Research

Pharmacological intervention of hippocampal CA3 NMDA receptors impairs acquisition and long-term memory retrieval of spatial pattern completion task

Laetitia Fellini,^{1,2,3} Cédric Florian,^{1,2,4} Julie Courtey,^{1,2} and Pascal Roulet^{1,2,5}

¹Université de Toulouse, UPS, Centre de Recherches sur la Cognition Animale, F-31062 Toulouse Cedex 9, France; ²CNRS, Centre de Recherches sur la Cognition Animale, F-31062 Toulouse, France

Pattern completion is the ability to retrieve complete information on the basis of incomplete retrieval cues. Although it has been demonstrated that this cognitive capacity depends on the NMDA receptors (NMDA-Rs) of the hippocampal CA3 region, the role played by these glutamatergic receptors in the pattern completion process has not yet been specified. In the present study, we investigated the function of the CA3 NMDA-Rs during the different memory stages (acquisition, memory consolidation, and retrieval) in a spatial pattern completion task (when some visual cues were removed from the environment) in comparison to a standard spatial water maze task (when all visual cues were available in the environment). Thus, we coupled a massed training with the injection of NMDA-receptor antagonist (AP5) into the CA3 subfield of the dorsal hippocampus of C57BL/6 mice. Our results show that NMDA-Rs are not implicated in a standard situation but are crucial during both acquisition and long-term memory retrieval in pattern completion. This work provides the first evidence of a specific role of CA3 NMDA-Rs during memory process involved in the reactivation of incomplete memory trace, particularly when the amount of environmental information available is strongly limited.

The ability to recall previous experiences is fundamental to make choices and adapt behaviors to actual situations. However, the environment and information it contains are permanently evolving, reducing the probability for humans or animals to be confronted twice with exactly the same situation. Nevertheless, complete single-event memories can be recalled even with a sparse amount of cues. This process of retrieving entire memories from partial or degraded cues is called pattern completion. It is a fundamental daily cognitive activity that allows us to remember our previous experiences despite environmental changes. For many years, computational models have studied the process of pattern completion. In 1970, Marr (1970) proposed that such a process needs a particular memory system sustained by a structure presenting three elemental particularities. First, the structure involved in pattern completion should present extensive connectivity so that the activation of one element can lead to the activation of all elements forming this structure. Second, as a memory is often formed by several types of information (i.e., noises, odors, tastes, and also feelings), the structure involved in pattern completion should be connected with other brain regions responsible for the processing of different types of information. And third, the structure should display synaptic plasticity phenomena so that all the different kinds of information perceived at the same time and processed by different brain regions can be bound into a unique memory trace. One of the candidate structures for this process is the CA3 area of the hippocampus (Rolls and Treves 1998; Wallenstein et al. 1998). This hippocampal region is one of the three major areas of the hippocampus, along with the dentate gyrus and CA1, and presents all the functional characteristics that

could be essential for pattern completion process. First, the CA3 region presents an extensive network of collateral fibers connecting with each other (Amaral and Witter 1989), so that the firing of a single neuron can lead to the activation of neighboring cells. According to Rolls and Treves (1998), this network would form an auto-associative matrix essential for reactivate memories from partial information. Second, this CA3 region receives directly or indirectly intra- and extra- hippocampal afferences from different parts of the brain and notably from cortical and subcortical regions via two main pathways: the mossy fibers and the perforant path (for review, see Amaral and Witter 1989). Finally, the recurrent collateral fibers exhibit Hebbian long-term potentiation (LTP) that depends on NMDA receptors (NMDA-Rs) (Harris and Cotman 1986; Zalutsky and Nicoll 1990; Ito et al. 1997; Debanne et al. 1998; Nakazawa et al. 2002).

Although these characteristics suggest that CA3 may be pivotal for pattern completion, only a few studies have tested this hypothesis. In a delayed matching-to-sample task, Gold and Kesner (2005) showed that the CA3 region plays an important role in spatial pattern completion. More specifically, it has been demonstrated that the role of the CA3 hippocampal region in this process is supported by NMDA-Rs. Likewise, Lee and Kesner (2002) found that NMDA-Rs in CA3 are involved in the reorganization of spatial information. Similarly, Nakazawa et al. (2002) showed that transgenic mice deficient in CA3 NMDA-Rs were impaired when performing pattern completion retrieval as tested in the spatial water maze task. However, the lesion and genetic approaches used in these two works prevented determining during which memory phase the role of these NMDA-Rs is crucial. By use of classical spatial memory procedures, previous studies showed that the CA3 region was not implicated in all memory phases but was particularly involved in the acquisition (Handelmann and Olton 1981) and early consolidation (Florian and Roulet 2004; Artinian et al. 2007), but not during retrieval, of spatial memory (Florian and Roulet 2004).

In the present study, we used intrahippocampal infusion of an inhibitor of glutamatergic receptors to specifically block CA3

Present addresses: ³Zentrum für Molekulare Neurobiologie, Universität Hamburg, D-20251 Hamburg, Germany; ⁴Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA.

⁵Corresponding author.

E-mail roulet@cict.fr; fax (33) 05-61-55-61-54.

