Stability of Retrieved Memory: Inverse Correlation with Trace Dominance

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compromised in its ability to initially establish an infection when competing with the corresponding wt strain (*22*). However, how might *Hp* or VacA meet T cells in the gastric mucosa? The number of lamina propria and intraepithelial T cells of the $CD4^+$ and $CD8^+$ subtype are significantly increased in *Hp*-infected versus noninfected patients (*23*), and *Hp* could directly contact such intraepithelial T cells. Because tight junctions can be opened by *Hp* (*24*), secreted bacterial products such as VacA can be found deep in the lamina propria (*25*). Thus, VacA might act as a "long distance weapon" to efficiently block proliferation of T cells in the local gastric environment. For *Hp*, classified as a type I carcinogen, a mechanism of local immune suppression might also be an important instrument for induction of malignant mucosa-associated lymphoid tissue (MALT) lymphoma and adenocarcinoma of the stomach.

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Supporting Online Material

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Stability of Retrieved Memory: Inverse Correlation with Trace Dominance

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In memory consolidation, the memory trace stabilizes and becomes resistant to certain amnesic agents. The textbook account is that for any memorized item, consolidation starts and ends just once. However, evidence has accumulated that upon activation in retrieval, the trace may reconsolidate. Whereas some authors reported transient renewed susceptibility of retrieved memories to consolidation blockers, others could not detect it. Here, we report that in both conditioned taste aversion in the rat and fear conditioning in the medaka fish, the stability of retrieved memory is inversely correlated with the control of behavior by that memory. This result may explain some conflicting findings on reconsolidation of activated memories.

We wanted to further elucidate the neurobiology of experimental extinction, which is the decline in the frequency or intensity of a conditioned response after its retrieval in the absence of the reinforcer (*1, 2*). We previously reported that in conditioned taste aversion (CTA)—in which taste (conditioned stimulus, CS) is associated with delayed visceral malaise (unconditioned stimulus, US) (*3*)—microinfusion of anisomycin, a protein synthesis inhibitor, into either the taste cortex (insular cortex) (*4*) or the basolateral amygdala (*5*) immediately after retrieval blocks extinction. This is congruent with the notion that extinction is relearning (of a CS-noUS or "inhibitory" association) rather than unlearning (of the CS-US association). Anisomycin, a universal consolidation blocker, did not disrupt the original CS-US trace. A similar effect of protein synthesis inhibition has been reported in inhibitory avoidance, in which extinction was blocked and the original trace spared (*6*). This finding contrasts with reports by several authors that application of consolidation blockers in retrieval leads to apparent amnesia of the original trace [(*7–10*), but see discussions in (*11, 12*)]. This discrepancy might be related to the difference in the ability of the retrieval protocol to initiate extinction (*13*).

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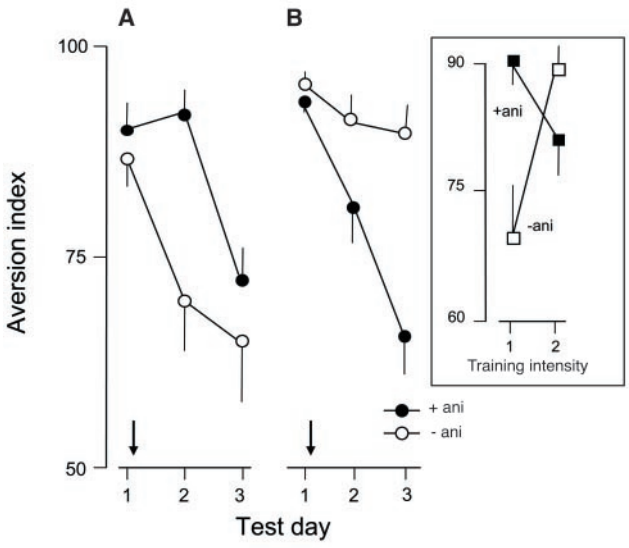

