# Reference memory, working memory and adaptive forgetting : a comparative study in rats

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par
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# Reference Memory, Working Memory and Adaptive Forgetting: A Comparative Study in Rats

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*'In principio erat Verbum, et Verbum erat apud Deum, et Deus erat Verbum'*

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*"When at a crossroads, my father was fond of saying "go with your gut." "Intuition," he said, "always has our best interests at heart." It is a voice that can tell us who is friend and who is foe…Which ones to hold at arm's length…And which ones to keep close."*

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*"In the moment we're born, we're drawn to form a union with others. An abiding drive to connect, to love, to belong. In a perfect union, we find the strength we cannot find in ourselves. But the strength of the union cannot be known until it is tested."*

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2. **Brain regions involved in forgetting during spatial working memory (Poster – Société Française des Neurosciences- Lyon- 2013)**

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3. **The neuronal populations involved in forgetting during spatial working memory. (Poster - 8th FENS Forum of Neuroscience – Barcelona, Spain, 2012)**

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4. **Is the inhibition of the Dentate Gyrus required for the management of interference in working memory? (Poster - Symposium NeuroMem, Cargèse, Corsica, 2012)**

**Joseph M.A.,** Fraize N, Arthaud S, Parmentier R, Salin P.A, Malleret G.

## Abbreviations

ABC: Avidin-Biotin Complex
aCC: anterior Cingulated Cortex
AMPA: α-amino-3-hydroxy-5-metyl-4-isoxazolepropionate
AMPAR: AMPA Receptor
ANOVA: Analysis of Variance
AP: Anteroposterior
Arc: Activity Regulated Cytoskeleton
BC: Before Christ
BrdU: 5-bromo-2´-deoxyuridine
BSA: Bovine Serum Albumin
C: Control
CA: *Cornu Ammonis*
CA1: the hippocampal *Cornu Ammonis* subregion 1
CA2: the hippocampal *Cornu Ammonis* subregion 2
$Ca^{2+}$: Calcium
CA3: the hippocampal *Cornu Ammonis* subregion 3
CAMK: Calcium/calmodulin dependent protein kinase
CaMKII: Calcium/calmodulin-dependent kinase II
CP: Caudate Putamen (Striatum)
CRE: Cyclic-AMP Responsive Element
CREB: Cyclic-AMP Response Element Binding
C-V-C: Consonant-Vowel-Consonant
DAB: Diaminobendizine
DAPI: 4',6-diamidino-2-phenylindole
DCX: Doublecortin
DG: Dentate Gyrus
DTT: Dithiothreitol
DV: Dorsoventral
EC: Entorhinal Cortex
ECL: Epirubicine-Cisplatine-5-Fluoro-uracile
EEG: Electroencephalography
Egr: Early growth response
EMG: Electromyogram
ERK: Extracellular signal-regulated kinase
GABA: γ-aminobutyric acid
GluR1: AMPAR subunit glutamate receptor 1 (also referred to as GluA1)
H.M.: Henry Gustav Molaison
$H_2O_2$: Hydrogen Peroxide
HIWM: High Interference Working Memory
HRP: Horse Radish Peroxidase
Hz: Herz
IEG: Immediate Early Gene
IL: Infralimbic area
LEC: Lateral Entorhinal Cortex

LIWM: Low Interference Working Memory
LPP: Lateral Perforant Path
LTD: Long-term Depression
LTM: Long-term Memory
LTP: Long-term Potentiation
MAPK: Mitogen-activated Protein Kinase
MEC: Medial Entorhinal Cortex
MGluR: Metabotropic Glutamate Receptor
ML: Mediolateral
mPFC: medial Prefrontal Cortex
MPP: Medial Perforant Path
NaCl: Sodium Chloride
NMDA: N-methyl-D-aspartate
NMDAR: N-methyl-D-aspartate (NMDA) receptor
PB: Phosphate buffer
PBS: Phosphate buffered saline
PBST: Phosphate buffered saline Triton
PCaMKII: Phosphorylated Calcium/calmodulin-dependent kinase II
PERI: Perirhinal Cortex
PFA: Paraformaldehyde
PFC: Prefrontal Cortex
PI: Proactive Interference
PKA: Protein Kinase A
PKC: Protein Kinase C
PP: Perforant Path
PP2A: Protein Phosphatase type-2a
PrL: Prelimbic area
PS: Paradoxical Sleep
PSD: Post Synaptic Density
PTL: Posterior parietal cortex
PV: Parvalbumin Interneurons
PVA-DABCO: Polyvinyl alcohol-1, 4-Diazabicyclo [2.2.2] octane
RAM: Random Access Memory
RI: Retroactive Interference
RM: Reference Memory
RM: Reference Memory
RSP: Retrosplenial cortex
S1: Primary Somatosensory cortex
SAFE: Scientific Animal Food and Engineering
SDS PAGE: SDS Polyacrylamide gel electrophoresis
SDS: Sodium DodecylSulphate
SEM: Standard Error of the Mean
SRE: Serum Response Elements
STM: Short-term Memory
SWS: Slow Wave Sleep
T: Trial

TA: Temporoammonic pathway
TBS: Tris Buffered Saline
TBST: Tris Buffer Saline Triton
vCA1: CA1 field of ventral hippocampus
vCA3: CA3 field of ventral hippocampus
VSCC: Voltage-sensitive Calcium Channel
VTA: Ventral Tegmental Area
WM: Working Memory
YHIWM: Yoked High Interference Working Memory
YLIWM: Yoked Low Interference Working Memory
YRM: Yoked Reference Memory
Zif268: Zinc finger binding protein clone 268

## French summary

Depuis de nombreuses années, les scientifiques ont étudié les bases neurales de la mémoire. Cependant, une question clé demeure: comment le cerveau distingue t'il les informations suffisamment importantes pour être consolidées en mémoire à long terme des informations stockées de manière temporaire en mémoire à court-terme/mémoire de travail, et qui doivent être effacées afin de ne pas saturer nos ressources cognitives. Contrairement à l'opinion populaire qui considère l'oubli comme nuisible à notre mémoire, de nombreux travaux suggèrent que l'oubli est un processus adaptatif essentiel permettant le filtrage des informations non-essentielles qu'on peut stocker de manière temporaire. Étonnamment, on connaît peu de choses des bases cellulaires et moléculaires de cet oubli adaptatif.

Le travail présenté dans cette thèse vise à déterminer les bases de cette forme d'oubli adaptatif, en particulier de celui nécessaire au traitement des informations en mémoire de travail. Pour ce faire, nous avons adopté une approche comparative en testant des groupes de rats dans un labyrinthe radial dans trois paradigmes comportementaux visant à évaluer trois processus cognitifs différents: 1) la mémoire de référence (RM - ou mémoire à long-terme), 2) la mémoire de travail (WM) impliquant, ou non, 3) le traitement des interférences en WM. Cependant, nous avons conçu nos tests comportementaux de telle sorte que chaque jour nos rats visitaient le même nombre de bras du labyrinthe permettant une comparaison claire entre les processus nécessitant le stockage à long terme ou à court terme d'informations (en RM ou WM) de ceux qui nécessitent l'oubli d'informations précédemment stockées en WM. En utilisant cette procédure, nous avons montré que les informations soi-disant stockées de manière temporaire à court terme en WM sont stockées de manière beaucoup plus longue, interférant plusieurs jours plus tard avec le stockage de nouvelles informations. Ce résultat remet donc en question l'existence d'un pur stock à court-terme de la WM. Ensuite, nous avons montré que le traitement de telles interférences pourrait nécessiter un contrôle spécifique et négatif du gyrus denté de l'hippocampe dorsal que nous avons visualisé sous la forme d'une inhibition de l'expression de marqueurs indirects de l'activité neuronale et de la plasticité synaptique, Zif268 et c-Fos (Chapitre II). Pour démontrer fermement le rôle du gyrus denté dans le traitement des interférences, nous avons lésé le gyrus denté de nouveaux rats et les avons testés dans nos trois paradigmes comportementaux. Nous avons montré que l'inactivation du gyrus denté perturbe bien la RM et WM, mais améliore le traitement des interférences en WM (chapitre IV). Enfin, nous avons essayé de déterminer les mécanismes présents au niveau du gyrus denté qui seraient impliqués dans la mémoire et l'oubli. Par conséquent, nous avons étudié l'implication des nouveaux neurones, des interneurones et des mécanismes de plasticité synaptiques en RM, WM et dans les processus d'oubli adaptatif (chapitre V).

Avec cette thèse, nous avons ainsi montré que le gyrus denté est une structure clé responsable du traitement des informations non pertinentes en mémoire, un processus essentiel qui permet une utilisation optimale de nos ressources cognitives. Nous pensons que ces travaux nous aident à mieux comprendre comment le cerveau gère les interférences, mais également à identifier les mécanismes responsables de l'oubli « utile » d'informations.

## English summary

For many years, scientists have been investigating the neural bases of memory. However, a key question remains unanswered: how does the brain distinguish information important enough to be consolidated into long-term memory from information required only temporarily, and that needs to be cleared away for not saturating our cognitive resources. In contrast to the popular view considering forgetting as deleterious to our ability to remember, forgetting might be an essential adaptive process allowing the filtering of non-essential information. Surprisingly, very little is known on the cellular and molecular bases of adaptive forgetting.

The work presented in this thesis aims to find a way to determine such bases of adaptive forgetting, in particular in the context of Working Memory processing. To do so, we adopted a comparative approach by training groups of rats in a three different radial maze paradigms aimed at testing three different cognitive processes: **1**) Reference Memory (RM), **2**) Working Memory (WM) and **3**) the processing of interference in WM. However, we designed these paradigms so that each day, rats in all conditions visited the same number of arms. This allows a clear comparison between processes requiring the long-term or short-term storage of information (in RM or WM) and those requiring forgetting of previously stored information in WM. Using this procedure, we first showed that information supposedly stored in short-term/WM can outlast their purpose by interfering, several days later, with the storage of newer information, thus questioning the existence of a pure short-term memory store. We then showed that the processing of such interfering previously stored information might require a specific and negative control of the dentate gyrus of the dorsal hippocampus materialized by an inhibition of the expression of indirect markers of neuronal activity and synaptic plasticity, *Zif268* and *c-Fos* (Chapter II). To firmly demonstrate the role of the dentate gyrus in the processing of interference, we lesioned the dentate gyrus of a new group of rats and tested them in our three behavioral paradigms. We showed that inactivating the dentate gyrus impairs both RM and WM, but improves the processing of interference (Chapter IV). Finally, we tried to unravel the mechanisms in the dentate gyrus possibly implicated in memory and forgetting. Consequently, we studied the involvement of new neurons, interneurons and plasticity mechanisms in processing memory over the long term, the short term and the forgetting of useless information (Chapter V).

With this thesis, we thus showed that the dentate gyrus is a critical node in processing the forgetting of irrelevant information, an essential process allowing optimal use of cognitive resources. Our work sheds light not only on the question of how the brain responds to interferences, but also on the mechanisms of "forgetting" what should be forgotten.

# CHAPTER I

# Literature review

*Far off from these, a slow and silent stream,*
*Lethe, the river of oblivion, rolls*
*Her wat'ry labyrinth, whereof who drinks,*
*Forthwith his former state and being forgets,*
*Forgets both joy and grief, pleasure and pain*
*(John Milton, Paradise Lost, 1674)*

When I was a little boy, my understanding of memory was as simple as the Monday school proverbs it hid behind. Neat, little cheerful slogans like 'We do not remember days; we remember moments' and 'Memory feeds imagination'. As I grew older, I realized that one of the keys to happiness is having a bad memory. For the truly wronged, real satisfaction can only be found in one of two places: infallible Memory or dreadful Oblivion.
This is not a story about Memory…

## I.1. Forgetting

Since the dawn of time, memory has intrigued and fascinated men. They tried to unravel the mystery of this faculty by which the mind stores and remembers information as defined in the Oxford Dictionary. The Greeks worshipped it as a goddess: Mnemosyne. She was a Titaness, the daughter of the sky and the earth. Zeus came to Mnemosyne on nine nights in a row and nine daughters, the muses, were the fruit of their unions. The muses were bestowed with many blessings: knowledge, eloquence, persuasion, mathematics, history, and astronomy. But Memory, Mnemosyne, was the mother of them all. The poet Hesiod (*c.* 700 BC) invoked the muses for inspiration; they revealed for him eternal truth and knowledge. Notwithstanding for most of us, who are not gifted the chance to mingle with the gods, remembering is a much more difficult and intricate process. At any time, our memory can fail us, and for many of us, forgetting is just the other facet of memory. For the Greeks, it was a distinct entity; the forgetfulness goddess Lethe is associated with the night. When a mortal is threatened by pain and suffering, he sought salvation and healing in forgetting.

If we now seek among philosophers those who have reflected on the interplay of memory and forgetting, we must move to a time as long after the birth of Christ as the Greek gods were before it. Moving through time we encounter Aristotle who likened the human mind to an empty slate and suggested that all humans are born without any knowledge and are only the result of their experiences. Such philosophical speculation was reformulated by 18$^{th}$ century British empiricist philosophers such as David Hartley who was the first to state that our memories were encoded through hidden movements in the nervous system.

In modern times there has been extensive research on memory and forgetting, mainly by psychologists who formulated several theories. In the late 19$^{th}$ century, fundamental facts about learning and memory were already discovered through empirical studies. Herman Ebbinghaus developed the first scientific approach to studying forgetting. By using himself as a subject, Ebbinghaus carried out a series of experiments where he memorized lists of meaningless three-letter nonsense syllables so that he could recall them by heart. Examples of such words are WUG, PIV or WAD. He used such nonsense words because relying on previously known words would have made use of his existing knowledge and associations in his memory. In order to test his recall of this new information, Ebbinghaus tried to relearn the list for periods of time variating from 19 minutes to 31 days. He measured the number of trials he needed to relearn the lists and then calculated the savings in relearning the lists he had learned earlier. The percent savings was the difference in trials for original learning (say, 10 trials) minus those required for later relearning (say 3) divided by the original learning trials (10-3/10 = 70%). He then published his findings in 1885 in 'Über das Gedächtnis' which means 'On Memory' translated later in English to 'Memory: A Contribution to Experimental Psychology'. The Ebbinghaus forgetting curve, a chart that resulted from this work, revealed a relationship between forgetting and time. Ebbinghaus noted that the forgetting curve appeared in a logarithmic shape (**Figure I.1**) (Ebbinghaus, 1964).

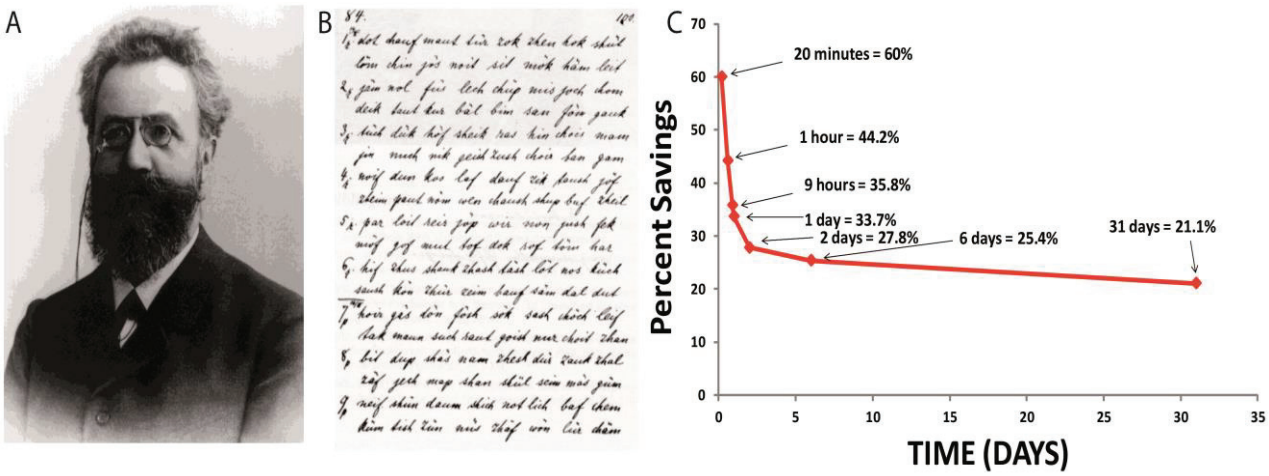


**Figure I.1: A bit of Ebbinghaus**. **(A)** German psychologist Hermann Ebbinghaus, a pioneer in the experimental study of forgetting. In 1885, he published his book Über das Gedächtnis ("On Memory", in which he delineated experiments he carried out on himself to analyze the processes of learning and forgetting. **(B)** Lists of nonsense syllables that are composed of a consonant-vowel-consonant (C-V-C) that does not spell anything in German. Ebbinghaus constructed 2300 of these items and then proceeded to memorize them in lists of 20. **(C)** The forgetting curve adapted from Ebbinghaus (1885/1964, 99. 67-76). The mathematical form of this curve is close to a power law, which declines rapidly at first but declines at slower rates as time goes on. Ebbinghaus found that after certain periods he remembered only a percentage of the original list he studied: after 20 minutes, he remembered only 60% of what he had learned, after an hour 44,2%, after 24 hours 33,7%, after 31 days 21,1% etc... In other words, within a month, nearly 80% of the learned content had been lost. Adapted from (Wixted and Ebbesen, 1991).

It seems that as much as we do remember, we forget even more. Experiences that were once salient and vivid in memory can become impossible to retrieve over time. One of the most intriguing aspects of human memory is undoubtedly forgetting. Our brain is jammed with an enormous amount of memories that we have formed over a lifetime of experiences. These memories range from the profound “who am I?” to the most trivial “where have I parked my car?” Our memory is constantly retrieving information but we are not usually aware of it; it is just forgetting that draws our attention (Ruiz-Vargas, 2010).

Many definitions were given to the term ‘forgetting’; personally I find Tulving’s definition the most plausible. Endel Tulving defined the term forgetting as “the inability to retrieve at present any information which was successfully retrieved in a previous occasion” (Tulving, 1974). In other words, forgetting refers to the loss of information or the inability to access previously encoded information within memory.

For more than a century, researchers have been trying to answer one big question: Why do we forget? Here are the main theories on the subject (**Figure I.2**).

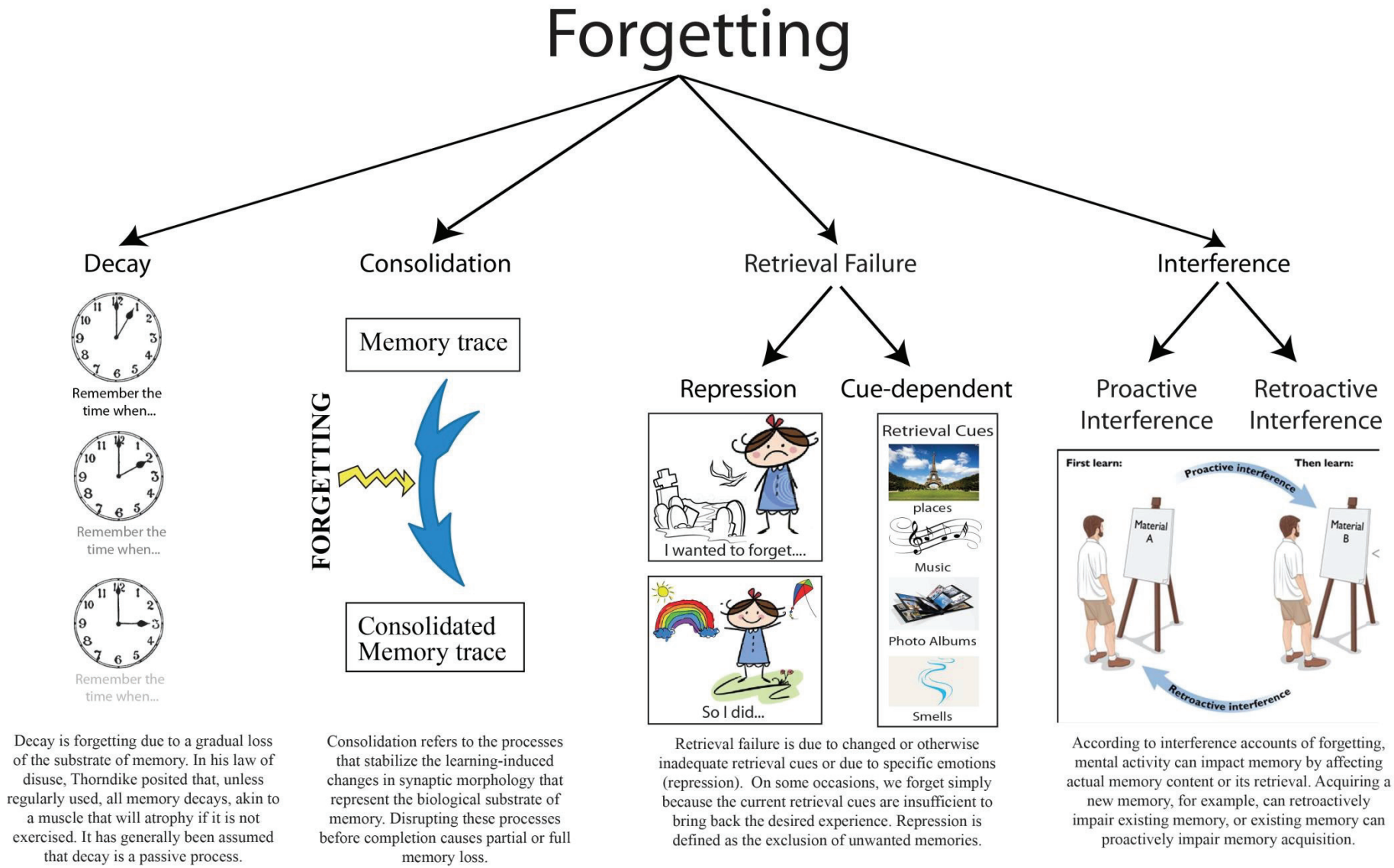


**Figure I.2: A taxonomy of forgetting theories.**

## I.1.A. Theories of forgetting

*The existence of forgetting has never been proved; we only know that some things do not come to our mind when we want them to' (Friedrich Nietzsche, 1844-1900)*

### a. Decay theory

Thorndike (1914) invented the term decay theory following the work of Ebbinghaus. The main idea driving his theory is that forgetting would be caused by the spontaneous weakening of memory representations with time. For Thorndike and his followers, memory disappears due to the mere passage of time. If information is not retrieved and rehearsed, it will eventually be lost (Thorndike, 1914). This theory is appealing because it is easy and simple to understand. However, it does not hold up. This theory tends to say that forgetting happens without specifying a mechanism by which it occurs. Time alone cannot be the answer; something else must happen (Lewandowsky et al., 2004). Take for example a fading pair of jeans. Their fading was not caused because these jeans have been around for a while. It was induced by chemical reaction with cleansing agents and light. So it is the case of memory: time alone is not responsible for the direct fading of memory traces. Forgetting must be induced by some mechanisms that occur during the passage of time. McGeoch (1932) attacked the decay theory and argued that when the passage of time is controlled, the amount of forgetting could be influenced by the quantity of interfering information given to the subject during that time; the more information, the greater forgetting (McGeoch, 1932). He pointed to Jenkins and Dallenbach's experiment (1924) showing less forgetting of nonsense syllables after resting (sleep) periods than after the same period of wakefulness. Although the role of sleep in memory and forgetting was not very clear at the time, Jenkins and Dallenbach's experiment delivered the last blow to the decay theory and scientists thus searched for other causes of forgetting (Jenkins and Dallenbach, 1924).

### b. Consolidation

Memory Consolidation refers to the progressive post-acquisition stabilization of long-term memory, as well as to the memory phase(s) during which it takes place (Dudai, 2002). It means that a memory trace is labile, may last only for a short time and if not consolidated, could be forgotten. John Wixted (2004) is one of the biggest defenders of the consolidation theory of forgetting. First, he argued that the secret of forgetting might lie in consolidation or more precisely in the lack of consolidation. "Consider the form of the forgetting curve" he said; "the forgetting rate is higher for recent - labile - memories and lower for older memories". He thus pointed to Jost's second law supporting the idea that older memories are more resistant to forgetting because they have had more time to consolidate (Wixted, 2004b, a). This theory comes from the fact that when two memory traces are equal in strength, forgetting appears to be faster for the recent of the two because this recent trace did not have the time to consolidate. Wixted also pointed to data from patients with retrograde amnesia, which involves impaired memories for events that occurred before the onset of the amnesia. The memory loss observed in these patients is larger for memory events occurred shortly before the onset of amnesia than for older events, a pattern usually referred to as the Ribot gradient. The most recently formed memories, not yet consolidated, are the most impaired.

However, like the decay theory, the consolidation theory seems to ignore an important and obvious fact about forgetting: that forgetting can be transient. I may forget today the lesson I have learned yesterday to finally be able to recall it tomorrow. This instance of transitory forgetting cannot be explained by permanent damage in the encoding or consolidation of the learned information as I would not be able to retrieve later the information I lost (not consolidated) today. Other factors must account for forgetting.

**c. Repression**

Austrian neurologist Sigmund Freud once wrote, "The essence of repression lies simply in turning something away, and keeping it at a distance, from the conscious" (Freud, 1915). Envisage a person living an extremely painful or threatening experience (an abuse or a death for example). The memory of this event associated with great anxiety could be forgotten in order to protect one's state of mind. Freud (1914/1957) argued that threatening memories are forced into the unconscious mind so that our conscious mind is protected from them. The repressed memory is temporarily forgotten but might still be accessible. The idea of repression played a central role in certain aspects of psychology; however it was confronted with many criticisms. One study showed that repressed memories of some patients turned out to be false (Lief and Fetkewicz, 1995). Moreover, repression theory is limited and cannot account for all types of forgetting, in that most of the information we forget everyday does not relate to traumatic events.

**d. Cue-dependent forgetting**

Cue-dependent forgetting means that information is stored in memory but there are no appropriate cues to retrieve this memory. In other words, the memory is available but not accessible. Many of us have had the experience of flashback memories when we return to our old house or school. Endel Tulving (1974) supported this theory of forgetting and said 'When we forget something we once knew, it does not necessarily means that the memory trace has been lost; it may only be inaccessible'. Tulving distinguished between state dependent forgetting (internal cues as physical state: happy, sad, tired… being present when the information was encoded are required to be present for recall of this information) and context dependent forgetting (external cues as environment: old house/school, music, smells… being present where the information was encoded are required to be present for recall of this information). In an experiment to test cue-dependent theory of forgetting, divers were asked to learn a list of words, either underwater or on land. When tested for retrieval, divers who learned the list underwater and tried to recall it underwater recalled much more words than those who learned it underwater but were tested on land (Godden and Baddeley, 1975) proving the importance of the environmental cues in helping the recall. However, our brain is flexible enough to recall in a context "B" information learned in a context "A". A child does not need to be in the classroom to tell his father what he learned at school today. Forgetting may thus depend on other factors than a difference between the training and testing contexts.

### e. Interference

*"Every time I learn something new it pushes some old stuff out of my brain. Remember when I took that home winemaking course, and I forgot how to drive?" (Homer Simpson, the Simpsons)*

While many theories have been developed and tested to explain why we forget, the interference theory has clearly dominated this field of research throughout the 20th century. The interference theory of forgetting suggests that we forget an item or event "A" because other information learned is interfering with our ability to recall this item or event. In other words, forgetting occurs because other memories interfere with and disrupt one another (Jenkins and Dallenbach, 1924). According to this theory, our ability to recall what we are learning now can be disrupted by either subsequent learning or by what we have learned before. In this context, we can differentiate between retroactive interference and proactive interference. Retroactive interference refers to new information interfering with the recall of information learned in the past. In contrast, proactive interference refers to past information interfering with the recall of new information more recently learned. For example, consider the deceptive task of a waiter in a restaurant, without any paper support, taking many orders in a day. If he has one or two tables to wait on, recalling the orders could be fairly easy. If he is jammed with plenty of customers, however, he may find himself giving mustard to the person who ordered ketchup, because a while ago another customer -maybe with similar features- asked for ketchup (here, mustard plays a role of Proactive Interference). Further, if the manager asked him what a client previously ordered, he would almost certainly fail to recall the command, as though the intervening posterior orders had overwritten the past ones (the new orders play a role of Retroactive Interference). Here, I will describe some of the designs used in humans and rats to examine these two most important culprits of memory: Retroactive interference and Proactive interference, the latter being the focus of the current dissertation.

#### i. Retroactive interference

Retroactive Interference (RI) refers to the forgetting induced by events that follows and interferes with the target memory to remember. In other words, addition of new information to memory results in a difficulty to retrieve older information.

Studies have shown that two significant factors could determine the amount of retroactive interference: the similarity of the interfering materials and the time between the target information and the interpolated material. Robinson (1920) tested the first factor and gave subjects a list of numbers. Between presentation and recall of these numbers, Robinson gave the participants another list of consonants, poetry, multiplication, photos or more numbers. He found that subjects who were given another set of numbers remembered a lot less of the original list than those who were given the different sets. Robinson concluded from this experiment that the interfering material alone does not induce forgetting, it is the quality of the material that does (Robinson, 1920). Other authors have found that as the interfering material increased in similarity to the target, the retroactive interference increases as well (Lund, 1926, Skaggs, 1926, Cheng, 1929). The amount of time between the original stimuli and the interfering material is also an important factor in inducing RI. Spencer (1924) found significant differences when he tested a lapse of 9 seconds or 20 minutes between the original

list of nonsense syllables and a second interfering list. Those who had just 9 seconds between the 2 lists exhibited more forgetting than those who had 20 minutes. He concluded that the amount of time between the original stimuli and the interpolated list is an important factor that affects RI (Spencer, 1924).

Probably the most well known experiment known to induce RI is the classic A-B, A-C paradigm (**Figure I.3**). On such paradigm, subjects first study a list of association between two words (e.g., Book-Water, Door-Car, etc…) until they can recall them perfectly. The test is to recall B (the second word) when given A (the first word) as a cue. Then, subjects learn a conflicting list A-C (e.g, Book-Room, Door-Dog, etc…) until perfect recall. So now, new responses C are paired with the same cues A. In a control condition, participants learn the A-B list until one perfect recitation but they were not given an interpolated list, or they were given a complete different list (say, C-D). After both groups have learned their lists, the test consists of receiving the original A cue (Book, Door…) with instruction to recall items from the first list B (Water, Car…). Authors found that subjects who have learned the A-B, A-C association recalled less word from the original list on the test condition than the control groups (McGeoch and Irion, 1942). This outcome defines RI.

Retroactive interference is not limited to human; rats can be sensitive to interfering activity as well. Rats can be tested on an eight-arm radial maze. The maze consists of an octagonal central platform from which radiates 8 identical arms. At the end of the arms, hungry rats are trained to find food in wells (Olton and Samuelson, 1976). It has been shown that rats use their spatial memory in order to respond correctly.

In a study, rats were allowed to visit four baited arms, and then they were submitted to different interpolated tasks (sounds, smells, odors and visiting four arms in a maze on a different room) before returning them to the maze to test their memory for the unentered arms. Control group was not subjected to the interpolated tasks. The authors found that the experimental rats were not different from the control and the interpolated tasks did not induce RI even the one in the different maze, which is a very similar activity (Maki et al., 1979). Many other authors did not find RI in rats placed in other mazes as an interpolated experience and this was particularly striking when compared to human studies (Beatty and Shavalia, 1980a). In some further experiments, W. Roberts (1981) forced rats to run in four rewarded arms in the maze. The interpolated condition consisted of placing the rat directly into the end of the remaining four arms it had not entered and allowing it to retrieve the food. At test, the rats now failed to choose the correct unentered arms. The author concluded from this result that significant RI could also be observed in rats (Roberts, 1981). However, one might object that rats having already retrieved the remaining food pellets during the interpolated condition might not be motivated to go back in the same arms during testing. The jury is thus still out concerning the role of retroactive interference in animals' forgetting. Unlike RI, Proactive Interference showed an important forgetting effect in humans as well as rodents.

### ii. Proactive interference

Proactive Interference (PI), the phenomenon by which information learned previously will disrupt subsequent learning, is a very common cause of forgetting and the focus of this thesis. It has been shown that subjects show great hindrance in recall when the number of prior trials increases. The magnitude of PI will thus depend on the strength of the interfering information previously stored in memory. Keppel and Underwood (1962) showed that when participants are presented with numerous lists, and when the number of these prior lists increased, their recall decreases substantially (Keppel and Underwood, 1962). The magnitude of this PI effect varies as a function of the resemblance and the time between the tests in the same way that RI does, with PI being most severe when lists share the same retrieval cues. Thus, the number of prior trials is more effective in inducing PI when the items presented are similar to the target list. Loess H. (1968) found that the build-up of PI is influenced by item similarity. He presented subjects with list of words from a certain category (e.g., plants) and then gave them subsequent lists from the same or different taxonomic category. He found that when the lists are similar, there was a large decrease in correctly recalled words showing that PI is influenced by items similarity (Loess, 1968). PI depends also upon the temporal proximity of prior items to the target one. Loess and Waugh (1967) varied the time between the previous lists of words and the to-be-remembered list. They found that with small intertrial interval the PI effect was high. On the contrary, there was little PI when the interval was prolonged and beyond 300 seconds interval, there was no PI (Loess and Waugh, 1967).
The A-B, A-C paradigm is also used to study PI and **Figure I.3** depicts the design that allows the study. The difference with the A-B, A-C paradigm used to study retroactive interference resides in presenting the list 1 (the A-B association), then the list 2 (participants are asked to give the target C, when given the word A), and test people's memory for the second list (A-C). In a control condition, no A-B list was studied. Researchers found that when participants had learned 20 lists before learning the last target list, they remembered only 20 % of this final list a day later. This design allows studying how previously learned information might disrupt our ability to recollect new knowledge (Underwood, 1957).

In rats on the other hand, in order to induce PI, massed trials were performed. Roberts and Dale (1981) found that the percentage of correct responses for rats collecting food in an 8-arm radial maze decreases as the number of prior trials increases. Thus, rats are less accurate in their arm selection in the radial maze after successive visits (Roberts and Dale, 1981). Furthermore, similarity between these trials plays an important role in inducing PI like in humans. It is important to recognize that PI was found in a situation in which a rat had to repeatedly choose among the same arms it had just entered on previous visits (Roberts, 1992). Cohen, Reid and Chew (1994) changed the visual characteristics of the distal landmark cues between different trials and found an attenuation of this disruptive effect of PI. Intertrial interval was shown to modulate PI in rats as well. The study from Cohen, Reid and Chew showed that increasing the intertrial interval from 2 minutes to 2 hours eliminated the impairment caused by PI and decreased the tendency of the rats to enter previously visited arms (Cohen et al., 1994).

PI in humans or animals thus share the same characteristics and could be responsible for forgetting. But another reason to be interested in PI as a cause of forgetting resides in the fact

that it could be adaptive, a fact that has not received much attention in research with animals (Kraemer and Golding, 1997). For example, we suffer from PI when we fail to recall our new Visa card PIN code because our old identification digits intrude during the recall process. Therefore, forgetting our old number is important, useful and adaptive so that we can successfully remember the new one and be able to retrieve money without getting our card blocked for security reasons. The adaptive role of forgetting has unfortunately been poorly studied and much work is still required to understand this crucial cognitive process.

**Retroactive Interference**

| Group | Learn | Learn | Test |
|---|---|---|---|
| Experimental | A-B (e.g. Book-Water) | A-C (e.g. Book-Room) | A-B (e.g. Book-Water) |
| Control | A-B (e.g. Book-Water) | - | A-B (e.g. Book-Water) |

**Proactive Interference**

| Group | Learn | Learn | Test |
|---|---|---|---|
| Experimental | A-B (e.g. Book-Water) | A-C (e.g. Book-Room) | A-C (e.g. Book-Room) |
| Control | - | A-C (e.g. Book-Room) | A-C (e.g. Book-Room) |

**Figure I.3: The A-B, A-C paradigm.** A method to test for retroactive and proactive interference. Note that on the test, only the first word is supplied and the participants must provide the second word (adapted from the book Psychology: An international perspective).

## I.1.B. Adaptive reasons for forgetting

*"If we remembered everything, we should on most occasions be as ill off as if we remembered nothing" William James, 1890*

Give her a date between 1974 and today and she can instantly tell you what day of the week it was, what she did on that day, and any major events that took place -or even minor events- as long as she heard about them that day. Jill Price is one of about 20 subjects positively diagnosed with the hyperthymesia syndrome. She is the woman who can't forget (Price and Davis, 2008). While most of us regard her memory as a gift, she, however, perceives it as "a burden". In contrast to the popular view considering forgetting as deleterious to our ability to remember, in Jill's case forgetting would have been -if it existed- an essential adaptive process allowing her to filter non-essential information. As early stated by Ribot (1882): "forgetting, except in certain cases, is thus no malady of memory, but a condition of its health and its life" (Ribot, 1882).

What we discussed above were theories of forgetting (trace decay, lack of consolidation, interference…) that try to explain why our memory fails us. However, an important question remains: do all instances of forgetting constitute processing failures? Obviously, the answer is "no". As we said before, irrelevant details about our daily lives are better left out for not cramming our brain system. Bjork and colleagues (1972) pointed to data from experiments showing retrieval inhibition by designated forgetting. They presented participants with items designated to-be-remembered and other items to-be-forgotten. They found that subjects decreased their recall when they were instructed to forget, and increased it for the to-be-remembered items. They concluded that this forgetting is adaptive and induce a suppression and destruction of outdated information in memory in order to remember current, more essential information effectively. "Designated forgetting" would thus naturally occur when we know that some information is to be discarded (Bjork, 1972).

Kraemer and Golding (1997) argued that some forms of forgetting in animals could be adaptive as well. They proposed that animals sometimes forget because they are designed to do so in order to enhance their behavioral responses. Take for example the extinction of freezing response in fear conditioning. After pairing neutral stimulus like a sound with an aversive stimulus like a foot shock, a fear response will be generated and measured behaviorally by a freezing response (immobility of the animal). However, later on, when the animal is presented repeatedly with the sound in absence of the shock, an extinction of the fear response will take place. The animal adaptively inhibits its freezing response because it is no longer necessary and the animal must discard the outdated information to respond to the change of the task (Bolles, 1985, Bouton, 1991, 1993). Finally, Kraemer and Golding pointed out to data from proactive interference in animals. They argued that after storing two conflicting memories in succession, adaptive forgetting reflects an efficient and powerful strategy for dealing with these conflicting memories. Thus, forgetting is not always deleterious and a failure process, animals -and humans- forget because they are designed to do so.

The main purpose of my dissertation is to study a form of adaptive forgetting when the animal is presented with too many similar trials. These trials will induce Proactive Interference and the best strategy for the rat is to forget (adaptively) previous irrelevant trials in order to

respond correctly to the ongoing one. Despite a growing emphasis on the study of forgetting, a differentiation and comparison on the interrelationship between forgetting and memorizing has not been sufficiently considered in recent animal research. In order to study and understand how forgetting works, it must be related and compared to the mechanism happening before the occurrence of forgetting. In order to study forgetting, we must understand Memory!

## I.2. Memory

*"You have to begin to lose your memory, if only in bits and pieces, to realize that memory makes our lives. Life without memory is no life at all… Our memory is our coherence, our reason, our feeling, even our action. Without it, we are nothing." Luis Buñel, Memoirs*

Just as there are two sides to every story, there are two sides to our Memory… Forgetting is one side that scares us. We yearn for the other side, for the comfort of Memory because it provides knowledge and experience, allowing us to recognize, to define what's before us. But what is it we are afraid of, really? Not the forgetting (process) itself… but losing memories. We always move forward with time. From the day we are born till death do us part, from our first breath till the last, we are young before growing old, and there is no going back to yesterday. The only exception is Memory. Memory is 'the ability to retain and utilize information or knowledge acquired in the past'. Thus, memory constitutes a fundamental basis of humans and animals behavior. It is an integral part of our existence that plays an important role in every aspect of our daily lives and enriches our experiences with meaning. Understanding the neuroanatomical substrate of memory is thus extremely important to find cures against diseases affecting memory functions.

Memory comes in different shapes, and each shape involves distinct neural systems and cellular changes that take time to emerge and then to persist (Nadel and Hardt, 2010). The first studies on memory date back to the nineteenth century with Ebbinghaus (1885) and his saving curve. William James (1890) was the first to distinguish between two memory systems one for the short-term and the second for the long-term. Müller and Pilzecker (1900) found that memories take time to consolidate and with them emerged the memory consolidation theory. Then, scientists started to investigate patients who suffered from brain damage and especially those who were amnesiac. These patients hugely advanced our knowledge about memory systems. Finally, in modern times, researchers started using animal models in order to study memory and its cellular and molecular underpinnings.

In this chapter, I will discuss the different forms of human memory with a focus on their temporal dimension, their analogy to rodents' memory and the experiments carried out in order to evaluate them. I will focus on spatial memory in rats and will not be able to discuss at length other forms of memory, such as emotional and procedural memories.

## I.2.A. Declarative Memory versus Procedural Memory

### The unforgettable amnesiac Henry Molaison (H.M.)

I will start my chapter to talk about a man who changed our understanding of memory systems and we shall be forever in his debt. Henry Gustav Molaison (1926-2008), known widely as the patient H.M., is a man without memory. Studies on this patient who underwent a medial temporal lobe resection as a treatment for epilepsy, led to the first insight that there are different memory systems in the human brain. At around the age of ten, Henry started to have epileptic seizures probably because of a bike accident. These seizures increased in intensity disrupting his performance at school and affecting his health and social life. As an adult, no anticonvulsant medications could alleviate his seizures, compromising his ability to work and to lead an independent life. In 1953, at 27 years old, Henry decided to undergo a surgery performed by the neurosurgeon William Beecher Scoville. The surgery was intended to remove portions of Henry's medial temporal lobes, including a large part of the hippocampus, which Scoville believed to be the source of his seizures. Following surgery, H.M. was virtually unable to form new memories for events and facts. He would meet a person he had talked to, at length, the day before and have no recollection of ever seeing him before. This condition that persists to his last day is known as anterograde amnesia (Scoville, 1954, Scoville and Milner, 1957, Corkin, 1984). Furthermore, Henry was reported to suffer from a temporally graded retrograde amnesia meaning that experiences recently formed prior his surgery were more severely impaired than remote memories he had formed years earlier (he only remembered events from his childhood).

In contrast to his inability to form new memories, H.M. had intact motor learning abilities. In a 1962 experiment, Brenda Milner trained H.M. in a mirror drawing task. This task involves tracing a five-pointed star on a paper by only seeing the mirror image of the drawing. Normal people found it a hard task at the beginning, but with practice, they tend to improve their results. Henry exhibited a very similar improvement although he could not explicitly remember the learning episodes (Corkin, 1968).

This study had a simple but revolutionary conclusion. As Henry was not able to consciously recall the events he is living through but seemed to be able to unconsciously remember how to perform certain motor tasks, our brain must contain at least two separate and independent memory systems. Cohen and Squire (1980) proposed a distinction between declarative and procedural memory (Cohen and Squire, 1980) (**Figure I.4**). Declarative memory depends on the integrity of the hippocampus and related structures as it was disrupted after H.M's surgery. Tulving (1972) further subdivided declarative memory into episodic memory and semantic memory. The latter relates to the memory for facts (e.g. knowledge about the world) and the former is the memory for events or experiences (e.g. knowledge about one's personal life) (Tulving, 1972). On the other hand, procedural memory is thought to be hippocampally-independent, is typically only expressed through performance and is not accessible through conscious faculties (Such as some skills and dispositions, priming, habitual and procedural behavior) (Squire and Zola, 1996). Numerous other similar distinctions have been made. A similar distinction was made between explicit memory (responsible for intentional or conscious recollection and impaired in amnesia) and implicit memory (responsible for non-intentional recollection tasks and spared in amnesia) (Schacter, 1987).

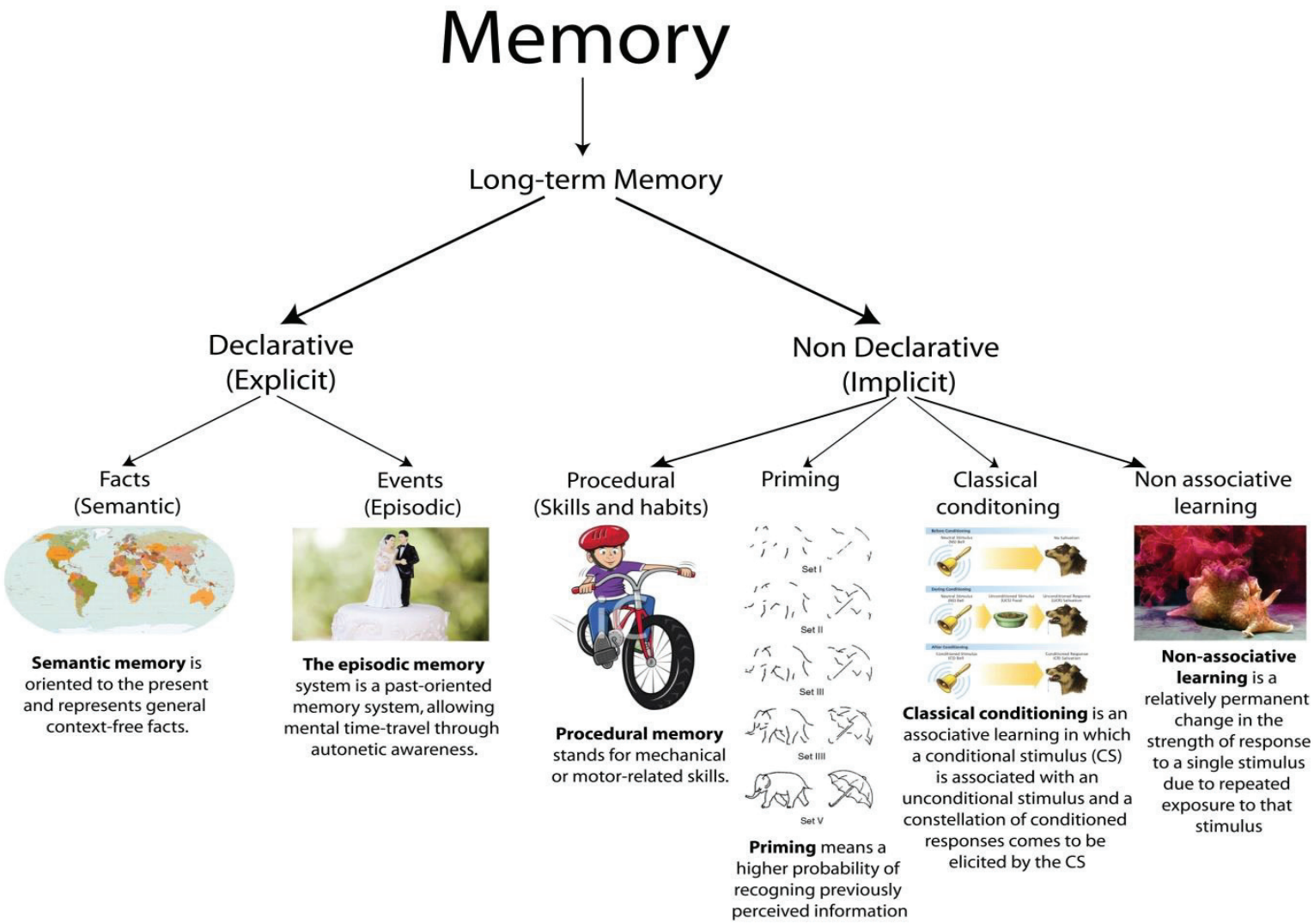


**Figure I.4: A taxonomy of memory.** It is generally accepted that non-declarative (procedural/implicit) memory is independent of the hippocampus. Which aspects of declarative (explicit) memory are hippocampally-dependent is somewhat controversial (Adapted from Squire, 2004).