Article is online at <http://www.learnmem.org/cgi/doi/10.1101/lm.1433209>.

NMDA-Rs with the aim to investigate the role of these receptors in the different phases of learning and memory (acquisition, consolidation, short-term, and long-term retrieval) and pattern completion. Because both animals and humans move around in a perpetually changing environment, and because most of the environmental modifications consist of a partial degradation of the previous state, we developed a new pattern completion paradigm using the Morris water maze task for testing pattern completion retrieval (see Fig. 1).

Results

An experimental design to study spatial pattern completion in mice

In Experiments 1–4, statistical analysis did not reveal any sex-related effect. Figure 2A illustrates the performance of the four experimental groups of mice, i.e., standard environment (SE), low impoverishment (LI), high impoverishment (HI), and very high impoverishment (VHI) across the massed-learning sessions in the water maze task. A two-way ANOVA with session as within factor and group as between-subject factor revealed a significant session effect ($F_{(3,81)} = 10.335$, $P < 0.001$) but no significant differences between the four experimental groups ($F_{(3,27)} = 0.428$, $P = 0.608$). In addition, the analysis showed no interaction between these two factors ($F_{(9,81)} = 0.408$, $P = 0.927$), suggesting that the four experimental groups of mice learned to locate and reach the exact location of the platform with the same level of performance.

In analysis of the 24-h probe trial data of Experiment 1 (group SE), an ANOVA indicated a strong quadrant effect ($F_{(3,28)} = 10.286$, $P < 0.001$). Post-hoc comparisons revealed a significant preference for the target quadrant ($P < 0.01$; Fig. 2B). Similarly, statistical analysis on the probe trial performed during Experiment 2 (group LI) and 3 (group HI) showed a significant quadrant effect ($F_{(3,28)} = 11.987$, $P < 0.001$ and $F_{(3,28)} = 10.375$, $P < 0.001$, respectively) and a difference on the number of annuli crossed between the target quadrant and the other three quadrants ($P < 0.001$). In contrast, in Experiment 4 (group VHI), no significant difference was observed on the exploration of the four quadrants ($F_{(3,24)} = 1.189$, $P = 0.335$), indicating that the mice failed to find the platform location.

These results showed that noncontrolled experimental cues (light gradient, noise, etc.) were insufficient to allow mice to find the platform location using a spatial strategy. On the contrary, mice were able to orient in the pool and find the spatial location of the platform using only one pattern and noncontrolled cues, independently of the size and shape of the remaining pattern. All together, this experiment revealed that a pattern completion situation could be achieved by removing some visual patterns surrounding the water maze. Also, this first experiment showed that a massed-learning session can be used to study spatial pattern completion.

Hippocampal CA3 NMDA receptors are not crucial for spatial memory formation

In Experiments 5–8, all the probe trials were realized in the SE (group SE) as described in Materials and Methods. In these experiments, statistical analysis did not reveal any sex-related effect.

First, in Experiment 5, we investigated the role of the hippocampal CA3 NMDA-Rs on the acquisition of the spatial information by using a single injection of AP5 prior to the training sessions. Figure 3A illustrates the mean latencies before escape onto the hidden platform across the four training sessions. A two-way ANOVA, revealed a significant session effect ($F_{(3,66)} = 6.706$, $P < 0.001$) but no treatment effect ($F_{(1,22)} = 0.075$, $P = 0.787$) and no significant interaction between these two factors ($F_{(3,66)} = 0.140$, $P = 0.935$). Immediately after the last training session, mice were submitted to a 60-sec probe trial. Both NaCl- and AP5-injected mice crossed significantly more of the annulus located in the target quadrant (Fig. 3A, inset). The two-way ANOVA revealed a significant quadrant effect ($F_{(3,88)} = 34.875$, $P < 0.001$) but no treatment effect ($F_{(1,88)} = 0.653$, $P = 0.421$) and no interaction between these two factors ($F_{(3,88)} = 0.806$, $P = 0.494$), indicating that the CA3 NMDA-Rs are not required for spatial learning.

Second (Experiment 6), we studied the role of the hippocampal CA3 NMDA-Rs on short-term memory by injecting AP5 directly into the CA3 area of the training and testing mice 30 min later. Concerning the latency to find the hidden platform during training, an ANOVA

A. FULL CONDITION

4 Visual patterns

B. PATTERN COMPLETION CONDITION

Figure 1. Schematic representation of the different experimental spatial environments. (A) During the training session, all the mice were submitted to the full pattern condition and tested 24 h later in the standard environment (SE), where all visual patterns were maintained. (B) With the low impoverishment (LI), only the largest pattern (110×74 cm) was maintained; with the high impoverishment (HI), only the smaller pattern (35×29 cm) was maintained; and with the very high impoverishment (VHI), all the patterns were removed.