Fig. 1. Effect of anisomycin on extinction of CTA in the rat as a function of the intensity of CTA training. Values are means ± SEM of the aversion index, defined as [ml water/(ml water + ml saccharin)] × 100 (*4*). (**A**) After a single training trial, the trace can be extinguished readily (–ani, $n = 8$); microinfusion of anisomycin into the insular cortex immediately after retrieval (arrow), under conditions that block consolidation of the original trace, blocks extinction (+ani, $n = 31$). The data are derived from replication of an experiment reported in (*4*). (**B**) After two training trials, 1 day apart, the trace becomes much more resistant to extinction (–ani, $n = 36$). The effect of microinfusion of anisomycin into the insular cortex immediately after retrieval (arrow), under the conditions of (A), is now reversed; the protein synthesis inhibitor leads to accelerated decline in the behavior guided by the original association (+ani, $n = 27$). Inset: Aversion indices on the second test day for the four groups described in (A) and (B) plotted as a function of training intensity. For statistical analysis, see text.

In CTA in the rat (*14*), although long-term memory is highly robust, extinction is readily obtained in a choice test situation after one trial training (standard training) but is significantly inhibited if two training sessions are used (intensive training) (*15*) (Fig. 1, A and B, open circles). This was verified by repeated-measures analysis of variance (ANOVA), which revealed a significant training intensity effect ($P < 0.001$) and Training Intensity × Test Day interaction ($P = 0.024$). Whereas microinfusion of anisomycin into the insular cortex immediately after retrieval of the memory obtained in the standard training protocol blocks extinction until the next extinction opportunity (Fig. 1A, anisomycin treatment effect $P = 0.008$), the same treatment immediately after retrieval of the trace obtained in the intensive training protocol leads to decline in the original memory rather than blockade of extinction (Fig. 1B, anisomycin treatment effect $P = 0.027$). Comparison of anisomycin treatment after intensive versus standard training (Fig. 1, A and B, closed circles, days 1 and 2) reveals a significant Training Intensity × Test Day interaction ($P = 0.006$). Moreover, anisomycin treatment after intensive training impairs memory on the following day significantly more than after standard training (*t* test, $P = 0.044$) (Fig. 1, inset). Two-way ANOVA reveals a significant Training Intensity × Anisomycin Treatment interaction (Fig. 1, inset, $P < 0.0001$). All in all, the data indicate that the sensitivity of the retrieved trace to the consolidation blocker is a function of either the intensity of the original training or the ability to sustain extinction after retrieval, which is related in this protocol to the intensity of the original training.

Is the phenomenon general? To approach this question, we have used another system: fear conditioning in the medaka fish (*Oryzias latipes*). Medaka, like other teleost fish, has a relatively simple brain, which could prove advantageous in the analysis of primitives of memory extinction in vertebrates (*16, 17*). Furthermore, the medaka is easily cultivated in large numbers and is suitable for neurogenetics and neurodevelopmental studies. In a fear conditioning protocol, the fish is conditioned to associate light (CS) with mild electric shock (US) to release a fear response, resulting in altered locomotion [unconditioned response (UR) or conditioned response (CR)] (*14*). The conditioned behavior is acquired within a few training sessions (Fig. 2A, one-way ANOVA, sessions 1 to 5, $P < 0.0001$). The CR is extinguished within a small number of training sessions when retrieved in the absence of the US (Fig. 2A, sessions 5 to 8, $P = 0.003$). Bath application of the anesthetic agent 3-aminobenzoic acid ethyl ester (MS222) immediately after training (*14*) blocks consolidation of long-term memory, tested at 24 hours (Fig. 2B, paired *t* test, $P = 0.007$), without affecting short-term memory, tested at 0.5 hour (Fig. 2B, $P = 0.9$). Memory and performance remained unimpaired relative to untreated fish when application of MS222 was delayed to 3 hours after retrieval (Fig. 2C, repeated-measures ANOVA, $P = 0.9$).

We investigated the effect of the same treatment on the retrieved fear memory. In our protocol, extinction in medaka is a graded function of the number of extinction (retrieval) trials. Whereas a single retrieval in the absence of the US does not result in significant extinction, increasing the number of extinction trials to 10 results in massive extinction on the subsequent day (Fig. 2D). Transient application of MS222 immediately after a single retrieval trial results in marked reduction in the CR at 24 hours (Fig. 2C). This is established statistically by comparison of the CRs before and after treatment (paired *t* test, $P = 0.017$) and of treated and untreated groups after anesthesia (two-sample *t* test, $P = 0.007$). In contrast, the same treatment after 10 retrieval trials blocks extinction, as is evident from the comparison of treated and untreated fish (two-sample *t* test, $P < 0.0001$, Fig. 2, A and C). The same treatment after five retrievals results in intermediate decline in the CR, as assessed by performance at 24 hours (Fig. 2C). All in all, two-way ANOVA unveils a significant Number of Extinction Trials × Drug Treatment interaction (Fig. 2D, $P < 0.0001$). This interrelationship parallels that described above for CTA, although here the interaction measured was with the number of extinction trials rather than the intensity of the original training.

