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The cellular, molecular and physiological mechanisms underlying the processing of proactive interference in the rat brain

Nicolas Fraize

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Nicolas FRAIZE

**The cellular, molecular and physiological
mechanisms underlying the processing of
proactive interference in the rat brain.**

Thèse réalisée sous la direction de Gaël Malleret

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Few words of introduction from the “*directeur de thèse*”:

Nicolas Fraize volunteered to spend some time in our team in the summer of 2009 because he was interested in the physiological aspects of learning and memory. He was then a Master1 student in Paris XI University. Even if Lyon was not an obvious work destination for him, he decided to pursue his Master2 training period in our lab in 2010. At this time, and in collaboration with Mickaël Joseph, another Master2 student in our team, Nicolas was involved in determining the neuroanatomical bases of adaptive forgetting, a work that is described in Chapter 1 of this manuscript. Unfortunately, Nicolas did not obtain a PhD fellowship to pursue this work at the time. After a year spent at the University of Pennsylvania in Philadelphia (2010-2011), Nicolas obtained an ARC (*Région Rhône-Alpes*) fellowship in 2011 and decided to come back to Lyon and work with us again. During his thesis, he was interested in determining the molecular bases of adaptive forgetting by studying markers of synaptic plasticity, but also the physiological bases of this type of forgetting by studying the potential role that sleep can have in the processing of interference. This work is described in chapter 2, 3 and 4 of this manuscript. All this work gave Nicolas the opportunity to submit different papers (chapter 1 and 3 are examples of such articles) and get acquainted with different challenging techniques that Nicolas never feared to experience. During this thesis, Nicolas was also involved in other studies that we decided not to include in this manuscript but that have been published in *Hippocampus* (work performed when he was in Philadelphia) and *Cerebral Cortex* (work that Nicolas quickly mentioned in chapter 4 of this manuscript).

We are now eager to share Nicolas work with you and hope you will appreciate reading this manuscript.

Gaël Malleret

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List of abbreviations

ABC: Avidin-Biotin Complex	MEC: Medial Entorhinal Cortex
aCC: anterior Cingulate Cortex	mPFC: medial Prefrontal Cortex
AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate	MPP: Medial Perforant Path
AMPAR: AMPA Receptor	NaCl: Sodium Chloride
ANOVA: Analysis of Variance	NMDA: N-methyl-D-aspartate
AP: Anteroposterior	NMDAR: N-methyl-D-aspartate (NMDA) receptor
Arc: Activity Regulated Cytoskeleton	PB: Phosphate buffer
BSA: Bovine Serum Albumin	PBS: Phosphate buffered saline
C: Control	PBST: Phosphate buffered saline Triton
CA: <i>Cornu Ammonis</i>	P-CaMKII: Phosphorylated Calcium/calmodulin-dependent kinase II
CA1: the hippocampal <i>Cornu Ammonis</i> subregion 1	PFA: Paraformaldehyde
CA2: the hippocampal <i>Cornu Ammonis</i> subregion 2	PFC: Prefrontal Cortex
Ca²⁺: Calcium	PI: Proactive Interference
CA3: the hippocampal <i>Cornu Ammonis</i> subregion 3	PKA: Protein Kinase A
CAMK: Calcium/calmodulin dependent protein kinase	PKC: Protein Kinase C
CaMKII: Calcium/calmodulin-dependent kinase II	PP: Perforant Path
CaMKIV: Calcium/calmodulin-dependent kinase IV	PP2A: Protein Phosphatase type-2a
CRE: Cyclic-AMP Responsive Element	PrL: Prelimbic area
CREB: Cyclic-AMP Response Element Binding	PS: Paradoxical Sleep
DAB: Diaminobenzidine	PSD: Post Synaptic Density
DG: Dentate Gyrus	PTL: Posterior parietal cortex
DTT: Dithiothreitol	PV: Parvalbumin Interneurons
EC: Entorhinal Cortex	RAM: Random Access Memory
ECL: Epirubicine-Cisplatin-5-Fluoro-uracile	REM sleep: Rapid Eye Movement sleep
EEG: Electroencephalogram	RI: Retroactive Interference
Egr: Early growth response	RM: Reference Memory
EMG: Electromyogram	RSP: Retrosplenial cortex
ERK: Extracellular signal-regulated kinase	S1: Primary Somatosensory cortex
GABA: γ -aminobutyric acid	SAFE: Scientific Animal Food and Engineering
GluA1: AMPAR subunit glutamate receptor 1 (also referred to as GluR1)	SDS PAGE: SDS Polyacrylamide gel electrophoresis
H.M.: Henry Gustav Molaison	SDS: Sodium DodecylSulphate
H₂O₂: Hydrogen Peroxide	SEM: Standard Error of the Mean
HIWM: High Interference Working Memory	STM: Short-term Memory
HRP: Horse Radish Peroxidase	SWS: Slow Wave Sleep
Hz: Herz	TBS: Tris Buffered Saline
IEG: Immediate Early Gene	TBST: Tris Buffer Saline Triton
IL: Infralimbic area	VSCC: Voltage-sensitive Calcium Channel
LEC: Lateral Entorhinal Cortex	WM: Working Memory
LFP: Local Field Potential	YHIWM: Yoked High Interference Working Memory
LIWM: Low Interference Working Memory	YLIWM: Yoked Low Interference Working Memory
LPP: Lateral Perforant Path	YRM: Yoked Reference Memory
LTD: Long-term Depression	Zif268: Zinc finger binding protein clone 268
LTM: Long-term Memory	
LTP: Long-term Potentiation	
MAPK: Mitogen-activated Protein Kinase	

French summary

Pendant de nombreuses années, les scientifiques ont étudié les bases neurales de la mémoire. Cependant, une question clé demeure sans réponse: comment le cerveau distingue-t-il les informations suffisamment importantes pour être consolidées en mémoire à long terme des informations requises pour un court instant, et qui doivent être supprimées pour ne pas saturer nos ressources cognitives. Contrairement à l'opinion populaire qui considère l'oubli comme néfaste à notre capacité à se souvenir, l'oubli pourrait être un processus adaptatif essentiel permettant le filtrage des informations non-essentiels. Étonnamment, très peu de choses sont connues sur les bases cellulaires et moléculaires de cet oubli. Le travail présenté dans cette thèse vise à déterminer les bases physiologiques de l'oubli adaptatif, en particulier dans celui en lien à nos capacités de mémoire de travail. Pour ce faire, nous avons adopté une approche comparative en testant des groupes de rats dans trois paradigmes comportementaux faisant appel à un labyrinthe radial et visant à tester trois processus cognitifs différents: 1) la mémoire de référence (MR), 2) la Mémoire de travail (MT) et 3) le traitement des interférences dans la MT. Cependant, nous avons conçu ces tâches comportementales de telle sorte que chaque jour, les rats dans toutes les conditions visitent le même nombre de bras, cette précaution permettant une comparaison claire entre les processus exigeant le stockage à long terme ou à court terme d'informations (en MR ou MT) de ceux nécessitant l'oubli d'informations précédemment stockées en MT. En utilisant cette procédure, nous avons montré que l'information supposée être stockée à court terme en MT pouvait perdurer plus longtemps que nécessaire et interférer, plusieurs jours plus tard, avec le stockage de nouvelles informations. L'oubli des essais précédents est donc nécessaire afin d'éviter de telles interférences. Pour comprendre les bases cellulaires, moléculaires et physiologiques de cet oubli, nous avons couplé cette approche comportementale à trois approches différentes:

1) Nous avons effectué une étude immunohistochimique visant à comprendre dans quelle région du cerveau ce traitement des interférences peut se produire. Dans cette étude, nous avons montré que ce traitement requiert une inactivation du gyrus denté de l'hippocampe dorsal matérialisée par une inhibition de l'expression de marqueurs indirects de l'activité neuronale et la plasticité synaptique, Zif268 et c-Fos (chapitre 1).

2) Nous avons ensuite effectué une analyse en western blot pour identifier les processus moléculaires à la base de cette inhibition du gyrus denté pendant le traitement des interférences proactives. Les résultats de cette étude montrent que, dans l'hippocampe, différents processus de plasticité synaptique pourraient se produire lorsque le traitement des interférences est nécessaire (Chapitre 2).

3) La troisième approche, a visé à comprendre à quel moment ce traitement des interférences se produit. Parce que le sommeil joue un rôle important dans les processus de mémoire, nous avons évalué la modulation du cycle de sommeil suivant nos trois tâches comportementales. Ici, nous avons utilisé une approche corrélacionnelle (chapitre 3) ainsi qu'une approche plus causale impliquant un protocole de privation de sommeil (chapitre 4). Ensemble, ces deux études ont révélé un rôle du sommeil lent (et peut être du sommeil paradoxal) dans les processus d'oubli des interférences proactives.

Pendant cette thèse, nous avons ainsi montré que des phénomènes de plasticité synaptique pouvaient intervenir dans le gyrus denté durant la réalisation d'une tâche impliquant le traitement des interférences. Nous avons aussi montré que cette gestion des interférences pourrait aussi faire intervenir des processus en lien avec les phases de sommeil. Ces travaux nous aident donc 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 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 showed that information supposedly stored in short-term/WM could outlast their purpose and interfere, several days later, with the storage of newer information. In order to avoid such interference, forgetting of previous trials is necessary.

To understand the cellular, molecular and physiological bases of this form of forgetting we used three different approaches:

1) We conducted an immunohistochemical study aimed to understand *where* in the brain this processing of interference may occur. In this study, we showed that such processing requires 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 1).

2) We then conducted a western blot analysis to identify *what* molecular mechanisms were lying behind this inhibition of the dentate gyrus during the processing of proactive interference. The results of this study showed that, within the hippocampus, multiple synaptic plasticity processes could occur when the processing of PI is necessary (chapter 2).

3) The third approach aimed to understand *when* this processing occurs. Because sleep plays an important role in memory processes, we assessed the modulation of sleep patterns following training in our three behavioral tasks. Here, we used a correlational (chapter 3) as well as a more causal approach using sleep deprivation protocols (chapter 4). Altogether, these two studies revealed a role of slow wave sleep (but maybe also REM sleep) in the forgetting processing of proactive interference.

With this thesis, we thus showed that adaptive forgetting may require synaptic plasticity mechanisms in the dentate gyrus, and that such form of forgetting may also depend on different sleep phases. Our work thus 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.

Scientific context

Forgetting

For me, it has always been a pleasure to see long-distance cousins, aunts or uncles during the traditional Christmas family reunion. It is a wonderful occasion to enquire on everyone's lives and whereabouts. This year, I was asked to explain the subject of my work. When I said that I study forgetting, everyone including my four-years old niece, knew exactly what I was talking about. Everyone had examples of things they forget: "my geography lesson" for the young cousin, "the wedding anniversary" for the funny uncle, "where I put my keys" for my grandmother, or even the question he was asked a few seconds ago for my grand dad. Forgetting seems to be a very common affliction but, surprisingly, very little is known about why and how we forget compared to the considerable knowledge accumulated these past decades on why and how we remember. A quick search for "Memory" in Pubmed gives more than 200 000 results while a search for "Forgetting" will only lead you to about 3000 articles. It seems clear that there is still a lot of work to do to understand fully the mechanism by which we forget.

The first question that needs to be asked is: what is forgetting? I will take the simplest and the most general definition of all: forgetting is the inability to recall information. Despite its simplicity, this definition raises a great deal of questions. Has this forgotten information been badly encoded? Has it been erased or replaced by something else? It is somewhere inaccessible to our conscious recollection? I will try to answer these questions in the following chapter by reviewing the main theories that had been formulated on forgetting. I will first present the theories that see forgetting as a default of memory and then I will review the theories suggesting that forgetting is in contrary a virtue.

1) Forgetting as a default of memory

1) Lack of encoding

Obviously, one cannot remember something one never knew. It seems logical to think that an information has to be stored before it can be forgotten. For example, forgetting where you left your keys may simply reflect a failure to pay attention to what you were doing when you set them down. Forming a memory of the location of your keys requires some attentional processes to encode this information. If somehow encoding does not occur, this event will never be memorized and therefore be forgotten forever. Encoding refers to the process allowing a perceived item to be converted into a construct that can be stored within the brain and recalled later from memory. The lack of encoding theory suggests that forgotten events are the one that have never been encoded. However, this theory seems to ignore an important

fact: some events can be encoded, remembered, recalled and then forgotten. For example, some students may learn their lessons overnight, can recall them properly the day after during the exam but once they are done with it, almost immediately forgot these lessons that have to be relearned the next year. Therefore, all instances of forgetting cannot be explained by a simple lack of encoding. Other mechanisms may explain forgetting.

2) Decay theory

It is common knowledge that events recently stored are easier to recall than older ones. For instance, you surely remember what you had for lunch today, probably what you had yesterday but surely not what you had last month. As early as in the 1880's the pioneer experimental psychologist Hermann Ebbinghaus was the first to study how much we forget over time. He realized his experiments on himself by learning series of meaningless three letter combinations (WAH, DUV...) and tried to recall them after different delays varying from 20 minutes to 31 days. He discovered that when the delay between learning and recall increased, the percentage of what he could remember of the original material decreased. Based on this work, Thorndike was the first to lay the foundation of the decay theory of forgetting. For him, forgetting is solely due to the mere passage of time. Thorndike argued that learning is the strengthening of connections during training and that, in contrast, forgetting is simply the dissipation of this strength due to time itself (Thorndike 1914). In other words, if and information is not presented once in a while it will simply fade away.

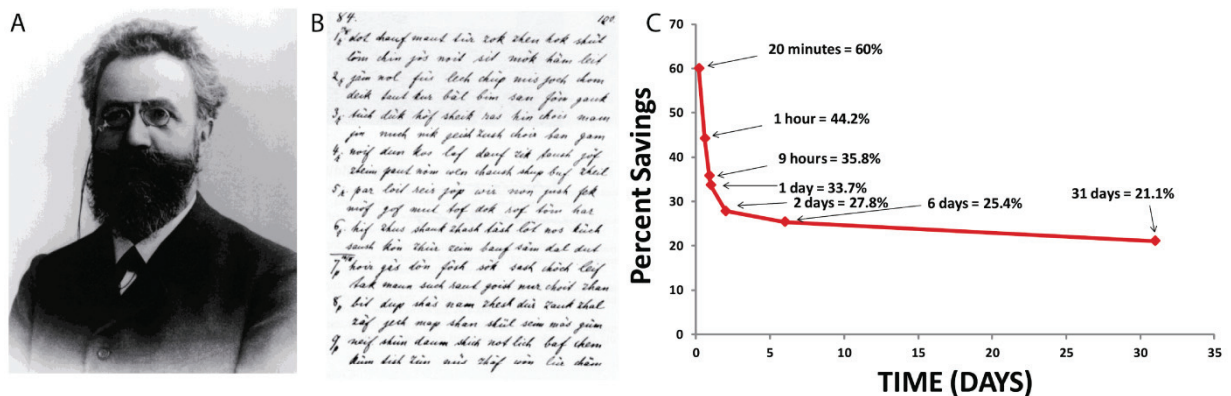


Figure 1: A bit of Ebbinghaus. (A) German psychologist Hermann Ebbinghaus, a pioneer in the experimental study of forgetting. In 1885, he published his book *Über das Gedächtnis* ("On Memory", in which he delineated experiments he carried out on himself to analyze the processes of learning and forgetting. (B) Lists of nonsense syllables that are composed of a vowel-consonant-vowel (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).

The beauty of Thorndike's theory lies in its simplicity. However, it is also one of its major flaws. As Thorndike's theory does not offer any mechanism on how this decay happens, it assumes that all memories will fade away with time at the same rate. It seems quite obvious that memories of certain events are stronger than others regardless of when they occurred. For instance, I can recall exactly what I drank for my 18th birthday (and most of all, the hangover that followed), but it is quite impossible for me to recall what I had for lunch last Tuesday. If time may be part of the answer, this simple example shows that some other processes must exist. Shortly after Thorndike expressed his decay theory, Jenkins and Dalenbach proved that time alone could not be the only cause of forgetting. They demonstrated that forgetting was more important after a period of active wake than after a period of equal time spent resting proving that forgetting was not only due to the amount of time spent between learning and recall but that a different process must be at play during forgetting (Jenkins and Dallenbach 1924). Even if the decay theory that accounts for the fact that some memory can be consolidated, recalled and then forgotten has recently known an interesting revival (see Hardt, Nader and Nadel 2013), this theory still does not account for the fact that forgetting can be transient. Sometimes a memory trace is inaccessible at one time but can be recalled afterwards. For example, I do not remember every single scientific article I read these past few years, but I will recall them as soon as my mentor talks about it. A memory can be stored somewhere in the brain and not be accessible when one needs it.

3) Cue dependent forgetting

According to the lack of encoding and the decay theories, forgetting occurs because a memory trace simply does not exist at the time of recall. This could be either because it has not been formed (lack of encoding) or because it has disappeared over time (decay). Sometimes however, when you try to remember something that was forgotten all you need is a little hint and the entire memory will come back to your mind. Some have thus argued that a memory trace that is forgotten must exist somewhere but that its access is impossible due to a lack of related cues. Godden and Baddeley designed a clever experiment: divers had to learn series of words underwater and then asked to recall them either under water or on land. When the retrieval site matched the learning site (for instance, learning underwater and recall underwater), the recall was more accurate than when the two sites differed. This experiment proved that better recall not only depends on the encoding of the information in itself but that the context of encoding also plays a role on how much we remember (Godden and Baddeley 1975). Endel Tulving thus established the "cue dependent forgetting" theory based on this observation. Tulving divided this kind of forgetting into two subtypes depending on the type of cues present at encoding and necessary for recall. Such cues could be related to the internal state of the subject (happy, tired, under the influence of some drugs, etc), and when inaccessible be the cause of "state dependent forgetting". They could in contrast be related

to external stimuli (childhood neighborhood, odors, sounds, etc) and the cause of “context dependent forgetting” (Tulving and Watkins 1977).

What we have seen so far are examples/theories explaining how forgetting is deleterious to our memory. However, in some cases forgetting may be a useful and active (purposeful) process allowing an optimization of our cognitive resources. Indeed, as Groucho Marx said “I never forget a face, but in your case I’ll be glad to make an exception”. I do not know who Marx was talking about but the intention and the value of forgetting appears clearly here and we shall see in the next section theories delineating such value of forgetting.

II) Forgetting as a virtue

As we have seen in the previous part, forgetting is often seen as a flaw in the natural process of remembering. This probably comes from our natural desire to remember every details of the past. However, from a biological perspective forgetting can be an adaptive process. Unlike storing photos on the hard drive of a computer, creating and storing memories in the brain is a process that requires energy and probably off line processing (during sleep) (as we will see in chapter 3 and 4). Remembering every single moments of our daily life would therefore be terribly costly and would thus be a clear evolutionary disadvantage. In 2013, 25 cases of patients remembering an abnormally vast number of their life experiences were reported. One of these hyperthymestic patients described her constant recollection of events as "non-stop, uncontrollable and totally exhausting" and as "a burden". This pathological aspect of remembering too many information highlights the necessity of forgetting irrelevant facts or events. Forgetting may thus be an adaptive process. To study this adaptive side of forgetting in normal functioning brain, Bjork and colleague designed a “directed forgetting” paradigm. During a learning phase, the patients were thus presented with items that they were subsequently asked to remember (items followed by the mention “to be remembered”) or forget (items followed by the mention “to be forgotten”). During the recall phase, patients were unexpectedly asked to recall both kinds of items. As anticipated, the authors of this study found that the recall of the “to be forgotten” items was significantly lower than the “to be remembered” ones (Bjork 1972). Here, the experimenters have induced forgetting of certain elements by marking them as irrelevant during the encoding phase. This directed forgetting paradigm thus suggests that forgetting may be an active and adaptive process allowing to tag information which may be useless in the future. Of course in everyday life, no one tells us what should be forgotten and this tagging may certainly occur through unconscious processes. In the next paragraphs, we will see some of the theories that explain what these processes may be.

FORGETTING

Lack of Encoding



Encoding refers to the process allowing a perceived item to be converted into a construct that can be stored within the brain and recalled later from memory. The lack of encoding theory suggests that forgotten events are the one that have never been encoded.

Decay



Decay is forgetting due to a gradual loss of the substrate of memory, in his law of disuse, Thorndike posited that unless regularly used, all memory decays, akin to a muscle that will atrophy if not exercised. It is generally assumed that decay is a passive process.

Cue dependant



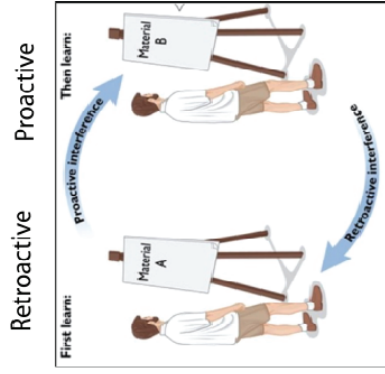
retrieval failure is due to changed or otherwise inadequate retrieval cues or due to specific emotions (repression). on some occasions, we forget simply because the current retrieval cues are insufficient to bring back the desired experience. Repression is defined as the exclusion of unwanted memories.

Retrieval failure



repression

Interference



According to the interference theory, mental activity can impact memory by affecting actual memory content or its retrieval. Acquiring a new memory, or existing memory can proactively impair memory acquisition

Figure 2: A taxonomy of forgetting theories

1) Repression

From a psychological perspective, the most obvious example of what is needed to be forgotten is traumatic events. For instance, if one has been in a car accident, and even if the event was painful, one would probably want to drive again someday. In such a case one might want to suppress the memories of the pain caused by the accident in order to overcome the problem and get behind the wheel again without too much anxiety. The first one to describe this repression phenomenon was the famous Austrian neurologist Sigmund Freud that stated that, in order to keep a sane conscious mind one needs to force his threatening memories into his unconscious mind. He presented the repressed memories as temporarily forgotten but that could be rendered accessible using hypnosis or analyzing dream. This repression theory was, according to Freud "the corner-stone on which the whole structure of psychoanalysis rests", however it has been challenged by many criticisms. Lief and Fetkewicz showed that some repressed memory turned out to be false (Lief and Fetkewicz 1997). Of course, everything we forget is not related to painful or traumatic events and there must be some other types of adaptive forgetting.

2) Interference

As we have seen in the previous parts, there are many theories that have been developed throughout the years to explain forgetting. However, one theory has dominated the field of forgetting since the 20th century: *the interference theory*. It was first postulated by McGeoch who clearly antagonized Thorndike's decay hypothesis. For McGeoch, the mere passage of time alone could not explain forgetting. Forgetting could thus be due to the quantity of stored information interpolated between the occurrence and the recall of an event (McGeoch 1932). In other words, forgetting of a particular memory occurs because other memories come to interfere during its recall. We have already mentioned Jenkins and Dallenbach's study showing that forgetting was more important after a period of active wake than after a period of rest and suggesting that forgetting was due to the interpolated (learning) experience occurring during the period of time spent awake and interfering with the recall of the previously learned information (Jenkins and Dallenbach 1924). In this case, past information were forgotten because of the presence of newly learned information playing the role of retroactive interference. However, the reverse process has also been described: a newly learned information can be forgotten because of the presence of previously learned information playing the role of proactive interference

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 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*).

In laboratory condition, the classical way of studying both proactive and retroactive interference in humans is to use the A-B/A-C paradigm. With this paradigm, subjects are asked to learn pairs of words (A-B) and then another pair containing one word of the first list (A-C). Later, people are given the cue word A and are asked to recall either the first word (B) to which it was associated (the second pair C playing the role of retroactive interference), or in the opposite are asked to recall the last association (C), the first pair (B) working as proactive interference (McGeoch and Irion 1942). In the next sections, I will give examples of tasks that allowed researchers to examine what might influence the intensity of retroactive and proactive interference in humans and then we will see how rodent models allow us to study the biological bases of this processing of interference. Lastly, I will see why forgetting due to proactive interference may be an adaptive process rather than a flaw in the process of remembering.

a) Retroactive interference

Retroactive interference (RI) is what happens when newly learned information interferes with and disturbs the recall of previously learned information. For instance, at the end of the semester, students usually remember better the last lesson than the first few chapters that they learned a couple months before. Each newly learned chapter can thus act as a retroactive interference for the recall of the previous ones.

Robinson (1920) wanted to better understand how interference works. He thus examined the factors that could make interference more disrupting. To do so, he gave to his subjects a list of numbers to remember. Before the recall he gave his patients another list to learn. This new list was either more numbers or something different such as a list of words, photos of just consonants. During the recall of the first list of numbers, he found that patients who had learned a new list of number remember far less the original list than patient who had words or photos as interfering material. He concluded that the more resemblance there is between two learned information the more likely they are to interfere with each other (Robinson1920). Of course, similarity is not the only factor to modulate the degree of RI. One other factor is the amount of time spent between the first learned item and the interfering one. This was shown by Spencer in 1924. Using the A-B/A-C paradigm and modulating the delay between the first (A-B) and the interfering (A-C) learning, Spencer discovered that a shorter delay between the two learning episodes caused more RI, and thus more forgetting of the previously learning material (Spencer 1924).

b) Proactive interference

Proactive interference (PI) occurs when what has been learned previously is interfering with new learning. A real life example of PI that probably most of us have experienced occurs while playing the game of “Hearts” (queen of spades). When playing such a game (or any other card game involving multiple rounds), one has to seek for the queen of spades and modifies his game plan according to whether it has yet been played or not. When the first round is over, the memory of seeing the queen of spades becomes irrelevant and needs to be erased. If not, during the following rounds, this irrelevant memory of having previously seen the queen may be recalled and one might apply the wrong game plan if the queen has not yet been presented during the ongoing round. In this example, memories of previous rounds interfere with the one being played in a proactive manner.

Keppel and Underwood (1962) took a more serious approach and studied the factors susceptible to increase the strength of PI and found out that the amount of previously learned information is a key factor. They asked participants to learn numerous lists of words and ask them to recall the last one. They demonstrated that as the number of previously learned lists increased, the recall of the last one diminished (Keppel and Underwood 1962). In addition, and similar to what occurs with RI, PI is influenced by resemblance and time between learning and recall. Loess and Waugh in 1967 showed that the influence of PI tends to decrease as a function of the length of the inter-trial interval (interval separating two successive learning episodes). When this interval exceeded 2 min, PI was considered negligible (Loess 1967) demonstrating the similar delay effect as seen in RI. Later, Loess asked his subject to learn a list of items belonging to a specific category (trees birds, president of the USA, etc) and then another list of either another or the same category. He found that the retention was maximal when the categories used were different and that mistakes were mostly attributable to prior items of the same category (Loess 1968)

demonstrating that, as for RI, two memories are more likely to interfere if they share common attributes.

The concepts of RI and PI are now well established in humans. However, little is known on the biological substrates of forgetting, and in particular of forgetting related to the processing of proactive interference. To identify such substrates cannot be done in humans and it is therefore necessary to use animal models such as rodents to achieve such aim that is at the center of this thesis.

c) Interference in rodents

Rodents are a very good model for studying the biological substrate of the processing of interference because 1) the neuroanatomy and cellular mechanisms underlying memory processes are quite well known (as we will see in chapter 3) and 2) because they can be tested for both PI and RI in many different kind of experimental paradigms. In the following section, I will focus mainly on studies that have been performed using the radial maze apparatus. This maze, originally designed by Olton and Samuelson (Olton and Samuelson 1976), allows a wide variety of behavioral assessments related to different forms of memory and has been used to study the influence of interference on memory. It consists in eight arms radiating from an octagonal central platform, at the end of which food wells containing or not food rewards are located. One of the great advantages of the radial maze model is that it can be used to test multiple types of memory depending on where the food rewards are placed and on when the animal has to retrieve them. It has been shown that rodents use spatial cues surrounding the maze in order to orientate within the maze and, thus, find the rewards (Olton and Samuelson 1976).

To study RI, Maki and colleagues conducted a radial maze experiment during which rats were allowed during a sample phase to visit four baited arms. After a retention interval, during a choice phase, rats were then asked to recall which arms they visited to visit the remaining four arms to retrieve the four remaining food rewards. This task represents a classical delayed-non-match-to-place task during which rats have to alternate their motion (non-match) in order to be reinforced after a delay. During the retention interval between the sample and choice phases, Maki and colleagues presented the rats with various interfering stimuli (smell, sounds or visit of a different radial maze). The authors found that, regardless of the interfering material, rats were able to retrieve the remaining four rewards without re-entering an arm already visited during the sample phase, suggesting that the rat's memory for spatial locations was immune to retroactive interference (Maki 1979). To explain why these results were at odd with human studies (as described before in the previous paragraph), Roberts argued that in Maki's study the interpolated tasks were too different from the original learning to interfere during the recall. We have thus seen previously that the "to be recalled" and interfering items have to be similar in order to interfere with each other. Roberts thus tried to induce retroactive interference within the same maze. Animals were

forced to enter four randomly selected arms as in the previously described learning experience. During the retention interval, they then were placed (passively) directly at the ends of four arms and allowed to eat a pellet placed there. The arms on which animals were placed were different, randomly determined, or the same to the arms previously entered. Placing the rats directly at the ends of four arms represented the interpolated task to trigger RI. After the retention interval, rats were placed back in the maze to visit the four remaining arms they had not previously visited actively. The outcome of this experiment indicated that RI could be produced only if the interpolated event involves placement in the arms which the rat must choose after the retention interval (Roberts 1981). However, this study did not really settle the debate on the existence of RI in rats. Indeed, because the rats were put back in the maze to retrieve the remaining four rewards during the interpolating task, they might have lacked motivation to visit these arms again after the retention interval.

Contrasting with RI, PI can easily be induced in rodents. To do so, Roberts and Dale used a massed trial protocol in a radial maze. Rats were thus allowed eight runs per trial to visit the eight arms of the maze and collect the eight food rewards located in this maze. This protocol was repeated multiple times a day. In order to get as much rewards as possible, rats had to remember which arms they visited during the ongoing trial in order not to enter (non-match rule) a previously visited (and therefore not baited) arm. Strikingly, Roberts and Dale showed that the rats tended to realize more errors (visiting unbaited arms) as the number of trials increased during the day (Roberts and Dale 1981) suggesting a progressive build-up of interference after multiple trials. During the first trial of the day, the rats thus came with a “clean slate” and no proactive interference disturbed their choice in the maze. Later on, as the number of visits increased, a buildup of interference due to the memories of previous visits was taking place, leading the rats to make more errors. This proactive interference effect was so strong that during the last trials rats could not make spatially based choices but had to switch their foraging strategy to a self-centered one (entering the adjacent arm each time). These results confirmed that if rats may be resistant to RI (at least to some extent), they are not immune and are most sensitive to the presence of PI. Like in humans, the similarity and delay between the learning sessions increases the level of proactive interference. This was shown by the study of Cohen and colleagues that used the same massed trial paradigm that we just described but in which they changed the distal cues on which the rats based their choices between trials. These authors found that such changes led to a diminution of mistakes made on the last trials, a result they interpreted as a diminution of the effect of PI. Similarly, increasing the delay between each trial decreased the power of PI (Cohen, Reid et al. 1994).

d) Proactive interference forgetting as an adaptive process?

The studies we just described suggest that interference in general, and PI in particular, could be responsible for the forgetting of an information acquired during an ongoing trial (wrong choice during a delayed-non-match-to-place task). On the contrary,

forgetting PI acquired during previous trials could be beneficial for the storage of the information related to a given trial. Dudchenko pointed out that in delayed-non-match-to-place tasks the memory of a given trial, supposedly used within seconds or minutes, could be stored longer than necessary and could thus play the role of PI on later trials (Dudchenko 2001). For instance, in the radial maze task presented previously, rats were victims of PI because their memories of earlier trials were accessed at the wrong time. Erasing, and therefore forgetting the memories of the first trials would therefore be an adaptive process to cancel PI. Preventing this forgetting/processing of PI would thus be essential to optimal memory processing, and errors due to PI would thus be seen as a forgetting malfunction more than a memory deficit. Kramer and Golding extended this view and stated that “the real world, in contrast to the laboratory, is inherently unstable, and the longer the time interval between successive contacts with a particular situation, the more likely that the situation will change. Forgetting processes that help map an animal's behavior to the instabilities inherent in a changing world could thus contribute to survival” (Kraemer and Golding 1997). Unfortunately, adaptive forgetting has not received much attention in animal research. The cellular and molecular mechanisms at stake during such process have thus been poorly studied and much work is still required to understand this crucial cognitive process. The main aim of this thesis is to identify such mechanisms.

In this chapter we have discussed the many theories of forgetting and emphasized the role of proactive interference as an adaptive process. Nevertheless, to understand how forgetting works, it is important to understand its opposite function: Memory. The next chapter will now review some important aspects concerning learning and memory.

Memory

In the previous chapter, dedicated to forgetting, we have seen the “dark side” of the memory process, the one that is considered as a failure of the brain to remember what happened. Even if we have seen that forgetting may be useful, remembering is generally considered most reassuring. Being able to remember what happened in the past allows us to predict what may happen in the future. This is why the ability to retain and utilize the knowledge from the past is a fundamental feature of animal behavior and evolution. In this chapter, I will review the different forms that memory can take and then I will explain the main theories explaining how memories are formed and stored safely in the brain.

I) Declarative Memory versus Procedural Memory

In 1953, the famous patient Henry Molaison (mostly known by his initials HM) underwent a bilateral resection of the medial temporal lobe including a significant brain area, the hippocampus. After the operation his physician, William Scoville, quickly realized that HM was suffering from severe anterograde amnesia: he was unable to form new memories (Scoville 1954; Scoville and Milner 1957; Corkin 1984). Despite this anterograde amnesia, HM had perfectly fine motor skills and was even able to learn new skills that he had never practiced before the surgery. For instance, HM was able to perform very well in a mirror drawing task even if he had no recollection of the multiple training sessions it took him to master that skill. This revolutionary case was one of the first observations that region-specific lesions can affect one specific type of memory without affecting others. This led the authors to propose the distinction between two type of memory, 1) *procedural memory*, the memory for skills and habits that can be acquired without conscious awareness, and 2) *declarative memory* that refers to memories that can be consciously recalled such as those related to facts and events (Cohen and Squire 1980).

The observation of HM memory impairments due to its cerebral lesion initiated the generation of the first anatomical organization of the memory systems. HM unaltered procedural memory led to the notion that procedural memory is now thought to be independent of hippocampal functions integrity (Squire and Zola 1996). On the other hand, declarative memory was described as dependent on the hippocampus as it was disrupted after HM’s surgery. Declarative memory was then subdivided into two sub-memory systems. Tulving thus made a distinction between the knowledge one has about the world (ie: “who is the president of the USA?”) that he called *semantic memory*, the memory for facts and concepts that can be acquired throughout different life experiences, and *episodic memory* related to an isolated event in time (and space) relevant to one’s personal life (episodic memory has often been confounded with *autobiographical memory*) (Tulving 1972).

Based on the same principle, other distinctions were proposed by different authors (Schacter 1987). Within declarative memory, a distinction was thus made based on how long an information is held in memory. Is this bit of information useful only for a short time? Or will this information be relevant multiple times in the future and therefore required to be stored more permanently in memory? For example, when meeting someone during a professional reunion, an information such as the phone number of this person has to be held in memory possibly for few seconds or minutes, only until it is saved on your phone. The name and position of this person however are bits of information that are likely to be useful in the future and are probably important to be stored safely for a while. In the next section, we will review literature concerning this distinction between short-term and long-term memory.

II) Short-term Memory versus Long-term Memory

In 1890, William James was the first to establish a theoretical distinction between 1) primary memory that he defined as the current state of mind, and 2) secondary memory that constitutes the knowledge of previous states of mind. The evidence for such distinction reemerged with the study of HM. After his ablation of the medial temporal lobe, HM was able to retain bits of information for a short time but forgot them as soon as he was distracted and therefore was unable to form new long lasting memory (Milner, Squire et al. 1998). This study showed that *short-term memory* could exist without leading to *long-term memories* and therefore pointed to the conclusion that short-term memory and long-term memory are distinct processes that can be studied separately. In the following section, we will examine the differences between these two forms of memory. For both types of memory, we will first see how human studies have permitted the establishment of these concepts of short-term and long-term memories, and then we will see how animal models allowed the study of the biological bases of these two forms of memory.

1) Short-term /working memory

In humans, it is possible to assess specifically short-term memory using very simple behavioral tasks such as the memory span task. With this task, subjects are presented an increasing series of numbers and have to recall them immediately following presentation. Using this paradigm, George Miller found in 1956 that the number of items a human subject can actually recalled in this task is quite constant and corresponds to approximately seven items (Miller 1956). However, this view of short-term memory as just a temporary storage location for memories is quite reductive. Indeed, it has been shown that processing the information that needs to be store on the short-term is possible. For example, Ericsson (REF?) has shown that grouping items together (in chunks) increases the short-term memory span. Short-term memory thus represents not just the storage of information meant to fade

away, but an actual work-space that allows a processing of this information. To explain this point of view, Miller compared short-term memory to a computer's random access memory (RAM), a very volatile artificial memory that is erased as soon as the computer shuts down (Miller 1986).

Following the same idea, Allan Baddeley defined what he called "*working memory*" (WM), a "limited capacity system allowing the temporary storage and *manipulation* of information necessary for such complex tasks as comprehension learning and reasoning" (Baddeley 1981). Baddeley designed a theoretical model to explain how WM may be functioning. His multicomponent model of WM is composed of three distinct parts, a central executive controlling two slave sub-systems, the phonological loop and the visuospatial sketchpad. 1) The role of the "*central executive*" is to focus on relevant information and to suppress whatever is useless and could interfere with the storage of the information. For example, when dialing a phone number, the digits are important to store in WM regardless of what material they were written on. The role of the central executive system is to extract whatever is important and to pass it on to one of the two other slave components. 2) The "*phonological loop*" role is to store phonological information (hence its name) such as vocal sounds and to prevent the decay of such information by rehearsing vocally its content. Following up with our phone number example, one can remember it for quite a while by continuously repeating it to oneself before dialing it. The other slave component is 3) the "*visuospatial sketchpad*". The visuospatial sketchpad is used to create virtual images in WM so that they can be manipulated mentally. (Baddeley 2000).

Animal models have greatly helped to determine the biological bases of WM. In the chapter dedicated to forgetting, we have mentioned the work of Olton and Samuelson who designed an eight-arm radial maze paradigm during which rats were allowed to complete eight runs per trial to retrieve eight food rewards placed at the end of each arm of the maze. These authors showed that rats were choosing an average of more than 7 different arms within the first 8 choices (Olton and Samuelson 1976). To perform so efficiently in their food-seeking behavior, the rat had to memorize for a limited time which arm has already been visited in order to avoid them during their search for other food rewards. However, once the eight rewards were found, these memories were irrelevant and faded away in order not to produce proactive interference that are detrimental to further storage of memory as we have seen in the first chapter. Based on this work, Olton proposed a model in which a certain kind of memory 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". This short-term memory system that allows flexible learning looks very much like Baddeley's WM model seen in the previous paragraph. This fact led Olton to conclude that the form of memory tested in his maze with rodents was WM.

long term memory

Declarative
(explicit)

Non declarative
(implicit)

Semantic
(facts)



Semantic memory is oriented to the present and represents general context-free fact.

Episodic
(events)



Episodic memory is a past oriented memory system, allowing mental time-travel through autotonic

procedural
(events)



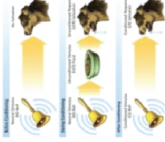
Procedural memory stands for mechanical or motor related learning

Priming



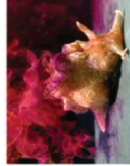
Priming means a higher probability of recognizing previously perceived information.

classical
conditioning



Classical conditioning is an associative learning in which a conditional stimulus (CS) is associated with and unconditional stimulus and a constellation of conditioned responses comes to be elicited by the CS

Non associative
learning



Non associative learning is a relatively permanent change in the strength of response to a single stimulus due to repeated exposure to that stimulus

Figure 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).

In laboratory conditions, WM is studied using the natural tendency that rodents have not to visit the same place twice, a behavior first described by Tolman in 1925 and called *spontaneous alternation*. One of the most commonly used task to study WM is the delayed-non-match-to-place paradigm we described earlier. In this task, the animal is first forced to visit one of two arms of a T or Y-shaped maze. After a short delay, the animal is placed back in the same maze and must visit the arm that has not been visited to get the food reward (Tolman 1925). The optimal food seeking strategy is to use WM to remember which arm was visited in the presentation (sample) phase in order to correctly choose the second arm in the choice phase. Such delayed alternation task have been widely used to study WM, and one of the main finding is that a lesion of the hippocampal region induces a decrease in performance in these tasks showing that WM is dependent on the hippocampus integrity (Dudchenko 2004).

WM is a very effective short term memory system but it loses its efficiency after a few minutes as shown in a study by Dudchenko in 2001. Increasing the delay between the sample and choice phases was thus found to greatly damage the rat WM abilities so that after a delay superior to 10 minutes the rat seems to choose randomly (Dudchenko 2001). If the information stored in WM is somehow considered relevant or required to be used further in time, the WM system is thus no longer effective and a long-term memory system has to take the lead.

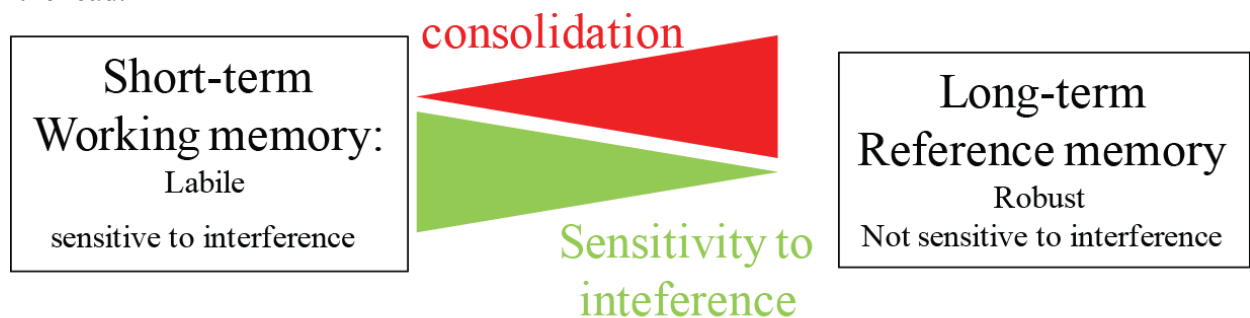


Figure 5: Memory temporal dimension. Dissociation between short-term and long-term memory is widely accepted. Researchers who work with rats distinguished between Working memory and Reference memory. Working memory is sensitive to prior knowledge interfering with the learning and recall of new incoming information, whereas Reference memory is thought to become more resistant to this proactive interference as the consolidation process advances

2) Long-term/reference memory

It takes no time and effort to remember significant episodes of our lives. We will probably always remember our wedding day or the terrible car accident we were involved in few years ago. But, fortunately and unfortunately, our lives are rarely made of these strong emotional events. That is why storing long-term memories such as a lesson or a speech usually takes time and effort (rehearsal). In the chapter dedicated to forgetting, we mentioned the work of Ebbinghaus who learned list of syllables and tried to recall them. He showed that the percentage of information he could actually recall decreased over time. Still, after 31 days, he was able to recall more that 20% of the original material. He had stored this

information in the long-term and was unlikely to ever forget it, suggesting that a long-term memory storage system must exist. Ebbinghaus also showed that the more he repeated the information to be learned, the better he could recall this information even after long periods of time. Recalling was more accurate when he rehearsed the original material multiple times until over-learning it. Later, Dempster (1988) showed that recall was enhanced when the multiple training sessions were separated (*spaced*) in time and over a long period rather than concentrated in a brief period (Dempster 1988). Some argued that this process, known as the “*spacing effect*”, is an adaptive process allowing an information appearing as a recurrent theme, and more likely to be relevant and useful in the future, to be preferentially stored in memory as compared to a onetime event. Indeed, it is more important to remember the building administrator that you see just once a day for a few seconds than the guy who was seated next to you during a 10-hour flight, but that you will probably never see again (Anderson and Schooler 1991). In 1975, Collins and Loftus proposed the term “semantic network” to define the long-term memory storage system. This term means that the information in long-term memory is stored as clusters of sensory information of the same categories (smells, sounds, etc) linked together by semantic relationship (Collins and Loftus 1975). Many studies have tried to find the locations of these “engrams” and to understand the biological mechanisms involved in this storage.

