NMDA-receptor blockade by CPP impairs post-training consolidation of a rapidly acquired spatial representation in rat hippocampus

Robert J. McDonald,1 Nancy S. Hong,2 Laura A. Craig,1 Matthew R. Holahan,2 Meira Louis2 and Robert U. Muller3,4
1Canadian Centre for Behavioural Neuroscience, Department of Psychology and Neuroscience, University of Lethbridge, Lethbridge, AB, Canada
2Department of Psychology, University of Toronto, Toronto, ON, Canada
3Department of Physiology, SUNY Downstate Medical Center, Brooklyn, USA
4MRC Centre for Synaptic Plasticity, University of Bristol, Bristol, UK

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Abstract
Recent evidence suggests that N-methyl-D-aspartate (NMDA)-receptor mediated plasticity in hippocampus has a more subtle role in memory-based behaviours than originally thought. One idea is that NMDA-based plasticity is essential for the consolidation of post-training memory but not for the initial encoding or for short-term memory. To further test this idea we used a three-phase variant of the hidden goal water maze task. In the first phase, rats were pretrained to an initial location. Next, intense, massed training was done in a 2-h interval to teach the rats to go to a new location after either an injection of the NMDA receptor antagonist (6)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) or of vehicle. Finally, under drug-free conditions 24 h after new location training, a competition test was done between the original and new locations. We find that N-methyl-D-aspartate (NMDA)-receptor blockade has little or no effect on new location training. In contrast, when tested 24 h later, the strength of the trace for the new location learned during NMDA-receptor blockade was much weaker compared with the trace for the new location learned after saline injection. Further experiments showed similar effects when NMDA-receptors were blocked immediately after the new location training, suggesting that this is a memory consolidation effect. Our results therefore reinforce the notion that hippocampal NMDA-receptors participate in post-training memory consolidation but are not essential for the processes necessary to learn or retain navigational information in the short term.

Introduction
In the context of the connectionist view of the nervous system, the key role of the hippocampus in certain forms of learning and memory (Grimwood et al., 2001; Martin & Morris, 2002) implies that the hippocampus should be the site of synapses whose strength depends on their activity. This prediction was satisfied by the discovery of long-term potentiation (LTP) and later of related processes at several synaptic sites in the hippocampus (Kandel, 2002) and other brain areas.

An especially fruitful line of work was initiated by the discovery that the induction of LTP at certain hippocampal sites could be prevented by blockade of NMDA-type glutamate receptors (NMDAR; Collingridge et al., 1983). If LTP plays an essential role in memory-based behavioural changes, blockade of NMDARs should also prevent learning.

This reasoning was corroborated by several studies in which NMDAR antagonists impaired acquisition of tasks sensitive to hippocampal damage in the rat (Morris et al., 1986; Morris, 1989; Robinson et al., 1989; Shapiro & Caramanos, 1990; Davis et al., 1992). Nevertheless, later work has challenged the idea that blockade of NMDAR necessarily leads to spatial memory deficits. Thus, peripheral or intraventricular administration of selective NMDA antagonists at doses that block LTP do not retard acquisition in the standard, hidden platform version of the water task if the animals are pretrained or are procedurally sophisticated (Bannerman et al., 1995; Saucier & Cain, 1995).

The ability of pretraining to greatly ameliorate learning deficits induced by NMDAR antagonists suggests that NMDAR-mediated LTP in hippocampus either has no role in hippocampal memory-based behaviours or that its role is more limited than previously thought. The hypothesis we have been pursuing is that NMDA receptor activation is necessary not for the initial acquisition of learning but instead for post-training consolidation of information initially stored in the hippocampus by some other mechanism. Evidence in this direction comes from two sources. (i) NMDAR blockade by CPP [(6)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid] leaves intact the formation of new place cell activity patterns in a novel environment but prevents the stabilization of the new patterns (Kentros et al., 1998). (ii) CPP does not interfere with short-term (~1–2 h) learning of extinction for fear conditioning but prevents consolidation of extinction learning (Santini et al., 2001).

The goal of the present study was to use the spatial navigation task to further examine the idea that NMDA-mediated LTP in hippocampus is important for consolidation but not for initial...
learning. We tested this idea with a three-phase procedure that included a prolonged pretraining phase, a brief, intense phase of retraining to a new location after injection of either saline or CPP, and a final competition test between the new and old location in drug-free conditions.

**Methods: general**

**Experimental protocol**

To further investigate the role of NMDAR in learning and remembering to swim to a hidden platform we designed a three-phase variant of the Morris swimming task (see Fig. 1).

**Phase 1**

In the first phase, which lasted 4 days, rats were taught to swim to a fixed location. No drug was administered during Phase 1 to avoid performance problems typically seen after administering NMDA antagonists to experimentally naive animals (Keith & Rudy, 1990).

