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Cellular and Systems Reconsolidation in the Hippocampus

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Summary

Cellular theories of memory consolidation posit that new memories require new protein synthesis in order to be stored. Systems consolidation theories posit that the hippocampus has a time-limited role in memory storage, after which the memory is independent of the hippocampus. Here, we show that intra-hippocampal infusions of the protein synthesis inhibitor anisomycin caused amnesia for a consolidated hippocampal-dependent contextual fear memory, but only if the memory was reactivated prior to infusion. The effect occurred even if reactivation was delayed for 45 days after training, a time when contextual memory is independent of the hippocampus. Indeed, reactivation of a hippocampus-independent memory caused the trace to again become hippocampus dependent, but only for 2 days rather than for weeks. Thus, hippocampal memories can undergo reconsolidation at both the cellular and systems levels.

Introduction

The formation of long-term memory (LTM) is generally believed to involve a process by which a labile short-term memory (STM) is converted into a lasting stable trace (Ebbinghaus, 1885; Hebb, 1949; Müller and Pilzecker, 1900). Evidence for this time-dependent process has come from numerous studies, showing that treatments such as electroconvulsive shock (ECS) produce amnesia shortly after learning, but the same treatment several hours later has no effect (Duncan, 1949; McGaugh, 1966). The dominant view of how the conversion from STM to LTM occurs is that new RNA and proteins are synthesized and transform temporary alterations in synaptic transmission into persistent modifications of synaptic architecture (Davis and Squire, 1984; Flexner et al., 1965; Goelet et al., 1986). This is called consolidation theory, or more precisely, cellular consolidation theory (Dudai and Morris, 2000).

Cellular consolidation theory was challenged by early studies demonstrating that amnesia could also occur if a fully consolidated and stable LTM was reactivated prior to amnesic treatments (Misanin et al., 1968). This phenomenon has been described in a large number of species, using a wide array of behavioral paradigms and amnesic agents (Sara, 2000). These findings suggested that old, reactivated memories undergo another round of consolidation, a process referred to as reconsolidation (Nader et al., 2000b; Przybyslawski and Sara, 1997). Consistent with the reconsolidation hypothesis is our recent demonstration that consolidated memories for auditory fear conditioning, which are stored in the amygdala (Fanselow and LeDoux, 1999; Maren, 2001; Schafe et al., 2000), undergo protein synthesis-dependent reconsolidation in the amygdala and that this process is contingent on memory reactivation (Nader et al., 2000a). Indeed, reconsolidation and consolidation have been found to share a number of common properties, including: (1) requirement of protein synthesis in order for the memory to persist, (2) time windows during which protein synthesis blockade is effective, and (3) that protein synthesis blockage in the same brain region, the amygdala, disrupts both. Given these similarities, it seemed parsimonious to conclude that a new memory and a reactivated, consolidated memory share a common memory state, as originally proposed by Lewis (1979). Thus, instead of just occurring once, memory storage may instead be a process that is reiterated with each use of the memory.

A key issue is whether reconsolidation also occurs in other brain systems. The most extensively studied memory system of the brain involves the hippocampus. Results from previous studies have suggested that memories for hippocampus-dependent tasks can undergo reconsolidation (Mactutus et al., 1979; Przybyslawski et al., 1999; Schneider and Sherman, 1968). For example, using a radial arm maze, systemic postreactivation injections of propranol were effective at producing amnesia if the memory was first reactivated (Przybyslawski et al., 1999). Because the treatment was systemic, however, it is not known whether the effects of the drug on reconsolidation occurred in the hippocampus or in some other structure that contributes to this task. Similarly, recent findings that disruption of CREB-mediated transcription in the forebrain interferes with the reconsolidation of contextual fear memories (Kida et al., 2002) suffer from the same drawback. In support of the possibility that memories stored within the hippocampus itself might undergo reconsolidation are the recent findings showing that reactivation of contextual memories induces the expression of *zif268,* a gene implicated in consolidation of new hippocampal-dependent memories (Hall et al., 2001).

In the present study, we first asked if hippocampal-mediated memories undergo protein synthesis-dependent reconsolidation in the hippocampus. The task we used was contextual fear conditioning, a variant of auditory fear conditioning in which the footshock comes to be associated with the chamber (context) in which the shock occurred. The hippocampus is thought to estab-

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lish the sensory/cognitive representation of the context that is then associated with the shock in the amygdala (Anagnostaras et al., 2001; LeDoux, 2000). Contextual fear conditioning is well suited for asking questions about cellular reconsolidation in the hippocampus since it is known that infusion of anisomycin into the hippocampus disrupts initial consolidation of such memory (Quevedo et al., 1999; Taubenfeld et al., 2001). The use of this paradigm in conjunction with targeted infusions of anisomycin into the hippocampus thus allowed the assessment of whether the reconsolidation findings from the amygdala apply to a different brain system (hippocampus) and for a qualitatively distinct kind of memory (sensory/cognitive representation of context) (O'Keefe and Nadel, 1978).

The term memory consolidation has a second meaning when applied to the hippocampus (Anagnostaras et al., 2001; Eichenbaum et al., 1994; Scoville and Milner, 1957; Squire and Alvarez, 1995). In addition to the cellular changes described above that occur within the hours immediately following learning, additional changes occur at the level of neural systems over a longer time frame (months in rats and years in humans), and these changes cause a memory that initially depends on the hippocampus to become independent of the hippocampus. One view of how this occurs is that initially the hippocampus forms a LTM (through a process of cellular consolidation). Over time, the memories become independent of the hippocampus and are stored in the neocortex (Anagnostaras et al., 2001; Eichenbaum et al., 1994; Marr, 1971; McClelland et al., 1995). For clarity, we will refer to a memory that has become independent of the hippocampus as a remote memory to distinguish it from a LTM that is still stored in the hippocampus. Thus, humans with hippocampal damage have better memory for old, rather than recent, memories, and lesions of the hippocampus in rats 1 day after training produce a severe impairment, but the same lesions 28 days afterwards have no effect (Kim and Fanselow, 1992; Scoville and Milner, 1957). The relative persistence of old over new memories is viewed as evidence for a temporal gradient of retrograde amnesia, and the restructuring of a memory from being hippocampus dependent to independent, is called systems consolidation (Dudai and Morris, 2000). Systems consolidation is obviously based on cellular consolidation in both the hippocampus and the neocortex. In addition to testing whether cellular reconsolidation occurs in the hippocampus, we therefore asked whether reactivation of a remote memory returns it to being hippocampus dependent again or not. If it does, systems reconsolidation would be demonstrated.

