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Extinction after fear memory reactivation fails to eliminate renewal in rats

Travis D. Goode [a], Crystal M. Holloway-Erickson [b], Stephen Maren [a,b,*]

[a] Institute for Neuroscience, Texas A&M University, College Station, TX 77843-3474, United States
[b] Department of Psychology, Texas A&M University, College Station, TX 77843-3474, United States

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ABSTRACT

Retrieving fear memories just prior to extinction has been reported to effectively erase fear memories and prevent fear relapse. The current study examined whether the type of retrieval procedure influences the ability of extinction to impair fear renewal, a form of relapse in which responding to a conditional stimulus (CS) returns outside of the extinction context. Rats first underwent Pavlovian fear conditioning with an auditory CS and footshock unconditional stimulus (US); freezing behavior served as the index of conditioned fear. Twenty-four hours later, the rats underwent a retrieval-extinction procedure. Specifically, 1 h prior to extinction (45 CS-alone trials; 44 for rats receiving a CS reminder), fear memory was retrieved by either a single exposure to the CS alone, the US alone, a CS paired with the US, or exposure to the conditioning context itself. Over the next few days, conditional freezing to the extinguished CS was tested in the extinction and conditioning context in that order (i.e., an ABBA design). In the extinction context, rats that received a CS + US trial before extinction exhibited higher levels of conditional freezing than animals in all other groups, which did not differ from one another. In the renewal context, all groups showed renewal, and none of the reactivation procedures reduced renewal relative to a control group that did not receive a reactivation procedure prior to extinction. These data suggest retrieval-extinction procedures may have limited efficacy in preventing fear renewal.

1. Introduction

Fear memories may last a lifetime (Bergstrom, 2016). Even with extensive clinical and pharmaceutical treatments, humans often exhibit relapse of pathological fear and anxiety (Borkovec & Costello, 1993; Hermans, Craske, Mineka, & Lovibond, 2006; Vervliet, Baeyens, Van den Bergh, & Hermans, 2013; Vervliet, Craske, & Hermans, 2013; Wicking et al., 2016). Fear relapse can be modeled in the laboratory using Pavlovian fear conditioning and extinction (Bouton, 1993, 2002, 2004, 2014; Bouton, Westbrook, Corcoran, & Maren, 2006; Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Goode & Maren, 2014; Haaker, Golkar, Hermans, & Lonsdorf, 2014; Hermans et al., 2006; Kim & Richardson, 2010; Maren & Holmes, 2016; Maren, Phan, & Liberzon, 2013; Vervliet, Baeyens, et al. 2013; Vervliet, Craske, et al., 2013), which may contribute to and interact with fear and anxiety disorders (Careaga, Girardi, & Suchecki, 2016; Nees, Heinrich, & Flor, 2015; Ribrough, Glenn, & Baker, 2016; Smith, Doran, Sippel, & Harpaz-Rotem, in press; Zuj, Palmer, Lommen, & Felmingham, 2016). Specifically, Pavlovian fear conditioning consists of pairing a harmless conditioned stimulus ("CS"; e.g., auditory tone) with a noxious unconditioned stimulus ("US"; e.g., footshock) (Konorski, 1948; Pavlov & Anrep, 1927; Rescorla, 1988). Following one or more pairings in a conditioning chamber, animals will come to express conditioned fear responses (e.g., freezing behavior, autonomic activity) to the CS alone (Fanselow, 1994; Izquierdo, Furini, & Myskiw, 2016; LeDoux, 2000; Maren, 2001). After conditioning, nonreinforced presentations of the CS result in the gradual reduction of fear responses to the CS, a process termed extinction (Bouton et al., 2006; Maren et al., 2013; Myers & Davis, 2007; Pavlov & Anrep, 1927). However, extinguished fear in humans and other animals is known to return under a variety of circumstances (Bouton, 1993, 2002, 2004, 2014; Bouton et al., 2006; Craske et al., 2014; Goode & Maren, 2014; Haaker et al., 2014; Hermans et al., 2006; Kim & Richardson, 2010; Maren & Holmes, 2016; Maren et al., 2013; Vervliet, Baeyens, et al. 2013; Vervliet, Craske, et al., 2013), including after encountering the CS outside of the environment or "context" in which extinction occurred (termed "renewal"; Bouton & Bolles, 1979). Thus, while fear responses to a CS generalize across contexts, extinguished fear

