An Unconditioned Stimulus Retrieval Extinction Procedure to Prevent the Return of Fear Memory

Jianfeng Liu, Liyan Zhao, Yanxue Xue, Jie Shi, Lin Suo, Yixiao Luo, Baisheng Chai, Chang Yang, Qin Fang, Yan Zhang, Yanping Bao, Charles L. Pickens, and Lin Lu

Background: Conditioned fear memories can be updated by extinction during reconsolidation, and this effect is specific to the reactivated conditioned stimulus (CS). However, a traumatic event can be associated with several cues, and each cue can potentially trigger recollection of the event. We introduced a technique to target all diverse cues associated with an aversive event that causes fear.

Methods: In human experiments, 161 subjects underwent modified fear conditioning, in which they were exposed to an unconditioned stimulus (US) or unreinforced CS to reactivate the memory and then underwent extinction, spontaneous recovery, and reinstatement. In animal experiments, 343 rats underwent contextual fear conditioning under a similar protocol as that used in the human experiments. We also explored the molecular alterations after US reactivation in rats.

Results: Presentation of a lower intensity US before extinction disrupted the associations between the different CS and reactivated US in both humans and rats. This effect persisted for at least 6 months in humans and was selective to the reactivated US. This procedure was also effective for remote memories in both humans and rats. Compared with the CS, the US induced stronger endocytosis of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptors 1 and 2 and stronger activation of protein kinase A, p70S6 kinase, and cyclic adenosine monophosphate response element binding protein in the dorsal hippocampus in rats.

Conclusions: These findings demonstrate that a modified US retrieval extinction strategy may have a potential impact on therapeutic approaches to prevent the return of fear.

Key Words: Extinction, fear memory, hippocampus, reconsolidation, retrieval, unconditioned stimulus

Anxiety disorders are often treated by exposure therapy that extinguishes or suppresses fear responses by repeatedly exposing subjects to the fear-inducing stimulus without harmful consequences (1,2). This therapy has been successfully modeled in humans and animals using Pavlovian fear conditioning and extinction, in which an originally neutral conditioned stimulus (CS) is associated with a noxious unconditioned stimulus (US), and the fear response is extinguished after repeated exposure to the CS without the US (3,4). However, although exposure therapy in individuals with anxiety disorders initially reduces fear responses, the reduced fear often returns in some conditions (5,6). The re-emergence of extinguished fear indicates that extinction normally leaves the original memory intact, which limits the long-term effectiveness of exposure therapy (5).

Exposure to a reminder of the conditioning experience, so that a memory is putatively re-encoded during a process termed "reconsolidation," has been shown to make a memory temporarily susceptible to disruption by several manipulations (7–9). Pharmacologic treatments have been used to disrupt reconsolidation for more than a decade (10–12), but side effects and the typical intracranial route of administration make these drugs more suitable for animal research than human treatment. More recent research showed that a drug-free procedure that uses reconsolidation to make extinction more effective disrupted both aversive and appetitive memories in animals and humans (13–18). However, extinction during reconsolidation permanently affects only memory for the reactivated CS and does not interfere with memory for other cues associated with the original learning event (16). A traumatic event is usually associated with several different cues, and each cue potentially triggers recollection of the event and elicits a fear reaction. Eliminating fear responses to all cues associated with the traumatic event through behavioral interference of reconsolidation is desirable. Presenting the US alone before extinction to trigger and disrupt US-specific reconsolidation could represent a promising avenue for treatment because pharmacologic manipulations after US-triggered reconsolidation can disrupt the conditioned memory for multiple CS associated with that US but not with other US (19). This specificity could make this approach useful to disrupt unhealthy emotional memories, while leaving other adaptive aversive memories intact. We investigated whether US retrieval extinction can disrupt representations of the US and persistently eliminate all US-associated memory traces and responses.

Methods and Materials

Human Experiments

Each participant signed a consent form approved by the institutional review board of Peking University and was paid for his or her participation.

