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THESIS

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by Sandra KLUR

Functional lateralization of spatial memory in the Rat: experimental approach by gene expression profiling and by reversible inactivation of the dorsal hippocampus in a spatial learning task

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THESE

Présentée pour obtenir le grade de
Docteur de l'Université Louis Pasteur de Strasbourg
Spécialité: Sciences du Vivant

par Sandra KLUR

Latéralisation fonctionnelle de la mémoire spatiale chez le Rat: approche expérimentale par analyse de l'expression des gènes et par inactivation réversible de l'hippocampe dorsal dans une tâche de mémoire spatiale

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“DNA makes RNA,
RNA makes proteins
and proteins make us.”
Francis Crick
in *the Central Dogma of Molecular Biology, 1958*

TABLE OF CONTENT

List of abbreviations

2-DG: 2-deoxyglucose

CA1: Cornu Ammonis 1 (a subregion of the hippocampus)

cDNA: complementary deoxyribonucleic acid

DNA: deoxyribonucleic acid

EST: expressed sequence tag

IEG: immediate early gene

Lido: lidocaine

LTD: long-term depression

LTP: long-term potentiation

mRNA: messenger ribonucleic acid

PBS: phosphate buffered saline

PCR: polymerase chain reaction

PF: platform

RNA: ribonucleic acid

TTX: tetrodotoxin

List of figures and tables

SUMMARY

1. French summary

Le rôle de l'hippocampe dans la mémoire spatiale est bien établi. Des approches reposant sur des techniques de lésions ou d'inactivations réversibles, ou encore l'analyse de l'activité des cellules de lieu dans cette région du cerveau ont permis de montrer, chez des rats, que l'hippocampe dorsal constitue un module fonctionnel jouant un rôle essentiel dans la mémoire spatiale. La première partie de mon projet de thèse avait pour but de déterminer s'il existe, dans l'hippocampe dorsal du Rat, des gènes qui sont différentiellement exprimés lors de la réalisation d'une tâche de mémoire spatiale dans le test de la piscine de Morris. De manière inattendue, nous avons mis en évidence que cette tâche cognitive pourrait reposer sur un processus neurobiologique latéralisé, et plus particulièrement que l'hippocampe droit pourrait présenter une implication plus forte que celle de son homologue gauche dans cette fonction. En effet, nous avons observé que le nombre de gènes exprimés différentiellement (activés ou réprimés) au cours de la tâche est nettement plus important dans l'hippocampe droit que dans l'hippocampe gauche. Phénomène n'ayant pas encore été clairement mis en évidence chez le rongeur avant nos travaux, la latéralisation de l'hippocampe – notamment le rôle prépondérant de l'hippocampe droit dans la « gestion » des informations spatiales – est bien décrite chez l'Homme, et a fait l'objet d'un étayage expérimental plus récent chez l'oiseau (pigeon). C'est donc tout naturellement que, dans la deuxième partie de notre travail, nous avons essayé de montrer que le phénomène de latéralisation observé au niveau moléculaire (modification du transcriptome) reflétait une latéralisation fonctionnelle de l'hippocampe. Pour ce faire, nous avons réalisé le test de la piscine de Morris en inactivant unilatéralement (à droite ou à gauche) ou bilatéralement l'hippocampe de rats. L'hypothèse était qu'une inactivation à droite aurait des conséquences plus marquées sur les performances mnésiques qu'une inactivation à gauche. Les résultats, quoique mitigés, tendent tout de même à confirmer que l'hippocampe droit aurait le rôle le plus important dans la gestion des tâches spatiales chez le Rat.

La première partie de notre travail a consisté à tester et valider une méthode permettant de préparer et d'analyser des microdissections de cerveau sur puces à ADN Affymetrix. Cette validation a été menée sur deux structures cérébrales de la Souris, à savoir l'hippocampe et le striatum. Une dissection manuelle classique ne nous permettant pas de prélever des échantillons avec le degré de finesse dont nous allions avoir besoin pour nos travaux chez le Rat, nous avons

eu recours à la microdissection au laser. Cette méthode consiste à prélever, grâce à un laser couplé à un microscope, des échantillons de petite taille sur des coupes du tissu d'intérêt. Après optimisation de cette technique, nous avons procédé à la quantification d'ARN total présent dans les microdissections. Nos microdissections contenant environ 50 ng d'ARN total, il ne nous était pas possible d'utiliser un protocole classique de préparation d'échantillon permettant d'analyser le transcriptome sur puces à ADN, ce protocole nécessitant encore, à ce moment-là, au minimum 5 µg d'ARN total. Sur des dilutions d'un ARN total de référence et sur des microdissections d'hippocampe et de striatum de souris, nous avons donc testé et comparé deux protocoles d'amplification différents, tous deux disponibles dans le commerce. A partir d'ARN total, le processus classique consiste à synthétiser un ADNc par transcription inverse à partir de l'ARN total présent dans l'échantillon. Puis, un ARNc est synthétisé à partir de l'ADNc, par transcription in vitro, et cet ARNc sera ensuite hybridé sur une puce à ADN. La première technique d'amplification, ou amplification linéaire, consiste à répéter 2 fois de suite le protocole classique. Le deuxième protocole consiste à amplifier l'ADNc par PCR, grâce à des amorces spécifiques. Après préparation et hybridation sur puce à ADN de nos échantillons de souris, nous avons réalisé une étude statistique qui nous a permis de conclure que la méthode par PCR permettait d'amplifier l'échantillon de manière plus rapide, plus fiable, plus proportionnelle à la méthode classique, et avec un ARNc de meilleure qualité que par la méthode par double amplification. Néanmoins, les deux techniques ont débouché sur des résultats quasiment identiques, et surtout, nous ont permis de mettre en évidence, dans les deux cas, la présence d'ARNm spécifiques à l'hippocampe et au striatum. Nous avons finalement décidé de poursuivre notre étude en utilisant le protocole d'amplification par PCR.

Dans un deuxième temps, après optimisation des protocoles, nous avons analysé le profil d'expression des gènes dans l'hippocampe de rats ayant réalisé le test de la piscine de Morris. Ce test permet d'évaluer les capacités du rat à gérer des informations spatiales dans une situation aversive. En effet, l'animal doit échapper à une situation inhabituelle et peu agréable (l'eau froide) en se réfugiant sur une plate-forme (PF) immergée sous la surface de l'eau. Les conditions de ce test peuvent être fixées de sorte qu'un rat ne puisse résoudre la tâche qu'en utilisant une stratégie d'orientation dans l'espace de type allocentrique, et donc basée sur son souvenir de la configuration de l'espace dans lequel il est testé. Les rats sont entraînés pendant 5 jours à raison de 5 essais consécutifs par jour. Le dernier jour, afin de vérifier si les rats ont bien appris la position de la PF immergée, le dernier essai est remplacé par un test de rétention pour lequel la PF est retirée de la piscine. Si le rat a bien appris la position de la PF, il nage principalement à proximité de l'endroit où se trouvait la PF lors des séances d'acquisition. Dans le cas contraire, il nage au hasard dans la piscine. Dans notre étude, nous avons testé 40 rats,

répartis dans 5 groupes. Tout d'abord, un groupe de rats entraînés à apprendre la position de la PF immergée, puis deux groupes de rats entraînés à trouver une PF visible (au-dessus de la surface de l'eau) et testés avec ou sans PF au cours de l'essai de rétention. Et finalement, nous avons testé un groupe de rats ayant nagé une minute sans PF et un groupe de rats naïfs n'ayant été exposés à aucun test comportemental. Après analyse statistique, nous avons identifié 623 gènes différentiellement exprimés dans l'hippocampe dorsal droit des rats entraînés à trouver la PF immergée ou visible, alors que seuls 74 gènes avaient un profil d'expression modifié dans l'hippocampe dorsal gauche de ces mêmes rats. Parmi les 623 gènes ayant un profil d'expression modifié dans l'hippocampe droit, 40% sont liés à la morphologie, la croissance et la réorganisation cellulaire et 25 % à la signalisation cellulaire. Ces résultats coïncident avec des travaux montrant que la plasticité cellulaire participerait à la mémoire spatiale et suggèrent donc que l'hippocampe dorsal droit pourrait avoir un rôle plus important que l'hippocampe dorsal gauche dans un processus de mémoire spatial tel que la piscine de Morris. Ces résultats ont été validés avec succès par PCR semi-quantitative pour 6 gènes.

Dans un troisième temps, nous avons poursuivi notre étude dans le but de confirmer la latéralisation observée précédemment. Pour ce faire, nous avons choisi de réaliser une étude comportementale associée à l'inactivation fonctionnelle réversible de l'hippocampe par microinjection d'un composé anesthésiant. L'inactivation repose sur l'injection intrahippocampique uni- ou bilatérale de tétrodotoxine (TTX) ou de lidocaïne, deux composés très largement utilisés et décrits dans la littérature, et qui bloquent l'un et l'autre les canaux sodiques. Toutefois, avant d'étudier les effets de microinjections hippocampiques sur la réalisation d'une tâche spatiale, nous avons évalué l'étendue et le degré de l'inactivation par ces deux composés en utilisant la technique du 2-désoxyglucose marqué au ^{14}C qui permet une visualisation qualitative et une quantification du métabolisme cérébral. Nous avons complété cette étude par l'évaluation des capacités de coordination sensori-motrices des rats après microinjection uni- ou bilatérale de ces deux substances. En effet, les performances relevées dans le test de la piscine de Morris reposant sur la nage, celles-ci ne peuvent être considérées comme pertinentes qu'à la condition de ne pas être biaisées par un déficit sensori-moteur. La TTX a été rapidement écartée de notre étude, en raison d'une zone d'action dépassant largement l'hippocampe et pouvant atteindre les régions adjacentes telles le cortex pariétal et le thalamus latéral, voire parfois l'hémisphère controlatéral. De même, il a été immédiatement établi que la TTX avait un impact négatif sur les capacités sensori-motrices des rats qui n'étaient plus capables de produire des performances sensori-motrices acceptables dans un test de franchissement d'une barre étroite surélevée (2.5 cm de large et 2 m de long). Exceptionnellement, les effets néfastes de la TTX se sont traduits par des crises convulsives. Ces

« effets secondaires » de l'inactivation étaient probablement liés à l'étendue très importante de l'inactivation cérébrale obtenue avec la TTX. Ces observations nous ont surpris, car nous avons injecté des doses de TTX couramment utilisées, publiées dans la littérature et pour lesquelles aucun effet secondaire de ce type n'avait été indiqué jusqu'à présent. Après microinjection de lidocaïne, la région inactivée se limitait au site d'injection de l'anesthésique, et nous n'avons observé aucun effet notoire sur les capacités sensori-motrices des rats.

Dans la dernière partie de notre travail, nous avons donc utilisé la lidocaïne afin de réaliser une inactivation réversible uni- ou bilatérale de l'hippocampe. Le but de cette expérience était de tester les capacités de navigation spatiale des rats après inactivation hippocampique lors de l'acquisition de la tâche ou du test de rétention. Pour des raisons pratiques, le protocole comportemental utilisé était légèrement différent du protocole de notre étude d'expression des gènes. Toutefois, le nombre d'essais lors de l'entraînement était identique. Les rats ont été entraînés sur une période de 6 jours à raison de 4 essais consécutifs par jour et le test de rétention a été réalisé le septième jour, soit 24 heures après le dernier essai. Tous les rats ont été entraînés à trouver une PF immergée, le groupe contrôle étant constitué de rats auxquels une solution de PBS était microinjectée bilatéralement. Dans un premier temps, nous avons réalisé une microinjection intrahippocampique unique, 5 minutes avant le test de rétention. Dans ce cas, nous avons remarqué que les rats ayant subi une microinjection à droite ou bilatérale présentaient une perte modérée, mais néanmoins significative, de leur capacité à se souvenir de la position de la PF immergée. Les rats ayant subi une microinjection de lidocaïne à gauche avaient des performances équivalentes à celles observées chez les témoins ayant reçu une microinjection bilatérale de PBS. Dans un deuxième temps, nous avons réalisé des inactivations tout au long de l'apprentissage de la tâche. Dans ce cas, la lidocaïne était administrée 5 minutes avant chaque session quotidienne d'apprentissage. Le jour du test de rétention, aucune microinjection n'a été réalisée. Nous avons observé que les rats ayant eu une microinjection du côté gauche avaient des performances indiquant une perte de leurs facultés de navigation spatiale, alors que les rats ayant reçu une microinjection bilatérale de lidocaïne du côté droit présentaient des performances proches des rats témoins ayant reçu une microinjection bilatérale de PBS. En conséquence, ces deux expériences nous permettent de proposer que l'hippocampe droit pourrait avoir une importance prépondérante lors du rappel de l'information spatiale au moment du test de rétention, alors que l'hippocampe gauche pourrait avoir un rôle plus marqué au moment de l'acquisition des informations spatiales.

En conclusion, notre étude d'expression des gènes a permis de mettre en évidence un argument indiquant une possible latéralisation de la mémoire spatiale chez le Rat, phénomène

qui, jusqu'à présent, n'était pas encore démontré chez un rongeur. Cette importance prépondérante de l'hippocampe droit pour la réalisation de tâches spatiales était toutefois connue chez l'Homme et décrite chez le Pigeon. Il est à noter qu'une asymétrie fonctionnelle a également été décrite chez la drosophile au cours d'un apprentissage associatif. L'importance de nos résultats est renforcée par le fait que les gènes présentant une expression différentielle dans l'hippocampe droit sont principalement liés à la morphologie, la croissance et la prolifération cellulaire ainsi qu'à la signalisation, processus déjà décrits comme étant fortement impliqués dans la mémoire, notamment spatiale. Par ailleurs, notre étude d'inactivation réversible tend à confirmer les résultats obtenus par puces à ADN et consolide partiellement le fait que les hippocampes droit et gauche contribuent de manière différente à la mémoire spatiale.

2. English summary

The role of dorsal hippocampus in spatial memory is well established. Approaches relying on lesion or reversible inactivation techniques or on activity analysis of place cells in this region of the brain showed that in the rat, dorsal hippocampus plays a major role in spatial memory. The first part of this thesis project was to determine if there are genes in the rat dorsal hippocampus, genes that are differentially expressed in relation to a spatial memory task in the Morris water maze. Unexpectedly, we could show that this cognitive task could rely on a lateralized neurobiological process and more particularly that the right hippocampus could present a stronger implication than the left hippocampus in this function. In fact, we observed that the number of genes differentially expressed (activated or repressed) during this task was higher in the right hippocampus than in the left one. A phenomenon still not clearly demonstrated for the rodent before our work – and more particularly the major role of the right hippocampus in the "management" of spatial information – lateralization is well described in humans and has recently been supported by experiments in the pigeon. In the second part of our work, we tried to show that the lateralization phenomenon observed at a molecular level (modification of the transcriptome) was reflecting a functional lateralization of the hippocampus. For this, we performed the Morris water maze test with rats subjected to unilateral (right or left) or bilateral inactivation of hippocampus. Our hypothesis was that right inactivation would have more extensive effects on mnesic performances than left inactivation. Results, although slightly controversial, tend to confirm that the right hippocampus could have the most important role for the management of spatial information in the rat.

The first part of our work consisted of testing and validating a method allowing preparation and analysis of brain microdissections on Affymetrix microarrays. This validation has been carried out on two cerebral structures of the mouse, namely the hippocampus and the striatum. Since classical manual dissection did not allow to dissect brain tissue as finely as necessary for our project in the rat, we employed laser capture microdissection. This method uses a laser coupled to a microscope to dissect out small-size samples of slices of the tissue of interest. After optimization of this technique, we quantified the amount of total RNA present in the microdissections. Given that our microdissections contained about 50 ng of total RNA, we were unable to use a classical sample preparation protocol to obtain sufficient cRNA to analyze the transcriptome on DNA microarrays, this protocol requiring, at that time, at least 5 µg of total RNA. Thus, using dilutions of a reference total RNA and microdissections of mouse hippocampus and striatum, we tested and compared two different amplification protocols

commercially available. The classical protocol consists of synthesizing, by reverse transcription, a double stranded cDNA using the total RNA present in the sample as template. Then a cRNA is synthesized by *in vitro* transcription and this cRNA is hybridized to a microarray. The first amplification protocol, also known as linear amplification, consists of repeating the classical protocol twice. The second procedure consists of amplification of the cDNA through a PCR reaction, thanks to specific primers. After preparation and hybridization of our mouse samples to microarrays, we performed a statistical analysis that enabled us to conclude that the method involving PCR amplification allowed faster and more reliable sample preparation; moreover this method was also more proportional to the classical method and produced a cRNA of better quality than linear amplification. Nevertheless, the two techniques gave similar results, and above all, allowed us to show in both cases, the presence of specific mRNA expression in the hippocampus and the striatum. We finally decided to continue our study by using the protocol based on PCR amplification.

In the second part of our project, we analyzed the gene expression profile of hippocampus of rats that were trained to the Morris water maze task. This test allows the evaluation of the rat's capabilities to manage spatial information in an aversive situation. Actually, the animal has to escape from an unusual and unpleasant situation (cold water) by climbing onto a platform (PF) submerged right under the surface of the water. The conditions of this test can be adjusted so that the rat can only solve the task by using an allocentric orientation strategy, based on its memory of the configuration of the space where the test is performed. Rats were trained for 5 days and had 5 consecutive trials per day. The last trial of the last day was replaced by a retention test, in which the PF was removed from the water maze, in order to verify that rats really learned the position of the PF. When a rat has really learnt the PF position, it swims mainly nearby the place where the PF was positioned during training. In the opposite case, the rat would swim randomly in the water maze. In our study, we tested 40 rats, divided into 5 groups. A group of rats was trained to learn the position of the submerged PF and two groups were trained to find a visible PF (above the surface of the water) and tested with or without visible PF on the retention trial. Finally, we tested a group of rats that only swam for one minute in the water maze (without the PF) and a group of naive rats that had not been submitted to any behavioral test. After statistical analysis, we identified 623 differentially expressed genes in the right dorsal hippocampus of rats trained to remember the position of a hidden or visible PF, whereas only 74 genes had a modified expression profile in the left dorsal hippocampus of these rats. Among the 623 genes differentially expressed in the right hippocampus, 40% were linked to morphology, cell growth and cell reorganization and 25% to cellular signaling. These gene expression data clearly show that spatial memory requires gene activity and more particularly

transcription. These results also support previous findings showing that cellular plasticity could play a role in spatial memory and suggest that the right dorsal hippocampus has a more important role than the left dorsal hippocampus in a spatial memory process like that incurred by the Morris water maze task. These results of gene expression profiling have been successfully validated on 6 genes using semi-quantitative PCR.

In the third part of our work, we extended our previous study in order to confirm the previously observed lateralization. For this, we decided to perform a behavioral study associated with reversible functional inactivation of the hippocampus by administration of an anesthetic compound. The inactivation procedure relies on uni- or bilateral intrahippocampal infusion of tetrodotoxin (TTX) or lidocaine, two widely used sodium channel blockers. However, before studying the effects of hippocampal microinjections on a spatial task, we evaluated the extent and degree of inactivation using these two substances. For this, we used the 2-deoxyglucose technique for visualization and measurement of cerebral metabolic activity. We completed this important control experiment with the evaluation of the rat sensory-motor coordination capabilities after uni- or bilateral infusion of the two compounds. This test was also of high relevance for our study, because the performances recorded in the Morris water maze rely on swimming and can only be considered as pertinent under the condition that they are not submitted to a bias due to a sensory-motor deficit. Therefore it was important to verify that rats can walk normally, thus swim normally, after intrahippocampal anesthetic injection. The TTX was not suited to our study because of its broad activity spreading far beyond the infusion site. Actually, the metabolic activity of adjacent regions such as the parietal cortex and the lateral thalamus, and even sometimes of the contralateral hemisphere was decreased. Likewise, it has been immediately established that TTX had a negative impact on the rat sensory-motor capabilities, because most of the animals were no longer able to walk on a narrow and elevated wooden beam (2.5 cm large by 2 m long). Moreover, in some extreme cases, harmful effects like convulsive seizures could be observed. These side effects of inactivation were probably linked to the extent of the cerebral inactivation obtained with TTX. We were astonished by these observations, because we injected commonly used TTX doses that were already published in the literature and for which no such drawbacks were previously described. In contrast to TTX, the region inactivated with lidocaine remained confined to the infusion site, and as a result we did not observe any obvious effect on the sensory-motor capabilities of the rat.

In the last part of our work, we therefore used lidocaine in order to perform uni- or bilateral reversible inactivation of hippocampus. The aim of this experiment was to test the rats' spatial navigation capabilities after hippocampal inactivation in acquisition or retention of a spatial memory task. For practical reasons, the behavioral protocol was slightly different from

the protocol used in our gene expression study. However, the number of training trials was similar. Rats were trained over 6 days, with 4 consecutive trials per day and the retention test was performed on the seventh day, that is 24 hours after the last training trial. All rats were trained to find a submerged platform, with the control group composed of rats receiving bilateral PBS infusions. First we applied a unique lidocaine microinjection, 5 min before the retention test. In this case, we noticed that rats receiving a right or bilateral injection had a mild but nevertheless significant impairment of their capabilities to remember the position of the submerged PF. Rats that received a lidocaine microinjection in the left hippocampus had performances close to the control rats that reveived bilateral PBS infusion. Then, we performed inactivation during the acquisition of the task. In that case, lidocaine was administered 5 min before each daily training session and there was no microinjection before the retention trial. We observed that rats receiving a left lidocaine infusion were impaired for their spatial navigation capabilities. In contrast, rats receiving a lidocaine infusion in the right hippocampus had performances close to those observed in control rats receiving bilateral PBS microinjection. In summary, these two experiments suggest that the right hippocampus might have a dominating importance for the recall of spatial information on the retention test, whereas the left hippocampus might be more involved in the acquisition of spatial information.

In conclusion, our gene expression study provides the first evidence demonstrating a possible lateralization of spatial memory in the rat, a phenomenon that has not yet been shown in rodents. The role of the right hippocampus in processing spatial information is well known in Human and also described for Pigeons with classical techniques. A functional brain asymmetry important for associative learning has also been described in Drosophila. The weight of our results is reinforced by the fact that genes presenting a differential expression in right hippocampus are mainly linked to morphology, growth and cellular proliferation as well as signaling, processes largely described as being cerebral markers for spatial memory. Finally, our behavioral study using reversible inactivation confirms the gene expression profiling results and suggests that there is, at some extent, a functional lateralization in rat brain. In summary, we showed that spatial memory/learning are processes which require transcription and we observed a lateralization of hippocampal function with right and left hippocampus contributing at a different level to spatial memory.

INTRODUCTION

1. General remarks

A human brain is constituted of about 100 billion neurons that build a complex network of synaptic connections that are continuously dealing with and exchanging large amounts of information. Learning and memory are fundamental for humans, as they are the basis for creating our identity by recording the experiences we live. Humans have been fascinated by their own learning and memory capabilities for a long time and many philosophical theories have been built on its functioning (e.g. St Augustin, 397). Since the 20th century, psychology and biology are bringing a more experimental view of this fascinating phenomenon. More particularly, biologists research which brain regions mediate learning and allow storage of memories. As the analysis of such a complex organ is a huge task and it is not possible to directly analyze human brain, except by non-invasive methods like Magnetic Resonance Imaging (MRI), Nuclear Magnetic Resonance (NMR) or on the basis of post-mortem dissection of brains, biologists tried to focus on model organisms such as rodents, frogs, birds or drosophila. By now, knowledge about memory and learning has grown and much work has been done in the field of associative learning or of management of spatial information. The hippocampus, a small region of the brain, plays a prominent role in spatial learning and memory. However, many processes are far from being completely understood, though thanks to the development of modern technologies allowing high-throughput analysis, researchers are now able to get large amounts of data about what is happening in the brain when we encode, consolidate or retrieve information. The understanding of the complex mechanisms underlying a well-operating brain will allow a deeper insight into brain disease and will potentially help to find new treatments to cure such diseases. The work presented here – showing data from investigation of spatial learning and memory in the rat, as a model - is our contribution to the huge task of understanding the still partly mysterious processes that are going on in the brain while we are behaving. How do animals and mammals build a reliable memory of their environment, which is essential for survival? Which regions of the brain are required for spatial navigation? Which molecular events could underlie spatial learning and memory? Some answers are partially known, but much work has to be done to understand how the brain is working. The focus of this thesis is the study of global gene expression related to spatial learning and memory in rat hippocampus. This molecular study has been complemented by a behavioral analysis that enabled us to show that the two hemispheres of a rat brain might, to some extent, play different roles in spatial memory,

even though one could think that both brain hemispheres are similar and have comparable functions in some species.

2. Objectives of the project

This thesis project has the challenging objective to apply an original approach using high-throughput molecular technologies in combination with behavioral tests to the study of spatial learning and memory in the rat. The aim of this work was to precise the molecular events occurring in the dorsal hippocampus of rats doing a spatial navigation task. Based on the results of this first experiment, the project evolved and the focus was refined to the study of lateralization of spatial learning and memory in rat dorsal hippocampus. Thus, the principal objectives of this work consisted of:

- developing and testing the molecular tools allowing taking out very precisely a small brain portion, followed by optimization of sample processing for analysis on DNA microarrays.
- application of these optimized techniques to the study of gene expression in the dorsal hippocampus (region CA1) of rats trained in a water maze task.
- characterizing the metabolic and behavioral effects of functional reversible inactivation of the dorsal hippocampus by intrahippocampal lidocaine or tetrodotoxin infusions.
- application of the optimized reversible inactivation technique to precise the role of the left and the right dorsal hippocampi in the resolution of a spatial navigation task.

3. Structure of the thesis

In the first part (“scientific context”), the main concepts addressed or used in this work will be presented. To begin with, we will expose the cognitive theories related to spatial learning and memory. Then, the molecular events supporting spatial memory and learning will be described. In the third paragraph, we will review the current knowledge about brain lateralization in humans and animals. Afterwards, data about lateralization of spatial memory will be presented. Then, we will present basic knowledge about the genome, the transcriptome and the proteome. This will be followed by the description of the technologies and tests used in this thesis work. Finally, we will describe the outline of our project.

In the second part (“technical optimization”), the reader might expect to read an exhaustive description of the methods used to explore the biological question addressed in this thesis. Actually the investigations resulted in the publication of two articles and in the recent submission of one manuscript for review by a scientific journal. The two published articles present technical data. Therefore, the classical “material and methods” has intentionally been removed and has been replaced by the first publication presenting the evaluation of protocols used to perform gene expression and by the second publication describing the comparison of functional inactivation strategies to avoid redundancy. The principles of the microarray technology, of reversible inactivation and of the main behavioral tests performed in this work will be presented in the “scientific context”.

The third part (“results”) will present our contribution to the understanding of spatial memory in the Rat. Most of the “biological results” are contained in the manuscript that is reported here in its submitted form. These data will be complemented by a supplementary experiment that was not published. Finally, the fourth part (“discussion”) contains a general discussion of our results followed by concluding remarks and perspectives.

SCIENTIFIC CONTEXT

1. Learning, memory and spatial navigation

Spatial navigation is essential for the survival of most animal species. In fact, animals must have reliable orientation skills for several reasons. For example, they must be able to locate and remember the localization of food resources, they have to remember the location of their nest to protect offsprings; they should be able to escape from predators by hiding in a safe place. All these capacities relying on spatial orientation contribute to the perpetuation of species.

This first paragraph aims at describing basic knowledge about memory, in humans and also in the Rat, which is the animal model used in this work to study spatial navigation. After a general definition of memory, some facts about memory systems in humans are mentioned. Finally, the hippocampal structure, playing a central role in building and retrieving memories is described and its role in (spatial) memory is presented.

1.1. General definition

Memory can be defined as a long-lasting modification of behavior resulting from learning. This modification can be observed and measured, and indicates that learning occurred. Memory is a process involving several discrete stages (for review, see Squire and Kandel, 1999): encoding, consolidation, storage, retrieval and forgetting. In the first step, the brain has to encode new information. Encoding refers to processes that allow the conversion of the perception of new information into a neural trace. Then, the organism must be able to store the memory. In this stage, the new memory trace has to be sustained by neural connections that are strong enough, otherwise the information can be lost and thus cannot be retrieved. Once the information is encoded and stored, it must be retrieved for remembering to occur. Retrieval involves the process of locating and activating the neural trace that represents the required information, by recapitulating the processes that were involved in the initial encoding of the information. Surprisingly, forgetting also plays an important role in memory. Actually, it is quite a usual and useful process: it might happen because new information overwrites old information and it also happens because time passes and therefore it protects the brain from an overflow of useless information. On the contrary, consolidation describes the capacity of neural information to resist forgetting. Consolidation occurs by conversion of short-term memory to long-term memory; consequently the neural traces become more resistant to loss.

1.2. Memory systems in humans

As early as in the 1950's, a large amount of data about memory was collected from the analysis of patients suffering from amnesia following cerebral lesions (ischemia, surgery, trauma, etc.). These data suggested that memory is not a unitary process, but that it is organized in several systems (Jaffard, 1994, for review) comprising different brain regions but in which a particular structure – the hippocampus – plays a central role.

The first theory, proposed by James in 1890, makes a distinction between short-term memory and long-term memory. Short-term memory (called primary memory by James) refers to a process maintaining information temporarily (from seconds to few hours), until it is forgotten (useless information) or incorporated into a long-term information stock, more stable and potentially permanent. The capacity of short-term memory is quite low and only 4 to 7 items can be maintained at the same time. In contrast, long-term memory (called secondary memory by James) can last for a few minutes to lifetime and there is apparently no limit to the amount of information that can be stored, the only constraint being the finite number of neurons present in the brain.