Results

Cellular Reconsolidation

Adult male Sprague-Dawley rats were placed in a conditioning chamber and given eight shocks at 62 s intervals (1.5 mA, 1 s duration). Three days later, they were returned to the conditioning chamber for a 90 s reactivation session and immediately afterwards infused with either ACSF or anisomycin 250 μg/2 μl/side into the hippocampus through implanted cannula. In order to

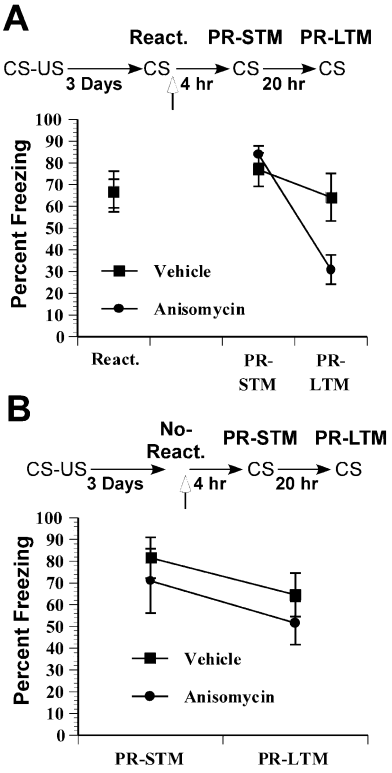

Figure 1. Hippocampal-Mediated Memories Undergo Protein Synthesis-Dependent Reconsolidation

(A–B) Schematic of the procedure used with the data presented below. Vertical open-headed arrows represent infusions. (A) Anisomycin infusions impaired PR-LTM, but not PR-STM. (B) Omitting memory reactivation protected the memory from being lost. This procedure was identical to (A) except that the contextual memory was not reactivated. Instead, animals were taken to a different room and given the infusions.

demonstrate a specific effect of anisomycin on consolidation of new memories, it is critical to demonstrate intact short-term memory (STM) and impaired long-term memory (LTM) (Schafe and LeDoux, 2000). Applying this logic to reconsolidation, we required intact behavior during a postreactivation short-term memory test (PR-STM) and impaired behavior in the same animals during a postreactivation long-term memory test (PR-LTM) (Nader et al., 2000a). During reactivation, the two groups exhibited comparable freezing scores (Figure 1A, $t(17) < 1$). An analysis of variance (ANOVA) comparing the drug treatment (anisomycin versus ACSF) and memory phase (PR-STM versus PR-LTM) revealed a significant interaction ($F(1, 17) = 9.4$, $p < 0.05$). Post hoc analysis revealed that in the PR-STM test, both groups were again comparable ($p > 0.05$); however, in the PR-LTM test, anisomycin-treated rats were impaired relative to the controls ($p < 0.05$). Given that in the same animals PR-STM was intact and PR-LTM impaired, this demonstrates that the hippocampus was functioning normally 4 hr after the expression of fear and the anisomycin infusions.

We considered two alternative interpretations of the deficit in the previous experiment. First, given that there are multiple time points during consolidation of new learning that require protein synthesis (Quevedo et al.,

1999), it is possible that anisomycin blocked a new late wave of protein synthesis that occurs 3 days after training and is required for the consolidation of the original trace. Second, anisomycin may simply have caused a lesion or other pathological change that took more than 4 hr to develop. This would explain the intact PR-STM and impaired PR-LTM. In order to test these two possibilities, we performed the same experiment as in Figure 1A except the contextual memory was never reactivated prior to drug infusions. Animals were given an infusion in a different room. Both of the alternate interpretations predicted an impairment in the PR-LTM test. Reconsolidation however, predicted no effect. Anisomycin infusions in the absence of memory reaction had no effect (Figure 1B). An ANOVA demonstrated there was no significant interaction between the groups and memory phases (F (1, 11) < 1), nor was there a main effect of group (F (1, 11) < 1). These findings are consistent with the proposal that hippocampal memories undergo cellular reconsolidation when reactivated.

It is possible that the drug spread into the brain's ventricles and affected reconsolidation by acting in some region other than the hippocampus, such as the amygdala. We tested this by performing the same experiment as experiment 1A but with the drug (same concentration and volume) infused directly into the ventricles. These infusions had no effect (Figure 2A). Both groups had comparable reactivation scores (t (13) < 1). An ANOVA revealed no significant interaction between the groups and memory phases (F (1, 13) < 1), nor was there a main effect of group (F (1, 13) < 1). These findings strongly suggest that the effects of anisomycin on reconsolidation were not due to anisomycin producing its effects on structures outside the hippocampus. This is not to say that intraventricular (ICV) infusions of anisomycin have no effect on reconsolidation in general. Rather, this low dose, which was effective when put directly in the hippocampus, was too dilute when put in the ventricles to produce reconsolidation by affecting other regions like the amygdala. Another explanation for the deficit seen in Figure 1A is that after the expression of fear induced by memory reactivation, anisomycin served as an unconditioned stimulus (US) to support a context-anisomycin association. During the PR-LTM test, the responses elicited by the context-shock and context-anisomycin association could have competed with each other, causing freezing to decrease. For example, if the context-anisomycin association produced conditioned hyperactivity only seen during the PR-LTM test, this could have compromised the animals' ability to freeze. To evaluate this possibility, rats were given a 90 s exposure to the environment during which a previously conditioned auditory fear cue was also presented (see Experimental Procedures). We used a protocol to condition the tone that leads to levels of freezing comparable to those obtained during contextual memory reaction. At the end of this 90 s period, rats received either vehicle or anisomycin injections. The next day, animals received fear conditioning and were tested for contextual freezing 3 days later. Directly pairing the anisomycin with the context after fear expression had no effect on the subsequent acquisition of contextual fear conditioning (Figure 2B). Both groups demonstrated comparable freezing during the 90 s tone presentation

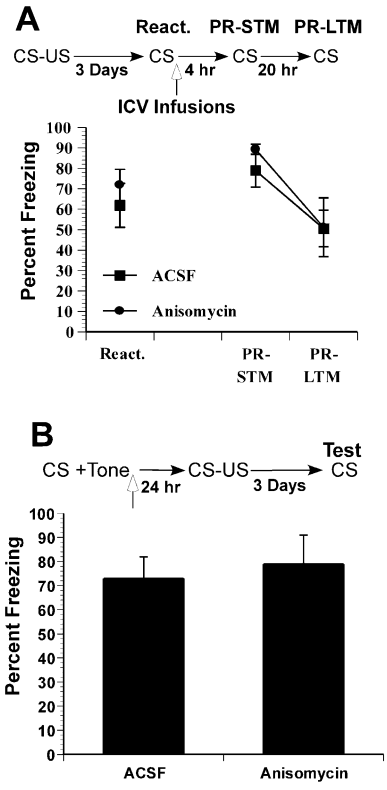

Figure 2. The Effects of Anisomycin Are Specific to the Hippocampus and Do Not Support Conditioned Competing Responses

(A) Anisomycin's effects are due to an action within the hippocampus. Intraventricular (ICV) infusions of anisomycin had no effects on reconsolidation.