Figure 2. (A) Mean latency to find the platform during the training phase in the spatial water maze task. (B) Number of annuli crossings during the 60-sec probe trial in the four environmental conditions. (SE) Standard environment; (LI) low impoverishment; (HI) high impoverishment; (VHI) very high impoverishment. The results are represented as mean $\pm$ SEM. *** $P < 0.001$ compared with the target quadrant.

for repeated measures revealed a significant session effect ($F_{(3,42)} = 12.335$, $P < 0.001$) but no pretreatment effect ($F_{(1,14)} = 0.016$, $P = 0.901$) and no interaction between these two factors ($F_{(3,42)} = 1.438$, $P = 0.245$). These data confirmed that prior to treatment, the two groups of mice (AP5 and NaCl) learned to find the exact position of the platform and displayed the same level of performance during the four training sessions. Figure 3B shows the effects of 5-min post-training AP5 and NaCl infusions on the number of annuli crossings during the probe trial. ANOVA revealed no treatment effect ($F_{(1,56)} = 0.596$, $P = 0.443$), a significant quadrant effect ($F_{(3,56)} = 43.798$, $P < 0.001$), and no treatment $\times$ quadrant interaction effect ($F_{(3,56)} = 0.047$, $P = 0.987$). Post-hoc comparisons revealed a significant difference between the target

quadrant and the other three quadrants for the two groups of mice ($P < 0.001$).

Third (Experiment 7), to study the role of the hippocampal CA3 NMDA-Rs on memory consolidation, injections of AP5 were given directly after the training and mice were tested 24 h later. A repeated-measures ANOVA on training revealed a significant session effect ($F_{(3,69)} = 12.760$, $P < 0.001$), no pretreatment effect ($F_{(1,23)} = 0.881$, $P = 0.358$), and no interaction between these two factors ($F_{(3,69)} = 1.059$, $P = 0.372$). During the probe trial, both NaCl- and AP5-injected mice crossed the annulus located in the target quadrant significantly more often than the three annuli remaining (Fig. 3C). The two-way ANOVA revealed a significant quadrant effect ($F_{(3,92)} = 35.501$, $P < 0.001$) but no treatment effect

Figure 3. Role of the hippocampal CA3 NMDA receptors in the different phases of spatial memory in full condition. Effect of AP5 injection on (A) acquisition, (B) short-term retrieval (SE), (C) memory consolidation (SE), and (D) long-term retrieval (SE) of the spatial information. Histograms represent the mean number of annuli crossings $\pm$ SEM during the 60-sec probe trial. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with the target quadrant.

($F_{(1,92)} = 0.530$, $P = 0.469$) and no interaction between these two factors ($F_{(3,92)} = 0.452$, $P = 0.717$). Subsequent comparison indicated a significant difference in the number of annuli crossings between the target annulus and the three other annuli for both control and experimental groups ($P < 0.001$).

Finally, Experiment 8 investigated the role of the hippocampal CA3 NMDA-Rs on long-term retrieval when mice received AP5 injections prior to the 24-h probe trial. A repeated-measures ANOVA on the training revealed a significant session effect ($F_{(3,57)} = 8.195$, $P < 0.001$), no pretreatment effect ($F_{(1,19)} = 0.030$, $P = 0.865$), and no interaction between these two factors ($F_{(3,57)} = 0.615$, $P = 0.608$). During the probe test (Fig. 3D), the two-factor ANOVA indicated a significant quadrant effect ($F_{(3,76)} = 20.414$, $P < 0.001$) but no treatment effect ($F_{(1,76)} = 0.482$, $P = 0.489$), and no treatment $\times$ quadrant interaction ($F_{(3,76)} = 0.692$, $P = 0.560$). Post-hoc comparisons revealed a significant difference in the number of annuli crossings between the target annulus and the three other annuli for the NaCl- and AP5-injected mice ($P < 0.05$ and $P < 0.001$, respectively).

These results indicated that hippocampal CA3 NMDA-Rs are not required for acquisition, consolidation, or retrieval of spatial memories when all the external visual cues are available around the experimental maze.

Hippocampal CA3 NMDA receptors are involved in the reactivation of a spatial memory trace

In Experiments 9–12, all the mice were trained in the SE (group SE) and submitted to a 24-h probe trial. In these experiments, statistical analysis did not reveal any sex-related effect.

First (Experiment 9), we investigated the role of the hippocampal CA3 NMDA receptors in long-term retrieval in pattern-completion in LI condition (group LI) by injecting AP5 30 min before the retrieval probe trial. A repeated-measure ANOVA on training revealed a significant session effect ($F_{(3,48)} = 7.617$, $P < 0.001$), no pretreatment effect ($F_{(1,16)} = 0.004$, $P = 0.949$), and no interaction between these two factors ($F_{(3,48)} = 0.328$, $P = 0.805$) (data not shown). The number of annuli crossings during the probe test for the NaCl- and AP5-injected mice is represented in Figure 4A. When only the largest pattern was present during the retrieval probe trial, AP5-injected mice showed the same high level of performance as animals from the control group. The two-factor ANOVA revealed a significant quadrant effect ($F_{(3,64)} = 36.327$, $P < 0.001$), no treatment effect ($F_{(1,64)} = 0.059$, $P = 0.809$), and no interaction between the two factors ($F_{(3,64)} = 0.424$, $P = 0.699$). Post-hoc comparisons showed a significant difference on the number of annuli crossings between the target quadrant and the other three quadrants for the two groups of mice ($P < 0.001$), suggesting that both NaCl- and AP5-injected mice searched the platform at its correct position.