Long-term memory is supported by at least four memory systems that differ by the type of information that is stored (Tulving, 1992; Squire and Zola-Morgan, 1988; Squire and Zola, 1996). Episodic memory is an autobiographic memory for events that have been personally experienced and stores the spatial and temporal references identifying time and place where these events occured. Semantic memory concerns the facts and conceptual knowledge. Procedural memory stores general know-how and sensory-motor capacities. Finally, the system of perceptive representation refers to the phenomenon of priming, which corresponds to the ability to detect or identify words or objects soon after having perceived them.

A distinction between explicit memory and implicit memory or between declarative memory and non-declarative memory has also been made (Squire and Zola-Morgan, 1988; Squire, 1992; Schacter et al., 1993, Squire and Zola, 1996). This difference relies on the fact that the remembering of the information can be conscious or unconscious. Therefore, non-declarative or implicit memory groups all cognitive operations that do not refer to a personal experience (procedural memory, perceptive representation and semantic memory, after Tulving, 1992). Conversely, declarative or explicit memory refers to episodic and short-term memory for which the subject knows in which spatial, temporal and emotional context an event happened.

Finally a distinction between working memory or reference memory can be made (Honig, 1978). Working memory is considered as an on-line system, working in parallel and which

allows the temporary maintenance of a set of information useful for a specific cognitive operation like language or reasoning, adding numbers, following directions, playing cards (Baddeley, 1992). Information supported by working memory is “erased” as soon as it becomes useless and therefore this information is never transferred into permanent memory. In contrast, reference memory can be compared to long-term memory because it refers to the ability to store information about a specific fixed situation. For example, reference memory allows the retention of the route from home to workplace, the rules of a card game.

1.3. Anatomical description of the rat hippocampus

The hippocampus is a structure in vertebrate brain that plays important roles in the formation of new memories. The anatomy of the rat hippocampal formation is described in the following part.

The hippocampus is a bilateral and symmetrical structure within the brain. It is part of the limbic system and is named for the resemblance of its curved shape to a sea horse (Figure 1A and 1C). The hippocampal formation is composed of five structures: the hippocampus, the subiculum, the presubiculum, the parasubiculum and the entorhinal cortex (Amaral et Witter, 1995). The hippocampus consists of two C-shaped interlocking principal cellular layers: the dentate gyrus (DG) and the hippocampus proper also named Cornu Ammonis (CA). The hippocampus is subdivided into three areas named CA1, CA2 and CA3 (Figure 1B).

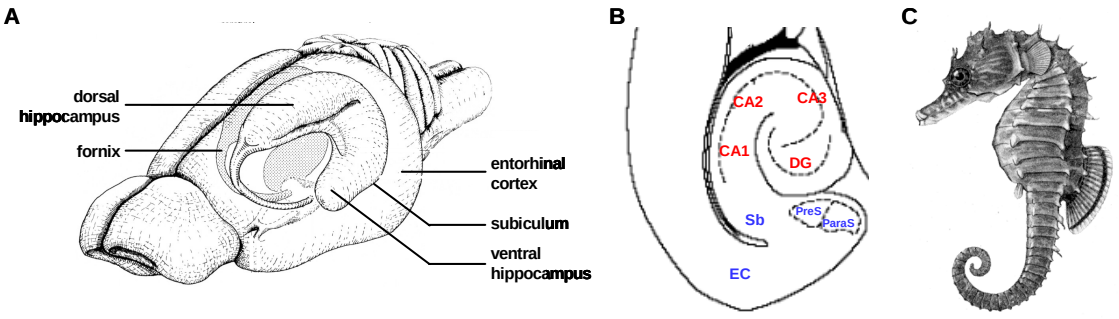

Figure 1. The hippocampus. **A.** Localization of the structures of the hippocampal formation on a lateral view of rat brain (from Amaral and Witter, 1995). **B.** Representation of a horizontal section of rat brain indicating the localization of the structures of the hippocampal formation (from Amaral and Witter, 1995). CA1, CA2 and CA3: Cornu Ammonis 1, 2 and 3; DG: dentate gyrus; Sb: subiculum; PreS: presubiculum; ParaS: parasubiculum; EC: entorhinal cortex. **C.** Sea horse, also named Hippocampus.

The hippocampal network including principal input, output and internal connections is depicted in Figure 2. The hippocampus forms a principally uni-directional network, with input

from the entorhinal cortex (EC) that forms connections with the dentate gyrus (DG) and CA3 pyramidal neurons via the perforant path. CA3 neurons also receive input from the dentate gyrus via the mossy fibers (MF). They send axons to CA1 pyramidal cells via the Schaffer collateral pathway, as well as to CA1 neurons in the contralateral hippocampus via the commissural pathway. CA1 neurons also receive input directly from the perforant path and send axons to the subiculum (Sb). These neurons in turn send the main hippocampal output back to the entorhinal cortex, and to other brain structures.

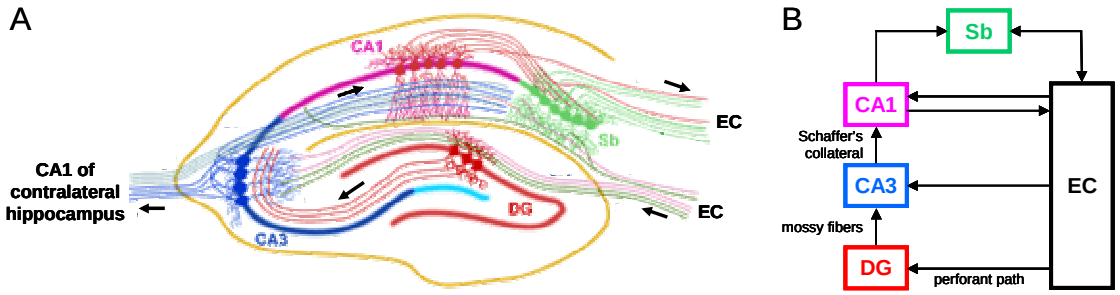

Figure 2. The hippocampal connections. **A.** Main hippocampal connections represented on a coronal section of dorsal hippocampus (adapted from www.bris.ac.uk). **B.** Schematic representation of the main hippocampal connections. CA1 and CA3: Cornu Ammonis 1 and 3; DG: dentate gyrus; EC: entorhinal cortex; Sb: subiculum.

In conclusion, the hippocampus has a pivotal position thanks to its numerous afferent and efferent neurons. The main afferent projections to the hippocampus arise from the entorhinal cortex, which relays information about the environment of the subject originating from several associative and sensorial cortical areas. Moreover, information related to emotion, motivation and physiology of the subject are also transmitted by the septum and the entorhinal cortex to the hippocampus. Finally, the hippocampus integrates all these data and forms various memories.

1.4. The hippocampus and spatial memory

The importance of the hippocampus in memory was first evidenced in the patient HM (Scoville and Milner, 1957). HM suffered from frequent treatment-resistant epileptic seizures. Therefore, he was subjected to a bilateral temporal lobe resection, including the hippocampus, the amygdala and the surrounding cortex, in order to stop epileptic episodes. Unfortunately and at that time somewhat unexpectedly, the surgery caused a profound inability of the patient to form new memories. However, his long-term memory was intact, except for a retrograde amnesia affecting recollection of events that happened between 2 and 10 years before surgery.

Three decades later, another case of amnesia with symptoms similar to HM was reported (Zola-Morgan et al., 1986). This patient, named RB, suffered from a stroke that affected his bilateral temporal lobes. A postmortem study of his brain showed that damages due to stroke were limited to the CA1 region from both lobes of RB's hippocampus.

Early experiments with rats in the 1970's provided much evidence that the hippocampus plays an important role in spatial navigation. First evidence was given by pioneering electrophysiological studies of single neurons (O'Keefe and Dostrovsky, 1971; O'Keefe et al., 1975). It was shown that some cells were only firing when rats entered specific and restricted areas of a familiar environment. From this finding, O'Keefe and Nadel (1978) built their theory of the "cognitive map", an expression introduced and used 30 years before by the american psychologist Edward Tolman (Tolman, 1948). At that time, lesions of the hippocampus and its connections induced deficits in spatial memory (O'Keefe and Nadel, 1978; Olton et al., 1978). Similarly, spatial navigation deficits due to reduction of the perforant path input to the hippocampus were also observed in senescent rats (Barnes, 1979). Finally, in 1984, Morris reported his first experiment using the water maze. In fact, he showed that total hippocampal lesions impair spatial navigation, with motor and motivational aspects being unaffected in this test situation.

Further evidence linking the hippocampus to spatial memory in animals comes from comparative neuroanatomy. Hippocampal volume has been shown to be related to spatial ability in several species of birds and small mammals (Krebs et al., 1989; Lee et al., 1998; Sherry et al., 1992; Healy and Krebs, 1993) in terms of their ability to keep track of a large number of stored foods items or a large home range. Furthermore, variations in the hippocampal volume in birds and some mammals were shown to track seasonal changes in the need for spatial memory (Smulders et al., 1995; but Lavenex et al., 2000). In some respects, comparable data were reported in humans. Actually, London taxi drivers have an extensive spatial orientation experience, since they know how to navigate between thousands of places in the city. It has been shown that taxi drivers had an increase in the volume of left and right posterior hippocampus in comparison to control subjects who lacked such extensive navigation exposure (Maguire et al., 1998). Finally, kinds of place cells have been identified in the human hippocampus (Ekstrom et al., 2003).

In conclusion, various approaches (clinical studies, neuroanatomy, electrophysiology, lesion studies in various animal models, etc.) have shown that hippocampus plays a major role in spatial learning and memory.

1.5. Experimental procedures used to test spatial navigation in rodents

Several experimental devices allow the evaluation of the spatial learning and memory capacities in rodents. The Morris water maze (Morris, 1984) and the 8-arm radial maze (Olton and Samuelson, 1976) are the most frequently used. Both tests were used in this thesis. Depending on the protocol, it is possible to test either the reference memory or the working memory.

1.5.1. Morris water maze

The orientation task in the water maze is one of the most eloquent tests where animals have to use their spatial navigation capacities. The rat is placed in a tank filled with opaque water and has to find a submerged platform in order to escape from water as fast as possible (Figure 3). The rat can orientate itself thanks to several visual cues external to the maze (e.g. a flag hanging on the wall). The test relies on water aversion and the motivation of the rats is to escape from hostile conditions (cold water at 20-22°C). Parameters like “escape time” or “distance swum” allow monitoring of orientation abilities of the rat. However, the distance is less sensitive to sensory-motor biases than the latency. It has been shown that rats, after some training sessions are able to locate very precisely (rapid and straight swimming) the position of the platform thanks to cues surrounding the pool.

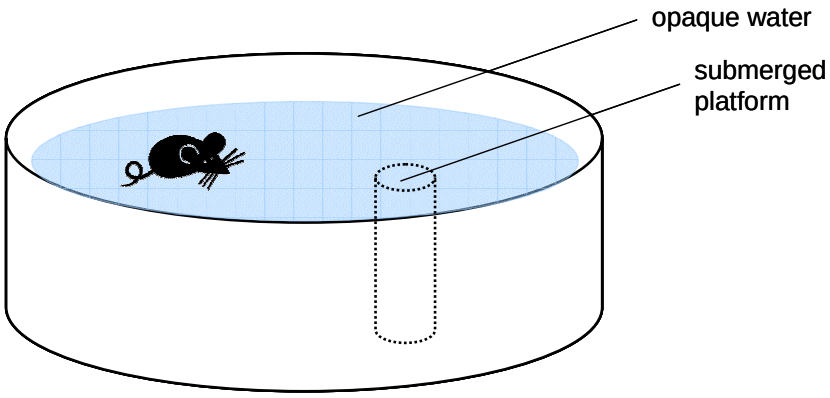

Figure 3. Representation of the Morris water maze. For each trial, the rat is released from another position and has to learn the position of a platform hidden 1 cm under the surface of the water. The maze is placed in a room containing visible cues allowing spatial orientation.

Various water maze protocols have been developed and we used a procedure allowing the evaluation of reference memory, which has been designed such as the spatial constraints of the task require the use of a cognitive map. In this test, the position of the escape platform is

constant from a trial to another; however the release position changes from trial to trial. This situation promotes research behavior relying on the use of allocentric cues, which are independent from the position of the rat (Whishaw, 1985). The decrease of escape latency and distance reflects learning. At the end of the learning period, a probe without platform trial is performed. Successful learning is characterized by the preference of the rat to swim in the area where the platform was located during learning.

1.5.2. Radial maze

The device is made of a central circular platform from which 8 or 12 arms (or even more) are radiating. In this test, rats have to find food pellets placed at the end of all or part of the arms (Figure 4). In this case, contrary to the water maze, the motivation is not due to an aversive condition but it is a positive reinforcement because rats are on food restriction before and during the test. The food pellets are not visible from the center of the apparatus and are not replenished after having been eaten. For the rat, the task consists of developing an optimized exploration scheme of the maze in order to find all food pellets by using the shortest way. The main issue for the rats is to distinguish visited from non-visited arms.

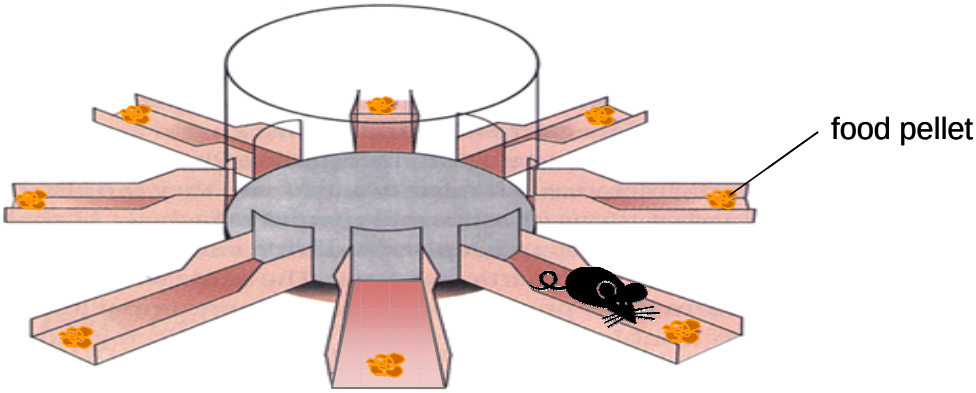

Figure 4. Representation of the 8-arm radial maze. For each trial, the rat is released from the central platform where it has access to the arms baited with food pellets. The maze is placed in a room containing visible cues allowing spatial orientation (adapted from www.ethologie.unige.ch).

The initial experiment (Olton and Samuelson, 1976) showed that rats do not use maze internal cues (i.e. odors), and consequently use external visual cues. In its original definition (Olton and Samuelson, 1976), this task allows testing of working memory. However, numerous protocols of radial maze have been developed and depending on the design of the test (number of baited arms, rotation of the maze between trials) it is possible to test different types of memory (i.e. working versus reference memory). We used a version where rats had to use reference memory to solve the test.

2. Molecular events related to spatial learning and memory

The first ideas regarding the ability of experience to shape and modify brain structures as well as synaptic junctions between neurons can be traced back to the end of the 19th century and were proposed by Ramon Y Cajal. More recently, Hebb proposed a cellular mechanism for associative learning, postulating that coincident activity at given synaptic junctions modifies the properties of those synapses, thereby increasing their efficiency (Hebb, 1949). This principle dominates thinking at present and presides over theoretical views on how use-dependent changes at synapses occur during learning. Investigations into the mechanisms underlying learning have thus primarily focused on the computational unit of the nervous system, namely the synapse. Synapses are not static; the cellular mechanisms of learning and memory, induced by experience, involve biochemical modification and morphological remodeling of existing synapses, as well as genesis of new synapses, resulting in activity-dependent physiological changes, which collectively define interactions between an organism and its environment. The majority of these investigations have focused on the mechanisms underlying changes in synaptic plasticity strength in one of the anatomical regions most relevant to learning and memory, the hippocampus. The study of synaptic plasticity in the hippocampus and its relationships to learning and memory has unveiled a labyrinth of potential mechanisms and molecular signals beyond the scope of the present contribution, and has been recently reviewed (Martin et al., 2000; Abel and Lattal, 2001).

The next paragraphs document the major molecular events supporting spatial learning and memory. In the first part, genes (and their products, mRNAs and proteins) that have already been shown to have an important role in spatial learning and memory will be presented. Then knowledge about the role of neurogenesis, long-term potentiation and long-term depression (LTP and LTD) and place cells activity will be described. Actually all these mechanisms cooperate and contribute simultaneously to the management of spatial information, and perhaps more fundamentally to the constitution of a spatial representation of the environment, a concept also called “cognitive map” by Tolman (1948).

2.1. mRNAs and proteins supporting learning and memory processes

The storage of long-term memory is associated with a cellular program of gene expression, modification of protein synthesis and growth of new synaptic connections (Kandel, 1997; Bailey et al, 1996). The duration of the memory is commonly divided into at least two temporally distinct components: short-term memory, lasting minutes to hours and long-term memory, lasting days, weeks, and, in some cases, even a lifetime. Studies of long-term memory indicate that it employs a cascade of molecular events that occurs during the consolidation period – the initial phase of memory storage – that is labile and highly sensitive to disruption. The conversion of a short-term form that requires only covalent modification of preexisting proteins, to a more stable and self-maintained long-term form that is accompanied by the growth of new synaptic connections, requires a cellular program of gene expression and increased protein synthesis. In this section, some facts about the role of specific mRNAs and proteins for learning and memory will be presented. Hundreds of genes and proteins participate in these processes, and have not yet been identified. Therefore only some examples will be described, for which their role in learning and memory is quite well understood or at least demonstrated. An exhaustive listing of these genes would be beyond the scope of this contribution.

2.1.1. Immediate early genes

Immediate early genes (IEGs) are activated transiently and rapidly in response to a wide variety of cellular stimuli. They do not require protein synthesis to be activated and, upon activation, can in turn activate downstream targets. IEGs can be divided into two functional classes (Figure 5). The first class encodes regulatory transcription factors (RTFs), which may indirectly influence cellular physiology by increasing or decreasing expression of specific downstream genes (Herdegen and Leah, 1998; O'Donovan et al., 1999; Tischmeyer and Grimm, 1999). The second class encodes a diverse range of biological effector IEGs, which have more defined and direct effects on cellular function than RTFs. Using subtractive hybridization techniques, Lanahan and Worley (1998) estimated that 30 to 40 genes could constitute the total neuronal IEG response (~10 to 15 RTFs and ~25 to 30 effector IEGs).

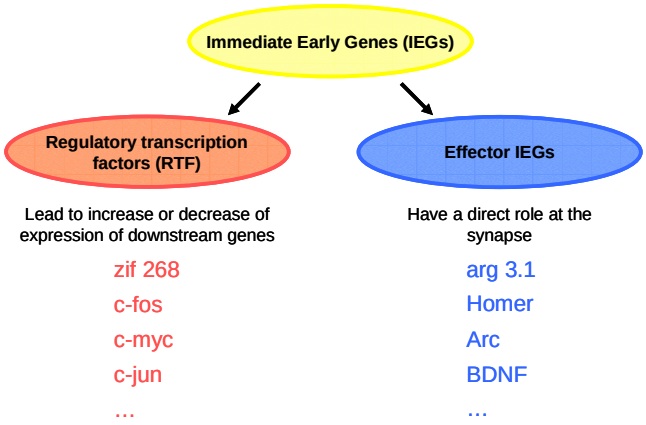

Figure 5. Immediate Early Genes (IEGs). There are two families of IEGs: (1) the regulatory transcription factors which modify expression of downstream genes and (2) the effector IEGs which play a role at the synapse.

The expression of specific immediate-early genes (IEGs) is induced by neural activity that produces stable changes in synaptic strength (Demmer et al, 1993; Worley et al., 1993). The expression of IEGs is also modified by behavioral training (Hess et al., 1995; Nagahara and Handa, 1995, Seeds et al., 1995; Vann et al., 2000). This has led to the hypothesis that expression of IEGs plays a role in the neuroplastic mechanisms required for memory consolidation processes (Robertson, 1992; Kaczmarek, 1993; Dragunow, 1996; Tischmeyer and Grimm, 1999).

Arc (an effector IEG), c-fos and zif 268 (two RTFs) have been analyzed in details. The regulation of the expression of these three IEGs in hippocampus follow a similar pattern and it has been observed that their mRNA levels increased in rats performing a spatial learning task in a water maze (Guzowski et al., 2001).

A more recent work showed the involvement of Zif 268 in reconsolidation in dorsal hippocampus of rats (Lee et al, 2004). Reconsolidation is a process allowing the stabilization of already consolidated memories that become labile once they are recalled. It has been shown that Zif 268 plays a key role in reconsolidation by inducing expression of new mRNA (and thus of the corresponding proteins), which stabilize memories. Another study, in birds, showed the importance of zenck (avian orthologue of Zif 268) in the memory processes associated with the acquisition, perception and production of song in forebrain structures (Metzger et al., 1998; Braun et al., 1999; Thode et al., 2005).

Finally, further experiments on the role of Arc in learning and memory showed that Arc interacts with the Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II). CaM kinase II is a multifactorial mediator of activity dependent on the increase in calcium levels in excitable cells (Bennett et al., 1983; Goldenring et al., 1983). It is highly concentrated in the brain and is one of the major proteins in the postsynaptic density (PSD) of the cerebral cortex and hippocampus (Goldenring et al., 1984; Kelly et al., 1984). CaM kinase II has been implicated in the

modification of nerve functions, including learning and memory (Kang et al., 2001; Lisman et al., 2002). More particularly, it has been shown that Arc potentiates the action of CaM kinase II for neurite extension.

In conclusion, the role of IEGs at various stages of memory formation is now well established. They contribute to learning and memory processes either by acting directly at the synapse or by inducing transcription of downstream genes having a role in synaptic plasticity.

2.1.2. Neurotrophic factors

Mechanisms of neuronal plasticity during brain development show striking similarities to those proposed to occur during learning in the adult nervous system. Actually, biological molecular signals are often conserved to serve similar functions in a diversity of roles, enhancing the evolutionary advantage they confer. Due to their established role during brain development, members of the family of neurotrophins are attractive molecular candidates for modulating synaptic plasticity during learning and memory processes. In addition, neurotrophins represent molecular signals initially establishing, and then allowing an organism's nervous system to remain in an unbound plastic state.

The mammalian neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), all have been shown to play an essential role in neuronal viability and differentiation, as well as synaptic plasticity in various brain regions relevant to learning and memory (McAllister et al., 1999). As an example of neurotrophin pathway signaling, we will summarize what is known about BDNF. It has been shown that hippocampus-dependent learning is associated with a rapid and transient increase in BDNF mRNA expression in the hippocampus (Hall et al., 2000). Several studies using either BDNF-mutant mice (Minichiello et al., 1999; Gorski et al., 2003), or the infusion of antisense BDNF oligonucleotides in rat brain (Mizuno et al., 2000), or treatment with anti-BDNF antibodies (Mu et al., 1999) have all shown the important role of BDNF for learning and memory and especially for spatial navigation. BDNF acts by inducing downstream pathways including signaling cascades (especially PI3-K and MAPK cascades) to enhance translation initiation and increase protein synthesis in neurons (Takei et al., 2001). Its activity is also mediated by glutamate receptors including NMDA and non-NMDA receptors (Zafra et al., 1990; Hughes et al., 1993). A summary of the pathway under the control of BDNF is illustrated in Figure 6, on the next page.

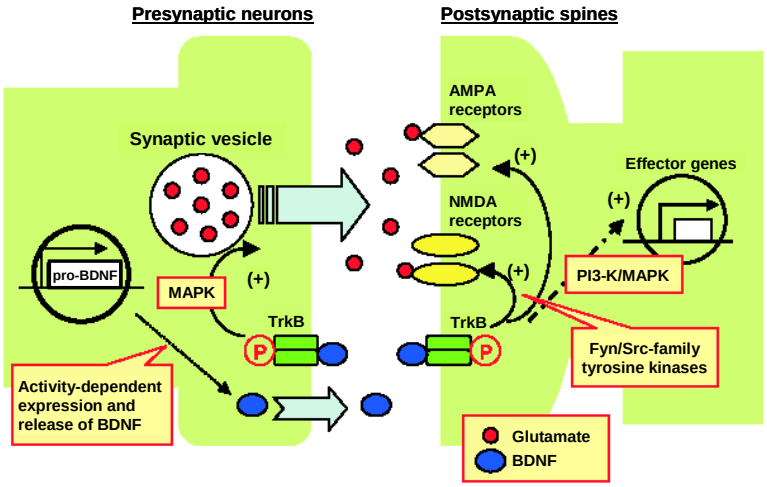

Figure 6. Interaction of BDNF/TrkB signaling with NMDA receptors in synaptic plasticity. Neuronal activity increases BDNF gene expression and stimulates BDNF release from presynaptic sites in an activity-dependant manner. BDNF binds to TrkB receptors located at presynaptic and postsynaptic sites leading to the activation of signal transduction pathways including MAPK, PI3-K and Fyn. Activation of BDNF/TrkB signaling induces phosphorylation of NMDA receptors, increases AMPA receptor expression and stimulates glutamate release (from Yamada and Nabeshima, 2004).

Extensive literature on the role of neurotrophins in synaptic plasticity, including neurotransmitter release and intracellular signaling cascades, has been reviewed in recent articles (Segal and Greenberg, 1996; Schinder and Poo, 2000; Thoenen, 2000; Poo, 2001; Tyler et al., 2002).

2.1.3. Translation factors

Studies of synaptic plasticity have shown a link between mRNA translation, learning and memory. Synaptic plasticity includes an early phase that depends on modification of pre-existing proteins and a late phase that requires transcription and synthesis of new proteins (Kandel, 2001; McGaugh, 2000). Activation of postsynaptic targets seems to trigger the transcription of plasticity-related genes. The new mRNAs are either translated in the soma or transported to synapses before translation.

Translation of eukaryotic mRNAs is regulated primarily at the level of initiation which is facilitated by the initiation factor 2 (eIF2) which in turn stimulates the mRNA translation of the transcriptional modulator ATF4 (Harding et al., 2000), which inhibits synaptic plasticity and behavioural learning in various animals. GCN2, an evolutionary conserved eIF2 kinase, enriched in the brain of flies and mammals (Santoyo et al., 1997; Berlanga et al., 1999; Sood et al., 2000), has been shown to regulate synaptic plasticity, as well as learning and memory, through modulation of the ATF4/CREB pathway (Costa-Mattioli et al., 2005). This confirms the regulating role of transcription for the formation of memory.

2.1.4. The postsynaptic density

The postsynaptic density (PSD) is a cellular structure specialized in receiving and transducing synaptic information. It is well established that the regulation of synaptic properties occurring at the PSD plays an important role in synaptic plasticity (Sheng, 2001; Yamauchi, 2002). One of the most abundant proteins in the PSD has been identified as the α-CaM kinase II. α-CaM kinase II phosphorylates a number of functional proteins in the PSD fraction, including glutamate receptors, membrane proteins, scaffold or adaptor proteins, cytoskeletal proteins and enzymes (Yoshimura et al., 2002). Therefore CaM kinase II may modulate synaptic transmission and plasticity through the regulation of these proteins in response to a rise in postsynaptic calcium levels.

In conclusion, many genes participate in the formation of memories as shown by the amount of data generated in the field of neuroscience. However, there is still much to do in order to understand how the different steps of learning and memory are operating in situ, from the moment at which a given event is experienced to the moment at which a memory is formed. Some pathways involved in learning and memory have already been identified and show that learning and memory are driven by fine processes occurring at the molecular level. Understanding how the brain works will help to understand brain disorders, and vice versa.

2.2. Neurogenesis

Neurogenesis - the birth of new neurons - occurs throughout life in mammals (Altman and Das, 1965; Kaplan and Hinds, 1977) including human (Eriksson et al., 1998). It has been associated with spatial memory and learning in birds and small mammals (Lavenex, 2000; Patel, 1997; Shors, 2001; Drapeau et al., 2003) and has been evidenced in adult primates (Gould et al., 1999) and postmortem tissue in the adult human hippocampus (Eriksson et al., 1998). The most active regions for neurogenesis in the mammalian brain include the subventricular zone and the subgranular zone of the hippocampus (Kempermann et al. 1997). Neurogenesis in the subgranular zone is highly responsive to enriched environments, exercise (van Praag et al., 1999) and hippocampus-dependent learning tasks (Gould et al., 1999). Moreover, recently nascent neurons seem to become integrated into the dentate granule cell circuitry and can have functional properties similar to those of mature dentate granule cells (van Praag et al., 2002). These data suggest that robust hippocampal activity may drive neurogenesis and thus increase hippocampal size and cognitive strength. In this paragraph, I will present some recent data about a possible link between neurogenesis and spatial learning and memory.