## I.2.B. Short-term Memory versus Long-term Memory

H.M. suffered from anterograde and retrograde amnesia after damage to the medial temporal lobe and in particular the hippocampal formation. However, according to early accounts of his condition, he seems to exhibit intact performance on short-term memory. Hence, he was capable of retaining immediate memories in his mind, but instantly when his attention was routed, they were gone (Milner et al., 1998b). These findings along with others have suggested a cardinal distinction between memories that last for a relatively short time and those that last for a long time period. These two types of memory are normally referred to as short-term (STM) and long-term memory (LTM). A century before, William James distinguished 1) primary memory, which constitutes our current conscious state of mind, and 2) secondary memory, which constitutes knowledge from previous state of mind (William, 1890). Since the conception of these two forms of memory, there has been ample evidence for a dissociation of memory systems according to a temporal gradient in the central nervous system (Gerard, 1949, Hebb, 1949).

### a. Short-term Memory

*"Memory and forgetting are inextricably intertwined. Any account of short-term memory (STM) should address the following question: If three, four, or five chunks are being held in STM, what happens after attention is diverted?" Paul Muter*

Short-term memory is a restricted capacity store that holds information for a short amount of time. It can be for example assessed with a memory span task. This task consists in presenting an increasing list of numbers, nonsense syllables or letters and asking subjects to recall them immediately after presentation. Researchers found that an average person can recall between 5 and 9 items in STM. This limited number of items seemed so constant that George Miller (1956) described the human STM capacity by the phrase "the magical number seven, plus or minus two" (Miller, 1956). Over the years, other studies have shown that our short-term storage capacity is more limited than Miller had suggested, and that the magical number is more likely to be four (Cowan, 2000). It has been found that this limited capacity of our STM can be increased by a phenomenon known as chunking. Chunking enables us to group different items together (chunks) in order to increase STM ability (Ericsson and Simon, 1980, Ericsson and Chase, 1982). Hebb (1949) was the first to propose a plausible neurobiological substrate to STM. He argued that a stimulus is encoded by neurons that interconnect with each others creating cell assemblies. When a memory is created, neurons will recurrently stimulate each other in order for this memory to persist for a short time. For Hebb, STM was sustained by this continuous reverberatory activity in the neuronal networks representing the information to be stored (Hebb, 1949).

Müller and Pilzecker proposed more than a century ago that information is initially encoded in a labile (short-lived) modifiable state and sensitive to disruption. Later, this information is coded in a more permanent, persistent and long-lasting state. In other words, short-term memory is changed into long-term memory through a process of stabilization. This process of

perseveration has become known as consolidation, for which multiple neuro-driven processes and mechanisms have been proposed (Müller and Pilzecker, 1900).

STM is very adaptive and economic eventhough it has a very limited storage that seems to be a disability. Just imagine yourself memorizing every name you heard, every phone number, all trivial information acquired during your day. Your mind will be crammed with nonsense information. If short-term memory had unlimited capacity, one would constantly be distracted. Therefore, it is very important to forget all nonsense data and to remember only the gist that we need. Any information when judged to be pertinent is transferred via consolidation to the long-term storage known as Long-term Memory.

### b. Long-term Memory

*"From birth to death, the average person stores five hundred times more information than the Encyclopedia Britannica" Mathematician John Griffith.*

Long-term memory (LTM) is a storage system that has the capacity to retain large amounts of information for a long period of time with virtually no limitation in capacity. Memorizing information over the long-term requires rehearsal effort. Ebbinghaus (1885) found that the more time he spent studying his lists of nonsense syllables, the better his memory was. Furthermore, Ebbinghaus and others found that recalling was better when they rehearse the material until overlearning it (Driskell et al., 1992, Semb et al., 1993). Long-term memory is also better when the rehearsal is extended over long intervals of time than when it is cluttered and concentrated in brief periods. This phenomenon is known as the "spacing effect" (Dempster, 1988). Anderson and Schooler (1991) argued that this spacing effect is adaptive because in our daily lives it is more important to remember names and faces of people that recur over spaced period of time than those concentrated in short period (Anderson and Schooler, 1991).

According to Collins and Loftus (1975), the information in our long-term memory is organized in clusters by categories. They proposed the term 'Semantic network' to describe the storage of our memories. In other words, items are linked together by semantic relationship (Collins and Loftus, 1975). Romney, Brewer and Batchelder (1993) presented a list of 17 words collected from various homogeneous semantic domains (fruits, vegetables, furniture, vehicle…). They found that subjects remembered items as a function of their domain similarity (Romney et al., 1993).

Many studies were carried out to find the physical trace of information stored in LTM also named 'engram'. Scientists have thus directed their attention to 1) localize the brain regions and structures involved in the long-term storage of memory, and 2) understand the neural and molecular changes responsible for this localized storage. To do so, exciting developments and techniques were elaborated and scientists directed their research toward studying animal's memory.

## I.2.C. Working memory and Reference memory

In rats, a similar distinction in memory is made between Working Memory (WM) and Reference Memory (RM). RM refers to the long-term storage of information that remains constant over time and that is gradually acquired over many training sessions, whereas WM is a specific form of short-term memory that refers to the ability to retain information within a single trial (Olton, 1979). Olton and Samuelson (1976) developed an eight-arm radial maze in order to test these two forms of memory. The radial maze is one of the most commonly used methods for testing spatial learning and memory in rats (Olton and Samuelson, 1976, Levin, 1988). The tasks involving this apparatus already presented in the first chapter consist in placing a hungry rat in the center of the maze to freely retrieve food pellets hidden in wells at the end of the radiating arms. Rats had to learn to avoid re-visiting already entered arms (delayed-non-match working memory tasks) or to remember which arms are consistently baited (reference memory tasks).

The aim of what follows is to discuss some of the work that has been done to study these two forms of memory.

### a. Working Memory

In Short-term memory, information is kept for a limited amount of time until it fades or is transferred to a permanent state. Many researchers are critical of this traditional view that considers STM as a passive storage depot (Crowder, 1993). To conceptualize STM as an active mental workspace where information is processed and manipulated rather than passively maintained for a certain duration, Miller, Galanter and Pribram (1986) preferred to use the term 'Working Memory' that was used in the 1960s in the context of theories that likened the mind to a computer. In this context, WM is compared to a computer's RAM, the volatile memory where stored information is lost when the power is removed (Miller et al., 1986). Later, Alan Baddeley (1986) defined human WM as "a system for the temporary holding and manipulation of information during the performance of a range of cognitive tasks such as comprehension, learning, and reasoning" (Baddeley, 1986). In 1974, Baddeley and Hitch introduced and made popular the multicomponent model of working memory. This theory proposes the existence of a central executive that, among other things, is responsible for directing attention to relevant information, suppressing irrelevant information and inappropriate actions, and for coordinating cognitive processes when more than one task must be done at the same time. The central executive is thus responsible for the supervision of information integration and for coordinating the slave systems that are truly the components responsible for the short-term maintenance of information. One slave system, the phonological loop, stores phonological information (that is, phonemes, the sound of language) and prevents its decay by continuously articulating its contents, thereby refreshing the information in a rehearsal loop. It can, for example, maintain a seven-digit telephone number for as long as one repeats the number to oneself again and again. The other slave system, the visuo-spatial sketchpad, stores visual and spatial information. It can be used, for example, for constructing and manipulating visual images, and for the representation of mental maps. In 2000, Baddeley extended the model by adding a fourth component, the episodic buffer, which holds representations that integrate phonological, visual, and spatial information, and possibly

information not covered by the slave systems (e.g., semantic information, musical information). The component is episodic because it is assumed to bind information into a unitary episodic representation. The episodic buffer resembles Tulving's concept of episodic memory, but it differs in that the Baddeley's episodic buffer is a temporary store (Baddeley, 2000).

The anatomical basis of WM has been extensively studied for the past decades and researchers found an implication of many structures most importantly the prefrontal cortex, thalamus, medial-temporal region and the parietal cortex. Although this model has dominated the field of memory for a long time, many other models were developed to explain our ability to manipulate information over the short-term (Cowan, 1988, Cowan, 1995).

It is clear that the model of WM created by Hitch and Baddeley cannot be adapted to animals because the linguistic information treated by the phonological loop cannot be processed in non humans (Nadel and Hardt, 2010). The term WM itself was mentioned first in animal literature with the studies of Werner Honig in pigeons (Honig, 1978) and later David Olton's works on rats (Olton, 1979). Olton hypothesized that WM is required when "different stimuli govern the criterion response on different trials, so that the cue that the animal must remember varies from trial to trial". Olton first tested rats in the 8-arm radial maze by placing a rat in the center of the maze and allowing it to explore all the arms until it retrieves all food from the wells. Olton noticed that, in order to optimize their search strategy, rats avoid re-entering arms already visited and empty of food. This type of WM was defined as a short-term storage that enabled rats to keep track of which arms they visited on a given trial and allowed the animal to remember which arm it had visited before. This type of memory was labeled short term because it was only needed for the duration of the trial, and therefore for a short time. Olton observed an inability to obtain all of the food rewards in the radial maze when rats had their hippocampus lesioned. These rats made numerous repetitive entries to previously visited arms where the baits have already been retrieved demonstrating the importance of the hippocampus in storing information for even a short time (Olton and Papas, 1979, Olton, 1983).

Delayed alternation is one of the most used tasks to test WM in rodents. In a T-maze, rats are placed at the base and allowed to visit an arm to retrieve a food reward. Then, in a subsequent trial, animals are placed once again at the base of the maze and must enter the arm it had not entered before. The same principle rules the behavior adapted to perform the Olton's WM tasks that I just described. Rats must remember their previous location in order to select an alternative response and to alternate. It has been found that rodents and in particular rats have a natural tendency to alternate their choices on repeated trials (Tolman, 1925). Consider a squirrel, another rodent, who stores nuts in different hides for the winter months. This animal also displayed spontaneous alternation by choosing to explore a different hide after having depleted the reserve of a previous visited one. Within this context, spontaneous alternation appeared as a perfect adaptive evolutionary strategy to optimize the search for food in the wild. More specifically, it has been found that rats tended to alternate spatial locations and not body turns (Montgomery, 1952). When the rat alternates, it remembers the previously visited arm based on the extramaze spatial cues and uses this memory to correctly solve the task. It has been proved that rats use extramaze landmarks present in the room where the maze is

placed to solve the task because rearranging these landmarks between the first presentation and the choice phase disrupted performance (Dudchenko, 2004). Delayed alternation tasks have been extensively used to test WM and have been shown to be sensitive to the effect of hippocampal damage. Many authors have shown that lesions to the hippocampus induced a decrease in the delayed alternation rates (Racine and Kimble, 1965, Olton, 1979, Dudchenko, 2004).

An important aspect to be noticed about WM is that once retrieved, the information temporarily stored is better forgotten in order not to overload the brain with irrelevant things. Once noted on a piece of paper, what happens to the phone number your friend just gave you to store momentarily in your WM? What happen to the many different orders the waiter at the restaurant has to take? In this context, forgetting would play a central role in processing WM as WM's task is to hold information that is relevant only momentarily. Paul Dudchenko (2004) argued that WM is a form of short-term memory that once used must be ignored or forgotten. He hypothesizes that forgetting the arms a rat visited during previous trials is adaptive and useful for this animal in order not to disrupt performance in subsequent trials. Other forms of short-term memory may not require forgetting if they do not interfere with subsequent learning. Furthermore, Dudchenko argued that WM might not actually be a type of memory, but a type of forgetting (**Figure I.5**).

### b. Reference Memory

In contrast to WM, RM can be compared to long-term memory because it refers to the ability to store information about a specific fixed situation (Olton, 1979). It is the memory for rules and procedures that re-occur across specific situations. RM involves storing information that remains constant over time, such as the animal's memory of the geography of its territory. Once stored, this form of memory is believed to be relatively stable and resistant to interference. Several theoretical distinctions can be made between WM and RM. One is that WM is useful for only one-trial in an experiment and the animal is better off discarding these information once retrieved, whereas RM is trial-independent as the information available for performing the task is constant from trial to trial (Santın et al., 2003).
Several authors have used the radial maze apparatus to assess the rodent RM's ability to incrementally learn during several repetitive trials the association between a distinct location in space and the location of a food reward. In the radial maze, this can be achieved by exposing rats or mice to tasks where only certain arms of the maze are baited from trial to trial, and where the rat must remember this invariable location during repeated trials. Olton and Papas (1979) used a 17-arm radial maze, always baiting one set of four arms and leaving the other set unbaited (Olton and Papas, 1979). In this context, rats had to learn to avoid the unbaited arms by using their RM. Rat soon learned to never enter the predetermined unbaited arms because the set of arms used was the same from day to day. The information regarding the significance of these unbaited arms is thought to involve RM. Therefore, when a rat entered these unbaited arms, it maybe because of altered processing of information in RM. The task used by Olton and Papas was able to assess rat's WM ability as well. In fact, the rat had to visit the four baited arms without re-entering a previously visited one, as the arms were not rebaited between trials. A re-entry into a previously visited arm was considered a WM error. This training procedure allows one to examine the effects of lesions and drugs on these

two different forms of memory (Olton, 1978, 1983). Olton (1979) found that lesions to the hippocampus disrupt only the utilization of spatial information in WM, whereas lesions to this area did not impair RM. In this study, animals were initially trained without any lesion until they have learned the task perfectly. The rats then underwent the hippocampal lesion after learning the task and were subsequently tested to see if the hippocampal damage altered recall of information stored in RM and WM. Scientists concluded from this experiment that the integrity of the hippocampal formation might not be required for the retention of RM information but was certainly required for the initial learning of which arms to avoid. To support this claim, Jarrard (1978, 1986) induced a hippocampal lesion before testing rats in a RM task and found impairment when the hippocampus was completely lesioned or when the lesion was selective to the dentate gyrus. Altogether these results showed that while the initial acquisition of RM depends on the hippocampus, once learned the information in RM is stored elsewhere. Barnes (1988) suggested that these already acquired memories could require a reorganization of the memory trace in other cortical areas (Barnes, 1988). This pattern of memory reorganization is known as 'system consolidation'.

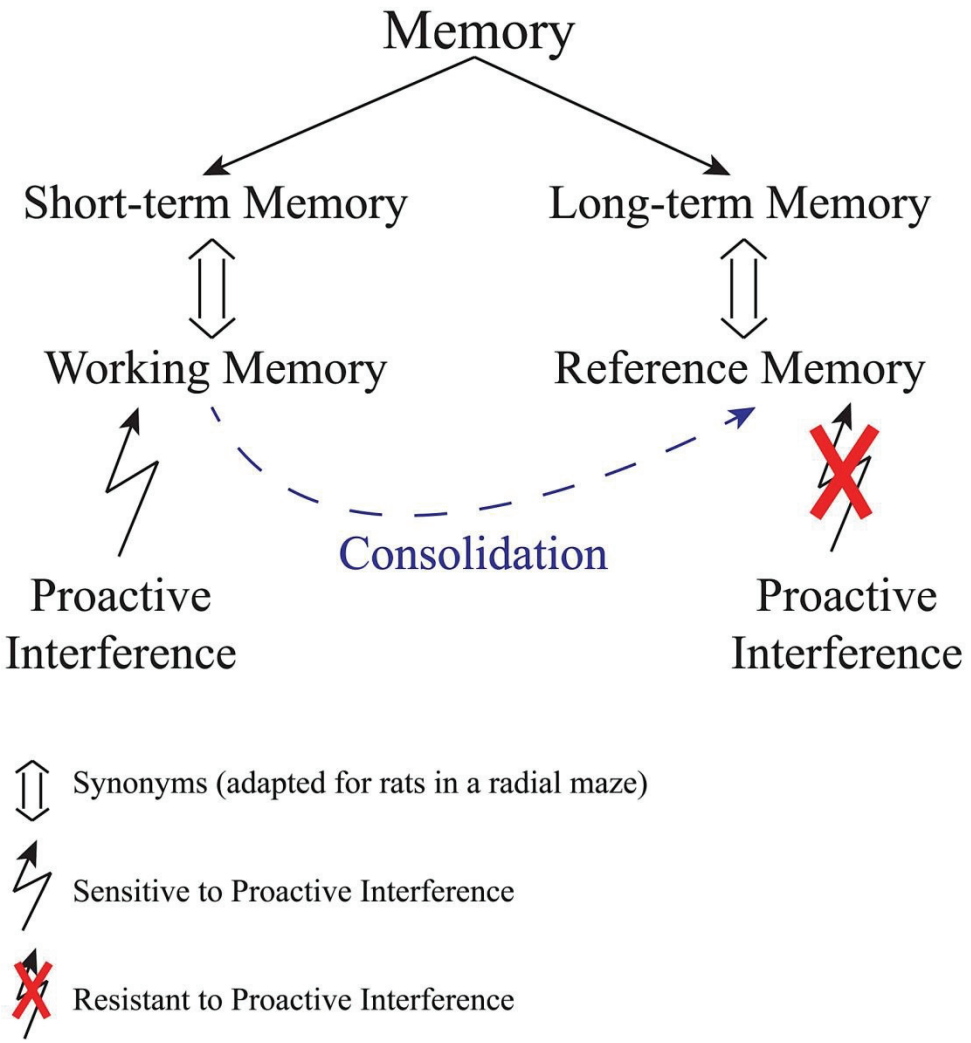


**Figure I.5: Memory temporal dimension.** A dissociation between short-term and long-term memory is widely accepted. Researchers who work with rats distinguished between Working memory and Reference memory. Working memory is sensitive to prior knowledge interfering with the learning and recall of new incoming information, whereas Reference memory is thought to be more or less resistant to this proactive interference.

### I.2.D. Theories of Memory consolidation

We have already seen that consolidation is a process by which labile memories are transferred into a stable state. Two theories are in the center of the consolidation debate: the "standard theory of consolidation" and the "multiple trace theory". The standard theory of consolidation assumes that the hippocampus is only required for a limited time in processing declarative memories until a complete consolidation process occurs in a more distributed neocortical network (Squire, 1984, Squire and Alvarez, 1995, Meeter and Murre, 2004). The data of Olton and Jarrard presented above seem to confirm such theory. The multiple trace theory, on the other hand, accepts the standard theory solely for semantic memories but believes that episodic memories always remain hippocampo-dependent and that a lesion of the hippocampal formation would always affect the recall of this type of memory, new or old (Nadel and Moscovitch, 1997, Fujii et al., 2000).

A study in rats that were tested in a reference memory task corroborated the standard theory of consolidation (Maviel et al., 2004). In order to find if the hippocampus remains active after consolidation of long-term memories, these authors mapped the regional expression of immediate early genes used as indirect markers of neuronal activity. They found that when rats acquired the task, the hippocampal neurons exhibited an increase in the expression of immediate early genes. However, when the hippocampus was observed after a recall of the task occurring a month later, the neuronal activation disappeared proving a disengagement of the hippocampus after the consolidation process. In parallel, an increase of neuronal activity was noted in some cortical areas.

Other data were in favor of the multiple trace theory (Nadel et al., 2000). For example, hippocampal lesions studies showed impairment in a contextual fear-conditioning task for recently acquired fear whereas no deficit was observed for a conditioning to unimodal stimuli (a tone, this task involved mainly amygdala not the dorsal hippocampus) or to remotely acquired fear (Kim and Fanselow, 1992). Rosenbaum and colleagues (2001) argued that tests dependent on relational context like the contextual fear conditioning task (analogous to episodic memories in humans) are impaired by hippocampal lesions whereas, tests that are less dependent on relational context like the tone-shock association (analogous to semantic memories in humans) are not disrupted (Rosenbaum et al., 2001).

A large number of studies support both theories and whether consolidation occurs according to the standard or the multiple trace theory is beyond the scope of this thesis.

### I.2.E. A caveat: Is STM really different from LTM?

Although the dissociation between short-term memory and long-term memory is widely accepted, some authors remain skeptic regarding its existence. Dudchenko (2001) argued that the distinction between WM and RM might not be absolute (Dudchenko, 2001). He presented data from Morris and colleagues (1986) suggesting that WM is not qualitatively different from long-term memory because animals could differentiate between information stored on a recent trial and use this information to guide their behavior on subsequent trials. In a water maze delayed match to place, a task usually used to test WM, rats had to find an immerged platform in 2 trials. The place of the platform did not change between the 2 trials that were separated by a long inter-trial interval (up to 30 minutes). Rats always exhibited a better performance on the second trial, suggesting that memories for the short-term could outlast

their use and were not totally forgotten (Morris et al., 1986).
Moreover, a study by Beatty and Shavalia (1980) showed that a one-trial WM could last for more than twenty-four hours in rats. These authors placed a rat in an 8-arm radial maze and allowed it to retrieve food from 6 out of the 8 baited arms. After different delays, the rat was placed in the maze to retrieve the remaining 2 food rewards. They found that the memory for the location of the two remaining food rewards supposedly stored in WM was highly accurate even after several hours (Beatty and Shavalia, 1980b). So the question is: when does short-term/working memory stops and when does long-term/reference memory starts? Consequently, separating memory in two opposite phases (short-term and long-term) could have been misleading.
According to many authors, if the dissociation between WM and RM is real there must be anatomically distinct memory stores that differentially support these two forms of memory (Atkinson and Shiffrin, 1968). Based on the studies of amnesiac patients, LTM was believed to specifically recruit the medial temporal lobe including the hippocampus, while WM was believed to be based outside of the medial temporal lobe due to the enduring capacity displayed by amnesic patients with medial temporal lobe damage such as H.M. to perform memory tasks with very short delays. Furthermore, evidence from primate studies originally implicated the prefrontal cortex (PFC) as being crucial for WM (Miller et al., 1991). For instance, damage to the PFC produces impairments in various WM tasks in humans and non-human primates. All these results led researchers to believe that LTM formation was hippocampal-dependent whereas WM was PFC-dependent. On the contrary, Ranganath and Blumenfeld (2005) in a review titled: “Doubts about double dissociations between short- and long-term memory” have argued that the evidence suggesting distinct neuroanatomical substrates for short and long-term memory may have been deceiving. They reviewed evidence demonstrating that short-term storage can be disrupted by damage to the medial temporal lobe. They explained that when the information to be stored is novel, patients with medial temporal lobe lesions show profound deficits in short-term retention. On the other hand, Maviel and colleagues (2004) identified prefrontal cortex as critical for the retrieval of remote long-term memories. Moreover, primates with frontal lesions can perform short-term retention tasks if the environment has minimal distractions. These results have led to the hypothesis that the frontal cortex is responsible for the resistance to distraction in WM tasks rather than supporting short-term memory storage itself (Ranganath and Blumenfeld, 2005). Furthermore, studies of hippoacampo-lesioned rats by Olton presented above suggested an implication of the hippocampus in WM but not in RM. Thus, it seems that RM and WM are not forcibly represented by entirely different substrates. However, to better judge and comprehend the potential role of these neuroanatomical substrates in these different forms of memory or forgetting, it is crucial to understand the way they are interconnected. In the next chapter, I will thus review the neuroanatomy of the hippocampal region and the prefrontal cortex, including both a description of connectivity within these regions and a summary of their afferent and efferent projections. Furthermore, I will be presenting data that may help on understanding the role of these different brain structures in memory and forgetting.

## I.3. Brain regions Involved in memory and forgetting

*"As long as our brain is a mystery, the universe, the reflection of the structure of the brain will also be a mystery" Santiago Ramón y Cajal (1852-1934).*

In this chapter, I will focus on the role of brain regions involved in memory and forgetting. Extensive evidence indicates a role of the hippocampal formation and the Prefrontal cortex in memory processing. First, I will be considering relevant aspects of the hippocampal complex and Prefrontal cortex anatomy and then considering some data that bear on understanding how these regions support memory and forgetting. Because our test subjects are rats, I will be focusing this part of the introduction on the rats' neuroanatomy.

### I.3.A. Neuroanatomy of the rat hippocampal formation

The hippocampal formation is possibly the most widely studied structure in the brain. The rat hippocampal formation is an elongated, banana-shaped structure. Its long axis extends in a C-shaped manner from the midline of the brain near the septal nuclei (rostro-dorsally) over and behind the thalamus into the incipient temporal lobe (caudo-ventrally) (**Figure I.6A**) (Witter and Amaral, 2004). Although the hippocampus is located underneath the cerebral cortex, it is not truly a subcortical structure. It is a real cortical area infolding itself, albeit much older and more primitive than the surrounding neocortex. Hence, it is also referred to as archicortex, or paleocortex. Thinking of the hippocampus along its long axis, one-end projects to the septum and the other borders the temporal lobe; hence "septotemporal" is technically the most accurate way to refer to the different ends of the hippocampus (**Figure I.6A**). However, "dorsal" and "ventral" hippocampi are more commonly used. The word hippocampus literally means 'seahorse' which precisely defines its aspect following visual examination (**Figure I.6D).**

The hippocampal complex terminology can be quite confusing. Consistent with Witter and Amaral (2004), the term "Hippocampal complex" includes both the hippocampal formation and the parahippocampal region.

- The hippocampal formation consists of three zones: the subiculum, the dentate gyrus (DG) and the hippocampus proper composed of Ammon's horn (*cornu ammonis* or CA).

- The parahippocampal region includes the entorhinal, the perirhinal and the parahippocampal cortices (Nadel and Moscovitch, 1998) (**Figure I.6C**).

#### a. Principle cells and layers

##### i. Cornu Ammonis (CA)

The principal cells of the CA field are the pyramidal cells. They are also located in one layer called *stratum pyramidale*. Based on the size of the pyramidal cells and their synaptic innervations, the *Cornu Ammonis* field is usually divided into three subfields: CA1, CA2 and CA3 (Lorente de Nó, 1934). The CA3 region contains pyramidal cells with relatively large *somata*. The apical dendrites of CA3 pyramidal cells traverse three *strata*: the *stratum*

*lucidum*, the *stratum radiatum* and the *stratum lacunosum moleculare*. The basal dendrites extend into *stratum oriens* towards the *alveus*. The *stratum lucidum* is unique to CA3 region. The other two regions of the *Cornu Ammonis* field receive no mossy fiber input, therefore they lack the *stratum lucidum*; otherwise they have the same laminar structure (**Figure I.6B**).

### ii. Dentate Gyrus

The DG is comprised of three layers. The molecular layer (*stratum moleculare*), occupied by the DG granule cells' dendrites, is localized superficially. The principal cell layer (granule cell layer) is situated beneath the molecular layer and is composed of granule cells. These neurons are the principles cells of the DG forming a condensed 'U' pattern. The polymorphic layer (*hilus*) is composed of glia and granule cells axons.

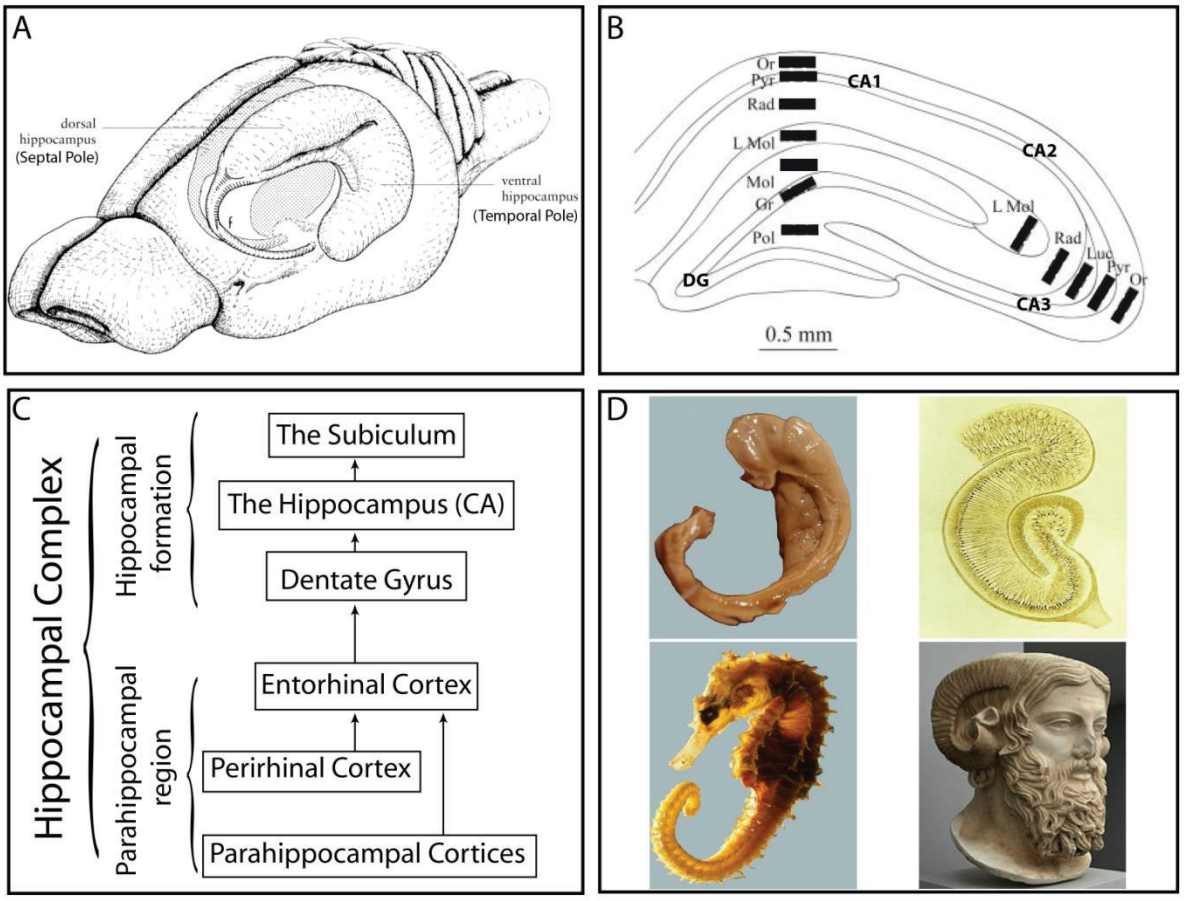


**Figure I.6: The Hippocampus. (A)** Drawing of the rat brain showing the C-shape septotemporal ends of the hippocampus. (**B**) Drawing of the hippocampal formation showing the different layers. In CA1: Or, Stratum Oriens layer; Pyr, Stratum Pyramidale; Luc, Stratum lucidum (only in CA3); Rad, Stratum radiatum; L, MOL, Stratum lacunosum-moleculare. In the DG, Mol, Stratum Moleculare; Gr, Stratum Granulosum; Pol, Polymorfic layer or hilus. (**C**) Schematic diagrams illustrating the standard view of projections within the medial temporal lobe, adapted from (Lavenex and Amaral, 2000). (**D**) Left top illustration shows a human hippocampus and fornix prepared by Laszlo Seress in 1980 compared to a seahorse left bottom. Right top illustration is a drawing by Camillo Golgi of a hippocampus stained with the silver nitrate method showing the cornu ammoni regions and compared to the horns of the Egyptian god Amun. The term Ammon's horn is a metaphor that refers to the ram shaped horns on the head representing the Egyptian God Amun.

### b. Intrinsic Hippocampal circuitry

In most of cases if not all, connections between cortical regions are reciprocal. If a region "A" projects to a region "B", region "B" will send projections back to region "A". However, this is not the case for the hippocampus which has a unique set of unidirectional non-reciprocated excitatory pathway. The Entorhinal cortex (EC) provides major inputs to the hippocampus through a fiber bundle called the Perforant path (Amaral et al., 1987, Suzuki and Amaral, 1990). Perirhinal and postrhinal cortices, which project heavily to the EC, also contribute to the perforant path projection. The EC's major excitatory circuits terminate mainly in the dentate gyrus. However it is to be noted that the axons from layer II project to the granule cells of the DG and the pyramidal cells of CA3. Axons from layers III project to pyramidal cells of CA1 and the Subiculum. The dentate granule cells project to CA3 via the mossy fibers. CA3 cells send a collateral, the Schaffer collateral, to CA1. These receiving cells project to the subiculum. CA1 and the subiculum both project back to the far down layers of the EC, predominantly layer V. Descriptions of the intrinsic circuitry of the hippocampal formation typically involve a reference to the "trisynaptic pathway," denoting a flow of information from EC to DG, CA3, and then CA1 (Kelso et al., 1986) (**see Figure I.7).**

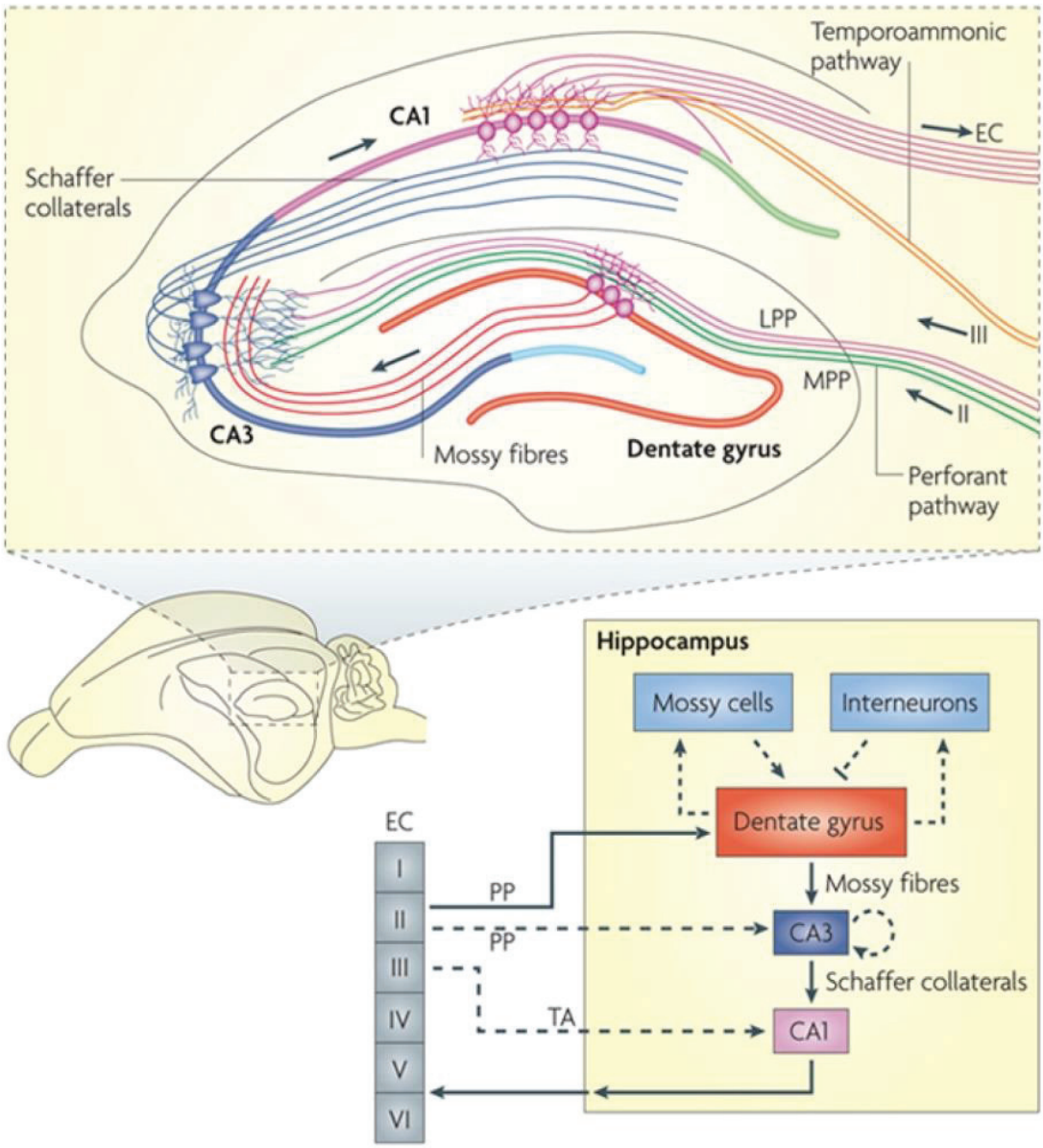


Diagram of the hippocampal neural network. The traditional excitatory trisynaptic pathway (entorhinal cortex (EC)-DG-CA3-CA1-EC) is depicted by solid arrows. The axons of layer II neurons in the entorhinal cortex project to the DG through the perforant path, including the lateral perforant path (LPP) and the medial perforant path (MPP). The dentate gyrus sends projections to the pyramidal cells in CA3 throufh mossy fibres. CA3 pyramidal neurons relay the information to CA1 pyramidal neurons through Schaffer Collaterals. CA1 pyramidal neurons send back-projections into deep-layer neurons of the EC. CA3 also receives direct projections from RC layer II neurons through the PP. CA1 receives direct input from EC layer III neurons through the temporoammonic pathway (TA). The dentate granule cells also project to the mossy cells in the hilus and hilar interneurons, which send excitatory and inhibitory projections, respectively, back to the granule cells

**Figure I.7: An illustration of the hippocampal circuitry,** from (Deng et al., 2010).

### c. Extrinsic connections

Although this chapter focuses on the hippocampal formation, scientists emphasize that no brain structure can be seen in isolation. The hippocampus sends projections to and receives projections from numerous other brain regions, and these interconnections are important to understand its function (Andersen et al., 2006). One of the most important features of the hippocampus connectivity is that most of its connections arise from within the hippocampus. Thus, extrinsic inputs are few in number and account for a relatively small number of synapses. Extrinsic inputs which modulate the hippocampal circuitry arise from (1) various cortical areas, (2) the amygdaloid complex, (3) the medial septal region, (4) the thalamus, (5) the supramammillary region and (6) monoaminergic brainstem nuclei (**Figure I.8**).

#### i. CA1

The CA1 subfield of the hippocampus receives its heaviest input from CA3. A relatively lighter projection originates from the EC via the temporo-ammonic pathway. Cells in the perirhinal cortex give rise to projections to the distal CA1 pyramidal cells (i.e., those located at the border with the subiculum). The septum provides light projections to CA1 (in term of number of fibers). The nucleus *reuniens* of the thalamus and the basal nucleus of the amygdala terminate in the CA1 as well (Amaral and Witter, 1995). On the other hand, CA1 pyramidal cells send projections to perirhinal, retrosplenial, medial and lateral entorhinal cortices. In rats, the ventral CA1 regions send the only direct projections to the prelimbic, medial orbital and infralimbic areas of the Prefrontal cortex (PFC) (Jay et al., 1989, Condé et al., 1995). Pyramidal CA1 neurons contact monosynaptically and form asymmetrical synapses with pyramidal cells and interneurons of the medial PFC (Jay and Witter, 1991).

#### ii. CA3

The CA3 subfield of the hippocampus receives input from the amygdaloid complex (Pikkarainen et al., 1999) and heavy projections from the Septum. CA3 area receives also noradrenergic and serotonergic inputs from the *locus coeruleus* and the *Raphe nuclei* respectively.

#### iii. DG

The DG receives its major input from the EC via the Perforant path (Ramon y Cajal, 1893). The DG receives few inputs from subcortical regions. One of the most important projections comes from the septal nuclei (Amaral and Kurz, 1985, Wainer et al., 1985). The major hypothalamic projections to the DG arise from the supramammillary area (Vertes, 1992, Magloczky et al., 1994). The DG receives important noradrenergic input from the *locus coeruleus*, a serotonergic projection from the *raphe nuclei* and a minor dopaminergic input from the ventral tegmental area (VTA). On the other hand, the DG appears to give rise to few if any extrahippocampal projections.

One of the major outputs of the hippocampus passes through the subiculum that sends projections to many cortical and subcortical structures such as the medial Prefrontal cortex (Infralimbic and Prelimbic), the amygdala and the thalamus. Connections to theses mnemonic and emotional areas reinforce the role of the hippocampus as a path integration system.

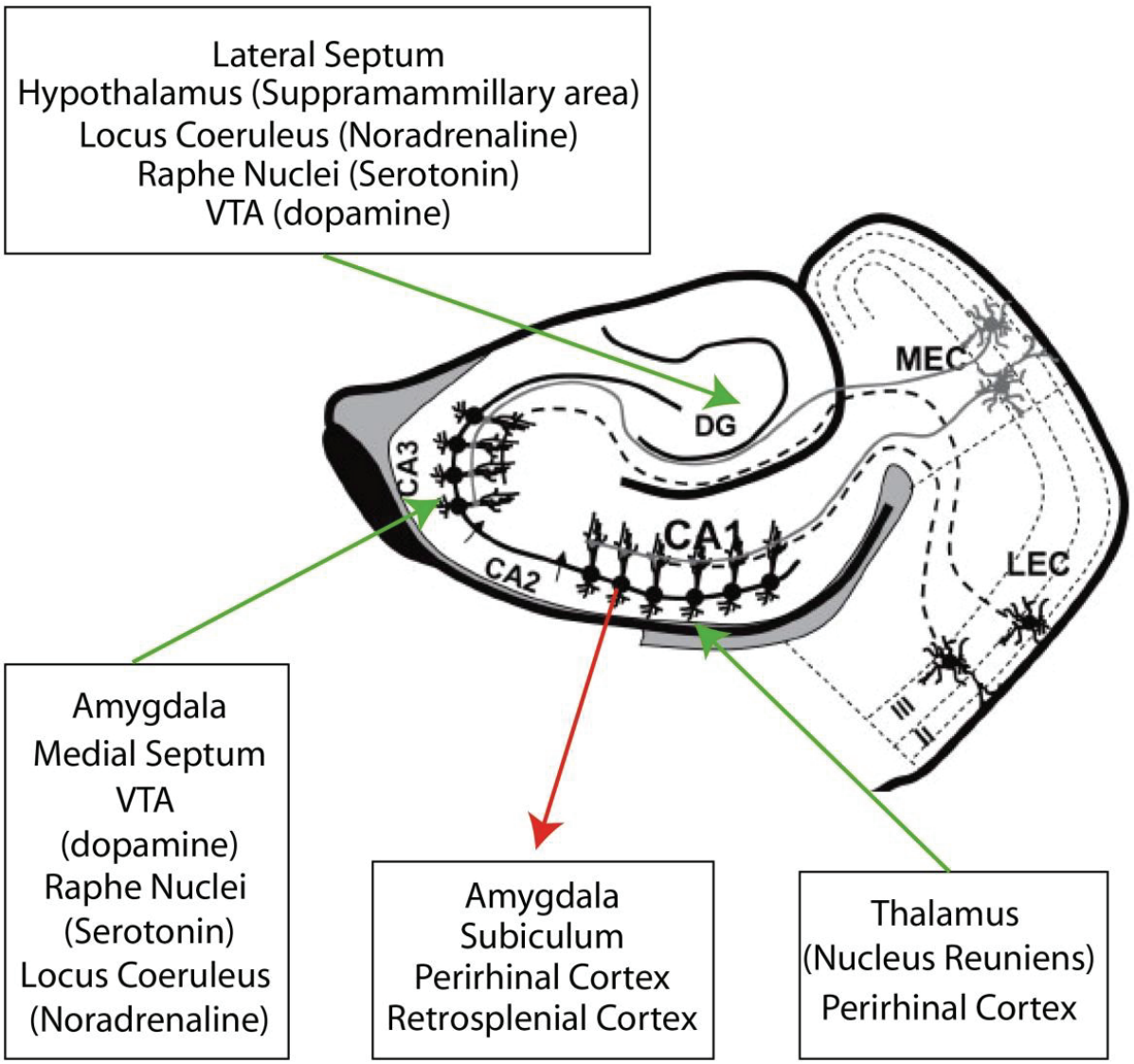


**Figure I.8: Intrinsic and extrinsic connections of the hippocampal formation.** Green arrows represent the structures that send projections to the different subfield of the hippocampus; the red arrow represents the brain regions that receive projections from the hippocampus.

## I.3.B. Neuroanatomy of the rat medial Prefrontal Cortex

The mammalian PFC has been classically described by anatomical basis such as cytoarchitectonic characteristics (granular vs. agranular characteristics). The PFC was defined by the area of frontal cortex which receives projections from mediodorsal thalamus (Rose and Woolsey, 1948). Neurons in the cerebral cortex are classically organized into six layers; however the rat's prefrontal cortex lacks a granular layer IV. Neurons in the mPFC are distributed across cortical layers I-III, V and VI. Each cortical layer comprehends different neuronal aspects, sizes and density along with distinct organizations of nerve fibers.
The rodent's mPFC is a cortex that can be broken down into three major regions (Heidbreder and Groenewegen, 2003, Dalley et al., 2004), a ventral region composed of the Prelimbic (PrL) and Infralimbic (IL) Cortices, and a dorsal region composed of the Anterior Cingulate cortex (aCC).

### a. Connections

Generally, the mPFC has a high amount of intrinsic connectivity. The IL cortex is highly connected with the PrL area and this one is highly connected with the aCC.
The mPFC is connected reciprocally to a large number of structures such as the thalamus (Bentivoglio et al., 1993), the Nucleus *Accumbens* (Gerfen and Paxinos, 2004, Voorn et al., 2004), the Subthalamic nucleus (Degos et al., 2008), the amygdala (Rosenkranz and Grace, 2001), to many cortical regions and more interestingly for our research, to the hippocampal formation. Anatomically, the ventral hippocampus sends direct projections to the mPFC. On the contrary, the dorsal hippocampus does not project directly to the mPFC. This brings up the question of how the medial prefrontal cortex can affect the dorsal hippocampal activity. In this regard, the nucleus *reuniens* of the thalamus represents an important source of input to the hippocampus and to the EC, and is strongly interconnected with the mPFC (McKenna and Vertes, 2004, Vertes, 2006) (**Figure I.9**). The Ventral part of mPFC sends sparse projections back to the CA1 area and the subiculum (Jones and Witter, 2007). The mPFC does, however, project strongly to the entorhinal cortex, particularly to pyramidal neurons in this area (Apergis-Schoute et al., 2006, Jones and Witter, 2007). This projection to the lateral entorhinal cortex is the primary input to the dorsal hippocampus. The lateral entorhinal cortex projects directly to the mPFC and this projection is reciprocal like many other inter-cortical connections.

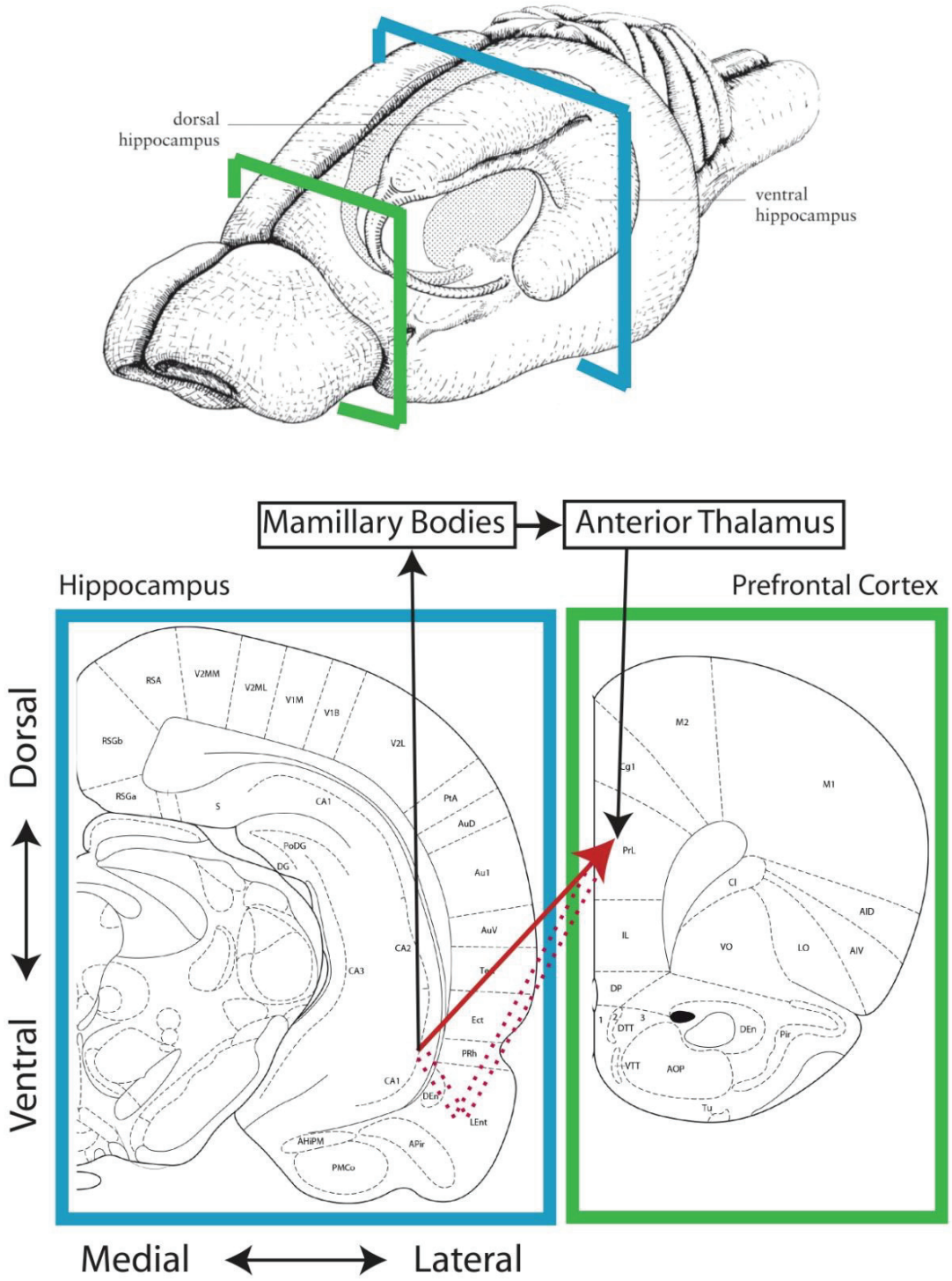


**Figure I.9: Interconnections between hippocampus, entorhinal cortex and medial Prefrontal cortex.** Upper: A rat brain in 3-D, including a view of the hippocampus under the neocortex (Witter and Amaral, 2004). Green and blue squares represent coronal slices depicted in bottom panels. Bottom: coronal slice showing the hippocampus and EC (left) and a coronal slice with the PFC (right), arrows describe connections between the regions. A direct projection exists from CA1/subiculum to the ventral mPFC (solid red arrow). An indirect reciprocal projection from hippocampus to mPFC also exists via the EC (dashed red lines). Finally, a third connection between hippocampus and PFC exists through the mammilary bodies and anterior thalamic nucleus (solid black arrows).