Second (Experiment 10), we studied the role of the hippocampal CA3 NMDA-Rs in long-term retrieval in pattern completion in the HI condition (group HI) by injecting AP5 only 30 min before the retrieval probe trial. A repeated-measures ANOVA on training revealed a significant session effect ($F_{(3,51)} = 14.330$, $P < 0.001$), no pretreatment effect ($F_{(1,17)} = 0.938$, $P = 0.346$), and no interaction between these two factors ($F_{(3,51)} = 1.071$, $P = 0.369$) (data not shown). However, when the smallest pattern was left during the probe trial, the experimental group performances decreased dramatically (Fig. 4B). ANOVA revealed no general effect of the treatment ($F_{(1,68)} = 0.148$, $P = 0.702$), a significant quadrant effect ($F_{(3,68)} = 14.452$, $P < 0.001$), and, more important, a treatment $\times$ quadrant interaction ($F_{(3,68)} = 3.824$, $P = 0.014$). Subsequent comparisons indicated a significant difference between the target annulus and the other three annuli for NaCl-injected mice ($P < 0.001$) but not for the AP5-injected mice ($P > 0.05$),

suggesting a failure in the platform research when only the small pattern was left during the long-term retrieval.

Together, these experiments indicated that NMDA-Rs of the CA3 region are implicated in the pattern completion process only when environmental impoverishment is strong. Therefore, we decided to keep only the smallest visual pattern (i.e., pattern N° 3) in the experimental environment during the retrieval probe trial from the following experiment.

First (Experiment 11), we studied the role of the hippocampal CA3 NMDA-Rs on short-term memory during a pattern completion retrieval (group HI), by injecting AP5 after the training and submitting mice to a probe trial 30 min later. A repeated-measures ANOVA on training revealed a significant session effect ($F_{(3,45)} = 14.850$, $P < 0.001$), no pretreatment effect ($F_{(1,15)} = 0.027$, $P = 0.871$), and no interaction between these two factors ($F_{(3,45)} = 0.462$, $P = 0.711$) (data not shown). During the retrieval session, the animals of the two groups searched preferentially for the platform at its correct position (Fig. 4C). The two-way ANOVA revealed a significant quadrant effect ($F_{(3,60)} = 27.924$, $P < 0.001$), no treatment effect ($F_{(1,60)} = 2.042$, $P = 0.158$), and no interaction between these two factors ($F_{(3,60)} = 0.657$, $P = 0.582$). Post-hoc comparisons showed a significant difference on the number of annuli crossings between the target annulus and the three other annuli ($P < 0.01$). Hence, animals belonging to the experimental group obtained the same high level of performances as the control group.

Second, Experiment 12 investigated the role of the hippocampal CA3 NMDA-Rs on memory consolidation during pattern completion retrieval (group HI) by injecting AP5 after the training and testing mice during probe trial 24 h later. Across the training sessions, there was a significant session effect ($F_{(3,45)} = 8.906$, $P < 0.001$), no pretreatment effect ($F_{(1,15)} = 2.673$, $P = 0.123$), and no interaction between these two factors ($F_{(3,45)} = 1.054$, $P = 0.378$) (data not shown). For the probe trial shown in Figure 4D, the two-way ANOVA revealed a significant quadrant effect ($F_{(3,64)} = 23.374$, $P < 0.001$), no treatment effect ($F_{(1,64)} = 0.216$, $P = 0.643$), and no interaction between these two factors ($F_{(3,64)} = 0.724$, $P = 0.541$). Subsequent comparison indicated a significant difference between the target annulus and the other three annuli for the control ($P < 0.01$) and for the experimental group ($P < 0.05$). Hence, animals of the two groups searched the platform preferentially at its correct position, and the two groups obtained the same high performances during the retrieval test.

In Experiment 5, we showed that CA3 NMDA receptors were not implicated during acquisition in full condition. However, we hypothesize that the integrity of these receptors during spatial learning might be essential later for retrieval when cues are not fully available. Thus, we blocked the NMDA receptors by injecting AP5 prior to the training and submitting mice to a probe trial 24 h later in the HI condition (Experiment 13). During the training, a two-way ANOVA revealed a significant session effect ($F_{(3,39)} = 4.620$, $P = 0.007$) but no treatment effect ($F_{(1,13)} = 0.739$, $P = 0.406$) and no significant interaction between these two factors ($F_{(3,39)} = 0.190$, $P = 0.903$), confirming that the CA3 NMDA-Rs are not required for spatial learning. During the probe trial performed 24 h later (Fig. 4E), ANOVA revealed no general effect of the treatment ($F_{(1,52)} = 0.058$, $P = 0.811$), a significant quadrant effect ($F_{(3,52)} = 13.976$, $P < 0.001$), and, more important, a treatment $\times$ quadrant interaction ($F_{(3,52)} = 4.298$, $P = 0.009$). Subsequent comparisons indicated a significant difference between the target annulus and the other three annuli for NaCl-injected mice ($P < 0.001$) but not for the AP5-injected mice ($P > 0.05$). These last results suggest also that the integrity of CA3 NMDA receptors is pivotal during spatial learning to reactivate a memory during long-term pattern completion retrieval.