The implication of growth factors in neurogenesis is well established. Indeed it has been shown that epidermal growth factor (EGF, Reynolds et al., 1992), fibroblast growth factor (FGF2, Ray et al., 1993), brain derived neurotrophic factor (BDNF, Kirschenbaum and Goldman, 1995) and erythropoietin (Shingo et al., 2001) play a role during neuronal differentiation and maturation. A recent study questioned the link between vascular endothelial growth factor (VEGF) and spatial learning and memory (Cao et al., 2004). Actually, VEGF is an hypoxia-inducible protein that promotes angiogenesis and its role in the vascularization of tumours is now well established (Ferrarra, 1999; Shweiki et al., 1995). Recently its neurotrophic factor functions were discovered and it was suggested that VEGF contributes to neurogenesis and has neuroprotective effects (Sondell et al., 1999; Jin at al., 2000). Cao et al., 2004 showed that expression levels of VEGF (mRNA and protein) in rats trained to find a hidden platform in the water maze task were increased compared to control rats. Moreover, overexpression of VEGF through injection of a recombinant adeno-associated vector expressing human VEGF induced better performances (than normal) in the water maze. Some hypotheses have been made on how VEGF might promote neurogenesis: perhaps through the establishment of a vascular niche that favors the proliferation and differentiation of neuronal precursors (Palmer at al., 2000), or by release of BDNF from endothelial cells (Louissaint et al., 2002), or by a direct mitogenic effect on neuronal precursors (Jin et al., 2002). In conclusion, VEGF may be a key mediator linking spatial learning and memory to neurogenesis.

Another series of experiments were conducted to elucidate the relationship between neurogenesis and spatial learning and memory. By comparing irradiated and non-irradiated rats tested in the Morris water maze task, researchers obtained data suggesting that neurogenesis could play a role in formation and/or consolidation of long-term memory (Snyder et al., 2005). A parallel can be made with the study of the songbird: ten years ago, Kirn et al. (1994) demonstrated that the birth and death of high vocal center neurons occurred at the same time as the acquisition and forgetting of different songs. Once more, there is great evidence that neurogenesis plays a role in the formation of memories and more particularly of spatial memory.

The functional significance of neurogenesis in adult, free-living animals has also been investigated. A recent study addressed this question and showed that grey squirrels which use multiple food storage sites have three times the density of proliferating cells in the dentate gyrus than chipmunks that hide food in a single cache (Barker et al., 2005). Interestingly, they also showed that, when aging, rodents using a single food storage site had a decrease in neurogenesis whereas animals storing food in multiple sites maintained elevated neurogenesis levels. This observation indicates that the realization of more complex spatial navigation tasks might lead to increased neurogenesis, or that the latter renders the former easier. Interestingly, a seasonal peak of neurogenesis has been observed in adult black-capped chickadees, a species of food-storing birds (Barnea and Nottebohm, 1994; Barnea and Nottebohm, 1996) and this supports nicely what has been shown for the grey squirrels (Barker et al., 2005).

In conclusion, even though the functional role of neurogenesis in spatial learning and memory is not entirely understood, it is likely that neurogenesis is an active process participating in the management of spatial information.

2.3. Place cells activity

As mentioned previously, in the beginning of the 1970's, O'Keefe and Dostrovsky (1971) discovered and provided the first electrophysiological evidence that the hippocampus can form an internal representation – a "cognitive map" – of its spatial environment. The orientation of an animal in a given space can be encoded in the firing pattern of the pyramidal cells of the hippocampus, the cells that support LTP.

Each of the pyramidal cells of the hippocampus encodes the characteristics of the environment and the relationship between these characteristics. Pyramidal neurons coding for spatial information are called "place cells". It has been observed that when an animal explores different regions of a familiar environment, several place cells are activated. The essential characteristic of the activity of place cells is that this activity is closely linked to the position of the animal's head in its environment. Therefore, place cells are specifically active as soon as the head of the rat enters into a precise region of the environment named the "firing field" or "place field" (Figure 7). These place cells, initially described in rats (O'Keefe and Dostrovsky, 1971) have recently been found in humans (Ekstrom et al., 2003).

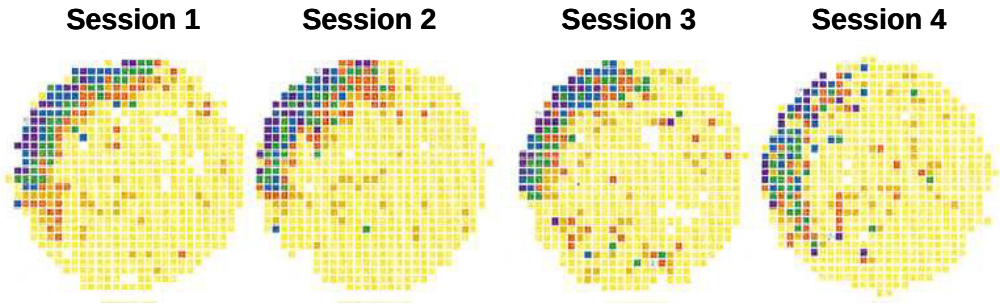

Figure 7. Firing fields of a place cell, recorded during 4 successive sessions. During each session, the animal explores similarly all zones of a circular open field. The place cell fires only when the animal is in a particular place and it is stable among the 4 sessions. Dark colors indicate high firing rate, light colors indicate low firing rates (after Rotenberg et al., 1996).

To summarize, an animal is able to create a spatial map of its environment. This map relies on the activity of hippocampal neurons, each cell identified as a place cell having at least one particular place field, which is stable from recording session to recording session. In a new environment, firing fields are established after 2-3 minutes (Hill, 1978; Bostock et al., 1991, Wilson and Mc Naughton, 1993). Actually, the neuronal response is stable after further exposition of the rat to the same environment, and can be recorded from a few hours to several months later (Thompson and Best, 1990), suggesting that the representation of the environment, once formed, may be consolidated.

The cognitive map reflecting the localization of the animal at any one time is not a topological view of the environment. Actually, the hippocampal place cells that have close cell bodies do not represent neighboring regions within the space (Muller et al., 1987). Moreover, place cell firing can persist even if pertinent visual cues have disappeared. If the animal is placed in a new environment, the activity of the cells is modified (O'Keefe and Conway, 1978; Muller and Kubie, 1987; Thompson and Best, 1989; Wilson and McNaughton, 1993). Cells can become completely silent, or, if they remain active in the new environment, they can generate new firing fields, mostly unrelated to the initial firing field. Such a modification, called "remapping", corresponds to a dynamic recoding of the new environment's properties (Bostock et al, 1991; Kubie and Muller, 1991; Jeffery and Hayman, 2004).

Several studies investigated the precise role of place cells in spatial navigation (Poucet, 1993; Jung and McNaughton, 1993; Poucet at al., 1994; 2001; Poucet et Benhamou, 1997). A relationship between spatial performance and place cells activity has been evidenced by combining cognitive tasks and electrophysiological recording (Wiener et al., 1989; Lenck-Santini et al., 2001; 2005). These results suggest a role for place cells in spatial memory: facilitation of orientation in space, storing of spatial localizations and rapid acquisition of new information (Poucet et al., 2000; 2001).

Which mechanisms allow for the consolidation of place fields? Interestingly, experiments conducted on rats have shown that place cells are reactivated during the period of sleep following spatial exploration (Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996). This place cells activity during sleep parallels the activity during spatial exploration: spatial information acquired during exploration is "re-expressed" within the hippocampal neuronal network during sleep. This neuronal reactivation and the long-term stability of this activity indicate that place cells contribute to immediate processing of spatial positions and to long-term spatial memory. More recently, Kentros et al. (2004) showed the importance of attention for an increased stability of place fields, especially in mice. In this work, the authors propose a model whereby attention provides the requisite neuromodulation to switch short-term to long-term memory. It has been shown recently that the long-term stability of new hippocampal place fields requires new protein synthesis (Agnihotri et al., 2004). Actually, these authors show that blocking protein synthesis in the brain of mice abolishes long-term stability of recently formed place fields, whereas short-term stability of new place fields and retention and recall of previously established fields is not affected.

In summary, place cells play an important role in the formation of spatial memories and several processes (more or less understood) allow for the establishment of a stable firing activity for the long-term storage of spatial information.

2.4. Long-term potentiation (LTP) and long-term depression (LTD)

Several lines of evidence have supported the hypothesis that memory storage involves persistent, experience-dependent modulation of the strength of synaptic connections among neurons. Synapses can be modified in many ways and the alteration of synaptic strength typically takes the form of long-term potentiation or depression (LTP or LTD, respectively) of excitatory synapses.

The hypothesis that information is stored in the brain as changes in synaptic efficiency emerged a century ago following the demonstration by Cajal, in the 1890's, that networks of neurons are not in cytoplasmic continuity but communicate with each other at specialized junctions, later termed the synapses by Sherrington (1897). The first synapses to be identified as showing LTP in the mammalian brain were the excitatory connections made by perforant path fibers onto the dentate gyrus of the hippocampus (Bliss and Lømo, 1973). This study reported that brief trains of high-frequency stimulation to synaptic excitatory pathways in the hippocampus caused a significant increase in the efficiency of synaptic transmission by production of a long-lasting enhancement of the extracellularly recorded field potential (Figure 8). Subsequent *in vivo* and *in vitro* studies showed that long-term potentiation (LTP) can last for weeks or months.

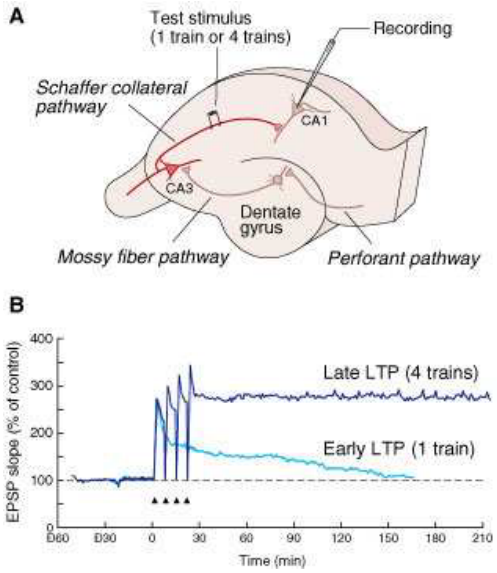

Figure 8. Long-term potentiation (LTP).
A. An electrical stimulation is applied on the Schaffer collateral pathway and a microelectrode records the excitatory postsynaptic potentials (EPSP) produced by CA1 neurons.
B. The early and late phases of LTP in the Schaffer collateral pathway. A single train of stimuli for one second at 100 Hz elicits an early LTP, and four trains at 10-minute intervals elicit the late phase of LTP. The early LTP lasts about two hours and the late LTP lasts more than 24 hours (from www.erickandel.org).

LTP is supported by the activation of receptors at the synapse, which allow electrical events at the postsynaptic membrane to be transduced into chemical signals, which in turn are thought to activate both pre- and postsynaptic mechanisms to generate a persistent increase in synaptic strength. Multiple pathways can lead to persistent synaptic enhancement, expression

and maintenance of LTP. Molecular substrates for LTP induction are, among others, NMDA receptors, metabotropic glutamate receptors, acetylcholine receptors, serotonin receptors or voltage gated calcium channels (Zhang and Linden, 2003). Maintenance of LTP over long periods of time is thought to be supported by gene expression and protein synthesis (Lynch, 2004). An extensive review on the molecular mechanisms underlying LTP has been written by Lynch (2004).

Another synaptic phenomenon, long-term depression (LTD) is thought to be the mechanism by which information is encoded in the cerebellum (Ito, 1986), as well as a process whereby LTP is reversed in the hippocampus and neocortex (Bear and Malenka, 1994). LTD is a weakening of a synapse that lasts from hours to days (Figure 9). It results from either strong synaptic stimulation (as in the cerebellum) or from prolonged weak synaptic stimulation (as in the hippocampus). LTD is thought to result from changes in postsynaptic receptor density, although changes in presynaptic release may also play a role.

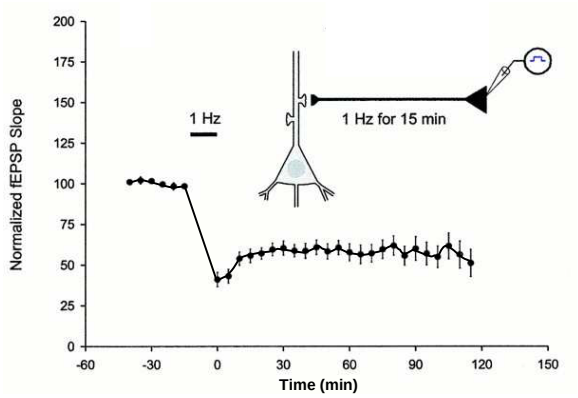

Figure 9. Long-term depression (LTD). An electrical stimulation (1 Hz for 15 min) is applied on the Schaffer collateral pathway and a microelectrode records the excitatory postsynaptic potentials (EPSP) produced by CA1 neurons. The stimulation resulted in a long-lasting depression (adapted from Kauderer and Kandel, 2000).

In summary, the strength of synaptic connection (regulated by LTP and LTD) plays a role in the formation of memories. There are several forms of LTP and LTD and many pathways by which they operate. For deeper insight into synaptic plasticity, LTP, LTD, their detailed functioning and their role in spatial learning and memory, refer to recent reviews by McGaugh (2000), Kandel (2001) Zhang and Linden (2003), Malenka and Bear (2004) and Lynch (2004).

2.5. Gene expression data about spatial learning and memory

Large numbers of studies preferred using a single-gene approach or exploration of a single pathway related to spatial memory and huge amounts of data have been generated. Genome-wide analysis using microarrays enables the measurement of transcriptional modifications occurring simultaneously in a specific process. Some studies already described transcriptional profiles from the hippocampus of non-spatially stimulated animals. For example, some studies aimed at analyzing the profile observed in aged or diseased rodents (Blalock et al., 2003; Roth et al., 2003). Other studies tried to differentiate sub-regions of the hippocampus by their transcriptional profile (Kamme et al., 2003; Lein et al., 2004; Datson et al., 2004). To broadly summarize, these studies demonstrated that it is possible to find markers for a diseased or aged state, it is possible to differentiate the structures forming hippocampus. Actually there is a vast amount of gene expression data addressing the transcriptional content of the hippocampus and describing all of this is beyond the scope of this work. However, only few studies investigated the transcriptional response to a spatial learning task in an animal's hippocampus. In this section, I will present and briefly comment on some studies that were recently published.

The first microarray studies addressing spatial learning were published in 2001. In a first attempt to identify maze-learning associated genes, rats were tested in a Stone T-maze in which they had to find the correct path to reach a goal in a certain time; if they failed they received a footshock (Luo et al., 2001). In comparison to control rats, 28 learning-associated genes having a significantly increased expression level were identified. Among these candidates, 4 genes were receptors and/or signaling molecules, 5 genes were linked to maintenance and remodeling of synaptic structure, 4 were growth-related molecules and the rest consisted of ESTs * (Expressed Sequence Tags) or genes with various functions including metabolism.

In the second study (Irwin, 2001), rats were trained in a Morris-like water maze task. They received a single training trial where they had to escape from a pool by gripping a rope. In a retention trial, it was observed that rats escaped the pool in less than 15 seconds. These performances showed learning of the task and remembering the position of the rope. Eleven genes and 39 ESTs were identified as being significantly regulated by learning. These 11 genes were principally related to gene regulation and energy metabolism.

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* ESTs (Expressed Sequence Tags): sequences that were identified in cDNA libraries and for which gene sequence is currently not defined.

Cavallaro et al. (2002), published a study where rats received a single training day (4 consecutive trials) in the Morris water maze. Hippocampi were removed at either one, six or twenty-four hours after training completion. In comparison with control groups, authors identified 345 genes significantly linked to physical activity and stress, and 140 genes significantly related to memory (out of which 110 were also influenced by physical activity). The 140 memory-related genes were classified into functional groups including cell signaling, synaptic proteins, cell-cell interaction and cytoskeletal proteins, apoptosis, enzymes and transcription or translation regulation. Further exploration showed that one particular gene - FGF18 (fibroblast growth factor) - which was not influenced by physical activity, presented an increased level of expression (mRNA) at the three time points after water maze training. Authors showed that an intracerebroventricular injection of FGF18 (protein) improved spatial learning performances in the water maze. Members of the FGF family have been shown to stimulate neurite outgrowth. Therefore FGF18 might be involved in spatial learning and memory through its function related to the growth of neurons.

In 2002, Leil et al. tested several mouse strains in the Morris water maze. Two hybrid strains and two inbred strains were trained in a water maze task. Actually the two inbred strains were known for their poor performances in spatial tasks and they also performed poorly in the study, whereas hybrid strains had normal scores. The authors could identify 27 significantly differentially expressed genes in whole hippocampus of hybrid compared to inbred strains indicating that spatial learning memory could be strain-specific. Most of the genes had an unknown function, however among the known genes that had an increased level of expression, three were interesting candidates and could play a role in learning. The first gene, Wolframin, has been implicated in mental retardation when mutated (Swift et al., 1998). The second gene, mbFZB, is thought to be strongly expressed in developing brain and adult hippocampus and piriform cortex. Finally, authors also identified overexpression of phophoribosylpyrophosphate (PPRP), a protein found to interact with a Na^+/H^+ exchanger in the hippocampus.

Finally, in 2003, Leil et al., continued the investigation of transcriptional profiles in hippocampus of mice performing the Morris water maze task. Mice were from a strain selected previously for showing excellent capacities in the maze test (Leil et al., 2002). Mice had 5 training sessions: three of which were performed on the first day and two on the second day. The hippocampi of mice were dissected 2 hours after completion of the last training session (no probe trial). The authors could identify three genes regulated during learning: (1) the alpha subunit of the platelet-derived growth factor (Pdgfra), (2) a gene homologous to DnaJ and CREB2 (cAMP response element-binding protein 2, also named ATF2 and (3) a novel gene with unknown function. Interestingly, Pdgfra has been shown to play a role in mice nervous system

development. Finally hypotheses have been elaborated about the role of the CREB 2 homologues: in *Aplysia*, derepression (a particular type of activation) of CREB 2 is required to switch from short-term to long-term facilitation of the connection between sensory and motor neurons. Therefore the homologues of CREB 2 might be induced as part of a mechanism to ensure consolidation of learning (Bartsch et al., 2000; Abel et al., 1998).

A common feature and perhaps weakness of these five studies is that transcriptional profiles were obtained from whole hippocampi. However, it has been shown that the hippocampus is not a homogenous structure (Lein et al., 2004, Datson et al., 2004). Therefore it might be possible that modifications of gene expression observed in these studies are not directly linked to spatial learning and memory and that relevant information might have been diluted or lost. Therefore it might be interesting to analyze the transcriptional profile of the sub-regions of the hippocampus.

These studies also point out that variations in gene expression related to the realization of a behavioral task are quite subtle. Actually modifications of gene expression in brain do not often exceed a two-fold difference between the experimental and control samples, contrary to massive changes occuring in other research fields. For example, changes related to the development of an organism are in the range of 1.5 to 5-fold or more (Michaut et al., 2003; Kelly et al., 2000). Modifications in expression can be even higher for disease states like cancer (Maxwell and Davis, 2000) or for response to tissue damage after ischemia (Roth et al., 2003) where variations usually are situated between 10- and 30-fold (Irwin et al, 2001). Interestingly, in the study of Leil et al. (2003), experiments were done on mice of an F1 hybrid strain whose major advantage is their isogeny, thus individual variations are avoided.

Finally, we would like to stress the fact that it is quite difficult to compare microarray studies. In fact the risk is high that differences observed between studies are due to experimental procedures, in particular the way animals are tested or the sample processing. So studies have to be appreciated in parallel, results might not be added up and differences do not necessarily reflect contradictions. These studies indicate which genes and pathways could contribute to learning and provide a basis for further experimentation. Moreover, it is not known whether increased levels of mRNA give rise to the production of increased levels of proteins. Therefore microarray data have to be manipulated carefully and this is even more important for experiments in which variations are very subtle.

3. Brain lateralization

The body plan of most adult animals is largely bilaterally symmetrical, although there are deviations from complete symmetry. Several organs such as the heart, the liver, stomach and spleen are present in only one side of the animal; however this aspect of lateralization is not the purpose of this work. The focus of this paragraph is the lateralization of the brain. Actually, the brain is present on both sides of the body of an organism but it often shows an asymmetrical organization. This kind of lateralization has been evidenced in the nervous system of several species, from invertebrates to human.

The expression "brain lateralization" refers to the fact that the two hemispheres of the brain are not functionally and morphologically alike. Thus, each hemisphere has a functional specialization; this means that some functions are localized on one side of the brain in preference to the other. Structures that are initially symmetrical might functionally diversify with a left-right specific bias to increase the functional capacities of the nervous system. This lateralized specialization is thought to originate from evolutionary, developmental, hereditary, experiential and pathological factors (Rogers and Sink, 1988; Bisazza et al., 1998; Thompson et al., 2001 Toga and Thompson, 2003). The aim of this paragraph is not to draw up an exhaustive list of structures of the brain that are lateralized. The focus will be on specific aspects and some examples, first in human and then in animals, to illustrate the phenomenon of lateralization. It is well established that lateralization is a rule rather than an exception among vertebrates and even in some invertebrates.

3.1. Lateralization of human brain is known since more than a century

The earliest functional specialization of human brain was discovered in the mid of the 19th century by Paul Broca (1861) and Carl Wernicke (1874). They identified, based on observations of brain-damaged patients, lateralization of speech and language abilities and they showed a left hemispheric dominance for these processes. This lateralization could be advantageous because it avoids competition between the two hemispheres for the control of the muscles involved in speech (Toga and Thompson, 2003) and it might be more efficient to transfer language information between a collection of focal areas in a single hemisphere. In the 1970's, some studies on postmortem brains (Geschwind and Levitsky, 1968; Rubens et al., 1976) showed that the *planum temporale*, a bilateral brain structure also involved in language, is asymmetric and that in most brains the left *planum temporale* is larger than the right (Habib et al., 1995). This reduction in structure size can be explained by the fact that there are fewer neurons

and this difference could be due to a differentiation early in the brain developmental process (Rosen et al., 1993).

A structural asymmetry is found in Heschl's gyrus and in the sylvian fissure, two structures that have a different orientation in the right or in the left hemisphere. Indeed, the asymmetrical trajectory of the sylvian fissure is known for more than one century (Eberstaller, 1884, Cunnigham, 1892) and the left sylvian fissure is longer and more horizontal than the right sylvian fissure, in most right-handed people (Figure 10). In left-handers, the fissures are more often symmetric. The left Heschl's gyrus (containing primary auditory cortex) is usually more oblique and less transverse than the right one. Likewise, magnetic resonance imaging studies showed that the right hemisphere protrudes anteriorly beyond the left and that the left hemisphere extends posteriorly beyond the right (LeMay and Kido, 1978; Kertesz et al., 1986).

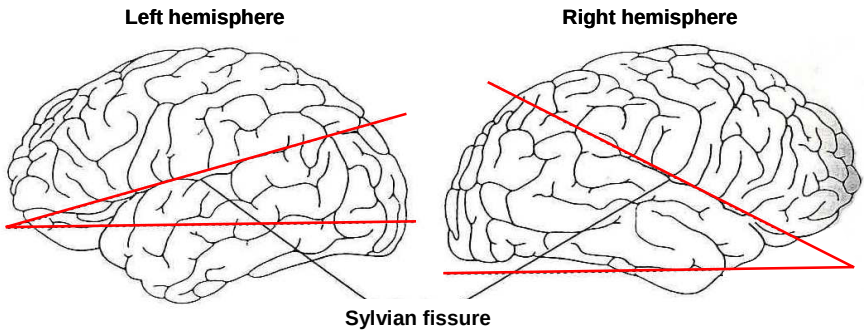

Figure 10. Differences between the temporal areas of the left and right hemisphere. In most right-handed people, the sylvian fissure in the left hemisphere is longer and runs at a shallower angle than the fissure in the right hemisphere (adapted from Geschwind, 1968).

Another phenomenon for which lateralization has been widely investigated is handedness. Surprisingly, hand preference correlates strongly with structural and functional asymmetries in language-processing structures: almost all right-handed people show a left specialization for language comprehension, whereas two thirds of left-handers have a right dominance for language processing. Similarly, a correlation between the size of the corpus callosum - the main fiber tract connecting the two cerebral hemispheres - hemispheric lateralization and handedness has been shown (Witelson, 1985). The corpus callosum is quite larger in left-handed and ambidextrous people and could originate from a greater anatomical connection between the two hemispheres necessary for dealing with a greater bihemispheric representation of cognitive functions in left-handed and ambidextrous people. In addition, we can notice that there is great evidence for predominance of the right hemisphere in processing of emotional information and anxiety (Richards et al., 1995). This can be related to observations previously reported in the rat

(Thiel and Schwarting, 2001). Interestingly, it has been shown recently that spatial memory can be lateralized to the right hippocampus in humans (Maguire et al., 1998; 2000; Burgess et al., 2002). These findings will be presented in section 4.1 (page 43). Some lateralized human brain functions are shown on Figure 11.

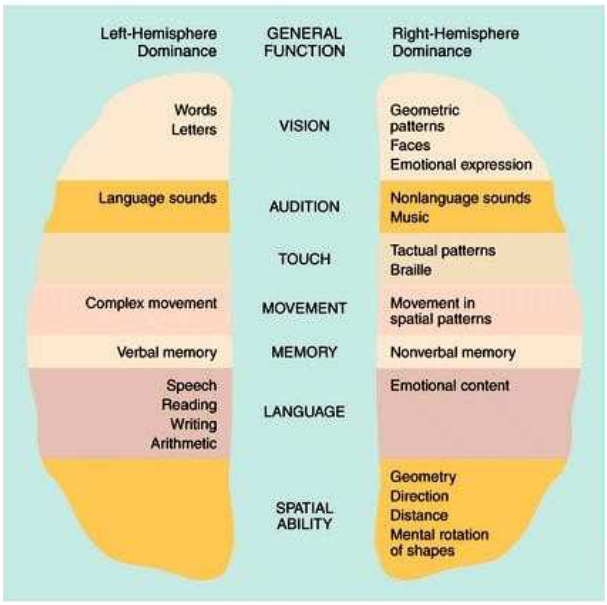

Figure 11. Example of functions which are lateralized to one hemisphere (after www.driesen.com).

Even some diseases can evolve asymmetrically or affect only one hemisphere. For example, magnetic resonance imaging showed that *planum temporale* volume asymmetry was reduced in dyslexic patients (Larsen et al., 1990). Greater asymmetry of cerebral function in males could also induce a greater incidence of stroke or focal lesions on language impairments and learning deficits. In patients with Alzheimer's disease, the grey matter loss usually originates in entorhinal and parietal cortices and then gains frontal and sensory motor cortices. This sequence occurs in both hemispheres but affects left side earlier than right side of the brain with right hemisphere following left hemisphere approximately two years later (Thompson et al., 2001, Thompson et al., 2003).

What are the origins of lateralization? A study suggests that lateralization of language perception could be influenced by fetal orientation (Previc, 1991). In fact, 66% of fetuses lay in a leftward position with their right side facing out. Thus lateralization of language perception might result from asymmetries in their auditory experience, the right ear being better positioned to discriminate speech sound. Asymmetrical vestibular stimulation *in utero* may produce behavioral asymmetries later in life (Previc, 1991).

The genetic determination of lateralization is more controversial: a study of monozygotic twins showed that there are twins who differ in handedness and also in *planum temporale* asymmetry (Steinmetz et al., 1994), which eliminates genetic origin. Moreover, considering handedness, the role of parental, cultural or environmental factors is apparently important and therefore can have a greater impact than genetics.

Asymmetries could also been driven by gender: it has been shown that the male brain might be more lateralized than the female brain (Grabowska et al., 1994). For example, gender differentiation is thought to underlie differences in motor and visuospatial skills or linguistic performance. Actually, gender related hemispheric specialization could be modulated by androgens provided their key role in inducing other neuroanatomical sex differences (Geschwind and Galaburda, 1985).

Recently, a gene expression study using serial analysis of gene transcription allowed the identification of 27 genes differentially expressed in left and right cerebral cortex from human embryos (these brains represent tissues discarded from pregnancy termination, Sun et al., 2005). This study, conducted on 12-14 weeks-old embryos (period of neuronal proliferation and migration) suggests that brain asymmetry is associated with early transcriptional asymmetries long before the onset of organized cerebral cortical functions.

3.2. What about lateralization in animals?

Cerebral lateralization has been long considered a uniquely human characteristic associated with the development of higher cognitive function. Recent studies, however, suggest that brain and behavioral lateralization is quite widespread among several animal classes. Evidences collected so far among invertebrates and vertebrates all point to a basic similarity in the pattern of specialization of the two halves of the nervous system. This paragraph gives a brief overview about the current knowledge on brain lateralization in animal models.

3.2.1. Lateralization of chemosensation in nematods

Asymmetry in the nervous system of *Caenorhabditis elegans* has been established and intensively studied from anatomy to characterization of molecular mechanisms underlying this phenomenon (Hobert et al., 2002). *C. elegans* is a nematode constituted of exactly 959 cells, among which 302 are neurons. It has been shown that despite a symmetrical nervous system development, some functions become lateralized and specific to a single neuron in a very subtle way. Recently, an elegant work showed that two morphologically bilateral taste receptor neurons

display an asymmetrical expression pattern of chemoreceptor genes. This receptor supports the capability of *C. elegans* to recognize various tastes. Johnston and Hobert (2003) showed that *lsy-6*, a microRNA, controls the asymmetry of chemosensory expression by regulating expression pattern of a family of chemoreceptor genes. The differential segregation of the chemosensory capacities in the left and right chemosensory neurons is required for intact behavior in complex sensory environments. In mutant animals that exhibit a partial lateralization of chemoreceptor expression, chemosensation *per se* is not affected, but the ability to discriminate between complex chemosensory inputs is lost.

