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Effects of memory age and interval of fear extinction sessions on contextual fear extinction

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HIGHLIGHTS

- An extinction of 5 consecutive days prevented the spontaneous recovery of fear memory.
- Fear memory was not affected by a passage of time.
- Older fear memory was more sensitive to fear extinction.
- A consecutiveness of extinction was not necessary for the prevention effect.

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ABSTRACT

Fear extinction is a major task in our understanding of the biological mechanisms of exposure therapy, one of the most used treatments for stress-related disorders. It was recently reported that an extinction of 5 consecutive days prevents spontaneous recovery of fear memory. Memory age and the timing of fear extinction influence the effect of fear extinction. In this study, we used contextual fear extinction in adult male mice to examine whether memory age influences an extinction of 5 consecutive days and whether consecutiveness is necessary to prevent spontaneous recovery. Our results showed that, although fear memory was not affected by the passage of time, the old fear memory (28 days after fear conditioning) was more sensitive to fear extinction than the young fear memory (7 days after fear conditioning). Additionally, we demonstrated that consecutiveness of extinction sessions is not necessary to prevent spontaneous recovery. Instead, fear extinction sessions at spaced intervals were found to be more effective than consecutive extinction sessions for young fear memory. Our results suggest that taking memory age and the interval of fear extinction sessions into consideration would help to optimize exposure therapy.

1. Introduction

Fear extinction is a major task in our understanding of the treatment of stress-related disorders such as exposure therapy. Although exposure therapy is highly effective in reducing fear and anxiety symptoms, some patients show return of fear [11,12]. One reason for this fear recovery is that the extinction memory may be labile and weak compared to the original fear memory. Fear extinction does not erase the original fear memory but forms a new memory of safety that inhibits fear expression [17]. This idea is supported by different types of fear recovery, including spontaneous recovery, in which conditioned fear responses can recover once a certain amount of time has passed after the acquisition of fear extinction [5]. Therefore, many researchers have focused on the prevention of fear recovery. Recently, it was reported that an extinction of 5 consecutive days prevented spontaneous recovery in adult male rats [19].

Memory age is one of the factors that influence memory stability [20,23]. Some researchers have reported that fear memory increases with time [7,15], while others reported no change [2]. On

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, analysis of variance; CS, conditioned stimuli.

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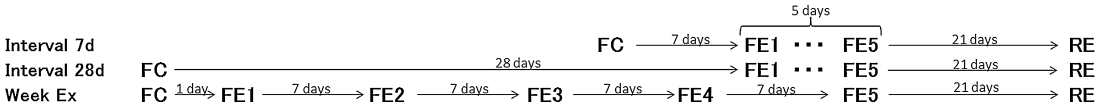

Fig. 1. Experimental protocol.

the other hand, older fear memory may be easier to mitigate by fear extinction [2,7]. The timing of fear extinction is also important. Spaced intertrial intervals during fear extinction have been shown to be more effective than fear extinction without intervals [13,22]. Inda et al. report that, although 2-day interval exposure to a conditioned fear context reduced fear response, 1-week interval exposure to the same context increased fear response [7]. These results suggest that it is essential to understand memory age and the timing of fear extinction in order to optimize exposure therapy.

Therefore, to study whether memory age influences an extinction of 5 consecutive days and whether consecutiveness is necessary to prevent spontaneous recovery, we changed the interval between fear conditioning and fear extinction, as well as the intervals between fear extinction sessions. Although a previous study used an avoidance task to investigate the effects of an extinction of 5 consecutive days [19], we used contextual fear extinction to confirm the prevention effects in other behavioral tasks.

2. Materials and methods

2.1. Animals

The procedures of animal use were approved in advance by the Guide for Animal Experimentation of Chiba University Graduate School of Medicine. C57BL/6J male mice (14 weeks of age) were purchased from SLC (Shizuoka, Japan). The mice were housed three per cage at a controlled temperature (23 ± 1 °C) and on a 12-h light/dark cycle (lights on at 07:00 h). The mice were provided food and water *ad libitum*. All behavioral testing was conducted between 12:00 and 17:00 h

2.2. Fear conditioning

All mice underwent handling for 5 days (1 min/day) one week before fear conditioning. At 15 weeks of age, the mice were placed in conditioning chambers for 20 min (habituation). One day after habituation, the mice received three foot shocks (2 s, 0.75 mA, 60–120 s intertrial interval) after a 180 s acclimation period in the chambers. The mice were subsequently returned to their home cages 180 s after the last foot shock. The activity of the mice was monitored using the Freeze Frame program (Actimetrics Software, Wilmette, IL, USA). Freezing (no visible movement except for respiration) was scored and converted into a percentage [(freezing observations/total observations) × 100]. We defined % freezing in first 30 s and 30 s immediately after third footshock as PRE and POST, respectively. The chambers were cleaned with 70% ethanol before and after each use.