Fear Conditioning Acquisition. We used a modified fear conditioning procedure (16). In all of the experiments, the positive CS (CS+) was paired with an electric shock (US) on a partial

From the Institute of Mental Health and Key Laboratory of Mental Health (JL, YL, CY, LL), Ministry of Health, the Sixth Affiliated Hospital of Peking University; National Institute on Drug Dependence (JL, LZ, YX, JS, YL, BC, YZ, YB, LL), Peking University; and Peking-Tsinghua Center for Life Sciences and Peking University-International Data Group-McGovern Institute for Brain Research (LL), Peking University, Beijing, China; Department of Pharmacy (LS), China MeiTan General Hospital, Beijing, China; School of Pharmacy and Affiliated Hospital of Guiyang Medical University (BC, CY, QF), Guiyang, China; and Intramural Research Program (CLP), National Institute on Drug Abuse, National Institutes of Health, Baltimore, Maryland.

Authors JL and LZ contributed equally to this work.

Address correspondence to Lin Lu, M.D., Ph.D., Institute of Mental Health and National Institute on Drug Dependence, Peking University, 38 Xue Yuan Road, Beijing 100191, China; E-mail: linlu@bjmu.edu.cn.

Received Oct 9, 2013; revised Feb 12, 2014; accepted Mar 10, 2014.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2014.03.027

reinforcement schedule (38% reinforced). In experiment 1, one CS was paired with the US (paired CS+ or unpaired negative CS [CS−] with US). In experiments 2, 3, and 5, two distinct CS were paired with the same US (paired CS1+ and CS2+ or unpaired CS− with US). In experiment 6, each of two distinct CS was paired with the different US (paired CS1+ with US1, paired CS2+ with US2, and unpaired CS−). See Methods and Materials and Table S1 in Supplement 1 for additional information.

Reactivation and Extinction. In reactivation and extinction, all of the CS were nonreinforced. In the animal experiment, we found that extinction after strong US reactivation, in which the intensity was same as the intensity used during acquisition, could not extinguish the fear response in rats (Figure S1 in Supplement 1). During US reactivation in humans, a weaker electric shock, in which the intensity was half of the one used during acquisition (200 msec), was administered. During CS reactivation, CS+ was presented once. During extinction, 10 CS+ and 10 CS− were presented. See Methods and Materials and Table S1 in Supplement 1 for additional details.

Test. In the test, all of the CS were nonreinforced. The spontaneous recovery test in experiments 1–3, 5, and 6 occurred 24 hours after the end of extinction. At the end of the spontaneous recovery test (experiments 1–3, 5, and 6) or the end of extinction (experiment 4), the response to the CS was thoroughly extinguished, and the participants then received three unsignaled US. The reinstatement test was followed by the unsignaled US. See Methods and Materials and Table S1 in Supplement 1 for additional information.

Psychophysiologic Stimulation and Assessment. Electric shock was delivered by a constant-current stimulator via a STM200 stimulator (BIOPAC Systems, Inc., Goleta, California). A stimulating electrode was attached to the right inner wrist or the right eyelid. Stimulus presentation was controlled by a computer using E-Prime software (Psychology Software Tools, Inc, Sharpsburg, Pennsylvania). Conditioning was assessed in terms of the skin conductance response, which was measured using a BIOPAC MP150 system and analyzed using AcqKnowledge software (BIOPAC Systems, Inc.). See Methods and Materials in Supplement 1 for additional details.

Statistical Analysis. We conducted mixed-model analysis of variance (ANOVA) for experiments 1–4 and repeated-measures ANOVA for experiments 5 and 6. To assess expectation of the reinforcer, only nonreinforced trials of the CS+ were included in the analysis. The differential fear response was assessed by subtracting the responses to the CS− from the responses to the CS+ in corresponding trials. Subjects who showed successful levels of fear acquisition and extinction were included in the analysis (see Methods and Materials in Supplement 1). Two-tailed tests and an α level of .05 were used for all of the statistical comparisons.

Rat Experiments

The fear conditioning procedure in rats was based on the work of Lubin and Sweatt (20), with some modifications. See the Methods and Materials in Supplement 1 for additional details.