3.2.2. Lateralization of associative learning in drosophila

Another very popular animal model is *Drosophila melanogaster* (fruitfly), and today little is known about its nervous system lateralization. Several mutants have been identified that show the existence of asymmetries of the body like position of specific organs or shape of the wings (Adam et al., 2003; Klingenberg et al., 1998). Some other drosophila mutants showed brain aberrations (Heisenberg et al., 1985; deBelle and Heisenberg, 1996); however, no “natural” asymmetry could be observed until recently. The first brain asymmetry of drosophila has been pointed out last year, from a fortuitous observation (Pascual et al., 2004). The authors built a model to study associative learning: the flies were trained to associate an odor with an electric shock. All flies performed well in a short-term memory test. However, all flies did not perform well in a long-term memory test. Therefore, the anatomy of the brain was analyzed and the authors showed that well performing flies presented an asymmetrical neural system whereas poor performing flies had a symmetrical brain. Actually, an asymmetrical body was present in the neural system of well operating flies while this structure was found bilaterally in the neural system of flies lacking long-term memory. In conclusion, asymmetry of drosophila’s brain may be required for the generation or the retrieval of long-term memory in this particular associative task.

3.2.3. Lateralized behavior in fishes

Behavioral lateralization of *Danio rerio* (zebrafish) has been shown when studying its reaction to the presentation of a range of objects (Guo, 2004, Miklosi et al., 2001). These small fishes use their right eye to survey and to react to an object encountered for the first time (i.e. to bite or not to bite). After habituation to this object, the left eye is used. It is known that nervous system of zebrafish is lateralized (Halpern et al., 2003). Thus it may be possible that eye preference is also lateralized and subsequently a suitable response to a specific stimulus may depend on a specific brain hemisphere.

3.2.4. Lateralization of aggressive behavior in reptiles

In *Anolis* (a lizard) such a lateralized behavior has also been shown, with left-eye use associated to aggressive responses. Some studies showed that aggressive responses are mainly activated by the right hemisphere, where the vast majority of retinal fibers from the left eye project (Butler and Northcutt, 1971).

3.2.5. Lateralization of vocalization in batracians

Studies of *Rana pipiens* (frog) put into evidence that vocalization is controlled by the left brain hemisphere and more particularly by neurons of the pretrigeminal area (Bauer, 1993). Even though vocalization in the frog is a quite primitive way of verbal communication, it is of high interest because it presents a similar asymmetric brain control of vocalization to that observed in birds, rodents, primates and humans.

3.2.6. Functional lateralization in birds

Lateralization has also been investigated in the bird brain. A behavior similar to that of the lizard *Anolis* has been observed in the chicken *Gallus gallus domesticus* (Howard et al., 1980): it has been shown that the left hemisphere suppresses aggressive behavior whereas the right hemisphere activates attack. Another study showed that hemispheric specialization allows chicken to do two tasks at the same time, namely finding food and being vigilant for predators (Rogers et al., 2004).

In pigeons, lateralization is known for the visual system that relies on a brain structure called thalamic nucleus *rotundus*. It has been demonstrated that the *rotundus* of the left hemisphere (dominant) receives input from both eyes whereas the right *rotundus* processes mainly information from the right eye (Folta et al., 2004; Güntürkün et al., 1998). And recently, it has been shown that there is a link between lateralization of brain in pigeon and spatial learning and memory (Bingman et al., 2003; Kahn and Bingman, 2004; Gagliardo et al., 2005). These data will be presented in section 4.

3.2.7. Functional lateralization in rodents

There is only little evidence for brain asymmetry in rodents. It has been observed, like in chickens and lizards, that right brain hemisphere of the rat activates aggressive behavior against the mice and that this behavior is repressed by left hemisphere (Garbanati et al., 1983, Denenberg et al., 1986).

Brain lateralization has been demonstrated for several neurotransmitter systems, especially dopamine. Functional implications of such neurochemical lateralization were

investigated by studying the relationship between dopaminergic asymmetries and lateralized motor behavior. For example, endogenous imbalances in striatal dopaminergic activity were related to the direction of thigmotactic behavior (right lateralization gives rise to right turn, Sullivan et al., 1994) and a right dopaminergic lateralization in the frontal cortex was associated with lower anxiety (Thiel and Schwarting, 2001).

Paw preference has been studied intensively in rodents and, as handedness in humans, it is thought to be hemisphere-dependent (Pence, 2002). Studies have shown that it could also be linked to brain dopamine asymmetries with dopamine dominance in the nucleus accumbens ipsilaterally to the preferred paw (Cabib et al., 1995).

Lateralization has also been investigated at the molecular level in rodents. For example it has been shown that the densities of dopamine receptors were not similar in right and left hemispheres of rodents (Schneider et al., 1982; Slopsema et al., 1982). More recently, it has been shown that this dopaminergic asymmetry can be related to behavioral asymmetry in rats (Thiel and Shwarting, 2001). In addition, it has been shown that certain subunits of NMDA receptors are asymmetrically expressed in the mouse hippocampus (Kawakami et al., 2003, Wu et al., 2005). In this case, the relation with cognition is not known, however it might be possible that this molecular lateralization reflects a functional lateralization.

3.2.8. Homologies in language areas of great apes and humans

Finally a study of Broca's area (equivalent to human language area) in *Pan troglodytes*, *Pan paniscus* and *Gorilla gorilla* showed that these species present a human-like asymmetry, with left hemisphere dominance (Cantalupo and Hopkins, 2001). Similarly, *planum temporale*, a portion of Wernicke's area involved in language, is larger in left compared to right hemisphere in great apes, as is the case in humans (Hopkins et al., 1998).

Altogether, these data suggest that brain lateralization is a quite common phenomenon, observed in many animal species along the phylum, from small invertebrates like nematodes to higher vertebrates like humans. Therefore hemispheric specialization could have an ancient origin and could be identified for various functions and structures for which there is currently no data. The traditional explanation for brain lateralization is that it avoids costly duplication of neural circuitry with the same function (Levy, 1977), as well as decreasing the interferences between different functions. For instance, dominance by one side of the brain may prevent the simultaneous initiation of incompatible responses in organisms with laterally placed eyes, such as fish (Vallortigara, 2000). The massive evolutionary expansion of the brain might have resulted in a level of complexity in which the duplication of structures was no longer efficient compared with the specialization of functions within a hemisphere (Toga and Thompson, 2003). In conclusion, lateralization could really bring an advantage for the execution of a specific task.

4. Brain lateralization, hippocampus, and spatial learning and memory

Only few data support the hypothesis that spatial learning and memory could be lateralized to one particular hemisphere and within this paragraph, some studies showing that there might be a hemispheric dominance for spatial navigation are presented.

4.1. Right hippocampus is preferentially used for spatial navigation in Humans

It has been shown few years ago that spatial navigation, in humans, is predominantly driven by one hemisphere of the brain, and more particularly the right hippocampus. A positron emission tomography (PET) analysis of subjects navigating to locations in a familiar virtual reality town showed that right hippocampus activation corresponds to a good knowledge of places location and an accurate capacity to navigate between them (Maguire et al., 1998). It has also been shown that patients with a right temporal lobectomy present an important impairment of spatial navigation (Kessels et al., 2001; Burgess et al., 2002; Maguire et al., 2000). Furthermore, a study investigating navigation capacities of London taxi drivers showed that their right hippocampus is predominantly activated when they have to recall complex routes (Maguire et al., 1997) and that the volume of the right posterior hippocampus increases with time spent working as a taxi driver (Maguire et al., 2000). These studies all suggest that both lobes of the hippocampus do not contribute similarly to spatial navigation and put into evidence that right hippocampus might have a more important role in this process than the left hippocampus.

4.2. Differential role of right and left hippocampi for spatial navigation in pigeons

The avian hippocampal formation has been extensively studied for its participation in a variety of navigational processes that support homing and more particularly in pigeons (Bingman and Able, 2002; Siegel et al., 2005). After displacement to a distant, unfamiliar location, the homing pigeon navigational map enables pigeons *(Columba livia)* to determine their location relative to the home (Papi, 1991; Wallraff, 1999). When learning a navigational map within an enclosed outdoor aviary (Ioale et al., 2000), lesions to the hippocampal formation of the left hemisphere impair acquisition while lesions to the right hippocampal formation do not (Gagliardo et al., 2001). Moreover, it has been recently established that the right hippocampus participates in the learning and memory for global, distally located spatial information whereas the left hippocampus is more sensitive to local cues (Bingman et al., 2003; Kahn and Bingman, 2004). In chicks, right brain hemisphere is also predominantly used for representing global

spatial information (Tommasi and Vallortigara, 2004). In addition to the navigational map and navigation by familiar landmarks, the homing behaviour of pigeons is dependant in their sense of direction determined by the sun compass and earth's magnetic field. The sun compass provides an essential framework in support to spatial learning (Bingman and Jones, 1994; Bingman et al., 1996). Recently, it has been shown that both the left and the right hippocampal formation are necessary in sun compass-based learning, however with the left hippocampus having a more important role (Gagliardo et al., 2005). To conclude, there is great evidence that right and left hippocampal formation make different but complementary contributions toward avian spatial navigation.

4.3. No evidence of lateralization of spatial navigation in rodents

As a comparison to humans and birds, functional lateralization of rodent hippocampus has not yet been established.

A set of studies using irreversible lesion of rat hippocampus showed that small remnants of hippocampus are sufficient to support spatial learning in the water maze (Moser et al., 1995; Moser and Moser, 1998, de Hoz et al., 2003). Recently a study showed that large lesions of rat hippocampus sparing small remnants of hippocampus located bilaterally or unilaterally had a more deleterious effect in a one-trial spatial task than in a water maze task. The magnitude of impairment was dependent on the total amount of hippocampal tissue spared, but not on whether the spared tissue was unilaterally or bilaterally located. Therefore, the use of permanent lesion could not answer about the lateralization of spatial navigation in rodents.

A series of experiments used reversible inactivation by microinjection of tetrodotoxin to study hippocampal lateralization. An early study could not establish hemispheric specialization of spatial navigation in the water maze: rats with only one active hippocampus learnt the location of the target as quickly and performed as well as normal animals provided the hippocampus that was active during training was also active during retrieval, whatever right or left hippocampus was inactivated (Fenton et al., 1993). Another experiment using irradiation and tetrodotoxin inactivation also failed to demonstrate the preferential use of one hippocampus for spatial navigation (Czeh et al., 1998). Once again, one intact hippocampus was enough to support spatial navigation. However, more recently, Cimadevilla et al. (2001) tested the ability of rats to avoid a stable room-defined place during dissociation of arena cues from room cues by rotation of the arena. Rats trained with both active hippocampi and tested for retrieval with a right inactivated hippocampus were impaired (Cimadevilla et al., 2001). This place avoidance task

requires that the rat separates the perceived stimuli into their respective coordinate frames and both hippocampi were necessary for a good performance. However, it was not possible to draw a conclusion about lateralization, because the effects of left infusions were not tested. Finally, Cimadevilla et al. (2005) tested consolidation and retrieval processes under right hippocampal inactivation and showed that consolidation was affected when inactivation was performed at the end of each training session. They also showed that when rats were trained in normal conditions (no hippocampal inactivation) retrieval was impaired following inactivation of the right hippocampus. Once again, the role of left hippocampus was not examined, thus no hypothesis of lateralization could be built.

In conclusion, using either permanent lesion or reversible functional inactivation, no study could demonstrate, in the rat, that spatial information is processed by the right, the left or both hippocampi. However, it is still not clear if spatial information is stored in the right, the left or in both hippocampi. Initially, this thesis work aimed at studying gene expression related to spatial learning and memory in the dorsal hippocampus of rats and was not expected to have to focus on lateralization of this brain function. However, the gene expression profiles established that the right hippocampus might be preferentially used for the management of spatial information in comparison to the left hippocampus. Therefore, we further explored the possible lateralization of the hippocampus for spatial learning and memory in rats by using reversible inactivation.

5. Molecular genomics

5.1. From the genome to the transcriptome and to the proteins

The genome of an organism is the whole hereditary information needed to build and maintain it alive. More precisely, the genome is the complete sequence encoded in the deoxyribonucleic acid sequence (DNA). However, some genomes (e.g. virus genome) are encoded in the ribonucleic acid (RNA) sequence, but they lay beyond the focus of this work. A strand of DNA contains the genes, areas that regulate genes, and areas that either have no function or a function we do not know yet.

The transcriptome is the set of all mRNA molecules (or transcripts) in one cell or in a population of cells for a given set of environmental circumstances and at a particular time point. Therefore unlike the genome, which is fixed for a given organism, the transcriptome varies from cell to cell or from cell population to cell population. The transfer of genetic information of DNA to RNA is the beginning of a process that ultimately leads to the translation of the genetic code into a functional peptide or protein. The genes are transcribed into several types of ribonucleic acids among which a vast majority of mRNA (messenger RNA) which will then be translated into proteins. Other RNA species include tRNA (transfer RNA), rRNA (ribosomal RNA), miRNA (microRNA), etc. A brief description of the major RNA classes and of their functions is detailed in the following table.

RNA type	Name	Function
mRNA	messenger RNA	RNA that are synthesized by transcription of the DNA and that are used as template for the biosynthesis of proteins (translation).
tRNA	transfer RNA	RNA that transfers a specific amino-acid to a growing polypeptide chain at the ribosomal site of protein biosynthesis during translation.
rRNA	ribosomal RNA	Primary constituent of ribosomes. Ribosomes are the protein-manufacturing organelles of cells and are located in the cell cytoplasm.
snRNA	small nuclear RNA	A class of small RNA molecules found within the nucleus of eukaryotic cells. They are involved in a variety of important processes such as RNA splicing (intron removal) or telomere stabilization.
snoRNA	small nucleolar RNA	A class of small RNA molecules that are involved in chemical modification of rRNA and other RNA, for example by methylation.
miRNA	microRNA	Small RNA molecules that are complementary to a portion of an mRNA. miRNA are thought to regulate the expression of genes by binding to their complementary mRNA so that this mRNA cannot be translated.
gRNA	guide RNA	RNA playing a role in editing, a process which modifies the nucleotide composition of an mRNA.

Table 1. Major RNA classes and functions.

In eukaryotes, mRNA are synthesized under the direction of several regulatory mechanisms, and several modifications are operated to obtain an active mRNA (e.g. capping, editing, polyadenylation, splicing, etc.). The three main stages are initiation, elongation and termination. A simplified scheme of translation is depicted on Figure 12.

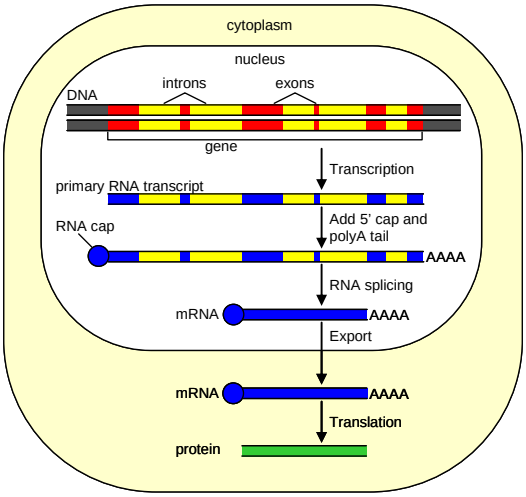

Figure 12. In eucaryotic cells, the initial RNA molecule produced by transcription (the primary transcript) contains both intron and exon sequences. Its two ends are modified, and the introns are removed by an enzymatically catalyzed RNA splicing reaction. The resulting mRNA is then transported from the nucleus to the cytoplasm, where it is translated into protein. Although these steps are depicted as occurring sequentially, they generally occur simultaneously. For example, the RNA cap is typically added and splicing typically begins before the primary transcript has been completed (from Alberts et al., 1995).

Finally, the proteome is the collection of proteins found in a particular cell type or population under a particular set of environmental conditions. The proteins are essential for the structure and function of all living cells.

A pluricellular organism can be made of hundreds to thousands of billions of cells and thus represents a very complex entity. In fact, one of the most fascinating phenomena in an organism is that the expression of the genes is executed very precisely so that the proper gene is activated or silenced in the right tissue, at the right place, and at the right time. The mechanisms controlling gene expression do not only ensure housekeeping functions (metabolism, cell growth and division, etc.) occurring in every living cell, they also participate to the processes leading to cellular differentiation and specialization involved in the development of pluricellular organisms. A system of tightly coordinated regulations, in which each gene (or group of genes) is expressed in a particular tissue, for a precise duration and at a specific rate, adapts the functioning and multiplication of the cells from an organ to the needs of the whole organism. Indeed, the cells of an organism receive continuously external signals (temperature, humidity, light, etc.) and internal ones (hormones, growth factors, etc.). These signals are emitted to the nucleus by the intermediate of signaling transduction pathways. In the nucleus, these signals can activate enzymes (kinases and phosphatases, in general) that, in turn, will modulate the activity of transcription factors by post-traductional modification (phosphorylation or dephosphorylation).

Thus, each cell expresses its own collection of factors allowing the expression of an ensemble of particular genes specifically characterizing this cell.

Therefore, analyzing the transcriptome of a specific cell population at a particular time point might give precise information about the processes driven by this cell population. Indeed, apart from increasing the knowledge about the general functioning of an organism, knowing which genes take part in a particular biological process might consequently help to build models enabling to address questions such as why a disorder occurs, how it evolves (etc.) and it might also provide some cues on how to cure it.

5.2. The Rat as a model

The laboratory rat (*Rattus norvegicus*) probably originated in central Asia and migrated to Europe around the eighteenth century (Barnett, 2002). Its success at spreading throughout the world can be directly attributed to its relationship with humans (Robinson, 1965). Rats are a reservoir of pathogens, known to carry over 70 diseases. They are involved in the transmission of infectious disease to man, including cholera, bubonic plague, typhus, leptospirosis, cowpox and hantavirus infection. However, *Rattus norvegicus* was the first mammalian species to be domesticated for scientific research, with work dating to before 1828 (Hedrich, 2000). The rat's contribution to human health has been readily demonstrated, from testing new drugs, to understanding essential nutrients, to increasing knowledge of the pathobiology of human diseases.

The recently published draft rat genome sequence (Rat Genome Sequencing Project Consortium, RGSPC, 2004) provides us with insights into both evolution and physiology of the rat. The rat genome is the third mammalian genome to deciphered and three-way comparisons with the human and mouse genomes resolve details of mammalian evolution. Calculations from the draft sequence suggest that the rat genome, at around 2.75-2.82 Gb is intermediate size between human (2.9 Gb) and mouse (2.6 Gb) (Hancock, 2004). The rat, mouse and human genomes encode similar number of genes, approximately 30,000. The RGSPC suggests that as many as 90% of rat genes have matches in both human and mice. This number is even higher than the 80% reported when comparing mice and humans. It has been observed that the vast majority of genes have persisted without deletion or duplication since the last common ancestor. Intronic structures are well conserved. Some genes in rat, but not in mouse, arose through expansion of gene families. These include genes producing pheromones, or involved in immunity, chemosensation, detoxification or proteolysis and can be easily associated with

biological features that make each species unique. For instance, human rely less than rodents on their sense of smell, and so have fewer olfactory-receptor genes. Of particular interest for the pharmacological studies are the detoxifying P450 genes, of which mice and rats have more than humans. So, it may be more difficult than thought to use the toxicity of drugs in rats as guide to their toxicity in humans, because rats may be better at removing toxins from their organism (Lindblad-Toh, 2004). Approximately 30% of the rat genome aligns only with mouse, a considerable portion of which is rodent-specific repeats. Of the non-aligning portion, at least half is rat-specific repeats. Finally, more genomic changes occurred in the rodent lineage than the primate. The rodent genomic changes include approximately 250 large rearrangements between a hypothetical murid ancestor and human. A threefold-higher rate of base substitution in neutral DNA is found along the rodent lineage when compared with the human lineage, with the rate on the rat branch 5-10% higher than along the mouse branch. Finally, microdeletions occur at approximately twofold-higher rate than microinsertions in both rat and mouse branches. A strong correlation exists between local rates of microinsertions and microdeletions, transposable element insertion, and nucleotide substitutions since divergence of rat and mouse, even though these events occurred independently in the two lineages.

To conclude, when considering modeling human behavior (stress, addiction, skills, etc.), rats are superior to mouse. Mice are difficult to work with because they scurry around so much; they are also slow and inflexible learners. Rats are also less aggressive than mice (Abbott, 2004). This is due to the species' social structure in the wild: mice live in groups where one highly aggressive alpha male monopolizes the females. Rats, by comparison, conform to the 1960s ideal for blessed-out free love, with widespread promiscuity and low levels of aggression. In addition, it is now possible to genetically manipulate rats. A transgenic rat has already been developed in 1990 by Mullins et al., however, cloning proved exceptionally difficult in rats. Recently, a method has been developed to overcome the cloning difficulties and knock-out and knock-in rats are now about to become available (Zhou et al., 2003). As rats are physiologically more similar to humans than to mice, the future availability of transgenic rats gives rise to new modeling possibilities in rats.

6. Technologies and tests used in this thesis project

This section aims at describing the main technologies used in the present thesis. Gene expression profiling and reversible functional inactivation are presented here. The behavioural tests are described in section 1.5 ("Experimental procedures used to test spatial navigation in rodents").

6.1. Gene expression profiling

Various techniques are available for detection or quantitation of RNAs. Traditional methods for gene expression measurements such as Northern blots or nuclease protection assay can be time-consuming and laborious and are not practical for application on a very large scale. So, the development of high-throughput systems like DNA microarrays - which enable monitoring of expression levels for hundreds, thousands or even tens of thousands of genes at the same time - allows researchers to generate a global view of the transcriptome at a given time point giving the opportunity to have a deeper insight into cellular activity (Hill et al., 2000; Hughes and Shoemaker, 2001).

Almost 10 years ago, microarray technology was established as a new tool for large-scale analysis of gene expression (Lockhart et al., 1996). Microarray technology evolved from Northern blotting, where an mRNA population is attached to a membrane and then probed with for a single gene (or fragment of gene). On the opposite, the microarray technology involves the determination of the relative concentrations of mRNA based on hybridization of mRNA population to high-density arrays of synthetic oligonucleotides. The microarray technology has developed very fast in the last years and this is underlined by the exponential number of publications dealing with experiments based on this technology (Figure 13). Microarrays have been used in various fields and for various animal models, in cancer investigation, cardiovascular disease research, and neuroscience or for validation of pharmaceutical compounds. There are several types of microarrays and we will focus on high-density oligonucleotide arrays (Lockhart et al., 1996) and more particularly on GeneChips® - arrays that are produced and commercialized by Affymetrix.

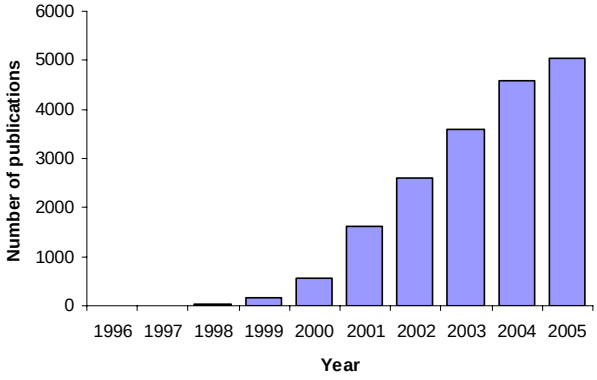

Figure 13. Plot of total microarray articles indexed in Medline. Search keywords are: DNA microarray OR oligonucleotide array OR DNA chip.

An oligonucleotide array consists of a small glass slide (1.5 x 1.5 cm) on the surface of which oligonucleotide probes are synthesized through photolithography and combinatorial chemistry. At the molecular level, the oligonucleotide probe must distinguish one sequence from millions of sequences (the sample), which requires that the probes are designed very accurately. Therefore, each array contains thousands of oligonucleotide probes allowing analysis of thousands of mRNA. The oligonucleotides are 25 bases long and are always paired. A pair consists of two different sequences: the first is perfectly complementary to a fragment of a particular mRNA (perfect match or PM); the second is identical to the perfect match, except for a single nucleotide at the central position (mismatch or MM). The PM probe provides measurement of the relative amount of mRNA whereas the corresponding MM probe measures any false or contaminant or non-specific hybridization and therefore is an internal control. Each gene is represented by a collection of 11 to 16 pairs of probes. This study used the GeneChip U34A, allowing the analysis of gene expression for 8,000 rat genes (out of which 1,000 are ESTs).

The mRNA population to be assayed on a microarray has to be labeled. So, after total RNA extraction, the whole mRNA is converted into cDNA (complementary DNA) by reverse transcription. Then, a biotinylated cRNA is produced by in vitro transcription and hybridized to a microarray. Only the sequences complementary to the oligonucleotide probe can hybridize on the microarray. After hybridization, the signal is amplified, by hybridization of fluorescently labeled streptavidin to the biotin residues. The fluorescence intensity is proportional to the number of biotinylated cRNA hybridized on the microarray. The intensity is measured by a scanner and the output signal is analyzed thanks to software. Finally, the expression level of the transcripts is determined and samples are compared. An example of scanned chip image with closer look at a probe set for one particular transcript is shown in Figure 14.

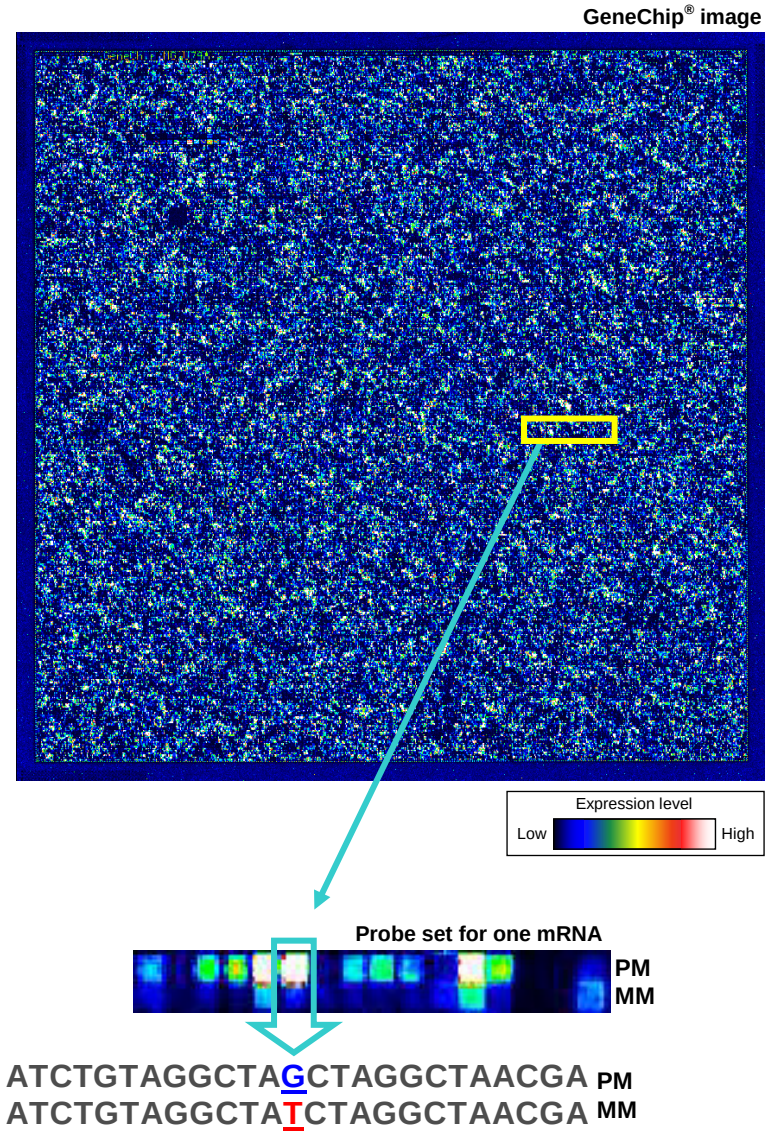

Figure 14. Image of a GeneChip® after scan. The different colors indicate relative expression of different genes. This microarray allowing rat genome analysis contains ~ 8000 probe sets for known genes and ~ 1000 for ESTs. Each probe set of the microarray is constituted of 16 perfect match and 16 mismatch probes. Expression level for each probe set is calculated as the average difference of the hybridization signal between PM and MM (perfect match and mismatch).

In our study, each brain sample was hybridized onto one microarray. Once all microarrays were scanned and output signal obtained, a statistical analysis was perfomed to determine which genes were differentially expressed. First, the quantile normalization (Bolstad et al., 2003) was applied to all microarrays. This mathematical transformation allowed comparing the expression levels of all genes, in the different testing conditions. Microarrays were then grouped by conditions and an average expression level was calculated for each gene in each condition. Finally, pairwise comparisons between the groups were performed using the

SAM algorithm (Tusher et al. 2001). A theoretical comparison between two conditions is shown on Figure 15. A gene was considered as induced or repressed if its level of expression was at least 50% higher or lower than in the control situation (fold change ≥ 1.5 or fold change ≤ -1.5, respectively). A supplementary criterion for being differentially expressed was a p-value < 0.1 which correspond to a false discovery rate of 10%. This p-value was computed by the comparison algorithm and automatically reported in the output file.

