Detection of a Temporal Error Triggers Reconsolidation of Amygdala-Dependent Memories

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Summary

Updating memories is critical for adaptive behaviors, but the rules and mechanisms governing that process are still not well defined. During a limited time window, the reactivation of consolidated aversive memories triggers memory lability and induces a plasticity-dependent reconsolidation process in the lateral nucleus of amygdala (LA) [1–5]. However, whether new information is necessary for initiating reconsolidation is not known. Here we show that changing the temporal relationship between the conditioned stimulus (CS) and unconditioned stimulus (US) during reactivation is sufficient to trigger synaptic plasticity and reconsolidation of an aversive memory in the LA. These findings demonstrate that time is a core part of the CS-US association and that new information must be presented during reactivation in order to trigger LA-dependent reconsolidation processes. In sum, this study provides new basic knowledge about the precise rules governing memory reconsolidation of aversive memories that might be used to treat traumatic memories.

Results and Discussion

Traumatic fear memories are strong and persistent and form the basis of several pathological disorders, including posttraumatic stress disorder (PTSD) and anxiety disorders. The search for procedures that may render these memories sensitive to pharmacological or behavioral treatments is thus critical. It is known that after memories have been consolidated into a long-term state, they can enter a new labile state when reactivated prior to being reconsolidated. During this lability window, it is believed that memories are updated and new elements are incorporated [4]. However, the exact rules governing the updating processes during reconsolidation are not yet understood. One proposal emerging from the literature is that reconsolidation processes are initiated when additional learning is involved during the reactivation procedure, forcing the original memory to be updated and reconsolidated [6, 7].

Pavlovian threat (fear) conditioning has been used extensively to study emotional learning and memory both in animals and humans [8], and the reconsolidation of auditory fear conditioning has been shown to be highly selective and dependent on plasticity mechanisms in the lateral nucleus of amygdala (LA) [9, 10]. Interestingly, only weaker fear memories have been observed to be susceptible to reconsolidation; recently formed stronger fear memories appear to be less susceptible to reconsolidation interference even when using a conditioned stimulus-unconditioned stimulus (CS-US) trial for reactivation [5, 11]. This is potentially problematic for the use of reconsolidation blockade as part of a therapy for traumatic memories because these memories by definition involve strong aversive experiences.

In Pavlovian conditioning, the subject not only learns that the CS predicts the arrival of the US but also learns when the US is expected to arrive [12]. Time is a critical element in associative learning [13, 14]. Here, we asked whether a change in CS-US time interval is necessary and sufficient to trigger an update of an aversive memory and its reconsolidation in an amygdala-dependent manner. To do so, we designed a temporal auditory fear-conditioning protocol in which a 60 s tone CS is associated with a foot-shock US delivered 30 s after the tone onset (US@30; see Figure S1 available online). This design permits the presentation of a single training trial in a reactivation procedure that alters only the temporal relationship between the CS and US while equating the total number and duration of stimuli presented (context, CS, and US).

We first verified that intra-LA infusion of a protein synthesis inhibitor immediately after reactivation is insufficient to interfere with the reconsolidation of a recently formed strong fear memory [5]. Rats were given ten CS-US@30 pairings followed by 2 hr later by a reactivation trial consisting of a single CS-US@30 pairing identical to the training condition. As expected, rats given intra-LA infusion of anisomycin following the reactivation trial showed equivalent levels of freezing during the reactivation trial [t(14) = 0.729, nonsignificant (n.s.)] and during the postreactivation long-term memory (PR-LTM) test 24 hr later [t(14) = 0.135, n.s.; Figure 1A] relative to vehicle-infused controls. Thus, in agreement with Wang et al. [5], recently formed stronger fear memories are less susceptible to reconsolidation interference using a protein synthesis inhibitor. In contrast, when the CS-US time interval was reduced from 30 to 10 s during the memory reactivation trial, rats infused with anisomycin showed significantly reduced freezing during the PR-LTM test relative to vehicle-infused controls [PR-LTM: t(12) = 8.403, p < 0.001; reactivation: t(12) = 0.488, n.s.; Figure 1B]. This suggests that the detection of a difference in the CS-US interval between training and reactivation is sufficient to induce reconsolidation of a stronger aversive memory. Importantly, both anisomycin and vehicle groups showed equivalent levels of freezing during a test of postreactivation short-term memory [PR-STM: t(10) = 0.040, n.s.; reactivation: t(10) = 0.785, n.s.; Figure 1C], suggesting that the impairment observed during PR-LTM was due to the disruption of reconsolidation processes and not due to damage to the amygdala. Furthermore, when anisomycin was infused into the central nucleus of amygdala (CeA), no
reconsolidation impairment was observed [PR-LTM: t(9) = 0.271, n.s.; reactivation: t(9) = 0.347, n.s.; Figure 1D]. Thus, reconsolidation of a fear memory following a temporal shift depends upon protein synthesis in the LA, but not in the CeA, as previously reported using standard fear conditioning and reactivation procedures [15]. In agreement with this observation, we observed that a change in CS-US interval triggers plasticity mechanisms in the LA, but not the CeA, as measured by retrieval-induced expression of the immediate early gene zif-268, a marker of synaptic plasticity that has been implicated in fear memory reconsolidation [16, 17] (Figure 2).

