrosplenial cortex (RSP) to adjust their representation to align with the new visual cue, resulting in bidirectional HD cells [387] [388].

In summary, while the head-direction system does not strictly require vision, a wealth of converging evidence indicates that visual information holds a remarkable level of influence and control over the functioning of the head-direction system.

5.2.2.2 Influence of head movements on visual processing

Advancements in virtual reality technology has taken a significant stride forward with the incorporation of rotating platforms designed for head-fixed animals. This innovation, combined with the capability to record data from freely moving animals while precisely tracking their eye and head movements, has ushered in a new era of research on the impact of spatial navigation signals on visual processing, both during passive observation and active movement scenarios [390] [385] [391] [392] [393] [394].

Neurons within the rodent visual thalamus and V1 exhibit strong reactions to both head and eye movements, displaying a notable affinity for head direction and posture [316] [395] [396] [397]. Through the utilization of a rotating platform, it was possible to dissect the sources of information that contribute to this tuning. These sources were investigated by comparing the modifications in visual responses prompted by head or eye movements under conditions of both darkness and light. This comparison allows for the assessment of the influence of reafferent visual input. Additionally, it helps to investigate the effects of passive rotations of head-fixed animals on rotating platforms or self-generated movements to study the impact of vestibular information with and without motor reference.

It has been demonstrated that under conditions of darkness, stimulation of the vestibular nerve can activate various brain areas, including visual regions [398]. Subsequent investigations revealed that these visual areas also respond to head movements in the dark, as shown by [398] [390] [396] [399]. Further exploration through electrophysiological studies of mouse V1 layers in response to rotational movements in darkness unveiled heterogeneous reactions within the V1 layers. Specifically, deep layer 6 neurons were observed to receive synaptic inputs reflecting the angular velocity of horizontal rotation [390]. Conversely, neurons in the superficial layers 2/3 of V1 exhibited a suppressive effect during passive rotation on a platform and active head movements in freely moving animals [399]. Additional supporting evidence suggests that this suppression occurs prominently when head movements coincide with gaze shifts, rather than maintaining a steady gaze [400]. This phenomenon potentially serves as an indicator of saccadic suppression facilitated by corollary discharge (more

detailed information about eye movements is explained below).

Interestingly, in the study conducted by Bouvier et al., 2020 [399], a noteworthy finding emerged. When the light was present, the layer 2/3 neurons that had previously shown suppression actually displayed an activation. This activation was observed in both head-fixed and passively rotated mice, as well as in freely moving mice. While a definitive explanation for the nature of this light-dependent shift in responses to head movements is not yet available, subsequent investigations suggest that gaze shifts and the associated changes in visual inputs might contribute to this phenomenon [400].

Recognizing the emergence of movement and posture-related signals only in the presence of light, without a clear visual stimulus, it was important to investigate the impact of self-motion signals on responses in the presence of visual input. Subsequent findings unveiled that the tuning curves of V1 neurons in response to self-generated visual motion information, appearing from either head movements [390] or saccadic eye movements [395], diverged from those triggered by external motion signals. This distinction holds the potential to facilitate the discernment of information between self-generated and external motion signals, along with their potential combinations, in downstream brain regions. In another study in freely moving mice navigating a patterned environment, it was proposed that the selective response of V1 neurons was modulated by eye or head movement rather than during self-motion [400].

These findings are also raising questions about how the brain's circuits handle signals from head movements in the visual cortex. Signals related to head movements that aren't connected to vision, like those from the vestibular signals, the neck's proprioceptive signals, and motor efference signals, are thought to come from areas like the RSP and the secondary motor cortex [396], as well as the higher-order visual thalamic pulvinar nucleus has been identified as a source that relays non-visual saccade-related activity to V1 [395]. However, more research is needed to really understand how self-generated motion can change how the brain deals with visual information, not just in the cellular but also in circuit levels.

5.3 Influence of vision on the object and boundaries representations

5.3.1 Role of the visual cues

An important component of any environment is its terrain and its boundaries which could be represented as physical boundaries (walls) and as a progressive change in landscape (from rocky desert fields to green fields). The other important components are objects inside the terrain which help to separate it into sub-environments and let easier navigation inside by calculating their location and distance from each other and oneself. In rodents, proximal objects are usually observed by limbs and whiskers, which are part of the somatosensory system. To estimate the relative or absolute distance of objects beyond the reach of the somatosensory system, however, vision can provide useful information.

5.3.1.1 Mechanism of the visual depth perception

From flat retinal images, the visual system has the ability to deduce details about the three-dimensional layout (depth) of surroundings by utilizing diverse depth cues, which involve factors like occlusion, shading, the relative sizes of objects, and defocus blur. Additionally, self-generated optic flow signals in combination with input related to head or eye movements contribute crucial information about the relative positioning of objects through a process known as motion parallax. Furthermore, for the area of vision that lies ahead and is seen by both eyes, the horizontal displacement between the two eyes enables the computation of depth by generating two distinct perspectives of the environment, a mechanism termed stereopsis.

In mammals, stereopsis relies on computations made by brain cells in the cortex that get information from both eyes. This information comes from a group of RGCs that don't cross over at the optic chiasm, but instead send signals to the same side of the brain they come from. These signals are sent to dLGN and end there. In mice, the part of the V1 that deals with input from both eyes extends across around 30% of this brain area. This region gets a mix of signals from the dLGN. Additionally, signals from the same side of the eye in this area are supported by connections between the two halves of the brain. This means that as information travels through the early stages of the mouse's central visual pathway, the small foundation of seeing with both eyes provided by the retinal cells gets greatly boosted in the dLGN and even more in V1. This suggests that binocular vision plays an important part in how the brain processes depth (see Nityananda and Read, 2017 [401] for a more detailed review of stereopsis). As it was mentioned before, rodents have eyes positioned on the sides of their heads, allowing them to see a wide area around them, even though there's not much overlap between what each eye sees. New studies have shown that the binocular area is widest in front of the animal and becomes narrower above and below. This emphasizes the significance of the space in front of a rodent's head for behaviors involving both eyes [402][391].

Various tasks that examine how rodents perceive space and distances through vision provide evidence that both stereopsis and motion parallax play crucial roles in their depth perception. For example, the cliff task 136 takes advantage of animals' instinct to avoid borders with steep drops. This task involves placing a transparent surface over platforms at different depths, creating the illusion of a cliff. The integration of information from both eyes is vital for mice to select the nearest platform, as shown by experiments comparing choices made with one eye closed to those with both eyes open [403]. Stereopsis is also important for capturing prey, particularly when estimating the distance accurately during the approach phase [404][405]. In tasks requiring spatial awareness, mice use various strategies. For instance, they make use of monocular cues and motion parallax to gauge visual depth. In certain situations, when mice need to jump specific distances across a gap, closing one eye doesn't affect their precision. Instead, they move their heads to create motion parallax and estimate distance [400].

To sum up, the examination of mice's behaviors in tasks related to spatial perception reinforces the concept that rodents use a blend of neural processing and specific behaviors to comprehend and interpret visual depth.

5.3.1.2 Role of visual depth in object perception and its influence on spatial navigation

The computation of depth information from visual input thus seems likely to be important for estimating distances to objects and boundaries in the environment and building an allocentric representation of space. Although direct evidence is lacking, indirect observations suggest that visual depth cues (providing information on the relative distances of objects) can influence place cells and other spatially selective cells.

Early works on the classical hippocampus-dependent spatial learning and memory task, the Morris water maze, were suggesting that distal spatial cues are the primary sources of information used by animals to solve the water task (and similar spatial tasks) and by place cells to generate their spatial specificity. Further, it was shown (see review Knierim and Hamilton., 2011 [406] for more examples) that distal cues appear to be mainly involved in binding the orientation of a spatially coherent, internal, cognitive representation of an environment to the external world via the head direction cell system, whereas local cues and apparatus boundaries primarily set the translation and scale of that coordinate system. Usually in the majority of studies, local cues provide more depth information. The proximal cues are usually texture cues placed on the floor of the environment [407][408] or they are objects placed in and/or around [409][410][273], while distal cues are usually features or patterns on the walls of the maze. Moreover, various studies have demonstrated that hippocampal activity during spatial navigation can be influenced by proximal cues. For instance, one study observed that when objects were removed or relocated within an environment, a subset of neurons in CA1 and CA3 displayed increased firing at the previous object locations. Some neurons exhibited this increase at a single location, indicating a conjunctive memory of object and place, while others displayed heightened firing at multiple locations where objects used to be situated [268]. In another study, a rotation of distal and proximal cue by 180 degrees resulted in mismatched responses in place cells. The majority of these cells reacted to the arrangement of both proximal and distal cues, with proximal cues exerting a more pronounced influence. This suggests that the order of responses is shaped by environmental adjustments [409]. A study by the Epsztein lab showed that virtual objects heightened the resolution of spatial coding within their proximity. This was evidenced by an increased proportion of place cells, smaller place fields, and improved spatial selectivity and stability. Additionally, objects enhanced temporal coding resolution through better theta phase precession and spike coordination on the theta timescale [273]. Collectively, these findings indicate that proximal cues can impact how the hippocampus represents space, reinforcing the notion that visual depth cues contribute to spatial representations.

Research has demonstrated that not only place cells but also various other types of spatial cells within the hippocampal formation can be influenced by visual depth cues. Among these are object vector cells, which have been identified both in the MEC [410] and the LEC [411][412]. Additionally, there are border cells in the RSP and boundary vector cells present in both RSP [413] and the subiculum [205]. As mentioned earlier, object vector cells respond when an animal is positioned at a consistent vectorial distance from objects. The evidence indicating their modulation by visual depth encompasses reduced responses in darkness and increased responses to three-dimensional objects that offer more comprehensive depth information about an object [414]. The border vector cells become more active when an animal approaches

specific boundaries within its surroundings. Similarly, boundary vector cells become active based on the animal's nearness and orientation relative to the boundaries in its environment. In a specific study, a significant portion of egocentric boundary vector cells were identified in the RSP, demonstrating increased firing rates when the animal maintained a particular distance and angle from a boundary. Remarkably, these firing patterns persisted even in environments without physical barriers [413]. Moreover, boundary vector cells situated in the subiculum react to features that are located at a distance away. Nevertheless, these cells have been shown to exhibit firing patterns that cover all directions, suggesting they might be more influenced by cues other than visual ones [205].

5.4 Eye movements as an important component of spatial navigation

To effectively navigate an environment, a crucial strategy involves evaluating one's surroundings and using that information to guide subsequent behavior. Eye movements are a fundamental mechanism central to this process. Certain species, including mice and other rodents, exhibit relatively immobile eyes that remain centered within their orbits and move conjointly during head-fixed conditions [415][416]. Nevertheless, recent years have seen a substantial increase in research aimed at elucidating the mechanisms and function of eye movements in mice.

In primates, two distinct types of voluntary eye movements substantially contribute to vision by aligning and stabilizing the visual object of interest onto the fovea – the region of the retina with the highest visual acuity. In contrast, mice are considered afoveate animals. Historically, eye movements in mice were primarily perceived as mechanisms for stabilizing or adjusting gaze following head movements, a phenomenon also observed in other species with forward-directed vision. Recent studies collectively showed the assertion that eye movements in mice possess a level of sophistication previously not anticipated.

The investigation of mice's eye movements began in 1976 with direct observations through a microscope by Mitchiner [417]. However, recent advancements, including the development of high-resolution, high-speed cameras for recording eye movements in head-fixed animals and head-mounted camera systems for freely moving animals, mark a significant leap forward in murine vision studies. This technology enables the detailed analysis of eye movements in both head-fixed and freely moving animals, encompassing a range of visual and naturalistic activities, such as hunting prey. Moreover, this technology opens up the possibility for concurrent electrophysiological or calcium recordings, facilitating explorations into the neural circuits that govern and modulate eye movements in mice. In the subsequent sections, I will describe the typical eye movements observed in head-fixed and freely moving mice (for more details see review by Giovannetti and Rancz, 2024 [418]).

5.4.1 Mice eye movements

Mammals display five primary categories of eye movements: vestibulo-ocular reflexes (VOR), optokinetic reflexes (OKR), saccades, smooth pursuit, and vergence movements. These can further be classified into subcategories, such as reflexive or voluntary, as well as gaze-stabilizing or gaze-shifting types (Figure 5.2). Additionally, they

can be differentiated based on their kinematic properties including speed, acceleration, amplitude, and direction [418].

Of particular importance is the distinction between gaze-stabilizing and gaze-shifting movements. Gaze-stabilizing eye movements work to counterbalance head and visual motion, thereby stabilizing the retinal image and ensuring clear vision [419]. These movements typically manifest as gradual, outward, or inward pursuit-like motions (slow phase of nystagmus), occasionally followed by a rapid, inward motion (fast phase of nystagmus or recentering saccade). This recentering saccade occurs when the eyes reach their maximum angle of deviation from the center of their orbit. In contrast, gaze-shifting eye movements redirect the eyes to focus on specific targets. In species with a fovea, these movements align the central high-resolution area of the retina (fovea) with the target, whether it lies within the same plane as the animal's forward-facing direction or at varying depths. Recent studies have revealed that even in the case of mice, which are afoveate animals, they are still capable of performing gaze-shifting eye movements [391][400].

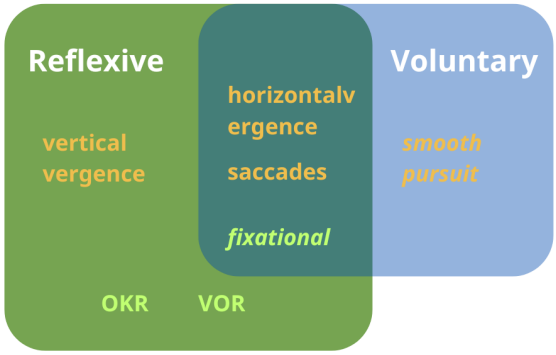


Figure 5.2: A schema illustrating types of eye movements categorized by their origin (reflexive or voluntary) and function: gaze-shifting (yellow) or gaze-stabilizing (light green). The eye movements in italics are observed in primates but not in rodents. (Adapted from Giovannetti and Rancz, 2024 [418])

5.4.1.1 Vestibulo-Ocular Reflexes (VOR)

From ancient fish to all modern vertebrates, the stabilization of visual images involves two fundamental reflexes: the vestibulo-ocular reflex (VOR) (Figure 5.3 A) and the optokinetic reflex (OKR) (Land, 2019). Imagine yourself as a passenger in a car that is making a turn. Your eyes instinctively move in the opposite direction of the turn, allowing you to maintain a fixed gaze on a point outside the vehicle. Similarly, consider dancing—when you spin in one direction, your eyes automatically adjust in the opposite direction to keep focus on a specific point. These instances highlight the workings of the vestibulo-ocular reflex. In the VOR, the semicircular canals within the inner ear play a crucial role by detecting head velocity. The oculomotor system translates this signal into instructions for the eye muscles, prompting the eyes to move at a speed equal and opposite to the head's movement. This coordinated response ensures that the gaze remains stationary [419]. The semicircular canals are responsible for sensing angular acceleration, known as the angular VOR (aVOR), while the otolith organs detect linear acceleration and the head's position relative to gravity, leading to the generation of the translational VOR (tVOR) [420].

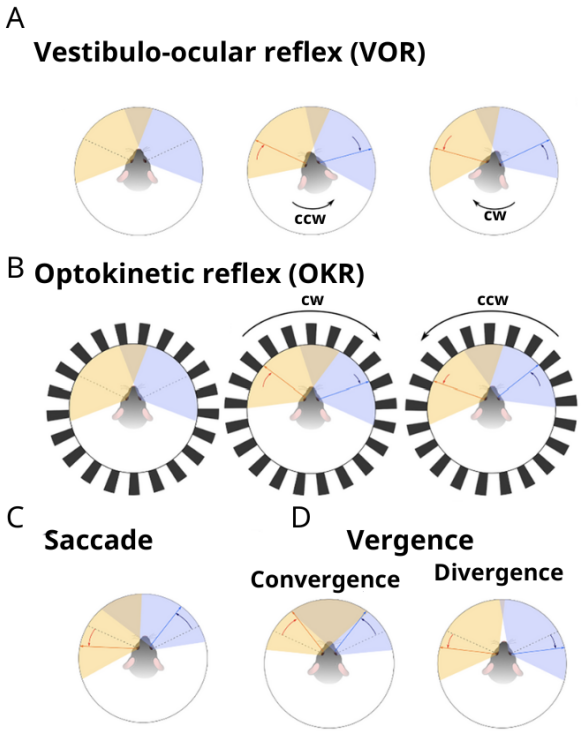


Figure 5.3: An illustration of types of eye movements in mice. **(A)** The vestibulo-ocular reflex (VOR) in head-fixed or body-restrained mice, triggered by a vestibular stimulus in the dark. The visual fields of the eyes are shaded in yellow and blue, with the dotted line indicating the visual axis. On the left, the eye position is at rest; in the center, a counterclockwise (ccw) head movement results in corresponding contraversive eye movements; and on the right, a clockwise (cw) head movement causes conjugate eye movements in the same direction. **(B)** The optokinetic response (OKR) in head-fixed mice induced by a rotating drum with black and white gratings. The left shows eye position at rest; the center illustrates that cw drum rotation produces cw eye movements; and on the right, ccw drum rotation results in ccw eye movements. **(C)** Saccades in head-fixed mice, with a representative ccw saccade shown on the left; red and blue lines indicate the final eye positions. **(D)** Vergence eye movements: convergence refers to non-conjugate movement toward the target (T-N direction), while divergence indicates non-conjugate movement away from the target (N-T direction). The T-N movement is faster and has a larger amplitude than the corresponding N-T movement, leading to an increased binocular field during VOR, OKR, and saccades. (Adapted from Giovannetti and Rancz, 2024 [418])

Notably, the VOR functions even in the absence of visual stimuli. However, in the presence of visual cues, the VOR and the OKR collaborate, giving rise to the visual-vestibular-optokinetic reflex (vVOR) [421]. This interaction heightens the stability of visual fixation and contributes to our seamless perception of the surrounding world.

5.4.1.2 Optokinetic Reflexes (OKR)

The OKR (Figure 5.3 B) is initiated by ganglion cells within the retina, which detect residual motion in the visual field. This detected motion signal is then looped back, amplified, and transmitted to the eye muscles, resulting in the eyes moving in alignment with the residual image motion. This mechanism effectively anchors the retina to the moving image. When the environment is typically static, the OKR remains dormant. However, during a leisurely stroll in nature, you might notice the flowing water in a river, observe clouds drifting across the sky, or watch a flock of birds soaring

together. In these instances, your eyes instinctively track these movements, triggering the OKR response. The OKR's responsiveness is not restricted by the dimension of vision—both binocular and monocular vision can activate it, operating within either the horizontal plane (fronto-parallel) or the in-depth plane (anteroposterior), known as the vergence OKR [422]. When visual motion aligns with the gaze (fronto-parallel OKR), the eyes converge in their movement. In contrast, when depth is involved (depth OKR), the eyes move asymmetrically.

Despite sharing foundational characteristics, the primate and murine OKR exhibit certain distinctions. Primates manifest two distinct phases: an open-loop phase (ocular following response or OFR) and a closed-loop phase. The OFR in primates is intrinsically linked with the cortical smooth pursuit system [423], absent in rodents due to the lack of a fovea. While the initial phase of the mouse OKR shares a resemblance with the primate OFR, discrepancies emerge [424]. Additionally, unlike primates, mice display consistent or declining eye velocity during extended presentations of stimuli in the closed-loop phase—an outcome attributed to an underdeveloped velocity storage system [421][425]. A shared phenomenon between both species is optokinetic after nystagmus (OKAN), where eye movements persist even after the cessation of the visual stimulus [426][427].

5.4.1.3 Saccades

As you read, your eyes swiftly jump from one word to another, engaging in a phenomenon known as saccades—an integral part of the oculomotor system (Figure 5.3 C). A saccade involves the rapid, coordinated movement of both eyes as they shift between various points of fixation in a single direction. These eye movements are vital for exploring our visual surroundings, allowing us to direct our attention to different objects or areas within our field of view.

While the saccade and fixation pattern is observed across vertebrates, the timing and extent of saccades can greatly differ between the two eyes. This ranges from tightly coordinated movements in primates to independent eye movements as seen in chameleons. Among lateral-eyed mammals like mice, as well as in fish, a common pattern emerges where both eyes move simultaneously, albeit not necessarily in perfect synchrony. For these lateral-eyed animals, which lack the ability to gauge distance through binocular disparity, the need for tightly coordinated eye movement seems less essential [428]. Also, in the case of mice, saccadic movements with rapid angular speed have been observed under both head-fixed and freely-moving conditions [421][391][405][429]. A purely kinematic perspective defines saccades as rapid, coordinated, and ballistic eye movements that occur in horizontal, vertical, or oblique directions. Describing saccades in terms of their kinematic properties involves understanding the relationship between their amplitude, duration, and velocity. In primates, this relationship exhibits a non-linear pattern with saturation, resulting in the duration of saccades increasing linearly with their amplitude [430]. Conversely, in mice, saccade speed scales linearly with amplitude, while the duration of saccades shows saturation in relation to their amplitude. Thus, murine saccades are notably faster than those observed in primates [431].

As mentioned earlier, mice exhibit horizontal eye movements similar to the quick eye shifts observed in primates, particularly under controlled head-fixed conditions. A study revealed that head-fixed mice displayed horizontal saccades triggered by natural scenes, influenced by image statistics. These saccades occurred in bursts interspersed

with fixation intervals, and their size correlated with the minimum distance required to elicit responses in specific modeled visual cells. Larger cortical receptive fields, which are linked to lower visual acuity, necessitated larger saccades to capture image diversity. Mice that experienced monocular deprivation made larger saccades, possibly due to reduced visual acuity [432].

