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Temporally Graded Requirement for Protein Synthesis following Memory Reactivation

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Summary

Learning of new information is transformed into long-lasting memory through a process known as consolidation, which requires protein synthesis. Classical theory held that once consolidated, memory was insensitive to disruption. However, old memories that are insensitive to protein synthesis inhibitors can become vulnerable if they are recalled (reactivated). These findings led to a new hypothesis that when an old memory is reactivated, it again becomes labile and, similar to a newly formed memory, requires a process of reconsolidation in order to be maintained. Here, we show that the requirement for protein synthesis of a reactivated memory is evident only when the memory is recent. In fact, memory vulnerability decreases as the time between the original training and the recall increases.

Introduction

New learning generates long-lasting memory through a process of consolidation, which transforms the acquired information into stable modifications. During consolidation, a new memory is initially labile and can be disrupted by a variety of interfering events and pharmacological treatments, including protein synthesis inhibitors (reviewed in Bailey et al., 1996; McGaugh, 2000). Indeed, profound memory impairment occurs when protein synthesis is temporarily blocked during or immediately after learning (Davis and Squire, 1984). A new memory becomes increasingly stable over time until it is finally insensitive to disruptive interferences. This evidence led to the hypothesis that once consolidation is complete, memory becomes permanent (Squire and Alvarez, 1995). This view of consolidated, permanently stored memories has been recently challenged. Several reports, in fact, have shown that when a consolidated memory is recalled, it becomes transiently sensitive to disruption by the same agents that affect consolidation, including protein synthesis inhibitors (Misanin et al., 1968; Mactutus et al., 1979; Judge and Quartermain, 1982; Lewis, 1979; Richardson et al., 1982; Sara, 2000; Nader et al., 2000a; Taubenfeld et al., 2001; Anokhin et al., 2002; Kida et al., 2002). These findings have led to a new hypothesis, which proposes that stored memories are not indefinitely stable; to the contrary, whenever recalled, memories become labile and need to undergo a protein synthesis-dependent reconsolidation in order to be to restabilized (Misanin et al., 1968; Nader et al., 2000b). The assumption of this view is that every time a memory is reactivated, it becomes susceptible to disruption by protein synthesis inhibitors. However, the risk of losing a memory every time it is recalled seems to be highly disadvantageous. An alternative explanation for these results is that the recalled memories being abolished via protein synthesis inhibition were recently acquired and not yet fully consolidated. Thus, older, more completely consolidated memories may not be sensitive to disruption when recalled. To test this hypothesis, we investigated whether the degree of vulnerability of a recalled memory changes as a function of the time elapsing between initial learning and recall.

Results and Discussion

Groups of rats were trained on an inhibitory avoidance (IA) task (Taubenfeld et al., 2001). Memory retention of different groups was tested at either 2, 7, 14, or 28 days after training (test one). This retention test recalls and, therefore, reactivates the IA memory. Immediately after test one, half of the rats received a subcutaneous injection of anisomycin (Davis et al., 1980), which inhibited >97% of the cerebral protein synthesis (data not shown). The other half received an injection of vehicle solution (0.9% saline). Forty-eight hours after test one, animals were retested (test two, Figure 1).

At test two, the anisomycin treatment caused profound retention impairment in posttraining reactivation times at days 2 and 7, but not at days 14 and 28. This impairment was not evident in rats that received the same treatment but in the absence of memory reactivation. A three-way analysis of variance (ANOVA) with treatment and time as between-subject factors and test as a within-subject factor revealed a significant treatment × time × test interaction ($F_{3,68} = 5.44$, $p < 0.05$). Newman-Keul posthoc analyses revealed that retention levels of the anisomycin-injected groups at 2 days (2d) (Figure 1A, 2d: 71.53 ± 10.42 s) and 7 days (7d) (Figure 1B, 7d: 217.71 ± 64.04 s) were significantly lower than those of their respective vehicle-injected controls (2d: 419.61 ± 62.49 s, $p < 0.001$ and 7d: 442.80 ± 64.87 s, $p < 0.05$) and of their corresponding test one (2d aniso: 484.65 ± 31.29 s, 7d aniso: 444.64 ± 44.83 s, $p < 0.001$ for both). The test one latencies of the groups that were injected with vehicle are shown independently (2d vehicle: 397.51 ± 63.34 s; 7d vehicle: 401.90 ± 54.43 s). Notably, the retention deficit of the 2 day reactivation group was significantly more severe than that of the 7 day reactivation group ($p < 0.05$; Figures 1B and 1A, respectively).

