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## Hippocampus and medial prefrontal cortex interplay throughout distinct learning processes

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## **Abstract**

The interplay between the hippocampus and the medial prefrontal cortex has emerged over the years as a fundamental artery in memory processes across species, notably spatial working memory and episodic-like memory. Recent studies, as this one, have focused on the direct monosynaptic projection from the ventral CA1 hippocampus to the medial prefrontal cortex.

The present study has now revealed that this projection plays a crucial role in spatial working memory, which involves the temporary storage of relevant spatial information, in the context of a DNMP task. These findings strengthen the evidence for the critical role of the hippocampal-prefrontal projection in constantly updating information in spatial working memory.

Additionally, our research permitted the development of a new paired-associates learning protocol for mice to assess episodic-like memory. Despite limited understanding of the role of the HPC to mPFC projection in episodic-like memory, this thesis provides a comprehensive overview of current knowledge and offers new perspectives on the contribution of these interactions to learning and memory processes in rodents.

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## Abbreviations

**AMPA receptors** -  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors

**CA1/2/3** - Cornu ammonis 1/2/3

**CNO** - Clozapine-N-Oxide

**DNMP** - Delayed non-matching to place

**DREADD** - Designer Receptor Exclusively Activated by Designer Drug

**GLMM** - Generalized Linear Mixed Models

**HPC** - Hippocampus

**IL** - Infralimbic cortex

**LTP** - Long Term Potentiation

**PA(L)** - Paired Associates (Learning)

**(m)PFC** - (medial) Prefrontal Cortex

**PL** - Prelimbic cortex

**MRI** - Magnetic Resonance Imaging

**SBEA** - Structure du bien-être animal

**(S)WM** - (Spatial) Working Memory

# Chapter 1

## Introduction

Aristotle treatises, Hume's theories and Ebbinghaus experiment are only a modest testament of the widespread interest in memory and its underlying mechanisms throughout the years. We observe by the late 20th century attempts at defining, fragmenting and classifying more precisely human memory in clear discontinuity of the previous monolithic view of memory. Quillian et al. (1966) first developed the now-common term semantic memory, basically encompassing human memory of general facts and knowledge. In contrast to this notion, Tulving (1972) formulated the concept of *episodic* memory, a notion that also prevails to this day. Simultaneously, procedural and *working* memory were introduced, contrasting with episodic memory but also refining slightly its characteristics (Miller et al., 1960).

The study of these different memory processes has been closely linked to studies of pathologies, especially psychiatric disorders and neurodegenerative pathologies in which memory impairments are strikingly common. Over decades of research, it gradually became apparent that patients diagnosed with major depressive disorder, post-traumatic stress disorder or even schizophrenia might have common underlying anomalies and abnormal functional coupling within the hippocampal-prefrontal circuit (Godsil et al., 2013). Insomuch as these pathologies' symptoms lie on a broad spectrum of cognitive impairment, a dysfunctional HPC-mPFC interplay could be the common factor of these pathologies (Godsil et al., 2013).

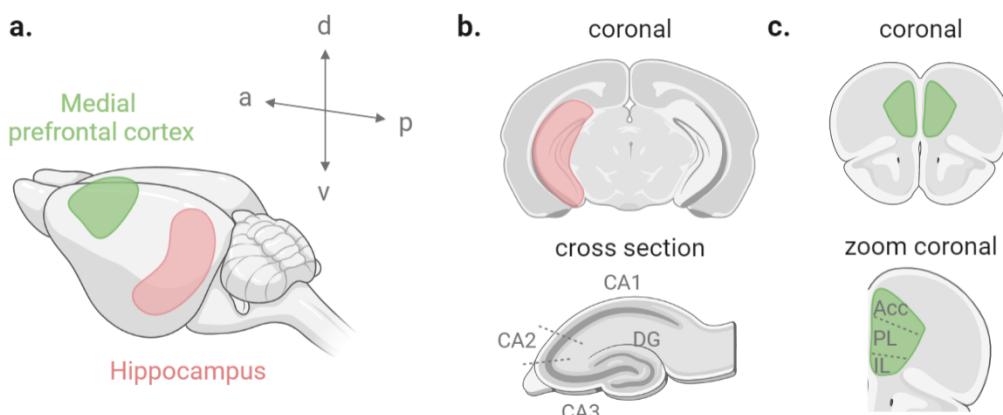
However, up to this day, studying the specifics of hippocampal to medial prefrontal cortical projections in humans remains tedious as current techniques are highly invasive and cannot be rationally applied (optogenetics, viral inhibition, knockouts...) (Parker et al., 2002). Such invasive approaches are usually restricted to either non-primate humans or rodents, causing them to be good models to

study such a specific projection. Over the years, the physiology of the hippocampus to prefrontal pathway has been extensively investigated in rodents, more specifically in mice (Godsil et al., 2013). For these reason, the present thesis will draw upon those findings, using the mouse as a model.

In rodents, the hippocampal formation (Hippocampus or HPC) extends caudally between the neocortex and diencephalon then curving ventrally approaching the temporal lobe. The hippocampus can be divided in disparate sub-areas based on morphology cell composition of tissues and functions: cornu ammonis (CA) 1, 2 and 3 and dentate gyrus (DG) (see Figure 1.1).

The HPC possess extensive afferent and efferent connectivity with frontal cortical and sub-cortical structures such as the medial prefrontal cortex including both multisynaptic projections via the thalamic nuclei (Cassel and Pereira de Vasconcelos, 2015) or via the entorhinal cortex (Swanson and Köhler, 1986; Insausti et al., 1997) and monosynaptic projections of ventral and intermediate HPC to prelimbic and infralimbic regions of mPFC (Laroche et al., 2000; Cenquizca and Swanson, 2007; Rajasethupathy et al., 2015) (Figure 1.2).

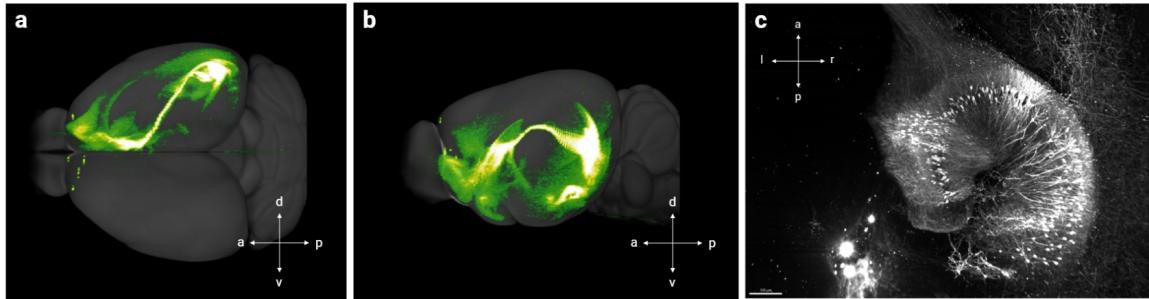
The medial prefrontal cortex (mPFC) is a region located in the medial anterior part of the brain, known to be thick in neuronal fibers (see Figure 1.1). The prefrontal cortex holds mainly pyramidal excitatory neurons (82%) that span multiple layers (Le Merre et al., 2021; Erö et al., 2018).



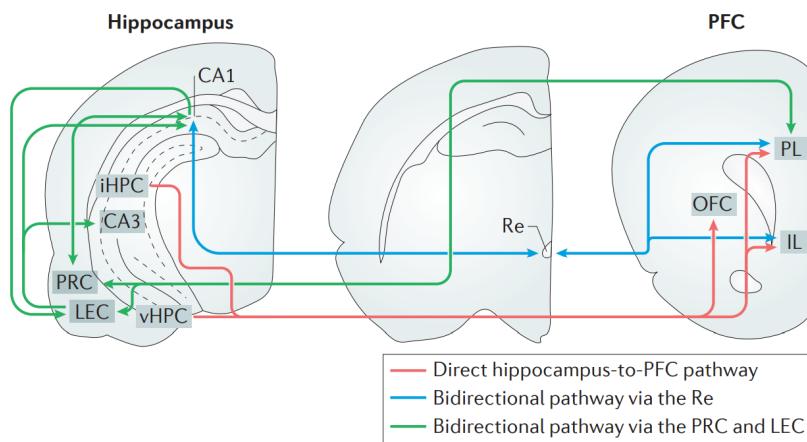
**Figure 1.1: The mouse hippocampus and medial prefrontal cortex** a. A lateral view of the mouse brain with the hippocampus in red and the medial prefrontal cortex in green. b. Coronal (top) and cross (bottom) section of the hippocampus with labels for the dentate gyrus (DG), and cornu ammonis (CA) subfields. c. Coronal (top) and zoomed view of the medial prefrontal cortex with labels for its sub-parts namely the anterior cingulate (ACC), prelimbic (PL), and infralimbic (IL) cortices.

These direct HPC to mPFC projections originate from the ventral hippocampal CA1 area onto the mPFC, with evidence showing stronger projections to ventral subregions and sparser ones from the intermediate third of the hippocampus (Cenquizca and Swanson, 2007; Hoover and Vertes, 2007) (see

Figure 1.3). Fibers of this pathway circulate through the fornix before ending their route in the IL, PL and ACC. According to electrophysiological studies in humans, this hippocampal to medial prefrontal projection makes synaptic contact with both glutamatergic pyramidal neurons and GABAergic interneurons within the medial PFC (Gabbott et al., 2002; Tierney et al., 2004).