While it remains uncertain whether retinal or cortical processes initiate these saccades, prior research has demonstrated the cortex's involvement in saccade generation in trained mice. These mice were taught to execute targeted saccades toward an LED marker [433]. During this task, variations in saccadic velocities and sizes based on direction were observed, with saccades towards the Temporo-Nasal (T-N) direction being swifter and larger than those towards the Naso-Temporal (N-T) direction. These saccades reached angular speeds of approximately 300 degrees per second, electrical stimulations were applied to the supplementary motor areas (MOs), it triggered saccades of varying sizes in mice. However, stimulating HVAs did not induce any response. Moreover, temporary silencing of the MOs disrupted the mice's ability to perform normal saccadic behavior [433]. The projections from the MOs were suggested to potentially influence saccade generation by modulating deeper brain regions involved in eye movements, such as the SC or the contralateral striatum. Despite an incomplete understanding of cortical control, it was verified that a specific component of the Superior Colliculus (SCm) indeed initiates saccade generation in mice, akin to primates [434][435]. Through the application of optogenetics, researchers could activate or inhibit the SCm, thereby impacting the frequency of contralateral saccades. Inhibition of the SCm resulted in saccades preferentially targeting specific areas, consistent with known patterns of eye movement control on each side of the brain. Furthermore, stimulating the SCm along the dorsoventral axis induced saccades with diverse amplitudes and directions. These findings underscore the similarity in saccadic control mechanisms between the mouse's brain, notably the role of the SCm, and those observed in primates [436][437].

In the mentioned studies, saccades were triggered by various stimuli, including LED light and optogenetic targeting. However, spontaneous eye movements were also observed. Notably, mice displayed intrinsic stimulus-oriented and coordinated horizontal saccades when exposed to tactile stimuli like air puff and whisker stimulation. Furthermore, researchers identified a link between these saccades evoked by stimuli and attempted head movements in the same direction. Interestingly, in some instances, these unplanned eye movements were preceded by gradual orienting head motions. This finding corresponds with previous observations indicating that mice utilize these orienting head motions to shift their visual focus. Essentially, these head movements embody a strategy, where mice anticipate the trajectory of their upcoming movement. A recent investigation conducted by Bergmann et al., 2022 [438] concentrated on saccades in relation to navigation among head-fixed mice navigating a plus-maze. The study revealed that saccades occurred concurrently with asymmetrical whisking motions, aimed at the anticipated future turn, irrespective of bodily movements.

Reflexive and voluntary saccades can be further categorized into various subtypes. Among reflexive saccades, five subtypes can be identified: stimulus-oriented, express, resetting, microsaccades, and compensatory. Interestingly, microsaccades and compensatory saccades have not been extensively characterized in mice (detailed insights available in the comprehensive review by Giovannetti and Rancz, 2024 [418]). Stimulus-oriented saccades, also known as visually-guided or reflexive saccades, are

initiated in response to an external stimulus. This type of saccade is exemplified when you observe movement at the periphery of your visual field while looking at a stationary scene. In this case, your eyes swiftly shift to focus on the moving object. Similarly, if an object unexpectedly falls or moves within your peripheral vision, your eyes promptly execute a rapid saccade to track the object's path. These saccades play a critical role in enabling us to swiftly adapt to environmental changes and direct our attention to novel or significant visual input. Notably, stimulus-oriented saccades can also occur when viewing static visual scenes. Research has shown a correlation between natural image statistics and saccade size in both primates and mice, underlining the role of environmental cues in influencing saccadic behavior [432].

Express saccades represent a subset of stimulus-oriented saccades characterized by an extraordinarily brief latency. These saccades exhibit an exceptionally rapid response, with the time between the onset of the stimulus and the initiation of the saccade typically falling within the range of 70 to 100 milliseconds. What sets express saccades apart is their remarkable speed, as they occur almost immediately after the presentation of a visual stimulus. Unlike regular saccades, which might show latency and velocity variations based on factors like stimulus intensity or complexity, express saccades maintain their swift and high-velocity nature regardless of the specifics of the stimulus. Express saccades are noteworthy for their association with rapid decision-making processes. They tend to emerge in tasks that demand quick reactions to visual targets or cues. Importantly, similar stimulus-oriented saccades with ultra-short latencies have also been documented in mice [435].

Resetting saccades plays a role in stabilizing the gaze during eye movements involved in maintaining visual stability, such as the OKR and the VOR. Specifically, these saccades are rapid and slight eye movements that swiftly follow larger saccades. Their purpose is to refine the eye's position after a significant movement, ensuring precise fixation on the intended target. After a substantial saccade, there may be a residual positional error due to the high velocity and momentum of the movement. Resetting saccades are corrective actions that rapidly counteract this lingering error, effectively fine-tuning the gaze and aligning it accurately with the desired target. Importantly, these corrective saccades are not consciously controlled by either mice or primates. Resetting saccades also referred to as "compensatory saccades" in recent mouse studies [391], are crucial for compensating for head movements in head-fixed mice and ensuring visual stability.

Microsaccades are tiny, involuntary eye movements that persist even when an aim is to sustain unwavering fixation on a specific point. These subtle shifts are too subtle to perceive without specialized tools, yet they are an inherent and continuous element of the oculomotor system. An essential purpose of microsaccades is to counteract visual adaptation. When a human‘s gaze remains fixed on a static point for an extended period, the photoreceptor cells in the retina gradually lose responsiveness to the unchanging input, leading to diminished perception. Microsaccades serve as a mechanism to introduce minor alterations in retinal stimulation, effectively refreshing the visual input and preventing the onset of adaptation.

Compensatory saccades, often referred to as corrective saccades or counter-saccades, are eye movements executed in reaction to external disturbances or internal sensory inconsistencies. The primary objective of these saccades is to rectify the gaze position and restore stability to the visual field. By making such adjustments, compensatory saccades ensure that a clear and sharply focused image is maintained on the retina, even in the face of disruptions. These saccades play a compensatory role within the

context of both the OKR and the VOR, acting as mechanisms that counteract perturbations and maintain visual clarity.

There are three distinct types of voluntary endogenously driven saccades: predictive, antisaccades, and memory-guided saccades. These saccadic types are frequently observed during cognitive processes like attention and memory. While these specific saccade types have not yet been extensively described in mice, the presence of evidence indicating higher cognitive functions, such as selective visual attention, offers the possibility of observing them under alternative experimental conditions. Predictive saccades, also known as anticipatory or proactive saccades, represent a unique category of eye movements that precede the occurrence of an anticipated event or the appearance of a target. Unlike reactive saccades, which react to stimuli that have already transpired, predictive saccades are instigated based on forecasts regarding forthcoming events, often guided by learned patterns or expectations. These saccades are shaped by past experiences and acquired associations. To illustrate, consider a tennis match between Alcaraz and Djokovic: the players demonstrate their ability to predict the potential trajectory of the ball, positioning themselves optimally for intercepting or returning it. This predictive prowess is especially evident when rapid visual stimuli direct the ball to their side of the court. This skill to adeptly track objects stems from their mastery of predictive saccades, enabling efficient object tracking and positioning.

Antisaccades constitute another class of deliberate saccades, demanding the suppression of the reflexive saccade towards a visual stimulus, and instead, necessitating the execution of a saccade in the opposite direction. In essence, antisaccades involve intentional shifts of gaze away from a visual stimulus [439]. This paradigm is widely employed in cognitive and neuropsychological research to investigate inhibitory control, working memory, attention, and executive function.

Memory-guided saccades, in contrast, rely on the information stored in memory to guide the eyes toward a location that is recollected, even in the absence of a visual stimulus. Movements directed towards a remembered object's location exhibit distinct dynamics and precision compared to saccades guided by visual input [440][441][442]. This capability hinges on the brain's spatial memory and its capacity to integrate past visual encounters.

In the field of human saccades studies, there's another interesting discovery: reward-modulated signals from the caudate nucleus aid in generating saccades by lessening the suppression of pre-saccadic burst activity in the superior colliculus, an essential component for starting saccades. This observation suggests that the anticipation of reward serves as a valuable variable for probing the cognitive control of saccades [443][444]. Additionally, further research indicates that monetary rewards accelerate saccades in humans in a manner analogous to how juice rewards expedite saccades in monkeys [414].

5.4.1.4 Smooth pursuit

Smooth pursuit is a specific type of eye movement that occurs when an individual visually tracks a moving object. This movement enables the eyes to smoothly trail a target as it traverses the visual field. However, for high-velocity targets, the continuity of smooth pursuit movements can be disrupted by rapid "catch-up" saccades. It's noteworthy that smooth pursuit is not symmetric: humans and primates tend to exhibit greater proficiency in horizontal smooth pursuit compared to their vertical

counterparts. This proficiency is determined by their ability to smoothly track without the need for catch-up saccades. In contrast to saccadic eye movements, which involve quick shifts between fixation points, smooth pursuit involves a continuous and relatively slow motion of the eyes. This motion is orchestrated to match the speed and direction of the moving object. It's important to distinguish smooth pursuit from the VOR, which operates solely during head movements and is aimed at stabilizing gaze on stationary objects. Analogous to the OKR, smooth pursuit can be divided into two phases: an open-loop phase and a closed-loop phase. In the open-loop phase, the acceleration of the eyes is exclusively influenced by the object's movement velocity on the retina. Conversely, the closed-loop phase takes eye movement into account, and the pursuit system endeavors to minimize the retinal slip of the target. This entails attempting to eliminate any difference between the velocity of the eyes and the velocity of the target. Notably, smooth pursuit movements have not been observed in rodents.

5.4.1.5 Vergence

The array of eye movements includes another important component known as vergence (Figure 5.3 D). Vergence involves the coordinated movement of both eyes in opposite directions, allowing the foveae of each eye to converge on a single object, often situated at a distinct depth within the visual field. This mechanism plays a pivotal role in depth perception facilitating the adjustment of eye positions. By working in conjunction with the saccadic system, both eyes can rapidly and symmetrically alter their fixation points. For instance, imagine holding a pen in front of your face while covering one eye. As your focus centers on the pen, the covered eye perceives only a vague blur, while the open eye zeroes in on the pen. Now, upon uncovering the second eye, you'll observe the previously blurred image in the covered eye transforming into clarity and aligning with the focused image perceived by the other eye. This alignment occurs due to vergence, wherein the uncovered eye subtly adjusts its position to align its fovea with the pen. This phenomenon provides you with a sense of depth and a distinct perception of the pen's position relative to you.

Vergence eye movements can be categorized into two distinct subtypes: convergence and divergence. Convergence signifies the inward disconjugate movement of the eyes, whereas divergence pertains to the eyes' ability to move outward in opposing directions [418]. Stimuli triggering vergence responses encompass retinal blur and target displacement [445].

Vergence movements are commonly observed in mammals with forward-facing eyes, such as carnivores and birds, as well as certain fish species that align a region of high visual acuity forward to aid in prey capture. In rodents, eye movements are generally non-conjugate, meaning the eyes move independently in various directions. Vergence eye movements in rodents emerge as a result of the 'ocular counter roll,' contributing to the torsional Vestibulo-Ocular Reflex (tVOR) [391] [405] [394] [416]. Mice also exhibit vergence eye movements in response to depth-related Optokinetic Reflex (vergence OKR), although with lower effectiveness compared to primates. These movements in mice are primarily driven by differences in interocular velocity (IOVDs). Recent studies [405] in freely moving mice hunting crickets showed that vergence tends to stabilize during approach periods towards the cricket due to a reduction of head movements. The combination of compensatory and non-compensatory eye movements with continuous change in head orientation resulted in a series of stable gaze positions

interspersed with abrupt shifts. This pattern of gaze stabilization interspersed with rapid gaze-shifting movements, known as 'saccade-and-fixate,' is present across the animal kingdom and likely represents a fundamental mechanism to facilitate visual processing during movement. Unlike primates, alterations in binocular disparity cues (CD) within the visual cortex do not induce vergence eye movements in mice [422].

5.5 Changes in pupil diameter as an important behavioral characteristic

Animals' neuronal activity is finely tuned to their behavioral state, allowing them to adapt their information processing effectively in various behavioral contexts. This phenomenon has been extensively observed across a wide range of animal species, encompassing both invertebrates and primates. The concept that an animal's sensory responses are influenced by its motor activity and internal state was initially substantiated through comprehensive investigations conducted on invertebrates. Since then, the modulation of sensory responses based on behavioral and internal states has been demonstrated in diverse animal species. An animal's actions and the responsiveness of its neurons are influenced by shifts in its brain's operational state. These states correspond to periods of neural network activity that align with specific behaviors like attention, alertness, movement, exploration, and focus [284][446].

For instance, during moments of heightened attention or engagement, rapid fluctuations occur in the electrical activity of specific brain cells, as well as in signals like the LFP and EEG in the sensory regions of the brain [447][448]. These alterations in network activity are closely associated with changes in neuronal responsiveness and the size of the pupil [449]. The dynamic changes in pupil size is correlated with small fluctuations of the membrane potential, neuronal responses evoked by sensory stimuli, salient information detection, error estimation, decision-making processes, and overall task performance [450][451][452][453][362][363][449][454][455]. This has also been confirmed through two-photon imaging data of intracellular calcium in awake mice, revealing a strong correlation between the animal's pupil size and the rate of calcium transients. Particularly noteworthy are the vasoactive intestinal peptide interneurons, which play a key role in enhancing cortical activity and attention [456]. Studies have highlighted a direct correlation between changes in pupil diameter and modifications of the level of intracellular membrane potential and the frequency of oscillations in vLFP recordings from cortical and hippocampal neurons in awake, head-fixed mice. Notably, pupil dilation corresponds to increased cortical activation and the suppression of slow waves, while pupil constriction is associated with elevated cortical low-frequency activity. Microdilations of the pupil align with the initiation of brief cortical up states during quiet wakefulness. This effect is generality underscored by the finding that pupil diameter correlates with the rate of hippocampal sharp-wave ripples. Moreover, in humans, pupil dilation can predict fluctuations in fMRI network structures that measure global state changes. Within cognitive neuroscience, pupil diameter is correlated with mental effort in decision-making tasks [450][457][453][458] as well as learning dynamics [452][459]. Recently, pupil diameter has emerged as a reliable indicator of network state fluctuations [362][460][449], allowing for noninvasive classification of network states.

As a result of these well-established correlations, the measurement of pupil di-

ameter has become a widely used technique for tracking brain states and uncovering the underlying neural mechanisms. The universality of neuronal activity patterns among different species and the recognition of shared neurophysiological mechanisms governing pupil dilation through both parasympathetic and sympathetic pathways in rodents and humans strongly suggest that the direct link between network states and pupil dilation is likely applicable to the human brain. Furthermore, valuable insights obtained from pupillometry studies on non-human subjects can be applied to enhance our understanding of human responses.

5.5.1 Pupil structure and neural circuits covering change in its diameter

The pupil, located within the iris of the eye, is a variable aperture that can change its size. Similar to the diaphragm in a camera lens, its primary function is to manage the amount of visual input that enters the retina of the eye. This management of light intake has two distinct effects on the eye: it controls the exposure, which is the quantity of light that reaches the retina, and the depth of field, determining whether objects at varying depths are sharply focused on the retina [455].

Early investigations into the correlation among pupil diameter, heart rate, and galvanic skin reflex highlight a robust connection to the peripheral nervous system [461]. The diameter of the pupil is governed by two distinct sets of muscles located within the iris, both subject to regulation by the sympathetic and parasympathetic divisions of the nervous system. The sphincter muscle, which is innervated by the parasympathetic nervous system and influenced by cholinergic signals, contracts to constrict the pupil. Conversely, the dilator muscle, driven by the sympathetic nervous system and noradrenergic signals, causes the pupil to dilate. As mentioned earlier, there is an existing correlation between the diameter of the pupil and the state of the cortical network. This relationship seems to be based on the fact that both are regulated by cholinergic and adrenergic projections [460].

The regulation of pupil dilation and constriction involves intricate neural pathways (Figure 5.4 D) situated within both the spinal cord and the brainstem. These pathways react to changes in light levels and play a pivotal role in the pupillary light reflex (PLR). When exposed to light, the pupil's constriction is primarily governed by parasympathetic activation, whereas, in response to darkness, pupil dilation is mainly orchestrated by sympathetic activation. The process of transmitting changes in light intensity involves specialized retinal ganglion neurons containing melanopsin, which inherently respond to light stimuli. These neurons project toward the pretectal olivary nucleus (PON) in the midbrain. This PON, in turn, extends its projections to the Edinger-Westphal nucleus (EWN), also located within the midbrain. In this network, a subset of cholinergic neurons located in the EWN provides parasympathetic innervation to the ciliary ganglion (CG), positioned just behind the eye. Inside the CG, these cholinergic inputs from the EWN connect with neurons that then relay signals to the eye, specifically targeting the sphincter pupillae. This circular muscle around the pupil triggers constriction when it contracts. On the sympathetic side, the input for pupil dilation originates from neurons within the intermediolateral (IML) cell column of the cervical and thoracic spinal cord. This input activates the superior cervical ganglion (SCG), which in turn directly stimulates the dilator pupillae, a radial muscle band that triggers pupil dilation (see for a more detailed review Joshi and Gold, 2020

[462]).

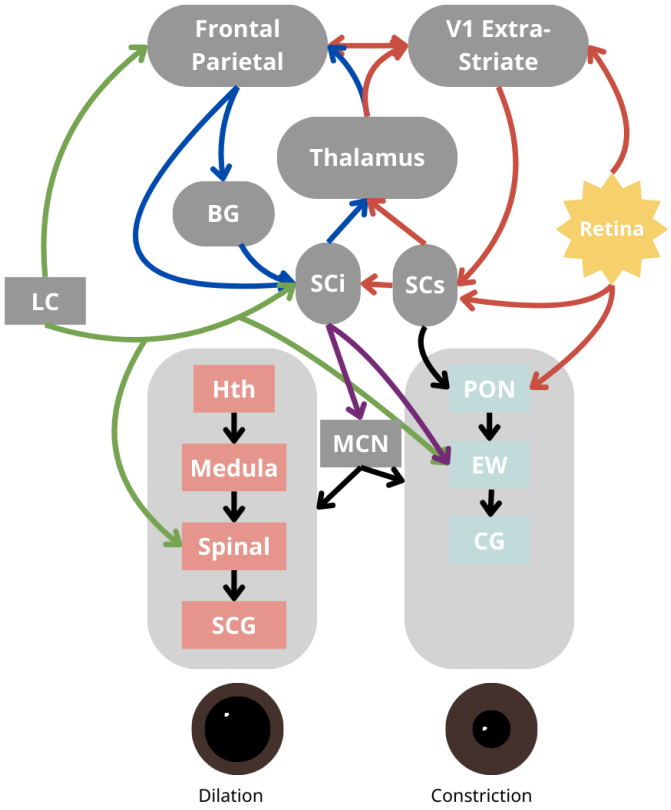


Figure 5.4: Schematic of the pupil orienting circuit, with pathway components color-coded for clarity. Light red represents the sympathetic system, while light blue indicates the parasympathetic system. Abbreviations: BG - basal ganglia; CG - ciliary ganglion; EW - Edinger–Westphal nucleus; Hth - hypothalamus; LC - locus coeruleus; MCN - mesencephalic cuneiform nucleus; PON - pretectal olivary nucleus; SCi - intermediate layers of the superior colliculus; SCs - superficial layers of the superior colliculus; SCG - superior cervical ganglion; V1 - primary visual cortex. (Adapted from Wang and Munoz, 2015 [437])

These neural circuits also receive input from higher brain regions that can influence pupil size through cognitive processes. A substantial portion of these inputs converge within three distinct nuclei: PON, LC, SC. Each of these nuclei is capable of directly modulating pupil size by projecting signals to the EWN. The interpretation of cognitive effects on pupil size often revolves around the activation of these three nuclei and the broader networks they function within. Next, I will explore evidence that establishes more direct and functional connections between neural activity within these systems and the resultant changes in pupil size.

5.5.1.1 Modulation of pupil size via the pretectal olivary nucleus

The PON is part of a cluster of nuclei within the pretectum that directly receives input from the retina. Significantly, the PON stands out as the singular nucleus that exhibits notable direct projections leading to the EWN. The PON's involvement in the PLR has been firmly established, evidenced by experiments wherein microstimulation of the PON prompts pupil constriction [462].

Moreover, there is also evidence supporting the existence of cognitive-driven modifications in the PLR. For instance, changes in the perception of a visual stimulus

can affect both the timing and magnitude of the PLR, even when the physical attributes of the stimulus, such as luminance, remain unaltered [463]. An interesting observation emerged when subjects were presented with photographs or abstract images of the sun, resulting in more pronounced pupil constriction compared to images with equivalent luminance [464][465]. Furthermore, additional studies demonstrate that the PLR can be influenced by variables including the load on working memory [466][467], the sensory context [464][465], spatial attention [468], preparation for eye movements [468][469].

The lesion studies indicated the involvement of cortical visual pathways [470], which are believed to convey advanced information about visual perception and attention to the PON, thereby influencing the PLR. These pathways likely involve the frontal eye field (FEF) in the prefrontal cortex and the lateral intraparietal area (LIP) in the parietal cortex. Both regions play crucial roles in guiding spatial attention and visually guided eye movements, projecting to the pretectal nuclei [471]. Supporting this notion, recent research demonstrated that electrical microstimulation in the FEF can specifically alter the PLR's magnitude in a spatially distinct manner [472]. Further investigations of this kind are essential to better understand the interactions between the FEF and the pretectum.