Figure 4. Role of the hippocampal CA3 NMDA receptors in the different memory phases in spatial pattern completion. Effect of AP5 injection on (A) long-term retrieval in low impoverishment (B) and in high impoverishment, (C) on short-term memory in high impoverishment, (D) on memory consolidation in high impoverishment, and (E) on the acquisition in high impoverishment. Histograms represent the mean number of annuli crossings $\pm$ SEM during the 60-sec probe trial. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with the target quadrant.

All together, these results showed that (1) NMDA-Rs of the CA3 hippocampal region are involved in the reactivation of spatial long-term memories only when the amount of environmental information available is strongly limited, (2) these glutamatergic receptors are implicated in spatial memory encoding that is later required for pattern completion retrieval, and (3) these receptors are also implicated during long-term retrieval, but not in short-term retrieval or in memory consolidation, of spatial pattern completion tasks.

Discussion

The first aim of this study was to develop a new setup to study pattern completion situation in the spatial water maze task, in which mice had to retrieve memory for the hidden platform location using limited information. We showed that mice tested in pattern completion retrieval, i.e., when only one visual pattern was maintained during the retrieval test, performed as well as the ones subjected to the classical retrieval independently of the size of the remaining visual pattern. On the other hand, when the four

controlled patterns were removed, mice displayed very poor performance. These results showed that mice were able to find the hidden platform during retrieval using a limited amount of information compared with the initial learning condition, but were not able to spatially orientate using uncontrolled cues only. The statistical comparison of searching behavior during classical and pattern completion retrievals did not reveal any significant differences between the two conditions. However the similar performances obtained by both groups of mice may be the result of different strategies notably because the amount of information available was reduced during pattern completion retrieval. These results showed that our new behavioral paradigm, that consisted in the reduction of the number of spatial information available in a complex environment made of controlled and of uncontrolled cues, was adapted to study pattern completion process.

The second part of this study aimed to block the NMDA-Rs selectively during acquisition, short-term retrieval, consolidation, or long-term retrieval phase in the Morris water maze when all the visual patterns were available in the environment. We showed that AP5-injected mice displayed the same high performance as

the control group injected with the saline, suggesting that CA3 NMDA-Rs are not required during the different phases of spatial memory, including memory consolidation. This result seems surprising given that some studies support the idea that the LTP induction in the CA3 collateral recurrent depends on the NMDA-Rs activation (Zalutsky and Nicoll 1990; Ito et al. 1997; Debanne et al. 1998). On the other hand, it is also conceivable that the CA3 NMDA-Rs are mainly involved in the initiation of the consolidation process. Thus, as AP5 injections were given immediately after the last training session (i.e., ~75 min after the beginning of the first learning session), it may be that the NMDA-Rs' blockage was inefficient to disrupt ongoing consolidation processes. Another possibility would be that the glutamatergic receptors are not required for memory formation following a massed training protocol. Indeed, Spreng et al. (2002) showed that spaced, but not massed, learning facilitated the formation of the spatial long-term memories in the Morris water maze, suggesting a different consolidation memory process between spaced and massed training. Our results obtained in standard condition are consistent with those obtained by Nakazawa et al. (2002). Indeed, they showed that CA3-NR1 knockout mice were able to correctly respond in a spatial task performed in the classical Morris water maze. In this study, the NMDA-Rs were nonfunctional during the different memory phases, and even so, retrieval performances of these KO mice were preserved. Thus, CA3 NMDA-Rs are not required during the different memory phases in the classical conditions of the Morris water maze task. However, a memory deficit was found following AP5 injections in the entire hippocampus (Morris 1989; Packard and Teather 1997). It has also been suggested there is an involvement of hippocampal CA1 NMDA-Rs in spatial memories (Tsien et al. 1996; Shimizu et al. 2001). Moreover, using the same behavioral massed procedure in the Morris water maze we showed that an inactivation of the CA3 region during acquisition or memory consolidation induced amnesia (Florian and Roulet 2004).

Together, these results indicate an implication of the CA3 network without NMDA-Rs activation in standard conditions in the water maze task, suggesting that these receptors may be activated in other hippocampal regions during these memory phases.

In contrast, our data demonstrated that, depending on the memory phase studied, NMDA-Rs can be involved in the pattern completion process. With a pharmacological approach, we showed that these receptors are not involved in the reactivation of a cognitive map during short-term retrieval or during memory consolidation. On the contrary, our results strongly showed that CA3 NMDA-Rs are crucial during acquisition and long-term retrieval in the pattern-completion condition. These results confirmed the involvement of the CA3 NMDA-Rs in pattern completion obtained with CA3 NMDA knockout mice (Nakazawa et al. 2002), but specify for the first time that these receptors are implicated in two memory phases: acquisition and long-term retrieval.

