Current Biology 23, 467–472, March 18, 2013 ©2013 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2013.01.053

Report

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

Lorenzo Díaz-Mataix,[1,*] Raquel Chacon Ruiz Martinez,[1,2] Glenn E. Schafe,[3] Joseph E. LeDoux,[1,4] and Valérie Doyère[1,5,6,*]

[1]Center for Neural Science, New York University, New York, NY 10003, USA
[2]Surgery Department, Medical School, University of São Paulo, LIM 26 HC-FMUSP, 01246 São Paulo, Brazil
[3]Department of Psychology, Yale University, New Haven, CT 06520, USA
[4]Nathan Kline Institute for Psychiatric Research, Orangeburg, NY 10962, USA
[5]Université Paris-Sud, Centre de Neurosciences Paris-Sud, UMR 8195, 91405 Orsay, France
[6]CNRS, 91405 Orsay, France

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 invoked during the reactivation procedure, forcing the original memory to be updated and reconsolidated [6, 7].

*Correspondence: llorensdiazmataix@gmail.com (L.D.-M.), valerie.doyere@u-psud.fr (V.D.)

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 $CS\text{-}US_{@30}$ pairings followed 24 hr later by a reactivation trial consisting of a single $CS\text{-}US_{@30}$ 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

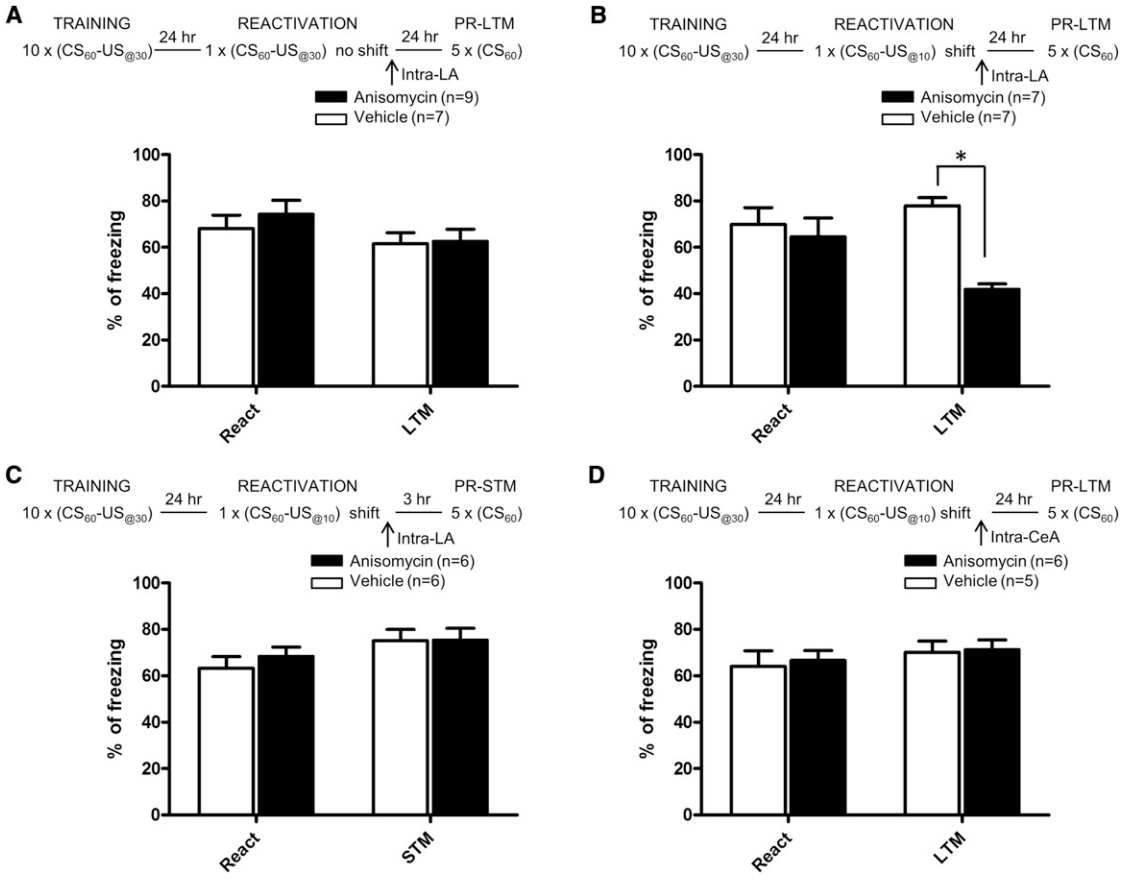

Figure 1. Changing the CS-US Time Interval during Reactivation of Strong Aversive Memories Triggers an LA-Dependent Reconsolidation Process