5.5.1.2 Modulation of pupil size via the superior colliculus

In the preceding chapter, I introduced the SC, a pivotal structure linked to saccadic eye movements and spatial attention [473]. The SC, situated in the midbrain, is composed of neurons forming a retinotopically coded map of the contralateral visual and saccade space. It consists of two functionally distinct layers: the superficial visual-only layers (SCs), which receive inputs from the retina and visual cortex, and the intermediate layers (SCi), which integrate cognitive, multi-sensory, and motor inputs [474][437]. The SCi directly projects to the brainstem premotor circuit for executing orienting responses. Recent studies have indicated that the SCi encodes stimulus salience and relevance to coordinate various aspects of orienting, including shifts of gaze, attention, and even pupil dilation [475]. The SC establishes direct connections to the pupil pathways. Specifically, the SCs project ipsilaterally to the PON. Meanwhile, the SCi receives inputs from the SCs, frontal-parietal areas, basal ganglia, and the LC. Importantly, the SCi projects directly and indirectly to the EWN, potentially activating and inhibiting parasympathetic pathways, respectively (reviewed in Wang and Munoz, 2015 [437]). The SCs receive direct retinal ganglion neuron input and project to SCi. Certain SCi neurons then project directly to the EWN and, thus, can influence the parasympathetic arm of pupil control [474]. A second, indirect pathway involves SCi projections to the mesencephalic cuneiform nucleus (MCN), which, in turn, influences parasympathetic tone via projections to the EWN and sympathetic tone via projections to the thoracic and cervical spinal cord (although the latter projection has to date been shown only in rats) [476]. The strength and nature of these direct and indirect projections in terms of their effect on pupil control circuits are unknown and, therefore, more work is needed to determine whether and how these pathways facilitate dilation, constriction, or both [477].

To investigate the link between SCi activation and pupil size, studies were conducted both in scenarios without the PLR and within the framework of the orienting reflex. This reflex involves shifts of gaze and attention, which are partly guided by the SC. In one study, researchers discovered that electrical microstimulation in the

SCi, responsible for processing sensory, motor, and cognitive inputs from the cortex, triggers pupil dilations. Interestingly, this effect was absent in the shallower SC layers primarily responsible for visual data processing [350]. These microstimulation-induced responses, more prominent in low-light conditions, are potentially linked to enhancing visual sensitivity or attention. In a subsequent study, both visual and auditory stimuli were observed to induce pupil responses and changes in SCi neural activity in a similar manner, influenced by contrast-based saliency—an essential factor for shifts in gaze and attention [475]. Another study further substantiated this idea by revealing connections between pupil diameter and both single-unit spiking and electrical microstimulation in the SCi [478]. While individual SCi spikes consistently correlated with momentary dilations, possibly stemming from direct SCi projections to brainstem nuclei controlling pupil size, prolonged higher SCi spiking activity (up to seconds) corresponded to more constricted pupils, rather than dilated ones. These findings imply the existence of multiple potential pathways driving associations between SCi and pupil dilations or constrictions in varying conditions and timeframes. The precise roles of these pathways are currently under exploration.

5.5.1.3 Modulation of pupil size via the locus coeruleus

The LC–noradrenergic (LC-NE) system has long been acknowledged as a pivotal regulator of arousal and attention [478]. It is widely accepted that fluctuations in pupil size are influenced by LC activity, primarily through changes in arousal levels. As a result, pupil diameter has emerged as a noninvasive indicator of LC activity. Within this circuitry, the LC directly projects to the IML, promoting excitatory sympathetic activity via α1-adrenergic receptors [479][480].The LC also exerts indirect sympathetic influence through reciprocal connections with the hypothalamus, which, in turn, projects to the IML [479]. Furthermore, the LC establishes direct connectivity with the SC, suggesting its potential role in modulating SC-mediated changes in pupil diameter. Additionally, the LC is believed to regulate pupil size by modulating neural activity within the EWN. The exact anatomical mechanism for this modulation remains debated and may involve direct connections between the LC and EWN, indirect pathways possibly involving shared input from other brainstem nuclei, or a combination of both, ultimately leading to EWN inhibition [479][481]. In this context, LC activation is hypothesized to reduce parasympathetic drive (via EWN inhibition) and enhance sympathetic drive (by exciting IML), resulting in pupil dilation [478][481].

Numerous studies suggest that LC activation is tied to changes in pupil size. For example, certain EEG studies show correlations between global arousal, pupil size, and LC activity [482]. Pharmacological studies, by modulating the LC-NE system, have successfully elicited alterations in pupil size [483]. More recently, studies have taken a more direct approach by simultaneously measuring both pupil size and LC activity. These investigations consistently reveal a pattern where pupil size changes align with LC neural firing rates. For instance, in one study, swift pupil dilations were associated with phasic LC-NE axon activity in the cortex of alert mice. In monkeys engaged in a basic fixation task, the activity of single neurons in the LC correlated with changes in pupil size over both short (associated with spiking of LC neurons) and extended (spanning seconds) timeframes. Interestingly, electrical stimulation of the LC in these monkeys consistently triggered pupil dilations [478], a phenomenon also observed in rats [480]. Another study focused on assessing neuromodulatory effects on effort and reward processing during goal-directed actions. Here, pupil dilation

also correlated with the firing rate of LC neurons f in monkeys, although the precise timing of pupil changes relative to LC neuronal firing was not explicitly outlined [484]. Collectively, these studies underscore the robust relationship between LC activity and pupil responses across various species and using diverse brain activity measures.

However, recent studies have revealed the contextual nature of the connection between pupil size and LC activity, suggesting that this relationship may not always be straightforward. In a recent study [485], researchers simultaneously recorded the activity of LC neurons using optogenetics and measured changes in pupil diameter as mice performed a tactile-detection task. They discovered a link between pupil size and LC activity, where pupil dilation tended to increase as LC neurons exhibited more spiking activity. However, the connection between pupil size and LC activity was found to predict only a fraction of LC spiking activity in real-time situations. Intriguingly, when optogenetic methods directly stimulated LC neurons, they observed significant variation in pupil responses across different sessions, despite consistent LC neuron responses to the stimulation. This emphasizes that while there is indeed a connection between pupil size and LC activity, it is not consistently straightforward. Therefore, the accuracy of pupil size as an indicator of LC activity is influenced by specific conditions and behavioral factors.

5.5.2 Pupil dynamics across behavioral states and sensory stimulus modulation

It is widely recognized that pupil size adapts to changes in ambient light levels. However, pupil size exhibits significant fluctuations even when lighting conditions remain constant, and decades of research have investigated these non-luminance-mediated changes in pupil size. Changes in pupil diameter have long been hypothesized to correlate with changes in brain state [486]. Studies have demonstrated that the pupil responds differently in states of wakefulness, sleep, locomotion, immobility and during various behavioral tasks, and associated cognitive processes. Despite the complexity of the factors influencing pupil modulation and the neural circuits associated with these changes, pupillometry serves as a valuable non-invasive tool for analyzing diverse brain states in both rodents and humans. Consequently, understanding the variations in pupil size across different states is essential for effectively designing experiments. In this section, I will describe several variables that are known to influence pupil size.

5.5.2.1 Pupil dynamics during wakefulness and sleep

The responses of the brain to external stimuli are significantly influenced by behavioral states and internal dynamics, which are organized around distinct substates that vary with different levels of wakefulness. These states include active wakefulness (characterized by arousal, locomotion, attention, and behavioral context), quiet wakefulness (periods of behavioral quiescence), and sleep periods [487][488][489].

Across various species, more active states are associated with cortical desynchronization. This desynchronization is reflected in a reduction in the amplitude of low-frequency oscillations measured through EEG, LFP, or intracellular recordings. For instance, in primates, the cortex exhibits more desynchronization during attentive states [490][491] and during REM sleep compared to deeper sleep stages [492]. Similarly, non-primate mammals display a spectrum of cortical dynamics during waking

periods, ranging from synchronized to desynchronized states, and this internal variability modulates responses to external stimuli [493][494].

Recent research in awake mice has revealed that the cortex undergoes desynchronization during exploratory behaviors like whisking [448][495] and running [496][358][361] when compared to stationary periods. During such active behavior there is an increase in pupil size (dilation) [362]. In a study by Reimer et al., 2014 [362], the researchers focused on cortical state switching during quiet wakefulness in head-fixed mice and its correlation with pupil fluctuations. They employed whole-cell patch-clamp recordings and two-photon calcium imaging during quiet wakefulness compared with running and whisking periods. One of the key findings was the occurrence of subtle, spontaneous fluctuations in pupil diameter on a remarkably short timescale of one to two seconds. Remarkably, during quiet wakefulness, these pupil dilations remain closely correlated with the desynchronization of the membrane potential in both visual and somatosensory cortices, Conversely, pupil constriction was linked to the activation of VIP+ interneurons and the inhibition of SOM+ interneurons. These and other observations highlight not only neural activity at the population level but also intracellular changes linked to alterations in pupil size during quiet awake and active awake states. This underscores the utility of pupillometry for non-invasively monitoring internal cortical states.

These changes in pupil diameter between different quiescent and locomotion states were also used in another study to identify and dissociate arousal and locomotion in V1 [363]. A third study, conducted by McGinley et al., 2015 [449], explored the cortical physiological signature of arousal during an auditory task in mice. They recorded the membrane potentials of auditory cortical neurons while mice performed a challenging tone-in-noise detection task. Pupil diameter, as previously discussed, was used as one marker of arousal states, alongside sharp-wave ripples in the hippocampus. Interestingly, these two parameters were not correlated before this study. In their work, they tested whether pupil diameter, when combined with sharp-wave ripples, could serve as a continuous measure of arousal. They examined the relationship between moment-to-moment pupil diameter and fast ripples associated with sharp waves and theta oscillations in the CA1 region of the hippocampus. Their findings demonstrated that a mouse's internal state fluctuates continuously and rapidly, with pupil diameter closely tracking the rate of occurrence of hippocampal sharp waves (Figure 5.5 A, B). There is a strong anticorrelation: as the ripple rate decreases, pupil dilation increases. This study confirms the potential of pupillometry as an efficient non-invasive method for studying arousal states during various behavioral tasks, complementing electrophysiological recordings and providing additional insights into cortical brain states.

Furthermore, changes in cortical states are not limited to quite wakefulness; they are also pronounced during different sleep phases when cognitive and sensory processes are significantly reduced. In a study by the Huber group [497], a setup was developed to allow mice to fall asleep under head-fixed conditions while their bodies remained in a natural position observed during quiet wakefulness and sleep. Electrocorticograms (ECoGs) and electromyography (EMG) of the neck muscles were used to record and quantify activity during awake, non-rapid eye movement (NREM) sleep, and rapid-eye-movement (REM) sleep.

The study demonstrated that pupil size dynamically changes during sleep and is closely linked to different sleep states. Specifically, as sleep deepens, the pupil constricts further. This coupling is primarily mediated through the parasympathetic

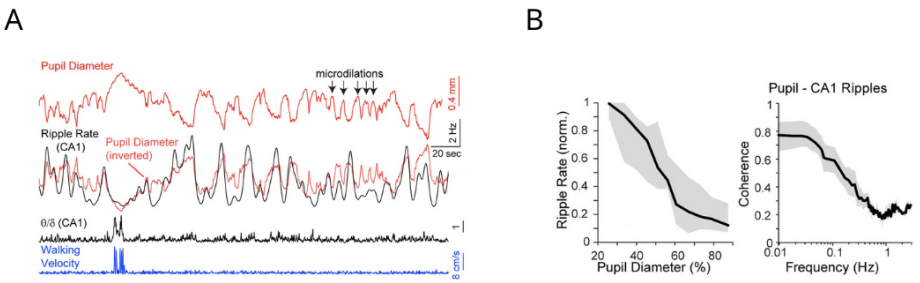


Figure 5.5: (A) Pupil diameter shows a strong correlation with the ripple rate in the hippocampal CA1 region. The light pink line represents the inverted pupil diameter (where upward movement indicates a smaller pupil size) overlaid with the hippocampal ripple rate. **(B)** An increase in pupil diameter corresponds with a significant reduction in hippocampal ripple rate. The coherence between pupil dilation and the CA1 ripple rate shows an average peak of 0.78 at low frequencies, indicating a strong relationship between pupil dilation and a decreased frequency of hippocampal ripples. (Adapted from McGinley et al., 2015 [449])

system and may serve a protective function by blocking visual input during deep sleep. Additionally, the study revealed that cortico-pupillary coupling is enhanced during NREM sleep compared to wakefulness or REM sleep states. This strong coupling during sleep can be attributed not only to the absence of external stimuli and locomotion but also to the cyclical fluctuations of various neuronal and physiological parameters during NREM sleep, which impose broad synchronicity on many processes.

In summary, the internal dynamics of the brain play a crucial role in modulating responses to external stimuli across different states of wakefulness and sleep. Pupillometry emerges as a valuable tool for non-invasively monitoring these internal cortical states, offering insights into arousal, cortical activity, and sleep-related processes.

5.5.2.2 Pupil dynamics during cognitive processing

In the previous chapter, I discussed the relationship between pupil reactions and various brain states, such as arousal, quiet wakefulness, and locomotion. While these insights have illuminated the dynamic interaction between pupil behavior and neural activity, it is equally important to understand how cognitive processes influence pupil responses during different behavioral tasks. Cognition plays a central role in shaping our reactions to the world around us, encompassing a wide range of mental functions, including perception, memory, decision-making, attention, learning, and more. As we navigate through life, our behavior is often guided by these cognitive processes, and pupils can serve as an additional indicator of various cognitive processes. In this chapter, I will showcase studies on attention and its connection to changes in pupil behavior as an illustrative example of pupil dynamics during cognitive behavior. One of the most crucial cognitive processes during wakefulness is attention. From our earliest years, parents and teachers emphasize the significance of "paying attention," underscoring the need to sustain focus on important tasks over extended periods. PhD students can undoubtedly relate to the challenge of maintaining focus while writing their manuscripts. Efficiently concentrating on specific information or stimuli while filtering out irrelevant distractions is crucial for making informed decisions and behavioral choices, not only for PhD students but also for other animals. Human attention encompasses multiple types, including sustained attention (maintaining focus on a task or stimulus over time), selective attention (focusing on a specific aspect

while ignoring distractions), executive attention (related to higher-level cognitive processes like planning and decision-making), spatial attention (prioritizing information from specific locations), and sensory-driven attention (focusing on specific features of stimuli).

Our understanding of attention in rodents often relies on observations of freely moving animals performing attentional nose-poke tasks [498][499]. In these tasks, animals can utilize visual cues to reduce the time required to make a choice in the presence of distracting stimuli. There is also substantial evidence suggesting that mice use visual cues to enhance their spatial attention capabilities [500]. For example, studies in mice have indicated that attending to specific spatial locations leads to improved behavioral and neural responses, mirroring findings observed in primates [501]. Furthermore, during locomotion, attention in mice enhances spatial resolution in V1, a phenomenon similar to what is observed in humans [502]. Through the use of pupillometry and electrophysiological recordings in the dorsal-parietal cortex, it has been demonstrated that attention segregates and enhances visual and motor signals during tasks demanding sustained attention [503]. In 2010, Fenton et al. [296] demonstrated that it is possible to decode attentional-like states during the ensemble discharge of hippocampal CA1 cells in rats performing spatial navigational tasks. Identifying these attentional-like periods also improved the reconstruction of the rat's path.

It was shown that pupil dilation occurs during attention and increased arousal states [504][488]. One of the reasons why pupils dilate during periods of heightened attention is due to an increase in arousal. This heightened state of arousal is related to the activation of the sympathetic branch of the autonomic nervous system, as was described in the previous chapter. The dilation of the pupil during attention also increases light reception. This increased light intake is beneficial for improving visual perception in low-light conditions or when trying to focus on small or detailed objects. It enhances the ability to gather visual information from the environment, which can be crucial for tasks that require close attention or precise visual discrimination. Pupil dilation is closely associated with improved visual acuity and a sharper focus. When pupils dilate, they expand the depth of field of the eye, thereby aiding individuals in discerning fine details and maintaining a clear view of the object they are focusing on [455].

Pupil dilation serves as a valuable window into the shifts in brain states during various cognitive tasks, rest, and sleep. Pupilometry, as a non-invasive and efficient method, offers an opportunity to observe these cognitive states. However, it is crucial to carefully control potential sensory stimuli to ensure accurate and reliable results.

Chapter 6

Hippocampus Role in Reinforcement Learning and Target-Finding Strategies

One of the key roles of navigation for any living organism is searching for food or a safe location, both of which can be considered rewards. To successfully navigate to a reward, an animal must integrate environmental features, past experiences, and knowledge of the environment's dynamics to predict the likelihood of obtaining a reward in a given space. Accounting for reward prediction, based on knowledge of the environment and its changes, is crucial for navigating effectively since real-world environments are dynamic (a nest can be destroyed, food sources can be depleted, and the environment can undergo seasonal or artificial changes). Therefore, to better understand spatial navigation in real-world environments, it is important to examine the role of reward coding and how the search for rewards influences navigation. In this chapter, I will briefly present general information on reward coding the role of the dopaminergic system, the representation of rewards in the hippocampal formation, and a model for reward coding in navigation studies.

6.1 Role of dopamine in reward coding

The midbrain dopamine system consists of two primary pathways, each with distinct functions. The nigrostriatal pathway originates in dopamine neurons of the substantia nigra pars compacta (SNc) and extends to the dorsal striatum, playing a crucial role in motor control. In contrast, the mesolimbic pathway begins in the ventral tegmental area (VTA) and projects to the ventral striatum, or nucleus accumbens (NAc) [505][506]. This pathway is involved in reward prediction error (RPE) signaling and encodes varying levels of confidence about reward outcomes [507][508], representing a range of expected rewards across a population of neurons [509]. The RPE is the difference between expected and received rewards [510]. Interestingly such RPE signal in dopaminergic neurons was predicted in temporal difference reinforcement learning (TD-RL) models [511]. TD-RL explains how dopaminergic neurons shift their activity from responding to the reward itself to responding to cues (such as sensory stimuli, decisions, actions, or locations) that predict the reward. With each outcome, the value of the cue is updated based on the RPE, refining future predictions. RPE signals are also observed in downstream areas like the NAc [512], where reward encoding and the

valuation of actions leading to rewards occur. The reward expectation signal in this region is modulated by higher dopamine concentrations as the animal approaches the reward [513][514].

Dopamine also plays a critical role in movement and motivation. In the 1970s, researchers found that damaging dopamine pathways caused a significant reduction in movement, similar to the effects of advanced Parkinson's disease (see more in Berke, 2018 [515]). However, both rats and humans retain the ability to move despite dopamine depletion. For example, rats with dopamine lesions can swim in cold water [516], and Parkinson's patients can move in critical situations where movement is essential ("paradoxical" kinesia) [517][515]. These findings suggest that while the ability to move is intact, there is a lack of motivation or effort to initiate movement. More recent evidence further supports dopamine's role in motivation to pursue future rewards. Dopamine levels rise at the start of actions aimed at obtaining rewards and are closely linked to both the value of each action and the vigor with which they are performed. This dopamine release pattern appears to be independent of VTA neuron activity, likely due to local regulation at dopamine terminals in the NAc [515]. Additionally, dopamine is co-released with noradrenaline from neurons in the locus coeruleus, the brain's noradrenergic center, which is involved in arousal, salience detection, and cognitive flexibility. LC neurons also respond to unexpected rewards and show firing patterns correlated with movement effort.

6.2 Reward coding in the hippocampus

One of the earliest examples of hippocampal cell activity related to goal location can be seen in a study where rats moved toward odor cues and reward ports during an odor-discrimination task. In this experiment, the firing rate of subpopulation of cells increased as the animals approached their goal (odor port and/or reward port) [518]. When the goal was known, this activity resulted in place-specific firing along the path to the goal. Another instance of reward-modulated spatial representation was evident in the clustering of place fields near reward locations, leading to an over-representation of those areas [304]. This clustering effect is primarily seen in the CA1 region, with variations depending on the type of reward, whether it's food, water, or an escape opportunity. Like goal-approach signals, the degree of place field clustering is influenced by the likelihood of receiving a reward at known goal locations. Larger and unexpected rewards result in a stronger representation of these areas [285]. As learning progresses, existing place fields shift toward reward sites [276], and after learning, place cells near the reward location become selectively stabilized [519][520]. Some place cells also show the ability to represent multiple goal locations [521]. In tasks where the goal location is separate from the reward delivery, such that animals have to go to an unmarked goal location to obtain a reward randomly distributed in the mazes, this over-representation is not observed, similar to the earlier described experiment with rats in odor-recognition task [287][286]. One explanation for the absence of place field clustering in these situations may be that the reward itself may serve as the primary trigger for reorganizing hippocampal place cells. As a result, place field clustering wouldn't occur in tasks where reward locations are unpredictable and change randomly. However, most of the recorded place cells discharged a few spikes at the goal location even if this was not their main place field suggesting a form of overrepresentation at the population level. Additionally, value-modulated firing has

been observed in CA1 neurons, where firing activity changes after probabilistic reward delivery and during departure from the reward site, depending on the reward outcome and its probabilistic value [522]. Overall, these findings suggest that the hippocampus processes reward-related signals across various phases of behavior associated with goal-directed navigation.

