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Hippocampal-Dependent Spatial Memory Functions Might be Lateralized in Rats: An Approach Combining Gene Expression Profiling and Reversible Inactivation

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ABSTRACT: The hippocampus is involved in spatial memory processes, as established in a variety of species such as birds and mammals including humans. In humans, some hippocampal-dependent memory functions may be lateralized, the right hippocampus being predominantly involved in spatial navigation. In rodents, the question of possible lateralization remains open. Therefore, we first microdissected the CA1 subregion of the left and right dorsal hippocampi for analysis of mRNA expression using microarrays in rats having learnt a reference memory task in the Morris water-maze. Relative to untrained controls, 623 genes were differentially expressed in the right hippocampus, against only 74 in the left hippocampus, in the rats that had learnt the hidden platform location. Thus, in the right hippocampus, 299 genes were induced, 324 were repressed, and about half of them participate in signaling and transport, metabolism, and nervous system functions. In addition, most differentially expressed genes associated with spatial learning have been previously related to synaptic plasticity and memory. We then subjected rats to unilateral (left or right) or bilateral reversible functional inactivations in the dorsal hippocampus; lidocaine was infused either before each acquisition session or before retrieval of a reference spatial memory in the Morris water maze. We found that after drug-free acquisition, right or bilateral lidocaine inactivation (vs. left, or bilateral phosphate buffered saline (PBS) infusions) of the dorsal hippocampus just before a delayed (24 h) probe trial impaired performance. Conversely, left or bilateral hippocampus inactivation (vs. right, or bilateral PBS infusions) before each acquisition session weakened performance during a delayed, drug-free probe trial. Our data confirm a functional association between transcriptional activity within the dorsal hippocampus and spatial memory in the rat. Further, they suggest that there could be a leftward bias of hippocampal functions in engram formation or information transfer, and a rightward bias in spatial memory storage/retrieval processes. © 2009 Wiley-Liss, Inc.

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KEY WORDS: DNA microarrays; hippocampus; lateralization; lidocaine; Morris water maze

INTRODUCTION

The hippocampal complex (hippocampus and related temporal lobe regions) plays a crucial role in episodic, context-dependent and spatial memory functions (Eichenbaum, 2000; Burgess et al., 2002; Eichenbaum, 2004; Moscovitch et al., 2005). Spatial learning refers to the construction of a representation of the topographical layout of an environment, and enables goal-directed navigation on the basis of a cognitive map (O'Keefe and Nadel, 1978). Implication of the hippocampus in such a function is supported by tangible arguments from lesion, reversible inactivation, early gene expression and electrophysiological studies (rev Frankland and Bontempi, 2005; Martin and Clark, 2007). In rodents, recordings from single neurons or assemblies of neurons within the hippocampal complex identified spatially selective firing patterns showing that navigation is based on positional and directional information processing within a distributed multi-structural network (Jeffery and Hayman, 2004; O'Keefe and Burgess, 2005). In humans, spatial memory appears to be functionally lateralized, the right hippocampus being predominantly involved in navigation–related processes (rev Burgess et al., 2002). Using a functional neuroimaging technique, Maguire et al. (1997, 1998, 2000) showed strong right hippocampal activation in both "normal" subjects and taxi drivers, associated with knowing place locations and navigating from one to another. Moreover, patients with right temporal lobectomy exhibit severe deficits in spatial navigation tasks (Nunn et al., 1998, 1999). Finally, hippocampal asymmetry also seems to depend on remoteness of memory (Maguire and Frith, 2003). Roles of the left and right hippocampi were also shown to be different in avian species (homing pigeons), the right hippocampus being more involved in the global representation of goal locations within geometric frameworks, and the left one being more sensitive to local cues (Kahn and Bingman, 2004). In

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most rodent studies, the left and right hippocampi are considered as two equivalent and functionally equipotent, sometimes synergistic, components of a large system serving memory functions. The question of left-right hippocampal specialization in the resolution of spatial tasks has thus seldom been addressed in the rat (Fenton and Bures, 1993; Czeh et al., 1998) and is still open. Herein, using gene expression analyses, functional inactivation techniques and water-maze testing, we examined, in the rat, if the contribution of the left and right hippocampus to spatial memory was strictly equivalent. Using laser microdissection and oligonucleotide microarrays to monitor learning-associated gene expression (Lockhart and Barlow, 2001), we found that spatial learning assessed in a water-maze task induced a differential modulation of the activity of 623 genes in the CA1 subregion of the right dorsal hippocampus, whereas only 74 genes were differentially modulated in its left counterpart (vs. untrained control animals). Then, using uni- or bilateral lidocaine-induced reversible functional inactivation of the dorsal hippocampus either before acquisition or before a 24 h-delay probe trial, we tried to establish a functional relationship between gene transcription and spatial learning of a reference memory water-maze task. Our data might point to a leftward bias in engram formation or information transfer, and to a rightward bias in spatial memory storage/retrieval processes.

MATERIALS AND METHODS

Animals

Male Lister Hooded rats aged 2–3 months (180–220 g) were obtained from Harlan's breeding colony (Netherlands). They were housed in a room at a controlled temperature (20–22°C), maintained on a 12 h light/dark cycle, and were allowed ad libitum access to food and water.

Experiments in Basel (behavioral approach in a water-maze task followed by mRNA expression analyses using microarrays) were performed in accordance with the Swiss Cantonal and Federal regulations. Experiments in Strasbourg (behavioral approaches using reversible inactivations in a water-maze task) were performed in accordance with the rules of the European Committee Council Directive of November 24, 1986 (86/609/EEC) and the French *Ministère de l'Agriculture et de la Pêche* (license no 67–215 to J-C.C., no 67–7 to A. PdV. and no 67–191 to RG; other authors from Strasbourg under the formers' responsibility). All efforts were made to minimize animal suffering and to reduce the number of animals used to a reasonable amount regarding statistical constraints.

Gene Expression Profiling

Spatial learning protocol (Basel)

The water maze consisted of a large circular white fiberglass pool (diameter 200 cm), positioned in the center of a room and filled with white opaque water (E308, Bronx Chemicals) maintained at room temperature (21°C). Visual cues were positioned on each wall around the maze. A computer tracking system (HVS Image Ltd., UK) was used to monitor and analyze swim paths online. During training, a circular platform (10 cm in diameter) was always positioned at the center of one quadrant, either hidden 1 cm below the water surface (spatial learning, (SP) group) or emerging from the water (1 cm above the water surface, the sides being covered with black tape to increase contrast) and thus being visible (control groups, CP and CN). For each trial, the rats were placed into the pool facing the perimeter wall. The starting position (north, south, east, west) was changed from trial to trial. Both control groups (CP and CN) were tested with the visible platform that was moved to another quadrant (Q1, 2, 3, 4; Q2 being the one where the hidden platform was submerged) for each new trial. Maximum trial length was 60 s. When a rat neither found nor climbed onto the platform by the end of the trial, it was gently guided onto it and allowed to remain there for 10 s. The inter-trial interval was less than 1 min. Rats were trained over 5 days (one session of five consecutive trials per day) and the last trial on the 5th day was replaced by a probe trial for which the platform was removed (SP group, $n = 8$ and CN group, $n = 8$) or left in place (CP group, $n = 8$). The distance swum during each trial of the acquisition phase, as well as the percent time spent within the target quadrant (i.e., where the platform was localized during training) during the probe trial were computed by the system and taken into account for statistical analysis. Two supplementary control groups consisted of naive rats (no test at all; they were kept in their home cage (HC) until euthanasia; HC group, $n = 8$) and of rats that were subjected to one swim-only session (one trial of 60 s in the water maze but no platform available, swim trial (SW) group, $n = 8$) according to Swiss Cantonal and Federal regulations.

Statistical analysis

Data from the water-maze task acquisition were analyzed using an analysis of variance (ANOVA) considering Group (CN, CP, SP) as the between-subject factor, and Day (1, 2,. . .5) as the within-subject factor. For the probe trial in CN and SP groups, we used a one-way ANOVA considering Quadrant as the within-subject factor. Analyses were followed by a multiple comparison test of Fisher when appropriate (Winer, 1971). ANOVA and Fisher test significance levels were taken at $P < 0.05$. In addition, to compare the probe trial performance within each group to a theoretical chance performance (25% of time spent in each quadrant, i.e., 15 s), a single sample Student's *t*-test was used (mean vs. 25%) with $P < 0.05$ for significance.

Tissue collection and microdissection

Immediately after the probe trial, all rats from the SP, CN and CP groups, and those from the SW, and HC groups were killed by decapitation. The brains were quickly removed, frozen on dry ice and stored at −80°C. Frozen brain sections of 30 µm were cut with a HM500 cryotome (Microm, Germany) and mounted on slides covered with a 1.35 µm thin polyethylene

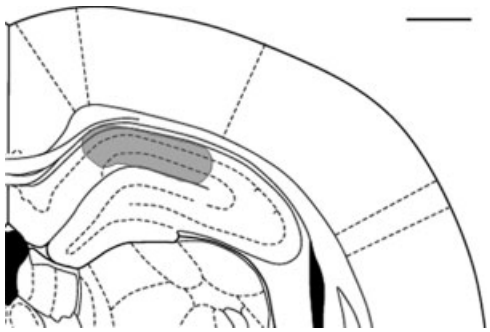

FIGURE 1. Location and limits of the region taken out by laser capture microdissection for gene expression analyses. Scale bar = 1 mm.

foil (PALM Microlaser Technologies, Germany). Slides were stained for 10 s in 0.5% toluidine blue, rinsed twice with DEPC-treated water and dehydrated for 10 s in 75 and 100% ethanol. They were left to dry at room temperature for 30 min and stored at −80°C with silica gel if not immediately used. To avoid any technical bias, an equivalent amount of slices from the left and right hippocampi was processed at the same time. The region of interest (i.e., the CA1 region of the dorsal hippocampus, Fig. 1) was cut with the laser as previously described (Klur et al., 2004) and microdissections were captured in one session using the PALM Robot-Microbeam system (Germany). The collected tissues (12 circular microdissection samples, diameter 0.5 mm, thickness 30 µm/sample) were solved in 1 ml of TRI Reagent® and stored at −80°C until further processing. To avoid RNA degradation during tissue collection and microdissection, all necessary precautions were taken. Thus, benches and all material containing/contacting samples including the cryotome, cryotome blades and boxes to store glass slides were cleaned with RNaseZap® (Ambion).

