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Research report

An isolated retrieval trial before extinction session does not prevent the return of fear

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HIGHLIGHTS

- We examined erasure effects of retrieval-extinction training on fear memory.
- High-intensity retrieval trial prior to extinction session did not erase fear memory.
- Juvenile and adult age mice retrieved fear memory after retrieval-extinction.
- Retrieval-extinction does not inhibit reconsolidation of consolidated fear memory.

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ABSTRACT

Several studies have shown that an isolated retrieval trial before the extinction session (retrieval-extinction) prevents the return of fear memory by inhibition of reconsolidation. Other studies have reported that retrieval-extinction did not prevent the return of the fear. To date, it is still unclear whether retrieval-extinction prevents the return of the original fear memory. A previous study revealed that reconsolidation of conditioned fear memory was not induced by the brevity of the retrieval session. Thus, we examined whether the number of retrievals in the retrieval-extinction paradigm was involved in the prevention of return of fear (Experiment 1). Furthermore, studies with different-age experimental subjects have shown conflicting results. We investigated the potential impact of age on the inhibitory effect of retrieval-extinction on the return of fear (Experiment 2). Our major findings were as follows: (1) Retrieval-extinction procedure did not prevent the return of fear, regardless of the intensity (number of presentations) of the stimulus inducing retrieval of fear memory. (2) The mice in both juvenile and adult age groups (4 and 8 weeks old) retrieved fear memory after retrieval-extinction. These results suggest the possibility that extinction after retrieval does not inhibit reconsolidation of previously consolidated fear memory.

1. Introduction

Fear extinction refers to the repeated presentations of a conditioned stimulus (CS) such as a tone in the absence of an aversive unconditioned stimulus (US) such as an electric footshock previously paired with it. It has been hypothesized that extinction does not erase the original fear memory but forms a new memory of safety that inhibits fear expression [1]. Reduced fear by extinction can be recovered spontaneously after the passage of time (spontaneous recovery) or renewed by the presentations of the CS alone in a context outside the extinction context (renewal) [2].

New memories are stabilized after an initial learning experience by a process called consolidation [3]. However, some studies have indicated that retrieval of a consolidated memory can induce a labile phase, during which the retrieved memory requires an active process called reconsolidation in order to be stabilized [4]. Additionally, several studies have shown that the injection of protein synthesis inhibitors into the basolateral amygdala after retrieval

Abbreviations: FC, fear conditioning; FE, fear extinction; CS, conditioned stimulus; US, unconditioned stimulus.

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Table 1
Inhibitory effect of an isolated retrieval trial on return of fear.

Author	Was return of fear prevented?	Animal
Monfils et al. (2009)	Yes	Rat
Clem et al. (2010)	Yes	Mouse
Chan et al. (2010)	No	Rat
Flavell et al. (2011)	No	Rat
Ishii et al. (2012)	No	Mouse
Stafford et al. (2013)	No	Mouse
Current study	No	Mouse

of a previously consolidated memory disrupted the original fear memory [5–9]. These studies suggest that the inhibition of reconsolidation after retrieval can disrupt consolidation of fear memory.

Monfils et al., Clem et al. and Schiller et al. reported that a single re-exposure to a CS (retrieval trial) prior to extinction training prevented the return of fear in rat, mouse and human, suggesting that the return of fear can be prevented by inhibiting reconsolidation of fear memory [10–12]. By contrast, Chan et al., Flavell et al., Ishii et al. and Stafford et al. reported that retrieval-extinction did not prevent the return of fear using the same protocol [13–15] (Table 1). In addition, our previous study showed that even increased intensity of fear extinction stimuli after a single re-exposure to a CS did not result in erasure of original fear memory [16]. Taken together, it is still unclear whether retrieval-extinction prevents the return of fear. For the potential clinical application of retrieval-extinction sessions, it is critical to determine whether retrieval-extinction can indeed prevent the return of fear; certainly the drug-free retrieval extinction paradigm which Monfils et al., Clem et al. and Schiller et al. have shown can prevent the return of fear would be beneficial for patients if found to be valid [10–12].

A previous report found that reconsolidation was not induced by a short retrieval session [17]. Additionally, our previous study showed that fear extinction after a single re-exposure to a CS did not have an erasure effect on original fear memory [16]. These studies suggest that an isolated retrieval trial is insufficient to induce a reconsolidation that destabilizes the original fear memory. To that end, we examined whether the number of retrievals in the retrieval-extinction paradigm affects the prevention of the return of fear (Experiment 1).