## I.3.C. Brain Regions involved in memory

### a. Role of the hippocampal formation in memory

There are now over a hundred of thousands studies using different memory paradigms in rats and they have consistently revealed a network of regions that are involved in memory, including the medial temporal lobe (hippocampal formation) and the Prefrontal cortex (Eichenbaum et al., 1994, Eichenbaum, 1999, 2000).

#### i. The Hippocampus

That the hippocampal complex plays a critical role in memory is no longer a matter of debate. The hippocampus plays a decisive role in the acquisition of new memories, and damage to this structure induces amnesic syndromes (Scoville and Milner, 1957). A general consensus is that damage to the hippocampus impairs declarative memory and more specifically episodic memory functions (Tulving, 1972, 2002). Since rats cannot speak, the animal counterpart of human declarative memory is referred to as relational memory (Dusek and Eichenbaum, 1997, Eichenbaum, 2004). Relational memory also involves the hippocampus, and is concerned with the storage of complex associations or relations between spatially and/or temporarily separated items. Therefore, in non-human primates or other mammals that do not use language, relational/declarative memory is generally assessed by testing spatial memory, the subjects' abilities for remembering a spatial location. Thus, the hippocampus is necessary for spatial navigation, especially in conditions in which going after a simple sensory stimulus or a well-learned path is not enough to decode the task (O'keefe and Nadel, 1978, Morris et al., 1982, Pearce et al., 1998). However, it has been suggested that the hippocampus is not involved alone in the process of spatial memory (Ross et al., 1984, Zola-Morgan and Squire, 1985, Sinden et al., 1986, Honey et al., 1998). As memories are organized into a 'relational network', the hippocampus along with cortical areas process the combination of stimuli (Eichenbaum, 1994, O'Reilly and Rudy, 2001, Bussey and Aggleton, 2002). Moreover, afferent and efferent projections from and to the hippocampus presented above give us an idea of the large circuit implicated in the memorization process. Along its dorso-ventral axis, the hippocampus plays different roles. The dorsal hippocampus is thought to process spatial information whereas the ventral pole is associated with emotion-related processing(Moser et al., 1995, Deguchi et al., 2011). The anatomical organization of these dorsal and ventral poles could explain their differential roles. The lateral entorhinal cortex (LEC) receives information from visual, auditory and somatosensory cortices and projects to the dorsal hippocampus, hence, processing this multi-sensory information (Witter and Groenewegen, 1984, Witter et al., 1989, Burwell and Amaral, 1998, Dolorfo and Amaral, 1998). On the other hand, the medial entorhinal cortex (MEC) receives information from the amygdala (Pitkänen et al., 2000, Petrovich et al., 2001) and projects to the ventral hippocampus, hence, processing information associated with fear, anxiety and emotions (Köhler et al., 1985). The MEC receives information from sensory cortex too and sends projections to the dorsal hippocampus (Andersen et al., 2006). Studying the activity of pyramidal neurons showed that each cell of the hippocampus encodes the characteristics of the environment. These neurons coding for spatial information are known as 'place cells' (O'keefe and Nadel, 1978). Place cells are defined as cells that fire in complex burst when a rat walks through a restricted and specific part of its environment. Poucet and colleagues (1994) found such place cells in both the dorsal

and ventral hippocampus (Poucet et al., 1994). However, the proportion of these cells is higher and much more selective in the dorsal than the ventral hippocampus (Jung et al., 1994). Furthermore, when the dorsal hippocampus was lesioned, animals' behavior in a Morris water maze spatial task was disrupted even though the ventral hippocampus was left intact (Moser et al., 1995). Other authors have seen the same result in a T-maze and radial maze tasks (Bannerman et al., 1999, McHugh et al., 2004). On the other hand, a selective lesion to the ventral part of the hippocampus impaired the animals' ability to develop a freezing response in a fear-conditioning task (Bannerman et al., 2002) confirming its role in emotional memory.

### ii. Role of the hippocampal subregions

As previously described, the hippocampus consists of structurally dissimilar processing subfields that are interconnected serially as well as directly with the EC (Witter and Amaral, 2004). This arrangement suggests that individual subfields may subserve discrete functions.

#### CA1

The CA1 area constitutes the primary output from the hippocampus to the neocortex. It is therefore not surprising that a lesion to this area would cut off the output from the rest of the hippocampus. It appears that CA1 plays a role in allocentric spatial tasks because specific lesions to this subregion induce impairment in such tasks (Nunn et al., 1991, Stubley-Weatherly et al., 1996). Dillon and colleagues (2008) applied restricted excitotoxic lesions to the dorsal CA1 and found a disruption of spatial memory. Lesioned mice thus exhibited spatial WM impairments in the Y-maze spontaneous alternation task, and displayed deficits in an eight-arm spatial discrimination-learning task (Dillon et al., 2008). Furthermore, CA1 was deemed to be implicated in the retrieval of spatial memory. CA1 lesioned rats were tested in a spatial delayed non-match to place task with intratrials delay ranging from 10 seconds to 5 minutes. Lesioned animals were only impaired when they were presented with the 5 minute delay giving the CA1 a potential role in intermediate memory retrieval (Kesner et al., 2002).

#### CA3

Unlike the CA1 area, CA3 does not seem to be critical in the acquisition of spatial tasks (Brun et al., 2001, Nakazawa et al., 2002, Okada and Okaichi, 2009). However, this subfield of the hippocampus seems to be critical for the retrieval, rather than the encoding of memory (Nakazawa et al., 2002, Rolls and Kesner, 2006). More specifically, it has been suggested that CA3 may support the process of "pattern completion" (Gold and Kesner, 2005, Lee et al., 2005b). Pattern completion is a mechanism by which a complete memory can be retrieved from only partial or degraded cues represented in this memory (Kesner and Hopkins, 2006). To investigate spatial pattern completion, Gold and Kesner (2005) used a delayed matching-to-sample task using spatial location. During the sample phase of the task, rats were trained to retrieve a reward by displacing an object covering a food well in one of five spatial locations on a cheeseboard apparatus. During the subsequent choice phase, the animals were required to find the same food well without the aid of the object, using only the four spatial cues available. Once the rats were trained in locating the reward based only on spatial cues, some received neurotoxin injections into the CA3 subfield of the hippocampus. After surgery, rats were tested on choice phases in which either zero, one, two, three, or four of the spatial cues

were removed. The control animals performed well in all choice conditions. The animals with CA3 lesions showed a linear increase in the number of errors as the number of spatial cues removed increased. These results suggest that CA3 lesions resulted in an impaired ability to perform spatial pattern completion (Gold and Kesner, 2005).

**DG**

The dentate gyrus is of special interest for researchers as it receives and processes the first projections from the entorhinal cortex to the hippocampus. Thus, this structure is in a key position to control the flow of information to the hippocampus (the detonator). Selective lesions to the DG resulted in impairment in RM tasks. These impairments were similar to the effects caused by a complete hippocampal lesion (Okada and Okaichi, 2009). This large impairment suggests that the DG might be more important for spatial memory processing than the other subfields of the hippocampus. This hypothesis was tested by many authors in tasks requiring allocentric spatial processing (Sutherland et al., 1983, Walsh et al., 1986, Xavier et al., 1999, Jeltsch et al., 2001). In line with these findings, the DG has been well placed in the role as an encoder of newly acquired spatial information (Lee and Kesner, 2004).
Moreover, the DG has the ability to separate or orthogonalize similar events, a process that has been named "pattern separation". When episodes that we experience during our daily lives are too similar, it is sometime important to be capable to carefully differentiate them. Many studies have shown that the DG may have a specific role in this pattern separation function (Schmidt et al., 2012).
There was a long belief that reaching adolescence we have a set of neurons that may persist during our entire lifespan (Rakic, 2002) and that, in the adult, new neurons cannot be further generated in the brain. The Dentate gyrus of the hippocampus turned out to be one exception. Even in adulthood, the DG can generate and incorporate new neurons. Adult neurogenesis represents a process by which adult neural stem cells proliferate, differentiate and integrate into the existing neural circuitry in the mature nervous system. Recently, the scientific community accepted the existence of adult neurogenesis after newborn neurons were identified due to technical advances, for review see (Alvarez-Buylla and García-Verdugo, 2002). Since the discovery of adult neurogenesis is mammals (Altman and Das, 1965, Altman, 1969) there have been extensive studies to understand the functional role of these new neurons. A growing body of evidence suggests that adult neurogenesis is involved (Magavi et al., 2005, Ramirez-Amaya et al., 2006, Kee et al., 2007, Trouche et al., 2009) and even necessary (Shors et al., 2001, Shors et al., 2002, Madsen et al., 2003, Rola et al., 2004, Bruel-Jungerman et al., 2005, Dupret et al., 2007, Imayoshi et al., 2008, Garthe et al., 2009, Hernandez-Rabaza et al., 2009) to memory acquisition and retrieval, and in particular to memory functions generally attributed to the DG (Bruel-Jungerman et al., 2007, Trouche et al., 2009). It is important to note that the DG is not the only region with capacity of neurogenesis. It has been shown that newborn neurons are present in the olfactory bulb as well and are necessary for olfactory learning (Veyrac et al., 2008, Moreno et al., 2009, Mandairon et al., 2011).

### b. Role of the Prefrontal cortex in memory

For a long time, the hippocampus was believed to have a limited role in working memory (Milner et al., 1998a), as the PFC was thought to be the major structure involved in this form of memory (Fuster, 2001). Recently, however, new studies seem to re-evaluate the role of the PFC in working memory (Curtis, 2006, Gisquet-Verrier and Delatour, 2006, Postle, 2006, D'Esposito, 2007) while others suggest that the hippocampus plays a significant role in it (Floresco et al., 1997, Lee and Kesner, 2003, Saxe et al., 2007). For instance, numerous tasks aimed at assessing working memory can be resolved by using proactive motor coding, a strategy known to involve the PFC (D'Esposito, 2007). In a maze, rats being released from the same starting point before and after the delay during a delayed non-match to place task can thus anticipate their motor response after the delay by preparing this response during the delay. To avoid the use of such strategy, test subjects can be placed before the delay in the sample arm in front of the reward (no motor action - going left or right - required), removed from the maze during the entire delay, and placed back in the maze after the delay from various orientations to complete the choice phase. The only way for the animal to locate the food reward after the delay is to remember the spatial emplacement of the previously visited arm and not the motor action needed to go back to it. This type of coding would less involve PFC sustained activation than the very brain systems that are responsible for the visual and spatial perception of these stimuli in the first place (Curtis, 2006, Postle, 2006).

In this context, it is therefore not surprising that the hippocampus, a structure heavily involved in visuo-spatial information processing, recently gained more recognition as an anatomical site for information storage during spatial working memory tasks. First, there is now good evidence for parallel processing of information between the PFC and the hippocampus in the storage of a multiple number of trial-unique items during a working memory task (Floresco et al., 1997). Lee and Kesner have recently confirmed this hypothesis using single and double inactivation of the PFC and the hippocampus in a delayed non-match to place task. When the working memory rule is applied to a small number of trial-unique items, these authors found that while double inactivation provoked a deficit in such task, single inactivation has no impact on the performance of pre-trained animals for delays up to 10 seconds, indicating that the inactivation of one structure can be compensated by the activity of the other. However, when the delay exceeded 10 seconds, single inactivation of the hippocampus, but not of the PFC, was sufficient to impair working memory. Altogether, these results indicate that for short-term delays the hippocampus and the PFC might process working memory information in parallel; but as soon as the system detects a longer delay, hippocampal memory may become essential, demonstrating more persistence than the PFC (Lee and Kesner, 2003).

On the other hand, the PFC has also been recognized as being critical in goal directed action (Corbit and Balleine, 2003, Hasselmo, 2005, Vertes, 2006) and the monitoring and flexible adaptation of future movements(Compton et al., 1997, Seamans et al., 2008). Studies have shown that the medial part of this structure is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats (Hannesson et al., 2004). Lesions of the medial prefrontal cortex have been found to impair reversal learning (Aggleton et al., 1995). Interestingly, PrL-IL inactivation was found to have an effect on an intermodal task shift: switching from a place strategy (go to a position) to a response preference (always turn right) on a four-arm radial maze was impaired (Ragozzino et al., 1999).

### I.3.D. Brain regions involved in forgetting

While the neuroanatomical bases of memory have been extensively studied over the last century, those of forgetting did not catch the attention of scientists until recently. The principle reason for that was that forgetting was largely seen as just an absence of memory or a failed process rather than an adaptive and important function for the optimization of cognitive resources. For many, studying how the brain reacts during impaired memory processing was sufficient to establish the bases of forgetting. However, we have seen in the first chapters of this introduction that forgetting is more complex than that and can be due to many potential mechanisms. The first studies conducted to really understand the neurobiological localization of forgetting used neuroimaging techniques in humans. Perhaps the most obvious fact revealed by these studies was that forgetting is not produced by a single mechanism. As we have seen in the first chapter, forgetting may arise from a lack of consolidation, from retroactive or proactive interference and decay just to name a few. I will now present some data attempting to localize the brain regions engaged in situations involving forgetting. First, as we have already seen, forgetting can be caused by a simple lack of consolidation. Consolidation occurs when a fragile hippocampo-dependent memory is transferred to become a stable cortico-dependent memory (Wixted, 2004b). After memories become consolidated, fMRI studies showed a disengagement of the medial temporal lobe and an activation of some cortical structures. When intervening experience interferes with the fragile hippocampo-dependent memory, forgetting can occur to impede this trace from being transferred into neocortical areas and thus, from being consolidated. Second, we have seen that forgetting can also be due to a potential competition existing between two similar memories. If the first memory competes and interferes with the second memory that is to recall, we talk of proactive interference. The reverse phenomenon is known as retroactive interference. Studies on interference theory have been carried out using the classic A-B, A-C paradigm (Anderson and Neely, 1996, Wixted, 2004b). In this paradigm competition arises between B and C because of the existence of a common cue A. Human studies have shown that overcoming retrieval competition is heavily dependent on the lateral PFC. Lesions to this area induce errors in this paradigm. For example, patients suffering form lesions to the PFC performed as well as controls in learning the A-B association. However, these patients made more errors in subsequently learning the A-C interfering pairs (Shimamura et al., 1995). More specifically, lesions to the PFC generally resulted in increased susceptibility to PI. Increased PI effects have been associated with damage to both left and right PFC (Moscovitch, 1992, Lou Smith et al., 1995). Finally, although decay theory is not considered a prominent cause of forgetting, some authors still attribute our lack of memory to be a simple decay of information with time. Frankland and Colleagues (2013) have recently postulated a hypothesis about hippocampal neurogenesis being the neurobiological mechanism behind forgetting and decay of information. These authors focused on how newborn neurons by integrating the circuits will impact already existing memories. They argued that in adult animals, increasing hippocampal neurogenesis should weaken existing hippocampal memories and lead to the natural decay of information. Consequently, in their animal model, the hippocampus was the culprit in forgetting and not the PFC (Frankland et al., 2013). While these data presented herein hold promise for an increasingly specified account of why we sometimes forget, the neural bases and molecular events occurring when we fail to remember our past is far from being elucidated in details. To understand these, we need to know the molecular bases of a

good memory first. In the next chapter, I will discuss now what we know of the molecular and cellular bases of memory.

## I.4. Cellular and molecular mechanisms underlying memory and forgetting

*"All the psychological matters that we are progressively formulating, will have to rely, on an organic substrate" S. Freud, Entwurf einer psychologie, 1895*

Regardless of the type of memory, the capacity to remember something is widely believed to be dependent on changes in synaptic efficacy (Martin et al., 2000). As is the case with memory and forgetting, synaptic plasticity is not a unitary phenomenon but is composed of two major types: long-term synaptic potentiation (LTP) and long-term synaptic depression (LTD) (Bear and Malenka, 1994, Bear and Abraham, 1996). Although these two processes are dependent on different signaling cascades, it is important to note that most synapses that exhibit LTP can also express LTD. It is believed that these two opposing forms of synaptic plasticity provide bidirectional regulation of synaptic strength *in vivo* and serve to keep neuronal activity under control.

### I.4.A. LTP and LTD phenomena

At the cellular level, encoding of memory is thought to involve an increase in synaptic efficacy through hebbian mechanisms. As postulated by Hebb, Long-Term Potentiation (LTP), a putative memory model that incorporates this concept, involves a persistent increase in synaptic transmission that can be observed after application of brief trains of high frequency stimulation. The most widely studied form of LTP is N-methyl-D-aspartate receptor (NMDAR) dependent LTP in the hippocampus. NMDAR-dependent LTP is triggered by activation of NMDARs during a period of strong post-synaptic depolarization, which leads to an increase in post-synaptic calcium concentration. It is believed that the level of intra-cellular calcium in the post-synaptic cell has to reach a certain threshold in order to trigger the signal transduction mechanisms responsible for LTP. Once this threshold is reached, calcium/calmodulin-dependent protein kinase II (CaMKII) undergoes auto-phosphorylation and in turn phosphorylates GluR1 (subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) at the Ser831 site. These "activated" AMPARs are trafficked to the post-synaptic membrane (also referred to as the post-synaptic density, PSD) and increase the conductance of AMPAR-mediated current (**Figure I.10A**). Although numerous other changes occur at the synaptic level during LTP induction, AMPAR trafficking is accompanied by structural changes on the dendrite, making it an attractive mechanism for LTP maintenance (Citri and Malenka, 2008), and therefore for the stabilization of the memory trace.

However, theoretical models have proposed that a reduction in synaptic strength should occur concomitantly to LTP, so that plastic neuronal circuits could work efficiently (Stent, 1973, Bear et al., 1987): if LTP was the only type of activity-dependent change in synaptic efficacy, then synaptic transmission in neuronal circuits would rapidly become saturated, losing its plasticity ability. Consequently, some authors have proposed the rather natural concept that "what goes up must come down'' (Willshaw and Dayan, 1990), or that neuronal circuits must also have a synaptic substrate of "forgetting" (Siegelbaum and Kandel, 1991).

Like LTP, the decrease of synaptic transmission can be persistent and in that case is known as Long-term Depression (LTD). Although their effects are opposite to that of LTP, the molecular mechanisms responsible for the induction of LTD remain quite similar to those of LTP and depend also on NMDA, AMPA, kainate and metabotropic glutamate receptors. Like LTP, LTD is triggered by a post-synaptic calcium signal, but the widely accepted hypothesis indicates that whereas LTP requires a rapid and large calcium increase beyond a certain threshold, LTD is induced by a slower and modest increase in calcium. A slow but steady increase in calcium can be experimentally induced by several protocols such as prolonged repetitive low frequency stimulation. It is argued that this modest increase in post-synaptic calcium concentration triggers not the activation of protein kinases such as CaMKII, as is the case for LTP induction, but activation of calcium-dependent protein phosphatases, the most well known being calcineurin (also called protein phosphatase 2B). LTD has also been shown to involve dephosphorylation of postsynaptic protein kinases A and C (PKA, PKC). More importantly, during LTD the gluR1 subunit of the AMPAR is dephosphorylated at the Ser845 site, which is a PKA substrate site (**Figure I.10B**). AMPAR dephosphorylation decreases AMPAR conductance and leads to the endocytosis of AMPARs from the PSD (Citri and Malenka, 2008), opposite to what is observed in LTP. As such, the phosphorylation state of the gluR1 subunit of the AMPAR has been a widely exploited molecular marker for LTP and LTD detection.

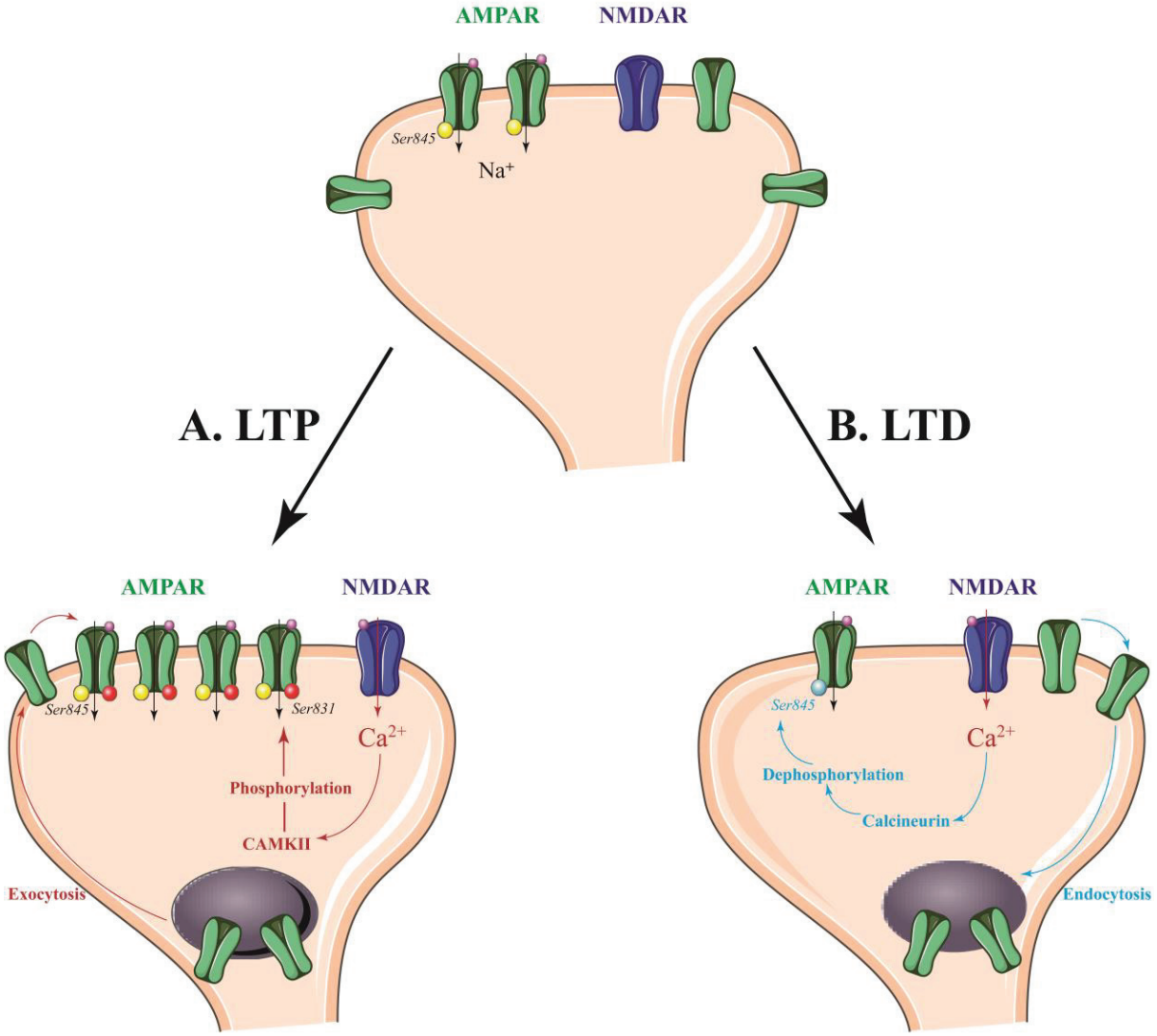


**Figure I.10: A schematic representation of the molecular changes during (A) LTP and (B) LTD.**

### a. A Million Dollar Question: LTP = Memory and LTD = Forgetting?

*Here's a million dollars. You have to bet it on whether the following statement will finally turn out to be true or false: "Long-term potentiation (LTP) and long-term depression (LTD) are cellular mechanisms underlying some forms of memory." Charles F. Stevens*

Since it has been suggested that memory formation is dependent on changes in synaptic efficacy, it is likely that new information is stored only when activity causes a change in a given neural circuit. In light of its similarities with the "requirements" for memory formation, LTP has been widely proposed, and supported with research, as a potential mechanism for information storage. Research has shown that mice with a defective NR1 subunit of the NMDAR in the hippocampus lacked NMDAR-dependent LTP and were unable to form remote memories (Frankland and Bontempi, 2005). Further work from our group has shown that a transient reduction in calcineurin, a vital protein phosphatase involved in synaptic plasticity, facilitated both *in vivo* and *in vitro* LTP. This LTP facilitation was associated with a strengthened ability to perform many hippocampal-dependent memory tasks (Malleret et al., 2001). In concordance with the hypothesis that LTP is necessary for long-term memory formation, it has been shown that the inhibition of LTP maintenance, but not LTP induction, produces a persistent loss of 1-day old spatial information (Pastalkova et al., 2006), implying that LTP maintenance is required for a period of 24 hours or more after exposure to an information in order for it to be stored in long-term memory. The most striking causal evidence for the link between LTP and memory formation comes from a relatively recent study in which the acquisition of an inhibitory avoidance-learning task was shown to induce LTP *in vivo* (Whitlock et al., 2006). It is widely accepted today that hippocampal LTP plays an integral role in the formation and consolidation of declarative memory, although we can scarcely say that it is the only molecular mechanism underlying such a complex and vast function such as memory as a whole.

Whereas it is widely accepted that long-term storage of information is dependent on LTP and LTP-like mechanisms, the role of LTD in memory formation is more nebulous. Restricted disruption of calcineurin phosphatase activity in the mouse adult forebrain was shown to decrease the magnitude of LTD and lead to an impairment of hippocampus-dependent episodic like/working memory (Zeng et al., 2001). These forebrain-specific Knockout mice displayed a total inhibition of calcineurin in selective cell type, in particular in the DG and the CA1 but not the CA3 area of the hippocampus. While leaving the classical forms of LTP (induced by 100Hz) intact, this deletion seriously impaired hippocampal LTD. This alteration in synaptic plasticity in these mutant mice had no consequences on RM task tested with the spatial version of the water maze, which consists of a regular training over days of an invariable location of the platform. In contrast, this alteration was accompanied by impaired performance in a delayed match to place task in the water maze. In this task, the platform position changes everyday and the mice were required to learn this position over 8 daily training sessions. While control mice learned readily each platform position everyday, no such learning was observed in the calcineurin knockout mice suggesting impairment in episodic-like memory (each platform location learned everyday representing a discreet episode isolated in space and time). Based on this finding, the authors then explored WM of these mutants in an eight-arm radial maze. During this task, the mice were given one trial per day to localize eight food rewards located in each arm of the maze. A re-entry in an already visited arm thus represented a working memory error. Here again, forebrain-specific

calcineurin knockout mice showed impaired performance in this working memory task. Following this study, Nakao and colleagues tested rats in a spontaneous alternation test aimed to assess spatial working memory and monitored thereafter LTD in response to low-frequency burst stimulation in the DG of the same rat under anesthesia. Interestingly their behavioral parameter of spatial working memory was positively correlated to the magnitude of LTD (Nakao et al., 2002). The authors concluded from this study that bidirectional synaptic modification seems to be critical for one-trial learning-based working/episodic-like memory and that LTD does not seem to be a mechanism that constrains memory, but that on the contrary, and in conjugation with LTP, constitute the basis for an effective distributed memory system. The question was what is the specific role of LTD in this effective distributed memory system?

Few years later, members from our team tried to answer this question by studying two new mutant mice models with altered LTD. The first model involved the forebrain expression of an inhibitor of the protein phosphatase 2A (PP2A) (Nicholls et al., 2008). This restricted inhibition of PP2A blocked the expression of a hippocampal NMDAR-dependent form of LTD while leaving intact other forms of plasticity. This blockade of LTD prevented the mice to forget old (previously learned) information (concerning a platform position learned in the water maze task) that was no longer relevant. However, this deficit of forgetting impaired the processing of proactive interference in a WM (T-maze) task by increasing the level of interference between trials. Unable to process and forget information related to previous trials, these mutant mice showed no resilience to the presence of proactive interference in the T-maze task and thus displayed altered performance in this WM task. This result thus showed for the first time that LTD may act by weakening previously encoded memory traces when new information is learned. In 2010, the same authors found in a second mouse model (with restricted hippocampal expression of an inhibitor of PKA) that increasing LTD enhanced both forgetting and WM abilities by decreasing the level of interference between highly identical trials of a radial maze task (Malleret et al., 2010). Altogether, the results described above suggest that the long-term storage of information into RM benefits from phosphorylation mechanisms increasing long-term synaptic potentiation (LTP), while forgetting and the processing of interference would depend on dephosphorylation and a decrease in long-term synaptic transmission (LTD).

While these results nicely illustrate the importance of LTP and LTD for synaptic plasticity, memory and potentially forgetting, they also raise important questions about the mechanisms involved. These are likely to include the regulation of *de novo* gene transcription, since this is required for persistent synaptic changes and long-term memory (Bailey, 1999). Of particular interest are immediate early genes (IEGs), which provide the first transcriptional response within minutes after neuronal activity and represent key effectors of cytoplasmic signaling cascades.

## I.4.B. Immediate early genes

*"It is now apparent that functional mapping techniques utilizing IEG products have secured their place in the repertoire of functional imaging techniques in the neurosciences" Reza Farivar*

The nervous system is in a constant dynamic state, changing synapses, forming new connections and modifying already existing ones. This driving force needs a turnover of new proteins and thus a perfectly organized genomic response. Some of these genomic responses are fast and activate a class of genes designated as Immediate Early Genes (IEGs). IEGs are the first class of genes that exhibit a rapid but transient expression after neuronal activation. Their mechanism of induction is protein synthesis-independent and when activated can in turn trigger "downstream" targets (Herdegen and Leah, 1998, O'Donovan et al., 1999, Tischmeyer and Grimm, 1999).

### a. Molecular cascade of IEG activation

The molecular cascade activating these IEGs starts from transmembrane receptors and channels, which may be activated by action potential. These transmembrane proteins include metabotropic glutamate receptors (mGluR), NMDA receptors and Voltage-sensitive calcium channels (VSCC). Calcium ($Ca^{2+}$) is liberated and its level is increased in the cytoplasm following membrane depolarization (for VSCC) or when glutamate binds to NMDA receptors. mGluR also contribute to the increase in intracellular calcium and this increase in calcium regulates the activation of protein kinases via the IP3 pathway. In addition, they can also lead to the phosphorylation of Protein kinase C (PKC) via the DAG pathway. Calcium orchestrates many enzymes including kinases (CaMKs, PKC) and phospholipases. Kinases activate the transcription factor CREB that has binding sites on the promotor region of many IEGs (Sheng and Greenberg, 1990). On the other hand, NMDA receptors might activate a second pathway involving extracellular signal-regulated kinases (ERKs) (Xia et al., 1996, Kaminska et al., 1999). In the nucleus, IEGs undergo transcription when various kinases or transcription factors like ElK-1 and CREB bind to IEGs' regulatory sites SRE and CRE respectively among others. After transcription, IEGs' mRNA are transported to the cytoplasm in order to be translated into proteins. IEG's proteins migrate rapidly into the nucleus where they can influence the expression of another set of genes known as the late-response genes (**Figure I.11)**.

At least three IEGs have been popularly used to map neuronal activity and plasticity: *c-fos, zif268* and *arc*. *c-fos* and *zif268* are "regulatory IEGs" and encode proteins that elevate or diminish the expression of other genes, whereas the latter is an "effector IEG" which encodes a protein that play a direct functional role at the synapse.

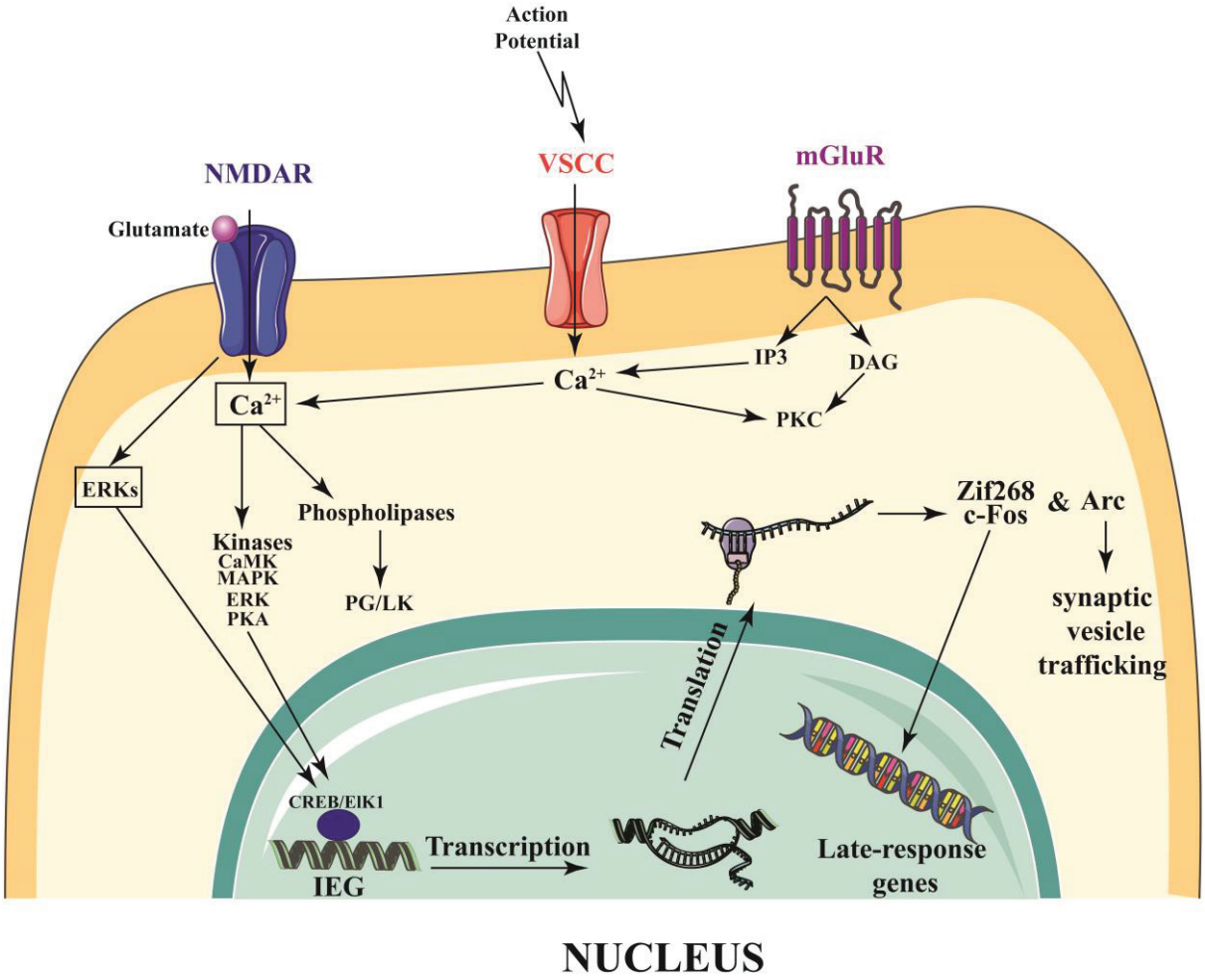


**Figure I.11: A simplified schematic representation of molecular processes involved in neuronal induction of Zif268, c-Fos and Arc.** The molecular cascade starts when an action potential activates a voltage sensitive calcium channel (VSCC) or induces a liberation of Glutamate that binds to NMDA receptors (NMDAR) or mGlu receptors (mGluR). Hence, inducing an increase in intracellular Calcium (Ca2+). This will activate kinases such as Ca2+/Calmodulin-dependent protein kinase (CaMK), mitogen-activated protein kinase (MAPK), extracellular signal-regulated proteins kinases (ERK) and protein kinase A (PKA). These kinases will allow several different signaling pathways, including ElK-1 binding to the serum response element site and CREB binding to the cyclic-AMP-responsive element for example. Thus, inducing transcription and translation of the IEG. IEGs encode for 1) inducible transcription factors, such as Zif268 and c-Fos, which regulate expression of late effectors involved in neuronal plasticity and for 2) effectors, such as Arc, involved in synaptic vesicle trafficking.

**b. The Immediate Early Gene c-fos**

*C-fos* was the first IEG to be characterized, and it remains as the most extensively used IEG activity marker. A single short stimulus typically initiates a rapid expression of *c-fos* mRNAs that peaks 20 to 60 minutes later and comes back to normal levels after 2 hours, and an expression of c-Fos proteins that peaks 1 to 2 hours after stimulus induction and that disappears more slowly (Herdegen and Leah, 1998, Clayton et al., 2000, Farivar et al., 2004). c-Fos is largely distributed throughout the brain and has been used as a neuronal activation marker (Dragunow and Faull, 1989). The hippocampus and other parahippocampal cortices showed an increased expression of c-Fos protein after training rats in a radial-arm maze task (Vann et al., 2000a, He et al., 2002). Moreover, it has been found that training in a radial-maze task was impaired after blocking *c-fos* expression in the dorsal hippocampus providing more direct evidence for the important role of this IEG (He et al., 2002). The protein c-Fos plays a role in morphological or synaptic remodeling of neurons during the later phases of memory formation (Patel and Stewart, 1988, Rose and Stewart, 1999, Clayton et al., 2000).

**c. The Immediate Early Gene *zif268***

***Z**if268* (aka **e**gr-1, **N**GFI-A, **K**rox24 and **Zenk**) was first discovered because of its response to nerve growth factor treatment. *Zif268* differs in many ways from *c-fos* in that it has a high level of basal expression in many neural structures whereas *c-fos* has a low basal expression level in most neural systems (Kaczmarek and Chaudhuri, 1997, Herdegen and Leah, 1998). Zif268 complements c-Fos as it is also largely distributed in many brain regions and, like c-Fos, has been linked to learning and memory (Jones et al., 2001a, Bozon et al., 2002, Davis et al., 2003). Due to its high basal expression, *zif268* possesses one important advantage over *c-fos* in that its down-regulation can also be studied (Farivar et al., 2004). Following behavioral training of a learning task (two-way active avoidance response), Zif268 expression was induced in the rat hippocampus as well as the visual cortex (Nikolaev et al., 1992). Furthermore, Zif268 induction is associated with LTP in hippocampal granule cells (Cole et al., 1989, Wisden et al., 1990, French et al., 2001). For all these reasons, scientists studying learning and memory and synaptic plasticity soon understood the importance of Zif268's involvement in memory-related neuronal activation. This was based on the idea that Zif268 expression may represent a marker of synaptic plasticity rather than general neuronal activity, differentiating it from other IEGs like c-Fos (Rosen et al., 1998).

**d. The Immediate Early Gene *arc***

The Activity Regulated Cytoskeleton (*arc*) factor has been studied extensively in relation to hippocampal function and memory consolidation (Guzowski et al., 1999, Guzowski et al., 2000, Guzowski et al., 2001), as it is expressed exclusively in neurons following spatial exploration (Vazdarjanova et al., 2006). *arc* codes for an effector protein whose RNA and protein are localized both in the soma and the dendritic tree (Lyford et al., 1995). *arc* expression and localization may thus be studied separately. Interestingly, when *arc* mRNA travels to the synapse, it facilitates endocytosis of AMPA receptors resulting in LTD of the synaptic response (Chowdhury et al., 2006). Behaviorally, it has been shown that after a

single exposure to a novel environment, mRNA expression is observed in 40% of CA1 neurons of the hippocampus (Guzowski et al., 2006). On the other hand, a simple spatial exploration is capable of inducing the expression of Arc in all the CA3 area (Miyashita et al., 2009). However, after a single spatial exploration, the upper blade of the DG expressed Arc albeit in just 2% of the cells (Chawla et al., 2005).

### I.4.C. IEGs involvement in Memory and Forgetting

IEG imaging techniques have been utilized to study brain activity in a variety of learning paradigms, such as the radial maze (He et al., 2002, Poirier et al., 2008). Altough the evidence suggests that IEGs are required for spatial learning in a radial maze, levels of IEG expression that take place in relevant brain regions after tasks requiring forgetting have not been carried out. Therefore, our experiments will seek to map out the changes that take place in a number of brain regions between different tasks taxing memory and forgetting using IEG expression as a marker of neuronal activity.

## I.5 Aim of this thesis

For many years, scientists have been investigating the neural bases of memory. We have seen in this introduction that a cardinal distinction lies between long-term/Reference Memory, and short-term/Working Memory. The mechanisms underlying these forms of memory have often been studied separately; some authors have studied the neural bases of WM while others have tried to determine the biological correlates of the long-term storage of information (Squire, 1992, D'Esposito et al., 1995). However, a key question remains: how does the brain distinguish information important enough to be consolidated into long-term memory from information required only temporarily, and that needs to be cleared away for not saturating our cognitive resources?

Some authors have suggested that WM would be more a form of forgetting than a form of memory (Dudchenko, 2004), and that WM and RM could simply be two antagonistic processes, one requiring forgetting and the other impaired by it (Malleret et al., 2010). During the past decades, numerous studies have considerably advanced our understanding of memory processes and their cellular and molecular underpinnings (Kandel, 1991, Tsien et al., 1996, Kandel, 2001). The concept of forgetting, however, remains elusive, probably because forgetting has often been seen as just a lack of memory, a failed process that happens to us involuntarily. We have seen that human studies suggest just the opposite and propose that forgetting is as important as memory, and that some forms of forgetting are adaptive and essential to secure optimal storage of information (Anderson et al., 1996, Kraemer and Golding, 1997). Surprisingly however, we hardly know anything of the cellular or molecular underpinnings of forgetting, and in particular of the adaptive forms of forgetting.

The overall objective of this thesis was to find a way to determine such bases of adaptive forgetting, in particular in the context of WM processing. To do so, instead of studying this process in an insolated way, we adopted a comparative approach by training groups of rats in a three different radial maze paradigms aimed at testing three different cognitive processes: **1**) Reference Memory (RM), **2**) Working Memory (WM) and **3**) the processing of interference in WM. The radial maze, requiring the use of spatial orientation and memory, was chosen as it allows training in both RM and WM tasks in one single spatial environment, and thus permits to determine a clear distinction between processes required for these different forms of memory. Whether they were tested in RM or WM, rats were placed in the same conditions and allowed for 10 consecutive days to complete eight runs (in one of the arms' maze) that were separated by a 15 seconds interval during which the rat was returned to its home cage. In the RM task, two baited arms remained constant across all trials, whereas in the WM task (Low Interference WM task - LIWM), the position of the target arm varied on each trial. The WM task consisted in a delayed-non-match-to-place task classically used in various models ranging from rodents to humans. In order to introduce considerably more PI in WM, we designed another WM task using the same pair of arms everyday for each trial (High interference WM task - HIWM). We have previously shown that this task promoted high level of interference, and involves the necessity to forget previous trials in order to correctly complete an ongoing trial (Malleret et al., 2010).

We designed these paradigms so that each day, rats in all conditions visited the same number of arms. This allows a clear comparison between processes requiring the storage of information (in RM or WM) and those requiring forgetting of previously stored information in WM. Behaviorally, we found that RM rats significantly improved their performance over days indicating a learning of the general rules and strategies required to solve the task. On the other hand, LIWM rats showed a good performance from the beginning of training, whereas, HIWM rats showed a decrease in performance over days indicating that accumulation of PI critically distorts WM performance with time.

## Chapter II: Brain regions differentially involved in memory and forgetting: A neuroanatomical approach

In the first chapter, we conducted an in-depth analysis of the contribution of a wide range of brain regions in each of the cognitive processes just described (RM, WM and the processing of interference). A number of brain regions which have been implicated in spatial learning was assessed, including the dorsal hippocampus and its subregions CA1, CA3 and the dentate gyrus, the lateral entorhinal cortex, and the medial prefrontal cortex. The method used to measure brain activation was IEG imaging of Zif268 and c-Fos protein, due to their established role in learning and memory. A significant increase in the density of Zif268 labeled neurons was observed in the Hippocampus, Entorhinal cortex and Prefrontal cortex in the three groups of animals compared to a control group composed of rats also exposed to the maze and trained to find food rewards but forced to go in pre-determined arms (and thus not involving cognitive choices). These results are consistent with the well-established roles of these brain areas in learning and memory. However, when examining more specifically the hippocampal formation, we found that the Dentate Gyrus of the dorsal hippocampus displayed the most unique pattern of activity, with expression of Zif268 (and c-Fos) remaining low, specifically after HIWM training. This result suggests for the first time that the non-activation of the Dentate Gyrus may be required to accomplish this task and overcome interference, and that the Dentate Gyrus might stay non-activated when forgetting/processing of previously learned (but no longer relevant) information is required.

## Chapter III: An analysis of Immediate Early Gene expression across two stages of learning and forgetting in the radial maze

In the first chapter, we examined IEG expression only upon completion of training (10 days), at which point the task has been mastered in RM and LIWM and deteriorated in HIWM. In this second study, we were interested in finding if a differential cerebral activity would occur during progressive learning of our tasks. More importantly, we were curious to find how the Dentate Gyrus responds to interference in an early learning stage when performance of the rats was not disrupted. The level of activity in different brain regions was assessed at an intermediate learning-stage in order to find if a differential cerebral activity would occur during progressive learning of our tasks. We ended the behavioral training of rats in the middle of the normal training (at 4 days of training) in order to carry out an analysis of IEGs expression across two stages of learning and forgetting in the radial maze. The results observed in this chapter were not conclusive because a higher expression of Zif268 and c-Fos

was observed in the control group on day 4 compared to day 10 in almost all the studied brain areas. Such increase in expression in controls is discussed in this chapter.

## Chapter IV: Dentate Gyrus lesion facilitates forgetting by reducing Proactive Interference

Our finding that the dorsal Dentate Gyrus shows no increase in Zif268 and c-fos expression after radial maze training involving forgetting is particularly striking. In this chapter, we sought to determine to what extent the Dentate Gyrus is required for WM and the processing of PI. To address this question, we examined the effects of ibotenic acid lesion of the dorsal Dentate Gyrus on our three behavioral tasks. Lesions of the DG significantly impaired RM and LIWM training with respect to the sham-operated rats. In sharp contrast, DG lesions did not affect HIWM performances. On the contrary, it produced a facilitatory effect on the last block of days. This result was in agreement with our IEG data suggesting that a non-activation of the DG is required during HIWM training. This facilitation may occur because DG lesions prevent the recall of similar but irrelevant information previously stored in memory from interfering with learning. This result confirms previous data showing that reference and working memory (requiring forgetting) are somewhat antagonistic processes as DG lesion impairs the consolidation of information but benefits to working memory by facilitating forgetting and the processing of interference.