RNA isolation and processing for hybridization on Affymetrix GeneChips®

Total RNA extraction was performed using the TRI Reagent® protocol adapted for small-size samples. The whole amount of total RNA extracted from rat hippocampal microdissections was used to produce a cDNA using the Microarray Target Amplification Kit (Roche Molecular Systems, Germany). This cDNA was purified using the Microarray Target Purification Kit (Roche Molecular Systems, Germany) and amplified by PCR in 24 cycles using the Microarray Target Amplification Kit (Roche Molecular Systems, Germany). The amplified cDNA was purified using the Microarray Target Purification Kit (Roche Molecular Systems, Germany). Subsequently, the cDNA was transcribed in vitro and labeled with the T7 MEGAscript™ kit (Ambion, USA). Twenty micrograms of labeled cRNA were hybridized on Rat Genome U34A GeneChips®. Detailed protocols are described elsewhere (Klur et al., 2004). Left and right hippocampi of each animal were hybridized separately on a microarray, and a total of 80 arrays were used. Three microarrays were removed from the study because of low correlation with other arrays in the same group (two arrays in the SW group, left hippocampus, and one array in the CP group, left hippocampus).

Statistical analysis of microarray data

The gene expression was normalized after scanning by using the quantile method on raw intensities without background subtraction (Bolstad et al., 2003). The aim of this normalization was to make the distribution of probe intensities on each array the same. To identify differentially expressed genes we used the Significance Analysis of Microarrays (SAM) statistical method (Tusher et al., 2001). The SAM algorithm is a permutation-based method that relies on variance information present in measurements obtained from all probes on a microarray. In our analyses, the HC group was considered as baseline and each hippocampus side (right or left) of the four other groups (SP, CN, CP and SW) was compared with this HC group. Following statistical analysis, we selected genes with a q-value lower than 0.1 to balance false-positive and false-negative findings, and with a change factor higher or equal to 0.5 or lower or equal to −0.5 (fold change $\geq$1.5 or $\leq$−1.5). In addition, we selected genes with an average call of at least 0.75 (gene flagged as "present" in at least six out of the eight arrays in each group) and a minimal group average intensity of 50. Gene lists that arose from these comparisons were analyzed through the use of GeneOntology annotations (www.geneontology.org), KEGG pathways (www.kegg.com) and the Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com).

Semiquantitative PCR

A real-time semiquantitative PCR was performed on all samples (n = 8 per group) using the ABI Prism 7,700 Sequence Detector system (Applied Biosystems). TaqMan® Universal PCR Master Mix (Applied Biosystems) was used and reactions were performed in a total volume of 25 µl. Two microliters of amplified cDNA (the same that was used for in vitro transcription during microarray analysis sample preparation) were used in each real-time assay. The oligonucleotide and TaqMan® reporter sequences are reported in Supporting Information Table 1. The 5′ reporter dye used was FAM and the 3′ quencher TAMRA. A passive reference dye (ROX) provided an internal standard for normalization of FAM fluorescence, correcting for fluctuations resulting from volume changes. For relative quantitation, a standard curve was constructed for each primer and reporter set. Tubulin was used for normalization. The possibility of genomic DNA contamination was excluded by designing primers so that their sequence was "bridging" an exon-exon junction. Each assay was performed in triplicate with a non-template control.

Spatial Learning and Reversible Inactivation Studies

The choice of a reversible functional inactivation technique over permanent lesion was dictated by one of the major draw-

backs of permanent lesions, i.e., the neural reorganization that may occur during recovery, to the extent that different strategies and even brain areas are able to compensate for what had been an initial deficit. Thus, partial irreversible lesions made before training might spare sufficient hippocampal tissue to support near-normal task acquisition and subsequent memory retrieval [see (Martin and Clark, 2007) for rev, personal unpublished data in rats subjected to uni- or bilateral NMDA-induced lesions of a restricted volume of the CA1 region]. In addition, the pre-acquisition irreversible lesions' technique precludes the analysis of the possible critical role played by discrete brain structures in the distinct stages of mnemonic processing (acquisition, consolidation, retrieval) during which these regions may be specifically but transiently active.

Surgery

Rats were anesthetized with a mixture of xylazine (0.85 mg/kg) and ketamine (6.38 mg/kg), and a standard stereotaxic technique was used to implant stainless steel guide cannulae (external diameter 0.4 mm) bilaterally into the dorsal hippocampus (Pereira de Vasconcelos et al., 2006). Implantation coordinates were in mm: AP −3.8 (from Bregma), ML ± 3.0 from midline, and DV −1.5 (from dura), according to Paxinos and Watson (1998). Guide cannulae were kept in place with acrylic dental cement tightly fixed to the skull by stainless steel screws. A stainless steel mandrel (0.28 mm in diameter) was placed into the guide cannula to avoid obstruction. Postoperatively, after recovering from anesthesia under a warm lamp, rats were returned to their home cages for a 7-day recovery period with ad libitum access to food and water.

Intrahippocampal microinfusions

After 3 days of handling by two experimenters, and familiarization with the infusion procedure, the rats were gently restrained by hand, the mandrels were removed, and an infusion needle was inserted into each guide cannula. The tip of the needle protruded 1 mm beyond the tip of the guide cannula, therefore infusion sites in the dorsal hippocampus were ~2.5 mm below the dura (Paxinos and Watson, 1998). Lidocaine (Lido, 1 µl, 1% in PBS, Sigma, St Louis, MO) was infused unilaterally (whereas PBS was infused at the same time in the contralateral side) or bilaterally at a rate of 1 µl/min, using a 10 µl Exmire syringe connected to a microinjection pump (CMA/100) by polyethylene tubing. Lidocaine was preferred to another voltage-dependent sodium channel blocker (i.e., tetrodotoxin) on the basis of previous comparative experiments (Pereira de Vasconcelos et al., 2006). Control animals received a bilateral infusion of PBS in the hippocampus. After infusion, the needle was left in place for another minute before it was slowly removed and the mandrels were placed back into the guide cannulae. Microinfusions were performed 5 min before either each training session (inactivation during acquisition but drug-free probe trial) or before the probe trial (inactivation during the probe trial after drug-free acquisition).

Spatial learning protocol (Strasbourg)

The water maze consisted of a large circular white fiberglass pool (diameter 160 cm), positioned in the center of a room with several distinct cues on the wall, and filled with white opaque water (milk powder) maintained at room temperature (21°C). During training, a circular platform (10 cm in diameter) was always positioned at the center of one quadrant and submerged 1 cm below the water surface. A computer tracking system (EthoVision®, Noldus, Netherlands) was used to record and analyze swim paths. Training trials during acquisition were performed as previously described (see paragraph on the Spatial learning protocol (Basel)). Rats were trained over 6 days (one session of four consecutive trials per day). Twenty-four hours after the last training session, the platform was removed and all rats were given a 60 s probe trial during which several variables were recorded. For performance analyses we considered the percent time spent in the target quadrant and two additional variables: we first defined an area corresponding to the platform surface enlarged by an annulus of 10 cm (defined hereafter as the "annulus area") and then we computed the frequency of annulus area crossings and the time spent in the annulus area during the probe trial. Two rats were removed from the study because they presented a very aggressive behavior interfering with the possibility to make reliable infusions and thus reliable performance on the probe trial. Groups were as follows: i) rats given drug-free acquisition and infused just before the probe trial with Lido either in the right hippocampus (Lido-Right, $n = 12$), in the left hippocampus (Lido-Left, $n = 16$), or bilaterally (Lido-Bilat, $n = 11$), controls receiving PBS bilaterally (PBS, $n = 15$), ii) rats infused before each daily acquisition session with Lido either in the right hippocampus (Lido-Right, $n = 13$), in the left hippocampus (Lido-Left, $n = 10$) or bilaterally (Lido-Bilat, $n = 8$), controls receiving PBS bilaterally (PBS, $n = 11$). All these groups were given a drug-free probe trial.

Paw preference test

We assessed rat handedness in order to keep, should it be needed, the possibility to analyze the effects of unilateral inactivation with respect to hemispheric dominance, i.e., the preferential use of one brain hemisphere for the resolution of a specific cognitive task, as shown in humans (Mesulam, 1999; Josse and Tzourio-Mazoyer, 2004). Thus, we determined paw preference, using a food-reaching test (Pence, 2002). A transparent testing cage, 20 cm × 20 cm × 40 cm in size, having a hole (3 cm in diameter) located equidistantly from the right and left sidewalls, was used in the study. The hole was 7 cm above the floor and a cylindrical tube was placed in it. Sugar pellets were available at the end of this tube and the rat could only reach the food by introducing one of its forepaws into the tube. Rats were habituated to the testing cage and trained to reach the pellets over 4 days before the test. They were food-deprived 1 day before starting the habituation period and weights were surveyed and adjusted to make sure that loss never exceeded 15% of initial weight. On the test day, rats had to reach for 50 sugar