Although the implication in long-term retrieval has not been evoked previously, the computational models of the CA3 may support this idea. According to Treves and Rolls (1992), the two main afferent pathways of the CA3 region, the mossy fiber and the direct perforant inputs, would play a distinct role during memory processing. Whereas the mossy fiber inputs play a role during the learning (Lassalle et al. 2000), the perforant pathway, divided into the lateral and the medial, would be crucial for the retrieval. Indeed, Treves and Rolls (1994) proposed that the retrieval can be initiated by an activation of the direct perforant pathway and subsequent reactivation of the CA3 recurrent collateral system. Assuming the fact that spatial information enters the dorsal hippocampus via medial perforant path (Hunsaker et al. 2007)

and that the strengthening of medial perforant path/CA3 synapses requires NMDA-Rs activation (Do et al. 2002), we hypothesized that NMDA antagonist injection into CA3 may block the retrieval process by inactivating the medial perforant pathway and/or recurrent collateral loop. Indeed, without the primary input initiated by the perforant path, the CA3 associative/recurrent collateral connection is not able to play its role of auto-associative network. Hence, during pattern completion retrieval, partial information may arrive via the medial perforant pathway, may activate the synapses of the CA3 recurrent collaterals, and complete the partial information inputs.

We also investigated the role of CA3 NMDA-Rs during acquisition in pattern completion retrieval 24 h later. Our results suggested that CA3 NMDA-Rs are involved in encoding of spatial memory that is later required for pattern completion retrieval when retrieval cues are not fully available. As mentioned above, the mossy fiber inputs play a major role during acquisition, whereas NMDA-Rs are poorly represented in mossy fiber synapses (Watanabe et al. 1998). On the contrary, the CA3/CA3 recurrent collateral synapses are mainly glutamatergic, suggesting an action of the NMDA-R antagonist on these CA3 recurrent synapses. These recurrent synapses appear to be required to encode memory traces in order for successful retrieval occurring during pattern completion.

So far, no study has investigated the role of CA3 NMDA-Rs in pattern completion retrieval in relation to the level of environmental impoverishment. Recently, Gold and Kesner (2005) showed that rats with CA3 ibotenic lesions showed impaired pattern completion in the spatial location task and, more important, that the level of errors was correlated with the reduction of available cues. Thus, we hypothesize that the ability to reactivate a memory from partial information depends on the activation threshold of the CA3 network that is determined by the intrinsic properties of its NMDA-Rs. For example, it is possible that the information from the environment has to be either meaningful enough or sufficiently numerous to activate CA3 pyramidal cells supporting the neuronal representation of the memory through NMDA-Rs.

In conclusion, this present study demonstrated a crucial role of CA3 NMDA-Rs in long-term memory retrieval in spatial pattern completion, but the mechanisms and pathways underlying this memory process are still unclear. For the future investigations of the CA3 area in learning and memory, it will be important to differentiate the role of the NMDA-Rs into the two main entries of this region (i.e., mossy fiber and perforant pathways) that may play complementary roles as suggested by Rolls and Treves (1998).

Materials and Methods

Subjects

Male and female C57BL/6 inbred mice born in our laboratory were used in this study. Mice were placed in cages of three to six animals of the same sex and same age, in standard breeding cages with ad libitum access to food and water, and were maintained in a temperature controlled colony room ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$) on a 12-h light/dark cycle (with lights on from 08:00 h). Every possible effort was made to minimize animal suffering, and all procedures were in strict accordance with European community and French national laws and regulations on the use of animals in research and NIH guidelines on animal care. All mice were between 12 and 16 wk old at the time of behavioral training. The behavioral tasks were performed between 10:00 h and 17:00 h.

Morris water maze and experimental environment

The water maze was an ivory circular pool (110-cm diameter, 30-cm high) filled to a depth of 15 cm with water maintained at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The water was made opaque by addition of a white

opacifier. A white-painted platform (9-cm diameter) was placed inside the pool, 15.5 cm away from the pool wall. The apparatus was surrounded by white curtains, preventing mice from using visual extra-maze cues, and was illuminated by three lamps heterogeneously placed behind the curtains (Fig. 1A). Four white and black visual landmarks approximately located at 50–100 cm from the pool, differing in shapes, motives, and size, were placed on the curtains (Fig. 1A). The swimming pool was surmounted by a video camera connected to a video recorder and a computerized tracking system (Ethovision, Noldus).

Behavioral procedure

The massed procedure in the spatial Morris water maze was used as described previously (Florian and Roulet 2004). Briefly, on day 1, mice were individually submitted to a single familiarization session of three trials with the platform located always in the same quadrant and protruding 0.5 cm over the surface of the water. The starting positions were determined in a pseudorandom order, such that each of them was used once in a single session. If the mouse did not find the platform within 1 min, it was gently guided to it. The animal stayed 1 min on the platform between the trials and before returning to the home cage. On day 2, mice were given four consecutive sessions of three trials with an intersession delay of 15–20 min, during which they were returned to their home cage. The procedure was the same as in the familiarization phase, except that the platform was submerged 0.5 cm beneath the surface of the water. On day 3, the platform was removed from the pool, and mice were submitted to the 60-min probe trial. Mice were randomly assigned to one of the 12 experiments described as follows, and the number of mice in each group was between eight and 12.