## Chapter V: What mechanism(s) in the Dentate Gyrus is (are) responsible for forgetting?

In this last chapter, we tried to unravel the cellular mechanisms at play in the dentate gyrus that are possibly implicated in memory and forgetting. Consequently, we studied the involvement of new neurons, interneurons, afferents to the DG and plasticity mechanisms in processing memory over the long-term (RM), the short-term (WM) and the forgetting of useless information (HIWM). Our results are discussed at the end of this chapter.

# CHAPTER II

## Brain regions differentially involved in memory and forgetting: A neuroanatomical approach

## II.1 Introduction

In this first chapter, we sought to determine what brain regions are responsible for learning and forgetting of spatial memory. Thus, we carried out a comparative study to determine the differences existing between the biological bases of long-term memory, short-term memory and those of adaptive forgetting in the context of WM processing. Therefore, we tested different groups of rats in an eight-arm radial maze in tasks involving reference (RM) or working memory (WM) with or without proactive interference (PI) (i.e. requiring or not forgetting of previous information). The radial maze, requiring the use of spatial orientation and memory, was chosen as it allows training in both RM and WM tasks in one single spatial environment, and thus permits to determine a clear distinction between processes required for these different forms of memory. Indeed, we designed these paradigms so that each day, rats in all conditions visited the same number of arms. This permits a clear comparison between processes requiring the storage of information (in RM or WM) and those requiring forgetting of previously stored information in WM. After 10 days of training in these tasks, rats were sacrificed to identify brain regions differentially involved in the processing of RM or WM with and without interference using an immunohistochemical method to target the expression of immediate early genes (*zif268* and *c-fos*) known as indirect markers of neuronal activity and plasticity.

## II.2 Materials and Methods

### Subjects

A total of 110 ten week old Dark Agouti male rats initially weighing 210-230g at the beginning of the experiment were purchased from Janvier France. They were kept in a 12/12h light/dark cycle at 22-24°C with a humidity of 45 to 55 %. Animals were kept in group of two in a type IV Macrolon cage on softwood particle bedding (Scientific Animal Food and Engineering (SAFE), Augy, France) under optimal hygienic conditions. SAFE pelleted standard diet was fed *ad libitum* until a week before pretraining, after which food was gradually restricted. Tap water from local supply was available *ad libitum* from polyethylene bottles. Rats were handled everyday upon arrival in our facility. Testing took place during the light period of the cycle.

### Ethics Approval

The procedures concerning animal care and treatment were in accordance with the regulations of the local ethical committee (Lyon 1 University CE2A-UCBL 55) for the use of experimental animals and of the European committee (2010/63/EU).

### Behavioral Apparatus

An eight-arm radial maze requiring the use of spatial orientation and memory was used throughout the entire experiment for all tasks. The apparatus consisted of an elevated radial maze **(Figure II.1).** Eight arms (87 cm long x 12 cm wide) were arranged around an octagonal central platform (34 cm diameter). Clear Perspex barriers (20 cm long) extended along each arm from the central hub to discourage the rats from jumping across arms. The entrance of each arm was blocked by opaque Perspex doors that could be automatically lowered (pneumatic system) by the experimenter located in a room directly adjacent to the testing room. Squared food wells of 2 cm diameter and 0.5 cm deep were fixed at 0.5 cm beyond the end of each arm. Food rewards (Dustless Precision Pellets; Bioserve, Frenchtown, NJ) could be placed in these food wells. Food rewards were odorless in order to impede olfactory cues. The maze was located in a room with a number of extra-maze cues (e.g., poster, door, furniture – see **Figure II.1B**). A video camera, connected to a video recorder and a monitor, was fixed above the maze to enable the experimenter to observe the rats from an adjacent room. Behavior of the rats in the maze was videotaped for later examination.

## Behavioral protocol

Rats were housed in cohorts of two such that each rat that was to acquire a high interference, low interference working memory or reference memory task was housed with its yoked control. Therefore, three groups learned a radial maze task (Reference Memory RM, Low Interference Working Memory LIWM and High Interference Working Memory HIWM group), and three groups served as their respective controls (Yoked RM or YRM, Yoked LIWM or YLIWM, and Yoked HIWM or YHIWM).
Rats were food deprived and maintained at 85% of their free-feeding weight throughout the experiment. Food deprived rats had to retrieve food rewards at the end of the maze's arms using spatial navigation and distal visual cues surrounding the maze **(Figure II.1).**

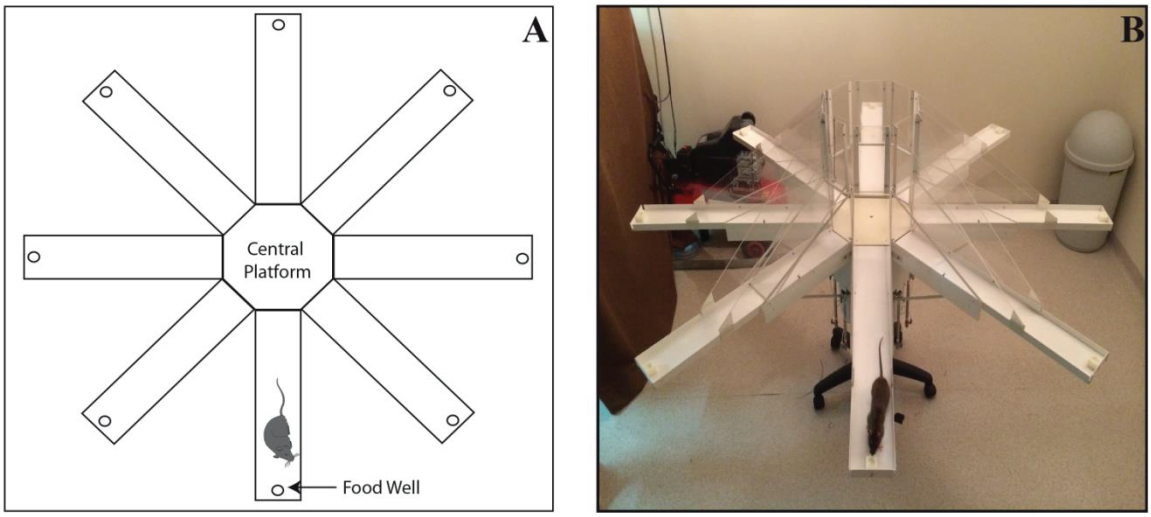


**Figure II.1: Behavioral apparatus: 8-arm Radial Maze** (**A**) Schematic of the 8-arm radial maze. Rats were trained to retrieve food pellets from the food wells. The task involved the rats leaving a pseudo-randomized starting arm and retrieving food from another arm. (**B**) A photograph depicting the 8-arm radial maze apparatus used in this study. The maze was placed centrally in a 3.20 x 3.00 m room which was lit with three 35-W halogen bulb lights to provide uniformly lit environment (to avoid shadows inducing internal cues), and to reduce stress and increase locomotor activity in rats.

## Pretraining

Rats underwent a 6-day habituation period during which they became accustomed to the radial maze environment and learned to find rewards in the arm wells. Rats were allowed to explore the maze for a 10 to 15 minute period each day. During these trials, food pellets were scattered throughout the maze (i.e., Day 1: 3 food pellets are located in the central platform, 1 food pellet in the center and two in the well of each arm; Day 2: idem; Day 3: 1 food pellet is located in the central platform, 1 food pellet in the center and 1 in the well of each arm;…; Day 6: food pellets were only located in the well of each arm, one pellet per arm well). Pretraining consisted in providing the animals with the experience of obtaining food in the maze, and in particular in the wells at the end of each arm. After habituation, they were pseudo-randomly assigned to one of the following groups. Whatever their group assignment, they were able to complete eight runs to an arm per day, making the three tasks strictly comparable in terms of motivational, emotional and motor processes.

**Reference Memory group**

Rats trained in the reference memory task had to retrieve food pellets in two arms of the maze (**Figure II.2A**). These two arms remain constant and were the same every day for the entire 10 days of training (Bontempi et al., 1999). Rats were initially placed in a pseudo-randomly chosen starting arm. A transparent blocker prevented rats from going backward to the food well of this starting arm. The rat was thus placed between this blocker and the closed door of this arm. Shortly after (the time for the experimenter to go the central command system – 1 sec), all arms of the maze were opened. The rat was thus allowed to enter the platform and choose an arm to visit. The use of a starting arm (instead of putting the rat directly on the central platform) prevented the rat to impetuously go to the first door that opened in front of him. From the starting arm, the rat thus takes the time to penetrate the central platform, and then make a choice among the seven other arms that were opened for visit. Once the rat chose one of the seven other arms (an arm selection was defined when the animal reached the arm's half way) the door to that arm was closed confining the rat in the chosen arm. After consuming the food reward in the case of a correct choice, or not in the case of an incorrect choice, rats were returned to a transfer cage adjacent to the maze for a short delay of 15 seconds, after which it was placed back in a new starting arm position for the next trial to begin. During subsequent trials, the doors to previously chosen arms remained closed until both food rewards were retrieved in order to prevent the rat to return into such arms (working memory errors). After both food pellets were retrieved, the two previously baited arms were re-baited and all arms were re-opened. Rats underwent eight trials per day (one trial = one run into an arm) and the maximum score per day was fixed at 8 pellets eaten. The latency to choose an arm as well as the number of correct choices were scored.

**Yoked Control RM (YRM)**

Each experimental RM rat was paired with a YRM that performed the same amount of motor activity and ate the same number of pellets. These yoked controls were forced to enter into pseudo-randomly chosen arms and were either reinforced or not depending on the performance of their experimental matched rat. The starting and destination arms varied between trials in such a way that yoked controls could not use motor memory to predict which direction they had to go. The position of food pellets in the maze varied also each day so that the rat could not predict (and memorize) the spatial location of such reinforcements. The use of yoked controls allows the experimenter to conclude that all differences seen between groups after inmmunohistochemistry analysis are inherent to learning processes during the task and not due to motivational, sensory or locomotor aspects of the task (Bontempi et al., 1999, Poirier et al., 2008).

**Low interference Working Memory (LIWM) group**

Rats trained in this task were submitted to four trials per day, each consisting of a sample and a choice phase (4 x 2 = 8 runs, matching the eight runs performed by the RM group). In the sample phase, rats were first allowed, from a starting arm, to enter one randomly chosen baited arm while all other arms remained closed. Rats were then returned to the transfer cage for a short delay of 15 seconds (identical delay than in the RM task). During the subsequent

choice phase, rats were presented with two adjacent arms, the arm that had just been visited and empty of food, and a new adjacent arm containing a second food reward (**Figure II.2B**). Rats had to choose the novel arm in order to be positively reinforced (classical delayed non-match to place task). Different pairs of arms were used for each trial **(Figure II.2B** indicates an example of trial sequence for a given day**)**.

**Yoked Control LIWM (YLIWM)**

Like for RM rats, each experimental LIWM rat was paired with a yoked control (YLIWM) that was exposed to the same radial maze arms to make sure that the two groups (LIWM and YLIWM) were exposed to the same spatial information. Like YRM rats, YLIWM rats were forced to visit only one arm during each run and were not exposed to any cognitive choice as compared to LIWM rats. Whereas LIWM rats had to learn a delayed non-match to place task rule in order to successfully complete the task, YLIWM rats were exposed to an equal number of non-match and match "forced" trials in a pseudorandom fashion in order to prevent YLIWM rats to predict the outcome of a trial and the presence, or not, of a food reward.

**High interference Working Memory (HIWM) group**

HIWM rats were exposed to an experimental procedure similar to the one used in the LIWM task, except that the same pair of arms was used everyday for each trial. This promoted high level of interference and repetition in order to make forgetting of previous trials necessary to complete an ongoing trial (Saxe et al., 2007, Malleret et al., 2010) (**Figure II.2C** indicates an example of trial sequence for a given day).

**Yoked Control HIWM (YHIWM)**

Each experimental HIWM rat was paired with a yoked control (YHIWM) that performed the same amount of motor activity and ate the same number of pellets as already described for the RM and YLIWM groups. Like YLIWM rats, YHIWM rats were exposed to an equal number of non-match and match forced trials in a pseudorandom fashion in order to prevent YHIWM rats to predict the outcome of a trial.

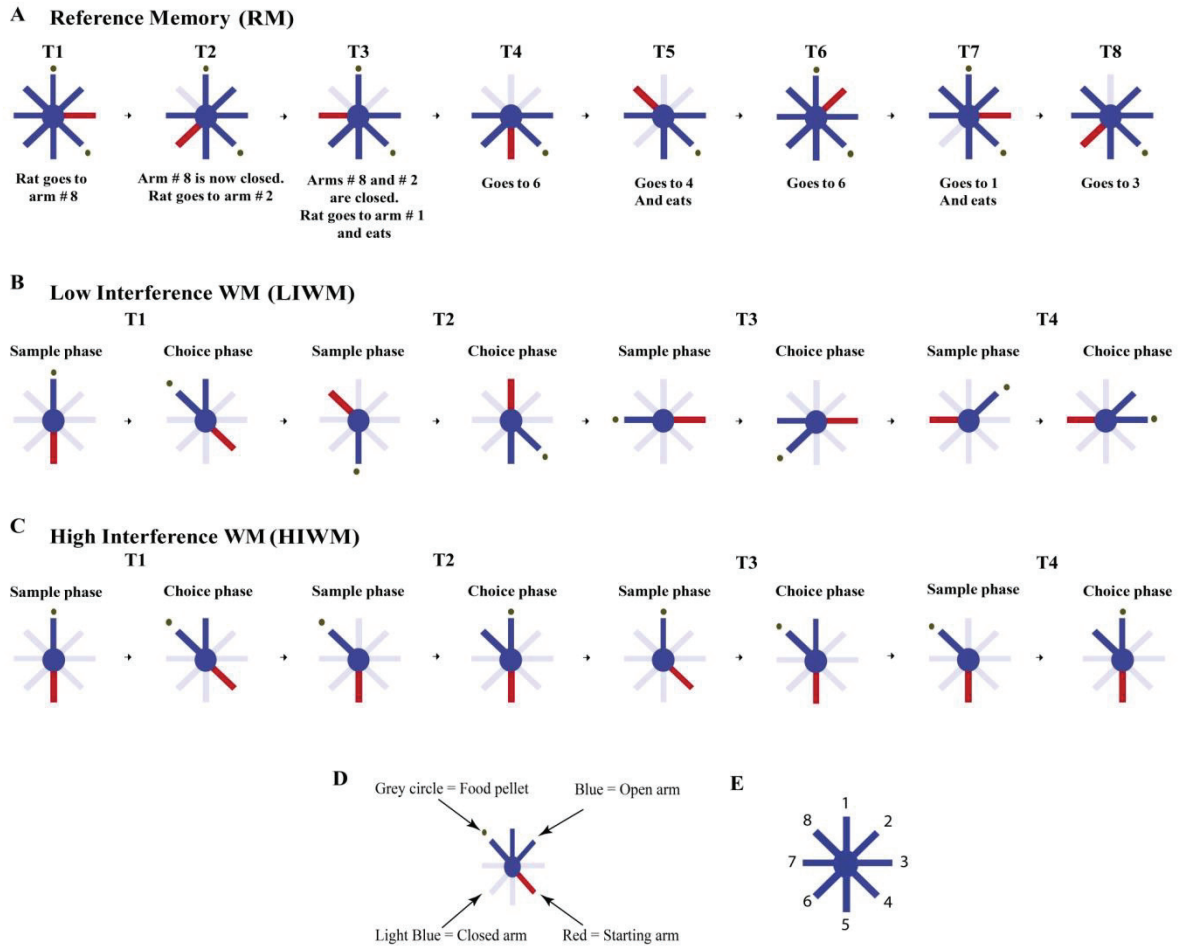


**Figure II.2: Behavioral paradigms.** A schematic representation of one day of training for each of the three different experimental groups. Each day consisted of 8 runs. (**D**) Blue represents open arms. Light blue represents closed arms. Red represents starting arm. Grey circles represent food pellets. (**A**) Schematic representation of one daily session of RM training for a given rat. The same two arms (here #1 and #4, (**E**)) are baited every day for each trial. Each daily session consisted of 8 trials (T1 to T8). On each trial, the rat can visit only one arm. Once the rat chose one of the arms, the door is closed. After both food pellets are retrieved, the two previously baited arms are re-baited and all arms are re-opened (in the example presented here, at trial 6). (**B**) Schematic representation of one daily session of LIWM training. Each day consisted of 4 trials (T1➔T4). Each trial (T) consisted in 2 phases. This task is a "delayed non-match to place" in which the animal must memorize a position in space (during the sample phase) and retain this information for a short time (15 seconds). To obtain the food reward during the choice phase, the animal must remember the information stored and visit a different place in space. In this task, different pairs of arms are used for each trial, consequently forgetting of previous trials is not necessary in order to have good performance on an ongoing trial. (**C**) Schematic representation of one daily session of HIWM training. This task is also a "delayed non-match to place" task except that the same pair of arms is used every day for each trial. Consequently, the trials are very similar to each other and it is therefore necessary to ignore previous trials (e.g. T1 and T2) in order to complete an ongoing trial (e.g. T3). In all three tasks, we matched our experimental rats (performing the tasks as described) with (yoked) control rats eating the same number of pellets, visiting the same number of arms, but that are not exposed to any cognitive choice (forced arm).

**Perfusion**

Ninety minutes after the last training session (time required to induce expression of *zif268* and *c-fos* (Kubik et al., 2007), a subset of rats (n = 44) were deeply anesthetized with an overdose of sodium pentobarbital (140 mg/kg, Sigma) that was warm to room temperature. This group of rats (n=44) was used for immunohistochemistry while the second subset of rats (n=66) was used for Western Blot study (see chapter V) in order to examine synaptic changes potentially responsible for long-term memory or the erasure of previous information (Fraize et al., in preparation). After pentobarbital injection, a vertical incision was made in the abdomen then a small hole was cut in the peritoneum by tenting the tissue. Scissors were then inserted to make a large vertical incision. After lifting the xyphoid and clamping it, a careful horizontal incision was made in the diaphragm in order not to damage the heart. After visualizing the heart and making sure it was still beating, the needle was inserted into the left ventricle of the heart and the pump was turned on. Immediately afterward an incision was made in the right atrium with a very small scissor to allow outflow of the blood. When the liquid running out of the atrium was clear (after flushing with about 200 ml heparinized ringer lactate (0.1%)), the pump was shut off and the end of tubing was placed into 4% paraformaldehyde (PFA) 0.1 M phosphate buffer (PB) (pH 7.4). Each rat was perfused with 400 ml of PFA. Brain was then removed from the skull and cryoprotected in 30% sucrose solution (in 0.1M PB) and then placed in a refrigerator maintained at 4$^{o}$C for two days.

To produce thin, high-quality frozen sections, the brain must be well prepared, the conditions of the cryostat (Microm, France) must be optimal and the block temperature must be correct for the tissue being cut. The brain was first freezed in a solution of isopentane (2-methylbutane) cooled by dry ice (carbonic ice) to approximately -50$^{o}$C for 2 minutes and then placed in a cryostat at -20$^{o}$C. Cryomount (Histolab AB, Gothenburg), was used to bind the brain to a specimen holder. The brain was surrounded and covered with cryomount embedding fluid (water, soluble glycols and resins). Brains were cut into 25 μm-thin coronal sections and placed in two blocks of 12 containers (wells) each, filled with PB saline triton (PBST), to obtain 8 columns of 3 wells each containing slices from the whole brain.

For immunohistochemistry, the use of Horse Radish Peroxidase (HRP) conjugated antibody may result in high, non-specific background staining. This non-specific background can be significantly reduced by pre-treatment of brain tissues with hydrogen peroxide ($H_2O_2$), prior to incubation with HRP conjugated antibody. Hydrogen peroxide ($H_2O_2$), a blocking agent, is commonly used to block endogenous peroxidase acitivity. Sections were thus pre-treated with 10% $H_2O_2$ for 10 minutes to induce an irreversible inactivation of endogenous peroxidase.

**Immunohistochemistry**

To minimize variability in staining attributable to the histological procedure rather than to the behavior, brain sections of representatives for all conditions were processed in a single batch. All immunohistochemical reactions were carried out on free floating sections under continuous gentle agitation. Serial sections were collected in PBST Azide (PB 10 mM pH 7.4, NaCl 0.9 %, Triton X100 0.3%, Azide 0.1 %) and then incubated at 4°C with the primary antibody (**Table II.1**). After incubation with primary antisera, sections were washed at room temperature (three times 10 min in PBST). Sections were then incubated with the appropriate biotinylated secondary antibody IgG for 2 hours at room temperature. Then, they were subsequently washed and processed with avidin-biotin horseradish peroxidase complex (ABC 1:2000, Elite Kit from Vector Laboratories) overnight at 4ºC. The next day, Sections were washed three times for 10 minutes in PBST and immunoreactivity was visualized with 0.025% diaminobenzidine chromogen (DAB, Sigma), 0.05 % Nickel and 0.03% $H_2O_2$ as reaction initiator. Sections were mounted on gelatin-coated slides, air dried for couple of days, dehydrated through a graded series of alcohols and toluene and coverslipped (**Figure II.3**)

**Table II.1: Primary and Secondary antibodies used in Immunohistochemistry**

| | Characteristics | Specie | Dilution | Reference |
|---|---|---|---|---|
| Zif268 | Polyclonal antibody (Primary Antibody) | Rabbit | DAB: 1/1000 | Santa Cruz Egr-1(588):sc-110 |
| C-Fos | Polyclonal antibody (Primary Antibody) | Rabbit | DAB: 1/5000 | Calbiochem Anti-c-Fos (Ab-5) |
| Anti-Rabbit | Biotinylated IgG (H+L) (Secondary Antibody) | Goat | DAB: 1/1000 | Vector Laboratories BA-1000, X0524 |

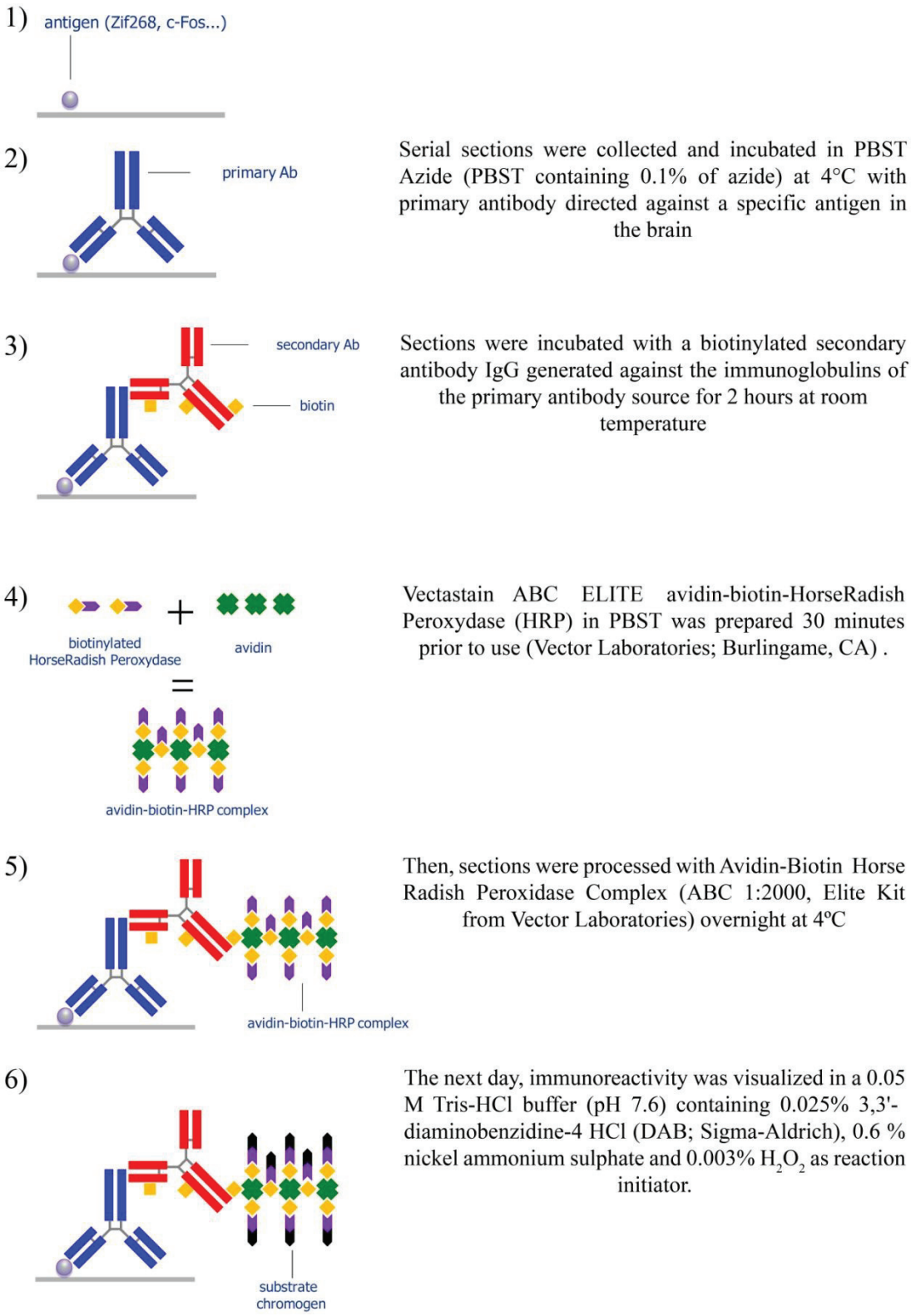


**Figure II.3: Immunohistochemistry Protocol**. In brief, the primary antibody is bound to the protein of interest (Zif268/c-Fos). A biotinylated secondary antibody is then bound to the primary antibody. In a separate reaction, a complex of avidin and biotinylated enzyme is formed by mixing the two in a ratio that leaves some of the binding sites on avidin unoccupied. This complex is then incubated with the tissue section after the antibody incubations. The unoccupied biotin-binding sites on the complex bind to the biotinylated secondary antibody. A reaction between DAB (3, 3'-diaminobenzidine) HRP substrate, Nickel and $H_2O_2$ produces a dark black product.

**Cell counts**

Quantitative analyses of Zif268 and c-Fos positive cells were performed by using a computerized image processing system (Mercator, Explora Nova ®) coupled to an optical upright microscope. Structures were defined according to the Paxinos and Watson atlas (Paxinos and Watson, 1996) (**Figure II.4**). Immunoreactive neurons were counted bilaterally using a minimum of four sections. Cells were counted throughout the different area of the sections with an objective of 20x magnification. Data from YLIWM, YHIWM and YRM were pooled as a unique control group of reference as no significant statistical difference in neither Zif268 nor c-Fos activation in all studied structures was found between these three groups. For each animal, Zif268 and c-Fos density was calculated by dividing cell counts of each area by the surface of the area. Each animal's areas density was then normalized by dividing the corresponding control density (% of control). The experimenter was blinded to experimental groups during counting.

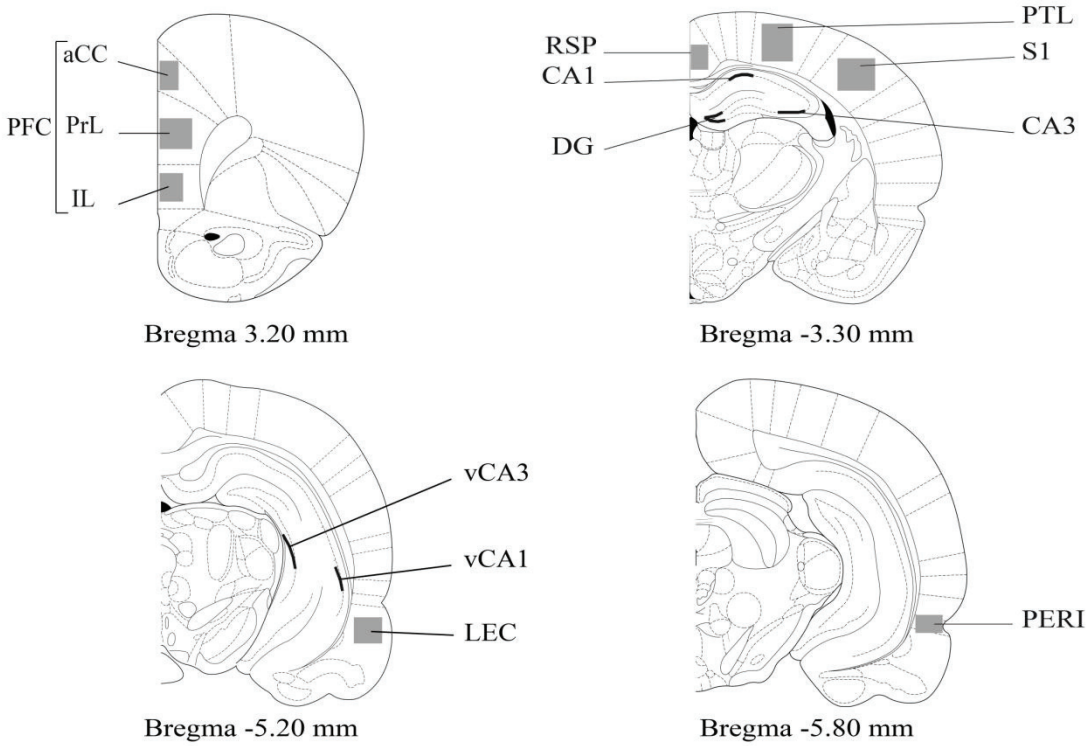


**Figure II.4: Diagrams of rat brain coronal sections depicting regions of interest (filled areas) where immediate-early gene cell counts were obtained**. The numbers indicate the distance (in millimeters) of the sections from bregma (Paxinos and Watson, 1997). aCC: anterior cingulated cortex; CA1: CA1 field of dorsal hippocampus; CA3: CA3 field of dorsal hippocampus; DG: dentate gyrus; IL: infralimbic cortex; LEC: lateral entorhinal cortex; PERI: Perirhinal Cortex; PFC: Prefrontal cortex; PTL: posterior parietal cortex; PrL: prelimbic cortex; RSP: retrosplenial cortex; S1: primary somatosensory cortex; vCA1: CA1 field of ventral hippocampus; vCA3: CA3 field of ventral hippocampus; IEG counts for the following brain regions were expressed as the pooled means of the listed subregions: Prefrontal cortex : IL, PrL, aCC.

**Statistical analysis**

- **Behavior**

The data collected from the behavioral experiment in the radial maze represent repeated measures, i.e. the same measures are obtained from the same rats repeatedly from day to day. Data which are not normally distributed, and which do not exhibit homogeneity of variance should be analyzed by non parametric methods. To our knowledge, non parametric methods for multiple dimensions analysis (two way) of repeated measures have not been developed. For this reason, behavioral data were analyzed using two-way ANOVAs (Analysis of Variance) for repeated measures with Block (2 days) and Group (RM, LIWM, HIWM) as main factors (Statview 5.0.). Further comparisons were performed by a *post hoc* (Scheffe) test for particular within-group comparisons. Data are expressed as means ± s.e.m.
A percent correct score was calculated for each rat's daily performance by dividing the number of correct arm choices by the total number of arm choices (= 8 for RM and 4 for WM). The chance level of performance for the two working memory tasks is simply 50 % because the rat always had to make a choice between two open arms. For the RM group, as the chosen arm in a trial is blocked during the subsequent trial, the probability to choose a baited arm varied from trial to trial. In this case, the chance performance is affected by the number of correct choices made by each animal. For example, the probability of selecting the correct arm on the first choice is 2/7 since there are 7 open arms and 2 baited ones (the rat is present in the $8^{th}$ starting arm). On the next trial, the visited arm is blocked and the rat is facing 6 open arms. But the chance performance for this trial is either 1/6 if the rats found a food reward on the previous trial, or 2/6 if it did not. A global chance performance for the RM task was thus calculated using a Python script made by François Nader, a PhD Student at INSA LYON (personal communication, May 2014). The script calculated the virtual performance for 10 million rats performing random choices. Using this procedure, we determined chance performance to be 2.7982298 for 8 trials per day which gives us a chance level of 34.9%.

- **Immunohistochemistry**

Zif268 and c-Fos immunoreactivity was statistically analyzed with non-parametric Mann-Whitney U-tests. Data are expressed as mean of normalized Zif268 and c-Fos density (% of control) ± s.e.m.

- **Correlation**

The density of Zif268 and c-Fos labeled neurons was also used to compare inter-regional brain activity. To better understand the functional connectivity between brain regions, we assessed the correlation matrix for each experimental group using the Spearman's rank correlation coefficient, a measure of statistical dependencies between non-parametric variables. A positive coefficient between two brain regions indicates that an increase in a region would result in a proportional increase in the other region.

## II.3 Results

### Proactive Interference induces a decrease in performance

After 10 days of training, we found that RM rats significantly improved their performance over time and reached 85% of correct choices on the last block of days (**Figure II.5A**) indicating a learning of the general rules and strategies required to solve the task. *Post hoc* analyses revealed that RM rats significantly improved their performance over time ($p < 0.0001$).

LIWM rats showed a good performance from the beginning of training, their scores increasing with time and reaching 100% on Block 5 ($p = 0.045$).

On the contrary, HIWM rats showed a decrease in performance over days indicating that accumulation of PI critically distorts WM performance with time. This effect was statistically significant ($p = 0.017$). More importantly, significant difference in scores was shown on the last block of training between RM, LIWM and HIWM ($p < 0.0001$) but all groups clearly performed above chance at the end of training ($p < 0.0001$).

Our next goal was to see whether rats' performance in WM declines from trial 1 to trial 4 but always returns to errorless performance on the first trial of the next day. This is known by the process of resetting, defined as the capacity of the ratsto erase or reset the contents of memory at the end of each trial. To do so, we analyzed rats' performance by trial rather than by day over the first (Day 1-5) and last 5 days (Day 6-10) of the experiment. We found that while there was a slight impairment in trial 4 in HIWM compared to LIWM (ANOVA $p = 0.0211$) at the beginning of training (Day 1-5), HIWM rats' performance dropped significantly during all the trials at the end of training (Day 6-10) ($p < 0.0001$) (**Figure II.5B, C**). From day 1 to day 5, the results showed intertrial proactive interference and probably a resetting on the next day. However, from day 5 to day 10, resetting phenomenon was absent as HIWM rats' performance was worse than LIWM in all trials.

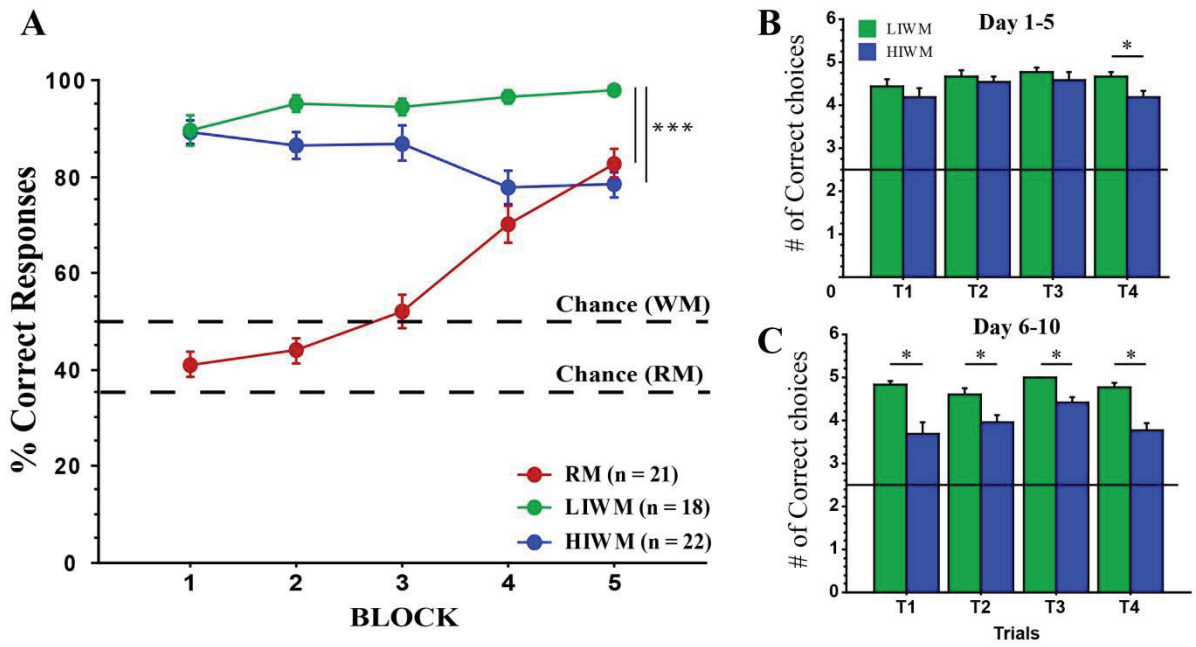


**Figure II.5: Rats tested in the HIWM task exhibit a decrease in performance after few days of training. (A)** Percentage of correct choices ± s.e.m per block (each block = 2 days of training) in a RM, LIWM and HIWM task. Black hatched line represents the chance level for the WM groups (50%) and for RM groups (34,9%). ANOVAs revealed a significant Group effect [$F(2, 58) = 147.97$; $p < 0.0001$], a significant Block effect [$F(4, 232) = 11.75$; $p < 0.0001$], as well as a significant Group x Block interaction [$F(8, 232) = 20.86$; $p < 0.0001$]. (**B** and **C**) Total number of correct choices made by animals described in (A) on trials 1, 2, 3, and 4 (T1, T2, T3, and T4) of days 1 to 5 (**B**) and days 6 to 10 (**C**). Black lines represent the chance level.

### The Dentate Gyrus of the dorsal Hippocampus is not activated in a HIWM task

To identify brain regions differentially involved in processing memory over the long term, the short term and the forgetting of useless information, we mapped the regional expression of the immediate early genes (IEG) *zif268* (**Figure II.6**) and *c-fos* (**Figure II.7**) used as indirect markers of neuronal activity and plasticity. These IEGs were studied because they are associated with spatial memory formation and their expression was examined following ten days of training. All groups of rats had increased number of Zif268 immunoreactive cells in the dorsal hippocampus as a whole, or more specifically in the CA1 and CA3 areas, compared to the control group (100% baseline – pooled yoked control groups YRM, YLIWM and YHIWM) composed of rats also exposed to the maze and trained to find food reward but forced to go in pre-determined arms (and thus not involving cognitive choices – see material and methods), (LIHM versus Control; HIWM versus Control; RM versus Control: all $P < 0.05$).

In sharp contrast, when examining more specifically the hippocampal formation, this increase in the number of zif268-positive cells was not observed in the dentate gyrus of HIWM rats and consequently did not differ significantly from control (**Figure II.6 A** and **B**). Zif268 expression was significantly higher for LIWM and RM rats as compared to control in the dentate gyrus (HIWM versus C: P = 0.1876, ** RM versus C: P= 0.0019, ** LIWM versus C: P = 0.004) and compared to HIWM (# HIWM versus LIWM: P = 0.01, ## RM versus HIWM: P = 0.0065).

We next investigated whether zif268 activation was induced in the prefrontal cortex (PFC) cortex, another region known to be involved in cognitive processes, of RM, LIWM and HIWM rats. We found that the density of zif268 positive cells was enhanced in the PFC as a whole (Prelimbic, Infralimbic and Anterior cingulated cortex) ($P = 0.0052$ for RM versus C, $P = 0.0048$ for LIWM versus C and $P = 0.0006$ for HIWM versus C).

The lateral entorhinal cortex (LEC) also showed an increased density of zif268 positive cells in the three groups ($P = 0.0011$ for RM versus C, $P = 0.0085$ for LIWM versus C and $P = 0.05$ for HIWM versus C).

As expected, the Primary Somatosensory cortex (S1) was not activated after training in any of the three tasks and consequently did not differ from the control ($P > 0.05$ for all conditions). This region is usually not specifically activated by higher order cognitive processes and thus serves as an "anatomical control" area.

**Absence of interregional brain activity correlation in HIWM**

The density of Zif268 labeled neurons was also used to compare inter-regional brain activity. To better understand the functional connectivity between brain regions, we assessed the correlation matrix for each experimental group using the Spearman's rank correlation coefficient, a measure of statistical dependencies between non-parametric variables. A positive coefficient between two brain regions indicates that an increase expression of Zif268 in a region would result in a proportional increase of Zif268 in the other region. In the control group, a high level of positive inter-regional brain correlation was specifically observed between the different areas of the hippocampus (between CA1 and CA3 $r = 0.709$; CA1-DG $r = 0.567$ and DG-CA3 $r = 0.858$, $p < 0.05$; **Figure II.6C**). In addition to the control group, numerous correlations were observed between brain regions in the RM (DG-CA1 $r = 0.792$; LEC-CA3 $r = 0.782$; LEC-PFC $r = 0.708$, $p < 0.05$) and LIWM groups (CA3-CA1 $r = 0.75$; LEC-CA1 $r = 0.893$; LEC-CA3 $r = 0.794$; LEC-PFC $r = 0.729$, $p < 0.05$). Specific correlations are evident between intrahippocampal areas but also between entorhinal and medial prefrontal cortices and the hippocampus. However, the pattern of correlated activity dramatically changed in the HIWM compared to the other groups of rats. No inter-regional brain correlation was observed between any of the studied structures suggesting a decoordinated activity of these structures during the processing of interference.

**c-Fos staining**

The patterns of c-Fos expression mimicked those of Zif268 in the CA1, CA3 and the DG of the hippocampus, the LEC and in S1 (**Figure II.7 A**). However in the PFC, c-Fos had a differential expression. PFC was only activated after a LIWM task (LIWM versus C, $p = 0.0091$), despite a close significance for RM and HIWM ($p = 0.094$).
As done previously with Zif268, the density of c-Fos labeled neurons was used to compare inter-regional brain activity. The patterns of c-Fos correlation also mimicked those of Zif268. We found correlations between different brain structures in RM, LIWM and the control condition. However, it is important to note the absence of correlation in the HIWM task confirming our previous observation of Zif268 inter-regional brain de-correlation (**Figure II.7 C**).

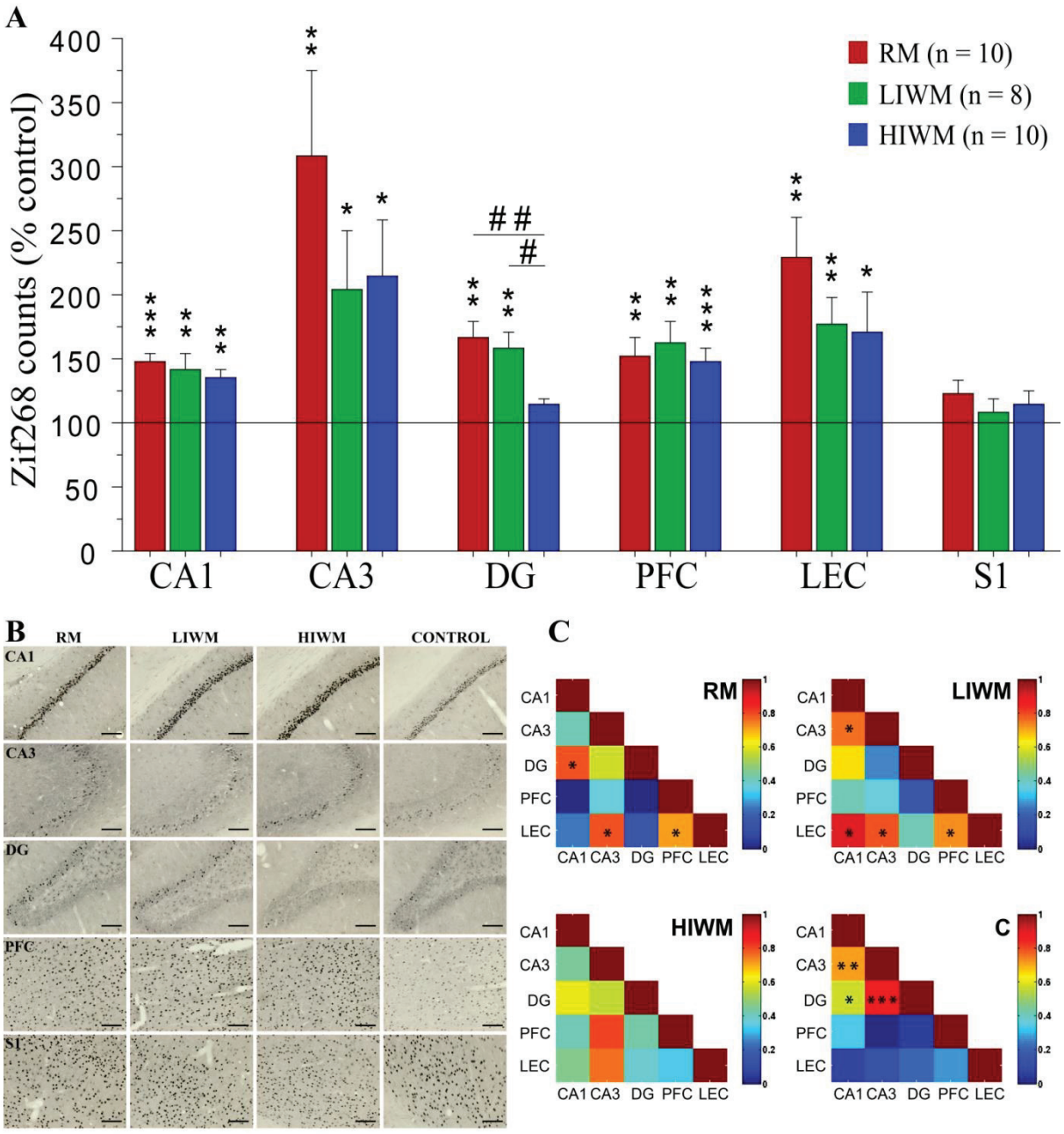


**Figure II.6: The dentate gyrus is not activated after training in the HIWM task**. (**A**) Zif268 counts relative to paired controls (black line) in the CA1, CA3 and Dentate Gyrus (DG) of the hippocampus, Prefrontal cortex (PFC), lateral Entorhinal Cortex (LEC) and primary Somatosensory (S1) after 10 days of training. All groups of rats had increased number of Zif268 immunoreactive cells in these areas compared to control animals (n = 16, 100% baseline) except the control structure S1. This increase in the number of zif268-positive cells was not observed in the dentate gyrus of HIWM rats. P < 0.05; **, ## P < 0.01; *** P < 0.001. (**B**) Representative Photomicrographs showing Zif268-stained nuclei in the dorsal dentate gyrus of the hippocampus in our four groups of rats. Scale bar, 100 µm. (**C**), Interregional Correlation matrices for Zif268 expression within each group. R-Spearman rank correlation coefficients are color-coded. Colors reflect correlation strength (scale, right). Significant correlations ($p<0.05$) are marked with (*).