(B) Anisomycin does not act as a US after fear expression to mediate conditioned responses that could compete with freezing. The CS is the context. The tone is a previously fear-conditioned tone that was presented for the duration of the preexposure period.

during context preexposure (ACSF = 62 $\pm$11.6 and anisomycin = 65.7 $\pm$ 5.3; t (13) < 1). This level of freezing was comparable to that seen in Figure 1A. On test, both groups again demonstrated comparable contextual freezing (t (13) < 1). This demonstrates that anisomycin was not acting like a US after fear expression to support conditioned responses that compete with freezing.

Together, these findings suggest that consolidated hippocampal sensory memories undergo cellular reconsolidation in the hippocampus as do auditory fear memories in the amygdala (Nader et al., 2000a). Consistent with this claim are the recent findings showing that reactivation of contextual memories induces the expression of *zif268*, which is implicated in consolidation of new hippocampal-dependent memories (Hall et al., 2001).

Does Reconsolidation Demonstrate a Retrograde Gradient?

Given that the hippocampus plays a time-limited role in consolidation of new memories, we asked whether the hippocampus would also show a time-limited effect during reconsolidation. To this end, we increased the time between training and reactivation from 3 to 15 or 45 days. By 45 days, memory for contextual conditioning

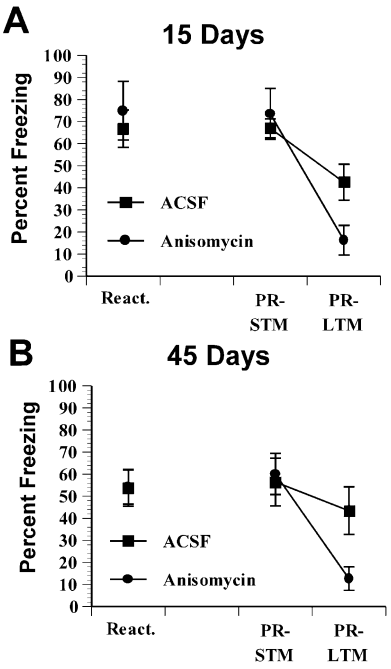

Figure 3. Increasing the Training-Reactivation Delay Has No Effect on the Memory's Ability to Return to a Labile State

Intra-hippocampus anisomycin blocks reconsolidation after memory reactivation (A) 15 or (B) 45 days after training. In both cases, a specific effect was found on PR-LTM, but not PR-STM.

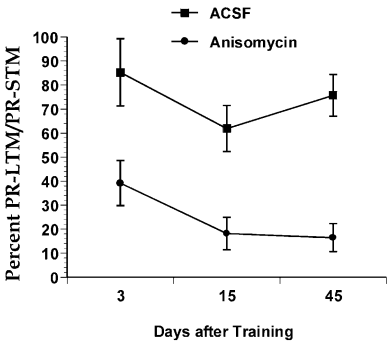

Figure 4. Reconsolidation Does Not Show a Temporally Graded Retrograde Amnesic Gradient

A memory index was computed for groups in the 3, 15, and 45 day experiment (PR-LTM/PR-STM %). Because the PR-STM test produces approximately 20% extinction, the controls lie at approximately 75%–80%.

is independent of the hippocampus (Anagnostaras et al., 1999; Kim and Fanselow, 1992) and presumably dependent on other cortical areas (Bontempi et al., 1999; Frankland et al., 2001). As a result, it was expected that anisomycin infusions into the hippocampus would have an effect at 15, but not 45, days.

Anisomycin infusions blocked the reconsolidation of a contextual memory that was reactivated 15 days after training (Figure 3A). The anisomycin and ACSF groups demonstrated comparable reactivation scores ($t(12) < 1$). An ANOVA comparing the groups with memory phases revealed a significant interaction ($F(1, 12) = 14$, $p < 0.05$). Post hoc analyses demonstrated that the groups only differed on their PR-LTM scores ($p < 0.05$).

Interestingly, even 45 days after training (when the contextual trace should have been hippocampus independent), intra-hippocampus anisomycin blocked the reconsolidation of the reactivated memory (Figure 3B). Both the anisomycin and ACSF groups demonstrated comparable reactivation scores ($t(20) < 1$). An ANOVA comparing the groups with memory phases revealed a significant interaction ($F(1, 20) = 14$, $p < 0.05$). As in experiment 1 and the previous experiments, the deficit was specific to PR-LTM ($p < 0.05$). In order to test whether at this time point, anisomycin's effects were being produced by an action in the hippocampus, we repeated the 45 day experiment and infused anisomycin into the ventricles. ICV infusions of anisomycin 45 days posttraining had no effect on either PR-STM or PR-LTM, suggesting that anisomycin produced its behavioral effects within the hippocampus itself (PR-STM scores, ACSF = 80 ± 6.7 versus ANISO = 88 ± 6.7; for PR-LTM scores, ACSF = 67 ± 9.3 versus ANISO 62 ± 14.3; data not shown). An analysis of variance comparing drug group and memory phase revealed no significant interaction ($F(1, 12) = 1$, $p > 0.05$) nor a group effect ($F(1, 12) < 1$). There was, however, a significant effect of memory phase, demonstrating that both groups showed significant extinction ($F(1, 12) = 12$, $p < 0.05$).

In order to compare the efficacy of anisomycin over time, the scores of groups in the 3, 15, and 45 day experiments were converted to a standardized memory index (percent of PR-LTM/PR-STM). Given that the PR-STM test produces approximately 20% extinction, control groups lie at 75%–80%. Reconsolidation did not show any temporally graded gradient across the time points (Figure 4). There was no significant interaction between time after training and drug treatment ($F(1, 49) < 1$). However, there was a main effect of group ($F(1, 49) = 50$, $p < 0.05$), demonstrating that recent (3 or 15 day old) as well as old (45 day old) contextual memories undergo protein synthesis reconsolidation in the hippocampus. This is particularly interesting for the 45 day time point since contextual fear memories are believed to be independent of the hippocampus at this point (Anagnostaras et al., 2001; Kim and Fanselow, 1992).

Systems Level Reconsolidation

There are two possible explanations for the apparent contradiction between the time-limited role of the hippocampus in consolidation versus reconsolidation. First, it is possible that in our particular paradigm the memory for the context is still hippocampus dependent after 45 days. Second, the memory might in fact be independent of the hippocampus after 45 days; however, reactivation returns it to being dependent on the hippocampus again. In order to distinguish between these two possibilities, we prepared rats with either sham or electrolytic lesions of the dorsal hippocampus 45 days after conditioning. Two other groups were treated identically except that immediately prior to surgery, they received a reactivation session. If the effects of anisomycin are due to the contextual memory still being hippocampus dependent after 45 days, then there should be a deficit in the lesioned animals regardless of whether they had received

a reactivation session or not. Conversely, the hypothesis that a memory returns to being hippocampus dependent after reactivation predicts that only animals that had their memories reactivated prior to lesions should show a deficit.