In Chapter 4 I described place cell modulation by goal. Reward-related changes have been also observed in the dentate gyrus and its projections. One study [523] recorded activity from granule cells and CA3 cells while rats performed a working memory task on an 8-arm radial maze. It was found that both the activity of dentate neurons and the frequency of SWRs increased during reward consumption. Additionally, reward-related SWR generation in CA3 was reduced without direct input from dentate granule neurons, indicating that DG modulates SWR reactivation in CA3 in a reward-dependent manner. Another study [524] showed that granule cells responded to reward-predicting olfactory cues, while mossy cells, known to express D2 dopamine receptors, were specifically activated by food, with their activation reducing food intake in mice [525].

The dorsal-ventral axis of the hippocampus also shows distinct reward-related properties. The vHPC, more associated with emotion and valence processing, receives greater catecholaminergic input and has stronger anatomical connections to reward-related regions such as the PFC and NAc [42][526]. The vHPC exhibits more frequent firing modulation around reward locations compared to the dHPC [527][528]. However, recent findings indicate that reward increases SWR rates in the dHPC more than in the vHPC, and the dHPC engages reward-related activity in the NAc to a greater extent than the vHPC [529].

In the medial entorhinal cortex, several studies suggest a reward influence on cell firing patterns. In a study conducted by Aronov et al., 2017 [530], rats performed a "sound manipulation task". In this task, they needed to manipulate a joystick to activate a pure tone produced by a speaker. To receive rewards, they had to release the joystick within a fixed frequency range. They found frequency-modulated cells in both the MEC and the hippocampus. Interestingly, there was no response during passive tone playback without a reward, but they displayed a weak response when a constant tone was followed by a reward. Recent studies also indicate that MEC firing patterns change with goal arrival during spatial navigation tasks. When a reward was delivered in an unmarked zone of an open field, grid cells increased their firing rates near the reward zone, and non-grid spatial cells shifted their active locations, leading to a population-level increase in firing near the reward site [531]. In another study, when rewards were delivered at multiple remembered locations in a cheeseboard maze, grid cells shifted their firing fields toward the reward sites [532]. These findings demonstrate that rewards can modulate MEC cell activity and that the MEC integrates spatial and reward information, adapting spatial representations to reward sites.

It's interesting that the dopamine system, which is involved in reward-seeking behavior, also plays a role in modulating synaptic plasticity in the hippocampus. The hippocampus contains both D1-type dopamine receptors (including D1 and D5) and D2-type receptors (including D2 and D4), with CA1 primarily expressing D1-type receptors [533]. It was shown that dopamine and D1-type receptor agonists enhance long-term potentiation at CA3–CA1 synapses without increasing the excitability of CA1 neurons, indicating that dopamine works together with glutamatergic inputs to promote plasticity [534][535][536]. It has also been shown that infusing dopamine

receptor antagonists into the hippocampus prevents rapid learning of novel locations and contexts [536].

The hippocampus and entorhinal cortex are known to receive inputs from the VTA [533][537][538]. However, it has also been shown that the LC can release dopamine to the hippocampus. In a spatial learning task by Takeuchi et al., 2016 [534], mice learned a new reward location each day. While memory degraded after 24 hours, optogenetic LC activation 30 minutes after location encoding preserved the memory beyond 24 hours. A similar effect was seen with exposure to novel environments, suggesting LC activation mimics the memory-enhancing effects of novelty. This enhancement was blocked by intra-hippocampal D1/D5 receptor antagonists, and the same results occurred with LC activation. Since VTA activation failed to restore memory, the findings suggest LC noradrenergic fibers (LC-TH+) release dopamine in the hippocampus. Further studies showed that activating LC-TH+ fibers in hippocampal slices boosted CA3–CA1 synaptic plasticity, consistent with dopamine release from noradrenergic terminals. Another study by Kempadoo et al., 2016 [539] used high-performance liquid chromatography to measure catecholamine release in hippocampal slices from mice expressing Channelrhodopsin-2 in LC-TH+ axons. They found a near fourfold increase in both noradrenaline and dopamine levels during photostimulation, confirming dopamine release by noradrenergic fibers, though dopamine was present at one tenth the concentration of noradrenaline.

Dopaminergic inputs from the VTA and LC to the hippocampus play critical roles in shaping and stabilizing spatial representations. The stability of the hippocampal map is enhanced by VTA axon stimulation [538] and reduced by inactivation of the VTA and LC [540][535], as well as by dopamine receptor antagonists [541]. In addition to promoting stability, dopaminergic transmission is essential for flexibly updating the hippocampal map to reflect the most relevant information for current tasks [541]. Blocking hippocampal dopamine receptors during learning impairs memory of reward-location associations [537]and prevents animals from learning to locate rewards based on new sensory cues [541]. Conversely, activating VTA input improves memory for goal locations [538]. Dopaminergic input also contributes to shifting place cell representations toward learned reward locations. Optogenetic activation of VTA axons can shift the firing of place cells toward the stimulation location, while inhibition tends to shift place fields away [277]. Activity from LC axons in the hippocampus signals upcoming rewards when the reward location changes [542]). Activating this LC projection can reorganize place fields around a fixed reward location but does not lead to reorganization at unrewarded locations or when the reward location is unpredictable, such as during random foraging [542]. It is important to note that the signaling mechanism behind LC-induced place cell reorganization remains unknown. Both noradrenaline and dopamine could be responsible for the observed effects.

Another indication that dopamine-induced plasticity in hippocampal neurons is essential for spatial navigation toward reward locations is the relationship between plasticity and reward-location encoding during sharp-wave ripples. It was shown that reactivated cell firing patterns during sleep-SWR are established through increased firing associations between cells active in similar locations during exploration, with greater overlap and co-activity of place cells during movement leading to more accurate reactivation in the correct order during subsequent sharp-wave ripples [543]. In the study by Dupret et al., 2010 [276], it was shown that in a goal-directed task, place fields tend to shift closer to goal locations. These cells also exhibit stronger co-firing and reactivation during SWRs. Such clustering of place fields may enhance the

precision of spatial reward memories consolidated through SWRs. Dopamine likely plays a significant role in this process, as stimulating VTA axons projecting to the hippocampus during learning aids in shifting place fields [277] and improves the fidelity of replayed place cell activity during sleep [538]. This increased reactivation during sleep, boosted by rewards, strengthens memory of the task and facilitates the later use of those place field maps [276][538].

The integration of behavioral and circuit-level evidence suggest the important role of the hippocampus in both spatial navigation and reward processing. The modulation of place cell activity and the involvement of the dopamine system highlights how the brain encodes and reinforces memories related to rewards and goal-directed behaviors. As place fields shift and stabilize in response to learned rewards, the interaction between dopamine and synaptic plasticity facilitates not only the learning of spatial relationships but also the retention of these memories over time. Moreover, the dynamics observed in both hippocampal and medial entorhinal cortex neurons demonstrate the intricate mechanisms through which environmental cues and reward signals influence neuronal firing patterns, shaping navigation strategies and enhancing memory accuracy.

6.3 Modelling of the reward prediction during navigation

A primary function of spatial navigation is for an animal to reach a desired goal, which can often be a reward destination, such as a food source, safe area, or nest. To comprehend how the brain accomplishes this task, several models can be proposed. One approach is to create a model of the environment that allows for long-term reward predictions through the simulation of future states, similar to reinforcement learning. However, in real-world settings, the vast number of potential outcomes makes this approach computationally complex [544]. Another method involves a trial-and-error approach, where a value function maps states to long-term reward predictions [505]. Yet, this model can also be challenging in dynamic environments, where changes in reward distribution would require a complete relearning of the value function. An alternative is learning a predictive map that outlines each state based on its potential successor states [545]. In this model, an animal's optimal actions often depend on its current location or state.

Here, I will briefly describe existing models and corresponding suggested hippocampal representations for efficient yet flexible goal-directed navigation.

6.3.1 Reinforcement learning

Reinforcement learning (RL) is a type of machine learning where an agent learns to make decisions by interacting with an environment to achieve specific goals. In RL, the agent takes actions and receives feedback in the form of rewards or penalties based on its performance. The goal is to maximize cumulative rewards over time by learning a policy π — a strategy for choosing actions that lead to the best outcomes [511]. This process typically involves exploring the environment to gather information (exploration) and using that information to exploit known strategies that yield higher rewards (exploitation). Reinforcement learning algorithms often utilize techniques to

update the agent's knowledge and improve decision-making. This approach is widely applied in various fields, including robotics, game playing, and autonomous systems. For example, AlphaGo, developed by DeepMind, which used RL to defeat human champions in the board game Go. It learned strategies by playing against itself and optimizing its moves based on the outcomes of each game or in robotics, RL is used to train robots to perform tasks such as walking, grasping objects, or navigating environments. There are two main families of reinforcement learning algorithms: model-free and model-based methods.

Model-free methods assess the value of a specific state $V_\pi(s)$ or a state-action pair $Q_\pi(s, a)$ by either running simulations to gather results and averaging them or through Temporal Difference (TD) learning. This allows them to derive the expected outcomes from a given state based on past experiences. The advantage of model-free methods is their speed: they allow for quick decisions by referencing pre-calculated value functions. When faced with a choice at a certain time t, this method simply selects the action associated with the highest Q-value in that state. However, when the reward associated with a transition changes, model-free methods require multiple experiences to update the corresponding values. This process relies on reward prediction errors (RPE). Model-free reinforcement learning has garnered significant attention in experimental neuroscience due to the influential hypothesis that midbrain dopamine neurons encode the reward prediction error necessary for value updates [505]. This hypothesis is supported by findings indicating that dopamine responses align with the fundamental principles of temporal difference learning [546] and by evidence of dopamine's causal role in reward learning [547][548][549]. It is believed that the striatum is primarily responsible for value computation [550][512].

In contrast, model-based methods construct or learn a representation of the environment, including transition probabilities $p(s_{t+1} \mid s_t, a_t)$ and reward probabilities $r(s_t, a_t, s_{t+1})$. These methods use this model to plan the best actions that maximize overall returns, employing techniques such as forward planning or dynamic programming. The key benefit of model-based methods is their adaptability; they can quickly adjust to changes in the environment, allowing for immediate updates in planning when the distribution of rewards shifts. However, for large-scale navigation there is a significant drawback since model-based approaches often require significant computational resources to construct and maintain a detailed model of the environment. This complexity can lead to increased processing time, especially in dynamic or large-scale environments where frequent updates to the model are necessary. Research on the planning abilities of humans and animals often focuses on tasks requiring flexible reassessment after changes in reward or transition structures. For example, outcome devaluation tasks examine whether animals stop performing previously rewarded actions when the reward is devalued through satiety or illness pairing. This single-step action-outcome association demonstrates planning capabilities [544]. While the devaluation paradigm and its more complex iterations [551] have offered significant insights, the precise neural mechanisms underlying model-based planning remain largely unknown [552].

While model-free and model-based RL methods are often presented as opposites, there are approaches that lie between these two extremes. One such approach is the Successor Representation (SR), which balances flexibility and efficiency by summarizing patterns in the environment without requiring costly forward simulations [545]. The SR arises naturally from the typical definition of value used in reinforcement learning. The value of a state s is defined as the expected sum of rewards from future

states s_t, with rewards being discounted by a factor $\gamma \in [0, 1]$, which reduces the weight of distant rewards:

$$V(s) = E\left[\sum_{t=0}^{\infty} \gamma^t R(s_t) \mid s_0 = s\right]$$

The value function can be expressed as the product of the reward function and a predictive state representation, the SR, denoted by M:

$$V(s) = \sum_{s'} M(s, s')R(s')$$

Here, the SR represents the expected discounted future visits to state s starting from state s:

$$M(s, s') = E\left[\sum_{t=0}^{\infty} \gamma^t I(s_t = s') \mid s_0 = s\right]$$

The function $I(s_t = s')$ equals 1 if $s_t = s'_t$ and 0 otherwise. This allows us to break down the expected discounted reward into two parts: the expected discounted future state occupancy and the reward at each state. The SR, denoted as M, can be updated incrementally using a variant of the temporal difference learning algorithm [553].

The SR blends elements of both model-free and model-based approaches. Like model-free methods, it allows for efficient policy evaluation. However, by splitting the value function into two components—one reflecting future state expectations and another focused on rewards—it gains some of the adaptability typical of model-based algorithms. This separation allows quick recalculations of value when rewards are modified, added, or moved, without the need to relearn the underlying state dynamics. In contrast, model-free systems must relearn value estimates for each state, while model-based systems rely on computationally demanding searches through their models to update predictions. Additionally, SR enhances generalization by linking states that predict similar future outcomes, resulting in comparable value assignments. This feature is especially beneficial in spatial tasks, where neighboring states tend to lead into each other. The SR inherently represents adjacent states similarly, smoothing out transitions over both time and space [553].

6.3.2 Successor Representation in the hippocampal system

In this framework, animals during navigation rely on a predictive map where each state represents future outcomes, not just the current position. This successor representation model suggests that place cells reflect likely future states rather than merely encoding the present location. States with similar predicted outcomes share representations, even if not physically adjacent, while nearby states leading to different outcomes have distinct representations. Unlike traditional temporal difference reinforcement learning, which focuses on reward prediction errors, the SR model updates transition probabilities based on prediction errors about future state occupancy [545]. This separation of learned transition structures from rewards enables efficient adaptation to changes without relearning. The SR model allows animals to learn transition probabilities without immediate rewards, such as during exploration [554].

It is suggested that hippocampal place cells represent the successor representation using a rate code, with peak firing when the animal is in a location a cell predicts

strongly. The decline in firing when moving away from that location is based on predictions about future states and the applied discount factor. A key prediction of the SR model is that place cell firing indicates the animal's upcoming locations, leading to more activity in cells corresponding to likely future visits. This is supported by evidence showing that when animals trained to run on a linear track exhibit symmetric firing fields, a bias occurs when training encourages movement in a specific direction [555]. Another experimental evidence shows that the SR model can predict various experimental observations as a higher density of place fields near goals [304]. The grid cells are thought to encode a low-dimensional representation of the successor representation, capturing the underlying structure of relationships between states [553][556]. It is also important to note that SR develops through learning: the more often a state is visited, the more likely it is to be predicted [557]. In open environments with randomly distributed rewards, the SR appears as an evenly spaced grid, since the animal does not consistently follow any particular path. However, when certain routes are taken frequently, such as between fixed goal locations, the increased visitation of states along these routes leads to a shift in grid fields, bringing them closer to the goals [532][557]. When states are visited more frequently without following a specific path, such as when searching for a hidden goal, grid cells may exhibit increased firing rates in those local areas instead [531].

The SR theory sheds light on how the hippocampus-entorhinal cortex system learns the sequence of state transitions to make value predictions, establishing a link between the hippocampus and value-based decision-making [558][559]. Replay mechanisms are believed to play a role in assigning value to these state sequences. In "prioritized replay," forward replay occurs during decision-making to assess the value of potential routes, which enhances the likelihood of making correct choices [560]. After a reward is obtained, reverse replay updates the values of prior states. Both types of replay may also function simultaneously to estimate and store values [561][562].

It is still unclear how value is assigned to each state in the SR at the neural level. If hippocampal cells solely represent SRs, they would need to integrate their activity with an external reward signal to compute value. Dopamine release in the NAc and the firing of reward-related neurons in the NAc or VTA, synchronized with hippocampal-entorhinal sequences, could link states to their respective values. In this framework, the basal ganglia would evaluate hippocampal sequences to select actions that lead to desired outcomes [563][564]. Additionally, neurons in the prefrontal cortex may assist in generalizing value across similar tasks [565][557]. Alternatively, the value might be computed locally within the hippocampus-entorhinal cortex, potentially through dopamine's effects on synapses, which could enhance spiking in CA1 neurons for higher-value sequences [566].

Thus, experimental work on hippocampal place cell modulation of reward probability in an environment that mimics real-world uncertainty would enhance our understanding of the intricate dynamics of successor representation in learning and decision-making. Interestingly, it was recently shown that grid cells in the MEC can have grid fields representing future locations during goal-directed behavior [567]. By examining how place cells encode information about future states based on current spatial experiences and reward expectations, we can gain deeper insights into the neural mechanisms underlying adaptive decision-making and the role of hippocampal circuits in navigating complex reward landscapes.

Chapter 7

Utilizing Virtual Reality to Study Spatial Navigation in Rodents

7.1 Virtual reality in scientific research

Animal behavior can be studied in various ways. The first one, which is also the most ancient, involves observing animals in their natural environment. This can take the form of observations or experiments conducted in naturalistic setting, such as introducing new objects or individuals into the animals' habitats. The second one involves conducting experiments in indoor laboratory conditions where animals are raised in cages and participate in various experiments. Both practices can provide answers to a variety of questions, but they also come with their own set of limitations. Studying animals in their natural environment can help us understand their true behavior. However, identifying the specific reasons behind certain behaviors can be challenging because natural environments are multisensorial spaces. Factors like touch, colors, odors, and sounds can all contribute to neural responses and behavior. Controlling these sensory inputs, their quantities, and their effects simultaneously or in isolation is nearly impossible in complex natural environments. Additionally, outdoor experiments may face unforeseen changes due to weather conditions or human disturbances, making it difficult to standardize reproducible protocols.

To overcome these challenges, indoor experiments in laboratory conditions offer a better opportunity. However, even in the lab, it can be challenging to control the sensory inputs that animals receive during tasks, preserve natural behavior, and combine multisensory protocols that can precisely measure these stimuli. Technological advancements, such as the incorporation of cameras for tracking behavior, speakers, and projectors, have significantly advanced behavioral studies and are utilized in innovative ways to manipulate the information animals receive about their surrounding environment.

A significant breakthrough has been achieved through the implementation of virtual reality (VR) in animal studies, bridging the gap between natural and laboratory environment studies. VR systems simulate real-world inputs to one or more of an organism's sensory neural circuits, then measure the subject's actions and apply updates to sensory stimuli in response. Virtual reality technology can be traced to its early beginnings in 1939, when the View-Master, a simple non-electric device designed to render three-dimensional environments from slides, was patented in the United States as the first VR device (Figure 7.1 A). Starting from there, VR technology advanced

significantly, allowing its application in various spheres from education and entertainment to scientific research and medical studies. It has already been successfully used in peri-operative planning, surgical training, and rehabilitation (see Scott et al., 2022 [568]). VR technology entered scientific research in the late 90s in the field of human psychology and behavior [569] after developing CAVE VR (Figure 7.1 B), which was used in human research to study human responses in environments with various visual stimuli and the ability to interact with them [570]. In this environment, the viewer enters a room where head position is tracked, and the stimuli are rendered on the walls from the perspective of the viewer. Two-dimensional figures can appear as three-dimensional objects when presented from a specific perspective. This study inspired researchers to adapt VR for human research to animal studies for studying the behavior of freely moving animals in VR.

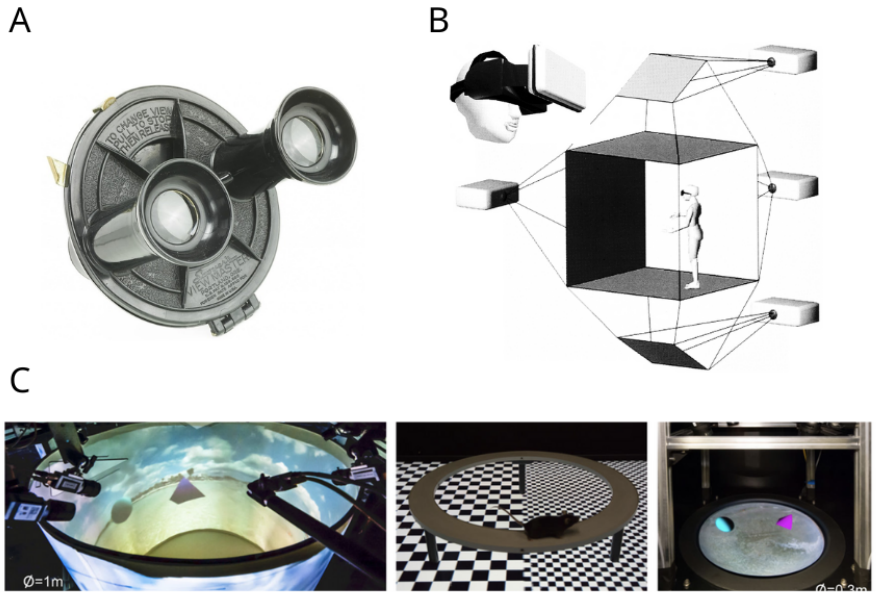


Figure 7.1: **(A)** Sawyer's View-Master stereoscopic viewer. The device displayed stereoscopic three-dimensional pictures for viewers. [From the National Museum of American History] **(B)** A schematic illustration of CAVE VR for humans. (Adapted from Cruz-Neira et al., 1992 [570]) **(C)** On the left is the 'Flycave,' a cylindrical flight arena measuring 1 meter in height and 1 meter in diameter. Virtual environments are projected onto the cylinder walls using three projectors, featuring a magenta pyramid and a cyan sphere against a photographic backdrop. In the center is 'MouseVR,' a setup for freely moving mice, utilizing a 1.9-meter television for displaying virtual environments. On the right is 'FishVR,' where zebrafish swim in a 32 cm diameter hemispherical bowl filled with 9 cm of water, with a virtual environment projected from below. (Adapted from Stowers et al., 2017 [571])

This has opened up new insights and possibilities for the study of animal behavior and neural systems, enabling researchers to observe brain responses during more complex behavioral tasks. Furthermore, the connection between sensory and motor inputs can be adjusted by changing the amplification or delay between an action and the subsequent modification of the virtual environment. Alternatively, they can be temporarily uncoupled from each other. This separation of sensory and motor variables allows the subject to engage naturally and actively with the sensory environment. Another advantage of using VR is its capacity to broaden the array of tools

accessible for monitoring neural activity. Since the subject is typically confined in VR setups, it becomes feasible to employ techniques that would either be impractical or yield lower-quality results when dealing with freely moving subjects. These techniques encompass functional magnetic resonance imaging, high-resolution fluorescence imaging, and electrophysiology.