**Phase 2**

The second phase consisted of new location training and was done entirely on Day 5, the day after completion of Phase 1. At the start of Day 5, rats were given i.p. injections of saline or 10 mg/kg CPP in Experiments 1, 2 and 4, or intrahippocampal (i.h.) 1.0-µL injections of artificial cerebrospinal fluid (ACSF) or 1.0 µL injections of 32 ng/µL CPP in Experiment 3. Retraining to swim to the new platform location was started 1 h after i.p. injections or 0.5 h after i.h. injections, and continued for 2 h of massed trials. In this way, all swims aimed at the new location were made in an interval during which CPP blocks primed-burst potentiation (Kentros *et al.*, 1998). Phase 2 allowed us to ask if rats could learn to go to a new location during NMDAR blockade.

**Phase 3**

The third phase was done 24 h after Phase 2 in Experiments 1, 2 and 3, and 48 h after Phase 2 in Experiment 4. No drug was given during Phase 3, and the interval of either 24 or 48 h was sufficient so that the CPP given on Day 5 would no longer block primed-burst potentiation (Kentros *et al.*, 1998; but see Discussion). For Experiments 1, 3 and 4, Phase 3 was done with the platform put back at its original (Phase 1) location so that we could ask how the massed new location training of Phase 2 affected relearning to the original location. In Experiment 2, testing for the effects of new location training was done with a probe trial during which no platform was present. The rats’ behaviour was assessed by measuring the time spent in the four quadrants of the pool.

In summary, the three-phase protocol allowed us to see how NMDAR blockade in procedurally sophisticated subjects affects the rapid acquisition of new spatial information and whether any newly acquired information is stabilized (consolidated) after the NMDAR blockade has ceased to be effective.

**Time course of CPP action**

The ability of rats to learn to swim to a new location shortly after a CPP injection but to not remember the new goal 24 h later reveals an important limitation of the role of NMDA-based plasticity processes. There is, however, conflicting evidence about the effective duration of CPP action. In anaesthetized rats, the same or similar doses of CPP used here continued to block LTP 24 h after administration although its effectiveness varied (Abraham & Kairiss, 1988; Abraham & Mason, 1988). In contrast, in alert rats, 10 mg/kg doses of CPP did not block primed-burst potentiation (an NMDAR-dependent increase in the population spike) 24 h after injection, although it was effective 2 h after injection (Kentros *et al.*, 1998). In the usual version of the 8-arm maze protocol, CPP impaired performance 2 h post-injection but not at 24 h, although it caused increased errors at 24 h in a more difficult version of the task (Ward *et al.*, 1990; also see Tan *et al.*, 1989). Overall, it therefore seems to be the case that the duration of CPP action is prolonged in anaesthetized rats. We conclude, however, that additional work on the pharmacodynamics of CPP is necessary to clarify the state of the brain at the 24 h time point.

**Subjects and handling**

Male Long–Evans rats from Charles River colonies were used for all four experiments. Upon arrival, animals were housed individually,

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**Fig. 1.** Three phases of the basic experimental design.
maintained on a 12 : 12 h light : dark cycle, and had food and water available ad libitum. Rats were allowed a week to acclimatize before handling. Each rat was handled 5 min per day for 4 days prior to water maze training. Exceptions to this protocol to allow implantation of cannulae for brain injection are stated in the procedure for Experiment 3.

Training room and pool
A white plastic pool 184 cm in diameter and 60 cm in height was filled with water (20–22 °C) to a level of 36 cm; the water was made opaque by adding non-toxic white paint (Tempra, Northvale, NJ, USA). The 12 x 12 cm clear Plexiglas platform was submerged 2 cm under water. Small holes drilled into the top of the hidden platform provided a grip for the animals. The room used for training was 1075 cm long x 655 cm wide. Extra-maze cues included three posters of different sizes and orientations mounted separately on three of the four walls, a computer rack, a door, an animal rack and the experimenter.

Data collection
A computer-based rat tracker (VP118, HVS Image) was used to collect and analyse data obtained from an overhead video camera. For Phases 1 and 2 of all five experiments the measure of performance was time spent near the original platform location. The latency between release and escape onto the platform. If a rat did not reach the platform 60 s after release, a latency of 60 s was assigned, the rat was captured and placed on the platform. Once a rat was on the platform it was allowed to stay there for 10 s, removed and put into a holding cage until the next trial. Swim time was also used to assess performance in Phase 3 of Experiments 1, 3 and 4. In contrast, for the probe swim in Phase 3 of Experiment 2 we measured the percentage of time spent by rats in the four pool quadrants during the first three 10-s intervals.

