

Memory Reconsolidation: Sensitivity of Spatial Memory to Inhibition of Protein Synthesis in Dorsal Hippocampus during Encoding and Retrieval

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Summary

Reconsolidation is a putative neuronal process in which the retrieval of a previously consolidated memory returns it to a labile state that is once again subject to stabilization. This study explored the idea that reconsolidation occurs in spatial memory when animals retrieve memory under circumstances in which new memory encoding is likely to occur. Control studies confirmed that intrahippocampal infusions of anisomycin inhibited protein synthesis locally and that the spatial training protocols we used are subject to overnight protein synthesis-dependent consolidation. We then compared the impact of anisomycin in two conditions: when memory retrieval occurred in a reference memory task after performance had reached asymptote over several days; and after a comparable extent of training of a delayed matching-to-place task in which new memory encoding was required each day. Sensitivity to intrahippocampal anisomycin was observed only in the protocol involving new memory encoding at the time of retrieval.

Introduction

The purpose of this study was to examine the generality of the concept of memory “reconsolidation” (Misanin et al., 1968; Nader et al., 2000; Przybyslawski and Sara, 1997). Memory consolidation refers to the progressive postacquisition stabilization of a memory item and to the temporal phase when this stabilization takes place. The classic consolidation hypothesis (Dudai and Morris, 2001; McGaugh, 1966; Muller and Pilzecker, 1900) postulates that consolidation starts and ends just once for each item in long-term memory. In contrast, the reconsolidation hypothesis holds that items in long-term memory can re-enter a consolidation phase when reactivated. Upon reactivation, the stabilized memory item transiently destabilizes and again engages neuronal mechanisms of restabilization. Accordingly, memory reactivation be-

comes susceptible to amnesic agents, such as protein synthesis inhibitors.

The notion of reconsolidation has been with us since the 1960s (Misanin et al., 1968) but has recently been revitalized by a series of experiments, triggered mostly by the studies of Nader et al. (2000) on fear conditioning in the rat. These authors reported that postretrieval infusions into the lateral amygdala of the protein synthesis inhibitor anisomycin, a widely used consolidation blocker, disrupted the subsequent retrieval of rapidly acquired fear memory. Anisomycin in the absence of memory reactivation had no effect. The amnesic effect of postretrieval anisomycin infusion was long lasting. In additional studies from the same group, hippocampal lesions disrupted contextual fear conditioning when given 45 days after training, again providing that the memory was reactivated shortly before the lesion was made (Debiec et al., 2002). Reconsolidation has also been reported in other memory paradigms and in species ranging from molluscs and bees to humans (Dudai and Eisenberg, 2004; Nader, 2003; Sara, 2000b).

Despite the growing number of reports that support the reconsolidation hypothesis, alternative interpretations have been raised to account for the postreactivation effects of amnesic agents. One suggestion is that reconsolidation is a lingering consolidation process associated with the original training (Alberini, 2005; Dudai, 2004). According to this view, consolidation may stop temporarily and then be restarted by memory reactivation, with some differences in the underlying neural mechanisms associated with training- and reactivation-associated forms of consolidation. Another possibility is that behavioral phenomena that may appear to indicate a “reconsolidation” process might actually reflect a reactivation-locked, temporary inability to access memory traces that dissipates over time (Lattal and Abel, 2004). A third possibility is that apparent amnesia is the consequence of blocking a retrieval-associated updating process that is required to keep old memories useful and retrievable (Dudai and Eisenberg, 2004). It is also becoming clear that reconsolidation is not a universal feature of memory retrieval, rendering the identification of its generality and boundary conditions all the more important (Eisenberg et al., 2003; Nader et al., 2005; Pedreira and Maldonado, 2003).

Relatively few recent studies have investigated reconsolidation in relation to allocentric spatial memory (e.g., Przybyslawski and Sara, 1997; Suzuki et al., 2004). Spatial memory is rapidly acquired, like fear conditioning and inhibitory avoidance (Milekic and Alberini, 2002; Nader et al., 2000), but certain training protocols can result in performance reaching an asymptote at which point no new memory encoding needs to occur because the spatial environment is adequately represented in memory for the task in hand. We hypothesized that this would be a point at which retrieval-associated reconsolidation would no longer be required unless, due to changes in the environment, new memory encoding was engaged. In other spatial learning protocols, the state of the environment may be changing all the time,

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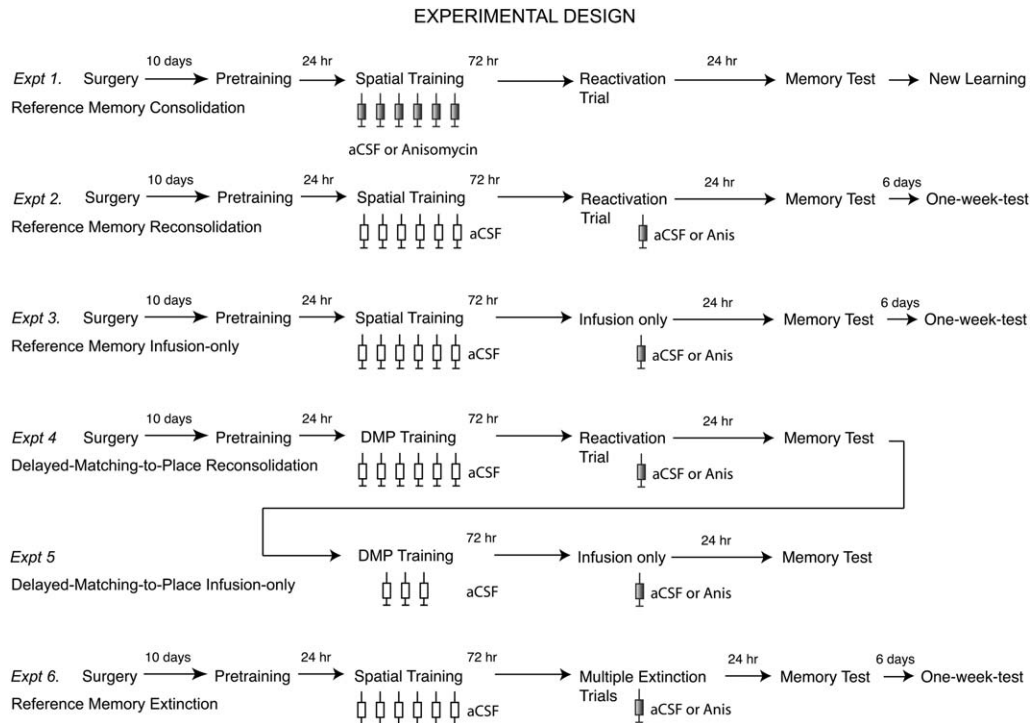


Figure 1. Experimental Design

The series of procedures for the six experiments are depicted, together with intervals of time between training stages. Bilateral intrahippocampal drug infusions are depicted as syringe cartoons of two types. Open syringes are aCSF infusions; shaded syringes refer to either aCSF or anisomycin infusions across groups. Reactivation trials, extinction trials, and test probes were all procedurally identical but are named in relation to their distinct purpose within each study. Means \pm 1 SEM.

with the brain systems responsible for representing allocentric space attempting to keep track of altered relationships. Under these circumstances, memory encoding will remain engaged at the time of retrieval, with the memory representations that have been subject to consolidation being rendered labile and, as a consequence, subject to reconsolidation. Securing positive findings of a differential effect of intrahippocampal anisomycin would establish boundary conditions that favor reconsolidation of spatial memory in a theoretically cogent framework using an intervention that acts in a regionally specific manner.