In animals, the equivalent of long-term memory is called reference memory (RM). RM refers to the long-term storage of information that remains constant over time and is gradually acquired over many trials presenting similar information. As opposed to WM, RM requires several training sessions but once stored, RM is believed to be stable and resistant to interference (Santini, Aguirre et al. 2003). In the radial maze apparatus, when only a constant subset of arms are baited during multiple sessions of learning, rats can learn to differentiate the always baited arms from the never unbaited ones by associating the location of the rewards to a location in the room. Olton tested this RM by using a 17-arms radial maze. He baited only 8 arms and allowed the rats to make eight runs a day for 10 days to retrieve the 8 food rewards (Olton and Samuelson 1976). The optimal strategy for the rat was thus to form a cognitive map of the maze and its surroundings and adding to it which arms were baited. This map stored in RM could be stored and used with virtually no limitation in time. Using the same task, Olton and papas were also able to assess both RM and WM by judging the nature of the errors made by the animal. If a rat went into an arm that has been visited previously in the same trial (and that was originally baited), it was considered as a WM error. On the opposite, going into an arm that was never baited was considered a RM error. Lesions studies permitted to make the distinction between these two forms of memory. A hippocampal lesion performed after initial RM training increased the number of WM errors but did not affect RM (Olton 1983). These results led to the conclusion that the hippocampus may be required for the learning of the association between the reward and the location but not for the subsequent utilization of this association stored in RM. In the next paragraphs, we

will see how these representations can be formed and which brain regions are involved in these processes.

III) Theories of Memory consolidation

Consolidation refers to the process by which labile memories initially stored in WM are transformed into stable memories that can be stored in long-term/RM. To explain how this process may work, two main theories are battling each other: the *standard theory of consolidation* and the *multiple trace theory*.

1) The Standard theory of consolidation

The standard theory of consolidation (McClelland, McNaughton et al. 1995; Squire and Alvarez 1995; Bontempi, Laurent-Demir et al. 1999) is based on the work of the theorist David Marr (Marr 1970; Marr 1971). It states that the hippocampus only provides a temporary storage of the information while allowing its transformation into a more robust neocortical-dependent form. According to this theory, a memory would be initially retained in a hippocampal-neocortical network for up to one week after initial learning, representing the hippocampus-dependent stage of the consolidation process. Over time, the hippocampus would allow the transfer and integration of this memory into a pure neocortical network. It has been proposed that the hippocampal-cortical network's successive reactivations of this memory (possibly during sleep) could strengthen cortico-cortical connections to form the long-term trace of this memory.

As time passes, this memory trace would thus gradually merge into existing cortical networks and eventually become independent of the hippocampus. This theory was confirmed by different functional brain imaging studies conducted in rodents and monitoring the expression of immediate early genes used as indirect markers of neural activity (Bontempi, Laurent-Demir et al. 1999; Frankland, Bontempi et al. 2004). Maviel and colleagues have thus trained rats in a radial maze RM task and tested their animals for recall either 24 hours or 30 days after the acquisition of the task. They showed that when tested at 24 hrs, recall induced activation of the hippocampus but not of the prefrontal cortex. In contrast, 30 days later, recall no longer induced activation of the hippocampus but of the prefrontal cortex (Maviel, Durkin et al. 2004). A recent study aimed to track the fate of individual neurons after contextual fear conditioning. After a conditioning phase, consisting in pairing a specific context with an aversive stimulus like an electric foot shock, a fear response is generated and measured behaviorally by a freezing response (total immobility of the animal apart from breathing motion). This freezing responses can be measured either immediately after the conditioning or after a retention period by putting the animal back in the conditioning context. In this study, Tayler and Tanaka used a fluorescent tag to mark the neurons that are activated during the encoding. Two days or two weeks later, they examined

the neurons involved during memory retrieval using a different tag. They found a large network of double-tagged neurons (involved in both encoding and recall) in the hippocampus and neocortex that were reactivated 2 days after learning. This result suggests that memory retrieval involves reactivation of individual neurons that were engaged during learning. However, two weeks after learning, the pattern of reactivation was altered in the hippocampus but remained largely unchanged in the cortex. These findings suggest that, over time, only the cortical memory trace persists while the hippocampus disengages (Tayler, Tanaka et al. 2013).

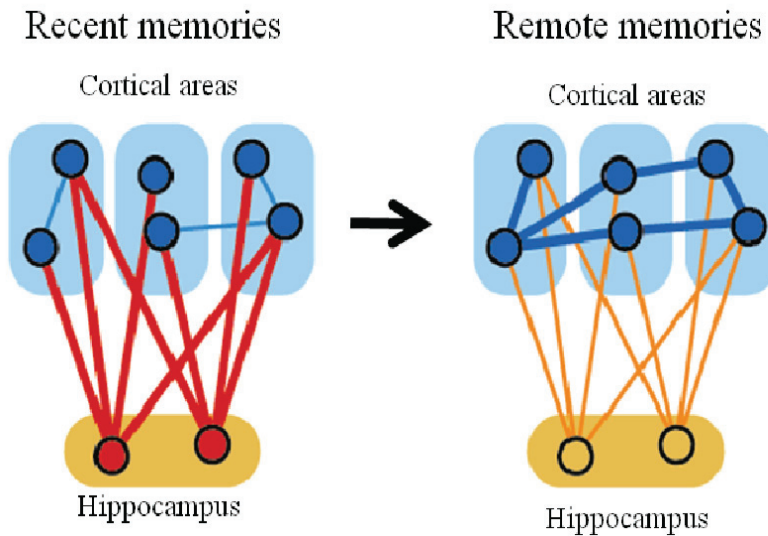


Figure 6: Standard theory of consolidation model: The encoding of information initially occurs in the specialized and related cortical areas. The information distributed in these modules is then integrated by the hippocampus to rapidly form a coherent memory trace. The successive reactivations of hippocampal - cortical connections lead to the gradual strengthening of cortico-cortical connections. Recent memories thus become independent of the hippocampus by being gradually integrated in the neocortex.

2) Multiple traces theory

On the contrary, the multiple traces theory suggests that the hippocampus is always required regardless of when the event to memorize occurred. It is based on the observation of patients with lesions of the medial temporal lobe that displayed, in some cases, a total retrograde amnesia (i.e. affecting even distant childhood memories supposedly acquired long before the time of the lesion) restricted to episodic or complex spatial memories. In addition, functional imaging studies in humans have shown that the recall of remote detailed episodic memories activates the hippocampus, suggesting that the hippocampus continues to store certain aspects of episodic memory in the long-term (Nadel and Moscovitch 1997). In order to integrate these observations, Nadel and Moscovitch proposed that the hippocampus would code only the spatio-temporal context of an event to remember. According to this theory, the reactivation of the hippocampal-cortical network would not lead to a complete disengagement of the hippocampus but in the generation of multiple interconnected traces in the hippocampus and neocortex. Retrieval of detailed episodic memories, whether remote or recent, would thus still depend on the hippocampus. In contrast, semantic (de-contextualized) memories would be stored at the cortical level and recall of these memories would not require the hippocampus.

There is a quite large number of studies that support both standard and multiple trace theories and the debate on the exact role of the hippocampus in the recall of remote memories is still open. I do not pretend to review them all in this manuscript as it is not the main topic of my work. Even if the concepts are still subject to debate, in the next chapter I will take a more “down to earth” approach and review the structural and cellular mechanisms that are playing a role in memory and/or forgetting.

Cellular and molecular mechanisms underlying memory and forgetting

In this chapter, I will focus on the biological mechanisms underlying memory and forgetting. I will first discuss the brain regions involved in these processes and then I will explain the molecular mechanisms necessary to form or erase memories. As the molecular mechanisms that I will describe in the second part are mainly studied in rodents, the anatomical consideration made in the first part will focus mainly on this animal model.

I) Structures

Among the many structures involved in memory and forgetting, two of them play a central role, the *hippocampus* and the *prefrontal cortex*. Here, I will focus on these two structures but it is important to keep in mind that there are other structures taking part in the memory processes such as the amygdala that plays an important role in emotional memory for instance.

1) Hippocampal formation

The hippocampal formation is a quite large structure located in the middle of each brain hemisphere that is known to play a key role in memory processes. I will now briefly describe its anatomy and connections before discussing its role in memory formation and forgetting.

a) Neuroanatomy

The hippocampal formation is a banana-shaped structure that extends from the septal nuclei and goes backward over and behind the thalamus to reach the temporal lobe in both hemispheres. The hippocampal formation in itself is composed of the hippocampus proper, in which we find the dentate gyrus and the Ammon's horns (CA1 and CA3 subfields), and the subiculum. It is important to note that some cortices are closely associated with the hippocampal formation, such as the entorhinal cortex (EC), the perirhinal cortex and the parahippocampal cortices. With the hippocampal formation, these cortices thus form a whole structure called the hippocampal complex (Amaral and Witter 1989). The *Ammon's horns* are layered structures composed mainly of pyramidal neurons. Based on the size and connection of these pyramidal cells, Ammon's horns are usually divided into three subfields: CA1, CA2 and CA3. The *Dentate gyrus* (DG) is a V-shaped structure that embeds the end of CA3. The DG is composed mainly of granules cells and interneurons. The life lasting capacity of the DG to generate new neurons from stem cells and integrate them into the mature nervous

system makes it a unique region in the brain. Along the rostro-caudal axis, the hippocampus can be divided into *dorsal hippocampus* and *ventral hippocampus* based on the different anatomical connections that we will discuss later.

b) Connections

Within the hippocampus the flow of information is mainly unidirectional. The first structure to receive inputs is the DG that receives projections from the lateral EC (LEC). This forms the first synapse of the trisynaptic pathway. The second synapse is located in the CA3 region where the projections from the granule cells of the DG meet the pyramidal cells of CA3 via the mossy fibers. The flow of information then goes from CA3 to CA1 via the Shaffer collaterals to form the third and last synapse of the “trisynaptic pathway” (Kelso, Ganong et al. 1986). These three synapses are all glutamatergic excitatory connections.

In term of extrinsic connections, the dorsal parts of CA1 mostly send projections to the cortices forming the hippocampal complex (perirhinal, retrosplenial, MEC and LEC), while the ventral hippocampus mainly sends projections toward the prefrontal cortex (prelimbic and infralimbic (Jay, Glowinski et al. 1989; Conde, Maire-Lepoivre et al. 1995). The DG receives its major inputs from the LEC via the Perforant path and some minor inputs from hypothalamic regions such as the supramammillary nucleus. On the opposite, the DG sends almost no connections outside of the hippocampus.

c) Role in memory and forgetting

The role of the hippocampus in cognitive processes has been first discovered after accidental lesions such as the one occurred by the famous patient HM who had both temporal lobes (including the hippocampus) removed and who showed no possibility to create new memories afterwards (Scoville and Milner 1957). However, as we have seen in the previous section, the hippocampus is a complex assembly of multiple structures, each one with different internal anatomy and specific connections. In this section, we will discuss the possible role of the ventral and dorsal parts of the hippocampus, but also the role of each sub-structure within the hippocampus in memory and forgetting.

Along the longitudinal axis, the hippocampus serves different functions. During a pre-doctoral training period at University of Pennsylvania, we used a predator odor contextual fear conditioning paradigm (here, the aversive stimulus used was a predator odor instead of an electrical foot shock) in combination with lesion restricted either to the dorsal or ventral part of the hippocampus. We showed that both lesions abolished the freezing response when animals were tested 24h hours after conditioning. However, during the presentation of the stimulus, the behavior of the two lesioned groups was different. Only the animals with ventral hippocampal lesions did not seem to express fear of the predator odor. This result, in accordance with previous studies showing that the ventral hippocampus is specifically connected to the amygdala suggest that, while the ventral hippocampus could be

involved in the processing of emotional information, the dorsal hippocampus would be specifically implicated in the storage of contextual information (Wang, Fraize et al. 2013).

Encoding contextual information requires the processing of spatial information related to a specific context. Studying the pyramidal neurons of CA1, O'keefe and Nadel observed that these neurons code for spatial characteristics of the environment. These so called "place cells" were defined as neurons that fire the most when the animal is in a restricted region of space (also called place field) (O'Keefe 1979). This specificity of the CA1 pyramidal neurons suggests that this subregion of the hippocampus may serve to encode the surrounding environment. CA1 is also the primary output from the hippocampus to the neocortex. Therefore, it is not surprising that a lesion to this area would cut off the output from the rest of the hippocampus. Taken together, these observations could explain why in a study by Dilon and colleague lesions restricted to the CA1 area impaired the performance of mice in a task that requires encoding and retrieval of spatial information such as a Y-maze spontaneous-alternation task (Dillon, Qu et al. 2008).

Unlike CA1, the CA3 subregion does not seem to be critical for the acquisition of spatial tasks (Brun, Ytterbo et al. 2001; Okada and Okaichi 2009). However, using a delayed-match-to-place paradigm, in which some cues present during the sample phase were removed during the retrieval phase, Gold and Kesner showed that CA3 may support the process by which a complete memory can be retrieved from only partial or degraded cues represented in this memory called "*pattern completion*" (Gold and Kesner 2005).

In contrast to CA3, Okada and Okaichi showed that a selective lesion of the DG lead to an impairment of long-term memory equivalent to the one seen after lesion of the entire hippocampus (Okada and Okaichi 2009). Given that the DG receives and processes the first projections from the EC to the hippocampus, this result did not come as a great surprise. However, the key and specific role of the DG is its function of "pattern separation". Pattern separation represents the ability to separate or orthogonalize similar events (and/or places), such as life episodes that we experience during our daily lives and that share similarities although they refer to different time (and/or space) bouts. Pattern separation is important to be capable to carefully differentiate such similar events and places (Schmidt, Marrone et al. 2012).

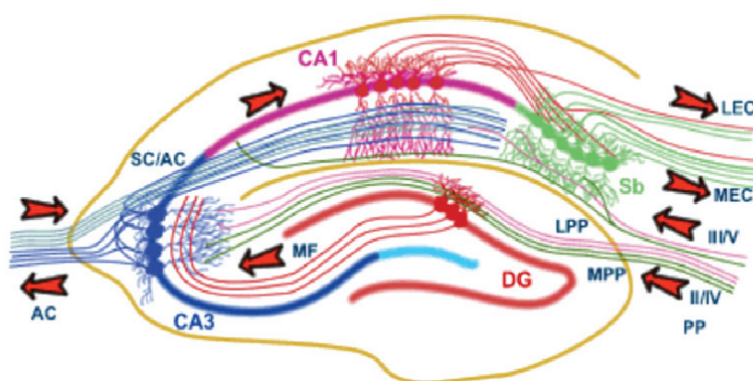


Figure 7 The hippocampal network. The hippocampus forms a mainly unidirectional network. The afferences from the Entorhinal cortex (EC) form synapses with the dentate gyrus (DG) via the perforant path (PP). The axons from the DG form synapses with CA3 neurons which project toward the CA1 area via the Schaffer collaterals (SC). CA1 sends projection back to the entorhinal cortex thus forming an activation loop (Sharma, Reams et al. 2007).

As we have seen in the anatomy part of this section, the DG has the ability to generate new neurons in adulthood. These new neurons are highly excitable compared to mature neurons (Saxe et al., 2006). While mature neurons may not respond to weak stimulation, immature neurons are more likely to be excited. That is why these new neurons have been proposed to be crucial for the encoding of new memories as they are the first neurons to respond to a new stimulus. On the contrary, old memories would be supported by old granule cells (Aimone and gage 2010). 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). Altogether, these results suggest that neurogenesis could be involved in the processing of PI (newly encoded information similar to one another). Exploring this idea, Saxe and colleagues (Saxe, Malleret et al. 2007) observed that suppressing DG neurogenesis caused not an impairment but an improvement of performance in a WM task when the animal has to process PI (i.e. same information presented twice on the same day). Surprisingly, these results suggest that suppressing neurogenesis reduces the impact of interference on WM performance. As similar events tend to be encoded by similar population of neurons, the same population of newly formed neurons would tend to encode similar trials when facing repeated information (PI). Activating new neurons would lead to the activation of overlapping and thus conflicting representations. Shutting down neurogenesis, and thus pattern separation function, would reduce the amount of overlap between the sets of neurons that represent similar spatial information during distinct trials and would therefore be beneficial for the processing of PI.

In line with the trace decay theory seen in chapter 1, some authors have also suggested a role for neurogenesis in the decay, and thus forgetting of memory over time. According to Frankland and Kohler, the newly formed neurons may be incorporated randomly into existing consolidated networks that represent remote memories. For these authors, this incorporation of new neurons could disrupt these consolidated networks. As time passes, more and more neurons are incorporated in these networks until the old consolidated memory disappears completely (Frankland, Kohler et al. 2013). In conclusion, while the DG and adult neurogenesis may be involved in the rapid encoding of new memories, they may become a burden when repeated overlapping and conflicting information need not to be process, but in the opposite deleted from memory.

2) Prefrontal cortex or PFC

a) Neuronanatomy and connections

The PFC was first defined as the area of the frontal cortex that receives projections from the mediodorsal thalamus (Rose and Woolsey 1948). In rodents, it can be subdivided into two major regions. The ventral region includes the prelimbic (Prl) and infralimbic (IL) cortices while the most dorsal region corresponds to the anterior cingulate cortex (aCC).

There are lots of intrinsic connections within the PFC. The IL is reciprocally connected to the PrL which is itself connected to aCC.

In terms of extrinsic connections, the PFC is strongly and reciprocally connected to the EC. The fact that this connection goes in both directions places the PFC in a key position to dialogue with the hippocampus. The PFC also receives direct connections from the ventral hippocampus, whereas the dorsal hippocampus sends only few projections towards the PFC and does it preferentially *via* the thalamus.

b) Role in memory and forgetting

The study of the patient HM by Milner (Milner, Squire et al. 1998)(REF 1998) who showed no WM deficits after ablation of his hippocampi misled researchers to think that the hippocampus played a limited role in this form of memory. According to Milner, the PFC (Milner 1998) was the key structure to process WM. However, more recent studies tend to re-evaluate that role (Gisquet-Verrier and Delatour 2006). Indeed, numerous tasks aimed at assessing WM can be resolved by using proactive motor coding, a strategy known to involve the PFC. For instance, in a delayed-non-match-to-place paradigm in a radial maze, rats being released from the same starting point before and after the retention interval can anticipate (proactively) their motor response after the delay by preparing (coding) their response during the retention interval. Along with this hypothesis and using a similar task, D'esposito suggested that the ventral part of the PFC is not involved in the temporary on-line storage but rather in the control of information required to prospectively organize the ongoing action (D'Esposito 2007). These results suggest that the PFC would be involved more in the short-term storage of a motor action rather than a fleeting experience in memory. To study the relationship between the hippocampus and the PFC during a WM task, Lee and Kesner used temporally restricted inactivation of either the hippocampus, the PFC or both structures during a delayed-non-match-to-place task. They have shown that, while a double inactivation provoked a deficit in this task whatever the delay used, 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. Lee and Kesner have thus proposed that the PFC and hippocampus work together to store information but that the time window of the type memory used is a key factor in dissociating multiple memory systems (Lee and Kesner 2003). When the retention delay is short, the hippocampus and PFC may be processing the information together, however when the delay increases the PFC is no longer essential.

As we said earlier, WM represents the ability to temporarily store and manipulate information. The PFC would have a preferential role in this manipulation rather than in the storage of the information. Human studies thus reveals that the PFC would be useful when there is a competition to resolve between two information to retrieve as it is the case in tasks

involving PI. Shimamura studied patients suffering from accidental PFC lesions in an A-B/A-C learning paradigm that we described earlier. He has shown that these patients learned as well as controls the A-B pairs but lesioned patients made more errors in subsequently learning the A-C interfering association (Shimamura 1995). Furthermore, patients with PFC lesions exhibited an increased susceptibility for PI. In other words, patients with damaged PFC had a hard time forgetting previously learned irrelevant information (Moscovitch 1992).

We just reviewed the role of two major brain regions involved in memory and forgetting processes. In the next session, we will take one more step down and envisage the molecular mechanisms at play in these structures that are essential for memory and forgetting.

II) Cellular mechanisms

In 1949, Donald Hebb proposed a model that explains how memory can be stored at the cellular level. Hebb proposed that information could be stored by cell assemblies, groups of neurons that code for a specific stimulus and that forms an assembly of cells more connected to each other than to other neurons (Hebb, 1949). According to him, the formation of such assemblies could rely on a more efficient synaptic transmission between distant cells forming these assemblies. The Hebbian theory proposes that “When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased”. In 1973, Bliss and Lomo were the first to demonstrate experimentally the existence of such phenomenon in the brain, and they did so in the hippocampus. They showed that high frequency stimulation (100 Hz, 3-4 sec) of the axons from the perforant path potentiates durably the synaptic responses of the granule cells of the dentate gyrus (Bliss and Lomo 1973). After that, it has been shown that this response can be modulated bidirectionally (in vitro and in few cases in vivo) by using different electrical stimulation patterns or pharmacological treatments. Potentiation and depression of the synaptic response is measured by the size of the excitatory postsynaptic potential (EPSP) induced in response to a pre-synaptic stimuli of constant intensity. It has been shown that experimental induction of facilitation or depression of the synaptic response can be maintained over time for hours or even weeks, and were therefore respectively called long-term potentiation (LTP) and long-term depression (LTD). It is now widely accepted that, learning and memory depends mainly on the regulation of these synaptic and plastic processes (Martin, Grimwood et al. 2000). The properties of LTP and LTD have been studied extensively *in vitro*, and have been found again *in vivo* (Doyere, Redinidelnegro et al. 1993; Burette, Jay et al. 1997; Hyman, Wyble et al. 2003).

Synaptic plasticity can involve many different processes. For instance, cyclic Adenosine Mono-Phosphate (cAMP) mediates a presynaptic form of LTP at cerebellar

parallel fiber synapses (Salin, Malenka et al. 1996). LTP and LTD, the two kinds of long-term synaptic plasticity the most commonly studied depends on postsynaptic NMDA (N-methyl-D-aspartate) glutamate receptors such as the ones located on the synapses formed by the projections from the EC to the granule cells of the DG (perforant path), or those formed by Schaffer collaterals onto the pyramidal neurons of the CA1 area of the hippocampus. The involvement of NMDA glutamate receptors was demonstrated by the inability to induce LTP or LTD in the presence of a selective antagonist of these receptors (Morris, Anderson et al. 1986; Dudek and Bear 1992). In the following paragraphs, we will describe the molecular mechanisms of induction and maintenance of NMDA-dependent LTP and LTD in the hippocampus, as well as the role of these two forms of synaptic plasticity in learning and memory.

1) Long-term potentiation or LTP

a) The mechanisms of induction of LTP: Ca^{2+} influx

The first experimental LTP-inducing protocols were based on high-frequency electrical stimulations (typically around 100 Hz), which are non-physiological to many neuronal populations (Barnes 1995). However, later studies have shown that LTP can also be induced by a pre-synaptic low frequency stimulation (1 Hz) when it coincides with a depolarization of the postsynaptic neuron (Gustafsson, Wigstrom et al. 1987). The induction of LTP is also possible when the stimulation of the postsynaptic neuron gives rise to a back propagation of the action potential in the dendritic arborization which usually succeed to the pre-synaptic stimulation of few ms (Magee and Johnston 1997; Markram, Lubke et al. 1997). This demonstrates that, what is relevant for the induction of LTP is the temporal coincidence of presynaptic glutamate release and the generation of an action potential in the postsynaptic neuron as Hebb predicted. The usual high frequency stimulation has the effect to dramatically increase the likelihood of such temporal coincidence by increasing neurotransmitter release and the generation of many action potentials in a very short time interval. The key molecule capable of detecting this coincidence of activity is the NMDA receptor, an ionotropic Na^+/Ca^{2+} channel which has the property of being activated by glutamate release only if the postsynaptic membrane is depolarized (Elgersma and Silva 1999). Under these conditions, the Mg^{2+} ion, that blocks the ion channel when the membrane is at resting potential, is then released (Herron, Lester et al. 1986). The opening of the channel induces a massive influx of Ca^{2+} in the postsynaptic compartment ($[Ca^{2+}]_i$). The result of this calcium flux is a rapid rise in intracellular calcium concentration that leads to postsynaptic responses which will induce LTP.

b) Transduction mechanisms of LTP: the central role of CaMKII

The first consequence of the $[Ca^{2+}]_i$ increase is the activation of protein kinases located in the proximity of NMDA receptors. The Ca^{2+} -calmodulin-dependent-kinase II (CaMKII) is one of the key protein kinase activated during the induction of the NMDA-dependent LTP (Malenka and Nicoll 1999; Soderling 2000). The massive influx of Ca^{2+} in the post-synaptic compartment leads to the formation of a calcium-calmodulin complex. Once coupled to calcium, calmodulin activates CaMKII, which is capable of maintaining its activation by autophosphorylation in presence of Ca^{2+} (Shonesy, Jalan-Sakrikar et al. 2014). The central role of CaMKII in the induction of LTP has been verified by numerous studies. First, autophosphorylation of CaMKII occurs after the onset of LTP (Barria, Derkach et al. 1997). Conversely, it has been shown that the induction of LTP fails in transgenic animals deficient for CaMKII (Silva, Stevens et al. 1992) or, more specifically in animals with CaMKII mutated at the autophosphorylation site (Giese, Fedorov et al. 1998). Furthermore, adding a peptide blocking the activation of CaMKII in the postsynaptic cell prevents the onset of LTP (Malenka, Kauer et al. 1989), whereas prior increase in the concentration of activated CaMKII results in saturation of the synaptic strength and occlusion of LTP (Lledo, Hjelmstad et al. 1995). CaMKII activation is the first step in the signal transduction initiating LTP, leading to the phosphorylation of other proteins as long as CaMKII remains active (Lisman, Schulman et al. 2002).

CaMKII plays a critical role in the cascades associated with the maintenance of LTP. However, other kinases have been implicated in the phosphorylation cascades but with less substantial effects. It has thus been suggested that activation of cAMP-dependent protein kinase (PKA) can indirectly promote the action of CaMKII via the activation of calmodulin-dependent adenylate cyclase (AC) (Lisman 1989; Makhinson, Chotiner et al. 1999). The role of PKA would be to reduce the activity of protein phosphatases such as protein phosphatase 1 (PP1). The action of PKA thus unbalances the competition between phosphorylation and dephosphorylation processes in favor of kinases (including CaMKII) dependent action. Other signaling pathways make use of GTP-bound proteins (G proteins), in particular the Ras protein as the effector of two distinct phosphorylation cascades. The first depends on mitogen-activated protein kinases (MAPK) such as the extracellular-regulated kinase (ERK) that is required for LTP and some forms of memory (Sweatt 2004; Thomas and Huganir 2004). The second involves the phosphoinositol 3'-kinase (PI3K), which activity increases during LTP (Sanna, Cammalleri et al. 2002). Finally, other molecular candidates have been suggested such as protein kinase C (PKC), tyrosine kinase and CaMKIV (Sheng and Kim 2002). The contribution of these protein kinases forms a complex molecular response including short- and long-term mechanisms to achieve the expression and maintenance of LTP itself.

c) The mechanisms of expression of LTP: modulation of the AMPA receptors trafficking

A widely accepted consensus establishes that the expression of LTP in the CA1 area of the hippocampus is largely based on mechanisms affecting AMPA (α -amino-3-hydroxy-5-methyl acid 4-isoxazole propionic acid) glutamate receptors. Like NMDA receptors, AMPA receptors are ionotropic channels activated by glutamate. However, the major difference between them is that AMPA receptors are mainly permeable to monovalent cations (Na^+/K^+). Most of AMPA receptors in the central nervous system are tetramers consisting of 4 subunits (GluR1 to GluR4). Despite significant homology between these four subunits, their mix in the composition of AMPA receptors affects the functional properties and cell trafficking of these receptors (Malinow and Malenka 2002; Collingridge, Isaac et al. 2004; Derkach, Oh et al. 2007). In the hippocampus of adult rodents, the two predominant forms of AMPA receptors are heteromeric GluR1/2 and GluR2/3 (Wenthold, Petralia et al. 1996). The membrane insertion of heteromeric receptors GluR2/3 has been shown to be relatively fast, while the insertion of AMPA receptors containing GluR1 subunits is slow under basal conditions, and greatly stimulated by the activation of NMDA receptors (Hayashi, Shi et al. 2000; Shi, Hayashi et al. 2001). These results suggest specific roles for each type of AMPA receptor subunits regarding their insertion at the synaptic membrane following activation and induction of LTP.

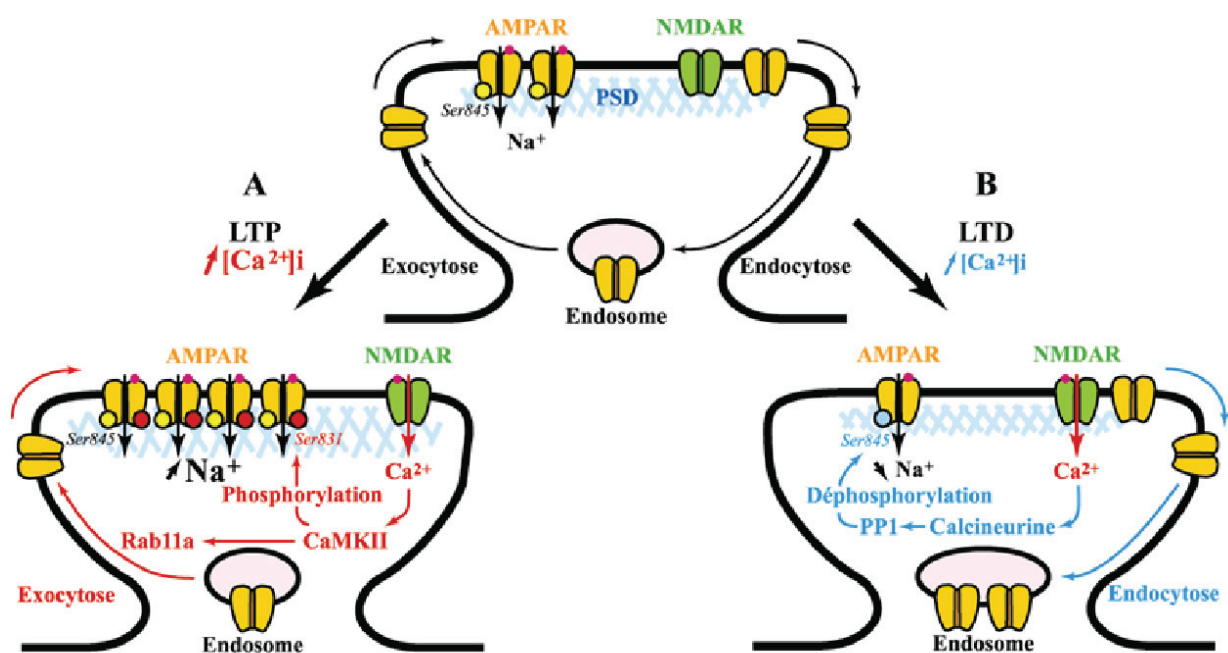


Figure 8: (A) Mechanisms of expression of LTP. In a naive synapse (top), the renewal of the AMPA receptors at the membrane is ensured by an endocytosis/exocytosis cycle of receptors stored in dendritic endosomes. At the induction of LTP (left), the increase in $[\text{Ca}^{2+}]_i$ active CaMKII will promote membrane insertion of AMPA receptors by two mechanisms. CaMKII phosphorylates Ser831 site of the GluA1 subunit, which stabilize the receptor at the postsynaptic membrane. This results in an increase of the sodium conductance and therefore the increase in post-synaptic response. (B) For the induction of LTD (right), the moderate increase in $[\text{Ca}^{2+}]_i$ activates calmodulin and protein phosphatase 1 (PP1). These phosphatases lead to dephosphorylation of the Ser845 site of GluA1 subunit and increases AMPA receptor endocytosis. The decrease in the number of AMPA receptors at the surface of the membrane results in the decrease of the post-synaptic response.

After induction of LTP, the increase in the amplitude of the postsynaptic response is the result of two main mechanisms that promote the entry of Na⁺ in response to a presynaptic release of glutamate. The first is the increase in the unitary conductance of AMPA receptors once they have been phosphorylated. As we have seen earlier, CaMKII plays a key role in LTP. One of the phosphorylation target of CaMKII is the serine 831 site of the GluA1 subunit. Such phosphorylation of GluA1 results in a conformational change that increases the unitary sodium conductance of GluA1 monomeric receptor (Derkach, Barria et al. 1999; Derkach, Oh et al. 2007). The second mechanism responsible for the increase of the post-synaptic response after induction of LTP involves the insertion of new AMPA receptors in the post-synaptic membrane, allowing the increase in the Na⁺ flux in response to the release of glutamate. Strong evidence for AMPA receptors stocks localized in dendrites endosomes have been reported (Park, Penick et al. 2004). These AMPA receptors are also phosphorylated at Ser831 by CaMKII. In this case, this phosphorylation is a signal for the externalization of these receptors and their stabilization at the synaptic membrane (Esteban 2003; Lee, Takamiya et al. 2003; Boehm, Kang et al. 2006). Quite surprisingly, the insertion of these new AMPA receptors do not take place in the immediate proximity of the post-synaptic density (PSD), but would be localized to peri-synaptic regions with the help of auxiliary proteins of the TARP (transmembrane AMPAR regulatory proteins) family (Nicoll, Tomita et al. 2006; Ziff 2007). Once at the surface of the plasmic membrane, these AMPA receptors are quickly captured by the protein complexes of the PSD. The best candidates for this lateral mobility are a family of proteins located in the PSD, the MAGUK (membrane-associated guanylate kinases), with PSD-95 as the leading actor in this family of kinases (Kim and Sheng 2004). More specifically, PSD-95 appears to be involved in regulating the number of AMPA receptors inserted in the postsynaptic membrane. The overexpression of this protein thus increases the synaptic strength and leads to occlusion of LTP (Ehrlich and Malinow 2004), while its subexpression reduces the synaptic response and expression of surface AMPA receptors (Ehrlich, Klein et al. 2007).

d) Long, long-term potentiation: *New protein synthesis, synaptic tagging and capture.*

The molecular cascade we just described guarantees LTP maintenance for the first two to four hours (early LTP, protein synthesis independent) after LTP induction. The extended persistence of NMDA-receptor-dependent LTP beyond this delay (late LTP) would be critically dependent on the synthesis of new proteins, such as new receptors that could be integrated at the activated synapse or participate to the formation of new synapses to reinforce transmission between the pre- and the post-synaptic neurons. Because this type of synaptic modification requires both gene transcription and translation, the problem of targeting gene products from the nucleus to the few activated synapses (and not the other synapses) in a vast dendritic tree has been solved by the neuron in ways that we do not yet fully understand. The *synaptic-tagging and capture hypothesis* has been put forward as a way to address this problem (Frey and Morris 1997). This hypothesis proposes that the

products of gene expression are delivered throughout the cell, but that they function to increase synaptic strength only at synapses that have been ‘tagged’ by previous synaptic activity. Despite strong evidence for some type of synaptic tag, the identification of such tag and the mechanisms by which the products of gene expression are ‘captured’ by tagged synapses have remained elusive. The validation of the synaptic-tagging and capture hypothesis thus rests on the identification of such tag(s). From a broad perspective, anything that provides a spatially restricted trace of activity is a candidate for the synaptic tag. Any of the synaptic changes we described earlier could potentially serve as tags. Many of these changes are related to the activity and strength of the synapse, such as the rapid addition of AMPA receptors to ionotropic glutamate receptor clusters or the lateral mobility of NMDA receptors between synaptic and extra-synaptic sites. Such events could serve as localized traces of previous synaptic activity able to produce synaptic strengthening on their own within a limited time period. However, to function as synaptic tags, they would need to be able to interact with cell-wide events to produce local and persistent increase in synaptic efficacy. Previously, we have shown the importance of phosphorylation state of key proteins in the maintenance of LTP. Persistently active kinases meet several of the criteria for a tag, as they allow a synapse to ‘remember’ previous activity in a spatially restricted and reversible manner. CaMKII that becomes autonomously and persistently active by autophosphorylation once activated could be such a tag. Likewise, the atypical protein kinase C known as protein kinase M ζ (PKM- ζ), the persistent activity of which requires protein synthesis, has been shown to be both necessary and sufficient for the maintenance of late LTP in the hippocampus (Frey and Morris 1997). Any disturbance in the PKM- ζ dependent cascade can lead to the loss (forgetting) of the biological substrate of the encoded memory (Hardt, Nader et al. 2013). Local, persistent and synaptic stimulation induced changes in the activity of these kinases could thus serve as synaptic tags that combined with products of gene expression would produce enduring but local changes in synaptic efficacy.

2) Long-term depression or LTD

We have just seen how high frequency stimulation could induce to the induction of LTP. Nevertheless, LTP cannot be the only cellular mechanism supporting memory. The sole increase in synaptic strength would quickly lead to the saturation of the whole network and to the impossibility to store new information. The formation of a memory trace can therefore only be realized if there is a relative modulation of synaptic weights across networks to create assemblies of interconnected neurons. That is why there must be a reverse mechanism to LTP that may promote a decrease in synaptic strength and a de-saturation of the neuronal network. Such decrease has been experimentally observed using two approaches. First, prolonged stimulation of low frequency (1-3Hz) can depotentiate synapses that have been previously reinforced by LTP, bringing them back to their basal state. Secondly, low frequency stimulation can also induce *de novo* NMDA receptor dependent

long-term depression (LTD) on naive synapses in the hippocampus (Dudek and Bear 1992). It has been shown that such LTD exists in many structures with an excitatory transmission (Citri and Malenka 2008). NMDA receptor-dependent LTD shares many characteristics with LTP. Its mechanisms of induction, expression and maintenance are very similar to the ones we have seen earlier for LTP.

The low frequency stimulation induces a slow but continuous release of glutamate that generates a postsynaptic depolarization below the threshold of generation of action potentials. *Via* NMDA receptor activation, such combination of molecular events leads to a slow and continuous entry of Ca^{2+} into the dendritic spine (Kemp and Bashir 2001). Thus, the induction of LTD, like LTP, is dependent on an increased concentration of intracellular Ca^{2+} . However, it is the amplitude and the speed of this increase that allows the differential occurrence of LTP or LTD. Citri and Malenka have shown that a low (nanomolar) Ca^{2+} concentration is sufficient for the induction of LTD while LTP requires concentration within the micromolar range (Citri and Malenka 2008). Thereby, modulation of this intracellular calcium concentration could allow the synapse to switch between LTP and LTD (Harney, Rowan et al. 2006). In the case of LTD, the low calcium concentration does not activate CaMKII but instead leads to the activation of calcineurin (or protein phosphatase 2B PP2B), a phosphatase that starts a cascade of activation of other phosphatases among which PP1. PP1's role is to decrease the activity of kinases that have been described previously (Kirkwood and Bear 1994; Morishita, Connor et al. 2001). PP1 thus prevents Ser831 phosphorylation of GluR1 subunits and the insertion of AMPA receptors to the membrane. Additionally, under normal conditions, the PKA phosphorylates the Ser845 site of the GluR1 subunit which leads to a stabilization of the interaction between GluR1 and PSD95 and thus its location in the PSD. During LTD, PKA loses its ability to phosphorylate Ser845, reducing GluR1-PSD95 interaction and leading to the internalization of AMPA receptors (Dierning, 2014). De-phosphorylation of Ser831 and Ser845 thus both lead to a net decrease in the number of functional AMPA receptors at the PSD and to a decrease of in synaptic transmission.

It is important to note that the molecular mechanisms governing LTP and LTD involve the same down-stream proteins but are opposed. CaMK2 is activated and GluR1 is externalized (phosphorylated at the ser831 site) during LTP while CaMKII is inhibited and GluR1 is now internalized (dephosphorylated at the ser845 site) during LTD. It is therefore possible to observe bidirectional regulations of the synaptic plasticity. For instance, Malleret and colleagues have shown that a strain of mice with decreased hippocampal PKA activity displays opposite changes in long-term synaptic plasticity. Whereas maintenance of LTP is impaired in these mice, consolidation of LTD is facilitated (Malleret, Alarcon et al. 2010). These physiological changes were associated with a deficit in long-term reference memory, but improved working memory abilities. This raises the question of the role of long-term changes in the synaptic efficacy in memory and forgetting.

3) The Key Question: “LTP = Memory and LTD = Forgetting?”

There is a quite large body of evidence showing the role of LTP in memory consolidation and memory formation. For instance, using genetic constructs, mice were generated so that NR1 subunit of the NMDA receptor could be deleted in CA1 by adding doxycycline to the drinking water. These mice expressed LTP under normal conditions, but not when given the drug. These mice were first trained off dox in two hippocampal-dependent learning tasks: the Morris water maze and contextual fear conditioning. Suppressing LTP (animals on dox) the week immediately after training drastically increased the escape latency in the water maze task and decreased freezing in presence of the conditioned context. Suppressing NMDA receptors functions at later time points did not affect learning in both tasks. These results suggest a transient role of NR1 in the consolidation of memories (Shimizu, Tang et al. 2000). Similarly, overexpression of a dominant-negative form of CaMKII in the forebrain the week immediately after training blocks the formation of remote contextual fear memories but do not have the same effect when overexpressed later after training. (Wang, Shimizu et al. 2003). Furthermore, work from Malleret and colleagues showed that mice with transient decrease in calcineurin expression showed increased LTP both *in vivo* and *in vitro*. When tested in the Morris water maze task, these mice showed enhanced memory for the escape route (Malleret, Haditsch et al. 2001). This shows that LTP is necessary to obtain good performances in long-term memory tasks. Using pharmacological injection in the rat hippocampus, Pastalkova has shown that it is LTP maintenance and not LTP induction that is responsible for the formation of remote memories (Pastalkova, Serrano et al. 2006). Nevertheless, even if these results taken together are convincing to suggest that learning and memory requires LTP, there was no causal link between LTP and memory formation. The most striking evidence of such a link came from the Bear’s lab in 2006. The authors trained rats in a single trial inhibitory avoidance-learning task and showed that such training produced the same changes in hippocampal glutamate receptors expression as an induction of LTP with HFS and caused a spatially restricted increase in the amplitude of evoked synaptic transmission in CA1 *in vivo* (Whitlock, Heynen et al. 2006). This result clearly demonstrated that learning induces LTP in the hippocampus. However, when looking closely at their results we can see that, at some recording sites the amplitude of the evoked response does not increase, but in the opposite slightly decreases after learning. This is just an observation and these results were barely mentioned in the article. But they raise the question of the role of LTD in learning and memory.