Examples of adapted human VR systems for animal studies could be the FreemoVR, immersive VR for mice, flies, and zebrafish (Figure 7.1C) [571], and the FlyVR for flies [572]. The technology used to develop these environments was similar to that used for human ones. However, the sensory perception of some animals differs from that of humans. For example, the color perception range, use of odors for navigation, and ultrasonic sounds. To address these issues, virtual realities for animal studies could explore new technological solutions, such as real-time tracking and realistic graphic rendering. From the moment of its establishment, VR has been used in multiple studies on animal navigation, including not only mammals but also insects [573], studies of visual systems (see subchapters below), and has opened up possibilities for data treatment [574] and replacement of animal models [575].

In the next sections, I will explore the available VR systems for rodents, their pros and cons, as well as consider potential future implementations.

7.2 Developing VR for rodent studies

Before the development of VR systems for studying optic flow in rodents, various visual learning and discrimination tasks were already in use within the research community. However, these systems had a significant drawback in that they lacked closed-loop functionality, which is an essential component of virtual reality systems. Notably, they did not provide a continuous update of visual stimuli based on the animal's movements, thus lacking interaction between the animal and the environment. The first VR system designed for rodents was introduced by Hölscher et al. in 2005 [576] (Figure 7.2 A). This pioneering setup drew inspiration from similar apparatus used to study insects in virtual environments. The fundamental elements of this setup included a panoramic display that covered a substantial portion of the rodent's field of view. Typically, these displays consist of toroidal or cylindrical screens on which a virtual scene is projected using a projector and a system of mirrors that includes an angular amplification mirror. In other setups, angular amplification is not used, and the VR is directly projected onto the screen [177] (Figure 7.2 B). Alternatively, computer screens have been employed in some setups [360] [175][314][577][273] (Figure 7.2 C).

The second critical component of these systems is a treadmill that enables the animal to navigate within the created virtual environment. In this setup, a Styrofoam sphere floated on a cushion of pressurized air to minimize friction and reduce the exhaustion of the animal. The animal's movements were captured using optical sensors, similar to those found in computer mice, and this information was sent to the VR system for real-time updates of the animal's position within the virtual world. Lee et al., 2007 [578] later enhanced this system by introducing a motorized linear belt treadmill and a back-projected dome screen.

Significant advancements were made when it was demonstrated in 2010 that the firing of neurons increased more than twofold when animals in motion were looking at static landmarks, compared to previous studies that focused on brain activity during anesthesia or immobility [358]. This observation highlighted the importance

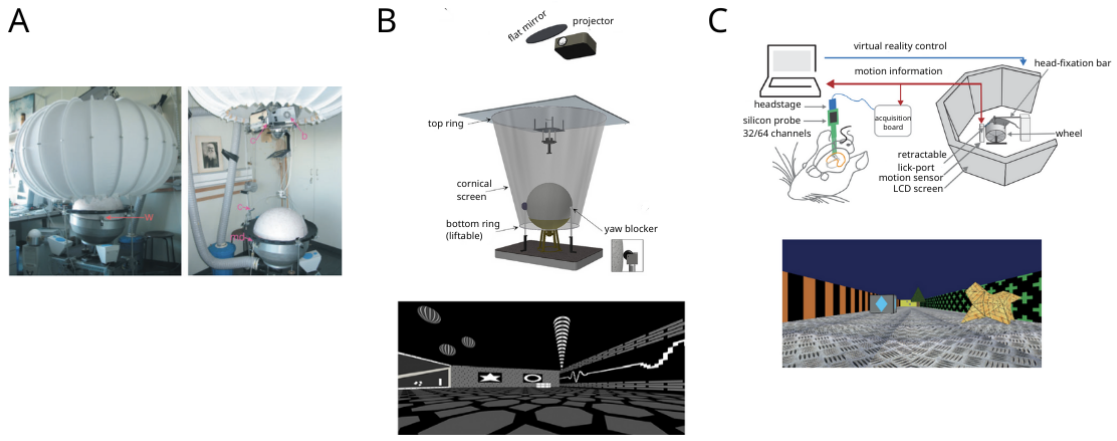


Figure 7.2: **(A)** Photos of the first VR system designed for rodents. On the left, the machine is shown in its operational state with the screen lowered. The red "w" indicates one of two wheels that prevent the sphere from rotating around the vertical axis. On the right, the machine is depicted with the screen raised. The red "b" marks the DDV 1800 (Liesegang) projector; "c" indicates two small video 'finger' cameras used to monitor the rat's behavior inside the screen; "md" represents the motion detector. (Adapted from Hölscher et al., 2005 [576]) **(B)** A schematic of the setup for 2D rat navigation in virtual reality, where the environment is projected onto a screen. The screen is shown as partially transparent, and the ceiling is tinted for clarity. A yaw blocker is in contact with the treadmill, preventing it from rotating around the vertical axis during the rat's movement. (Adapted from Aronov and Tank 2014, [177]) **(C)** A schematic of the setup for 1D mouse navigation in virtual reality, where the mouse is head-fixed and moves on a wheel. The virtual environment is displayed directly on LCD screens. (Adapted from Bourboulou et al., 2019 [273])

of locomotion as a critical parameter for accessing neural activity. Consequently, there arose a need to record neural activity while animals navigated in the VR environment, leading to the development of head-fixation systems that provided better stability while awake animals moved within the VR. These head-fixation systems allowed researchers to conduct various electrophysiological and imaging recordings, such as photon calcium imaging [367][579][360][577], patch-clamp studies of place and grid cells [580][581][582], and acute multisite recordings [175][583][314]. To incorporate vestibular input for head rotations and body movements, researchers introduced free movement in VR navigation. One approach was to use rotation platforms to induce passive head and body movements while allowing for head fixation for various electrophysiological techniques [584]. Another method for enabling free movement involved placing the animal within a bowl-shaped structure, using the walls as projection screens for the VR. Head-tracking systems were employed to record the animal's movement within the maze [585].

Before constructing and preparing a VR setup for experiments, a critical step involves designing the virtual environment to align with the goals of the investigation. For studies related to spatial navigation or visual perception, a 1D corridor with infinite length or defined length with teleportation is commonly used. In some cases, a square maze shape with the ability to navigate in a 2D virtual environment was employed. The walls of the environment typically featured simple dot or stripe patterns, along with distal and/or proximal objects and cues to aid in orientation and address specific research questions [586].

The choice of colors for the virtual environment depended on the visual properties of mice or rat vision systems, often utilizing black and white patterns or wavelengths

ranging from 370 to 510 nm (see Chapter 5 for more details on mouse vision systems). To achieve real-time updates of the virtual scene, scientists utilized modern graphics software that performed the necessary calculations directly on the graphics hardware (GPU). This could involve software for video game development, such as Unity Engine [580][587][175], specific 3D graphics software like Blender [582], OGRE [583], OpenSceneGraph (FlyVR; [572]), and Vizard (WorldViz,; [588]), or direct use of OpenGL, for instance, accessed via Matlab or Python [576][314][177][585].

In addition to visual stimuli, other sensory inputs could be introduced into the virtual environment to investigate different sensory modalities or multisensory environments. For instance, tactile stimuli were incorporated to study whisker-guided locomotion. In this setup, head-fixed mice ran through corridors with movable walls on both sides [589]. Two walls maintained a fixed 30mm distance from each other, simulating a corridor with a constant width. The walls moved in response to the mouse's movement on the ball, simulating curvature in the virtual corridor. In another study, a flat-floored air-lifted platform was employed to provide closed-loop sensory stimulation. In this setup, mice could move within the air-lifted mobile cage, which could be enriched with various visual, tactile, and olfactory cues [590]. To explore the effect of auditory stimuli on navigation, researchers introduced auditory cues in the virtual Morris water maze task [591]. These cues were presented using a 7-speaker ambisonic surround sound system, with the intensity and orientation of auditory stimulation being modifiable based on the rat's navigation within the maze. Another group of researchers developed an olfactory VR system for mice [592], introducing an olfactory virtual landscape. This landscape distributed precise odors to head-fixed mice as they explored a virtual environment. This study revealed that, besides visual cues, other sensory stimuli could engage place cell coding and potentially contribute to the cognitive map structure. In a recent study [593], scientists investigated the influence of cognitive map building and place cell coding in a multisensory environment using two behavioral tasks in which mice navigated the same multisensory virtual environment by either pursuing a visual landmark or tracking an odor gradient.

7.3 Limitations of VR for rodents

Besides the discussed possibilities in research that have been explored with the implementation of VR in various studies, there are also certain limitations related to the redundant and current functionality of existing systems. These limitations are associated with the possibility of implementing 2D navigation and vestibular input, tracking animals during navigation, and the latency of system performance. Firstly, the incorporation of additional hardware for multisensory navigation and real-time closed-loop interactions with the environment, along with the simultaneous recording of animal responses and stimuli, may introduce synchronization challenges. This is particularly crucial as different setups are used for neural recording, and ensuring precise timing between neural firing, animal position, and stimulus presentation within VR is essential for subsequent analysis. Furthermore, the multiple electrical components of VR systems could potentially introduce additional electrical noise during electrophysiological recordings, necessitating careful consideration in the design of future setups.

Secondly, when studying navigation, vision, or decision-making systems in rodents, it becomes important to provide the animals with the option of movement in a 2D

space to facilitate more natural interactions with their environment. Specifically, 2D navigation is essential in spatial navigation studies. Allowing unrestricted movement results in position-coding activity of place and grid cells similar to that observed in the real world. However, this coding is disrupted when body movement is restricted or the head is fixed. To address this, researchers may use head fixation with a rotation platform or navigation via piezo-sensors on a belt, offering passive 2D navigation. In the first scenario, animals can explore the environment with vestibular input but lack control over their path. In the second scenario, animals can choose their direction using sensors, but vestibular input is eliminated. Also, using sensors and a rotation platform could prolong the training time for the animal in the VR. Using a styrofoam ball could be more challenging for animals to navigate in 2D environments, primarily due to the need to control the ball's motion. Cameras used to sample ball motion must operate at higher frame rates than the ball's rotation speed. The ball's rotation mechanism must also be sensitive to variations in animal movement to avoid unwanted disruptions. For instance, it is crucial that the ball accelerates and decelerates in harmony with the animal's movements; otherwise, it could introduce undesirable disturbances for the animal [594]. Implementing 3D tracking necessitates the use of multiple cameras, introducing technical challenges related to camera calibration and synchronization. Additionally, the use of additional infrared lighting for tracking the ball without affecting the VR environment adds further complexity. Navigation without head fixation also demands head tracking using additional LED lights and is not feasible for imaging experiments.

Thirdly, latency in a closed-loop system refers to the overall delay between an animal's movement and the corresponding stimulus change on the display. Numerous computational steps, such as image processing, data storage, and graphics rendering, introduce time delays in the system. These delays result from both software and hardware components. Achieving a low latency is crucial for conducting interactive experiments in real-time. Addressing hardware-related challenges, such as fast computation, high-bandwidth data transmission, and responsive displays, is difficult, and researchers must consider achievable overall latency when designing future experiments.

VR systems provide valuable insights in a variety of neuroscientific research. Nevertheless, it's crucial to bear in mind that, much like other reductionist methodologies, VR involves a balance between improving experimental accessibility and maintaining consistency with natural processes. The choice of the optimal setup depends on the specific research question being addressed.

7.4 Future perspectives in VR for rodents

Despite some possible limitations, VR is a powerful tool for addressing specific research questions. It has opened doors to the study of complex animal behavior in real-time, including their neural responses to various stimuli. The feasibility of applying modern recording techniques, which require high stability when tracking mice, has undeniably benefited from VR and makes it an exceptional tool for probing neural responses. Nevertheless, rodent VR systems certainly possess great potential that extends beyond being merely a tool for head fixation. Considerable progress has been made in the past two decades, but support in terms of technology development is required to expand the use of this technology in neuroscientific research. In addition to the previously

discussed implementations in VR setups, there have been new advances thanks to collaborative efforts between XR developers and neuroscientists. For example, there is a recent development called "MouseGoggles," a miniaturized virtual reality display designed for head-fixed mice. It provides independent binocular visual stimulation over a wide field of view, is cost-effective, and has a compact size, allowing for broader adoption of VR methods in neuroscience. These goggles also offer a greater degree of immersion in the virtual environment by effectively blocking irrelevant and conflicting visual stimuli when compared to panoramic screens.

Another implementation involves pupil tracking while animals navigate in virtual reality and solve various spatial and visual tasks while recording electrophysiology data. Moreover, modern video game engines can facilitate the fast and flexible implementation of similar virtual environment tasks for humans, non-human primates, and rodents, enabling interspecies studies that are crucial for fundamental research. Similarly, adapting traditional psychophysical tasks from human research for VR can further enhance our understanding of perception.

One more valuable approach is the study of attention in a multisensory setting. Tasks can be developed in a multisensory VR environment in which an animal is required to focus on one sensory modality at a time in a reward-based task. In addition to spatial VR experiences, interactions with virtual objects can provide opportunities for research in truly interactive situations. For example, a study might involve detailed observation of whisker movements during the exploration of different objects and then replicate similar whisker movements through multiprobe stimulation [595][596][597]. This virtual object setup can be used to study object recognition and allow for a systematic investigation of varying stimulus components and their influence on recognition.

Advancements in rodent VR technology could include methods to incorporate motion parallax, enabling animals to use depth and distance cues. Such systems would require precise head tracking, and efforts in this direction are already underway [585]. Additionally, the use of displays or projectors that emit short-wavelength light, such as blue/UV light, would enhance the visual component of rodent VRs by better aligning with the short wavelength-sensitive photoreceptors of rodents. Furthermore, the flexibility of VR systems can facilitate changes in the shape and length of the virtual environment, making it a valuable tool for investigating long-distance navigation in complex environments similar to the real world.

Part II

Experimental Contribution

Reward seeking behavior in Unpredictable Environments: Navigating without Cognitive Maps

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Abstract

A population of hippocampal CA1 pyramidal cells, commonly known as place cells, encode spatial information within an environment by forming place fields, serving as the neural substrate for spatial memory. In small-scale environments, place cells typically form one place field, while studies in larger-scale settings have demonstrated that they display multiple place fields exhibiting multiscale coding. However, previous research has involved rodents exploring environments with uniformly distributed cues and reward locations, which are not representative of natural settings characterized by inhomogeneous landscapes and stochastically distributed goal locations. In this study, we utilized a semi-naturalistic virtual reality (VR) environment with stochastically distributed rewards to examine mouse behavior and recorded CA1 cell activity through multi-site electrophysiology. Although the mice were able to learn the task and navigate successfully within the environment, we did not observe strong spatial tuning of pyramidal cells. Our findings suggest that, depending on the task, mice can employ alternative spatial navigation strategies and utilize rule abstraction to form a schematic representation of space in large-scale environments, enabling them to navigate effectively.

Keywords Virtual reality, Navigation, Electrophysiology

1. Introduction

Successful navigation in the natural world is crucial for animal survival. Animals constantly forage for food, encountering environments with varying degrees of predictability in resource location. While some resources may be consistently found in specific locations (e.g., a reliable watering hole), others, like food sources, are often distributed stochastically, with their location varying across time and space (Bruno and Lovari, 1989). This uncertainty necessitates cognitive flexibility and the ability to utilize available cues to guide behavior.

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Despite the ecological importance of navigating environments with uncertain resource locations, much of our understanding of spatial navigation in rodents comes from studies using fixed reward locations within relatively small environments (O'Keefe and Dostrovsky, 1971, Wilson and McNaughton, 1993). This approach, while valuable, may not fully capture the complexities of real-world navigation where animals must integrate spatial information with inherent uncertainty.

Furthermore, studying navigation in small environments with fixed rewards presents another challenge. Animals quickly learn the optimal path to reach the reward, leading to a cessation of exploratory behavior (Hölscher et al., 2003). To promote exploration in larger environments, researchers often distribute rewards throughout the space. Although this ensures full coverage (Harland et al., 2021, Lee et al., 2020, Rich et al., 2014), a homogenous reward distribution has lower ecological relevance.

Therefore, a key question emerges: How do the brain circuits underlying navigation encode reward uncertainty and the structure of the environment? Investigating navigation in environments with stochastic reward locations within larger, linear tracks can provide a more ethologically relevant framework for studying these processes. This study addresses this gap in knowledge by employing a head-fixed virtual reality environment with a stochastic reward distribution on a long (14m) linear track. We analyze the behavior of mice in this environment and investigate the neural correlates of navigation in the face of reward uncertainty.

1.a. Background

The hippocampus plays a crucial role in spatial navigation and memory. Pyramidal neurons in the CA1 area of the hippocampus are known to play an important role in how the brain encodes the environment. In particular, the discovery of “place cells” – hippocampal neurons that are activated at specific locations in the environment – and their corresponding “place fields” (areas where place cells are active) has led to intensive research into the neural mechanisms underlying spatial coding. Place cells are believed to be the fundamental building blocks of the internal cognitive map (O'Keefe and Nadel, 1978) that allow animals to flexibly navigate their environment.

Multiple studies have demonstrated that place cell activity can be modulated by the goal location and its variability in an environment. For example, during rewarded navigation, place cells in the CA1 region shift their fields toward the goal (Dupret et al., 2010). Similarly, local clustering of place fields around goals has been linked to place preference behavior (Mamad et al., 2017), with multiple active fields associated with specific goals. However, no single cell responded exclusively to all potential goals, suggesting a more complex encoding of space than a simple goal representation (Ainge et al., 2007). In another study, (Gauthier and Tank, 2018) identified a small subset of cells that activated exclusively at goal locations, even when the goal was moved within the same virtual environment or transferred to a different one.

Further investigations into whether place cells encode goal value have shown mixed results. It was shown (Tabuchi et al., 2003) no evidence of hippocampal encoding of goal volume, while subsequent studies (Duvelle et al., 2019, Lee et al., 2012, Tryon et al., 2017) revealed that CA1 place cells could reflect the animal’s choice of goal and its outcome across trials (Lee et al., 2012). In tasks designed with two goals offering

varying amounts of food, animals were able to distinguish between goals and navigate preferentially toward the one with a greater reward. Interestingly, place cells did not consistently encode goal value or show a significant clustering of fields at goal locations. In one goal-directed task where the goal and reward were dissociated, place cells formed secondary firing fields at the goal location, but there was no over-representation of the goal itself (Hok et al., 2007).

In larger environments where rewards were randomly distributed, place fields did not overrepresent rewards. However, when rewards were fixed, place fields tended to accumulate around these areas (Lee et al., 2020). Another study (Aghajan et al., 2015) showed that hippocampal neurons with no spatial selectivity during a random foraging task in virtual reality became place-selective after fixed cued goals were introduced in the arena. Given the evidence of goal-driven modulation of place cells, it would be important to explore how changes in the spatial predictability of goals—similar to natural settings with stochastically distributed food sources—affect place cell coding.

To effectively navigate toward a goal, animals can employ various navigational strategies. One of the simplest is beaconing, a stimulus-response navigation method that requires the constant perception of a beacon or goal landmark (O'Keefe and Nadel, 1978). While computationally simple, this strategy is limited by the need for the beacon to always be present, making navigation impossible when the beacon is absent. Another navigational strategy is path integration. Path integration allows an animal to track its position relative to a reference point, such as a nest, using internal signals derived from its own movements (Etienne and Jeffery, 2004, Gallistel, 1990). This strategy does not rely on external cues and can even be used in complete darkness. However, over longer distances, the integration errors accumulate at each step, leading to significant inaccuracies in position estimation. These errors can be corrected if the animal incorporates external cues, such as environmental landmarks, to recalibrate its position. A different strategy involves navigation using cognitive maps (O'Keefe and Nadel, 1978, Tolman, 1948). Unlike the idiothetic information used in path integration, cognitive maps rely on allothetic information to construct a map of the environment. This allows animals to navigate flexibly in an environment. The hallmark of cognitive map navigation, an animal's ability to find novel shortcuts, was demonstrated in a study on Egyptian fruit bats (Toledo et al., 2020). However, it remains unclear how and when rodents utilize cognitive maps, as most studies have been limited to small mazes and short 1D tracks. Similar to cognitive maps, animals may use spatial schemas for navigation. Unlike cognitive maps, which represent detailed spatial relationships in a specific environment, schemas capture general patterns or spatial relations that can be applied across different environments. In the context of navigation, schemas summarize recurring spatial elements, such as landmarks or paths, enabling animals to navigate new environments by recognizing familiar structures (Farzanfar et al., 2023). Spatial schemas have been documented in rodent (Bethus et al., 2010; Tse et al., 2007, 2011) and primate studies (Baraduc et al., 2019).

To understand spatial navigation in large-scale environments and the impact of various factors (such as visual, olfactory, and auditory cues) on navigation strategies and their neural basis, it is essential to use environments with controllable and easily manipulable features. In our study, we leveraged virtual reality (Aronov and Tank,

2014, Cohen et al., 2017, Gauthier and Tank, 2018, Harvey et al., 2009, Ravassard et al., 2013) to precisely control and manipulate stochastic reward distribution combined with semi-realistic environmental features.

