Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus

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Evidence that protein synthesis inhibitors induce amnesia in a variety of species and learning paradigms indicates that the consolidation of newly acquired information into stable memories requires the synthesis of new proteins. Because extinction of a response also requires acquisition of new information, extinction, like original learning, would be expected to require protein synthesis. The present experiments examined the involvement of protein synthesis in the hippocampus in the extinction of a learned fear-based response known to involve the hippocampus. Rats were trained in a one-trial inhibitory avoidance task in which they received footshock after stepping from a small platform to a grid floor. They were then given daily retention tests without footshock. The inhibitory response (e.g., remaining on the platform) gradually extinguished with repeated testing over several days. Footshock administered in a different context, instead of a retention test, prevented the extinction. Infusions of the protein synthesis inhibitor anisomycin (80 μg) into the CA1 region of the hippocampus (bilaterally) 10 min before inhibitory avoidance training impaired retention on all subsequent tests. Anisomycin infused into the hippocampus immediately after the 1st retention test blocked extinction of the response. Infusions administered before the 1st retention test induced a temporary (i.e., 1 day) reduction in retention performance and blocked subsequent extinction. These findings are consistent with other evidence that anisomycin blocks both the consolidation of original learning and extinction.

re-consolidation | anisomycin | reminder treatments

Extensive evidence indicates that the extinction of a learned response, like the original learning, requires acquisition of new information (1, 2). During extinction of an aversively based response, for example, a cue formerly associated with a particular consequence (e.g., footshock) becomes associated with a new consequence (e.g., no footshock). The considerable evidence that many drugs have comparable effects on the acquisition and extinction of responses indicates that acquisition and extinction share common mechanisms. Systemic injections of the GABAergic (related to γ-aminobutyric acid) antagonist picrotoxin enhance retention of many kinds of tasks, including fear-based tasks when administered posttraining (3) and, additionally, enhance extinction of fear conditioning when administered after an extinction session (4). Intrahippocampal administration of the GABAergic agonist muscimol, which impairs consolidation of different forms of contextual fear (5), also impairs context-specific extinction (2). The *N*-methyl-D-aspartate (NMDA) antagonist APV blocks the consolidation of many tasks (5–8) and also blocks both the consolidation (5) and the extinction of conditioned fear tasks (9). Recent findings indicate that infusions of the protein synthesis inhibitor anisomycin or the β-adrenoreceptor antagonist propranolol administered into the insular cortex block both the consolidation (10) and the extinction of conditioned taste aversion (11).

The present experiments examined the involvement of protein synthesis in the hippocampus of rats in the learning and extinction of one-trial inhibitory avoidance, a form of contextual fear conditioning used extensively in studies of the pharmacology and biochemistry of memory consolidation (6, 7). The learning of this task is known to require hippocampal protein synthesis (12). The role of protein synthesis in extinction is of interest because some findings, both older (13) and recent (14), have suggested that memories reactivated by retrieval cues, like newly formed memories (3), may be susceptible to treatments known to disrupt memory consolidation. Memory reactivation may, it is suggested, initiate ''re-consolidation.'' Despite previous evidence questioning this suggestion (15, 16), there is renewed interest in it (14, 17). This issue is important because the idea that a reminder cue initiates re-consolidation of memory for the original training (13, 14) clearly conflicts with the extensive evidence that presentation of a reminder cue without reinforcement (e.g., footshock) initiates extinction of responses associated with that cue (1). It was recently reported that anisomycin infused into the amygdaloid nucleus immediately after presentation of a cue previously used in fear conditioning impaired subsequent retention (14). In contrast, as noted above, other recent findings indicate that anisomycin infused into the insular cortex blocked extinction of conditioned taste aversion (10) when the cue used in training was presented without reinforcement (11). We report here that the protein synthesis inhibitor anisomycin infused into the CA1 region of the dorsal hippocampus blocks both the consolidation and the extinction of memory for inhibitory avoidance training.