Results

To investigate whether a reconsolidation process is specifically engaged when the act of retrieval of spatial memory is, or is likely to be, associated with new memory encoding, we compared two distinct hippocampus-dependent water maze tasks. Only one of these tasks was likely to involve new memory encoding in association with retrieval at the equivalent point in training on each when reconsolidation was being investigated.

The design of our training protocols was predicated on the capacity of the paradigms that have been used to date to reveal reconsolidation, coupled with the desire to calibrate any findings against effects upon consolidation and/or the extinction of spatial learning (Abel and Lattal, 2001; Lattal and Abel, 2004). We began by developing a *single* training protocol in the water maze (Figure 1) that could be used to study consolidation (experi-

ment 1), reconsolidation (experiment 2), and nonspecific drug effects (experiment 3). This used the “on-demand” (Panakhova et al., 1984) or Atlantis Platform (Spooner et al., 1994) procedure in the first training trial of each day to monitor memory retrieval unaffected by new learning or reminding. In this procedure, the escape platform only becomes available after 60 s of trial 1 on each day, allowing that trial to serve as a reinforced memory probe trial during training and post-memory reactivation treatments. A change in the behavioral training protocol to a delayed matching-to-place (DMP) procedure was then introduced for experiments 4 and 5, with the extent of overall training kept equivalent. The relevant feature of DMP is that, irrespective of the extent of training, new memory encoding is required each day as the location of the escape platform changes. Importantly, the same extent of training (6 days) was used in both the reference memory and DMP experiments. Finally (experiment 6), we examined the impact of intrahippocampal anisomycin on extinction. Separate autoradiographic measurements of the uptake and incorporation of [14 C]L-leucine were also made to establish that anisomycin was successful in inhibiting protein synthesis locally when infused into the dorsal hippocampus.

Blockade of Protein Synthesis in the Dorsal Hippocampus

Autoradiographic imaging and quantitative densitometric analysis of [14 C]L-leucine uptake into the brain enabled the extent and spatial distribution of the inhibition of protein synthesis to be visualized and quantified.

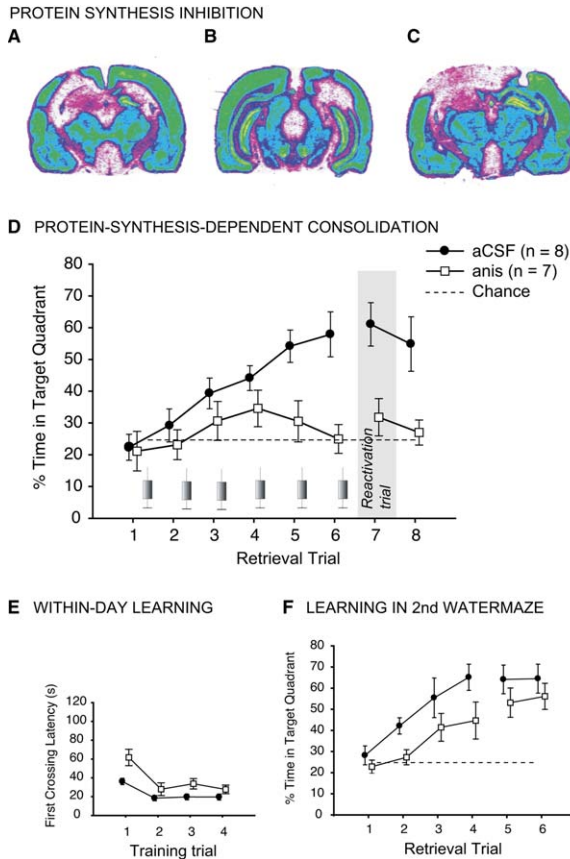


Figure 2. Protein Synthesis Analysis and Consolidation of Spatial Reference Memory—Experiment 1

Autoradiographic images of [¹⁴C]-leucine localization reflect greater signal density over the cell bodies of hippocampal subfields in the absence of anisomycin. Representative brain sections were chosen to reflect the following: (A) Localized inhibition of protein synthesis in the dorsal hippocampus at the site of infusion on the left of the image (white regions). (B) In the same animal as (A), the limit of this inhibition, with near normal protein synthesis in the posterior and ventral hippocampus. (C) In a different animal, the maximal extent of neocortical involvement around the injection site. (D) In the consolidation control study, the time spent in the target quadrant during the first trial of each session's training is plotted for retrieval tests 1–6, the memory reactivation trial (test 7), and the postreactivation memory test (test 8). Drug infusions are given after each training session. Note that anisomycin-treated rats failed to show overnight memory of the location of the platform (chance = 25%). (E) First crossing latency (s) for the four trials of each session averaged across sessions 1–6. Animals treated with anisomycin after each session are capable of learning and show a striking reduction in latency between training trials 1 and 2. (F) The proportion of time spent in the target quadrant during new learning in the second context (water maze 2). No drug infusions are given. The previous anisomycin-treated animals can learn, catch up the previous aCSF group, and show equivalent levels of performance during retrieval tests 5 and 6. Means ± 1 SEM.

Typical examples of the impact of unilateral intracerebral anisomycin are shown in Figure 2. Close to the injection site, at the septal pole of the dorsal hippocampus (Paxinos and Watson, 1998), protein synthesis inhibition was well localized at the infusion site (Figure 2A). Inhibition in parts of the contralateral hippocampus was seen in three out of the six animals, but this is unimportant in the context of the bilateral infusions of anisomycin

Table 1. Uptake of [¹⁴C]-leucine into Hippocampus following Local Infusion of Anisomycin or aCSF

Hippocampal Area	Tracer Concentration (nCi/g)		Percentage Change
	aCSF	Anisomycin	
Septal (Dorsal)			
CA1	98 ± 18	6 ± 4*	-94
CA2	113 ± 6	5 ± 2*	-96
CA3	116 ± 17	5 ± 2*	-96
Dentate	145 ± 20	9 ± 5*	-94
Total hippocampus	123 ± 13	4 ± 3*	-97
Temporal (Ventral)			
Subiculum	70 ± 15	18 ± 12*	-74
CA1	99 ± 6	108 ± 16	+9
CA2	102 ± 6	109 ± 10	+6
CA3	133 ± 15	128 ± 18	-4
Dentate	125 ± 20	64 ± 48*	-49
Total hippocampus	110 ± 16	91 ± 16	-17

Data are presented as mean ± SD (n = 6 in each group). *Significantly different from hippocampus injected with aCSF (p < 0.05, paired t-test).

used in the behaviorally trained animals. At a more posterior and temporal location of the longitudinal axis, the area of inhibition was more circumscribed, with much of the ventral hippocampus unaffected (Figure 2B). There was some evidence of inhibition in extrahippocampal regions—including overlying neocortex. Figure 2C shows the animal in which this was most marked. However, there also appeared to be leakage of anisomycin from the injection site into the ventricular system, and in all animals, there were some indications of partial inhibition in periventricular tissues, such as hypothalamus (Figures 2A and 2C) and periaqueductal gray matter (Figure 2B). The patterns of inhibition found in contralateral hippocampus suggest that here too anisomycin gained access via the ventricular system, rather than spreading by bulk flow.