Hippocampal lesions caused memory impairments only in animals that had received a reactivation session (Figure 5A). Groups CS/lesion and CS/sham demonstrated comparable freezing scores during reactivation of 84 ± 7 and 80 ± 10, respectively (data not shown, $t(12) < 1$). There was a significant three-way interaction between reactivation (no CS versus CS), surgical procedure (sham versus lesion), and test day, 1–4 ($F(3, 63) = 4.1$, $p < 0.05$). Indeed, post hoc analysis revealed that the CS/lesion group was different from all other groups (p's < 0.05) on day 1, while all other groups demonstrated comparable freezing (p's > 0.05). The finding that hippocampal lesions had no effect ($F(3,30) = 1.1$, $p > 0.05$) in the absence of memory reactivation is consistent with the general tenet of systems consolidation theory, that the hippocampus has a time-limited role in memory (Anagnostaras et al., 2001; Eichenbaum et al., 1994; McGaugh, 2000; Scoville and Milner, 1957; Squire and Alvarez, 1995). Furthermore, it demonstrates that the hippocampus is not necessary for the expression of contextual fear at this time point. The finding that memory reactivation immediately prior to the same lesions caused a large impairment demonstrates that reactivation returns a hippocampus-independent memory to being hippocampus dependent again. In addition, testing animals daily for 4 days and again after a week did not cause a putative latent neocortical memory to recover (Zinkin and Miller, 1967). Animals that were amnesic remained amnesic across all tests, with the level of freezing over all retests comparable (p's > 0.05). These data suggest that reactivation of remote neocortical traces causes some critical plasticity to return to being hippocampus dependent again. Given that the effects of the lesions were contingent on memory reactivation, it is difficult to interpret in terms of nonspecific effects, such as impaired memory expression, increased locomotion interfering with freezing (McNish et al., 1997), or state-dependent learning (Millin et al., 2001).

In order to further test whether the effects of the infusions and lesions are due to actions in the hippocampus versus the overlying cortex, we repeated the above experiment in animals with lesions of the overlying cortex. Animals were trained and returned to their home cage. Forty-five days after training, rats received a reactivation session and either the sham or electrolytic lesions of the neocortex overlying the dorsal hippocampus. Neocortical lesions destroyed the majority of the trunk region of the primary somatosensory as well as the parietal association area extending from −2.5 mm posterior to bregma to −4.5 mm. Freezing scores were similar in sham and lesioned animals during the reactivation session (sham = 65 ± 7, lesion = 66 ± 11, $t < 1$). Similarly, freezing scores did not differ between sham and lesioned rats during the postlesion test (sham = 37 ± 4, lesion = 40 ± 7, $t < 1$). These data, together with the fact that ICV infusions of anisomycin had no effect on reconsolidation 45 days after conditioning, strongly suggest that reconsolidation occurs in the hippocampus itself.

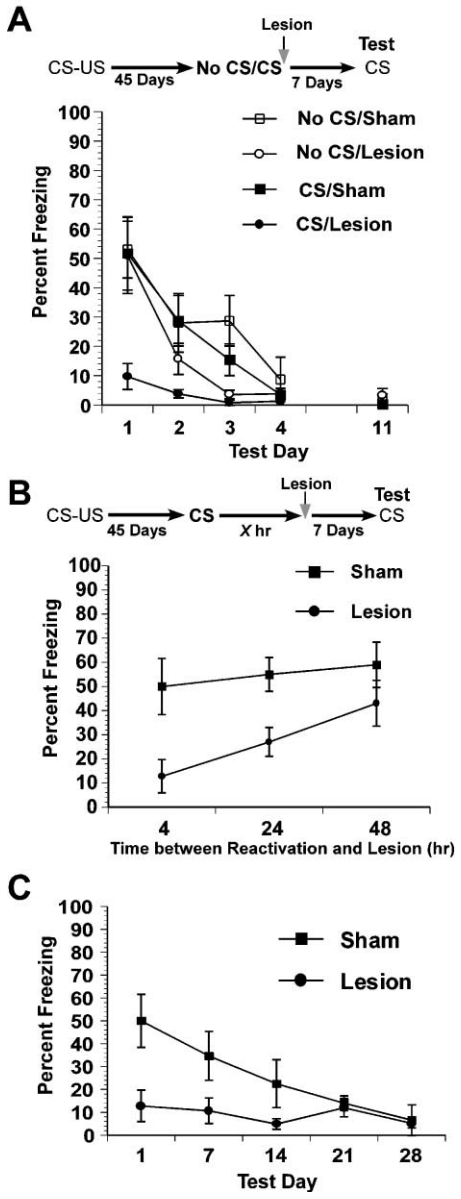

Figure 5. Hippocampal Memories Undergo Systems Reconsolidation

(A–B) Schematic of the procedure used with the data presented below. (A) Remote hippocampus-independent memories return to being hippocampus dependent after memory reactivation. The score of the CS/lesion group did not show any spontaneous recovery across all test days. The no CS/sham and no CS/lesion group did not significantly differ as there was no significant interaction between group and test days. (B) The hippocampus plays a time-limited role in the restabilization of a reactivated remote memory. This second temporally graded retrograde amnesic gradient was only 2 days after which time the trace once again became hippocampus independent.

(C) Retesting animals in the 4 hr group weekly for up to 28 days did not cause any spontaneous recovery.

The Second Temporally Graded Retrograde Amnesic Gradient

We next asked how long the hippocampus is required to stabilize the reactivated remote memory. Animals received

contextual fear conditioning and were undisturbed for 45 days to allow the memory to become independent of the hippocampus. Their remote memory was reactivated and dorsal hippocampus lesions were performed 4, 24, or 48 hr later. In all cases, the sham and to be lesioned groups reactivation scores were comparable with each other (p's $>$ 0.05; 4 hr scores, sham = 91 $\pm$ 5 versus lesion = 71 $\pm$ 13; 24 hr scores, sham = 50 $\pm$ 11 versus lesion = 62 $\pm$ 8; 48 hr scores, sham = 69 $\pm$ 8 versus lesion 69 $\pm$ 9; data not shown). Animals that received lesions 4 or 24 hr postreactivation demonstrated an impairment, but the 48 hr group was intact (Figure 5B). An ANOVA revealed a significant interaction between the postreactivation time of lesions and the lesion type (sham or electrolytic) (F (2, 37) = 5.4, p $<$ 0.05). Post hoc analysis indicated that only the 4 and 24 hr lesioned animals were significantly different from their respective controls (p's $<$ 0.05). In order to test for the presence of a latent neocortical trace, the 4 hr group was tested once weekly for 4 weeks. Again, there was no recovery of behavior over 4 weeks (p's $>$ 0.05) (Figure 5C). Thus, whereas the duration of hippocampus involvement for new learning (first retrograde amnesic gradient) in this task is typically on the order of weeks (Anagnostaras et al., 2001; Kim and Fanselow, 1992), the duration of hippocampal involvement for remote reactivated memories (second retrograde amnesic gradient) is 1–2 days.