2. Results

2.a. Mice and navigate the large environment and learn the reward location

statistics

To investigate how mice navigate a stochastic environment, we employed a head-fixed virtual reality (VR) system (Figure 1 A). The VR environment was designed using the Unity rendering framework . Briefly, the mouse was head-fixed, and neural activity was recorded from the hippocampus using a 64-channel Buzsaki probe connected to an Open Ephys recording system. Behavioral data, including the animal's position within the VR environment, was continuously monitored via a custom Python pipeline (see Code Availability section for details).

The VR environment itself consisted of a linear track stretching 14 meters in length (Figure 1 B). While not a perfect replica of a natural environment, the track incorporated semi-realistic features to provide the animal with basic spatial cues. This approach aimed to balance ecological relevance with the ability to precisely control and manipulate features of the environment.

The virtual environment was designed with two distinct zones: a reward zone (Zone 1) and a non-reward zone (Zone 2) (Figure 1 B). Rewards were distributed stochastically within Zone 1 using a Poisson point process, meaning the probability of encountering a reward increased progressively within the zone and then decreased towards the end (Figure 1 B). Zone 2 contained no rewards. To prevent the motor sound from the delivery system from introducing bias into task performance, we introduced a second identical motor with a reversed distribution compared to the real one. This motor produced sound uniformly across the track, covering both zones (Figure 1 C). After completing the 14-meter track, the mouse was automatically teleported back to the beginning for the next lap (trial).

Mice successfully navigated the entire 14-meter track multiple times per session (Figure 1). Their behavior within the environment demonstrated learning across sessions. Over time, mice spent progressively less time stopped in Zone 2, suggesting a reduction in unproductive exploration. Conversely, they exhibited increased stopping behavior within Zone 1, likely to search for the stochastically placed rewards.

To assess reward intake, we monitored tongue contact with the lick port, focusing on the number of lick bouts rather than individual licks as mice typically lick in bursts. Interestingly, observations revealed that mice generally stopped running before initiating a lick bout. While occasional additional lick bouts occurred after stopping, these were considered part of the same episode. Therefore, only lick bouts associated with running stops were analyzed.

To quantify how effectively mice learned the reward distribution, we employed the Kullback-Leibler (KL) divergence. This metric measures the difference between the observed distribution of lick bout positions and the underlying reward density. A pro-

gressively decreasing KL divergence across sessions demonstrates that mice were learning the spatial distribution of rewards within the environment (Figure 2 A). We analyzed the lick bouts around real and fake rewards to verify whether the animals could distinguish between the two sounds (Figure 2 B). (Figure 2 C) During the training sessions, animals progressively learned the reward distribution along the track, resulting in few or no licks in Zone 2, which contained no rewards, during the later training stage. (Figure 2 D).

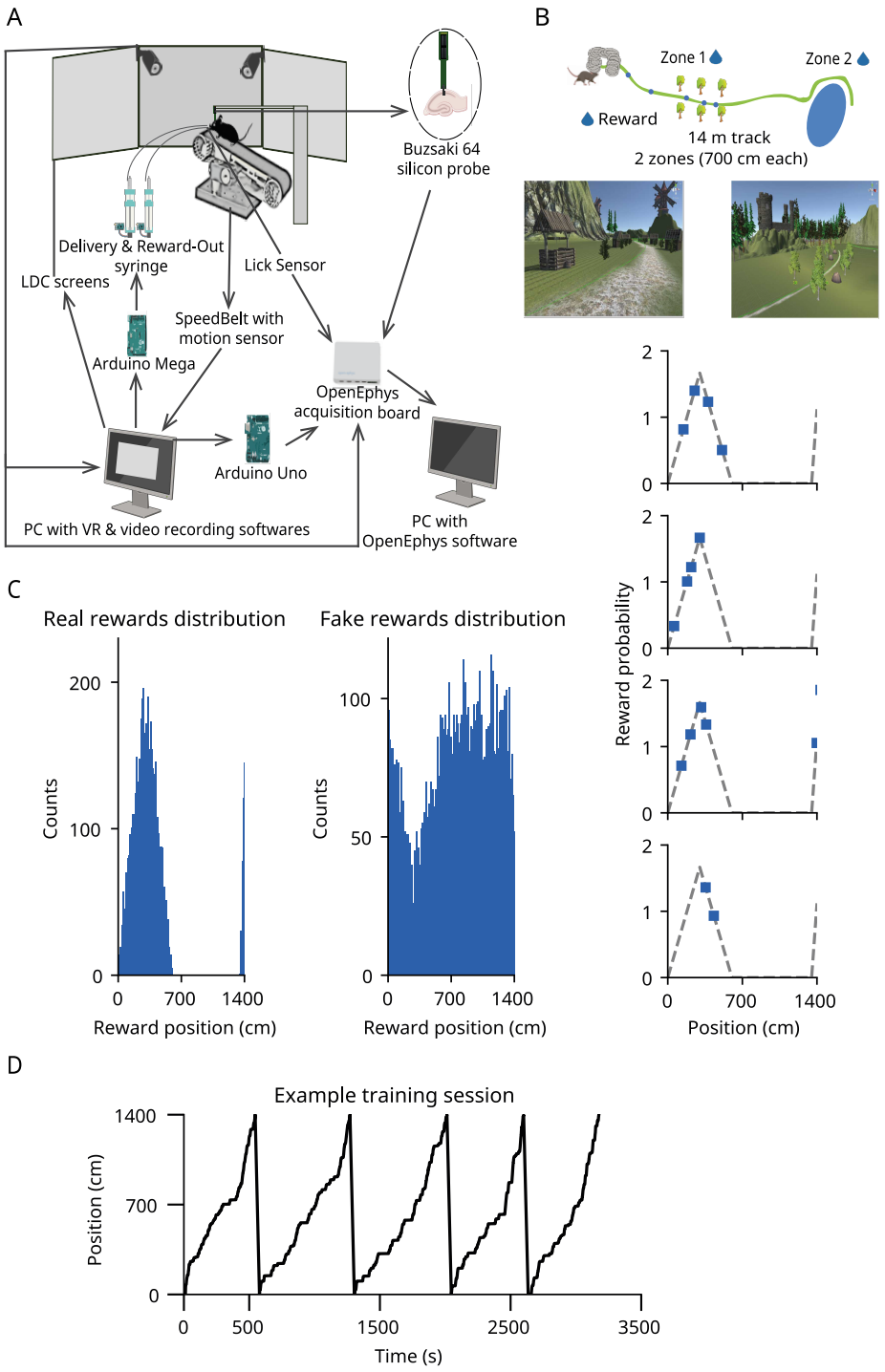


Figure 1: Animals are able to navigate in the semi-realistic large-scale virtual environment. A. Schema of the virtual reality set-up. B. Virtual Reality. General schema and a first-person view. C. Behavioral Task with Reward Probabilities. D. Example of Animal's Position and Speed as a Function of Time.

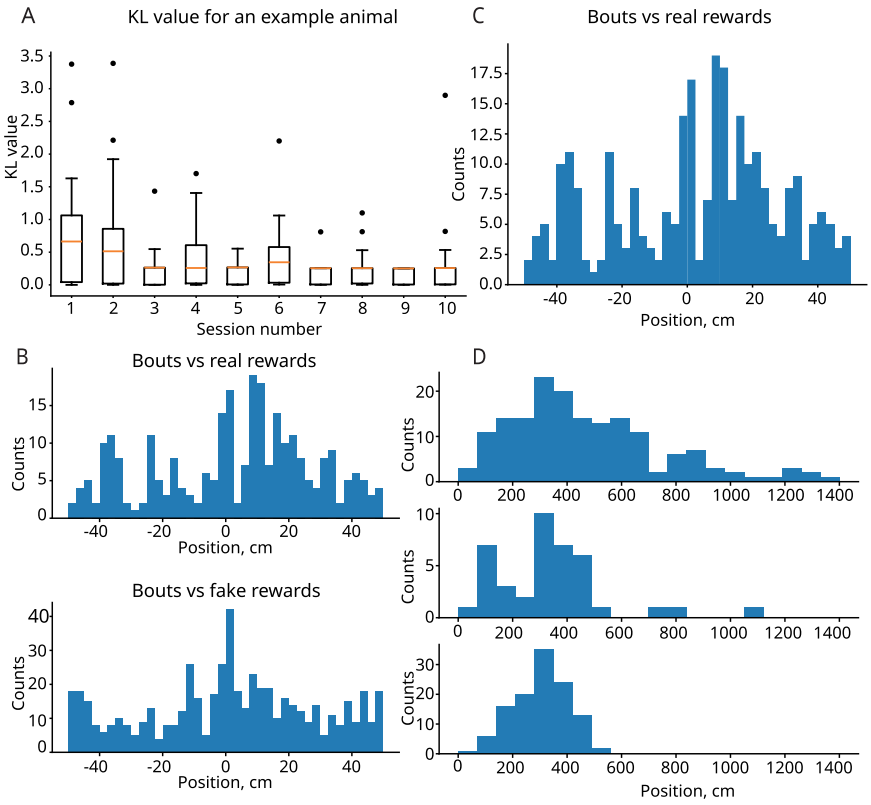


Figure 2: Animals are able to navigate in the semi-realistic large-scale virtual environment. A. Schema of the virtual reality set-up. B Virtual Reality. General schema and a first-person view. C. Behavioral Task with Reward Probabilities. D. Example of Animal's Position and Speed as a Function of Time. E. Example of Animal's Lick Distribution along a Track.

2.b. Mice react to visual cues and adapt their velocity

To quantify the amount of information available to the animal at any point along the track, we use a metric called Fisher information. We then compare the evolution of this metric with the animal's behavior. Our observations reveal a negative correlation between the animal's velocity and the Fisher information; specifically, when the environment provides more information, the animal's velocity decreases (Figure 3 A). Additionally, we analyzed the temporal dynamics of the Fisher information before and after the animal made a stop, further supporting this observation (Figure 3 B).

Given the amount of information available to the animal, we estimate the precision of the animal's localization. We also introduce the Cramér-Rao bound, which defines the theoretical limit on localization precision based on the available information. Moreover, we present an alternative approach to estimate localization precision using a position regression model (Figure 9). This feedforward model infers the animal's position on the track from the images it sees during navigation. We then compare the standard deviation of the position regression model to the theoretical localization bound (Figure 3 C).

Interestingly, we observe that, although the final part of the track is relatively poor in terms of available information, the theoretical bound remains low due to rapid envi-

ronmental changes in the curved track. However, the limited information in this section causes the model to struggle (Figure 3 D).

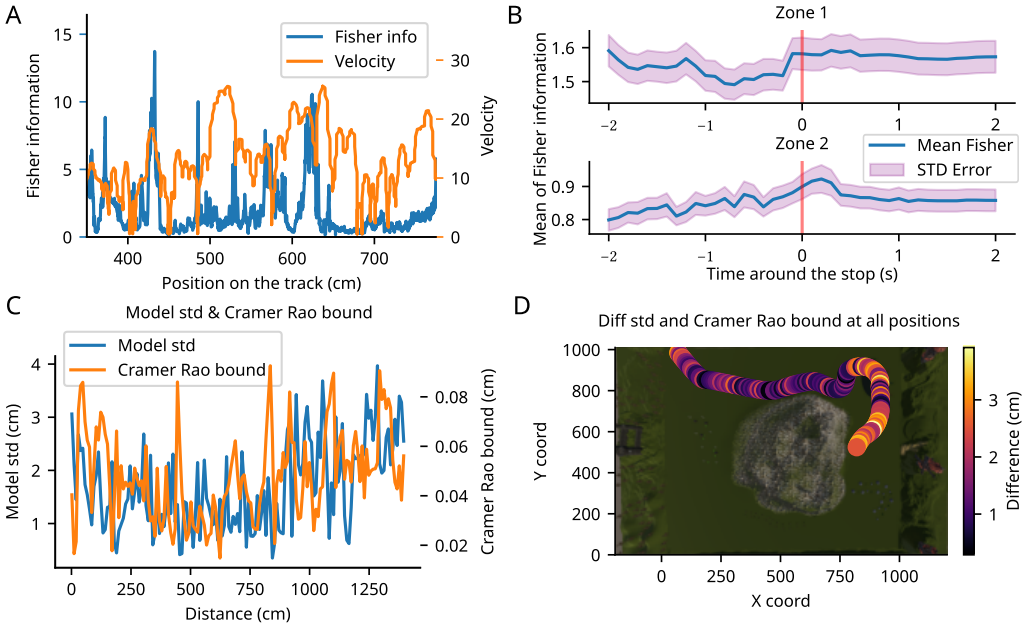


Figure 3: Visual lanscape and behavior interaction. A. Evolution of the Fisher information and velocity of the animal along the track. B. Temporal dynamic of Fisher information before and after the animal made a stop separated in the two defined zones depending on where the animal made the stop (n animals = 6, n sessions = 14). C. Position regression model standard deviation and theoretical localization bound. D. Difference between the theoretical localization bound and the standard deviation of the position regression model.

During the animal's navigation, both eyes were recorded using two cameras. To further analyze the eyes movements, we used a U-Net model (Figure 7) to segment the pupils from the eyes, allowing us to track the center of the eyes and measure the pupil size (Figure 4 A). Based on the results from Figure 3 A and B, we can test whether the animal behavior is in line with its attentional state by using its pupil size as a proxy. To do this, we examined the temporal dynamics of the pupil size around stops (Figure 4 B). We observed an increase in the pupil size before animals make a stop followed by a decrease when they start running again. Taken together with the result from Figure 3 B, animals identify informative areas and focus on them to gain information for localization. Building on this, we aimed to determine what specific cues the animal uses to gather information by examining the correlation between the position of the most informative cue in the environment and the position of the eyes' centers. We focused only on moments when the animal's eyes were making saccades (Figure 7 B), as the animal does not need to track the cue throughout the entire track. Considering the experimental setup, we expect these two positions to be negatively correlated. However we observed only a weak negative correlation, making it difficult to conclude whether the animal is specifically focusing on the most informative cue.

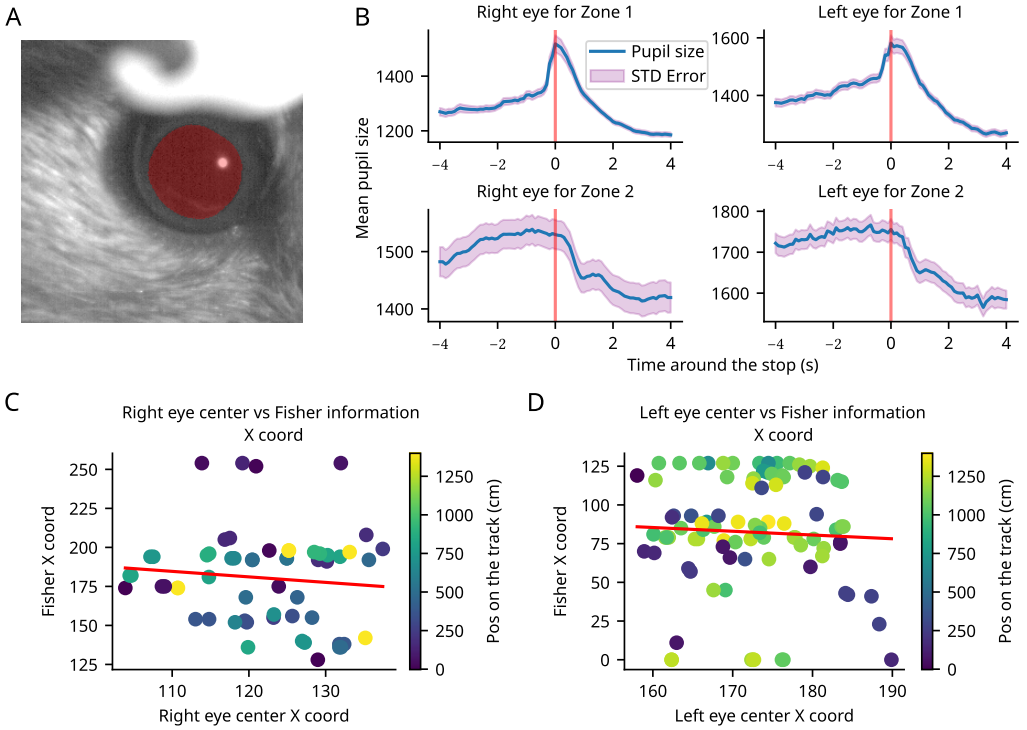


Figure 4: Animal's pupil size as attention proxy and visual landscape interaction. A. Example of a segmented pupil using a U-Net model. B. Temporal dynamic of the pupil size before and after the animal made a stop separated in the two defined zones depending on where the animal made the stop (n animals = 6, n sessions = 14). C. Position of the most informative cue against the pupil center for the right eye. D. Position of the most informative cue against the pupil center for the left eye.

2.c. The hippocampal cell population is not tuned to spatial location nor local travelled distance

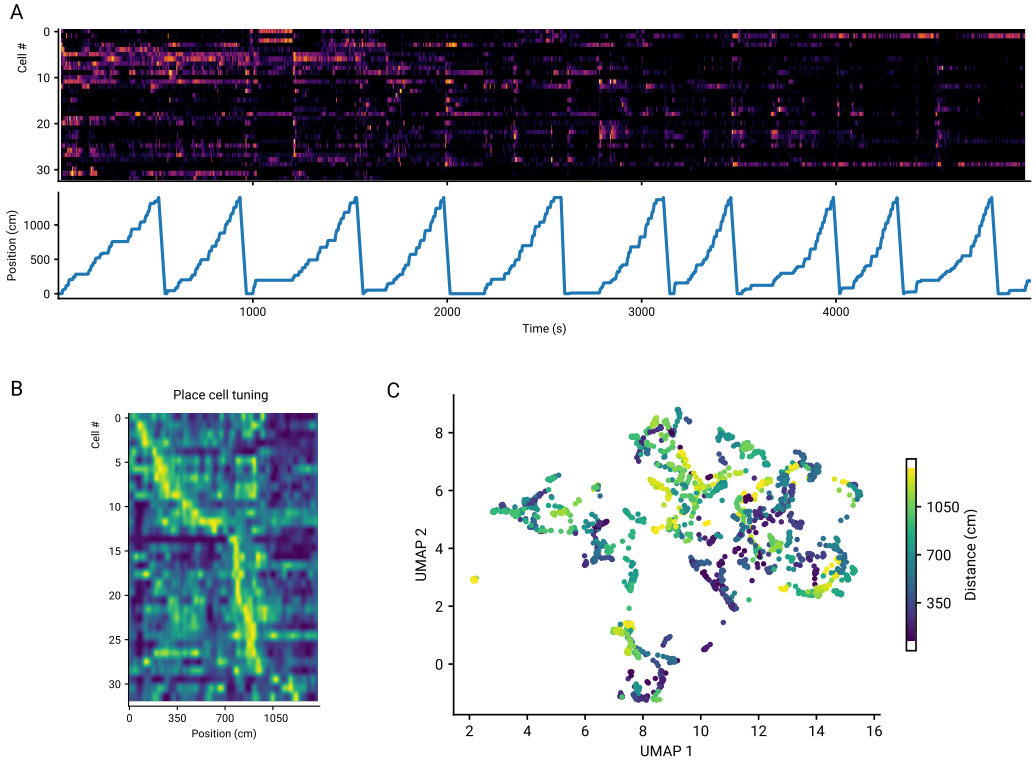


Figure 5: The neural activity is not tuned to the spatial location of the animal. A. Example of activity traces along the track. B. Tuning curves ranked by the position of the maximum. C. UMAP dimensionality reduction of the activity vectors.