To determine whether a retrieval-extinction session prevents the return of fear, it is important to compare the differences among previous studies which may have contributed to the conflicting results. Our previous study and previous ones used different-age rodents. Clem and Huganir [10] used early adolescent mice at postnatal days 30–50. On the other hand, our previous study used 8-week-old mice and Stafford et al. used 8- to12-week-old mice [15,16]. There is a possibility that age differences caused the conflicting results [15,16]. Accordingly, we examined what impact age had on the inhibitory effect of a retrieval-extinction session on the return of fear (Experiment 2).

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

Thirty-eight C57BL/6J male mice (8–9 weeks old) were housed one per cage at a controlled temperature (23 ± 1 °C) on a 12-h light/dark cycle (light on at 07:00 h). The animals were provided food and water *ad libitum*. All behavioral testing was conducted between 09:00 and 13:00 h. Mice were randomized and used only once. Research and animal care were carried out according to the Guide for Animal Experimentation of the Chiba University Graduate School of Medicine.

2.1.2. Behavioral experiments

To begin exploring how the number of retrievals in the retrieval-extinction paradigm impacts the prevention of return of fear, we used a modification of the technique described in Clem et al. [10] (Fig. 1A). All mice were then returned to their home cages located inside the colony room after each session.

2.1.3. Fear conditioning

On day 1, mice were placed in conditioning chambers (context A) 22.8 × 19.7 × 13 cm, with transparent walls in the front and back, stainless-steel bars, and a metal-grid floor connected to a shock scrambler and generator in a sound-attenuating box, and received 6 pairings (100 s interstimulus interval) of a CS (20 s, 80 dB, 2.8-kHz tone) and a US (2 s, 0.75-mA scrambled footshock), after a 200-s acclimation period. The US was presented during the last 2 s of the CS. After a 180-s no-stimulus consolidation period after the final CS–US pairing, mice were returned to the home cage. After matching for equivalent levels of freezing, conditioned mice were divided into six groups: Ret-1 ($n = 11$), Ret-5 ($n = 9$), Ret-10 ($n = 10$) and No-retrieval ($n = 8$). Chambers were cleaned with 70% ethanol before and after use.

2.1.4. Retrieval

On day 2, all mice were placed in novel chambers (context B), with a square-shaped base without a shock grid and four triangular profiles, one of which was made of a clear plastic wall. After an initial 650-s pre-explore period (Pre), the Ret-1 group received one presentation of the CS (20 s, 80 dB, 2.8-kHz tone) alone. After an initial 450-s pre-explore period (Pre), Ret-5 group received five presentations of the CS alone (interstimulus interval: 30 s). After an initial 200-s pre-explore period (Pre), the Ret-10 group received 10 presentations of the CS alone (interstimulus interval: 30 s). The no-retrieval group was exposed solely to context B without the CS. Chambers were cleaned with a 79.5% water/19.5% ethanol/1% vanilla-extract solution.

2.1.5. Extinction

Thirty minutes after the retrieval trial, all groups received extinction training divided into two sessions (extinctions 1 and 2) separated by 30 min in context B. In extinction 1, the Ret-1 group received 19 presentations of the CS alone (interstimulus interval: 10 s) after an initial 250-s pre-explore period. The Ret-5 group received 15 presentations of the CS alone (interstimulus interval: 10 s) after an initial 450-s pre-explore period. The Ret-10 group received 10 presentations of the CS alone (interstimulus interval: 10 s) after an initial 700-s pre-explore period. On the other hand, the No-retrieval group received 20 presentations of the CS alone after an initial 200-s pre-explore period. To balance the total number of CSs presented, 1, 5 and 10 CS were omitted from extinction 1 in the Ret-1, Ret-5 and Ret-10 group, respectively. Apart from the fear-conditioning session, a total of 20 CS was delivered during each extinction session, except the session 1 for the Ret-1, Ret-5 and Ret-10 groups. Chambers were cleaned with a 79.5% water/19.5% ethanol/1% vanilla-extract solution.

2.1.6. Spontaneous recovery and renewal tests

On day 3, all mice were returned to context B to test spontaneous recovery of fear. After an initial 200-s pre-explore period, all groups received four presentations of the CS alone at an interval of 30 s (spontaneous recovery test). Chambers were cleaned with a 79.5% water/19.5% ethanol/1% vanilla-extract solution. Thirty minutes later, all mice were placed in the context A and presented with four CS to measure renewal (renewal test).