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 (CS_{60} – $US_{@30}$). 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 (CS_{60} – $US_{@30}$) 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 (CS_{60} – $US_{@10}$) 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.

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 10 s after the tone onset ($US_{@10}$). Twenty-four hours later, rats received intra-LA infusion of anisomycin or vehicle immediately after a CS-US reactivation trial with a US foot shock delivered 30 s after tone onset. During the PR-LTM test, rats infused with anisomycin showed a significant decrease in the level of freezing compared to vehicle-infused rats [PR-LTM: t(13) = 4.487, p < 0.001; reactivation: t(13) = 0.169, n.s.; Figure 3A]. Therefore, a change in CS-US time interval, either shorter or longer, triggers an amygdala-dependent reconsolidation process. Remarkably, the temporal pattern of freezing during PR-LTM seemed to differ among the three experimental groups: no shift-$US_{@30}$, shift-$US_{@10}$, and shift-$US_{@30}$. Although no statistical comparison can be made between experimental groups because they belong to independent experiments (Figures 3B–3D), we can note that

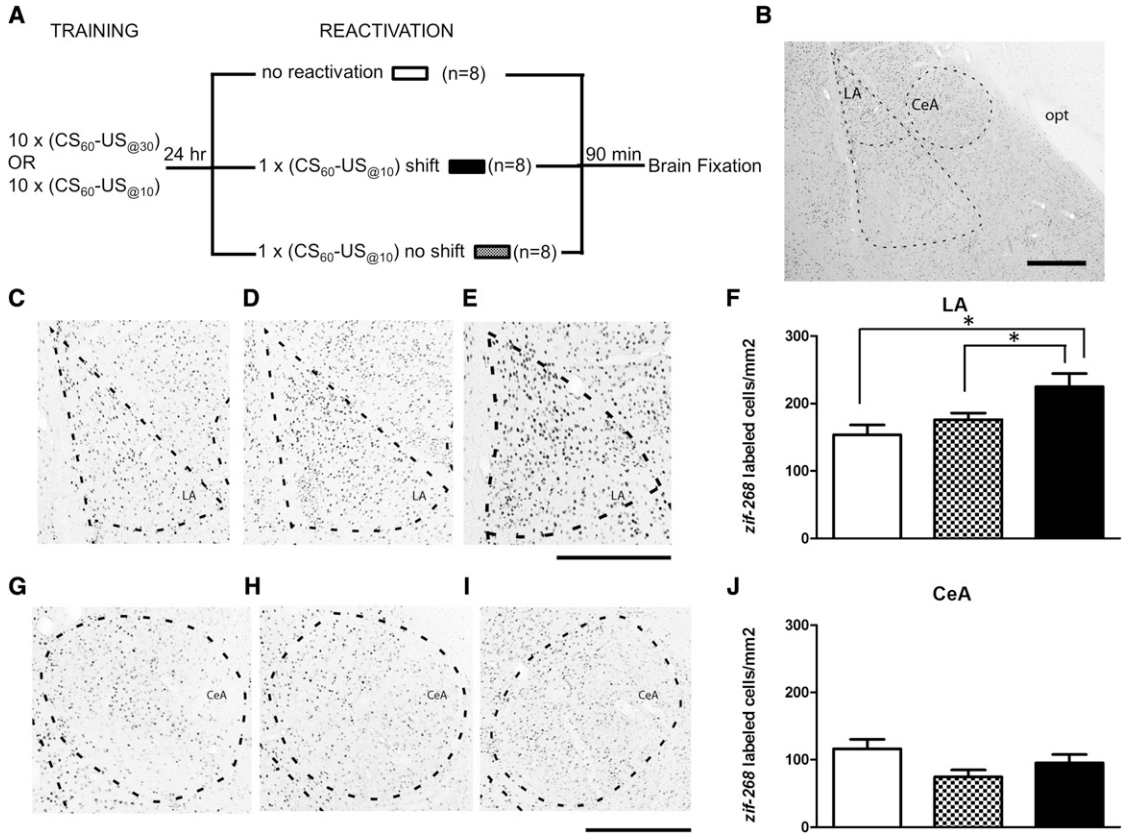

Figure 2. Memory Reactivation Induces Synaptic Plasticity in LA Only when the CS-US Time Interval Is Shifted

(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.