2.d. The hippocampal cell population encodes the reward seeking behavior

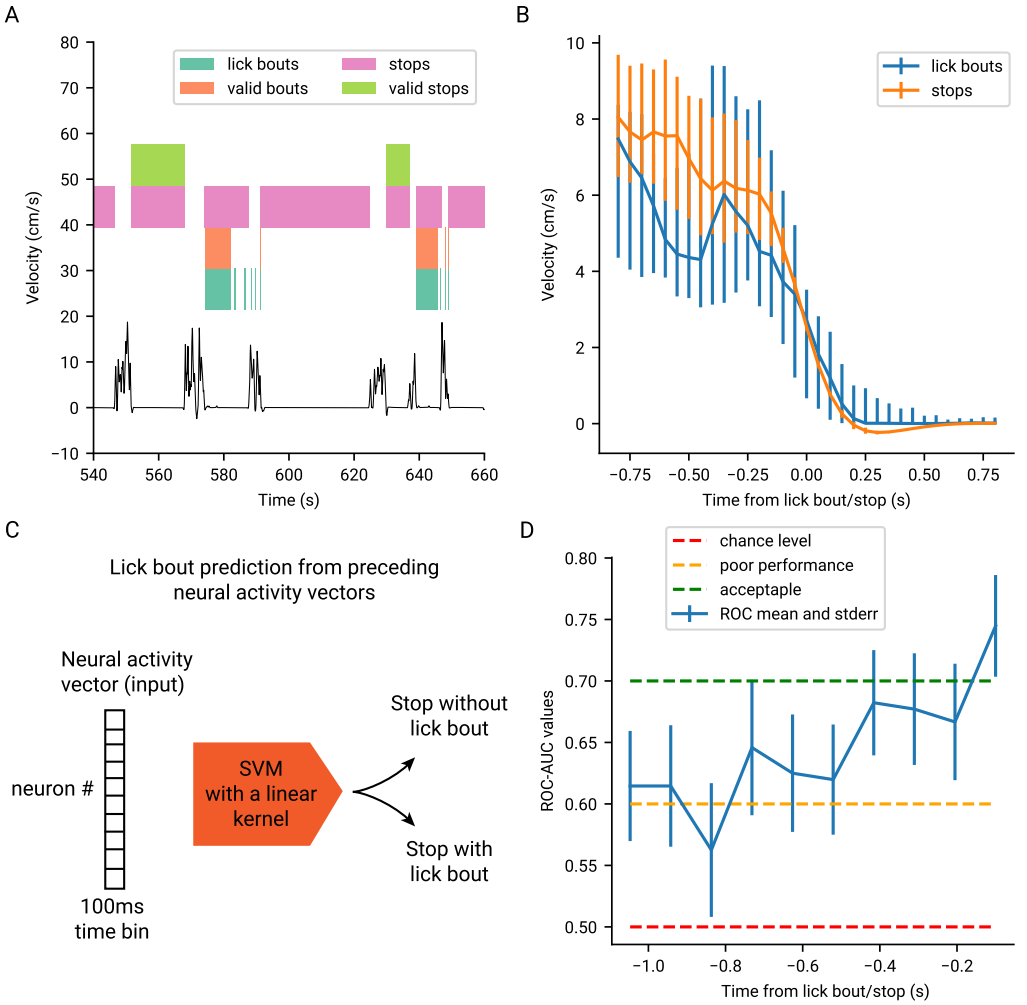


Figure 6: Preceding neural activity vectors predicts stops associated with licking behavior. A. Example of velocity and licking behavior time course. Only lick bouts associated with stops are considered valid. Annotated valid stops are stops that are **not** associated with licks. B. Velocity distribution around lick bouts and stops without lick. Notice that the distribution strongly overlap. C. Schematics of the machine learning framework used to predict lick bouts associated with stops. D. Prediction acuracy as a function of time preceding the stop.

3. Discussion

In this work, we investigated how dorsal hippocampal CA1 pyramidal cells encode space in an environment with stochastic reward locations and semi-naturalistic features. Specifically, we aimed to understand how place cells adapt to environments where rewards are unpredictable, similar to real-world situations where food locations may change over time. To explore this, we recorded extracellular activity from dorsal CA1 cells of mice navigating a large 1D virtual track, with rewards stochastically distributed in one zone and no rewards in the other. Compared to other environments used in navigational tasks, our setup—while benefiting from the stability of a head-fixation system—introduced passive 2D navigation by allowing turns on the VR track,

mimicking natural settings. The environment lacked clear borders like walls and included various cues (e.g., trees, a windmill, a lake) placed at different distances along the track. This design balanced ecological relevance with precise control over environmental features. Additionally, we recorded both eyes during navigation to study eye movement and pupil dynamics, assessing the animal's attentional state.

Mice successfully navigated the environment over multiple trials, demonstrating progressive learning of the space. Their behavior revealed an increase in reward-seeking in the reward zone. These findings align with previous studies showing that mice can effectively navigate virtual environments and perform spatial tasks like foraging in VR (Aghajan et al., 2015, Aronov and Tank, 2014, Chen et al., 2018). They also spent progressively less time stopped in Zone 2 while exhibiting increased stopping behavior in Zone 1, likely in search of the stochastically placed rewards. This confirmed that the mice could distinguish between the two zones. Furthermore, a progressively decreasing KL divergence across sessions demonstrated that the mice were learning the spatial distribution of rewards. We also quantified the amount of information available to the animal using Fisher information and compared this with their behavior. We observed a negative correlation between the animal's velocity and Fisher information: when the environment provided more information, the animal's velocity decreased, indicating that the mice were utilizing visual information from the VR. Moreover, we examined the temporal dynamics of both Fisher information and pupil size before and after the animal stopped, observing an increase in both as the animal came to a stop. This suggests that the mice stop to utilize visual information when it becomes relevant.

Overall, these behavioral findings indicate that the animals effectively learned the locations of the stochastically distributed rewards, navigated within the virtual reality environment, and utilized visual cues to successfully complete the task.

Compared to other studies conducted in large-scale environments, we did not observe multi-scale place cell coding of the environment (Fenton et al., 2008, Harland et al., 2021, Lee et al., 2020, Rich et al., 2014, Tanni et al., 2022, Toledo et al., 2020). Additionally, we did not find evidence of place cell coding or overrepresentation of reward locations (Dupret et al., 2010, Gauthier and Tank, 2018, Lee et al., 2020, Mamad et al., 2017, Sato et al., 2018, Zaremba et al., 2017), nor enhanced coding around the more visually informative parts of the environment, as compared to Fisher information, or around objects along the track, as seen in previous studies (Bourboulou et al., 2019, Deshmukh and Knierim, 2013). However, other studies have shown that the specificity of the behavioral task can lead to a lack of goal overrepresentation in hippocampal CA1 cells (Duvelle et al., 2019, Grieves et al., 2016, Trullier et al., 1999). Similarly, overrepresentation was absent in studies where the reward location was moved (Kobayashi et al., 2003, Speakman and O'keefe, 1990), which could explain the difference in our results, as the reward location was new for the animal in each trial, even though it remained within a relatively long part of the track (7 meters).

It is unclear whether learning plays a role in the overrepresentation of reward locations, as some studies did not observe it (Fyhn et al., 2002), while others reported reward overrepresentation after extensive training (Danielson et al., 2016, Lee et al., 2020, Mamad et al., 2017). In our study, animals were recorded after progressive learning, but we did not observe any specific locations with a higher probability of re-

ceiving a reward. However, our behavioral analysis of stops and licking behavior, along with neural activity predicting licking, suggests that neural activity reflects the animal's awareness of potential reward locations and correlates with its stopping behavior during reward-seeking.

As mentioned, we did not observe neural activity tuned to space or distance in the environment, which excludes the possibility that the animal was navigating using cognitive mapping or path integration. However, our results show that the animal could distinguish between the two zones and successfully learned the reward distribution. This raises the question of what prevented the formation of place cell encoding in this environment and what alternative navigational strategies the animal might have employed. In a study where rats navigated a 2D VR maze (Aghajan et al., 2015), there was a significant loss of spatial selectivity during random foraging in VR compared to real-world conditions, with only slight improvement when objects were introduced into the maze. However, when rewards were fixed and associated with these objects, the spatial selectivity of cells increased, approaching that of real-world conditions. Similarly, another study (Moore et al., 2021) involving rats performing a task similar to the Morris water maze in a 2D VR environment showed that, while rats were able to successfully complete the task, only 30% of the recorded cells exhibited allocentric spatial selectivity, and these cells demonstrated significantly lower stability compared to those in real-world navigation tasks. In our study, one possible explanation for the lack of spatial selectivity in hippocampal cells could be the stochastic presentation of rewards. Since the reward location changed with each trial, the animal may have struggled to associate the reward with a fixed spatial location or specific environmental cues, as seen in Aghajan's study (Aghajan et al., 2015). The added complexity of a curved path, combined with the large scale of the VR environment, the absence of other sensory modalities (olfactory, tactile cues), and stochastic rewards, might have further hindered the animal's ability to form a stable spatial map of the environment. Additionally, the randomness of the goals could have made it difficult for the animal to use path integration, which relies on idiothetic cues from its movements, as there was no consistent goal location. It would be interesting to investigate whether anchoring fixed and stochastic reward locations to visual cues could enhance the spatial selectivity of hippocampal cells.

Based on our observations, it appears that the mice developed a schematic representation of the environment with stochastically placed rewards through repeated exposure to the task. The decreasing KL divergence suggests that they were refining their spatial knowledge, gradually integrating the learned reward distribution into a cohesive schema. The changes in velocity and pupil dynamics in response to visual cues further indicate that the mice were utilizing this schema to prioritize attention on key environmental features likely to lead to rewards. This schema-driven behavior enabled them to differentiate between reward and non-reward zones and apply learned generalizations to optimize their search strategies across trials. It would be intriguing to test whether maintaining the same stochastic distribution in a similarly complex environment, as well as in a simpler one (like a 1D track with few or no objects), would replicate these findings. Such an investigation could provide valuable insights into whether animals employ schema-based navigation in these types of tasks.

VI. Conclusion

In this study, we examined how dorsal hippocampal CA1 pyramidal cells encode spatial information in an environment with stochastic reward locations. Our findings suggest that mice can develop a schematic representation of their surroundings through repeated task exposure, refining their spatial knowledge over time. Importantly, the mice distinguished between reward and non-reward zones, using visual features of the environment to optimize their search strategies, indicating they employed a schema-driven approach to navigation.

These findings challenge traditional views of spatial coding in rodents and propose that, in flexible and dynamic environments, rodents, similar to primates and humans, can also rely on abstract knowledge structures, such as schemas, for navigation. Further studies are needed to investigate this hypothesis and the potential neural mechanisms involved.

4. Acknowledgments

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5. Declaration of interests

The authors declare that no competing interests exist.

6. STAR Methods

6.a. Animals

All experiments were conducted in accordance with the guidelines and regulations set forth by the Institut National de la Santé et de la Recherche Médicale (INSERM) animal care and use committee and authorized by the Ministère de l’Enseignement supérieur et de la Recherche, following evaluation by a local ethical committee (agreement number 02048.02), in compliance with the European Community Council directives (2010/63/UE). For the experiments, male mice born from a crossbreeding of CD1 female (8 weeks old, Janvier/Charles River) and C57BL/6J male (8 weeks old, Janvier/Charles River) in the laboratory animal facility were utilized. The pups were weaned at P21 and initially housed in groups of 2 or 3 per cage, transitioning to individual housing with a 12-hour inverted light/dark cycle post the first surgery. Training and recording sessions were conducted during the dark phase. Data were collected from 7 male mice aged between 8 and 12 weeks during the recording phase. Before surgeries, mice had *ad libitum* access to water and food. Following recovery from the head-fixation bar implantation surgery, food continued to be provided *ad libitum*, while water intake was restricted to 2 mL. Their weight and health were monitored daily, and

water was provided *ad libitum* if their weight dropped below 80% of the pre-surgery weight.

6.b. Surgery for Implantation Head-Fixation Bar

For further training and recording of the head-fixed animals in the VR environment, surgery was conducted to implant a fixation bar. The animals were anesthetized using isoflurane (3%) along with a subcutaneous injection of buprenorphine (0.06 mg kg^{-1}). Continuous monitoring ensured the absence of reflexes and maintained a stable breathing rate, while their body temperature was regulated at around 37 ℃ using an electrical heating pad. For eye protection, a cardboard mask and eye gel were applied. The surgical area was locally anesthetized with Lurocaine (100 µL) and sterilized with a Vetadine solution. The skull was cleansed with an H_2O_2 solution. Two jeweler's screws with wired pins were carefully inserted above the cerebellum into the skull to serve as reference and ground. These pins were affixed to the front side of the skull. Additionally, two screws were placed in the frontal part of the skull to further secure the cement fixation. A dental cement Super-Bond Universal was used to cover the screws, pins, and the exposed skull, creating a cement 'hat' while leaving the area above the hippocampi untouched for future craniotomy surgery. Subsequently, a small titanium bar (0.65 g; 12*x*6 mm) was affixed onto the hat above the cerebellum, serving as a fixation point for a larger head plate used for head fixation during training sessions and recordings. Following this, acrylic dental cement Paladur was applied over the previously created cement hat. The exposed skull was covered with silicon elastomer (Kwik-Cast, World Precision Instruments). Postoperative care involved subcutaneous injections of buprenorphine (0.06 mg kg^{-1}) administered every 8 hours for 2-3 days, with close monitoring of the animal's weight and health twice daily.

6.c. Virtual Reality Setup

A custom-made setup was developed for the experiments. A commercially available rodent running belt (PhenoSys SpeedBelt), enabling 1D motion, was utilized for animal navigation within the VR environment. An optical motion sensor within the belt was connected to the computer to transmit motion information. The belt was positioned in front of three LCD screens, Dell P2219H, offering a general field of view of 270 degrees, corresponding to the mouse visual system. The monitors were elevated so that the mice's eye level aligned with the lower third of the screen height, considering the upward bias in rodents' field of view. In front of the animals, below their line of sight, a custom-made reward system was set up. This system comprised two straight feeding needles (Drencher, 1.6 x 102 mm) connected to individual stepper motors, one for each needle. The reward delivery syringe was linked to a 3D-printed syringe driver with a NEMA17 stepper motor, while the syringe for dispensing the reward was connected to another 3D-printed syringe driver featuring a 28BYJ-48 stepper motor. The third syringe driver, identical to the reward one, was not connected to any syringes and served to simulate the sound of the reward delivery system. Arduino Mega controller regulated the syringe drivers, receiving commands from the Unity Virtual Environment. The Arduino code and schematics for the 3D-printed syringe drivers can be accessed on the Git repository (https://github.com/centuri-engineering/syringe_drivers). A silver wire was soldered to the reward tube, which was connected to the lick sensor system, designed as described in the work by ((Slotnick, 2009)). The lick sensor was linked to the I/O board of the OpenEphys Acquisition system. The head fixation system (Luigs

and Neumann) was positioned behind the animal to avoid interference with the display of the virtual reality environment. Two Basler ace acA2000-340km cameras with additional 75mm lenses were mounted on top of the LCD screens. Between them, the LED IR Illuminator from Thorlabs was installed. The cameras were connected to the PC and the I/O OpenEphys board for frame acquisition. The recording process was controlled using custom-developed software.

6.d. Virtual Reality

The Virtual Reality Environment was developed using Unity 3D version 2021.2.0f1. The VR terrain spanned an area of 100 square meters, enclosed by four mountains featuring windmills, a castle, a forest, and a field, each corresponding to one of the four sides. A large rock hill was placed at the center of the terrain to polarize the environment. The entire 1D curved track measured 35 meters and was divided into five zones. Animals traversed these zones, receiving rewards and being teleported back to the beginning of the track at the end of each trial. Each zone had its own probability level for an animal to receive either a reward or no reward. Within the reward zones, the location of the reward was always randomized, following the same distribution within each zone. Additionally, both reward and non-reward zones featured simulated motor sounds, with an inverted probability, aimed at isolating the influence of sound on animal perception of the reward. If an animal attempted to take a reward beyond 20 cm from the delivery point, a reward retrieval system would remove the reward. Each zone was designed with sections containing varying levels of local visual cues, achieved using naturalistic objects from the freely available Unity assets. The environmental colors were chosen from a naturalistic palette, primarily using green and blue shades, which are within the visible spectrum for mice.

6.e. Training

After 3 days of the animal's recovery from the head-fixation bar surgery, it was gradually habituated to the experimental room and to an experimenter. During this period, the animal underwent 20-minute handling sessions in the experimental room, which continued throughout the entirety of the experiments. Once the animal regained its pre-surgical weight, it was restricted to a water intake of 2ml per day, with daily weight monitoring. Access to water was restored if the animal's weight dropped below 80% of its pre-surgery weight at any point during training. Following another 3-day period, the animal underwent 1-2 days of habituation to head-fixation. Subsequently, it engaged in running on the Speed belt within a dark room with black screens, receiving a reward (5% sucrose) upon consecutive running on the belt. For the current experiments, only the first two zones, which cover the initial 14 meters of the track, were utilized for training and recording purposes. Zone 1 was designated as the reward zone, while Zone 2 had no rewards. Once the animal could consistently run on the belt and access the reward, it underwent training in the virtual reality environment within Zone 1 (the first 7 meters). A 2-minute break in the dark room followed each trial. After the animal successfully obtained rewards and completed a minimum of 6 trials within, the remaining 7 meters of Zone 2 without rewards were introduced. Upon achieving a minimum of 6 trials within 1 hour on the 14-meter track, the animal progressed to the recording phase. Behavior and eye movements were recorded starting from the first day of training in virtual reality.

6.f. Recording procedure

After the animals successfully completed the training session, they proceeded to the recording phase. Acute recordings were conducted using a silicone probe (8 shanks; A-64 Buzsáki Probe, Neuronexus) on both sides of the hippocampi. In the first recording session, a craniotomy was performed on one side of the hippocampus. Animals were anesthetized with isoflurane (3%) and received a subcutaneous injection of buprenorphine (0.06 mg/kg). The craniotomy was centered at coordinates –2 mm posterior and ±2.1 mm lateral from bregma. The drilled area was covered with agarose (2% in physiological saline) and sealed with elastomer (Kwik-Cast, World Precision Instruments). Recordings from the first craniotomy were conducted over 1-3 consecutive days, with probe adjustments made each new recording day. To accurately locate the probe sites for post-hoc histological analysis after the recordings, the back side of the probe shanks was coated with a thin layer of red-fluorescent dye (DiI, Life Technologies). The procedure was then repeated for the second craniotomy. During the recording session, the probe was gradually lowered into the animal's brain while the animal was walking on the track in the dark room. Recording commenced when sharp-wave ripples and multiple units displaying complex spike bursts were identified, confirming the area as the CA1 pyramidal cell layer. Together with video recordings of the environment were initiated. A 2-minute pause in the dark room followed each trial. Upon the animal completing more than 3 trials under the same conditions as during training, a probe trial was initiated. In this trial, the animal ran the same task with simulated sound from a fake reward syringe drive, but no actual reward was delivered. After the probe trial, the animal continued running under regular task conditions.

6.g. Data acquisition and pre-processing

The movement of the belt was detected by an optical sensor integrated into the speed belt. The position increments of the belt were transmitted to Unity, where it was translated into coordinates within the virtual environment. To synchronize the virtual environment (VR) with electrophysiological recordings, we established a real-time communication channel between the VR system and the OpenEphys recording box. This ensured that VR position data was time-locked with neural activity measurements. The positions were transmitted through a custom serial communication protocol that facilitates the transmission of VR position data, represented as 16-bit integers, onto a dedicated analog signal of the OpenEphys recording box. Data transmission begins with a start bit signaled by a voltage transition from below a defined threshold to above it. Conversely, a stop bit signifies the end of transmission with a voltage transition from above the threshold back down to below it. Between the start and stop bits, the protocol transmits the 16-bit integer representing the VR position. This information is encoded using a series of five distinct voltage levels within a defined range. The first four voltage levels encode the 16-bit integer using a 4-bit x 4-bit format. The final voltage level serves as a checksum value (CRC-4) to verify the accuracy of the decoded integer upon reception. Theoretically, the custom serial communication protocol offers high position resolution. With 16-bit integers representing the position data, the protocol can potentially distinguish between values on a very fine scale (65536 possible values for a 14-meter track). However, in practice, the overall accuracy of the system is ultimately limited by the resolution of the optical sensor integrated into the speed belt. In our experiments, the sensor's limitations resulted in an actual position resolution of approximately 2 centimeters.

Simultaneously, the lick signal was sent as a 5V step to the same I/O board. Camera frames were acquired at a rate and also transmitted to the I/O board. Neurophysiological signals were continuously recorded using a 256-channel recording system (Open Ephys, Intan Technologies, RHD2132 amplifier board with RHD2000 USB interface board) at a sampling rate of 25,000 Hz. The video data was captured and saved in the Zarr format. Both neurophysiological and behavioral data were saved in the .nwb format. After the electrophysiological recordings, the data was converted to the dat file format. Spike sorting was automated using SpyKING CIRCUS (Spampinato et al., 2018), followed by manual cluster verification, which involved cluster quality assessment, auto- and cross-correlograms, cluster waveforms, and similarity matrix analysis.

6.h. Lick spatial distribution and reward density comparison

The Kullback-Leibler divergence between two Poisson distributions of rates resp. λ_1 and λ_2 has the following expression:

$$D_{\mathrm{KL}}(f_1 \parallel f_2) = \lambda_1 \log\left(\frac{\lambda_1}{\lambda_2}\right) + \lambda_2 - \lambda_1 \tag{1}$$

If we split the track in K bins, one would estimate the Poisson rate of licks for each bin with the formulas given in the first document. The associated theoretical λ_k of each bin would be:

$$\lambda_k = \frac{N}{K} f_k \tag{2}$$

where f_k is the reward distribution for each bin and N is the total number of observed licks.

6.i. Pupil segmentation

To track the animal's eye movements within the VR environment, we employed a deep learning model for pupil segmentation. This model automatically segmented the pupils in each video frame, allowing us to analyze gaze direction and potential pupil dilation during navigation.

6.i.i. *Model Architecture and Training:*

The pupil segmentation model utilized a U-Net architecture, a standard choice for image segmentation tasks (Ronneberger et al., 2015). We implemented the U-Net architecture using the PyTorch deep learning framework (Paszke et al., 2017).

For training the model, we first established a diverse dataset of eye images. We employed a two-step approach: initial pupil segmentation using a watershed algorithm followed by manual correction to ensure accuracy. To enhance the model's generalization capabilities and robustness to variations encountered during data collection, we incorporated data augmentation techniques during training (details provided in Figure 7). These techniques included variations in lighting conditions, simulated head movements (through image rotation). With this approach, approximately a total 5,000 training images from both eyes were sufficient to achieve robust pupil segmentation across a wide range of scenarios (performance metrics for training and evaluation can be seen in Figure 8).

6.j. Saccade detection

Following pupil center extraction from the segmentation masks, we employed a velocity-based approach for saccade detection. This method analyzes the instantaneous changes in pupil position between consecutive video frames. Saccades, characterized by rapid eye movements, were identified by exceeding a pre-defined position change threshold. The threshold was set to 3 times the standard deviation of the position change between consecutive frame throughout the entire recording session.