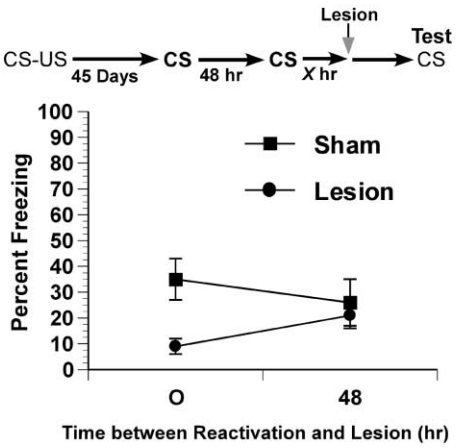

Figure 6. Contextual Memories Return to a State of Hippocampus Dependence for a Third Time

Top shows a schematic of the procedure used with the data presented below. Fourty-five days after conditioning, a time when the first temporally graded retrograde amnesic gradient is complete and the contextual memory has become hippocampus independent, the memory was reactivated. Two days later, when the second retrograde gradient was complete and the memory was once again hippocampus independent, the memory was reactivated again, and animals received sham or electrolytic lesions of the dorsal hippocampus. Animals that received lesions immediately, but not 48 hr, after reactivation showed a deficit in contextual freezing. Thus, the duration of the third retrograde gradient is comparable to the second.

The Third Temporally Graded Retrograde Amnesic Gradient

Next we tested whether a contextual memory that has become hippocampus independent twice could return to being hippocampus dependent for a third time after being reactivated. The previous experiment (Figure 5B) demonstrated that lesions of the hippocampus 48 hr after reactivation were ineffective. Forty-five days after training, rats received a reactivation test and 48 hr later, received a second reactivation session. Rats then received hippocampal lesions either immediately or 48 hr after the second reactivation test. As can be seen in Figure 6, reactivation of a hippocampus-independent memory returned it to being hippocampus dependent for the third time. This memory trace remained hippocampus dependent for less than 2 days. The reactivation scores were comparable between sham and lesioned groups (first reactivation: sham = 74 $\pm$ 8, lesion = 75 $\pm$ 11; second reactivation: sham = 66 $\pm$ 8, lesion = 60 $\pm$ 12, F's $<$ 1). Because the freezing levels in the control and lesion groups were so low, there was no significant interaction between time (0 hr and 48 hr) and surgical condition (sham or lesion) F (1, 29) = 2.8, p = 0.1. However, post hoc tests revealed that lesion and sham groups differed in the 0 hr condition (p $<$ 0.05) but were the same in the 48 hr condition (p $>$ 0.05). These data demonstrate that memory reactivation could return a memory to being hippocampus dependent for a third temporally graded retrograde amnesic gradient that is of comparable duration to the second.

Discussion

Using targeted infusions of anisomycin into and specific lesions of the hippocampus, we have demonstrated that hippocampal memories undergo cellular as well as systems reconsolidation. Specifically, we have demonstrated that intra-hippocampus anisomycin causes an impairment in PR-LTM, but not PR-STM, when infused after reactivation of contextual fear memory. This effect was not due to diffusion to a distal site of action such as the amygdala or the overlying cortex. Further, anisomycin's effects were contingent on memory reactivation. In addition, anisomycin did not function as a US after fear expression to support competing conditioned responses. Thus, the most parsimonious interpretation of these data is that memories stored in the hippocampus undergo cellular reconsolidation when reactivated.

In contrast to these findings, it has recently been demonstrated that systemic anisomycin infusions blocks the consolidation, but not reconsolidation or extinction, of contextual fear conditioning (Guzowski and McGaugh, 1997; Lattal and Abel, 2001). Furthermore, infusions of anisomycin directly into the hippocampus, which were sufficient to block consolidation of inhibitory avoidance, were ineffective in blocking reconsolidation (Taubenfeld et al., 2001). One of the likeliest explanations for these lacks of effects on reconsolidation is that the doses of anisomycin used were not high enough to affect reconsolidation. The dose used in the present study is twice that required to block consolidation. There are three main reasons why reconsolidation in the hippocampus could have a different dose response curve. First, over time the contextual memory may become more spatially dispersed, requiring a higher dose of anisomycin to inhibit protein synthesis over a larger area. Second, anisomycin may be acting in the cell nucleus to block transla-

tion of proteins required for consolidation and in the dendrites to block translation involved in reconsolidation. Therefore, different doses may be required to affect translation in these two compartments. Third, during consolidation a large amount of proteins are presumably required to sustain the presumed synaptic growth underlying the consolidation of long-term memories. We have argued that during reconsolidation new proteins are required to restabilize an already existing reactivated synapse (Nader et al., 2000a, 2000b), which may be accomplished through the production of a small number of proteins. Thus, in order to block reconsolidation, higher doses of anisomycin would be required to shut down protein synthesis to the point where even the small number of proteins required for restabilization cannot be formed.

Two studies have demonstrated that anisomycin infusions after reactivation blocked the extinction produced by the reactivation session (Berman and Dudai, 2001; Vianna et al., 2001). This is the opposite of our findings with reconsolidation in which behavior was lost after reactivation and protein synthesis challenge. One intriguing difference between those two studies and our own is that the reactivation session in our studies did not cause any significant extinction (Nader et al., 2000a). However, in both the studies by Vianna et al. (2001) and Berman and Dudai (2001), reactivation produced significant extinction. Thus, it is possible that extinction and reconsolidation compete on a molecular level. If extinction is expressed, it may be the dominant protein synthesis-dependent process, which in turn will be blocked by anisomycin infusions. On the other hand, in cases where a single reactivation session is not sufficient to induce significant extinction, reconsolidation may be the dominant protein synthesis-dependent process. Thus, in our paradigm, anisomycin infusions would block reconsolidation and not extinction.

Anisomycin infusions into the hippocampus blocked the reconsolidation of a reactivated contextual trace over a 45 day period, showing a lack of any temporally graded retrograde amnesic gradient. This was not due to the specific parameters of our paradigm that might lead to an ungraded retrograde amnesia. Rather, it was due to reactivation causing a remote memory to return to being dependent on the hippocampus. This conclusion is based on the findings that lesions of the hippocampus 45 days after conditioning had no effect on the expression of contextual fear conditioning. However, when the memory was reactivated for as short as 90 s immediately prior to the induction of surgical anesthesia for the production of those same lesions, a large impairment was seen. These findings extend Land et al.'s (2000) study of avoidance conditioning. However, unlike contextual conditioning, the avoidance task used by Land et al. depends on the hippocampus for retrieval, but not the initial learning. This difference may account for the fact that memory could be recovered in the Land et al. study, but not in our study.