An attempt to explore the role of LTD in memory was done with the characterization of the serum response factor (SRF) KO mice, which showed normal hippocampal LTP but impaired LTD. Like control subjects, SRF KO mice learnt perfectly well non-hippocampal-

dependent tasks but showed a profound deficit in hippocampal-dependent spatial learning tasks, such as contextual habituation or water maze training, suggesting a major role for LTD in the acquisition of spatial context. These results were in agreement with *in vivo* studies exploring changes in synaptic plasticity in the rat hippocampus after exposition to new spatial context. Manahan-Vaughan and colleagues thus showed that novel empty space reinforces the expression of LTP in the CA1 region and impaired LTD expression. In contrast, modification of this environment by placing new objects in it facilitates LTD and impaired LTP (Braunewell and Manahan-Vaughan 2001; Kemp and Manahan-Vaughan 2004). These results thus suggest that LTD is required for updating information from and to the hippocampus. Updating information is a key feature of short-term/WM. In 2001, Zeng and colleagues used a strain of mice in which the disruption of calcineurin activity specifically in the DG and the CA1 area of the hippocampus led to a restricted decrease in the magnitude of low frequency stimulation induced LTD. In terms of behavior, this LTD deficit had no consequence on a long-term memory tasks such as a contextual or cued fear conditioning or the spatial version of the Morris water maze. It had in contrast a serious impact on working memory when tested in a delayed-match-to-place task or in an eight arms radial maze (Zeng, Chattarji et al. 2001). Following this work, Nakao used rats and showed that the amplitude of LTD correlates with an alternation ratio (index of performance) in a WM Y-maze task (Nakao, Ikegaya et al. 2002). In light of Zen's and their findings, Nakao and colleagues concluded that LTD thus serves as an index of WM, suggesting that LTD is not a constraint that limits memory but a necessary process for some forms of learning. However, the question of the exact role of LTD in WM remained, at the time, opened.

Few years later, two studies tried to answer this question using genetically modified mice. First, Nicholls and colleagues used a model that expresses an inhibitor of the protein phosphatase 2A (PP2A) in the forebrain. This inhibitor blocked NMDA-dependent LTD without affecting LTP or other kinds of LTD (mGluR-dependent LTD or DHPG-induced internalization). These mice were trained during 10 days to learn the position of a first platform in the Morris water maze. The platform was then switched to the opposite quadrant of the maze, and the mouse had to learn this new position for 7 days. At the end of each training phase (day 10 and day 17), a probe trial consisting in letting the mouse swim freely in the water maze without platforms and assessing the percentage of time spent in each quadrant was conducted. When looking at the path length, control mice and genetically modified ones showed no differences and they learned both platform positions. However, during the second probe trial, mice lacking LTD spend significantly more time than wild type controls in the quadrant corresponding to the previously learned platform position showing that they were unable to forget the first location of the platform that was no longer relevant (Nicholls, Alarcon et al. 2008). The authors further showed that deficit in LTD and forgetting was associated with a deficit in WM. More specifically, this deficit in WM was observed when repeated trials were given to the mice. These results thus showed for the first

time that LTD may serve to weaken memory traces when new information needs to be stored. Two years later, the same group used a new genetically engineered strain of mice expressing an inhibitor of PKA. These mice exhibited enhanced and prolonged LTD. When tested in the water maze, they showed no differences in learning abilities, but when a probe trial was conducted a week after the end of the training (instead of immediately after), the mutants were not able to locate the target quadrant suggesting increased forgetting. When tested after learning a second location, mutant mice displayed enhanced flexibility as they spent more time searching for the newly acquired platform than controls. The consequence of this increased flexibility was tested in an eight arm radial maze. Mutant mice were thus tested in a WM task involving a high or a low level of PI. The authors found that enhanced LTD and flexibility was associated with enhanced performance in the task requiring a high level of PI (Malleret, Alarcon et al. 2010). Altogether, these two studies reveal a specific role of LTD in the treatment of previously learned information that need to be forgotten.

Altogether, these results showed nicely that synaptic potentiation resulting from phosphorylation processes benefits to the long-term storage of information, whereas LTD processes would be necessary to weaken previously learned memory traces in order to forget irrelevant information. To sort out which information is relevant and which one has to be forgotten, neurons have to detect events occurring at the same time (for example, the food reward and a specific location in the maze) and strengthen their connections during such experience. However, such coincidental activity usually happens briefly while memory consolidation takes time and energy. That is why consolidation probably occurs when no sensory inputs could create new and maybe contradictory coincidences that could disturb the consolidation of the relevant event. Sleep is a period of rest during which the subject is not submitted to distractive new experience and has thus been proposed to be a perfect state to allow memory consolidation. In the next section, we will see how sleep could be beneficial not only for memory but also for forgetting.

The role of sleep on memory

In the chapter dedicated to memory, we have seen that consolidation requires the reactivation of neuronal networks in order to transform a labile trace initially stored in WM into a stable and long-lasting memory. We have also seen in the chapter dedicated to forgetting the necessity of selecting which information are consolidated and which are better to be forgotten. This raises quite a serious issue. If consolidation of remote information occurs during the encoding of new information, these two processes could collide and interfere with each other leading to unpredictable results. One of the many functions of sleep would be to isolate us from the external world and therefore to allow memory consolidation to take place without being disturbed by the presence of external stimuli. In this chapter, after a brief overview of the different vigilance states existing in mammals, we will see the evidence for a role of sleep in memory consolidation and lastly we will see the different models that have been advanced to explain such role.

I) The sleep wake cycle

The sleep-wake cycle results in alternating different vigilance states that can be identified phenotypically by four criteria: a specific activity and/or posture, a characteristic electroencephalographic signature (EEG), a threshold response to environmental stimuli and a rapid reversibility (Dement and Kleitman 1957; Jouvet and Michel 1959; Jouvet, Michel et al. 1959). In rodents, EEG and electromyogram (EMG) are sufficient to differentiate the sleep-wake cycle. In laboratory, rodents being mostly active in the dark, 60% wake and 40% sleep are usually recorded the 12-hours dark phase whereas 70% sleep and 30% wake are mostly described during the 12 hours light phase.

During arousal, the animal is active. The EMG thus indicates a high muscle activity with bursts of activity corresponding to the animal's movements. The EEG is called "activated", that is to say, characterized by the presence of low amplitude fast β oscillations (20-30 Hz) and γ oscillations (30-60 Hz). When recorded with LFP electrodes within the hippocampus, theta waves can be observed during attentive waking periods of intense exploratory activity (7-8Hz), food intake behavior or grooming (5-10Hz).

On the other hand Sleep in rodent is classically divided into two states, slow wave sleep (SWS) and paradoxical sleep (PS).

1) Slow wave sleep (SWS)

SWS (also called non-REM sleep by opposition to REM sleep, see after) is the first stage of sleep and represents about 90% of the total sleep time during the dark phase and

80% during the light phase. During this stage, the animal is inactive. Its muscle tone measured with the EMG is weak, with no occurrence of twitches. The EEG mainly presents a slow wave activity (SWA) characterized by slow oscillations and delta waves (2-4Hz) of large amplitude accompanied by short duration spindles (1-3sec) of higher frequency (10-14 Hz) (Rasch and Born 2013). The CA3 and CA1 regions of the hippocampus generate "sharp wave ripples" (SW-R) that are complex transient events consisting in 40-120ms wave peaks that are superimposed with local oscillations of high frequency (100-300 Hz) from CA1 (Girardeau, Benchenane et al. 2009).

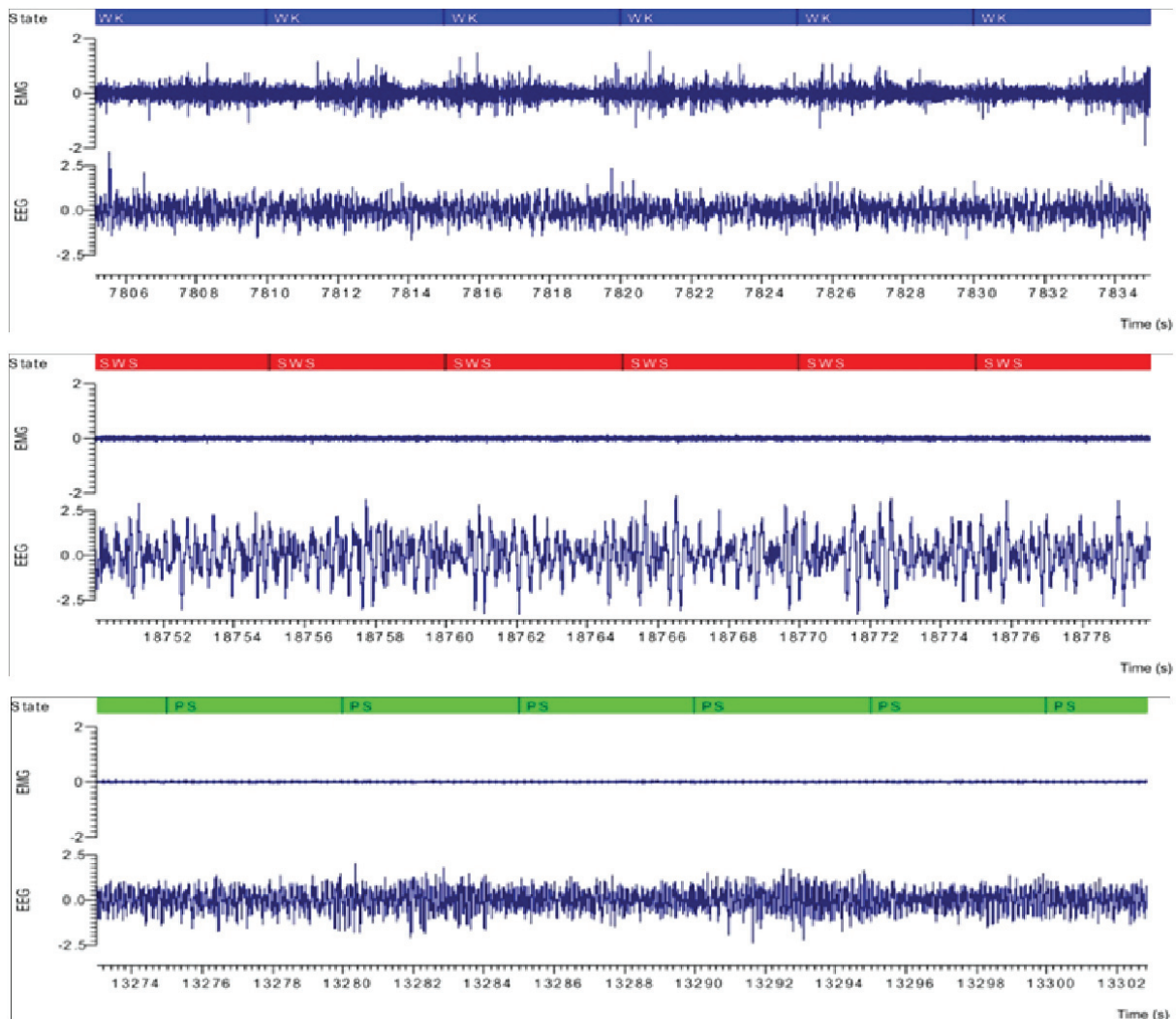


Figure 9. polysomnographic recordings in different vigilance states in rats : Plotted electromyographic (EMG) and electroencephalographic (EEG) recorded in rats during wakefulness (blue), SWS (in red) and REM sleep (green). During wakefulness, EMG indicates the presence of a significant muscle tone which may vary depending on the behavioral activity of the animal. The EEG shows fast and low amplitude cortical activity. During SWS, we see a decrease in muscle tone and an absence of phasic phenomena in the EMG. The EEG mainly presents slow waves of high amplitude. During REM sleep, the EEG present a predominance in theta waves. The flat EMG reflects the muscle atonia, a characteristic of this sleep state.

2) Paradoxical or Rapid Eye Movement (REM) Sleep

REM sleep (also called Paradoxical sleep) is characterized by rapid oculomotor phasic activity (hence its name Rapid-Eye-Movement sleep) and spasms (twitches) in the muscles of the extremities and the face. REM sleep represents 10% of the light phase (20% of total sleep time) and 5% of the dark phase (10% of the total sleep time). It is characterized by a total weakness of posture muscles with no notable posture change as compared to the preceding SWS episode. Paradoxically, this sleep stage is characterized by an "activated" EEG, very similar to the one observed during waking. That is why REM sleep is also called paradoxical sleep (PS). This activated EEG is characterized by tonic oscillations of small amplitude and theta frequency (5-10Hz) to which can be superimposed transient fast theta events (10-15 Hz) (Sakai, Sano et al. 1973) that are most prominent in the molecular layer of the DG (Montgomery, Sirota et al. 2008).

SWS generally precedes a REM sleep episode. This SWS- REM sleep *ultradian* cycle (that is to say 'less than one day') is repeated several times during the sleep-wake cycle with varying durations of episodes and more or less frequent transitions to different states of vigilance depending on the time of day or night. Note that at the end of an episode of SWS, there may be a transition toward a REM sleep episode or to wake state. However, a REM episode is always preceded by a SWS episode and generally ends with a waking phase, even if this one is short-lasting and the animal falls back into SWS just after.

II) The evidence for a role of sleep in memory

The first evidence for a role of sleep in memory was brought by Jenkins and Dallenbach in 1924. They asked their participants to learn lists of words, and ask them to recall these lists at a later time. Jenkins and Dallenbach noted that if subjects were allowed to sleep during the retention period after learning they exhibited better performances than subjects who stayed awake during that time (Jenkins and Dallenbach, 1924). As mentioned in the introductory chapter on forgetting, Jenkins and Dallenbach interpreted their results as a simple protective role of sleep against external interference that could be caused by new learning that might occur during an equivalent period of wakefulness. This passive role of sleep was then challenged by the discovery of REM sleep that, as we have seen, is characterized by an intense cortical activity and dreamlike content suggesting an active role of sleep in memory consolidation. This aspect has been studied extensively in the years that followed the discovery of REM sleep and a great number of data was acquired in rodents (for review see (Rasch and Born 2013)). In rodents, there is a significant increase in REM sleep amount following a wide variety of learning tasks or after exposure to an enriched environment (Smith 1995; Rasch and Born 2013). Conversely, an artificial increase in REM sleep amount by administration of pharmacological agents (or induced by rebound after specific REM sleep deprivation) enhances animal performance in a spatial memory test

(Wetzel, Wagner et al. 2003; Ravassard, Hamieh et al. 2014), while a loss of post-learning sleep causes impairment of memory performance, confirming a role of REM sleep in memory (Smith and Rose 1996). Altogether, these data prove a clear active role of REM sleep on memory consolidation. Other data suggest similar role for SWS. During SWS, an increase in the density and number of SW-R and spindles events has thus been reported after an olfactory discrimination task (Eschenko, Molle et al. 2006). This change in SWS events is not observed in animals that have failed to learn the task (Eschenko, Ramadan et al. 2008), suggesting a cognitive reason for these changes in the architecture of SWS.

However, it is interesting to note that not all forms of memory do seem to be affected the same way by sleep. In animals, both REM sleep deprivation and the increase of REM sleep quantity seem crucial when learning is complex, whereas performances during simple tasks are not affected by changes in REM sleep quantities (Hennevin, Hars et al. 1995). On the other hand, REM sleep seems to mainly promote hippocampal-dependent memory consolidation (Prince and Abel 2013). As mentioned earlier, spatial learning in the Morris water maze is generally divided in two stages: a “visible platform” phase that does not depend on the hippocampus and during which the animal learns to swim toward a visible platform, and a spatial hippocampal-dependent phase during which the animal must use spatial cues to locate a hidden platform submerged in opaque water. It has been shown that only the performances in the spatial version of the task are altered by a post-learning REM sleep deprivation (Smith and Rose 1996; Smith and Rose 1997). Similarly, REM sleep deprivation causes a decrease in performance in a contextual fear conditioning task, which is known to be dependent on the hippocampus, but not in the hippocampal-independent cued version of the task (McDermott, LaHoste et al. 2003; Ruskin, Liu et al. 2004).

In humans, studies involving partial deprivation of the first part (rich in SWS) or the second part (richer in REM sleep) of a night of sleep have helped us determine the specific role of SWS or REM sleep in the consolidation of different forms of memories. In 2007 Born and colleagues studied the recall of two different tasks: a mirror tracing skill (procedural memory) and the learning of paired-associate lists (declarative memory). In this study, retention rates following undisturbed periods of early and late nocturnal sleep were compared. If learning was followed by a 4-hr retention interval placed in the early half of nocturnal sleep, the recall of paired-associate lists improved more than if the retention interval was placed during the late phase the night. Conversely, the recall of procedural skills was enhanced if the retention interval occurred during the period of the night that contains more REM sleep. The effects may reflect different influences of SWS and REM sleep on two principal types of memory, declarative and procedural. (Plihal and Born 1997).

Even if all these evidence prove that sleep actively contributes to memory processes, they also show a differential effect depending on the type of sleep and the type of memory

that is studied. This clearly demonstrates a link between these two phenomena but the mechanisms involved in the link between sleep and memory are far from being understood. A number of theories explaining these results have been proposed and are discussed below.

III) Models for the role of sleep on memory

1) Memory trace reactivation

Just like learning a motor skill requires a lot of practice, declarative learning seems to require a replay of the information to be consolidated multiple times. The memory trace reactivation (MTR) theory suggests that such replay and the consolidation of memory traces occur during sleep. The reactivation of the neuronal network involved in learning would convert mnemonic traces from a labile state (susceptible to interference) to a stable state as described in the standard consolidation model we mentioned earlier. These reactivations occurring as a repeated pattern during quiet wakefulness and sleep could promote progressive integration of these new memory traces in the existing long-term memory networks without overwriting the oldest memories. This assumption comes from different electrophysiological studies on place-cell activity in the hippocampus recorded during and following spatial navigation tasks. When the animal wanders in a maze, there is formation of a specific sequence of place cell activation for storing the space environment. This specific pattern of activity observed in these neurons can be "replayed" backwards during quiet arousal following exploration (Foster and Wilson 2006) or in the same sequence during the sleep phases after exploration (Wilson and McNaughton 1994; Skaggs and McNaughton 1996). This suggests that learning during wakefulness induces an early stage of stabilization of mnemonic traces which treatment would be completed during subsequent sleep-dependent reactivation. Such reactivation is however much less accurate than the original recorded pattern, occurs at a quicker pace and takes place preferentially during SWS (Nadasdy, Hirase et al. 1999). In addition, these replays are observed mainly in conjunction with SW-R (Nakashiba, Buhl et al. 2009). However, some studies have also shown neuronal reactivation during REM sleep with the same time course as during wakefulness (Louie and Wilson 2001). Interestingly, the location of cells encoding a familiar information are preferably reactivated during the descending phase of REM sleep theta oscillations, while cells encoding new information discharge during the ascending phase of these oscillations. It has also been shown that theta rhythm synchronizes the activity of populations of hippocampal pyramidal cells by promoting excitatory transmission and thus, the modulation of synaptic weights during the ascending phase of theta (Kamondi, Acsady et al. 1998). Thus, in the hippocampus, REM sleep might promote the deletion of information that has now become familiar by depotentiating the synaptic circuits associated to these memories but could also potentiate neuronal circuits that encode new information (Poe, Nitz et al. 2000). Plus, if the

onset of SW-R is blocked during post-learning sleep, there is a significant decrease in the neuronal reactivations in the hippocampus and performance during the recall of a contextual fear conditioning. (Nakashiba, Buhl et al. 2009). This suggests a cognitive role of these reactivations for memory consolidation.

However, if neuronal reactivation promotes the redistribution of memory traces from the temporary store (hippocampus) to the long-term store (neocortex), this should happen not only in the hippocampus but also in other structures (neocortex?). In rats, reactivations in the PFC, parietal, visual cortices and in subcortical regions (ventral striatum, thalamus and nucleus accumbens) were thus also observed following spatial learning tasks (Qin, McNaughton et al. 1997; Ribeiro, Gervasoni et al. 2004; Euston, Tatsuno et al. 2007; Ji and Wilson 2007; Ahmed, McFarland et al. 2008). As these reactivations shortly follow those observed in the hippocampus, this reinforces the idea of a gradual transfer of memory traces from the hippocampus to cortical areas.

2) temporal/sequential treatment hypothesis

The global increase in synaptic potentiation induced by different learning experience at wake, whether or not reinforced during sleep, cannot continue unchecked forever. Some authors have thus assumed that the brain has a way to keep the overall synaptic strength balanced in the brain. Relying heavily on research done on animals, Tononi and Cirelli proposed the synaptic homeostasis hypothesis or SHY (Tononi and Cirelli 2003; Tononi and Cirelli 2006), that proposes a role for sleep in the down-regulation of global synaptic strength. According to this theory, daily activities during wakefulness (sensory stimulation, cognitive processes, etc) constitute new information that produces an increase (or potentiation) in synaptic strength in various neuronal networks (synaptic upscaling). Sleep would then promote synaptic depotentiation (synaptic downscaling) across these distributed networks in order to counter the synaptic potentiation induced during wakefulness. This homeostatic process (hence the name) would prevent the saturation of the whole network that could be costly energy-wise and that could interfere with the encoding of new information.

Slow wave activity during SWS would thus reflect the regulation of synaptic processes. Indeed, slow wave power is maximum at the beginning of the sleep phase. This is the sign of a high synchrony in a highly potentiated network. Slow wave power then gradually decreases during SWS. Slow waves could also participate to depotentiation as their frequency and their repeated nature could contribute to a decrease in synaptic strength. Therefore, sleep would have a restorative function in the brain, allowing neurons to prepare to receive and process new motor, sensory and cognitive information the next day. It would also promote the deletion of irrelevant information that can be stored during wakefulness.

The positive effects of sleep would thus to increase the signal-to-noise ratio for “to be consolidated” relevant memory traces in comparison to “to be forgotten” irrelevant material.

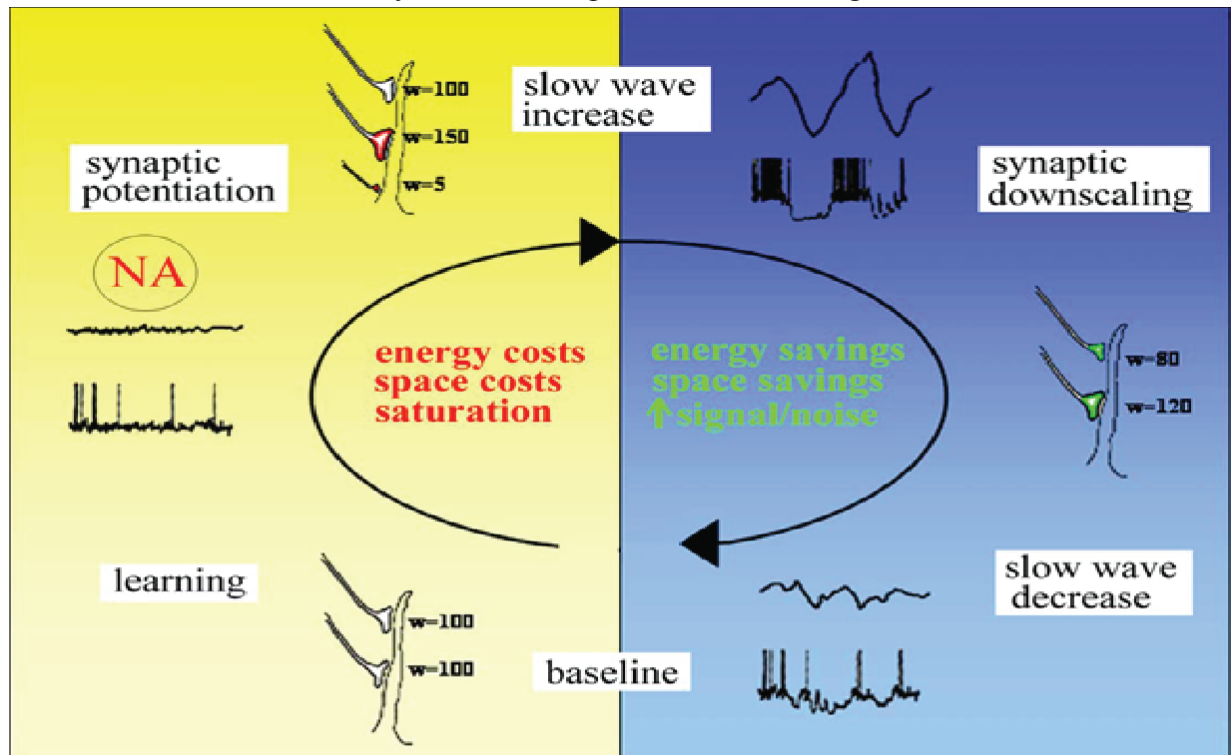


Figure 10. Synaptic homeostasis hypothesis (Tononi and Cirelli 2006). This model states that SWS decreases synaptic strength (synaptic downscaling). During arousal, the different learning processes increase synaptic strength (synaptic potentiation). It is proposed that the slow waves occurring during sleep are homeostatically regulated according to the level of potentiation that occurred during the previous awake (the more synaptic potentiation is important during wakefulness, the greater the activity of slow waves will be important during sleep) to reduce the strength of synapses to a basal level. Synaptic strength would thus be recalibrated during sleep. Such synaptic recalibration eliminates the weak and ineffective synapses (red), leaving only the most potentiated ones. This recalibration allow the conservation of the most important memory traces but erasing non-essential information.

The SHY was supported by multiple studies in *Drosophila*, rodents and human (for review, see (Tononi and Cirelli 2014)). In the rat frontal cortex, the slope and amplitude of responses of the response evoked by transcallosal stimulation were thus shown to increase over the wake phase and to decrease during sleep, and this sleep-related decline was found to correlate with the extent of the decline in slow-wave activity (Vyazovskiy, Cirelli et al. 2008; Vyazovskiy, Faraguna et al. 2009). However, more recent studies tend to nuance these results. For instance, in the frontal cortex the density of synapses tends to increase during slow wave sleep after a motor activity (Yang, Lai et al. 2014). Also, in cats the responses of the somatosensory cortex evoked by medial lemniscus fibers stimulation increases during wakefulness following the first episode of SL and are not modified by the following episodes of SL (Chauvette, Seigneur et al. 2012). Furthermore, in rat, neuronal discharge rates in the hippocampus gradually increase during SWS episodes and are accompanied by an increase in the peak synchrony during SW-R (Grosmark, Mizuseki et al. 2012). These results suggest that if a global synaptic depotentiation exists during sleep, it must coexist with local

mechanisms of potentiation and stabilization of memory traces that would occur during special events such as hippocampal SW-R (Born and Feld 2012).

3) Systemic consolidation model

According to the memory consolidation theories we have seen earlier, memory consolidation is the result of a dialogue between the hippocampus and the neocortex (Buzsaki 1996; Buzsaki 1998; Diekelmann and Born 2010; Rasch and Born 2013). One of the major hypotheses advanced to explain the current consolidation of memories during sleep is the “active systemic consolidation model”. This model assumes that acquisition and encoding of information, as well as the initiation of memory consolidation, would happen during wakefulness mainly in the hippocampus. Repeated reactivation of memory traces during sleep would then allow their stabilization via cellular mechanisms (synaptic potentiation) and their integration and progressive transfer to the neocortical memory networks (systemic consolidation). During SWS, SW-R in the hippocampus would trigger the neuronal reactivation during the ascending phase of the slow waves. The thalamo-cortical dialogue would then be possible during spindles that would allow a wide systemic redistribution of memory traces. Indeed, in humans, sleep has been shown to promote the integration of new representations in an existing network (during lexical tasks), and this effect was associated with an increase in the density of sleep spindles (Tamminen, Payne et al. 2010). In rats, there is also an increase in the number of spindles after learning or recall of an olfactory discrimination task (Eschenko, Molle et al. 2006; Rasch and Born 2013). In addition, SW-R have been shown to be associated with the hippocampal “through” of sleep spindles (Vyazovskiy, Faraguna et al. 2009). Altogether, these results clearly suggest a synchrony of learning networks (SW-R, spindles) crucial to the reorganization of memory traces in different memory stores (from the hippocampus to the neocortex).

The present model of active systemic consolidation also integrate the SHY by postulating that slow waves would allow the alternation between two processes: 1) global homeostasis promoted during their descending phase and 2) consolidation of memory traces in long-term memory during their ascending phase allowing a true dynamic optimization of the signal-to-noise ratio (Born and Feld 2012).

One major feature of the active systemic consolidation theory is that it is selective. Indeed, it would not make much sense to consolidate globally all newly acquired memory traces as the system would quickly be saturated. REM sleep would participate to the consolidation of memory on a more local scale (synaptic) (Siegel 2001; Ribeiro, Shi et al. 2007; Rasch and Born 2013) by promoting the increase in synaptic strength due to theta oscillations in the hippocampus (Buzsaki 2002). Theta oscillations during REM sleep would permit the stabilization and strengthening of synapses that are reactivated during SWS. Consistent with this idea of a selective consolidation, many studies have shown that sleep does not benefit in the same way all memory traces. In humans, performance in a lexical or spatial learning, are improved by post-learning sleep only if subjects are informed that there

will be re-tested later (Wilhelm, Diekelmann et al. 2011; van Dongen, Takashima et al. 2012). Performances in a finger-tapping task are also improved by the promise of monetary rewards (Fischer and Born 2009). This suggests that only newly formed memories that are expected to be useful in the future or associated with a reward, are consolidated.

Aims of this thesis

We have seen in the introduction the fundamental distinction between working/short-term memory and reference/long-term memory. RM is gradually acquired across several trials and can last for month whereas WM depends on the short-term storage of a single trial (Olton et al. 1979). These two types of memory are often studied separately. However, the frontier between the two can easily be crossed. Beatty and Shavalia (1980) (Beatty and Shavalia 1980) showed in a delayed-non-match-to-place task that the memory of a specific trial supposedly temporarily stored in WM (short-term) could last more than a day in rats. Dudchenko argued that in a WM task, the memory of a single trial that is supposed to be used within minutes, could be stored longer than necessary and disturb the acquisition or recall of later trials (Dudchenko 2001). Forgetting the information acquired in previous trials would therefore be necessary to perform a WM task, whereas RM requiring the long-term storage of information would be impaired by it. Forgetting and memory could be two antagonist processes supporting respectively WM and RM, two forms of memory that would therefore oppose each other (Malleret, Alarcon et al. 2010). Since the discovery of LTP by Bliss and Lomo in 1973 (Bliss and Lomo 1973), the neural bases of RM have been extensively studied (Squire 1992; D'Esposito, Detre et al. 1995) and the work from Mark Bear's lab in 2006 suggested a causal link between LTP and memory (Whitlock, Heynen et al. 2006). However, very little is known about the neural correlates of adaptive forgetting as the one at play in the processing of information in WM.

As mentioned previously, some of our team members have shown in transgenic mice models that hippocampal LTD might be responsible for adaptive forgetting and the weakening of previously stored memory traces, in particular during WM tasks when such information can interfere with the recall of newer information (Nicholls, Alarcon et al. 2008). However, it was important to demonstrate such findings in intact, non-transgenic, animals, and to show that the processing of previously stored and interfering information in WM can, in reverse, lead to hippocampal LTD. The first objective of my thesis was thus, to determine the neural correlates of adaptive forgetting during the processing of proactive interference. To do so, and instead of studying adaptive forgetting as an isolated process, we adopted a comparative approach allowing us to directly compare the biological substrates of RM and WM involving or not the processing of interference. We thus designed three tasks in an 8-arms radial maze. The RM task consisted in placing two food rewards always at the same place for 10 days of training. This protocol consists in presenting during multiple trials the same invariant information to be remembered in the long-term. On the contrary, the WM tasks consist in a series of unique trials in a delayed-non-match-to-place paradigm. The modulation of interference was made possible by using the same or different pairs of arms across trials as done before (Malleret, Alarcon et al. 2010). Using the same pair of arms in a repetitive manner for several days and trials induces a great amount of overlap between

trials. We previously showed that performance in this high interference working memory (HIWM) task involving the processing of PI, may depend on LTD-dependent forgetting of previously stored information/trials (Malleret, Alarcon et al. 2010). As a comparative control task, we developed a second WM task during which different pairs of arms were used for each trial. As compared to the HIWM task, this task involves a low load of interference. This low interference working memory task (LIWM) thus requires the same cognitive (WM – short-term storage of information) process as the HIWM task but involves less if any forgetting. 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

We used these behavioral paradigms to identify the molecular bases of memory and forgetting, and the results of our findings are described in chapter 1 and 2 of this manuscript. We also used them to determine the link existing between sleep, memory and forgetting, and the results of our research are presented in chapter 3 and 4. Below, I give a short overview of these 4 chapters.

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

During the first few months of my thesis, I collaborated with Mickaël Joseph on a neuroanatomical study aimed to understand where in the brain this processing of interference may occur. 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). To do so, we measured brain activation by using imaging of Zif268 and c-Fos protein, two immediate early genes (IEG) commonly used as indirect markers of neuronal activity and synaptic plasticity. IEGs undergo transcription when various kinases or transcription factors such as those involved in LTP like EIK-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 a new set of genes known as the late-response genes influencing the long-term synaptic changes required to express LTP. It has been shown extensively that Zif268 and c-Fos have important roles in learning and memory processes for review see (Veyrac, Besnard et al. 2014).

The paper presented in this chapter presents many data gathered almost entirely by Mickaël Joseph. I shortly participated to this study during my Master2. This is why I will not discuss the results of this article in depth in this manuscript. However, this work was the starting point of the work I pursued afterwards as it strongly suggests that the processing of PI requires specific processing in the brain. Indeed, the main finding of this work was that the DG 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 DG may be required to accomplish this task and overcome interference, and that the DG might stay non-activated when forgetting/processing of previously learned (but no longer relevant) information is required.

Chapter II Differential increase in hippocampal CaMKII and GluA1 activity after memory training involving or not the processing of interference

We showed that the DG is specifically inactivated during the HIWM task. The first step of my thesis was thus to understand what molecular processes lies behind this inhibition of the DG during the processing of PI. As we have seen in the introduction, some results suggest that LTD and LTP are opposite phenomenons underlying respectively forgetting and memory. We thus conducted a western blot analysis to identify the involvement of molecular markers of LTP and LTD in the different areas of the hippocampus of rats submitted to the three radial maze paradigms described above. Our results show that, in the DG, the HIWM task induces a selective increase in the phosphorylation of CaMKII as compared to controls and rats tested in the RM or LIWM tasks. This increase was not observed in the CA1 and CA3 area of the hippocampus. We also observed a specific DG decrease in ser845 phosphorylation of GluA1 after LIWM . This decrease suggests that, when the load of PI is not strong enough to induce a drop in behavior performances (LIWM), LTD processes can occur. However when the load of interference becomes too high to be dealt with efficiently (drop in performances), the level of phosphorylation of these GluA1 subunits in the dentate gyrus increase (at both phosphorylation sites) as compared to LIWM. This suggests that LTP occurs after the HIWM task.

These results, combined with the ones obtained using immunochemistry, suggest that both LTP and LTD could occurs selectively in the DG during the processing of proactive interference, and that, in this task that requires a high level of cognitive flexibility, the memory trace must be quickly formed as well as quickly erased.

Chapter III Sleep patterns are differentially affected by the processing of information into long-term or short-term memory, involving or not forgetting of previously stored information.

Behavioral data from the first two studies showed that rats trained in the HIWM task displayed a significant and slow decrease in performance over 10 days of training. This result indicates that information supposedly stored in short-term/WM outlasts their purpose and interfere several days later with the recall of new trials. This progressive build-up of interference occurs on the long-term and might be happening when the brain offline, during sleep. Plus, I have conducted an additional behavioral study comparing the effect of the delay between two training sessions showed that when rats were allowed to sleep between trainings, the effect of PI was enhanced. Altogether, these results led me to question the role of sleep in the processing of interference. In this third study, I thus tried to understand how sleep patterns can be modulated by the processing of PI.

In this study, we first observed a transient increase in REM sleep amount the day the animal has learned the RM rule, and, in contrast, a positive correlation between the performance of rats in the HIWM (but not in the LIWM) task and SWS amount and slow wave activity. In addition, oscillatory events known to be involved in memory processing were differentially modulated by the different cognitive processes involved in the three behavioral tasks. Notably, SWS spindles increase with RM training; rapid theta bursts during REM sleep after HIWM training, while sharp-wave ripples increase with all types of training.

This study demonstrates that SWS would not only be involved in memory, but also in forgetting. This result could thus partially confirm Tononi and Cirelli's synaptic homeostasis hypothesis. However, one important question remains unanswered: Could forgetting and the processing of interference be impaired by SWS alteration?

Chapter IV Dissecting the role of paradoxical and slow-wave sleep in the processing of proactive interference during working memory tasks in rats.

In the previous study, we found that SWS quantity and slow wave activity is correlated to HIWM performance suggesting that SWS is involved in forgetting processes. In this last chapter, we wanted to confirm such role by assessing the effects of a selective REM sleep or total sleep (SWS+REM sleep) deprivation on the processing of proactive interference. Because sleep deprivation is not possible over 10 days of training due to the stress and fatigue that such long deprivation would induce in the animal, we had to reduce our behavioral protocol to only two days of training and thus increase the number of trials on a given day. As observed in the 10 days version (presented in the previous articles), rats displayed lower performance when trained in the HIWM as compared to the LIWM task.

Strikingly, the presence of interference became only visible only on the second day of training confirming the role of sleep in the processing of interference. A four-hours REM sleep or total sleep (SWS+REM sleep) deprivation was conducted immediately after the first day of training.

Although such sleep deprivation method did not seem to alter the performance of rats on day 2 whether they were tested in the LIWM or HIWM task, a positive correlation between the amounts of SWS, but also REM sleep, and the improvement of performance on the second day of training was observed only in rats that performed the HIWM task. These results suggest that SWS but also REM sleep could cooperate to process proactive interference.

Chapter I

Differential involvement of the dentate gyrus in reference versus working memory requiring, or not requiring, the processing of proactive interference.

During my master 2 internship, I collaborated with Mickaël Joseph on a neuroanatomical study aimed to understand where in the brain the processing of interference may occur. To do so, we assessed brain activation by using imaging of Zif268 and c-Fos protein. We conducted an in-depth analysis of the contribution of a wide range of brain regions. The results presented in this chapter were the starting point of most of the analyses I conducted during my thesis. However, I only participated shortly to this study and in consequence will not discuss these results in depth. Chapter 1 is an article submitted to *Cerebral cortex* and describes thoroughly the behavioral tasks used in the other chapters of this thesis. This is why it was important to present this work in this manuscript as a kind of introduction to the other parts of this thesis.

Differential involvement of the dentate gyrus in reference versus working memory requiring or not the processing of proactive interference.

Abbreviated title: Inhibition of the dentate gyrus is required for adaptive forgetting

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Author contributions:

G.M., P.A.S, and M.A.J. designed research; all experiments were performed by M.A.J.; P.A.L and S.A. provided technical training and advice on data analysis. G.M. and P.A.S. co-directed the experiments. M.A.J. and G.M. wrote the manuscript. All authors contributed to discussion and interpretation of the findings.

Abstract

How does the brain discriminate essential information aimed to be stored permanently from information required only temporarily, and that needs to be cleared away for not saturating our precious memory space? To answer this question, we designed a comparative paradigm allowing us to differentially test in rats reference (RM) and working memory (WM) with and without the requirement of forgetting information from previous trials. RM refers to the long-term storage of invariable information whereas WM depends on the short-term storage of trial-unique information. WM tasks are very sensitive to proactive interference defined as old information interfering with the storage of new ones. To prevent such interference, irrelevant old memories must be forgotten to give new ones the opportunity to settle. Here, we show that the processing of such interfering previously stored information might specifically require a negative control of the dentate gyrus of the dorsal hippocampus materialized by an inhibition in the expression of indirect markers of neuronal activity and synaptic plasticity, Zif268 and c-Fos. Conversely, we show that inactivating the dentate gyrus impairs both RM and WM, but improves the processing of interference. These results strongly suggest for the first time that the dentate gyrus could be a key structure involved in adaptive forgetting.

Introduction

For many years, scientists have been investigating the neural bases of memory. A cardinal distinction lies between long-term/Reference Memory, and short-term/Working Memory. Reference Memory (RM) refers to the long-term storage of invariable information gradually acquired over many training sessions whereas Working Memory (WM) depends on the short-term storage of trial-unique information (Olton and Papas 1979). 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 (Maviel, Durkin et al. 2004; Poirier, Amin et al. 2008). 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, Alarcon 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; Chen and Tonegawa 1997; 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 (Della Sala ; Levy, Kuhl et al. 2010). 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 (Kraemer and Golding 1997). For instance, forgetting previous orders for a waiter taking many similar orders during a shift seems essential for the storage of relevant (e.g. new orders) information (Roberts and Dale 1981; Anderson, Neely et al. 1996; Rosenzweig, Barnes et al. 2002; Dudchenko 2004; Levy, Kuhl et al. 2010). Such examples of the adaptive role of forgetting are legion, but surprisingly we hardly know anything of its cellular or molecular underpinnings. Our goal was to find a way to determine such 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 three different radial maze paradigms to test three different cognitive processes: RM, WM and 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 storage of information (in RM or WM) and those requiring forgetting of previously stored information in WM. Using this procedure, we showed that the processing of such interfering information in WM might require a specific and negative control of the dentate gyrus (DG) of the dorsal hippocampus materialized by an inhibition of the expression of indirect markers

of neuronal activity and synaptic plasticity, Zif268 and c-Fos. Conversely, we showed that inactivating the DG impairs both RM and WM, but seems to improve the processing of interference. We thus here show that the DG is a critical node in processing the forgetting of irrelevant information, an essential process allowing optimal use of cognitive resources.

Materials and Methods

Subjects

A total of 95 three months old Dark Agouti rats initially weighing 200-250g at the beginning of the experiment were purchased from Harlan France. They were kept in a 12/12h light/dark cycle with ad libitum access to food and water but were subsequently food deprived and maintained at 85% of their free-feeding weight throughout the experiment. For the behavioral and immunohistochemical experiment (experiment 1), rats (n = 44) 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 (High Interference Working Memory HIWM, Low Interference Working Memory LIWM and Reference Memory RM group), and three groups served as their respective controls (Yoked HIWM or YHIWM, Yoked LIWM or YLIWM, and Yoked RM or YRM). For the behavioral lesion study (Experiment 2), the animals (n = 51) were also housed in pairs so that each lesioned rat was housed with its sham-operated control. Six groups of rats thus learned a task (DG lesioned RM, LIWM, and HIWM rats and their respective controls sham-operated RM, LIWM, and HIWM rats). 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 (Olton and Papas 1979) requiring the use of spatial orientation and memory was used for both experiment 1 and 2. The apparatus consisted of an elevated radial maze from which eight arms (87 cm long x 12 cm wide) radiated from an octagonal central platform (34 cm diameter). 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 2cm diameter and 0.5cm deep were fixed at 0.5 cm at the end of each arm. Food rewards (Dustless Precision Pellets; Bioserve, Frenchtown, NJ) could be placed in these food wells. The maze was located in a room with a number of extra-maze cues (e.g., poster, door,

furniture). A video camera, connected to a video recorder and a monitor, was fixed above the maze to record the animal's behavior.

Behavioral protocol

Food deprived rats had to retrieve food rewards (sugar pellets, Bioserv) at the end of the maze's arms using spatial navigation and distal visual cues surrounding the maze. 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. After habituation, they were pseudo-randomly assigned to one of the following groups (see below). 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 RM task had to retrieve food pellets in two arms of the maze (Fig 1a). These two arms remained constant and were the same every day for the entire 10 days (= 5 Blocks of 2 days) of training (Bontempi, Laurent-Demir et al. 1999). Rats were initially placed in a pseudo-randomly chosen starting arm at which point all arms of the maze were opened. A transparent blocker prevented rats from going to the food well of the starting arm. Once a rat chose one of the 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. 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). Once the two 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. For experiment 1, each experimental RM rat was paired with a yoked control (YRM) rat 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 use of yoked controls allows the experimenter to conclude that all differences seen between groups after immunohistochemistry analysis are inherent to learning processes during the task and not due to motivational, sensory or locomotor aspects of the task (Maviel, Durkin et al. 2004).

Low interference Working Memory (LIWM) group

The WM tasks consisted in a delayed-non-match-to-place task classically used in various models ranging from rodents to humans to assess WM. Rats trained in the LIWM task were submitted to four trials per day, each consisting of a sample and a choice phase (matching the eight runs performed by the RM group – see Fig 1b). 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 (Fig 1b). 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 (Fig 1b indicates an example of trial sequence for a given day). For experiment 1, as described above 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. 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 trials in a pseudorandom fashion in order to prevent YLIWM rats to predict the outcome of a trial. 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.