Materials and Methods

Subjects. Male Wistar rats (age, 2–3 months, weight, 220–260 g) were used. The animals were housed in plastic cages, five to a cage, with water and food available ad libitum, under a 12-h light/dark cycle (lights on at 7:00 a.m.) at a constant temperature of 23°C.

Behavioral Protocols. For the inhibitory avoidance training (17, 18, 19), rats were gently placed on a 2.5-cm-high, 8.0-cm-wide platform (CS) at the left of a 50.0 × 25.0 × 25.0-cm yellow acrylic training apparatus, whose floor was a series of parallel 0.2-cm-caliber bronze bars spaced 1.0 cm apart. Latency to step down onto the grid with all four paws was measured with an automatic device. The animals received a 0.5-mA, 2.0-s scrambled foot

Abbreviations: RS, reminder shock.

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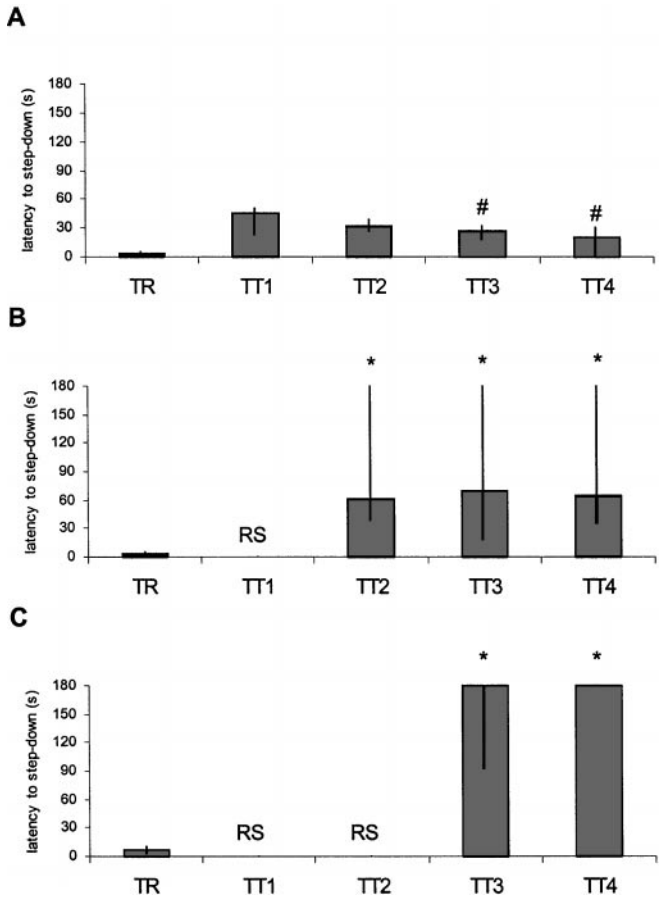

Fig. 1. Repeated exposure to the training apparatus without reinforcement induces extinction of one-trial avoidance. In this and following figures, retrieval values are expressed as median (interquartile range) step-down latency, in seconds. Rats were submitted to one training session (TR) and 3 or 4 test sessions (TT1, TT2, TT3 and TT4) of step-down inhibitory avoidance. Intervals between sessions were 24 h. $n = 9$–11 per group. In the experiments shown in this figure, unoperated animals were used. In *A*, a decline in retention tests performance is seen with repeated testing (extinction). #, Significant difference between TT1 and TT3 or TT4 at $P < 0.03$ level in Mann–Whitney *U* tests, two-tailed. When the *B* or the two *C* test sessions were substituted by a reminder shock (RS), extinction was abolished. Asterisks indicate significant differences in performance from the groups shown in *A*, at a $P < 0.05$ level in Mann–Whitney *U* tests, two-tailed.