**Figure 1.2: The hippocampal ventral CA1 to medial prefrontal monosynaptic direct and unilateral projection** a. and b. Images from the Allen Brain Atlas Mouse Connectivity explorer showcasing the pathway of interest. Mouse line: Syt17-Cre\_NO14 with A rAAV injection. Injection site volume: 0.185. c. mCherry fluorescent labeling of the hippocampal neurons directly projecting to the medial prefrontal cortex. Acquisition under a light sheet microscope in a transparent brain via the iDISCO method (Villet and Sanchez, 2022). Here the focus is made on the hippocampus, and neurons of interest can be seen in white. Scale: 300 $\mu$ m



**Figure 1.3: Indirect and direct prefrontal–hippocampal pathways in rodents** Both the ventral hippocampus (vHPC) and the intermediate hippocampus (iHPC) have direct pathway to various regions of the prefrontal cortex, including the orbital PFC (OFC), the prelimbic cortex (PL) and infralimbic cortex (IL). There exists also two bidirectional connections between the PFC and the hippocampus: one via the thalamic nucleus reunions (Re) to HPC CA1 and the other via the perirhinal cortex (PRC) to HPC CA1 and lateral entorhinal cortex (LEC) to HPC CA1 and CA3. Adapted from Eichenbaum (2017).

This thesis will explore the interplay between the hippocampus and the medial prefrontal cortex via this direct monosynaptic projection in two distinct learning processes: spatial working memory and episodic-like memory. Its aim is plural: providing a comprehensive overview of what has been established so far, contributing in collecting further evidences, developing new protocols and getting a broader understanding of the role of this projection in more global memory processes.

## **Chapter 2**

# **HPC-mPFC involvement in the learning of a spatial working memory schema**

### **2.1 Spatial working memory: a general characterization**

Derived from the concept of short-term memory, working memory (WM) was first used in 'Plans and the Structure of Behaviour' by Miller, Galanter and Pribram in 1960. In the following years, attempts at defining and describing this concept flourished. Baddeley and Hitch (1974) suggested a multi-component working memory model that, still to this day, is indubitably one of the most notorious and cited model. This model basically contradicts the overly simplified view of short-term memory being restricted to a short-term information storage and stipulates that working memory is a multi-component system managing information storage for higher cognitive utility (Baddeley and Hitch, 1974).

In animals studies, the first occurrence of the term WM can be found in Olton, Collison, and Werz (1977) in which the term WM was used to describe rats' aptitude for performing in a radial arm maze. In this experiment, the animal is placed on the center platform, while a food reward is obtainable at the end of each arm. In order to be as efficient as possible in getting the rewards, the animal would have to mentally maintain the information that one arm of the maze has already been checked or it will be endlessly rechecked without getting rewarded. This capacity of upholding precise information

for a certain period of time within an experiment is what Olton and colleagues described as WM.

Olton and colleagues latter definition actually referred to what will be later labeled spatial working memory (SWM), a sub-system of working memory. Spatial WM requires retention and manipulation of visuo-spatial information, in their case the spatial information of the radial maze. More recently, Eichenbaum and Cohen (2004) completed this definition, emphasizing the "working" feature of this memory. Indeed, while working memory is the capacity to hold information briefly in one's consciousness it also comprises a cognitive effort and processing, in others words an active mental manipulation with the purpose to draw relational and inferential judgments.

This latter memory system is one of interest in this thesis. I will follow the latter definition of spatial working memory: a short term storage of visuo-spatial information, used within a testing session, helping the animal performing a task at hand. It is also to be noted that WM both provide and incorporate information to and from long-term memory. Therefore, in this definition, it is primordial acknowledging that spatial working memory foster the creation of a long term memory rule.

SWM will be tested using a widely common paradigm: the T-maze delayed non-match to place task.

### **2.1.1 The T-maze delayed non-match to place task**

Years of research on spatial WM allowed the development of many protocols on rodents. Maze paradigms are, by nature, of great interest in such experiments. The delayed non-match to place task (DNMP), remains one of the most extensively used paradigm and thus has been universally validated. This maze capitalise on rodents' natural tendency to visit arms in a left/right alternating manner, making it a convenient and pertinent experiment. For these reasons, the DNMP is a perfectly appropriate tool to tackle to problem at hand.

In this task, the rodent is trained to enter a maze's arm (the choice phase) opposite to the one it had visited in the previous run (the sample phase), to get rewarded. The precise behavioral procedure will be developed in the Materials and Method section of this thesis.

## 2.2 Neural substrates

### 2.2.1 The Hippocampus (HPC)

While the hippocampus functions are not limited to the spatial realm, there exists ostensibly a consensus on its significant role in memory for place.

The hippocampus has been long known for encoding spatial information and location thanks to its "place fields" in animals (Wilson and McNaughton, 1993; O'Keefe, 1976; O'Keefe and Dostrovsky J., 1971). More precisely, O'Keefe has shown that there exists specific pyramidal neurons in the hippocampus, namely place cells, firing at a specific location the animal evolves in. Each of these place cells cover a specific place field and altogether help the animal develop a "cognitive map" of its environment. This theory of cognitive map proposes that the activity of rodents' hippocampus depict the animal's environments, their constituents and the animal own location within these environments.

Hippocampal lesions have been proven to affect performance in various spatial memory paradigms such as the delayed non-match to place task in a T-maze or in the radial arm maze (Olton and Papas, 1979; Rawlins and Olton, 1982). Globally, animals with extensive hippocampal lesions end up performing typically at chance level on spatial memory tasks (Bannerman et al., 1999). Aside from that, such lesions impair the animals' ability to associate a spatial location with a food reward (Deacon et al., 2002), or a non-spatial cue to a reward location (Murray and Ridley, 1999). For instance, in the Morris water maze task, lesions impaired the animals' ability to associate a location with the escape (Morris et al., 1982).

In tasks requiring more specifically the spatial working memory, hippocampal lesions resulted in similar outcomes. Delayed non match to place, such as in the T-maze, has been extensively proven to be dependent on dorsal hippocampus (Czerniawski et al., 2009, Dudchenko et al., 2000).

If the hippocampus' role in the spatial aspect of DNMP tasks appears evident, the actual precision of place fields to encode location has been proven to vary across the structure itself. Indeed, it appears that moving along the dorsal-temporal axis, place fields become gradually larger (Jung et al., 1994) up to the point one can theorize that ventral hippocampal fields might encode global context rather than precise location (Kjelstrup et al., 2008).

Thus, it appears clear that the hippocampus as a whole is essential in forming an internal representation of one's environment, a capacity of great importance in problems that involve a spatial cue and

a spatial response.

### 2.2.2 The medial Prefrontal Cortex (mPFC)

The initial portrayal of the functions of the prefrontal cortex, as for more generally any other region, was heavily supported by ablations and lesions, whether these were naturally occurring or endogenous to an experiment. The early experiments on animals considerably participated in this understanding, complementing the results obtained thanks to psychometric testing and clinical neurology. In 1874, Hitzig et al. observed that a frontal ablation in dogs resulted in an incapacity to remember an exercise learned prior to the surgery but also to learn it again. In that sense, Hitzig work is among the earliest to hint at a role of the prefrontal cortex in both long-term memory and working memory. Abounding experiments followed such as Bechterew (1911), Pavlov's (1949) or Jacobsen (1935) pinpointing, in various animal models, the role of the prefrontal cortex in goal-directed behavior, for which a functioning working memory is essential.

Delayed response tasks are a classic paradigm for studying working memory in both humans and animals as they require the maintenance in memory of a certain information for a given lapse of time. In such paradigms, animals with bilateral prefrontal lesions lean to an impediment to perform the task that has been whilom identified and highly documented in various species, including rodents (Wikmark et al., 1973; Larsen et al., 1978).

Later on, experiments involving narrower and more precise lesions and electrophysiology recording in rodents have permitted to identify that, within the prefrontal cortex, the medial prefrontal cortex (mPFC) is actually a critical cortical sub-area for behavioral performance on spatial working memory tasks (Brito et al., 1982, van Haaren et al., 1988). In a T-maze DNMP paradigm, prelimbic lesions on rats resulted in chance levels performance on the first postoperative session (Brito et al., 1982). However, animals performance increased with continued experience, suggesting that they could eventually relearn the task. Thus, the prelimbic area, a subpart of the mPFC, is critical in T-maze DNMP task. Latterly, studies on neuronal encoding provided further evidence towards this conjecture (Horst et al., 2012; Yang S.T. et al., 2014; Yang Y. et al., 2018). For instance, during a Y-maze DNMP paradigm, electrophysiological recordings of rats' pyramidal mPFC neurons shows that 93.7% mPFC cells (449 out of 479) displayed changes in spiking frequency temporally locked with the task events (choice, reward, delay, running back) (Yang et al., 2014). Some of these cells were tuned with spatial information, suggesting that they have a role in the spatial aspect of memory. Interestingly, some mPFC neurons

even displayed differential synchronization in firing during the delay. The latter results advocate for a role of mPFC neurons in working memory encoding, and even spatial working memory encoding, via either synchronization or increasing firing activity (Yang et al., 2014).