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. 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 (Roberts and Dale 1981; Saxe, Malleret et al. 2007; Malleret, Alarcon et al. 2010) (Fig. 1c indicates an example of trial sequence for a given day). For experiment 1, 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 YRM and YLIWM groups.

Immunohistochemistry (experiment 1):

Ninety minutes after the last training session (time required to induce expression of zif268 and c-Fos (Kubik, Miyashita et al. 2007)), a subset of rats ($n = 44$) were deeply anesthetized with an overdose of sodium pentobarbital (140 mg/kg, Sigma) and then transcardially perfused with 100 ml heparinized ringer lactate, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were then removed from the skull and were cryoprotected in 30% phosphate buffered sucrose. Brains were cut (25 μm

thin sections) on a freezing cryostat. Serial sections were collected in PBST Azide and then incubated at 4°C for 6 days with rabbit polyclonal antibody for Zif268 (1:1000, Santa Cruz Biotech) or 3 days with rabbit polyclonal antibody for c-Fos (1:5000, Calbiochem). Sections were incubated with a biotinylated secondary antibody IgG (donkey-anti-rabbit, 1:1000, Rockland, Tebu-bio) overnight at 4°C. The next day, sections were processed with avidin-biotin horseradish peroxidase complex (ABC 1:2000, Elite Kit from Vector Laboratories). Finally, immunoreactivity was visualized with 0.025% diaminobenzidine chromogen (DAB, Sigma), 0.05 % Nickel and 0.03% H₂O₂ as reaction initiator. Sections were mounted on gelatin-coated slides, dehydrated through a graded series of alcohols and coverslipped.

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). 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 was found between these groups in either Zif268 or c-Fos activation in all studied structures. 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).

Surgery – Dentate Gyrus lesion (experiment 2):

As the result of the immunohistochemical study revealed that the Dentate Gyrus may play a differential role in memory and forgetting, we conducted a lesion experiment. 51 rats were thus allocated to either the bilateral Dentate Gyrus (DG) lesion group (n = 24) or the sham operated group (n = 27). Surgery was performed under Isoflurane anesthesia in a standard stereotaxic apparatus. The rats were pre-anesthetized in a rectangular (30x20x15cm) chamber in order for them to endure positioning on the stereotaxic frame. Anesthesia was maintained via an inhalation nose cone affixed to the mouth bar on the frame. Inspired concentrations of 2-3 % Isoflurane with oxygen are required 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 opened the incision. Holes were drilled into the skull bilaterally over the DG. 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. The lesion coordinates for the dorsal DG group are 2.7 mm posterior to bregma, 1.2 mm lateral to

midline, and 3.4 mm ventral from skull, and 3.5 mm posterior to bregma, 1.9 mm lateral to midline, and 3.0 mm ventral from skull. Glass tubing with microcapillary (Harvard apparatus) was used for iontophoresis ejections. They were pulled in a single step on a Sutter PE-2 vertical puller (Narishige, Japan). The heat was programmed to give a tip of a less than 1 μm diameter size. Then, this tip was broken at 5 μm diameter to allow ibotenic acid ejection. Ibotenic acid (Tocris, bioscience) dissolved in sodium chloride to 10 mg/ml (pH = 8) was ejected at 4 sites in each hemisphere. The pipette was left in place for 5 min before ejection. 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 ejection, the pipette was left in place for at least 5 minutes to avoid leakage of the ibotenic acid along the pipette track. After surgery, the skin was sutured and local antiseptic was applied to prevent post-surgery infection. 2 ml of a 5g/l glucose solution was injected subcutaneously. The animals were then allowed a post-surgical recovery period of 10 days before starting behavioral testing in the radial maze as described above.

Statistical analysis

- *Behavior (experiment 1)*

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 \pm s.e.m.

- *Immunohistochemistry*

Zif268 and c-Fos immunoreactivity was statistically analyzed with Mann-Whitney U-test. Data are expressed as mean of normalized Zif268/c-Fos density (% of control) \pm s.e.m.

- *Correlation*

The density of Zif268 and c-Fos labeled neurons was also used to compare inter-regional brain activity and to better understand the functional connectivity existing between brain regions during the cognitive processes we dealt with. A correlation matrix was thus constructed based on this Zif268 and c-Fos density 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.

- *Dentate Gyrus lesion*

Behavioral data from the lesion study (experiment 2) were analyzed using two-way ANOVAs (Analysis of Variance) for repeated measures with Block (2 days) and Group (RM lesioned versus sham; LIWM lesioned versus sham; HIWM lesioned versus sham) 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 \pm s.e.m. Subsequent histological analyses revealed that lesions were limited to the dorsal dentate gyrus with negligible damages in other areas (Figure 6 a and b).

Results:

Experiment 1

Behavior:

Figure 2 shows the learning curves for RM, LIWM and HIWM rats. ANOVAs revealed a significant group effect [$F(2, 25) = 108.305$; $p < 0.0001$], a significant block effect [$F(4, 100) = 4.575$; $p = 0.002$], as well as a significant group \times block interaction [$F(8, 100) = 8.157$; $p < 0.0001$]. Post-hoc analyses revealed that RM rats significantly improved their performance over time [$F(9, 36) = 18.413$; $p < 0.0001$] and reached 85% correct choices on the last block of days, indicating a learning of the general rules and strategies required to solve the task. In WM groups, we investigated how proactive interference affected learning in an eight-arm radial maze. At the beginning of training, both LIWM and HIWM groups started at almost 90% of correct choices. LIWM rats kept high scores throughout the entire experiment, slightly increasing with time and reaching 100% on Block 5. On the contrary, rats trained in the HIWM task showed a decrease in performance over days indicating that accumulation of PI critically distorts WM performance with time (significant difference in score was shown on the last block of days between LIWM and HIWM $p = 0.0026$).

Immunohistochemistry:

To identify brain regions differentially involved in processing memory over the long term, the short term and the forgetting of previously stored information, we mapped the regional expression of the immediate early genes (IEG) *zif268* (Fig. 3a) and *c-Fos* (Fig. 4a) used as indirect markers of neuronal activity and plasticity (Kubik, Miyashita et al. 2007). These IEGs were studied because they are associated with spatial memory formation and

their expression was examined following ten days of training. A significant increase in the density of Zif268 labeled neurons was observed in the Hippocampus (CA1: $p = 0.0001$ for RM versus C, $p = 0.0085$ for LIWM versus C and $p = 0.0016$ for HIWM versus C; CA3: $p = 0.002$ for RM versus C, $p = 0.0321$ for LIWM versus C and $p = 0.0114$ for HIWM versus C), Entorhinal cortex ($p < 0.05$) and Prefrontal cortex ($p = 0.0052$ for RM versus C, $p = 0.0048$ for LIWM versus C and $p = 0.0006$ for HIWM versus C) in all 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 into pre-determined arms (Maviel, Durkin et al. 2004). These results are consistent with the well-established roles of these brain areas in learning and memory (Kubik, Miyashita et al. 2007). As expected, no such increase was visible in “control regions” such as the Primary Somatosensory cortex (S1) ($p > 0.05$) that are typically not activated by higher order cognitive processes (Poirier, Amin et al. 2008). However, we found that the Dentate Gyrus (DG) of the dorsal hippocampus displayed the most unique pattern of activity, with expression of Zif268 remaining low after HIWM training ($p = 0.0019$ for RM versus C, $p = 0.004$ for LIWM versus C, $p = 0.1876$ for HIWM versus C, $p = 0.01$ for HIWM vs LIWM and $p = 0.0065$ for RM vs HIWM; Fig. 3a and b). When mapping the regional expression of c-Fos, similar results were found. A significant increase in the density of c-Fos labeled neurons was observed in the Hippocampus (CA1: $p = 0.29$ for RM versus C, $p = 0.0118$ for LIWM versus C and $p = 0.0401$ for HIWM versus C; CA3: $p = 0.0055$ for RM versus C, $p = 0.0142$ for LIWM versus C and $p = 0.035$ for HIWM versus C), Entorhinal cortex ($p = 0.0047$ for RM versus C, $p = 0.0426$ for LIWM versus C and $p = 0.0002$ for HIWM versus C) and Prefrontal cortex ($p = 0.2643$ for RM versus C, $p = 0.0091$ for LIWM versus C and $p = 0.094$ for HIWM versus C) in all three groups of animals compared to the control group, although this increase was only marginal (and not statistically significant) in CA1 and the PFC for the RM group. As for Zif268 expression, no increase in c-Fos expression was seen in the S1 area ($p > 0.05$), but more importantly, the same pattern of expression was observed in the DG of the dorsal hippocampus, with c-Fos expression remaining low after HIWM training ($p = 0.0003$ for RM versus C, $p = 0.0005$ for LIWM versus C, $p = 0.3181$ for HIWM versus C, $p = 0.001$ for HIWM vs LIWM and $p = 0.0012$ for RM vs HIWM; Fig. 4a and b). Altogether, these results suggest that a non-activation of the DG might be required to accomplish this task and overcome interference.

Using the regional expression of zif268 (Fig. 5a) and c-fos (Fig. 5b), we also compared inter-regional brain activity to better understand the functional connectivity between brain regions (Poirier, Amin et al. 2008). In the control group, a high level of positive inter-regional brain correlation was specifically observed between the different areas of the hippocampus (for Zif268: between CA1 and CA3 $r = 0.709$; CA1-DG $r = 0.567$ and DG-CA3 $r = 0.858$, $p < 0.05$; Figure 5a). In addition to the control group, numerous correlations were observed between brain regions in the RM (For Zif268: 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 thus evident between intrahippocampal areas but also between the entorhinal and medial prefrontal cortices and the hippocampus. Most interestingly, we found that the pattern of correlated activity dramatically changed in the HIWM group as compared to the other groups. No inter-regional brain correlation was observed between any of the studied structures ($p > 0.05$) suggesting that forgetting and the processing of PI may require decoupling within these memory circuits. Together with a non-activation of the dentate gyrus, this inter-regional brain de-correlation might specifically promote forgetting of previous trials required to perform the HIWM task.

Experiment 2 - Lesion study:

Our findings that the dorsal DG shows no increase in Zif268 or c-Fos expression after radial maze training involving forgetting are particularly striking. To what extent is the DG required for WM and the processing of PI? To address this question, we examined the effects of inactivating the DG by ibotenic acid lesion on performance of the three behavioral tasks we already described. We first verified that the lesion was restricted to the DG. While the CA1 and CA3 subfields of the dorsal hippocampus were spared by the ibotenic acid injection in the DG, granule cells of the DG were almost completely eliminated comparing to sham rats (Figure 6a and b). Figure 6c shows the acquisition curve of the rats trained in the RM task. In this task, DG lesioned animals exhibited a marked impairment in performance as compared to the Sham-operated group. ANOVAs 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 Sham-operated rats significantly improved their performance over time (significant Block effect; $p < 0.0001$) unlike DG lesioned rats (marginal Block effect; $p = 0.0489$). Similarly, DG lesioned rats showed a net impairment when trained in the LIWM task (Fig 2b) as compared to sham-operated rats. ANOVAs 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; $p < 0.05$). Post hoc analyses revealed that LIWM lesioned rats exhibited impaired performances over time ($p = 0.0191$). In sharp contrast, the lesion of the DG seems to enhance performance when rats were trained in the HIWM protocol (Fig 2c). ANOVAs revealed a significant Group x Block interaction [$F(4, 64) = 2.784$; $p = 0.0339$]. Scheffe's post hoc analysis revealed that sham-operated rats exhibited impaired performances over time due to the build-up of interference [significant Block effect, $p = 0.0162$] as observed before in experiment 1. In contrast, DG lesioned rats were immune to interference and did not exhibit impairment in performance [no significant Block effect, $p = 0.1810$]. This result is in agreement with our IEG data suggesting that a non-activation of the DG is required for the processing of interference.

Discussion

Altogether, the results presented here confirm previous studies showing that the hippocampus is involved and required for RM and WM storage (Dolan and Fletcher 1997). However, we show for the first time that within the hippocampus, the processing of PI and forgetting of previously stored, but non-relevant information results in a selective absence of activation of the DG. We also show that inactivation of this structure (by lesion) does not impair, but could facilitate the processing of PI suggesting that the inactivation of the DG could be required to process and forget non-useful information.

Behavioral results of experiment 1 show that rats could effectively learn the RM task we designed. RM rats thus showed a significant increase in performance over the 10-day 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 delayed-non-match rule is certainly due to innate spontaneous alternation, a behavior that naturally causes rodents to choose a different option (visit arm#2) than one previously adopted (visit arm#1) and in consequence to alternate exploration between two open arms (Whishaw 1995). This tendency for spontaneously alternate between radial maze arms facilitates correct non-match responses. Nevertheless, spontaneous alternation requires memory storage of the previously visited arm in order to alternate to a different arm, and has long been shown to be dependent on the hippocampal formation functional integrity (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 present in the HIWM task. This modification in WM performance due to the ever-increasing buildup of PI has already been observed by our team in previous experiments using similar WM paradigms (Saxe, Malleret et al. 2007; Nicholls, Alarcon et al. 2008). This result highlights an often overlooked issue concerning WM; more precisely, that information supposedly stored temporarily in WM can have an impact on the long-term when it becomes an interference for subsequent WM information as seen here with the decrease in performance over days (not seconds or minutes) in the HIWM group. Information supposedly stored in short-term/WM thus outlast their purpose by interfering, several days later, with the storage of newer information. In consequence, this result questions the existence of a pure short-term memory store (Ranganath and Blumenfeld 2005) and rose doubts about a dissociation between short-term and long-term memory. Ranganath and Blumenfeld thus argued that the evidence suggesting distinct neuroanatomical substrates for short and long-term memory may have been misleading, and reviewed evidence demonstrating that short-term memory might be simply considered a temporary activation of some information already stored in long-term

memory. Various studies have thus 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, Dolcos et al. 2001). Our data seem to confirm such findings as both RM, LIWM and HIWM training induces a similar pattern of IEGs expression, with the exception of the dentate gyrus that was found to be non-activated by HIWM.

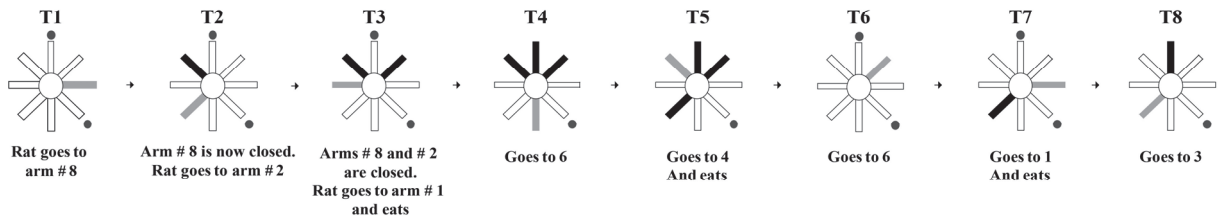
Zif268 expression in pyramidal cells of the CA1 and CA3 areas of the dorsal hippocampus, the entorhinal cortex and the prefrontal cortex were significantly elevated after RM and WM training, and this elevation was not altered by the presence of proactive interference. Slight difference with the pattern of expression of c-Fos in these structures could be observed. RM thus failed to increase expression of c-Fos in CA1 or the PFC. Differences in the expression of Zif268 and c-Fos have been previously reported and may explain such discrepancies (Davis, Bozon et al. 2003; Maviel, Durkin et al. 2004). In rats, we have thus repeatedly observed that CA1 expression of c-Fos is much weaker compared to the one of Zif268 (PA Salin and MA Joseph's personal communication). Such weak expression of c-Fos in CA1 may thus account for a failure to show a significant increase in c-Fos expression in this area for RM rats. However, overall, the pattern of expression of c-Fos largely reflects the one of Zif268. 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, Okada et al. 2008). In contrast, our data revealed a potential contribution of the DG of the dorsal hippocampus in processing PI. While a classic WM task (LIWM) and a RM task increased the activation of IEGs in the dentate gyrus, a WM task involving the processing of PI (HIWM task) caused a non-activation of this structure. In addition, such task also causes a de-coupled functional connectivity as no inter-regional brain correlation was observed between any of the studied structures. This result suggests that forgetting and the processing of PI may both require an inactivation of the DG and a functional de-coupling within the memory circuits. We thus assessed the effect of a specific inactivation of the DG on behavioral performance of rats tested in our three different radial maze paradigms, and found that a lesion of the DG impairs RM and LIWM training, but in contrast seems to improve the processing of interference in the HIWM task. This facilitation may occur because lesion of the DG prevents the recall of similar but irrelevant information previously stored in memory from interfering with learning. This result confirms previous data from our team showing that RM and WM (requiring forgetting) are somewhat antagonistic processes (Malleret, Alarcon et al. 2010) as DG lesion impairs the consolidation of information but benefits WM by facilitating forgetting and the processing of interference. In addition, confirming our immunohistochemical findings, these data also seem to demonstrate that DG inactivation is required to overcome and forget interfering non-relevant information.

The DG has largely been shown to be involved in pattern separation (Schmidt, Marrone et al. 2012), a process by which the amount of overlap between two representations stored in memory can be reduced. 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, Moser et al. 2008). 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 DG-dependent pattern separation function 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 DG, the animal may thus be able to forget previous similar representation/trial stored in memory and thus perform correctly an ongoing trial. Our results suggest that processing interference in a WM task could specifically require an inhibition of the DG, a site where adult neurogenesis is known to occur. They are thus in agreement with work from our group showing that DG inhibition of neurogenesis improves WM performance, especially in tasks where repetitive information were presented as it is the case in a HIWM task (Saxe, Malleret et al. 2007). Future experiments are required to establish if the number of activated DG newborn neurons decreases selectively in the HIWM task.

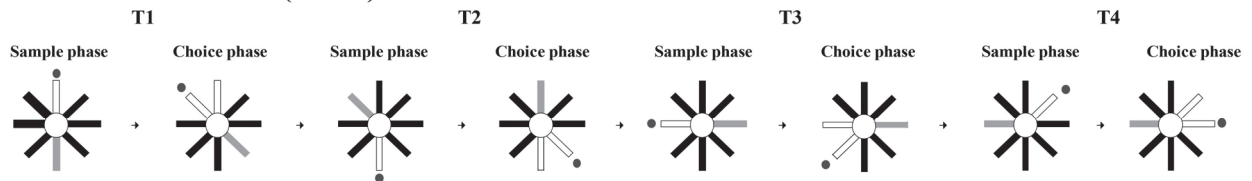
Given the importance of forgetting of irrelevant information for optimal use of memory in everyday life, it is now crucial to understand the molecular and cellular mechanisms underlying this essential cognitive process. Much work still needs to be done to achieve this goal, but the results presented in this study provide new insights in the molecular bases of forgetting by asserting the DG as a critical node in this process.

Figures

a Reference Memory (RM)



b Low Interference WM (LIWM)



c High Interference WM (HIWM)

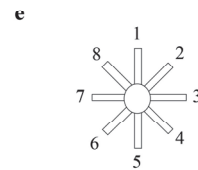
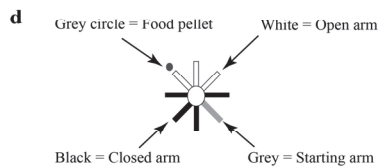
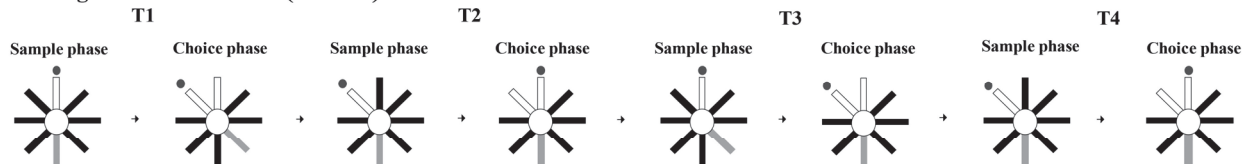


Fig. 1. Behavioral paradigms (see material and methods for more details). A schematic representation of one training day for each of the experimental groups. (a) RM training. The same two arms (here 1 and 4, (e)) are baited every day for each trial. Each daily session consisted of 8 trials (T1 to T8). (b) LIWM training. Each day consisted of 4 trials (T) of 2 phases each. (c) HIWM training. 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 in order to complete an ongoing trial. (d and e) schematic representations of the maze with the arms' attributed numbers and legends.

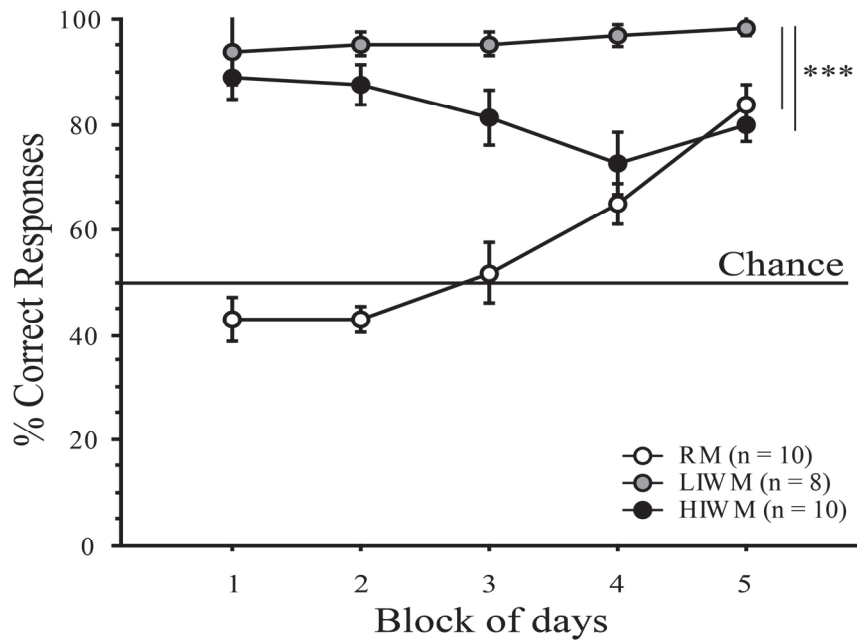


Fig. 2. Behavioral performances of each group of rats. Percentage of correct choices \pm s.e.m per Block (each Block = 2 days of training) in RM, LIWM and HIWM tasks. Black line represents the chance level for both WM tasks.

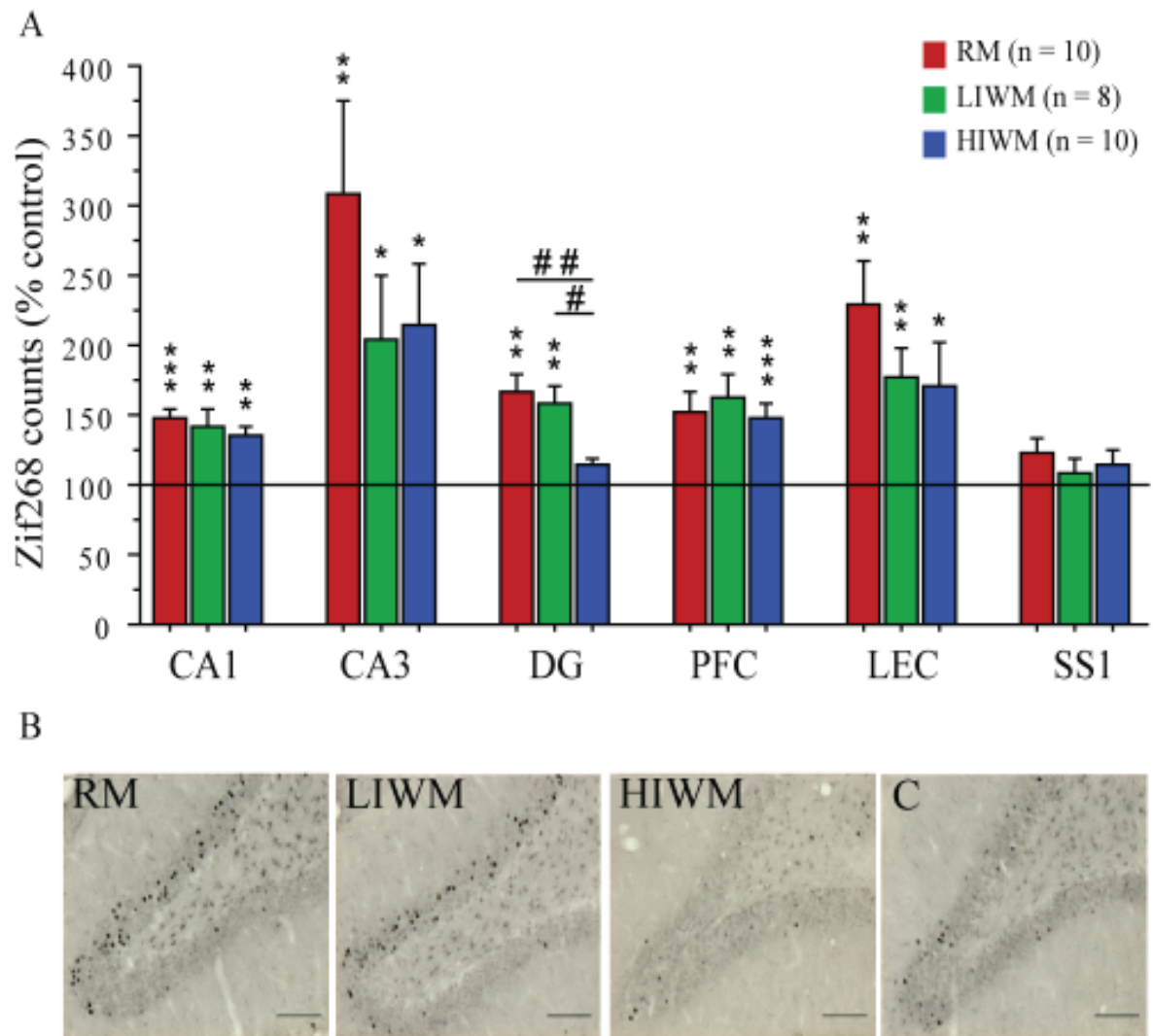


Fig. 3. (a) Zif268 counts relative to paired controls (black line) in the CA1, CA3 and DG of the hippocampus, Prefrontal cortex (PFC), lateral Entorhinal Cortex (LEC) and primary Somatosensory cortex (S1), after 10 days of training. All groups of rats expressed an increased number of Zif268 immunoreactive cells in these areas compared to control animals (n = 16, 100% baseline) except the control structure S1. This increase was not observed in the DG of HIWM rats (HIWM versus C: Mann–Whitney U-test, P = 0.1876, RM versus C: ** P = 0.0019, ** LIWM versus C: P = 0.004, HIWM versus LIWM: # P = 0.01, RM versus HIWM: ##P = 0.0065). * P < 0.05; **, ## P < 0.01; *** P < 0.001. (b) Representative Photomicrographs showing Zif268-stained nuclei in the dorsal DG. Scale bar, 100 μ m.

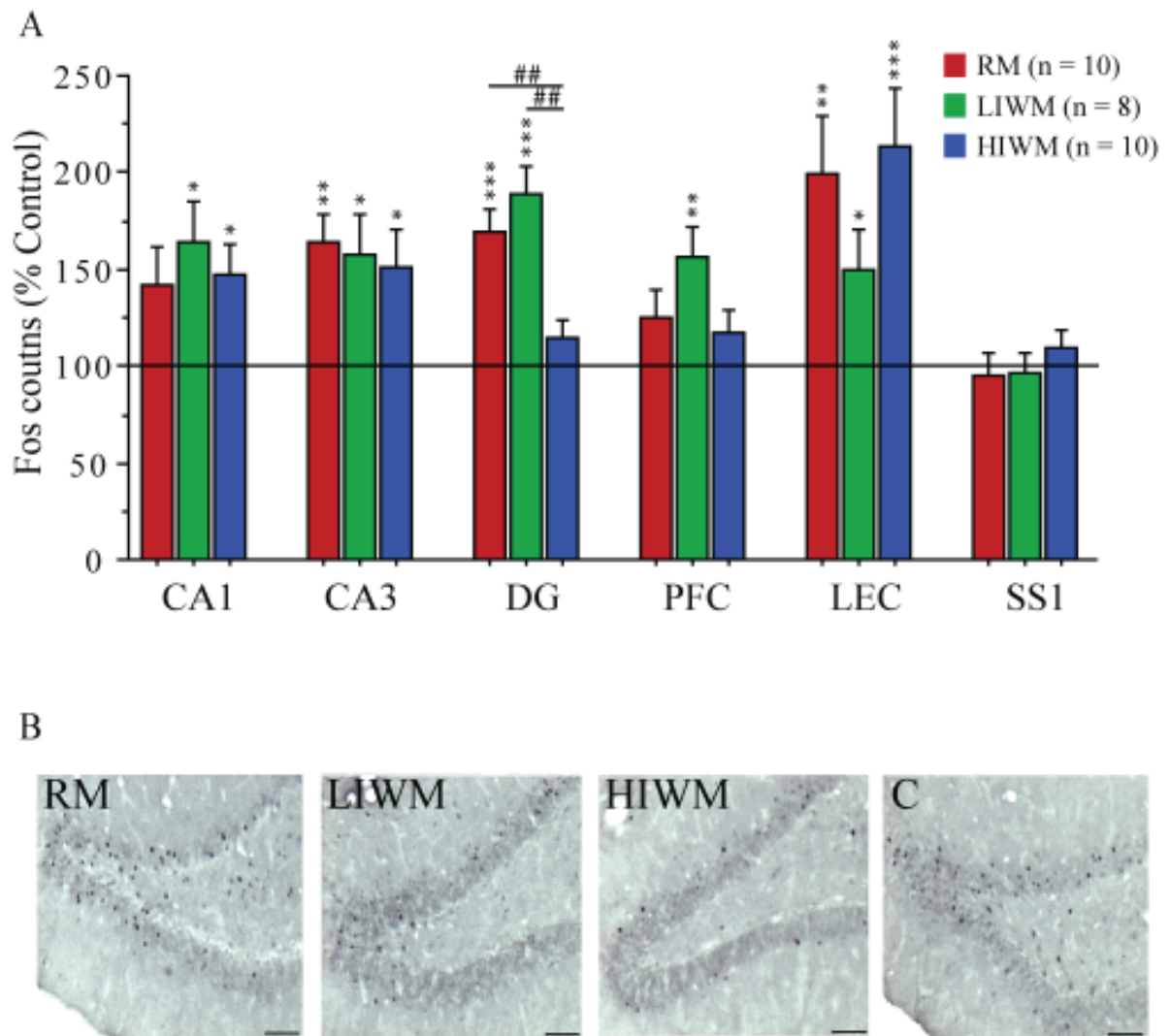
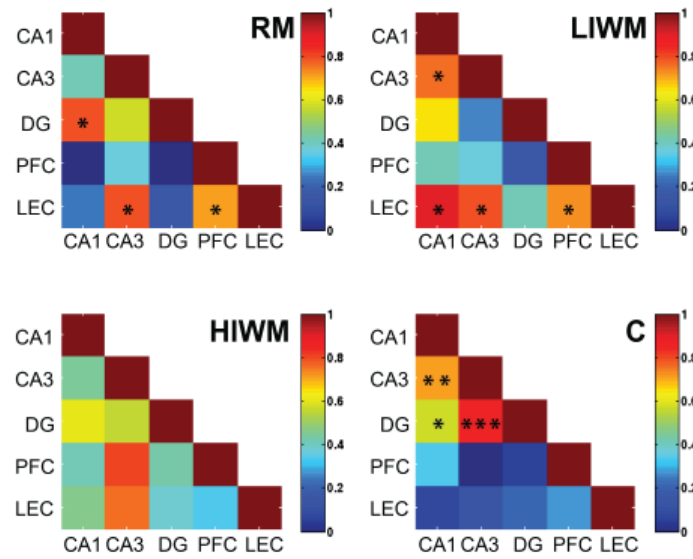


Fig. 4. (a) c-Fos counts relative to paired controls (black line) in the CA1, CA3 and DG of the hippocampus, Prefrontal cortex (PFC), lateral Entorhinal Cortex (LEC) and primary Somatosensory cortex (S1), after 10 days of training. No increase in c-Fos expression was observed in the DG of HIWM rats (HIWM versus C: Mann–Whitney U-test, $P = 0.3181$, RM versus C: *** $P = 0.0003$, *** LIWM versus C: $P = 0.0005$, HIWM versus LIWM: ## $P = 0.001$, RM versus HIWM: ## $P = 0.0012$). * $P < 0.05$; **, ## $P < 0.01$; *** $P < 0.001$. (b) Representative Photomicrographs showing c-Fos-stained nuclei in the dorsal DG. Scale bar, 100 μm .

A (Zif268)



B (c-Fos)

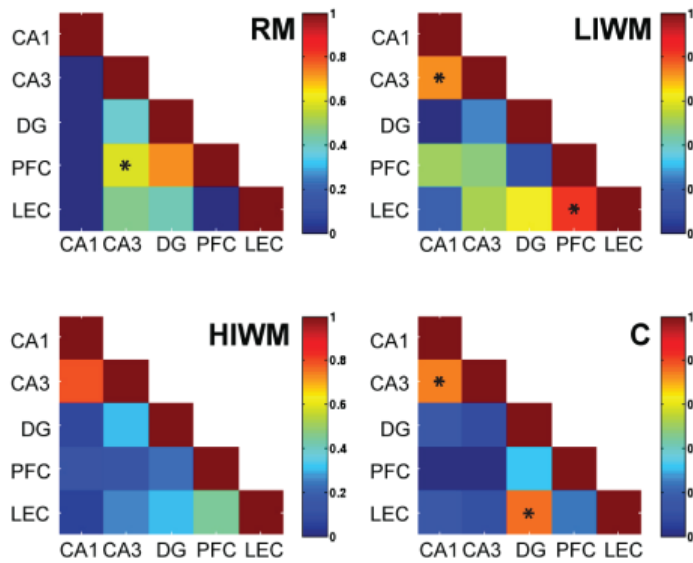


Fig 5. Functional connectivity analyses. (a) Interregional Correlation matrices for Zif268 expression within each group. (b) Interregional Correlation matrices for c-Fos expression within each group. R-Spearman rank correlation coefficients are color-coded. Colors reflect correlation strength (scale, right). Significant correlations ($p < 0.05$) are marked with (*).

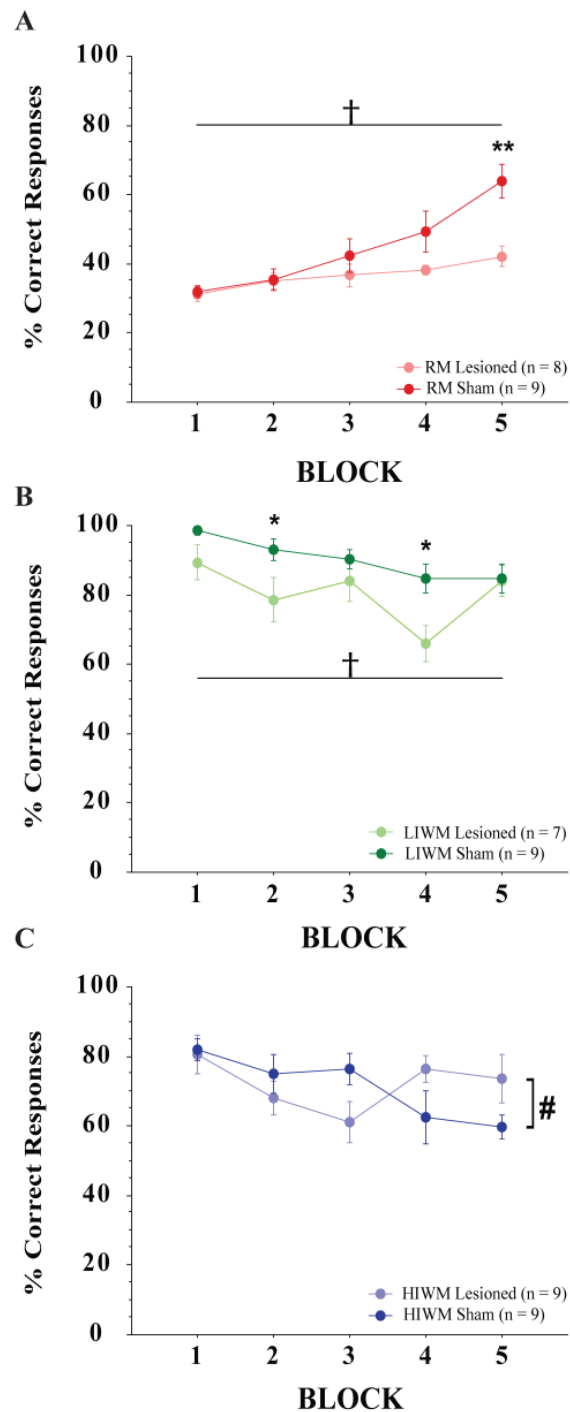


Fig. 6 Ibotenic acid lesion of the DG impairs RM and WM but improves the processing of interference. (a) Illustration showing the extent of the lesions to the Dentate Gyrus. The largest and the smallest tissue damage produced by ibotenic acid in the dorsal hippocampus are shown in gray and black respectively. The numbers represent distance (mm) from bregma. (b), Photomicrograph of Dentate Gyrus in a lesioned animal (bottom) and a sham animal (top) stained with NeuN. In this example, infusions of ibotenic acid produced a loss of tissue of the dentate gyrus. Scale bar, 150 μ m. Atlas sections are from the Paxinos and Watson (1997). (c-e) Percentage of correct choices \pm s.e.m per Block of days in RM (c), LIWM (d) and in HIWM (e) for ibotenic acid lesioned and control animals. † Group effect; * Block effect; * $P < 0.05$; ** $P < 0.01$; # interaction $p < 0.05$.

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Chapter 2

Differential increase in hippocampal CaMKII and GluA1 activity after memory training involving or not the processing of interference.

We showed in Chapter 1 that a lack of activation of the dentate gyrus of the dorsal hippocampus might be required for the processing of proactive interference in WM. We wanted to determine the molecular mechanisms underlying such inhibition. With chapter 2, we thus address this problem using a western blot approach to quantify key molecular targets of synaptic plasticity after training a new group of rats in the radial maze tasks described in chapter 1. Chapter 2 is written as an article to be submitted. This work has been done in collaboration with Al Mahdy Hamieh. The reader of this manuscript may skip the description of the behavioral tasks used in this study as they are strictly identical to the ones described in chapter 1.

Differential increase in hippocampal CaMKII and GluA1 activity after memory training involving or not the processing of interference.

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Abstract:

Working Memory (WM) and Reference Memory (RM) are two processes aimed to store information. WM is a specific form of short-term memory that refers to the ability to retain information within a single trial. This information can then be stored into long-term/reference memory. RM refers to the long-term storage of information that remains constant over time, that is gradually acquired over many training sessions and which is widely believed to be dependent on long-term potentiation (LTP) of the synaptic efficacy. However, not all information that comes from WM needs to be transferred into RM. Insignificant data is better to be erased in order not to overload the brain with irrelevant things. It has been demonstrated that WM is very sensitive to proactive interference (PI) which is a phenomenon whereby information learned in the past interferes with the learning of more recently presented material. Consequently, forgetting this old information would be necessary to perform everyday tasks requiring WM abilities. Previous work from our team suggest that long term depression (LTD) of synaptic efficacy may serve to weaken previous memory traces, thus preventing them to interfere with new ones. In order to assess post-synaptic changes in the expression and trafficking of biological markers of synaptic plasticity

involved in LTP or LTD, we performed a selective western-blot analysis in the different areas of the hippocampus in rats trained in a RM or WM tasks involving or not the processing of PI. Our western-blot analysis revealed that the processing of PI in WM may involve specific synaptic plasticity changes in the DG, while plastic changes after RM training occur mainly in the CA1 subregion. These changes involve an increase in the expression of AMPA receptor GluA1 subunit and in the phosphorylated/active state of CaMKII. Our results also suggest that the three sub-regions of the hippocampus may have antagonistic roles in memory processing.

Introduction

Hippocampal-dependent memory formation is thought to require changes in synaptic efficacy (Bliss and Lomo 1973; Bliss and Collingridge 1993; Kandel 2001). The two major kinds of hippocampal based synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD) (Bear and Malenka 1994; Bear and Abraham 1996). A major form of LTP in the hippocampus and the neocortex is dependent on NMDAR activation (Citri and Malenka,). LTP can be artificially induced by application of brief trains of high frequency stimulation and corresponds to an increase in synaptic transmission dependent on the phosphorylation state of key proteins such as glutamatergic N-Methyl-D-Aspartate (NMDA) receptors and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors subunits. NMDA receptors are ionotropic $\text{Na}^+/\text{Ca}^{2+}$ channels that have the property of being activated by glutamate release only if the post-synaptic membrane is depolarized (Elgersma and Silva 1999). Under these conditions, the Mg^{2+} ion that blocks the NMDA receptor channel when the membrane is at resting potential is released, liberating the channel and promoting a flux of Ca^{2+} through it (Herron, Lester et al. 1986). During the induction of LTP, a rapid rise in post-synaptic intracellular Ca^{2+} concentration thus occurs in the post-synaptic compartment. This increase in Ca^{2+} leads to the activation of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII). Once activated, CaMKII that is localized predominantly in the PSD (post-synaptic density), can become autonomously and persistently active by autophosphorylation, thus acting as a synaptic tag to drive new proteins to the activated synapse and contributing to a local and lasting increase in synaptic transmission. CaMKII autophosphorylation occurs at the Thr286 site of the α subunit (Fukunaga, Muller et al. 1995; Barria, Derkach et al. 1997). Once this site becomes phosphorylated, the enzyme can phosphorylate itself even in the absence of Ca^{2+} (Miller and Kennedy 1986). Consistent with this hypothesis, genetic deletion of Thr286 has been shown to block LTP and memory (Giese, Fedorov et al. 1998) and non-competitive inhibition of CaMKII during the maintenance phase of LTP has been shown to reverse LTP (Sanhueza, McIntyre et al. 2007). There is thus considerable evidence showing that CaMKII is critical for NMDAR dependent form of LTP. It has thus been shown that CaMKII, once activated, phosphorylates AMPA receptors GluA1 subunits at their Ser831 sites. Such phosphorylation allows the integration of new AMPA receptors at the PSD (Barria, Muller et al. 1997), further potentiating synaptic transmission. Recently, beta CaMKII was also shown to interact with Arc/Arg3.1 gene product to produce “inverse” synaptic tagging of inactive synapse (Okuno, Akashi et al. 2012). By this process, beta CaMKII could lead to LTD by promoting AMPA receptors endocytosis, thus preventing undesired enhancement of weak synapse in potentiated neurons.

Unlike LTP, NMDAR dependent form of LTD corresponds to a decrease in synaptic transmission, is generally induced by low frequency stimulation protocols and involves dephosphorylation processes. Whereas hippocampal-dependent memory studies suggest that the long-term storage of information requires LTP-like mechanisms (Kandel 2001; Malleret, Haditsch et al. 2001; Martin and Morris 2002), the role of LTD is less clear (Bear and Malenka 1994; Braunevel and Manahan-Vaughan 2001; Etkin, Alarcon et al. 2006). It has been proposed that LTD could increase the signal-to-noise ratio of a memory trace. In agreement with this notion, we found that LTD could function to weaken previous memory traces. We thus inhibited the expression of a protein phosphatase, PP2A (Nicholls, Alarcon et al. 2008) in transgenic mice. Inhibition of PP2A prevented the dephosphorylation and internalization of AMPA receptors and in consequence, the expression of a NMDA-dependent form of LTD while leaving intact other classical forms of synaptic plasticity (LTP, depotentiation). This mouse model was therefore particularly suited to study the role of LTD. We found that these mice had difficulties in forgetting previously stored information suggesting that LTD could function to weaken previous memory traces, thereby preventing those traces from interfering with newly encoded information when new conditions demands it as it is the case in a working memory (WM) task (Nicholls, Alarcon et al. 2008).