The outcome of an extinction trial can be regarded as the sum of multiple, sometimes conflicting time-dependent processes (*15*) involving at least two traces: the "excitatory" original CS-US trace, and an "inhibitory" or new CS-noUS trace. These traces compete for the control of behavior.

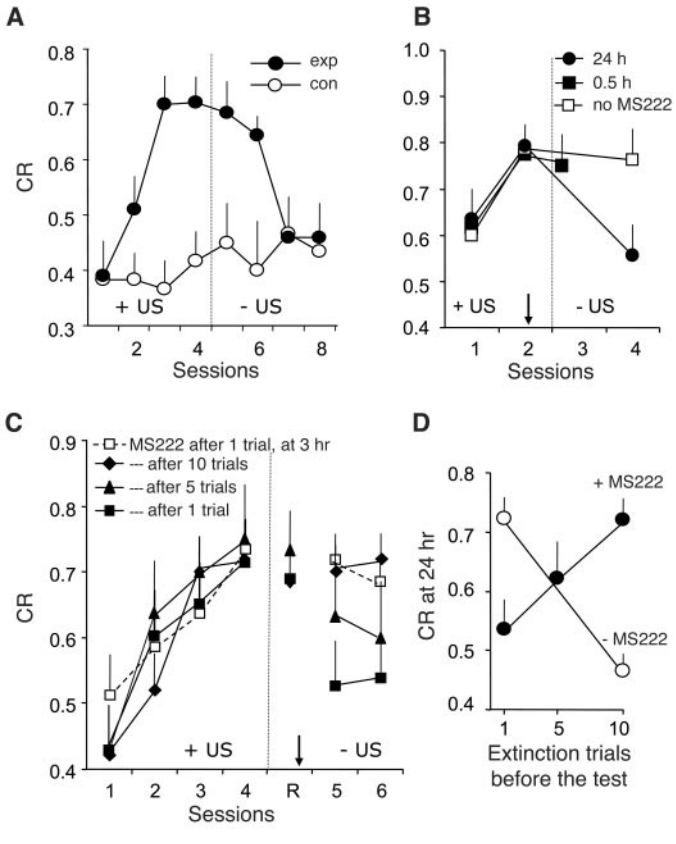

Fig. 2. Fear conditioning in medaka, its extinction, and the effect of transient anesthesia with MS222 on extinction as a function of the number of extinction trials. The *y* axis depicts the probability of the CR (mean + SEM), which is fear-induced alteration of locomotor behavior (*14*). (**A**) Acquisition and extinction of fear conditioning (exp, $n = 20$; pseudoconditioned fish, con, $n = 12$). Fish were trained for 2 days (four sessions of five trials each) with the US, followed by four extinction sessions in the absence of the US. (**B**) MS222 as a consolidation blocker in medaka. Application of MS222 (arrow) immediately after two training blocks (10 trials each) impaired the original memory at 24 hours but not at 0.5 hour ($n = 8$ each). (**C**) Effect of MS222 administered immediately after retrieval as a function of the number of retrieval trials. Fish acquired the conditioned response during 2 days (blocks 1 to 4). On day 3, they were subjected to 1 ($n = 16$), 5 ($n = 12$), or 10 ($n = 19$) retrieval trials (R); they were tested for their memory on day 4. No significant effect in performance was detected on the retrieval day, but a marked effect was detected 1 day later. Whereas the transient treatment with MS222 (arrow) led to apparent amnesia of the original conditioned behavior at 24 hours if applied after one retrieval trial, it completely blocked extinction if applied after 10 extinction trials; the results for 5 extinction trials were intermediate. Note that application of MS222 3 hours after retrieval ($n = 12$) had no effect on memory. (**D**) Memory at 24 hours as a function of the number of extinction trials before the application of MS222. In intact fish, the behavior guided by the CS-US association declines along the extinction session (–MS222), whereas in the presence of the consolidation blocker (+MS222) the opposite is the case. For statistical analysis, see text.