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 in the absence of new information. Rats were conditioned with two pairings of the CS with the $US_{@30}$, followed by reactivation with a single CS-US trial with no change in the CS-US interval. Both anisomycin- and vehicle-infused rats showed similar levels of freezing during reactivation [$t(10) = 0.537$, n.s.] and during PR-LTM [$t(10) = 0.365$, n.s.; Figure 4A]. In contrast, when the CS-US interval was reduced to 10 s during the reactivation trial, anisomycin-treated animals showed impaired PR-LTM compared to vehicle rats [PR-LTM: $t(11) = 2.231$, $p < 0.05$; reactivation: $t(11) = 0.031$, n.s.; Figure 4B]. Similar findings were observed when a single CS-US pairing was used during training [no-shift condition: PR-LTM: $t(14) = 1.523$, n.s.; reactivation: $t(14) = 0.019$, n.s., Figure 4C; shift condition: PR-LTM: $t(9) = 2.294$, $p < 0.05$; reactivation: $t(9) = 0.443$, n.s., Figure 4D]. Thus, fear memories appear to require new information during reactivation for the memory to become destabilized. Furthermore, these findings suggest that the CS-US time interval in auditory fear conditioning, like the CS-US association, can be learned in a single trial; the learning of the timing (see Figure 3) thus does not depend on the prior learning of the association. These findings extend those of a previous study [18] by showing that an interval as short as 30 s can be learned after a single CS-US training trial in an auditory fear-conditioning paradigm and that the precision of the US timing at the outset of learning is at least 20 s, because a difference between 10 and 30 s was detected. This adds empirical support to the concept of temporal maps as prerequisite for associative learning [14].