Experiment 1. Effects of peripherally administered CPP on post-training consolidation of rapidly acquired place information: assessment by retraining

Experiment 1. Methods
Phase 1: original location training
All 16 rats were treated the same way during Phase 1. The hidden platform was located at the centre of the south-east quadrant of the pool. Each rat was given two four-trial blocks per day for 4 days, for a total of 32 trials. Each trial within a block started at one of the four cardinal points: N, E, S and W. The start order was randomized for each rat on each day, but was the same for the two blocks for each rat on a given day. For a trial, the rat was put in the water facing the wall at the starting point and allowed to swim until it located the platform or until 60 s had elapsed. If a rat did not find the platform by the end of the 60 s ‘termination interval’ the experimenter placed it on the platform. Following escape or placement on the platform, the animal was left there for 10 s and then put into a holding cage while other animals were trained.

Phase 2: new location training (drug day)
The hidden platform was moved to the north-west pool quadrant (although its location was closer to the west start point) for new location training. The rats were divided into two groups of eight and given i.p. injections of either 10 mg/kg CPP or saline 1 h before training began. On each trial, the animal was placed in the water maze facing the wall and was released from a start position chosen randomly from one of three possibilities: N, E, S; the west start point was eliminated during this phase of training because it was closest to the platform location. The same start position sequence was used five times for each rat, with the first position used an additional time to make a total of 16 new location trials within a 2-h period on the same day. As during the initial training, the rat was allowed up to 60 s to locate the platform. If this interval was exceeded the rat was put onto the platform. As before, the rat remained on the platform for 10 s regardless of whether it escaped or was placed.

Phase 3: competition test
In this phase the hidden platform was put back in its original position and rats received eight trials (two blocks of four trials) in a single day. The order was randomized for each rat, but was the same within each block of four trials.

Experiment 1. Results
Phase 1: original location training
Rats were identified as either pre-CPP or pre-saline depending on the treatment they would receive on the drug day. Figure 2A shows that both groups learned the platform location at equal rates. On average, all rats were escaping in less than 10 s by the third and fourth training days. A two-way repeated-measures ANOVA for Day by Group showed that the decrease of latency with training was significant \(F_{3,30} = 79.81, P < 0.001\), but there was no Group or interaction effect.

Phase 2: new location training (drug day)
The ability of pretrained rats injected with either saline or CPP to learn the new platform location is demonstrated in Fig. 2B. For both groups, the escape latency is long during the first block of two trials because of time spent near the original location and because of the time required to find the new location. Saline-injected rats showed considerable improvement during the second two-trial block and performed at asymptote during the third and subsequent blocks. CPP-injected rats did not escape as rapidly during the second and third two-trial blocks, but caught up with the saline-injected rats by the fourth block, after which performance was the same for both groups. This pattern suggests that both saline- and CPP-injected rats can learn during massed training to go to the new platform location but that acquisition is somewhat slowed with NMDAR blockade induced by peripheral injection.

These impressions were mainly confirmed by a two-way ANOVA with repeated-measures on Trial Block. The ANOVA indicated significant effects for Trial Block \(F_{2,34} = 38.85, P < 0.001\) and for the Group–Trial Block interaction \(F_{1,12} = 3.09, P < 0.01\). Comparisons planned to interpret the interaction revealed a significant group difference on Trial Block three \(F_{1,12} = 6.4, P < 0.05\), showing that CPP animals were slower near the start of new location phase. The longer latency for CPP rats in the second block points in the same direction, but the difference is not significant due to the high variance for the CPP rats. The poorer performance was, however, transient; the latencies in block 4 and afterward are virtually the same for CPP and saline rats. Thus, by the end of the drug day, rats in both groups were performing at equal levels.

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Phase 3: competition test

When rats were retrained to the original location 1 day after the drug day, the initial performance of the CPP rats was superior to that of the saline rats, as shown in Fig. 2C. The difference is especially striking in Trial 1 when no information was available that the platform had once again been moved. Thus, CPP-injected rats swam directly to the original platform location so their average escape latency was only 5.18 s. In contrast, saline-injected rats took much longer, 21.11 s to escape at the original location. Their increased latencies were due to time spent searching in the new location as well as travel time from this point to finding the platform in the original location. These impressions were confirmed by an analysis performed on the first swim indicating a significant Group effect $F_{1,12} = 4.89$, $P < 0.05$.

With successive trials, the saline-injected rats escaped more rapidly at the original location so that by Trial 4 of Phase 3 they performed as well as the CPP rats. An ANOVA for all of Phase 3 showed a significant Trial effect $F_{7,84} = 3.1$, $P < 0.01$ and a significant Trial–Group interaction $F_{7,84} = 2.5$, $P < 0.05$.