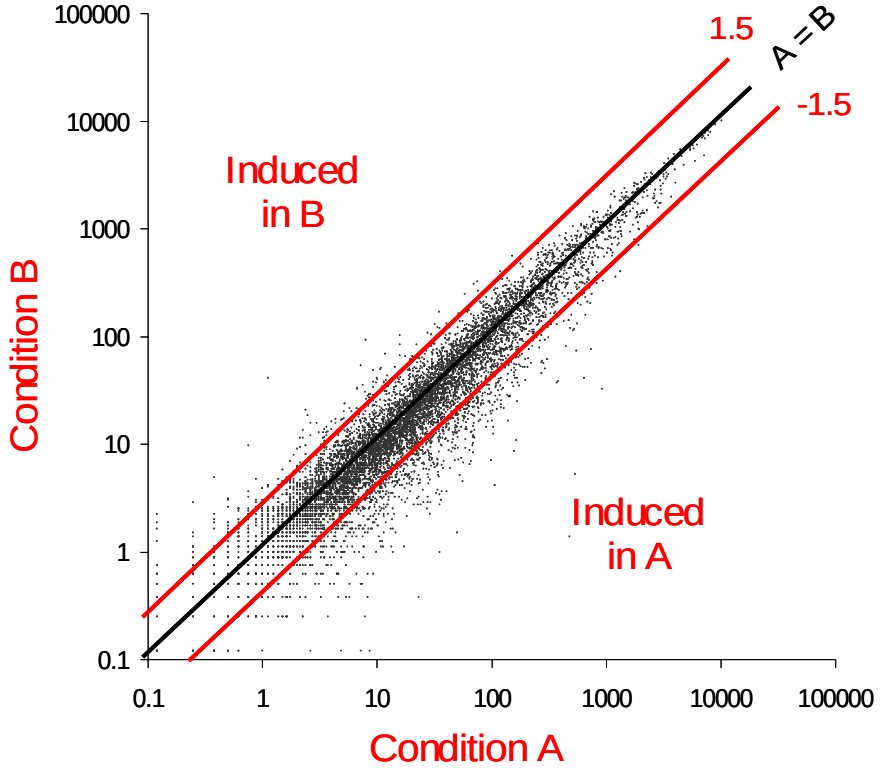

Figure 15. Analysis of microarrays: scatter plot showing the correlation between theoretical conditions A and B. Each point on the graph represents a gene tested by the microarray and therefore, about 9000 points are reported on the graph, representing the 8000 genes and 1000 ESTs tested by the microarrays. The coordinates of each point correspond to the expression level of the corresponding gene in condition A (x-axis) and in condition B (y-axis). The two red lines represent the threshold above which a gene was considered as differentially expressed in one or another condition. All genes above the upper line were considered as induced in condition B, whereas genes below the lower line were considered as repressed in condition B. The genes that were between the two red lines were considered as similarly expressed in both conditions.

We performed 4 pairwise comparisons between our 5 conditions, one of these conditions being always the control situation. We then identified the genes that were similarly expressed in the 4 testing conditions compared to the control situation. On this basis, we then grouped the genes by their expression pattern in the different conditions. Finally, from the different lists that

were obtained, we made an interpretation of the potential role of some genes in the different stages necessary to create a spatial memory.

The current work is dealing with rat brain tissue microdissections, containing much less total RNA than usual samples, therefore the first part of this thesis aimed at optimizing and validating new protocols for the analysis of small-size samples on microarrays.

6.2. Reversible inactivation and behavior

The traditional approaches to study the role of specific regions of the brain use electrolytic, aspiration or radiofrequency lesions, or local injections of excitotoxins such as glutamate analogues in high concentrations, which have the main advantage of sparing fibers passing through the damaged structure (Jarrard, 2001, 2002). These irreversible lesion techniques are very useful to identify which processes are supported by a particular brain region. However, it is sometimes difficult to draw precise conclusions about the time course of a cognitive process by using such lesions (Izquierdo and Medina, 1998; Ambrogi Lorenzini et al., 1999). Actually, permanent lesions do not always allow identifying precisely which stage of memory processes is disabled (encoding, consolidation, retrieval). Moreover, neuronal reorganization may partially compensate for the deficit due to the lesion and thereby induce a bias in the interpretation of results (Cassel et al., 1997; Ambrogi Lorenzini et al., 1999).

The use of reversible inactivation might circumvent some of the permanent lesion's drawbacks. Reversible inactivation relies on the microinjection of drugs which blocks temporarily a particular brain region (Ambrogi Lorenzini et al., 1999). The main advantage of such methods is that they provide information not only on "where", but also on "when" and for "how long" the processes involved in a given function may take place. In addition, the effects induced by the inactivation cannot be readily compensated for since the inactivation lasts only a short time (Bures and Buresova, 1990).

Reversible inactivation has already been used to study the different stages involved in the formation of memory. Thus, infusion of a drug before acquisition or before retrieval was used to show that recall could be altered independently from learning (Bohbot et al., 1996; Moser and Moser, 1998b; Parron et al., 2001; Cimadevilla et al., 2001). Similarly, reversible inactivation experiments showed that retrieval of recent or remote memories might involve different brain structures (Frankland and Bontempi, 2005; Maviel et al., 2004). Also, chronic inactivation for several days beginning after acquisition was used to disrupt long-term consolidation (Riedel et al., 1999).

Thus, we explored the possible lateralization of spatial learning and memory in rat brain by performing reversible inactivation of right and/or left hippocampus. Rats received infusions either before the acquisition of a spatial task or just before recall.

7. Description of the project

7.1. Preliminary technical study

Our spatial memory/learning study required focusing on a very small and precise region of the hippocampus, the dorsal part of CA1. This region was chosen since it corresponds to an area where place cells were recorded when rats had to resolve spatial problems (Lenck-Santini et al., 2002). Therefore, before starting our gene expression analysis of the dorsal hippocampus of rats doing a spatial learning task, we had to optimize several techniques, in order to adapt them to small-size samples. For this, we designed a small preliminary study, which allowed testing protocols for sample collection, total RNA extraction and sample processing for hybridization on microarray. This preliminary study was performed on mice and resulted in the development of a new and reliable protocol for the gene expression analysis of microdissected samples that has been used in our subsequent experiments on rats.

As a classical manual dissection did not allow us to take out small pieces of the dorsal hippocampus very precisely, we used laser microdissection. In one of the laboratories (PRBD-N, F. Hoffmann – La Roche Ltd., Basel), the PALM® MicroLaser Systems technology was available (Figure 16).

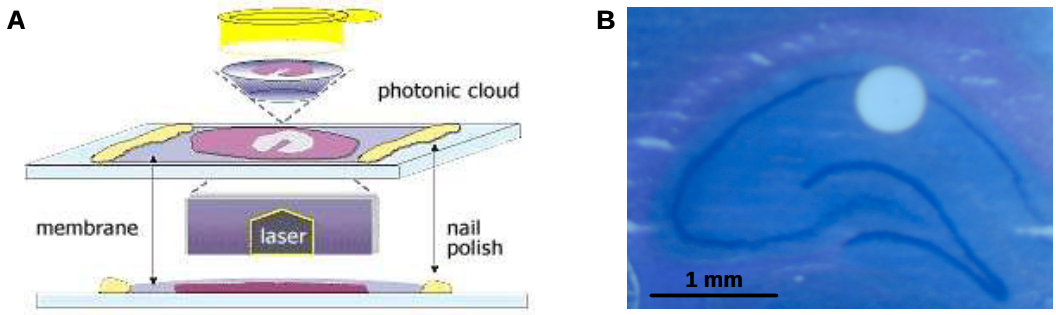

Figure 16. Laser Capture Microdissection. **A.** General principle of microdissection. The system consists of a laser beam, coupled to a microscope, which allows cutting out small parts of a slice of tissue of interest. The tissue slice is mounted onto a thin polyethylene naphtalene membrane, which is attached to a glass slide. The laser cuts the specimen and also the underlying membrane. After microdissection the entire membrane-tissue stack is ejected from the object plane, without mechanical contact, and catapulted directly into a microfuge cap with one single laser shot (from PALM Gmbh). **B.** Example of a circular microdissection taken out from dorsal hippocampus. Tissue slice was 30µm thick and was stained with toluidine blue 0.5%.

With the help of the Laser Capture Microdissection technology, we took out small pieces of the striatum and the hippocampus of mice (Figure 17). These regions were chosen only for the

purpose of technical validation of our approach. After total RNA extraction, samples were quantified and we were able to collect ~ 50 ng of total RNA per sample.

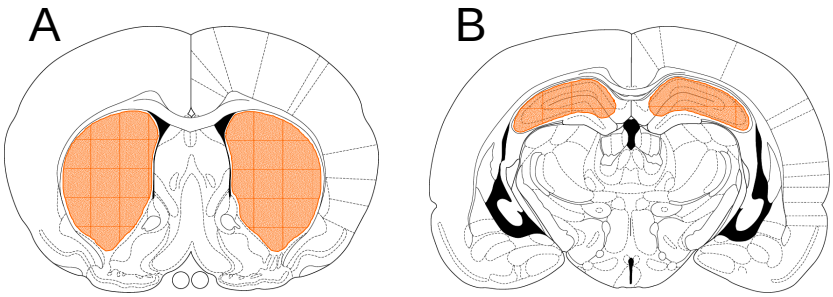

Figure 17. Schematic drawings of coronal sections of mouse brain showing regions where microdissections were taken out for protocol optimization. **A.** Striatum. **B.** Hippocampus.

These microdissections and dilutions of a commercial solution of mouse brain total RNA were used as templates to evaluate the performance of two protocols for hybridization of small-size samples on microarrays. We used the "Microarray Target Amplification" protocol developed by Roche, involving a PCR amplification step, and the "GeneChip® Eukaryotic Small Sample Target Labeling" protocol commercialized by Affymetrix (Figure 18). Gene expression profiles will be obtained from cRNA produced with these two methods by using the murine genome U74Av2 microarray from Affymetrix. This type of microarray allows the analysis of ~6000 functionally characterized Mouse Unigene Sequences (Build 74) and ~6000 EST clusters. Quality of microarray data were verified and expression profiles were compared thanks to a in-house software (RACE-A).

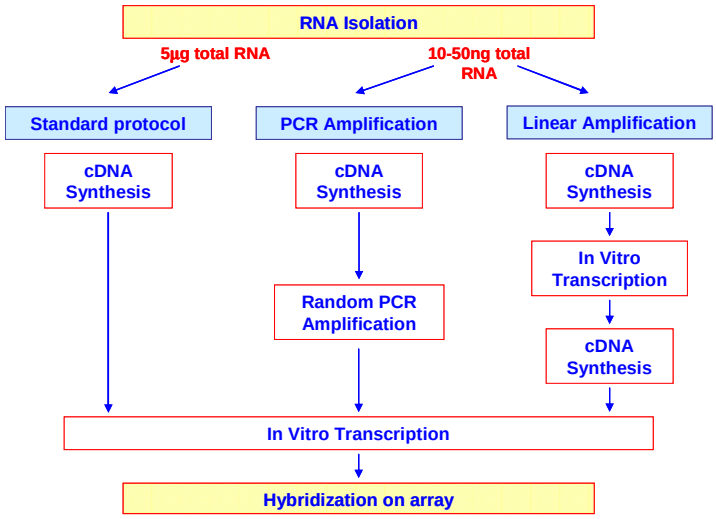

Figure 18. Protocols used for the preparation of samples for hybridization on microarrays. Two protocols are available for small samples: PCR amplification (Microarrays target Amplification Kit, Roche) or Linear amplification (GeneChip® Eukaryotic Small Sample Target Labeling, Affymetrix).

7.2. Gene expression profiling of hippocampus of rats doing a spatial learning task

After achievement of the technical optimization and validation, the "Microarray Target Amplification" protocol was selected for processing of samples from the study of spatial memory in the rat. Our aim was to investigate the molecular events, and more particularly the changes in mRNA expression, occurring in the brain when rats perform a spatial learning task. To this end, we trained male Lister Hooded rats in a Morris Water maze task.

Several reasons sustained the choice of this test to study spatial learning/memory in the rat. First, it is a test that has been used for decades to study the role of the hippocampus in spatial memory and it is now well recognized as a test of choice to assess the effect of various types of lesions, functional inactivation or any other experimental manipulations on spatial memory. Its specific involvement in the resolution of spatial tasks does however not exclude the contribution of its afferent and efferent systems. The second reason is that this test relies specifically on the use of working or reference memory (depending on the training protocol) for the resolution of the task. On the contrary, a test like the arm-maze may also imply procedural memory, which infers that rats develop a particular strategy, based on egocentric choice patterns, to succeed in the test (e.g. always visit first the arm in front of the entry arm and then always go on the arm situated on the left). A third reason concerns the fact that, unlike other spatial memory tasks such as the radial maze, rats do not require to be food deprived to induce motivation towards the aim of the task. Finally, the water maze test does not require much pre-training and can be performed in a rather short period of time (about 5 days), which may be a real advantage when large samples of animals have to be tested.

After the rats had completed the training and the probe trial in the water maze, the right and left dorsal CA1 were taken out with the help of microdissection (Figure 19).

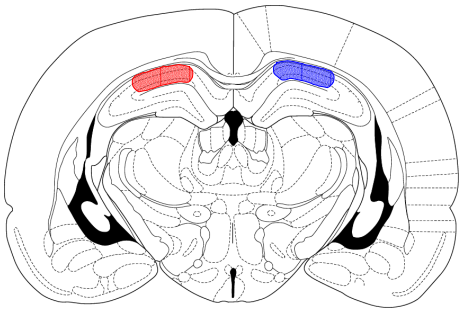

Figure 19. Schematic drawing of a coronal section of the rat brain illustrating the hippocampal regions where the microdissections were collected. This region was chosen according to Lenck-Santini et al. (2002).

After extraction of total RNA, the samples were prepared and hybridized on Rat U34A GeneChips® allowing the analysis of ~7000 characterized Rat Unigene Sequences (Build 34) and ~1000 EST clusters. Expression profiles were also obtained for right and left dorsal CA1 of water maze control rats (trained to find a visible platform), swimming controls and naïve rats. This allowed us to examine differential expression related to spatial learning in the right and the left dorsal hippocampus.

7.3. Reversible inactivation of hippocampus of rats doing a spatial learning task

This study has been set up on the basis of the results of expression profiling of rats doing the water maze task. In order to confirm the possible lateralization of spatial memory, we performed reversible functional inactivation of the dorsal hippocampus of rats doing a spatial learning task. For this, we injected two anesthetic compounds directly into the rat dorsal hippocampus. The design of the study required that we implanted guide-cannula in the rat brain in order to deliver the inactivating compounds directly into the dorsal hippocampus. Implantation sites are shown on Figure 20. Before running the behavioral experiment in order to explore the potent role of left and right hippocampus in spatial memory, we optimized the reversible inactivation method. For this, two widely used drugs, lidocaine and tetrodotoxin (TTX), were tested and characterized. The 2-deoxyglucose technique was used to show the extent and degree of inactivation of both substances and the beam-walking test was performed to explore the effects of uni- or bilateral inactivation on sensory-motor coordination capabilities of the rats. Lidocaine was preferred to TTX for performing reversible inactivation of dorsal hippocampus of rats. Actually, inactivation through lidocaine remained localized to the microinjection site in dorsal hippocampus and did not alter sensory-motor capabilities of the rats. In contrast, TTX microinjection induced extensive metabolic decrease far beyond the infusion site associated with major impairments in sensory-motor coordination.

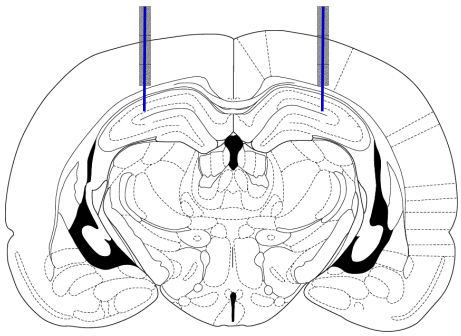

Figure 20. Schematic drawing of a coronal section of the rat brain showing guide-cannula implantation and infusion sites. Guide-cannula were implanted at AP -3.8, ML +/- 3.0, DV -1.5, with microinjection cannula protruding 1 mm below guide-cannula.

Two spatial learning tests were performed. First, we trained rats to a Morris water maze task. For this test, reversible inactivation of dorsal hippocampus was performed either during acquisition or just before the probe trial. Then, rats were tested in a 5-arm maze, where they had to learn and remember the location of a baited arm. In this case, inactivation was only done before the probe trial.

7.4. Summary of the doctoral work

1. Preliminary optimization of small-sample processing for analysis on DNA microarrays

Optimization of microdissection and sample extraction. →

Comparison of two amplification protocols for the analysis of small-size samples on microarrays: "microarray target amplification" (Roche) and "GeneChip® Eukaryotic Small Sample Target Labeling" (Affymetrix).

These 2 protocols were used to analyze the expression profiles of mouse striatum and hippocampus. →

Both protocols allowed production of sufficient material; however, "microarray target amplification protocol" produced samples of better quality. Therefore it has been used for the analysis of spatial memory in rats.

↓

2. Gene expression profiling of the dorsal hippocampus of rats doing a spatial navigation task

Training of the 5 groups of rats in the water maze.

- a spatial learning group trained to the hidden platform (SP)
- two control groups trained to a visible platform and tested with (CP) or without the platform (CN)
- a swimming group, no platform at all (SW)
- a group of naïve rats (HC, homecage). →

Preparation of samples and hybridization to microarrays.

Statistical analysis and comparison of the groups of rats.

Functional classification of the induced and repressed genes. →

Transcriptional activity is higher in the right hippocampus suggesting a more important role of the right dorsal hippocampus in the management of spatial information.

Therefore a functional analysis was performed in order to assess the roles of the right and the left hippocampus in the different stages of spatial information management.

↓

3. Optimization of reversible functional inactivation of the dorsal hippocampus

Optimization of reversible inactivation:

Comparison of the effects of two anesthetic compounds: lidocaine and tetrodotoxin. →

Characterization of the degree and extent of the inactivation with the 2-deoxyglucose method:
- lidocaine: inactivated region is localized to the infusion site
- TTX: metabolic decrease extends far beyond the infusion site and even contralaterally

Effects of inactivation on sensory-motor capabilities of the rats doing the beam-walking test:
- lidocaine: no effect
- TTX: major sensory-motor impairments →

Lidocaine is more appropriate than tetrodotoxin for reversible inactivation of the dorsal hippocampus of rats

↓

4. Reversible functional inactivation of the dorsal hippocampus of rats doing a spatial learning task with lidocaine

Test of paw preference.

No behavioral difference between left- and right-pawed rats. →

Reversible inactivation of the dorsal hippocampus of rats doing a Morris water maze task. Lidocaine was infused uni- or bilaterally, either before probe trial or during acquisition of the task.

Right dorsal hippocampus has a greater role in retention of the task, whereas acquisition might rather involve the left hippocampus. →

Reversible inactivation of the right or the left hippocampus of rats doing a spatial navigation task in the 5-arm maze. Unilateral lidocaine infusions were performed just before the probe trial.

Retention is unaffected, it might be possible that spatial information is consolidated elsewhere (e.g. prefrontal cortex).

TECHNICAL OPTIMIZATION

1. Evaluation of amplification protocols (Publication 1)

Evaluation of procedures for amplification of small-size samples for hybridization on microarrays.

Sandra Klur, Karen Toy, Mickey P. Williams, and Ulrich Certa.

Genomics 83 (2004), 508–517

This publication reports the first part of our work, which consisted of testing and validating a method allowing the preparation and the analysis of brain microdissections on Affymetrix microarrays. The validation has been carried out on two structures of the mouse brain, namely the hippocampus and the striatum. Laser capture microdissection was employed to dissect brain tissue very precisely. It was possible to extract approximately 50 ng of total RNA from these microdissections. Therefore, the classical sample preparation protocol to analyze the transcriptome on DNA microarrays could not be used, this protocol requiring, at that time, at least 5 µg of total RNA. Thus, using dilutions of a reference total RNA and microdissections of mouse hippocampus and striatum, we tested and compared two different commercially available amplification protocols. The classical protocol consists of synthesizing, by reverse transcription, a double stranded cDNA using the total RNA present in the sample as template. Then a cRNA is synthesized by *in vitro* transcription and this cRNA is hybridized to a microarray. The first amplification protocol consisted of repeating the classical protocol twice. The second procedure consisted of amplification of the cDNA through a PCR reaction, thanks to specific primers. After preparation and hybridization of our mouse samples to microarrays, a statistical analysis enabled us to conclude that the method involving PCR amplification allowed faster and more reliable sample preparation; moreover this method was also more proportional to the classical method and produced a cRNA of better quality than linear amplification. Nevertheless, the two techniques yielded similar results, and above all, allowed us to show in both cases, the presence of hippocampus and striatum specific mRNA expression. We finally decided to continue our study by using the protocol based on PCR amplification.

Evaluation of procedures for amplification of small-size samples for hybridization on microarrays

Sandra KLUR, Karen TOY, Mickey P. WILLIAMS and Ulrich CERTA

Genomics, 2004, vol. 83, numéro 3, pages 508-517

2. Evaluation of reversible inactivation strategies (Publication 2)

Reversible inactivation of the dorsal hippocampus by tetrodotoxin or lidocaine: a comparative study on cerebral functional activity and motor coordination in the rat.

Anne Pereira de Vasconcelos, Sandra Klur, Christophe Muller, Brigitte Cosquer, Joëlle Lopez, Ulrich Certa, and Jean-Christophe Cassel.

Neuroscience 141 (2006), 1649-1663

This publication reports the comparison of metabolic and motor effects of two drugs, namely lidocaine and tetrodotoxin (TTX), used for the reversible inactivation of hippocampus. Actually, this part of the project has been done in regard to the gene expression profiling analysis of rats doing a spatial learning task, which showed a possible lateralization of spatial learning and memory to the right dorsal hippocampus. The aim of the experiment was to associate reversible functional inactivation of hippocampus to a behavioral study addressing the relative implication of right and left hippocampus for the resolution of spatial navigation tasks. However, before studying the effects of hippocampal microinjections on the realization of a spatial task, we evaluated the extent and quality of inactivation using these two substances. For this, we used the technique where radiolabeled 2-deoxyglucose allows visualization and measurement (on a semi quantitative basis, in this case) of cerebral metabolic activity. This important control experiment was completed with the evaluation of the rats' sensory-motor coordination capabilities after uni- or bilateral infusion of one or the other compound. The data demonstrates that an intrahippocampal injection of TTX induced an inactivation that was not only localized to hippocampus but also spread to other brain structures (e.g. parietal cortex and thalamus) and even to the side contralateral to the infusion side. Moreover, it was established that TTX had a negative impact on the rat sensory-motor capabilities, most of the animals being no longer capable to walk on a thin and elevated wooden beam (beam-walking test). Conversely to TTX, lidocaine showed the expected characteristics: the inactivated region was close to the compound infusion site, and, as a result of this inactivation, we did not observe any obvious effect on the sensory-motor capabilities of the rats. Therefore, in the final experiment of this thesis, lidocaine was used to induce the reversible inactivation of hippocampus.

Reversible inactivation of the dorsal hippocampus by tetrodotoxin or lidocaine: a comparative study on cerebral functional activity and motor coordination in the rat

A. PEREIRA DE VASCONCELOS, S. KLUR, C. MULLER, B. COSQUER, J. LOPEZ, U. CERTA and J.C. CASSEL

Neuroscience, 2006, vol. 141, numéros 4, pages 1649-1663

3. Paw preference test

The present test was carried out to investigate the distribution of paw preference in rats and to search for a difference, if any, between right and left -pawed rats in the Morris water maze test, and also to enable, should it be necessary, correction of the effects of left or right hippocampal inactivation on the basis of each rat's preferred side.

3.1. Experimental procedure

To determine paw preference, we applied the food-reaching test described by Pence, 2002. A transparent testing cage, 20 x 20 x 40 cm in size, having a hole (3 x 3 cm) situated equidistant from right and left sidewalls, was used in the study (Figure 21). The hole was 7 cm above the floor and the rat could only reach the food with the right or left paw. A cylindrical tube was placed in the hole, in which sugar pellets were available. Rats were habituated to the testing cage and trained to learn to reach the pellets during the 4 days preceding the test. Rats were deprived for food one day before starting the habituation period and weights were surveyed so that rats did not lose more than 15 % of their initial weight. On test day, rats had to reach for 50 sugar pellets (in 50 trials) and the sequence of right or left paw reaching was scored.

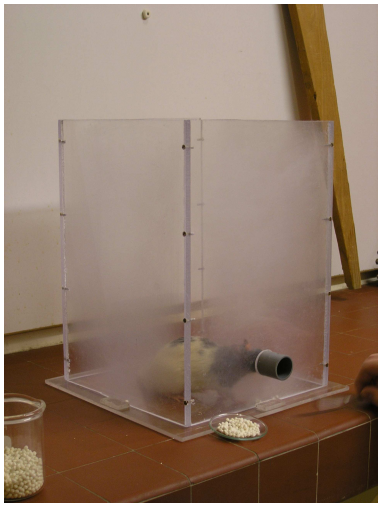

Figure 21. Photo of the paw preference test cage

After completion of the task, a preference ratio was calculated for each rat: the number of right-paw reaches was divided by the total of right- and left-paw reaches. A rat was classed as showing right preference when the ratio exceeded 0.6, left preference when it was lower than 0.4. When the ratio was in between both, the rat was considered ambidextrous.

3.2. Results

We tested a total of 149 male Lister Hooded rats out of which 43.6 % were right-pawed, 51.0 % left-pawed and 5.4 % ambidextrous (Figure 22). This repartition was quite surprising with regard to previous studies of paw preference in rats that showed a dominance of right-pawed animals (Pence, 2002; Elalmis et al., 2003; but Whishaw, 1992). However, it may be possible that paw-preference differs from one lineage to another. Indeed, Pence, 2002 and Elalmis et al., 2003, tested Sprague-Dawley and Wistar rats, respectively. Whishaw, 1992 studied Long-Evans rats and our focus was on Lister Hooded rats. Another reason might be the influence of test procedure on the preferential use of one paw; however, the test applied in this study was the one developed by Pence, 2002. In conclusion, the difference is more likely due to the lineage.

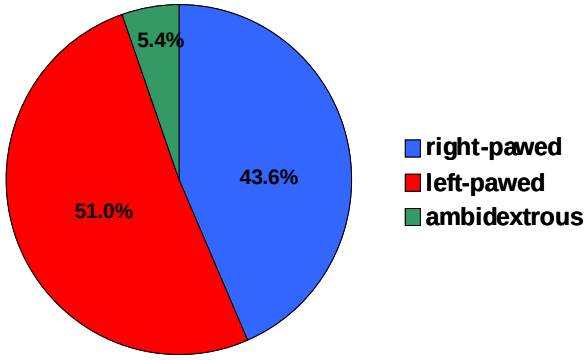

Figure 22. Distribution of paw preference for 149 Lister Hooded rats

After the paw preference test, these rats were tested in the Morris water maze task. Data from right and left-pawed rats were analyzed and no difference related to pawedness could be evidenced. In conclusion, there is no link between hemispheric dominance related to "pawedness" (a word introduced by Rosen et al., 1990, which is used to design the rat equivalent of "handedness" in humans) and preferential use of one hippocampus for the resolution of the water maze task, at least for rats of the Lister Hooded lineage.

RESULTS

1. Gene expression profiling and reversible inactivation of rat dorsal hippocampus (Manuscript 1)

Hippocampal-dependent spatial memory functions may be lateralized in rats: an approach combining gene expression profiling and reversible inactivation.

Sandra Klur[1,2], Christophe Muller[2], Anne Pereira de Vasconcelos[2], Theresa Ballard-Yardy[3], Jean-Christophe Cassel[2*] and Ulrich Certa[1*].

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* Contributed equivalently to this work

Submitted to *Journal of Neuroscience*.

This manuscript reports the results of gene expression profiling and reversible inactivation of dorsal hippocampus of rats doing a spatial navigation task in the water maze. The tools developed in this doctoral work and presented in the technical part of this thesis were used to study spatial memory. The first experiment consisted of high-throughput monitoring of mRNA expression in rat dorsal hippocampus, in order to identify genes that are differentially expressed in association with spatial memory. Thus, we examined laser capture microdissections from the dorsal CA1 region of rats that were trained in the Morris water maze. After statistical analysis, it was established that the number of genes that were differentially expressed in comparison with the control situations was much larger in the right than in the left hippocampus. These data show clearly that spatial memory requires gene transcription and suggest that the right dorsal hippocampus might have a more important role than the left dorsal hippocampus in a spatial memory process like that incurred by the Morris water maze task. This analysis was completed by reversible inactivation of dorsal hippocampus of rats doing the water maze task through unilateral and bilateral lidocaine infusions. The aim of this experiment was to test the rats' spatial navigation capabilities after hippocampal inactivation in acquisition or retention of the task. Retrieval was impaired, though not prevented, by inactivation of the right (not the left) hippocampus. In conclusion, the experiments presented here support defined roles in spatial memory processes for the left and right dorsal hippocampus in rats.

Hippocampal-dependant spatial memory functions might be lateralized in rats: an approach combining gene expression profiling and reversible inactivation.

KLUR S., MULLER C., PEREIRA DE VASCONCELOS A., BALLARD T., LOPEZ J., GALANI R., CERTA U., CASSEL J.C.