The lesion data demonstrate that reactivation of hippocampus-independent memories cause them to become critically dependent on the hippocampus again. Furthermore, this can happen more than once (we have demonstrated it three times). These findings are analogous to cellular consolidation except that they occur at the systems level. In cellular consolidation, a memory trace is stabilized from a labile state to a consolidated state with the synthesis of new proteins. Cellular reconsolidation is the demonstration that reactivation of the consolidated state returns the trace to a labile state that requires protein synthesis in order to be restored. Systems consolidation is the restructuring of a trace from being hippocampus dependent to independent. Systems reconsolidation is the demonstration that reactivation of a remote memory returns the trace to being hippocampus dependent again for a period of time before once again becoming independent of the hippocampus. The second and third retrograde gradients are on the order of 1–2 days. Although we have not tested the duration of the first systems consolidation gradient in this study, all studies using contextual fear conditioning have shown effects of lesions weeks after training if not longer (Anagnostaras et al., 2001). Thus, the duration of the first and subsequent gradients seem quite different.

Before accepting the above interpretations, however, there are two alternate interpretations that need to be considered. First, it is possible that what we view as being a blockade of reconsolidation is in fact facilitated extinction. This is unlikely for a number of reasons. First, extinction is new learning (Bouton, 1993). One of the most fundamental universals throughout the field of memory consolidation is that the production of new proteins is required for induction of normal long-term memory (Davis and Squire, 1984; Dudai and Morris, 2000; Flexner et al., 1965; Goelet et al., 1986). To say that anisomycin injections facilitated extinction is the equivalent of stating that inhibition of protein synthesis enhances memory formation. There is no evidence that blockade of protein synthesis enhances memory in any system. Indeed, the studies described above show that when anisomycin affects extinction, it does so by blocking rather than facilitating extinction. Second, our unpublished findings with auditory fear conditioning demonstrate that anisomycin blocks reconsolidation when the memory is reactivated with a reinforced training trial (S.D., J.E.L., and K.N.). Third, in the current lesion experiments, it could be argued that lesions of the hippocampus facilitated extinction. Explicitly speaking against this are the findings that the no CS/lesion and no CS/sham demonstrated comparable levels of extinction over the test days. Thus, no facilitated extinction was seen. This is consistent with previous data, demonstrating that lesions of the hippocampus do not affect extinction of fear conditioning (Frohardt et al., 2000).

Another interpretation of the lesion data is that the neocortical trace becomes labile again, and the lesions produced nonspecific neocortical disruption which, in turn, blocked neocortical cellular reconsolidation. According to this interpretation, there is no need to invoke plasticity returning to the hippocampus. This interpretation predicts that anisomycin injected into the hippocampus should have had no effect on day 45 because reconsolidation would be occurring in the neocortex and not in the hippocampus. However, intra-hippocampal infusions of anisomycin on day 45 blocked reconsolidation. Furthermore, anisomycin did not produce its effect by diffusing through the ventricles to a site distal to the hippocampus because intra-ventricular infusions of the

same dose, time, and volume had no effect. Similarly, the effect of hippocampal lesions were not due to damage to the overlying neocortex because lesions of this area had no effect on reconsolidation. Lastly, if our manipulations were producing nonspecific effects on the neocortical storage sites for the contextual representation, then that should have produced a flat retrograde gradient because the neocortical areas involved in storage would have been destroyed and a behavioral deficit should have been seen at any reactivation-surgery interval regardless of reactivation condition. In contrast, we found a very specific pattern of deficit. Specifically, in order for lesions of the hippocampus to affect behavior, the remote memory must first be reactivated. Furthermore, in contrast to the flat gradient predicted by the nonspecific interpretation, a temporally graded retrograde amnesia gradient that is limited to 48 hr after memory reactivation of a remote memory was found. Therefore, the most parsimonious interpretation of these data is that upon reactivation of a remote contextual memory, some plasticity critical to the remote memory returns to being hippocampus dependent.

Consolidation of a New Trace or Reconsolidation of an Old Trace?

One of the most central points within the consolidation/reconsolidation debate is whether the amnesic agents (anisomycin, lesions, ECT, etc.) block the original trace from being reconsolidated or whether reactivation produces a second new trace that has to be consolidated. Blockade of the new trace would be said to be a case of impaired consolidation instead of reconsolidation. There are multiple lines of evidence that favor the reconsolidation interpretation. Take the case where animals have their contextual memories reactivated and challenged with anisomycin. According to the consolidation view, anisomycin would block the consolidation of a new memory formed through reactivation. If this were true, however, then on test day, animals should have simply retrieved their original memory and performed at control levels. The fact that the animals are impaired speaks against the new memory interpretation of the findings. The second line of evidence comes from the durations of the first and second retrograde amnesic gradients. The first gradient for consolidating new memories is typically on the order of weeks. If reactivation was producing a second new memory, then the consolidation of the second new memory should have been on the order of weeks because this is how long a new contextual trace takes to undergo systems consolidation. However, the second retrograde amnesic gradient was only 2 days long. This short duration is more parsimoniously explained by positing that when remote memories are reactivated, the hippocampus is temporarily necessary to reinforce or modify the original neocortical trace.

It could be argued that the original trace still exists, but the amnesic agents are interfering with the ability of the trace to be retrieved. Speaking against this possibility is the lack of spontaneous recovery using two different protocols of retesting the animals. However, we do acknowledge that Miller's claim, that the absence of spontaneous recovery does not mean there is no latent trace (Miller and Springer, 1974). This claim is equally applicable to amnesia for both new and reactivated memories. The issue of whether amnesia for new information is due to a retrieval or storage failure was debated for decades and led to a stalemate (Cherkin, 1972; Davis and Rosenzweig, 1978; Davis and Squire, 1984; Gold and King, 1974; Gold et al., 1973; Miller and Springer, 1974; Miller and Matzel, 2000; Quartermain and McEwen, 1970). Both camps came to develop arguments and counterarguments that explained the vast majority of the findings concerning the durability of amnesia, how reminder treatments affect memories, spontaneous recovery from amnesia, etc. (e.g., Gold and King, 1974; Miller and Springer, 1974). Thus, in referring to the reconsolidation phenomena, our intent is not to make a qualitative statement that reconsolidation is necessarily a storage deficit. Rather, as consolidation is a time dependent process that is engaged by new learning (McGaugh, 1966), we are using reconsolidation to refer to another time-dependent process that is engaged by reactivation of a consolidated memory. Furthermore, we feel that the time-dependent processes engaged by consolidation and reconsolidation are of the same qualitative state. Thus, if the nature of amnesia for new learning (consolidation) is determined to be a retrieval deficit, then we suggest reconsolidation should also be a retrieval deficit. Conversely, if as is assumed, that consolidation ultimately is determined to be storage process, then we suggest that reconsolidation is also a storage process. Given the large degree of similarity between consolidation and reconsolidation, there is no reason to assume that they represent different qualitative processes.