Experiment 1. Discussion

There are two main results of the first experiment. (i) Rats can rapidly learn to escape at a new platform location after i.p. injections of either...
saline or 10 mg/kg CPP. Although the initial learning rate is slower in this experiment after CPP administration, asymptotic performance is the same. Moreover, in similar experiments that include identical retraining to the new location there was no slowing of learning for rats injected with CPP (see below). (ii) When tested the next day with the platform moved back to its initial position, rats injected with CPP acted as if the new location training had not taken place; they swam directly and consistently to the original position. In contrast, rats injected with saline showed a marked tendency on the first trial to swim to the new platform location; they revealed the impact of new location training by increases of escape latency. Despite the close agreement of this experiment with place cell (Kentros et al., 1998) and extinction of cued fear conditioning (Santini et al., 2001) studies, it is less than ideal because the performance measure is set by the first encounter with the platform on each trial. Accordingly, we repeated the experiment in every way except that for Phase 3 a probe trial was substituted for retraining to the original location.

Experiment 2. Effects of peripherally administered CPP on post-training consolidation of rapidly acquired place information: assessment by probe trial

Experiment 2. Methods

Phase 1: original location training

In this experiment, 14 rats (later divided into two groups of seven each) were used. Otherwise, Phase 1 was exactly the same as in Experiment 1.

Phase 2: new location training (drug day)

The same as Phase 2 of Experiment 1.

Phase 3: probe trial

The escape platform was removed from the pool. Rats were put into the pool at a randomly selected cardinal location and allowed to swim for 30 s. By tracking the rat it was possible to assign its position to one of the four pool quadrants at 1 s resolution. To characterize the temporal aspects of the probe trial behaviour, we measured the percentage of time the rat spent in each quadrant during the first three 10-s intervals (Devan et al., 2003).

Experiment 2. Results

As expected from the identical design, the outcome of Phases 1 and 2 in this experiment are very similar to those in Experiment 1. As shown in Fig. 3A, escape at the original location (Phase 1) improves progressively over the 4 days and reaches an asymptote of about 10 s by Day 4. An ANOVA confirms the existence of a Day effect \( F_{3,36} = 70, P < 0.001 \), but the F-value for neither Group \( F_{1,12} = 4.13 \) nor Group \( \times \) Day \( F_{3,36} = 1.38 \) approaches significance. Similarly, improvements of performance during new location training are evident for both saline- and CPP-injected rats during the massed training of Phase 2 (Fig. 3B), but in this replication there is no evidence of slowed acquisition by CPP-injected rats; NMDAR blockade does not impair learning in the short time interval of 2 h. The impressions gained by inspection of Fig. 3B are corroborated by an ANOVA indicating significant Trial Block effects \( F_{7,84} = 13.61, P < 0.001 \). In contrast to the equivalent performance of CPP- and saline-injected rats on the drug day, the two groups differed distinctly the next day in the probe trial of Phase 3, as summarized in Fig. 4A–C. Saline-injected rats spent 41.0% of the first 10-s time interval in the new location quadrant and only 9.9% in the original quadrant. A paired \( t \)-test revealed that this search time difference was just short of significant (\( t_6 = 2.16; P = 0.073 \). In the second 10-s time interval the swim time distribution for saline-injected rats switched so that they spent 9.3% of the time in the new location quadrant and 57.4% of the time in the original quadrant, a difference that was highly significant according to a paired \( t \)-test (\( t_6 = 5.92; P = 0.0010 \)). Crucially, for saline-injected rats, the fraction of the first 10 s in the new location quadrant was significantly higher than in the second 10 s (\( t_6 = 3.26, P = 0.017 \)), whereas the fraction of the first 10 s in the original quadrant was significantly lower than in the second 10 s (\( t_6 = 4.49, P = 0.0042 \)). This switch of search strategy by saline-injected rats indicates an initial preference for the new location quadrant with a strong secondary preference for the original quadrant.

The situation is markedly different for CPP-injected rats, but the exact pattern of preference is not as clear. A day after NMDAR blockade, rats on average showed a numerical preference for the original quadrant (46%) over the new location quadrant (23.7%), but this difference was not reliable (\( t_6 = 1.05; P = 0.334 \)). Inspection of individual rat scores shows marked variability in quadrant preference during the first 10 s: three rats spent no time in the new location quadrant and the bulk of their time in the original quadrant, two spent considerably more time in the new location than the original quadrant, and the remaining two subjects spent about equal time in these two quadrants. CPP-injected rats also did not show a strong switch of swim time between the new location (24.1%) and original (33.0%) quadrants during the second 10-s interval. Therefore, the effects of NMDAR blockade on initial recall of the new location training show up, on average, as a loss of quadrant specificity that replaces the clear early preference for the new location quadrant shown by saline-injected rats. Corresponding to this distinction, \( t \)-tests for the first 10-s epoch show a trend for saline-injected rats to spend more time than CPP-injected rats in the new location quadrant (\( t_{14} = 1.42; P = 0.177 \)), and to reliably spend less time in the original quadrant than CPP-injected rats (\( t_{14} = 2.41; P = 0.030 \)).