Experimental groups

In the first part of this study, we developed a new pattern completion paradigm in which mice had to find a hidden platform location in the water maze with a limited amount of information. During the training phase, the experimental environment mainly consisted of the four visual patterns surrounding the pool as well as uncontrolled cues. During the probe trials, we modulated the experimental impoverishment as follows:

Experiment 1

This experiment involved a SE group in which all four visual patterns were maintained around the pool (Fig. 1A).

Experiment 2

This experiment involved a LI group in which all visual patterns were removed except the largest one (i.e., adjacent right to the platform location; number 1 in Fig. 1A,B).

Experiment 3

This experiment involved a HI group in which only the smallest one was maintained (i.e., adjacent left to the platform location; number 3 in Fig. 1A,B).

Experiment 4

This experiment involved a VHI group in which all four visual patterns were removed from the experimental environment (Fig. 1B).

To investigate the role of hippocampal CA3 NMDA-Rs in the different phases of spatial memory, we realized a second set of experiments in which mice received injection of NMDA antagonist, D,L-2-amino-5-phosphonopentanoic acid (AP5) in the CA3 area. The behavioral protocol was the same as described in the training procedure part. After training, mice were randomly submitted to one of the four different experiments design described as following:

Experiment 5 (acquisition)

Injections were performed 15 min before the training sessions, and mice were submitted to the probe trial immediately after the last training session.

Experiment 6 (short-term retrieval)

Injections were performed 5 min after training, and the probe trial was 30 min later.

Experiment 7 (memory consolidation)

Injections were performed 5 min after training, and the probe trial was 24 h later.

Experiment 8 (long-term retrieval)

Injections were performed 30 min before the 24-h probe trial.

In a third set, we investigated the role of CA3 NMDA-Rs in the reactivation of spatial memory trace. Thus, the behavioral protocol was the same as described above, with the exception that the experimental environment was impoverished during the probe trial (LI and HI conditions) by keeping only a single visual pattern (small or large). Like previous experiments, mice were randomly assigned to different experimental groups that underwent one of the four following possible protocols:

Experiments 9 and 10 (long-term retrieval)

Mice were submitted to a 24-h post-training trial either in the LI condition (Experiment 9) or in the HI condition (Experiment 10). All injections were performed 30 min before the retrieval probe trial.

Experiment 11 (short-term retrieval)

Injections were performed 5 min after training, and mice were submitted to the probe trial 30 min after in the HI condition.

Experiment 12 (memory consolidation)

Injections were performed 5 min after training, and mice were submitted to the probe trial 24 h later in the HI condition.

Experiment 13 (acquisition)

Injections were performed 15 min before training, and mice were submitted to the probe trial 24 h later in the HI condition.

Surgery

Mice were placed in a stereotaxic apparatus (David Kopf Instruments) with mouse adapter and lateral bars. The head skin was cut longitudinally, and bilateral guide cannulae (0.56 mm in diameter) were implanted bilaterally 1.2 mm above the CA3 region. The following coordinates with lambda and bregma in the same horizontal plane were used: posterior to bregma, -1.6 mm; lateral to midline, ± 2.5 mm and 1.5 mm beneath the skull surface, according to the method of Franklin and Paxinos (1997). The subjects were then left in their home cage for a recovery period of 7–8 d.

Drug and intracranial infusion procedure

AP5 (Sigma-Aldrich) was dissolved in sterile saline (0.9% NaCl) at a concentration of $0.60 \mu\text{g}/\mu\text{L}$. Mice received a bilateral infusion of AP5 or NaCl ($0.25 \mu\text{L}/\text{side}$) into the hippocampal CA3 region through an injector (0.25 mm in diameter), which was connected with a polyethylene tubing to a $1\text{-}\mu\text{L}$ Hamilton syringe. The injection rate was kept at $0.1 \mu\text{L}/\text{min}$ by using a microsyringe pump (Bioblock). After injection, the needle was left in place for an additional 60 sec to allow diffusion.

Cannulae placement verification

On completion of the experiment, mice were sacrificed, and their brains were removed and frozen at -20°C . The cannulae position was determined for each mouse by examination of serial coronal sections ($40 \mu\text{m}$) stained with thionine. Only mice with both needle tracks terminating within the CA3 hippocampal region were included in the behavioral analysis.

Behavioral and statistical analysis

The training sessions and the retrieval probe trials were video recorded and analyzed with the software Ethovision (Noldus). Across the training sessions, the latency to find and climb onto the platform was used to measure the spatial learning. During the probe trial, the number of annuli crossings, i.e., the number of times a mouse crossed an ideal circle (14-cm diameter) located around each of the four possible platform positions in the four quadrants, was determined.

SYSTAT 9.0 statistical software package was used for data analysis. The results were expressed as mean $\pm$ SEM. Depending on the experiment, learning as well as probe trial data were analyzed using a one-way or a two-way ANOVA for repeated measures. Post-hoc multiple comparisons were carried out when allowed, using Tukey's honestly significant distance (HSD) test. Significance of results was accepted at $P < 0.05$.