### 2.2.3 The monosynaptic HPC-mPFC projection throughout learning

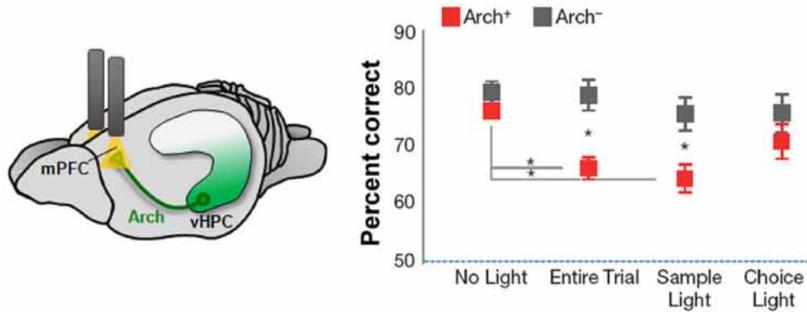
Hippocampal-prefrontal communication is reasonably expected to be fundamental in behaviors requiring both structures and the coordination of their distinctive functions, such as spatial working memory.

Investigating the projection between HPC and mPFC has been done using functional disconnection, lesions but also electrophysiological studies and optogenetics tools.

Functional disconnection of the unilateral HPC-mPFC projection consists in physical or chemical inactivation of the mPFC in one hemisphere and the HPC in the other hemisphere. As the connections between HPC and mPFC are unilateral, this would result in one intact HPC side and one intact mPFC side but no communication pathway between the both of them (Floresco et al., 1997). Such an approach has been widely used to establish that HPC-mPFC interplay is necessary for spatial WM in various paradigms such as the Morris water maze (Wang and Cai, 2008), the Hebb-Williams maze (Churchwell et al., 2010) or the DNMP (Wang and Cai, 2006). In the delayed non-match to place task, which is the paradigm of interest in this thesis, rats' performance has been shown to be impaired when either HPC or mPFC is damaged or inhibited bilaterally (Wang and Cai, 2006). More precisely, bilateral muscimol injection in the ventral hippocampus, in the prelimbic area or unilateral injection in the hippocampus together with contralateral injection in the mPFC undermined DNMP performance. Those results perfectly pinpoint the important role of this circuit in spatial working memory. Moreover, because HPC-mPFC disconnection effects are practically identical as bilateral mPFC inactivation (Wang and Cai, 2006; Euston et al., 2013), those results hint at the fact that mPFC is dependent upon the input from the hippocampus through the hippocampal-mPFC pathway.

More recently, efforts have been made to target more specifically the unidirectional monosynaptic projection of interest in this thesis. By expressing Archaerhodopsin (Arch) in ventral hippocampal neurons, optical stimulation of their synaptic buttons in mPFC will selectively and reversibly silence synaptic transmission of the HPC-mPFC projection of interest. This technique was used with a meticulous temporal precision on mice performing the DNMP in a T-maze, allowing to decipher the

HPC-mPFC projection role throughout the stages of learning a SWM task (Spellman et al., 2015). It appears that silencing of the HPC-mPFC inputs during the sample phase of the task, unlike the choice phase, impaired the mice memory performance. Thus, hippocampal-prefrontal direct afferent pathway is decisive in continuously encoding and updating spatial cues in spatial working memory paradigms, but not their retrieval or short-term maintenance in memory (Spellman et al., 2015). Adding onto those findings, it appears that optogenetic silencing of the HPC-mPFC also disrupt gamma oscillatory synchrony during the sample phase, a synchrony that is thus likely to be critical for encoding such spatial cues. Indeed, Spellman et al. detected an increased gamma synchrony during the sample phase (the encoding), contrasting with the well-studied hippocampal-prefrontal theta synchrony during the choice phase. The found increase in gamma synchrony foreshadowed animals' performance in the task, hinting at the fact that contrasting frequency bands synchrony support different memory processes (Spellman et al., 2015). While gamma synchrony between the two structures would support encoding, theta synchrony would back retrieval.



**Figure 2.1: Spellman et al. results of the inhibition of the HPC-mPFC pathway in the delayed T-maze task (2015).** During the task, ventral HPC inputs to the mPFC were optogenetically silenced during the different phases of the T-maze delayed non-match to place task. Performance was impaired when optogenetic silencing was operated during the entire trial or in the sample phase. Adapted from Spellman et al. (2015)

A variety of other electrophysiological studies have investigated hippocampal-prefrontal interplay across other spatial working memory paradigms, all confirming the critical role of oscillatory synchronization between HPC and mPFC. It appears that enhancement of theta-slow gamma coupling subserves working memory, preserving SWM performance when the task difficulty is incremented (Tamura et al., 2017).

While it appears clear that the HPC-mPFC projection is critical for SWM, the nature of the information exchange during the interplay remains difficult to identify. Altogether, current findings (See 2.2.1. and 2.2.2.) advocate for two potential roles for the hippocampus input towards the prefrontal cortex:

to supply contextual information or to allow for quick associative learning (Euston et al., 2012), both of them required to perform properly in a spatial working memory task. In support of the first role, evidence from place fields studies hint at the possibility that indeed, hippocampal input may be a plausible source for spatial context.

Regarding rapid associative learning, it appears that the rapid formation and consolidation of spatial cues may depend on hippocampus while the long-term storage and retrieval is mostly arbitrated by the mPFC (Frankland and Bontempi, 2005; Frankland et al., 2004).

Ultimately, HPC-mPFC interplay still need to be examined in terms of their contribution to behavior across a different timescale, while keeping the variables of the task constants. As a matter of fact, while Spellman et al. elegantly demonstrated that the direct monosynaptic HPC-mPFC projection is needed during the "sample" phase of the task, one might wonder if this projection is involved in a wider time range not only including the task itself but the following decisive hours during which consolidation seemingly happens. Moreover, Spellman et al. varied the delay between the sample and the choice phase, therefore making the results arduous to interpret.

This thesis will focus on filling out this blank by examining the role of the monosynaptic HPC-mPFC projection during the learning sessions thanks to viral inhibition techniques, thus contributing in deepening the general understanding of this cortical projection.

## Chapter 3

# HPC-mPFC involvement in the learning of episodic-like memory

Part of the late 20<sup>th</sup> century school of thoughts opposing the previous monolithic view of memory, Quillian et al. first developed the common term *semantic* memory, basically encompassing human memory of general facts and knowledge. In contrast to this notion, Tulving (1972) formulated the concept of *episodic* memory, a notion that also prevails to this day. In his work, episodic memory not only takes in and stores information about episodes or occurrences that are temporally dated, but also the spatio-temporal relationships among these events. According to this definition, a perceptual event would consistently be stored in respect of autobiographical reference to the previously existent contents of the episodic memory store (Tulving, 1972). Overtime, this definition evolved until reaching somewhat of a consensus among psychologists and neuroscientists on its form of retrieval: the query directed towards the system is of the form "what did you do at time T in place P" (Tulving, 1984). Later on, Dere added on to this definition stating that, altogether, human episodic memory is the conscious recollection of "what", "where" and "when" elements, which can eventually be verbally communicated (Dere, 2005). Another specificity of episodic memories is that they enclose a trace of the individual's internal state (emotions, thoughts...) during the encoding process (Dere et al. 2008, 2010). Lastly, in human episodic memory, retrieval of information can arise even when encoding is incidental and memory assessment is unanticipated (Tulving, 1999).

Because episodic memory impairments are detected in a number of neurodegenerative diseases (Alzheimer's disease, Huntington's Disease, Parkinson's Disease), psychiatric diseases and disorders that remains

somewhat drug-resistant (Schizophrenia, Major Depression) or in other cognitive impairments (Pause, 2013), it remains crucial to use the animal model to conceive appropriate and effective treatment and therapies (Dere, 2005). In the case of episodic memory, the use of the rodent is of particular interest. This model permits the production of a meticulous analysis of the cellular and neurophysiological mechanisms that are uncommon in non human primates or human models for numerous ethical and practical reasons (Hasselmo, 2011). Eventually, this model allows a more thorough and precise comprehension of the behavioral and physiological mechanisms of episodic memory.

### 3.1 Episodic-like memory: a general characterization

While much genetic conservation exists between rodents and humans, it is essential not to draw excessively broad analogies between the two models, as distinct biological pathways show extensive divergence between them (Yue et al., 2014). Conceivably, this applies to episodic memory.

Tulving (2001) argued that, as animals cannot communicate verbally on their past experiences, nor possess autoneotic awareness (the ability to mentally place oneself in the past), nor a sense of subjective time and self-perspective, they cannot by definition posses an episodic memory similar to humans. However, experiments have shown that non-humans animals possess memory of objects they have seen, places they visited, and a notion of time intervals and delays, which reassembles the "what", "when", "where" of human episodic memory. For instance, when scrub-jays where asked to cache larvae or peanuts in sand, the birds have shown not only to remember where they cached an item after some delay, but when given the choice between the two items, chose the freshest and tastiest one according to the time passed between their caching and the retrieval of food (Clayton and Dickson, 1998). This demonstrates that scrub-jays not only knew what item had been cached, but in which site and how long ago. In such a way, Clayton and Dickinson provided the first conclusive behavioural evidence of episodic-like memory in non-human animals.

