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Fear of the unexpected: Hippocampus mediates novelty-induced return of extinguished fear in rats

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ABSTRACT

Several lines of evidence indicate an important role for the hippocampus in the recovery of fear memory after extinction. For example, hippocampal inactivation prevents the renewal of fear to an extinguished conditioned stimulus (CS) when it is presented outside the extinction context. Renewal of extinguished responding is accompanied by associative novelty (an unexpected occurrence of a familiar CS in a familiar place), the detection of which may require the hippocampus. We therefore examined whether the hippocampus also mediates the recovery of extinguished fear caused by other unexpected events, including presenting a familiar CS in a novel context or presenting a novel cue with the CS in a familiar context (e.g., external disinhibition). Rats underwent Pavlovian fear conditioning and extinction using an auditory CS and freezing behavior served as the index of conditioned fear. In Experiment 1, conditioned freezing to the extinguished CS was renewed in a novel context and this was eliminated by intra-hippocampal infusions of the $GABA_A$ agonist, muscimol, prior to the test. In Experiment 2, muscimol inactivation of the hippocampus reduced the external disinhibition of conditioned freezing that occurred when a novel white noise accompanied the extinguished tone CS. Collectively, these results suggest that the hippocampus mediates the return of fear when extinguished CSs are unexpected, or when unexpected stimuli accompany CS presentation. Ultimately, a violation of expectations about when, where, and with what other stimuli an extinguished CS will occur may form the basis of spontaneous recovery, renewal, and external disinhibition.

1. Introduction

A major challenge to behavioral therapies for anxiety disorders, including post-traumatic stress disorder, is the relapse of fear that occurs outside of the clinical context. One account of relapse builds on the observation that the extinction of Pavlovian conditioned responses (CRs), including fear responses, is context-specific (Bouton & Bolles, 1979; Bouton, Mineka, & Barlow, 2001; Craske et al., 2008). For example, if a conditional stimulus (CS) that once predicted an aversive footshock unconditional stimulus (US) is presented alone several times in a distinct context, fear to the CS will be lost—but only in the extinction context (Bouton & Bolles, 1979). That is, the fear CR will return or 'renew' if the CS is encountered outside of the extinction context, including the conditioning context ('ABA' renewal, where the letters denote the contexts used for conditioning, extinction, and testing) or an equally familiar context in which the CS has never been experienced ('AAB' or 'ABC renewal', which is often found to be weaker than ABA renewal). This reveals that extinction does not erase the CS–US association but encourages the formation of a new inhibitory CS–'no US' memory (Konorski, 1967; Pavlov, 1927). It has been posited that these inhibitory memories are particularly context-bound, and that animals use contexts as retrieval cues to determine how to respond to a CS (Bouton, 1993; Bouton, Westbrook, Corcoran, & Maren, 2006). By this view, inhibition acquired during extinction (e.g., the formation of a CS–'no US' association) is linked to the extinction context, and is not retrieved outside of that context. In other contexts, fear is supported by the context-independent expression of the CS–US association—as a result, conditioned fear is broadly generalized, whereas extinction is not.

Another possibility is that the inhibition acquired during extinction fails to generalize to other contexts because the circumstances of the test situation produce "inhibition of an inhibition" (Pavlov, 1927). Pavlov observed that novel or unexpected events (including his own presence during a colleague's experiment) caused a spontaneous return of an extinguished salivary response, a phenomenon he termed 'external disinhibition' (Pavlov, 1927). The loss of inhibition that occurs when novel stimuli accompany an extinguished CS might account for other forms of CR recovery that are accompanied by unexpected events, including renewal. In this case, extinguished CSs are unexpectedly encountered in contexts in which they had never been presented (AAB, ABC), or had not

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been encountered recently (ABA). Hence, renewal may be a form of disinhibition produced by associative novelty when testing occurs in a familiar context. This account has found recent support in a computational model of extinction that includes attentional mechanisms and novelty in determining conditioned responding (Larrauri & Schmajuk, 2008).

Interestingly, there is considerable evidence in both rats and humans that the hippocampus plays an important role in novelty detection, particularly in the detection of associative novelty (Honey & Good, 2000; Knight, 1996; Kumaran & Maguire, 2006; Vinogradova, 2001). For example, hippocampal lesions in rats reduce the orienting response to the novel pairing of habituated stimuli, a phenomenon that requires the detection of an associative mismatch among familiar cues (Honey, Watt, & Good, 1998). In addition, human neuroimaging experiments have revealed that hippocampal activity is elevated under conditions in which unexpected sequences of familiar stimuli are encountered (Kumaran & Maguire, 2006; Wessel, Danielmeier, Morton, & Ullsperger, 2012). Moreover, it is now apparent that the hippocampus and its afferents are critical for the renewal of extinguished fear that occurs when an extinguished is unexpectedly encountered outside the extinction context (Corcoran, 2004; Corcoran & Maren, 2001; Corcoran, Desmond, Frey, & Maren, 2005; Hobin, Ji, & Maren, 2006; Ji & Maren, 2005; Ji & Maren, 2008a; Ji & Maren, 2008b; Maren & Hobin, 2007; Zelikowsky, Pham, & Fanselow, 2011). While most authors have interpreted these hippocampal lesion effects in terms of contextual retrieval processes and occasion setting (Maren, 2005; Maren & Holt, 2000), a more parsimonious account appeals to the role of the hippocampus in novelty detection. That is, the hippocampus may mediate the renewal effect, because it is detects associative novelty during the retrieval test that leads to the disinhibition of extinguished fear. If true, hippocampal inactivation should limit the return of extinguished fear that accompanies exposure to a novel stimulus (external disinhibition) or presentation of the extinguished CS in a novel context (renewal). To examine this question, we examined the effect of hippocampal inactivation with the $GABA_A$ agonist muscimol on the expression of fear to an extinguished CS presented in either a novel context (Experiment 1) or after delivery of a novel stimulus (white noise) in the extinction context (Experiment 2). Insofar as the hippocampus is required for detecting novel or unexpected events, we hypothesized that hippocampal inactivation would impair the return of fear driven by the presence of novel contexts or cues.