Quantification of the autoradiographic images (Table 1) shows that the uptake and incorporation of [¹⁴C]-leucine was substantially reduced in hippocampal cell populations close to the injection of anisomycin. In dorsal CA1, for example, tissue tracer concentrations were reduced from a mean of 98 nCi.g⁻¹ on the aCSF-injected side to 6 nCi.g⁻¹ on the anisomycin-injected side, a reduction of 94%. In the septal pole of the hippocampus as a whole, tracer levels were reduced by 97%. Given that residual levels of tracer in trunk blood remained quite high at the time of sacrifice (93 ± 2 nCi/ml), the contribution of the blood compartment in brain to the total tissue concentrations will prevent the measured values ever reaching zero. Thus, the primary site of action was the dorsal and middle regions of the hippocampal formation.

Histological Analysis

All behaviorally trained rats were sacrificed at the end of the experiment, and the brains were carefully removed from the skull and associated “headcap” containing the cannulae. Once stained, brain sections were carefully examined for tissue damage. The tips of bilateral cannulae were found to be located in the dorsal hippocampus of all rats reported above, with minimal tissue damage affecting the target structure. All animals

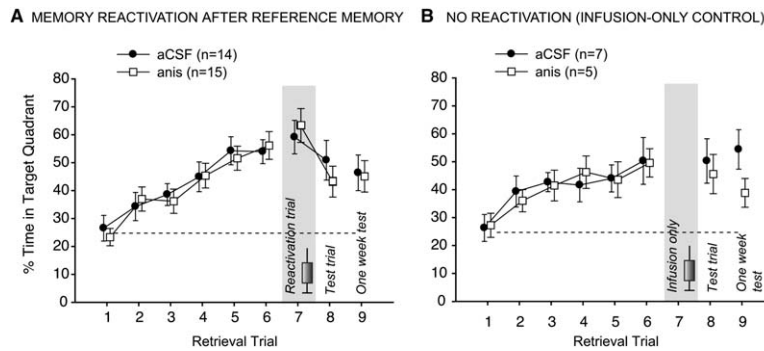


Figure 3. Reconsolidation of Spatial Reference Memory and Infusion-Only Control—Experiments 2 and 3

(A) Time spent in the target quadrant during training, memory reactivation, and the retrieval test and 1 week test trials. The drug infusions occurred immediately after retrieval test 7 (memory reactivation). Anisomycin failed to disrupt memory tested on session 8. (B) In the infusion-only control task, drug infusions occurred on session 7, but exposure to the water maze was omitted. There was no change in performance in retrieval test 8 from that observed at the end of training, reflecting the omission of the memory reactivation/extinction trial. Means \pm 1 SEM.

infused with aCSF were rated as having brain damage scoring 0 or 1 (see [Experimental Procedures](#) for the scoring system). Three anisomycin-treated animals were rated as having scores of 2 or more in experiment 1, and one animal with a score of 3 was excluded from the data analysis, with the threshold for inclusion set at unilateral damage only. Five anisomycin animals were rated as having a score of 2 in experiment 2, and all were included in the analysis. In experiment 4, two anisomycin animals were rated as having scores of 3 or above and were excluded from the data analysis. The group numbers in each of the studies are of animals *included* after histological assessment.

Consolidation of Reference Memory Is Impaired by Anisomycin—Experiment 1

As both experiments 2 and 4 (below) used four trials of training per day, a control study was conducted to confirm that daily post-training injections of anisomycin into the dorsal hippocampus would preclude the consolidation of spatial memory trained in this way. Impaired overnight memory consolidation was revealed in an analysis of the proportion of time spent in the correct quadrant of the water maze during the daily probe tests ([Figure 2D](#)). Over days 1–6, the ANOVA showed no overall difference between groups ($F = 3.55$, df 1/13, $p = 0.08$), a significant improvement across successive retrieval tests ($F = 9.36$, df 5/65, $p < 0.001$) and, critically, a highly significant groups \times retrieval tests interaction ($F = 5.05$, df 5/65, $p < 0.001$). A separate analysis was conducted of performance in the memory reactivation and post-reactivation memory tests (sessions 7 and 8), revealing an impairment in the anisomycin-treated group ($F = 8.27$, df 1/13, $p < 0.025$). All analyses were also conducted using the measure introduced by [Moser et al. \(1995\)](#) of time spent in a 40 cm diameter zone around the correct location; these revealed an identical pattern of statistically significant effects (data not shown).

Within-session learning (averaged across all six training sessions) indicated that the anisomycin group took longer to cross the correct location of the platform on trial 1 but nonetheless showed effective within-session learning ([Figure 2E](#)). The ANOVA revealed a decline in latency across the four daily trials ($F = 35.23$, df 3/39, $p < 0.001$) and a significant groups \times trials interaction ($F = 3.35$, df 3/39, $p < 0.05$). There was a significant difference between groups on trial 1 ($F = 6.16$, df 1/14, $p < 0.05$), pointing to overnight forgetting in the anisomy-

cin-treated animals as indexed by time to reach the platform.

Training in WM2 without drug infusions revealed successful learning by the group previously treated with anisomycin ([Figure 2F](#)). Both groups showed a steady improvement in memory retrieval across the four probe trials ($F = 15.54$, df 3/39, $p < 0.001$). The groups did not differ on sessions 5 and 6 of the new learning phase ($F < 1$). The modest trend reflecting apparently faster learning by the group previously given aCSF ($F = 3.41$, df 1/13, $p > 0.05$) most likely reflects the benefit of the animals’ successful earlier training in WM1 rather than any (nonsignificant) deficit in the anisomycin group. Indeed, a comparison of performance on session 4 of the new learning protocol ([Figure 2F](#)) with session 4 of training in the initial spatial training period ([Figure 2D](#)) confirms that the animals previously treated with anisomycin were now learning at a similar rate to the aCSF group during initial training (percent time in target quadrant: aCSF = $44.1\% \pm 3.9\%$; Ani = $47.5\% \pm 9.6\%$). Thus, the group previously treated with anisomycin could learn a spatial location in a new environment normally; i.e., it displayed no lasting functional deficit, a “behavioral histology” finding compatible with the exacting selection of animals based on true histological criteria. A deficit might have been seen had animals with partial brain damage been included, and the failure to use such exacting histological criteria could, in turn, have compromised the interpretation of experiments 2 and 4 below.

Retrieval of Asymptotic Spatial Reference Memory Is Unaffected by Anisomycin—Experiment 2

[Figure 3A](#) shows performance in the daily probe tests (trial 1 of each day). The groups did not differ during training ($F_s < 1$) and showed equivalent excellent search performance during the memory reactivation trial of session 7 (72 hr after session 6). In absolute terms, the proportion of time spent in the target quadrant during the retrieval test of session 7 ($57.78\% \pm 3.29\%$ averaged across groups) was equivalent to that obtained by the aCSF group in experiment 1 ($61.06\% \pm 6.8\%$).