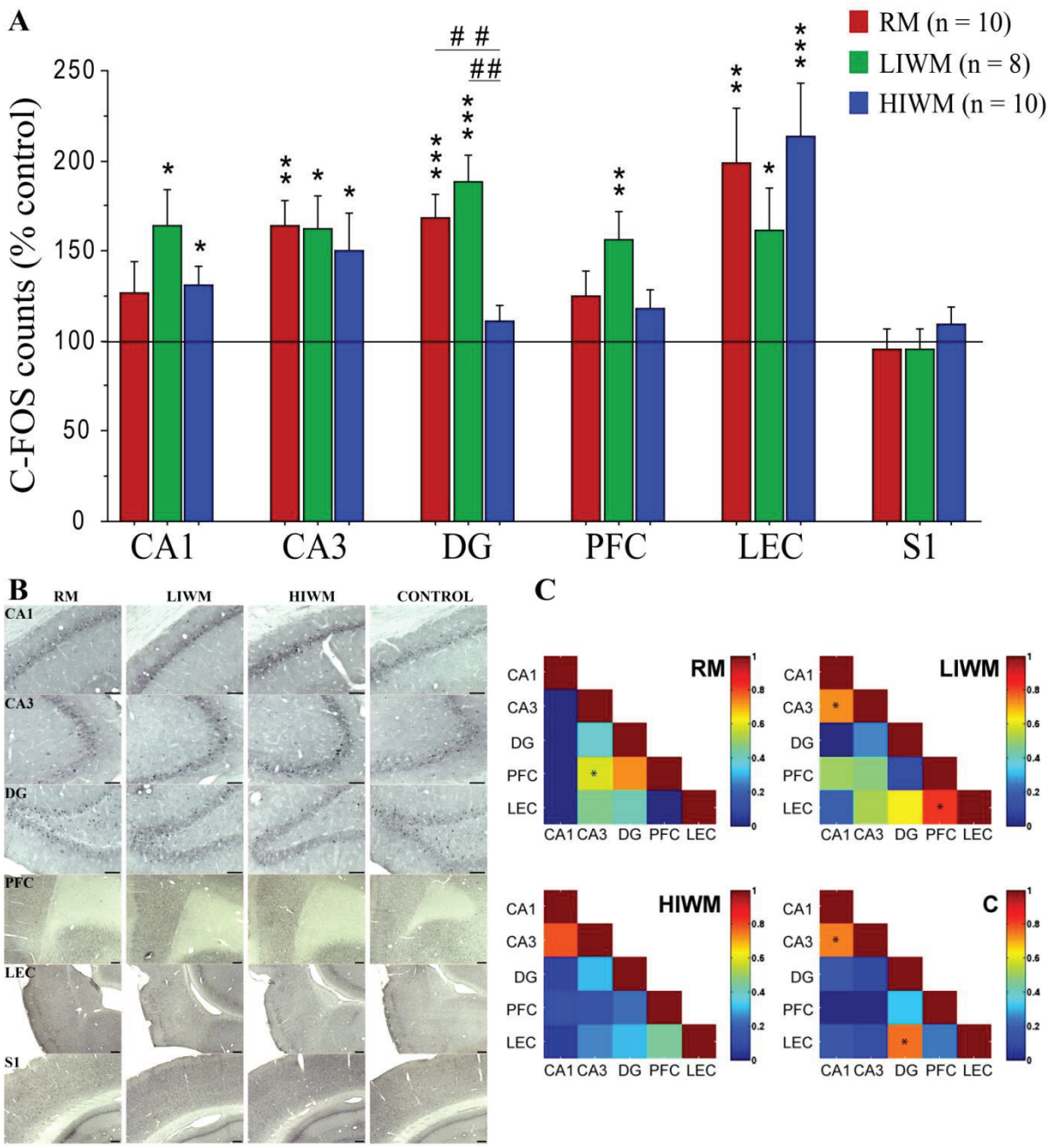


**Figure II.7: c-Fos counts relative to paired controls (black line)** (**A**) in the dorsal CA1, CA3 and Dentate Gyrus (DG) of the hippocampus, Prefrontal cortex (PFC), lateral Entorhinal Cortex (LEC) and primary Somatosensory cortex (S1) after 10 days of training. The patterns of c-Fos expression mimicked those of Zif268 in the CA1, CA3 and the DG of the hippocampus, the LEC and in S1. However in the PFC, c-Fos had a differential expression. This could be due to the fact that these two IEGs are differentially implicated in synaptic plasticity-related mechanisms (Davis et al., 2003) * P < 0.05; ** P < 0.01 versus control group (100% line); # *P* < 0.05 versus group. (**B**) Representative Photomicrographs showing c-Fos-stained nuclei in the dorsal dentate gyrus of the hippocampus in out four groups of rats. Scale bar, 100 µm. (**C**) Interregional Correlation matrices for c-Fos expression within each group. R-Spearman rank correlation coefficients are color-coded. Colors reflect correlation strength (scale, right). Significant correlations: * P < 0.05. The patterns of c-Fos correlation mimicked those of Zif268. We can found correlations between different brain structures in RM, LIWM and the control condition. However, it is important to note the absence of correlation in HIWM task.

Very recently, using the same quantification of the Zif268 protein as an indicator of neuronal activation, we studied the involvement of the ventral hippocampus, associative cortices and other structures in our three spatial memory tasks (**Figure II.8**).

The ventral Hippocampus counts were taken from the same coronal sections and involved the corresponding portions of CA1 and CA3. In ventral CA1, counts of nuclei stained for Zif268 were significantly higher in both RM and HIWM but not in LIWM (RM vs C, p = 0.0002; HIWM vs C, p = 0.0305 and LIWM vs C, p = 9485). Moreover, there were highly significant differences for all experimental groups versus Control in the CA3 area of the ventral hippocampus resulting in the greatest zif28 activation (All $p < 0.05$).

The other cortical regions examined comprised all the layers of the posterior parietal cortex (PTL), the medial and dorsal parts of the retrosplenial cortex (RSP) and the perirhinal Cortex (PERI). There was an increased number of Zif268-postive nuclei in animals performing the RM task compared to the control condition. This difference was significant for the PTL (RM vs C, p = 0.04), the RSP (p = 0.0034) and PERI (p = 0.0269). Performing a HIWM task also resulted in greater numbers of Zif268-stained nuclei for the PTL (HIWM vs C, p = 0.0028) and RSP (p=0.0013) but not for the PERI (p = 0.3991). In LIWM group, a greater number of Zif268 positive cells was only observed in the PTL and differed significantly from Control group (LIWM vs C, p = 0.0005). No difference was observed neither in the RSP nor the PERI. A final region was included by virtue of its involvement in procedural memory. This region was the Caudoputamen (CP). There was no evidence of a difference in *zif268* activation between the experimental animals and the control group (All $p > 0.05$).

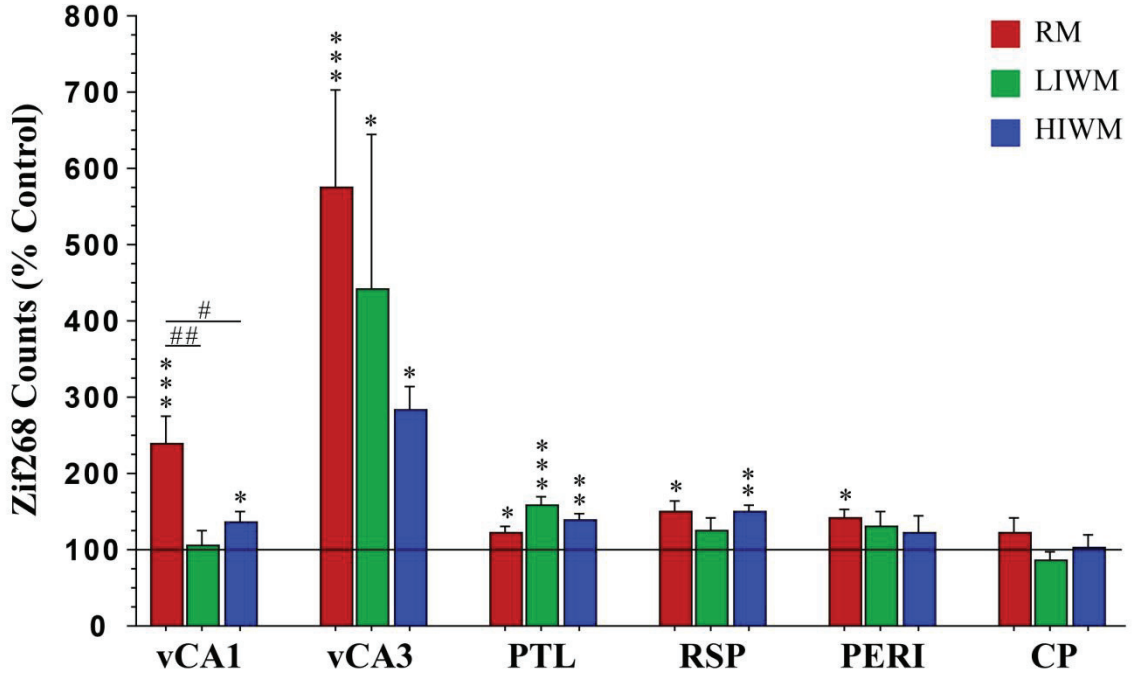


**Figure II.8: Normalized counts of Zif268-positive nuclei for the ventral hippocampus and other cortical regions**. Zif268 counts relative to paired controls (black line) in the CA1, CA3 of the ventral hippocampus, Parietal cortex (PTL), Retrosplenial Cortex (RSP), Perirhinal Cortex (PERI) and caudoputamen (CP) after 10 days of training. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$ versus control group (100% line); # $P < 0.05$; ## $P < 0.01$ versus group.

## II.4 Discussion

### The effects of Proactive Interference on Working Memory

This study allowed us to characterize the pattern of activity of different brain regions involved in RM, in normal WM and more particularly when WM is influenced by high level of interference. Our behavioral results showed that rats could effectively learn a RM task as we designed it. Indeed, RM rats showed a significant increase in correct responses over the 10-day training period and were well above chance during the last half of the training period. In contrast, all WM rats displayed a high percentage of correct responses from the first blocks of training. This immediate learning of the WM task is certainly due to innate spontaneous alternation, a behavior that naturally causes rodents to alternate between two open arms (Whishaw, 1995). This tendency to spontaneously alternate between radial maze arms facilitates correct WM responses. Spontaneous alternation requires memory storage of the previously visited arm in order to alternate to a different arm, and is dependent on the medial temporal lobe functional integrity, including the hippocampus (Ellen and Deloache, 1968). Most interestingly, while LIWM rats kept high scores throughout the entire experiment, HIWM rats showed a significant decrease in their performance over the course of training. This significant decrease is attributed to the high level of interference and repetition that is present in the HIWM task. The evolution of WM performance due to ever-increasing buildup of PI is a result that has been seen by our team in previous experiments using similar WM paradigms (Saxe et al., 2007, Nicholls et al., 2008). Nevertheless, HIWM rats maintained a score significantly superior to chance throughout the experiment and performed on the same level as RM rats on the last day of training. Interestingly, mice exposed to the same LIWM/HIWM tasks exhibit a learning curve similar to that seen here with RM rats (Malleret et al., 2010). These mice made an important number of errors during the first days of training and needed two weeks to reach an acceptable level of performance and learn the WM tasks. These results indicate that rats and mice may not be equal when facing training cognitive tasks requiring the use of WM.

### Does a short-term storage really exist?

Intertrial interference in a radial maze Working Memory task was shown to occur in mice (Aultman and Moghaddam, 2001). These authors suggested that information learned during past experienced trials could impair performance on subsequent trials due to the buildup of interference. Mice exhibited normal performance on the first trial of a day but their behavior was disrupted on subsequent trials. This pattern is thought to reflect the influence of previously learned information from previous trials (PI) on performance (Nicholls et al, 2008). Nicholls and Colleagues argued that on the first trial of each day, interference does not occur because the interfering material has been forgotten during the 24 hours. This ability called resetting of WM has been proposed by Olton in 1978 (Olton, 1978). However, Nicholls and colleagues have only observed such resetting in transgenic mice in which LTD was blocked. On the other hand, in wild type mice, this phenomenon was not observed. Resetting of WM was not observed in our HIWM task and our rats were impaired even on the first trial over the last five days of the experiment. These results confirmed earlier findings by Roberts and Dale (Roberts and Dale, 1981) arguing that such resetting of WM may not occur, at least

in rats. They also suggest that information supposedly stored in short-term/WM can outlast their purpose by interfering, several days later, with the storage of newer information, thus questioning the existence of a pure short-term memory store. This possible lack of short-term memory storage has also received recent support from the work of Ranganath and Blumenfeld (2005) that rose doubts about a double dissociation between short-term memory and long-term memory. These authors have argued that the evidence suggesting distinct neuroanatomical substrates for short-term memory (requires only the PFC) and long-term memory (requires only the hippocampus) may have been misleading. They reviewed evidence demonstrating that short-term memory store might be simply considered a temporary activation of some portion of the long-term store (Ranganath and Blumenfeld, 2005). Moreover, other studies have shown similarities in the neural correlates of long-term memory and WM in a way that these different cognitive functions activate overlapping brain regions (Cabeza et al., 2001). Our data seem to confirm such findings… but with a little "twist".

**Processing Interference requires a negative control of the dorsal DG**

Zif268 expression in pyramidal cells of CA1, CA3 of the dorsal hippocampus, the entorhinal cortex and the prefrontal cortex were significantly elevated after RM and WM training, and this elevation was not altered by the presence of proactive interference. These results suggest that like RM, WM depends on the activation of the hippocampal complex and of the prefrontal cortex. These data are consistent with previous studies that found an implication of the dorsal hippocampus as well as the prefrontal cortex in WM (Yoon et al., 2008). However, while a normal WM task (LIWM task) and a RM task increased the activation of the dentate gyrus, a task involving the proessing of proactive interference (HIWM task) caused a non-activation of this structure. This suggests that the non-activation of the dentate gyrus is necessary to accomplish the task and overcome interference.

It has been demonstrated that the dentate gyrus provides pattern separation which is the process of reducing the average overlap between two representations. By using electrophysiology and functional anatomy, it has been shown that the population of activated neurons is different when rats are placed in slightly different environment (Colgin et al., 2009). Thus, the function of pattern separation is to make different, but quite similar representations more distinct in order to afford rapid learning without inducing interference and retrieval errors (Vago and Kesner, 2008). Shutting down this function (dentate gyrus-dependent pattern separation) may be necessary for the subject to focus on an ongoing trial, especially in task involving a high level of overlap between different trials (HIWM task). By reducing the number of active cells in the dentate gyrus, the animal may thus be able to forget previous similar representation/trials stored in memory and therefore be better prepared to perform correctly an ongoing trial. Processing interference in a WM task could thus specifically induce and require an inhibition of the dentate gyrus, a site where adult neurogenesis has been described. This hypothesis is in agreement with work from our group showing that inhibition of neurogenesis in the dentate gyrus improves WM performance, especially in tasks where repetitive information were presented as it is the case in a HIWM task (Saxe et al., 2007). Other experiments are required to establish if the number of new neurons activated in the dentate gyrus decreases selectively in the HIWM task. The results of such experiments are discussed in chapter V.

**Processing of Interference may require a decorrelation in brain synchronized activity**

The density of Zif268 labeled neurons was also used to compare inter-regional brain activity. To better understand the functional connectivity between brain regions, we assessed the correlation matrix for each experimental group. In the control group, a high level of positive inter-regional brain correlation was specifically observed between the different areas of the hippocampus indicating a synergy between the different parts of the hippocampus of rats placed in a spatial context but supposedly not performing any (or performing very basic) cognitive activity. In addition to the control group, numerous correlations were observed between brain regions in the RM and LIWM groups. Specific correlations are evident between intrahippocampal areas but also between entorhinal and medial prefrontal cortices and the hippocampus suggesting some multi-collinearity between the regions of interest (Poirier et al., 2008). However, the pattern of correlated activity dramatically changed in the HIWM compared to the other groups of rats. No inter-regional brain correlation was observed between any of the studied structures suggesting that forgetting and the processing of PI may require functional de-coupling within these memory circuits. This inter-regional brain de-correlation might specifically promote forgetting of previous trials as required in the HIWM task. These data, however, need to be complemented with electrophysiological studies of coherence. Functional interactions between distributed brain areas, known as functional connectivity, give rise to coherent patterns of brain oscillations that can be recorded and studied. We expect to see a decoupling between the DG oscillations and those recorded in other structures only during and/or after (during post-training sleep?) HIWM training.

**Activation of the CA1 area of the ventral hippocampus in a Reference memory task**

Numerous experiments have demonstrated the critical nature of the hippocampus in WM tests but also in a more general manner, in spatial memory tests (Olton, 1979). However, several studies in rodents suggest a functional unbundling within a septo-temporal axis of the hippocampus (Bannerman et al., 2004). It has thus been shown that lesion or inactivation of the dorsal hippocampus induces spatial memory deficits in a radial maze (Pothuizen et al., 2004). In contrast, transient inactivation or lesion of the ventral hippocampus selectively disrupts fear conditioning without affecting performance in spatial memory tests (Fanselow and Dong, 2010). In conclusion, a double dissociation is observed between the respective roles of the dorsal and the ventral hippocampus. The dorsal hippocampus would thus play a preferential role in spatial learning while the ventral hippocampus would not be as essential for performance on spatial tasks (Bannerman et al., 2004), but would rather play a role in emotional learning and in motivation. Our results seem to contradict this idea as they show a specific involvement of ventral CA1 in our spatial HIWM and, in particular, RM tasks. We thus observed an activation of ventral CA1 area in RM animals as compared to control and to LIWM animals whereas CA1 area of the dorsal hippocampus was activated in the three groups which give an idea about the respective involvement of these two substructures to long-term and short-term retention. It is known that the ventral hippocampus plays a role in motivation and that the motivational system implicates dopamine. It has been shown that in the CA1 region of the hippocampus, LTP can be enhanced by several neurotransmitters, including dopamine. The ventral hippocampus receives the most dopaminergic innervation

and the ventral CA1 contains more dopamine than the dorsal CA1 (Hörtnagl et al., 1991). The activation of the CA1 area of the ventral hippocampus by RM training could thus be due to the dopaminergic system. We now need to verify if the dopaminergic system is more active in RM than in WM, and in particular LIWM. However, such possibility would be surprising as we controlled motivational aspects of the task (control rats received same number of reinforcements than experimental rats). Moreover, at the end of training (day of sacrifice), RM and HIWM rats reached the same score eating the same number of pellets.

Anatomically, the prefrontal cortex is directly connected to the ventral hippocampus and indirectly connected to the dorsal hippocampus via the thalamus (Laroche et al., 2000). The lack of monosynaptic projections between the PFC and the dorsal hippocampus, a region implicated in spatial information processing (Colgin et al., 2009), suggests that perhaps they belong to separate and parallel memory systems participating in spatial memory tasks. Therefore, there may be a direct "on-line" hippocampo-cortical dialogue between the prefrontal cortex and the hippocampus via the ventral CA1 region, reflecting a critical and specific role of ventral CA1 in the consolidation of information (Maviel et al., 2004). If it appears quite likely that the ventral hippocampus may participate in the stabilization of memory traces in RM through projections and reciprocal dialogue with the prefrontal cortex, the ventral hippocampus could also be more specifically involved in the integrative transfer of spatial information distributed at the cortical level necessary for the recall of certain information stored in long term memory. With this in mind, a specific and large activation of ventral CA1 during RM training may not be surprising.

**Role of parietal, retrosplenial and perirhinal cortices in our three tasks**

An increased Zif268 counts in the parietal cortex (PTL) was also observed during our three tasks and compared to control. Our present results are in line with previous findings showing that lesions of the PTL impair performance in short-term memory (Kolb and Whishaw, 1983) and RM (Kesner et al., 1987) tasks. PTL receives inputs from the somatosensory area (S1) giving it a role in vestibular and proprioceptive information treatments (Giannetti and Molinari, 2002). Save and Poucet showed that the PTL is involved in spatial orientation and integrates both self-motion and external cues in order to convert egocentric into allocentric information (for review, (Save and Poucet, 2009)). This brain area could be very important in our three tasks in order to transform the perception of the animals into spatial representation.

The retrosplenial cortex (RSP) was also found to be more activated in RM and HIWM rats compared to controls. The LIWM task did not cause such increase in activation. It would, therefore, appear that the RSP is more involved in the long-term storage of information and the processing of interference. Many studies suggested a role of the RSP in mnemonic processes. Such evidence comes from lesions inducing deficits associated with various tests of spatial learning (Harker and Whishaw, 2002). Vann and colleagues showed that rats with retrosplenial lesions were impaired on the acquisition of a RM task (Vann et al., 2003). On the other hand, the lack of activation seen after LIWM training agrees with Maviel and Colleagues finding showing that c-Fos expression in the RSP did not differ from control after a WM task. However, the increase of activity in the RSP cortex in rats that underwent a HIWM task designed specifically to capitalize on the necessity to forget previous trials, is in

line with experiments showing an implication of this structure in cognitive control to disambiguate conflicting responses by inhibiting responses to task-irrelevant cues (Hindley et al., 2014).

Many studies suggested a role of perirhinal cortex (PERI) in mnemonic processes (Wiig and Bilkey, 1994a, b). Lesions of the PERI produce spatial memory deficits in both RM and WM tasks on the radial maze (Liu and Bilkey, 1998). However, Maviel and Colleagues found an increase of c-Fos expression in the PERI after a RM task but not after a WM task which is in line with our zif268 expression.

Finally, one of the most important factors for our team in designing the three tasks was that regardless of the evolution of their learning curve, rats must rely only on spatial information to navigate the maze without adopting any algorithm (e.g. turning clockwise) nor egocentric strategies. The use of different starting arms from trial to trial secured the animals to rely only on spatial memory. This prevented the rat to develop stereotypical turnings by relying on their procedural memory. Furthermore, the use of multiple trials within a day reduced the use of intramaze odor trails. It has been suggested that the caudate putamen (CP) known as the striatum facilitates learning and memory when a situation demands the use of egocentric-response hypothesis (Kesner et al., 1987). Chang and Gold found after infusing lidocaine in the dorsal CP, impairment in acquisition of a response discrimination in which a rat was required to take the same direction without relying on spatial locations (Chang and Gold, 2004). Our results did not show any significant difference in the activation of the CP nuclei in the four groups of rats suggesting that this structure is not specifically activated by any of our cognitive tasks (the CP is certainly activated by the procedural aspects of the tasks, but these procedural aspects being equal for all tasks including control conditions, we do not find a specific involvement of CP in any of our behavioral training condition). These results confirm in some way that our rats did not develop any egocentric strategies to perform the cognitive tasks they were submitted to.

**Conclusion**

Our goal was to find a way to determine the neurobiological bases of adaptive forgetting, in particular in the context of WM processing. To do so, we adopted a comparative approach by training groups of rats in a three different radial maze paradigms aimed at testing three different cognitive processes: RM, WM and the processing of interference in WM. Using this procedure, we first showed that information supposedly stored in short-term/WM can outlast their purpose by interfering, several days later, with the storage of newer information, thus questioning the existence of a pure short-term memory store. We then showed that the processing of such interfering previously stored information might require a specific and negative control of the dentate gyrus of the dorsal hippocampus materialized by an inhibition of the expression of indirect markers of neuronal activity and synaptic plasticity, *Zif268* and *c-Fos*. Although we recently observed other differences in the pattern of expression of *Zif268* in other cortical structures (RSP, PERI, vCA1), we believe this chapter shows that the dentate gyrus could be a critical node in processing the forgetting of irrelevant information, an essential process allowing optimal use of cognitive resources.

# CHAPTER III

## An analysis of Immediate Early Gene expression across two stages of learning and forgetting in the radial maze

## III.1 Introduction

In our previous chapter, we have seen that during our RM task, rats exhibited a gradual progression of their performance level. They started from a very low level of performance on the first days of training to reach a great mastery of the task after 10 days of training. The LIWM group also showed a significant, albeit small, increase in performance. However, our HIWM group exhibited a decrease in performance starting from the third block of days. We have shown that training rats in RM and WM tasks produced an increase of Zif268 and c-Fos expression in the hippocampus, the entorhinal cortex and the prefrontal cortex after 10 days of training. We then showed that the processing of interfering previously stored information in HIWM might require a specific and negative control of the dentate gyrus of the dorsal hippocampus that was materialized in our experiment by an inhibition of the expression of indirect markers of neuronal activity and synaptic plasticity, *Zif268* and *c-Fos*. We concluded that this non-activation of the DG could be required and necessary to accomplish this task and overcome interference, and that the Dentate Gyrus might stay non-activated when forgetting/updating of previous information is required.

Nevertheless, the previous study examined IEG expression only upon completion of training, at which point the task has been mastered in RM and LIWM and deteriorated in HIWM. In the present chapter, we were interested in finding if a differential cerebral activity would occur during progressive learning of our tasks. More importantly, we were curious to find how the Dentate Gyrus responds to interference in an early learning stage when performance of the rats was not disrupted.
To this aim, the level of activity in the same brain areas studied earlier was examined at an intermediate learning-stage in a new group of animals we tested two years after the ones used in the first experiment (chapter II). The methods used for this work were the same than the ones described in chapter II. We chose day 4 (2 Blocks) of training to end the behavioral experiment in this new group of animals as we have observed in the first group that HIWM rats' performance was not yet disrupted and RM group started to consolidate information at this stage of the experiment. Levels of Zif28 and c-Fos were measured in various brain regions and compared between 4 and 10 (previous findings) days of training.

## III.2 Results

### Partial acquisition of the tasks over a 4-day period

47 male Dark Agouti rats were used for this experiment. **Figure III.1A** shows the learning curves for RM, LIWM and HIWM rats after 4 days of training (2 Blocks). Analysis revealed that RM rats slightly improved their performance [F (7, 1) = 3.943; p = 0.0875] and reached 38 % of correct choices on block 2. RM and LIWM rats exhibited a small positive evolution in their number of correct choices over the 2 days whereas HIWM rats appeared to show a slight decrease in performance. This effect was not yet statistically significant however [F (7, 1) = 2.333; p = 0.1705]. More importantly, LIWM and HIWM rats were at the same level of performance upon sacrifice as indicated by the lack of a significant difference on Block 2 (HIWM versus LIWM, p = 0.1655). WM scores were above chance at all time points.

Like in chapter II, our next goal was to see whether rats' performance in WM declines from trial 1 to trial 4 but returns to errorless performance on the first trial of the next day (resetting). To do so, we analyzed their performance by trial rather than by day (Day 1-4). We found an impairment in performance at trial 4 in HIWM rats as compared to LIWM subjects (ANOVA p = 0.0486) similar to the one we observed during the first 5 days of training in our 10-day experiment (see **figure II.5B** from chapter II). This result suggests a start of a build-up of Proactive interference on cognitive performance in the HIWM group **(Figure III.1B)**.

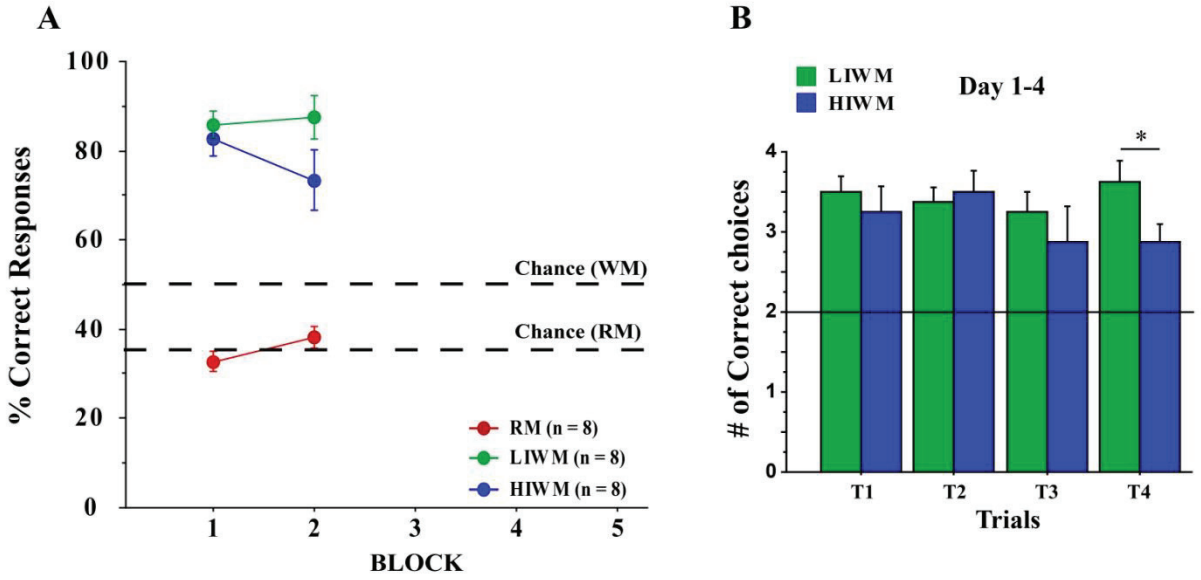


**Figure III.1: Behavioral analysis. (A)** Curves illustrating the percentage of correct choices ± s.e.m per block (each block = 2 days of training) in a RM, LIWM and HIWM task. Black hatched line represents the chance level for the WM groups (50%) and for RM groups (35%). ANOVAs revealed a significant Group effect [F (2, 21) = 61.696; p < 0.0001]. No significant Block effect was found [F (1, 2) = 0.085; p = 0.7732], nor a significant Group x Block interaction [F (2, 21) = 2.756; p = 0.0865]. (**B**) Total number of correct choices made by animals described in (A) on trials 1, 2, 3, and 4 (T1, T2, T3, and T4) of days 1 through 4.

**Zif268 and c-Fos immunoreactivity**

As before (chapter II), we normalized the value of Zif268 and c-Fos positive cells assessed in our three experimental groups at day 4 relative to control levels (% of control). Using these normalized values, we did not find any significant difference between groups for any of the structures studied here (see **Figure III.2** and **III3**) suggesting that, **as compared to control**, none of our cognitive tasks seem to activate (or inactivate) a brain area. However, when comparing these normalized value to those observed earlier in chapter II (10 day), we observed a significant increase of activity in the CA1 and CA3 areas of the hippocampus, the PFC and the LEC on day 10 compared to day 4. In the DG of the dorsal hippocampus, Zif268 and c-Fos counts relative to control were higher after 10 days as compared to 4 days of training in both the RM or LIWM tasks. In contrast, this increase of activity (% control) was not observed in HIWM rats. No significant change in Zif268 or c-Fos was found between day 4 and day 10 in S1 cortex in all groups of rats. Here is a breakdown of our findings:

**Hippocampus**

As shown in **Figure III.2A** and **B** and **Figure III.3A**, a significant increase in Zif268 and c-Fos activity was observed for all experimental conditions (RM, LIWM and HIWM) on day 10 compared to day 4 ($P < 0.05$) of training in the dorsal hippocampus as a whole. More specifically, in the CA1 area, this increase was significant for Zif268 ($p = 0.0007$) and c-Fos (even if it was only marginally non-significant ($p = 0.079$) for RM rats). In the DG of the dorsal hippocampus, RM and LIWM rats were significantly more labeled than their respective control on day 10 compared to day 4 ($p < 0.05$). However, Mann Whitney U test pointed out that Zif268 and c-Fos expression did not change in HIWM rats between these two time points.

**Other cortices**

In the PFC, the immunoreactivity of Zif268 in RM, LIWM and HIWM rats was significantly higher on day 10 compared to day 4. This increase in RM rats was not observed with c-Fos ($p = 0.2481$). In S1, whatever the learning stage, no difference was observed between the different groups of rats whether with Zif268 or c-Fos ($p > 0.05$).

**Correlations**

Spearman's correlation tests were applied to the number of c-Fos and Zif-268 positive neurons in each area for all four groups of rats. These correlations are shown in **Figure III.2C** for Zif268 and **Figure III.3B** for c-Fos. As shown in the matrices, in the RM task, an important positive correlation between LEC and CA3 area is observed ($p = 0.014$ for Zif268 and $p = 0.0442$ for c-Fos). We have seen that such correlation was also present on day 10. Additionally, a positive correlation between PFC and CA3 was detected in LIWM ($p = 0.0438$ for Zif268 and $p = 0.0198$ for c-Fos). In contrast, after training in the HIWM task, no correlation was observed between any of the studied structure. This result is in line with our previous study showing a lack of correlation between the hippocampus and other cortices after completion of 10 days of training in the HIWM task. Finally, as in 10 days, the control group only exhibited an intra-hippocampal correlation.

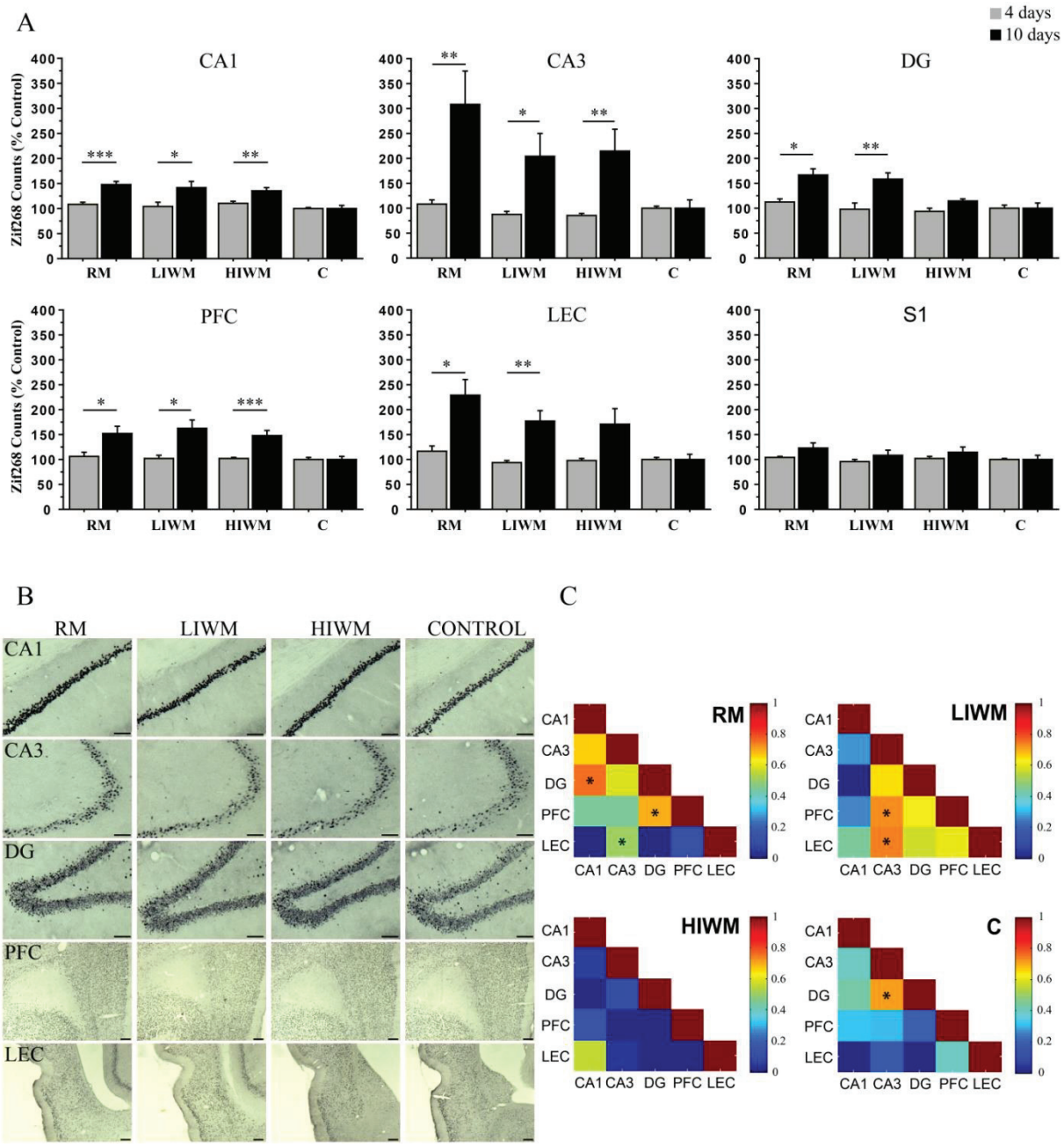


**Figure III.2: Chronological analysis of Zif268 expressioin. (A)** Histograms illustrating Zif268 density relative to the Control group (mean ± s.e.m) in CA1, CA3, DG of the dorsal hippocampus, the prefrontal cortex (PFC), the entorhinal cortex (LEC) and the somatosensory cortex (S1) in RM (n=8), LIWM (n=8), HIWM (n=8) and control group (C) (n=23) on day 4 (grey) and day 10 (black) of training. **(B)** Photomicrographs of Zif268 expression in different brain areas on day 4 in our four groups of rats. Scale bar = 100 µm. **(C)** Interregional Correlation matrices for Zif268 expression (day 4) within each group. R-Spearman rank correlation coefficients are color-coded. Colors reflect correlation strength (scale, right). Significant correlations ($p<0.05$) are marked with (*).

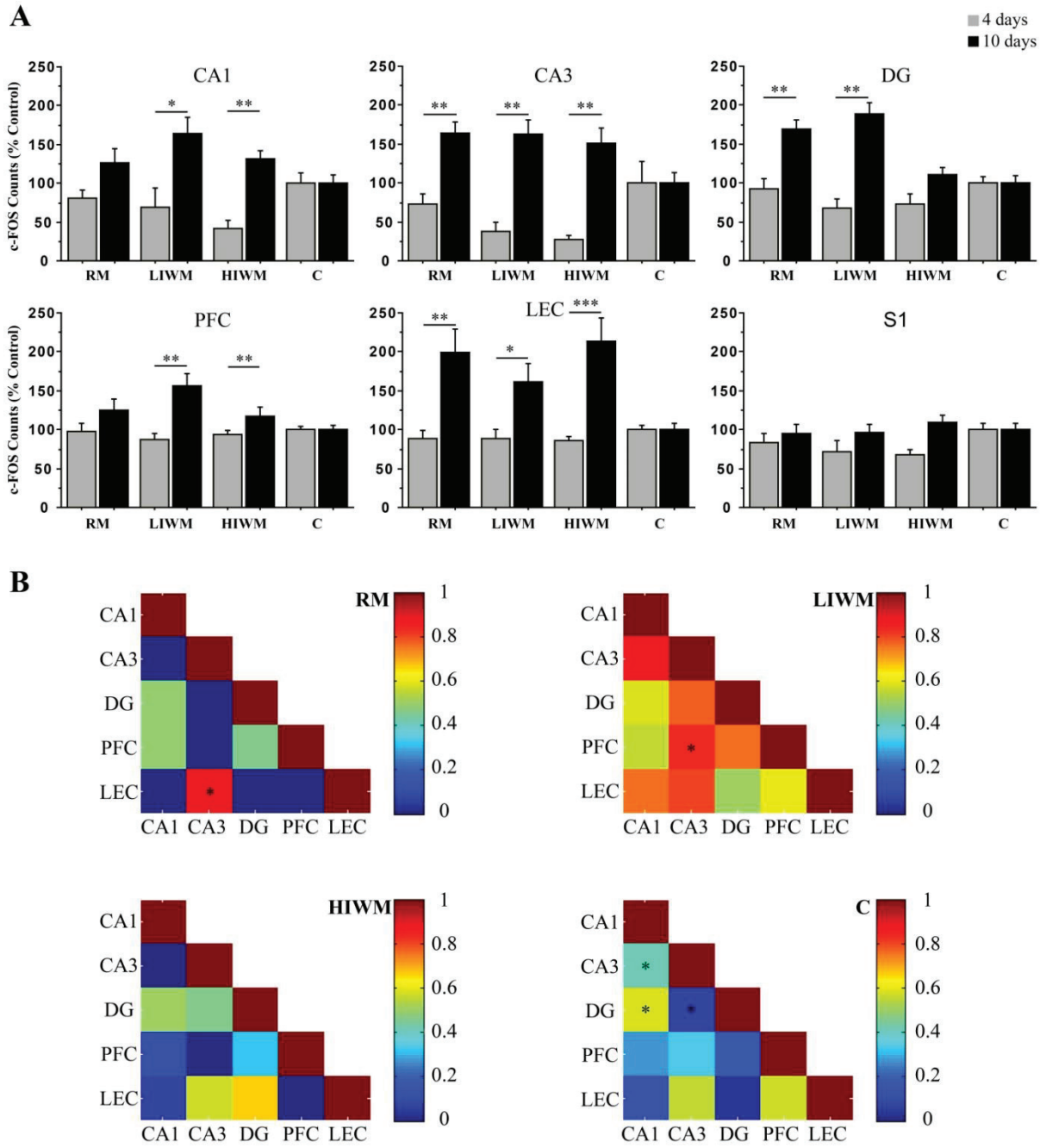


**Figure III.3: Chronological analysis of c-Fos expression. (A)** Histograms illustrating c-Fos density relative to the Control group (mean ± s.e.m) in CA1, CA3, DG of the dorsal hippocampus, the prefrontal cortex (PFC), the entorhinal cortex (LEC) and the somatosensory cortex (S1) in RM (n=8), LIWM (n=8), HIWM (n=8) and control group (n=23) on day 4 (grey) and day 10 (black) of training. **(B)** Interregional Correlation matrices for c-Fos expression (day 4) within each group. R-Spearman rank correlation coefficients are color-coded. Colors reflect correlation strength (scale, right). Significant correlations ($p<0.05$) are marked with (*).

**The level of IEG expression was much higher in the 4-day Control group**

We were greatly puzzled by the fact that 4 days of training in any of our three experimental conditions do not activate any change in the expression of IEGs. One possibility is that the level of expression of these IEGs is much higher at the beginning of training for all experimental conditions, including control conditions, due to the novelty of the tasks for all rats. If that was true, no subtle changes in IEG expression could be observed as compared to controls. We thus decided to conduct a comparative analysis of the level of expression of Zif268 and c-Fos between our two control groups (4 versus 10 days control conditions). We found that the level of Zif268 **(Figure III.4A**) and c-Fos (**Figure III.4B**) expression was always much higher in the 4 day-control condition compared to the 10 day-control. Mann Whitney U test pointed out that Zif268 and c-Fos expression was not similar in Control rats through the two learning-stages. Thus, 4 day-Control rats had a significantly higher number of labeled neurons compared to 10 day-Control rats in all the studied brain regions (in all brain areas $p < 0.001$ except the S1 for c-Fos as no statistical difference was observed between control groups). This result could thus explain why no activation can be observed relative to controls in any of our experimental conditions.

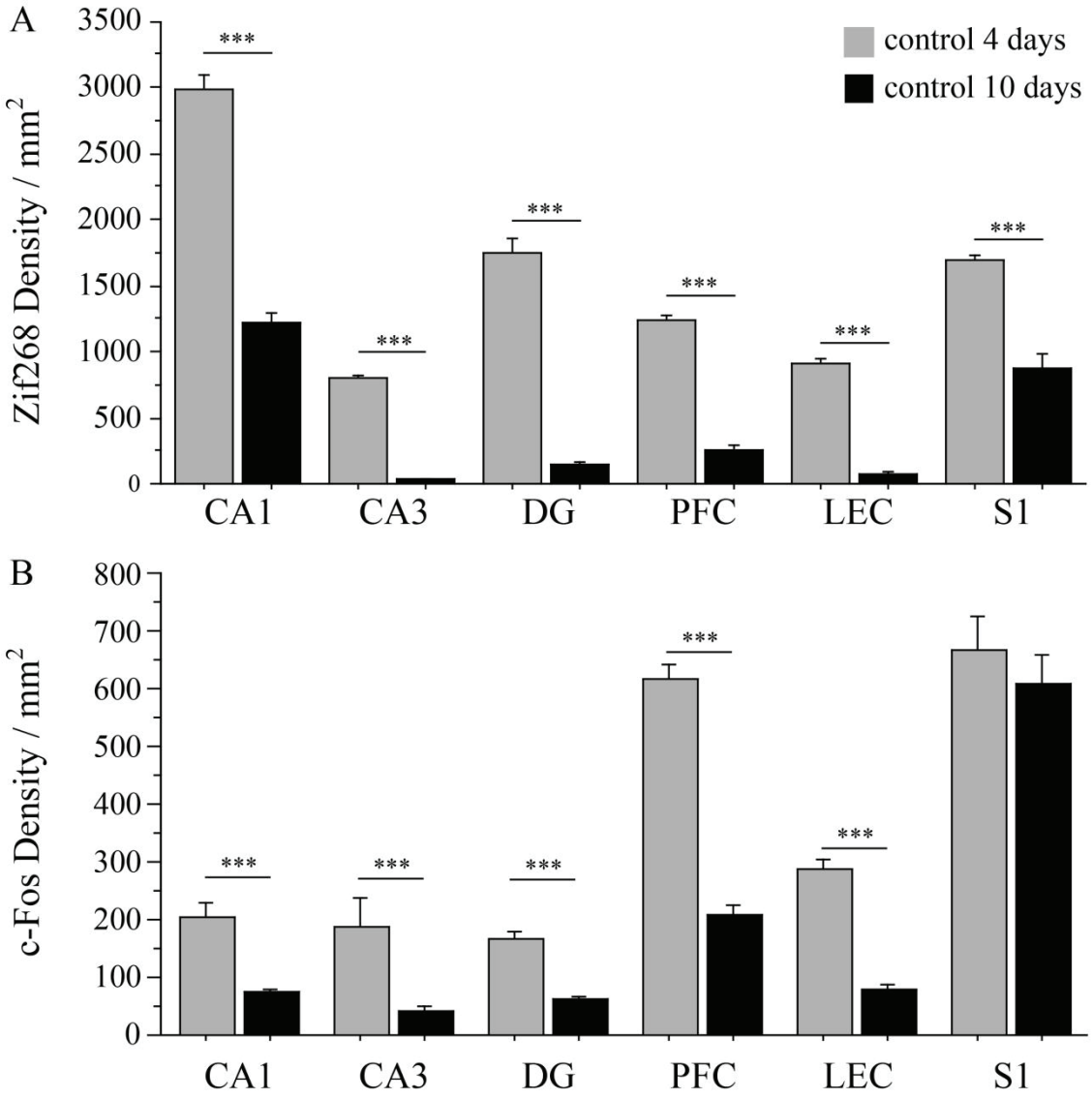


**Figure III.4: Chronological analysis of (A) Zif268 and (B) c-Fos density in the Control group.**

### III.3 Discussion

With the present experiment, we were interested in finding if a differential cerebral activity would be observed during the progressive learning of our three behavioral tasks. More importantly, we were curious to find how the Dentate Gyrus would respond to interference in an early learning stage, when performance of the rats was not yet disrupted by the presence of interference. Therefore, we evaluated whether our three different tasks produce similar or different expression in the levels of IEG expression at two different stages of learning in the 8-arm radial maze. After 4 days of training, we did not find any significant difference between groups for any of the structures studied here suggesting that, **as compared to control**, none of our cognitive tasks seem to activate (or inactivate) a brain area. This result was probably due to the higher expression of IEGs at the beginning of training for all experimental conditions, including control conditions. This higher expression seen in all rats in the beginning of training may simply reflect the experience of novelty of the radial maze environment. However, we found a greater Zif268 and c-Fos expression after 10 days of training when compared to 4 days in the dorsal CA1, CA3, PFC and LEC in all experimental groups (when average to their respective control). As expected, the Dentate Gyrus displayed the most unique pattern of activity, with expression of both Zif268 and c-Fos significantly higher for RM and LIWM on day 10 when compared to day 4. This supports the studies showing that the DG has been implicated in RM and WM tasks (Kesner, 2013). However, HIWM rats did not show such increase and IEGs expression remained low across the two learning stages and not significantly different from the control group. In consequence, most of the brain regions increased their activity when compared to control group after mastering the task, except the DG that stayed non-activated.