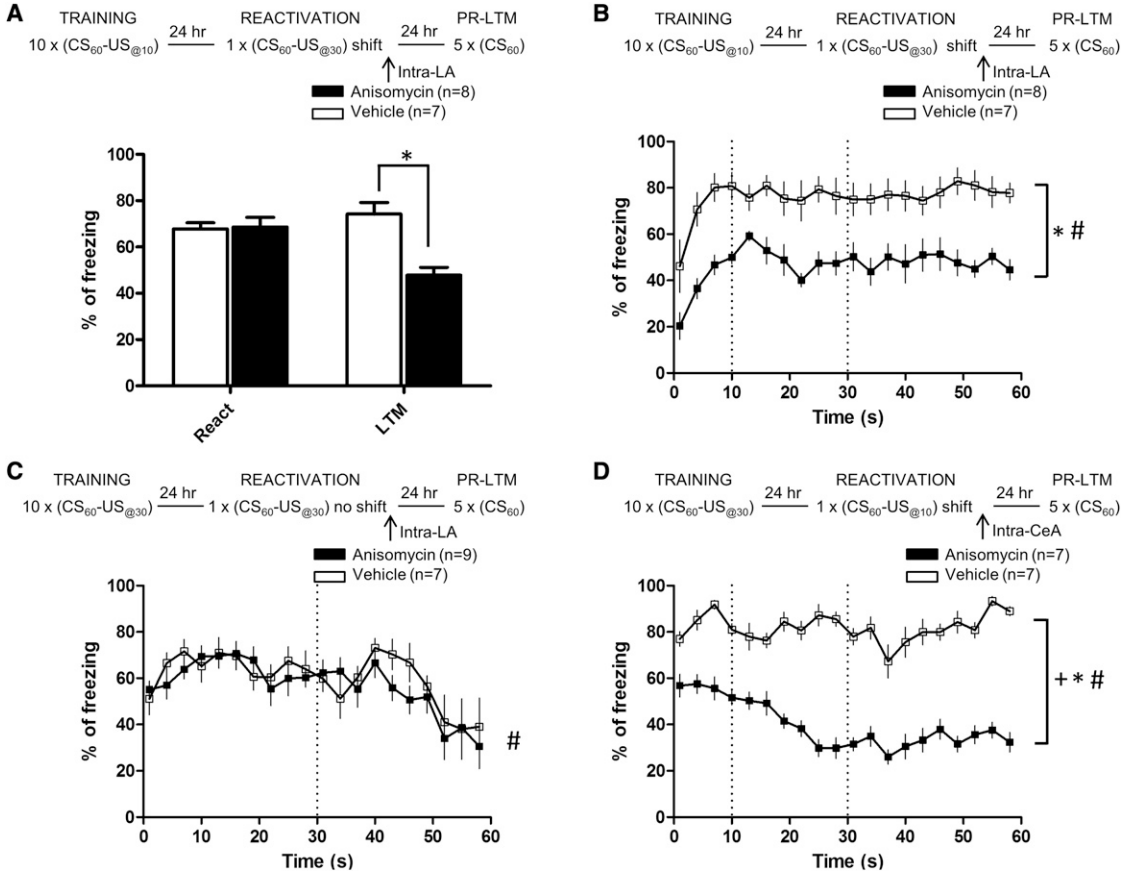

Figure 3. Time Is a Critical Parameter of the CS-US Association that Triggers the Update of Strong Fear Memory

Schematic of the experimental design for each experiment is shown at the top of each panel.

(A) Percentage of freezing (mean ± SEM) to the CS during reactivation (React) with a longer CS-US interval ($US_{@30}$) than the one learned during training ($US_{@10}$) and during PR-LTM test in rats infused in the LA with vehicle (white bars) or anisomycin (black bars) (bottom). Freezing during reactivation was equivalent between groups. Rats given intra-LA anisomycin showed an impairment of memory during PR-LTM.

(B–D) Temporal pattern of freezing during PR-LTM tests (mean ± SEM in 3 s bins), for rats nonshifted (C), shifted with a US delivered later (B), or shifted with a US delivered earlier (D) during reactivation. In all experiments, there was a significant effect of time [B: F(19,280) = 4.62; C: F(19,260) = 2.91; D: F(19,240) = 4.45; #p < 0.0001]. Only when a change in CS-US interval was imposed during reactivation, the anisomycin produced a significant reduction of freezing [B: F(1,260) = 234.7; C: F(1,280) = 2.24, n.s.; D: F(1,240) = 1019.6; *p < 0.0001]. When anisomycin was infused after reactivation with a shifted CS-US time interval from 30 to 10 s, the temporal pattern of freezing was different from vehicle controls [D: time × group interaction F(19,240) = 3.50; +p < 0.0001].

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 Duvarci 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