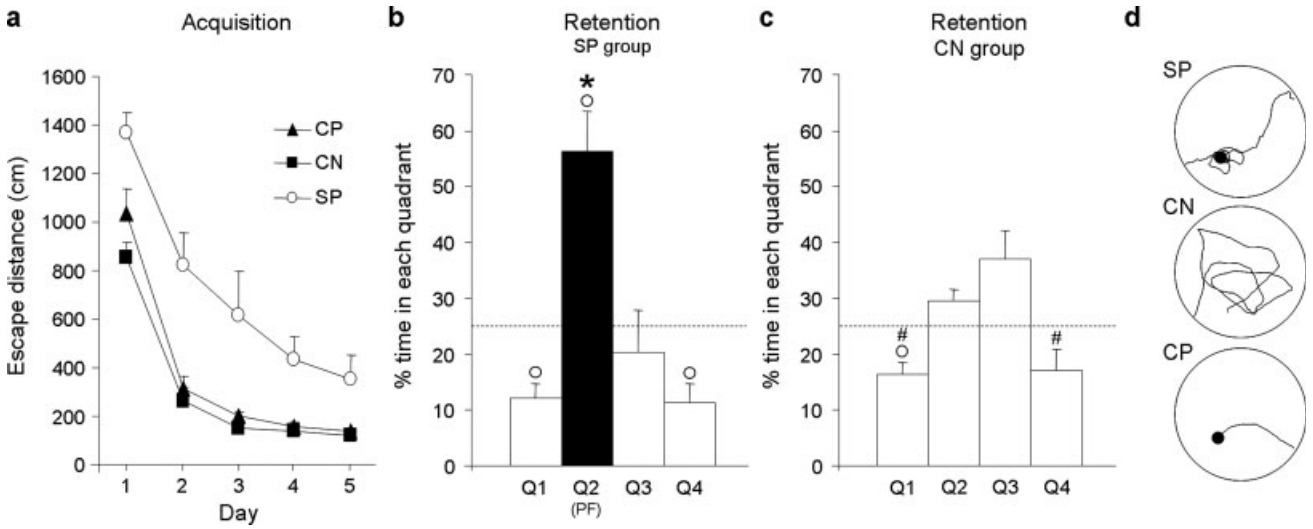

FIGURE 2. Water-maze performances. (a) Acquisition. Escape distances to the platform. SP rats were tested with a hidden platform, whereas the platform was visible for CN and CP rats. Values are expressed as mean (+ s.e.m) escape distance (cm). Data are averaged in blocks of five trials of the daily session. (b) Probe trial at the end of the acquisition period (day 5, fifth trial) expressed as % mean time (+ s.e.m) spent in the four quadrants for SP rats (acquisition and probe trial with hidden platform). (c) Probe trial at the end of the acquisition period expressed as % mean time spent in the target area (+ s.e.m) for CN rats (acquisition with visible platform, probe trial without platform). (d) Representative swim paths of rats from SP, CN, and CP (acquisition and probe trial with visible platform) groups. The black-filled circle indicates the former location of the platform. *$P < 0.001$, vs. the three other quadrants. °$P < 0.01$, vs. chance (25%).#$P < 0.05$, $P < 0.001$, vs. quadrants 2 and 3, respectively.

pellets and an experimenter scored which paw was used. A rat was classified as showing right or left preference when the % of right or left paw reachings exceeded 60%. When the % of right or left reaching was under 60%, the rat was considered ambidextrous. Forty-five rats selected randomly among the 54 rats tested in the water maze were used for the paw preference test.

Statistical analysis

Data from the water-maze acquisition task were analyzed using an ANOVA considering Treatment (PBS, Lido-Right, Lido-Left, Lido-Bilat) as the between-subject factor, and Day (1, 2,. . .., 6) as the within-subject factor. The probe trial performance data were analyzed using a one-way ANOVA considering Treatment as the between-subject factor. An effect was considered significant at $P < 0.05$. Analyses were followed, when appropriate, by multiple comparisons based on a Fisher test (Winer, 1971) with significance taken at $P < 0.05$. For the comparison of the probe trial performance with a theoretical chance performance, see the previous statistical analysis paragraph on the spatial learning protocol (Basel). The frequency of annulus area crossings and the time spent in the annulus during the probe trial were analyzed using a one-way ANOVA considering Treatment as the between-subject factor.

For the analysis of a possible role of paw preference in water-maze performance during acquisition, we used an ANOVA considering Paw preference as the between-subject factor (right vs. left) and Day (1, 2,. . .., 6) as the within-subject factor. For the probe trial performance, data were analyzed using a two-way ANOVA considering Treatment (PBS, Lido-Right, Lido-Left, Lido-Bilat) and Paw preference (right vs. left) as factors, followed by a one-way ANOVA (Treatment) for each group (left and right) and a Fisher test when appropriate. Significance levels for the ANOVA and the Fisher test were set at $P < 0.05$. For the comparison of the probe trial performance with a theoretical chance performance, see the previous statistical analysis paragraph on the spatial learning protocol (Basel).

Histological verification of implantation sites

The rats were euthanized with an overdose of pentobarbital (200 mg/kg, i.p.) and perfused transcardially with a 4% paraformadehyde (PFA) solution at +4°C. The brains were removed and post-fixed 2–4 h in 4% PFA and cryoprotected with a 20% saccharose solution (+4°C) before being cut in a cryostat in 30 μm thick sections. These sections were stained with cresyl violet and used to check for appropriateness of the infusion sites.

RESULTS

Spatial Information Processing Induces Differential Modulation of Gene Expression

Behavioral data

The data are shown in Figure 2. The analysis (Group [CN, CP, SP] X Day [1–5]) of the escape distance (cm) over acquisition trials showed that all rats improved performance across days (Fig. 2a; $F_{(2,84)} = 116.1$, $P < 0.001$). There was also a significant Group effect ($F_{(2,21)} = 13.38$, $P < 0.001$) which reflected longer distances in the SP group as compared with

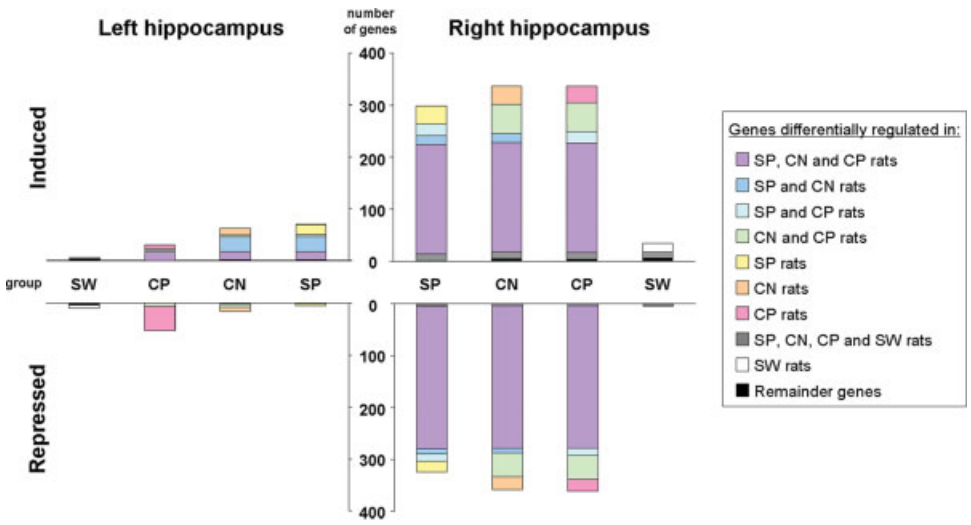

FIGURE 3. Gene expression variations in the left and right hippocampi. Histograms point to the number of genes that underwent differential regulation in SP, CN, CP (abbreviations as in Fig. 2), and SW (no platform, only swimming) rats as compared with HC rats. Color codes read as follows: purple for differentially regulated genes common to SP, CN, and CP rats (N-RSIP in the text, for navigation-related spatial information processing genes). Dark blue are common to SP and CN. Light blue are common to SP and CP. Green are common to CN and CP. Yellow, orange, and pink are specific to SP, CN, and CP, respectively. Gray are common to the four groups, white specific to SW, and black refers to the remainder. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

each of the control groups (SP versus CN or CP, $P < 0.0001$). Analyses of the latencies yielded similar conclusions (not illustrated). In the probe trial, rats trained with the hidden platform (group SP) spent more time in the target quadrant (Q2), as compared with the other three quadrants ($F_{(3,28)} = 14{,}07$, $P < 0.0001$), indicating that SP rats had learned the location of the platform (Fig. 2b). In addition, in SP rats, the time spent in the target quadrant was significantly above chance (i.e., 25%; $P < 0.01$), but it was significantly below chance in Q1 and Q4 ($P < 0.01$), whereas it was not significantly different from chance in Q3 ($P = 0.54$). CN rats, which were tested with a daily change of the visible platform position during acquisition, showed significant differences as regards the time spent in the different quadrants ($F_{(3,28)} = 7.56$, $P < 0.001$): the time spent in Q1 and Q4 was significantly shorter than that recorded in Q2 and Q3 ($P < 0.05$ and $P < 0.001$, respectively). In addition, the time spent in Q1 was significantly below the 25% chance level, and in Q3 it tended to be above this level ($P = 0.06$), probably because of an anticipatory behavior of the rats, this quadrant being located on the side of the room where the experimenter entered to take the rat out of the pool at the end of each acquisition trial.

Gene expression analysis

The statistical criteria identified genes that were differentially expressed in rats trained with the hidden platform (SP) or the visible platform (CN and CP), as compared with the HC controls. There was a marked difference between the right and the left hippocampi (Fig. 3). Indeed, in the SP group, 623 differentially expressed genes were identified in the right hippocampus against only 74 in its left counterpart. Among those 623 genes, 299 were induced and 324 were repressed. In addition, in the right hippocampus of rats from the three groups trained with either the hidden or the visible platform (SP, CN and CP), we found 211 identical genes that were upregulated and 274 that were downregulated relative to the HC condition (purple in Fig. 3). These genes were thus considered as genes linked with navigation-related spatial information processing (N-RSIP, Supp. Info. Table 2), with no particular relation to platform memorization processes. In the left hippocampus, only 20 N-RSIP genes were evidenced (Fig. 3, Supp. Info. Table 3). Apart from these common genes, we also identified in the right and left hippocampus genes that were specific to a particular training program. Thus, 56 and 23 genes in the right and left hippocampus respectively, underwent a differential regulation in the SP rats but in none of the four other groups (SP genes, Fig. 3, yellow, Supp. Info. Tables 2 and 3), and about 80 genes were induced or repressed in rats trained to swim to a visible platform (CN and CP groups vs. HC, Fig. 3, green). None of them were modulated in the SP group. Interestingly, the comparison between rats that only swam without a platform (SW) and HC rats showed that 39 genes underwent differential expression in the right hippocampus (15 in the left), indicating that one 60 s-swimming session had relatively little impact in terms of differential gene regulation (Fig. 3, white). These results confirm that the sensitivity of mRNA detection through DNA microarrays in the rat hippocampus is sufficient to discriminate between more or less pronounced testing condition-related differences in gene regulation (e.g., CN vs. CP, or SW vs. HC rats).