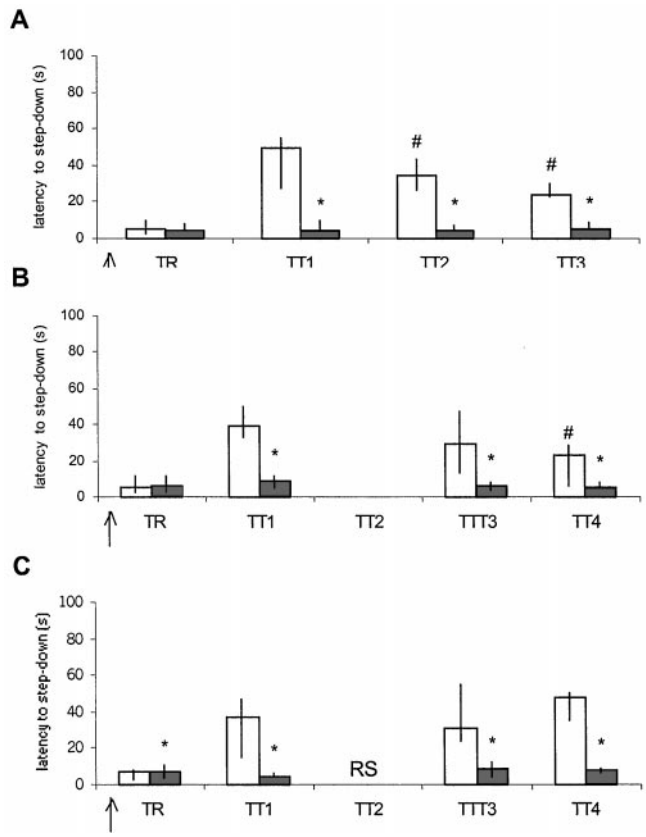

Fig. 2. Intrahippocampal anisomycin administration blocks the consolidation of one-trial avoidance. In this and the following figure, animals cannulated in CA1 were used; open columns show values from animals that were treated with saline, and filled columns show values of animals treated with anisomycin (80 μg/side). The treatments were given 10 min before training. Asterisks indicate differences from the corresponding saline group at $P < 0.05$ in Mann–Whitney *U* tests, two-tailed. (*A*) As shown previously (8), pretraining intrahippocampal anisomycin caused anterograde amnesia. Here, the amnesia persisted across three test sessions. Extinction was observed in the saline group (difference between performance in TT1 and TT2, TT2 and TT3, and TT1 and TT3 was $P < 0.05$, 0.05, and 0.001 level, respectively). Omission of TT2 (*B*) or its substitution by a reminder shock (*C*) did not reverse the amnesia caused by pretraining anisomycin. The RS hindered extinction (TT4 was not different from either TT1 or TT3 at $P = 0.1$ level in Mann–Whitney *U* tests, two-tailed).

shock (US). They were then tested for retention 24, 48, 72 and in most experiments, also 96 h later. On the test sessions the foot-shock (US) was not administered. The difference in step-down latency between the training session and the 1st test session was used as a measure of retention (i.e., retrieval) of the learned response (5, 6, 12, 18, 19). In some experiments (Figs. 1 *B* and *C* and 3*E*), one or more test sessions were replaced by a nonspecific "reminder" shock (RS). The RS was identical to the US except that it was given in different apparatus.

Surgery and Infusion Procedures. In the experiment shown in Fig. 1, unoperated animals were used. In all others, rats were implanted under deep thiopental anesthesia with 30-gauge cannulae in the CA1 region of the dorsal hippocampus at the coordinates of the atlas of Paxinos and Watson (20): anterior, -4.3; lateral, ± 4.0; ventral, 2.6. The cannulae were fixed to the skull with dental acrylic (8, 18, 19, 21). After recovery from surgery, these animals were submitted to training in inhibitory avoidance. They received bilateral hippocampal 0.5-μl infusions of saline or of anisomycin (80 μg) dissolved in saline. Infusions were given 15 min before training (Fig. 2), 15 min before or immediately after the test session, or instead of the test session (Fig. 3). A 27-gauge infusion cannula was fitted into the guides at the appropriate time, and the treatments were delivered through it by a 1.0-μl microsyringe connected to the infusion cannula by polyethylene tubing. Infusions were carried out over 30 s, first on the left and then on the right side; the infusion cannula was kept in place for 1 additional minute to aid diffusion. Thus, the entire procedure took 3 min (18, 19).