Possible Mechanisms

We have previously suggested that the simplest mechanism that could induce a trace to return to a labile state in cellular reconsolidation is that reactivation of consolidated synapses causes them to become unstable (Nader et al., 2000a, 2000b). In the absence of new protein synthesis, the reactivated synapses remain functional for at least 4 hr (based on intact PR-STM) but become dysfunctional over longer time points. Such a mechanism allows for reconsolidation effects on specific memories by ensuring that only the memories that the reactivated synapses contributed to return to a labile state while other nonreactivated synapses would remain in a consolidated state. While the physiological events that cause cells to once again require protein synthesis is unknown at this point, it is possible that insertion of a molecular tag during synapse reactivation may contribute (Frey and Morris, 1997; Martin et al., 1997). In addition, the new proteins could be due to dendritic (Steward et al., 1998) or nuclear (Goelet et al., 1986) translation. However, recent evidence that CREB is required for reconsolidation suggests that nuclear protein synthesis is required (Kida et al., 2002). This proposed mechanism for cellular reconsolidation is biologically conservative. Indeed, one theory of the mechanisms mediating LTM postulates that new proteins are required for the normal maintenance of a trace after synapses have been active (Dudai and Morris, 2000). Thus, a very dynamic memory system could arise from a very

simple mechanism that is already posited to play a role in the maintenance of LTM.

Based on the pattern of results obtained following hippocampal lesions, we can infer a possible mechanism mediating reconsolidation at the systems level. The findings that the no CS/sham and no CS/lesion groups performed the same over multiple tests suggests that hippocampal lesions themselves do not affect the integrity of the remote trace. The only difference between the no CS/lesion and CS/lesion, which showed a deficit, was that the latter had an intact hippocampus during reactivation. Therefore, an intact hippocampus seems to be necessary to produce a labile neocortical trace. Thus, reactivation seems to be doing two things: (1) it creates a hippocampal trace that is labile and undergoes protein synthesis-dependent reconsolidation in order to persist in the hippocampus, and (2) it renders the neocortical trace labile via the backprojections to the entorhinal, perirhinal, and parahippocampal cortices and onward to the neocortex (Suzuki and Amaral, 1994). Interestingly, these projections synapse in superficial layers of the cortex, where the NMDA class of glutamate receptors (which are believed to play a crucial role in memory [Rosenblum et al., 1997]) are abundant (Monaghan and Cotman, 1985). These backprojections have been proposed to be involved in updating neocortical information (McClelland et al., 1995; Rolls, 1989). If after reactivation, which renders the neocortical trace labile, the hippocampus is lesioned or prevented from synthesizing the proteins required for its cellular reconsolidation, then the neocortical trace is deprived of reinforcing feedback and thus decays. The neocortical trace requires between 1 and 2 days of feedback from the hippocampus (second and third retrograde gradient).

Consistent with this mechanism, recent work has shown that the hippocampus can be activated after retrieval of remote memories in humans (Cipolotti et al., 2001; Mayes and Roberts, 2001; Ryan et al., 2001) and rats (Bontempi et al., 2000, Soc. Neurosci. Abstr.). Additional support comes from studies of false memories in amnesics. Reconsolidation has been proposed as a mechanism by which false memories occur (Loftus and Yuille, 1984). Specifically, reactivation of the trace returns it to a labile state, where its contents can be changed through suggestion or other means (Loftus and Yuille, 1984). The mechanism described above predicts that for cases of amnesia produced by hippocampal damage, the remaining remote memories should be more resistant to false memories than in normal subjects. This is because amnesics do not have a hippocampus to trigger the neocortical traces to return to a labile state. Indeed, recent preliminary findings have shown that the amnesic H.M. tends to have better memory for famous faces for the time period prior to his amnesia than controls (Corkin, 2002). One extremely counterintuitive implication of this postion is that amnesics should make for the best witnesses for events they can remember because those memories should be resistant to change for the reasons described above. Finally, these findings have novel implications for strategies to address memory loss. Given that the hippocampal backprojections are required to trigger the cortical trace to return to a labile state, then a drug that prevents this pathway from triggering the neocortical trace to become labile should help keep neocortical memories intact.

Theoretical Implications

Systems consolidation theory predicts that the hippocampus has a time-limited role in memory storage, after which time memories are independent of the hippocampus (Anagnostaras et al., 2001; Eichenbaum et al., 1994; McClelland et al., 1995; Scoville and Milner, 1957; Squire and Alvarez, 1995). The fact that hippocampal lesions had no effect in the absence of reactivation is consistent with this theory. However, systems consolidation theory cannot explain why the hippocampus again becomes critically involved after reactivation or why there is more than one retrograde gradient. Further, systems consolidation theory cannot explain the disruptive effects of anisomycin on memory at 45 days (when the memory is hippocampus independent).

An alternate view of hippocampal function is the multiple trace theory (MTT) (Nadel and Moscovitch, 1997). This model states that the retrograde gradient is not due to the memory becoming independent of the hippocampus, but instead to the fact that over time multiple copies of the memory are made and stored in the hippocampus. Lesions of the dorsal hippocampus are effective at blocking behavior mediated by a small number of copies of the memory, but not the large number of copies that accumulate with time. Therefore, it could be argued that our effects are due to the creation of a new copy of the contextual memory during reactivation, a memory which then has to undergo consolidation. If this were so, then the contextual memory should have been more resistant to the effects of our dorsal hippocampal lesions because there would be more copies of the memory created by the reactivation session and presumably stored in other regions of the hippocampus. However, we see the opposite pattern of results. Reactivation prior to the lesion rendered the memory susceptible to disruption. Furthermore, given the different durations of the first and second retrograde gradient, reactivation cannot be creating a copy of the memory that is acting like a new memory.

Consolidation theory and the MTT are two positions that have not been able to be reconciled so far. Our work on systems reconsolidation may be able to move this debate forward. The majority of the data supporting the consolidation theory derives from lesion studies, demonstrating that lesions of the hippocampus/medial temporal lobe region have decreasing effects with time (Anagnostaras et al., 2001; Scoville and Milner, 1957; Squire et al., 2001). Conversely, the majority of the experimental support for the MTT comes from imaging studies that show hippocampal activation for both recent and remote memories (Cipolotti et al., 2001; Mayes and Roberts, 2001; Ryan et al., 2001). Systems reconsolidation can incorporate both of these data sets. Our findings, that lesions of the hippocampus 45 days after training had no effect, are consistent with the consolidation view that the hippocampus is not involved in the expression of the remote memory (although the memory is likely to be less flexible than normal). On the other hand, the fact that reconsolidation occurs in the hippocampus after remote memory reactivation can explain

the hippocampal activation seen in imaging studies with remote memory recall. Thus, systems reconsolidation offers a way forward from the debate between the consolidation and MTT views of hippocampal contributions to memory.

Existing theories of memory cannot easily account for these results. Any theory of hippocampal memory must explain the following: (1) reactivation of consolidated hippocampus-dependent memories requires protein synthesis-mediated changes, an instance of cellular reconsolidation; (2) reactivation of consolidated, hippocampus-independent memories causes them to again depend on protein synthesis- mediated plasticity in the hippocampus in order to persist, which is an instance of systems reconsolidation; and (3) the existence of multiple, distinct retrograde gradients. Cognitive psychologists have long known that memories, even autobiographical memories acquired during childhood, are very dynamic and in fact can be reconstructed at the time of retrieval (Bartlett, 1932; Loftus and Yuille, 1984; Schacter, 1999). An understanding of reconsolidation at the cellular and systems level may help to explain these dynamic aspects of memory.