* Corresponding author at: Institute for Neuroscience, Texas A&M University, College Station, TX 77843-3474, United States.
E-mail address: maren@tamu.edu (S. Maren).

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responses are context-dependent. Renewal and other relapse phenomena (e.g., shock-induced reinstatement and time-dependent spontaneous recovery of fear) reveal that extinction is not typically a fear-erasing process, rather extinction results in a new competitive memory that is thought to suppress the expression of conditioned fears (Bouton, 1993, 2002, 2004, 2014; Bouton et al., 2006; Maren, 2011). Given that extinction learning is thought to be an important factor in common forms of cognitive-behavioral therapy (e.g., exposure therapy; Graham, Langton, & Richardson, 2011; Graham & Milad, 2011; Hermans et al., 2006; Kaplan, Heinrichs, & Carey, 2011; Wicking et al., 2016), there is considerable interest in identifying new methods to enhance fear extinction and erase pathological fear memories selectively (Dejean et al., 2015; Fitzgerald, Seemann, & Maren, 2014; Goode & Maren, 2014; Herry et al., 2010; LeDoux, 2015; Maren, 2011; Maren & Holmes, 2016; Maren et al., 2013; Morrsion & Ressler, 2014; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014).

One possible method for the selective erasure of maladaptive fear memories involves disrupting memory *reconsolidation*. After conditioning, encountering fear conditioning-related stimuli (the CS, US, and/or conditioned context) can trigger the previously consolidated conditioned memory to enter a labile state that requires reconsolidation (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Clem & Schiller, 2016; Kredlow, Unger, & Otto, 2016; Schiller & Phelps, 2011). Behavioral, pharmacological, or neural manipulations during this postretrieval period allows for modification of the fear memory, including weakening or potentially erasing the memory (Auber et al., 2013; Giustino, Fitzgerald, & Maren, 2016; Kindt, Soeter, & Vervliet, 2009; Kindt & van Emmerik, 2016; Lattal & Wood, 2013; Meir Drexler & Wolf, 2017; Monfils, Cowansage, Klann, & LeDoux, 2009; Nader, 2003, 2015; Nader, Schafe, & LeDoux, 2000; Quirk et al., 2010; Schiller et al., 2010; Schwabe, Nader, & Pruessner, 2014; Soeter & Kindt, 2011). Of particular interest, it has been shown that reactivating or retrieving fear memories prior to extinction training can lead to a loss of responding to the CS that does not exhibit renewal, reinstatement, or spontaneous recovery (Monfils et al., 2009). This effect was time-dependent, such that the retrieval trial was found to enhance extinction only if it preceded normal extinction by 1 or 6 h but not 24 h (i.e., during the "reconsolidation window"; Monfils et al., 2009). Similarly, time-dependent postretrieval extinction has been shown to prevent relapse in humans (Schiller et al., 2010). In these studies, it has been proposed that the CS reminder engages a reconsolidation process that can be disrupted (and the labile memory erased) by extinction trials delivered shortly after memory enters a malleable state (Monfils et al., 2009; Schiller & Phelps, 2011; Schiller et al., 2010).