Bilateral intrahippocampal infusion of anisomycin following the memory reactivation trial of session 7 did not result in poorer performance in the postreactivation test probe the next day ($F < 1$). Performance declined between sessions 7 and 8 ($F = 15.62$, df 1/27, $p < 0.001$), reflecting extinction, but this decline did not differ across groups ($F = 1.90$, df 1/27, $p > 0.10$; performance in

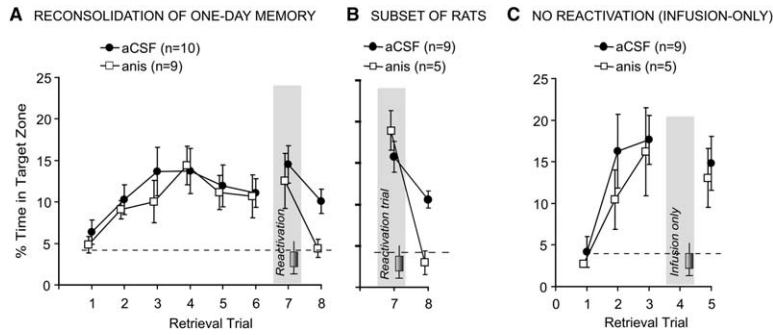


Figure 4. Reconsolidation of 1 Day Memory (DMP) and Infusion-Only Control—Experiments 4 and 5

(A) Time spent in the target zone during training (sessions 1–6), the memory reactivation, and test probe trials. The drug infusions occurred immediately after probe test 7 (memory reactivation) scheduled 72 hr after session 6 (as in experiment 2). Anisomycin caused a clear disruption of memory to chance level (4%) when tested 1 day later. (B) The subset of animals that individually showed effective memory (twice chance level) during retrieval test 7. The decline to chance in test 8 is confirmed.

(C) In the infusion-only control, which could be run within-subjects for the DMP task, drug infusions occurred on session 4, but exposure to the water maze was omitted. There was no effect of anisomycin in the absence of memory reactivation. Means \pm 1 SEM.

retrieval test 8: aCSF = $46.9\% \pm 5.4\%$; ANIS = $44.6\% \pm 4.2\%$). While there was a trend for the decline to be greater in the anisomycin group, it did not approach significance, despite the large group sizes. When tested again 1 week after the reactivation trial, the two groups again did not differ ($F < 1$).

Anisomycin Infusion without Memory Reactivation Also Has No Effect on Spatial Reference Memory—Experiment 3

Experiment 3 was conducted to examine the nonspecific effects of intrahippocampal infusions of anisomycin on long-term spatial memory. Figure 3B shows that neither group differed during the 6 days of training with respect to time spent in the target quadrant ($F < 1$). An ANOVA revealed no difference between groups across retrieval sessions 8 and 9 ($F < 1$) and, despite an apparent trend in the 1 week test, no interaction across sessions. Importantly, performance in probe test 8 showed no decline from the end of training.

Retrieval of 1 Day Spatial Memory Is Impaired by Anisomycin—Experiment 4

In the DMP task, the animals learn a platform location during the four daily trials and a new location on each of the days thereafter for as long as training continues. An effective strategy is acquired across days, in which the rats show a small but highly significant tendency to search in the previous session's platform location on the first trial of each day (retrieval) and immediately after encoding information about the new platform location (Steele and Morris, 1999). Accordingly, memory retrieval reaches an above chance asymptotic mean that remains relatively stable across days (Figure 4A). In a target zone of 40 cm diameter centered on the varying locations of the escape platform in a 1 meter radius pool, the ratio of surface areas is 25:1, leading to a chance level of 2.5 s spent searching in the target zone (i.e., 4%; chance may actually be slightly higher, as the animals quickly develop a tendency to search away from the side walls, and the area ratio calculation underestimates this). Target search rises from 4% to 6% to a relatively stable mean of 10% to 14% across days 1 to 6, reflecting modest but significant memory (ANOVA: days $F = 3.80$, $df 3.8/64.6$ $p < 0.01$; Greenhouse-Geisser correction) with no difference between groups as a function of their subsequent drug treatment ($F < 1$).

The critical comparison is the change in performance between sessions 7 and 8, each consisting of a memory retrieval trial without the possibility of escape onto the platform (Figure 4A). The ANOVA showed a significant decline between these sessions ($F = 7.24$, $df 1/17$, $p < 0.025$) and that the groups did not differ on session 7 (conducted 72 hr after session 6 as in experiment 2; $F < 1$) but did differ on session 8 ($F = 9.56$, $df 1/17$, $p < 0.01$). The group treated with anisomycin after memory retrieval on session 7 showed apparently complete forgetting in retrieval test 8. Relative to the estimated chance level, both groups showed above chance memory on session 7 ($ps < 0.05$), but only the aCSF group was above chance on session 8 ($p < 0.01$). This pattern is consistent with a reconsolidation effect, with the caveat that the Groups \times Days interaction for sessions 7 and 8 did not quite reach significance. The reason for this may have been because the mean target search performance of sessions 1 through 6 masks day-to-day variability in the learning of each session's new location by individual animals and their memory of it the next day. As it is not possible to evaluate reconsolidation in an individual animal that performed poorly on session 7 just prior to drug treatment (even if that same animal did well on previous days), we considered only the subset of rats that reached a threshold of session 7 search time at least twice the chance level (i.e., $>8\%$ searching in the target zone). This reduced the numbers of animals assessed (from 19 to 14) and revealed a significant Group \times Days interaction for sessions 7 and 8 ($F = 4.94$, $df 1/12$, $p < 0.05$; Figure 4B). The group mean consequently shows higher values of time spent searching the target on session 7 (16.5%) but shows chance performance again on session 8 by the anisomycin group. This legitimizes the selection of animals and argues against it having caused a mere regression to the mean. In addition, if the zone measure is calculated in a different way, looking only at the proportion of time spent in each of the six zones used throughout training, the Groups \times Days interaction for days 7 and 8 remains significant ($F = 5.79$, $df 1/13$, $p < 0.05$; denominator df increases by 1 as an additional anisomycin animal could then be included). In absolute terms, the mean search time in the target zone for the anisomycin group dropped from 43.0% to 17.2% between days 7 and 8.

Given this apparent "reconsolidation" effect, it would have been desirable to assay whether the poor memory of the anisomycin group on session 8 was transient

(e.g., a retrieval deficit) and might have recovered over 7 days. Unfortunately, the DMP protocol does not lend itself to such an analysis as, over time, all animals revert to searching all over the pool as they have been trained to do with the daily relocation of the platform.

Anisomycin Infusion without Memory Reactivation Has No Effect on 1 Day Spatial Memory—Experiment 5

This control study used the same subset of 14 animals of experiment 4 that had shown good memory retrieval on day 7 of experiment 5. After an interval of 10 days, these animals were retrained on the DMP task for 3 days, given aCSF or anisomycin the next day without memory being reactivated, and then a probe trial on session 5 (Figure 1). No differences between groups were found over days 1 through 3 ($F < 1$) or on day 5 ($F < 1$; Figure 4C).

Extinction of Spatial Reference Memory Is Unaffected by Anisomycin—Experiment 6

Figure 5 shows performance in the daily probe tests for the extinction experiment. The groups did not differ during the 6 days of training or during extinction ($F_s < 1$). A high proportion of time was spent searching in the target quadrant on the first memory retrieval trial of session 7 ($52.5\% \pm 4.1\%$ averaged across groups). This declined across the eight repeated unrewarded probe trials of extinction (probe tests 7 through 14; $F = 8.82$, $df\ 7/70$, $p < 0.001$). Infusion of anisomycin or aCSF immediately after these multiple extinction trials had no differential impact upon performance the next day. Target search was much lower for both groups in retrieval test 15 (session 8; mean = $34.9\% \pm 2.7\%$ averaged across groups), although still above chance ($t = 3.65$, $df\ 11$, $p < 0.005$). During a further memory retrieval trial conducted 6 days later (test 16), no spontaneous recovery was seen for either group (mean across groups = $25.2\% \pm 3.4\%$; $F = 1.39$, $df\ 1/11$, $p > 0.10$), although there was a nonsignificant trend for the group given anisomycin earlier to perform slightly better (Groups \times Tests interaction, $F = 2.07$, $df\ 1/10$, $p > 0.10$).

