



RESEARCH HIGHLIGHT

The Secret of Fear Memory Attenuation: Facing Fears

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Exposure therapy, an effective treatment for fear disorders, requires memory retrieval of previous horror experiences in controllable environment. However, it remains unknown whether previous fear memories have been updated [1] or inhibited [2] during the formation of new memories, as well as the underlying neural mechanisms involved (*e.g.* the type of cells engaged in this process).

Memory traces or engrams represent learning-activated ensembles of neurons [3]. Accumulating evidences have demonstrated that memory traces at certain stages can be visualized by dynamic labeling of neurons accurately with temporal precision [4–6]. A recent study published in *Science* by Khalaf *et al.* [7] demonstrated that the attenuation of a fearful memory depends on reactivation of the original fear memory trace. Using double transgenic TetTag mice (*c-Fos::tTA/TRE::τ-LacZ*), the authors first identified the ensembles of neurons (β -gal⁺ neurons) involved in the recall of long-lasting memory. Specifically, double transgenic TetTag mice provided a *c-Fos*-based labeling system of neuronal activity in which the *Fos* promoter drives expression of *tTA* after neuronal activation, which binds to the *TRE* site, resulting in the expression of β -gal. Then the ensembles of *c-Fos*⁺ neurons recruited in memory attenuation were identified following extinction.

The β -gal⁺*c-Fos*⁺ neurons therefore suggested co-activation by both long-lasting memory recall and extinction training. Notably, the reactivation rate (β -gal⁺*c-Fos*⁺/total β -gal⁺) in the dentate gyrus (DG) was much higher than chance level; there was a positive correlation between the reactivation rate and the extent of fear memory attenuation. Furthermore, the authors obtained the same results using different extinction paradigms (spaced extinction and massed extinction) and other techniques for visualizing neuronal ensembles. All these results indicated that fear memory attenuation may be related to the memory retrieval induced activation of recall-encoding neurons.

The authors further manipulated these “reactivated neurons” with chemogenetic approaches, such as Daun02 inactivation or DREADD for continuous excitation (Fig. 1). They found that memory attenuation (Δ freezing) was lower in Daun02-treated TetTag mice than in vehicle-treated TetTag mice. In contrast, continuous activation of these neurons in *c-Fos* *tTA* mice (DREADD expression in the DG, local infusion of clozapine-N-oxide) showed facilitated memory attenuation compared to vehicle-treated animals. These results suggest that long-lasting fear memory attenuation is largely dependent on the reactivation of recall-induced neuronal ensembles.

In fact, the fear memories generated by traumatic events are difficult for early extinction procedures and might incubate long-lasting memories [8], which makes it important to investigate the mechanism of their attenuation [9]. Previous studies have shown that retrieval can open a time window in which some manipulations contribute to memory attenuation. In the study of Auchte *et al.*, freezing time in the “retrieval + extinction” group was less than in the “extinction alone” group during the post-reinstatement test [10]. Another study showed that extinction training in an appropriate reconsolidation window provided by

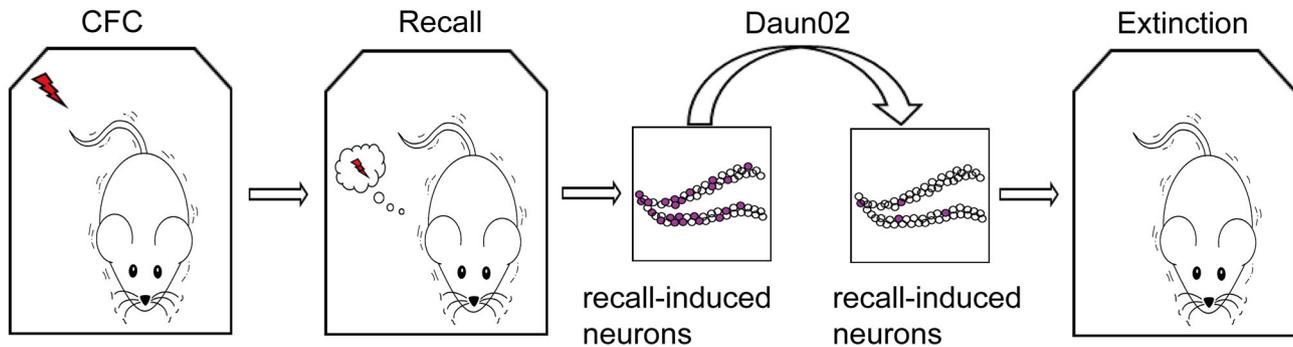
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A Daun02 Inactivation Method