Experiment 2. Discussion

Results from Phases 1 and 2 of this experiment mainly reproduce the corresponding results of Experiment 1, although no slowing of acquisition of the new platform location was seen in rats injected with CPP. The overall pattern of results from Phase 3 are also in agreement with those from Experiment 1, although quadrant preferences by CPP-injected rats during the probe trial render the outcome somewhat less satisfactory in regard to the hypothesis that NMDAR blockade should induce amnesia for the new platform location. Specifically, the pattern of results indicates that amnesia for the new location is not complete. Nevertheless, the lack of a clear preference for the new location quadrant by CPP-injected rats compared with saline-injected rats is compatible with the idea that consolidation is poorer after new location training is done during NMDAR blockade.

Experiment 3. Effects of infusing CPP into the dorsal hippocampus on consolidation of rapidly acquired place information

Experiment 3. Methods

Phase 1: original location training

Training to an initial location for 16 rats was exactly the same as Experiment 1.
Phase 2: new location training (drug day)
Injecting CPP via a peripheral route leaves open the site of drug action. The purpose of this experiment was to see if injecting CPP directly into the dorsal portion of CA1 would mimic the effects of i.p. injections. Specifically, we asked if new location training would be unaffected by i.h. CPP, whereas consolidation of such training would be disrupted on testing the next day. Apart from the route of CPP administration, the only difference from Experiment 1 was that the interval between injection and the start of new location training was 0.5 h instead of 1 h.

Based on earlier studies of the mnemonic effects of i.h. CPP injections (Ohno et al., 1992, 1996; Riekkinen & Riekkinen, 1997), we administered 1.0 µL of either ACSF or 32 ng/µL CPP in ACSF into each hippocampus.

Phase 3: competition test
Training back to the original location was the same as in Experiment 1.

Animals and handling
The preparatory part of this experiment was extended by a week to permit surgical implantation of cannulae into dorsal CA1. Specifically, surgery was done 1 week after arrival and an additional week was allowed for recovery before rats were handled 5 min per day for 4 days in preparation for water maze training.

Surgery
Surgical operations were done according to guidelines provided by the Canadian Council on Animal Care (CCAC). Rats were bilaterally implanted with 26-gauge cannulae into the dorsal hippocampus. The tip coordinates for the cannulae were AP: −4.0; ML: ± 2.5; V: −1.9; the coordinates are in mm relative to the skull surface and to bregma. To introduce CPP or ACSF into the brain parenchyma, a 32-gauge injector tip was passed through the cannula to extend 1.0 mm beyond the cannula tip.
Surgery was performed under isoflurane anaesthesia in a standard stereotaxic apparatus. During and following surgery animals were given buprenorphine (Temgesic) as an analgesic. After surgery, animals were monitored until they became active.

Histology

Upon completion of Phase 3 the rats were anaesthetized with sodium pentobarbital and perfused intracardially with 0.9% saline followed by 4% formalin. The brains were sectioned at 40 microns and stained with cresyl violet. Upon examination, the cannula tips for all 16 rats were found to be in the dorsal hippocampus, allowing all behavioural results to be used. The visualized cannula tip locations are superimposed in Fig. 5 on an atlas figure of the dorsal hippocampus.

Experiment 3. Results

Phase 1: original location training

Rats that were eventually assigned to the ACSF and CPP groups learned the position of the hidden platform over the same time course, as illustrated in Fig. 6A; by Day 4 their escape latency was about 8 s. As expected from the appearance of the curves in Fig. 5A, a two-way ANOVA with repeated-measures on training day indicated a significant effect of Day $F_{3,36} = 81.7$, $P < 0.001$, but no Group or interaction effect were present.

Phase 2: new location training (drug day)

I.h. injections of CPP had no detectable impact on the ability of rats to learn the new platform location (Fig. 6B). A two-way ANOVA with repeated-measures on Trial Block indicated a significant effect of Trial Block $F_{7,84} = 88.44$, but no Group or interaction effect were observed.

Phase 3: competition test

The progress of relearning to go to the original platform location is shown in Fig. 6C. As further described in the text, saline-injected rats showed a clear switch in preference from the new to the original quadrant between the first and second 10-s interval. In contrast, CPP-injected rats preferred the original quadrant during the first 10-s interval and also during the second 10-s interval, although the preference was not very great during either time span.

The results of Experiment 3 confirm that NMDAR blockade induced by hippocampal injections has little discernable effect on the ability to learn a new platform location but that it disrupts consolidation of memory for new location training when tested 24 h later. The new information is that the blocked NMDARs are probably local to the hippocampus and may be on principal cells in Ammon’s Horn or the dentate gyrus or both.