To determine whether this inhibition was specific to reactivated memories rather than being related to the consolidation process per se or to nonspecific effects, groups of rats received anisomycin at the same times after training (2 or 7 days) in the absence of test one. Newman-Keul posthoc analyses revealed that the retention levels of rats that received anisomycin without memory reactivation were not significantly different from those of vehicle-treated controls (2d: 287.08 ± 71.26 s,

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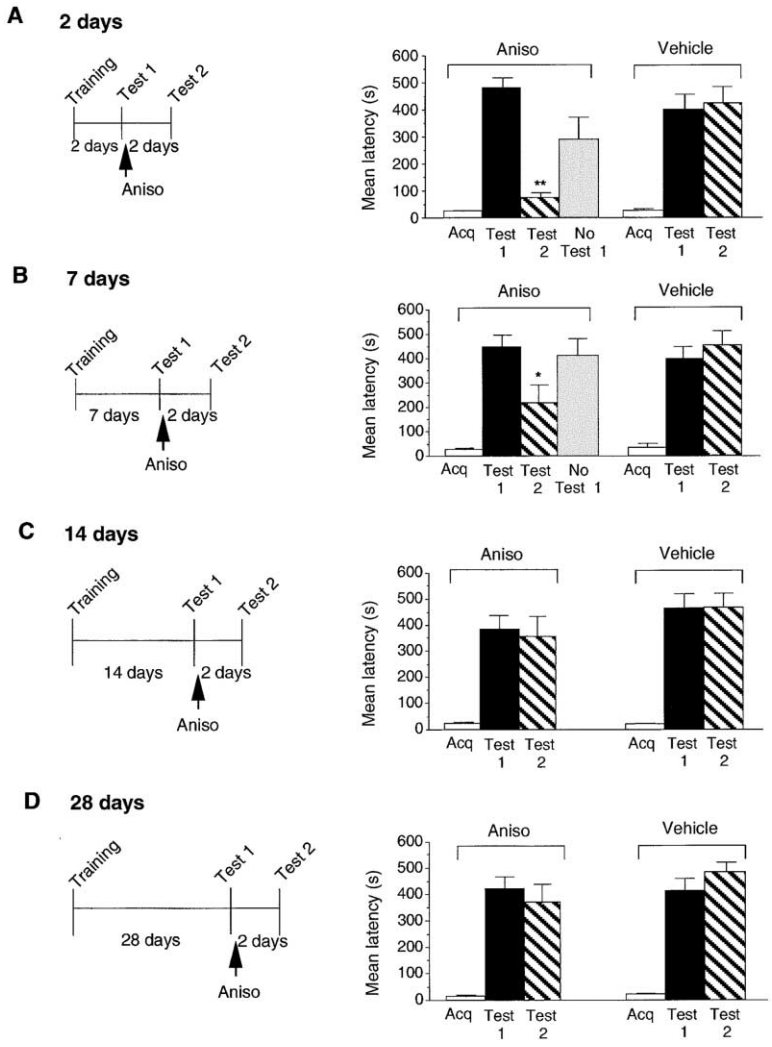

Figure 1. Anisomycin-Induced Amnesia following Inhibitory Avoidance (IA) Reactivation Is Temporally Graded

IA training was administered. Latency to enter the shock chamber was taken as a measure of acquisition (Acq). Retention, which recalled the memory, was performed by returning the rat to training context and measuring the latency (in seconds, [s]) to enter the dark chamber. Memory was recalled (test one) at 2 days (A), 7 days (B), 14 days (C), and 28 days (D) after training. Subcutaneous injections of anisomycin or vehicle (saline) were delivered immediately following test one. Memory was retested 2 days later (test two). (A) IA memory reactivated 2 days after training was significantly impaired (**$p < 0.001$) by anisomycin ($n = 15$) compared to vehicle controls ($n = 8$) at test two; anisomycin injection at the same time without reactivation ($n = 10$) had no effect. (B) IA memory reactivated 7 days after training was significantly impaired by anisomycin (*$p < 0.05$) at test two ($n = 11$) compared to vehicle-injected controls ($n = 8$); anisomycin injection without reactivation showed no effect ($n = 8$). (C) IA memory reactivated 14 days after training was not affected by anisomycin ($n = 9$) compared to vehicle-injected controls ($n = 8$) at test two. (D) IA memory reactivated 28 days after training was not affected by anisomycin ($n = 10$) compared to vehicle-injected controls ($n = 10$) at the time of test two. The latencies at test one are shown independently for each group.

7d: 412.51 ± 66.48 s). Furthermore, it revealed that the latencies of these anisomycin/no-reactivation groups were significantly different from those of rats that at the same time points received anisomycin injection after reactivation ($p < 0.05$ for both).