The number of zif-268-positive cells was significantly higher in the LA in rats reactivated with a change in the CS-US time interval relative to rats reactivated with the initial CS-US time interval and those in the nonreactivated group [F(2,21) = 6.011, p < 0.01; Figures 2C–2F]. No significant differences were observed among the three groups for the number of zif-268-positive cells in the CeA [F(2,21) = 2.892, n.s.; Figures 2G–2J].

The aforementioned findings demonstrate not only that a change in the CS-US interval can return strong aversive memories to a labile state but also that the CS-US interval itself has been learned during the conditioning session. If a temporal discrepancy is the critical parameter that triggers reconsolidation, then increasing the CS-US interval should be equally effective as decreasing it. In our next experiment, we therefore trained rats with a 60 s tone CS paired with a US foot shock delivered 30 s after tone onset (CS60 s US@30). Freezing during reactivation was equivalent between vehicle and anisomycin rats in all four experiments. *p < 0.05. LA, lateral nucleus of amygdala; CeA, central nucleus of amygdala.

Figure 1. Changing the CS-US Time Interval during Reactivation of Strong Aversive Memories Triggers an LA-Dependent Reconsolidation Process. Each panel shows schematic of the experimental design (top) and percentage of freezing (mean ± SEM) to the CS during reactivation (React) and postreactivation long-term memory test (PR-LTM) in rats infused with vehicle (white bars) or anisomycin (black bars) (bottom). All four experiments consisted of training with ten trials of 60 s tone paired with a US foot shock delivered 30 s after tone onset (CS60 s US@30). Freezing during reactivation was equivalent between vehicle and anisomycin rats in all four experiments. *p < 0.05. LA, lateral nucleus of amygdala; CeA, central nucleus of amygdala.

(A) Rats reactivated with the same CS-US time interval as the one learned during training (CS60 s US@30) and given intra-LA anisomycin did not show an impairment of memory during PR-LTM.

(B and C) Rats infused with anisomycin in the LA and reactivated with a CS-US interval shifted to 10 s (CS60 s US@10) showed an impairment during PR-LTM (B), but not when memory was tested 3 hr (postreactivation short-term memory; PR-STM) after reactivation (C).

(D) The infusion of anisomycin in the CeA did not induce an impairment of memory during LTM in the rats reactivated with a shifted CS-US interval.
whereas vehicle-treated shifted animals tend to express freezing at an equivalent level throughout the CS duration, the anisomycin-treated shifted rats show different patterns depending on their shifted conditions. In particular, anisomycin-treated rats shifted to a shorter CS-US interval show a higher level of freezing at the beginning of the CS and a progressive decay as time elapses during the CS, indicating a stronger contribution of the fear related to the new CS-US interval. Thus, although further experiments are needed to specifically address this issue, the present data suggest that when a change in the temporal parameters during reactivation is detected, the new temporal relationship between the CS and the US during reactivation is acquired in a single trial and possibly in a manner independent of the LA.

We next asked whether a strong fear memory established with fewer training trials is susceptible to reconsolidation interference with anisomycin. The present study, however, only provided evidence for the influence of the US timing at the outset of learning. Anisomycin-treated rats that were shifted to a shorter CS-US interval showed a higher level of freezing at the beginning of the CS and a progressive decay as time elapses during the CS, indicating a stronger contribution of the fear related to the new CS-US interval. Thus, although further experiments are needed to specifically address this issue, the present data suggest that when a change in the temporal parameters during reactivation is detected, the new temporal relationship between the CS and the US during reactivation is acquired in a single trial and possibly in a manner independent of the LA.