Histology

Upon completion of Phase 3 the rats were anaesthetized with sodium pentobarbital and perfused intracardially with 0.9% saline followed by 4% formalin. The brains were sectioned at 40 microns and stained with cresyl violet. Upon examination, the cannula tips for all 16 rats were found to be in the dorsal hippocampus, allowing all behavioural results to be used. The visualized cannula tip locations are superimposed in Fig. 5 on an atlas figure of the dorsal hippocampus.
Experiment 4. 48 h delay between new location training and retraining to the original location

**Experiment 4. Methods**

**Phase 1: original location training**
Training to an initial location for 16 rats was the same as Experiment 1.

**Phase 2: new location training (drug day)**
These procedures were identical to those in Experiment 1.

**Phase 3: competition test**
Retraining to the original location was the same as in Experiment 1, with the key exception that Phase 3 was performed 48 h instead of 24 h after Phase 2. The purpose of this change was to ask if the interference of consolidation caused by CPP was persistent after 48 h. The extra delay between new location training and retraining was motivated by the finding in an earlier study (Santini et al., 2001) that extinction memory impaired at 24 h could be recovered after 48 h.

**Experiment 4. Results**

**Phase 1: original location training**
As in earlier experiments, rats later assigned to the saline- and CPP-injected groups learned to go to the original hidden platform location over equivalent time courses (Fig. 7A); escape latency was less than...
10 s by the third training day. An ANOVA verified the impression of a significant effect of Day $F_{3,18} = 56.68$, $P < 0.001$, but neither a Group nor an interaction effect was observed.

Phase 2: new location training (drug day)

Learning of the new platform location during massed training is shown in Fig. 7B. Here, the saline-injected rats show a longer escape latency during the first trial block (trials 1 and 2) than the CPP-injected rats. This may be due to a reduced tendency for the CPP-injected rats to search in the vicinity of the original platform location. In any case, performance is equal for the two groups after the first trial block. This overall description of new location training is confirmed by a two-way ANOVA with repeated-measures, which reveals a significant effect of Trial Block $F_{7,42} = 43.2$, $P < 0.001$, and Trial Block by Group interaction $F_{7,42} = 5.34$, $P < 0.001$. Planned comparisons indicated a trend for better performance by CPP-injected rats on the first trial block but the effect was not significant.
Phase 3: competition test
When rats were retrained to the original location 48 h after new location training we once again saw the same lack of impact of the massed training during Phase 2 (Fig. 7C) for CPP-injected rats; the average latency on trial 1 was only 9.7 s. In contrast, saline-injected rats at first took much longer (27.3 s) to arrive at the escape platform. An ANOVA for the first swim (the competition test) revealed a significant Group effect $F_{1.6} = 10.4, P < 0.05$. An ANOVA for the remaining swim trials showed no significant effects. The only change across trials is the rapid learning by the saline-injected rats that the platform was back in its original location.

Experiment 5. Post-training injections of CPP: immediate vs. delayed
The previous experiments suggest that NMDAR blockade has a deleterious effect on the representation acquired during massed training to the new platform location. It is unclear, however, if the NMDAR blockade weakened the new representation or if it impaired post-training memory consolidation processes. A third possibility is the preference for the old location during the competition test in CPP-injected rats is a state-dependent effect such that CPP is a conditional stimulus that signals that the old platform location is the correct choice. To distinguish among these possibilities CPP injections were made immediately after new location training or 6 h later. If the acquired representation is defective or if the drug effect is due to state dependence, post-training injections should have no effect during the competition test. In contrast, if the choice of the old location is due to interference with consolidation, the immediate CPP injection should have the same effect as injection during new location training.

To determine which of these scenarios is valid, an experiment was completed in which the CPP injection was given at different times after the massed training session (immediate or 6 h delay). This will test the idea that NMDAR inactivation disrupts post-training memory consolidation processes in the hippocampus and that these effects are not state dependent.

Experiment 5. Methods
Phase 1: original location training
Training to an initial location for 16 rats was the same as Experiment 1.

Phase 2: new location training (drug day)
These procedures were identical to those in Experiment 1, except that separate groups of rats were either given an injection of CPP immediately after training or 6 h after the massed training session.

Phase 3: competition test
Retraining to the original location was the same as in Experiment 1.