Hippocampus, 2009, vol. 19, numéro 9, pages 800-816

Supplementary Table 1
Semi-quantitative PCR

Gene		Comparison				Semi-quantitative PCR		
		SP vs. HC - Left Hippo		SP vs. HC - Right Hippo				
Name	Identifier	Affymetrix	Taqman	Affymetrix	Taqman	Oligo sequence		Amplicon size
Beta actin	V01217_at	0.44 ± 0.34	0.56 ± 0.18	0.15 ± 0.31	0.05 ± 0.04	FW primer	5'-GACCCAGATCATGTTTGAGACCTT-3'	165 nt
						RV primer	5'-GCATGAGGGAGCGCGTAA-3'	
						TaqMan probe	5'-CCGGAGTCCATCACAATGCCAGTG-3'	
Gamma synuclein	X86789_at	1.45 ± 0,11	2.26 ± 0.36	0.42 ± 0.27	0.15 ± 0.19	FW primer	5'-AGTACGCAAGGAGGACTTGGAA-3'	117 nt
						RV primer	5'-AGCCTCTGTGGTTGACTGGAA-3'	
						TaqMan probe	5'-CGAAGAGGCCAAGAGTGGAGGTGACTAGAA-3'	
MAP Tau	X79321_at	1.27 ± 0.03	2.35 ± 0.01	0.29 ± 0.01	0.22 ± 0.00	FW primer	5'-CCAAGAAGGAGACATGGACCAT-3'	140 nt
						RV primer	5'-CGTCATTTCCTGTCCTGTCTTTG-3'	
						TaqMan probe	5'-AAAGCTGAAGAAGCAGGCATCGGAGAC-3'	
Alpha Thalassemia	D64059_at	0.55 ± 0.1	0.58 ± 0.02	1.06 ± 0.02	0.37 ± 0.01	FW primer	5'-GCCTTCACATACTGGATTTTGTCA-3'	96 nt
						RV primer	5'-GGATCATTGTCGTCGTCATCAT-3'	
						TaqMan probe	5'-TCTCTAAATCAGTGCCTGCCACAGTGGAT-3'	
Nuclear Receptor 4, a2	U01146_s_at	0.08 ± 0.02	0.02 ± 0.06	0.69 ± 0.08	0.23 ± 0.09	FW primer	5'-TGCCTTCTCCTGCATTGCT-3'	71 nt
						RV primer	5'-CCACTCTCTTGGGTTCCTTGAG-3'	
						TaqMan probe	5'-CCCTGGCTATGGTCACAGAGAGACA-3'	
ATPase, Ca2+ transporting, 2b1	L04739cds_s_at	0.79 ± 0.15	2,05 ± 0.42	0.64 ± 0.23	0.51 ± 0.07	FW primer	5'-AGGCCTGAACAGAATCCAAACA-3'	78 nt
						RV primer	5'-CTTAGAGCCCCCTGAATGGAA-3'	
						TaqMan probe	5'-TGTAGTGAATGCTTTCCAGAGTGGAG-3'	

Reference Gene				
Tubulin	V01227_s_at	FW primer	5'-GGAGATCATTGACCTTGTCTTGG-3'	71 nt
		RV primer	5'-AGAAGCCCTGGAGACCCGT-3'	
		TaqMan probe	5'-CAGAATTCGCAAGCTGGCTGACCA-3'	

FW = forward
RV = reverse

Trancripts levels for the genes that were assayed by semi-quantitative PCR to validate microarray data. The sequence of primers used and length of PCR product are indicated. Results are mean of change factor ± sem when comparing expression in spatial learning rats vs. control rats, in the left and right hippocampus respectively.

Supplementary Table 2

Genes differentially expressed in the right dorsal hippocampus

SIP related genes are genes related to spatial information processing and were differentially regulated in the SP, CN and CP rat groups, compared to the HC rats.

SP related genes are genes differentially expressed only in the SP rats, compared to the HC rats.

The genes which are in **bold characters** are represented several times on the microarray and, for most of them, are assayed by 2 or 3 different probe sets. In our case, the modulation of expression of a gene calculated for each of the probe sets was similar. Also, the expression level given by the different probe sets were in a similar range. The fold change, however, could vary depending on the probe set.

The *change factor* which is reported for each gene in the following tables is the one that was calculated by the algorithm when comparing the spatial learning group (SP) to the homecage group (HC).

A. SIP related genes

Induced

Apoptosis

Identifier	*Symbol*	*Name*	*Change factor*
S76511_s_at	BAX	Bcl2-associated X protein	0.91
X75856_at	TEGT	testis enhanced gene transcript	0.78

Cell growth

Identifier	*Symbol*	*Name*	*Change factor*
rc_AA899106_at	CCND2	cyclin D2	1.95
U66470_at	CGREF1	cell growth regulatory with EF-hand domain	0.86
rc_AA892378_g_at	TTC11	tetratricopeptide repeat domain 11	0.78
AB014722_at	SART1	squamous cell carcinoma antigen recognized by T-cells 1	0.59

Cell morphology

Identifier	*Symbol*	*Name*	*Change factor*
U49099_at	GOSR1	golgi SNAP receptor complex member 1	1.05
rc_AA892310_at	AP1M1	adaptor-related protein complex 1, mu 1 subunit	0.87
rc_AA891441_s_at	MAP1LC3A	microtubule-associated protein 1 light chain 3 alpha	0.85
X62160_at	DCTN1	dynactin 1	0.72
rc_AI070848_f_at	ACTB	actin, beta	0.71
rc_AA963447_at	PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	0.69
AFFX_Rat_beta-actin_M_at	ACTB	actin	0.65
rc_AA866454_g_at	COL1A2	procollagen, type I, alpha 2	0.64
rc_AI179012_s_at	ACTB	actin, beta	0.51

General processes

Identifier	*Symbol*	*Name*	*Change factor*
AB013454_at	RPL17	ribosomal protein L17	2.10
rc_AA849648_g_at	**RPL21**	**ribosomal protein L21**	**2.05**
rc_AA849648_at	**RPL21**	**ribosomal protein L21**	**1.76**
X15216cds_s_at	**RPL21**	**ribosomal protein L21**	**0.55**
rc_AA892791_at	ERCC1	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	1.58
AF052042_s_at	RLZFY	zinc finger protein Y1 (RLZF-Y)	1.58
X52619_at	RPL28	ribosomal protein L28	1.34
U30186_at	DDIT3	DNA-damage inducible transcript 3	1.19
X58465mRNA_at	RPS5	ribosomal protein S5	1.06
rc_H31907_at	PP3111	embryo-related protein	1.03
AB012234_g_at	NFIX	nuclear factor I/X	1.01
U30381_at	ZNF148	zinc finger protein 148	1.00
rc_AI013194_at	EIF5	eukaryotic initiation factor 5 (eIF-5)	0.97
U60882_at	HRMT1L2	heterogeneous nuclear ribonucleoproteins methyltransferase-like 2 (S. cerevisiae)	0.96
L08814_at	SSRP1	Structure specific recognition protein 1	0.80
U05014_g_at	EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1	0.72
rc_AA799672_s_at	RPL6	ribosomal protein L6	0.69
AF003926_at	NR2F6	nuclear receptor subfamily 2, group F, member 6	0.68
AF058791_at	G10	maternal G10 transcript	0.66
rc_AI229637_at	MYBBP1A	MYB binding protein 1a	0.61
rc_AA860024_at	EEF1G	eukaryotic translation elongation factor 1 gamma	0.59
X14210cds_at	RPS4X	ribosomal protein S4, X-linked	0.57
M89646_g_at	RPS24	ribosomal protein S24	0.56

Immune response

Identifier	*Symbol*	*Name*	*Change factor*
D45249_g_at	PSME1	protease (prosome, macropain) 28 subunit, alpha	0.74
X71127_at	C1QB	complement component 1, q subcomponent, beta polypeptide	0.73
rc_AI178135_at	C1QBP	complement component 1, q subcomponent binding protein	0.64
rc_AI009801_at	MIF	macrophage migration inhibitory factor	0.60
rc_AI011179_at	IGBP1	immunoglobulin binding protein 1	0.60
X13983mRNA_at	A2M	alpha-2-macroglobulin	0.59

Metabolism

Identifier	*Symbol*	*Name*	*Change factor*
AFFX_Rat_GAPDH_5_st	**GAPD**	**glyceraldehyde-3-phosphate dehydrogenase**	**2.83**
AFFX_Rat_GAPDH_3_st	**GAPD**	**glyceraldehyde-3-phosphate dehydrogenase**	**2.25**
X56228_g_at	**TST**	**thiosulfate sulfurtransferase**	**2.40**
X56228_at	**TST**	**thiosulfate sulfurtransferase**	**0.89**
X08056cds_s_at	GAMT	guanidinoacetate methyltransferase	1.96
rc_AI172411_at	GPX3	glutathione peroxidase 3	1.41
rc_AA893065_at	NPEPPS	puromycin-sensitive aminopeptidase	1.39
D86641_at	FKBP1A	FK506-binding protein 1a	1.32
M10068mRNA_s_at	POR	P450 (cytochrome) oxidoreductase	1.23
rc_AA859529_at	DGAT1	diacylglycerol O-acyltransferase 1	1.22
E05646cds_s_at	PBP	phosphatidylethanolamine binding protein	1.09
rc_AA963839_s_at	**DIA1**	**diaphorase 1**	**1.06**
D00636Poly_A_Site#1_s_at	**DIA1**	**diaphorase 1**	**0.87**
rc_AI229440_s_at	**DIA1**	**diaphorase 1**	**0.82**
Z36980_at	**DDT**	**D-dopachrome tautomerase**	**1.04**
Z36980_g_at	**DDT**	**D-dopachrome tautomerase**	**0.86**
S72594_s_at	TIMP2	tissue inhibitor of metalloproteinase 2	1.03
D89069_f_at	CBR1	carbonyl reductase 1	0.93
D86642_at	FKBP1B	FK506 binding protein 1b	0.90
L19998_g_at	SULT1A1	sulfotransferase family 1A, phenol-preferring, member 1	0.84
rc_AA892808_at	IDH3G	isocitrate dehydrogenase 3, gamma	0.83
rc_AI171844_at	ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	0.79
U55938_at	SIAT8C	sialyltransferase 8 C	0.78
rc_AA859837_at	GDA	guanine deaminase	0.78
J04792_at	ODC1	ornithine decarboxylase 1	0.68
U86635_at	GSTM1	glutathione S-transferase, mu 5	0.67
M38135_at	CTSH	cathepsin H	0.67
U00926_g_at	ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta	0.66
X73653_at	GSK3B	glycogen synthase kinase 3 beta	0.61
U50185_at	PPP1R12A	myosin phosphatase, target subunit 1	0.51

Nervous system

Identifier	*Symbol*	*Name*	*Change factor*
M24852_at	PCP4	Purkinje cell protein 4	1.78
U57391_g_at	SH2B	SH2-B PH domain containing signaling mediator 1	1.49
D28110_g_at	MOBP	myelin-associated oligodendrocytic basic protein	1.43
rc_AA875659_s_at	INA	internexin, alpha	1.34
M59980_s_at	KCND2	potassium voltage gated channel, Shal-related family, member 2	1.31
AB007690_s_at	HOMER2	homer, neuronal immediate early gene, 2	1.24
AF037071_at	CAPON	C-terminal PDZ domain ligand of neuronal nitric oxide synthase	1.23
D13125_at	HPCAL4	neural visinin-like Ca2+-binding protein type 2	1.02
M64780_g_at	AGRN	agrin	1.00
M55291_at	NTRK2	neurotrophic tyrosine kinase, receptor, type 2	0.99
AF016296_at	NRP1	neuropilin	0.93
U16845_at	HNT	neurotrimin	0.89
X54656_at	**GRIA3**	**glutamate receptor, ionotropic, AMPA3 (alpha 3)**	**0.77**
M36420_s_at	**GRIA3**	**glutamate receptor, ionotropic, AMPA3 (alpha 3)**	**0.74**
rc_AA874794_at	NGFRAP1	nerve growth factor receptor associated protein 1	0.76
X76985_at	LXN	latexin	0.70
D64059_at	ATRX	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S.cerevisiae)	0.69
L09119_g_at	NRGN	neurogranin	0.65
M15880_at	NPY	neuropeptide Y	0.65
M88751_at	CACNB3	calcium channel, voltage-dependent, beta 3 subunit	0.57

Signaling and transport

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI172499_at	ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	3.29
rc_AI073204_at	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	2.72
M83678_at	RAB13	RAB13	1.76
U35775_at	ADD3	adducin 3, gamma	1.68
E00698cds_s_at	**NPPA**	**natriuretic peptide precursor type A**	**1.51**
X01118_at	**NPPA**	**natriuretic peptide precursor type A**	**0.54**

Signaling and transport (continued)

Identifier	*Symbol*	*Name*	*Change factor*
U75917_at	AP2S1	clathrin-associated protein 17	1.49
U36444cds#1_g_at	PCTK1	PCTAIRE-1 protein kinase, alternatively spliced	1.48
rc_AI231807_g_at	**FTL**	**ferritin light chain 1**	**1.34**
rc_AI231807_at	**FTL**	**ferritin light chain 1**	**1.02**
AA799389_g_at	RAB3B	Rab3B protein	1.29
M57664_g_at	CKB	creatine kinase, brain	1.22
J04024_at	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	1.11
AF033027_at	YKT6	prenylated SNARE protein	1.05
AJ007291_at	**PARK7**	**fertility protein SP22**	**0.95**
AJ007291_g_at	**PARK7**	**fertility protein SP22**	**0.71**
AB009463_g_at	LRP3	low density lipoprotein receptor-related protein 3	0.93
U36482_at	**C12orf8**	**endoplasmic retuclum protein 29**	**0.92**
U36482_g_at	**C12orf8**	**endoplasmic retuclum protein 29**	**0.72**
M12672_at	GNAI2	GTP-binding protein (G-alpha-i2)	0.91
rc_AA819338_g_at	**SSR4**	**signal sequence receptor 4**	**0.90**
rc_AA819338_at	**SSR4**	**signal sequence receptor 4**	**0.85**
rc_AI104225_at	LAMB2	laminin, beta 2	0.89
rc_AA800015_at	STXBP1	syntaxin binding protein 1	0.88
rc_AA944397_at	**HSPCA**	**heat shock protein 86**	**0.88**
rc_AA819776_f_at	**HSPCA**	**heat shock 90kDa protein 1, alpha**	**0.79**
X55572_at	APOD	apolipoprotein D	0.86
AB006013_at	RGS8	regulator of G-protein signaling 8	0.85
U43175_at	ATP6V1F	ATPase, vacuolar, 14 kD	0.85
M94918mRNA_f_at	**HBB**	**hemoglobin beta chain complex**	**0.84**
rc_AI179576_s_at	**HBB**	**hemoglobin beta chain complex**	**0.59**
U03390_at	GNB2L1	guanine nucleotide binding protein, beta polypeptide 2-like 1	0.79
rc_AI013472_at	PPAP2B	ER transmembrane protein Dri 42	0.78
X56325mRNA_s_at	HBA1	hemoglobin, alpha 1	0.78
rc_AA875291_at	HRASLS3	HRAS like suppressor	0.74
X14265_at	CALM3	calmodulin 3	0.73
rc_AI175208_at	GOSR2	golgi SNAP receptor complex member 2	0.71
L21711_s_at	LGAL5	lectin, galactose binding, soluble 5	0.69
AF090867_at	GMPR	guanosine monophosphate reductase	0.67
rc_AA946313_s_at	**SPARC**	**secreted acidic cysteine rich glycoprotein**	**0.65**
U75928UTR#1_s_at	**SPARC**	**secreted acidic cysteine rich glycoprotein**	**0.52**
M77245_at	AP1B1	adaptor protein complex AP-1, beta 1 subunit	0.64
rc_AI102205_s_at	1G5	vesicle-associated calmodulin-binding protein	0.61
rc_AI231821_at	STMN1	stathmin 1	0.58
rc_AA866257_at	PGEA1	cytosolic leucine-rich protein	0.56
rc_AA892422_at	MRPL11	mitochondrial ribosomal protein L11	0.55
rc_AI175539_at	PVALB	parvalbumin	0.54

Unknown

Identifier	*Symbol*	*Name*	*Change factor*
U77931_at	-	-	3.36
S77900_g_at	-	-	2.36
rc_AA891054_at	-	-	1.81
rc_AI638989_at	-	-	1.66
rc_AI008074_s_at	-	-	1.54
rc_AA799633_at	-	-	1.46
AA685112_at	-	-	1.38
L38482_g_at	-	-	1.24
rc_AI639294_at	-	-	1.22
rc_AI639435_at	-	-	1.19
rc_AA799475_at	-	-	1.08
M13100cds#4_f_at	-	-	1.07
rc_AA891069_at	-	-	1.06
M13011cds_at	-	-	1.06
rc_AA859909_at	-	-	1.05
rc_AA875523_i_at	-	-	1.04
U30788_g_at	-	-	1.01
rc_AA799474_at	-	-	0.99
rc_AA874999_at	-	-	0.99
rc_AA799732_at	-	-	0.94
X07686cds_s_at	-	-	0.93
rc_AA875268_at	-	-	0.91

Unknown (continued)

Identifier	*Symbol*	*Name*	*Change factor*
rc_H33750_at	-	-	0.90
rc_AA685974_at	-	-	0.88
C06598_at	-	-	0.87
rc_AI102044_at	-	-	0.87
rc_AA799788_s_at	-	-	0.86
rc_AA799526_at	-	-	0.86
X62660mRNA_at	**-**	**-**	**0.85**
X62660mRNA_g_at	**-**	**-**	**0.53**
rc_AA893237_at	-	-	0.85
rc_AA892505_at	-	-	0.82
rc_AA799762_g_at	-	-	0.81
rc_AA892378_at	-	-	0.80
rc_H33656_at	-	-	0.80
rc_AA875552_at	-	-	0.78
rc_AA893603_at	-	-	0.78
rc_AA925556_at	-	-	0.77
rc_AA799616_at	-	-	0.77
rc_AA891631_at	-	-	0.75
AA684963_at	-	-	0.74
rc_AI639079_at	-	-	0.74
rc_AI013297_at	-	-	0.74
rc_H33459_g_at	-	-	0.73
rc_AA893741_at	-	-	0.72
rc_AA800175_at	-	-	0.72
X05472cds#3_f_at	-	-	0.70
rc_AA818726_at	-	-	0.70
X61295cds_s_at	-	-	0.69
rc_AA874832_at	-	-	0.68
rc_AA799479_g_at	-	-	0.68
rc_AA858588_g_at	-	-	0.66
rc_AI010580_s_at	-	-	0.66
rc_AA859612_f_at	-	-	0.64
D17521_at	-	-	0.64
rc_AA800199_at	-	-	0.64
rc_H31964_at	-	-	0.63
rc_AA800198_at	-	-	0.62
rc_AA891796_at	-	-	0.61
rc_AA891818_at	-	-	0.59
L25387_at	**-**	**-**	**0.58**
L25387_g_at	**-**	**-**	**0.55**
rc_AA892132_at	-	-	0.58
M13101cds_f_at	-	-	0.58
rc_AA892507_at	-	-	0.54
rc_AA874995_at	-	-	0.54
rc_AI177096_at	-	-	0.53
D49446_at	-	-	0.51
rc_AA800881_at	-	-	0.50
rc_AI639120_at	-	-	0.50

B. SIP related genes

Repressed

Apoptosis

Identifier	*Symbol*	*Name*	*Change factor*
rc_AA799641_at	TDE1	tumor differentially expressed 1	-2.97
rc_AA850781_at	PPID	peptidylprolyl isomerase D (cyclophilin D)	-1.46
M86564_at	PTMA	prothymosin alpha	-1.21
rc_AA893611_s_at	MXI1	MAX interactor 1	-1.07
rc_AA799664_at	AKT1	v-akt murine thymoma viral oncogene homolog 1	-0.97
X73411mRNA_s_at	SNRPN	small nuclear ribonucleoprotein polypeptide N	-0.65

Cell growth

Identifier	*Symbol*	*Name*	*Change factor*
AF067795_g_at	**CTBP1**	**C-terminal binding protein 1**	**-1.64**
AF067795_at	**CTBP1**	**C-terminal binding protein 1**	**-1.54**
U34843_g_at	D123	D123 gene product	-1.57
rc_AA892598_at	NS	nucleostemin	-1.02
U83883_at	SND1	p105 coactivator	-1.00
X70871_at	CCNG1	cyclin G1	-0.99
L21192_at	GAP43	growth associated protein 43	-0.98
U41803_at	U711	mitofusin 2	-0.85
rc_AI227887_g_at	CDC42	cell division cycle 42 homolog (S. cerevisiae)	-0.82
AJ131902_g_at	GAS7	growth arrest specific 7	-0.74
rc_AI232379_at	PDGFRA	platelet derived growth factor receptor, alpha polypeptide	-0.72
rc_AA801130_at	GRB2	growth factor receptor bound protein 2	-0.70

Cell morphology

Identifier	*Symbol*	*Name*	*Change factor*
U39044_at	DNCI2	dynein, cytoplasmic, intermediate polypeptide 2	-3.66
D13985_g_at	CLNS1A	chloride channel, nucleotide-sensitive, 1A	-2.86
rc_AA892918_at	TJP1	tight junction protein 1 (zona occludens 1)	-2.20
K00750exon#2-3_at	**CYCS**	**cytochrome c, somatic**	**-1.51**
rc_AI008815_s_at	**CYCS**	**cytochrome c, somatic**	**-1.47**
rc_AI144767_s_at	TPM1	tropomyosin 1, alpha	-1.44
rc_AI169005_at	CLNS1A	chloride channel, nucleotide-sensitive, 1A	-1.31
rc_AA892498_at	TM4SF8	transmembrane 4 superfamily member 8	-1.21
rc_AA799791_at	STK25	serine/threonine kinase 25 (STE20 homolog, yeast)	-1.16
S63521_i_at	HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	-0.95
D26154cds_at	M-RIP	Rho interacting protein 3	-0.89
rc_AA944422_at	CNN3	calponin 3, acidic	-0.67
S78556_at	HSPA9B	heat shock protein 9B (mortalin-2)	-0.62
rc_AI105348_i_at	CFL1	cofilin 1	-0.56

General processes

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI177751_at	TCEB1	elongation factor SIII p15 subunit	-3.98
D78303_at	YT521	splicing factor YT521-B	-2.75
L13635_s_at	SFRS5	splicing factor, arginine/serine-rich 5	-2.68
rc_AI177986_at	**EIF5**	**eukaryotic initiation factor 5 (eIF-5)**	**-2.38**
rc_AI012604_at	**EIF5**	**eukaryotic initiation factor 5 (eIF-5)**	**-2.17**
rc_AA799582_at	**HNRPK**	**heterogeneous nuclear ribonucleoprotein K**	**-2.30**
D17711cds_s_at	**HNRPK**	**heterogeneous nuclear ribonucleoprotein K**	**-0.97**
S59892_f_at	**SSB**	**Sjogren syndrome antigen B (autoantigen La)**	**-1.95**
X67859_at	**SSB**	**Sjogren syndrome antigen B**	**-0.76**
U20796_at	NR1D2	nuclear receptor subfamily 1, group D, member 2	-1.81
AF030091UTR#1_g_at	**CCNL1**	**cyclin L**	**-1.69**
AF030091UTR#1_at	**CCNL1**	**cyclin L**	**-1.44**
U95052UTR#1_s_at	EIF4G2	eukaryotic translation initiation factor 4 gamma, 2	-1.49
rc_AI008641_at	RPL22	ribosomal protein L22	-1.48
rc_AA892297_at	HDAC2	histone deacetylase 2	-1.41
X16933cds_at	HNRPD	RNA binding protein p45AUF1	-1.19
AF090306_s_at	RBBP7	retinoblastoma binding protein 7	-1.16
M63485_at	MATR3	matrin 3	-1.11
U67081_at	MYT1L	myelin transcription factor 1-like	-0.99
D37951UTR#1_at	HIVEP2	human immunodeficiency virus type 1 enhancer-binding protein 2	-0.98

General processes (continued)

Identifier	*Symbol*	*Name*	*Change factor*
J03179_at	DBP	D site albumin promoter binding protein	-0.95
rc_AI104524_s_at	HNRPAB	heterogeneous nuclear ribonucleoprotein A/B	-0.92
rc_AA893307_at	NCBP2	nuclear cap binding protein subunit 2, 20kDa	-0.91
M36074_g_at	NR3C2	nuclear receptor subfamily 3, group C, member 2	-0.79
rc_AA799995_g_at	RPL14	ribosomal protein L14	-0.77
Z71925_at	POLR2G	polymerase (RNA) II (DNA directed)polypeptide G	-0.74
rc_AI178207_at	RPS21	ribosomal protein S21	-0.69

Immune response

Identifier	*Symbol*	*Name*	*Change factor*
rc_AA818025_g_at	CD59	CD59 antigen	-1.79
rc_AI171462_s_at	**CD24**	**CD24 antigen**	**-0.92**
U49062_g_at	**CD24**	**CD24 antigen**	**-0.91**
rc_AI170268_at	B2M	beta-2 microglobulin	-0.67
AB008538_at	ALCAM	activated leukocyte cell adhesion molecule	-0.64

Metabolism

Identifier	*Symbol*	*Name*	*Change factor*
AF009656mRNA_s_at	HPRT	hypoxanthine guanine phosphoribosyl transferase	-4.97
U52663mRNA#3_s_at	**PAM**	**peptidylglycine alpha-amidating monooxygenase**	**-4.79**
rc_AA891068_f_at	**PAM**	**peptidylglycine alpha-amidating monooxygenase**	**-2.62**
rc_AA799575_f_at	**PAM**	**peptidylglycine alpha-amidating monooxygenase**	**-1.69**
E03358cds_g_at	**PSMA2**	**proteasome (prosome, macropain) subunit, alpha type, 2**	**-4.18**
rc_AI170403_at	**PSMA2**	**proteasome (prosome, macropain) subunit, alpha type 2**	**-2.83**
AJ000347_g_at	BPNT1	3(2),5-bisphosphate nucleotidase	-4.16
X16043cds_at	**PPP2CA**	**protein phosphatase 2a, catalytic subunit, alpha isoform**	**-3.50**
rc_AI012595_at	**PPP2CA**	**protein phosphatase 2a, catalytic subunit, alpha isoform**	**-0.68**
U07971_at	GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	-3.36
S81353_s_at	PSAP	prosaposin	-3.17
D83538_at	PIK4CA	phosphatidylinositol 4-kinase	-2.97
E03229cds_s_at	CDO1	cytosolic cysteine dioxygenase 1	-2.90
rc_AA892828_at	PDHB	pyruvate dehydrogenase (lipoamide) beta	-2.83
rc_AI176595_s_at	CTSL	cathepsin L	-2.35
rc_AI169417_s_at	PGAM1	phosphoglycerate mutase 1	-2.33
L27843_s_at	PTP4A1	protein tyrosine phosphatase 4a1	-2.23
rc_AA891286_at	TXNRD1	thioredoxin reductase 1	-2.21
rc_AA818593_at	PPAP2A	phosphatidate phosphohydrolase type 2a	-1.88
X78593_g_at	GPD2	glycerol-3-phosphate dehydrogenase 2	-1.86
X52625_at	HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	-1.78
rc_AI237731_s_at	LPL	lipoprotein lipase	-1.73
M62763complete_seq_at	SCP2	sterol carrier protein 2	-1.66
rc_AA799980_at	PPM1B	protein phosphatase 1B, magnesium dependent, beta isoform	-1.62
D14418_at	PPP2R1A	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR	-1.54
rc_AA801286_at	IMPA1	Inositol (myo)-1(or 4)-monophosphatase 1	-1.54
rc_AA893325_at	OAT	ornithine aminotransferase	-1.40
rc_AI105448_at	HSD11B1	hydroxysteroid 11-beta dehydrogenase 1	-1.36
rc_AA849722_at	PSMB1	proteasome (prosome, macropain) subunit, beta type 1	-1.34
D30666_at	ACSL3	fatty acid Coenzyme A ligase, long chain 3	-1.30
rc_AA892314_at	IDH1	isocitrate dehydrogenase 1	-1.15
rc_AI176504_at	GLS	glutaminase	-1.14
rc_AA924326_s_at	ALDOA	aldolase A	-1.06
U57050_g_at	PSMD1	26S proteasome, subunit p112	-0.85
AB011068_at	DIO2	deiodinase, iodothyronine, type II	-0.85
rc_AI168942_at	BCKDHB	branched chain keto acid dehydrogenase E1, beta polypeptide	-0.81
U54632_g_at	UBE2I	ubiquitin-conjugating enzyme E2I	-0.79
D13907_at	PMPCB	mitochondrial processing peptidase beta	-0.75
M91652complete_seq_at	GLUL	glutamine synthetase 1	-0.73
rc_AI112173_at	NME7	nucleoside-diphosphate kinase 7	-0.72
rc_AI013834_s_at	**HSD17B4**	**peroxisomal multifunctional enzyme type II**	**-0.66**
S83279_g_at	**HSD17B4**	**peroxisomal multifunctional enzyme type II**	**-0.61**
rc_AI231519_at	SIAT7C	sialyltransferase 7c	-0.65
M89945mRNA_at	FDPS	farensyl diphosphate synthase	-0.59
U38379_at	GGH	gamma-glutamyl hydrolase	-0.56

Metabolism (continued)

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI010083_at	PRDX1	peroxiredoxin 1	-0.56
AF093773_s_at	MDH1	malate dehydrogenase 1	-0.55
rc_AI231292_g_at	CST3	cystatin C	-0.54
rc_AI231500_at	PRPSAP2	phosphoribosyl pyrophosphate synthetase-associated protein 2	-0.53