2.3. Fear extinction and recall test

After fear conditioning, the mice were divided into three groups based on POST (Fig. 1): first group which underwent fear extinction per week for 5 weeks (Week Ex, $n = 12$), second group which underwent a consecutive 5-day fear extinction after 7 days after fear conditioning (Interval 7d, $n = 8$), and third group which underwent a consecutive 5-day fear extinction after 28 days after fear conditioning (Interval 28d, $n = 12$). All mice were re-placed in the chambers for 20 and 2 min as fear extinction session (FE) and recall test (RE), respectively.

2.4. Statistical analysis

For fear conditioning, we used the % freezing data from PRE and POST, and two-way (time and group) repeated analysis of variance (ANOVA). For fear extinction, we used the % freezing data from every 2 min period, and three-way (time, day, and group) ANOVA. We defined % freezing during 18–20 min in FE5 as Last FE. For recall of fear extinction, we used the % freezing in RE and one-way ANOVA. For spontaneous recovery, we used the % freezing in Last FE and RE, and two-way (day and group) repeated ANOVA. For all analyses, the level of statistical significance was set at $p < 0.05$. We used post hoc *Bonferroni* tests for multiple comparisons. All statistical analyses were performed using SPSS 19.0 J for Windows (SPSS Japan, Inc., Tokyo, Japan). The data are presented as the mean ± SEM.

3. Results

3.1. Fear conditioning

All groups showed differences in % freezing between PRE and POST (time $F_{(1,29)} = 74.91$, $p < 0.01$). Post hoc revealed that all groups showed higher % freezing during POST compared to PRE, indicating that all groups acquired fear memory (Fig. 2A). To study whether fear memory increases with a passage of time, we investigated % freezing during 0–2 min in extinction session 1. There was no difference in % freezing among three groups (Week Ex: 29.06 ± 5.34%, Interval 7d: 29.82 ± 6.66%, Interval 28d: 35.91 ± 5.51%; group $F_{(2,31)} = 0.46$), indicating that fear memory did not increase with a passage of time.

3.2. Fear extinction

Next, we investigated whether fear memory age or intervals of fear extinction sessions affected 5-day extinction. All groups showed differences in % freezing during fear extinction (time × day $F_{(28,999)} = 3.74$, $p < 0.01$). Post hoc revealed that % freezing during 18–20 min was lower than that in 0–2 min until Session 3 (Session 1, $p < 0.05$; Sessions 2 and 3, $p < 0.01$), and no reduction was observed in Sessions 4 and 5. Percent freezing in fear extinction also differed among the three groups (group $F_{(2,145)} = 5.02$, $p < 0.01$). Week Ex and Interval 28d showed lower % freezing compared to Interval 7d ($p < 0.05$; Fig. 2B). Notably, these differences were observed within extinction sessions (time × group $F_{(14,999)} = 2.50$, $p < 0.01$) but not between sessions (day × group $F_{(8,145)} = 0.71$). Post hoc revealed that Week Ex showed lower % freezing during 6–12 min compared to Interval 7d ($p < 0.05$). Moreover, Interval 28d also showed lower % freezing during 8–12 min and 18–20 min compared to Interval 7d ($p < 0.05$). Thus, memory age and interval of extinction sessions affected 5-day extinction.

3.3. Recall test

To study whether differences in fear extinction were persistent for long periods, we investigated % freezing at 21 days after the last extinction session, and found differences in % freezing among the three groups ($F_{(2,31)} = 5.13$, $p < 0.05$). Post hoc revealed that Interval 7d showed higher % freezing than Interval 28d and Week Ex ($p < 0.05$), indicating that differences in fear extinction were persistent (Fig. 2C). Finally, we studied whether memory