Results

Extinction Interferes with Reconsolidation Triggered by US Presentation

We first examined whether extinction during US retrieval-triggered reconsolidation can disrupt fear memory in experiment 1 (Figure 1A). All of the participants in the three groups (10 min, 24 hours, and no retrieval) achieved successful acquisition [$F_{1,51} = 98.04$, $p < .05$] and extinction [$F_{1,51} = 86.66$, $p < .05$]. No significant difference was found between groups in either acquisition or extinction (all $p > .05$).

Spontaneous recovery was assessed using mixed-model ANOVA, with the between-subjects factor group and the within-subjects factor test (first trial of spontaneous recovery vs. last trial of extinction). This analysis showed main effects of group [$F_{2,51} = 29.56$, $p < .05$] and test [$F_{1,51} = 102.60$, $p < .05$] and a significant interaction [$F_{2,51} = 14.05$, $p < .05$]. The post hoc analysis showed that spontaneous recovery occurred in the no-retrieval group and 24-hour group (both $p < .05$) but not in the 10-min group ($p > .05$). One-way ANOVA showed that fear responses in the last trial of spontaneous recovery in all of the groups were similar ($p > .05$).

During reinstatement, mixed-model ANOVA, with the between-subjects factor group and the within-subjects factor test (last trial of spontaneous recovery vs. first trial of reinstatement), showed main effects of test [$F_{1,51} = 80.44$, $p < .05$] and group [$F_{2,51} = 17.52$, $p < .05$] and a significant interaction [$F_{2,51} = 13.79$, $p < .05$]. Follow-up t tests showed that only the fear response in the 10-min group was not reinstated ($p > .05$) (Figure 1B). Additionally, we found that extinction 10 min after US exposure prevented spontaneous recovery and reinstatement of extinguished fear in rats (Figure S2 in Supplement 1). Altogether, these findings showed that extinction during US-triggered

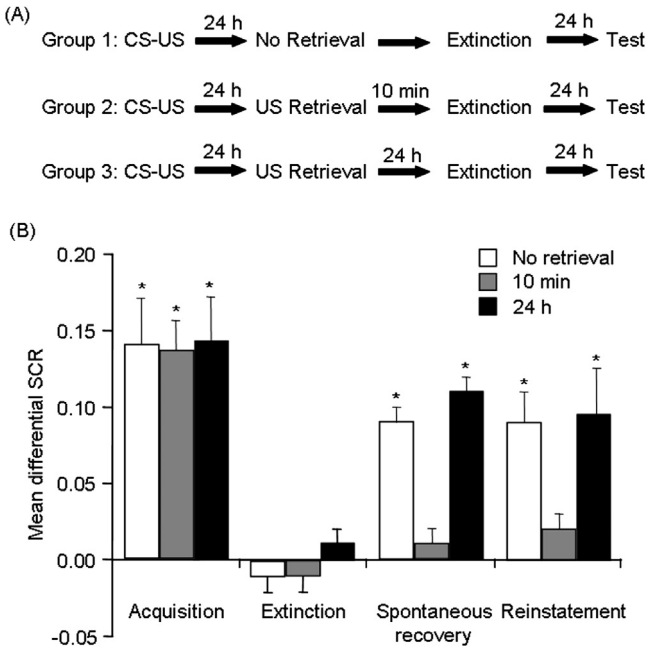

Figure 1. Extinction 10 min after unconditioned stimulus (US) exposure prevented spontaneous recovery and reinstatement of extinguished fear in humans. **(A)** Experimental design and timeline. **(B)** Mean differential skin conductance response (SCR) (positive conditioned stimulus [CS] minus negative CS) during acquisition (late phase), extinction (last trial), test for spontaneous recovery (first trial), and reinstatement (first trial) for each of the experimental groups (10 min, 24 hours, and no retrieval). Spontaneous recovery (first trial of this test vs. last trial of extinction) and reinstatement (first trial of reinstatement vs. last trial of spontaneous recovery) were found in the 24-hour and no-retrieval groups. No spontaneous recovery or reinstatement was found in the 10-min group. *$p < .05$, comparisons between acquisition and extinction, between extinction and first trial of spontaneous recovery, and between last trial of spontaneous recovery and reinstatement (all within-group). Data are expressed as mean ± SEM ($n = 16$–19 per group).