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Synapse-specific reconsolidation of distinct fear memories in the lateral amygdala

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When reactivated, memories enter a labile, protein synthesis–dependent state, a process referred to as reconsolidation. Here, we show in rats that fear memory retrieval produces a synaptic potentiation in the lateral amygdala that is selective to the reactivated memory, and that disruption of reconsolidation is correlated with a reduction of synaptic potentiation in the lateral amygdala. Thus, both retrieval and reconsolidation alter memories via synaptic plasticity at selectively targeted synapses.

Newly formed memories are initially sensitive to disruption for a certain period of time before being stored in a long-term stable state. Later memory retrieval triggers a new phase of lability, during which the memory may be updated and stabilized again for long-term storage. This 'reconsolidation' process has attracted much attention because of its potential application to the treatment of psychiatric disorders, including post-traumatic stress disorder and drug addiction. To date, however, we still know relatively little about the extent to which reconsolidation is a selective process or how it is mediated at the cellular level[1,2].

Much of the recent research on reconsolidation has focused on Pavlovian fear conditioning, a paradigm in which a stimulus becomes fearful after its association with an aversive event. The acquisition of fear conditioning induces synaptic potentiation at lateral amygdala synapses[3–6]. Whether retrieval of a fear memory also induces synaptic potentiation in the lateral amygdala, and whether disruption of reconsolidation is mediated through a reduction in potentiation at reactivated synapses remains unknown. To address these issues, we developed a fear conditioning paradigm allowing selective reactivation, and hence reconsolidation, for one of two distinct auditory fear memories.

Two very distinct conditioned stimuli, a pure 1-kHz tone and a more complex frequency-modulated sound, were paired with the same aversive unconditioned stimulus (**Supplementary Methods** online; approved by the New York University Animal Care and Use Committee). One day after training (**Fig. 1a** and **Supplementary Fig. 1** online), rats were given an intra–lateral amygdala infusion of either vehicle or the MAPK inhibitor U0126, a pharmacological agent known to block both consolidation[7,8] and reconsolidation[9], before a reactivation trial with one of the conditioned stimuli (CSr, reactivated conditioned stimulus). The other conditioned stimulus (CSn, not reactivated conditioned stimulus) was not reactivated. Rats were then tested for memory retention of both CSr and CSn shortly after retrieval (post-reactivation short-term memory, PR-STM, 3 h) and also 24 h later (post-reactivation long-term memory, PR-LTM). The two groups of animals (vehicle and U0126) showed equivalent levels of freezing to CSr during reactivation and to CSn and CSr during the PR-STM test (**Fig. 1b** and **Supplementary Analysis** online). During the PR-LTM test, however, a memory impairment for the CSr relative to CSn was observed in the U0126-infused group, but not in the vehicle group (**Fig. 1c** and **Supplementary Analysis**). Thus, the effect of U0126 on memory is limited to the reactivated conditioned stimulus (CSr). These findings demonstrate that two associative memories can be independently reconsolidated even though they share the same aversive outcome.

To examine the reconsolidation process at the neurophysiological level, we recorded freezing and auditory-evoked field potentials (AEFPs) from the lateral amygdala and the areas of the auditory thalamus that project to the lateral amygdala in rats fear conditioned and treated, as above, with U0126 before memory reactivation (**Supplementary Fig. 1** online). The selective impairment of fear memory reconsolidation by intra–lateral amygdala U0126 for the reactivated conditioned stimulus observed behaviorally above was replicated in these animals (**Fig. 2a**). Thus, there was a difference in freezing to CSn and CSr in the PR-LTM test, but not in the PR-STM test. As previously reported, fear conditioning led to an enhancement of the

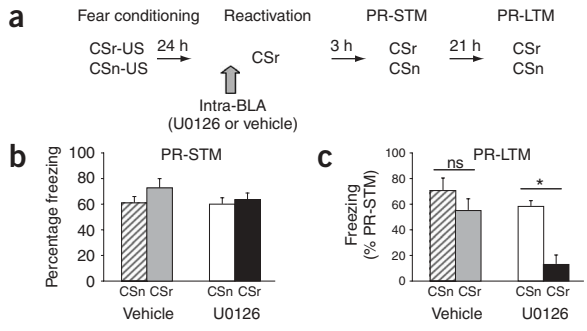

Figure 1 Selective reconsolidation of a fear memory. (**a**) Schematic of the experimental design. After animals were trained in a two–conditioned stimuli fear-conditioning, each paired with the same aversive unconditioned stimulus (US), one conditioned stimulus was reactivated (CSr), whereas the other was not (CSn). Thirty min before reactivation, animals received either U0126 or vehicle. (**b**) Percent freezing (mean + s.e.m.) observed during PR-STM tests. (**c**) Freezing observed during PR-LTM tests expressed as a percent of PR-STM. U0126 animals showed impaired PR-LTM only to the reactivated conditioned stimulus ($*P < 0.05$, contrast analysis).