However, there was no explicit evidence that the jays consciously recollected their personal past experiences, a criterion of human episodic memory. Drawing on those results, Dere (2004, 2005) argued that animals might possess an inherent model of episodic memory labelled episodic-like memory, which might be experienced at the behavioral level according to the "what", "when" and "where" components.

This multifaceted and controverted definition renders the demonstration of episodic-like memories

in rodents quite challenging. Overall, there exists two predominant methodological approaches to study episodic-like memory in animal: training-based and training-free models (Chao et al., 2020). Training-free models mostly revolve around the exploitation of natural and instinct behaviors that do not require specific learning: they are by nature based on one-trial learning. Among those paradigms lies the spontaneous object exploration task (Bevins et al., 2006), an extensively studied approach to test episodic-like memory. On the other hand, training-based models are akin to Clayton and Dickinson experiments (1998) in which animals are guided towards the learning of specific "what-when-where" rule(s), reinforced by either positive or negative outcomes. This thesis will focus on such learning as we focused on developing a task that has been widely validated in human experiments testing episodic memory: the Paired-Associates task.

### 3.1.1 Paired-associate learning

Paired-associate learning (PAL) was introduced by Mary Whiton Calkins in 1894 as an episodic memory paradigm in which pairs of items are presented during one or more learning trials. For instance, in humans one prominent use of the Paired-associate learning is the Verbal Paired Associates Learning sub-test from the Cambridge Neuropsychological Test Automated Battery in which participants have to learn a set of word-pairs (CANTAB, Cambridge Cognition, Ltd; Robbins et al., 1994). In animals, a variety of PAL derivatives were studied, ranging from Object-in-Place Learning (Kim et al., 2011; Kesner et al., 2015; Sakon et al., 2014), to odor-context reward assignments (Rajji et al., 2006) or various cue-combination tasks (Terada et al., 2017).

Because, in both animals and humans, PAL is a widely used tool in order to test episodic and episodic-like memory, the experiment developed in this thesis is based on this paradigm. However it remains to specify the nature of our cues, as well as the essence of our task.

Recently, in animals, various studies reproduced the Visual Paired Associates Learning sub-test from the CANTAB, a touch-screen based task that requires the animal to learn that a specific image is only rewarded if selected in a specific location (Talpos et al., 2014; Bartko et al., 2011; Kim et al., 2015; Smith et al., 2022). This approach has a lot of potential, not only because the use of a touch-screen allows for automation and flexibility of the task but also because of the important similarities between the human and the animal's tasks allowing for translational studies. Nonetheless, the learning of such task is a lengthy process, nearing 50 days for a mice, which heavily restrict its use (Kim et al., 2016). On another note, while the touch-screen based PAL is not a spatial task, it has been shown that the

visuo-spatial PAL test is the preferred test for discriminating patients with Alzheimer's disease from healthy controls (Swainson et al. 2001; Blackwell et al. 2004).

As the touch-screen based PAL is neither a spatial task nor a time efficient one, it appears there is room for the development of a more effective episodic-like memory task.

The paired associates (PA) task used in this paper, the duration-texture combination task, is a new paradigm created to combine the spatial aspect of CANTAB visuo-spatial PAL test and the team expertise of maze paradigms and its automation, allowing the study of episodic-like memory, time and stimulus encoding, but also differentiation of spatial and non-spatial aspects of memory representation by the hippocampus. The duration-texture combination task will be described in the Materials and Methods.

## 3.2 Neural substrates

The first glance at both the brain's mechanisms of episodic memory and the concrete existence of multiple memory systems arise from the study of brain lesions, the first striking case being Henry Molaison (Scoville and Milner, 1957). In order to cure a intractable epilepsy, HM had undergone a surgery extracting two-thirds of his hippocampus, his entire entorhinal cortex, parts of the perirhinal and parahippocampal cortices as well as the amygdala and white matter connections of these regions (Scoville and Milner, 1957; Hasselmo, 2011). Subsequently, HM presented an extensive memory impairment to the point that he was unable to form any new memory moving forward while his past episodic memories before his surgery remained intact. Numerous patients followed, like RB (Zola-Morgan et al., 1986), who presented a lesion selective to the CA1 region, the first region in the hippocampal circuit, hinting at a selective effect of hippocampal damage on long-term episodic memory. As damage limited to the hippocampus for RB was sufficient to generate anterograde memory impairment, the hippocampus quickly became the dominant region to investigate in terms of episodic memory in humans. As a result, it has logically become an area of great interest in rodent's studies, drawing the parallel between the two species.

### 3.2.1 The Hippocampus (HPC)

As mentioned in the preceding chapter, the hippocampus plays a crucial role in providing spatial information about events, however it appears that it also provides a temporal context such as the order of stimuli or the sequence in which they occur (Fortin et al. 2002; Kesner et al. 2002). While spatial and temporal context appear to be quite different in nature, they actually both consist of sequential representations (of events or locations). That being said, it appears clear that the hippocampus must be crucial for paired associates learning, a sequence of events and/or locations, at least for the "where" and "when" elements of the rodents' episodic-like memory.

Following studies have shown that lesions of the hippocampus actually debilitate the capacity to integrate the three "what", "when" and "where" components of episodic-like memory (Day et al., 2003; Eacott and Norman, 2004; Egoru and Eichenbaum, 2004; DeVito and Eichenbaum, 2010). In Day et al., a paired associates task consisting of flavors of food and their spatial locations, the inhibition of hippocampal  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors impaired both encoding and recall of the PA while the inhibition of N-methyl-D-aspartate (NMDA) receptors in the rats' hippocampus undermined memory encoding only. Eacott and Norman (2004) also replicated these results, showing that rats can learn a flavour-location association in a single trial and that their capability is highly dependent upon the hippocampus.

More recent investigations on the rate and temporal coding of hippocampal neurons further confirms the latter inferences. Hippocampal neuronal activity has been shown to reflect the discrete event sequences of a cue-combination task (Terada et al., 2017). Terada et al., trained animals in a cue-combination, analogous to a paired associates task, with sound and odor cues. The animal was required to pull one of two levers depending on the combination of the sound and odor cues in order to be rewarded. Analysis of the neuronal activity of those animals indicate that the majority of hippocampal CA1 neurons displayed sensory cue-, combination-, or choice-specific elevated discharge activities. The combination-selective neurons activity represent information of the two cues and the choice, thus reflecting that the hippocampus is coding the complete paired associates task in itself, hence episodic-like memory.

Going back to humans studies, it has been shown that the involvement of other regions beyond the hippocampus such as the prefrontal cortex (Buckner et al., 2000) are also critical for episodic memory.

### 3.2.2 The medial Prefrontal Cortex (mPFC)

Studies in both humans and animal models pinpoint that the prefrontal cortex controls the retrieval of context-appropriate memories by suppressing competing, context-inappropriate memories (Eichenbaum, 2017). In animal studies, prefrontal lesions lead to deficits in learning object-context or object-place associations (Browning et al., 2005; Kesner et al., 2003; Barker et al., 2007). In a classical object-place learning paradigm, rodents have to remember which object (first cue) and location (second cue) combination is linked to a reward. In this paired associates task, one object is associated with a reward in one location but not in another location. Rats with prelimbic and infralimbic lesions (regions part of the medial prefrontal cortex) do not learn the task: they cannot encode this episodic-like schema.

In a contextual conditioning paradigm, rats with bilateral mPFC lesions did not succeed in retrieving the contextual and temporal information (the what-when-where components) in order to retrieve memory of the conditioning event (Li et al., 2011). More precisely, such lesions have shown to lead to an extinction of conditioning to a fearful context (Giustino et al., 2015).

While it appears that both the medial prefrontal cortex and the hippocampus are essential for episodic-like memory, they also have been shown to operate interactively in memory processing. As previously stated, I will focus here on the monosynaptic HPC to mPFC projection (detailed in Introduction).

### 3.2.3 The monosynaptic HPC-mPFC projection throughout learning

Direct testimony of the interaction between the HPC and the mPFC in memory arose from disconnection procedures, lesions or early gene expression studies, and oscillatory synchronizations observations akin to memory processing.