Prefrontal cortex activity showed a slight increase in Zif268 expression across the two stages of training in the RM group. However, this increase was not observed for c-Fos activity. This result could be in agreement with previous studies showing no involvement of PFC during the early stage of RM training. These studies showed that the PFC only becomes important in the long-term recall of remote information (Frankland and Bontempi, 2005, Leon et al., 2010). Maviel and colleagues (2004) compared the activity of the PFC during the recall of either recent or remote memories in a five arms maze. They trained mice to locate a single baited arm in a maze for 10 consecutive days, like in our study. They tested these mice either one day or 30 days later to assess recent or remote spatial memory respectively. Zif268 and c-Fos proteins' expression in the PFC was low at first (one day), but increased in response to the remote memory recall (30 days). The activation of PFC (Zif268) that we observed after 10 days of training in RM is thus in contradiction with this study. In fact, immediately after the end of training in their maze, Maviel and Colleagues did not find any activation of the PFC (Zif268 counts) as compared to control mice (placed in a similar condition than our control rats). The activation of the PFC that we observed in RM could be explained by the initiation of the consolidation process described in Maviel study at 30 days, where the information undergoes a gradual transfer from the hippocampus to the neocortex, and specifically to the PFC. However, this initiation of consolidation would be surprising as we chose not to overtrain our rats (minimal training of 10 days). We may also hypothesize that the systemic

consolidation process (hippocampal-neocortical transfer of the information) begins much sooner in the rat than in mice.
In the Somatosensory cortex no difference of Zif268 and c-Fos expression was detected between the groups. In consequence, changes in IEGs expression in the other brain regions cannot be interpreted as a general activation. Interestingly, overall, a higher expression of Zif268 was observed in the control group on day 4 compared to day 10 in almost all the studied brain areas. This is probably due to the novelty of the experiment protocol for all rats (including controls) that could account for the higher Zif268 labeling on day 4. Even though control rats were exposed and habituated to the radial maze one week before the 4 day-training, the motivation of these rats changed during the experiment itself. As mentioned in the material and methods section, control rats found food and ate only when their corresponding experimental match rat (RM, HIWM or LIWM) found food pellets. In our experiment, control rats become progressively habituated to the radial maze and become aware with time that no learning is needed in order to accomplish this task. Such "learning" that "no learning" occurs for them may be present at day 10, but not at day 4, when these rats may still try to learn and adopt an optimum strategy to obtain more food (as their runs in the maze are not always rewarded). This could explain why IEG expression was high for control rats at day 4 and decreased thereafter. Many studies have shown that IEGs decrease their expression after familiarization to a context (Zhu et al., 1995, Montag-Sallaz and Buonviso, 2002, Roullet et al., 2005). We can thus assume that when the impact of novelty has attenuated (at day 10), IEGs expression was also attenuated in most brain regions.

Using IEGs expression pattern, we investigated possible co-activations between brain regions as we did earlier for the 10 days experiment. In RM and LIWM, a positive correlation between the hippocampus and the cortices was found. This result is in agreement with our previous study and with other studies indicating an integrated role of these regions in long-term and short-term storage of information (Vann et al., 2000a, Vann et al., 2000b). However, we did not find any positive correlation during HIWM training at day 4 confirming our previous results (10 days). This result thus suggests that forgetting of PI starts early on during training. Interestingly, to our knowledge, this is the first study to show a lack of correlation between brain areas when forgetting is needed. It is important to note, however, that correlation data do not imply any causal link between these structures. These data do not tell us if a region activates another one. However, these data are informative to potentially suggest the existence of a dialogue between distant structures that more direct *in vivo* electrophysiological experiments could reveal.

**Conclusion**

In summary, because the control group at day 4 expressed a high level of IEGs, we could not be conclusive whether a differential cerebral activation would occur during the progressive learning of our three tasks. However, this experiment provided further support for a critical decorrelation of the hippocampo-cortical network when forgetting is required.

# CHAPTER IV

## A lesion of the Dentate Gyrus facilitates forgetting by reducing Proactive Interference

## IV.1 Introduction

Our previous work suggested that the dentate gyrus of the dorsal hippocampus is not activated when forgetting is needed during training in a HIWM task on the eight-arm maze. While a RM and LIWM tasks both increase the expression of indirect markers of neuronal activity and synaptic plasticity Zif268 and c-Fos compared to controls, training in the HIWM task does not induce such increase in expression. We concluded that the non-activation of the DG of the dorsal hippocampus may promote forgetting and improves the processing of information in WM. The DG is of special interest as it receives excitatory synapses from the entorhinal cortex via the perforant path and projects to the pyramidal cells of the CA3 subfield of the hippocampus (Amaral and Witter, 1995). The position of the DG thus allows a control of the flow of information within the hippocampus (Xavier et al., 1999). Several studies have shown that selective lesions of the DG in rats caused severe impairment in the acquisition of RM and WM as compared to sham lesions or lesions induced in other hippocampal subregions (Aggleton et al., 1995, Xavier et al., 1999, Okada and Okaichi, 2009). In contrast, studies have shown that ablation of hippocampal neurogenesis, occuring selectively in the DG, impaired LTP and long-term memory (Saxe et al., 2006, Bruel-Jungerman et al., 2007) but improved the processing of PI in WM as tested in a radial maze (Saxe et al., 2007). Therefore, the present study directly examined the impact of a restricted lesion of the dorsal DG in spatial RM and WM with or without interference using our three different behavioral paradigms that permits a clear comparison between processes requiring the storage of information (in RM or WM) and those requiring forgetting of previously stored information in WM. The primary aim of this study was to determine whether the lesion of the DG will impair RM and low Interference WM, but will improve the performance in a HIWM task. Impairment on a RM and WM tasks would provide further support for the role of the DG in memory processing while an improvement on a High Interference WM task would demonstrate that the DG of the hippocampus is a critical node that needs to be silenced in order to process the forgetting of irrelevant information. A secondary aim of this work was also to examine whether the DG lesion could modulate a compensatory expression of Zif268 in other brain regions, as there are no previous studies on the impact of DG lesions upon Zif268 expression.

## IV.2 Materials and Methods

### Subjects

51 male Dark Agouti rats weighing 210-230 g at the beginning of the experiments were used. Light was provided from 08:00-20:00 h, and room temperature was maintained at 22 ± 2ºC. Rats were allocated to either the bilateral DG lesion group (n = 24) or the sham surgery group (n = 27) and were housed singly in plastic cages in the laboratory facility. Food and Water were available *ad libitum* at the beginning of the experiment. One week after their arrival, rats underwent surgery. Behavioral tests were carried out 3 weeks after surgery. A week before the behavioral tests, food was restricted to achieve a 20 % weight loss.

### Surgery – Dentate Gyrus lesion

Surgery was performed under Isofluorane anesthesia in a standard stereotaxic apparatus. The rats were pre-anesthetised in a rectangular (30x20x15cm) chamber for them to endure the fixation on the stereotaxic frame. Anesthesia was maintained via an inhalation nose cone affixed to the mouth bar on the frame (Oxygen supplied with required amount of isoflurane, around 2-3 % for the induction and later on around 1.5 % for the maintenance of the narcosis). As preparation for surgery, ophthalmic liquid gel was applied to the rat's eyes for protection, the hair was shaved from the top of the rat's head with an electric shaver and the scalp was cleaned with Betadine. A 2 cm midline incision was made and the skull disclosed. The skin was retracted with 4 Bulldog clamps to expose the skull and hold open the incision. Holes were drilled into the skull bilaterally over the Dentate Gyrus. The *dura* was removed using a small syringe. For the lesion animals, 4 holes were drilled bilaterally over the DG using coordinates derived from pilot experiments (see coordinates in **Table IV.1**). Glass tubing with microcapillary (Harvard apparatus) used for iontophoresis ejections. They were pulled in a single step on a Sutter PE-2 vertical puller (Narishige, Japan) programmed for heat and delay to give a tip pipette of less than 1 µm diameter size. This tip was broken at 5 µm diameter to allow ejection. Ibotenic acid (Tocris, bioscience) dissolved in sodium chloride to 10 mg/ml (pH = 8) was injected at 4 sites in each hemisphere. The pipette was left in place for 5 min before ejections. For iontophoresis, the pipette was connected by a silver wire immersed in the ibotenic acid solution to a current generator (CS4, Transkinetics, MA) that delivered pulsed negative current (7 seconds on/7 seconds off) for 4 minutes. At each site, ibotenic acid or NaCl were administered iontophoretically using currents of -12 µA. At the end of the ejection, the pipette was left in place for at least 5 minutes to avoid leakage of the ibotenic acid along the pipette track.

**Table IV.1: Stereotaxic Coordinates for Ibotenic acid Injections (Paxinos and Watson, 2005).**

| AP | ML | DV (dura = 0) |
|---|---|---|
| **- 2.7** | **± 1.2** | **-3.4** |
| **- 3.5** | **± 1.9** | **-3.0** |

AP, anteroposterior; ML, mediolateral; DV, dorsoventral

After the injections, the wound was sutured and the animals were kept under surveillance for couple of hours then returned to their cages in the facility to recover for 2 weeks. 27 control rats received the same surgery but were treated with sodium chloride (sham-operated control group). Seizures were not observed in any animal during the experimental period.

**Post-lesion behavioral procedures**

After surgery, rats were left to recover for a period of two weeks. Then, the rats were kept on a food deprivation schedule at 80 % of their free-feeding weight by limiting access to food. After one week, the rats underwent a habituation period where they explored the maze to the pellets from the eight arms of the radial maze. After habituation, the behavioral procedures used were exactly the same as in chapter II and III. The behavioral paradigms used to test RM, LIWM and HIWM were carried out in the same test room, apparatus, and spatial cues as the experiments in chapter II and III.

**Histological procedures**

90 minutes after the end of behavioral testing, the animals were deeply anesthetized with intraperitoneal injection of 2 ml sodium pentobarbital and perfused intracardially with heparin followed by a 4 % paraformaldehyde solution. The brains were removed and stored in a 30 % sucrose solution at 4ºC. Brains were frozen and cut at 25 µm on a cryostat and placed in a 12-well block. One set of sections of each rat was mounted on glass slides and stained with cresyl violet in order to be examined for histological verification of the lesion specificity. Another set of sections underwent immunohistochemical procedures and was immune-labeled for Zif268 (1:1000, Santa Cruz). Immunohistochemistry was performed as already described in chapter II.

**Data Analysis**

*Behavior*

Repeated measure ANOVAs were performed for each group (RM, LIWM, HIWM) with Lesion (lesioned versus sham-operated) and Blocks as main factors (Statview 5.0). Further comparisons were performed by a *post hoc* (Scheffe) test for particular within-group comparisons. Data are expressed as means ± s.e.m.

*Immunohistochemistry*

Estimated number of Zif268-positive neurons was counted using an automated cell counting procedure (Mercator, Explora Nova – see chapter II). In all conditions, counting procedures were blinded and without knowledge of the group condition. This number of positive-stained neurons was then divided by the surface area in order to generate a density. Zif268 immunoreactivity was statistically analyzed with Mann-Whitney U-test. Data are expressed as mean of Zif268 density ± s.e.m.

*Correlation*

As before (see chapter II and III), we assessed the correlation matrix for each experimental group using the Spearman's rank correlation coefficient.

## IV.3 Results

### Histology – Ibotenic acid-induced DG lesions

Verification of lesions using cresyl violet-stained sections revealed cell loss throughout the dorsal dentate gyrus. While the CA1 and CA3 subfields of the dorsal hippocampus were spared, there was neuronal loss in the DG with damage detected in the *hilus* (CA4). Granule cells of the DG were almost completely eliminated comparing to sham rats (**Figure IV.1C** and **E**). There was very little damage in CA3 pyramidal cells (**Figure IV.1B** and **E**). A small non-significant loss of pyramidal cells in the CA1 subfield was noted in all lesioned groups (**Figure IV.1A**). This small loss was also observed in other studies with DG colchicine-lesioned rats (Xavier et al., 1999, Lee and Kesner, 2003, 2004). **Figure IV.1D** represents the extent of dentate gyrus damage in the subjects with the largest and the smallest reconstructed lesions.

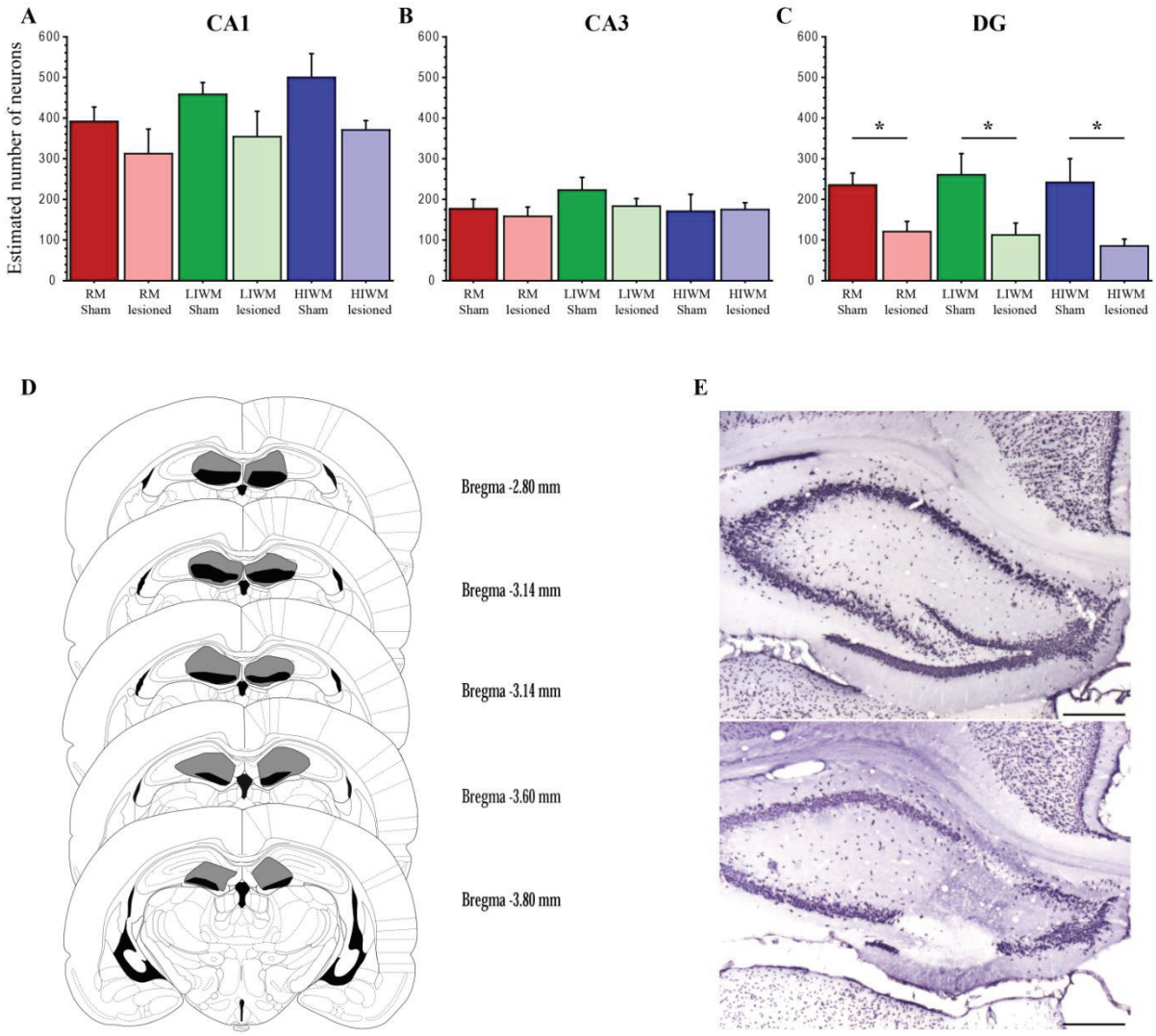


**Figure IV.1: Ibotenic acid-DG lesion.** Mean estimated number of neurons in the hippocampus counted with Zif268 staining for ibotenic acid lesioned rats and Sham control in the CA1 (**A**), CA3 (**B**) and DG (**C**). (**D**) Illustration showing the extent of the lesions to the Dentate Gyrus. The largest and the smallest tissue damage produced by ibotenic acid in the dorsal hippocampus are shown in gray and black respectively. The numbers represent distance (mm) from bregma. (**E**), Photomicrograph of Dentate Gyrus in a lesioned animal (bottom) and a sham animal (top) stained with Cresyl violet. In this example, infusions of ibotenic acid produced a loss of tissue of the dentate gyrus. Scale bar, 150 µm. Atlas sections are from the Paxinos and Watson (1997).

## Behavior

### Lesion of the DG impairs Reference Memory

**Figure IV.2A** shows the acquisition curve of the DG lesioned and Sham-operated rats trained in the RM task. The RM lesioned group exhibited marked impairment as compared to the RM Sham group. ANOVAs repeated measures revealed a significant Group effect † [$F(1, 15) = 4.89$; $p = 0.0429$], a significant Block effect ** [$F(4, 60) = 16.10$; $p < 0.0001$], as well as a significant Group x Block interaction ($p = 0.0026$). Scheffe's post hoc analyses revealed that RM Sham rats significantly improved their performance over time (significant Block effect; $p < 0.0001$) as compared to RM lesioned animal (marginal Block effect; $p = 0.0489$). However, RM lesioned rats performed at chance level (34.9% - see chapter II) during the 10 days of training. In contrast, RM sham group performed above chance level on the last two Blocks of days.

### Lesion of the DG impairs Low Interference Working Memory

**Figure IV.2B** shows the performance of the DG lesioned and Sham-operated rats trained in the LIWM task. Note the deficit of the DG lesioned LIWM group. ANOVAs repeated measures revealed a significant Group effect † [$F(1, 14) = 8.05$; $p = 0.0131$] and a significant Block effect * [$F(4, 56) = 5.84$; $p = 0.0005$] (on Block 2 and Block 4, LIWM lesioned versus LIWM Sham; $p < 0.05$). Split by analyses revealed that LIWM lesioned rats exhibited impaired performances over time ($p = 0.0191$).

### Lesion of the DG improves High Interference Working Memory

**Figure IV.2C** shows the acquisition curve of the DG lesioned and Sham-operated rats trained in the HIWM task. While sham-operated rats show a decrease in performance over days comparable to the one seen in HIWM trained rats studied in chapter II, lesioned rats showed enhanced performance on the last block of HIWM training. ANOVAs on repeated measures revealed a significant Group x Block interaction # [$F(4, 64) = 2.784$; $p = 0.0339$]. Split by analyses revealed that HIWM Sham rats exhibited impaired performances over time due to the build-up of interference [significant Block effect, $p = 0.0162$]. In sharp contrast, DG lesioned rats were immune to interference and did not exhibit impairment in performance [no significant Block effect, $p = 0.1810$]. * $P < 0.05$; ** $P < 0.01$; # interaction $p < 0.05$.

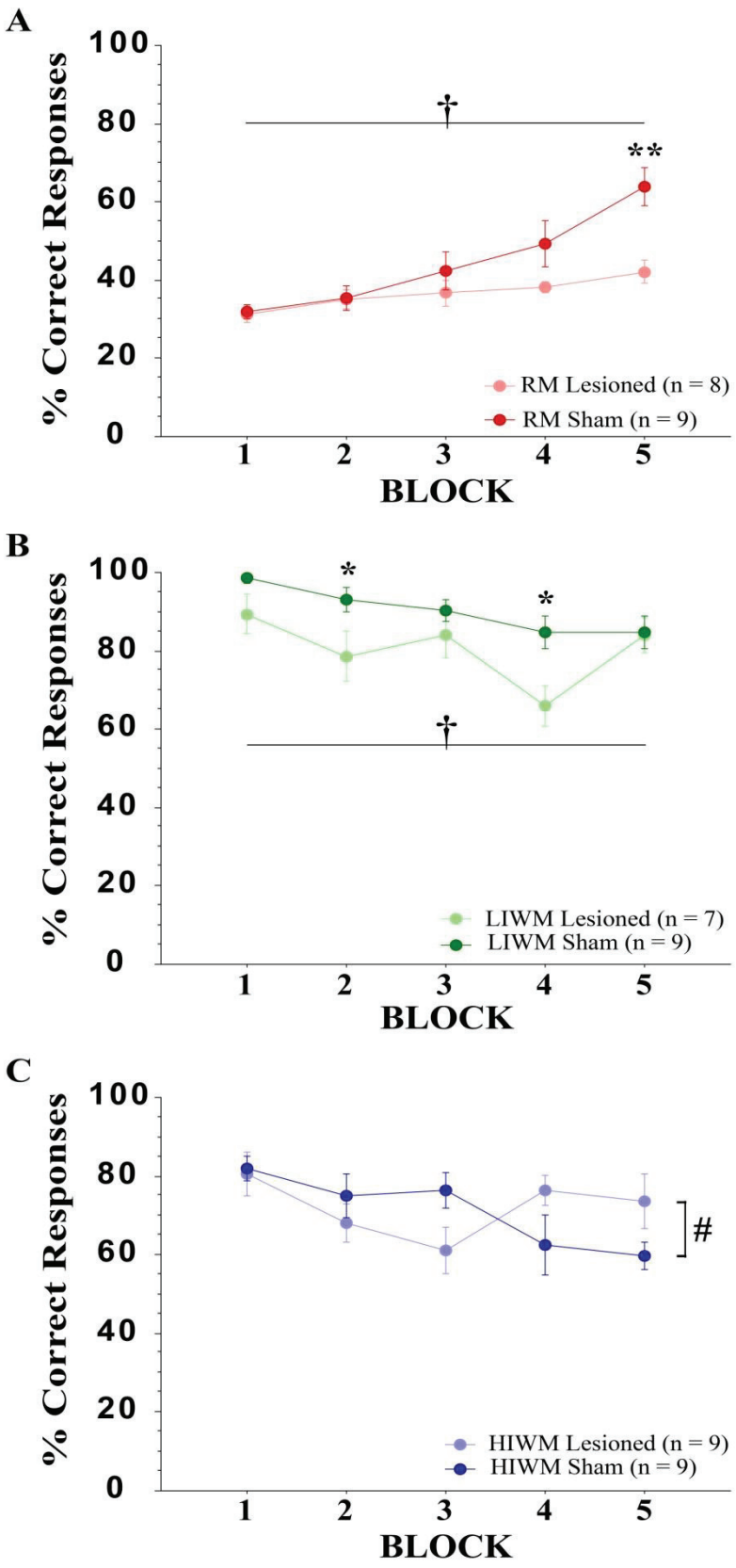


**Figure IV.2: Effect of Dentate Gyrus ibotenic acid lesion or NaCl (Sham) injections on the % of correct responses in the three groups of rats.** Percentage of correct choices ± s.e.m per Block of days in a RM (**A**), LIWM (**B**) and HIWM (**C**) tasks for lesioned and control animals.

**No change in Zif268 counts in the hippocampus, the striatum or the primary somato-sensory cortex after DG lesion**

We then asked whether lesioning the DG would induce compensatory changes in the activity of other brain areas. Counts of Zif268 protein were thus performed in the hippocampus, the striatum or the primary somato-sensory cortex (**Figure IV.3**). Counts of Zif268-positive cells in these the CA1 and CA3 subfields of the dorsal hippocampus failed to find evidence of a change in Zif268+ cells number following DG-ibotenate lesion (For all groups, $p > 0.05$) **(Figure IV.3A, B, E** and **F)**. Examination of the dorsal sriatum revealed no significant difference in Zif268 counts between all groups of rats (**Figure IV.3C)**. The cortical sensory region examined was the primary somatosensory cortex. No significant difference in Zif268 expression was observed between lesioned and Sham groups (**Figure IV.3D)**.

**Lesion of the DG increases the number of Zif268 positive cells in the Prefrontal Cortex**

Zif268 counts were also performed in the Prelimbic cortex, the infralimbic cortex and the anterior cingulate area (**Figure IV.4)**. An increase in the number of Zif268 positive cells across the whole PFC was observed in the DG lesioned animals trained in the HIWM task as compared to animals of other groups (HIWM lesioned versus RM Sham, $p = 0.0071$; HIWM lesioned versus RM lesioned, $p = 0.0124$; HIWM lesioned versus LIWM lesioned, $p = 0.039$; HIWM lesioned versus HIWM Sham, $p = 0.06$). This is the only area studied, and the only experimental condition (HIWM), that showed an increase of activity after DG lesion (**Figure IV.4 B** and **C**). Zif268+ cells were counted in the entorhinal cortex (LEC) as well. Zif268 positive cells in the LEC were not significantly different between lesioned and control groups (**Figure IV.4A**).

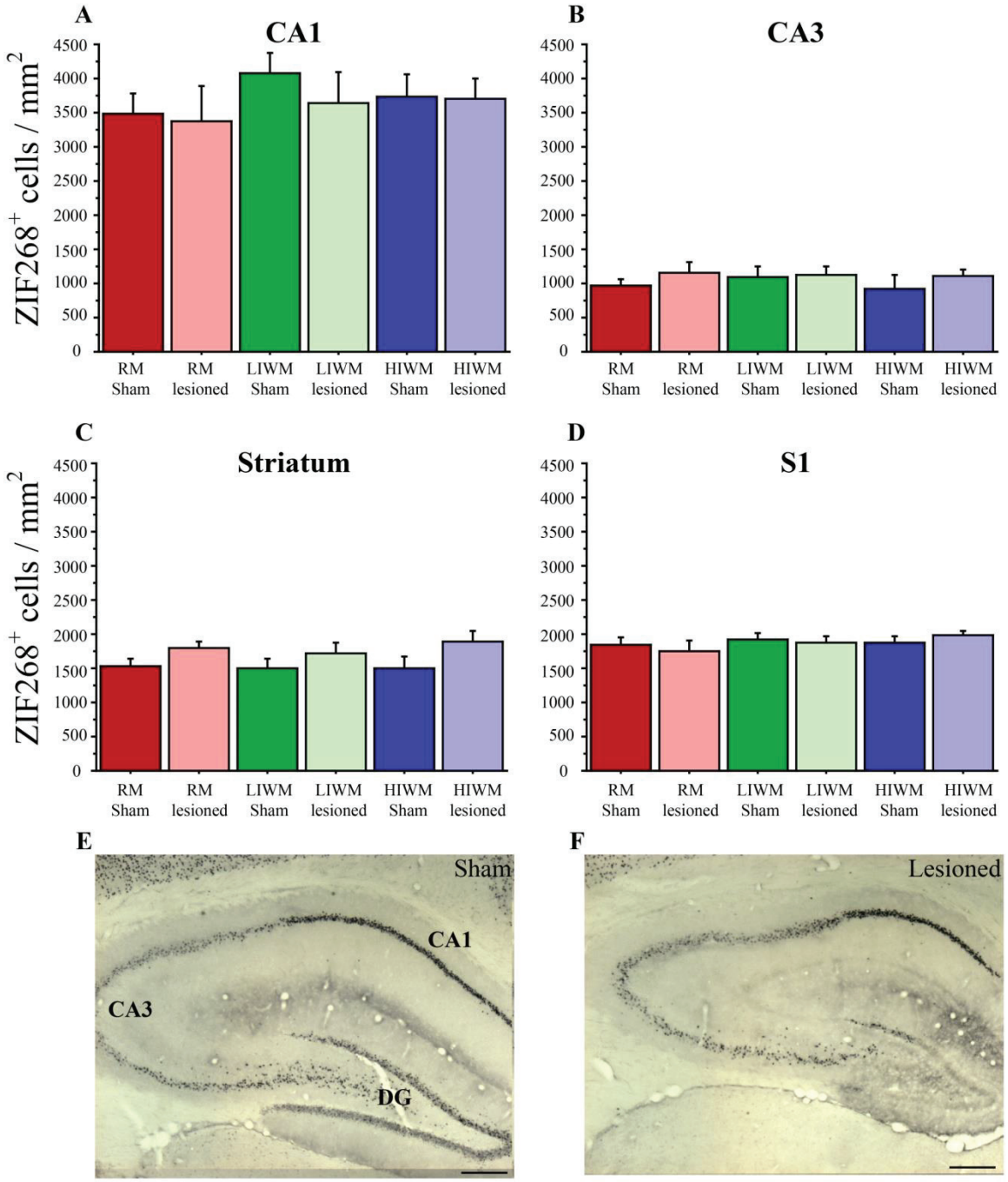


**Figure IV.3: Zif268 level after DG-lesion and after control surgeries (Sham) of rats trained in our three tasks.** Density of Zif268 positive cells per mm$^2$ is shown for CA1 (**A**) and CA3 (**B**) areas of the dorsal hippocampus, the striatum (**C**) and the somatosensory cortex (S1) (**D**). E and F show photomicrographs comparing Zif268 levels in the hippocampus with either a DG lesion (**F**) or Sham surgery (Control) (**E**). Scale bar, 100 µm.

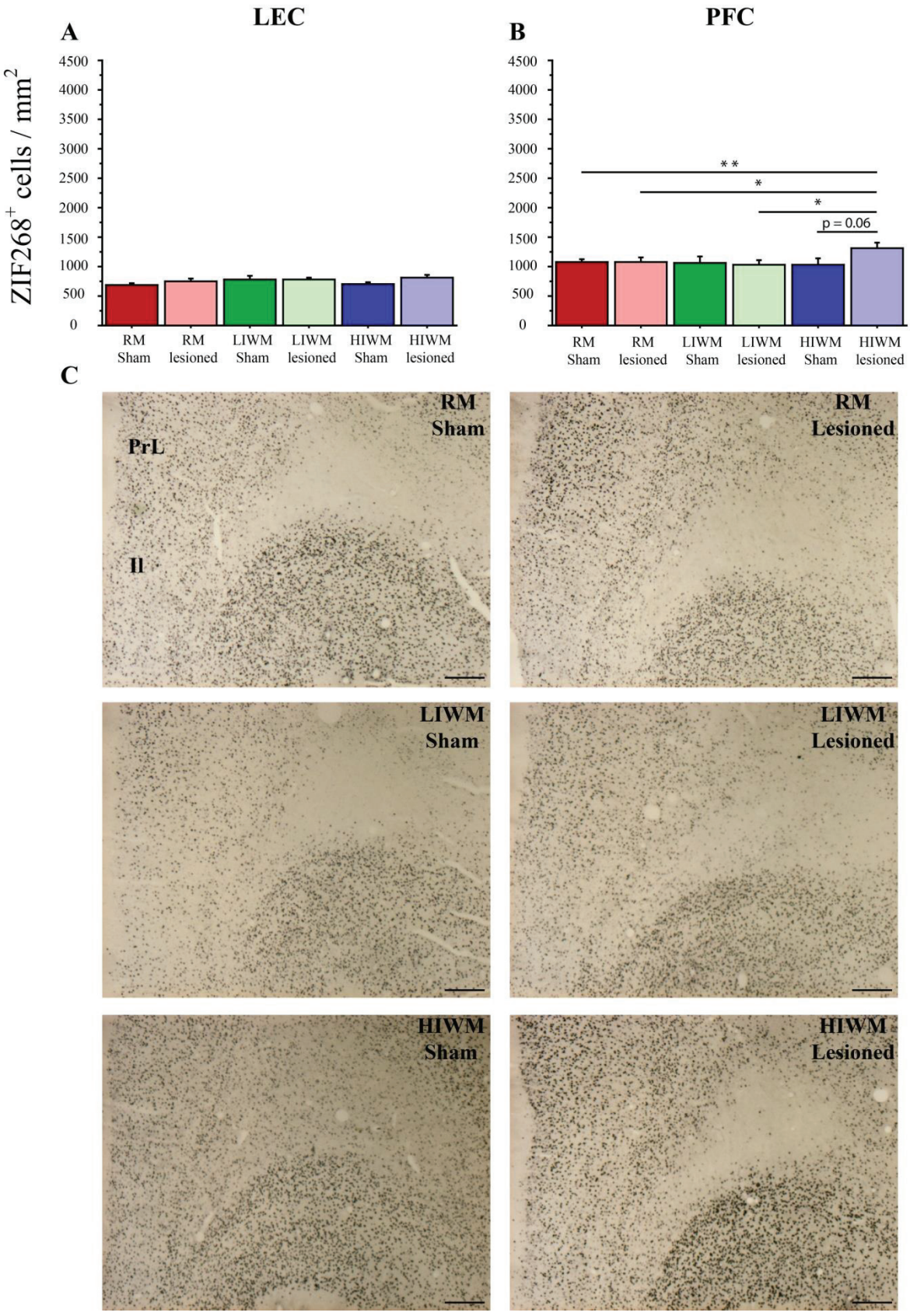


**Figure IV.4: Zif268 level increases in the PFC after DG lesions.** Density of Zif268 positive cells per $mm^2$ is shown for the lateral entorhinal cortex (LEC) (**A**) and Prefrontal Cortex (**B**). Photomicrographs comparing Zif268 levels in the PFC (PrL: Prelimbic cortex and Il: Infralimbic cortex) with either a DG lesion (Right) or Sham surgery (Left) (**C**). Scale bar, 100 µm.

**Loss of Correlation between brain structures after DG lesion.**

Although there were no differences in Zif268 counts after DG lesions (except for the PFC in the HIWM task), this null result does not mean that co-activation between brain regions (within the same animal) may not have changed with lesion and experience. The correlation matrices for regional Zif268 activity for each behavioral group were thus studied and are summarized in **Figure IV.5**. In RM Sham group, a single correlation was found between the CA3 area of the hippocampus and the DG ($p = 0,0209$). In LIWM, an important number of correlations was observed between the hippocampus areas on one hand and the PFC and LEC on the other hand. Surprisingly, and in contrast with our previous results (see chapters II and III), in the HIWM Sham group, intrahippocampal correlations were also found between CA1-CA3 ($p = 0,0233$), CA1-DG ($p = 0,0198$) and CA3-DG ($p = 0,0198$). These correlations were all lost after DG-lesion as shown in **Figure IV.5**. No single correlation between the studied structures was seen in all experimental lesioned groups.

The significant correlations found in the HIWM Sham group are difficult to interpret given that in this experimental condition no interregional brain correlation was observed in the first two studies (Chapter II and III). It is, therefore, particularly interesting to understand these discrepancies in the Sham HIWM group between our different studies. Consequently, I carried out another correlation study between the three experiments: the first 10-day experiment (Chapter II), the second 4-day experiment (Chapter III) and the DG-lesioned versus DG-Sham experiment (Chapter IV). Correlations coefficients were calculated between error rate (measures of the total number of errors made by each group of rats) and functional correlation between two structures (measures of correlation's coefficient). The data are presented in **Figure IV.6**.

In the RM group, we found a negative correlation between the error rate and the correlation coefficient between the hippocampus and the cortices (**Figure IV.6 A, B, C, D** and **E**). In other words, performance increases with an increase in the correlation coefficient. On the contrary, for the HIWM group, an opposite result emerged. We found a positive correlation for all studied structures, which means that the performance of the animals in this task decreases with an increase in the correlation coefficient (**Figure IV.6 L, M, N, O, P** and **Q**). Finally, in a LIWM group, a mixed result was observed. Both negative (**Figure IV.6 F, G** and **H**) and positive (**Figure IV.6 I, J** and **K**) correlations were noted. When these results are more closely viewed, we can see that positive correlations are only seen when the DG of the hippocampus is implicated in the correlation (**Figure IV.6 I, J** and **K**). However, with the other brain regions, a negative correlation was noted.

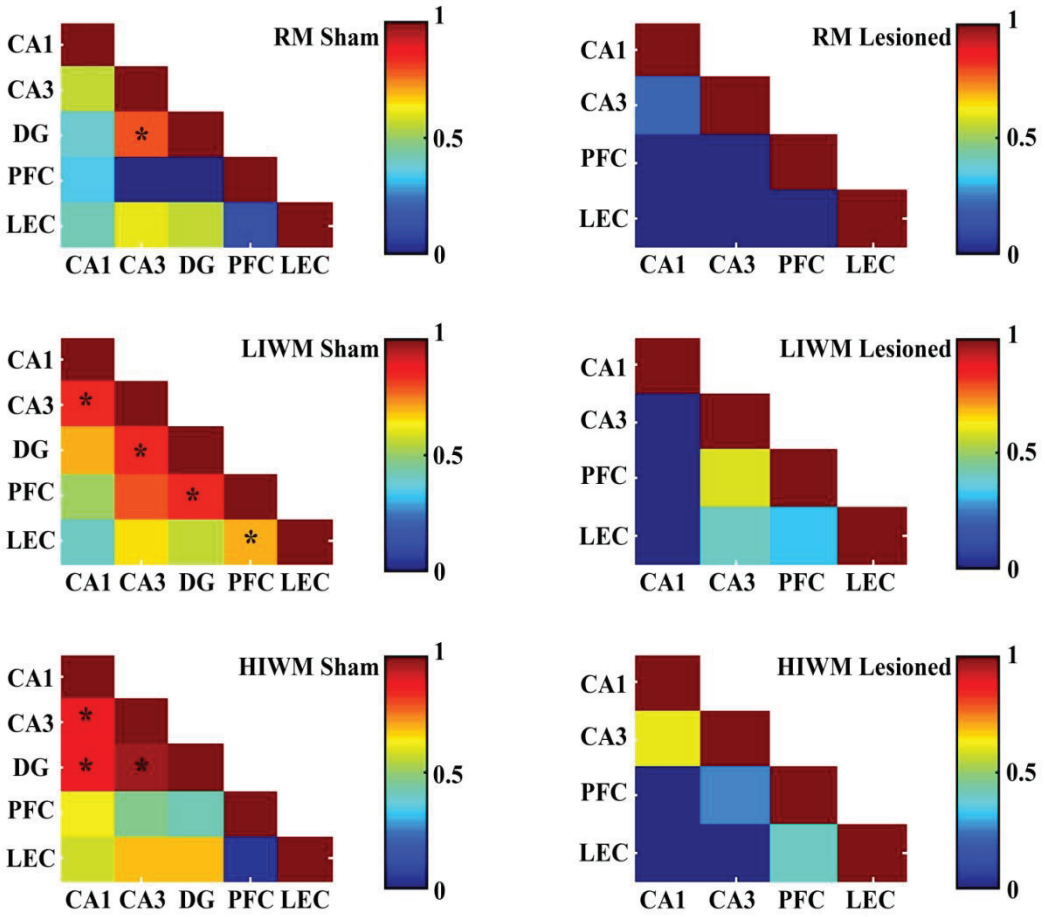


**Figure IV.5: Interregional correlation matrices for Zif268 expression within each group (Sham and lesioned).** R-Spearman rank correlation coefficients are color-coded. Colors reflect correlation strength (scale, right). Significant correlations ($p<0.05$) are marked with (*).

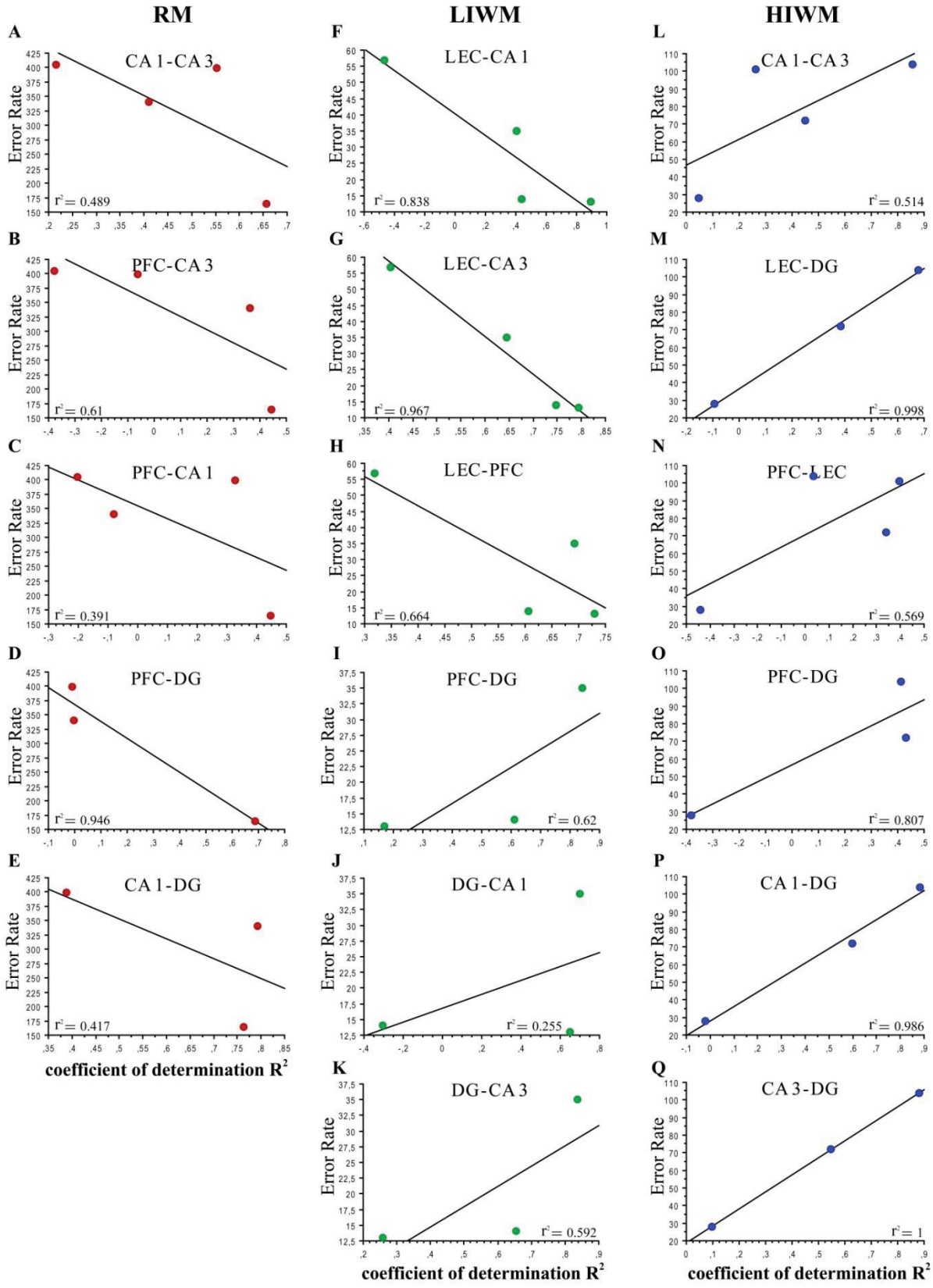


**Figure IV.6: Correlations between the error rate and the correlation coefficient within different structures.** Global error number was calculated for each group of rats in the three experiments of chapter II (10-day group), chapter III (4-day group) and chapter IV (Sham and Lesioned groups). This error number was correlated with the coefficient of correlation obtained earlier between different brain regions (hippocampus and cortices). A negative correlation was observed in RM group (**A** to **E**). On the contrary, a positive correlation was noted in HIWM group (**L** to **Q**). A mixed result was noticed in the LIWM group, i.e.; a positive correlation was only perceived when the DG is implicated (**I** to **K**).

## III.4 Discussion

Our findings described in chapter II that the dorsal DG shows no increase in Zif268 expression after HIWM training were particularly striking. To what extent the DG is required for WM and the processing of PI? To address this question, we examined the effects of ibotenic acid lesion of the DG on our three behavioral tasks. Lesions of the DG significantly impaired RM and LIWM training with respect to the sham-operated rats. In sharp contrast, DG lesions did not affect HIWM performances. On the contrary, it produced a protection against PI on the last block of days. In addition, such lesion increased the number of Zif268 positive cells in the Prefrontal Cortex of rats trained specifically in the HIWM task.

First, our results showed an impairment of RM after ibotenate-DG lesions. This result agrees with previous studies showing a significant disruption in the (reference) memory for places in the eight-arm radial maze after intra-dentate gyrus injection of colchicine (Jarrard et al., 1984). The effects of DG lesion have also been investigated using other behavioral tasks believed to assess the same cognitive process. Xavier and colleagues (1999) showed a disruption in the acquisition of a RM water maze task after colchicine injection into the DG (Xavier et al., 1999). Nevertheless, these authors still found a slow improvement in spatial search strategies of the task, a result that agrees with the slight (marginally significant Block effect) improvement that we observed in the 8-arm radial maze indicating that DG-lesioned rats could still acquire some relevant information about the requirements of the task. However, it is important to note that the pseudo-random variation of the starting arms used throughout the 10-days of training (see methods chapter II) was performed to preclude the adoption by the rats of egocentric orientation strategies; performance in our RM task are thus believed to rely solely on spatial cues. In addition, even if a slight improvement in performance is observed in our lesioned animals, they did not significantly deviate from chance level (34.8%), indicating that should there was “learning”, such learning was not beneficial to resolve the RM task.

Lesions of the DG significantly impaired LIWM training with respect to the sham-operated rats. Freeman and Stanton found that a lesion of the fimbria-fornix, a bundle of fibers heavily connected to the hippocampus and the DG in particular, interferes with the performance of non-matching to place and spontaneous alternation tasks (Freeman and Stanton, 1991). In line with our results, Emerich and Walsh showed that rats with colchicine-induced DG damage exhibit a transient deficit of performance in a non-matching to place T-maze task. With time however, all these rats were able to reacquire the task to control level, possibly due to the incomplete lesion of the DG (Emerich and Walsh, 1989). Furthermore, Costa and Colleagues found that DG lesion disrupted performance in a delayed non-match to place task, however, DG-lesioned rats recovered control levels of performance during repeated training when the inter-trial interval was between 3 seconds and 4 minutes (Costa et al., 2005). These results could explain why our DG-lesioned subjects still perform above chance level in our LIWM task, even if their scores are lower than those of sham-operated animals.

We found that when the same pairs of arms were used as in the HIWM task, lesioned rats performed more accurately than control rats at the end of training. For control rats, the use of the same pairs of arms produced an increase in proactive interference inducing a substantial

reduction in discrimination performance consistent with our results observed previously in chapter II with non-operated subjects or with previous studies on inter-trial proactive interference effects (Wright et al., 1986). In contrast, lesioned rats appeared to be immune to this proactive interference build-up effect, even if they may still experience transient WM deficits. This WM deficit can be seen on the third block of training when the performance of DG-lesioned rats seems lower than those of sham-operated controls. This WM deficit however, could lead to the rapid fading (in less than 20 seconds) of stored information relative to previous trials. Consequently, lesioned rats would not be affected by proactive interference from previous trials, permitting performance superior to that of control rats in later blocks. In line with our results, Han and colleagues found that total hippocampal lesions enhance configural learning by reducing proactive interference. Indeed, these authors trained rats on an operant conditional discrimination in which an ambiguous stimulus (X) indicated both the occasions on which responding in the presence of a second cue (A) would be reinforced and the occasions on which responding in the presence of a third cue (B) would not be reinforced. When training was spaced, both control and lesioned group learned the discrimination pretty well. However, when training was massed, lesioned group acquired the task more rapidly than the control group. These authors argued that hippocampal lesions might have improved learning or performance on a given trial by reducing proactive interference from previous trials (Han et al., 1998). Our results suggest that this control of proactive interference could reside in the inhibition of the DG specifically.

The main IEGs' findings of this study are very clear. Lesions of the rat's DG resulted in a striking increase in the counts of Zif268-positive cells in the Prefrontal cortex only after training in the HIWM task. This novel result shows that DG lesions leave the PFC hyperactivated when forgetting is needed, raising the possibility that PFC might also contribute to the processing of interference when the DG is damaged. Studies on the impact of PFC lesions on WM have shown that this area implements the protection of the contents of WM from disruptive effects of proactive interference (Knight et al., 1999, Postle et al., 2004). The involvement of the PFC in the processing of PI is well described in humans(Feredoes et al., 2006, Nee et al., 2007), while the implication of the hippocampus in processing PI is largely observed in animals (Han et al., 1998, Fortin et al., 2002, Saxe et al., 2006). Our result is the first to show a possible implication of the PFC in processing PI in DG-lesioned animals. This result could suggest the rat needs to inactivate the DG and activate the PFC in order to respond accurately to the HIWM task demands.

Interestingly, correlations between the different brain structures and correlations between the error rates and the coefficient of correlation gave us more insight about how the brain processes information when performing our three different behavioral tasks. We found that a higher coefficient of correlation between brain regions was associated with higher performance in RM. In contrary, a higher coefficient of correlation between brain areas was associated with poorer performance in HIWM. Strikingly, in LIWM, when the coefficient of correlation involving the DG was high, this coefficient was associated with poorer performance; but when it did not implicate the DG, a high coefficient was associated with higher performance. In other words, altogether, these results suggest that in order to respond correctly to a WM task, there must be an uncoupling of the DG. Our results are in agreement with Poirier, Amin and Aggleton who tested rats in an 8-arm radial maze for spatial working

memory and found that higher expression of Zif268 protein in the DG was associated with poorer performance (Poirier et al., 2008).

The DG possess the ability 1) to disambiguate spatial environments (Leutgeb et al., 2007), a role known as spatial pattern separation (O'reilly and McClelland, 1994, McClelland and Goddard, 1996, Rolls, 1996, Lörincz and Buzsáki, 2000, Gilbert et al., 2001), 2) to detect spatial novelty (Lee et al., 2005a), and 3) to process “errors” (Lörincz and Buzsáki, 2000). In agreement with these studies delineating a role for this area in pattern separation, silencing the DG could reduce the amount of overlap between the sets of neurons that represent distinct (different trials) but very similar (same pair of arms) spatial information during consecutive WM trials. We believe these findings not only provide striking evidence about an implication of the DG in forgetting previously stored useless information, but also help to understand how connected regions and hippocampal subfields interact to support memory and forgetting.