When the findings of experiments 2 and 6 were compared, we observed that the overall level of performance on session 9 was significantly higher after one trial of memory “reactivation” than after eight trials of “extinction” ($F = 10.47$, $df\ 1/36$, $p < 0.005$), with no difference between groups across the two studies ($F = 1.18$, $df\ 1/36$, $p > 0.10$). Thus, eight trials of extinction did cause a greater decrease in spatial memory than a single “reactivation/extinction” trial. However, extinction was unaffected by anisomycin.

Discussion

The key new finding is that the sensitivity of reactivated spatial memory in the water maze to local inhibition of protein synthesis in dorsal hippocampus is a function of the kind of spatial memory task that has been trained. In separate groups of animals all given an equivalent extent of training over 6 days, reactivation of a spatial memory acquired in four trials in the immediately preceding session (DMP task) was sensitive to anisomycin when tested the next day, whereas a memory that had been acquired over the full 6 days was not. The difference cannot just have been a matter of memory

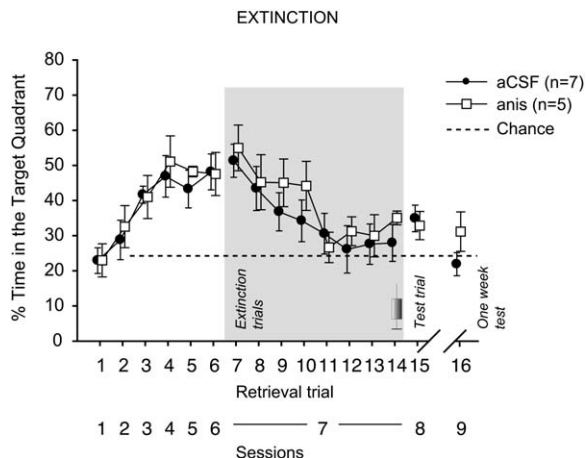


Figure 5. Extinction of Spatial Reference Memory—Experiment 6
Time spent in the target quadrant during training, and the subsequent extinction trials, memory test, and 1 week test probe trials. The drug infusions occurred immediately after the single session in which extinction trials 7–14 were scheduled. Both groups showed extinction of spatial memory in the retrieval test (session 15) relative to that shown in session 7 (both are first trials of the day) and compared to retrieval test 8 of experiment 2 above. There was no spontaneous recovery of spatial memory shown during the 1 week test. Means \pm 1 SEM.

“updating,” as post-trial anisomycin in the DMP task returned performance to chance levels. No significant effect of anisomycin on allocentric spatial memory was detected in the absence of memory reactivation in either task. Key control studies revealed that the four trials/day protocol used in experiments 2 and 5 was sensitive to anisomycin, that extinction of spatial memory was unaffected, and that the uptake and incorporation of [^{14}C]L-leucine following intrahippocampal infusions of anisomycin revealed regionally localized inhibition of protein synthesis in the hippocampus. This reversible inhibition did not impair the ability of the treated animals to learn a second water maze task in a different room later.

The central theoretical issue that these data raise is whether they support or argue against a process of “memory reconsolidation” with respect to spatial memory. We shall argue that these data are consistent with the reconsolidation hypothesis, but at the same time constrain the conditions under which the reconsolidation of spatial memory takes place. Specifically, they suggest that the engagement of a memory-encoding mode during the act of spatial memory retrieval may be one requirement for reconsolidation to be observed. This occurs in spatial tasks when animals are confronted by spatial novelty or “mismatch” that triggers exploration and the updating of their cognitive representation of space (O’Keefe and Nadel, 1978).

The Value and Limitations of Local Anisomycin Infusion

Some recent experiments on reconsolidation have used microinfusions of anisomycin into targeted brain areas (Debiec et al., 2002; Eisenberg et al., 2003; Nader et al., 2000; Runyan and Dash, 2005), whereas others have used peripheral injections of this and other less-specific

drugs (Anokhin et al., 2002; Litvin and Anokhin, 2000; Suzuki et al., 2004; Taubenfeld et al., 2001). Peripheral administration has the merit of being neutral regarding the cerebral localization of the putative reconsolidation process, but the twin disadvantages of precluding the identification of the main target of action and potentially increasing the likelihood of unwanted effects on physiology and behavior (e.g., the transient sickness that often accompanies systemic injections of anisomycin).

Central administration of drugs overcomes these problems but is not without its own difficulties—including potential tissue damage and an uneven diffusion through the target brain area. One potential artifact to consider was the possibility that infusions of anisomycin cause permanent damage in the hippocampus and that this, rather than direct effects of the drug, impairs spatial performance. However, not only can this not explain the differential outcome of experiments 2 and 4 (which both had a single infusion), but also our ratings of the extent of brain damage indicate that any infusion-associated damage was both minimal and equivalent in the two studies. Moreover, multiple infusions of anisomycin in experiment 1 did not prevent subsequent learning of a second water maze in the drug-free state.

A separate issue is that local infusion may affect only one or a subset of the brain areas that subserve a neuronal process, an issue that is analytically complicated, as one area may be important during encoding and initial storage (e.g., the hippocampus) with others being more important for certain types of consolidation (e.g., selected regions of the neocortex). These are potential shortcomings of our experiments, but, overall, we consider local drug administration to be more informative than systemic administration in the long run. Our autoradiographic observations revealed protein synthesis inhibition to be very substantial, but it did not extend the full length of the longitudinal axis of the hippocampus. The more posterior and ventral regions were unaffected. Given that learning occurred while the animals were untreated, resulting in the likely potentiation of a sparse network of synapses throughout the entire hippocampus (Moser and Moser, 1998b), it is possible that sufficient protein synthesis could take place in the unaffected regions to enable reconsolidation to occur normally. However, there are three reasons to question this criticism. First, if sufficient protein synthesis is possible in the ventral hippocampus to enable reconsolidation in experiment 2, it should also have been sufficient to enable consolidation in experiment 1. Second, the DMP task is exquisitely sensitive to lesions of the dorsal hippocampus, suggesting it to be the likely site of initial memory trace formation within the hippocampus (de Hoz et al., 2005). Third, lesion studies reveal that when damage occurs after learning, lesions of as little as 30% of hippocampal volume are sufficient to limit subsequent memory retrieval (Moser and Moser, 1998a), whereas the same small lesions may have a lesser effect when given before training. This is presumably due to the dispersed nature of the sparse representation of the spatial information acquired by a normal brain when learning the task. It seems parsimonious to assert that similar constraints should apply to other post-training treatments (lesions and drugs) that may affect memory consolidation and reconsolidation.

Theoretical Implications: A Dual Encoding and Retrieval State Is Required for Reconsolidation of Spatial Memory

The reconsolidation hypothesis postulates that items in long-term memory undergo new cycles of consolidation upon their reactivation (Misanin et al., 1968; Nader, 2003; Przybyslawski and Sara, 1997). This need not imply that each consolidation cycle is mechanistically identical to the preceding or subsequent consolidation cycles (Bahar et al., 2004; Lee et al., 2004; Taubenfeld et al., 2001; Tronel et al., 2005; von Herten and Giese, 2005), but still, the reactivated memory is assumed to become transiently unstable and, as during learning, prone to disruption by at least some amnesic agents. Data in line with the reconsolidation hypothesis have been accumulated over the years in many systems involving multiple species and learning paradigms (Dudai, 2004; Nader, 2003; Sara, 2000a).