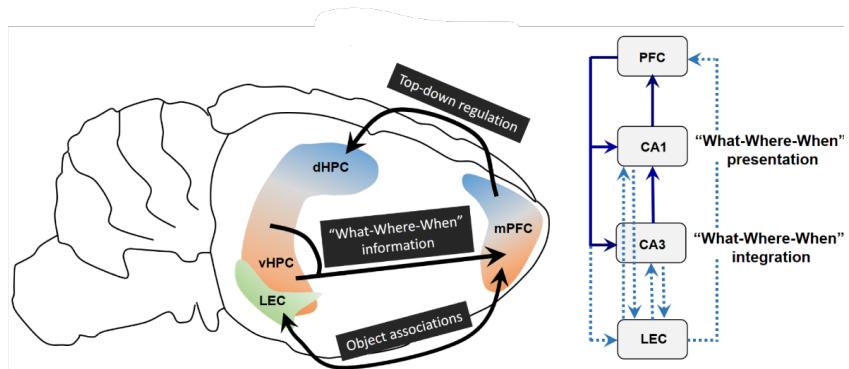
The disconnection approach proposes that if two brain regions communicate with each others, their concomitant disruption should result in a functional deficit. Disconnection of the HPC-mPFC monosynaptic projection has been studied in various episodic-like memory paradigms. Such disconnection impaired memory of object-location associations in both tests of memory for temporal order and Object-in-place preference memory (Barker and Warburton, 2011). Altogether, these findings imply that the HPC-mPFC interaction is critical for "what-when-where" memories. However, it remains to understand whether the HPC-mPFC interplay contributes to episodic-like memory of only its three

sub-components separately namely "what" "when" and "where". Moreover, it is to be noted that, while disconnection approaches have paved the way for plentiful discoveries, they are not only inhibiting the monosynaptic projection of interest but also unilaterally disrupting both areas' activity, which cannot be conceivably considered identical. For this reason, early genes studies, pharmacogenetic deactivation or viral inhibitions techniques are crucial to understand the role of the direct monosynaptic pathway of interest.

In another episodic-like paradigm, namely conditioned-training of licking behavior, Veyrac et al. (2015) detected higher expression of immediate early genes c-Fos and Zif268 in a large distributed hippocampal-prefrontal cortex network, associated with the correctness of episodic memory recollection. c-Fos and Zif268 are transcription factors involved in brain plasticity and activated by learning and memory (Filipkowski et al., 2006). More particularly, Zif268 is crucial for hippocampal LTP (long term potentiation) maintenance thus its presence has been commonly linked with memory processes (Gonzalez et al., 2019).

It appears that the precise pharmacogenetic inhibition of the HPC to mPFC pathway impaired episodic-like memory tested via training-free episodic-like memory paradigms using spontaneous object exploration (ELM4-2 test for e.g.) (Barker et al., 2017). More specifically, Barker et al. were able to distinguish between the actual two hippocampal-mPFC pathways. While inhibition of the dorsal CA1 to mPFC projection selectively impaired temporal order judgments, the deactivation of the intermediate CA1 to mPFC pathway disrupted spatial memory. This disjunction of functions within the hippocampal CA1 neuronal population projecting directly to the mPFC might be key in the discretizing contextual information in episodic-like memory.

Drawing on those insights, an hypothetical system of episodic-like memory has been recently developed by Chao et al. (2020) (Figure 3.1).



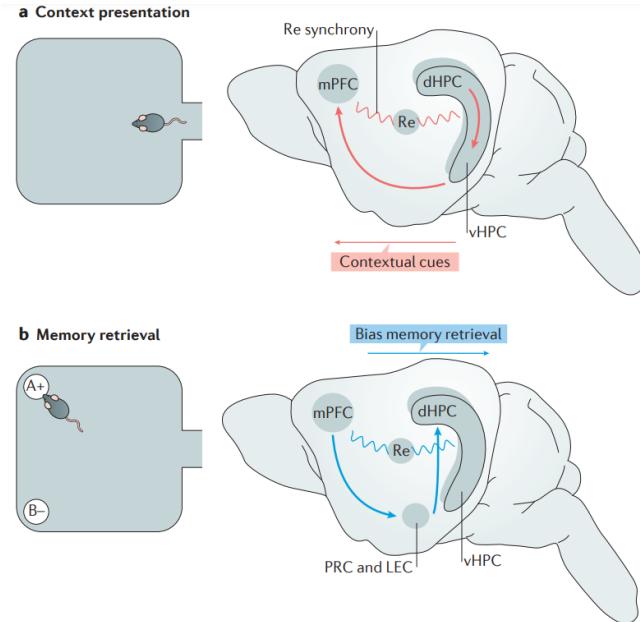
**Figure 3.1: Chao et al. prefrontal-hippocampal interactions hypothetical model in episodic memory (2020).** The PFC and HPC interplay govern episodic-like memory. The HPC is responsible for transmitting specific “What-Where-When” information unto the PFC that coordinates information selection. The role of CA1 and CA3 differ, while the CA1 account for processing the presentation of “What-Where-When” components, the latter is responsible for the integration of “What-Where-When” representation. Adapted from Chao et al. (2020)

However this remains an hypothetical model in need of further experimental evidences.

In humans, functional MRI studies have uncovered an increased functional connectivity between the HPC and mPFC during episodic memory encoding and retrieval (Zeithamova et al., 2012), while magnetoencephalography data reflects that hippocampal theta power and coherence with mPFC theta increased during the learning of new associations (Backus et al., 2016).

Parallel have been shown in animals studies across various episodic-like memory paradigms (Shirvalkar et al., 2010; Kim et al., 2011; Place et al., 2016; Malkow et al., 2022). In Place et al. (2016), animals were to learn that two different contexts cued different object-reward associations. In context 1, object A was rewarded and object B was not, and the other way around for context 2. This task can be considered analogous to an object-place paired associates task. Throughout the task, theta activity in both the mPFC and HPC were found to be strongly coherent. Moreover, while the animal entered the cue environment, hippocampal theta preceded that in the mPFC by 30ms hinting at a flow of contextual information from the HPC to the mPFC. Contrariwise, upon decision of the animal, the flow of information reversed, with mPFC theta leading hippocampal theta by 30ms. However, it appears that a delay of order of 30ms is a period that exceeds the delay for a monosynaptic connection, hinting that, the actual synchronization of the two areas arise from a more complex, indirect, network phenomenon (Eichenbaum, 2017). Drawing on those observations, Eichenbaum developed a conditional model of mPFC-hippocampal interactions in episodic memory based on a context-guided memory task (Figure 3.2). In this task, a contextual cue or another recent event leads to the retrieval of specific memories. According to this model, the ventral HPC pass on information

about contextual cues, the general context, to the PFC which will then exercise top-down regulatory control over the HPC via a cortical pathway. In this model, the oscillatory synchrony between the two sub-regions is mediated by the thalamic nucleus reuniens only, and not the monosynaptic projection of interest.



**Figure 3.2: Eichenbaum prefrontal-hippocampal interactions hypothetical model in episodic memory (2017).** When presented with a contextual cue, the nucleus reuniens (Re) onset oscillatory synchrony directed from the HPC to the mPFC for the HPC to pass on information about context to the mPFC. During retrieval, the Re reverse the flow of information by engaging synchrony from the mPFC to HPC pathways, the mPFC restrains the retrieval of context-inappropriate memories in the entorhinal and perirhinal cortices (LEC and PRC), providing top-down control of retrieval of appropriate memory in the dorsal HPC. Adapted from Eichenbaum (2017)

This hypothetical model is consistent with bountiful findings and has yet to be proven inaccurate.

It appears almost unequivocal that the neuronal system governing episodic-like memory involve not only the hippocampus and the medial prefrontal cortex, but also the hippocampal CA1 to medial prefrontal cortical unilaterial projection. However, to this day it remains unclear how the whole system functions, especially during the learning phase.

Concisely, there is not only a need for better episodic-like memory paradigms but also for further investigations on the episodic-like neural mechanisms. The experiment developed in this paper cannot pretend to answer those needs but aims at providing a first step in this lengthy and tedious inquiry.

# Chapter 4

## Materials and Methods

### 4.1 Materials

#### 4.1.1 Animals

##### 4.1.1.1 Spatial working memory task

We used ten 7-weeks-old male C57BL/6JRj mice weighting 22-28,3g at the experiment's onset. They were housed in groups of 4 per cage and kept on a 12-h light on/off schedule at 23°C. Experiments were performed during the light phase of the cycle. Animals were food restricted to 1.5-3g of food per day per animal during the whole experiment, ensuring that their weight does not drop below -10% of their initial weight before the experiment onset. Water was kept ad libitum. Procedures were performed in accordance with the national guidelines animal care and approved by the local institutional ethical committee and the SBEA.

##### 4.1.1.2 Episodic-like memory task

We used ten 7-weeks-old male C57BL/6JRj mice weighting 21,3-24,3g at the experiment's onset. They were housed in groups of 5 per cage. Other elements were kept similar as in the spatial working memory experiment previously described.

## 4.1.2 Apparatus

### 4.1.2.1 Working memory task

Spatial working memory was assessed using a delayed T-maze test of alternation.

The T-maze is a T-shaped apparatus composed of a central arm, two lateral goal arms (left and right) and an opaque start box named "home box" containing clean litter and closed by a removable door (Figure 4.1.). This enclosure, in addition to being used to encase the animal before the task onset, will also be used to isolate the animal between the two phases of this protocol. The transparent Plexiglas labyrinth also includes three corridors of 35 cm joining in the middle, forming a T. At each end of the arms lies a small metal cup used to place a kibble of reward food ( $\frac{1}{8}$  Weetos Weetabix®). Around the maze, visual cues were arranged to help the animal orientate itself. This maze was located in a room isolated from the noise of the animal facility and low lighting was used in order not to stress the animals.