# CHAPTER V

## What mechanism(s) in the Dentate Gyrus is (are) responsible for forgetting?

## V.1 Introduction

Our immunohistochemistry and lesion studies have demonstrated an essential role for the DG in processing proactive interference. Precisely, the inactivation of this hippocampus subfield seems to be crucial for the forgetting of previously stored, but non-relevant information. Now that the importance of this inactivation has been established, the important step was now to understand what triggers and induces this inhibition, and to determine whether a specific population of DG's cells could be implicated in this process. Several hypotheses were thus envisaged. The DG is an integral part of the hippocampal formation, possesses a multiple number of cell types and receives inputs from different regions. The non-activation of the DG could be induced either from intradentate cells or from afferences to the DG. Therefore, the main focus of this chapter was to identify what type of neural populations is involved in the forgetting of previous information and consequently what are the cellular mechanisms underlying such function.

Our first hypothesis was that interneurons of the DG might be potential candidates suited to specifically inhibit the DG granule cells. With granule cells, DG interneurons are a heterogenous group of neurons that use GABA as their primary neurotransmitter. In the DG, interneurons contain high calcium-binding proteins such as Parvalbumin (PV) (Freund et al., 1990, Braak et al., 1991, Nitsch and Ohm, 1995). These interneurons play an important role in normal hippocampal function by inhibiting the activity of granule cells and other dentate neurons (Buckmaster and Schwartzkroin, 1995). Thus, we first carried out a double fluorescent labeling of PV-positive interneurons and Zif268-positive cells in order to see if these interneurons are particularly activated in our HIWM group.

A unique feature of the DG is its remarkable ability to generate continuously during adulthood new neurons that become functionally integrated into existing neural circuits. Members from our team have shown that reducing neurogenesis in mutant mice could improve WM task involving the processing of proactive interference (Saxe et al., 2007). Consequently, we tested the hypothesis that the population of newborn neurons might be specifically inhibited when rats are trained in the HIWM protocol. Newborn neurons can be identified with various labeling strategies. For example, endogenous proteins such as doublecortin (DCX) can be labeled using immunohistochemical techniques. DCX is a protein expressed in immature neurons from the time of cell division until approximately 21 days of age (Brown et al., 2003, Couillard-Despres et al., 2005). Thus, a double labeling Doublecortin and Zif268 was carried out in order to assess the implication of newborn neurons. The results of this experiment are presented below.

A possible source of the inactivation of the DG might also be coming from outside this structure. It has been shown that the DG receives a number of extrinsic inputs, principally from the entorhinal cortex, the septum and the supramammillary region. Most of the septal afferents to the DG terminate in the polymorphic layer (Swanson and Cowan, 1976) and we already mentioned the role of the entorhinal cortex before and failed to demonstrate a specific role of this structure in forgetting processes. In contrast, the supramammillary region of the hypothalamus is of particular interest as it sends projections to the molecular layer and the

granule cells' layer of the DG (Wyss et al., 1979). A lack of activity in the supramammillary region could thus be responsible for the inhibition of the DG. We thus investigated if the activity of the supramammillary region is differentially modulated by our three tasks and whether the activity in this region could be correlated with the activity of its efference, the DG. We found that the supramammillary area was activated in synergy with the DG only when forgetting was required. This result, presented in this chapter, suggests a possible implication of the supramamillary area in processing proactive interference.

Finally, a last goal of our study was to examine plastic synaptic changes potentially responsible for the processing of interference. Using transgenic mice models, our team and other groups have previously shown that the long-term storage of information into RM benefits from phosphorylation mechanisms increasing long-term synaptic potentiation (LTP) (Lee et al., 2003), while flexible learning such as the one at play in WM, and HIWM in particular, would depend on dephosphorylation mechanisms involved in the expression of long-term synaptic depression (LTD) (Malleret et al., 2010; Nicholls et al., 2008). It has thus been shown that long-term plasticity and long-term memory require the activation of the calcium/calmodulin-dependent protein kinase II (CaMKII) and reversely, that the expression of this kinase is particularly activated by the induction of LTP and memory processes (Sanhueza et al., 2007, Pi and Lisman, 2008). Recently however, CaMKII was also shown to interact with Arc/Arg3.1 gene product to produce "inverse" synaptic tagging of inactive synapse (Okuno et al., 2012). By this process, CaMKII could lead to LTD by promoting AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors endocytosis, thus preventing undesired enhancement of weak synapse in potentiated neurons. Therefore, we investigated the expression and phosphorylation state of molecular markers of synaptic plasticity such as CaMKII and glutamate AMPA receptor subunit GluR1 in a new group of rats trained in the three behavioral tasks already described. To do so, we performed Western Blot assays of these molecular markers in the three sub-regions of the hippocampus (Fraize et al., in preparation). In addition, we assessed the expression of Arc in the dentate gyrus by immunohistochemistry. Strikingly and in concordance with our previous data suggesting a specific role of the DG in forgetting, we found that long-term synaptic plasticity occurs selectively in the DG during the HIWM task.

## V.2 Materials and Methods

### Animals

To study the expression of Arc and the implication of interneurons, newborn neurons and DG afferences in our tasks, we carried out immunohistochemical experiment by using different series of brain slices from the same rats we used in the chapter II. However, in order to tag the phosphorylation state of molecular markers mentioned above (CaMKII, GLUR1), a new group of rats needed to be tested in the three behavioral tasks and sacrificed immediately after behavioral training. Western Blot assays of these molecular markers were performed in the three sub-regions of the hippocampus.

### PV/Zif268 double labeling

Brain slices were washed with PBST (PB 10 mM pH 7.4, NaCl 0.9 %, Triton X100 0.3%) and transferred to Zif268 primary antibody solution made with Rabbit anti-Egr-1 and with Mouse anti-Parvalbumin in PBST Azide to be incubated for 72 hours at 4°C (**Table V.1**). After three rinses with PBST, slices were incubated in Zif268 secondary antibody solution, which consisted of Goat anti-Rabbit ALEXA 546 and Donkey anti-Mouse ALEXA 488 in PBST for two hours at room temperature. Brain slices were washed with PBST three times. After rinsing with PBST, tissue slices were mounted onto glass slides and cover-slipped with PVA DABCO.

### DCX/Zif268 double labeling

Brain slices were washed with PBST (PB 10 mM pH 7.4, NaCl 0.9 %, Triton X100 0.3%) and transferred to Zif268 primary antibody solution made with Rabbit anti-Egr-1 in PBST Azide to be incubated for 24 hours at 4°C. After three rinses with PBST, brain slices were incubated in Zif268 secondary antibody solution, which consisted of Goat anti-Rabbit ALEXA 546 in PBST, for 2 hours at room temperature. Brain slices were then washed with PBST three times. The DCX primary antibody solution was prepared with guinea pig anti-DCX in PBST azide and brain sections were incubated in this solution for three days at 4°C. After rinsing with PBST, a secondary antibody solution containing Donkey anti-guinea pig in PBST was used to incubate brain slices for two hours at room temperature. After rinsing with PBST, tissue slices were counterstained with DAPI (1/50), mounted onto glass slides and cover-slipped with PVA DABCO (**Table V.1**).

### ARC labeling

The immunohistochemical procedure of ARC was the same used in the previous studies for Zif268 and c-Fos. Briefly, an additional series of slices were incubated in ARC primary antibody solution for three days at 4°C (**Table V.1**). Then, slices were incubated in secondary antibody for two hours at room temperature. The reaction product was visualized using the nickel-DAB technique (see chapter II).

**Table V.1: Primary and Secondary antibodies used in Immunohistochemistry**

| | | Characteristics | Specie | Dilution | Reference |
|---|---|---|---|---|---|
| Primary Antibody | Zif268 | Polyclonal antibody | Rabbit | Fluo: 1/500 | Santa Cruz Egr-1(588):sc-110 |
| | Parvalbumin | Monoclonal antibody | Mouse | Fluo : 1/400 | Sigma immune chemicals PA-235 |
| | Doublecortin | Polyclonal antibody | Guinea pig | Fluo : 1/500 | Merck Millipore VPA 2253 |
| | ARC | Serum albumin | Rabbit | DAB: 1/10000 | Synaptic System Cat. No. 156 003 |
| Secondary Antibody | Fluo anti-rabbit | Alexa Fluor 546 conjugated (Polyclonal) | Goat | Fluo : 1/1000 | Invitrogen A 11010 |
| | Fluo anti-Mouse | Alexa Fluor 488 conjugated (Polyclonal) | Donkey | Fluo : 1/400 | Life technologies A 21202 |
| | Fluo anti-guinea Pig | Alexa Fluor 488 conjugated (Polyclonal) | Donkey | Fluo : 1/1000 | Interchim Bs-0358D-A488 |
| | Anti-Rabbit | Biotinylated IgG (H+L) | Goat | DAB: 1/1000 | Vector Laboratories BA-1000, X0524 |

**Western Blot**

A new group of rats (n = 66) was trained as described earlier (see chapter II for the behavioral results concerning these rats). After the last trial on the last day of training, these animals were immediately decapitated and their brains were rapidly removed on a bed of dry ice. Brains were immediately plunged into isopentane at -50°C and were soaked for 10-15 minutes. All brains were stored at -80°C. Each brain was then individually dissected with the aid of a Cryostat (Microm HM550) kept at -14°C. 300µm thick sections containing the medial Prefrontal Cortex (mainly prelimbic area), and the dorsal hippocampus (CA1, CA3 and the DG) were sliced and structures were micropunched under microscope guidance by using small trocars adapted to the size of these structures. Overall protein concentrations were obtained using the Bradford method (1976). Briefly, using precise concentrations of bovine serum albumin (BSA), samples with known protein concentrations were prepared and scanned using a spectrophotometer ($\lambda$ = 595nm) in order to establish a standard curve. A given sample was combined with homogenization buffer and Bradford Reagent (4.5% Coomassie Blue G250, 10% ortho-phosphoic acid) (Bradford, 1976). Samples were scanned using the spectrophotometer and protein concentration readings were recorded. Samples were then individually diluted with homogenization buffer and were denatured at 65°C during 5min in denaturation buffer (125mM Tris pH6.8, 50mM dithiothreitol (DTT), 1% sodium dodecylsulphate (SDS), 0.005% bromophenol blue, 8% glycerol) in order to contain a final protein concentration of 10µg/10µL. Denatured samples were aliquotted and stored at -80°C until further analysis. Each sample was then deposited on an electrophoresis precast gel (4-12%tris bis -SDS PAGE Biorad). Gels were run at constant voltage 80V for 15min in order to compress the bands of protein and then changed to 110V for 90min to separate the proteins

according to their size. Gels were then cut into 3 bands each containing a group of relevant protein (250-150 Kda for the NMDAR; 150-80 Kda for the AMPAR; and 80 30 Kda for β-Tubulin and CamKII) as described by (Kiyatkin and Aksamitiene, 2009). These bands were then deposited on a nitrocellulose membrane (whatman) and transferred (Criterion Blotter, BIORAD) at 100V for 40 minutes at 4°C. Once the transfer completed, membranes were soaked in Red Ponceau to verify the good transfer of protein from the gel to the membrane. Membranes were blocked in TBS (Tris buffer saline) +5% milk for at least one hour under agitation before exposure to antibodies. Membranes were then cut and incubated in primary antibodies anti-phosphorylated CaMKII (Tebu-Bio, 1:100), anti-CaMKII (AB Cam, 1:6000), anti-glutamate receptor 1 phosphoSer831 (Millipore, 1:500), anti-glutamate receptor 1 phosphoSer845 (Millipore, 1:500), anti-glutamate receptor 1 (Millipore, 1:10000) or anti-β-Tubulin III (SIGMA, 1:2000), in TBS-T (tris buffer saline-0.1% Tween 20) +3% milk overnight at 4°C under agitation. The next morning membranes were removed from the primary antibody solution and were washed for 3x10min (2x TBS-T 1x TBS) before incubation for 120 minutes in secondary antibody (in TBS + 3% milk) all at 4°C and under agitation. Following secondary antibody incubation, membranes were washed for 3x10 min (2x TBS-T 1x TBS) under agitation and were then exposed to fluorescent ECL substrate (Epirubicine-Cisplatine-5-Fluoro-uracile) to cause a fluorescent reaction between the secondary antibody and the ECL. Band fluorescence was captured by a FluorImager (Molecular Dynamics). After revelation membranes were reused using a stripping solution for 90min and washed for 3x10min (2x TBS-T 1x TBS) before blocking and antibodies exposure.

It is important to note that this Western Blot experiment was carried out by Nicolas Fraize, a PhD student in the laboratory.

**Cell counts**

An experimenter blind to treatment conditions performed all quantifications. Quantitative analyses of DCX-positive and ARC-positive cells were performed by using a computerized image processing system (Mercator, Explora Nova ®) coupled to an optical upright microscope. Structures were defined according to the Paxinos and Watson atlas (Paxinos and Watson, 1996). Immunoreactive neurons were counted bilaterally using a minimum of four sections. Cells were counted throughout the different area of the sections with an objective of 20x magnification. Data from YLIWM, YHIWM and YRM were pooled (control group) as no significant statistical difference in neither DCX or ARC activation in all studied structures was found between these groups. For each animal, DCX and ARC density was calculated by dividing cell counts of each area by the surface of the area. Each animal's areas density was then normalized by dividing the corresponding control density (% of control).

A subset of slices of the four groups of rats was arbitrarily chosen for the PV/Zif268 double labeling. The percentages of cells that co-express PV and Zif268 were determined by examining Zif268-labeled cells located in the DG and observing whether these cells also expressed PV. Zif268/DCX double labeling was assessed in a similar manner. However, in this experiment all the set of rats was included.

**Statistical analysis**

- **Immunohistochemistry**

Immunoreactivity was statistically analyzed with Mann-Whitney U-test. Data are expressed as mean of normalized density (% of control) ± s.e.m.

- **Western Blot**

The quality of each step was controlled by running each experiment twice. When values for the two duplicates varied for more than 25% the sample was not taken into account into the statistical analysis. Each band was then normalized to its corresponding loading control band, the housekeeping protein β-Tubulin. Western Blot analysis was done with ImageJ (NIH) and statistical results were obtained using StatView 5.0. Mann-Whitney U-test was performed to analyze Western Blot results. Data are expressed as means ± s.e.m.

- **Correlation**

The density of Zif268 labeled neurons was also used to compare inter-regional brain activity between the supramammillary area and the DG. To better understand the functional connectivity between these two brain regions, we assessed for each experimental group the Spearman's rank correlation coefficient, a measure of statistical dependencies between non-parametric variables. A positive coefficient between two brain regions indicates that an increase in Zif268 expression in a region would result in a proportional increase in the other region.

## V.3 Results

### Are Parvalbumin interneurons implicated in the inhibition of the DG?

First, we sought to examine whether interneurons could be implicated in our tasks. Interneurons might inhibit granule cells during HIWM training leading to the non-activation of the DG we observed earlier, and consequently to the beneficial forgetting of previously stored information. A double staining of Parvalbumin/Zif268+ cells was thus performed to reveal if interneurons were activated, especially during HIWM training. We expected to see a critical mass of double-labeled neurons in the HIWM group of rats. However, we failed to show PV labeled cells that expressed Zif268 in any of the three tasks or the control condition (**Figure V.1)**.

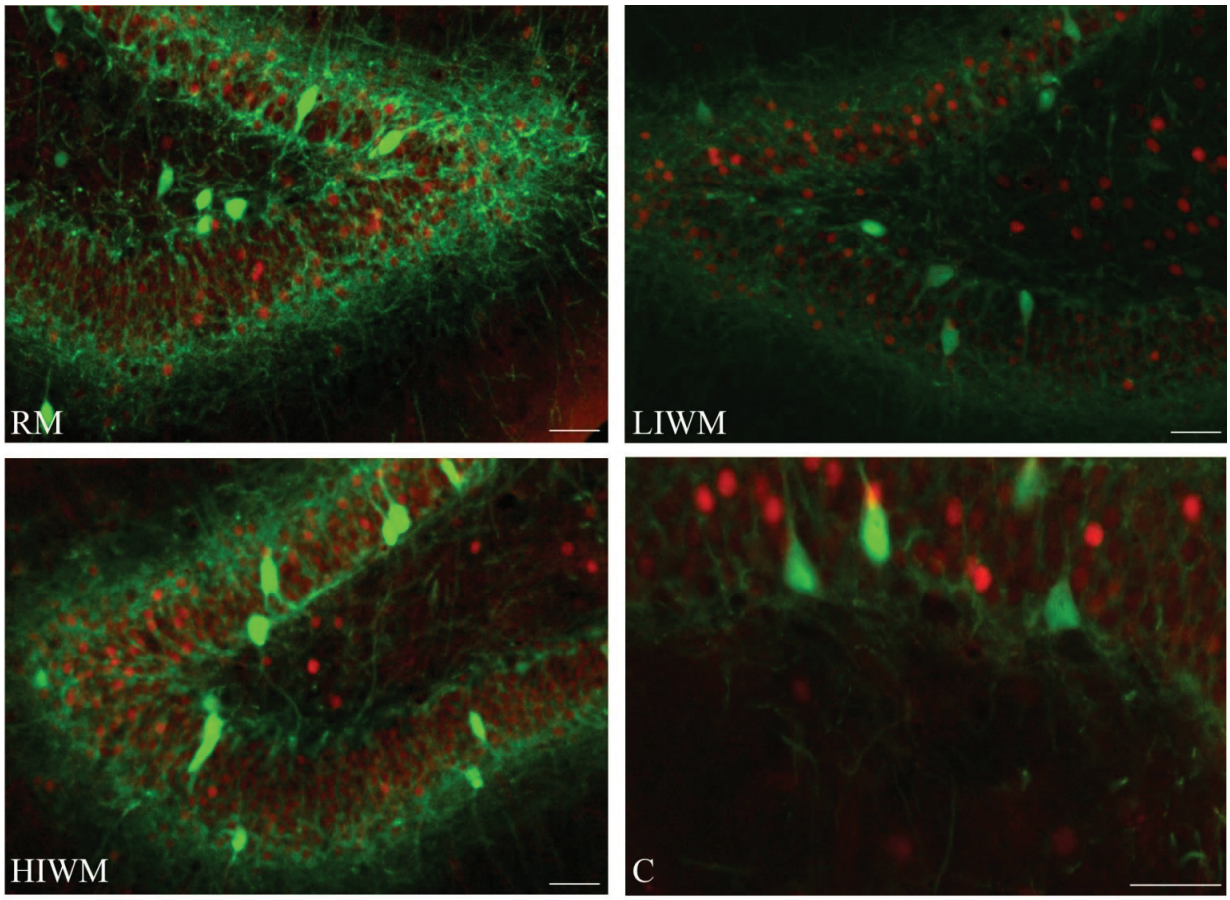


**Figure V.1: Photomicrographs of Zif268/PV double labeling.** These pictures show Parvalbumin (green) and Zif268 (red) labeling in the granule cell layer of the dentate gyrus. Scale bar: 50 µm for RM, LIWM, HIWM and 100 µm for the Control group.

**Are new neurons specifically inhibited during HIWM training?**

We next examined the specific involvement of newborn DG neurons in the processing of interference. We carried out a double staining Doublecortin/Zif268 in order to see 1) whether a change in the number of newborn neurons in the DG might be related to the changes of the behavioral tasks and 2) if these immature neurons were activated. We expected to see less new neurons and/or no double-labeled DCX/Zif268 in our HIWM compared to the other tasks. First, we found that the density of DCX positive cells in the dorsal DG was similar in our three experimental conditions (RM, LIWM, HIWM) (**Figure V.2**).

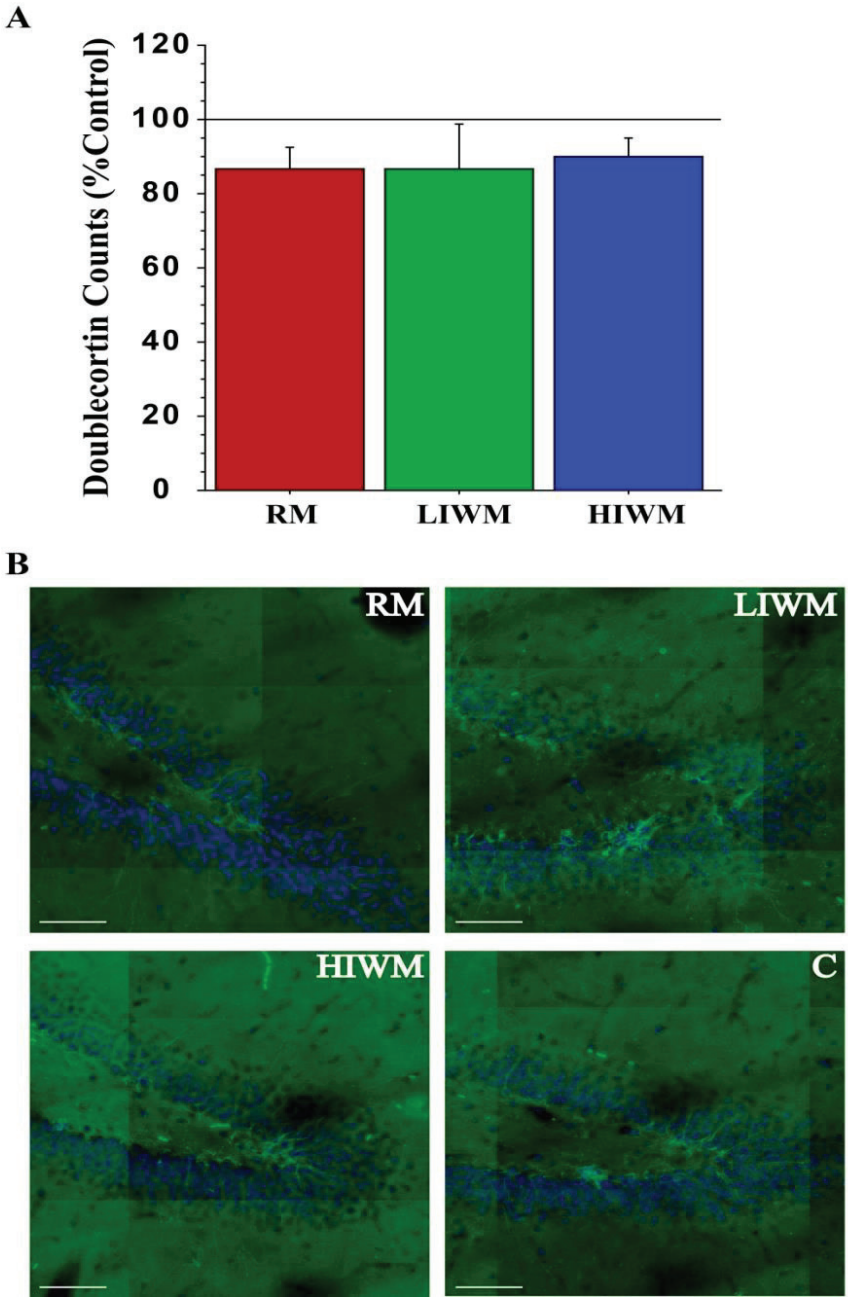


**Figure V.2: Radial maze training did not affect the rate of expression of the immature neuron marker doublecortin.** (**A**) DCX counts in the three groups of rats relative to paired controls (black line) in the dorsal Dentate Gyrus (DG) of the hippocampus after 10 days of training. (**B**) Representative photomicrographs depicting DCX positive cells (green) and DAPI (blue) in the DG (Scale bar; 100 µm).

The activation of immature neurons was determined using immunohistochemical double labeling with fluorescent detection. Unfortunately, we failed to observe any double-labeled Zif268/DCX cells in the DG of experimental and control rats (**Figure V.3**).

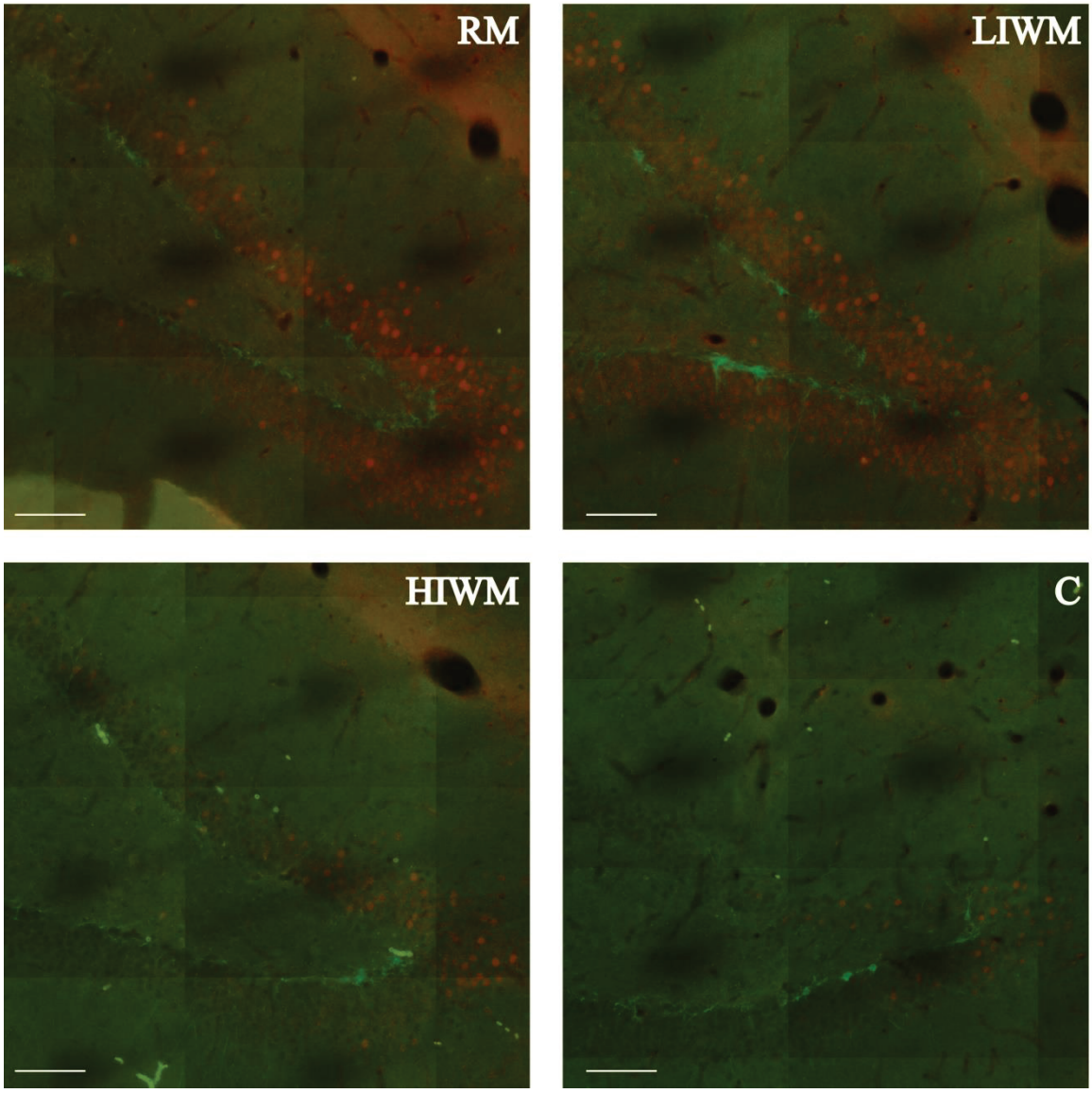


**Figure V.3: Failure to show any activation of the immature neurons in all experimental conditions.** Representative photomicrographs depicting double staining for DCX (green) and Zif268 (red), (Scale bar; 100 μm).

**Is a lack of activity in the supramammillary nucleus responsible for the non-activation of the DG?**

In view of the importance of the DG for our HIWM task, the present experiment sought to identify a region that constitutes an afference to the DG and which may also contribute to its non-activation. It has been shown that the supramammillary neurons project to the DG. We thus compared the expression of the three IEGs zif268, c-fos and arc in our four groups of rats in the supramammillary area. RM and HIWM training resulted in an increase of Zif268 and c-Fos counts in the supramammillary area. For Zif268 (** RM versus C: P = 0.0032, ** HIWM versus C: P= 0.0011, LIWM versus C: P = 0.8065) and compared to LIWM (# RM versus LIWM: P = 0.0209). For c-Fos (* RM versus C: P = 0.0203, * HIWM versus C: P= 0.0204, LIWM versus C: P = 0.3272). However, no expression of ARC was observed in the supramammillary area for all conditions (**Figure V.4**).

The density of Zif268 and c-Fos labeled neurons was also used to explore the correlation of activity between the DG and the supramammillary area (**Figure V.5**). The only significant correlation observed was for the HIWM group and for Zif268 ($r = 0.735$; $p = 0.03$). An important correlation was observed with c-Fos ($r = 0.232$) but this correlation was not statistically significant. This correlation shows that when the supramamillary area is activated, the DG is activated as well. This correlation suggests a concerted action of the supramammillary area and the DG in the processing of proactive interference (**Figure V.5**).

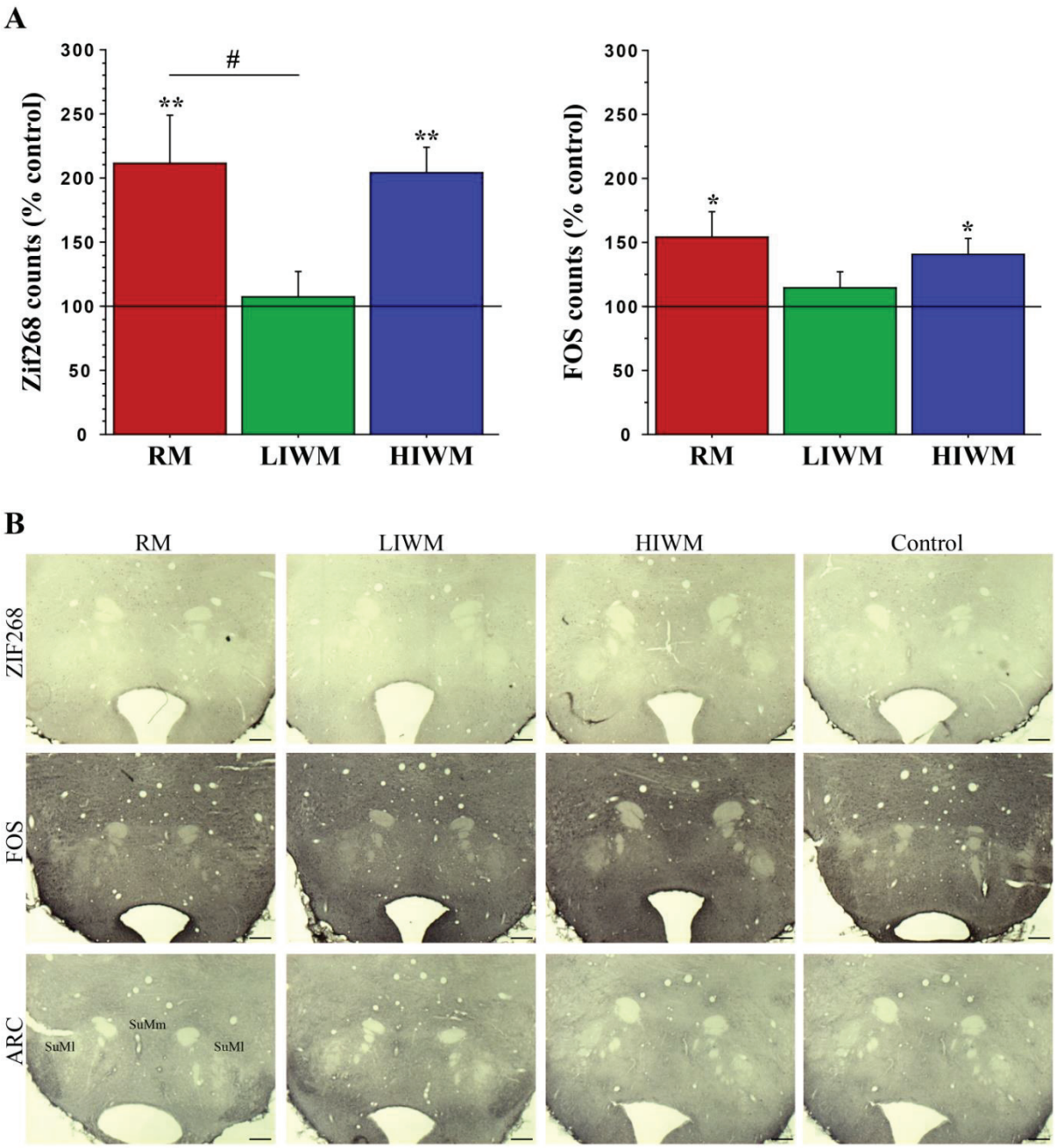


**Figure V.4: the supramammillary area is activated after training in RM and HIWM tasks, but not after LIWM.** (**A**) Zif268 (left) and c-Fos (right) counts relative to paired controls (black line) in the supramammillary area after 10 days of training. RM and HIWM groups of rats had increased number of Zif268 and c-Fos immunoreactive cells in this area compared to control animals (n = 16, 100% baseline). This increase in the number of zif268 and c-Fos positive cells was not observed in LIWM rats. *, # $P < 0.05$; ** $P < 0.01$. (**B**) Representative Photomicrographs showing Zif268, c-Fos-stained nuclei in supramamillary area of the hypothalamus in our four groups of rats. For ARC, note the absence of staining in the supramammillary area. Scale bar, 100 µm.

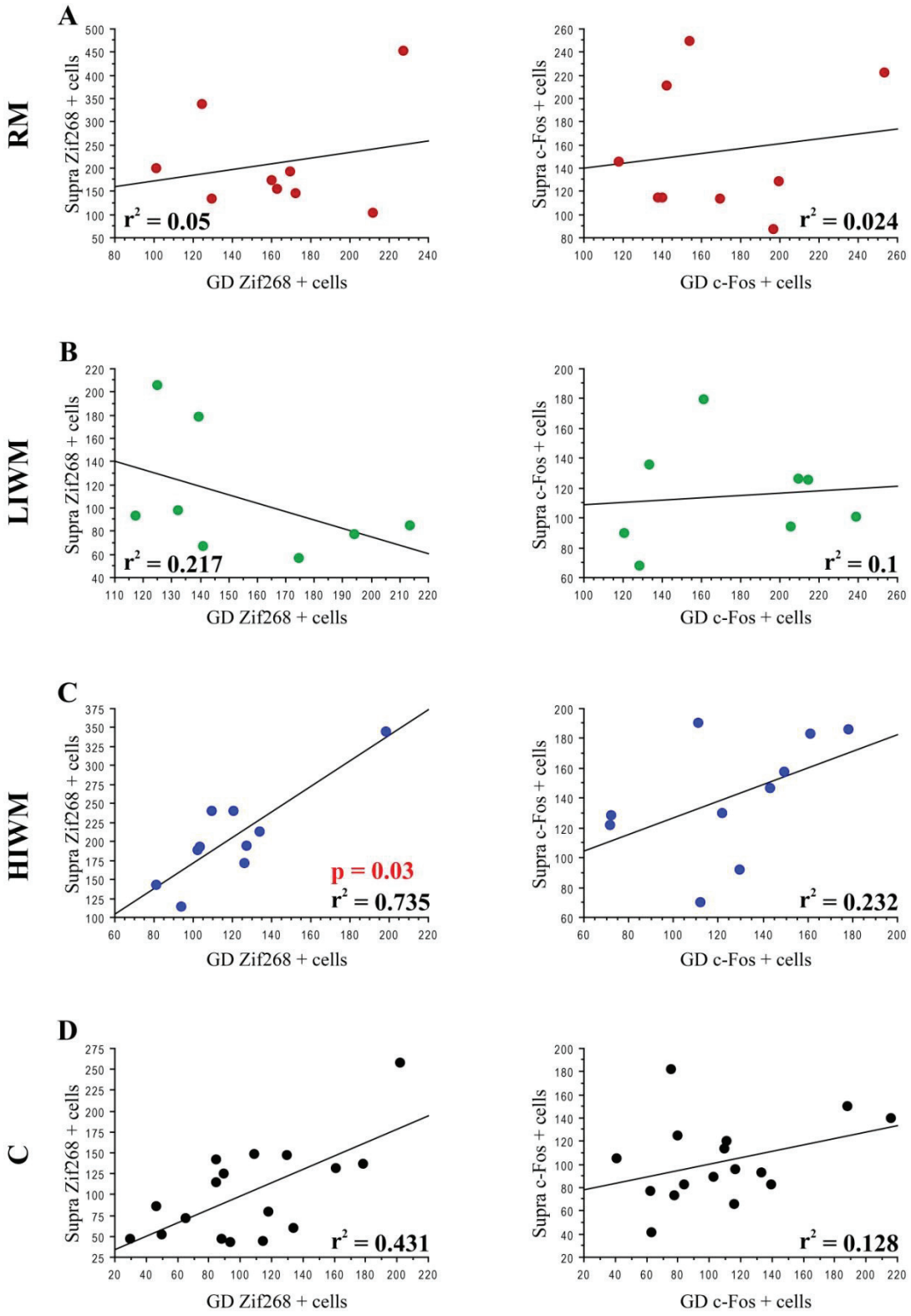


**Figure V.5: Correlations between positive cells counts in the DG and positive cells counts in the Supramammillary area for Zif268 (Left column) and c-Fos (Right column) in our four groups of rats.** Note the high correlation of IEG positive cells between these two areas only in HIWM group.

**Long-term synaptic plasticity occurs in the DG after training in the HIWM task**

Finally, we wanted to determine if different plasticity changes were at work in the dorsal hippocampus, and in particular in the DG, after training for RM or WM involving or not forgetting. To answer this question, we tested a new group of rats in the same behavioral tasks and assessed the expression and phosphorylation state of molecular markers of synaptic plasticity such as calcium/calmodulin-dependent protein kinase II (PCaMKII) and glutamate AMPA receptor subunit GluR1 in the DG as well as in the CA1 and CA3 region of the dorsal hippocampus.

Our results revealed that in the CA3 and CA1 regions of the hippocampus, no change in the expression or phosphorylation of CaMKII or GluR1 was observed, with the exception of an increase in the level of CaMKII activation (determined by calculating the ratio of phosphorylated form of CaMKII (PCaMKII) to the total CaMKII) seen in CA1 after RM training (but decreased after LIWM training) (**Figure V.6 A**). On the contrary, in the Dentate Gyrus, it is HIWM training that dramatically increases the quantity of PCaMKII. In addition, in this area, overall GluR1 expression, and the quantity of phosphorylated forms of GluR1 (ratio Ser P-831 and Ser P-845 over total GluR1), increased only after HIWM training (**Figure V.6 B, C** and **D**). Altogether, these results suggest that long-term synaptic plasticity occurs selectively in the DG during the WM task involving a high level of interference.

**Arc expression is enhanced after a HIWM task in the DG**

CaMKII was also shown to interact with Arc/Arg3.1 gene product to produce "inverse" synaptic tagging of inactive synapse. We thus examined Arc expression in the DG of the dorsal hippocampus. In contrast to Zif268 and c-Fos expression that was not activated by HIWM training, Arc expression was significantly higher in the three experimental groups, including HIWM, compared to the control condition ($P < 0.05$ for RM, LIWM and HIWM compared to C) (**Figure V.7**). We also showed that this increase in Arc+ cells was also visible in the PFC ($P < 0.05$ for RM, LIWM and HIWM compared to C), but not in the striatum (CP) (all $p > 0.05$), a region expected to show no difference between the tasks if they have been appropriately matched for non-spatial demands. Due to the weakness in Arc expression in CA1 and CA3 (Daniel Barry, thesis, 2013), no counts of Arc+ cells were performed in these areas.

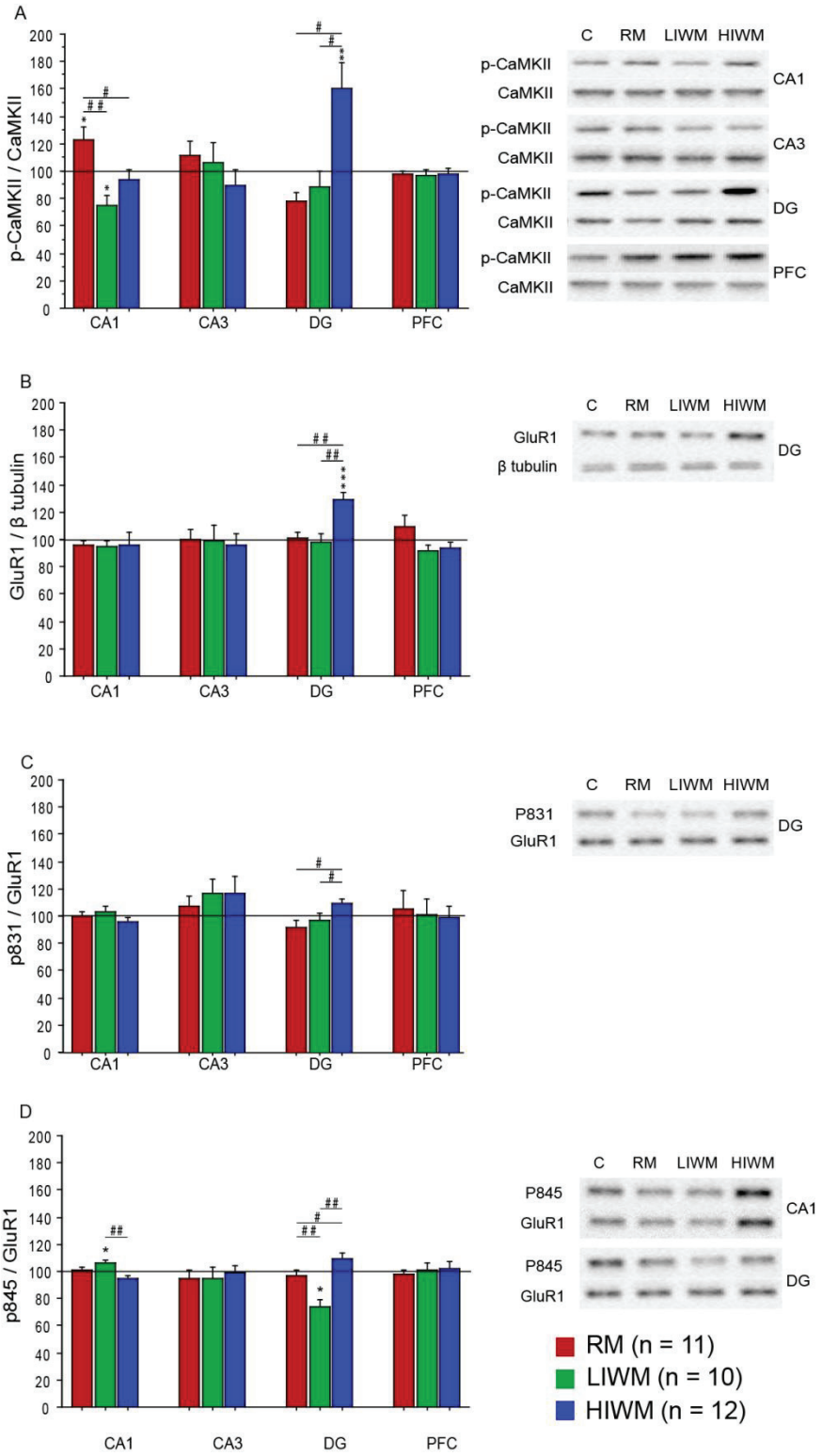


**Figure V.6: Long-term synaptic plasticity occurs selectively in the DG during HIWM task.** Western Blot quantification of the ratio CaMKII phosphorylation to the total CaMKII (**A**), overall GluR1 (**B**), Serine831 phosphorylation of gluR1 (**C**) and Serine845 phosphorylation of gluR1 (**D**) in the CA1, CA3, DG areas of the hippocampus and PFC. Representative immunoblots and quantification of the gels are shown. Note that compared with RM and LIWM, the HIWM group showed increased absolute levels of CaMKII phosphorylation (**A**) and overall GluR1 (**B**) specifically in the DG of the hippocampus. Data are expressed as mean ± s.e.m., experimental group values are expressed as 100% ± s.e.m. of average Control group (n = 33). *,# $P < 0.05$; **,## $P < 0.01$; *** $P < 0.001$ (Mann-whitney U-test).

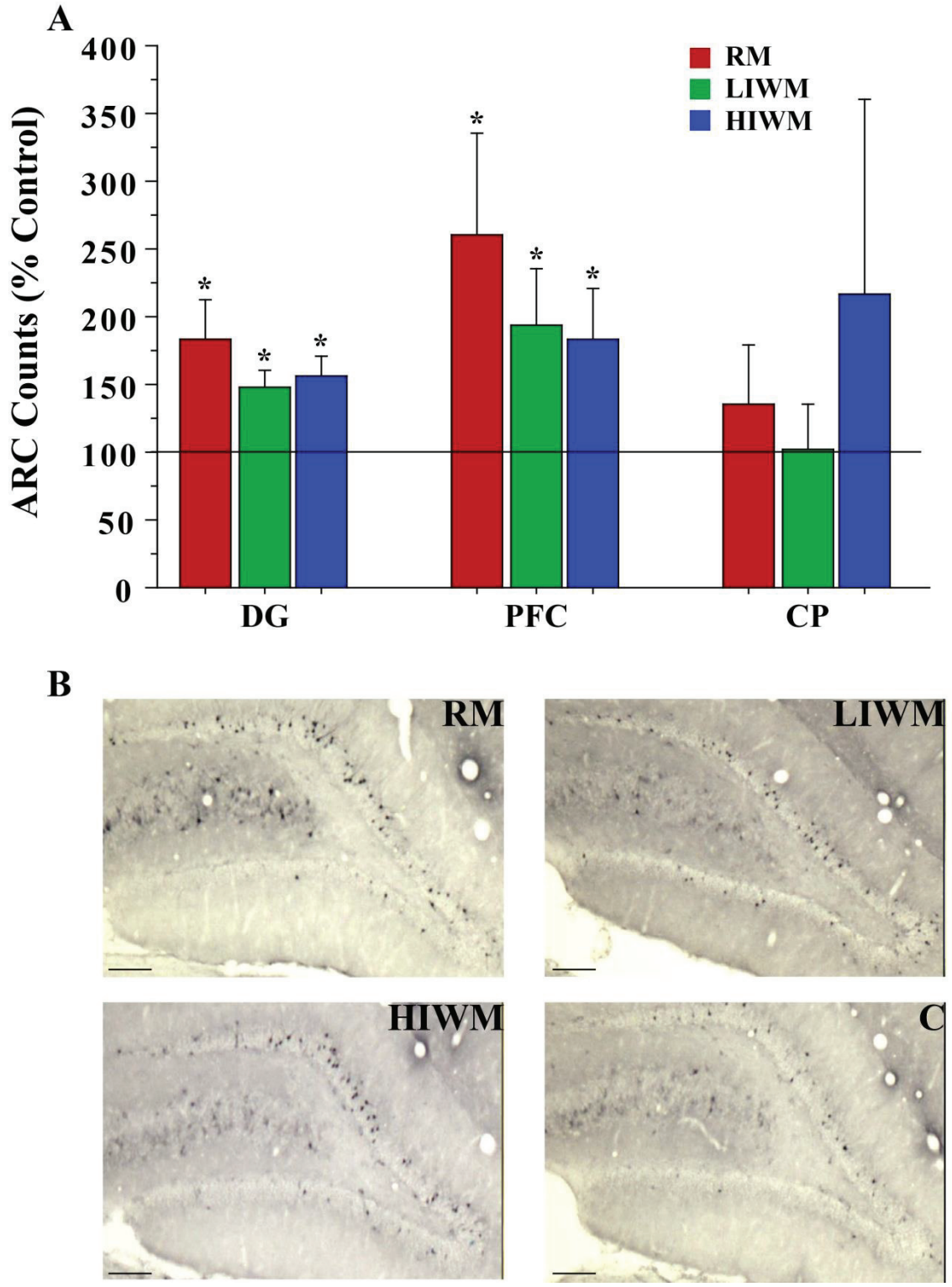


**Figure V.7: Arc expression in the three behavioral tasks. (A)** Normalized counts of Arc postive cells for the Dentate Gyrus (DG), the Prefrontal Cortex (PFC) and the Striatum (CP). Data are shown as mean ± s.e.m. *, $p < 0.05$. (**B**) Photomicrographs of the 4 groups showing Arc positive cells in the DG. Scale bar, 100 µm.