B hM3Dq Activation Method

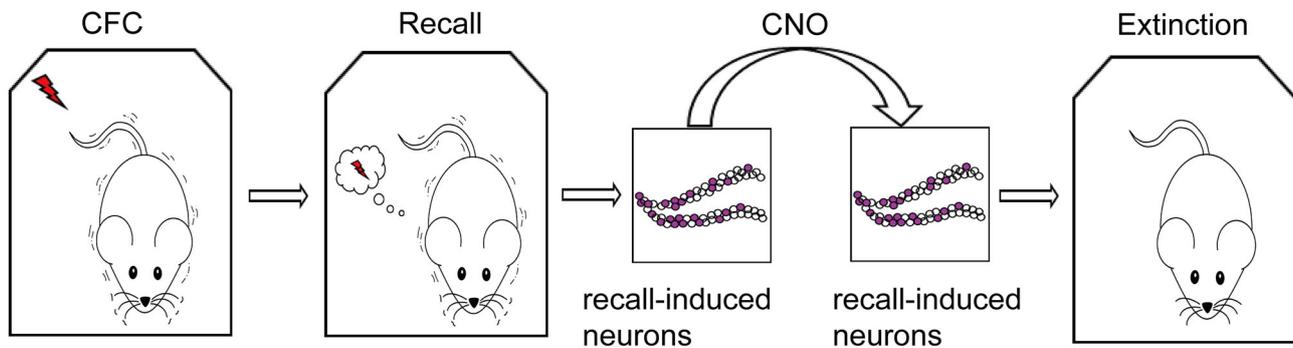


Fig. 1 Manipulation of reactivated neurons. **A** Fear memory attenuation was impaired by reduced neuronal excitability of recall-induced neurons in the DG *via* Daun02 inactivation. **B** Fear memory

attenuation was facilitated by activated recall-induced neurons in the DG continuously *via* hM3Dq activation. CFC, contextual fear conditioning. CNO, clozapine-N-oxide.

memory retrieval might weaken fear memory persistently, which suggested that reactivation-induced reconsolidation of memory might update fear memory toward safety following memory retrieval [11]. These findings are in line with the present study showing that neuronal ensembles in the DG are co-activated by memory recall and extinction training; there was a positive correlation between the reactivation rate and the extent of fear memory attenuation. However, the reactivation rate is not high; the effect of extinction training on fear memory attenuation was impaired rather than completely disappearing when the activity of recall-activated neurons was inhibited. Therefore, there might be other mechanisms underlying fear memory attenuation.

Several interesting questions that need to be investigated in the future are raised by the present study. First, fear memory involves many brain areas. Although the authors focused on the DG, which plays a critical role in memory recoding, recall, and attenuation, the number of neurons in the basolateral amygdala reactivated in memory retrieval is correlated with fear memory strength [6]. In addition, recall-induced reactivation opens a window for memory reconsolidation; neuronal ensemble reactivation by

presenting extinction training in this window contributes to fear memory attenuation, but the upstream and downstream mechanisms of the reconsolidation-updating remain to be investigated. Last but not least, it is still unknown whether fear memory coding in the DG is synapse-specific [12] and the changes in synaptic plasticity [13] of these “reactivated neurons”.

Fear memories are often reinstated after extinction learning [14]. The present study shed light on the mechanism of remote fear memory attenuation based on extinction training at the level of neuron ensembles. It is possible to manipulate neurons to make false memories. For instance, Ramirez *et al.* showed that when DG neurons were activated by the exposure to a certain context during fear conditioning, reactivation of these neurons in a different context could still increase the freezing behaviors [15]. Furthermore, specific manipulation of these reactivated neurons could promote therapeutic targeting of fear-related disorders, such as post-traumatic stress disorder.

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