Semi-quantitative PCR. Results of gene expression analyses based on microarrays are usually confirmed by a second

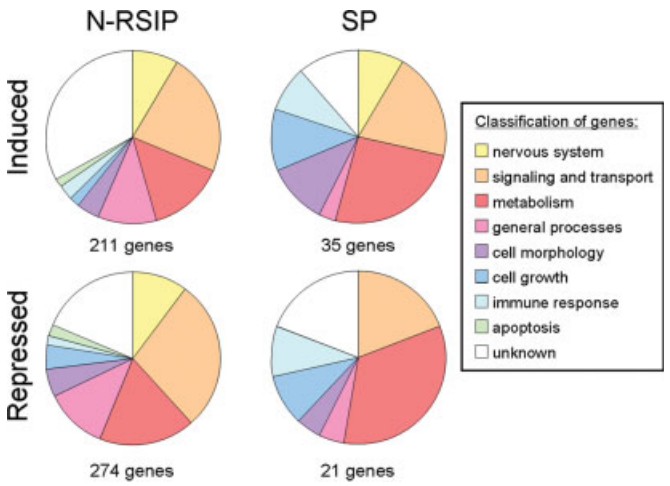

FIGURE 4. Classification of N-RSIP-related and SP genes differentially expressed in the right hippocampus in different functional classes. Based on GeneOntology annotations and on the Ingenuity Pathways database, genes were classified as genes playing a role in: nervous system functions (yellow), signal transmission and molecule transport (orange), cellular metabolism (red), general processes such as replication, translation, or transcription (pink), maintenance of cell morphology (purple), cell growth and proliferation (dark blue), immune responses (light blue), and cell death and apoptosis (green). For some genes, function was not defined (white). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

method. Thus, we selected five genes for which the differential expression was further validated by real-time PCR quantification (TaqMan® assays), which was performed on the remaining unamplified cDNA. As illustrated in Supporting Information Table 1, microarrays and real-time PCR yielded comparable results for these five genes.

Functional analyses of differentially expressed genes. One goal of this study was to examine the expression of genes in the right vs. left dorsal hippocampus during spatial learning in the Rat. As described earlier, we identified in the CA1 region of the right dorsal hippocampus a set of 56 genes that were exclusively modulated in the SP group whereas 485 genes (N-RSIP) were modulated in the three groups, i.e., SP, CN and CP, as compared with the HC group. Only a few genes were modulated by a single swimming session, as previously shown (Irwin, 2001) (Fig. 3). We performed a computer-assisted pathway analysis with commercial (Ingenuity Pathway Analysis) and publicly (GeneOntology, KEGG) available databases (see methods section) to cluster the SP and N-RSIP-related genes according to their functional implications. Both groups fell into eight functional classes (Fig. 4). Twenty-six percent and 15% of N-RSIP- and SP genes, respectively, that were differentially expressed in the right hippocampus, comprised genes not yet annotated in the public databases. The identified N-RSIP and SP-related genes expressed in the right hippocampus were, for the most part, involved in signaling/transport or metabolism (36–52%), nervous system functions (9–10%, except for repressed SP genes, 0%), and in cell morphology (4–5% except 11% in SP-induced genes). Genes associated with immune responses and cell growth represented 2 and 3% of N-RSIP genes and 9 and 11% of SP-related genes, respectively, whereas genes involved in general processes represented 10–11% of N-RSIP genes and 3–5% of SP genes in the right hippocampus. In addition, more N-RSIP genes were repressed than induced (274 vs. 211) in the right hippocampus, while SP genes were preferentially upregulated (35 vs. 21 downregulated). Complete listings of all differentially expressed SP and N-RSIP-related genes in the right dorsal hippocampus are outlined in Table 1 and Supporting Information Table 2.

In the left hippocampus, only a few genes were modulated by the N-RSIP (16 genes) and SP conditions (23 genes), and these were mostly upregulated. Thirty one percent and 22% of these N-RSIP and SP genes, respectively, are unknown. Within identified N-RSIP genes, most belong to general processes (38%) and to signaling/transport or metabolism (19%), while SP-induced genes were related for the most part to signaling/transport or metabolism (35%), to nervous system functions (20%) and general processes (15%) (Supp. Info. Table 3).

Spatial memory gene expression/pathways altered with training condition (SP)

Tables 1 and 2 summarize our data concerning SP genes in both the right and left hippocampi, respectively. Genes were regrouped and classified in several categories previously described as important in cerebral plasticity and learning and memory, i.e., cell growth, structure/cytoskeleton, DNA/chromatin/transcription, protein synthesis/degradation, signaling/transport, metabolic/catabolic enzymes and immune response.

Right hippocampus (Table 1). Within genes showing specific modulation in the SP condition in the right hippocampus, 21 (17 known genes) were repressed, while 35 (30 known genes) showed upregulation, as compared with the HC condition (Supp. Info. Table 2).

Cell growth. Three genes showed specific modulation in SP rats; these were Tnxa, SART1 and CYCS.

Structural/cytoskeleton. Within genes important for the structural reorganization of neurons following synaptic plasticity, most of them were upregulated, such as the genes coding for beta-spectrin 3 (STPBN2), microtubule associated protein (MAP1A), and MAPT (two proteins that link membrane proteins to the actin cytoskeleton), and ITIH3 which codes for a stabilizing factor of the extracellular matrix. In addition, upregulation of the MOBP gene, which plays a role in the compaction or stabilization of myelin and in the control of axonal diameter, supports a role for myelination in cerebral plasticity. Only the KIF3C gene was downregulated in SP rats; this gene codes for the microtubule-dependent motors, involved in anterograde axonal transport.

TABLE 1.

Differential Expression of Spatial Memory Identified (SP) Genes in the Right Dorsal Hippocampus (CA1) After Learning a Spatial Task in the Morris Water-Maze

Functional group	Genes upregulated	Gene symbol	Genes downregulated	Gene symbol
Cell growth	-tenascin XA	TNXA	-cytochrome c, somatic	CYCS
	-squamous cell carcinoma antigen recognized by T-cells 1	SART1		
Structural/ cytoskeleton	-myelin-associated oligodendrocytic basic protein	MOBP	-kinesin family member 3C	KIF3C
	-microtubule-associated protein 1A	MAP1A		
	-beta-spectrin 3	SPTBN2		
	-microtubule-associated protein tau	MAPT		
	-pre-alpha-inhibitor, heavy chain 3	ITIH3		
DNA/chromatin/ transcription	-Transformation/transcription domain-associated protein	TRRAP	-nucleaosome assembly protein 1-like1	NAP111
Protein synthesis/ degradation/ regulation	-proteasome 26S subunit, non-ATPase 4	PSMD4	-calnexin	CANX
	-proteasome subunit, beta type 4	PSMB4	-peptidylprolyl isomerase A	PPIA
	-eukaryotic translation initiation factor 3, subunit 7 zeta	EIF3S7		
Signaling/ transport	-solute carrier family 1 member 2	LC1A2	-protein kinase, cAMP-dependent, catalytic, alpha	PRKACA
	-mitogen activated protein kinase 2	MAP2K2	-A kinase (PRKA) anchor protein 11	AKAP11
	-protein kinase C, zeta	PRKCZ	-protein phosphatase 2, regulatory subunit B (PR52)	PPP2R2B
	-kinase substrate HASPP28	PDAP1	-chemokine (C-X3-C motif) ligand 1	CX3CL1
	-protein phosphatase 1, regulatory subunit 1A	PPP1R1A	-ras-related portein rab 10	RAB10
	-GNAS complex locus	GNAS	-ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	ATP2A2
	-cyclic nucleotide phosphodiesterase 1	CNP		
	-neurotensin receptor 2	NTSR2		
	-guanosine momophosphate reductase	GMPR		
	-solute carrier family 1, member 2	SLC1A2		
	-lysozyme	LYZ		
Metabolic, catalytic enzymes	-diaphorase	DIA1	-lipoprotein lipase	LPL
	-isovaleryl coenzyme A dehydrogenase	IVD	-peroxiredixin 3	PRDX3
	-acetyl-coenzyme A acyltransferase 2	ACAA2	-steroyl-Coenzyme A desaturase 2	SCD2
	-phosphofructokinase, muscle	PKFM	-dihydrolipoamideacetyltransferase	DLAT
	-ATPase class II, type 9A	ATP9A	-glycerol-3-phosphate dehydrogenase 2	GPD2
	-hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-coenzyme A hiolase/enoyl-Coenzyme A hydratase, alpha subunit	HADHA		
Immune response	-MHC classI RT1.O type 149 processed pseudogene	LOC360231	-B-cell translocation gene 1	BTG1
	-CD63 antigen	CD63		
	-properdin P factor, complement	PFC		

DNA/chromatin/transcription. Two SP genes code for proteins involved in epigenetic mechanisms: TRRAP, a component of many histone acetyl transferase (HAT) complexes, showed upregulation, while Nap1l1, a nucleosome assembly protein, member of the histone chaperone group, was downregulated.