## V.4 Discussion

In this chapter, we sought to identify the mechanisms involved in the inhibition of the DG observed after HIWM training. Using double fluorescence labeling, we tried to determine if interneurons and newborn neurons were involved in forgetting previously stored information but failed to do so. On the other hand, we found that the supramammillary area seems to play a role in synergy with the DG in processing proactive interference. Our results also revealed that the processing of interference in WM might involve specific synaptic plasticity changes in the DG. These changes involve an increase in the expression of AMPA receptor GLUR1 subunit and in the phosphorylated state of CaMKII. All these findings are discussed below.

### Are Interneurons involved in the processing of interference?

Interneurons of the DG have the particularity of using GABA as their primary neurotransmitter (Houser, 2007). In consequence, these interneurons are the main source of inhibition in the DG. Finding if these interneurons are activated differentially in our tasks could be achieved through a simultaneous fluorescent immunostaining for Zif268 and the calcium-binding protein parvalbumin for instance. We found no evidence of the involvement of PV interneurons in any of our three behavioral training. However, a possible role of interneurons cannot be ruled out because PV-expressing cells are not the only interneurons present in the DG. Additionally to these cells, other types of GABAergic interneurons, expressing cholecystokinin or somatostatins just to name a few also provide inhibition to the DG granule cells. Consequently, this result does not allow us to conclude about a possible lack of implication of interneurons in our tasks and more specifically in inhibiting granule cells in order to better process proactive interference. Further experiments should be carried out.

### Are newborn neurons involved in the processing of interference?

The finding of adult neurogenesis in the DG of the hippocampus has favored the idea that newborn neurons might subserve cognitive functions (Verret et al., 2007). It has been shown that reducing hippocampal neurogenesis disrupts hippocampal dependent memory (Martinez-Canabal et al., 2013). On the contrary, other studies have shown that new neurons are not obligatory for memory formation (Jaholkowski et al., 2009). Paradoxically, Saxe and colleagues found an improvement of hippocampal-dependent WM when repetitive information was presented (HIWM training) after ablating adult neurogenesis by irradiation (Saxe et al., 2007). On the other hand, as we said earlier, it has been demonstrated that the DG provides pattern separation which is the process of reducing the average overlap between two representations (Colgin et al., 2008). Shutting down this function relying on the functional integrity of the DG may be necessary for the subject to focus on an ongoing trial, especially in task involving a high level of overlap between different trials (HIWM task). It has been suggested that newborn neurons could promote pattern separation (Clelland et al., 2009). Consequently, we expected to see less newborn neurons activated after our HIWM task. We assessed the number of newborn neurons by staining DCX protein by immunohistochemistry.

New studies have shown that DCX is an effective marker to analyse the absolute number of newly generated neurons in the adult DG (Rao and Shetty, 2004). This method could be used without the cumbersome of multiple intraperitoneal BrdU (5'-bromo-deoxyuridine, which incorporates into the DNA and used as a primary evidence for dentate neurogenesis) injections. We could not be conclusive as our experiment showed no difference in the number of immature neurons activated between the different behavioral conditions, a very surprising result. We probably should carry out a new experiment changing our antibody incubation protocol for instance.

**The supramammillary area could be involved in processing proactive interference**

The supramammillary area provides a substantial projection to the DG (Segal and Landis, 1974). It has been shown that this structure is involved in several hippocampal dependent cognitive functions (Pan and McNaughton, 2002). More specifically, Vann and Colleagues found an increase of Fos-positive cells in this area after radial maze training (Vann et al., 2000a). In our radial maze tasks, the supramammillary area was activated in RM and HIWM but not in LIWM. These results are in agreement with many studies showing a role of the supramammillary area in long-term mermory but not in short-term memory. For example, Shahidi and colleagues have found that an inactivation of this area would induce impairment in the consolidation of RM tested by a Water maze task (Shahidi et al., 2004). On the other hand, Santin and colleagues found no effects on spontaneous alternation after lesioning the supramammillary region suggesting that this area might not be implicated in basic WM processes such as those tested with our LIWM protocol (Santın et al., 2003). More interestingly, we found that the activity of the supramammillary area is correlated with the activity of the DG only in our HIWM task. This result suggests that the supramammillary region must be non-activated as well when processing repetitive information. We can thus hypothesize that the lesion of the supramammillary region would induce the same result as a DG lesion; more precisely, it would enhance performance when repetitive information is presented. However, Aranda and colleagues tested rats with supramammillary lesion in a WM task with high interference load. The task consisted in an openfield delayed-matching-to-position paradigm where the rat must retain information about the reward on a sample phase and then make a correct choice on the choice phase. Proactive interference was generated by increasing the number of trials per day. They have found a deficit in performance in the lesioned group compared with the sham group that kept a steady performance (Aranda et al., 2006). Although several authors consider the supramammillary area-hippocampus pathway to be glutamatergic, Soussi and colleagues have revealed the existence of a GABAergic pathway (Soussi et al., 2010). Based on these studies, we might speculate that in HIWM, the supramammillary GABAergic pathway might be activated to induce an inhibition of the DG. Further studies must be done in order to verify this hypothesis. However, the positive correlation of activity between the supramammillary nucleus and the DG that we observed during HIWM argued against such hypothesis.

**Processing of interference might involve specific synaptic changes in the DG**

Our westernblot analysis revealed that the processing of interference in WM might involve specific synaptic plasticity changes in the dentate gyrus. These changes involve an increase in the expression of AMPA receptor GLUR1 subunit and in the phosphorylated state of CaMKII.

It has extensively been shown that LTP requires an increase in the number of AMPA receptors at the synaptic level (Schmitt et al., 2005). The increased expression of GLUR1 observed in the DG after HIWM training might thus be responsible for an increase in synaptic efficacy (LTP). However, it is worth noting that the level of phosphorylation of these GLUR1 subunits in the DG of HIWM rats was unchanged compared to controls' level (even if it was increased as compared to the one of RM and LIWM rats). We may thus hypothesize that the increase in GLUR1 expression could reflect changes in the cytosol store of this subunit rather than an increase integration of new AMPA receptors at the synaptic (membrane) level. AMPA receptors may be massively available in the DG of animals trained to be extremely flexible in their information processing (HIWM task), but this extreme cognitive flexibility could require fast relocation of these receptors from the cytosol to the post-synaptic density (PSD) for rapid memory storage, and from the PSD to the cytosol for fast forgetting of this information once it had been retrieved. HIWM training could thus require both the recruitment and internalization of functional AMPA receptors at the synaptic (PSD) level.

On the other hand, CaMKII has long been shown to be involved in LTP and long-term memory storage, and we here showed that rats trained in the long-term/reference memory paradigm did expressed an elevated amount of active (phosphorylated) CaMKII in the CA1 area of the dorsal hippocampus. Recently, however, CaMKII was also shown to be involved in LTD and the inverse synaptic tagging of inactive synapse via interaction with the IEG *Arc* protein (Okuno et al., 2012). Using immunohistochemistry, we have shown that HIWM training induced an increased expression of *Arc* in the DG, but of an inactivation in the same area of *zif268* and *c-fos*. The increase of CaMKII in the DG could thus reflect two processes. The first one implies that HIWM training would require CaMKII-dependent phosphorylation mechanisms leading to short-term synaptic potentiation benefiting to the short-term memory storage of the information required in this task. One can postulate that this increase in synaptic potentiation (LTP) and phosphorylation of CaMKII (but also GLUR1) would be short-lived (lasting few hours?) and that, for forgetting purposes, synaptic transmission and phosphorylation levels could go back to controls levels after training, possibly during sleep, a period favorable for synaptic downscaling (Tononi and Cirelli, 2006). The other hypothesis implies that CaMKII could interact with *Arc* gene product to reverse synaptic potentiation, promoting depotentiation of synapses that were potentiated during HIWM training, and therefore forgetting. However, it was the β form of CaMKII that was shown to interact with Arc for inverse synaptic tagging (Okuno et al., 2012), and the antibody used in our study was supposed to specifically target α-CaMKII. Additional work now needs to be done. Does LTP occur during HIWM training? Why? And why in the DG? Can it be subsequently depressed (depotentiated) by a synaptic downscaling process yet to show (possibly during sleep)? These are questions that need to be answered.

# CHAPTER VI

# General Discussion

**Summary of the findings of this thesis**

These past decades, numerous studies have greatly advanced our understanding of memory processes and of their cellular and molecular underpinnings. The concept of forgetting, however, remains elusive and its molecular and physiological bases are poorly understood. This thesis set out to determine the biological bases of such forgetting using a comparative study between three groups of rats in an 8-arm radial maze trained using different conditions. We first sought to identify brain regions differentially involved in processing reference (RM) or working memory (WM) with and without interference (involving or not forgetting) using an immunohistochemical method in rats performing three different behavioral tasks. We thus tested rats in an eight-arm radial maze requiring the use of spatial orientation and memory. This maze was chosen as it allows training in both RM and WM tasks in one single spatial environment with the same spatial cues. In order to segregate processes differentially involved in RM, WM and in the processing of proactive interference, we used 1) a modified version of a WM task with highly repetitive trials inducing a high level of interference (HIWM task) in order to make forgetting of previous trials necessary to complete an ongoing trial (Malleret et al., 2010), 2) a low interference WM (LIWM) task in which the information presented is different from trial to trial (forgetting of previous trials being unnecessary), as well as 3) a RM task (Bontempi et al., 1999) that involves the long-term storage of invariable information that is not sensitive to interference. In order to identify brain structures and areas specifically activated by the three behavioral conditions, we mapped the anatomical distribution of neurons expressing the inducible immediate early genes (IEG) *zif268* and *c-Fos*, indirect markers of neuronal activity known to play a role in synaptic plasticity and memory formation (Jones et al., 2001b). Overall, we found that IEG expression increased in the hippocampus but also in the entorhinal and medial prefrontal cortices of rats trained in the RM, LIWM and HIWM tasks as compared to yoked controls. However, when examining more specifically the hippocampal formation, we found that the expression of IEG was decreased in the Dentate Gyrus of the dorsal hippocampus of rats that were exposed to the WM task involving a high level of proactive interference (HIWM) as compared to rats that performed a WM task involving a low level of interference (LIWM) or rats that performed a RM task. This result thus suggests for the first time that this area might be inactivated when forgetting/updating of previous information is required. We also compared inter-regional brain activity to better understand the functional connectivity between brain regions. We found that the pattern of correlated activity dramatically changed in HIWM as compared to the other groups. No inter-regional brain correlation was observed between any of the studied structures suggesting that forgetting and the processing of PI may require de-coupling within these memory circuits. Together with a non-activation of the DG, this inter-regional brain de-correlation might specifically promote forgetting of previous trials as required in the HIWM task (ChapterII). Our next step was to find whether a differential cerebral activity would be observed at an earlier stage of training in our tasks. More importantly, we were curious to find out how the DG responds to interference when performance of the rats was not yet disrupted by the presence of interference. We thus carried out an immunohistochemical study in a new group of rats in order to identify brain regions involved in processing RM and WM with and without interference at an intermediate stage of learning. The immediate early genes *zif268* and *c-fos* were used and their expression was examined following four days of training. Our

results were not decisive since a higher expression of IEGs was observed in the control condition clouding any difference of activity between our groups. However, this high level of IEG expression in the control group does not affect brain regions correlations. Consequently, we did not find any positive correlation during HIWM training. These results were in agreement with our previous study (10 days) and consistent with the hypothesis that forgetting of proactive interference starts early on during training (Chapter II). Subsequently, in order to firmly demonstrate the role of the dentate gyrus, we performed a lesion of this area in rats submitted to the same HIWM, LIWM and RM tasks. We showed that this lesion of the DG specifically altered performance of rats tested in LIWM and RM tasks, but on the opposite, improved performance of rats tested in the HIWM task. Lesions of the rat's DG resulted in a striking increase in the counts of Zif268-positive cells in the Prefrontal cortex only after training in the HIWM task (Chapter III). Finally, our last goal was to identify what type of neural populations is involved in the forgetting of previous information and consequently what are the cellular mechanisms underlying such function. We found an activation of synaptic plasticity in the DG expressed by an increase in the expression of AMPA receptor GLUR1 subunit and in the CaMKII phosphorylation level. This increase could occur to cope with the increasing level of difficulty (reflected by the decrease of performance in the HIWM group) across days, although this synaptic plasticity induction might be detrimental to optimal processing of PI. In this discussion, we will first discuss some methodological aspects relative to our work. I will then provide a general overview of the scientific contributions of the current research and identifies possible future research directions. More in depth discussion of individual experimental findings was already provided in each experimental chapter (see chapter II, III, IV and V discussions).

**Methodological aspects:**

**The Radial Arm Maze: comparative study**

The radial arm maze was developed by Olton and Samuelson (1976) and has become an essential tool for testing memory in rats. Since its invention, animals' performance in this maze has been shown to be a true measure of spatial memory. The radial maze was chosen as it allows training in both RM and WM tasks in one single spatial environment, and thus permits to determine a clear distinction between processes required for these different forms of memory. Whether they were tested in RM or WM, rats were placed in the same conditions and were allowed to complete eight runs per day. All our tasks are presumed to tax allocentric processing and there was no evidence of using any egocentric strategies. First, the use of different starting arms from trial to trial guaranteed that animals had to rely only on spatial memory, and thus the hippocampus, to find food rewards. In addition, during the intertrial delay, rats were removed from the maze and then placed to a transfer cage before being placed back in the maze. This procedure was used to prevent any prospective motor coding, the ability for the animal to predict its next move relying not on spatial (perceptual) memory but on motor (procedural) memory. Being removed from the maze and then placed back in a different starting arm prevented the animal to adopt such strategy, and therefore to use an egocentric strategy. In a similar manner, Roberts and Dale (1981) forced rats to enter in four randomized predetermined baited arms before allowing them to search for the remaining food rewards in all the maze's arms. As the first 4 arms were chosen by the experimenter, rats

could not use an algorithmic strategy. In our WM tasks, the rats were always forced to enter a rewarded arm in the sample phase, this method allowed us to control the starting arm and the predetermined arm in order to make the use of an algorithm strategy impossible for the rats. Moreover, the use of many trials in a day reduced the use of any intramaze odor cues. In addition, during the intertrial interval, the maze was wiped with water in order to disperse odors in the maze. We have also used odorless food pellets. These manipulations allowed covering up any olfactory cues or trails left by the rats on visited arms. Moreover, many authors have ruled out any possibility for the rat to use odor cues in the radial maze resulting from the placement of a rats scent to "mark its territory" as a sign that it has been there (Olton and Samuelson, 1976, Olton and Papas, 1979). Zoladek and Roberts made rats temporarily anosmic and rat's performance on the maze was unaffected (Zoladek and Roberts, 1978). Therefore, the radial maze is a true memory paradigm. Importantly, in our tasks, rats were matched in all experiments in terms of number of runs (8 runs per day), exposure to the maze and motivational aspects. Consistent with this, there was no evidence that any of the control areas (Somatosensory cortex, Striatum…) differed among the groups. Additional studies of the motor cortex and the visual cortical cortices activity could confirm that our tasks are not solved by egocentric strategies.

**The control group**

*"Because the task constrains behavioral parameters such as arm choice and the number of arm entries, yoked animals provide close behavioral controls" (Poirier et al., 2008).*

Our work attempted to provide a clear comparison between three groups of rats performing different training in the same radial maze that taxes both WM and RM. IEGs activation was used as a tool to assess brain activity. The use of a control group was therefore essential in order to correctly compare IEGs activation between different groups of rats. We found that using yoked control provides excellent tools to conclude that any changes in IEGs expression compared to these controls reflects purely an involvement of a cognitive process. In fact, IEG activation can be triggered by general sensory stimulation and motor activity. Consequently, commonly used cage control rats may not be appropriate when assessing memory and learning related IEG changes. Moreover, these cage controls are not exposed to the environment of the maze the same way than those engaged in a memory task. In consequence, the somatosensory cortex and motor cortices express reduced level of IEG activation in these cage controls, which can shadow the interpretation of IEG discrepancies in the experimental groups. Shires and Aggleton trained two groups of rats in the Morris Water maze. A first group was exposed to the active learning task, whereas the second performed only the procedural aspects of the task. At the end, both groups crossed the same distance in the maze and swam the same amount of time. These authors have found that the PFC expressed lower levels of activation in the procedural controls compared to the active group. Surprisingly, the hippocampus activity was the same in both groups even though solving a Morris Water maze task is considered to rely on hippocampal functions (Shires and Aggleton, 2008). This unexpected finding highlights the importance of using yoked control for IEG experiments in order to give a clear picture of what changes in IEGs are learning dependent. These rats performed 8 trials per day for 20 consecutive days. After this long training duration, one would expect a habituation of the control group to the water maze and consequently a

decrease in IEG expression, as it is the case in our 10-day training control group. In this study, the procedural group was trained to find a hidden platform that was placed always close to the perimeter of the water maze and which position changed from trial to trial. This technique allowed the rat to use the wall of the maze to guide its behavior. However, on the last training day, in order to make procedural control more comparable to the training group, the platform remained in the same location on all 8 trials. This could explain the significant hippocampal activity of the control group that was trying to understand a possible new rule during the last day of training. Although the pool wall was the most salient proximal cue to guide performance in the procedural control, the emergence of a strategy based on the use of distal cues which may significantly tax spatial navigational abilities cannot be ruled out, and appears to be the most parsimonious explanation of these results. This same explanation could be applied to our 4-day training experiment (chapter III) as no clear differences in IEG expression was observed in the hippocampus or other regions between the control group and the animals performing the tasks. On the other hand, other factors associated with early training, such as stress or attention directed to stimuli dimensions that do not predict successful spatial learning, as demonstrated by spatial overshadowing (Diez-Chamizo, 1985), could readily interact with hippocampal activity and thus increase IEG activity in the early stages of training in all groups including controls.

**The build-up of Proactive interference**

We have designed the HIWM task on the radial maze in order to study the processing of proactive interference in rats. This task was created based on the fact that rats choose an arm of the radial maze by building a representation of the surrounding environment. Each arm of the radial maze is associated with a specific landmark such as curtains, doors, posters, windows and other object in the experimental room. Each arm is recalled as a specific cue (Olton, 1978, Brown, 1992). In order to induce PI, we used the same pairs of arms, hence associated with the same spatial cues. In a control condition, LIWM training, we used all the available arms of the radial maze so the rat had no difficulty in discriminating the different spatial cues (**Figure VI.1**). We found that the High interference task revealed significant PI and disrupted performance. On the contrary, the low interference task revealed not to be vulnerable to PI (Saxe et al., 2007, Nicholls et al., 2008, Malleret et al., 2010). We observed a decrement in performance in HIWM over days, hence PI affected rats' performance from day to day. We analyzed rats' performance by trial rather than days over the first and last 5 days of the experiment. We found that while HIWM rats performed normally on the first trial of the first 5 days, when the most recent trial was 24 hour earlier, their performance dropped during the last (fourth) trial, when the most recent trial was only couple of minutes earlier. This pattern is consistent with the phenomenon of intertrial interference and suggests that information learned by these animals during recently experienced trials impaired their performance on subsequent trials (Aultman and Moghaddam, 2001). This is also consistent with David Olton's study (1978) who suggested that memory of each visited arm is held in WM and would be reset at the end of each trial by deleting its contents; that way, PI does not interfere with retention of events within subsequent trial. In order to prove his hypothesis, Olton have carried out an experiment in which rats were examined repeatedly for eight trials, with a 1 minute interval between trials. Within each trial, rats were allowed to make four free choices on the radial maze. Then, rats were placed in the center of the maze for a minute

before resuming the trial. Rats had to choose the remaining four arms between all 8 opened arms. Each entry was labeled as a choice, with entries into previously un-entered arms considered as correct choices and entries into previously entered arms considered as errors. Olton plotted the performance over choices made on each trial and found that accuracy of the rat decreased as a function of choices within each trial but returned to errorless performance on the initial choices of the subsequent trial (Olton, 1977, 1978). This return to errorless performance at the beginning of each trial was interpreted as evidence for the resetting mechanism. These results are consistent with our first 5 days as all the three first trials were errorless and HIWM rats were only disturbed in the fourth trial. However, these results seem at odd with our last 5 days of behavioral experiment when the build-up of PI becomes more difficult to handle. It is clear that for the last 5 days of training, rats' performance was disrupted even for the first trials. This result suggests that information learned the day before is still present and disrupts the current trials. This result is in agreement with other studies. For instance, Roberts and Dale have demonstrated that PI can occur under some conditions. Rats received five trials per day on an eight arm radial maze. In one trial, rats were placed in the center of the maze with all arms opened and baited. They had to retrieve all the eight pellets (in eight runs) without revisiting previous arms. Roberts and Dale examined error patterns within each trial. The rats never made errors on their first or second run in any of the five trials. However, the probability of making errors on the third through the fifth runs clearly differed between the second and subsequent trials. On the first trial, performance remained error free until after the fifth run. On subsequent trials, performance started to decline much sooner (after the second run), a finding consistent with the presence of proactive interference and incompatible with the notion of a resetting mechanism (Roberts and Dale, 1981). Similarly, Beatty and Shavalia tested memory in a two phase's memory task: after forced visits to four randomly chosen arms in phase one, rats were required to visit the four previously unvisited arms in phase two of training. This study was similar to Olton's with the only difference that the authors have imposed a four-hour delay between the fourth (first phase) and fifth (second phase) visit. They have demonstrated that WM performance on the radial maze remains above 90% after a 4-hour retention interval. Beatty and Shavalia repeated the experiment with a 24-hour retention interval and they found that rat's performance exceeded chance even after 24 hours (Beatty and Shavalia, 1980a). This result thus indicate that information stored in a delay non-match to place task, used throughout the world to study short-term/working memory, can be stored for a much longer time (hours) that the time period usually associated with short-term/working memory retention (seconds). This result is thus coherent with our finding and suggests that a memory trace supposedly stored for a short time, can outlast its purpose (persisting for several days) and interfere with subsequent learning. The discovery of PI in rat spatial memory argues against the use of a resetting mechanism. If the content of WM was deleted after each trial, no PI should be observed. This observation suggests further that rats remember the events of a preceding trial and cannot simply erase this information from WM in preparation for the next trial. For this reason, an active mechanism underlying an adaptive form of forgetting is required.

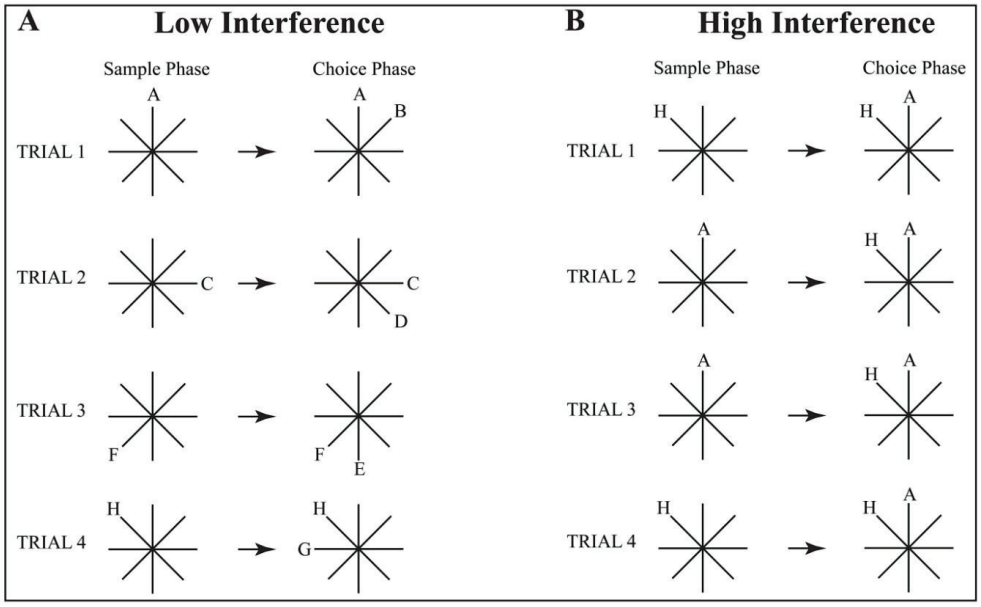


**Figure VI.1: Schematic Diagrams of the testing for a single day (4 trials).** These diagrams show the position of extramaze stimuli A through H in Low Interference versus High Interference training. Each arm is associated with a spatial cue. Thus, each arm location is learned by its relationship to the full array of landmarks in the room. (**A**) In the low Interference task, rats are allowed to visit one arm on a sample phase, then after a delay of 15 seconds rats had the choice between two adjacent arms, the one visited before empty of food and a new baited arm. Rats must select the unentered arm to be positively reinforced. On each trial a different pair of arms was used. (**B**) On the contrary, the high interference task consisted of using the same pairs of arms (A and H). On each trial, animals may confuse early and late arm visits because they have difficulty discriminating the points in time at which they entered arms on the current trial and the immediately preceding trials (Roberts and Dale 1981). Thus, the best strategy for the rat is to forget previous trials and focus on the current one. This is known as adaptive forgetting

## Adaptive Forgetting

*"It is natural for people to think that learning is a matter of building up skills or knowledge in one's memory, and that forgetting is a matter of losing some of what was built up. From that perspective, learning is a good thing and forgetting is a bad thing. The relationship between learning and forgetting is not, however, so simple, and in certain important respects is quite the opposite: conditions that produce forgetting often enable additional learning, for example, and learning or recalling some things is a contributor to the forgetting of other things (Robert A. Bjork, 2010)"*

Given the frustration that we express when we are subjected to forgetting, this quote by Robert Bjork must seem strange. Regarding this failing process how can forgetting be beneficial? Even though many authors have tried to explain forgetting by uncovering its source as trace decay, cue-dependent or interference theory, Bjork's idea is certainly plausible and not all instances of forgetting constitute processing failure (Anderson and Levy, 2010). For example, when an animal is subjected to a large number of similar information as it is the case in our HIWM task, the failing process of forgetting will occur when the rat could not make clear distinction between the current information and the previous ones. Rosenzweig, Barnes and McNaughton have argued that in order to prevent such interference, irrelevant old

memories must be forgotten in order to give new ones the opportunity to settle (Rosenzweig et al., 2002). Kraemer and Golding were one of the few researchers to address the question of adaptive forgetting in animals (Kraemer and Golding, 1997). Their theory relies on the fact that when the animal is subjected to two competing episodes that need a different response but share all other similarities, forgetting of a more recent event (H) is enhanced by the presence of some prior memory (A) (**Figure VI.1 B**). For example, our rats trained in two arms of the radial maze learnt first to choose arm A, followed by reversal training in which they learn to choose arm H. According to Kraemer and Golding, that exposure to conflicting experiences induces ambiguity. An account of this form of PI in the context of adaptive forgetting assumes that as long as the behavioral consequences associated with retrieval of memory "H" are in agreement with its content, the prior validity of "A" is irrelevant. In other words, subjects must discard previous irrelevant episodes and focus on present (ongoing) valid ones. This same theory applies also to us. Robert and Elizabeth Bjork were interested in adaptive forgetting in humans. These authors argued that learning contributes to forgetting. When we learn new information, we create the potential for competition with other related information that already exists in our memory. As a consequence, recalling information from memory requires not only that the information be recalled but also that other information associated to the same cues is forgotten. They thus proposed the "theory of disuse". This theory states that the key to adaptive forgetting is when an information becomes inhibited due to retrieval of competing memories (Bjork and Bjork, 1992). According to the Bjorks, we have a constant burden to keep our memories current, valid "in the now". We need to remember our current phone number, not our prior one; we need to remember where we parked our car today, not yesterday or a week ago. Such updating requires some mechanism to set aside, inhibit or erase information that is out of date and, hence, a source of errors and confusion.

**The role of the DG in adaptive forgetting**

Imagine yourself parking your car everyday in the same parking lot but each time in a different spot. For the first couple of days, you will find it an easy task to retrieve your car. However, many days later, you will be subjected to the frustrating experience of forgetting where you have parked your car most recently. As we have seen before, this forgetting is attributable to interference from memories of former stops at this lot. Previous memories can restrain active memory processing, contributing to short-term memory deficits. Proactive interference is most pronounced when older memories share similarities with the new ones (Wickens et al., 1963). Nonetheless, when two memories are similar, our brain possesses a mechanism allowing it to differentiate one from the other. Pattern separation is this mechanism of processing partially overlapping patterns of neural activation and separating them into discrete representations. This process is necessary for attenuating interference that can take place when different memory representations have resembling components. Many studies have suggested that the DG of the hippocampus facilitates the mechanism of pattern separation. By orthogonalization of various inputs, distinct representations are created, thus facilitating accurate encoding and subsequent retrieval (Gilbert and Kesner, 2006, Kirwan and Stark, 2007). Many studies in humans and rodents have demonstrated that the DG is particularly implicated in the creation of dissimilar representations of resembling or interfering inputs. Restricted lesions to the DG induce impairment when the resemblance between environments is very important (Gilbert and Mack, 1998, Gilbert et al., 2001, Goodrich-Hunsaker et al., 2008, Hunsaker and Kesner, 2008, Hunsaker et al., 2008). The recent (2014) Nobel Prize winners, Edvard and May-Britt Moser, argued that the brain prevents interference between similar memories by forming non-overlapping representations in the hippocampus, a phenomenon known as global remapping of place. Global remapping is defined as changes in firing rates and firing fields of place cells. The Mosers explained that when an animal is subjected to similar behavioral contexts, remapping might happen in order to create independent representations of these resembling stimuli. "*Remapping also serves as a pattern separation mechanism that is likely important for reducing interference between related memories…that might originate in the Dentate Gyrus" (Colgin et al., 2008).* On the other hand, Yassa and Stark argued that pattern separation is typically considered as mediating one-trial rapid learning in a similar context (Yassa and Stark, 2011). Thus, the DG is required to store a pattern and differentiating it from other similar patterns. However, when we have massed presentations of similar contexts and the individual needs to differentiate between large panels of similarities, what would be the best strategy to be adopted by the DG? As suggested by Rosenzweig, Barnes and McNaughton (2001), a substantial number of information will lead to an overlap of stored data and the incapacity of recovering memories correctly. A possible way to prevent this to happen would be an active elimination of old memories during the setting of new ones. In other words, when we have too many similar inputs to deal with, it would be advantageous to overwrite all old memories of events as new ones occur. These arguments agree with our results showing an absence of activation of the DG of the hippocampus in rats that were exposed to the HIWM task. In agreement with studies delineating a role for the DG in pattern separation (Kesner et al., 2004), this result thus suggests for the first time that this area might be inactivated when forgetting/updating of previous information is required. Silencing the DG could reduce its pattern separation function and the processing of overlapping information existing between the sets of neurons

that represent distinct (different trials) but very similar (same pair of arms) spatial information during consecutive WM trials. In fact, in the HIWM task, the goal is not to remember past information and compare them with new ones (pattern separation), but to do exactly the opposite: to focus on an ongoing trial while ignoring and forgetting past ones. One can thus consider that pattern separation is an impediment in resolving interference in such condition. Therefore, shutting down pattern separation function (by inactivating the DG) would be beneficial to process a high level of repeated and very similar information. In such cases, forgetting (of previous trials) is bliss!

Another study coherent with our findings that the DG could be disadvantageous for certain aspects of WM was carried out by Poirier, Amin, and Aggleton (2008). In this study, Zif268 expression in response to training in the radial arm maze was assessed in a spatial discrimination training protocol. Although no overall difference was found between spatially-trained and yoked control rats as regards to the level of hippocampal IEG activation, the authors found a positive correlation between performance errors and Zif268 expression in the DG of the spatial group, suggesting that this area may be involved with error choices. An opposite pattern was found in CA3, where IEG expression correlated with successful performance. Structural equation modeling also revealed a loss of DG efferents and uncoupling of CA3 and CA1 with additional training, whereas yoked controls showed no such pattern. The dynamic changes in activity of a number of brain regions observed in this study suggests that similar changes may also occur over the course of learning in our radial maze. An increase of DG activity could store additional information disrupting HIWM and inducing more errors. Nevertheless, an increase of CA3 activity could be favorable for successful choices.

**Role of neurogenesis in adaptive forgetting**

Once the importance of the DG in separating similar patterns started to increase in the scientific community, neurogenesis' specialists began to wonder about the role of newborn neurons in this phenomenon. Given that the generation of new neurons in adulthood is a particularity of the DG (with the subventricular zone), researchers have predicted an important role of neurogenesis in pattern separation and processing interference. Clelland and colleagues (2009) have tested mice with damaged neurogenesis in a radial maze delayed-non-match to place task. Mice were impaired when the arms presented were very close in space (little separation). However, no impairment was observed when arms were presented at a greater distance. Moreover, computational studies have attributed to newborn neurons the capacity to cope with interference between memories formed in the DG. Deng, Aimone and Gage (2010) argued that new neurons encode new memories, whereas older memories are represented by old granule cells. According to these authors, this could facilitate the formation of new memories while avoiding catastrophic interference. Given that our most important finding occurred in the DG, we suggested a role of newborn neurons in processing interference. This hypothesis emerged from the fact that immature neurons are highly excitable compared to mature neurons (Saxe et al., 2006) and as a result may confer a degree of excitability to the DG. Thus, while mature neurons may not respond to weak stimulation, immature neurons are not under the same type of inhibition and are more likely to be excited. Consequently, when the rat is subjected to similar information (HIWM), it is more likely that

these small changes in the task succeed to stimulate neurons that are already highly excitable (newborn neurons) rather than less excitable ones (mature granule cells). Moreover, a study conducted by Aimone and colleagues (2006) have suggested that similar events that occur close together in time will activate a similar population of new neurons (Aimone et al., 2006). Furthermore, our doubts of an implication of newborn neurons in processing interference come from a study that used a focal x-irradiation procedure resulting in a permanent loss of neurogenesis within the DG of adult mice. Saxe and colleagues (2007) observed in irradiated mice an improvement in WM in a radial maze task due to a more efficient processing of proactive interference (enhancement observed when the same pair of arms was repeated during successive trials). Such results are in agreement with ours. Diminishing neurogenesis and thus DG activity, decreases pattern separation and optimizes the processing of a high level of repeated and very similar information. However, in this thesis, we failed to show a difference in Doublecortin expression after learning our three tasks. This result could not be conclusive about the implication of newborn neurons in processing interference. BrdU administration could give us more information on how proactive interference effects cell survival dependent on the age of the immature neurons being examined. In addition, future studies using optogenetics in order to increase or decrease the activity of newborn neurons will be more accurate in testing this hypothesis and elucidating the involvement of new neurons in processing interference. When neurogenesis is decreased, we expect to see a better performance in HIWM task and a better processing of interference.

**Role of Prefrontal Cortex**

The DG is probably not the only region involved in the processing of interference. Our lesion study showed that the activity of the PFC increased after HIWM training when the DG is absent. Along with the hippocampus, the PFC is another brain area highly implicated in memory and probably forgetting (Benoit and Anderson, 2012). Functional relations between the hippocampus and the PFC have been largely investigated (Eichenbaum, 2000, Frankland and Bontempi, 2005). Shimamura (2000) has argued that the PFC plays a role of a dynamic filter that coordinates internal information by activating or inhibiting the system (Shimamura, 2000). This theory predicts that the PFC possesses the ability to inhibit some inputs when we are subjected to interference. Moreover, our findings lend well to data in humans showing that reduced ability to recruit brain prefrontal regions has explained difficulties to avoid interference (Clapp et al., 2011, Solesio-Jofre et al., 2011, Clapp and Gazzaley, 2012, Solesio-Jofre et al., 2012). In rodents and more particularly in mice, Marighetto and colleagues (2011) have found that a lesion of the prelimbic and infralimbic cortices induced an important disruption of WM performance when proactive interference was in process (Marighetto et al., 2012). This result is congruent with our findings that the PFC could take over the inhibition of the DG in order to process interference. On the other hand, the PFC is also implicated in RM. The observed increase of Zif268 and Arc in the medial prefrontal cortex after RM training is likely to be attributable to increased involvement of this area in long-term memory. Maviel and colleagues (2004) compared the activity of the hippocampus and medial prefrontal areas at recent and remote retention in a test of spatial discrimination memory, using the five-arms maze. Mice were trained to locate a single, baited arm in the maze based on surrounding cues for 10 days, and were tested either one day or 30 days later to assess recent or remote spatial memory respectively. While performance levels across the two sessions were comparable,

Zif268 protein expression increased between recent and remote memory in the medial prefrontal and retrosplenial cortices. Increased c-Fos protein expression was also observed in the medial prefrontal cortex on remote, but not recent memory recall. The hippocampus exhibited an opposite pattern with Zif268 expression decreasing between recent and remote retention, when it was significantly lower than paired controls. Temporary inactivation of the hippocampus confirmed the IEG results, revealing a functional disengagement of this region over time. Infusions of lidocaine into the hippocampus impaired recent memory retrieval, but spared remote memory. Conversely, inactivation of the medial prefrontal cortices did not affect retrieval on day one, but impaired retrieval on day 30. Levels of Zif268 in the medial prefrontal cortex positively correlated with labeling of Growth Associated Protein 43 during remote retention. These results suggest a restructuring of cortical networks involved in remote recall of the RM radial arm maze task and suggest that the PFC is necessary for the recall of remote memories. After 10 days of training, our RM rats had learnt the task. Based on Maviel's results, we should not have observed any PFC activation at the end of training (recent memory condition). Surprisingly however, we found that the hippocampus along with the PFC expressed a high level of Zif268. This result could mean that the information stored in RM is still hippocampo-dependent (recent), but in the same time is becoming consolidated and thus PFC-dependent (remote).

## Outlook

Based on the data gathered in chapters II and IV, we have proposed a summary of our findings that illustrates the role of the DG in our three tasks. This summary is presented in **Figure VI.2**. It was developed by consolidating our new data and is notably hypothetical. However, it suggests the benefits of having an active DG in RM and LIWM and the advantage of its absence of activity in HIWM.

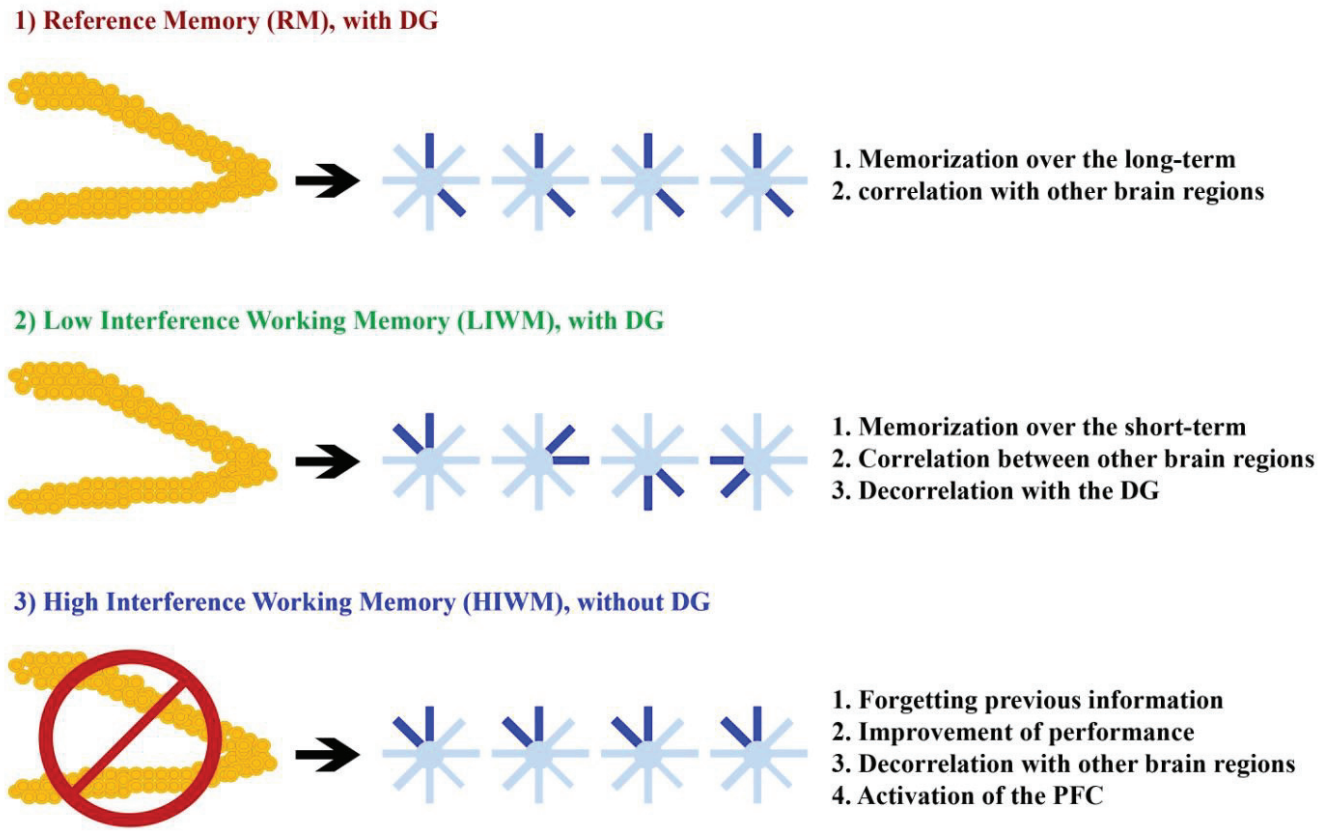


**Figure VI.2: Take home message.** In RM and LIWM, the DG along with a correlation between the hippocampal formation and the PFC are essential for Memory. However, in HIWM, the DG is not required and its absence is beneficial for the forgetting of previously stored information. A decorrelation of the DG with other brain regions is important for the good functioning of working memory.

## Future directions

*"Sleep is the price we pay for plasticity" (Tononi and Cirelli, 2014)*

The experiments presented in this thesis represent only the beginning of a long-term study in our lab aimed to determine the biological bases of adaptive forgetting. Among the many physiological functions controlling such a process, sleep could have an important role. It has been suggested that sleep plays an important role in synaptic homeostasis (Tononi and Cirelli, 2006) and can modulate memory storage accordingly. Our long-term goal is to examine the molecular and electrophysiological changes in the hippocampus as well as in the prefrontal cortex during, but also after acquisition of the memory tasks we described in this manuscript. What happens in the brain of a rat processing the flow of information while retrieving food rewards in a radial maze is as important to what is happening in its brain after completing such task. It has long been shown that after training, sleep can be beneficial to the processing of information into memory. Like memory however, sleep is not a unitary process. In mammals, EEG and EMG recordings differentiate two distinct sleep states. The first state is characterized by a predominance of high amplitude low frequency oscillations and is therefore called slow-wave sleep (SWS). SWS is followed by paradoxical sleep (PS), also called rapid-eye-movement (REM) sleep. PS is a deep sleep state characterized by low-voltage waves of higher frequency, and in that respect strangely similar to waking (hence its name), but with an absence of muscle tonus (Jouvet, 1965). The notion that a good night of sleep improves memory is widely accepted by the general public. Among scientists, however, the idea has been hotly debated for decades (Vertes, 2004, Frank and Benington, 2006). Among the numerous possible roles of sleep, its role in memory consolidation is certainly the most studied (Ellenbogen et al., 2006). On one hand, we know that while sleep deprivation has been suggested to impair memory consolidation, exposure to new environments or cognitive tasks has been reported to alter subsequent sleep variables and next-day improvements in learning tasks. Learning has thus been shown to increase PS duration, number of PS episodes, SWS duration, and localized increases in EEG slow-wave activity in SWS (Benington and Frank, 2003, Ellenbogen et al., 2006, Rasch and Born, 2013). On the other hand, it has been proposed that plastic phenomenon occurs during sleep. For instance, it has been extensively shown by our team and others that sleep, and in particular PS deprivation inhibits the induction or maintenance of LTP *in vivo* and *in vitro* (Benington and Frank, 2003, Ravassard et al., 2009), whereas LTP and neuronal expression of LTP-related genes could be more easily induced during PS (Bramham and Srebro, 1989, Ribeiro et al., 2002, Ribeiro et al., 2007). In contrast, it has been suggested that SWS facilitates the induction of LTD (Muzur, 2005). As we mentioned, SWS is characterized by low frequency oscillations and several works show that low frequency stimulation can induce LTD in various neuronal networks within the hippocampal pathways (Brandon et al., 1995, Alarcon et al., 2004, Etkin et al., 2006). Cirelli and colleagues have also shown that sleep is associated with the upregulation of molecules implicated in LTD (Cirelli et al., 2004). The synaptic homeostasis hypothesis (Tononi and Cirelli, 2006) predicts that plastic processes occurring during wakefulness and resulting in a net increase in synaptic strength would be reduced (synaptic depression or downscaling) during SWS in order to reduce synaptic efficacy to a baseline level that is energetically sustainable and beneficial for learning and memory processes. This is how non-adaptive, "useless" or "non-usable" memory traces would be

eliminated (Giuditta et al., 1995, Muzur, 2005). Given the phenomenological differences between SWS and PS, these two sleep states must have different roles, most notably in learning and memory processes (Rasch and Born, 2013). With the sequential hypothesis of the function of sleep, Giuditta and colleagues hypothesized that these roles would also be inter-dependent (Giuditta et al., 1995). For them, elaboration of memory traces acquired during the waking period is assumed to require two sequential steps taking place during SWS and then during PS. During SWS, the first processing operation would consist in a weakening of non-adaptive memory traces, whereas the remaining traces would be stored again under a better configuration during the ensuing PS episode. In other words, SWS would facilitate forgetting and PS memory consolidation. We further proposed that these two sleep states have a differential role in the antagonistic forms of memory (RM and WM) that we previously described. Therefore, we think that synaptic depression induced during SWS leads to forgetting, thus improving WM abilities, whereas PS (but also SWS oscillations of higher frequency such as hippocampal ripples and cortical spindles) facilitates synaptic potentiation and long-term consolidation of information into RM. To test this hypothesis, our team chronically implanted rats (for EEG/EMG and hippocampal recordings) that were exposed to the three types of training regimen in the radial maze we described earlier (RM, HIWM, and LIWM tasks). We observed a transient increase in PS the day the animal has learned the RM rule, and, in contrast, a positive correlation between SWS amount and the performance of rats trained in the HIWM, but not in the LIWM or RM tasks. Confirming our hypothesis, these results thus suggest that PS contributes to the long-term storage of information whereas SWS would be required for proper treatment of memory interference and therefore forgetting of irrelevant information required for WM (Fraize et al., in preparation). However, we now want to show how such processes occur physiologically. Does PS induce LTP in neuronal networks responsible for the storage of RM? Does SWS induce LTD in synapses involved in the processing of interference? Such questions could be answered by ongoing work in our lab.

## Conclusion

*"There is a goddess of Memory, Mnemosyne; but none of Forgetting. Yet there should be, as they are twin sisters, twin powers, and work on either side of us, disrupting for sovereignty over us and who we are." Richard Holmes, A Meander through Memory and Forgetting.*

By using the behavioral paradigms in this thesis, numerous experiments can be designed to investigate how memory and forgetting operate in more detail, especially as concerns the neuroanatomical organization of the underlying processes. Several aspects of memory and forgetting remain mysterious and fascinating. Given the importance of forgetting of irrelevant information for optimal use of memory in everyday life, it is now crucial to understand the molecular and cellular mechanisms underlying this essential cognitive process. Much work still needs to be done to achieve this goal, but the results presented in this thesis provide new insights in the molecular bases of forgetting by asserting the dentate gyrus as a critical node in this process.

# CHAPTER VII

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