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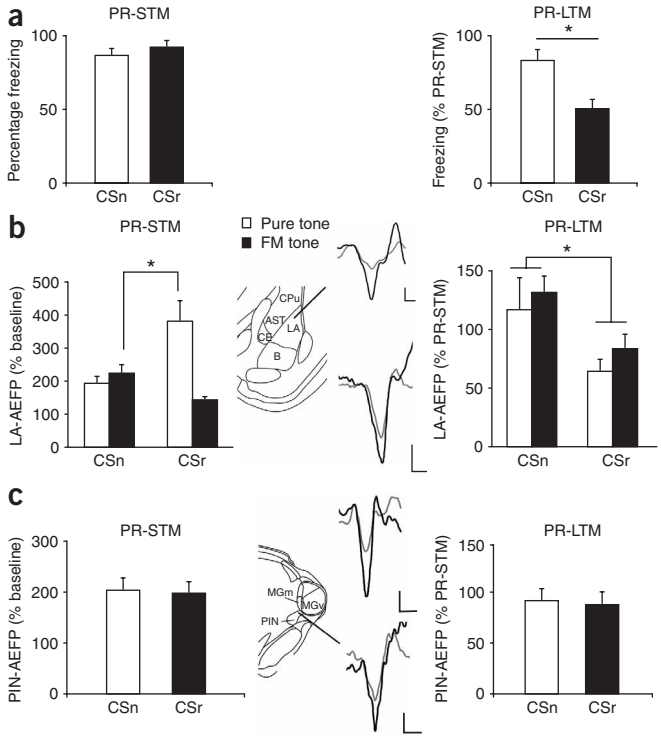

Figure 2 Retrieval and reconsolidation alter memories via synaptic plasticity in lateral amygdala (LA). (**a**) Replication of selective behavioral impairment of reconsolidation by U0126 ($P < 0.05$, one-way ANOVA). (**b**) Changes in short-latency AEFPs in lateral amygdala. A selective additional potentiation was observed during PR-STM (left panel, $P < 0.05$, contrast analysis) after reactivation with the pure tone conditioned stimulus. U0126 produced a depotentiation of lateral amygdala responses to the reactivated conditioned stimulus (CSr), regardless of tone type (right panel, $P < 0.05$, two-way ANOVA). (**c**) Changes in AEFPs in the auditory thalamus. No differential effect of reactivation or intra–lateral amygdala infusion of U0126 on either memory test was observed. Data are mean + s.e.m. Insets are examples of AEFPs evoked by pure tone (top) and frequency-modulated (FM) tone (bottom) recorded in lateral amygdala (**b**) and posterior intralaminar nucleus, PIN (**c**) before conditioning (light gray) and 3 h after its reactivation (thick black). Scale = 10 μV, 5 ms.

short-latency conditioned stimulus–elicited AEFPs in the lateral amygdala[4,5] and in the auditory thalamus[5]. This is readily observed in the PR-STM test for the conditioned stimulus that was not reactivated (see **Fig. 2b,c** left, CSn).

Surprisingly, for the CSr, the level of potentiation in the lateral amygdala during the PR-STM test differed between the two kinds of stimuli (pure tone versus frequency modulated). Reactivation by the pure tone conditioned stimulus led to a further potentiation of AEFPs (**Fig. 2b** left and **Supplementary Analysis**). Combined with the fact that AEFPs in the thalamic areas that project to lateral amygdala were not modified by conditioned stimulus presentation during the reactivation session (**Fig. 2c**), these results show that retrieval itself can produce plastic changes in the lateral amygdala independent of alterations in afferent processing in the thalamus. These findings are in agreement with studies showing that frequency-modulated sounds used as a conditioned stimulus require cortical processing[10,11] and thus are likely to use cortico-amygdala rather than thalamo-amygdala pathways. They also suggest that initial encoding and updating of long-term representations of distinct auditory fear memories may not rely on the exact same networks. In spite of the differences we observed during reactivation, there was a similar disruption of AEFPs in lateral amygdala responses during PR-LTM for the two tone types (**Fig. 2b** right and **Supplementary Analysis**). This neurophysiological result parallels the impairment of reconsolidation seen in behavior during PR-LTM, and suggests that the failure to reconsolidate following treatment with U0126 was the result of reduction of potentiation at reactivated synapses.

Our protocol provides a tool to observe, within the same animal and at the same site of recording, the selective effects of retrieval and reconsolidation. We show that retrieval triggers two potentially distinct processes. The first produces a synapse-specific potentiation in the lateral amygdala selective to the reactivated memory. This retrieval-induced synaptic potentiation may reflect mechanisms of updating memories because the reactivation trial triggers new learning as well as reconsolidation of the initial memory[12]. Whether the potentiation induced by retrieval and by initial learning share similar characteristics remains to be examined. The second process triggered by reactivation renders the synaptic modifications associated with initial learning labile. In effect, when reconsolidation is disrupted, a reduction of potentiation at thalamo-amygdala synapses is observed, but only to the tone presented during reactivation. This in turn suggests 'de-consolidation' of the fear memory trace in the lateral amygdala; that is, possibly an erasure of initial encoded plasticity[13]. These findings provide the neurophysiological basis for content-limited modifications during the updating of fear memories. They also lend some validity for therapeutic use of agents that disrupt reconsolidation to reduce the fear-arousing aspects of emotional memory in post-traumatic stress disorder, as such treatments may have highly specific and potentially permanent effects.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

V.D. designed the experiments, conducted the electrophysiological experiments, analyzed the data, interpreted the results, wrote the initial manuscript and was involved in the revision process. J.D. was involved in the design of the studies, conducting the experiments, the data analysis, the interpretation of the results and the preparation and revision of the manuscript. M.-H.M. was involved in data analysis, interpretation of the results and preparation and revision of the manuscript. G.E.S. was involved in the design of the experiments, conducted histological analysis on cannula and electrode placements and participated in preparation and revision the manuscript. J.E.L. was involved in the design of the studies, the interpretation of the results and the preparation and revision of the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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