Protein synthesis/degradation/regulation. Three genes coding for proteasome subunits (PSMB4 and PSMD4) and one subunit of a translation initiation factor (EIF3S7) were upregulated in SP rats, whereas two genes were downregulated. These were PPIA (cyclophylin A) and calnexin (CANX), which are chaperone molecules playing a role in the folding of neuron-specific membrane proteins and in the assembly of receptor complexes.

Signaling and transport. Genes involved in the balance between kinase and phosphatase activity represent about 50% of this gene category, supporting the well-known major role of protein kinases and phosphatases for processing information in the brain. Accordingly, we found that the expression of the genes coding for MAP2K2, PKC zeta, PP1R1A, the kinase substrate HASPP28 (upregulated), PRKACA, AKAP11, PP2R2B, and CX3CL1 (downregulated) was modulated in the right hip-

TABLE 2.

Differential Expression of Spatial Memory Identified (SP) Genes in the Left Dorsal Hippocampus (CA1) After Learning A Spatial Task in the Morris Water-Maze

Functional group	Genes upregulated	Gene symbol
Cell growth	-cyclin D2	Ccnd2
Structural/ cytoskeleton	-proteolipid protein	Plp
	-adducin 3, gamma	Add3
DNA/chromatin/ transcription	-NonO/p54nrb homolog	SFPQ
	-splicing factor, arginine/ serine-rich 10	Sfrs 10
	-acidic nuclear phosphoprotein 32 family, member A	Anp32a
Protein synthesis/ degradation/ regulation	-suppression of tumorigenicity 13 HP70-interaction	St13
	-proteasome subunit, beta type 8	Psmb8
	-eukaryotic translation initiation factor 4E binding protein 1	Eif4ebp1
	-Nopp 140 associated protein	Nap65
Signaling/transport	-interleukin 6 signal transducer	Il6st
	-calcium binding protein 1	Cabp1
	-synapsin 2	Syn2
	-phospholipase C, delta 4	Plcd4
	-protein kinase C-like 1	Pkn1
	-transferrin	Tf
	-XLas protein	Gnas
Metabolic, catalytic enzymes	-monoglyceride lipase	Mgll

pocampus after spatial learning. In addition, three upregulated genes code for protein involved in the cGMP or cAMP signaling cascade: these were GMPR, CNP and GNAS. Two upregulated signaling genes were the glial glutamate transporter GLT1 (SLC1A2) and NTSR2, a gene coding for a neurotensin receptor. Furthermore, RAB10, coding for a GTP-binding protein involved in vesicular trafficking, and ATP2A2, which codes for an ATPase playing a role in the maintenance of Ca^{2+} storage in the endoplasmic reticulum, were downregulated.

Metabolic, catalytic enzymes. A group of genes involved in several metabolic pathways were also modulated in SP rats. One upregulated gene, DIA, codes for diaphorase, identified as NO synthetase. Other genes participate in lipid metabolism and were either downregulated, such as LPL involved in cholesterol homeostasis, and SCD2, a regulatory enzyme in lipogenesis, or were upregulated, such as HADHA and ACAA2. Three genes code for enzymes of glucidic metabolism: DLAT and GDP2 were downregulated, and the PFKM gene was upregulated. Lastly, IVD, a mitochondrial enzyme involved in leucine catabolism, and PRDX3, involved in mitochondrial homeostasis, were up- and down-regulated, respectively.

Immune response. Within upregulated genes were LOC360231, coding for a MHCclass I protein involved in the activity-dependent remodeling and plasticity, CD63 and PFC (properdin P factor, complement), while BTG1 was downregulated in SP rats.

Left Hippocampus (Table 2). As opposed to the right hippocampus, 20 genes modulated by the SP condition showed upregulation (function was known for 18 genes), as compared with the HC condition; the three repressed genes had unknown functions (Supp. Info. Table 3).

Cell growth. Ccnd2, coding for cyclin D2, a cell cycle protein playing a critical role in adult neurogenesis within the hippocampus, was the only gene upregulated in SP rats.

Structural and cytoskeleton. Two genes showed upregulation, i.e., Add3 coding for a cytoskeleton protein that promotes association of spectrin with actin, and Plp coding for proteolipid protein, the major constituent of myelin.

DNA/chromatin/transcription. Two genes were upregulated in the SP condition. They code for splicing factors (Sfrs10, SFPQ) that control gene expression, through activity-dependent regulation of transcription factors, or genes such as NMDAR1 or nuclear receptors and Anp32a, an important member of the transcriptional repression machinery inhibiting histone acetylation and thus gene silencing.

Protein synthesis/degradation/regulation. Within the four upregulated genes in SP rats was the gene St13, coding for Hip, a cofactor of HsP70 whose function is to facilitate the chaperone role of HsP70 in protein folding, assembly, transport, degradation and control of the activity of regulatory proteins. In addition, Psmb8 codes for one proteasome subunit gene involved in protein degradation, while the binding protein (repressor) for EiF4e (EiF4ebp1), a dendritic protein associated with synapsin 1, controls local protein synthesis at potentiated synapses. Finally, Nap65, a Nopp140 associated protein, plays a role in modification of rRNA involved in protein synthesis.

Signaling and transport. Four upregulated genes in SP rats were closely associated with neuronal activity and synaptic-dependent changes linked to cerebral plasticity. These genes code for (1) calcium binding protein 1 (Cabp1), involved in the activity-dependent regulation of neuronal calcium influx; (2) synapsin 2 (Syn2), a regulator of synaptic vesicle mobilization and hence, neurotransmitter release; (3) one isoform of phospholipase C (Plcd4) required for structural and functional changes in spine actin, PSD scaffolding and glutamate receptor trafficking and (4) protein kinase C-like 1 (Pkn1). In addition, two genes also showed upregulation in SP rats: Tf, coding for transferrin, and Gnas for XLas protein. Finally, modulation of Il6st,

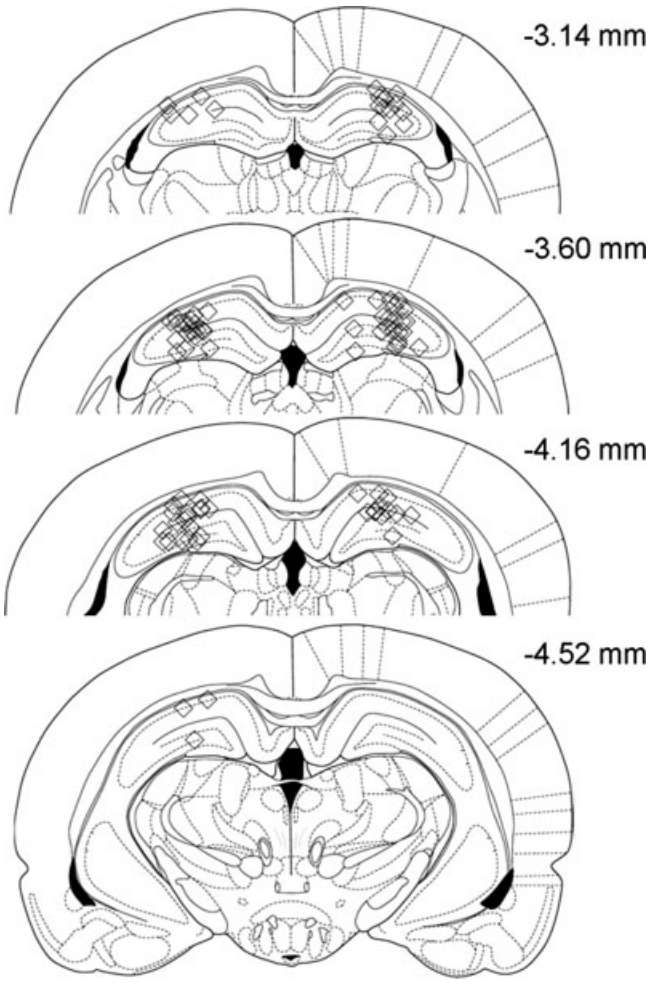

FIGURE 5. Schematic representation of infusion sites on coronal sections through the dorsal hippocampus in all rats infused with PBS or Lido in the right, the left or both hippocampi before the probe trial. Coordinates are given in mm from Bregma according to Paxinos and Watson (1998).

the interleukin 6 signal transducer confirms a physiological role of brain cytokines in memory consolidation through phosphorylation of synaptic substrates.

Metabolic, catalytic enzymes. The only gene in this category codes for monoglyceride lipase (Mgll), an enzyme responsible for the hydrolysis of 2AG, an endogenous cannabinoid.

Reversible Hippocampal Inactivation by Lidocaine in the Water Maze

Histology

The infusion sites were examined under light microscopy by an experimenter who was blind to the experimental treatment. The infusion sites for all rats were reported on corresponding plates of the stereotaxic atlas of Paxinos and Watson (1998). The infusion sites from the rats that received the infusions before the probe trial are illustrated in Figure 5. Almost all infusion sites were found to be localized in the anterior pole of the dorsal hippocampus, for most between −3.60 and −4.16 mm from Bregma. One rat was discarded from the statistical analyses because of inappropriate infusion sites (one site was too medial, the other too lateral). Comparable locations were found in the rats that were subjected to the infusion before the acquisition trials.

Behavioral data

Lidocaine infusions before the probe trial. Acquisition: An ANOVA using a Group (PBS, Lido-Right, Lido-Left, Lido-Bilat) X Day (1–6) design was performed. Rats improved their performance, as indicated by swim distances that became significantly shorter over the days (Fig. 6a; $F_{(5,250)} = 42.5$, $P < 0.0001$). In addition, there was no overall Group effect for acquisition performance ($F_{(3,50)} = 2.11$, ns), and no significant Group X Day interaction ($F_{(15,250)} = 1.42$, ns). Analyses of the latencies led to similar conclusions (not illustrated).