In striking contrast, when the reactivation event occurred 14 (351.95 ± 75.65 s) or 28 days (367.99 ± 63.90 s) after training, the latencies of anisomycin-injected rats did not differ significantly from those of vehicle-injected controls (14 d: 460.94 ± 57.47 s, 28 d: 479.41 ± 37.24 s) (Figures 1C and 1D) or their corresponding test one (14d aniso: 378.77 ± 54.38 s, 28d aniso: 420.93 ± 52.14 s). The groups that received injection of vehicle had the following test one latencies: 14d vehicle: 458.10 ± 55.67 s, 28d vehicle: 409.28 ± 49.79 s.

Finally, posthoc tests revealed that the latencies of anisomycin-treated rats that underwent memory reactivation at 2 and 7 days posttraining were significantly different from those that received reactivation at 14 ($p < 0.001$ and $p < 0.05$, respectively) and 28 days ($p < 0.001$ and $p < 0.05$, respectively).

If the retention of anisomycin-treated IA memories (test two) are expressed as a percentage of their correspondent memory retention before treatment (test one) according to the formula [(mean latency(s) test one − mean latency[s] test two)/(mean latency[s] test one)] × 100, then we obtain the percent susceptibility to disruption by anisomycin. As depicted in Figure 2, the percent

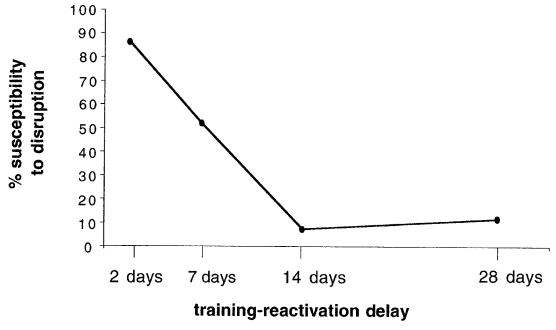

Figure 2. Temporally Graded Decrease of Susceptibility to Disruption of a Reactivated Memory

The percent (%) susceptibility to disruption by anisomycin of IA reactivated memory was calculated using the following formula: [(mean latency[s] test one − mean latency[s] test two)/(mean latency[s] test one)] × 100.

susceptibility of a reactivated IA memory to disruption by protein synthesis inhibitors is inversely proportional to the amount of time elapsed between initial training and reactivation.

From these data, we conclude that as the time interval from training increases, there is increasing resistance to postreactivation interfering disruptions. The results suggest that old, well-consolidated memories do not return to a labile state after reactivation and that recall, per se, does not place stable memories in a complete state of vulnerability. Conversely, recently acquired memories, although already insensitive to protein synthesis inhibition, become unstable if reactivated and do require protein synthesis to be later recalled.

These results appear to be in disagreement with the findings of Nader et al. (2000a), who showed that the requirement for protein synthesis (within the amygdala) lasts much longer. These authors, using classical auditory conditioning, reported that memories reactivated 2 weeks after training were disrupted by posttesting injection of anisomycin into the amygdala. The discrepancies between this and our findings may be due to the different experimental conditions used (e.g., amygdala versus systemic injection, different learning tasks); however, it is also possible that different tasks have different temporal requirements for protein synthesis after reactivation. Further studies should clarify this aspect.

In addition, it is still unclear whether memory reactivation induces a protein synthesis-dependent process similar to that required for consolidation. Task-related neuroanatomical differences between consolidation of initial learning and stabilization of reactivated memories exist. Lesion studies have revealed that IA requires both hippocampus and amygdala, while classical auditory conditioning is dependent on amygdala, but not hippocampus (Liang et al., 1982; Munoz and Grossman, 1981; Fendt and Fanselow, 1999). Nader et al. (2000a) found that amygdala protein synthesis is required not only after initial learning, but also after memory reactivation. In contrast, in IA, hippocampal protein synthesis is essential following initial learning, but not after recall (Taubenfeld et al., 2001; Vianna et al. 2001). Notably, IA memory reactivated 2 days after training is sensitive to systemic, but not hippocampal, administration of anisomycin. This indicates that protein synthesis in regions other than the hippocampus (perhaps amygdala) is required for stabilizing reactivated IA memories. Similarly, Berman and Dudai (2001) found that protein synthesis in insular cortex is necessary after learning, but not after retrieval of conditioned taste aversion. On the other hand, although the anatomical regions in which protein synthesis is required after learning and reactivation differ, transcriptional mechanisms such as CREB activation seem to be critical for both (Kida et al., 2002).

Our conclusions do not exclude the possibility that reactivation of fully consolidated memories is accompanied by a phase of de novo protein synthesis. However, protein synthesis induced by the reactivation of a fully consolidated memory does not appear to be required for later recall. Authors reporting the vulnerability of reactivated memories have proposed that the protein synthesis induced by memory reactivation allows for the incorporation of new information into old memories (Sara, 2000) and, indeed, it seems intuitively obvious that memories need to be continuously updated with new learning. Nevertheless, our data suggest that editing of completely consolidated memories may occur without jeopardizing their stability.