Experiment 5. Results
Phase 1: original location training
As evident in Fig. 8A, all groups of rats learned to find the hidden platform over the 4 days of training, although the control group was faster at reaching the platform than the other groups. A two-way ANOVA with repeated-measures indicated significant effects of Group

General discussion
To test the idea that NMDA receptor activation is involved with post-training consolidation of rapidly acquired spatial information and not the acquisition of such information we designed a three-phase variant of the hidden platform water maze task. To ensure that rats were procedurally sophisticated before blockade of NMDARs they were pretrained during Phase 1 to asymptotic performance to an
Phase 2 consisted of rapid (< 2 h) massed training to a second, new platform location that was used for two reasons. First, previous water maze experiments (Saucier & Cain, 1995; Hoh et al., 1999) showed that pretrained rats could learn, during rapid massed training, to go to a hidden platform during blockade of NMDARs. Second, earlier work with place cells (Kentros et al., 1998) and extinction of cued fear conditioning (Santini et al., 2001) showed that other sorts of plastic changes could take place in the time span of 2–3 h after injection with an NMDAR antagonist while NMDAR-dependent forms of plasticity were blocked (Abraham & Mason, 1988; Kentros et al., 1998; Villarreal et al., 2002).

Phase 3 was designed to test if learning to swim to the new platform location affected initial choices when the platform was returned to its original location (Experiments 1, 3 and 4) or removed from the pool to permit a probe trial (Experiment 2). In this sense, the first trial of Phase 3 is a competition test; it measures the degree to which the swimming behaviour is controlled by the most recently acquired spatial location compared with control by a previously acquired location. A similar competition test was used previously in the water task (McDonald & White, 1994), where the idea was to test the relative ability of learned habits encoded in the dorsal striatum vs. spatial representations encoded in the hippocampus to guide behaviour.

Fig. 8. Outcome of Experiment 5. (A) Phase 1. Time course of learning to go to the original platform location in the south-east quadrant in the pool. The swim latency decreased for the (pre-CPP) rats designated to be injected with (6)-3-(2-carboxy-piperazin-4-yl) propyl-1-phosphonic acid (CPP) (immediately or 6 h after training) in the same manner over the 4 days. However, the (pre-saline) rats designated to be injected with saline immediately after training learned the platform location more readily than the other groups. (B) Phase 2. Rats learned to go to the new location during rapid acquisition training and were subsequently given i.p. injections of 10 mg/kg CPP immediately or 6 h after training, or were given injections of saline immediately after massed training. Each Trial Block represents data for two training trials (± SE). (C) Phase 3. Twenty-four hours after Phase 2, rats were retrained to the original platform location. The first swim shows a shorter latency for the rats injected with CPP immediately after training to traverse the original platform location. The remaining seven swims also show that rats given CPP injections immediately after training were faster to locate the platform position.
Using this design, we report two main findings. First, in five very similar repetitions of the basic protocol, rats administered the NMDA receptor blocker CPP are able to learn to go to a new platform location during a 2 h massed training session as fast as vehicle-injected rats; overall, there was no sign of retarded learning due to interference with NMDAR based transmission. This result is in agreement with those of Sauzier & Cain (1995) and Hoh et al. (1999), but is in conflict with the interpretation that performance deficits in the swimming task are due to sensorimotor difficulties.

The second main finding is that CPP administration interferes with consolidation of memory for the new platform location. At the beginning of retraining to the original location (Phase 3), the performance of control rats was distinctly slower than before new location training. This pattern of behaviour suggests that in the competition test of Phase 3 the most recently acquired spatial representation dominated control over location preference. In contrast, at the beginning and throughout retraining to the original location, CPP-injected rats performed as well as they did before new location training. Thus, the most recently acquired spatial representation had little influence on goal choice, as if they had little or no memory of the intervening massed training of Phase 2.

Three additional findings should be noted. First, the amnesia for the new location is as clear after 48 h of delay between Phase 2 and Phase 3 of Experiment 3 as it was for the 24 h delay in the other experiments. Thus, the additional time between drug administration and testing does not allow for recovery of memory, as was the case for extinction of cued fear conditioning (Santini et al., 2001). This difference may be due to the much greater complexity of the memory required for swimming to a certain goal.

Second, the overall pattern of results is the same after bilateral injections of CPP directly into the hippocampus as it was after i.p. injection, strongly implying that the hippocampus is a critical site for post-training memory consolidation of rapidly acquired spatial information. At first glance, the difference in the rate of new location learning for CPP-treated rats in Phase 2 of Experiments 1 and 3 suggests that the transient impairment after peripheral injections is due to CPP action at extra-hippocampal NMDARs. The normal learning rate during Phase 2 of Experiments 2 and 4 implies, however, that such receptors do not contribute to the ability to learn the new goal location or to the ability to perform properly. Indeed, the normal learning and performance during NMDAR blockade suggests that this component of glutamatergic transmission is not essential for the sensory, motor or motivational systems that support navigation or spatial memory (Keith & Rudy, 1990).