24-h delayed retention: All groups infused before the probe trial (PBS, Lido-Right, Lido-Left, Lido-Bilat) showed a significant preference for the target quadrant ($P < 0.01$, in each group vs. chance, Fig. 6b). Further analysis using a one-way ANOVA showed a significant Group effect for the percent time spent in the target quadrant ($F_{(3,50)} = 3.30$, $P < 0.05$). In Lido-Right rats, the percent time spent in the target quadrant was significantly lower than in PBS and Lido-Left rats ($P < 0.05$). Interestingly, there was no difference between rats infused bilaterally with PBS and those infused with Lido in the left hippocampus ($P = 0.88$). Rats given bilateral Lido infusions were impaired in comparison with PBS and Lido-Left rats ($P < 0.05$), but they were not different from Lido-Right rats ($P = 0.75$). Most interestingly, when the frequency of the annulus area crossings or the time spent in this area was considered (see Table 3), we confirmed the impairment observed in Lido-Right and Lido-Bilat rats. Indeed, besides the ANOVA, which showed a significant overall Group effect for each variable (frequency: $F_{(3,50)} = 3.7$, $P < 0.05$; time: $F_{(3,50)} = 4.9$, $P < 0.05$), multiple comparisons indicated that performance of PBS and Lido-Left rats was significantly above that found in Lido-Right ($P = 0.05$, for frequency and $P < 0.05$ for time) and Lido-Bilat rats ($P = 0.05$, for frequency and $P < 0.05$ for time). Representative swim paths (Fig. 6c) illustrate the more focalized search paths within the target quadrant in PBS and Lido-Left groups, as compared with Lido-Right and Lido-Bilat groups.

Lidocaine infusions before the acquisition sessions. Acquisition: An ANOVA using a Group (PBS, Lido-Right, Lido-Left, Lido-Bilat) X Day (1–6) design was performed. There was no significant Group effect for acquisition performance (Fig. 6d, $F_{(3,38)} = 0.5$, ns). Despite left, right or bilateral hippocampus inactivation before each acquisition session, rats were able to improve performance, as indicated by the significant decrease of the distances to reach the platform over time ($F_{(5,190)} = 54.34$, $P < 0.001$) and the lack of a significant Group X Day

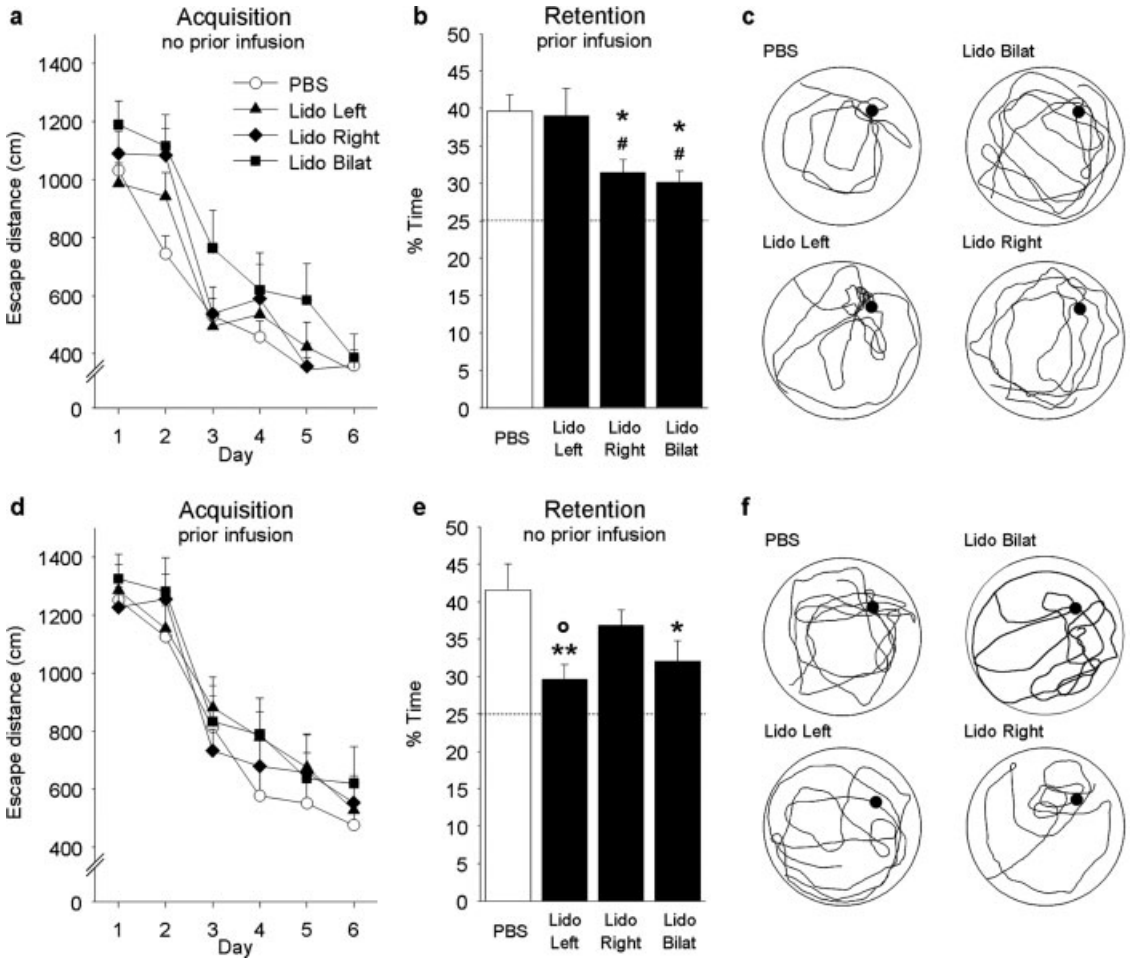

FIGURE 6. Water-maze performances. (a) Acquisition of the task (no prior Lido infusion). Values are expressed as the mean (+ s.e.m) of the escape distance (cm). Data are averaged in blocks of four trials of the daily session. (b) Probe trial (prior Lido infusion) at a 24 h delay. Performance was expressed as % mean time (+ s.e.m) spent in the target quadrant (where the platform was located during acquisition): in all groups there was a preference for the target quadrant ($P < 0.05$), but Lido infused bilaterally or in the right hippocampus impaired performance as compared to PBS and Lido-Left groups. (c) Examples of swimming paths during the probe trial. (d) Acquisition of the task (prior Lido infusion) (see legend in a). (e) Probe trial (no prior Lido infusion) at a 24 h delay (see legend in b). In all groups there was a significant preference for the target quadrant ($P < 0.05$). Lido infused bilaterally or in the left hippocampus impaired performance compared with PBS, and the Lido-Left group was also significantly impaired compared with the Lido-Right group. (f) Examples of swimming paths during the probe trial. The black-filled circle indicates the former location of the platform. *$P < 0.05$, **$P < 0.005$, vs. PBS. #$P < 0.05$, vs. Lido-Left. °$P < 0.05$, vs. Lido-Right.

TABLE 3.

Frequency of Platform Annulus Crossings and Time Spent in the Annulus Area (mean ± SEM) During the Probe Trial in Rats Subjected to Drug-Free Acquisition but Receiving an Intrahippocampal Infusion Right Before the Probe Trial (Infusion Before Probe Trial) and in Rats Subjected to the Intrahippocampal Infusion Before Each Acquisition Session but Then Given a Drug-Free Probe Trial (Infusion Before Acquisition)

	Infusion before probe trial		Infusion before acquisition	
Treatment Group	Frequency of annulus crossings	Time in annulus	Frequency of annulus crossings	Time in annulus
PBS	5.11 ± 0.48	5.53 ± 0.89	5.50 ± 0.59	5.06 ± 0.82
Lido-left	5.70 ± 0.73	6.30 ± 1.30	3.22 ± 0.61[a,b]	2.18 ± 0.46[a,b]
Lido-right	3.36 ± 0.47[a,c]	2.80 ± 0.51[a,c]	5.43 ± 0.42	4.89 ± 0.47
Lido-bilat	3.36 ± 0.79[a,c]	2.49 ± 0.60[a,c]	3.63 ± 0.69[a,b]	3.65 ± 0.80

Statistical analysis:
[a]$P < 0.05$, vs. PBS.
[b]$P < 0.05$, vs. Lido-Right.
[c]$P < 0.05$, vs. Lido-Left.

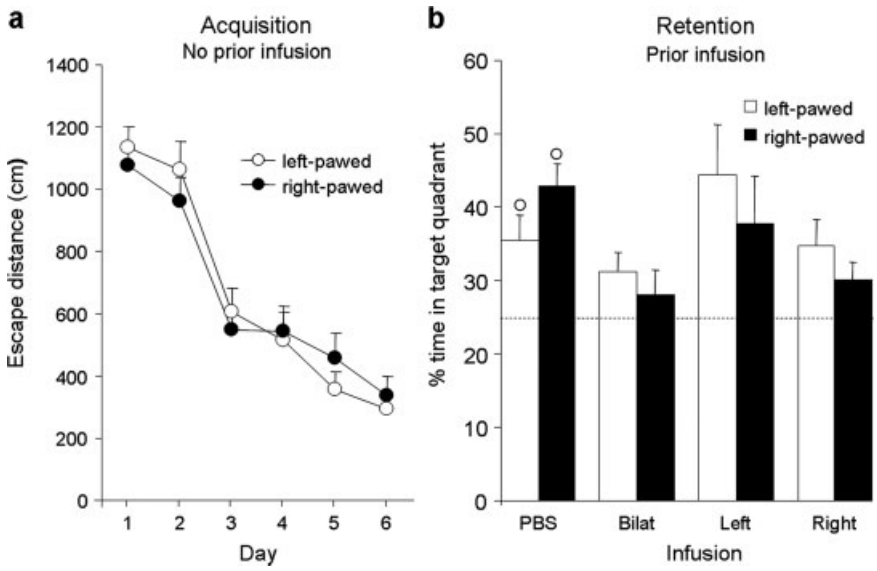

FIGURE 7. Water-maze performance in right- and left-pawed rats. (a) Acquisition of the task (no prior Lido infusion). Values are expressed as mean (+ s.e.m) escape distance (cm). Data are averaged in blocks of four trials of the daily session. (b) Probe trial (prior Lido infusion) at a 24 h delay. Performance was expressed as % mean time (+ s.e.m) spent in the target quadrant (where the platform was localized during acquisition): in right- and left-pawed PBS groups there was a preference for the target quadrant (°$P < 0.05$ vs. chance), but right- and left-pawed Lido groups infused, in the right or left hippocampus, or bilaterally, showed performance not significantly different from chance.

interaction ($F_{(15,190)} = 0.4$, ns). Analyses of the latencies led to similar conclusions (not illustrated).