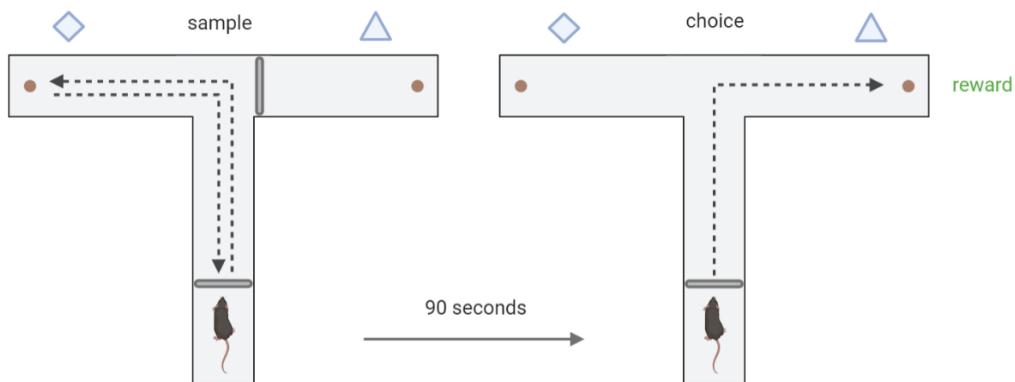


Figure 4.1: **Delayed T-maze test of alternation**

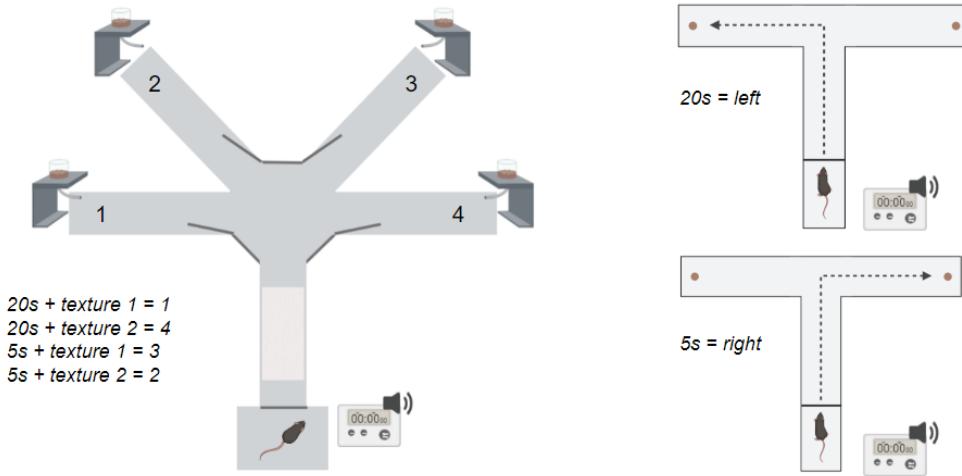
### 4.1.2.2 Episodic-like memory task

Episodic-like memory is to be assessed using a new maze.

Derived from a 8-arms radial maze, an automated 5-arms maze has been developed to test the duration-texture paired associates on mice (episodic-like memory). This "dragonfly"-shaped opaque maze includes corridors of 35 cm joining in the middle. It is composed of 1 central stem with a home box at its end and four arms that can be closed at their base by computer-controlled hinged doors. At each end of each of the four arms lies an automated pellet dispenser, also programmable (see Figure 4.2).

Duration discrimination was assessed using a T-maze.

See 4.1.2.1 for the description of the apparatus and Figure 4.2 for a schematic view.

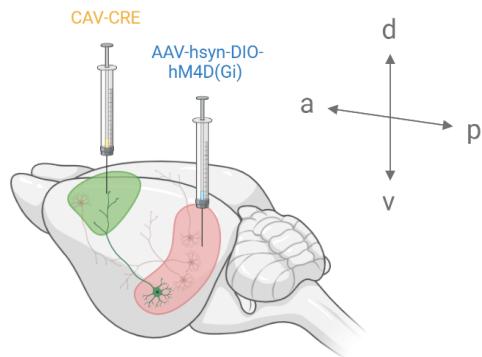


**Figure 4.2: Episodic-like protocol's mazes** The automated "dragonfly" maze (left) and the duration discrimination T-maze (right)

## 4.2 Methods

### 4.2.1 Surgery and viral injection for the spatial working memory task

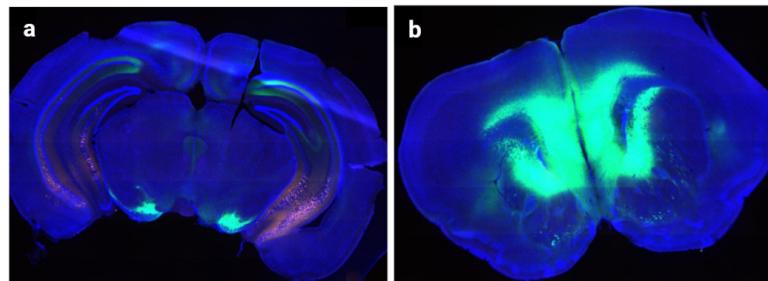
Anesthetized mice were submitted to stereotaxic surgery (0,75 ml ketamine 15% ; 0,25ml xylazine 5% ; 4ml saline solution) to inject viral suspension aimed to the ventral hippocampus (AP, -2,46; ML, +2,75; DV, -4,56 in mm) and prefrontal cortex (AP, +1,7; ML, +0,27; DV, ±2,5). The virus used were either AAV-Hsyn-EGFP (an adeno associated virus incorporated with GFP with a specific neuronal promoter), or an AAV-Hsyn-(DIO)-hm4-mcherry (an adeno associated virus incorporated with hm4 neuronal inhibitor with specific promotor under potential dependence of CRE-recombinase and carrying mCherry) or a CAV-CRE (canine adeno associated virus carrier of CRE recombinase). This viral construction allow the use of the DREADD technique. DREADD (Designer Receptor Exclusively Activated by Designer Drug) method allows inhibition of specific cerebral structures that present a hm4 G protein-coupled muscarinic receptor. Under intraperitoneal injection of clozapine-N-oxide (CNO), the monosynaptic projection from the HPC to the PFC will be inhibited.



**Figure 4.3: DREADD method** In dark green, the inhibited monosynaptic projection from the HPC to the PFC.

#### 4.2.2 Histology for the spatial working memory task

Following behavioral testing, all animals were administered an overdose of sodium pentobarbital and perfused transcardially with 0.01 M phosphate-buffered saline succeeded by 4% formalin. Brains were then removed, fixed and cryoprotected, so that coronal sections (**40 µm**) can be cut using a freezing microtome. Sections were mounted with mowiol and imaged through an epifluorescence microscope. For each mice, representative sections in both the ventral HPC and medial PFC were chosen to determine the accuracy of the viral injections (See 4.2.1.1).



**Figure 4.4: Histology, showing the vCA1 neurons projecting to the mPFC** a. In red (mCherry stained), the neurons of the projection, located in the vCA1. b. In green (GFP stained) the localization of the viral injection of CAV CRE. Here we confirmed that the injection was properly made in the medial prefrontal cortex.

#### 4.2.3 Behavioral Procedure

##### 4.2.3.1 Habituation procedure

For both experiments, mice were handled by the experimenter(s) for two days before training began. During this period, Weetos Weetabix® (the food reward used in the protocols), were sprinkled in their

home cages for the animals to become familiar with it. Mice were habituated to the mazes without doors for 10 minutes a day for the two consecutive days. Weetos Weetabix® were sprinkled across the mazes to encourage exploration, especially at the arms extremities. The very first day, all mice from a same cage were put together in the maze in order to lower anxiety. Then, the second day, each mice on its own was exposed to the maze. Only after completion of these two days the experiment can start.

#### 4.2.3.2 Spatial working memory task

Starting every trial, the animal was placed in the home box, with the guillotine door closed, waiting for the trial to start. In this task, each trial is divided into three phases. First off, the *sample phase* in which the mice is liberated from the home box, and one of the two arms is blocked by a sliding door, forcing the animal to move towards the arm that remains open. During this phase the animal must encode or learn the path it has taken. Subsequently, there is a *delay phase* where the mouse return to the home box and ought to keep in memory the visited arm for a period of 90 seconds. At the end of this delay, the home box is opened once again and the animal must visit the previously closed arm, the arm it did not visit in the sample phase, in order to get a food reward and pass the test: this is the *choice phase*. The animal is to be rewarded only if it chose the correct arm, else no food were given. Eventually, after the animal has consumed the reward, it was manually put back in the home box, and the next trial could start.

To avoid olfactory cues that might help the animal chose the arm not based on memory but scent, the maze was cleaned with water and ethanol in between each of the phases.

All of these phases are repeated 10 times per day and per mouse. Each day the sequence of the 10 trials was pseudo-randomly distributed with an equal number of left-right trials, which means that the animal performs a different sequence each day. The learning criterion was defined as 7 out of 10 successful attempts (70% success) over three consecutive days and the experiment ends as soon as all animals from one of the two groups reach this criterion.