At first sight, our findings seem to extend the generality of the reconsolidation phenomenon to rapidly but not slowly acquired forms of allocentric spatial memory, echoing findings on reconsolidation of hippocampal-dependent tasks in studies using peripheral injections of anisomycin (Suzuki et al., 2004). This also parallels the presence of hippocampal-dependent reconsolidation following the retrieval of context fear conditioning that is also learned in 1 day (Debiec et al., 2002). However, with longer training that is subject to a hippocampally localized protein synthesis-dependent consolidation process (as shown in experiment 1), retrieval no longer seems to engage a protein synthesis-dependent mechanism in the dorsal hippocampus (experiment 2). Re-testing memory 7 days later also fails to reveal any effect of anisomycin that might have been masked in the first test. In contrast, if animals are required to learn a new location each day, they can retain new spatial information overnight, but the retrieval of such a memory continues to reactivate a protein synthesis-dependent consolidation-like process (experiment 5). Reconsolidation may therefore occur in relation to rapidly acquired spatial memory, with extent of training of a specific location being a key parameter. However, “extent of training” is merely an operational description, and it would be better to understand why this parameter influences the engagement of reconsolidation.

The difference between the memory processes challenged in experiments 2 and 5, respectively, provides a clue to the nature of this condition. Recent data have provided evidence for ideas such as the behavioral dominance of the memory trace and the balance between extinction and nonextinction (Eisenberg et al., 2003; Pedreira and Maldonado, 2003), the strength of the original memory (Suzuki et al., 2004), and its age (Eisenberg and Dudai, 2004; Litvin and Anokhin, 2000; Milekic and Alberini, 2002) as factors influencing the likely engagement of reconsolidation. The delayed matching-to-place (DMP) task (Steele and Morris, 1999) points to another possibility related to its simultaneous demands for both retrieval and new encoding. This analytically powerful task is sometimes incorrectly categorized as “working memory,” but it is really one in which parts of an animal’s experience are stable over time (e.g., *that* escape is possible) while other parts are not (e.g., *where* it is possible). Lower absolute levels of performance are typically

observed compared to a reference memory paradigm, even when extent of training is matched as here, because the protocol is such that in recalling on day $N + 1$ where the platform was on day N , the animal finds that the escape platform has moved (or on a probe trial is absent). Thus, over the training period, trial 1 involved both the *retrieval* of where the platform was in the previous session and the immediate opportunity to *encode, store, and consolidate* a new platform location. Engaging each of these processes during the 6 days of DMP training would then be likely to extend to include the memory retrieval trial of session 7 conducted 72 hr later. Session 7 was a nonrewarded probe trial, but the cognitive acts of retrieval, platform searching, attending to extramaze cues, and even failing to find the platform are likely to engage memory-encoding processes, just as they do during training. That it is nominally an “extinction” trial is no reason to assume that new encoding could not occur (Berman et al., 2003).

The hippocampus has been proposed to honor the distinction between memory encoding and retrieval, by sometimes rapidly recruiting different neuronal mechanisms at different phases of the theta cycle (Hasselmo et al., 2002). These differential activity configurations could render the circuit differentially sensitive to amnesic agents, with the engagement of an encoding phase favoring a plastic, transiently unstable state in the coherently activated network that encodes the representation. It is noteworthy that mismatch between expected and actual events in a retrieval session can trigger memory extinction or reconsolidation of contextual fear conditioning in the crab *Chasmagnathus* (Pedreira et al., 2004). Such mismatch is expected to drive encoding (Rescorla and Wagner, 1972). Similarly, exposure to a novel context together with a learned stimulus from a different trained context will trigger second-order conditioning whose consolidation and reconsolidation are sensitive to anisomycin (Tronel et al., 2005).

Alternative Accounts

An alternative way of conceptualizing our findings might be to suppose that the rat is engaged in no more than ongoing learning and/or updating of what it has retrieved (Dudai and Eisenberg, 2004). If so, what would be affected by the protein synthesis inhibitor after retrieval on day 7 is the consolidation of memory updating, a proposal that fits within the conceptual framework of the classical consolidation hypothesis and may not require an explicit reconsolidation process (Rodríguez-Ortiz et al., 2005). However, our data argue against this parsimonious interpretation. As can be seen in Figures 4A and 4B, inhibition of protein synthesis returns the performance to the pretraining chance level, a finding difficult to reconcile with disruption of memory updating only. We therefore suggest that the memory reactivation session somehow induces protein synthesis-dependent plasticity in the *original* trace and not only in its updated portions. In other words, the engagement of a memory-encoding state during reactivation triggers reconsolidation or is at least a necessary condition for its occurrence.

A separate issue is that session 7 of experiment 5 is an extinction trial. Experiment 6 was therefore important, as it established that a hippocampal-dependent protein

synthesis-dependent process is not directly engaged by extinction of the spatial reference memory task despite the consolidation of this task during acquisition being dependent on protein synthesis. It is therefore unlikely that the sensitivity of memory reactivation in the DMP task to anisomycin has to do with extinction. Indeed, to the contrary, the postsession application of anisomycin *decreased* the performance seen on session 8 rather than protected it. That the extinction of water maze spatial reference memory can be sensitive to peripheral injections of anisomycin (Suzuki et al., 2004) raises the possibility that its long-lasting memory traces are actually in cortex rather than hippocampus, a view compatible with the concept of systems-level consolidation.

One could argue that there is a confounding factor across the two spatial tasks between relative memory strength (Suzuki et al., 2004) and the requirement for new encoding (the view expressed here). The spatial memory developed over 6 days of the reference memory task is quite strong and directs highly localized spatial search, whereas that following 6 days of the DMP task reflects only the learning that has occurred in the previous session. We recognize this inescapable constraint on the experimental design but felt that matching the numbers of days and trials of training across the two distinct tasks was vital. Further work might investigate the impact of anisomycin on retrieval after 24 trials of reference memory training given within a single session or increasing the numbers of trials per day of the DMP task relative to the reference memory task. Even if the animals reach some asymptote of low escape latency and optimal search during a single session of the reference memory task, there is no guarantee that new memory encoding will not be engaged on session 2. This is because systems-level consolidation is a time-dependent process that cannot be short-circuited by extended training in 1 day. However, even with extended training, we predict that retrieval in the DMP task would remain sensitive to protein synthesis inhibition irrespective of the number of trials of training given each day or its dependent consequence, memory strength, because the dual retrieval/encoding mode would be automatically engaged. It would also be valuable to investigate whether reactivation of spatial memory in the DMP task engages the synthesis of C/EBP β and, like Tronel et al. (2005), use this to dissociate the relative contributions of consolidation and reconsolidation.

To summarize, our findings point to a differential contribution of hippocampal protein synthesis to the consolidation, reconsolidation, and extinction of spatial reference memory in a water maze. They suggest that postretrieval sensitivity to protein synthesis inhibition can be observed in circumstances that favor a new encoding mode during memory reactivation. Memory encoding is therefore proposed as a further boundary condition on reconsolidation.

Experimental Procedures

Subjects

The data from a total of 86 experimentally naive male Lister Hooded rats were used. The animals were housed individually in plastic cages with ad libitum access to food and water. A 12 hr light/dark cycle was maintained, with all the testing carried out in the light phase (7 am to 7 pm). The series of studies were run in replicates,

each consisting of up to 16 animals. Each replicate included a balanced number of rats treated with aCSF or anisomycin.