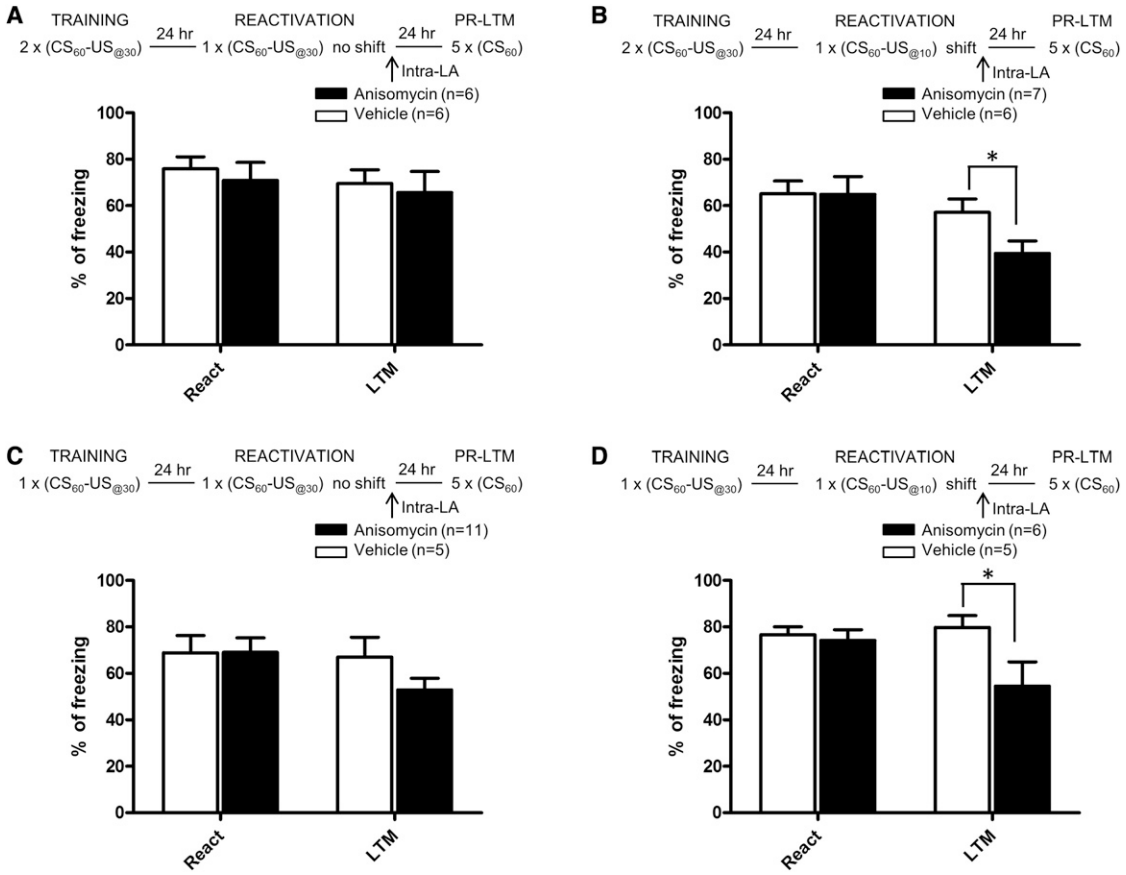

Figure 4. The CS-US Time Interval Is Learned at the Same Time as the CS-US Association

Each panel shows schematic of the experimental design (top) and percentage of freezing (mean ± SEM) to the CS during reactivation (React) and PR-LTM test in rats infused in the LA with vehicle (white bars) or anisomycin (black bars) (bottom). Freezing during reactivation was equivalent between vehicle and anisomycin rats in all experiments. *$p < 0.05$.

(A and B) Rats trained with two CS-US pairings. Rats reactivated with the same CS-US interval as the one learned during training ($US_{@30}$) and given intra-LA anisomycin did not show an impairment of memory during PR-LTM (A); in contrast, when memory was reactivated with a different CS-US time interval ($US_{@10}$), anisomycin-infused rats showed an impairment of memory (B).

(C and D) The same effect was observed after one-trial training.

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 (CS_{60} − $US_{@30}$) or 10 s (CS_{60} − $US_{@10}$) 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.

Acknowledgments

We thank Claudia Farb for her excellent assistance with histology and Begoña Brotons for her help in the design of the graphical abstract. R.C.R.M. was the recipient of grants CAPES #2350/09-2 and FAPESP #11/08575-7 and 12/06825-9 from the government of Brazil. V.D. was supported by Agence Nationale de la Recherche grants. V.D. and J.E.L. were recipients

of collaborative grants from CNRS and the Partner University Fund. J.E.L. was the recipient of NIH grants R01 MH038774 and R01 MH46516 funding this research.

Received: October 10, 2012
Revised: December 23, 2012
Accepted: January 18, 2013
Published: February 28, 2013