Placement of the cannulae was checked histologically post mortem as described elsewhere (8, 18). Two to twenty-four hours after the last test session, 0.5 μl of a 4% solution of methylene blue was infused as above in each animal. Only the behavioral data from animals with correct infusion placements were used. Infusions were considered correct when the spread of the dye in the infusion sites was within 1 mm^3 of the intended site (18, 21).

Statistical Analysis. The retention test session latencies were recorded for a maximum of 180 s (17, 18, 21). Because of this "ceiling" cutoff, nonparametric statistics were used to analyze the data. First, a Kruskal-Wallis analysis of variance was applied

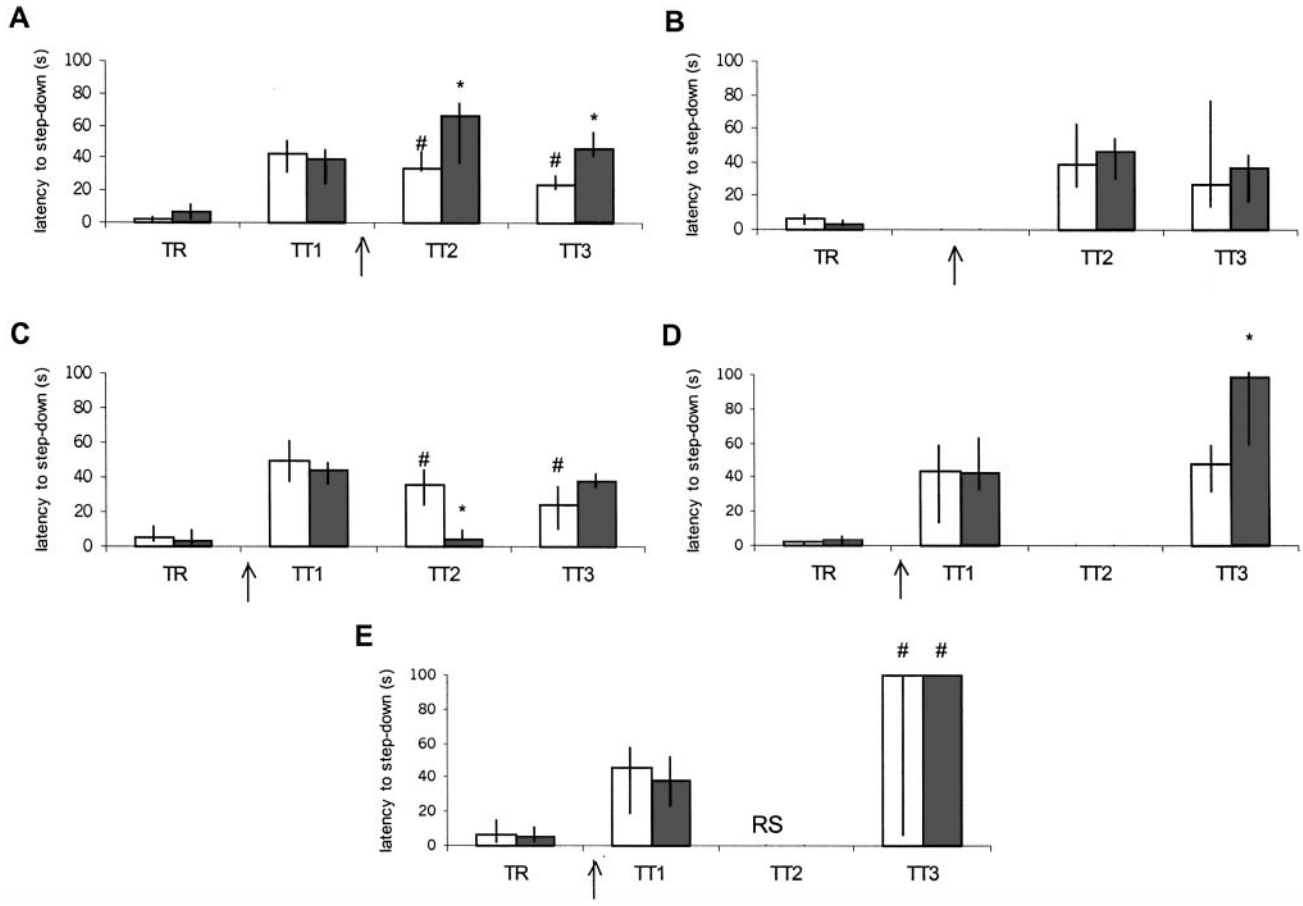

Fig. 3. Intrahippocampal anisomycin administration blocks the extinction of one-trial avoidance. In this experiment, intrahippocampal infusions were given either immediately after TT1 (*A*), instead of TT1 (*B*), or 15 min before TT1 (*C*, *D*, and *E*). In *B*, no differences in retention test performance between or within groups were seen. In the animals in which anisomycin was given either after TT1 (*A*) or before TT1 (*C*), a blockade of extinction was observed. In the corresponding saline groups, the difference between TT2 and TT1 was significant at a $P < 0.05$ level in *A*, and at a $P < 0.05$ level in *C*. There was no evidence of extinction in control groups in *D* or *E*. Anisomycin given before TT1 caused an impairment of retrieval in TT2 ($P < 0.01$ vs. TT1 or TT3), with full recovery in TT3 (*C*). If TT2 was omitted (*D*) or replaced by a RS (*E*), anisomycin given before TT1 actually enhanced retrieval in TT3 ($P < 0.02$ and < 0.001, respectively, relative to TT1). The RS given instead of TT2 enhanced retrieval in the saline group, too (significant difference between TT3 and TT1 at $P < 0.01$ level).