The outcome of this competition depends in part on the intensity of the original training and the number of extinction trials; if the original training is highly robust and/or the number of extinction trials is too small, the "inhibitory" or CS-noUS trace may not gain appreciable control of behavior. Our findings, using two different species, different memory paradigms, and different consolidation blockers with different molecular targets, suggest that the trace that retains or is in the process of gaining appreciable control over behavior after the retrieval session (i.e., becomes dominant) is the one that displays transient sensitivity to the consolidation blocker. In other words, the stability of the trace, as judged by insensitivity to consolidation blockers, is inversely correlated with trace dominance. It remains possible that the drugs block extinction under some conditions but accelerate it under other conditions. This differential effect is highly unlikely, particularly in light of the aforementioned prevalent view that extinction is relearning (*2, 18–20*) and that consolidation blockers disrupt the formation of the long-term trace (*21–23*); acceleration of extinction by the two different consolidation blockers might have implied that these agents enhance memory formation, for which there is no evidence.

Our findings could be construed in the context of the reconsolidation hypothesis, which posits that after retrieval the activated trace must undergo a process of stabilization to enter again into a long-term phase (*7–10*). A caveat is, however, appropriate. We do not yet know whether the effect of consolidation blockers after retrieval is identical to their effect after acquisition. The possibility that the effect might be different is hinted at by reports that molecular and circuit mechanisms that subserve the acquisition of original and extinguishing traces share components but are nonidentical (*4, 5, 15*). For example, although we did not detect spontaneous recovery of the blocked memory [tested at 8 days in rat CTA and 2 days in medaka fear conditioning (*24*)], we cannot exclude the possibility that other manipulations might unveil a latent, depressed trace. This could indicate performance or retrieval deficit rather than storage deficit. It is noteworthy that although a predominant assumption is that blockade of consolidation after acquisition leads to storage deficit, explanations based on retrieval deficits were not abandoned [e.g., (*11*)].

The proposed relationship between the susceptibility to disruption and the dominance of the trace after retrieval, which probably is an experimental manifestation of the theoretical dichotomy "active-inactive" memory (*25*), may explain some lingering discrepancies in the literature on the effect of consolidation blockers after retrieval. It may also be of potential value in designing protocols for selective modification of different long-term associations of a target item in memory.

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Materials and Methods

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Encoding Predictive Reward Value in Human Amygdala and Orbitofrontal Cortex

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Adaptive behavior is optimized in organisms that maintain flexible representations of the value of sensory-predictive cues. To identify central representations of predictive reward value in humans, we used reinforcer devaluation while measuring neural activity with functional magnetic resonance imaging. We presented two arbitrary visual stimuli, both before and after olfactory devaluation, in a paradigm of appetitive conditioning. In amygdala and orbitofrontal cortex, responses evoked by a predictive target stimulus were decreased after devaluation, whereas responses to the nondevalued stimulus were maintained. Thus, differential activity in amygdala and orbitofrontal cortex encodes the current value of reward representations accessible to predictive cues.

An organism's ability to predict future events, such as food or danger, on the basis of relevant sensory cues is emblematic of associative learning. This phenomenon can be studied with classical conditioning, whereby a previously neutral item (the conditioned stimulus, CS+) acquires importance after being paired with a biologically salient reinforcer (the unconditioned stimulus, UCS). The efficacy of conditioning depends on establishing CS-UCS links, but evidence suggests that a CS+ can invoke multiple, unique UCS representations, including sensory properties, reward value, or associated affective states (*1*). Clarifying the neural substrates that support these associative links has important implications for biological models of reinforcement learning (*2*, *3*).

Neuroimaging studies emphasize the roles of amygdala and orbitofrontal cortex (OFC) in human classical conditioning (*4–7*), but no experiment has characterized the psychological underpinnings of these activations. Reinforcer devaluation offers a means of dissociating among the various central representations that a CS+ may engage. This approach has been applied to animal studies of appetitive learning, which show that damage to amygdala and OFC

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