Twenty-four hour delayed retention: All groups infused before each acquisition session (PBS, Lido-Right, Lido-Left, Lido-Bilat) showed a significant preference for the target quadrant during the drug-free probe trial, i.e., more than 25% time spent in the target quadrant ($P < 0.05$ for Lido-Bilat and Lido-Left groups and $P < 0.001$ for PBS and Lido-Right groups, vs. chance, Fig. 6e). However, further analysis using a one-way ANOVA showed a significant Group effect for the percent time spent in the target quadrant ($F_{(3,38)} = 4.02$, $P < 0.05$). In the rats subjected to left or bilateral Lido infusions, the percent time spent in the target quadrant was significantly lower than in PBS rats ($P < 0.005$ and $P < 0.05$, respectively). In addition, rats infused in the left side with Lido were also impaired as compared with rats infused with Lido in the right side ($P < 0.05$). Finally, there was no difference between rats infused bilaterally with PBS and those infused with Lido in the right hippocampus ($P = 0.185$). When the frequency of the annulus area crossings or the time spent in this area was considered (see Table 3), some of the aforementioned between-group differences were also found. Indeed, beside the ANOVA showing a significant overall Group effect for each variable (frequency: $F_{(3,38)} = 4.5$, $P < 0.05$; time: $F_{(3,38)} = 4.3$, $P < 0.05$), multiple comparisons indicated that performance of PBS and Lido-Right rats was significantly above that found in Lido-Left rats ($P < 0.05$, for frequency and time). In Lido-Bilat rats, however, only the frequency of annulus area crossings was significantly below that found in PBS and Lido-Right rats ($P < 0.05$ for each comparison). Representative swim paths (Fig. 6f) illustrate the more focalized search within the target quadrant for both the PBS and Lido-Right groups, as compared with the Lido-Left and Lido-Bilat groups.

Paw preference test. Because lateralization might also depend on hemispheric dominance, which may be reflected in handedness, 45 rats were tested using a paw-reaching test before the water-maze task. Among these rats, 17 were left-pawed (preference score 92 ± 3%), 20 were right-pawed (preference score 92 ± 3%), 3 were ambidextrous (preference score 47 ± 4%), and in five rats preferential paw use could not be defined (animals not using one of their forepaws to try to reach the pellet).

Behavior of right- and left-pawed rats. Acquisition: Data from the water maze of rats being infused with Lido or PBS before the probe trial (Fig. 7a) were then analyzed with additional consideration of left or right paw preference (ANOVA: Handedness [left, right] X Day [1–6]). There were no differences in acquisition performance (distances) between left-pawed and right-pawed rats ($F_{(1,35)} = 0.35$, ns), but the analysis showed a significant Day effect reflecting learning ($F_{(5,175)} = 50.92$, $P < 0.0001$). Similar conclusions were drawn from the analyses of latencies (not illustrated).

24-h delayed retention: A two-way ANOVA (Treatment x Paw preference) of data from the probe trial performance (Fig. 7b) showed that there was no effect of paw preference ($F_{(1,29)} = 0.70$, ns) and only a tendency towards an effect of Treatment ($F_{(3,29)} = 2.27$, $P = 0.10$), with no significant interaction ($F_{(3,29)} = 0.41$, ns). In addition, in the probe trial, right- and left-pawed rats given PBS bilaterally just before the probe trial showed performances that were significantly different from chance, i.e., more than 25% time spent in the target quadrant

($P < 0.05$ for both groups), whereas left- and right-pawed rats infused with Lido performed at a level which did not differ from chance, most probably because of the smaller sample sizes. *P*-values were $P = 0.07$ and 0.11, respectively for left- and right-pawed rats infused with Lido in the left hippocampus, $P = 0.07$ and 0.08 for rats infused with Lido in the right hippocampus, and $P = 0.10$ and 0.45 for Lido-Bilat rats (Fig. 7b).

DISCUSSION

Right Versus Left Hippocampus: Gene Expression

Previous studies exploring hippocampal transcriptional activity in response to spatial learning pooled both hippocampi (Luo et al., 2001; Cavallaro et al., 2002; Leil et al., 2003), studied CA1 uni- (Blalock et al., 2003) or bilaterally (Burger et al., 2007), or focused on the left dorsal hippocampus (Rowe et al., 2007). None paid attention to functional lateralization. Our gene expression data point to a stronger implication of the right (623 genes) vs. left dorsal hippocampus (74 genes) in rats having acquired a spatial navigation task vs. untrained rats. The high number of genes modulated in the spatial memory (SP) condition confirms previous data showing changes in a broad spectrum of genes associated with spatial learning and memory (Luo et al., 2001; Cavallaro et al., 2002; Leil et al., 2003). Furthermore, about half of the genes were downregulated (324 vs. 299 upregulated), confirming that memory processes also involve negative regulatory mechanisms (Cavallaro et al., 2002; Blalock et al., 2003; Burger et al., 2007, 2008), with decreased expression of proteins exerting inhibitory constraints on memory (Abel et al., 1998a,b). It is also noteworthy that a single swimming session had little effects on the modulation of gene expression, indicating that the modulation found under other conditions had little to do with an acute effect. The high number of *N-RSIP* genes might be explained by the fact that all rats swimming to a platform, whether hidden or visible, have processed (spatial) information. This viewpoint seems acceptable because hippocampal place cells code spatial information regardless of whether rats walk around in a no-goal environment or have to learn a particular place/goal therein (Moser and Paulsen, 2001; Knierim, 2003; Poucet et al., 2004). In addition, physical activity induces expression changes of a large number of genes associated with neuronal activity, synaptic structure and neuronal plasticity in the hippocampus (Tong et al., 2001; Cavallaro et al., 2002; Molteni et al., 2002). Our data also show that, in the right hippocampus, a majority of genes modulated by the SP condition were also modulated by the N-RSIP condition (485 out of 541, i.e., 90%), as previously described by Cavallaro et al. (2002), who found an 80% overlap between the so-called "spatial memory" and the "physical-activity-related" genes.

Concerning the functional categories of genes involved in learning and memory, the training conditions, type of task, and brain regions vary widely across studies. A consistent overview has not emerged yet (rev Blalock et al., 2005). Within factors responsible for these discrepancies, profound changes in hippocampus gene expression have been found to depend on the delay between training and euthanasia (Cavallaro et al., 2002; Igaz et al., 2002; Ressler et al., 2002; Levenson et al., 2004) or on the subregion analyzed (Robles et al., 2003). Moreover, in the right hippocampus, upregulated SP genes in the same category were different from downregulated ones (Table 1), indicating differential modulation of cellular processes (Burger et al., 2007), while in the left hippocampus all identified SP genes were upregulated (Table 2). These qualitative and quantitative differences in gene expression suggest different contributions of the left vs. right hippocampus in spatial memory processes. It should, however, be emphasized that the microarrays provide estimated changes in mRNA levels and not in protein synthesis. Thus, modifications of functionally relevant gene products and protein turnover cannot be inferred from gene expression analysis alone. Finally, as the rats were killed right after a probe trial at the end of training, once they could perform the task efficiently, the present study did not allow to distinguish whether (and which of) these changes were related to ongoing learning, to recent or ongoing trace consolidation (and perhaps even reconsolidation), or to retrieval of the latter. In fact, the modifications in gene expression might reflect cumulative alterations, which have been triggered by different phases of memory processing, but do not necessarily lead to transcription or translation.

Right Versus Left Hippocampus: Functional Groups of Spatial Memory Genes (SP condition)

Many genes previously implicated in hippocampus-dependent spatial memories were not revealed in our experiments and vice versa. However, a certain number of processes identified (Tables 1 and 2) were previously implicated in other spatial memory paradigms. Thus, genes coding for proteins linked to *synaptic, dendritic and axonal structures* are essential in synapse formation, differentiation and stabilization (Yuste and Bonhoeffer, 2001; Matsuzaki et al., 2004; Matsuzaki, 2007) and represent 15% of the genes modulated in the right hippocampus (mostly upregulated) and 11% in the left one. The MAP1A and STPBN2 were upregulated in the right hippocampus, as previously shown in the amygdala after fear conditioning (Mei et al., 2005). Furthermore, the gene coding for kinesin (KIF3C) was downregulated in the right hippocampus of SP rats. This family of genes is modulated during hippocampal synaptic plasticity (Park et al., 2006), spatial learning and fear conditioning (Levenson et al., 2004; Mei et al., 2005; Rowe et al., 2007). In addition, two important genes in myelin formation or stabilization, MOBP and Plp, showed increased expression in the right and left hippocampi, respectively, as found in the hippocampus and amygdala after fear conditioning (Levenson et al., 2004; Mei et al., 2005), or in the hippocampus of aged rats subjected to a spatial task (Blalock et al., 2003; Rowe et al., 2007). The Add3 gene, which participates in the reorganization of cytoskeletal structures during long-term synaptic plasticity (Gruenbaum et al., 2003), was upregulated in the left hippocampus. *Protein synthesis and degradation* that