Out of the ten mice in this experiment, five were used as a control group and five as the inhibited group. Mice in the inhibited group were administered via intraperitoneal injection Clozapine N-oxide (CNO) approximately 30 minutes before the onset of their first trial, a delay that corresponds to the peak concentration of the solution in the organism. Mice in the control group were given a saline solution in a similar manner, at the same time intervals. Mice in both groups were handled in a perfectly similar

fashion so that the only changing variable was the actual product administered through intraperitoneal injection.

#### 4.2.3.3 Episodic-like memory task

This protocol has been inspired by the PAL task of the Cambridge Neurological Test Battery (see 3.1.1.). Two different cues were chosen according to research interest, team knowledge and practicality. The first type of cue is the duration of a sound. The sound, either lasting 20 seconds or 5 seconds, is a constant tone of 2000Hz and displayed in the home box. This cue was highly inspired by Sabariego et al. (2020) work on the role of hippocampus in the experience of elapsed time. Choosing a sound duration as a cue potentially allow the study of hippocampal time cells (MacDonald et al., 2011), and their dynamics throughout learning, adding furthermore support for the "when" component of the episodic-like memory, often criticized for being a vague concept. The second chosen cue is a texture located on the maze floor, varying in its degree of granularity for the animal to easily differentiate. Mice being nocturnal animals, they have developed an extremely acute sense of touch, which makes such a cue highly convenient.

Mice start each training session placed in the home box of the "dragonfly" maze (see Figure 4.2. left). Just above the home box is located a speaker, from which the tone is played using winsound.Beep(frequency, duration) function from python's winsound module (Python 3.10.8). Frequency was kept at 2000Hz, mimicking Sabariego et al. experiment (2020). As soon as the sound ends (the first cue), the door of the home box opens automatically, allowing access to the central arm. In order to progress in the maze, the animal has to walk on a texture (light beige in Figure 4.2.), this is the second cue. According to the combination between the sound cue and the texture cue, the animal has to chose a specific arm to get a food reward (see Figure 4.2. for the four specific sequences).

However before testing this protocol, one had to verify if mice could actually differentiate between a 5 and 20 seconds sound. While this has been shown on rats, it remains to be proven with mice. For this reason, a pre-test was performed on a T-maze, testing only for the sound duration cue.

Mice start each training session placed in the home box of a T-maze (see Figure 4.2. right). Just above the home box is located a speaker, from which the tone is played using winsound.Beep(frequency, duration) function from python's winsound module. Frequency was kept at 2000Hz, mimicking Sabariego et al. experiment (2020). As soon as the sound ended, the guillotine door of the home box was opened, giving access to the central arm of the T-maze in which the animal ventured itself in. If the preceding

tone was 5 seconds, the animal was forced to visit the right arm to get rewarded (the left arm being closed), and if the preceding sound lasted 20 seconds, the animal was forced to turn to the left arm to be successfully rewarded (the right arm being closed).

All of these phases are repeated 10 times per day and per mouse. Each day the sequence of the 10 trials was pseudo-randomly distributed with an equal number of left-right trials, meaning that the animal performs a different sequence each day. After 5 days of forced training, animals moved onto the free-learning phase, in which both arms were accessible and the animal was not forced in its choice. Mice were only rewarded if they correctly turned right after a 5sec sound or right after a 20sec tone. The learning criterion was defined as 7 out of 10 successful attempts (70% success) over two consecutive days.

Due to time constraints, solely the second protocol described here (the T-maze test with the sound cue) was concluded.

#### 4.2.4 Data analysis

Data analysis was completed using RStudio Version 1.4.1717 on a Microsoft Windows (Version 21H2) computer.

For the spatial working memory experiment, the average number of successful trials in each session and the single trial performance were both analyzed using a Generalized Linear Mixed Model (glmer function in lme4 package, version 1.1-31). Of all ten mice, the data of one animal has been removed because histology revealed a flawed viral injection.

# Chapter 5

## Results

### 5.1 Spatial working memory task

Within ten days of daily training, all mice in the saline group reached the learning criterion. The experiment stopped with only one CNO mice achieving the criterion, showing off somewhat a flat mean learning curve across the inhibited group (see Figure 5.1). Thus, if one solely consider this highly subjective learning criterion, one can conclude that the HPC to mPFC projection appears to have a role across learning of such spatial working memory task. That said, a proper statistical analysis is necessary to legitimately being able to draw conclusions.

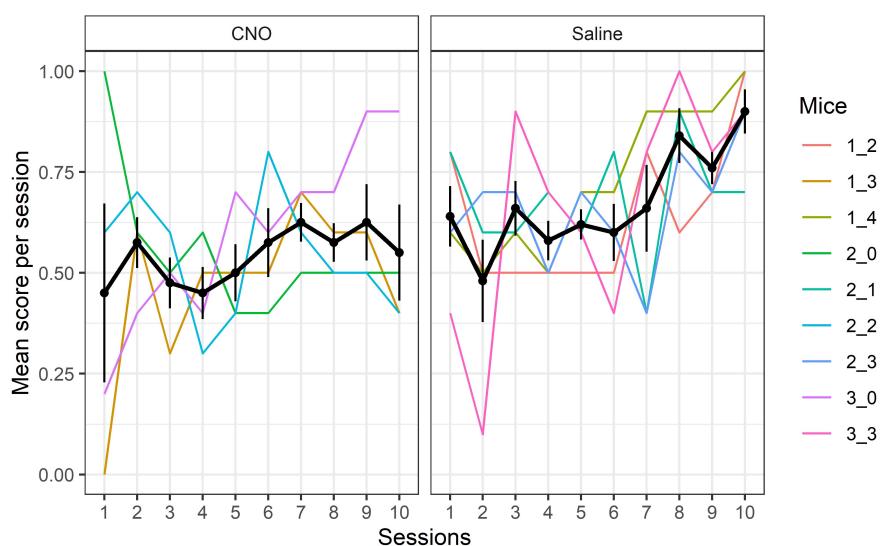


Figure 5.1: **Learning rate evolution of both groups throughout the experiment** In black: mean of all the mice of the group

We aim at answering the following question: is there a general effect of the inhibition on the animals' performance over the days?

The most appropriate statistical tool to analyze such longitudinal data with non-independence is Generalized Linear Mixed Models (GLMM). Predictor variables here are both the group (Saline/CNO) and the day number, while the criterion variable is the animals' performance. In such a setting, GLMM allows to take into consideration the entire time frame while accounting for the variability between individuals.

GLMM with logit link and binomial error distribution was computed on the daily mean score of each mice (the percentage of correct trials) such that (see Figure 5.3):

$$glmer(\text{MeanScore} \sim \text{Treatment} + \text{Day} + (\text{Day}|Mice), \text{family} = \text{"binomial"})$$

with

$$\text{logit}(p) = \ln\left(\frac{p}{q}\right) = \sum_{j=1}^p \beta_j X_{ij}$$

Results indicates a coefficient relative to the Saline group superior to 0 ( $\approx 0.7962$ ) with corresponding p-value statistically significant ( $\approx 0.0200$  after Bonferroni correction), meaning that the average daily performance is higher with saline treatment than with Clozapine-N-Oxyde. The treatment (CNO/Saline) significantly predicted the performance, there is a statistical evidence of a difference in performance between the groups.

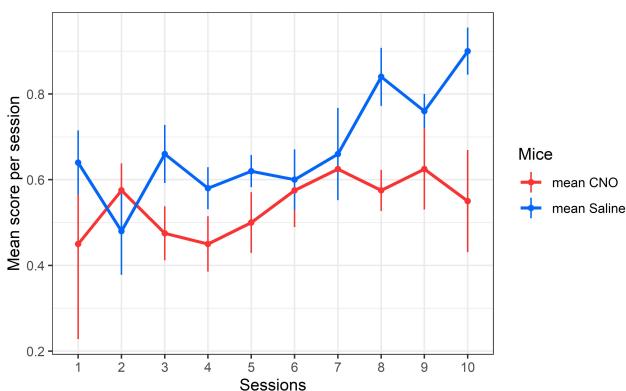


Figure 5.2: Mean learning rate by day evolution throughout the experiment

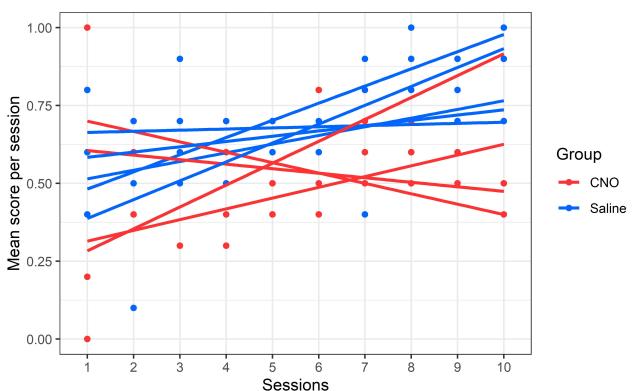


Figure 5.3: GLMM on the mean learning rate per day per mouse

Because no significant p-value was obtained for the variable  $\text{Day}$ , and in order to be statistically more powerful, a GLMM with logit link and binomial error distribution was computed on the binary suc-

cess/failure (1/0) score of each mice for each trial such that:

```
glmer(BinaryScore ~ Treatment + Day + (Day|Mice), family = "binomial")
```

This allows to avoid working with means and have a higher number of data points, two steps towards an upgraded analysis of the data at hand.