2. Materials and methods

2.1. Subjects

The subjects were 64 (Experiment 1) and 32 (Experiment 2) adult male Long-Evans rats (200–224 g) obtained from a commercial supplier (Harlan Sprague Dawley, Indianapolis, IN). After arrival, the rats were housed individually in Plexiglas hanging cages on a 14:10 h light/dark cycle (lights on at 7:00 a.m.) and were allowed unlimited access to food and water. After being housed, the rats were handled (10–20 s per rat per day) for 5 days to habituate them to the experimenter. All experiments were UCUCA-approved and conducted at the University of Michigan.

2.2. Surgical procedures

Rats underwent surgical procedures before any behavioral testing. Rats were treated with atropine methyl nitrate (0.3 mg/kg, i.p.), anesthetized with sodium pentobarbital (Nembutal; 65 mg/kg, i.p.), and mounted in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). The scalp was incised and retracted, and the head was positioned to place bregma and lambda in the same horizontal plane. Small holes were drilled through the skull for bilateral placement of stainless steel guide cannulas (23 gauge; 10 mm in length; Small Parts, Inc., Miami Lakes, FL) into the DH (3.8 mm posterior, 2.5 mm lateral, 2.5 mm ventral to bregma) and three jeweler's screws. Cannulas were affixed to the skull and the scalp incision was closed with dental acrylic. After surgery, stainless steel obturators (30 gauge; 10 mm in length; Small Parts) were placed in the guide cannulas. Obturators were replaced every other day throughout the remainder of the experiment.

2.3. Behavioral apparatus

Eight identical observation chambers (30 × 24 × 21 cm; MED-Associates, St. Albans, VT) were used in all of the experiments. The chambers were constructed from aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door) and were situated in sound-attenuating cabinets located in a brightly lit and isolated room. The floor of each chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center-to-center). Rods were wired to a shock source and solid-state grid scrambler (MED-Associates) for the delivery of footshock USs. A speaker mounted outside a grating in one wall of the chamber was used for the delivery of acoustic CSs. Video cameras mounted above each chamber were used to visualize the behavior of each rat, and each chamber rested on a load-cell platform that was used to detect locomotor activity and quantify freezing behavior.

Two contexts were used in these experiments. For one context, the overhead lights in the room and the houselights (15 W) in each observation chamber were illuminated. The chambers were cleaned with a 2% acetic acid solution, and stainless steel pans containing a thin film of the same solution were placed underneath the grid floors to provide a distinct odor before the rats were placed inside. Ventilation fans in each cabinet supplied background noise (65 dB). Rats were transported to this context in white plastic boxes. For the second context, all room and chamber houselights were turned off; a pair of red lights (40 W) provided illumination. Additionally, the doors on the sound-attenuating cabinets were closed, the ventilation fans were turned off, and the chambers were cleaned with a 1% ammonium hydroxide solution. To provide a distinct odor, stainless steel pans containing a thin film of this solution were placed underneath the grid floors before the rats were placed inside. Rats were transported to this context in black plastic boxes.

2.4. Behavioral procedure for Experiment 1

Rats were given one-week recovery after surgery, then submitted to three phases of training: fear conditioning, extinction, and retrieval testing. We used a two-context design (AAB or ABB; each letter refers to the context for the three phases of training); all contexts were counterbalanced. Unlike our previous designs of this nature (Corcoran, 2004), we did not familiarize the animals in the AAB condition with the B context prior to testing. In other words, we arranged the test context to be novel for the AAB animals, whereas it was familiar for the ABB animals insofar as it had hosted extinction for them.

For fear conditioning, rats were transported in squads of eight and placed in the conditioning chambers; chamber position was counterbalanced for each squad. The rats received five tone (10 s; 80 dB; 2 kHz)-footshock (1 s; 1 mA) trials (70 s intertrial interval, ITI) beginning 3 min after being placed in the chambers. Sixty seconds after the final shock, the rats were returned to their home cages.

The extinction phase began 24 h after the conditioning session; it was conducted in a different context from conditioning. During