Apparatus

Spatial training was conducted using two water mazes (Morris, 1981, 1984), each consisting of a large circular tank (2.0 m diameter) of water (depth, 0.6 m; temperature, $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) made opaque by the addition of 150 ml of latex solution. Each water maze was located in a separate room (upstairs pool, downstairs pool) distinguished by their extramaze cues. A 12 cm diameter Atlantis Platform (Spooner et al., 1994) was hidden at a fixed location in each water maze. This platform is initially submerged such that its top surface is >30 cm below the surface and then is raised automatically and at an appropriate time to its normal position of 1.5 cm below the water. This enables (1) the selective reinforcement of swimming to and dwelling at the correct location in the pool during training through the use of suitable animal tracking software; and (2) rewarded probe tests to be scheduled on trial 1 of each training day by allowing the rat to escape onto the now raised platform after a 60 s swim. The animals' swimming behavior was monitored by an overhead video camera, a video recorder, and a commercially available online data acquisition system that used video frame-grabbing software (Watermaze Software, Edinburgh, UK) that digitizes the path taken and computes various parameters. This allowed the collection of objective measures of the paths taken by the rats as they searched for the platform (e.g., latency, path length, swim speed, time in quadrant of the pool, or zone around platform, etc.) from which it is possible to make inferences about their knowledge of its spatial location. A separate computer-based image analysis system (MCID, Ontario, Canada) was used for autoradiographic analysis of protein synthesis inhibition.

Behavioral Protocols

The primary aim was to examine, successively, the impact of intrahippocampal anisomycin infusions upon the *acquisition/consolidation* of spatial reference memory acquired over 6 days (experiment 1), the *reconsolidation* of such memory when it was reactivated 72 hr after training (experiment 2), and upon memory after *nonreactivation* (experiment 3). These were followed by the experiments using the DMP paradigm to examine the impact of intrahippocampal protein synthesis inhibition upon the *reconsolidation* of a spatial memory that was retrieved 72 hr after having been acquired in 1 day (experiment 4) and upon subsequent memory after *nonreactivation* (experiment 5). Finally, the *extinction of spatial memory* was studied after prior training in the reference memory protocol (experiment 6). The series also included quantitative measurements of the extent of inhibition of protein synthesis in the hippocampus.

Surgery

Bilateral guide cannulae for subsequent infusion of drugs (Plastic One Inc, 26 gauge with stylets) were implanted bilaterally into the dorsal hippocampus (AP, -4.5 mm; lateral, ± 3.0 mm; ventral, 3.0 mm from dura) under tribromoethanol (Avertin) anesthesia using standard stereotaxic techniques. The cannulae were secured by means of dental cement and small skull screws, and the animals were allowed to recover for at least 14–21 days prior to the start of all behavioral training.

Drugs and Infusions

Anisomycin was dissolved in equimolar HCl, diluted with aCSF and adjusted to pH 7 with NaOH to produce a final concentration of anisomycin of $125 \mu\text{g} \cdot \mu\text{l}^{-1}$. The artificial cerebrospinal fluid (aCSF) was made using pyrogen-free (injectable) water and consisted of NaCl (150 mM), KCl (3 mM), CaCl_2 (1.4 mM), MgCl_2 (0.8 mM), Na_2HPO_4 (0.8 mM), and NaH_2PO_4 (0.2 mM). During post-training or post-memory retrieval drug infusions, the rats were restrained lightly in a towel and drug or aCSF infused at a rate of $0.25 \mu\text{l} \cdot \text{min}^{-1}$ over 8 min. The infusion cannulae (bilateral) were left in place for a further 2 min, and the animal then returned to its home cage. The times when these infusions were given are noted for each experiment below.

Cued Pretraining

Prior to starting any procedures in each experiment, all rats were handled extensively and "habituated" to the water maze by giving them a single 60 s swim trial in water maze 1 (WM1) with no escape platform present. On the following day, the animals were given eight

trials of cued pretraining in WM1 (curtains were drawn around the pool to occlude extramaze cues, hidden escape platform at four separate locations in the pool, four start locations, Atlantis Platform lowered at the start of each trial, rat had to swim for 1 s below a hanging cue that indicated the platform position in order to raise it to a position near the surface that would afford escape from the water, max trial duration = 120 s, 30 s on platform at the end of each trial). All animals concluded this phase effectively, and, for clarity, the data are not presented.

Spatial Training

All animals were then trained over 6 days to locate a hidden platform in a fixed or varying location within WM1. The platform position was counterbalanced (NE for half the animals, SW for the remainder) in the reference memory experiments (experiments 1–3 and 6), but occupied many different locations in the DMP experiments (experiments 4 and 5). Except where noted, the Atlantis Platform either came up automatically after 60 s on a probe test (trial 1 of each day) or after the rats had swum for "dwell" periods of 1 to 2.5 s in a zone whose dwell radius could be specified by the experimenter. Training was for 6 days (four trials per day, max trial duration = 120 s, Atlantis Platform on all trials, a probe test lasting 60 s on trial 1 throughout training with, thereafter, the platform raised only by localized swimming, 30 s on the platform after each swim trial). The dwell radius was set at 20 cm (Dwell times: day 1 = 1 s; day 2 = 1.5 s; day 3 = 2 s; days 4–6 = 2.5 s). Trial 1 of each day enabled the gradually developing memory for the platform location to be tested daily. As trial 1 of each day was a rewarded probe test, the latency to the "first crossing" of the platform's position was used in analyses of time taken to reach the platform's location instead of escape latency.

Experiment 1: Consolidation of Spatial Reference Memory

The aim of the first "control" study ($n = 15$) was to confirm that bilateral, intrahippocampal infusions of anisomycin ($250 \mu\text{g}$ per hippocampus) would block the consolidation of long-term spatial memory. To do this, either anisomycin ($n = 7$) or aCSF ($n = 8$) was infused immediately (<5 min) after the end of each animal's four trials of training over sessions 1–6 of spatial training (as described above). A 60 s nonrewarded probe test was conducted on session 7 (72 hr after session 6), without any post-testing drug infusions, and again on session 8 (24 hr after session 7). For probe test 7—the memory reactivation trial—the animals were started from either the nominal adjacent-left or adjacent-right quadrants of the pool (i.e., never the target or opposite quadrants) and lifted out from the pool while swimming when the 60 s swim period was completed without any opportunity to climb onto the hidden platform. Training in WM1 was followed by training in WM2 to check whether anisomycin infusion caused any lasting functional disruption of the brain. No drug infusions were given. One hour after completion of the probe test on session 8, animals were taken to WM2 to learn an escape location in a novel environment. The rats were then trained for 4 days using a similar protocol to that used in WM1. The dwell radius was set at 20 cm (the dwell times were: day 1 = 1 s; day 2 = 1.5 s; day 3 = 2 s; days 4 = 2.5 s). Tests of the animals' ability to retrieve a memory of the location of the platform, or to extinguish the memory, were then conducted 72 and 96 hr after completion of training (sessions 5 and 6).

Experiment 2: Reconsolidation of Spatial Reference Memory

The aim of this study ($n = 29$) was to examine whether bilateral, intrahippocampal infusions of anisomycin blocked reconsolidation of long-term spatial memory. To do this, only aCSF was infused immediately (<5 min) after the end of each animal's four trials of training over sessions 1–6 of spatial training (as described above). Memory retrieval was then examined by means of 60 s nonrewarded probe test on session 7, 72 hr after session 6. Half the animals were given an immediate postretrieval infusion of anisomycin ($n = 15$) and the other half given aCSF ($n = 14$). Start locations were as in experiment 1. A memory probe test was scheduled on day 8. On session 7, as in experiment 1, the animals were lifted from the pool when the 60 s swim period was completed, without any opportunity to climb onto the hidden platform.