Memory is not unitary and a distinction is often made between long-term/reference and short-term/WM. Unlike the well-known long-term memory, WM represents the short-term acquisition of trial-unique information (Baddeley 1981; Cowan 2008). It has been proposed that WM is thought to be a short-term form of a memory that, once used, is better to be forgotten or ignored in order not to interfere with later learning (Dudchenko 2004; Delaney and Sahakyan 2007). A consequence of this view is that forgetting would be a process required for WM. However, unlike memory, forgetting processes and their biological bases are poorly understood, probably because forgetting has often been seen as just a lack of memory, a failed process that happens to us involuntarily (Levy, Kuhl et al. 2010; Sala 2010). Human studies, using directed forgetting, 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 (Rauchs, Feyers et al. 2011). For instance, a waiter taking many similar orders during a shift would find forgetting of previous orders essential for the storage of relevant (e.g. new orders) information (Roberts and Dale 1981; Anderson, Neely et al. 1996; Rosenzweig, Barnes et al. 2002; Dudchenko 2004). Common examples of this adaptive role of forgetting such as these are legion, but surprisingly we hardly know anything of its cellular or molecular underpinnings. In an attempt to determine the biological bases of such forgetting, we recently tested different groups of rats in an eight-arm radial maze in tasks involving reference (RM) or WM with or without proactive interference (PI) (i.e. requiring or not the forgetting of previous information) (Joseph, *et al* submitted). Using the Immediate Early Genes *zif268* and *c-Fos* as markers of neuronal

activity, we found that many brain structures are activated by RM or WM processes involving or not forgetting, except the Dentate Gyrus (DG) of the dorsal hippocampus that remains non activated when forgetting is needed. Conversely, we showed that inactivating the DG promotes forgetting. We therefore wanted to determine if different plasticity changes were at work in the dorsal hippocampus, and in particular in the DG, after training in RM or WM processes 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 CaMKII (P-CaMKII) and glutamate AMPA receptor subunit GluA1 in the DG as well as in the CA1 and CA3 region of the dorsal hippocampus. The results of this study are described below. And the medial prefrontal cortex

Material and methods

Subjects

A total of 66 three months old Dark Agouti rats initially weighing 200-250g at the beginning of the experiment were purchased from Harlan France. They were kept in a 12/12h light/dark cycle with *ad libitum* access to food and water but were subsequently food deprived and maintained at 85% of their free-feeding weight throughout the experiment. Rats were housed in cohorts of two such that each rat that was to acquire a high interference, low interference WM or RM task was housed with its yoked control. Therefore, three groups learned a radial maze task (High Interference Working Memory HIWM, Low Interference Working Memory LIWM and Reference Memory RM tasks), and three groups served as their respective controls (Yoked HIWM or YHIWM, Yoked LIWM or YLIWM, and Yoked RM or YRM). 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. Eight arms (87 cm long x 12 cm wide) were arranged around an octagonal central platform (34 cm diameter). 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 2cm diameter and 0.5cm deep were fixed at 0.5 cm beyond the end of maze arms. Food rewards (Dustless Precision Pellets; Bioserve, Frenchtown, NJ) could be placed in these food wells. The maze was located in a room with a number of extramaze cues (e.g., poster, door, furniture). A video camera, connected to a video recorder and a monitor, was fixed above the maze. Behavior of the rats in the maze was videotaped for later examination.

Behavioral protocol

Food deprived rats had to retrieve food rewards (sugar pellets, Bioserv) at the end of the maze's arms using spatial navigation and distal visual cues surrounding the maze. 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.

Reference Memory Task

Rats trained in the RM task had to retrieve food pellets in two arms of the maze. These two arms remain constant and were the same every day for the entire 10 days (or 5 Blocks of 2 days) of training (Bontempi, Laurent-Demir et al. 1999). Rats were initially placed in a pseudo-randomly chosen starting arm (red arms, Fig. 1A) at which point all arms of the maze were opened (light blue arms, Fig.1D). A transparent blocker prevented rats from going to the food well of the starting arm. Once a rat chose one of the arms (an arm selection was defined when the animal reached the arm's half way), the door to that arm was closed. 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. 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 (WM errors). After both food pellets were retrieved (For instance, on trial T5 in the example given in Fig. 1A), the two previously baited arms were re-baited and all arms were re-opened (T6, Fig. 1A). Rats underwent eight trials per day 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. Each experimental RM rat was paired with a YRM rat 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 use of yoked controls allows the experimenter to conclude that all differences seen between groups after western blot analysis are inherent to learning processes during the task and not due to motivational, sensory or locomotor aspects of the task (Bontempi, Laurent-Demir et al. 1999; Poirier, Amin et al. 2008).

Low interference Working Memory (LIWM) task

Rats trained in this task were submitted to four trials per day, each consisting of a sample and a choice phase. In the sample phase, rats were first allowed to enter from a starting arm 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 and

procedure than in the RM task). During the subsequent choice phase, rats were presented with two adjacent arms, the familiar arm that had just been visited and empty of food, and an adjacent arm containing a second food reward (**Figure 1B**). 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. 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. 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 trials in a pseudorandom fashion in order to prevent YLIWM rats to predict the outcome of a trial. 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.

High interference Working Memory (HIWM) task

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 every day 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 (Roberts and Dale 1981; Saxe, Malleret et al. 2007; Malleret, Alarcon et al. 2010) (**Figure 1C**). 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 YLIWM group.

Western Blot

After the last trial on the last day of training, rats 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 individually dissected with a Cryostat (Microm HM550) kept at -14°C. 300µm thick sections containing the medial Prefrontal Cortex (mainly prelimbic area), and the dorsal hippocampus (dCA1, dCA3 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 (Bradford 1976). Briefly, using precise concentrations of bovine serum albumin (BSA), samples with known protein concentrations were prepared and scanned using a spectrophotometer ($\lambda = 595\text{nm}$) 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-phosphoric acid). 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 run at 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 in (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 was 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 3x10 min (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 3x10min (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.

Statistical analysis

Behavior

Behavioral data were analyzed using two-way ANOVAs (Analysis of Variance) for repeated measures with Block (corresponding to two consecutive days of training) and Group (RM, LIWM, HIWM, YRM, YLIWM, YHIWM) 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.

Western Blot

The quality of each step was controlled by running each experiment twice. Western Blot analysis was done with ImageJ (NIH). 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 normalized to its corresponding loading control band: the housekeeping protein β -Tubulin. As no significant statistical difference in any proteins expression and in any of the structures was found between the three control groups (YLIWM, YHIWM and YRM) they were pooled as one single control group. The ratio protein of interest/ β -Tubulin was calculated for each duplicate and for each protein of interest. Each animal average value for the two duplicates was then expressed as a percentage of the control group mean. Statistical results were obtained using StatView 5.0. Mann-Whitney U-test was performed to analyze Western Blot results. Data are expressed as means \pm S.E.M.

Correlations

Linear regression analysis was performed to assess correlation between CaMKII phosphorylation ratio and GluA1 phosphorylation. We used the Spearman's rank correlation coefficient, a measure of statistical dependencies between non-parametric variables. A positive correlation coefficient between two phosphorylation states indicates that an increase in phosphorylation of one protein (i.e. PCaMKII/CaMKII) results in a proportional increase in the phosphorylation of the other protein (i.e. Ser831/GluA1).

Results

Behavior

The performance of rats trained in the three different tasks (RM, LIWM and HIWM), are represented in Figure. 1F. ANOVAs revealed a significant Group effect [$F(2, 30) = 57.472$; $p < 0.0001$], a significant Block effect [$F(4, 120) = 9.317$ $p < 0.0001$], as well as a significant Group x Block interaction [$F(8, 120) = 13.762$ $p < 0.0001$]. Post-hoc analyses revealed that RM rats significantly improved their performance over time [$F(9, 36) = 18.413$; $p < 0.0001$] and reached 85% correct choices on the last block of days, indicating a learning of the general rules and strategies required to solve the task. In WM groups, we investigated how proactive interference (PI) affected WM performance. At the beginning of training, both LIWM and HIWM groups started at almost 90% of correct choices. LIWM rats kept high scores throughout the entire experiment, slightly increasing with time and reaching 100% on Block 5 (no significant Block effect). On the contrary, rats trained in the HIWM task showed a decrease in performance over days indicating that accumulation of PI critically distorts WM performance with time (significant difference in score was shown on the last two blocks of days between LIWM and HIWM $p = 0.0055$ for block 4 and $p = 0.0005$ for block 5) (**Figure 1F**). This result confirmed our previous findings (Joseph, *et al* submitted) and showed that information supposedly stored in WM can have long-term impact on the subsequent storage of newer information, questioning the existence of a pure short-term memory store.

Western-Blotting

After 10 days of training, rats were sacrificed and we carried out western blot assays of the molecular markers of synaptic plasticity CaMKII and AMPA receptor GluA1 subunit (Glu A2 ?). We also quantified the level of NMDA receptor subunits GluN1, GluN2A and GluN2B. The western blot analysis was carried out in the CA1 and CA3 region of the dorsal hippocampus as well as in the DG and the prefrontal cortex.

Global constitution of NMDA receptors

First, we quantified the expression level of the total form of the different subunits constituting NMDA receptors within both the PFC and the dorsal hippocampus. No changes in the overall expression of these subunits were observed as compared to control rats just exposed to the maze after training in any of the three behavioral tasks and whatever the brain structure studied. For GluN1 the expression level was: for RM vs controls in CA1 $p = 0.466$, in CA3 $p = 0.408$, in the DG $p = 0.9389$, in the PFC $p = 0.6353$; for LIWM vs Control in CA1 $p = 0.7156$, in CA3 $p = 0.1676$, in the DG $p = 0.2638$ in the PFC, $p = 0.7518$; for

HIWM vs controls in CA1 $p = 0.9353$, in CA3 $p = 0.5899$, in the DG $p = 0.8301$ in the in the PFC $p = 0.9386$ (**Figure 2B**). For GluN2A: for RM vs controls in CA1 $p = 0.9863$, in CA3 $p = 0.8121$, in the DG $p = 0.9389$, in the PFC $p = 0.5971$; for LIWM vs Control in CA1 $p = 0.7735$, in CA3 $p = 0.6950$, in the DG $p = 0.6933$ in the PFC, $p = 0.546$; for HIWM vs controls in CA1 $p = 0.8916$, in CA3 $p = 0.8533$, in the DG $p = 0.8857$ in the in the PFC $p = 0.8172$ (**Figure 2C**). For GluN2B: for RM vs controls in CA1 $p = 0.8155$, in CA3 $p = 0.9789$, in the DG $p = 0.8011$, in the PFC $p = 0.9643$; for LIWM vs Control in CA1 $p = 0.7911$, in CA3 $p = 0.2398$, in the DG $p = 0.5206$ in the PFC, $p = 0.2875$; for HIWM vs controls in CA1 $p = 0.7548$, in CA3 $p = 0.7513$, in the DG $p = 0.1015$ in the in the PFC $p = 0.3690$ (**Figure 2D**).

GluA1 phosphorylation ratio is modulated in the DG after WM training

The GluA1 subunit of the AMPA receptor is a key molecule involved in the expression of both LTP and LTD. Our results revealed no changes in the expression level of GluA1 in all groups and in all studied structures (for RM vs controls in CA1 $p = 0.3072$, in CA3 $p = 0.542$, in the DG $p = 0.6353$, in the PFC $p = 0.560$; for LIWM vs Control in CA1 $p = 0.1509$, in CA3 $p = 0.8631$, in the DG $p = 0.6744$ in the PFC, $p = 0.1426$; for HIWM vs controls in CA1 $p = 0.359$, in CA3 $p = 0.8815$, in the in the PFC $p = 0.569$), except for a 140% increase observed specifically in the DG after HIWM training as compared to the control group (**Figure 2A**) ($p=0.0005$). We also analyzed the phosphorylation state of GluA1. We showed that the phosphorylation ratio of the GluA1 subunits at the ser831 and ser845 sites does not differ from the control group after training in any behavioral condition in CA1, CA3 or the PFC (For the ser831 site phosphorylation: for RM vs controls in CA1 $p = 0.9863$, in CA3 $p = 0.6547$, in the PFC $p = 0.946$; for LIWM vs Control in CA1 $p = 0.5845$, in CA3 $p = 0.7737$, in the PFC $p = 0.7737$; for HIWM vs controls in CA1 $p = 0.3102$, in CA3 $p = 0.7968$, in the in the PFC $p = 0.7581$; For the ser845 site phosphorylation: for RM vs controls in CA1 $p = 0.6454$, in CA3 $p = 0.7917$, in the PFC $p = 0.7968$; for LIWM vs Control in CA1 $p = 0.2890$, in CA3 $p = 0.9108$, in the PFC $p = 0.8857$; for HIWM vs controls in CA1 $p = 0.0611$, in CA3 $p = 0.5612$, in the in the PFC $p = 0.7002$) (**Figure3 and Figure S2**). In the DG and after RM training, we did not observe any change in the phosphorylation ratio at either phosphorylation site (for ser831 site $p = 0.3953$; for the ser845 site $p = 0.7807$). However, we observed a significant decrease of the ser845 phosphorylation ratio after LIWM training and as compared to controls ($p=0.0072$) (figure 3). In the same structure and after HIWM training, we did not observe any significant change in the phosphorylation ratio of GluA1 as compared to control, but we observed an increase of both ser831 and ser845 phosphorylation ratio as compared to the LIWM group (for ser831 site $p = 0.0494$; for the ser845 site $p = 0.0015$) (**figure 3**). No other significant differences between groups were observed.

HIWM training induces an increase in CaMKII activation.

Because CaMKII is critical in modulating synaptic strength (Hell 2014; Shonesy, Jalan-Sakrikar et al. 2014), we first estimated the expression level of CaMKII after training in the radial maze. We did not observe any significant changes in any studied structure and after any behavioral training as compared to the control group (for RM vs controls in CA1 $p = 0.9097$, in CA3 $p = 0.7760$, in the DG $p = 0.5460$, in the PFC $p = 0.4171$; for LIWM vs Control in CA1 $p = 0.8525$, in CA3 $p = 0.8631$, in the DG $p = 0.7422$, in the PFC $p = 0.9208$; for HIWM vs controls in CA1 $p = 0.8976$, in CA3 $p = 0.4723$, in the DG $p = 0.542$, in the in the PFC $p = 0.7454$) (**Figure S1**). We then assessed the level of CaMKII activated form by quantifying the phosphorylation ratio of CaMKII (P-CaMKII/total-CaMKII) and observed, in the DG, a large increase of 160% (as compared to controls) of CaMKII activated form after HIWM training ($p = 0.0046$), and a significant decrease after RM task ($p = 0.0474$) (Fig 4). In contrast, in CA1, we observed an increase of 122% of CaMKII activated form as compared to controls after RM training ($p = 0.0428$), but a significant decrease after LIWM training ($p = 0.0148$). We did not observe any other changes in any group or for any structures. (for RM vs controls in CA3 $p = 0.3225$, in the PFC $p = 0.6189$; for LIWM vs Control in CA3 $p = 0.8631$, in the DG $p = 0.4576$, in the PFC $p = 0.3374$; for HIWM vs controls in CA1 $p = 0.6656$, in CA3 $p = 0.3784$, in the in the PFC $p = 0.4138$).

CaMKII activation is correlated with GluA1 phosphorylation.

We wanted to confirm that the increase in CaMKII activation observed in the DG after the HIWM task might be indeed related to synaptic plastic changes. We thus assessed for each subject the correlation between the phosphorylation ratio of GluA1 (ser831 and ser845) and the phosphorylation of CaMKII. We found a positive correlation between Ser831 phosphorylation and CaMKII activity specifically after HIWM training ($p = 0.0209$) confirming the key role of CaMKII in the phosphorylation of GluA1 at the ser831 site. No other significant correlation was observed between GluA1 phosphorylation and CaMKII activity (after RM training, for ser831 phosphorylation vs CaMKII activity: $p = 0.7528$, for ser845 phosphorylation vs CaMKII activity: $p = 0.7414$. After LIWM training, for ser831 phosphorylation vs CaMKII activity: $p = 0.0965$, for ser845 phosphorylation vs CaMKII activation: $p = 0.2554$. After HIWM training, for ser845 phosphorylation vs CaMKII activity: $p = 0.2568$).

Discussion

Here, we show that behavioral training involving the long-term or short-term storage of information and/or the processing of PI differentially affects the expression and the phosphorylation state of biological markers of synaptic plasticity in the three main areas of the dorsal hippocampus. Our western-blot analysis 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 GluA1 subunit and in the phosphorylated/active state of CaMKII.

It has extensively been shown that LTP processes within the hippocampus require a sustained increase in the number of AMPA receptors at the synaptic level (Malinow 2003). Consequently, our finding that RM training does not induce any change in GluA1 expression may seem surprising. However, this may be due to the fact that this task requires a gradual and long-term storage of information acquired over several days. Our animals have been sacrificed after 10 days of training, a time when probably the acquisition of the task rules has already been achieved. This fact may explain why no change in GluA1 expression has been observed after 10 days of RM training. In fact, a new (unpublished) study from our group seems to support this hypothesis. This study shows that a transient increase in REM sleep (known to be linked to LTP and memory processes) is observed only after the fifth day of RM training.

Contrasting with the RM task, the LIWM task requires the short-term storage of information. In this delayed-non-match-to-place, different pairs of arms are used for each trial. The deleterious action of PI is thus negligible in this task (as reflected by the behavioral performance indicating no decrease in performance such as the one seen after HIWM training). After LIWM training, we observed a decrease in ser845 phosphorylation in the DG. The dephosphorylation of ser845 has been described as a signal for GluA1 internalization and lysosomal degradation leading to LTD (Lee, Barbarosie et al. 2000). Our results thus suggest that, when the interference load is not strong enough to induce a drop in behavioral performances (as it is the case in HIWM), LTD processes may occur to erase memories of the trials that have just been performed, and that are no longer useful. After the HIWM task, on the other hand, when the load of interference is too high to be dealt with efficiently (and when a decrease in performance is observed), the level of phosphorylation of these GluA1 subunits in the DG of HIWM trained rats was found to be unchanged as compared to controls level. We may thus hypothesize that the increase in GluA1 expression after HIWM training reflects changes in cytosolic stores of AMPA receptors, but may not be a sign of an increased integration of new AMPA receptors at the synaptic level. These

receptors may just be massively available in the DG granule cells of animals trained to be extremely flexible in their information processing (HIWM task). This extreme cognitive flexibility could require fast relocation of these receptors from the cytosol to the PSD for rapid memory storage, and from the PSD to the cytosol for fast forgetting once this information becomes irrelevant. HIWM training could thus require both the recruitment and internalization of functional AMPA receptors at the PSD level. It has been shown that blocking hippocampal AMPA-receptors-dependent LTP could impair WM (Reisel, Bannerman et al. 2002; Schmitt, Sprengel et al. 2005). On the opposite, work from our team showed that the inhibition of GluA1 dephosphorylation and LTD processes impaired the processing of interference in WM (Nicholls, Alarcon et al. 2008). Altogether, these results suggest that WM may rely on both hippocampal synaptic potentiation and depression. Indeed, it is worth noting that the level of phosphorylation of GluA1 subunits, even if not increased as compared to the yoked control group, was in fact (although modestly) increased in the DG of HIWM rats as compared to rats trained in the LIWM tasks suggesting that the processing of PI could in fact require the phosphorylation of AMPA receptors, and therefore synaptic potentiation. This increase in the phosphorylation of GluA1 was also correlated to CaMKII activity. In fact, CaMKII activity was massively increased in the DG of rats trained in the HIWM task. As mentioned above, CaMKII has long been shown to be involved in LTP and long-term memory storage (for review see (Lisman, Yasuda et al. 2012)), and we here showed that rats trained in the long-term/RM paradigm did expressed an elevated amount of activated (phosphorylated) CaMKII in the CA1 area of the dorsal hippocampus. Recently, however, CaMKII was also shown to be involved in LTD and inverse synaptic tagging of inactive synapse via interaction with the IEG *Arc/Arg3.1* protein (Okuno, Akashi et al. 2012). Using immunohistochemistry, we have shown that HIWM training induced an increased expression of *Arc/Arg3.1* in the DG (Joseph, personal communication), but of an inactivation in the same area of *Zif268* and *c-Fos* (Joseph *et al.*, submitted), two IEGs strongly involved in LTP and long-term memory storage (Dragunow 1996). The increase of CaMKII in the DG could thus reflect two processes. First, 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, the form of CaMKII involved in LTD (β -CaMKII) is not the same as the one we observed to be increased (α -CaMKII). The other hypothesis 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 the increase in synaptic potentiation induced by the phosphorylation of CaMKII (but also GluA1) would be short-lived 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 2003).

Further work should determine which of these two possible processes is involved in forgetting and the processing of PI. However, we here confirmed that the DG of the dorsal hippocampus is a crucial area involved in these processes. Our most significant results were found in this brain region for rats trained in the HIWM paradigm. This result is concordant with our previous study (Joseph *et al.*, submitted) showing that HIWM training was responsible for a very restricted and specific inactivation of *Zif268* and *c-Fos* (but not *Arc*) expression in the DG, while inactivating this structure was beneficial to the processing of interference. It is therefore not surprising to see important changes in the expression of synaptic plasticity markers in this brain area after HIWM training. Our results can thus be explained as followed: processing of proactive interference could involve and require the inactivation of the DG and its pattern separation function, a result in agreement with previous findings showing that ablation of hippocampal neurogenesis (occurring in the DG) lead to improved processing of proactive interference (20). However, this same processing of interference could result in an increase of synaptic plasticity in the DG to cope with the increasing level of difficulty (reflected by the decrease of performance in the HIWM group) across days, although this increase in plasticity might be detrimental to optimal processing of interference. Alternatively, the increase of synaptic plasticity in the DG could involve not synaptic potentiation, but synaptic depression (inverse synaptic tagging) that could participate to the inactivation of the DG required for forgetting and the processing of interference.

Our study also highlights interesting aspects on hippocampal function and physiology. Our results show that a form of memory (i.e. RM) may induce opposite changes in two different areas of the hippocampus (i.e. CA1 and DG) suggesting that the hippocampus can no longer be seen as a whole, but as different sub-structures having sometimes antagonistic roles in memory processing. CA1 has long been shown to be implicated in the retrieval of spatial memory (Dillon, Qu et al. 2008) CA1 lesioned rats were tested in a spatial delayed non-match to place task with intratrial delays ranging from 10 seconds to 5 minutes (Kesner, Gilbert et al. 2002). Lesioned animals were only impaired when they were presented with the 5 minute delay giving to CA1 a potential role in intermediate memory retrieval. In line with these findings, our study shows that, in CA1, CaMKII is activated after RM training. On the opposite, CaMKII activity was found to be decreased in the DG after training in this task.

It has been shown that CA3 may support the process by which a complete memory can be retrieved from only partial or degraded cues represented in this memory, a function called "*pattern completion*" (Gold and Kesner 2005). In our study, the visuo-spatial cues did not change or move throughout the whole experiment, and therefore the absence of activity observed in this sub-region may not be surprising. More surprisingly, the PFC does not seem to be involved in LIWM, HIWM or RM training. Historically, the PFC was thought to be the

most important structure involved in WM (Fuster 2001). Recently, however, new studies seem to re-evaluate its role (Curtis 2006; Gisquet-Verrier and Delatour 2006; Postle 2006; D'Esposito 2007). These studies suggest that the PFC is not involved in the temporary on-line storage of information but rather in the control of motor activity required to prospectively organize an ongoing action. In particular in our WM tasks, we prevented such “proactive motor coding” by removing our animals from the maze between the sample and the choice phases of the two delayed-non-match-to-place HIWM and LIWM tasks. This may explain why we did not observe plastic changes in the PFC after training in any of the tasks involved in our study.

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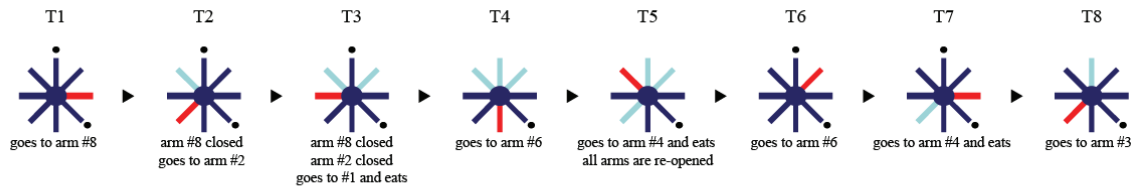
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Acknowledgments:

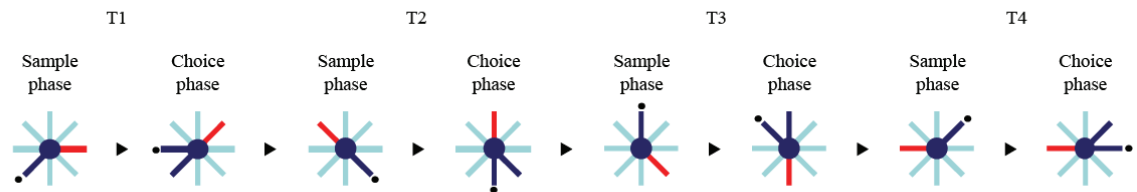
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Figures

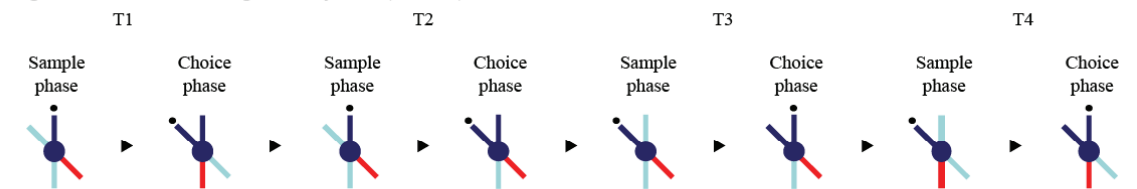
A Reference Memory (RM)



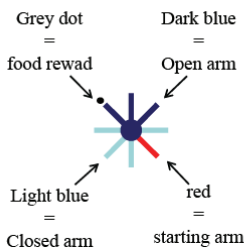
B Low interference Working memory task (LIWM)



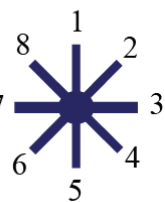
C High interference Working memory task (HIWM)



D



E



E

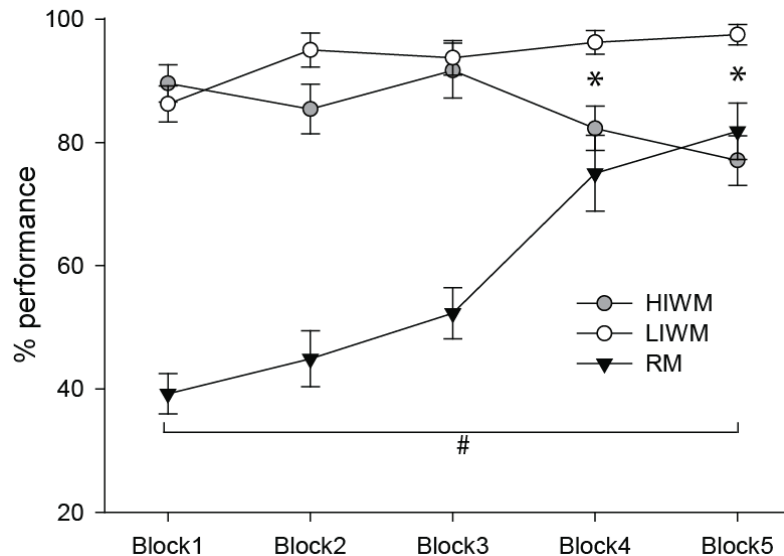


Figure 1. The build-up of proactive interference impairs the performance of rats trained in the HIWM task over time. (A-E) Behavioral paradigms (see Material and for details) and schematic representation of one training day for each of the experimental groups. (A) Reference memory (RM) training. The 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). (B) Low Interference Working Memory (LIWM) training. Each day consisted of 4 trials (T) of 2 phases each. (C) High Interference Working Memory (HIWM) training. 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 in order to complete an ongoing trial. (D and E) schematic representations of the maze with the arms' attributed numbers and legends. (F) Behavioral performances of each group of rats. Percentage of correct choices \pm s.e.m per Block (each Block = 2 days of training) in RM, LIWM and HIWM tasks. Black line represents the chance level for both WM tasks. * indicate significant difference between HIWM and LIWM groups * $P < 0.05$ (ANOVA), # indicates a significant block effect for the RM group (*Sheffe* $p < 0.0001$).

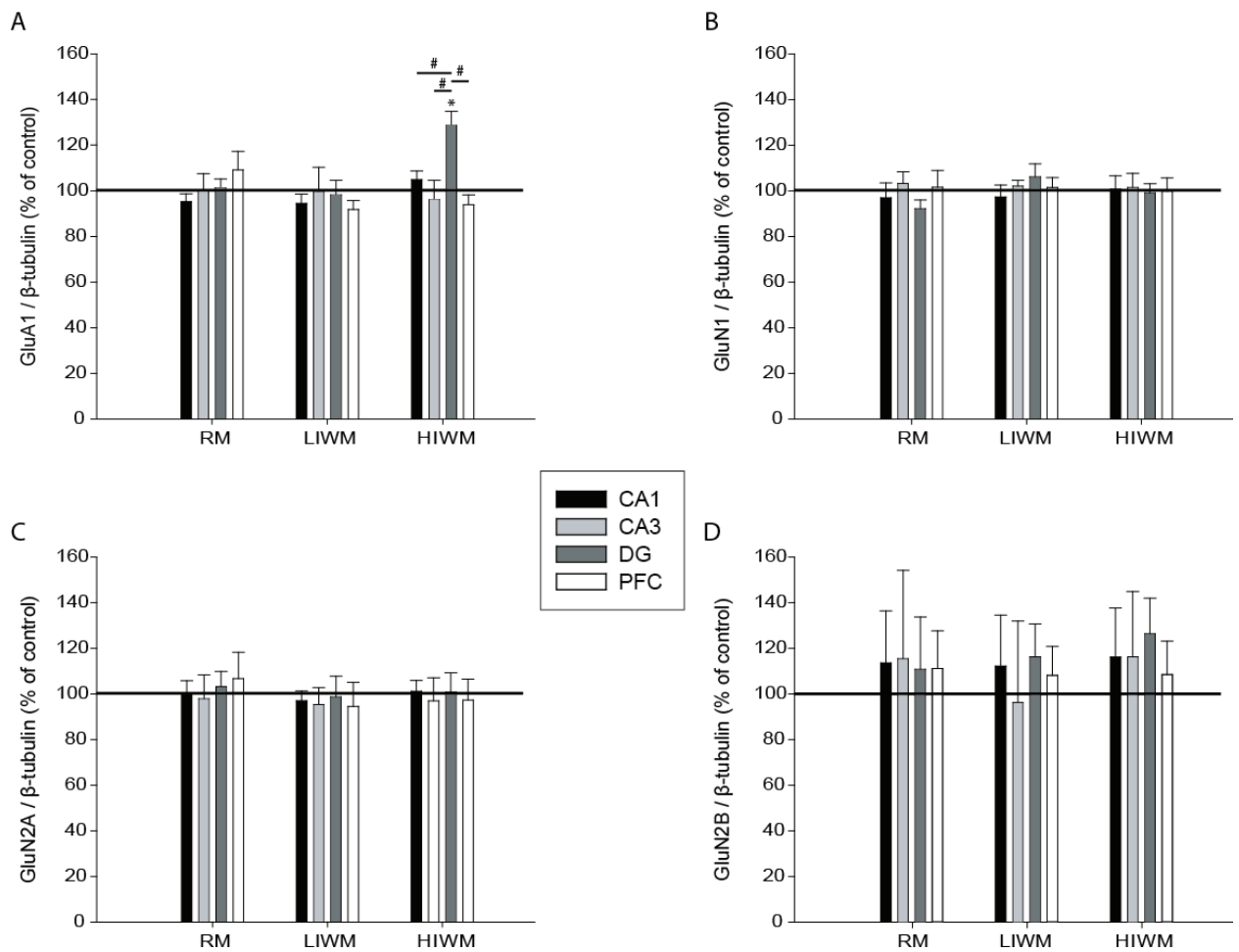


Figure 2. Western blot quantification of the overall GluN2A, GluN2B, GluN1, and GluA1 in the CA1, CA3, DG areas of the hippocampus and the PFC. Note that no change was observed in the overall quantity of GluN (NMDA receptors) subunits (**B**, **C**, **D**). We observed a significant increase in the overall GluA1 quantity after HIWM training (**A**). Data are expressed as mean \pm s.e.m.. Experimental group values are expressed as 100% \pm s.e.m. of average control group (n = 33). * indicates difference with the control group, # indicates difference between groups * P < 0.05; (Mann-whitney U-test).

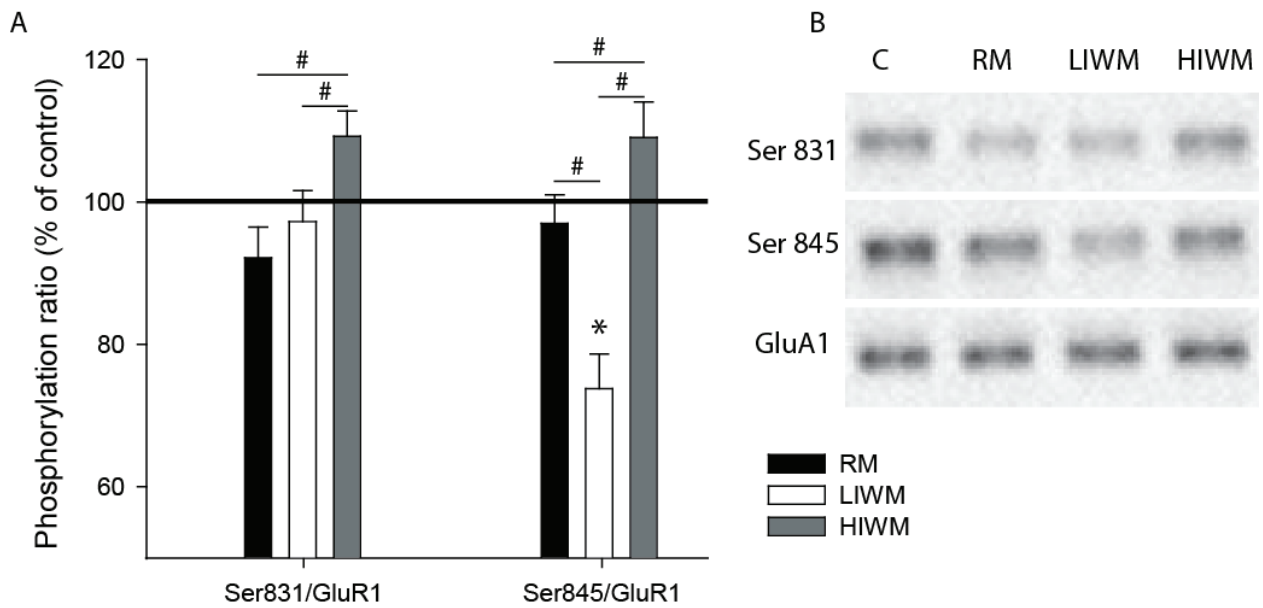


Figure 3. Western blot quantification of the phosphorylation ratio of GluA1 at the ser831 and ser845 sites in the DG. After LIWM training, Ser845 phosphorylation ratio is decreased as compared to controls and as compared to the HIWM group. Ser831 phosphorylation is increased in the HIWM group as compared to the LIWM group. Representative immunoblots and quantification of the gels are shown. Data are expressed as mean \pm s.e.m.. Experimental group values are expressed as 100% \pm s.e.m. of average control group (n = 33). * indicates significant difference compared to the control group: $p < 0.05$; (Mann-whitney U-test). # indicates significant differences between experimental groups : $p < 0.05$; (Mann-whitney U-test)

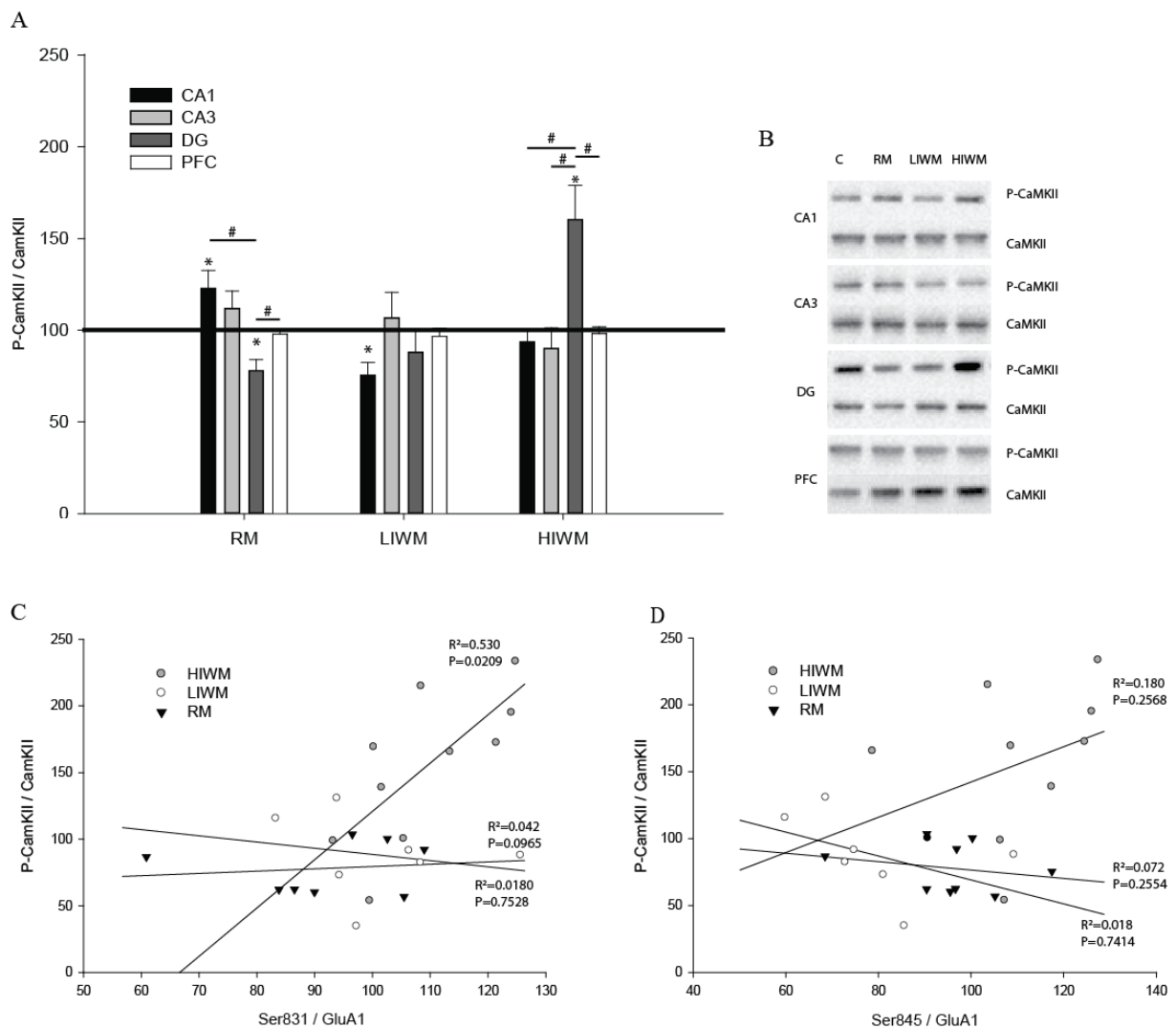
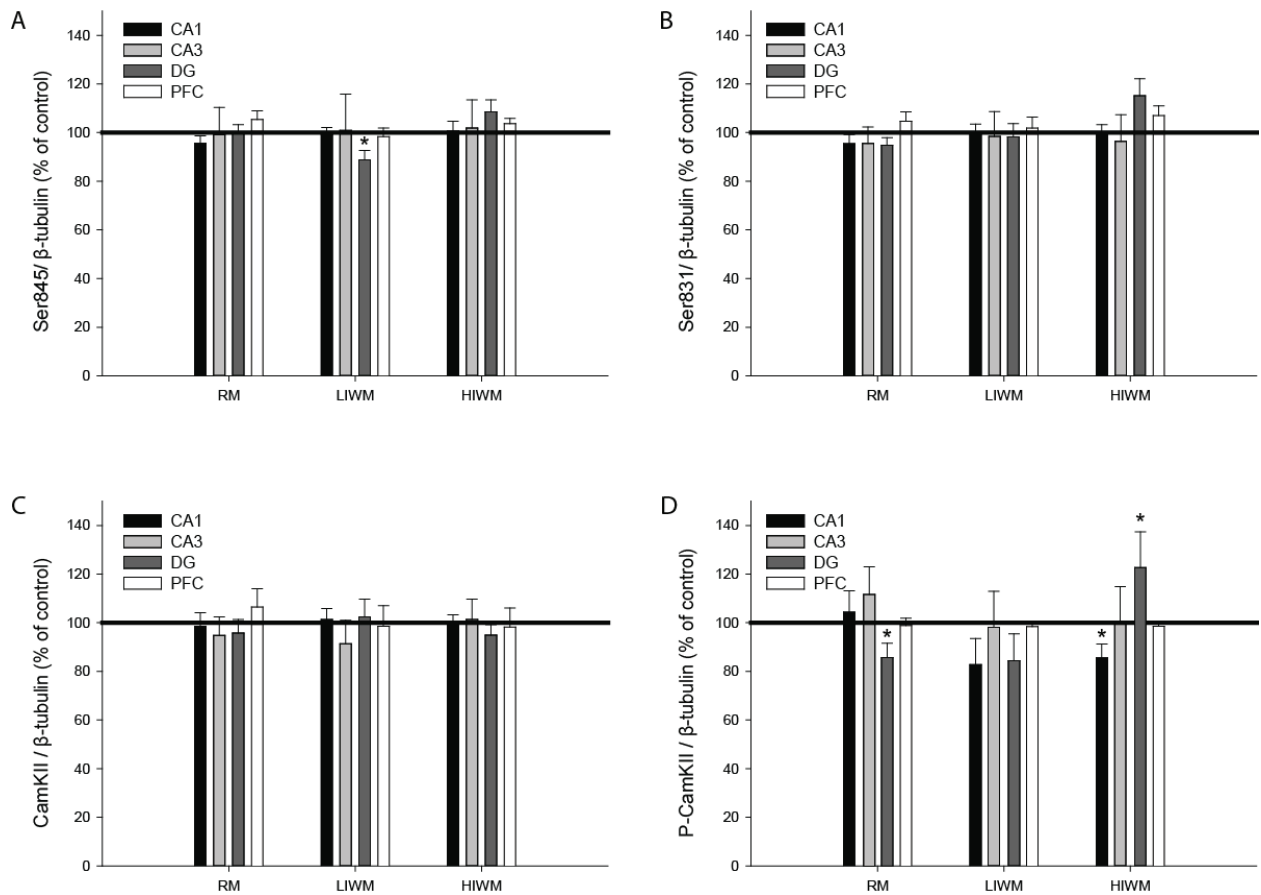
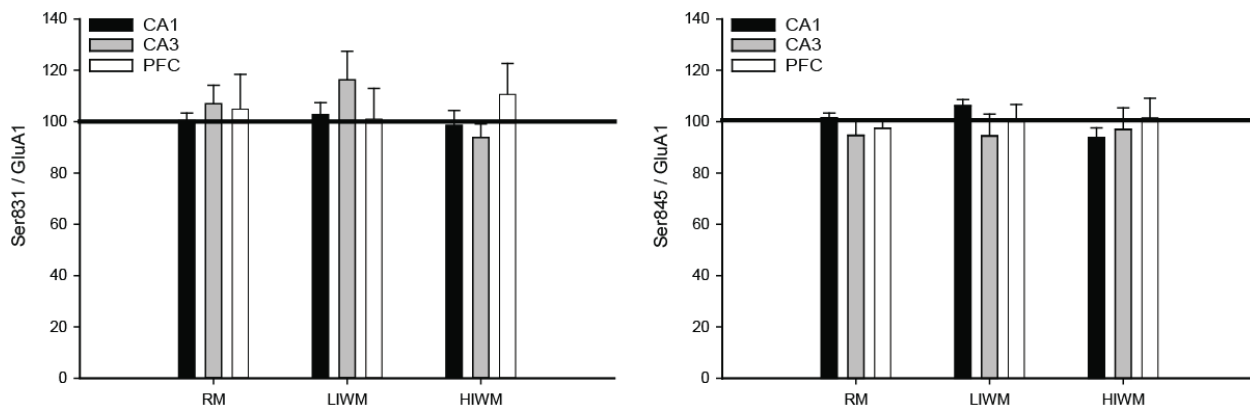


Figure 4. (A) Western Blot quantification of the phosphorylation ratio of CaMKII (P-CaMKII/ CaMKII). After RM training, phosphorylation of CaMKII is increased in CA1, but decreased in the DG as compared to controls. In contrast, after HIWM training, CaMKII phosphorylation is specifically increased in the DG. Representative immunoblots and quantification of the gels are shown. Data are expressed as mean \pm s.e.m., experimental group values are expressed as $100\% \pm$ s.e.m. of average Control group ($n = 33$). * indicates significant difference compared to the control group: $p < 0.05$; (Mann-whitney U-test). # indicates significant differences between experimental groups: $p < 0.05$; (Mann-whitney U-test). (B,C) correlation between CaMKII activity (phosphorylation ratio) and GluA1 phosphorylation at the ser831 (B) and the ser845 (C) sites. Data are expressed as mean \pm s.e.m., experimental group values are expressed as $100\% \pm$ s.e.m. of average Control group * $p < 0.05$; (Spearman correlation-test).