References

1. Nader, K., Schafe, G.E., and Le Doux, J.E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature *406*, 722–726.
2. Johansen, J.P., Cain, C.K., Ostroff, L.E., and LeDoux, J.E. (2011). Molecular mechanisms of fear learning and memory. Cell *147*, 509–524.
3. Alberini, C.M. (2011). The role of reconsolidation and the dynamic process of long-term memory formation and storage. Front. Behav. Neurosci. *5*, 12.
4. Dudai, Y. (2009). Predicting not to predict too much: how the cellular machinery of memory anticipates the uncertain future. Philos. Trans. R. Soc. Lond. B Biol. Sci. *364*, 1255–1262.
5. Wang, S.H., de Oliveira Alvares, L., and Nader, K. (2009). Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. Nat. Neurosci. *12*, 905–912.
6. Lee, J.L. (2009). Reconsolidation: maintaining memory relevance. Trends Neurosci. *32*, 413–420.
7. Sevenster, D., Beckers, T., and Kindt, M. (2012). Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. Neurobiol. Learn. Mem. *97*, 338–345.
8. LeDoux, J.E. (2012). Rethinking the emotional brain. Neuron *73*, 653–676.
9. Doyère, V., Debiec, J., Monfils, M.H., Schafe, G.E., and LeDoux, J.E. (2007). Synapse-specific reconsolidation of distinct fear memories in the lateral amygdala. Nat. Neurosci. *10*, 414–416.
10. Díaz-Mataix, L., Debiec, J., LeDoux, J.E., and Doyère, V. (2011). Sensory-specific associations stored in the lateral amygdala allow for selective alteration of fear memories. J. Neurosci. *31*, 9538–9543.
11. Duvarci, S., and Nader, K. (2004). Characterization of fear memory reconsolidation. J. Neurosci. *24*, 9269–9275.
12. Pavlov, I.P. (1927). Conditional Reflexes (New York: Dover Publications).
13. Balsam, P.D., and Gallistel, C.R. (2009). Temporal maps and informativeness in associative learning. Trends Neurosci. *32*, 73–78.
14. Balsam, P.D., Drew, M.R., and Gallistel, C.R. (2010). Time and associative learning. Comp. Cogn. Behav. Rev. *5*, 1–22.
15. Debiec, J., Díaz-Mataix, L., Bush, D.E., Doyère, V., and Ledoux, J.E. (2010). The amygdala encodes specific sensory features of an aversive reinforcer. Nat. Neurosci. *13*, 536–537.
16. Maddox, S.A., Monsey, M.S., and Schafe, G.E. (2011). Early growth response gene 1 (Egr-1) is required for new and reactivated fear memories in the lateral amygdala. Learn. Mem. *18*, 24–38.
17. Lee, J.L. (2010). Memory reconsolidation mediates the updating of hippocampal memory content. Front. Behav. Neurosci. *4*, 168.
18. Davis, M., Schlesinger, L.S., and Sorenson, C.A. (1989). Temporal specificity of fear conditioning: effects of different conditioned stimulus-unconditioned stimulus intervals on the fear-potentiated startle effect. J. Exp. Psychol. Anim. Behav. Process. *15*, 295–310.
19. Finnie, P.S., and Nader, K. (2012). The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. Neurosci. Biobehav. Rev. *36*, 1667–1707.
20. McNally, G.P., Johansen, J.P., and Blair, H.T. (2011). Placing prediction into the fear circuit. Trends Neurosci. *34*, 283–292.
21. Sutton, R.S., and Barto, A.G. (1981). Toward a modern theory of adaptive networks: expectation and prediction. Psychol. Rev *88*, 135–170.
22. Moses, S.N., Houck, J.M., Martin, T., Hanlon, F.M., Ryan, J.D., Thoma, R.J., Weisend, M.P., Jackson, E.M., Pekkonen, E., and Tesche, C.D. (2007). Dynamic neural activity recorded from human amygdala during fear conditioning using magnetoencephalography. Brain Res. Bull. *71*, 452–460.
23. Belova, M.A., Paton, J.J., Morrison, S.E., and Salzman, C.D. (2007). Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. Neuron *55*, 970–984.
24. Bucci, D.J., and Macleod, J.E. (2007). Changes in neural activity associated with a surprising change in the predictive validity of a conditioned stimulus. Eur. J. Neurosci. *26*, 2669–2676.
25. Herry, C., Bach, D.R., Esposito, F., Di Salle, F., Perrig, W.J., Scheffler, K., Lüthi, A., and Seifritz, E. (2007). Processing of temporal unpredictability in human and animal amygdala. J. Neurosci. *27*, 5958–5966.
26. Metereau, E., and Dreher, J.C. (2013). Cerebral correlates of salient prediction error for different rewards and punishments. Cereb. Cortex *23*, 477–487.