The possibility that fear memories can be erased has generated enormous excitement in the clinical community (Careaga et al., 2016; Kroes, Schiller, LeDoux, & Phelps, 2016; Post & Kegan, 2017; Quirk et al., 2010; Smith et al., in press), but the efficacy of "reconsolidation update" procedures in preventing fear relapse is mixed (Auber et al., 2013; Clem & Schiller, 2016; Kredlow et al., 2016; Schiller & Phelps, 2011). A critical variable that has not yet been fully explored might be the procedure used to reactivate the fear memory prior to extinction. For example, reconsolidation windows can be opened by the presentation of the CS alone, the US alone, a conditioned context, or even a conditioning trial (CS + US) and protein synthesis inhibitors delivered after these forms of reactivation lead to impaired retention of conditioned fear memories (Duvarci & Nader, 2004). Moreover, recent work in humans and rats indicates that *weak* US-alone exposure prior to extinction prevents fear reinstatement and spontaneous recovery (Liu et al., 2014; Thompson & Lipp, 2017). However, the relative efficacy of these manipulations in preventing relapse phenomena, including renewal, have not been explored.

In the present study, we examined the efficacy of four different retrieval procedures in preventing fear renewal after extinction. We hypothesized that retrieval procedures that produced prediction errors (CS-, US-, or shock-associated context-alone reminders; Rescorla & Wagner, 1972) would be more effective than a CS + US trial in promoting reconsolidation update and in preventing fear renewal (provided animals were sufficiently extinguished). This hypothesis is based on work by Sevenster, Beckers, and Kindt (2012, 2013, 2014), which highlight the importance of prediction error in engaging reconsolidation (Fernández, Boccia, & Pedreira, 2016). Accordingly, rats were conditioned and underwent extinction 1 h after brief or single exposure to the CS, US, a CS + US trial, or the conditioning context; another group of rats did not receive any retrieval procedure to serve as a control. To assess relapse, we tested animals to the extinguished CS outside of the extinction context (renewal). None of the retrieval procedures attenuated fear renewal—in fact, retrieval with a US-alone or CS + US trial facilitated fear expression during renewal. These results challenge the efficacy of retrieval-extinction procedures in preventing fear relapse.

2. Materials and methods

2.1. Subjects

Subjects were sixty-four adult male Long-Evans (Blue Spruce) rats (200–225 g) obtained from Harlan Sprague-Dawley (Indianapolis, IN). Subjects were individually housed in a climate-controlled vivarium at the University of Michigan where the present experiment was conducted. Rats were kept on a reverse light (14 h)-dark (10 h) cycle. Food and water were accessible *ad libitum*. Rats were handled once a day for ~1 min for 5 consecutive days prior to the start of behavior. The University of Michigan Animal Care and Use Committee approved all experimental procedures.

2.2. Behavioral apparatuses

All training and testing procedures occurred in rodent observation chambers (MED-Associates, St. Albans, VT) of identical size (30 × 24 × 21 cm) and construction (Plexiglas ceilings, rear walls, and doors, aluminum side walls, and stainless steel grid floors). Observation chambers were contained within external sound-attenuating cabinets. Grid floors of the observation chambers (consisting of 19 stainless steel rods) were connected to shock sources and solid-state grid scramblers (MED-Associates) for delivering footshock (US). Small speakers were attached to the chambers and provided auditory tones (CS). The observation chambers were also fitted with 15-W house lights and ventilation fans. Each observation chamber rested upon a load-cell platform (connected to load-cell amplifiers) that would respond to cage displacement as a result of a rat's movements (load-cell amplifiers were calibrated to a standardized degree of chamber displacement prior to behavioral training). Load-cell activity output (+/−10 V) was transformed into values of 0–100 and captured every 200 ms using Threshold Activity software (MED-Associates). Smaller values indicated less cage displacement and freezing was quantified as transformed load-cell activity values of ≤10 for 1 s or more.

Sensory features of the chambers were manipulated to obtain three unique contexts (A, B, and C) for the current study. For context A, 1% acetic acid was used to wipe down the chambers (grid floors were dried) and a small volume of the odor was poured into the pans beneath the grid floors. Chamber house lights remained lit, room lights were on, chamber fans were on, cupboard doors encasing the chambers were left open, and rats were transported to and from the chambers in white plastic transport boxes. For