References

- Amaral DG, Witter MP. 1989. The three-dimensional organization of the hippocampal formation: A review of anatomical data. *Neuroscience* **31**: 571–591.
- Artinian J, De Jaeger X, Fellini L, de Saint Blanquat P, Roulet P. 2007. A reactivation with a simple exposure to the experimental environment is sufficient to induce reconsolidation requiring protein synthesis in the hippocampal CA3 region in mice. *Hippocampus* **17**: 181–191.
- Debanne D, Gähwiler BH, Thompson SM. 1998. Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. *J Physiol* **507**: 237–247.
- Do VH, Martinez CO, Martiner JL Jr, Derrick BE. 2002. Long-term potentiation in direct perforant path projections to the hippocampal CA3 region in vivo. *J Neurophysiol* **87**: 669–677.
- Florian C, Roulet P. 2004. Hippocampal CA3- region is crucial for acquisition and memory consolidation in Morris water maze task in mice. *Behav Brain Res* **154**: 365–374.
- Franklin KB, Paxinos G. 1997. *The mouse brain in stereotaxic coordinates*. Academic Press, San Diego, CA.
- Gold AE, Kesner RP. 2005. The role of the CA3 subregion of the dorsal hippocampus in spatial pattern completion in the rat. *Hippocampus* **15**: 808–814.
- Handelmann GE, Olton DS. 1981. Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: Impairment and recovery with preoperative training. *Brain Res* **217**: 41–58.
- Harris EW, Cotman CW. 1986. Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. *Neurosci Lett* **70**: 132–137.
- Hunsaker MR, Mooy GG, Swift JS, Kesner RP. 2007. Dissociation of the medial and lateral perforant path projections into dorsal DG, CA3, and CA1 for spatial and nonspatial (visual object) information processing. *Behav Neurosci* **121**: 742–750.
- Ito I, Futai K, Katagiri H, Watanabe M, Sakimura K, Mishina M, Sugiyama H. 1997. Synapse-selective impairment of NMDA receptor functions in mice lacking NMDA receptor $\epsilon 1$ or $\epsilon 2$ subunit. *J Physiol* **500**: 401–408.
- Lassalle JM, Bataille T, Halley H. 2000. Reversible inactivation of the hippocampal mossy fiber synapses in mice impairs spatial learning, but neither consolidation nor memory retrieval, in the Morris navigation task. *Neurobiol Learn Mem* **73**: 243–257.
- Lee I, Kesner RP. 2002. Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. *Nat Neurosci* **5**: 162–168.
- Marr D. 1970. A theory for cerebral neocortex. *Proc R Soc Lond B Biol Sci* **176**: 161–234.
- Morris RG. 1989. Synaptic plasticity and learning: Selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. *J Neurosci* **9**: 3040–3057.
- Nakazawa K, Quirk MC, Chitwood RA, Watanabe M, Yeckel ME, Sun LD, Kato A, Carr CA, Johnston D, Wilson MA, et al. 2002. Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* **297**: 211–218.
- Packard MG, Teather LA. 1997. Double dissociation of hippocampal and dorsal-striatal memory systems by posttraining intracerebral injections of 2-amino-5-phosphonopentanoic acid. *Behav Neurosci* **111**: 543–551.
- Rolls ET, Treves A. 1998. *Neural networks and brain function*. Oxford University Press, Oxford, UK.
- Shimizu E, Tanq YP, Rampon C, Tsien JZ. 2001. NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. *Science* **290**: 1170–1174.
- Spreng M, Rossier J, Schenk F. 2002. Spaced training facilitates long-term retention of place navigation in adult but not in adolescent rats. *Behav Brain Res* **128**: 103–108.
- Treves A, Rolls ET. 1992. Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus* **2**: 189–199.
- Treves A, Rolls ET. 1994. Computational analysis of the role of the hippocampus in memory. *Hippocampus* **4**: 374–391.
- Tsien TJ, Huerta PT, Tonegawa S. 1996. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* **87**: 1327–1338.
- Wallenstein GV, Eichenbaum H, Hasselmo ME. 1998. The hippocampus as an associator of discontinuous events. *Trends Neurosci* **21**: 317–323.
- Watanabe M, Fukaya M, Sakimura K, Manabe T, Mishina M, Inoue Y. 1998. Selective scarcity of NMDA receptor channel subunits in the stratum lucidum (mossy fibre-recipient layer) of the mouse hippocampal CA3 subfield. *Eur J Neurosci* **10**: 478–487.
- Zalutsky RA, Nicoll RA. 1990. Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* **248**: 1619–1624.

Received March 25, 2009; accepted in revised form April 6, 2009.

Pharmacological intervention of hippocampal CA3 NMDA receptors impairs acquisition and long-term memory retrieval of spatial pattern completion task

Laetitia Fellini, Cédric Florian, Julie Courtney, et al.

Learn. Mem. 2009, **16**:

Access the most recent version at doi:[10.1101/lm.1433209](https://doi.org/10.1101/lm.1433209)

References

This article cites 24 articles, 3 of which can be accessed free at:
<http://learnmem.cshlp.org/content/16/6/387.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).