Why does protein synthesis dependence of a reactivated memory decrease as time from the original training increases? A dominant cellular/molecular view of memory storage hypothesizes that the consolidation of a new memory is accompanied by the growth of new synapses (Bailey and Kandel, 1993; Andersen and Soleng, 1998; Engert and Bonhoeffer, 1999; O'Malley et al., 2000; Geinisman et al., 2001). Thus, it is believed that, as time from the original training elapses and consolidation proceeds, the number of newly formed synapses increases until it reaches a plateau. One could speculate that when a memory is reactivated, a given number of the same newly formed synapses is reengaged and, therefore, destabilized and reorganized in order to incorporate the new information. As a result, if memory reactivation occurs soon after training, it can potentially destabilize a large part (perhaps most) of the new synapses. On the other hand, if reactivation occurs later, the proportion of the synapses that will be reorganized will decrease. Hence, over time, the vulnerability of that memory will progressively diminish.

Another hypothesis to consider is that the initial phase of both consolidation and reorganization after reactivation may physically share a process of encoding; the former because it encodes new memory traces, and the latter because, as suggested by Nadel and Land (2000), it reorganizes recalled memory traces in conjunction with new information. In support of this hypothesis, training-driven, time-dependent changes in the topography of firing activity, possibly related to memory consolidation, have been described in rabbit avoidance learning by Freeman and Gabriel (1999). Similarly, Ambrogi Lorenzini et al. (1999) reported that different brain structures are required during different temporal phases of memory formation in rat. Thus, it is possible that the initial phase of consolidation is driven by modifications of encoding circuits, which, over time, may lead to long-lasting changes in physically distinct storage circuits. In the same way, new information produced by the reactivation of a memory would also engage the same or overlapping encoding circuits, which, in turn, would modify storage circuits. Therefore, the encoding of a reactivated memory would interfere with the stability of that memory only if the initial phase of consolidation is active, that is, when the same encoding circuits are still engaged.

Indeed, the simplistic view of synapse modification needs to be integrated into a more comprehensive, system-level understanding of memory. Within the context of the present results, the central question is: why is the time between learning and recall critical for generating a temporally graded resistance to disruption of a reactivated memory? One possibility is that as discussed above, more time simply allows for more memory consolidation. However, because the time scale is on the order of weeks, a second, nonmutually exclusive explanation is that what is critical is not the original consolidation of newly acquired information but, rather, the further integration of this information into aspects of other memories or behavioral representations (Squire and Al-

varez, 1995; Sutherland and McNaughton, 2000). Such elaboration and integration may be mediated by modulatory hormonal and/or neuronal pathways and involve different areas of the brain (Gold and McGaugh, 1975; McGaugh, 2000). Testing these hypotheses will provide further understanding of the memory reactivation process.

Experimental Procedures

IA Behavioral Training

This procedure has been described in Taubenfeld et al. (2001). Ninety-seven adult male Long Evans rats (200–250 g) were used in these experiments. Animals were individually housed and maintained on a 12 hr on/12 hr off light/dark cycle. All rats were allowed free access to food and water. The IA chamber consisted of a rectangular-shaped Perspex box divided into a safe compartment and a shock compartment. The safe compartment was white and illuminated; the shock compartment was black and dark. Footshocks were delivered to the grid floor of this chamber via a constant current scrambler circuit. The apparatus was located in a sound-attenuated, nonilluminated room. During training sessions, each rat was placed in the safe compartment with its head facing away from the door. After a period of 10 s, the door separating the compartments was automatically opened, allowing the rat access to the shock chamber. Latency to enter the shock chamber was taken as a measure of acquisition (Acq). The door closed 1 s after the rat entered the shock chamber, and a brief footshock (0.6 mA for 2 s) was administered to the rat. The rat was then removed from the apparatus and returned to its home cage. Retention tests, which also recalled and reactivated the memory, were performed either 2, 7, 14, or 28 days (test one) later by placing the rat back in the safe compartment and measuring the latency to enter the shock chamber. Footshock was not administered on the retention test, and testing was terminated at 540 s. Forty-eight hours after test one, animals were retested for retention (test two). Statistical analysis of the behavioral data was performed using three-way ANOVA followed by Student Newman-Keuls posthoc tests. All protocols complied with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Mt. Sinai School of Medicine Animal Care Committees.

Anisomycin Administration

Systemic injections of anisomycin were performed similar to those described in other studies (Davis et al., 1980). Anisomycin (Sigma, St. Louis, MO) was dissolved in 0.9% saline and adjusted to pH 7.2 with 1 N HCl. Rats were injected subcutaneously with 210 mg of anisomycin/kg body weight or an equivalent volume of 0.9% saline (vehicle).

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