As for the precedent GLMM, the coefficient relative to the Saline group superior to 0 ( $\approx 0.7856$ ) with corresponding p-value statistically significant ( $\approx 0.00747$  after Bonferroni correction).

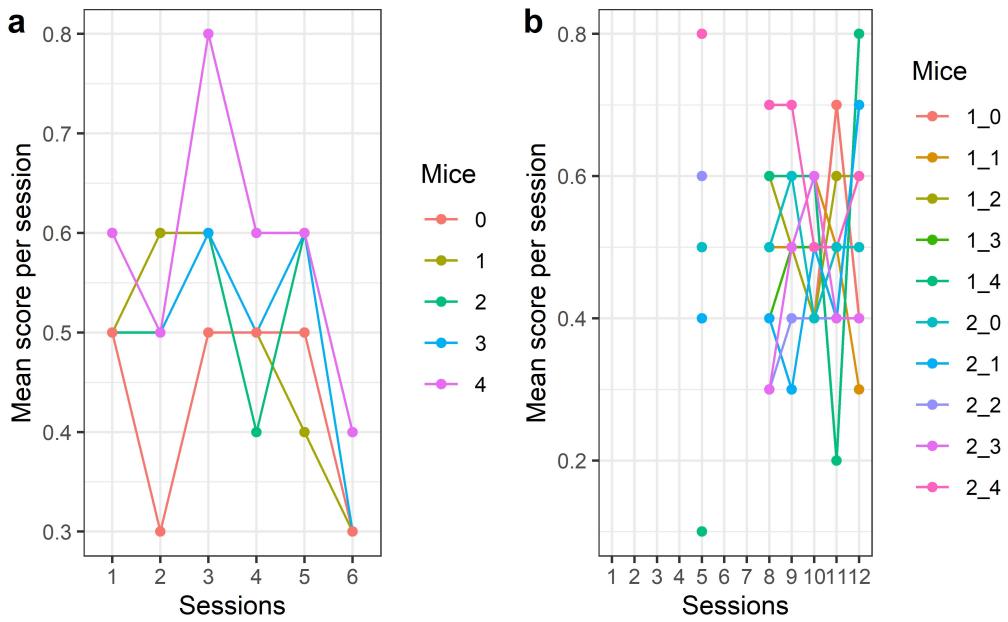
The p-value associated with the variable *Day* is significant ( $\approx 0.0369$  after Bonferroni correction) with an estimates of 0.5316.

Overall, the treatment (CNO/Saline) significantly predicted the DNMP performance, there is indeed a statistical evidence of a difference in performance between the groups. Our hypothesis is therefore corroborated by statistics: the hippocampal-mPFC projection is necessary for learning DNMP, a spatial working memory task.

## 5.2 Episodic-like memory task

The duration discrimination T-maze task remains inconclusive to this day. Out of the ten mice used in this experiment, none reached the criterion. The mice's performance persistently stuck at chance level across the days, and their behaviors was reflective of their bewilderment (Figure 5.4).

These results and their implications are discussed in the next chapter.



**Figure 5.4: Learning rate evolution for the two duration discrimination experiments** a. First experiment performed on non-naive mice, that were trained 30 days prior on the DNMP task. Mice developed a stereotypical alternating left-right behavior and could not discriminate between the two sound duration. b. Second experiment performed with completely naive mice. Days without data points are days of forced training during which the mice was only forced to visit the correct arm and rewarded. Performance across days varied greatly, showing that it was mostly dependent on the sequence within the trial rather than on appropriate learning.

# Chapter 6

## Conclusion

This thesis reviewed current knowledge and have provided insights into how interactions between the hippocampus and the medial prefrontal cortex in rodents contribute to both spatial working memory and episodic-like memory. Collectively, electrophysiological, lesions and optogenetics studies have revealed the relevance of hippocampal-prefrontal interactions in rapid associative learning and memory processes.

When it comes to spatial working memory, it appears clear that the hippocampal to medial prefrontal direct pathway is necessary for rapid learning of the T-maze delayed non-match to place task, as emphasized in this thesis' experiment. One must emphasize that the preceding experiment does not allow to conclude that this specific projection is necessary for learning, but only for rapid and effective learning.

Indeed, while mice with an inhibited HPC-mPFC projection did not reach the learning criterion as the saline group did, one might hypothesize that the inhibited group could still learn the task with supplementary time. Indeed, as described in this thesis, there exists other routes between the HPC and the mPFC, indirect pathways, that might eventually take the lead when the direct projections are impaired. While this hypothesis remains to be proven by experiments, this functional plasticity would explain the inhibited group's incapacity to learn as fast as the saline group, while still someday reaching the learning criterion.

The role of the HPC to mPFC projection in episodic-like has yet to be examined thoroughly. The sound duration discrimination task, supposedly a pre-test to the paired associates task developed in this thesis, has not been proven to work on mice. Multiple factors can explain such a result. Firstly,

mice might be too distracted in their home box (digging their litter, try to break away from the box, grooming etc.) to pay attention to the sound displayed. To address this issue, one can think of restraining mice much more, and removing the litter in the box. However, this has to be "wisely" considered as this home box is also there to create a safe environment to reduce the animals' overall stress. Another explanation is that the difference between the 5 seconds and 20 seconds sound is not striking enough. 20 seconds is also a quite long duration, which might surpass mice's attention. The opposite can be true, and 5 seconds might not be long enough to be taken into consideration as a cue by the animal. If this factor is an issue, one might consider sticking to duration that mimic more accurately sound duration to which mice can be naturally exposed to (another mouse squeak, a predator sound etc.). Yet another factor that might have influenced these results is that while one mouse was performing the task, the rest of the mice in the cage waited in the same experimental room. Thus, the mice in the cage could also hear the sound displayed for the mouse currently performing the task. Lastly, and maybe the most self-evident consideration: mice simply might not be able to learn this task in a reasonable amount of days because of how complex it is. Overall, it is too optimistic to think that one can identify the factor behind this lack of success, if one. Thus, further work is critical to identify the precise role of the HPC-mPFC pathway.

## 6.1 Discussion and Critics

While the latter results of the SWM task are worth noting, one must emphasize that HPC-mPFC interactions are also detected in rule-based spatial tasks not requiring working memory. For instance, Benchenane and colleagues (2010) showed that hippocampal-prefrontal synchrony increased at choice points in a Y-maze, more specifically consecutive to the acquisition of a new rule. Remondes and Wilson uncovered the same results in animals performing a sequence of decisions in a "wagon-wheel" maze. Altogether, these results advocate for the role of hippocampal-prefrontal interaction in decision-making in a spatial setting (Yu and Frank, 2015; Sigurdsson et al., 2016) that does not necessarily require working memory. It is thus necessary to be alert when mentioning the role of this projection in spatial working memory, because this would be restricting its function to lesser than it actually is.

The episodic-like protocol presented in this thesis (paired associates) is a training-based model, in which animals are steadily directed towards the learning of "what-when-where" components of the episodic-like memory task by means of positive reinforcement (food reward). However, instead of

involving learning of episodic-like memories such training-based procedures might arguably require the learning of facts, essentially presenting a "semantisation" of "what-where-when" memory. Thus, such training approaches have been criticized for being nothing but semantic memory procedures giving rise to well-learned expectations about the sequence of events, in broad contrast with episodic-like memory (Zhou et al., 2011).

A crucial element of human episodic memory is that retrieval might occur when encoding is incidental (unconscious) and retrieval unanticipated. That said, training-based episodic-like procedures such as the one developed in this thesis do not meet these criteria, cause they likely give rise to the learning of rule and expectations about the sequence of events (knowing when the memory is necessary) (Zhou et al., 2012). Thus, this participates in saying once again that animals might use semantic rules in order to perform well on the episodic memory test, and not their episodic-like memory.

I do too consider that there might be a "semantisation" of the memory in such episodic-like protocols. However, one has to question if this might not be inevitable. I believe that the complexity of episodic-like paradigms such as PAL, that needs days of training, leads to a natural semantisation at least in animal models, simply because it is not a "one shot" learning, at that the animal must eventually learn facts about the protocol in order to perform properly. In a PAL setting, this might have to be considered as a additional different between animals' episodic-like memory and human episodic memory.

## 6.2 Future Work

Our general understanding of how hippocampal-prefrontal interactions support memory processes still remain to be deepen.

There exists a need for better understanding of the rodent prefrontal cortex, to conceive an generally agreed upon model of how the rodent's prefrontal cortex compare to non-human primates and humans (Godsil et al., 2013). Extensive debates on the homology of the prefrontal cortex between species have lead to differing conclusions. For instance, the rat IL and PL has been hypothesized to correspond to the vmPFC and dorsal anterior cingulate cortex in humans (Milad et al., 2009, Milad et al., 2007) while others have linked the IL and PL with the omPFC and dlPFC in humans (Hoover and Vertes, 2007). The homology between rodents and humans is made especially difficult since the prefrontal cortex in humans is highly developed compared to rodents. However, improving our understanding of cross-species differences in PFC homology would be undeniably helpful in integrating findings from

animal models and understanding the function of the HPC-mPFC pathway in species for which invasive methods cannot be reasonably used such as us, humans.

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