to the various sets of groups. If significance levels were found to be below the $P = 0.05$ level, individual Mann–Whitney *U* tests, two-tailed, were carried out where appropriate.

Results

Animals Learned the One-Trial Task. All animals, except those treated with pretraining anisomycin (Fig. 2), displayed clear evidence of retention of the training. The difference in step-down latency between the training session and the immediately subsequent test session was significant at a $P < 0.001$ level (Figs. 1, 2, and 3).

Repeated Testing Induced Extinction. The experiment of Fig. 1 shows that testing over several days resulted in extinction (Fig. 1*A*). Also, see controls in Fig. 2 *A* and *B*, Fig. 3 *A* and *C*). RS, given rather than the retention test, attenuated the extinction (Fig. 1*B*; also see Fig. 2*C*). RS given on two sessions enhanced retention (Fig. 1*C*).

Anisomycin Induced Amnesia for the Original Training. Anisomycin infused bilaterally into the CA1 before training induced amnesia. Fig. 2*A* shows that the amnesia was not attenuated by repeated testing (i.e., repeated exposure to the training context alone). Fig. 2 *B* and *C* shows that the amnesia was also not attenuated by omission of one of the test sessions, or by replacing it with an RS.

Anisomycin Infused at the Time of the 1st Test Session Inhibited Extinction. Anisomycin infused into CA1 immediately after the 1st test enhanced retention test performance in the following two tests; i.e., it blocked extinction (Fig. 3*A*). Anisomycin or saline given *instead* of the 1st test had no effect on retrieval performance in the subsequent test sessions (Fig. 3*B*). Anisomycin infused before the 1st test did not block retrieval performance on that test but blocked extinction. In these animals, retention performance was impaired on the second test but recovered spontaneously on the following test (Fig. 3*C*) where it was enhanced relative to controls that showed extinction.