Supplementary figure 1. Western Blot quantification of overall ser845 (A), ser831 (B), CaMKII (C), and P-CaMKII (D) in the CA1, CA3 and DG areas of the hippocampus and the PFC. Data are expressed as mean ± s.e.m., experimental group values are expressed as 100% ± s.e.m. of average control group (n = 33). * indicates difference with the control group, # indicates difference between groups * P < 0.05; (Mann-whitney U-test).

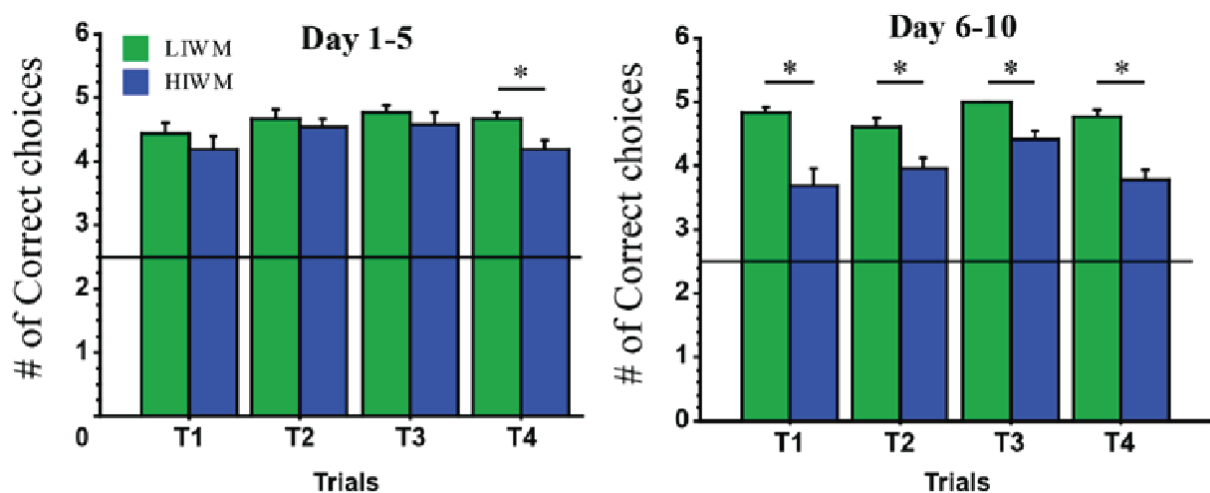


Supplementary figure 2. Western blot quantification of the phosphorylation ratio of GluA1 at the ser831 and ser845 sites in the CA1, CA3 and DG areas of the hippocampus and the PFC. Data are expressed as mean \pm s.e.m., experimental group values are expressed as 100% \pm s.e.m. of average control group (n = 33). * indicates difference with the control group, # indicates difference between groups * P < 0.05; (Mann-whitney U-test).

Chapter 3

Sleep patterns are differentially affected by the processing of information into long-term or short-term memory, involving or not forgetting of previously stored information.

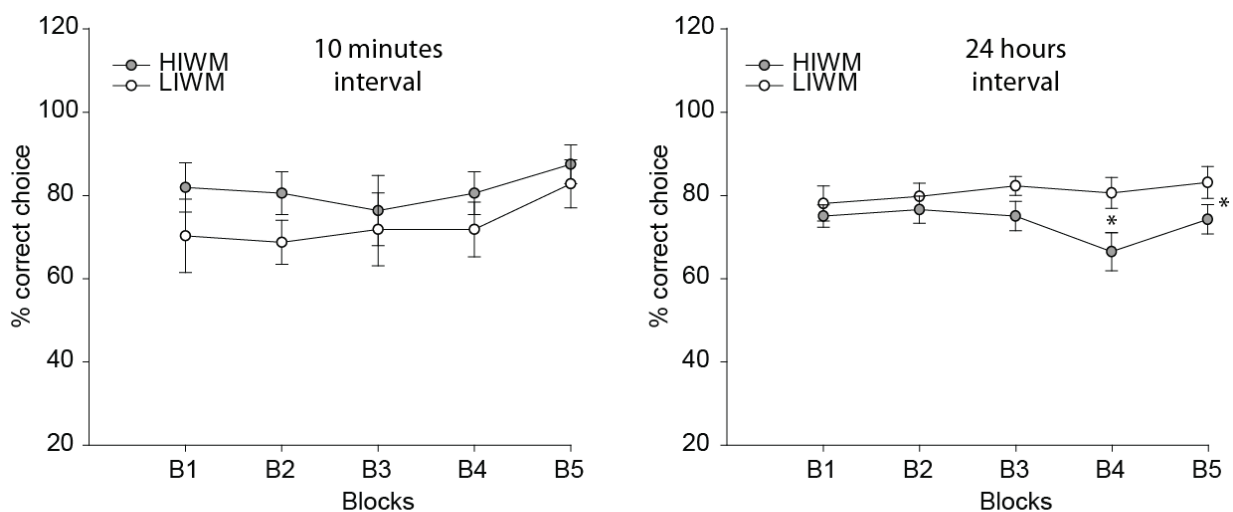
Behavioral data from the first two studies showed that rats trained in the HIWM task displayed a significant and slow decrease in performance over 10 days of training. This result seems to indicate that PI is building up over days and more precisely, that information supposedly stored in short-term/WM outlasts their purpose and may interfere several days later with the recall of newer information. However, we did not know if this PI effect occurred within a session (a day) or if the drop of performance observed during the last days of training was due to interfering memories from previous days. We thus wanted to see if rats' performance in WM declines from trial 1 to trial 4 and always returns to errorless performance on the first trial of the next day (after a 24h delay between this first trial and the fourth trial of the day before). This ability called “*resetting*” of WM has been proposed by Olton in 1978 (Olton, 1978). Resetting is defined as the capacity of the rats to erase or reset the contents of memory at the end of each daily session. To assess resetting in our rats, we next analyzed their performance not by day (as done in chapter 1), but by trial. We thus compared the percentage of correct choices made at each trial during the first five days (when the effect of PI is negligible) to the percentage of correct choices made at each trial when the effect of PI is more significant (Days 6 to 10) (see below).



Resetting does not occur at the end of training. Total number of correct choices made by animals described in on trials 1, 2, 3, and 4 (T1, T2, T3, and T4) of days 1 to 5 (left) and days 6 to 10 (right). Black lines represent the chance level.

We found that while there is a decrease in performance in trial 4 for rats trained in HIWM as compared to rats trained in LIWM (ANOVA $p = 0.0211$) at the beginning of training (Day 1-5), HIWM rats' performance were significantly lower than those of LIWM rats for all trials (including trial 1) at the end of training (Day 6-10) ($p < 0.0001$). This result indicates that at the end of training, no resetting occurs for rats trained in HIWM impairing the performance of the rats from the very first trial of the day. This result thus confirmed what we anticipated: that information supposedly stored in short-term/WM outlasts their purpose and interfere days later with the recall of newer information.

This progressive build-up of interference thus occurs on the long-term and might be happening when the brain is offline, during sleep. In order to assess if the build-up of interference is indeed more important if the animal is allowed to sleep, I conducted a new behavioral experiment. Instead of separating each training session by a 24-hour delay (during which the animals were allowed to sleep, as done in the previous studies), I reduced this delay to only 10min (during which rats did not sleep). When rats were tested with a 10 min delay between sessions, no difference was found between HIWM and LIWM trained rats contrasting with results obtained before (chapter 1 and 2) when rats were allowed to rest for 24 hours between sessions. The same amount of PI was presented in the two sets of experiments the two groups were, the only variable being the possibility for the rat to sleep between sessions.



Reducing the inter-session interval reduces the effect of proactive interference. Behavioral performances of each group of rats. Percentage of correct choices \pm s.e.m per Block (each Block = 2 days of training) in RM, LIWM and HIWM tasks. No statistical difference between LIWM and HIWM were observed with a 10minutes interval.

This new study shows that the build-up of interference occurs during the 24hours delay, and possibly during sleep phase. As we have seen in the introduction, sleep plays an important role in memory processes. These data thus made us think that sleep also probably plays a role in the consolidation of PI. Others have suggested on the opposite that sleep may be crucial for the offline processing and deletion of non-relevant and interfering information acquired during wakefulness (Tononi and Cirelli 2014). With chapter 3, we thus asked if

sleep is indeed a promoter or an impediment to forgetting. The results of this study described here in chapter 3 are written as an article we recently submitted for publication to *Journal of Neuroscience*.

In the next two chapters dedicated to the study of sleep pattern during the processing of PI, we switched to a different radial maze. This maze has larger dimensions and is located in a different room which could explain slight differences in the behavioral results.

Sleep patterns are differentially affected by the processing of information into long-term or short-term memory, involving or not forgetting of previously stored information.

Abbreviated title: Sleep, memory and forgetting in rats

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Abstract

It is commonly accepted that sleep is beneficial to memory processes, but it is still unclear if this benefit originates from improved memory consolidation or enhanced information processing. It has thus been proposed that sleep may also promote forgetting of undesirable and non-essential memories. Here, we tested the hypothesis that slow wave sleep (SWS) promotes forgetting of irrelevant information, more specifically when processing information in short-term/working memory (WM), while paradoxical sleep (PS) facilitates the consolidation of important information. We thus recorded sleep patterns of rats trained in a radial maze in three different tasks engaging 1) WM with high level of proactive interference (HIWM) requiring the storage of variable information and forgetting of previous trials, 2) WM with low level of interference (LIWM) not requiring such forgetting, or 3) reference memory (RM) requiring the long-term consolidation of invariable information. We observed a transient increase in PS amount the day the animal has learned the RM rule, and, in contrast, a positive correlation between the performance of rats in the HIWM (but not in the LIWM) task and SWS amount and slow wave activity. In addition, oscillatory events (SWS spindles, sharp-wave ripples) known to be involved in memory processing were differentially modulated by the three types of training. These results suggest that PS, but also rapid oscillations occurring during SWS would be specifically involved in the long-term storage of information whereas SWS and slow oscillations could be implicated in the proper treatment of memory interference and the forgetting of irrelevant information required for WM.

Introduction

Of all the possible functions of sleep, its role on learning and memory is certainly the most studied. Many studies have shown that brain structures engaged in mnemonic tasks can be reactivated during post-training sleep reactivation (Diekelmann, Buchel et al. 2011; Bendor and Wilson 2012; Oudiette, Antony et al. 2013). Others have shown that memory deficits can be observed after sleep deprivation (Albouy, Vandewalle et al. 2013; Inostroza, Binder et al. 2013). Some interpreted these results as the positive evidence of a selective function of sleep in memory consolidation (Peigneux, Laureys et al. 2004; Albouy, King et al. 2013; Rasch and Born 2013). On the opposite, others have suggested that these were manifestations of forgetting. Sleep has thus been hypothesized to promote forgetting, and in particular adaptive forgetting of undesirable memories, by wiping out weak synaptic connections randomly created during wakefulness (Crick and Mitchison 1983; Tononi and Cirelli 2014). Forgetting of irrelevant information could thus occur during sleep to promote a more efficient and selective recall of more important information.

Whereas numerous studies suggest that the long-term storage of information in memory requires long-term synaptic potentiation (LTP), notably in the hippocampal formation (Morris et al., 1982; Kandel, 2001), we and others have shown that long-term depression (LTD) may function to weaken previous memory traces thereby preventing those traces from interfering with newly encoded information, in particular in tasks requiring working memory (WM), a flexible form of short-term memory (Nicholls et al., 2008; Malleret et al., 2010). LTP and LTD being dependent on different neuronal firing pattern (high frequency stimulation preferentially facilitating LTP; and low frequency LTD), we hypothesized that memory is differentially modulated by the different sleep stages and their specific oscillations. Like memory, sleep is not unitary. Slow-wave sleep (SWS) is mainly characterized by low frequency oscillations, and on the opposite higher frequency oscillations are predominant during paradoxical sleep (PS - or Rapid Eye Movement REM sleep). The synaptic homeostasis hypothesis (Tononi and Cirelli, 2006) predicts that plastic processes occurring during wakefulness would be reduced (synaptic depression or downscaling) during SWS in order to decrease 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). Based on this hypothesis and our previous work (Nicholls et al., 2008; Malleret et al., 2010), we propose that the consolidation of information into long-term memory would preferentially engage PS that facilitates LTP possibly *via* its rapid oscillations (Bramham and Srebro, 1989), and that in an opposite manner forgetting of irrelevant information such as the ones temporarily stored in WM would selectively involve SWS potentially promoting synaptic depression (Tononi and Cirelli, 2014). Such hypothesis has never been tested before. To test this hypothesis, we trained rats in behavioral tasks

engaging the consolidation, or the forgetting of spatial information, and recorded their sleep patterns each day after training. Our results indicates that the long-term storage of information induces specific changes in the amount of PS, but also SWS-dependent rapid oscillations, whereas the proper treatment of memory interference and the forgetting of irrelevant information required for WM is correlated to an increase in slow wave activity.

Material and Methods

We trained different groups of rats in three different radial maze tasks for ten days. The radial maze allows testing in both reference memory (RM – long-term form of memory) and working memory (WM) in one single spatial environment and thus permits to determine a clear distinction between processes required for these different forms of memory. In order to segregate processes differentially involved in RM, WM and forgetting, we used 1) a modified version of a WM task with highly repetitive trials inducing a high level of proactive interference (High Interference Working Memory or HIWM task) in order to make forgetting of previous trials necessary to complete an ongoing trial (Malleret et al., 2010), 2) a low interference working memory task (LIWM) in which the information presented is different from one trial to the other (making forgetting of previous trials less required), 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 (see Figure 1). The three tasks thus involved different cognitive processes but were strictly comparable in terms of motor, emotional or motivational demands. Each day after training, a pool of implanted rats was placed in recording chambers in order to assess changes in sleep patterns initiated by the long-term or the short-term memory task and the flexible storage of information.

Subjects

A total of 173 Dark Agouti rats (200-250g) were purchased from Janvier, France. They were kept in a 12/12h (7am-7pm) light/dark cycle with *ad libitum* access to food and water. Rats were food deprived and maintained at 85% of their free-feeding weight throughout the whole radial maze experiment. Rats were housed in individual cages under the same environmental conditions as used for the behavioral protocol. Three groups learned a radial maze task (High Interference Working Memory HIWM, Low Interference Working Memory LIWM or Reference Memory RM task), and three groups served as their respective controls (Yoked HIWM, LIWM or RM). The animal care and treatment procedures were in accordance with the regulations of the local (Lyon 1 University CE2A-UCBL 55) and European (2010/63/EU) ethics committee for the use of experimental animals. Every effort was made to minimize the number of animals used or any pain and discomfort occurred during surgical or behavioral procedures.

Surgery and polygraphic recordings

Within these 173 animals, 37 rats were submitted before behavioral testing to surgical implantation of brain electrodes for sleep EEG and local field potential (LFP) recordings. These rats were anesthetized with Isofluran (5% induction, 1.5-2.5% for maintenance). Rimadyl (carprofen 5mg/kg) and Xylocain were injected subcutaneously before incision for analgesia. The skull was exposed, cleaned and burr holes were drilled for the insertion of screws (0.6mm diameter 1 mm long) for subdural EEG recordings. These screws were located over the right prefrontal cortex (AP +1; ML +1.2) and the cerebellum (reference electrode; AP, -9.0; L, ~1.0). For LFP recording, a 100µm diameter electrode (stainless steel, BioMedical Instruments, Germany) was inserted into the hippocampal CA1 region (AP, -3.3; L, 2.8; D, -2.4). Electromyogram (EMG) activity was assessed from the neck muscles by two wires embedded with a ~1 mm diameter gold plated tin sphere. Electrodes were linked to a sub-miniature plastic-one connector cemented to the skull. Skin was then sutured around the cement and local antiseptic was applied to avoid post-surgery infection. 2 ml of a 5g/l glucose solution was injected subcutaneously. During a post-surgery recovery period of 10-days, animals were placed in a sound-attenuated, ventilated and electrically isolated chamber (60 cm side cube) and connected by a tether to a rotating connector (Plastics One Inc., CT). After recovery, animals were habituated to the radial maze apparatus for 5-days. Each day after habituation or training, animals were placed in their recording chambers and EEG/EMG activity was amplified (1000×), filtered (0.3-500 Hz for EEG and 3-500 Hz for EMG, A-M systems) and recorded continuously using a National instrument data acquisition card (NI USB6353) and Matlabsoftware. The signals were sampled at 2 kHz. Data were stored on a computer for off-line analysis.

Behavioral Apparatus

An eight-arm radial maze was used throughout the entire experiment for all tasks. The apparatus consisted of an elevated radial maze (Poirier et al., 2008). Eight arms (65 cm long x 12 cm wide) ended by rectangular platforms (17 cm x 25 cm) were arranged around an octagonal central platform (33 cm diameter). Each arm could be automatically moved in an upward (open) or a downward position (close) by the experimenter monitoring rat movements using a video camera above the maze while located in a room directly adjacent to the testing room. Lowering (closing) an arm prevented access to the platform located at its end. Food rewards (Dustless Precision Pellets; Bioserve, Frenchtown, NJ) could be placed in squared food wells of 2 x 2 cm and 0.5 cm deep located on each platform. The maze was located in a room with a number of extramaze cues (e.g., door, furniture...) allowing the rats to use spatial (hippocampal-dependent) memory to remember locations of food rewards. All rats' movements in the maze were recorded for off-line examination.

Behavioral protocol

Food deprived rats had to retrieve food pellets at the end of the maze's arms using spatial navigation and distal visual cues surrounding the maze. Rats underwent a 5-day habituation period during which they became accustomed to the radial maze environment and learned to find rewards in the platform wells. At the end of this habituation procedure, rats were randomly assigned to one of the six groups (RM, YRM, LIWM, YLIWM, HIWM and YHIWM) described below.

-Reference Memory (RM) group

Rats trained in the RM task had to retrieve two food pellets placed each day invariably in the same two out of eight arms for the entire 10 days of training (see Figure 1a, e.g. arms #1 and #4). A rat was thus initially placed in a pseudo-randomly chosen starting platform (on which the food well had been removed), all arms of the maze being in the upward (open) position. Once the rat has left this platform to reach a new ending platform (consisting in a "visit" or "run"), the arm leading to this new platform was lowered in order to block the rat on the platform. After consuming the food reward in the case of a correct choice, or not (in the case of an incorrect choice), the rat was placed back in a transfer cage adjacent to the maze for a short inter-trial delay of 15 seconds. The arms leading to the previously chosen platform(s) remained lowered (close) in order to prevent working memory use and errors. After both food pellets were retrieved, the two previously baited arms were re-baited and all arms were replaced in the upward (open) position. Rats underwent eight trials (runs) per day and the maximum score per day was thus fixed at 8 pellets eaten. The latency to choose an arm as well as the number of correct choices were scored. Each experimental RM rat was paired with a yoked control (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 (RM) matched rat. The starting and destination platforms varied between trials in such a way that yoked controls could not use motor memory to predict the arm location they were forced into or anticipate a reward. Using yoked controls allows the experimenter to conclude that all differences in sleep patterns observed between groups are inherent to cognitive aspects of the task and not due to emotional, motivational or locomotor effects (see (Bontempi et al., 1999)).

-Low interference Working Memory (LIWM) group

Rats trained in the LIWM task (Figure 1b) were submitted to four trials per day, each consisting of a sample and a choice phase (4 trials x 2 phases = 8 runs matching the 8 runs performed by RM rats). In the sample phase, a rat was first allowed to enter one randomly

chosen baited arm/platform with all other arms being lowered (closed). This rat was then returned to a transfer cage adjacent to the maze for a short delay of 15 seconds (same cage and delay than in the RM task). During the subsequent choice phase, the rat was allowed to visit one of two adjacent arms, the familiar arm that had just been visited and empty of food, or an adjacent arm (right or left chosen pseudorandomly) containing a new food reward. The rat had to choose the novel arm in order to be positively reinforced (classical delayed non-match to place task, DNMT). Different pairs of arms were used for each trial. LIWM rats were also matched to a yoked control group (YLIWM). The same radial maze arms were used for the LIWM and YLIWM rats to make sure that the two groups were exposed to the same spatial information. Whereas LIWM rats had to learn a DNMT rule in order to successfully complete the task, YLIWM control rats were exposed to an equal number of non-match and match trials in a pseudorandom order to make sure that these rats could not predict the trial outcome. They were also forced to visit only one arm during each phase of the task and were not exposed to any cognitive choice as compared to LIWM rats.

-High interference Working Memory (HIWM) group

The HIWM task procedure (Figure 1c) was the same as the one described above for the LIWM task, except that the same pair of arms was used for every trial during the entire 10 days of training. This promoted a high level of interference and repetition in order to make forgetting of previous trials necessary to complete an ongoing trial (Malleret et al., 2010). Each experimental HIWM rat was paired with a yoked (YHIWM) control that performed the same amount of motor activity and ate the same number of pellets (see above the YLIWM group).

Signal and Statistical analysis

Overall behavioral performance and sleep analysis

The vigilance states were scored by 5 sec epochs. Double-blind off-line analysis was independently carried out using the classical following criteria: for wakefulness (Wk), low voltage/fast cortical EEG and high amplitude EMG; for NREM sleep, high voltage (>200 μ V) slow wave EEG (1-5 Hz) and low amplitude EMG; and for REM sleep, low voltage and predominant theta frequency (5-10 Hz) with an absence of muscle tone (Parmentier et al., 2002). For sleep-wake cycle parameters, duration of each vigilance state was assessed for each hour and during the first 12 hours post-training each day. These durations were expressed in minutes. Durations and behavioral performances were analyzed using two-way ANOVAs (Analysis of Variance) allowing repeated measures analyses with Days, Hours and Group (RM, LIWM, HIWM and controls) as main factors (Sigmaplot). Data from YLIWM, YHIWM and YRM were pooled (as a global control group) as no significant statistical

differences were found between these three groups for all considered analyses. *post hoc* (Scheffe) tests were further performed for particular within-group comparisons. In the RM task, the first day of rule learning was assessed by fitting performances of each rat to a classical sigmoidal curve (dose response EC50 analysis). The EC50 95% confidence interval was calculated (Sigmaplot) indicating for all rats the average period of highest increase in performance, i.e. the point in time when animals learn the rule of the RM task. This confidence interval (in days) also represents statistically the start and the end of learning. We also designed a simple Matlab routine that uses conditional operators (“if”, “then”, “else”) to virtually replicate the behavioral RM test using the same two rules as our real experiment: 1) not visiting the same arms twice unless both baited arms were visited, and 2) not going back in the starting arm. The starting arm and the visited arm were chosen randomly. The scores at the end of each session of 8 trials were recorded. This routine allowed us to calculate the chance level for the RM experiment by creating a virtual group of animals composed of the same number of individuals (n=12) that performed completely randomly. We then compared this virtual group with our experimental RM group and performed a Man-Whitney statistical test to determine the first day of learning for RM rats, *ie* when our experimental RM group deviated from chance (virtual group performance) level. In contrast, for HIWM and LIWM, two classical delay non-match tasks, chance level is simply fixed at 50%.

Spectral analysis

For spectral analysis, EEG power spectra were computed for 5 consecutive sec epochs within the frequency range of 0–500 Hz (total spectrum) using a multitaper Fourier transform analysis with the help of the chronux toolbox (Mitra and Bokil, 2008) (Chronux data analysis platform from <http://chronux.org>) and custom-written Matlab script (MatWorks, Inc.). The data were collapsed in 0.25 Hz bins. To prevent misinterpretation on epochs corresponding to transition phases between two vigilance states, the first and last epochs of PS and the first and last two epochs of SWS were omitted. Power densities obtained for each state were summed over the total band of 0–500 Hz (total power). To standardize the data, all power spectral densities at the different frequency ranges, i.e., delta, 0.5–5 Hz; theta, 5–10 Hz; sigma (spindles) 10–14 Hz; beta, 14–30 Hz; and gamma, 30–60 Hz, were expressed as a percentage relative to the total power of the same epoch (relative spectral power). All power spectral analyses were conducted for the first hour of concatenated SWS episodes and the first 20 minutes of concatenated PS episodes. Transient rapid theta (9-14Hz), spindles (10-14 Hz) and sharp wave ripples (SW-R; 100-300 Hz) were detected using a mean + 2 SD threshold of the integrated spectral power from the corresponding band calculated from EEG (rapid theta and spindles) and CA1 signal (SW-R). This analysis was conducted within the first 20 minutes of concatenated PS episodes after training for rapid theta and 60 minutes of SWS for spindles and ripples.

Correlation analysis

Increase in performance was calculated as the difference between the performance of a given day (day n) and the performance of the same rat the day after (day n+1). For all oscillatory parameters, the difference between the relative spectral power on one day (day n) and the relative spectral power on the next day (day n+1) was calculated (increase - or decrease - in relative spectral power). The same calculation was performed for the overall vigilance state parameters (duration, number of episodes average duration). A linear regression analysis was then performed to assess correlation between behavioral performances and sleep variables (duration, spectral power, progression of spectral power). Pearson's correlation coefficient was used to detect significant regressions. To compare the different regressions, Fisher's Z transform was calculated on the correlation coefficient to obtain a normally distributed population of values. t-tests were then performed (Sigmaplot) to compare the transformed r values for the different frequency bands. All data are expressed as means \pm S.E.M. and statistical difference was assessed with probability under 0.05 after checking for normality and homogeneity of variance.

Results

Proactive interference alters working memory performance.

Figure 1f represents the performance of our three experimental groups of rats. The increasing amount of interferences building up after numerous trials and days in the HIWM group provoked a significant decrease in performance observed during the last days of training for rats trained in this task as compared to rats trained in the LIWM task that improved their performance over time (80.0 ± 3.7 vs 97.1 ± 1.6 , $p < 0.0001$; see Figure 1,f). This first result highlights an often overlooked issue concerning WM; more precisely, that an information supposedly stored temporarily in WM can have an impact on the long-term when it becomes an interference for subsequent WM information as seen here with the decrease in performance over days (not seconds or minutes) in the HIWM group. We previously showed that forgetting such interference in this task is essential to process information in WM more efficiently (Malleret et al., 2010). In contrast to both WM groups, RM rats gradually increased their performance across successive days to reach a level of 83% of correct choices on the last day of training (Day effect $F(9, 270) = 30.20$; $p < 0.0001$; see Figure 1,f).

Learning the reference memory task transiently increases the amount of paradoxical sleep.

The sleep patterns of a subset of rats in the three groups described above were recorded ($n = 7$ in RM, $n = 8$ in HIWM and $n=8$ in LIWM - see performance for these specific rats in Figure 2a). Each day after training, these rats were placed in recording chambers to assess changes in the amount of SWS and PS that the different types of training initiated in the rats (their sleep recordings were systematically compared to those of yoked control animals – see methods). Overall, we found that the amount of SWS increased over days in all groups including the control group (significant Day effect; $F(9, 172) = 2.15$; $p = 0.028$), suggesting that non-purely cognitive aspects of the tasks could be responsible for changes in SWS patterns. However, noticeable changes in sleep patterns that could be attributed to cognitive processes were observed. First, an increase in PS was seen in the RM group on day 5 of training ($p = 0.0276$; Figure 3). Statistically, the 95% confidence interval (4.8 to 8.8 day) calculated from the sigmoid model (EC50 analysis) shows that the 5th (4.8 exactly) day is critical for learning the RM task as the majority of RM recorded rats start to reach a score greater than half of the maximum score ($> 4/8$ correct trials) on that day (Figure 2b). In addition, using a simple Matlab routine mimicking our behavioral test, we created a group of virtual rats that make random choices (performing at chance level – the Matlab routine estimated this chance level to be 34.2%). Compared to such a group, we found that the RM group started to have significantly higher performances ($p < 0.05$) on day 5. Altogether, these data show that day 5 is critical for learning the RM task. We can thus assume that the increase in PS is observed when the rats started to integrate and memorize

the RM task rule. In addition, the analysis of the cumulative curve of PS quantity on day 5 shows that this increase in PS began shortly after training as significant difference is observed between RM rats and controls (and the other groups) only three hours after the end of training ($p = 0.0039$; see Figure 4).

The processing of interference in WM is associated with an increase in SWS quantity and slow wave activity.

Another noticeable change in sleep patterns was specifically observed in the HIWM group. In this group, SWS increased by 12.8% as compared to controls levels after the second day of training ($p = 0.0264$, see Figure 3), a day before the peak (see Figure 2a) of performance seen for this group of rats (81.3 ± 0.05 % of correct responses). No such significant change was observed in the LIWM group suggesting that the increase in SWS may facilitate the processing of interference thus improving the performance of rats in WM the day after. Therefore, we asked whether there was any correlation between the time spent in SWS on a given day (day n) and the performance in HIWM the day after (day $n+1$). As hypothesized, SWS amount during the 12 hours post training (day n) were positively correlated to the level of performance during the first five days of training in the HIWM task (day 1-5; see Figure 5, a), a time window during which the performance of the animals were equivalent to the performance of rats trained in the LIWM task and not yet altered by the presence of interference (see Figure 1 and Figure 2a). Such correlation was neither found between performance and PS amount (Figure 5, c and d) in this group nor between performance and SWS or PS amount in the RM and LIWM groups (see Table 1), suggesting that SWS may specifically promote an efficient processing of proactive interference. Moreover, at the end of training (Day 6-10), when the performance of rats in HIWM was clearly affected by the accumulation of interference, such correlation between SWS amount and HIWM performance was not observed anymore.

SWS, and in particular slow wave activity (SWA) have been hypothesized to decrease synaptic strength to a baseline level that is beneficial for learning and memory (Giuditta et al., 1995; Tononi and Cirelli, 2006). On the other hand, another form of decrease in synaptic efficacy may promote forgetting of previous irrelevant information allowing the brain to acquire new information (Nicholls et al., 2008). We then asked if SWA may promote forgetting and the processing of proactive interference, and found that an increase in delta power (0-5 Hz) on the EEG signal during SWS could predict an increase in performance of rats in the HIWM task over two consecutive days (Figure 6a). Next given that the tasks implicate the hippocampus (Olton and Papas, 1979; Dolan and Fletcher, 1997) we carried out the same analysis on hippocampal LFP signals and found that the correlation between the increase in Delta power in the hippocampus and the increase in performance of rats trained in the HIWM task was even stronger suggesting that, during SWS, local process in the hippocampus are specifically involved (Figure 6d). Strikingly, no such correlation was found for the other groups of rats or for any other frequency band during SWS (see Figure 6

and Table 2) or for all frequency band during PS suggesting that, as predicted, a more efficient processing of proactive interference in a spatial working memory task depends on a very specific increase in SWA, that may facilitate hippocampal LTD and forgetting (Nicholls et al., 2008; Vyazovskiy et al., 2008; Malleret et al., 2010).

The processing of interference in the HIWM task is associated to a cumulative increase in rapid theta oscillation, sharp wave ripples but not spindles.

Previous work has shown that transient increases in theta frequency and spectral power during PS are associated with other phasic activity notably rapid eye movements, changes in heart rate and respiration, ponto-geniculo-occipital waves, or muscle twitches (Sakai et al., 1973; Rowe et al., 1999; Karashima et al., 2005), and are most prominent in the molecular layer of the dentate gyrus (Montgomery et al., 2008). We observed that such transient increases in rapid theta power (9-14 Hz) were superimposed with increases in high oscillations (100-200Hz, see Figure 7). Using a threshold of mean + 2 SDs, we detected 92.5 ± 1.5 transient epochs of rapid theta (per 20 minutes of PS recording for each animal), lasting 672 ± 15 ms in average for all groups. Although no changes in the number of rapid theta epochs (Figure 7b) was observed between groups, a significant cumulative increase in duration of these epochs was seen over days in the HIWM group (Figure 7c) as compared to controls ($p = 0.0025$) or LIWM group ($p = 0.0179$). This result suggests that rapid-theta-oscillations could be specifically involved in forgetting and processing of interference in WM.

Other sleep oscillatory activities have been found relevant for learning and memory processes. During SWS, hippocampal sharp waves corresponding to depolarizing events superimposed with fast ripple activity (100–300 Hz) in CA1 occur and form sharp wave ripples (SW-R) events. Recent studies showed that SW-Rs are critically involved in the replay of hippocampal activity and in consolidation of hippocampal-dependent memories during sleep, the selective suppression of SW-Rs during sleep periods resulting in a decrease in memory performance (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010). We analyzed high frequency events from the CA1 local field recordings and found 2713 ± 44 SW-R epochs per 60 minutes of SWS recording, lasting 30.1 ± 0.7 ms in average. Although no statistical changes in the number of SW-R epochs (Figure 8b) was observed between groups, a significant cumulative increase in the duration of these epochs was seen over days in all experimental groups (Figure 8c) as compared to controls. This increase was particularly prominent for HIWM rats ($p = 0.0311$), and more discrete for RM and LIWM rats, only occurring on the second, third and eighth days ($p < 0.05$ vs control). This result suggests that SW-R could be involved indiscriminately in the storage of spatial information.

A large body of literature indicates the importance of SWS spindles oscillations for learning and memory processes (for review see (Rasch and Born, 2013)). Notably, rats present increases in sleep spindles (12–15 Hz) density after a passive avoidance training

(Fogel et al., 2009) or after an odor-reward association learning (Eschenko et al., 2006). Using a threshold of mean + 2 SDs for the 10-14 Hz band, we found 549 ± 34 spindle epochs (per 60 minutes of SWS recording), lasting 709 ± 7 ms in average for all groups of trained rats. Although no statistical changes in the mean duration of spindle epochs (Figure 9c) was observed between groups, a significant cumulative increase in the number of these epochs was seen over days in the RM group (Figure 9b) as compared to controls ($p = 0.0239$), potentially confirming the role of spindles activity in the long-term consolidation of information.

Discussion

Many authors have suggested that sleep is beneficial to memory consolidation (Diekelmann and Born, 2010; Binder et al., 2012; Albouy et al., 2013a). Other have hypothesized that sleep may promote forgetting (Crick and Mitchison, 1983; Hardt et al., 2013; Welberg, 2013; Tononi and Cirelli, 2014) but it has never been clearly shown. This work represents the first comparative study aimed to assess how different cognitive processes involving long-term consolidation of information or in the contrary forgetting, can influence sleep patterns. We found that RM training involving the long-term storage of spatial information lead to a transient increase in PS the day the animal reach a significant level of performance. In contrast, we found that the processing of proactive interference in a HIWM task previously shown to depend on synaptic depression-dependent forgetting (Malleret et al., 2010) is linked to an increase in SWS quantity and SWA. These results seem to confirm Giuditta's sequential hypothesis of the function of sleep proposed two decades ago (Giuditta et al., 1995) and stating that, after learning, SWS would consist in the weakening of non-adaptive (useless) memory traces, while PS would deal with the storage of the remaining memory traces into long-term memory.

It has long been hypothesized that PS promotes learning and memory by facilitating consolidation of newly acquired information into long-term storage (Maquet, 2001; Siegel, 2001; Hobson and Pace-Schott, 2002). Replay of activity pattern seen in hippocampal neuronal networks during spatial learning was thus observed during PS episodes following learning (Louie and Wilson, 2001). In addition, it has been shown that training in various memory tasks transiently increases PS amount within a short time window, during which consolidation is sensitive to PS loss (Smith and Lapp, 1986; Smith and Rose, 1996; Datta et al., 2004). Our results confirm such findings as we found that training rats in a RM task transiently increases PS. In contrast, no such increase was found in SWS or in rats trained in WM or control tasks suggesting that PS may be specifically linked to the consolidation of information into long-term storage, whenever this information is consolidated (the day the animal learn the information to be consolidated; here, on day 5 for most of the subjects). Expression of the immediate-early gene *Egr1* (also known as *Zif268/Krox-26*), a marker of synaptic plasticity (Bozon et al., 2002), was found to be increased in hippocampal and

neocortical neurons when exploration of a novel environment or the induction of LTP were followed by PS (but not SWS) (Ribeiro et al., 1999; Ribeiro et al., 2002). We have recently found that PS amounts modulate hippocampal Egr1 expression, LTP and the consolidation of contextual information acquired during fear conditioning (Ravassard et al., 2009; Ravassard et al., in press). Therefore, it is reasonable to assume that the increase in PS transiently seen in rats trained in the RM task induces the same changes providing a cellular and molecular ground for consolidation of important information required for solving the RM task the day following the increase in PS.

In contrast to PS, SWS is known to be a non-permissive sleep stage for LTP induction (Bramham and Srebro, 1989), and it has been suggested that SWS, characterized by low frequency oscillations, facilitates instead the induction of LTD (Muzur, 2005). Cirelli and colleagues have thus shown that sleep is associated with the upregulation of molecules implicated in LTD (Cirelli et al., 2004). Such molecules include protein phosphatases such as calcineurin (PP2B) or PP1. Also, SWS is associated with higher levels of insulin, which promotes the internalization of glutamate AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and LTD (Cirelli and Tononi, 1998; Man et al., 2000), and *in vivo* and *in vitro* electrophysiological results suggest that SWS induces LTD in CA3 and CA1 regions of the hippocampus (Lubenov and Siapas, 2008) by internalization of specific AMPA receptor subtypes (Lante et al., 2011). We previously showed that blocking NMDAR-dependent hippocampal LTD results in an increase in behavioral flexibility and WM performance in a T-maze task, suggesting that NMDAR-dependent LTD is required for behavioral flexibility and may act by weakening previously encoded memory traces when new information is learned (Nicholls et al., 2008). On the opposite, we showed that inhibition of protein kinase A (PKA) leads to an increase in hippocampal LTD, cognitive flexibility and WM performance, specifically when repetitive information (proactive interference) are presented as it is the case in a HIWM task (Malleret et al., 2010). It is therefore not surprising that performance in this task is linked to an increase in SWS and SWA as observed here. Altogether, these data suggest that processing (forgetting) of proactive interference may request SWS-dependent synaptic depression mechanisms promoting de-phosphorylation and internalization of AMPA receptors.

This forgetting function of SWS is of course in stark contrast with the many studies suggesting the consolidation function of SWS (Maquet, 2001; Peigneux et al., 2001; Wilson, 2002). Nevertheless, as we have said earlier, post-training sleep reactivation of brain structures engaged in mnemonic tasks, as well as memory deficits observed after sleep deprivation, can also be seen as the positive evidence of a selective forgetting function of sleep. Forgetting is essential to our daily lives (Kraemer and Golding, 1997; Dudchenko, 2004; Levy et al., 2010). By promoting forgetting, SWS may sort information important to be consolidated from the one to be discarded. This hypothesis is in agreement with recent findings (Rauchs et al., 2011). Rauchs and colleagues have studied the impact of total sleep deprivation on directed forgetting. Directed forgetting is an experimental approach

consisting in presenting “to be remembered” and “to be forgotten” information that allows selectively decreasing or increasing the strength of individual memory traces according to the instruction provided at learning. The authors found that human participants to this study remember more “to be forgotten” items after sleep deprivation than after a normal night of sleep suggesting that sleep promotes the erasure of irrelevant information. Although this work does not conclude on the nature of the sleep phase involved in forgetting, our data suggest that SWS, and more specifically SWA, might be responsible for such a process. Indeed, SWA is a cardinal feature of SWS. However, other higher frequency oscillations characterize SWS. For instance, SWS-dependent hippocampal reactivation is suggested to occur mainly during bursts of activity known as sharp-wave ripple events (SW-Rs) (Buzsaki, 1986; Wilson and McNaughton, 1994; Buzsaki, 1998; King et al., 1999; Kudrimoti et al., 1999). Girardeau and colleagues found that a selective elimination of SW-Rs during post-training SWS resulted in performance impairment in rats trained in a radial maze on a RM task similar to ours (Girardeau et al., 2009). These results thus suggest that SWS, with its SW-Rs, may also facilitate memory consolidation through a different mechanism than SWA. High frequency oscillations during SWS may thus facilitate LTP and, by so, the storage of spatial information (Born, 2010) regardless of the training involved to learn this information, as we found here that both RM and WM training increased SW-Rs duration as compared to control levels. The common feature of our three tasks is that they all require the formation and the use of a spatial map of the behavioral room whereas the control group does not need such spatial map to obtain food rewards. That is why our results suggest a specific role of SW-Rs in spatial memory formation (Figure 8).

SWS spindles have been shown to play a key role in the reactivation of memory during sleep. Notably in humans, reactivations thus occur not only in temporal synchrony with spindles (12-14 Hz) but are also modulated by the amplitude of spindle events (Bergmann et al., 2012). Spindles have also been hypothesized to promote the hippocampo-neocortical transfer of new memory traces acquired during prior waking for long-term storage (Tamminen et al., 2010). It is therefore not surprising that the increase in spindle activity seen in our experiments was specifically observed during RM training, a task requiring the long-term storage of information. We can thus hypothesize that spindle oscillations could be involved in the hippocampo-neocortical transfer of invariable information useful for RM training (Rasch and Born, 2013).

In contrast, HIWM training requires both the flexible short-term storage of relevant information in combination to a forgetting of previously stored interfering material. We found that HIWM training was associated to a cumulative increase in the duration of rapid theta epochs during PS. It has been shown that during theta frequency oscillations synapses can either be depressed or potentiated in the hippocampus (Huerta and Lisman, 1993; Holscher et al., 1997). Rapid theta oscillations could thus promote bidirectional changes in synaptic plasticity required for the short-term storage of new information and at the same time the forgetting of previously stored information as required by HIWM training.

Altogether, these results also strongly suggest that it is probably cerebral oscillations (high versus low frequency) more than sleep phases (SWS versus PS) that control the consolidation or the forgetting of information.