Experimental Procedures

Subjects

Subjects consisted of adult male Sprague-Dawley rats obtained from Hilltop Labs, Scottdale, PA. Rats were housed individually in plastic Nalgene cages and maintained on a 12:12 hr light/dark cycle. Food and water were provided ad libitum throughout the experiment.

Surgery and Histology

Cannulation: under Nembutal anesthesia (45 mg/kg), rats were implanted bilaterally with 22-gauge stainless steel cannulas into the dorsal hippocampus and angled 10° away from the midline. Coordinates, taken from Paxinos and Watson (1986) and adjusted according to pilot data were: 3.6 mm posterior to bregma, 3.1 mm lateral to the midline, and 3.4 mm ventral to the skull surface. For cannulas aimed at the ventricles, the coordinates were 0.4 mm posterior to bregma, 1.5 mm lateral to the midline, and 4.4 mm ventral to the skull surface. The lesion procedure was based on Kim and Fanselow's procedures (Kim and Fanselow, 1992). Electrolytic lesions were made by passing positive current (1.0 mA, 20 s) through a monopolar electrode insulated with epoxy to within 200 μm of the tip. The coordinates for the four sites were: 2.8 mm posterior to bregma, 2 mm lateral to the midline, and 4 mm ventral to the skull surface and 4.2 mm posterior to bregma, 3 mm lateral to the midline, and 4 mm ventral to the skull surface. Rats were given at least 7 days to recover prior to experimental procedures. All animals included in the analysis had extensive damage to the dorsal hippocampus and were comparable to those shown by Kim and Fanselow (Kim and Fanselow, 1992). Lesions of the overlying neocortex used the identical protocol except for the ventral coordinate, which was −2 mm from the skull at bregma.

At the termination of the experiment, using standard histological methodologies, animals were perfused and their brains sectioned at 50 μm thickness. The sections were stained using Cresyl violet and examined with light microscopy for cannula penetration into the hippocampus and lesion size. Only animals that had bilaterally placed cannula in the hippocampus were included in the statistical analysis. All procedures were in accordance with the NIH Guide and were approved by the NYU Animal Care and Use Committee.

Infusions

Drugs were infused slowly via infusion pump at a rate of 0.25 μl/min. Following drug infusion, injectors were left in place for an additional minute to allow diffusion of the drug away from the cannula tip. Anisomycin (Sigma, Cat#A9789) was dissolved in equimolar HCl, diluted with artificial cerebrospinal fluid (ACSF), and adjusted to pH 7.4 with NaOH.

Apparatus

Conditioning took place in a Plexiglas rodent conditioning chamber with a metal grid floor (Model E10-10, Coulbourn Instruments, Lehigh Valley, PA) that was enclosed within a sound attenuating chamber (Model E10-20). The chamber was dimly illuminated by a single house light. Auditory fear conditioning took place in a different room with distinctly different conditioning Plexiglas chambers (ENV-001, MedAssociates, Inc., Georgia, VT).

General Behavioral Procedures

Rats were habituated to the conditioning chamber for 5 min each on day 0. On day 1, they were placed into the chamber and after 2 min received eight shocks at 62 s intervals. Each shock was 1.5 mA and 1 s duration. Rats were left in the conditioning chamber for 30 s after termination of the procedure and then returned to their home cage. For all testing, an animal was placed into the conditioning chamber and observed for 5 min. The last half of each minute was scored for immobility. An average of those five scores was obtained for each rat, which was then used for the analysis. For reactivation, animals were returned to the conditioning chamber for 90 s.

Experiment 1

A: Three days after conditioning, rats were immediately infused with either 250 μg/2 μl/side (n = 12) anisomycin or ACSF (n = 7) after a reactivation session. B: In this experiment, rats were transported to a distinctive room and received an infusion of ACSF (n = 6) or anisomycin (n = 7).

Experiment 2

A: This was identical to experiment 1A; however, the anisomycin (n = 8) and ACSF (n = 7) infusions were made into the ventricles. B: the day after habituation to the training context, animals were placed into a second distinctive environment and receive two pairings of a 30 s, 75 dbl, 5 kHz tone that coterminated with 1 mA, 1 s footshock. The following day, all rats were returned to the conditioning chamber for 90 s, during which time the auditory CS was played. This equated for how intensely and for how long the fear system was driven during context preexposure and memory reactivation in experiment 1A. After this period, all rats were given an intrahippocampal infusion of either anisomycin (n = 8) or ACSF (n = 7). The next day, they were conditioned and 3 days later tested for expression of contextual fear memories as described above.

Experiment 3

A: 15 or 45 days were inserted between conditioning and reactivation. After CS reactivation, rats received either anisomycin (15 day, n = 7; 45 day, n = 12) or ACSF (15 day, n = 7; 45 day, n = 10) infusion. B: Rats were conditioned and left in their home cage for 45 days. On day 45, they received either ACSF (n = 7) or anisomycin (n = 7) infusions into the ventricles immediately after memory reactivation.

Experiment 4

A: Forty-five days after conditioning, rats received either sham or electrolytic lesions of the dorsal hippocampus. Half of each group received a reactivation session immediately prior to surgery, while the other half simply received surgery. The groups were comprised of no CS/sham (n = 6), no CS/lesion (n = 6), CS/sham (n = 6), and CS/lesion (n = 7), where the first word of the name refers to whether the animals received a reactivation session or not, and the second word indicates the nature of the surgery administered. After a 7 day recovery period, all animals were tested daily for 4 days to test for any spontaneous recovery of the memory. Animals were then left for 1 week, after which they received a test session. B: Forty-five days after conditioning rats received a reactivation session and then either sham or electrolytic lesions of the dorsal hippocampus 4 (sham, n = 8; lesion, n = 7), 24 (sham, n = 6; lesion, n = 7), or 48 (sham, n = 7; lesion, n = 6) hr later. C: Animals from the 4 hr group of B were retested on a weekly basis for 4 weeks. See Figure 5.

Animals that received lesions of to the neocortex overlying the dorsal hippocampus underwent the identical surgical protocol that was used to lesion the hippocampus; however, the ventral coordinate used was −2 mm (sham, n = 7; lesion, n = 9).

Experiment 5

Forty-five days after conditioning, rats were returned to the conditioning chamber and received a reactivation session. Forty-eight hrs later, they were again given a reactivation session and then immediately received sham (n = 5) or electrolytic lesions to the dorsal hippocampus (n = 7). Two other groups treated identically received either sham (n = 8) or electrolytic lesions to the dorsal hippocampus (n = 12) 48 hr after the second reactivation session.

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