removes excessive or damaged proteins participate in remodeling synapses and receptor structure following synaptic plasticity (Ehlers, 2003). Furthermore, the proteasomal pathway is involved in long-term memory formation in rats (Lopez-Salon et al., 2001). Three genes (*PSMB4*, *PSMD4* in the right side, *PSMB8* in the left one) were upregulated in SP rats, as previously shown (e.g., Blalock et al., 2003; Burger et al., 2007; Rowe et al., 2007). In addition, eukaryotic translation initiation factors (EiF) and their binding proteins are modulated during synaptic plasticity (Park et al., 2006) and are implicated in various learning and memory tasks (Mei et al., 2005; Rowe et al., 2007; Burger et al., 2008). In the present study, EiF3S7 and EiF4ebp1 were upregulated in the right and left hippocampi, respectively. Within important *signaling/transport pathways* in learning and memory processes, those involving kinases and phosphatases are essential for the processing of information and synaptic plasticity underlying learning and memory (Kandel, 2001; Sweat, 2001). Modulation of hippocampal genes coding for these protein categories were previously evidenced in spatial discrimination (Robles et al., 2003) and water-maze tasks (Cavallaro et al., 2002; Blalock et al., 2003; Verbitsky et al., 2004; Rowe et al., 2007; Burger et al., 2007, 2008). They were also evidenced in both the amygdala and the hippocampus after fear conditioning (Mei et al., 2005). Accordingly, we found that quite a high number of genes (17%) modulated in the right hippocampus belong to the MAPK and kinase/phosphatase signaling pathways. In addition, interleukins are modulated by synaptic plasticity (Park et al., 2006) or spatial learning (Cavallaro et al., 2002; Blalock et al., 2003; Rowe et al., 2007). In the present study, the interleukin 6 signal transducer, Il6-st, showed upregulation in the left hippocampus of SP rats. In the left hippocampus, four upregulated genes (Plcd4, Syn2, Tf, Cabp1) belong to gene families shown to be modulated in the hippocampus by spatial learning in young (Cavallaro et al., 2002) and aged rats (Blalock et al., 2003; Rowe et al., 2007), in middle-aged mice (Verbitsky et al., 2004), as well as after fear conditioning (Levenson et al., 2004). *Epigenetic mechanisms* (i.e., DNA methylation, histone acetylation and methylation), which produce lasting alterations in chromatin structure and thus regulation of critical gene transcription, are involved in synaptic plasticity and memory formation (rev Martin and Sun, 2004; Levenson and Sweatt, 2004, 2005). Thus, in the right hippocampus, the regulator of histone acetylation TRRAP is upregulated, while the histone chaperone Nap1l1 is downregulated. Moreover, in the left hippocampus, the upregulated Anp32a gene participates in the repression of transcription (Seo et al., 2002) and the two other genes (*Sfrs* 10 and *SFPQ*) code for splicing factors, which regulate gene expression and are involved in synaptic plasticity (Daoud et al., 1999; rev Ule and Darnell, 2006). The involvement of other splicing factors has been previously evidenced in both the amygdala and hippocampus after fear conditioning (Mei et al., 2005), and in the hippocampus after spatial memory in middle-aged mice (Verbitsky et al., 2004) and aged rats (Burger et al., 2007).

Therefore, we have shown that most SP genes in both the right and left hippocampi participate in synaptic plasticity, learning and memory. The difference between the right and left hippocampus relies not only on the number of genes undergoing expression changes, but also on their type. In the right hippocampus, most genes code for cytoskeleton proteins and proteins involved in signaling pathways (kinases/phosphatases). In the left hippocampus most genes participate in the repression of transcription, or in signaling/transport pathways other than those involving kinases/phosphatases. This pattern of gene modulation in the right vs. left hippocampus points to a possible differential role of each hippocampus in spatial learning, perhaps more precisely in the consolidation/use of a recently acquired spatial memory.

Right Versus Left Hippocampus: Inactivation Study

To further the question of a possible lateralization of spatial memory processes within the hippocampus, we used a Morris water-maze task to assess the effects of uni- or bilateral reversible inactivation induced by lidocaine (Lido) infused into the dorsal hippocampus. We showed that the right hippocampus may be preferentially involved in the delayed (24 h) retrieval of spatial information and precise locations, whereas its left homologue may be necessary for optimal acquisition, for encoding and/or early consolidation. Previous studies using unilateral tetrodotoxin infusions also reported deficits, but did not pay attention to the infusion side (Fenton and Bures, 1993; Fenton et al., 1995). Herein, reversible inactivation of one hippocampus, whatever the side, was insufficient to obliterate improvement of performance during training, presumably because the other hippocampus had done alone what both normally do in a cooperative way. Such a conclusion is in line with previous reports showing that a lateralized spatial memory can be rapidly transferred from the trained hippocampus to the one blocked during acquisition, once inactivation has dissipated (Fenton and Bures, 1993; Fenton et al., 1995; Czeh et al., 1998). The absence of effects of the bilateral inactivation of the dorsal hippocampus regarding between-day acquisition performances is discrepant with previous permanent lesion studies pointing to the major role of the dorsal hippocampus and particularly the CA1 region in acquisition of a spatial memory (Moser et al., 1993, 1995; Duva et al., 1997; Vago et al., 2007). However, one may take into account that in those latter studies, the lesion extent, i.e., including all fields of the dorsal hippocampus (Moser et al., 1993; Broadbent et al., 2004), or the entire CA1 region from the ventral to the dorsal pole (Duva et al., 1997), was probably much larger than the inactivation volume achieved in our study. In addition, a dorsal hippocampus permanent lesion size of about 10–20% in the Moser et al. (1993) study, which approximately corresponds to the lidocaine spread in our study (unpublished data), and even a lesion targeting up to 40% of the dorsal hippocampus (Moser et al., 1995; Broadbent et al., 2004) had no effect on the latency to reach the platform during acquisition of a reference memory task in the Morris water maze. Moreover, it cannot be excluded that when partial hip-

pocampus reversible inactivations are in fact repeated several times, a takeover of the acquisition function by other intra- or extra-hippocampal areas such as the cingulate cortex (Sutherland et al., 1988; Meunier et al., 1991), the posterior parietal cortex (Kesner and DiMattia, 1987) or the entorhinal cortex (Schenk and Morris, 1985) might become possible. Finally, in case of permanent lesions, different strategies could be used (O'Reilly and Rudy, 2001; Winocur et al., 2005), supporting the concept of a "competitive interaction" between different memory systems (Kim and Baxter, 2001). However, despite improving performances over training, retrieval performance was impaired under two conditions: after drug-free acquisition, when Lido was infused into the right hippocampus or bilaterally before the delayed probe trial, and in a drug-free probe trial after Lido infusions before each acquisition session in the left side or bilaterally. Fenton and Bures (1993) reported that a place representation engram established with only one hippocampus during acquisition became unavailable for retrieval when this hippocampus was blocked before a probe trial, demonstrating that place learning can be lateralized to one hippocampus. Based on our present data, it is possible that the right and left hippocampi played different and perhaps complementary roles in the processes involved in the establishment of a spatial representation and/or in its efficient use in a water-maze task. Thus, during formation of a memory trace, and more particularly a representation of the topographical interrelations of allothetic stimuli, the operations initially leading to trace formation and transfer, i.e., during encoding, might preferentially require the left hippocampus. The specific engagement of the left hippocampus in spatial short-term memory suggested by Poe et al. (2000), who used hippocampal tetracaine inactivation in aged rats, does not contradict this view. During retrieval, however, the operations necessary for memory recall might preferentially require the participation of the right hippocampus, as shown by Cimadevilla et al. (2005) and Cimadevilla and Arias (2008). Indeed, whereas right inactivation before the probe trial impaired retrieval, performance was still above chance. Moreover, as rats with bilateral inactivation were not more impaired than those subjected to inactivation of the right hippocampus, it is possible that memory retrieval already partly depended on ongoing reorganizations within extra-hippocampal networks, perhaps involving prefrontal cortical connections (rev Frankland and Bontempi, 2005), and compensating to some extent the effects of right or bilateral hippocampus blockade. It is also noteworthy that we cannot exclude that an intrahippocampal infusion of larger amounts of lidocaine (e.g., 40 or 80 μg), such as in the Broadbent et al. (2006) experiment, had resulted in marked performance impairments during training. Nevertheless, based on our gene expression data in the right hippocampus and on the fact that, once the task was learned, inactivation of the right, not the left hippocampus before the probe trial impaired performance, the right hippocampus could be regarded as the region where the spatial memory system is preferentially storing a more stable, though still relatively recent, hippocampus-dependent spatial memory.

The concept of a differential contribution of the left and right hippocampi in some behaviors also finds some support in recently described hippocampus asymmetries in the rat. Within them, the left-right difference in the ϵ2 subunit of the NMDA receptor at the Schaffer collateral-CA1 pyramidal cell synapse results in asymmetry of NMDA receptor content and synaptic plasticity (Kawakami et al., 2003; Wu et al., 2005). Interestingly, Belcheva et al. (2007) reported that rats were more impaired in an avoidance task when a 5-HT_{1A} agonist, namely 8-OH-DPAT, was infused into the right vs. left hippocampus. These authors concluded on a possible rightward bias of memory processing in the rat. Thus, our data could be an additional support to this viewpoint.

In conclusion, our results do not demonstrate that hippocampus-dependent stages of learning or construction of a spatial representation are strictly lateralized. Rather, they are compatible with a view that would confer a preferential role of the left dorsal hippocampus in engram formation and information transfer (to the right hemisphere) underlying a spatial representation, and a preferential role of the right hippocampus, perhaps in interaction with other nonhippocampal structures, in either storage or retrieval of a spatial trace, or in other operations involving configural trace processing once learning is achieved. This can be inferred from our inactivation data. Regarding gene expression, the maximal changes at the end of learning appear in the right hippocampus, suggesting that part of them could be involved in memory consolidation. However, the inactivation of the right hippocampus during training had no impact on probe trial performance, which may therefore seem paradoxical. At least, this apparently discrepant observation suggests that the interaction between both hippocampi is complex, and indicates that the study must be furthered. It is the occasion to remind an important remark: a change in the expression of a gene is not obligatorily reflected in protein synthesis, and thus reflected in an organism's behavior.

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