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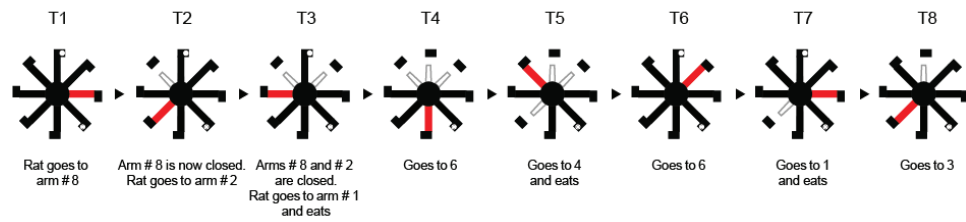
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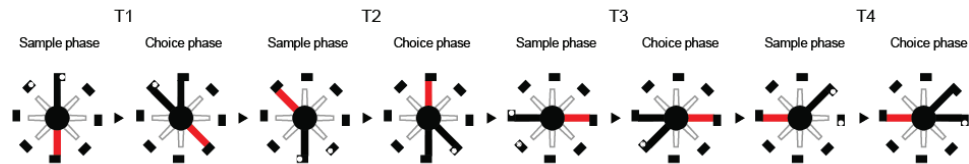
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Legends:

a Reference Memory (RM)



b Low Interference WM (LIWM)



c High Interference WM (HIWM)

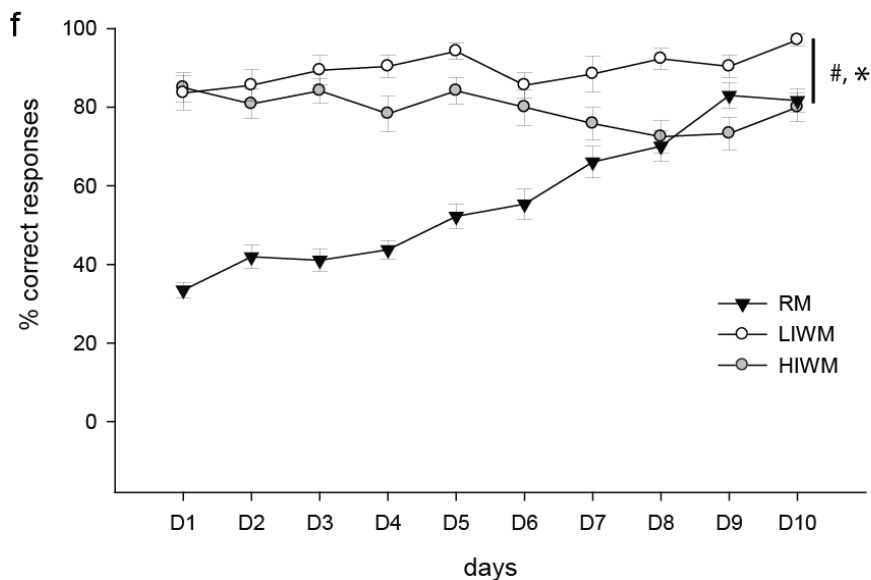
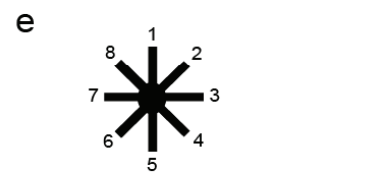
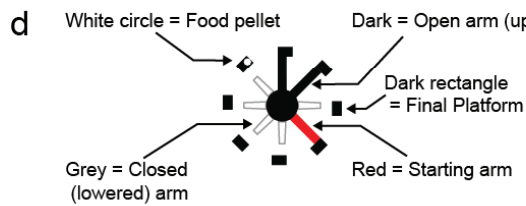
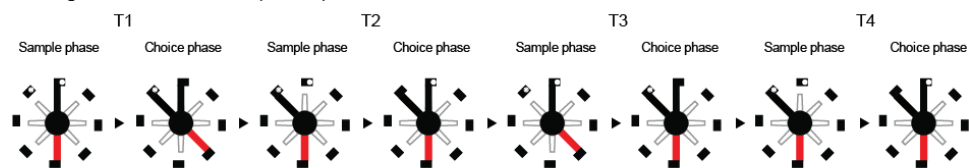


Figure 1. Behavioral paradigms and performance. a-e, A schematic representation of one day of training for each of the three different experimental groups. a, One daily session of RM training for a given rat. The same two arms (here 1 and 4, see e) were baited every day for each trial. Each daily session consisted of 8 trials (T1 to T8). During a given trial, the rat is able to visit one arm only. Dark represents open arms. Grey represents closed arms. Red represents starting arm. Circles represent food pellets (d). b, One daily session of LIWM training. Each day consisted of 4 trials (T1 to T4). Each trial (T) consisted of 2 phases. This task is a “delayed non-match to place” task during

which the animal must memorize a position in space (acquired 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 (arm) in space. In this task, different pairs of arms are used for each trial, so that forgetting of previous trials is not necessary in order to have good performance on an ongoing trial. **c**, 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 forget/ignore previous trials (e.g. T1 and T2) in order to complete an ongoing trial (e.g. T3). **f**, **Proactive Interference induces a decrease in performance in WM.** Percentage of correct choices \pm s.e.m per day in the RM (dark triangles, $n = 31$), LIWM (white circles, $n = 32$) and HIWM (grey circles, $n = 30$) tasks. ANOVAs revealed a significant Group effect [$F(2, 96) = 81.51$; $p < 0.0001$], a significant Day effect [$F(9, 729) = 10.72$; $p < 0.0001$], as well as a significant Group \times Day interaction [$F(18, 864) = 13.36$; $p < 0.0001$]. *Post hoc* analyses revealed that RM rats significantly improved their performance over time [$F(9, 288) = 45.15$; $p < 0.0001$] and reached 83% correct choices on the last day of training. In WM groups, we investigated how proactive interference affected learning. At the beginning of training, both LIWM and HIWM groups started at almost 85% of correct choices. Overall, rats kept high scores throughout the entire experiment, but HIWM rats showed a decrease in performance over days. This effect was statistically significant [$F(9, 306) = 2.00$; $p = 0.038$]. On the other hand, scores of LIWM rats slightly increased with time and reached 96% on day 10 [$F(9, 270) = 3.00$; $p = 0.002$]. More importantly, a significant difference in performance was observed at the end of training between LIWM and HIWM rats ($p < 0.0001$).

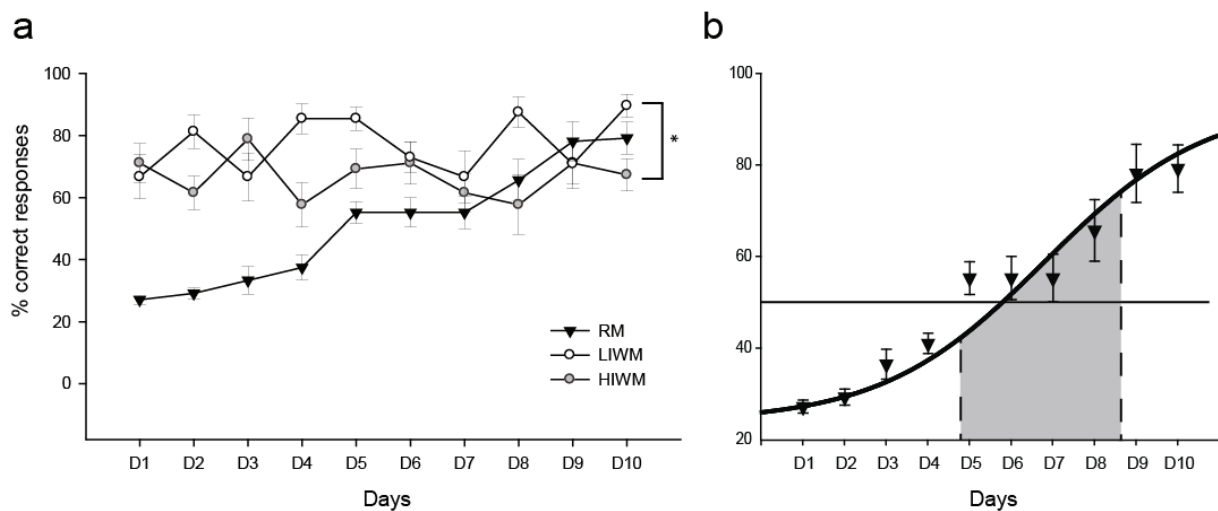


Figure 2. Performance of implanted and sleep-recorded rats, and modeling of performances in the reference memory task. **a**, Percentage of correct choices \pm s.e.m per day in the RM (dark triangles, $n = 7$), LIWM (white circles, $n = 8$) and HIWM (grey circles, $n = 8$) tasks. ANOVAs revealed a significant Group effect [$F(2,20) = 24.971$; $p < 0.0001$], a significant Day effect [$F(9, 180) = 4.46$; $p < 0.0001$], as well as a significant Group \times Day interaction [$F(18, 180) = 2.99$; $p = 0.0001$]. As observed in Fig. 1, RM rats significantly improved their performance over time [$F(9, 54) = 15.11$; $p < 0.0001$]; this subset of RM rats reached 79% correct choices on the last day of training. Concerning WM, at the beginning of training, both LIWM and HIWM groups started at almost 70% of correct choices. Overall, these subset of rats kept high scores throughout the entire experiment, but HIWM rats showed lower performance as compared to LIWM rats (*significant Group effect ($p=0.0071$)). **b**, The first day of rule learning was assessed by fitting performances of each rat to a classical sigmoidal curve (dose response EC50 analysis). The 95% confidence interval was calculated (Sigmaplot, grey area, from 4.82 to 8.78) indicating the highest increasing period for performances, ie the period when animals learn more. Note that the start of this area corresponds to a score up to 50% of correct choices (black line), and that the first day of learning was thus determined as the 5th (4.82 exactly – see above) day of training.

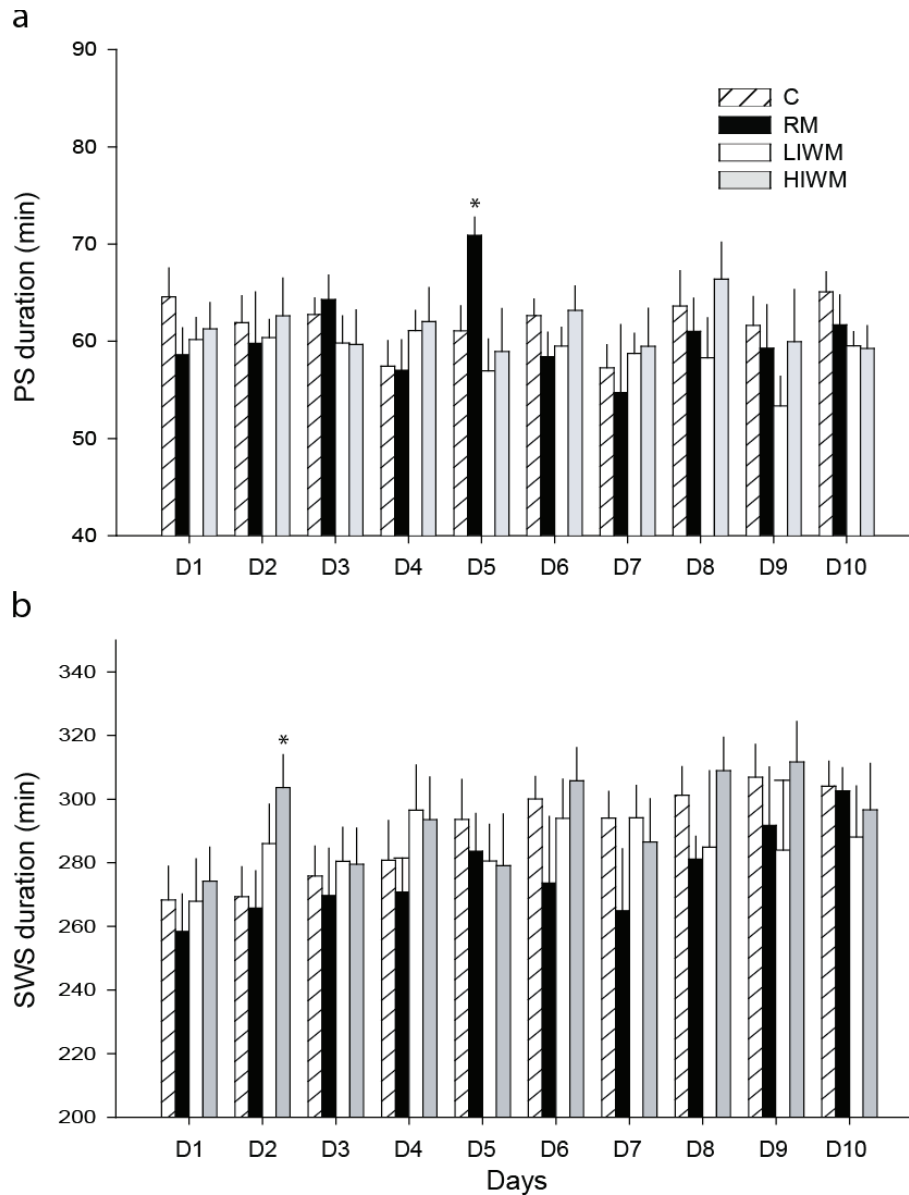


Figure 3. Transient increase in PS after training in the RM task, and in SWS after HIWM training. Quantitative variations of sleep states in RM (n=7, dark bars), LIWM (n = 8, white bars), HIWM (n= 8, light grey bars) and control (n = 14, hatched bars) rats. Data are expressed as mean \pm SEM (min.) over the 10 days of training. ANOVAs revealed a significant Day effect in all groups including the control group for SWS [$F(9, 172) = 2.15$; $p = 0.028$] suggesting that all behavioral procedures led to a general increase in SWS over the 10 days of training. However, a transient increase of PS was seen in the RM group on day 5 of training ($p = 0.0276$ vs Control group), and of SWS in the HIWM group the second day of training ($p = 0.0264$ vs Control group).

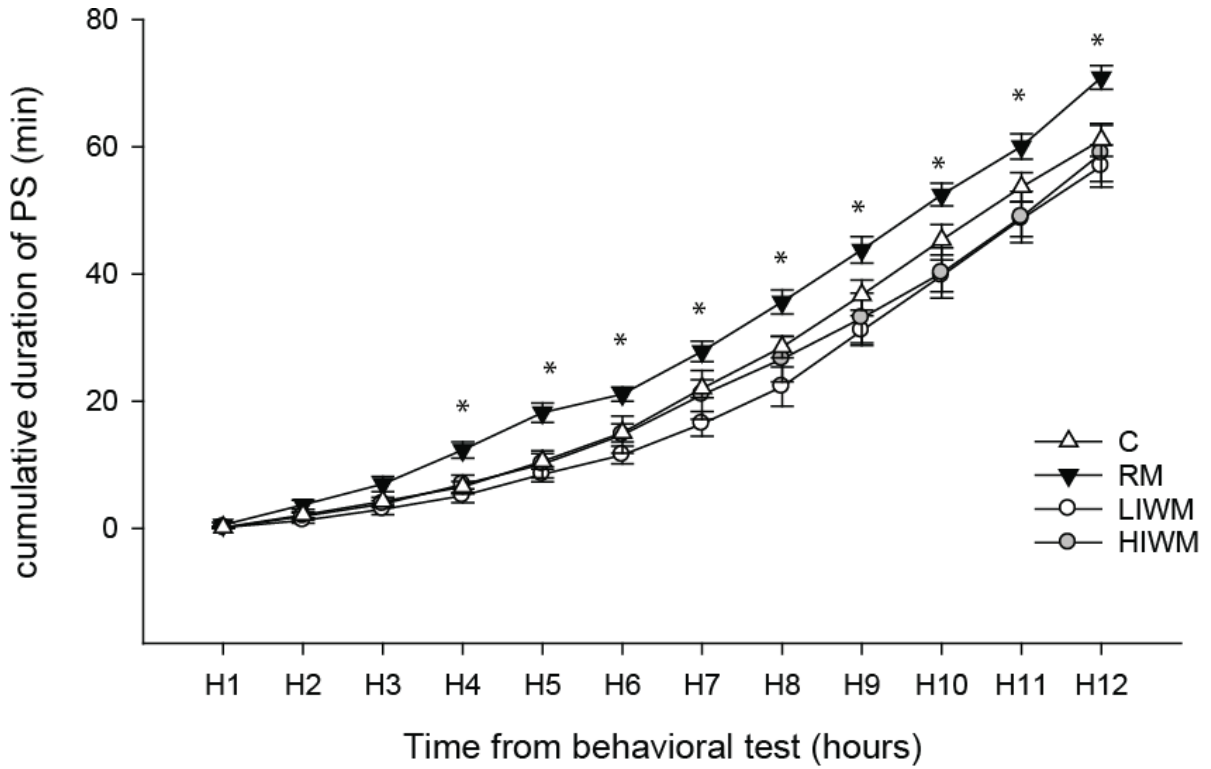


Figure 4. The increase in PS on day 5 of RM training is observed shortly after training. Mean cumulative duration (\pm SEM) of PS during the 12 hours following day 5 of training. ANOVAs revealed a significant Group effect [$F(3, 33) = 4.872$; $p = 0.065$] as well as a significant Group \times Hour interaction [$F(33, 363) = 1.827$; $p = 0.0045$]. *Post hoc* analyses indicate that the significant increase of PS quantity appeared on the 4th hour of recording (* $p < 0.05$, RM vs Control ; # $p < 0.05$, RM vs LIWM or HIWM).

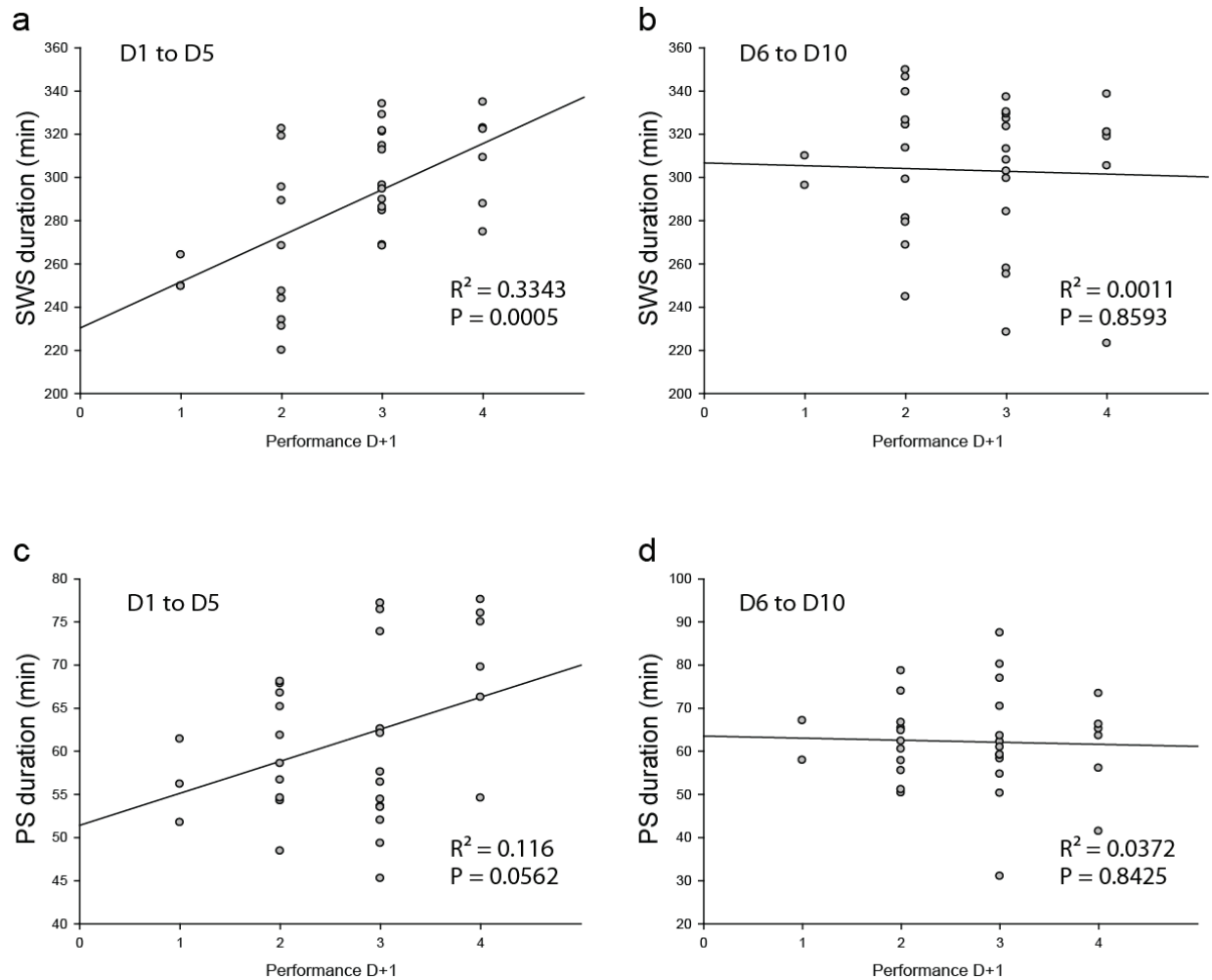


Figure 5. Performance of rats trained in the HIWM task is correlated to SWS amount. Correlation between SWS or PS recorded during 12 hours post training (day n) and performance of the rats in behavioral testing (HIWM task) on day n+1. a, A significant correlation (Pearson coefficient tested; $r^2 = 0.3343$, $p = 0.0005$) between SWS duration and performance was seen at the beginning of training (day 1 to 4) when performance was maximum and equivalent to performance of rats trained in the LIWM task (see Figure 1). No such correlation was found at the end of training (b), with PS duration (c and d) or in the other groups (Table 1).

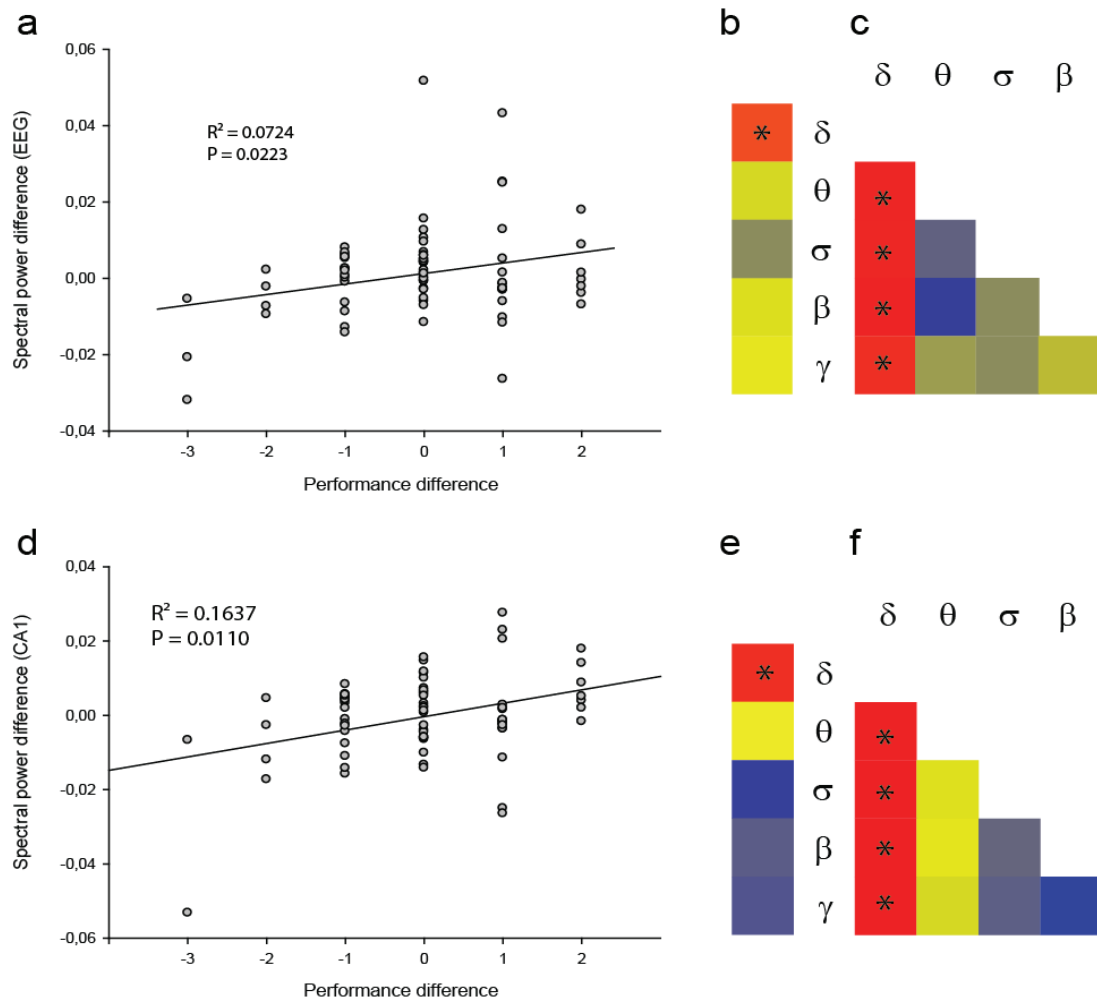


Figure 6. An increase in delta power is correlated to an increase in performance over two consecutive days of HIWM training. (a) A positive correlation was found ($r^2 = 0.0724$, $p = 0.0223$) between Delta band spectral power increase (calculated as the difference between the relative spectral power in EEG recordings on one day (day n) and the relative spectral power on the next day (day $n+1$)) and performance increase (calculated as the difference between the performance of a given day (day n) and the performance of the same rat the day after (day $n+1$)) for rats trained in the HIWM task. Relative spectral power was calculated based on the first hour of SWS for each animal and each day from EEG recordings. b-c, In color scale (red $p = 0$; blue $p = 1$), the Pearson correlation coefficients for each regression curve (b) showed that the increase in the delta band spectral power is the only oscillation variable correlated to the increase in performance in the HIWM task. The Fisher tests for equality of slopes (c) between each frequency band showed that the positive correlation between the increase in delta power spectrum and the increase in performance was significantly different from all increases in other frequency band and performance. d, Same analysis as in a) based on intrahippocampal CA1 local field recordings; a positive correlation was found ($r^2 = 0.1673$, $p = 0.011$) between Delta band spectral power and performance increases for rats trained in the HIWM task. e-f, Pearson correlation coefficients show significance only in the delta band and Fisher test for equality of slopes indicates the positive correlation in delta band statistically different from the other frequency band correlations in the HIWM task (see also Table 2 for the other tasks and PS).

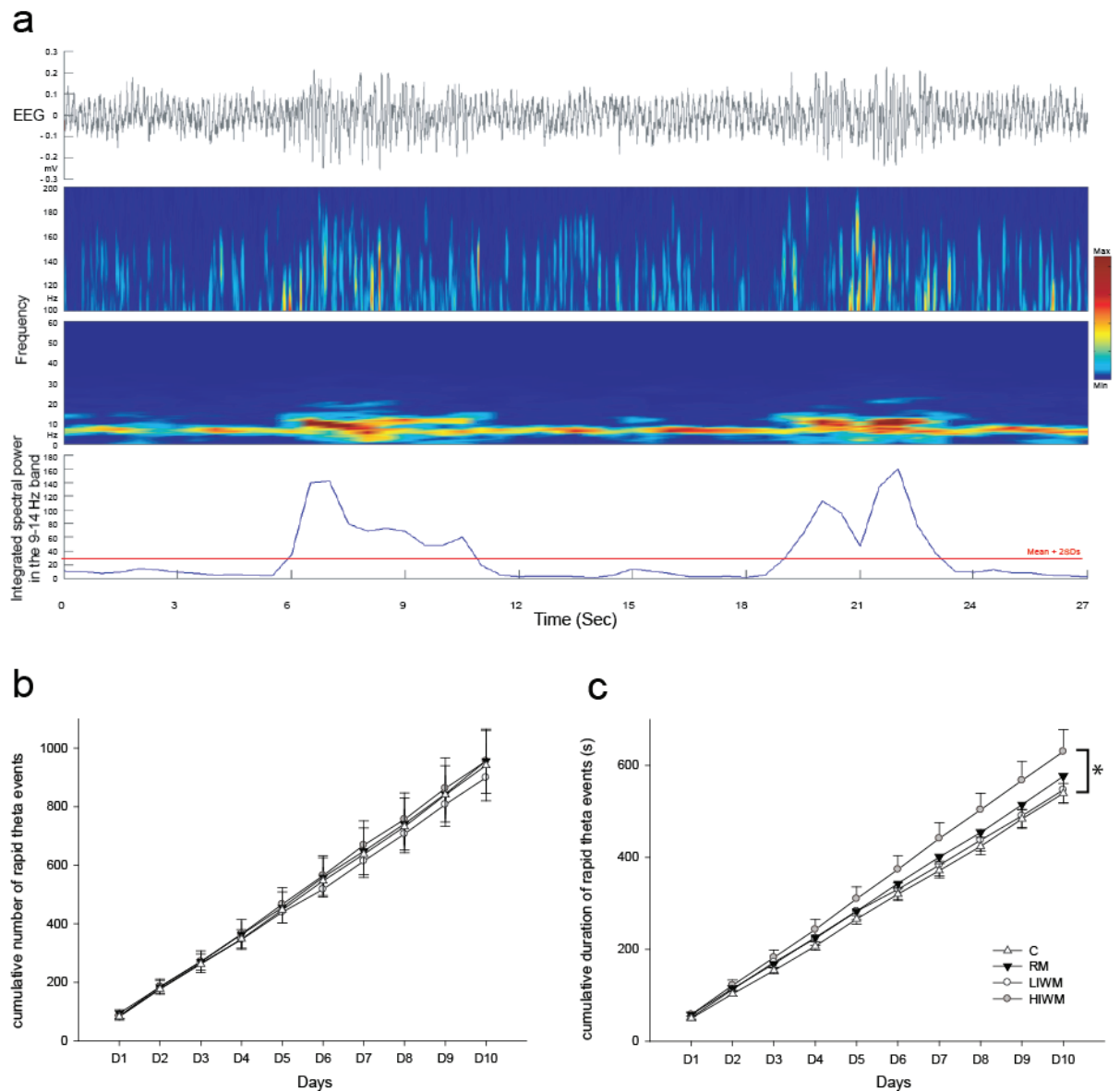


Figure 7. Rapid theta oscillations increases during PS in HIWM trained rats. **a**, Sample of EEG recording during detected bursts of theta activity superimposed with rapid oscillations (100-200 Hz). Transient rapid theta bursts of activity were detected using a threshold of mean + 2 SDs (red line) from the filtered (9-14 Hz) EEG trace. **b-c**, Cumulative number of events and duration of rapid theta oscillations in the four groups of animals over 10 days of training. ANOVAs on repeated measures show a significant Group x Day interaction [$F(31, 279) = 1.963$; $p = 0.0038$], and *post hoc* analyses indicate a significant difference between HIWM and Control and LIWM groups ($p = 0.0025$ and 0.0179 respectively) for duration while no differences between groups were observed in the number of episodes of rapid theta bursts.

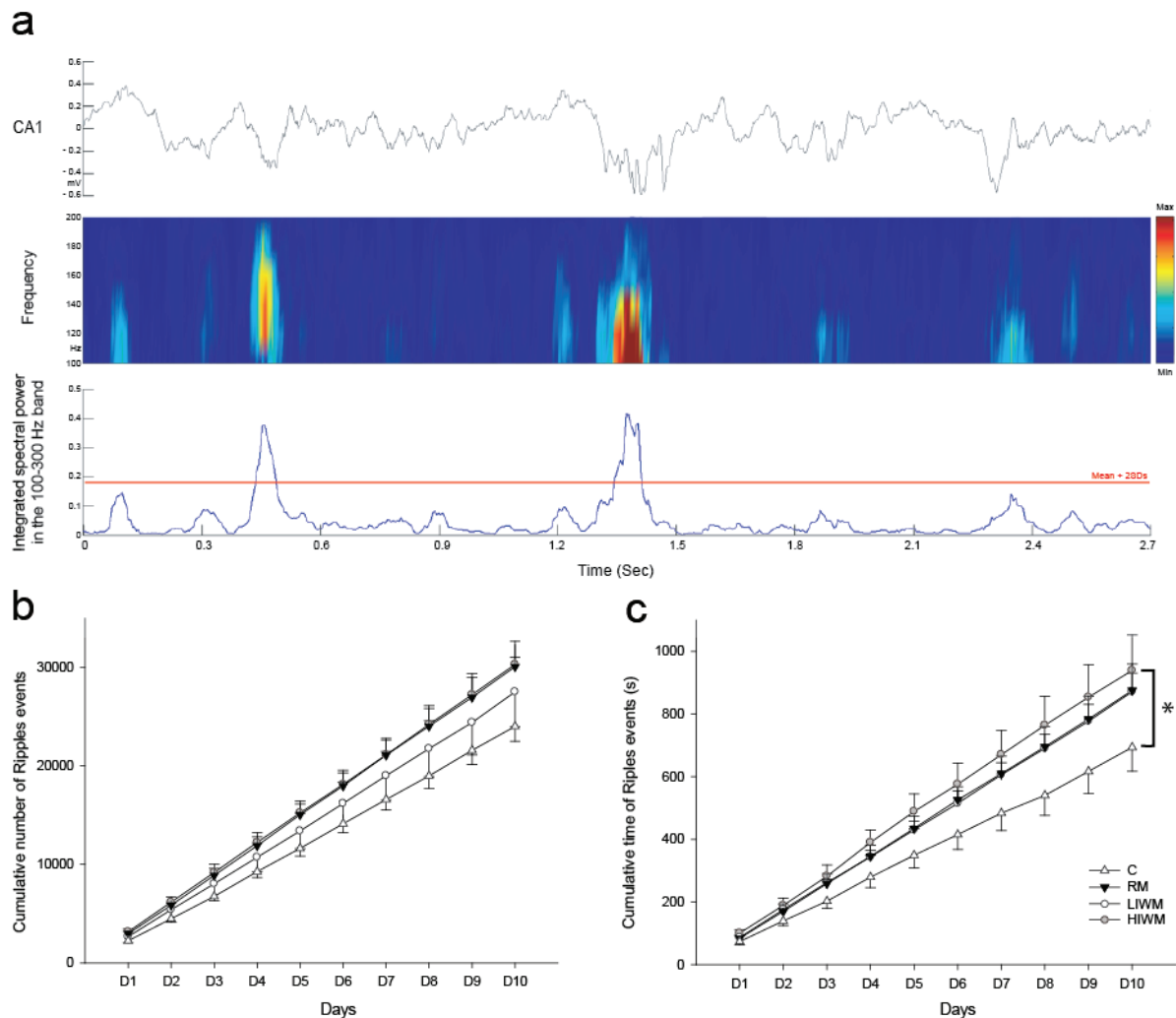


Figure 8. Ripples oscillations increases during SWS in all experimental trained rats and more specifically in HIWM trained animals. a, Sample of CA1 recording showing detected sharp wave ripples activity using a threshold of mean + 2 SDs (red line) from the filtered (100-300 Hz) CA1 trace. b-c, Cumulative number of events and duration of the ripples activity in the four groups of animals over 10 days of training. ANOVAs on repeated measures show a significant Group x Day interaction effect [$F(27, 288) = 1.615$; $p = 0.0306$], and *post hoc* analyses indicate a significant difference between control and the HIWM group ($p = 0.0311$) while no differences between groups were observed in the number of episodes of ripples.

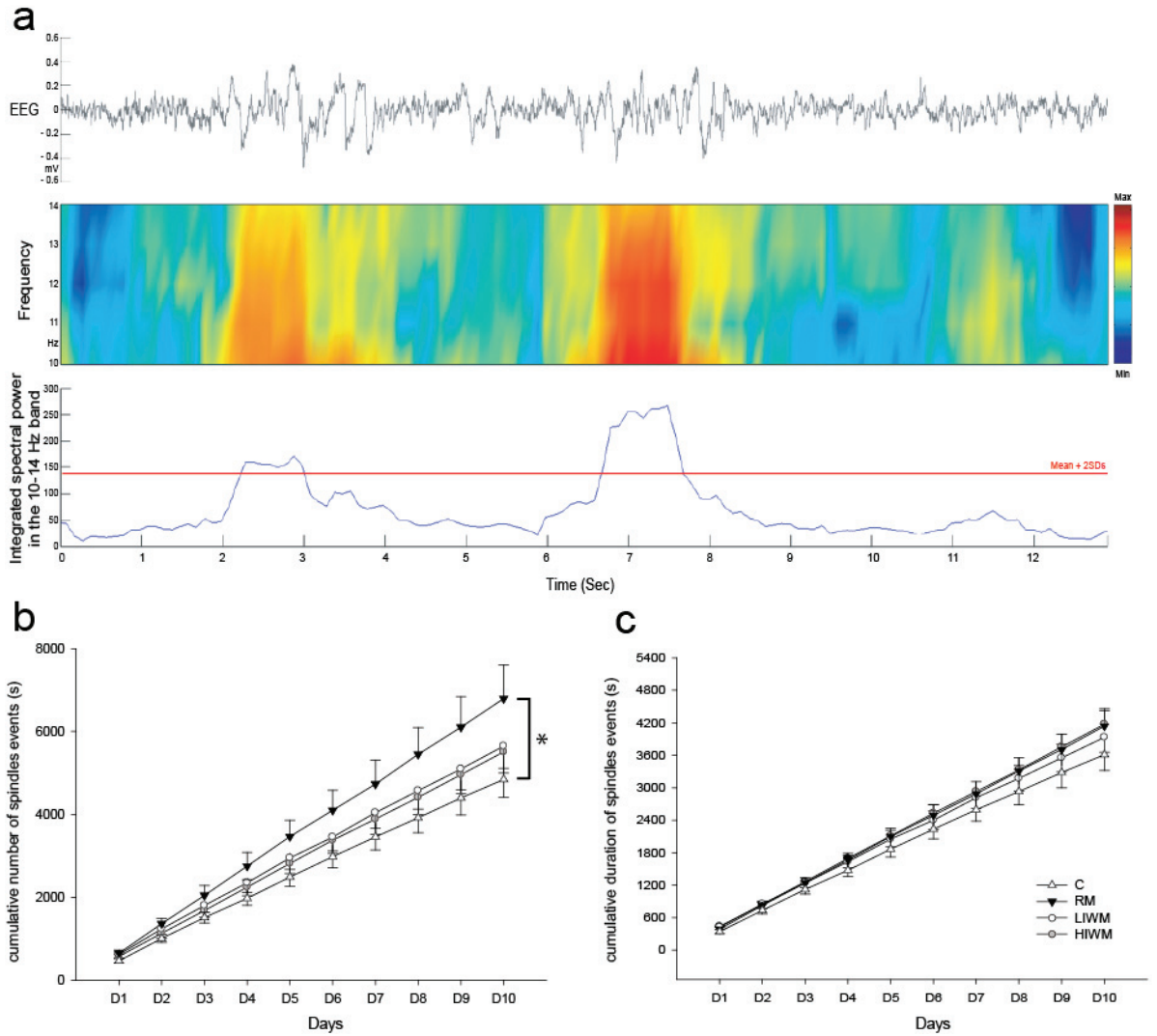


Figure 9. Spindle oscillations increase during SWS in RM trained rats. **a**, Sample of EEG recording during detected transient spindle activities using a threshold of mean + 2 SDs (red line) from the filtered (10-14 Hz) EEG trace. **b-c**, Cumulative number of events and duration of spindle oscillations in the four groups of animals over the 10 days of training. Repeated measures ANOVAs shows a significant Group x Day interaction effect [$F(27, 288) = 1.846$; $p = 0.0079$], and post hoc analyses indicate a significant difference between RM and Control groups ($p = 0.0239$) in the number of spindles while no differences between groups were observed in the duration of the episodes.

		D1-5	D6-10
SWS	RM	0.015 (0,54)	<0,001 (0,99)
	LIWM	0,004 (0,74)	<0,001 (0,99)
	HIWM	0,334 (<0,01)**	0,001 (0,86)
PS	RM	0,001 (0,88)	0,016 (0,52)
	LIWM	0,043 (0,25)	0,024 (0,39)
	HIWM	0,116 (0,06)	0,037 (0,84)

Table 1. Correlation between performance of rats and sleeps amount showed by the regression coefficient and the Pearson probability of significance (in parentheses). Correlation between SWS or PS amounts recorded during 12 hours post training (day n) and performance of rats in behavioral testing on day n+1. A significant correlation (Pearson coefficient tested; $r^2 = 0.3343$, $p = 0.0005$) between SWS duration and performance was seen at the beginning of training (day 1 to 5 see Figure 4) when performance was maximum and equivalent to performance of rats trained in the LIWM task (see Figure 1 and 2). No such correlation was found at the end of training, with PS duration or in the other groups.

	RM		LIWM		HIWM		
	SWS	PS	SWS	PS	SWS	PS	
EEG	delta	<0,001 (0,95)	0,041 (0,12)	0,008 (0,46)	0,060 (0,54)	0,072 (0,02)*	0,019 (0,25)
	theta	<0,001 (0,96)	0,011 (0,42)	0,010 (0,42)	0,010 (0,39)	0,022 (0,21)	0,007 (0,50)
	sigma	0,011 (0,45)	0,026 (0,22)	<0,001 (0,97)	0,021 (0,22)	0,020 (0,24)	0,001 (0,81)
	beta	0,007 (0,55)	0,055 (0,07)	0,022 (0,22)	0,057 (0,08)	0,015 (0,16)	0,007 (0,50)
	gamma	0,022 (0,28)	0,018 (0,31)	0,010 (0,41)	0,031 (0,14)	0,007 (0,50)	<0,001 (0,93)
CA1 LFP	delta	0,007(0,56)	0,029(0,20)	0,004(0,61)	0,047(0,07)	0,164(0,01)**	<0,001 (0,91)
	theta	0,022(0,30)	0,014(0,37)	0,033(0,12)	0,007(0,23)	0,044(0,10)	0,005 (0,57)
	sigma	0,040(0,16)	0,011(0,43)	0,008(0,45)	0,006(0,54)	0,002(0,85)	0,006 (0,56)
	beta	0,004(0,67)	0,036(0,15)	0,023(0,20)	0,026(0,18)	0,002(0,68)	0,015 (0,34)
	gamma	0,046(0,13)	0,001(0,80)	0,041(0,09)	0,003(0,67)	0,003(0,71)	0,011 (0,42)

Table 2. An increase in delta power is correlated to an increase in performance over two consecutive days of HIWM training. A positive correlation was found ($r^2 = 0.0724$, $p = 0.0223$ and $r^2 = 0.1673$, $p = 0.011$ from EEG and CA1 LFP recordings respectively) between Delta band spectral power increase (calculated as the difference between the relative spectral power on one day (day n) and the relative spectral power on the next day (day n+1)) and performance increase (calculated as the difference between the performance of a given day (day n) and the performance of the same rat the day after (day n+1)) for rats trained in the HIWM task (see also Figure 6). No such correlation was found with the other frequency bands, in PS or in the LIWM or RM groups. Relative spectral power was calculated based on the first hour of SWS for each animal and each day.

Chapter 4

Dissecting the role of REM sleep and slow-wave sleep in the processing of proactive interference during working memory tasks in rats

In chapter 3, we have seen that training rats in protocols involving or not forgetting differentially alters sleep patterns suggesting that SWS and REM sleep could have very different roles in information processing. In this fourth part of the manuscript, I now ask if altering sleep can in turn induce very specific changes in behavioral performance of rats trained to remember and/or forget. The results of this study are still preliminary and have thus not been submitted for publication yet. This is why chapter 4 is not built as an article as it was the case for chapters 1, 2 and 3.

Introduction

In chapter 3, we have seen that behavioral training involving or not forgetting processes, differentially affect sleep patterns. Our results have shown that information that has to be remembered in RM could be preferentially (but transiently) processed during REM sleep whereas SWS seems to be preferentially involved in forgetting. These results are in accordance with the synaptic homeostasis hypothesis that states that sleep is a way to forget non-pertinent information and restore the synaptic homeostasis challenged by LTP linked to learning episodes occurring during wake (Tononi and Cirelli 2014). This renormalization would be achieved through LTD-like processes during sleep (Cirelli and Tononi 2004; Vyazovskiy, Cirelli et al. 2008). With this hypothesis, forgetting processes during sleep are seen as an adaptive force allowing an optimization of cognitive resources by erasing previously learned information that could interfere with new learning (Kraemer and Golding 1997; Levy, Kuhl et al. 2010).