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Figure 2. Memory Reactivation Induces Synaptic Plasticity in LA Only when the CS-US Time Interval Is Shifted
(A) Schematic of the experimental design.
(B) Photomicrograph showing the amygdala nuclei analyzed for zif immunoreactivity. The broken lines delineate the nuclei borders. LA, lateral nucleus of amygdala; CeA, central nucleus of amygdala; opt, optic tract. Scale bar represents 200 μm.
(C–E and G–I) Photomicrographs of transverse zif-stained sections from representative cases illustrating animals placed in the context but not presented with any CS-US (no react; C), no shift (D), and shift animals (E) in the LA or in the CeA (G–I) at 2.8 mm posterior to bregma. The broken lines delineate the nuclei borders. Scale bars represent 200 μm.
(F and J) The average of zif-648-positive cells per square millimeter (mean ± SEM) across three anterior-posterior levels. Only the rats whose memory was reactivated with a CS-US time interval different than the one used during training showed an increase in the expression of zif-648-positive cells in the LA (F), but not in the CeA (J). *p < 0.05 by Newman-Keuls post hoc test.
Conclusions

In sum, our results demonstrate that when a change in the temporal relationship between CS and US is detected, an update of the previously acquired aversive memory and its reconsolidation is triggered in an amygdala-dependent manner. Our results also demonstrate unequivocally, and for the first time with amygdala-dependent memories, that when the association is well learned, changing the temporal association architecture is sufficient to trigger reconsolidation even of recently acquired strong aversive memories [5]. In contrast, if nothing novel is added and no additional learning is elicited, the aversive memory trace is not rendered labile [6, 7, 19]. In contrast to the findings of Duvarcı and Nader [11], in our study the freezing level reached its maximum after a single training trial, indicating that the CS-US association was fully learned; the additional CS-US trial during reactivation with no change in the temporal structure was therefore not sufficient to trigger additional learning, as in Wang et al. [5]. Given that learning the time interval and learning the association may be tightly intertwined, and because time is a critical element of the US expectation [20, 21], changing the temporal relationship between CS and US appears to elicit an update of temporal expectancy rules (e.g., modifying the previously consolidated temporal association) and therefore may be the most powerful tool to trigger reconsolidation. Our results also demonstrate that changes in the temporal relationship between CS and US trigger synaptic plasticity and reconsolidation processes in the LA. Neurophysiological studies in human and nonhuman primates, as well as in rodents, have suggested that the amygdala may be involved in the detection of prediction error, both in appetitive and aversive Pavlovian situations. In effect, the amygdala shows anticipatory neurophysiological activity [22, 23, 24], as well as reactivity to surprising temporal irregularities or unexpected events [25, 26]. Whether temporal processing (timing, CS-US interval storage, and comparison between experienced and expected US value) is computed in the LA is not known. Our results strongly suggest that aversive prediction error detection—whether processed in part in the amygdala itself or only transmitted from upstream neural structures—is
Error Detection Triggers Memory Reconsolidation

A fundamental mechanism in triggering reconsolidation of aversive memories in the amygdala. Collectively, our findings provide precise boundary rules for effective destabilization of strong aversive memories.

**Experimental Procedures**

**Behavioral Experiments**

Adult Sprague-Dawley rats provided by Hilltop Lab Animals and weighing 250–300 g at the beginning of the experiments were used. All procedures were in accordance with the NIH Guide for the Care and Use of Experimental Animals and were approved by the New York University Animal Care and Use Committee.

After recovering from surgical implantation of cannulae (see Supplemental Experimental Procedures for details and Figures S2 and S3 for cannulae placements), rats were fear conditioned with a novel protocol that allowed us to change the time of arrival of the US. The CS was a 60 s, 5 kHz, 80 dB SPL sine wave tone. The US (1 s, 1 mA foot shock) was delivered 30 s (CS60-US@30) or 10 s (CS60-US@10) after the onset of the tone, depending on the experiment (see Figure S1). Memory reactivation session took place 24 hr after fear conditioning by presenting one reinforced trial. The US was delivered either at the same time after the tone onset as during conditioning (no-shift groups) or at a different time (shift groups). Immediately after, the rats received an infusion of anisomycin or vehicle in the LA or in the CeA, depending on the experiment. The memory-retention tests were performed either 3 hr (PR-STM) or 24 hr (PR-LTM) after reactivation and involved five tone-alone presentations in a modified context (see Figure S4 for contextual freezing). Freezing was used to measure the conditional emotional fear response.

**Immunohistochemistry**

In separate groups of animals 90 min after the reactivation session, brains were taken, cut, and processed for zif-268 immunohistochemistry (see Supplemental Information).

**Statistical Analysis**

ANOVA and post hoc tests were performed using GraphPad Prism 5.0 software. The significance level was set at \( \alpha = 5\% \).

**Supplemental Information**

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.01.053.

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References