Experiment 3: Nonreactivation (i.e., Infusion-Only Control)

This study ($n = 12$) was identical to experiment 2 in every respect except that exposure to the water maze on session 7 (memory

reactivation) did not occur. There were a total of 12 animals that were accepted after testing and blind histological assessment: aCSF ($n = 7$), anisomycin ($n = 5$).

Experiment 4: Reconsolidation of 1 Day Memory

This study ($n = 19$) examined whether bilateral, intrahippocampal infusions of anisomycin blocked reconsolidation of spatial memory acquired in 1 day. As in experiments 2–4, aCSF was infused immediately (<5 min) after the end of each animal's four trials of training over sessions 1–6 of spatial training. The key difference to experiment 2 was that the hidden platform moved location between sessions. Memory retrieval for the previous session's training location was examined throughout training by means of 60 s rewarded probe test on trial 1. A single, nonrewarded memory retrieval trial was given on session 7, at the same 72 hr interval after session 6, followed by an immediate infusion of anisomycin ($n = 9$) or aCSF ($n = 10$). A memory probe test was scheduled on day 8.

Experiment 5: Nonreactivation of 1 Day Memory (i.e., Drug Infusions Only)

This study ($n = 14$) used the same subset of the animals in experiment 5 that had shown good retention on day 7 of that task. After a 10 day delay, these animals were retrained on the DMP task for three sessions, then given aCSF ($n = 9$) or anisomycin ($n = 5$) infusions on session 4 without exposure to the water maze (i.e., no memory reactivation). A memory retrieval test was given as session 5. As in experiment 3 above, the aim was to examine whether bilateral, intrahippocampal infusions of anisomycin had nonspecific effects on spatial memory unconnected with reconsolidation.

Experiment 6: Extinction of Spatial Reference Memory

This study ($n = 12$) examined whether bilateral, intrahippocampal infusions of anisomycin blocked the extinction of long-term spatial memory. Initial training was as in experiment 2. The key difference was that a series of eight successive extinction trials were scheduled on session 7, beginning 72 hr after session 6, followed by an immediate infusion of anisomycin ($n = 5$) or aCSF ($n = 7$). These eight extinction trials lasted for 60 s each. They all started from the adjacent-left and adjacent-right quadrants of the pool in semirandom sequence and terminated with the animals being lifted from the pool. A further memory probe test was scheduled on session 8, and 6 days later, on session 9.

Protein Synthesis Inhibition

A semiquantitative assay of de novo protein synthesis in the brain (Smith, 1991) was conducted using a subset of six animals that had previously been trained behaviorally (the aCSF animals of experiment 4). To enable each animal to serve as its own control, one hippocampus was infused with anisomycin and the other, simultaneously, with aCSF. Otherwise, the injection protocol followed exactly the procedure of the behavioral experiments. Twenty minutes following the end of the intracerebral injections, a bolus of [14 C]-leucine (Amersham Biotech; specific activity 59 mCi.mmol $^{-1}$) was injected into the tail vein (7.5 μ Ci.100 g $^{-1}$) and the animals returned to their home cage for the subsequent 60 min. At the end of this period, the animals were decapitated and trunk blood collected into heparinized centrifuge tubes. The brains were dissected intact and rapidly frozen in precooled 2-methylbutane (-45° C). Frozen brains were mounted onto specimen holders with embedding medium (Lipshaw) and stored overnight at -80° C. Whole-blood samples were centrifuged (13,000 \times g for 60 s), and 20 μ l aliquots of plasma were taken for liquid scintillation analysis to determine blood concentrations of tracer at the end of the experiment.

The brains were sectioned (20 μ m) in the coronal plane using a cryostat maintained at -22° C. Three consecutive sections from every 100 μ m cut throughout the rostro-caudal axis of the hippocampus were thaw mounted onto a glass coverslip and rapidly dried on a hot plate (75° C). In areas of the brain more rostral and caudal to hippocampus, three sections were collected from every 400 μ m of tissue sectioned. Autoradiograms were prepared by applying these sections, together with a series of eight precalibrated [14 C]-standards (40–1069 nCi/g tissue equivalents: Amersham Biotech, UK), to X-ray film (Kodak, SB-5) in light-tight cassettes for 7 days. At the end of the exposure time, the films were processed according to the manufacturer's instructions. Sections adjacent to those

used for autoradiography were mounted onto gelatine-coated slides and stained with cresyl violet.

Analysis of the autoradiograms was performed using a computer-based image analysis system (MCID/M5+). The background density of the films was measured, and local tissue isotope concentrations were derived from the optical density of autoradiographic brain images, following background subtraction, relative to the [14 C]-standards. Measurements of tracer levels in hippocampal subfields were taken from three sets of consecutive sections at the level of the habenula (bregma -3.30 mm approx.) and at the level of the medial geniculate (bregma -5.80 mm approx.). Thus for each subfield at each level, tracer levels were derived from the mean of nine measurements for each side of the brain separately. To determine the concentrations of tracer found in the hippocampus as a whole, the outline of the structure was delineated using cresyl violet sections, the area stored on the computer, and then superimposed upon the adjacent autoradiographic images.

Histology

At the end of the experiments, all rats were deeply anesthetised, and their brains were removed and postfixed in 10% formalin. They were sectioned in the coronal plane throughout the region of the cannula placements (40 μ m sections) and stained with cresyl violet. The sections were assessed "blind" with respect to the behavioral data by at least two observers (J.I., J.A.A., and R.G.M.M.); rats included in the statistical analysis had to have (1) the cannulae terminating in the dorsal hippocampus bilaterally; and (2) a maximum of slight unilateral tissue damage at the termination site. The histological ratings were conducted quantitatively with ratings given as follows: 0 = no brain damage other than that inevitably caused by the cannulae; 1 = minimal damage to the hippocampus at the cannulae tips; 2 = infusion-associated but strictly unilateral hippocampal damage; 3 = extensive unilateral and minor contralateral hippocampal damage; 4 = extensive bilateral damage associated with infusions of aCSF or anisomycin. Animals were only included in the study with scores of 2 or less.

Data Analysis

All data were analyzed using ANOVA, with group sizes determined after the blind histological assessment. Conventional measures of performance in water maze experiments include escape latency, path length, and swim speed during training, and percent quadrant or target zone occupancy during post-training probe tests. Here, our use of the Atlantis Platform to encourage focused searching in the correct target location necessitated different measures of performance during training and memory probe trials. On training trials, the platform would not rise if a rat swam accurately to the target area but stayed there for a slightly shorter period than that session's required dwell time. Typically, the animal returns to the correct area and then remains long enough to activate raising of the platform. First crossing latency is then a more appropriate measure than overall escape latency. Swim speeds did not differ across groups that always received their drug infusions after behavioral testing, and path length was not then used, as it tends to show slightly greater variability than latency scores due to occasional tracking errors. Thus, the three main measures of performance used were (1) first crossing latency (s) during training; (2) percent time spent swimming in the target quadrant during reference memory retrieval tests; and (3) percent time spent swimming in a target zone during DMP probe tests. The retrieval tests were always on the first trial of each session and, as described above, involved the hidden platform being raised to within 1.5 cm of the water surface only after 60 s had passed.

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