Nervous system

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI014163_at	IFRD1	interferon-related developmental regulator 1	-4.30
AF007758_g_at	SNCA	synuclein, alpha	-3.97
AF009329_at	BHLHB3	basic helix-loop-helix domain containing, class B3	-3.44
U47315_s_at	BRP44L	brain protein 44-like	-3.21
X52817cds_s_at	RTN1	reticulon 1	-3.02
X57573_at	GAD1	glutamate decarboxylase 1	-2.59
rc_AI228669_at	SLC6A1	GABA transporter protein	-2.16
D32249_s_at	PJA2	protein carrying the RING-H2 sequence motif	-2.01
X66366_at	GPHN	gephyrin	-1.85
M38061_at	GRIA2	glutamate receptor, ionotropic, 2	-1.54
X67877_at	**PHAX**	**cytosolic resiniferatoxin-binding protein**	**-1.50**
X67877_g_at	**PHAX**	**cytosolic resiniferatoxin-binding protein**	**-0.81**
AF078779_at	VGCNL2	voltage gated channel like 1	-1.46
M25888_at	PLP1	proteolipid protein	-1.21
rc_AA944324_at	ARF6	ADP-ribosylation factor 6	-1.13
rc_AI639484_at	SV2B	synaptic vesicle glycoprotein 2 b	-1.00
X95466_at	SYNE1	CPG2 protein	-0.96
M92076_at	GRM3	glutamate receptor, metabotropic 3	-0.87
AF031880_at	NEFL	neurofilament, light polypeptide	-0.83
L34262_at	PPT	palmitoyl-protein thioesterase	-0.83
M84725_at	TAGLN3	transgelin 3	-0.74
U16802_at	CADPS	Ca2+-dependent activator protein	-0.70
M73049_at	INA	internexin, alpha	-0.69
X51992_at	GABRA5	gamma-aminobutyric acid A receptor, alpha 5	-0.65
K00512_at	MBP	myelin basic protein	-0.62
D12519_s_at	STX1A	syntaxin 1a	-0.62
AF058795_at	GPR51	G protein-coupled receptor 51	-0.61
Y09000_at	DDN	dendrin	-0.51

Signaling and transport

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI059508_s_at	TKT	transketolase	-4.78
L12380_at	ARF1	ADP-ribosylation factor 1	-4.52
rc_AI233225_at	GUCY1B3	guanylate cyclase 1, soluble, beta 3	-4.02
U12402_at	**ARL1**	**ADP-ribosylation factor-like 1**	**-4.02**
rc_AA875253_at	**ARL1**	**ADP-ribosylation factor-like 1**	**-2.64**
X06889cds_at	RAB3A	RAB3A, member RAS oncogene family	-3.62
rc_AI009605_at	RHEB	Ras homolog enriched in brain	-3.57
rc_AI235358_at	UQCRC2	ubiquinol-cytochrome c reductase core protein II	-3.54
X74401_at	GDI2	guanosine diphosphate dissociation inhibitor 3	-2.77
U95727_at	**DNAJA2**	**DnaJ (Hsp40) homolog, subfamily A, member 2**	**-2.63**
rc_AI170685_g_at	**DNAJA2**	**DnaJ (Hsp40) homolog, subfamily A, member 2**	**-1.15**
L01702_at	PTPRA	protein tyrosine phosphatase, receptor type, A	-2.46
D30740_g_at	**YWHAZ**	**tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide**	**-2.39**
D30740_at	**YWHAZ**	**tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide**	**-2.12**
rc_AI180424_at	**YWHAZ**	**tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide**	**-1.71**
rc_AA892373_at	SDCBP	syntenin	-2.37
rc_AI237592_at	EDD	progestin induced protein	-2.35
U68562mRNA#2_s_at	**HSP60**	**heat shock protein 60 (liver)**	**-2.32**
X54793_at	**HSP60**	**heat shock protein 60 (liver)**	**-1.08**
D12771_s_at	SLC25A5	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	-2.29
rc_AA866460_at	RNP24	coated vesicle membrane protein	-2.21
AF045564_g_at	NDR4	N-myc downstream regulated 4	-2.21

Signaling and transport (continued)

Identifier	*Symbol*	*Name*	*Change factor*
U69109_s_at	PTK2B	protein tyrosine kinase 2 beta	-2.12
rc_AI228247_at	GNAI3	guanine nucleotide binding protein, alpha inhibiting 3	-2.09
L12383_at	ARF4	ADP-ribosylation factor 4	-2.09
D84346_s_at	NCKAP1	NCK-associated protein 1	-2.08
rc_AA859520_at	GNAQ	heterotrimeric guanine nucleotide-binding protein alpha q subunit	-2.01
X55812complete_seq_at	OAZ-1-PS	cannabinoid receptor 1	-1.97
M93669_at	SCG2	secretogranin 2	-1.90
rc_AA799784_at	RAB6A	RAB6, member RAS oncogene family	-1.90
rc_AA900505_at	RHOB	rhoB gene	-1.84
X53363cds_s_at	CALR	calreticulin	-1.75
rc_AA891035_at	BECN1	beclin 1 (coiled-coil, myosin-like BCL2-interacting protein)	-1.64
rc_AA799893_g_at	HNRPA1	heterogeneous nuclear ribonucleoprotein A1	-1.64
rc_AA800296_at	PAPOLA	poly(A) polymerase alpha	-1.60
S55305_s_at	YWHAG	tyrosine 3-monooxgenase/tryptophan 5-monooxgenase activation protein, gamma polypeptide	-1.57
U55816_at	SLC12A5	solute carrier family 12, (potassium-chloride transporter) member 5	-1.54
rc_AI170613_at	HSPE1	heat shock 10 kDa protein 1	-1.52
D79221_at	SCFD1	vesicle transport-related	-1.51
U02096_at	FABP7	fatty acid binding protein 7	-1.45
X63744_g_at	SLC1A3	solute carrier family 1, member 3	-1.43
rc_AA957510_s_at	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	-1.31
U25281_at	CR16	SH3 domain binding protein CR16	-1.31
M24542cds_at	UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	-1.20
rc_AA893443_at	**RAP1B**	**RAP1B, member of RAS oncogene family**	**-1.19**
rc_AA944856_at	**RAP1B**	**RAP1B, member of RAS oncogene family**	**-0.74**
D50093_s_at	PRNP	prion protein	-1.17
D10926_s_at	TFPI	tissue factor pathway inhibitor	-1.14
Y15068_at	STIP1	stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein)	-1.14
X89968_at	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	-1.11
rc_AA894317_s_at	CHN1	chimerin (chimaerin) 1	-1.01
rc_AA819500_g_at	RFC4	replication factor C (activator 1) 4, 37kDa	-1.00
D13124_s_at	ATP5G2	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	-1.00
U21101_at	PDE2A	cyclic GMP stimulated phosphodiesterase	-0.99
X82021cds_at	ST13	suppression of tumorigenicity 13 (colon carcinoma) Hsp70-interacting protein	-0.95
rc_AA943387_at	CAMLG	calcium modulating ligand	-0.94
D17614_at	YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	-0.92
U90725_s_at	HDLBP	lipoprotein-binding protein	-0.92
D17445_at	**YWHAH**	**tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide**	**-0.90**
D17445_g_at	**YWHAH**	**tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide**	**-0.85**
D28557_s_at	CSDA	cold shock domain protein A	-0.90
U48246_at	NELL1	protein kinase C-binding protein NELL1	-0.84
M28647_g_at	ATP1A1	ATPase, Na+K+ transporting, alpha 1	-0.83
S55223_s_at	YWHAB	tyrosine 3-monooxgenase/tryptophan 5 monooxgenase activation protein, beta polypeptide	-0.81
rc_AI234604_s_at	HSPA8	heat shock protein 8	-0.80
S83025_s_at	NSEP1	nuclease sensitive element binding protein 1	-0.78
D30739_s_at	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	-0.76
rc_AA859954_at	VMP1	vacuole Membrane Protein 1	-0.73
rc_AI010725_at	CANX	calnexin	-0.71
L14323_at	PLCB1	phospholipase C, beta 1	-0.67
U53922_at	DNAJA1	DnaJ-like protein	-0.64
AF044581_at	STX12	syntaxin 12	-0.60
rc_AI145044_at	MAPK8IP2	glycine receptor, alpha 2 subunit	-0.59
Y16774_at	SLC30A4	Dri 27/ZnT4 protein	-0.57

Signaling and transport (continued)

Identifier	*Symbol*	*Name*	*Change factor*
J05510_at	ITPR1	inositol 1,4,5-triphosphate receptor 1	-0.56
X13933_s_at	CALM1	calmodulin 1	-0.54
rc_AI237836_g_at	GNAS	GNAS complex locus	-0.50

Unknown

Identifier	*Symbol*	*Name*	*Change factor*
U64705cds_i_at	**-**	**-**	**-9.03**
U64705cds_f_at	**-**	**-**	**-6.53**
rc_AA891049_at	-	-	-8.82
rc_AA892842_at	-	-	-4.98
rc_AA893217_at	-	-	-4.09
rc_AA891476_at	-	-	-3.76
rc_AA891734_at	-	-	-3.04
rc_AA891580_at	-	-	-2.50
rc_AI639501_s_at	-	-	-2.42
rc_AA892863_at	-	-	-2.24
rc_AA892570_at	-	-	-2.10
rc_AA800290_g_at	-	-	-1.77
rc_AI639447_at	-	-	-1.67
rc_AA799636_at	-	-	-1.63
rc_AA891872_at	-	-	-1.61
rc_AA859897_at	-	-	-1.55
rc_AA848545_at	-	-	-1.55
S63233_g_at	**-**	**-**	**-1.51**
S63233_at	**-**	**-**	**-1.15**
rc_AA875084_at	-	-	-1.40
S59893_f_at	-	-	-1.36
rc_AA799607_at	-	-	-1.35
U83880UTR#1_at	**-**	**-**	**-1.32**
U83880UTR#1_g_at	**-**	**-**	**-0.97**
rc_AA894305_at	-	-	-1.28
rc_AA891969_at	-	-	-1.26
rc_H31897_at	-	-	-1.24
AA848268_at	-	-	-1.17
rc_AA874928_g_at	-	-	-1.16
rc_AA891800_g_at	**-**	**-**	**-1.16**
rc_AA891800_at	**-**	**-**	**-0.64**
rc_AA866364_at	-	-	-1.08
rc_AA893584_at	-	-	-1.01
rc_AA892376_at	-	-	-1.00
rc_AA894090_at	-	-	-1.00
X52815cds_f_at	-	-	-0.99
rc_AA893984_at	-	-	-0.95
rc_H33629_at	-	-	-0.92
rc_AA893939_g_at	-	-	-0.91
D87991_at	-	-	-0.90
rc_AI177919_at	-	-	-0.84
rc_AA892860_at	-	-	-0.82
rc_AA874803_at	-	-	-0.78
rc_AA891171_s_at	-	-	-0.78
rc_AA893173_at	-	-	-0.75
rc_AA799641_g_at	-	-	-0.73
rc_AA859832_at	-	-	-0.73
rc_AA894345_at	-	-	-0.70
rc_AA891881_at	-	-	-0.69
rc_AA859688_at	-	-	-0.67
rc_AA892829_at	-	-	-0.64
S85184_g_at	-	-	-0.60
rc_H31456_at	-	-	-0.59
rc_AA891789_at	-	-	-0.59
rc_AA892851_g_at	-	-	-0.55
rc_AA799599_at	-	-	-0.51

C. SP related genes

Induced

Cell growth

Identifier	*Symbol*	*Name*	*Change factor*
U26541_at	PDAP1	kinase substrate HASPP28	0.87
U24489_at	Tnxa	tenascin XA	0.67
rc_AA800513_at	TRRAP	transformation/transcription domain-associated protein	0.53
AB014722_g_at	SART1	squamous cell carcinoma antigen recognized by T-cells 1	0.51

Cell morphology

Identifier	*Symbol*	*Name*	*Change factor*
X79321_at	MAPT	microtubule-associated protein tau	1.35
L16532_at	CNP	cyclic nucleotide phosphodiesterase 1	0.64
M83196_at	MAP1A	microtubule-associated protein 1 A	0.56
AB001347_s_at	SPTBN2	beta-spectrin 3	0.51

General processes

Identifier	*Symbol*	*Name*	*Change factor*
rc_AA891553_at	EIF3S7	eukaryotic translation initiation factor 3, subunit 7 zeta, 66/67kDa	0.54

Immune reponse

Identifier	*Symbol*	*Name*	*Change factor*
X61654_at	CD63	CD63 antigen	0.74
L40364_f_at	LOC360231	MHC class I RT1.O type 149 processed pseudogene	0.63
rc_AI639534_g_at	PFC	properdin P factor, complement	0.53

Metabolism

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI102838_s_at	IVD	isovaleryl coenzyme A dehydrogenase	0.74
X05341_at	ACAA2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	0.72
J03867_s_at	DIA1	diaphorase 1	0.69
X83231_at	ITIH3	pre-alpha-inhibitor, heavy chain 3	0.58
AB017188_g_at	PSMD4	proteasome (prosome, macropain) 26S subunit, non-ATPase,4	0.54
U25651_at	PFKM	phosphofructokinase, muscle	0.54
U78977_at	ATP9A	ATPase, Class II, type 9A	0.54
L17127_at	PSMB4	proteasome (prosome, macropain) subunit, beta type 4	0.54
D16478_g_at	HADHA	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A hiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	0.50

Nervous system

Identifier	*Symbol*	*Name*	*Change factor*
X97121_at	NTSR2	neurotensin receptor 2	0.62
D28111_at	**MOBP**	**myelin-associated oligodendrocytic basic protein**	**0.58**
D28111_g_at	**MOBP**	**myelin-associated oligodendrocytic basic protein**	**0.57**

Signaling and transport

Identifier	*Symbol*	*Name*	*Change factor*
AF090867_g_at	GMPR	guanosine monophosphate reductase	0.75
U15098_at	SLC1A2	solute carrier family 1, member 2	0.64
rc_AA963674_g_at	MAP2K2	mitogen activated protein kinase kinase 2	0.60
M18332_s_at	PRKCZ	protein kinase C, zeta	0.56
J05592_at	PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A	0.55
rc_AA892775_at	LYZ	lysozyme	0.51
L10326_at	GNAS	GNAS complex locus	0.50

Unknown

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI639203_at	-	-	1.04
rc_AI638949_s_at	-	-	0.70
rc_AA891940_at	-	-	0.68
rc_AA892753_s_at	-	-	0.59

D. SP related genes

Repressed

Cell growth

Identifier	*Symbol*	*Name*	*Change factor*
K00750exon#2-3_g_at	CYCS	cytochrome c, somatic	-0.81
X57986mRNA_s_at	PRKACA	protein kinase, cAMP-dependent, catalytic, alpha	-0.51

Cell morphology

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI013107_at	KIF3C	kinesin family member 3C	-0.62

General processes

Identifier	*Symbol*	*Name*	*Change factor*
AF062594_g_at	NAP111	nucleosome assembly protein 1-like 1	-0.53

Immune response

Identifier	*Symbol*	*Name*	*Change factor*
AF030358_g_at	CX3CL1	chemokine (C-X3-C motif) ligand 1	-0.72
L26268_g_at	BTG1	B-cell translocation gene 1	-0.57

Metabolism

Identifier	*Symbol*	*Name*	*Change factor*
X78593_at	GPD2	glycerol-3-phosphate dehydrogenase 2	-5.27
D10655_at	DLAT	dihydrolipoamide acetyltransferase	-1.61
U48288_at	AKAP11	A kinase (PRKA) anchor protein 11	-1.12
L03294_at	LPL	lipoprotein lipase	-0.63
rc_AA818858_s_at	PPIA	peptidylprolyl isomerase A	-0.57
rc_AA799650_g_at	PRDX3	peroxiredoxin 3	-0.57
rc_AA894282_at	SCD2	stearoyl-Coenzyme A desaturase 2	-0.50

Signaling and transport

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI235707_g_at	CANX	calnexin	-1.01
rc_AI230406_at	RAB10	ras-related protein rab10	-0.58
AA799276_at	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	-0.57
rc_AI103238_at	PPP2R2B	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta isoform	-0.54

Unknown

Identifier	*Symbol*	*Name*	*Change factor*
rc_AA893002_at	-	-	-1.03
rc_AA800222_at	-	-	-0.66
rc_AA893821_at	-	-	-0.64
rc_AI639132_s_at	-	-	-0.63

Supplementary Table 3

Genes differentially expressed in the left dorsal hippocampus

SIP related genes are genes related to spatial information processing and were differentially regulated in the SP, CN and CP rat groups, compared to the HC rats.

SP related genes are genes differentially expressed only in the SP rats, compared to the HC rats.

The genes which are in **bold characters** are represented several times on the microarray and, for most of them, are assayed by 2 or 3 different probe sets. In our case, the modulation of expression of a gene calculated for each of the probe sets was similar Also, the expression level given by the different probe sets were in a similar range. The fold change, however, could vary depending on the probe set.

The *change factor* which is reported for each gene in the following tables is the one that was calculated by the algorithm when comparing the spatial learning group (SP) to the homecage group (HC).

A. SIP related genes
Induced

General processes

Identifier	*Symbol*	*Name*	*Change factor*
D64059_at	Atrx	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S.cerevisiae)	1.27
AB012234_g_at	Nfix	nuclear factor I/X	0.83
U01146_s_at	Nr4a2	nuclear receptor subfamily 4, group A, member 2	0.79
rc_AA849648_g_at	**RPL21**	**ribosomal protein L21**	**0.78**
rc_AA849648_at	**RPL21**	**ribosomal protein L21**	**0.71**
rc_AI013472_at	Ppap2b	ER transmembrane protein Dri 42	0.65

Cell growth and proliferation

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI176461_s_at	Glg1	selectin, endothelial cell, ligand	0.60

Metabolism

Identifier	*Symbol*	*Name*	*Change factor*
M64755_at	Csad	cysteine-sulfinate decarboxylase	1.16

Nervous system

Identifier	*Symbol*	*Name*	*Change factor*
AB007690_s_at	Homer2	homer, neuronal immediate early gene, 2	1.00

Signaling and transport

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI172499_at	-	ATPase, Ca++ transporting, plasma membrane 1	1.14
M12672_at	Gnai2	GTP-binding protein (G-alpha-i2)	0.60

Unknown

Identifier	*Symbol*	*Name*	*Change factor*
X61296cds#2_f_at	-	-	1.21
X53581cds#3_f_at	-	-	0.70
M13100cds#4_f_at	**L1Rn**	**-**	**0.64**
M13100cds#3_f_at	**L1Rn**	**-**	**0.56**
rc_AI638989_at	-	-	0.63

B. SIP related genes
Repressed

no repressed genes were detected

C. SP related genes
Induced

Apoptosis

Identifier	*Symbol*	*Name*	*Change factor*
X82021cds_at	St13	suppression of tumorigenicity 13 (colon carcinoma) Hsp70-interac	0.57

Cell growth and proliferation

Identifier	*Symbol*	*Name*	*Change factor*
rc_AA899106_at	Ccnd2	cyclin D2	0.50

Cell morphology

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI072770_s_at	Plp	proteolipid protein	0.88

General processes

Identifier	*Symbol*	*Name*	*Change factor*
AF036335_g_at	SFPQ	NonO/p54nrb homolog	0.68
AF069782_at	Nap65	Nopp140 associated protein	0.58
rc_AA851749_s_at	Sfrs10	splicing factor, arginine/serine-rich (transformer 2 Drosophila homolog) 10	0.56

Immune response

Identifier	*Symbol*	*Name*	*Change factor*
D10729_s_at	Psmb8	proteosome (prosome, macropain) subunit, beta type 8	0.53

Nervous system

Identifier	*Symbol*	*Name*	*Change factor*
rc_AA892864_at	Mgll	monoglyceride lipase	0.70
Y17048_at	Cabp1	calcium binding protein 1	0.65
U35775_at	Add3	adducin 3, gamma	0.56
rc_AI145494_at	Syn2	synapsin 2	0.51

Signaling and transport

Identifier	*Symbol*	*Name*	*Change factor*
U05014_at	Eif4ebp1	eukaryotic translation initiation factor 4E binding protein 1	0.69
D32209_at	Anp32a	acidic nuclear phosphoprotein 32 family, member A	0.62
M92340_at	Il6st	interleukin 6 signal transducer	0.54
X84047cds_at	Gnas	XLas protein	0.51
D38380_g_at	Tf	Transferrin	0.51
U16655_at	Plcd4	phospholipase C, delta 4	0.51
D26180_at	Pkn1	protein kinase C-like 1	0.50

Unknown

Identifier	*Symbol*	*Name*	*Change factor*
S65091_g_at	-		0.57
U83119_f_at	-		0.51

D. SP related genes
Repressed

Unknown

Identifier	*Symbol*	*Name*	*Change factor*
rc_AI639347_at	-	-	-0.81
rc_AA893664_at	-	-	-0.69
rc_AA799964_at	-	-	-0.50

Supplementary data

Supplementary file containing important data for microarray analysis, Minimal Information About Microarray Experiment (MIAME compliant).

Experiment design

- Type of experiment: analysis of the effect of a spatial learning task on gene expression profiles in dorsal hippocampus of rats
- Experimental conditions: 5 groups of 8 male Lister Hooded rats, 2-3 months old
    - Group 1: rats trained to the water maze task (hidden platform version) and tested without platform
    - Group 2: rats trained to a visible platform and tested without platform
    - Group 3: rats trained to a visible platform and tested with platform
    - Group 4: rats submitted to a single swim session, without platform
    - Group 5: naïve rats, untrained
- Number of hybridizations: 80 (2 microarrays per rat)
- Reference for hybridization: NO
- Quality control steps: no quality control of total RNA (too small amount). Agarose quality check of cDNA after amplification and of labeled cRNA.

Samples used

- Origin of biological sample: microdissections of dorsal hippocampus of rats (region CA1). Microdissections were taken out from rat brain slices (30 μm thick) stained with 0.5% toluidine blue.
- Protocol for sample preparation and labeling:
    - Total RNA extraction: thanks to TRI-reagent (Sigma)
    - cDNA synthesis and amplification: Microarray target amplification kit (Roche Applied Sciences)
    - in vitro transcription: MEGAscript® T7 Kit (Ambion)

  The detailed protocol is described in Klur et al., 2003.
- External controls (spikes): NO

Hybridization procedures and parameters

- Standard Affymetrix procedures were used for hybridization on microarrays

Array design

- Commercial array from Affymetrix: U34A rat genome GeneChip® (www.affymetrix.com). This microarray allows the analysis of the expression level of ~7000 known genes and of ~1000 ESTs

Measurement data and specifications

- Standard Affymetrix specifications were used to detect gene expression levels with the Affymetrix Scanner 3000. Data were collected and analyses were done thanks to an in-house software. We performed the quantile normalization on the CEL files (Bolstad, 2003) and the SAM algorithm was used to compare conditions (Tusher, 2001).
- We selected genes for which p value was smaller or equal to 0.1, change factor higher or equal to 0.5 or lower or equal to -0.5 (fold change ≥ 1.5 or ≤ -1.5). Supplementary criteria were an average call of at least 0.75 and an intensity on microarray of at least 50.

2. Complementary test using another learning situation

To examine whether reversible hippocampal inactivation could affect the retrieval of memory in another spatial navigation test, lidocaine was injected into the dorsal hippocampus of rats before information retrieval in a 5-arm maze test (according to Bontempi et al., 1999). Rats that were tested in this situation were those that were previously trained and tested in the water maze.

2.1. Experimental protocol

The device consisted of a modified version of the 12-arm radial maze and allowed testing of reference memory (Figure 23). Only 5 arms of the maze were open and one of them was baited with chocolate chips, which were not visible from the center of the maze. A position of baited arm was arbitrary assigned to each rat. Rats always entered the maze by the arm facing the 5 open arms and could orientate themselves on the basis of different fixed objects (rat cages, forms hanging on the walls, etc.) disposed in the room.

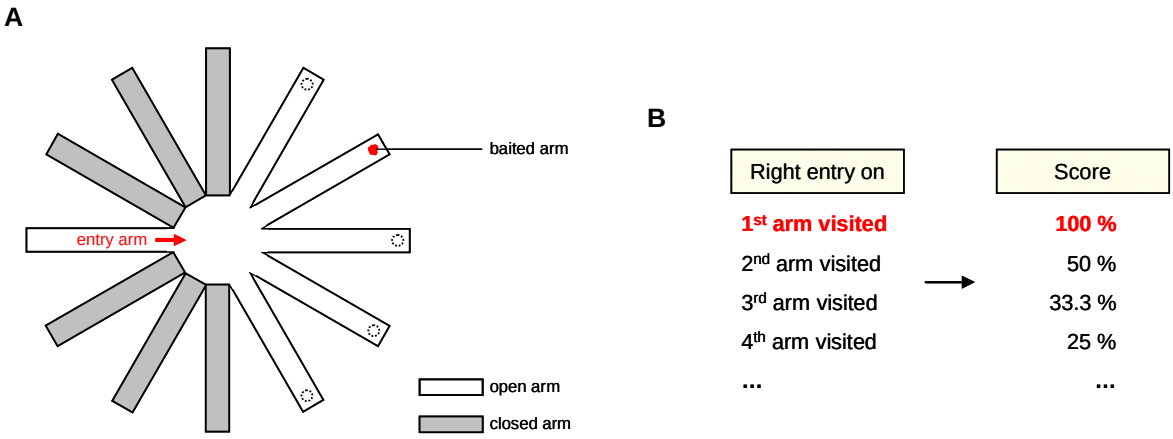

Figure 23. The modified 12-arm maze. **A.** Experimental device. **B.** Scoring chart.

Rats received a daily session of 4 consecutive trials and were trained as long as necessary so that they were able to visit first the baited arm over 2-3 consecutive days. On each trial, rats were scored as indicated on Figure 23.

Retention of the position of the escape platform was tested one day after the last acquisition trial. Five minutes before probe trial, lidocaine (1 µl, 10 µg/µl) was infused unilaterally into the left or right dorsal hippocampus of rats, and PBS was infused in the contralateral side. As a control, rats received PBS bilateral infusions (PBS).

2.2. Results

All rats presented a similar acquisition curve and were able to reach stable performance after 16 to 19 training days (Figure 24). Some rats could only reach a maximal performance of 50% over 2-3 consecutive days, which was then considered as stable. For such rats, the baited arm was always the second arm visited.

Therefore rats were submitted to the probe trial depending on their individual performance and were tested on day 17, 18, 19 or 20. Rats were equivalently distributed among the 3 infusion groups (PBS, Left and Right). By comparing the performances of the rats on probe trial and on the day before, it was not possible to show an effect of the lidocaine microinjection (Figure 23). As the rats were trained over 15 days, it might be possible that spatial information has been consolidated elsewhere, and more particularly in the prefrontal cortex (Maviel et al., 2004; Hok et al., 2005).

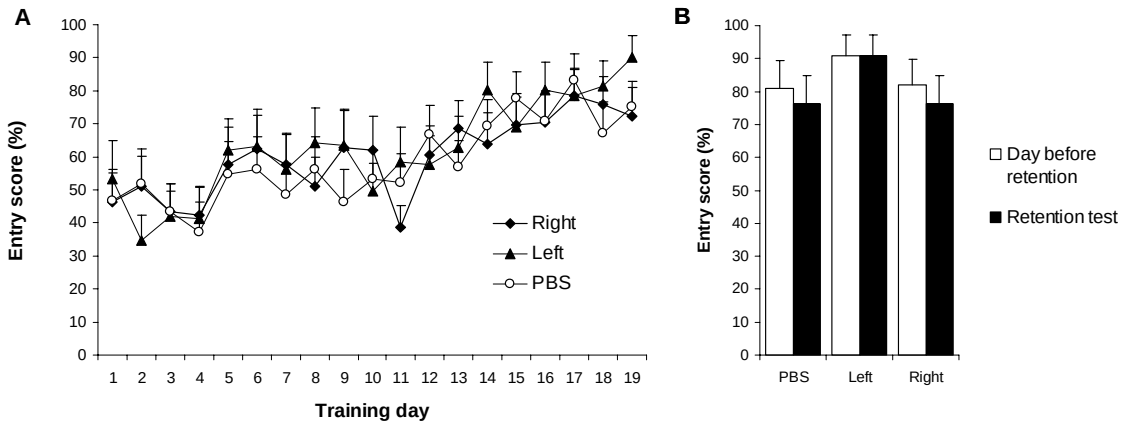

Figure 24. Performance of rats in the 5-arm maze. **A.** Acquisition. Entry score during acquisition of the task (mean + sem). **B.** Retention. Entry score on retention test (mean + sem).

GENERAL DISCUSSION

This thesis work aimed at applying molecular genomic technologies and functional reversible inactivation to the study of spatial learning and memory in the rat. The investigations presented here included the development and testing of the technologies and their subsequent use for the analysis of rat spatial navigation. The main findings are summarized below:

- Microdissection allowed for the precise excision of small brain areas, and it was possible to extract enough RNA for subsequent gene expression analysis. Two protocols for the preparation of samples for analysis on microarrays were compared: one involved random PCR amplification and the other relied on linear amplification. Both protocols allowed for the production of good quality data, with a small advantage for random PCR amplification. The latter technique was applied to the study of spatial learning and memory.

- By comparing gene expression profiles of rats doing a spatial navigation task in the water maze to expression profiles of several types of control rats, it was possible to show that transcriptional activity is increased in right dorsal hippocampus of rats trained in the water maze to a much larger extent than in the left hippocampus. Indeed, 623 genes were significantly induced or repressed in the right hippocampus, whereas only 74 were differentially regulated in the left dorsal hippocampus (compared to untrained rats). These results suggest that the right dorsal hippocampus has a more important role than the left dorsal hippocampus in spatial memory processes such as those required to perform a Morris water maze task.