Discussion

Repeated testing for inhibitory avoidance every 24 h after training resulted in gradual extinction of the response. Anisomycin administered into the CA1 induced amnesia for inhibitory avoidance training when given before training but not when administered at the time of testing. In this task (12) and in conditioned taste aversion (10), anisomycin produces anterograde amnesia. In this task, anisomycin is also amnestic when given several hours posttraining (12). In other tasks, its effect can also be seen when given shortly after training (7).

The most critical finding of this study is that anisomycin administered into the hippocampus at the time of the 1st test inhibited subsequent extinction. This effect was obtained when the drug was given either before or after the 1st retention test. Thus, the present findings indicate that inhibiting protein synthesis in the CA1 region of the hippocampus prevents the learning of new information that would lead to extinction of the inhibitory avoidance response. The inhibitory avoidance task (6, 7), like many others, particularly those involving contextual fear (2), depends on the hippocampus and its connections (7, 18). The inhibition of extinction found in the present study is similar to the effect obtained when anisomycin was infused into the insular cortex before testing for conditioned taste aversion (11).

Extinction of the inhibitory avoidance response was also prevented if a nonspecific RS was given instead of the 1st and/or the 2nd retention test session. It should be noted that, in all cases, substitution of a RS for an extinction trial enhanced retention performance on subsequent tests. The effectiveness of the RS was most likely due to generalization resulting from its similarity to the training context. The RS had no effect on retrieval when memory was disrupted by pretraining infusions of anisomycin (Fig. 2), and the animals were thus effectively prevented from consolidating the context–shock association.

It is known that memory for inhibitory avoidance training critically involves the CA1 region of the hippocampus and is strongly modulated by the amygdala (3, 6, 7, 22). Additionally, the amygdala has been suggested as a site of consolidation (9) and ''re-consolidation'' (14), after reactivation, of memory of fear conditioning. Because the present findings indicate that anisomycin infused into the hippocampus impairs extinction, they do not support a general view that memory retrieval activates memory re-consolidation processes that are susceptible to anisomycin-induced amnesia. Additionally, other findings (e.g., ref. 23) have questioned the hypothesis that fear-based memories are consolidated and stored in the amygdala.

In the present experiments, anisomycin infused into the hippocampus before the 1st retention test session impaired retrieval on the subsequent test 24 h later, but did not affect retrieval performance on the following tests. This temporary disruption of retrieval might be due to a delayed influence of protein synthesis inhibition on the biochemistry of retrieval (12), an influence that does not last beyond 24 h. In a recent study reporting that anisomycin infused into the amygdala after a retrieval test for conditioned fear impaired subsequent memory (14), the animals were given only a single retrieval test 1 day later. Thus, it was not determined whether there was any subsequent spontaneous recovery of the original response. This is a critical issue because of evidence of spontaneous recovery from retention performance impairments induced by treatments administered after a retrieval test (24, 25). Additionally, of course, it remains to be determined whether memory retrieval effects involving the hippocampus differ from those in the amygdala.

The evidence that many drugs have comparable effects on original learning and extinction strongly suggests that the learning involved in acquisition and extinction share some common mechanisms (2–11). To date, however, relatively little is known about the relationship between the biochemistry of retrieval and that of extinction. Biochemical observations made in the first 3 h after a retrieval test of one-trial avoidance reveal very few changes, aside from a rapid initial activation of p42 and p44, two enzymes of the mitogen-activated protein kinase (MAPK) pathway (19). The biochemistry of retrieval is partly reminiscent of, but indeed far from identical to, that of memory consolidation (18, 19). Retrieval of memory for one-trial step-down inhibitory avoidance requires the integrity of glutamate metabotropic receptors and the cAMP-dependent protein kinase and MAPK pathways in the hippocampus and in various cortical structures of the rat (13, 14).

Note Added in Proof. Taubenfeld *et al.* (26) recently reported that anisomycin infused into the hippocampus immediately after inhibitory avoidance training impaired retention but when infused after a retention test did not impair subsequent retention performance.

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