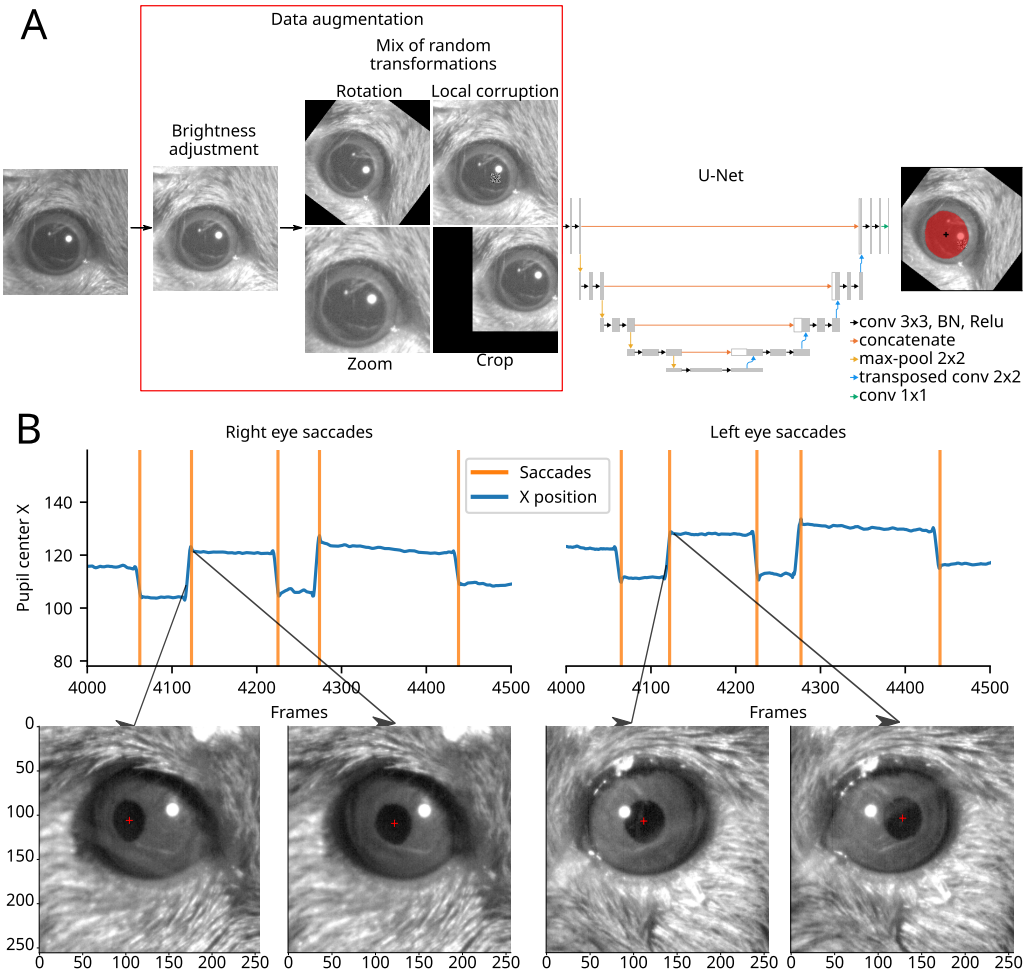


Figure 7: Pupil segmentation using a U-Net model and saccade detection.

We assessed the performance of the pupil segmentation model with commonly metrics for segmentation tasks in Figure 8. Our results show that the model is able to accurately segment the pupils of both eyes while converging quickly.

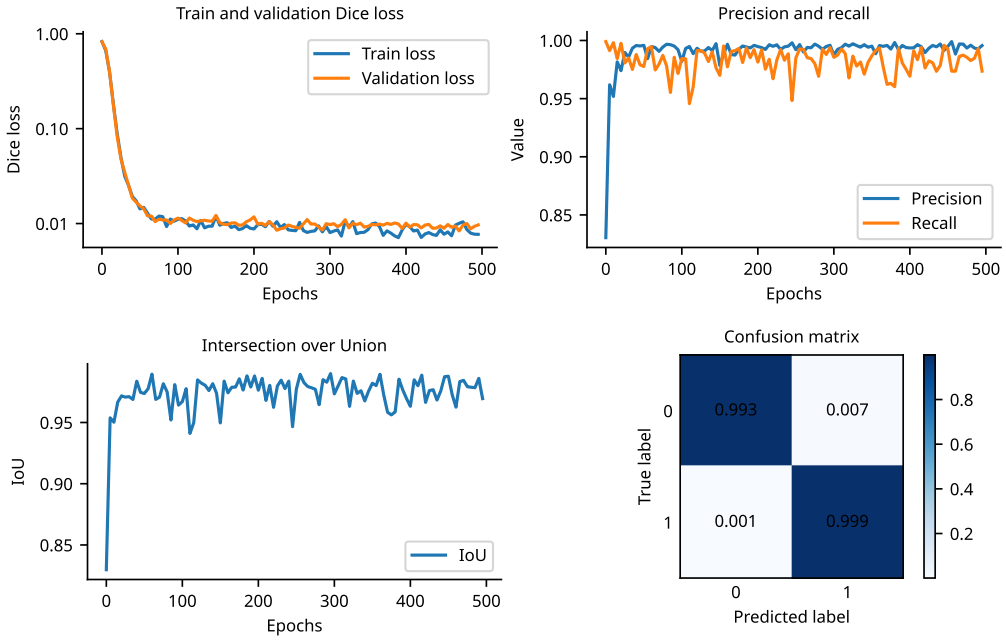


Figure 8: Pupil segmentation model performance metrics.

6.k. Position inference model from images seen by the animal

We propose a feedforward neural network to infer the position on the track from the images of the environment presented to the animal. In order to train the network, we first extract the images displayed on the 3 cameras from the Unity virtual environment. We then do a pre processing on the acquired image by adding both gaussian blur and noise to be closer to the animal visual capabilities (Geng et al., 2011) and account for motion blur.

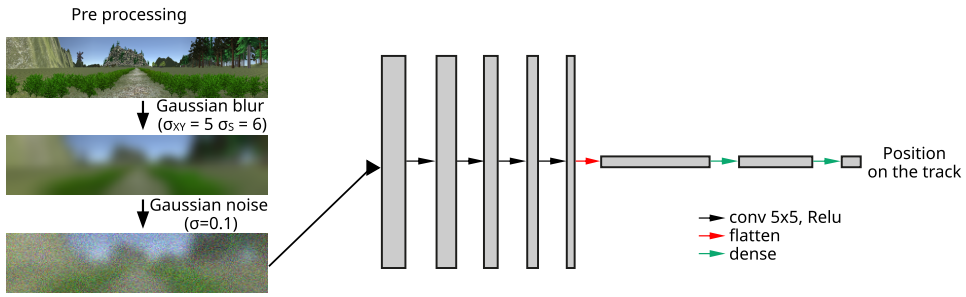


Figure 9: Position inference model from images seen by the animal.

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Part III

Discussion

Discussion

In this thesis, we aimed to investigate how dorsal hippocampal CA1 pyramidal cells encode space in an environment with stochastic reward locations and semi-naturalistic features. Specifically, we sought to understand how place cells adapt to environments where reward presence is variable, similar to real-world situations where food locations may change over time. To explore this, we recorded extracellular activity from dorsal CA1 cells of mice navigating a large (14 m) 1D virtual track with rewards stochastically distributed in one zone and no rewards in the other.

Compared to other environments used in navigational tasks, our setup, while benefiting from the stability of a head-fixation system, introduced passive 2D navigation using turns on the VR track, mimicking natural settings. The environment lacked clear borders like walls and included various cues (e.g., trees, a windmill, a lake) placed at different distances along the track. This design balanced ecological relevance with precise control over environmental features. Additionally, we recorded both eyes during navigation to study eye movement and pupil dynamics, assessing the animal's attentional state.

Mice successfully navigated the environment over multiple trials, showing progressive learning of the space. Their behavior revealed an increase in reward-seeking (licking) in the reward zone and fewer or no licks in the non-reward zone. They also progressively stopped less in the second zone, while exhibiting increased stopping behavior in the first one, likely searching for the stochastically placed rewards. This confirmed that the mice could distinguish between the two zones. Furthermore, a quantification of the Kullback-Leibler (KL) divergence between the real distribution of rewards and the observed lick distribution showed that KL progressively decreased across sessions demonstrating that the mice were learning the spatial distribution of rewards. These findings align with previous studies showing that mice can effectively navigate virtual environments and perform spatial tasks like foraging in VR [177][585][360][175][314][577][273].

We also quantified the amount of visual information available to the animal about its location using Fisher information and compared this with their behavior. We observed a negative correlation between the animal's velocity and Fisher information: when the environment provided more information, the animal's velocity decreased, indicating that the mice were using visual information from the VR. It is also known that velocity affects pupil dilation, with dilation increasing as velocity increases [502], our observation of pupil dilation around stops suggests that this is not related to the animal's speed since the correlation was inverse before rewards, but rather to internal state changes, likely attention to rewards. We also compared the position of the most informative cue with the eye centers during saccades to test whether the animals were tracking cues while navigating, but we didn't find strong evidence for the tracking of the most salient visual features. Together, these behavioral results demonstrate

that the animals learned the stochastically distributed reward locations, navigated in virtual reality, and used visual information to successfully perform the task.

In our study, we expected several outcomes. First, similar to previous research on rodent navigation in large-scale environments [151][152][305][306][308][153], we anticipated numerous place fields showing multi-scale coding of space, with both small and large place fields, and most cells exhibiting multiple place fields. Previous studies have also shown that in environments with homogeneously distributed landmarks [306][308][153][305] or their absence [151][152], place cells exhibit a homogeneous distribution of place fields, following a gamma-Poisson distribution. This distribution suggests that each place cell has a varying propensity to form place fields, influenced by intrinsic cell properties and environmental contexts like reward presence or salient visual cues which could induce place field formation [302][152][305].

In our task, we expected place fields to be inhomogeneously distributed, with more numerous, narrower, and stable fields in the more informative zone, where visual cues and rewards were more frequent. This expectation aligns with previous findings that show place fields tend to accumulate around reward zones [305] and proximal visual cues [273] in virtual reality mazes. We hypothesized that this spatial coding strategy would enable the animal to encode the reward zone with higher resolution, using narrower and more numerous place fields, while encoding the non-reward zone with lower resolution, using wider and fewer fields. This kind of inhomogeneous distribution would allow the animal to efficiently encode the large-scale environment.

However, in our study, we observed no evidence of place cell coding in the environment. Neural activity was neither tuned to the animal's spatial location nor to the distance traveled—both key signals expected during spatial navigation. Despite behavioral evidence that the animals could distinguish between the two zones and navigate the environment, an important question arises: what prevented the animal from using place cell coding to form a cognitive map of the environment? Which alternative navigational strategies could the animal have used, and what neural circuits might be involved in these processes?

A study by Aghajan et al., 2015 [176] provides some clues. In their study, rats were performing a random foraging task in the real world and in a virtual reality environment. They showed that in the virtual reality maze, the cells underwent a significant loss in spatial selectivity. When the objects (floating pillars) were introduced to the maze, the spatial selectivity was only slightly improved. In a third condition, where floating pillars were fixed to reward location the spatial selectivity of cells was close to real world conditions. This suggests that the repetition of locomotion cues—such as step counting and optic flow—at consistent positions allowed these cues to become spatially informative. Over time, these locomotion cues were paired with distinct visual cues at specific locations, reinforcing spatial selectivity. In our study, one possible explanation for the lack of spatial selectivity in hippocampal cells could be the stochastic presentation of rewards. Since the reward location changed with each trial, the animal may have struggled to bind the reward to a fixed spatial location or environmental cues, as was the case in Aghajan's study. Additionally, the scale of the environment in our study was considerably larger (14 m track in our case to a 220 x 10 cm linear track in a 3x3x3 meter room in the case of another study), where the complexity of an environment with multiple cues could introduce further ambiguity and affect the possibility of an animal to bind a location/cue to the reward location. In a study by Lee et al., 2020 [305] where mice were also navigating in a large-scale environment the mouse navigating in a 1D straight track with homogeneously distributed

rewards and visual cues along the track or fixed reward location which probably also allowed cells to be anchored to space and become spatially selective with showing an overrepresentation of reward locations where they were fixed in the environment (in comparison with a curved track which allowed passive 2D navigation in our study).

In a study by Moore et al., 2021 [598] which modified the 2D virtual reality setup similar to that used in Aghajan's study, rats were trained to perform a virtual navigation task similar to the Morris water maze, while recording the activity of dorsal CA1 cells. This setup allowed them to remove non-specific cues and human intervention, ensuring navigation relied solely on distal visual cues. The rats were able to successfully perform the task. However, consistent with Aghajan et al., 2015 study [176], only 30% of cells exhibited allocentric spatial selectivity, and these cells had significantly lower stability compared to rats navigating in a real-world environment.

While analyzing the influence of distance traveled, they found that almost 50% of neurons were significantly modulated by path distance—specifically, the distance traveled from the start of a trial, regardless of the allocentric start position. Few cells showed clear tuning to path distance measured from the goal, and this distance tuning could not be explained solely by motor signals from turning. Similar to findings from two-dimensional random foraging tasks in both real-world and virtual reality environments, approximately 40% of neurons were significantly modulated by the angle relative to distal visual cues. Notably, the study showed that the proportion of neurons modulated by two or more variables was roughly equal to chance, suggesting that individual CA1 neurons can multiplex, simultaneously encoding angular, allocentric, and path-centric information, rather than functioning as distinct, segregated populations.

Comparing this with our study, the larger space and stochastic reward locations likely prevented the animals from efficiently using proximal and distal cues, unlike the real-world environments where multiple sensory modalities are available to aid allocentric input. Additionally, in classical 1D linear VR tracks, as seen in previous studies, animals typically run towards a fixed goal at the end of the track or within uniformly distributed reward zones, facilitating distance coding. However, in our study, the animals needed to make variable stops along the 7 m reward zone to search for randomly appearing rewards. This could disrupt the efficient calculation of distance from the start of the track to the reward location, impairing distance coding. Moreover, the absence of a salient cue at the end of the track likely affected the encoding of the track's start-to-end distance.

It would be interesting to test whether distance and allocentric-like coding would emerge in the same environment if fixed reward locations were marked by salient cues, along with a clear indicator of the track's end.

As mentioned earlier, the study by Moore [598] demonstrated that dorsal hippocampal pyramidal cells can simultaneously store and represent allocentric space, angle, and path distance. This suggests that, depending on the task and environmental rules, animals can switch between strategies to choose the most efficient navigation method for successfully completing a task. It was also shown that some animals, like primates, can navigate environments without clear place cell activity (Rolls, 1998). Another possible strategy, proposed for primates in tasks similar to random foraging, involves navigation using schemas [599], where the hippocampus utilizes a schema that incorporates both spatial and non-spatial components to effectively perform the task.

A schema is an abstract knowledge structure that integrates common patterns

from multiple experiences into a flexible reference framework (see more details in [30]. Firstly introduced by Henry Head in 1926 [600], describes how knowledge is organized and consolidated over time. Neurobiological studies suggest that pre-existing knowledge accelerates systems consolidation by stabilizing memories originally formed in the hippocampus into neocortical regions such as the mPFC and vmPFC [601]. This process generalizes memories, transforming them from specific instances to broader, gist-based representations. Sleep plays a crucial role in this by facilitating hippocampal-neocortical interactions during memory replay [602], aiding in schema learning and enhancing both specific and generalized spatial memories. However, the precise neural mechanisms behind spatial schema integration remain to be fully understood.

Unlike cognitive maps, which represent detailed spatial relations within a specific environment, schemas capture general patterns or spatial relations that can be applied across different environments. In the context of spatial navigation, schemas summarize recurring spatial elements, like landmarks or paths, allowing animals and humans to navigate new environments by recognizing familiar spatial structures. This process involves the hippocampus and neocortical regions, which interact to form schemas by extracting and integrating these patterns from past experiences (see details in [30].

In a study using primates [599] researchers aimed to determine if hippocampal cells in monkeys could represent spatial abstractions rather than just specific environmental features. They hypothesized that these abstractions involved schemas that extracted commonalities across experiences, allowing for flexible behavior in varying contexts. Monkeys were trained to navigate a virtual maze with a joystick, searching for an invisible reward that had to be triangulated based on visible landmarks. Once proficient, they were tested in a novel maze with different landmarks and adapted quickly, indicating the formation of a task schema rather than simple stimulus-response associations. To determine whether the observed process stemmed from environment-specific memories or a single schema applicable to both spaces, the researchers investigated neural activity. They found that while some cells discriminated between differences in the environments, similar to the "remapping" seen in rodent place cells, a significant portion of cells was active in both familiar and novel environments. Further investigation revealed that a subpopulation of neurons exhibited similar firing patterns when their maps were realigned with respect to reward position. These neurons, termed "schema cells," displayed a complex pattern of activity in position and state spaces across both environments, indicating that the reward was not signaled by specific cues but had to be triangulated from landmarks, aligning with the general rule. Additionally, their activity was not anchored to specific stimuli, further supporting these observations.

Similar to early research on cognitive maps, the neurobiological mechanisms underlying schemas have primarily been examined in rodents using spatial learning paradigm [603][604][537]. In one of the studies [603], rats were trained on a paired-associate memory task, where they learned the spatial arrangement of various flavor-place associations in a test environment. New paired associates consistent with previously learned associations could be acquired in just one trial. Interestingly, hippocampal lesions performed more than 24 hours after paired-associate learning did not impair the recall of either original or novel paired associates. However, HPC lesions administered before the representation had time to undergo rapid consolidation (within 24 hours) disrupted initial acquisition. In a subsequent study, an increased expression of immediate early genes in several neocortical regions, including the medial prefrontal cortex and retrosplenial cortex, was linked to a quicker acquisition of new paired associates in non-lesioned rats previously exposed to the original paired

associates [604]. More recently, the 'HexMaze,' a complex, large-scale maze used with mice, demonstrated that the passage of time, rather than training frequency, drives the accumulation of spatial knowledge into long-term representations [605]. The learning gains observed in these studies were attributed to the formation of a spatial schema [603][604]. An earlier experiment utilized a 'village maze,' a two-story, enriched environment containing multiple rewards that may facilitate schema formation [606]. After several weeks in the village maze, rats' abilities to flexibly navigate to different rewarded locations were assessed. Rats with HPC lesions but substantial experience in the maze learned those associations as quickly as the controls, while inexperienced HPC-lesioned rats took significantly longer. This rapid updating observed in experienced HPC-lesioned rats aligns with the idea that novel paths to goals were integrated into a schema of the environment rather than being newly learned. One hypothesis is that this quick adaptation of new paths is guided by schema information-processing circuits in the dorsal striatum, which updates a stored transition structure linked to specific goals [607]. Further investigation is needed to clarify the roles of other extra-HPC structures in schema formation and utilization.

Following our observations, it is likely that mice developed a schematic representation of the environment with stochastically placed rewards through repeated exposure to the task. The decreasing KL divergence suggests that the mice were refining their spatial knowledge, integrating the learned reward distribution into a schema over time. Changes in velocity and pupil dynamics, in response to visual cues, further indicate that the mice were using a schema to prioritize and focus attention on key environmental features most likely to lead to rewards. This schema-driven behavior enabled the mice to distinguish between the reward and non-reward zones and apply learned generalizations to optimize their search strategies across trials.

The reliance on a schema-based strategy, rather than on a cognitive map, can be attributed to the nature of the task. In each trial, the environment changed unpredictably, requiring the mice to learn general statistical rules rather than detailed allocentric relationships. While some spatial features were relevant, the stochastic placement of rewards did not necessitate the formation of a cognitive map, which typically encodes fixed spatial relations. Instead, schemas allowed the mice to generalize from past experiences in the reward zone and associate certain visual landmarks with potential rewards, supporting flexible learning across environments with shared characteristics, even when specific details varied.

In contrast, studies involving bats [306] navigating large-scale naturalistic environments, such as foraging for fruit trees, have shown that bats likely use cognitive maps to navigate. In these cases, the goal (e.g., a tree) remains static, and the bats must learn the fixed locations of multiple targets. The absence of schematic behavior in bats might be due to the need for precise spatial representations in environments with stable, fixed goals, whereas the mice in our study dealt with a dynamically changing environment.

Further investigation could test this hypothesis by introducing fixed rewards associated with visual cues in the same environment, which might induce the formation of place cells. This would allow for the study of how place field accumulation around reward zones and visual objects occurs in large-scale environments. Additionally, it would be valuable to replicate several 1D virtual tracks with simpler geometric objects and shapes while maintaining the same stochastic representation of rewards to see whether we observe successful behavioral outcomes alongside a schematic representation of the environment taking away an ambiguity of visual cues inside track

used in a present study. Hippocampal lesioning studies or studies that inactivate hippocampal formation activity could further confirm if animals can rely on schemas in such navigational tasks.

Conclusion

In summary, this study provides insights into the mechanisms underlying spatial encoding in dorsal hippocampal CA1 pyramidal cells within an environment characterized by stochastic reward locations. Our findings suggest that while mice demonstrated the ability to navigate effectively and learned to distinguish between reward and non-reward zones, they did not rely on traditional place cell coding to form a cognitive map of the environment. Instead, the observed behavior aligns more closely with the development of a schematic representation, where the animals utilize general statistical rules and visual cues to optimize their search strategies. This highlights the adaptability of spatial navigation strategies in response to dynamic environments and suggests that the hippocampus may facilitate flexible learning through schema-based processing rather than fixed spatial representations.

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