The ability of i.h. injections of CPP to act in precisely the same way as peripheral injections also militates against the idea that swimming to the original goal location in the competition test is a conventional state-dependent effect. The usual explanation of state-dependent learning is that the drug acts as a discriminative stimulus such that the context of learning during drug action is different than the context of testing after drug action. The localized distribution of CPP to the hippocampus suggests that synapses in sensory (or motor) systems are unaffected by the same manipulation that limits consolidation of the new location learned in Phase 2.

To explicitly test the idea that the preference for the original location during Phase 3 testing is not a state-dependent effect, we administered CPP after new location training in Experiment 5. The fact that this manipulation had the same effect as CPP administered before the start of new location training strongly militates against a state-dependent explanation and moreover argues that the apparent amnestic effect is not due to a failure to generate a fully competent representation during massed new location training after CPP injections.

Comparison with related results

In line with inferences drawn from the remapping of place cells in a novel environment (Kentros et al., 1998) and the extinction of cued fear conditioning (Santini et al., 2001), the work described here is a third example in which adhering to a protocol of training, rapid retraining and testing at 24 h leads to the same conclusions: NMDARs need not be active for retraining to be possible but, if they are not, a stable trace of the retraining fails to be formed.

In rats injected with CPP, the normal remapping of place cells after exposure to a novel environment was interpreted as most likely due to the operation of a non-NMDA-dependent form of plasticity, but it was impossible to exclude an alternative view in which the new firing patterns were taken as sensory-like responses to a new set of stimuli without any need to invoke plasticity. The water maze data presented here demonstrate that an unstable form of spatial, hippocampal-dependent learning is possible during NMDAR blockade. Our results therefore tend to support the idea that remapping during CPP intoxication depends on a form of plasticity and is not merely a shift in response to sensory stimulus changes. The neuropharmacological basis of this other form of plasticity is not currently known, but likely candidates include other glutamate receptors including subtypes of AMPA and metabotropic receptors. Further research is needed to understand this form of plasticity and its relationship to NMDAR plasticity.

Despite a somewhat different protocol, a similar outcome is found when the NMDAR antagonist AP5 is injected into perirhinal cortex, a brain region important in recognition memory (Brown & Aggleton, 2001). AP5 injections leave intact the preference at 20 min but abolish the tendency to prefer the novel object at 24 h, a result parallel to our work, even though there is no initial pretraining phase.

In general, the delayed match-to-position results of Steele & Morris (1999) are consistent with our report. In the delayed match-to-position study a rat must learn that the platform position it finds on the first swim of each day is the same on the subsequent three swims for that day. If the interval between the first and second trials is short (15 s), rats administered i.h. AP5 do as well as rats administered ACSF. In contrast, interposing 20 min or 2 h delay interferes with performance on trial 2. Thus, NMDAR blockade has little effect over a very short retention interval but causes performance deficits over longer intervals. The problem here is that the 20 min interval (and probably the 2 h interval) is within the time range where we find that rats are able to learn at a normal rate to go to a new position, a process similar to a 1-day version of delayed match-to-sample. It is unclear how to reconcile this different time dependence unless the repetitive nature of the delayed match-to-sample task shifts the burden of information storage from a non-NMDAR-dependent to an NMDAR-dependent form. A similar difference in the time over which the initial learning was stable is seen after APV blockade of NMDAR in contextual fear conditioning where post-training freezing lasted for only 3 min (Kim et al., 1992).

In another important study (Shimizu et al., 2000), expression of the NMDAR1 subunit in CA1 was switched by addition of a drug to the drinking water of mice and then back on by removal of the drug. In a key experiment, 7 days of training was followed by 7 days of deletion in this way of NMDAR1 subunits followed by an additional 7 days of incubation time during which the NMDAR1 subunit population recovered. Testing 15 days after the end of training revealed swimming deficits according to swim latency, and a probe test performed without the platform. Later deletion of NMDAR1 subunits from Day 9 to Day 14 did not interfere with performance on Day 15. In this work, therefore, memory problems are found even if NMDARs are active during learning and testing so long as they are inactive.
during a consolidation period starting just after training. If consolidation is allowed to proceed normally for 7–8 days, removal of NMDAR function before and during testing does not induce difficulties. Differences in protocol between our work and that of Shimizu et al. (2000) are sufficiently great that it would be valuable to repeat their experiment with repetitive injections of CPP or AP5.

In summary, our results suggest the following. (i) The acquisition and short-term storage of spatial information rely on a non-NMDAR-dependent form of memory. We imagine but cannot show that the storage site is hippocampal. (ii) NMDAR-mediated plasticity in the hippocampus is required during training for post-training consolidation of spatial information. We conclude that the role of NMDARs in memory is more limited than originally proposed and that the overall process of memory storage is bound to be very complex (Martin & Morris, 2002).

Abbreviations

ACSF, artificial cerebrospinal fluid; CPP, (6)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate; NMDAR, N-methyl-D-aspartate acid receptors.

References