However, the study delineated in the previous chapter is purely correlative. To prove a causal link between sleep and forgetting, a sleep deprivation study was required. More precisely, we wanted to assess the effects of a selective REM sleep or total sleep (SWS+REM sleep) deprivation on the performance of a new group of rats trained in our three behavioral tasks. Unfortunately, depriving our animals of sleep during 10-days of training would induce a lot of stress and fatigue that could cause a misinterpretation of our results. To avoid such confounding factors, we decided to adapt our behavioral paradigms to a 2-days experiment. Instead of submitting our rats to one session of 8 runs per day during 10 days (as previously done in the other studies), we massed-trained them with 10 sessions of 8 runs per day, during two days. At the end of training on day 1, rats were immediately sleep-deprived and were tested again on day 2 to assess the influence of sleep-deprivation on memory performance. Members from our team have recently shown that a 4-hours REM sleep deprivation impairs both the induction of LTP and the consolidation of contextual fear conditioning (Ravassard, Hamieh et al. 2014). This result combined with the results from the previous study showing that the REM sleep increase observed after RM training occurs within a 4-hours window, led us to sleep-deprive our rats for 4hoursimmediately after training on day 1. After deprivation, rats were allowed to recover from this sleep-deprivation before being tested the next day (24 hours later).

It is important to keep in mind that the results presented in this chapter are preliminary and should be interpreted as such. The conclusion drawn in this chapter may change as the results become more accurate in the future. We present at the end of this chapter further directions for this work that could precise the interpretations of the results presented here.

Material and Methods

Subjects

Ten Dark Agoutis rats (from Janvier) were kept in individual cages with a 12/12h (9am-9pm) light/dark cycle with *ad libitum* access to food and water. The animal care and treatment procedures were in accordance with the regulations of the local (Lyon 1 University CE2A-UCBL 55) and European (2010/63/EU) ethics committee.

Surgery and polygraphic recordings

Surgery was performed on these rats as previously described (chapter 3) for polygraphic recordings. Briefly, two EEG (electroencephalogram) electrodes were implanted above the left prefrontal cortex and left parietal cortex; four LFP (local field potential) electrodes were implanted in the right prefrontal cortex, and in the DG, CA1 and CA3 areas of the hippocampus and two EMG (electromyogram) electrodes were inserted between neck muscles. After surgery, rats were allowed to recover during seven days in a sound-attenuated, ventilated and electrically isolated chamber.

Behavioral protocol

The behavioral apparatus used for behavioral training was the elevated eight-arm radial maze described in chapter 3. The experimenter could automatically move each arm either in an upward (open), or a downward (closed) position preventing the rat from accessing the platform at its end. On each of the eight platforms, a squared food well could contain odorless food rewards undetectable for the rat from the central platform. The testing room contained extramaze visual cues allowing rats to use their spatial hippocampus-dependent memory to remember the position of food rewards.

Each of the ten rats was trained in the three behavioral tasks (LIWM, HIWM and RM tasks) described earlier in this manuscript. Instead of training a rat in only one behavioral task, each individual rat was thus trained for LIWM, HIWM and RM, sleep-deprived or not. The order of the tasks was pseudo-randomly determined by the experimenter so that one rat could start by being tested in HIWM and later in LIWM, and a second rat could start by being tested in LIWM and later in HIWM. In contrast, RM training was always assessed after LIWM and HIWM training as RM training demands to learn a fixed location and can only be tested once. In the present study, rats were submitted to ten sessions of eight runs per day during two days, allowing a single sleep-deprivation session between day 1 and 2 of training. They were run between 10am and 13pm, during the light phase of the rats 12h-cycle. Each rat finishing a 2-days experiment (i.e. HIWM) was allowed at least three days of rest before being tested in a new paradigm (i.e. LIWM). In the present study, we did not use yoked control animals as analysis of performance was made by comparing each rat performances after undisturbed sleep to its performances after sleep (total sleep or REM sleep) deprivation.

Sleep deprivation protocol

Immediately after the first day of training, rats were placed back in their attributed recording chamber, and connected to the data acquisition system. Signals were stored for off-line analysis. During the first four-hours post-training, while their sleep oscillations were being recorded, rats were either allowed to sleep *ad libitum* (non deprived), or were totally sleep deprived (TS-D) or selectively REM sleep deprived (REMs-D). It is important to notice that as SWS necessarily precedes REM sleep, SWS deprivation always fosters a REM sleep deprivation. Therefore, a selective SWS deprivation is impossible to achieve. However, the effect of such deprivation can be assessed indirectly by comparing the impact of REMs-D to TS-D. In order to sleep deprive rats, sleep oscillations were monitored by the experimenter, and each time the start of a SWS or REM sleep phase was detected, the cage was manually inclined to gently wake the rat (Ravassard, Hamieh et al. 2014)

Signal and statistical analysis

Behavioral data were analyzed using two-way ANOVAs (Analysis of Variance) for repeated measures with Block (of two consecutive sessions) and Group (RM, LIWM, HIWM) as main factors (Statview 5.0.). Sleep analysis was performed off-line on the data recorded on a custom Matlab routine, and vigilance states were scored by 5 seconds epochs to define wake (W), SWS and REM sleep periods using the previously described criteria of frequency and amplitude (see chapter 3 material and methods). The total duration of each sleep phase was estimated from 1 to 4 hours post-training (HPT) for day 1 and day 2. In order to make sleep quantities comparable to each other, these data are expressed as a percentage of the baseline corresponding to the sleep quantities observed the day before the behavioral test on the same period (2PM-5PM). Sleep quantities were statistically analyzed with Mann-Whitney U-test. Data are expressed as mean \pm S.E.M..

For the correlation analysis, the increase in performance was calculated as the difference between the performance the last block of day 1 (block 5) and the performance of the first block of day 2 (block 6). A linear regression analysis was performed to assess correlation between behavioral performances and sleep quantity. Pearson's correlation coefficient was used to detect significant regressions. All data are expressed as means \pm S.E.M. and statistical difference was assessed with probability under 0.05 after checking for normality and homogeneity of variance.

Results

Sleep analysis shows the effectiveness of sleep deprivation.

Data from our team showed that four hours of REMs-D immediately following contextual fear conditioning were sufficient to impair the consolidation of hippocampal-dependent information in rats (Ravassard, Hamieh et al. 2014). We thus used the same sleep-deprivation protocol and time scale (but for both REMs-D and TS-D) in the present study. This short lasting sleep deprivation was shown to be non-stressful for the rats compared with previous protocols in which animals were REM sleep deprived for several days (Youngblood, Zhou et al. 1997; Vertes 2006). Our protocol of sleep deprivation was effective and selective, as on HPT 1 to 4, the average percentages of SWS (9%) and REM sleep (0%) were significantly reduced compared to baseline percentages (37% and 4%) for the TS-D group, whereas only the percentage of REM sleep (0.1%) was decreased for REMs-D rats (**Figure 1**). No significant differences were observed between baseline and the non deprived group.

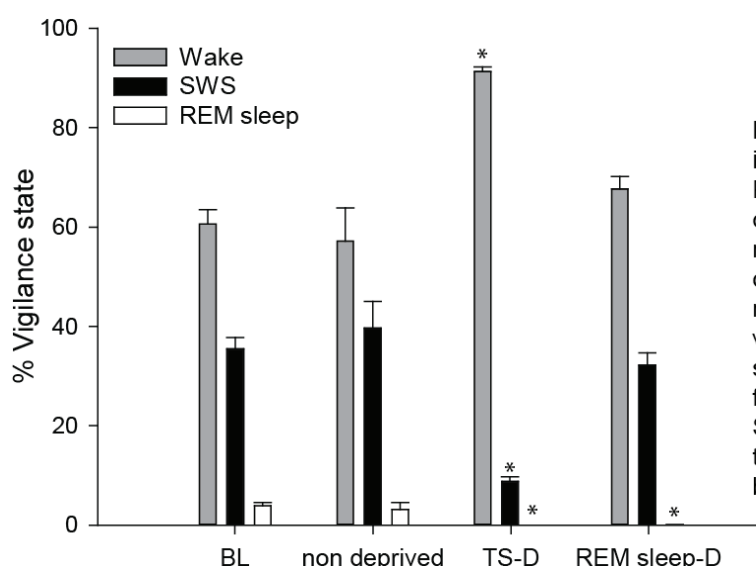


Figure 1: The sleep deprivation protocol is specific of the targeted sleep phase. Percentages of wake, SWS and REM sleep quantified between 1 and 4 HPT on the data recorded during baseline (BL) and training day 1 (D1). After training day 1, TS-D reduced both SWS and REM sleep, whereas REMs-D specifically reduced REM sleep while leaving wake and SWS unaffected. Data are expressed as mean \pm S.E.M, and significant differences compared to the baseline percentages were detected by Mann-Whitney U-test (* $P < 0.001$).

Effect of the 2-days training on behavioral performances of non deprived rats

The performance of rats trained in the three different tasks (RM, LIWM and HIWM), are represented in Fig. 2. ANOVAs revealed a significant Group effect [$F(2, 40) = 13.547$; $p < 0.0001$], a significant Block effect [$F(9, 360) = 35.351$; $p < 0.0001$], as well as a significant Group x Block interaction [$F(9, 360) = 11.913$; $p < 0.0001$]. *Post-hoc* analyses revealed that RM rats significantly improved their performance over time [$F(9, 81) = 55.555$; $p < 0.0001$] and reached 100% correct choices on the last two blocks of days, indicating a learning of the general rules and strategies required to solve this task. In WM groups, we investigated how proactive interference (PI) affected WM performance. When comparing the HIWM and LIWM, we observed no group effect [$F(1, 31) = 0.908$; $p <$

0.3481] but a significant Block effect [$F(9, 279) = 6.407$; $p < 0.0001$], as well as a significant Group x Block interaction [$F(9, 279) = 3.986$; $p < 0.0001$]. At the beginning of training, both LIWM and HIWM groups started at almost 90% of correct choices. LIWM rats kept high scores throughout the entire experiment, increasing with time and reaching 100% on Block 9 and 10 [$F(15, 135) = 8.519$; $p < 0.0001$]. On the contrary, rats trained in the HIWM task did not show such increase in performance over days indicating that accumulation of PI distorts WM performance with time (no significant block effect [$F(16, 144) = 8=1.843$; $p = 0.0654$]). Significant difference in score was shown for blocks 2, 8 and 9 between LIWM and HIWM ($p = 0.0406$, $p = 0.0010$ and $p = 0.0009$) (**Figure 2**)

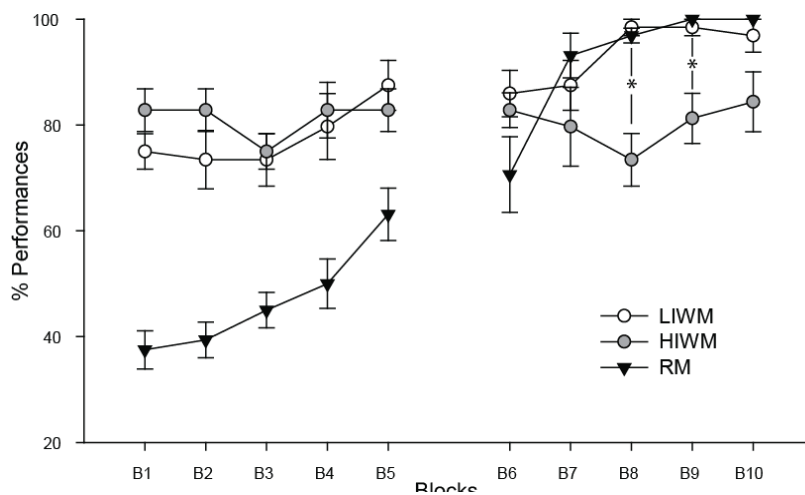


Figure 2: PI induce a drop of performances on day 2. Percentage of correct choices \pm SEM per blocks (B1-10) of two sessions, with B1 to B5 on day 1 and B6 to B10 on day 2 of non-sleep deprived rats trained in the RM, LIWM or HIWM tasks. Scheffe post hoc tests (* $P < 0.05$).

Effect of sleep deprivation on behavioral performance

We then asked if sleep deprivation could impair the consolidation process in the RM task and the processing of PI especially in the HIWM task. On day 2 of training (following sleep deprivation), we observed that TS-D rats trained in the RM task learned the invariable position of the food rewards significantly less accurately than non-deprived and REMs-D animals. TS-DRM trained rats never reached the 100% maximum performance observed in the two other groups. Significant differences were found for blocks 8 and 9 ($p = 0.0110$ and $p = 0.007$) (**Figure 3C**). However, no statistical difference was observed between non-deprived, TS-D or REMs-D rats trained in the other LIWM (**Figure 3A**) or HIWM (**Figure 3B**) tasks suggesting that only TS-D impairs RM training.

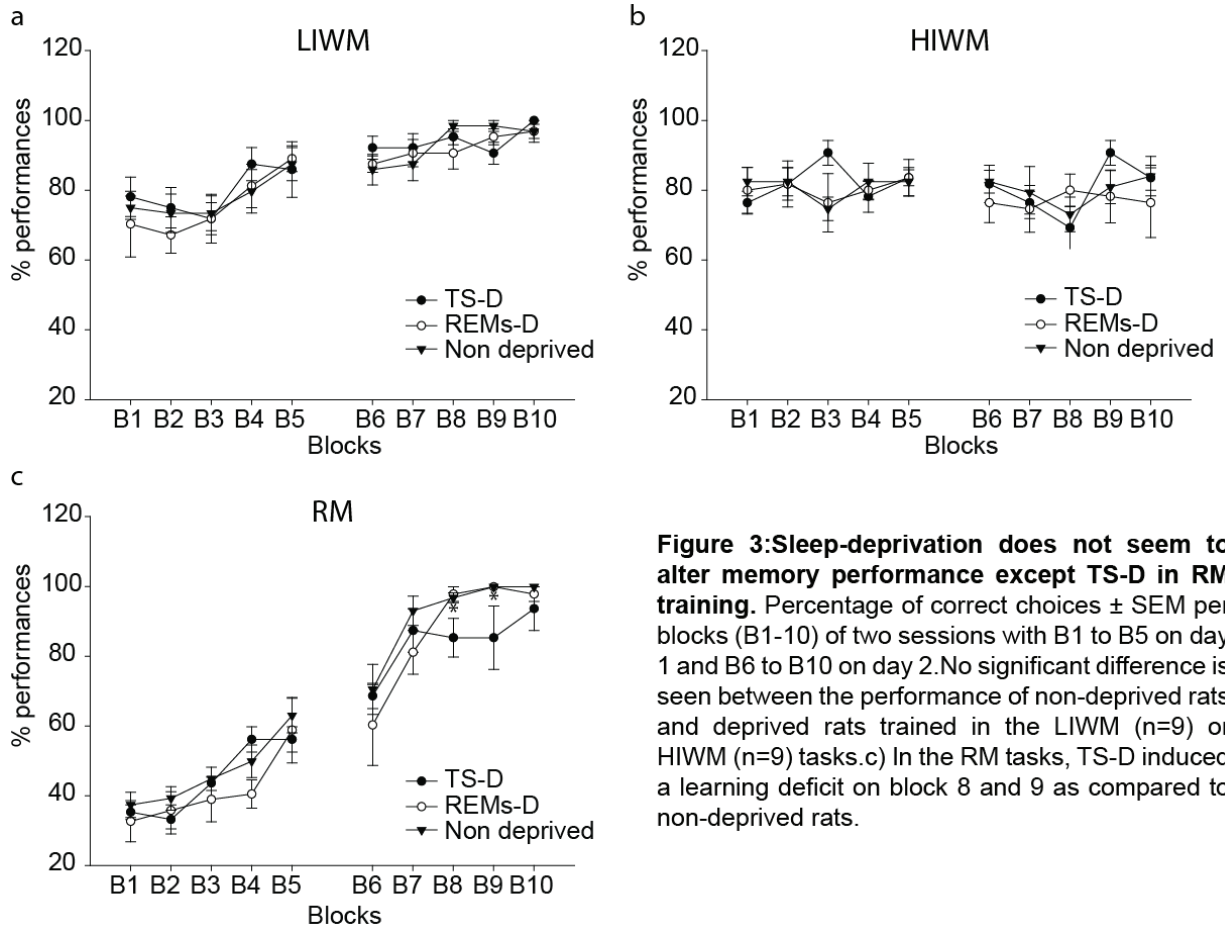


Figure 3: Sleep-deprivation does not seem to alter memory performance except TS-D in RM training. Percentage of correct choices \pm SEM per blocks (B1-10) of two sessions with B1 to B5 on day 1 and B6 to B10 on day 2. No significant difference is seen between the performance of non-deprived rats and deprived rats trained in the LIWM (n=9) or HIWM (n=9) tasks. c) In the RM tasks, TS-D induced a learning deficit on block 8 and 9 as compared to non-deprived rats.

SWS and REM sleep quantities are correlated to improved performance for rats trained in HIWM.

The quantities of SWS and REM sleep were quite different in the three groups (non-deprived, TS-D and REMs-D) of rats. Given that our behavioral tasks are quite complex, we assumed that they were sensitive to small changes of sleep quantities. We thus decided to further study the impact of sleep deprivation based on potential correlations between the exact percentages of SWS or REM sleep and the performance of rats. For each rat (and in all conditions), we normalized the percentages of SWS and REM sleep to their relative quantities during baseline (recorded the day before each training phase). This normalization was performed between 1 and 4 HPT, corresponding to the period of sleep deprivation, when differences of sleep quantities between the different groups was the highest. No significant correlation between the amounts of SWS and REM sleep and changes of rat performance between day 1 and 2 was observed for rats trained in the LIWM task (**Figure 4**), suggesting that simple processing of information in WM (with a negligible level of interference) is probably not sleep-dependent.

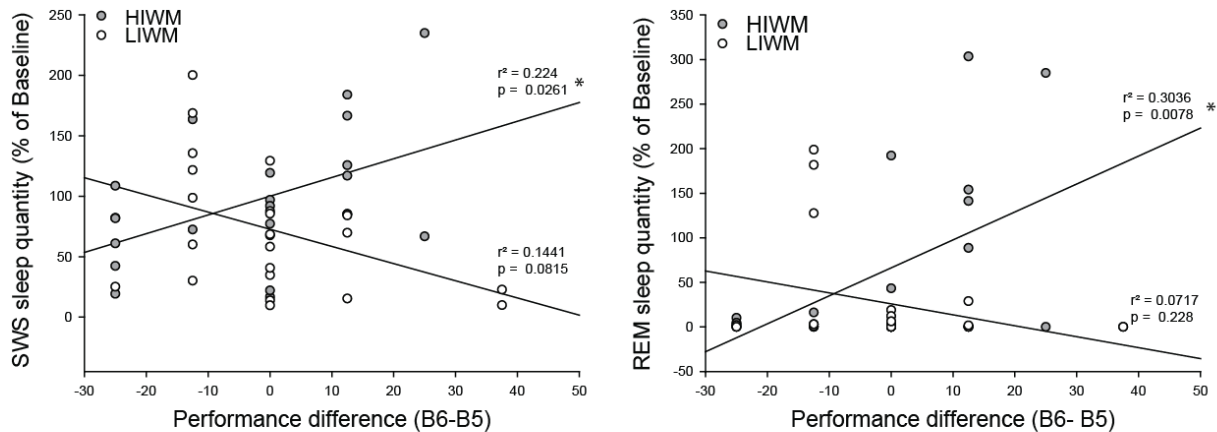


Figure 4: Positive correlations are observed between 1) the increase of SWS and REM sleep quantities and 2) the improvement of performance between day 1 and 2 for rats trained in the HIWM task. A-B) Correlation between the percentages of SWS (left) or REM sleep (right) (normalized to the mean baseline percentages for each rat in all conditions, during 1 to 4 HPT) and the corresponding difference of performance of the rats between block 5 on day 1 and block 6 on day 2. Pearson coefficients were calculated for each of the regression curves. No significant correlation for rats trained in the LIWM task was observed for both SWS and REM sleep, contrary to the HIWM tasks.

In contrast, a significant positive correlation between the amounts of SWS, but also REM sleep, and changes of performance between day 1 and day 2 was observed in rats trained for HIWM (**Figure 4**), suggesting that the more the rat spend time in SWS or REM sleep after training on day 1, the more its performance will improve 24h later, on day 2. Therefore, these results suggest that both SWS and REM sleep seem to be involved for efficient processing of interference. Correlations for the RM group have not been computed because of the very small number of data points obtained so far for this group that can only be tested once (see material and methods).

Discussion

Although we did not observe any impact of REMs-D or TS-D on behavioral performance, performance in HIWM seems to be positively correlated to increased REM sleep and SWS quantities. Therefore, contrary to our initial hypothesis that poses that forgetting of non-relevant information would specifically occur during SWS while the consolidation of long-term memory would occur during REM sleep, the preliminary results of the present study seem to show here that the processing of PI could involve both SWS and REM sleep. Also contrary to our initial hypothesis, RM memory performances are affected by a TS-D but not by a more specific REM sleep specific deprivation. The absence of effect after REM sleep deprivation suggests that, contrary to our previous results (chapter 3), SWS also participates to memory consolidation. This role of SWS in consolidation could be due to sharp-wave ripple events that are fast depolarizing events occurring in the hippocampus and whose inhibition was shown to impair RM performance of rats in a radial maze similar to ours (Girardeau, Benchenane et al. 2009). However, this result is in disagreement with a

previous study from our group showing that a 4-hours REM sleep deprivation impairs the consolidation of information in contextual and hippocampal-dependent memory in rats trained in a fear-conditioning paradigm. However, this discrepancy may be due to the fact that the radial maze task used in our study is purely spatial whereas the contextual fear conditioning includes an emotional component that may be processed specifically during REM sleep as suggested by other authors (Goldstein and Walker 2014).

The adaptive forgetting of irrelevant information, more specifically required for HIWM processing, was expected to be more strongly linked to SWS as suggested by our previous study (chapter 3) showing a link between the processing of interference in HIWM task and an increase in SWS quantity and SWA. The involvement of REM sleep we found here in the processing of PI is thus more surprising, given that many evidence point towards a role of REM sleep in the consolidation of long-term memory rather than in WM. Indeed, data presented in chapter 3 indicates a transient increase in REM sleep after a RM task while no such increase was observed after WM training. However, these data also showed that a significant cumulative increase in the duration of rapid theta (9-14 Hz) epochs during REM sleep could be observed over days of training in the HIWM task. This result might explain the involvement of REM sleep in HIWM in this fourth study.

Overall, the results presented here highlight the fact that long-term storage of spatial information could specifically rely on SWS, a result at odd with our previous findings showing a very specific REM sleep increase after RM training. They also showed that the processing of PI seems to be more complex and probably involves both SWS and REM sleep. This study however only presents preliminary data and more animals should be now tested to confirm our findings. The discrepancies seen between the third and this fourth study could thus be explained by the fact that we used two different types of training regimen, a spaced (chapter 3) and a massed (chapter 4) training. Moreover, our behavioral tasks are quite complex and probably involve complex processing of information. Therefore, the idea that such processes depend only on one sleep phase seems to be over-simplistic. Indeed, SWS and REM sleep are composed of multiple phasic oscillatory events in different frequency bands and with different spectral power. Consequently, when considering the role of sleep in cognition, one should reason in terms of specific events rather than in terms of specific sleep phase. To confirm the involvement of such specific oscillatory events in the processing of information in our radial maze tasks, rats of this study were implanted with local field potential (LFP) recording electrodes within the hippocampus and the PFC. The analysis of the data obtained with these recordings have not been computed yet. But such analysis should allow us to dissect more precisely the role of each sleep phase by assessing the involvement of transient events such as spindles, ripples or fast-theta oscillatory activities.

Lastly, the data presented in chapter 1 and 2 suggest that the hippocampus cannot be considered as a unique structure. The DG was shown to be specifically inactivated during HIWM training and the western blot analysis showed that different processes may occur at the same time in the different sub-regions of the hippocampus. However, these studies only focused on the molecular and cellular processes occurring after 10 days of training. A coherence analysis of the signal obtained from LFP recordings (CA1, CA3, DG and PFC) could allow a real time analysis of the processes occurring within and with the hippocampus.

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General discussion

Over the past decades, many studies have advanced our knowledge about memory processes. Both the molecular and cellular underpinnings of memory and consolidation are now quite well understood even if some grey areas still remain such as on the role of the hippocampus during the recall of remotes memories or the exact role of sleep in the consolidation of relevant information. In contrast, the processes at stake during forgetting have not been studied as thoroughly as those related to memory. The overall goal of my thesis was to understand the molecular and cellular processes playing a role in the forgetting of irrelevant information. To do so, we used a comparative approach testing three groups of rats trained in an 8-arms radial maze. In the first part of the manuscript, we sought to identify the brain regions involved in the processing of PI and found that the DG seems to play a critical role in these processes. In the second chapter, we tried to understand what molecular mechanisms occur during the processing of PI and showed that, in the DG, activation of the molecular cascades related to long-term plasticity may be involved. Even if LTD might be involved in forgetting (Nicholls, Alarcon et al. 2008), LTP may also be observed during tasks requiring forgetting, a fact that could account for the decrease in performance seen in such tasks. In the last two parts of the manuscript, we focused on the role of sleep in forgetting. First, we tried to determine how different training regimen could impact sleep patterns and then we studied the consequence of sleep-deprivation on such training. These two studies tended to show that SWS plays an important role in forgetting, even if we cannot rule out that REM sleep also participates to the processing of PI. In this discussion part of the manuscript, I will first discuss some methodological aspects, and then talk about the theoretical aspects raised by our studies. I will finally present some future possible directions to take in order to better understand the processing of PI.

Methodological aspects

Behavioral protocols

The radial maze apparatus was developed about 40 years ago and has become an essential tool for testing memory in rodents. It has been shown that performance in this kind of tasks requires spatial memory abilities. The main advantage of using a radial maze is that it can be used for a multitude of behavioral tasks performed in a same and unique environment. During my thesis, we mainly focused on the comparison between two WM tasks involving or not the forgetting of previously encoded information and a RM task that requires the long-term storage of information. In order to make the comparison between the three tasks possible, we had to make sure that rats used their spatial memory and therefore did not use unrelated memory processes. To do so, we took several precautions. First, to ensure that the rats did not use any intramaze odor cues, we wiped the maze with water (instead of ethanol) between each session in order to disperse odors in the maze. This action allowed a saturation of the maze with dispersed animal odors preventing the rats to use such cues to locate the presence of rewards. Also, we used odorless pellets as food rewards. These manipulations allowed us to prevent the rats to use any olfactory cues or trails left by them on visited arms. Moreover, many authors have ruled out any possibility that the rat use scent to “mark its territory” as a sign that it has been there and use it as odor cues to navigate in the radial maze (Olton and Samuelson, 1976, Olton and Papas, 1979). Proactive motor coding is the ability for the animal to predict its next motor response relying on procedural (motor) learning. To force the rats to use spatial (perceptual) memory rather than motor (procedural) memory, we removed the rat from the maze and placed it in a transfer cage at the end of each run. Placing the rat in such cage between trials also allowed us to use different starting arms for each trial. The starting arms and the target arms were predetermined in a pseudo-random order so that the rat could not predict the next choice based on an algorithmic motor strategy.

The control group

The biological changes observed in the first three chapters (IEG activation, phosphorylation processes, and sleep regulation) could be triggered by any general sensory stimulation. It was thus essential to use a control group to make the comparison between groups possible. In other studies (Zoladz, Park et al. 2012; Kumazawa-Manita, Hama et al. 2013; Park, Yoon et al. 2014; Seip-Cammack and Shapiro 2014), cage controls are commonly used. These cage controls however are not exposed to the environment of the maze the same way than animals engaged in a memory tasks and therefore do not receive the same sensory inputs than experimental animals. In consequence, we considered that using such cage controls was not appropriate for the study of the specific biological mechanisms involved in our three cognitive tasks. We thus wanted our control group to match as closely

as possible our experimental group with the exception of the cognitive process involved. We found that using yoked control provided excellent tools to conclude that any physiological change observed in the experimental groups as compared to these controls reflected purely an involvement of a given cognitive process. Indeed, these groups were designed in such a way that each (control and experimental) rat experienced at all times the same number of runs in the maze. Each yoked control rat was also paired with an experimental rat, and was given a reward according to the performances of its paired experimental rat. This procedure allowed us to control the number of food pellets and therefore to avoid any reward-associated effect. In the sleep deprivation study (chapter 4), the use of such controls was not considered pertinent as each rats had to undergo the three tasks (HIWM, LIWM and RM) and the three types of sleep deprivation that followed (non-deprived, TS-D and REMs-D). In such condition, each rat was considered its own control and comparisons between performances were made by comparing the performance of each rat after undisturbed sleep to its performance after sleep deprivation.

Significance of the effect of PI

In the first three studies however, the use of yoked controls and the design of the behavioral protocols allow a direct comparison between different cognitive processes in order to identify the processes specifically involved in forgetting and the processing of PI. Despite its apparent complexity, the task used to assess WM is a simple delayed-non-match-to-place task with only two possible outcomes: a correct or a wrong choice. Throughout this manuscript, we presented the behavioral data as percentage of performance and we interpreted the drop of performance as a deleterious effect of PI. This effect may appear quite small. When we pooled together all the animals trained in these tasks, the difference between the performance of LIWM and HIWM trained rats is “only” of 15%. In addition, the performance of HIWM trained rats (oscillating roughly from 80 to 60%) may seem quite high. This subtle behavioral effect has generated much discussion about the significance of our results. However, we are confident that this very statistically significant and reproducible decrease in performance is indeed due to the effect of PI. First, a rat performing at chance in these tasks would still have a 50% chance of success. Moreover, the delayed-non-match procedures used in these WM tasks heavily rely on the natural tendency that rodents have not to visit a same place twice, a behavior first described by Tolman in 1925(Tolman 1925) and called *spontaneous alternation*. This behavior facilitates training as confirmed by the performances on the first days of training already significantly above chance (85% success in the first two sessions for both HIWM and LIWM trained rats). Also, in the LIWM task the memory processes involved are the same as in the HIWM (short term storage of a one trial presentation) but effect of PI is negligible. The animals in this group reach almost 100% performances at the end of training suggesting that rats are perfectly capable of encoding and retrieving the information in this kind of task. Therefore, we interpreted the errors made by

the animals trained HIWM as mainly due to the effect of PI. In contrast, a correct choice is more difficult to interpret; obviously it can be the result of an efficient memory storage and retrieval. But, if due to PI the encoding or the retrieval is not successful, the choice is then made randomly and can result in a wrong choice (as seen before) but also in a correct response. Some successful choices are thus the result of that 50% chance of success in choices made randomly. When looking at our behavioral data, it is important to keep in mind that half of the effect of PI is masked by such an effect. That is why analyzing behavioral data based only on performance is not enough. It will be interesting to analyze more precisely the behavior of the rats in the maze during training. This will allow us to see more subtle differences between the different conditions such as an increase in the choice-latency or hesitations before making the final decision, which could be the reflection of the effect of PI. With the help of Paul Antoine Libourel, we recently developed a video tracking software that will allow such analysis.

The western blot analysis

In the western blot study, we assessed the level of phosphorylation of AMPA receptors and interpreted such phosphorylation as an indirect marker of the integration of these receptors at the synaptic (PSD) level. One could argue that PSD isolation protocols would be a more direct approach to observe such changes. First, it is now well accepted that ser831 and ser845 phosphorylation are accurate markers for plasticity changes (Song and Huganir 2002; Lee, Takamiya et al. 2003; Thomas and Huganir 2004; Lee, Takamiya et al. 2010; Huganir and Nicoll 2013). Second, isolation of the PSD is a long process that involves many steps, each of them potentially inducing variability in the results. Because the impact of PI on cognitive performance is very subtle (between LIWM and HIWM rats), we expected very subtle changes in the molecular cascades associated with it. The variability induced by the PSD enrichment protocol could therefore shadow these changes and lead to false negative results. Recently however, discussions with Pierre de Rossi in the Oncoflam team of the CRNL about a new (and more efficient) PSD enrichment protocol lead me to rethink such assumption. However, such protocol would generate twice more samples because the western blot assay would have to be performed on the PSD enriched fractions as well as on the other cellular compartments. From a technical point of view, I doubt that running a western blot assay on a large number samples would be effective and give better results. Due to the size of our electrophoresis chamber, multiple migrations would have to be run and this process could lead to more variability and give again false negative results. Nevertheless, this is a promising lead and I do not rule out the possibility of running such an experiment in the future.

Theoretical aspects

Is LTP or LTD involved in the processing of PI?

The study presented in chapter 1 shows an inactivation of the DG after HIWM training using immunohistochemistry of C-Fos and Zif268. On the other hand, the western blot study (chapter 2) described an increase of indirect markers of LTP in the same sub-region of the hippocampus and after the same training. These results may seem contradictory. Zif-268 expression was thus shown to be associated to LTP in the granule cells of the hippocampus (French et al 2001). Therefore, the question that remains is how can it be possible to observe LTP without observing Zif 268 expression? This discrepancy could be explained as followed. In the two experiments, the animals were not sacrificed at the same moment after the last trial of the last day of training. In the IEG study, animals were perfused 90 min after the last trial in order to reach the peak of expression of the proteins c-Fos and Zif268 whereas in the western blot study, the animals were euthanized immediately after the last trial in order to visualize short-term but unstable modifications (phosphorylations) of the synaptic markers. Even though Zif268 expression and GluA1 phosphorylation may be linked, they are two very different processes. First, they do not occur in the same neuronal compartment. The plasticity processes we observed in the western-blot study certainly occur specifically at the synaptic level whereas Zif-268 expression occurs in proximity to the nucleus (as confirmed by the characteristic nuclear rounded-shape labeling of Zif268 and also c-Fos). Therefore, we can conclude that the changes in plasticity observed immediately after learning may only last for a short period of time and may not be converted in changes in the pattern of protein expression. One possibility is that, during HIWM training and across the dendritic tree, the signal is not transferred as it usually is during LTP and the long-term storage of information. The signaling cascade between the activation of CaMKII and the one of Zif-268 is quite complex and could be suppressed at many critical levels. A good candidate for such regulation is the extracellular signal regulated kinase (ErK). When phosphorylated, this kinase activates the translation of IEGs such as Zif-268 and c-Fos. We tried to assess the level of ErK phosphorylation by westernblot, but due to technical reasons we could not obtain a good labeling resulting in inconclusive results concerning this kinase.

There are other leads that we could follow in order to further investigate the molecular mechanisms involved in the processing of PI. First, an immunohistochemistry analysis on sample collected 90minutes after training could reveal in which population of neurons the extra GluA1 are located, and if these receptors are indeed located at the synaptic level. Further studies using proteins chips arrays could also reveal new candidates for the down-regulation of the synapse/nucleus signaling cascade. The signaling pathways involved in forgetting processes are not as well known as the ones for memory and the use of wide range arrays could give new insight on the molecular processes involved in forgetting.

Involvement of the neurogenesis in the processing of PI

We have seen in chapter 1 and 2 that the DG is critically involved and downregulated in HIWM training. One particularity of the DG is to generate new neurons in adulthood. Some scientists have predicted an important role of adult neurogenesis in pattern separation and the processing of interference, two important processes at play in our HIWM task. For instance, 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. 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 the processing of 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), small variations between trials detected by the animal in the task may stimulate neurons that are already highly excitable (newborn neurons) rather than less excitable ones (mature granule cells). Saxe and colleagues (2007) thus observed a more efficient processing of proactive interference in WM in irradiated mice with impaired DG neurogenesis. An enhancement of performance is observed when the same pair of arms was repeated during successive trials, similar to the conditions of our HIWM task. Such results are in agreement with ours. Diminishing neurogenesis, and thus DG activation, may decrease pattern separation function and the recall of a high level of repeated and very similar information that is in fact useless in the resolution of the HIWM task. In this task, the animal is not required to recall and compare similar trials that happened in the past with an ongoing trial, but in the contrary is asked to focus on an ongoing trial while ignoring (and forgetting) similar past trials.

Following our first study, my colleague Mickael Joseph has conducted an experiment aimed to determine if new neurons could be activated, or more precisely inactivated during HIWM training. Unfortunately, his experiment gave inconclusive results (due to a poor labeling) when the animals were sacrificed 90 minutes after learning. However, adult DG neurogenesis has been shown to be down-regulated by sleep deprivation suggesting a role of sleep in the generation of new neurons. Recent data favor the hypothesis that a decrease in cell proliferation are related to a reduction in REM sleep, whereas a decrease in the number of cells that subsequently develop into adult neurons may be related to reductions in both

SWS and REM sleep (Abel 2013). This suggests that each sleep phase (REM sleep and SWS) regulate differentially specific molecular and cellular mechanisms. The build-up effect of PI we observed when the inter session delay increases from 10 min vs 24 hours in our HIWM task could be related to a sleep-dependent up-regulation of neurogenesis in the DG. This up-regulation of neurogenesis during sleep could have the counterproductive effect of increasing pattern separation function that may be deleterious to optimal processing (deletion) of PI.

Involvement of REM sleep and SWS in the long-term storage of information

In chapter 3, we showed that RM training induces a transient but strong increase in REM sleep. In contrast, in the sleep deprivation study, RM performances are impacted by a total sleep but not by a more selective REM sleep deprivation. Contradicting our previous findings (chapter 3), this last result suggests that SWS may also participate to memory consolidation in the RM task. Such role of SWS in consolidation has been strongly defended by Jan Born (Molle and Born 2011) who postulated that SWS fosters the replay of information encoded during wake in order to favor their transfer to the neocortex for systemic consolidation. However, the role of SWS in consolidation could also be due to sharp-wave ripple events that are fast depolarizing events occurring in the hippocampus and whose inhibition was shown to impair RM performance of rats trained in a radial maze similar to ours (Girardeau, Benchenane et al. 2009).

The absence of an effect of a REM sleep selective deprivation on RM performance is in disagreement with a previous study from our group showing that a 4-hours REMs-D impairs the consolidation of information in contextual and hippocampal-dependent memory in rats trained in a fear-conditioning paradigm and re-tested 24 hours after learning. In our sleep deprivation study, we used the exact same time course (learning followed by a 4 hours REMs-D and testing 24 hours later) but did not observe any deficits in learning after such deprivation. This result came as a surprise for two reasons: first, because the role of REM sleep in long-term memory consolidation is widely accepted and second, because in chapter 3 we observed a very transient increase in the quantity in REM sleep following the day the rats learned the RM rule (days 5) and no effect on SWS patterns suggesting that our RM task is mainly dependent on REM sleep related processes. In contrast, in chapter 4 and at the end of day 2, non-deprived animals had acquired the tasks perfectly (100% performance on the last 2 blocks of training). The massed training used in this experiment thus probably induced an over-learning that may have rendered the rats immune to a short REM sleep deprivation. As mentioned before, it is important to keep in mind that the number of animals run in this task is quite small and further analyses will be necessary to confirm our results that may change in the future.

Involvement of REM sleep and SWS in the processing of PI

The idea that SWS may be involved the processing (erasing) of irrelevant information after HIWM training appears quite clearly in our two sleep studies. In both studies, we found that an increase in performance of rats trained in this task was positively correlated to an increase in SWS. These results are in accordance with the SHY hypothesis proposed by Tononi and Cirelli that states that SWS may be a perfect candidate for erasing useless information by fostering LTD-like mechanism. However, the absence of an effect of TS-D on HIWM performance seems contradictory to this hypothesis. We may have two possible explanations for such conflicting results. First, the performance of non-deprived animals reached a quite low level (30% of errors) on day 2. It is therefore quite unlikely that disturbing SWS by TS-D would have induced a further decrease in performance. Second, the processing of PI may have been only delayed by the sleep deprivation. In fact, during the 4-hours following TS-D, we observed a slight (but not significant – not shown) increase in the quantity of SWS as compared to the non-deprived group. This SWS rebound could allow the processing of PI later in the day as compared to non-deprived rats and explain the absence of effect of a TS-D on HIWM performances on day 2. Further analyses will be performed on the sleep quantities following the sleep deprivation and may reveal that the increase in SWS during this rebound is correlated with the performances of rats trained in HIWM on day 2. Such result would confirm the role of SWS in forgetting processes and would show that, unlike memory consolidation that needs to occur immediately after learning during a specific time window (Breton and Robertson 2014), forgetting (a more essential process?) could be delayed and happen whenever SWS occurs.

Another point of disagreement between our two studies is that, in chapter 4, REM sleep quantities were positively correlated to an increase in performance of rats trained in HIWM whereas we did not observe such an effect in chapter 3. The role of REM sleep in memory consolidation has been extensively studied and consistent evidence for such role of REM sleep in memory was provided by studies in animals (Abel, Havekes et al. 2013). With the use of a variety of tasks including classic, aversive, an appetitive conditioning procedures, a large number of studies consistently revealed increases in REM sleep after learning in rats, mice, and cats. It was also shown that increasing REM sleep pharmacologically (by administration of carbachol into the pontine reticular formation and of corticotrophin-like intermediate lobe peptide) or by rebound (after prior REM sleep deprivation) post-training improved memory for a Y-maze discrimination task (Wetzel 2003). However, the role of REM sleep in forgetting has been poorly (if not at all) studied. Even if we did not observe a direct modulation of REM sleep in the HIWM task in our first study, the analysis of the fast theta oscillations revealed that these specific events occurring during REM sleep may be implicated in the processing of PI. However, given the difference between our two studies (positive correlation between REM sleep and performance in

HIWM in the second but not the first studies), further work is necessary to better understand the mechanisms linking REM sleep and forgetting.

Future directions

It seems clear that the biological bases of adaptive forgetting, and more precisely of the processing of PI are still widely unknown. Future investigations are required to determine the key physiological actors playing a role in such cognitive functions. The western blot analysis suggested that complex plasticity processes are occurring within the DG. However, the markers analyzed in the first two studies are indirect ones and more direct approach could help clarifying the mechanisms involved during HIWM. Mark Bear's group has recorded evoked potentials in the CA1 region of the hippocampus and has shown that learning induces LTP. However, such study has never been performed on forgetting. That is why in a near future (ongoing work not presented in this manuscript but to which I participated), we want to verify that different types of learning involving forgetting, or in contrast the long-term storage of information, modulate in a differential fashion *in vivo* hippocampal synaptic plasticity. More specifically, we plan to show that RM causes LTP and on the opposite, that training in a WM task requiring the processing of interference can induce LTD. In our laboratory, Felix Lallemand has shown (preliminary results) that HIWM training induces both LTP and LTD. Julien Carponcy has recently developed excellent tools to record evoked potentials and to assess the induction and maintenance of LTP and/or LTD during sleep. He has also designed a new software that allows on-line analysis of such data. Using these tools, we might find that HIWM training does indeed induce LTD, but only during sleep.

Quite recently, Benchenane and colleague have studied the interplay between the hippocampus and the medial prefrontal cortex (Pfc) and its importance for learning and memory consolidation. They measured the coherence in theta oscillations between these two structures in rats learning new rules on a Y maze. They showed that hippocampal/prefrontal coherence could lead to synchronization of reward predicting activity in prefrontal networks, tagging it for subsequent memory consolidation. It would thus be interesting to observe that, in our behavioral paradigm, the effect of PI induces a decrease in coherence between the DG and CA1. In our laboratory, Francesca Billwiller has developed optogenetic recording tools. Using optogenetic to increase or decrease the activity of a specific neuronal population in the DG (newborn or inhibitory neurons?) will be very particularly suited to elucidate the involvement of the DG in the processing of PI.

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