- In order to evidence functional lateralization of the management of spatial information, we performed a behavioral study associated with reversible functional inactivation of hippocampus by administration of an anesthetic compound. Two widely used sodium channel blockers were tested in a prior experiment. The results showed that the activity of tetrodotoxin spread widely around the microinjection site, and therefore altered the sensory-motor capabilities of the rats. Contrary to tetrodotoxin, the anesthetic effects of lidocaine remained localized to the microinjection site and thus sensory-motor capabilities of the rats were unaltered. Lidocaine was therefore used to perform uni- or bilateral inactivation of the dorsal hippocampus of rats performing a spatial navigation task in the water maze.

- When lidocaine was injected during acquisition of the task (before each trial), rats almost performed normally in the probe trial (no prior infusion), except for those that were injected on the left hippocampus, which showed an impairment (random behavior on probe trial). On the opposite, when rats were infused in the right hippocampus (or bilaterally) before the probe trial, recalling the position of the hidden platform was impaired.

- An infusion of lidocaine, whatever the side, before retrieval of a 5-arm maze task had no influence on the spatial navigation of the rats. One possible explanation, though it is improbable, is that a structure different from the hippocampus could be involved in the resolution of this particular task. Another more probable account would be that the trace has been stored elsewhere because of a longer acquisition period (e.g. in the prefrontal cortex).

- There is no relationship between left- and right-"pawedness" and lateralization of spatial navigation capabilities to one particular brain hemisphere.

In conclusion, the results tend to show that the right hippocampus could have a more important role for the management of spatial information in the rat and particularly for retrieval, whereas the left hippocampus could be more engaged during encoding operations and information transfer.

1. Gene expression profiling of small-size samples

Studying small sample preparations is now possible thanks to specific tools enabling the dissection of small tissue pieces including a small number of cells and the assessment of the expression profile of minute amounts of mRNA. These methods allow the purification of cells from complex cellular samples like brain tissue, tumors, organisms (or tissue) in development, or immune cells. Our focus was the brain and it has been a big challenge to obtain an expression profile from this tissue since variations in gene expression were reported to be quite subtle there (Mirnics et al., 2001). Actually changes in gene expression in brain are usually less than two-fold between the experimental and control samples. Therefore, careful experimental design and

precise tissue selection can lead to successful gene expression profiling in brain, as shown in the present work.

Micromanipulations, fluorescence-activated cell sorting (FACS) and laser microdissection (Bohm et al., 1997; Emmert-Buck et al., 1996; Simone et al., 1998) are the classical techniques used to isolate small numbers of purified cells. Micromanipulation can be tedious and is not adapted to rapid isolation of small samples or individual cells. FACS is appropriate to some goals (such as sorting different cell types from a mixture, study of cell division cycle, etc.) and works efficiently, however it requires cell suspensions. Thus laser microdissection is the most adapted method for isolation of minute fragments from a tissue sample and was perfectly suited for this thesis project aimed at studying a specific region of the rat dorsal hippocampus. In addition, this latter technique combined to microarray already lead to the production of microarrays data (Luo et al., 1999; Scheidl et al., 2002; Walch et al., 2001).

Our focus was the analysis of gene expression and therefore the RNA population had to be extracted from the hippocampal microdissected samples. As RNA is unstable, it is very important to use a method allowing the efficient recovery of these samples without degradation due to RNases. Thus, several RNA extraction protocols were tested and the most efficient of them was selected. Once the RNA was isolated, one had to process it for analysis on microarrays. As standard protocols for sample preparation were inefficient with total RNA amounts smaller than 5 µg, it was evident that an amplification step was necessary for analyzing minute amounts of total RNA extracted from microdissections. There are two different methods currently used for sample amplification: linear amplification or PCR. Both were tested in this work and it was shown that the two protocols gave good quality microarray data, with a small advantage for random PCR amplification. Moreover, the results were biologically relevant and showed that amplification can be used for gene expression analysis because no major distortion was introduced into the sample, thus allowing a reliable study of gene expression. Surprisingly, PCR amplification, often described as introducing bias (Lockhart and Winzeler, 2000; Eberwine et al., 1992), gave results comparable to those of other methods: when performing a relatively low number of PCR cycles (around 25), almost no bias was introduced. Moreover, whatever bias may be introduced into a sample, it was compensated for since all samples were prepared with the same protocol and were therefore subjected to the same bias. The relative expression level between samples is preserved, even though the absolute expression level may be slightly altered.

In conclusion, several important issues have to be taken into account when designing a study using microarrays. No experimental technique is perfect and one has to find the best compromise of reliability, quality and ease of use (especially because we wanted to use it on a

relatively large sample of rats). In our preliminary study, it was established that the protocol using PCR amplification worked slightly better than the one involving linear amplification, therefore the first one was used for gene expression profiling in a subregion of the rat dorsal hippocampus.

2. Reversible inactivation relying on infusion of lidocaine and tetrodotoxin

Inactivation of a brain structure can be achieved by local infusion of drugs. In this thesis, reversible hippocampal inactivation has been induced by infusion of tetrodotoxin or lidocaine, two compounds that block sodium channels, and thus prevent action potential initiation and propagation. However, to our knowledge, the effects of tetrodotoxin and lidocaine on both cerebral metabolic activity and motor coordination have not been studied in detail before we started this work. Therefore the effects of their infusion in the hippocampus on glucose uptake and on a beam-walking test have been assessed prior to the study of spatial learning and memory in the rat.

The rates of glucose utilization predominantly reflect nerve terminal activity (Nudo and Masterton, 1986; Sharp et al., 1988; Kurumaji et al., 1993). Therefore, the 2-deoxyglucose (2-DG) technique is an excellent tool for assessing cerebral functional activity, as it has been shown that the final glucose utilization rates mainly reflect the cerebral metabolism occurring during the first 15-20 min after the radioactive tracer injection (Sokoloff et al., 1977). This method has already been applied to the hippocampal formation of rats (Sokoloff, 1981; Wree et al., 1988) So, in order to assess and compare the levels of functional inactivation achieved by intrahippocampal infusions of tetrodotoxin and lidocaine, the 2-DG technique was used (for limits of our semi-quantitative method, see the discussion in our corresponding manuscript).

Actually, inactivation of a brain region might affect several processes participating in the functioning of an organism. In particular, the motor processes constitute an essential basis of behavior, and their disruption may affect the formation and expression of memory, or may bias evaluation of memory performance for which animals have to produce a motor response (e.g., press a lever, enter an arm of a radial maze, or swim to a hidden platform). Therefore, the beam-walking test was used to verify that the sensory-motor capabilities remained unaffected by reversible inactivation of the dorsal hippocampus.

The maximal inactivation through lidocaine is achieved between 10-20 min post-infusion, before a gradual recovery occurs (Martin, 1991; Fenton and Bures, 1994; Tehovnik and Sommer, 1997; Malpeli 1999). Therefore, the 2DG method used for the quantification of cerebral

metabolism covered completely the inactivation period of the lidocaine when the radioactive tracer was injected 5 min after the drug. However, when 2-DG was injected 30 min after Lido infusion, metabolic data only partially reflect the degree of inactivation due to quite short-lasting effects of this drug. The metabolic data demonstrate that lidocaine, at the dose of 10 µg, induced a 15-20% functional inhibition localized around the infusion site, and with only limited effects outside of the dorsal hippocampus. This limited metabolic decrease confirms the relatively small diffusion of the drug from the infusion site, which was shown to be less than 1.5 mm for doses between 10 and 40 µg in a volume of 1µl, in both cortical and subcortical tissue (Sandkuhler et al., 1987; Martin, 1991; Malpeli 1999; Boehnke and Rasmusson, 2001; Daumas et al., 2005). Furthermore, the magnitude of functional inactivation observed in the present work (about 20%) is comparable to that attained with a selective antagonist of AMPA/kainate glutamate receptor for hippocampal inactivation (Riedel et al., 1999) or after suppression of synaptic transmission by pentobarbital (Crane et al., 1978), and appears sufficient to induce impairment of both the encoding and retrieval of spatial memory, as well as trace consolidation or long term storage (Riedel et al., 1999). Moreover, several data have shown that inactivation of a small area of the dorsal hippocampus yields significant effects on learning in various tasks assessing spatial or fear memory (Maviel et al., 2004; Moser et al., 1995; Lee and Kesner, 2003; Hammond et al., 2004).

The full blockade after tetrodotoxin infusion is reached after 10 min and lasts approximately 90 min with, subsequently, an exponential decrease reaching control levels within 24 h (Zhuravin and Bures, 1991). It is noteworthy, nevertheless, that this kinetic was described after infusions of TTX into the Edinger-Westphal nucleus, which is located in the brain stem. Therefore, tetrodotoxin metabolic effects were more pronounced when the delay between drug infusion and radioactive tracer injection was of 30 min. Compared to lidocaine, tetrodotoxin showed more widespread reductions of cerebral functional activity, not only localized around the infusion site, but also spreading to other ipsilateral and even contralateral brain areas. All observations in tetrodotoxin rats revealed extended functional inactivation far beyond the usually recognized spatial limits of the drug diffusion, which translated into metabolic decreases far away from the infusion site, particularly affecting the whole hippocampal region from its anterior (infusion site) to its posterior part, and up to the ventral hippocampus, especially at the 30 min delay.

As shown by the beam-walking test, lidocaine has no motor consequences at 5 or 30 min post-infusion. These data are in accordance with the very limited effects of lidocaine on cerebral metabolism, which were mostly restricted to the infusion site. On the opposite, and this was also unexpected, tetrodotoxin infusions into rat dorsal hippocampus had major consequences on

sensory-motor coordination. Actually, and to the best of our knowledge, no study using intrahippocampal infusions of tetrodotoxin did mention this type of effect (Fenton and Bures, 1993; Klement et al., 2005; Wesierska et al., 2005). However, there are several reasons why tetrodotoxin infusions might affect sensory-motor capabilities of the rats. Actually, the hippocampus has been associated with motor processes involving control of motor responses to sensory stimuli, and especially locomotor activity (Bast and Feldon, 2003, Bast et al., 2001). Therefore, the metabolic data showing a decrease in functional activity within the whole hippocampus might also reflect the alterations of performances recorded in the beam-walking test. In addition, the more severe motor deficits observed after 10 ng of tetrodotoxin as compared to 5 ng are in accordance with a greater reduction in hippocampal metabolic activity at this high dose. The motor alterations observed in tetrodotoxin-infused rats might also be linked to the metabolic changes observed in the thalamus and other associated structures. Finally, it cannot be excluded that the decrease in cerebral glucose metabolism observed in parietal and visual cortices also played some role in the alterations of motor coordination after tetrodotoxin infusion, and this might be resulting from drug diffusion along the guide cannula track (but not only, as other extrahippocampal regions close to the infusion site, e.g. dorsal thalamus, also exhibited weaker activity, and these were not in contact with the cannula track). In addition, it has been recently shown that lesions of the associative parietal cortex modify activity of hippocampal place cells, therefore pointing to the fact that hippocampus and parietal cortex are functionally related (Parron and Save, 2004). More precisely, the associative parietal cortex may be involved in the processing of spatial information based on the use of proximal landmarks.

Our metabolic data support the severe motor deficits observed in tetrodotoxin treated rats that showed large metabolic decreases, not only within the hippocampus, but also in some thalamic and cortical areas. These behavioral effects are particularly pronounced at 30 min post-infusion. On the contrary, Lidocaine, which showed metabolic effects restricted to a limited area around the infusion site, did not affect motor coordination. Therefore lidocaine was used for the behavioral experiments with an infusion-to-test delay of about 5 min.

3. Lateralization of spatial navigation in rat hippocampus

The role of the dorsal hippocampus in spatial memory is well established (O'Keefe and Nadel, 1978; Olton et al., 1978). Approaches relying on lesion or reversible inactivation approaches, or on activity analysis of place cells in this region of the brain showed that, in the rat, the dorsal hippocampus is an essential center for spatial memory (encoding, consolidation and at least short-term retrieval). However, it has not been established yet that, in the rat, one hippocampus might be preferentially used for resolving spatial navigation tasks. The model used in this work was the Morris water maze task, which is an established model for studying spatial learning and memory in rodents (Morris, 1984; D'Hooge and De Deyn, 2001). In this thesis, a novel approach was used, which is the combination of global transcription analysis in the dorsal hippocampus of rats while they were performing a spatial navigation task. This study was completed by a more precise characterization of the learning and memory stages that could be, to some extent, lateralized.

Several studies using microarrays have already been conducted to explore changes in mRNA expression related to spatial navigation (Luo et al., 2001; Leil et al., 2003; Cavallaro et al, 2002). However, these studies were centered on the whole hippocampus. Therefore an expression profile of the entire hippocampus might not only reflect transcriptional activity related to spatial memory since hippocampus is constituted of thousands of cells of different types (Kamme 2003) and also supports other memory processes (Eichenbaum 1999). In addition, there are functional differences between the dorsal part (which is more involved in spatial aspects of information treatment) and the ventral part (which is more involved in the treatment of emotional aspects) of the hippocampus. Consequently, the focus of this thesis was on CA1 region of the dorsal hippocampus, in regard to studies of place cells activity that previously showed that this brain region is implicated in the management of spatial information (Rotenberg 1996, Lenck-Santini 2002, Lenck-Santini 2005). Thanks to careful tissue selection by laser capture microdissection, which targeted exactly the same region as the one in which Poucet and his colleagues had recorded place cells (Lenck-Santini et al., 2005), it was possible to detect different expression patterns in the right and left hippocampus; such patterns could not be detected previously in the whole hippocampus.

A set of 211 induced and 274 repressed genes common to the spatial learning rats and maze control groups could be identified in the right dorsal hippocampus, compared to untrained rats. This set of genes could be related to the role of the hippocampus for navigation in the environment. The hypothesis is that the hippocampus might be used spontaneously when an animal is evolving in a particular place, independently from the need to use spatial cues to orient

a motivated navigation (such as finding an escape platform). The role of the hippocampus for spatial navigation can easily be related to the concept of the cognitive map, which, among other indications, refers to the capacity of specific neurons – place cells – to fire only when the rat is in a restricted area of the environment (O'Keefe and Nadel 1978). Therefore, rats evolving in the water maze could build a cognitive map of their environment, even if they do not have to orientate themselves to escape from aversive surroundings. Actually, rats that are exposed repeatedly to the same environment over several consecutive days might record the organization of the room by building a cognitive map, and this map construction could be comparable regardless of whether they have to swim to a visible or a hidden platform. Indeed, from previous studies on place cells, it is known that it is not necessary that a particular behavior in a given environment be motivated (reinforced) for place-cell activity to be detected (Poucet et al., 2004). The absence of a relationship between place-cell activity and motivation clearly indicates that spatial coding may operate independently from the aim of a reinforced task. This could explain why very similar transcriptional events occurred in the dorsal hippocampus of rats trained to remember the hidden or the visible platform.

The data resulting from reversible inactivation of the hippocampus with lidocaine infusions show that the right and the left hippocampus could participate in different ways in the processing of spatial information. Indeed, performance accounting for retention of the position of a hidden platform in a water maze task was weakened when lidocaine was infused into the right dorsal hippocampus, or bilaterally, just before a delayed probe trial. When infused during acquisition, lidocaine did not prevent performance improvement over days, whether infused on the left or the right side. When tested without prior drug infusions after a 24-hour delay, however, rats in which the left hippocampus had been inactivated during acquisition were impaired, not those in which the right side was. Therefore right and left hippocampus might play different roles in the processes involved in spatial learning assessed in the Morris water maze. Although the respective testing protocols were not exactly the same as in the microarray approach, these observations can be linked to the results of our gene expression profile analysis showing a larger differential transcriptional activity in the right hippocampus when the rats are performing a spatial learning at the end of an acquisition period.

The data also demonstrate that despite reversible inactivation restricted to the right or left dorsal hippocampus, acquisition of spatial information in a water maze task was normal, which partly corroborates that hippocampal blockade slowed down but did not prevent acquisition in normally trained rats, and had no effects in overtrained ones (Czéh et al., 1998; Fenton and Bures, 1993). Thus, Fenton and Bures (1993) concluded that there was no left-right hemispheric specialization in acquisition. The authors used TTX, however, which might have had more

widespread effects than lidocaine as shown in our comparison of anesthetic compounds for functional reversible inactivation.

Based on the present data we have built a speculative model in which the right and the left hippocampus play different and perhaps complementary roles in the processes involved in the establishment of a spatial representation and/or of its efficient use in a water maze (Figure 25). As stated in the introduction of this work, memory is a process that involves discrete stages including encoding, consolidation, storage and retrieval. Our results suggest that, when rats have to build a spatial memory trace, the processes resulting in encoding and information transfer (i.e. during encoding) may require a more important participation of the left hippocampus. In contrast, the rats might preferentially require the use of the right hippocampus for memory consolidation and perhaps even more recall after several days of acquisition. However, participation of the left hippocampus or of other structures (e.g. prefrontal cortex) cannot be excluded at this stage of our investigations. Our model implies that a time-dependent information transfer between both hippocampi is possible (with the left one being more involved in encoding and the right one in retrieval), and this transfer possibility has been demonstrated earlier in rats (Fenton and Bures, 1994), though not on the basis of a lateralized distribution of operations.

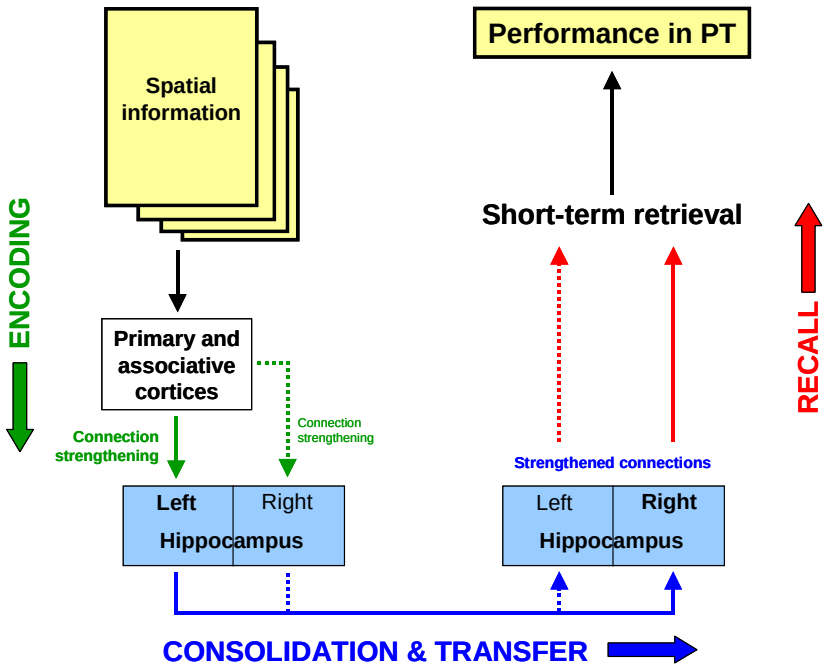

Figure 25 Theoretical model showing the putative roles of the left and the right hippocampi in the management of spatial information. Spatial information is first encoded in primary and associative cortical areas. Then, it is integrated in the hippocampus. Repeated testing could be accompanied by strengthening of intrahippocampal connections and maybe extrinsic connection with cortical areas. This process may finally result in the establishment of a memory. Then, the right hippocampus might take the relay to process spatial memory trace leading to retrieval.

The deficit in the probe trial observed in rats having the left hippocampus inactivated during acquisition and both active hippocampi during retrieval may then be interpreted as reflecting an insufficient connection strengthening to get a strong enough trace or an inability to operate the transfer of information. The gene expression data showing increased transcriptional activity in the right hippocampus, as well as the fact that following drug-free acquisition, the inactivation of the right hippocampus impaired performance during the probe trial both converge to the possibility that the right hippocampus could be the anatomical region where the (relatively recent) spatial memory is preferentially stored. However, it was noticed that rats with right or bilateral inactivation before the probe trial showed weaker performances but were not completely impaired, nor were significantly different from each other. This could be explained by the fact that other structures may have participated in the memory retrieval process. The participation of the left hippocampus cannot be completely excluded, however such a possibility would not be compatible with the finding that rats with bilateral inactivation were not more impaired than those subjected to inactivation of only one hippocampus. An alternative possibility would be that memory retrieval was already partly depending upon reorganizations within hippocampo-cortical networks, perhaps particularly of prefrontal cortical connections (Frankland and Bontempi, 2005), and that, to some extent, these reorganizations were able to compensate for the effects of right or bilateral hippocampal blockade.

Additionally, rats were tested in another spatial learning test (12-arm maze with only 5 arms used as potential targets), in which they had to learn the position of an arm baited with food pellets. Infusion of lidocaine in the left or right hippocampus before the probe trial did not affect retrieval. One important difference with the water maze task, in addition to the type of motivation (positive vs. negative), was the duration of training. Actually, in the water maze, rats were trained over a 6-day period to learn the position of a platform hidden under the surface of the water, whereas they were trained for a period of at least 16 to a maximum of 19 days in the modified 12-arm maze. At a 6-day post-acquisition delay, the retrieval process was still dependent upon hippocampal functions, as was demonstrated in recent experiments relying upon hippocampal vs. prefrontal inactivation or upon early gene expression (Frankland and Bontempi, 2005; Maviel et al., 2004), whereas at longer delays, as in our arm-maze test, retrieval processes seem to depend much more upon other brain structures, in particular the prefrontal cortex and the anterior cingulated cortex (Maviel et al., 2004). Therefore, it is possible that due to the longer acquisition period, inactivation of the hippocampus in our arm-maze study had no impact on retrieval of spatial information and thus this result neither invalidates nor supports our model.

Our model can be reexamined in comparison with the current knowledge about spatial memory, especially in human and avian. In humans for example, several neuroimaging studies

support the idea that the right and the left hippocampus play different though complementary roles in memory processes. Studies of declarative memory have shown that functional lateralization seems to depend on the type of information to be processed, stage of processing, meaningfulness and mnemonic strategies. Thus, at least for some particular material that might comprise spatial information, encoding preferentially activates the left hemisphere whereas the right is used in retrieval (Tulving et al., 1994; Nyberg et al., 1996; Schacter et al., 1995). Several studies that focused on spatial memory showed that the right hippocampus is predominantly used for spatial orientation (Burgess 2002; Maguire et al., 2000; Maguire et al., 1998; Maguire et al., 1997; Riedel et al., 1999). In addition, it has been shown recently that left and right hippocampus of pigeons play different though complementary roles in spatial orientation (Kahn and Bingman, 2004; Bingman et al., 2003; Gagliardo et al., 2005), the right hippocampus being involved in the management of global and distally located spatial information and the left hippocampus being mainly dealing with local cues. Once more, it is evident that left and right hippocampus play different but complementary roles in the formation of spatial memories. Finally, our data support for the first time a possible specialization of the right and the left hippocampus in processing particular stages of the construction of a spatial memory. Such a lateralization has also been observed in other brain structures and more particularly in the amygdala (Baas et al., 2004). Authors showed that the left amygdala is more often activated for emotion processing in humans. This is also the case in the rat, where is has been demonstrated that the right amygdala has a greater involvement than the left one in fear conditioning tests (Baker and Kim, 2004).

In summary, previous efforts using gene expression profiling made to understand how spatial information is managed in the hippocampus failed to show lateralization of spatial learning/memory in the rats principally because these studies focused on the whole hippocampus and not on a particular subregion. In addition, the question addressed was not the possible lateralization of hippocampus in most cases. Therefore, it is really important to carefully select the tissue to be analysed. Similarly, previous studies using intrahippocampal infusions of drugs to analyze the role of right or left hippocampus in spatial learning /memory also failed to show lateralization. In one study (Fenton and Bures, 1993), authors used TTX (which may be contestable, at least to some extent, in regard to our observations showing the negative effects of TTX on general behaviour). In another study, more recently, only the right hippocampus was studied (Cimadevilla et al., 2005), so no hypothesis about differential roles of right and left hippocampi could be proposed. An important issue is also the duration of test. If testing is maintained too long, it might be possible that the information is overtaken by another region (i.e. the prefrontal cortex, as reviewed by Frankland and Bontempi in 2005). Finally, lateralization of

hippocampal function could not be evidenced in lesion studies (de Hoz et al., 2005). In that case, plasticity of neuronal cells could have compensated for the altered regions, thus the right hippocampus might have overtaken the role of left hippocampus (and vice versa). In this study it was shown that learning was slower, however not completely abrogated, and at the end no conclusion about lateralization could be made. In our study, we both combined gene expression analysis of a specific hippocampal region and use of reversible inactivation with lidocaine (a compound having more neuroanatomically circumscribed effects than TTX) in a mid-term spatial learning task (therefore hippocampal dependent).

In conclusion our findings suggest that left hippocampus might have a preferential role in the formation (encoding) and transfer to the right hippocampus of a spatial memory trace. In contrast, the right hippocampus could be more particularly implicated in either retrieving spatial information or in operations involving the processing of the memory trace. These results do not mean that hippocampus-dependent spatial learning is completely lateralized, but rather that the different stages leading to establishment of a spatial memory are more supported by the left hemisphere at the beginning (encoding and transfer) and more by the right hippocampus during later processes (storage and retrieval). An important concept emerges from our work in rats, which might also apply to other studies in rats, birds and humans: lateralization is not absolute, but may rather exist as being exhibited in a pattern of asymmetric activations, with one side, but not necessarily exactly the same along the whole process of information treatment, being more active than the contralateral one, and this may be true at different stages of memory formation or recall operations.

CONCLUSION & PERSPECTIVES

This thesis project consisted in the study of spatial navigation in the rat, by using an approach combining high-throughput molecular biology technologies and a behavioral approach using reversible functional inactivation of the dorsal hippocampus. The role of the regulation of mRNA expression in spatial memory could be characterized and these investigations led to new insights into the functioning of the rodent brain. In particular, they point toward a possible lateralization of some hippocampal functions required for spatial memory formation.

The investigations carried out in this thesis work allowed to establish new methods for the analysis of gene expression in specific areas of the brain. Actually the combined use of laser microdissection and DNA microarrays enabled the precise characterization of transcriptional events occurring in the dorsal hippocampus of rats doing a spatial learning task in the water maze as compared to home cage or swim with no goal controls. Even though it is well established that dorsal hippocampus plays a prominent role in spatial navigation, the precise transcriptional profile related to spatial learning and memory was not published. Actually, several systems allow for accurate processing of spatial information. These systems function in parallel and are complementary. However, the role of transcription remained unclear. The investigations presented here showed, for the first time, that transcriptional activity is increased in the right compared to the left dorsal hippocampus of rats that are submitted to a water maze task in which they have to encode spatial information. The significance of our results is reinforced by the fact that genes presenting a differential expression in the right hippocampus are mainly linked to morphology, growth and cellular proliferation as well as signaling, i.e., processes largely described as being cerebral markers affected by or necessary for spatial memory (Mizuno and Giese, 2005; Yamada and Nabeshima, 2004). This preferential use of the right hippocampus for the management of spatial information could not be demonstrated before.

The unexpected discovery of lateralization of the rodent brain for spatial navigation could be confirmed by using reversible functional inactivation of dorsal hippocampus of rats. Two sodium channel blockers, lidocaine and tetrodotoxin, were used to perform reversible inactivation. However, the use of tetrodotoxin was given up, because it generated negative effects on sensory-motor coordination of the rats and widespread effects, in fact far beyond the infusion site, on glucose metabolism. To the best of our knowledge, these negative effects were not documented before this study in the literature. The infusion of lidocaine into the dorsal

hippocampus of rats could strengthen the important role of the right dorsal hippocampus for recalling spatial information and provides some evidence for a preferential role of the left hippocampus in encoding and transferring spatial infromation. By testing rats in another spatial learning test, namely a modified version of the 5-arm maze used by Bontempi et al. (1999), it was possible to show that retrieval performance was not altered by unilateral hippocampal inactivation, regardless of the side in which Lido was infused. This could be explained by the fact that, due to a longer training period, spatial information may have been consolidated in another brain region, the prefrontal cortex, as shown previously (Bontempi et al., 1999; Maviel et al., 2004).

In perspective, this thesis work could be completed by the analysis of transcriptional profiles of the dorsal hippocampus of rats during a spatial task at different time points. Actually, the present study focused on expression profiles immediately after the probe trial, thus reflecting more or less the storage of spatial information since the delay was too short to assess the transcriptional modifications due to recall. Therefore, it might be interesting to analyze global mRNA expression at later times, for example at 1, 3 or 6 hours after the probe trial. One could also investigate earlier transcriptional changes, for example during the first days of training.

Further experimentations using the reversible inactivation strategy could also be performed. First it could be interesting to characterize more precisely the time course of the inactivation with lidocaine. How long does the inactivation last? How long does it take until the tissue has totally recovered from anesthesia? Higher doses of lidocaine that would perhaps increase the inhibition level could also be tested. Thus, with perhaps further adaptations of our inactivation tool (e.g., by using higher doses of Lido), one could tax more precisely the involvement of one or the other hippocampus in encoding, consolidation, or short- vs. long-term recall of spatial information. It could be interesting also to study the transfer of spatial information from the hippocampus (short- or mid-term storage) to the prefrontal cortex (long-term storage), and to check whether this information transfer also follows some lateralization.

By using the tools developed or adapted in this thesis, numerous experiments can be designed to investigate how memory, and perhaps more precisely spatial memory, is operating in more detail, especially as concerns the temporal and neuroanatomical organization of the underlying processes. Several aspects of spatial navigation and brain function remain still mysterious and fascinating. This thesis is a contribution to the effort of understanding the brain, and brings new or at least complementary insights into the role of the rat dorsal hippocampus in spatial navigation.

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