Explicit Disassociation of a Conditioned Stimulus and Unconditioned Stimulus During Extinction Training Reduces Both Time to Asymptotic Extinction and Spontaneous Recovery of a Conditioned Taste Aversion.

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Abstract

Conditioned taste aversions (CTAs) may be acquired when an animal consumes a novel taste (CS) and then experiences the symptoms of poisoning (US). When later given a choice between the poisoned taste and water, the animal will avoid the taste previously associated with malaise. This aversion may be extinguished by repeated exposure to the CS alone. However, following a latency period in which the CS is not presented, the CTA will spontaneously recover (SR). Thomas et al. (2005) have used an explicitly unpaired (EU) procedure to thwart renewal of a conditioned emotional response (CER) following extinction. We applied similar procedures to the CTA paradigm. Sprague-Dawley rats acquired a CTA [3 pairings of oral saccharin (SAC) and i.p. lithium chloride (LiCl)] followed by extinction training (EXT) consisting of either (a) CS-only exposure or, (b) exposure to SAC and LiCl on alternate days (i.e., explicitly unpaired: EU). Both extinction procedures resulted in ≥ 90% reacceptance of SAC although the EU-EXT procedure significantly decreased the time necessary for rats to reach this criterion (compared to CS-only controls). Rats were subsequently tested for SR of the CTA upon re-exposure to SAC following a 30-day latency period of water drinking. Rats that acquired a CTA and then underwent the CS-only extinction procedure exhibited a significant suppression of SAC drinking during the SR test (as compared to their SAC drinking at the end of extinction). However, animals in the EU extinction group (EU-EXT) did not show such suppression in drinking compared to CS-only controls. These data suggest that the EU-EXT procedure may be useful in reducing both time to extinction and the spontaneous recovery of fears. The findings are clinically relevant as we seek the development of treatments for deficits in fear extinction [e.g., Post Traumatic Stress Disorder (PTSD), phobias].

Introduction

- Fears may be acquired through associations of previously neutral stimuli with painful or aversive experiences.
  - Phobias
  - PTSD
- Fears may be reduced through various exposure therapies in which the object of fear (CS) is presented again, this time without the aversive stimuli (US), in an attempt to disassociate the CS – US connections (Foa, 2000).
- Fear extinction may be temporarily successful; however, spontaneous recovery (SR) and renewal of the fear (e.g., flashbacks) impede therapeutic progress (Bouton, 2002).
- Thus, it would be of great therapeutic value to develop a means by which spontaneous recovery of conditioned fears could be reduced or eliminated.
- Pre-clinical studies employing a conditioned emotional response (CER) paradigm suggest that using an extinction procedure in which the CS and US are explicitly unpaired may be able to thwart renewal of learned fears (Thomas et al., 2005).
- Our laboratory has been studying a different model of learned fears – the conditioned taste aversion (CTA) paradigm – in which a novel taste (CS) is associated with the symptoms of poisoning (US) (Mickley et al., 2004; 2005).
- The resulting defensive aversion to, and avoidance of, the feared taste can slowly be extinguished by repeated exposure to the CS alone. However, this CS-only extinction procedure allows spontaneous recovery of the CTA (Mickley et al., 2007).
- The current study sought to determine if, following acquisition of a CTA, employment of specifically unpaired presentations of the CS and US during extinction training (EU-EXT) might reduce or eliminate the spontaneous recovery of the CTA.

Methods

Table 1. Group Nomenclature and Treatments

	Group Designation	Conditioning Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Extinction[3]		30-day SR test	Number of rats per group[4]
CTA	CS-Only Extinction	SAC+LiCl[2]	Water	SAC+LiCl	Water	SAC+LiCl	Water	SAC		SAC	11 / 5
CTA	Explicitly Unpaired Extinction (EU-EXT)	SAC+LiCl	Water	SAC+LiCl	Water	SAC+LiCl	Water	SAC	Water+LiCl	SAC	12 / 6
EU	EU+ CS-Only	SAC	Water+LiCl	SAC	Water+LiCl	SAC	Water+LiCl	SAC		SAC	23
EU	EU+EU	SAC	Water+LiCl	SAC	Water+LiCl	SAC	Water+LiCl	SAC	Water+LiCl	SAC	12

[1]SAC = 30min exposure to the 0.3%SAC solution
[2]LiCl = Injection of lithium chloride (81mg/kg, i.p.)
[3]Extinction = the split cells for animals designated as EU Extinction refer to the 2 different treatments received on alternate days throughout the extinction phase; the single cells refer to the single treatment that was administered daily to animals designated as CS-Only Extinction.
[4]The first number indicates the total number of rats in each group. The second number represents the number of rats that had the spontaneous recovery test.

Subjects: Male, Sprague-Dawley rats

CTA Aquisition

- Animals were habituated to a 23h water deprivation schedule for 2 days prior to the start of the experiment.
- **CTA animals:**
  - On days 1, 3, and 5 of CTA conditioning, animals were presented with a 0.3 % saccharin solution (SAC) for 30 min. During a 15min latency animals were injected with lithium chloride (LiCl; 81mg/kg, i.p.) and subsequently presented with tap water for 30 min to prevent dehydration.
  - Days 2, 4, and 6 served as rest days during which the animals received two 30 min presentations of water separated by 15 min.
- **Explicitly Unpaired (control) animals:**
  - On experimental days 1, 3, and 5, rats were presented with the SAC for 30 min. Following a 15 min latency they were presented with water for 30 min.
  - On experimental days 2, 4, and 6, rats were presented with two 30 min presentations of water, separated by a 15 min. latency during which LiCl (81mg/kg, i.p.) was administered.

CTA Extinction

- **CS-Only Extinction:** Animals received 30 min SAC exposure and 15 min later were presented with water for 30 min every-other day until reaching asymptotic extinction (90% baseline SAC consumption levels).
- **Explicitly Unpaired Extinction (EU-EXT):** Animals received 30 min SAC exposure followed 15 min later by 30 min water presentation every-other day of the extinction phase until reaching asymptotic extinction. On alternate days these animals received two 30 min presentations of water separated by a 15min latency during which LiCl (81mg/kg, i.p.) was administered.

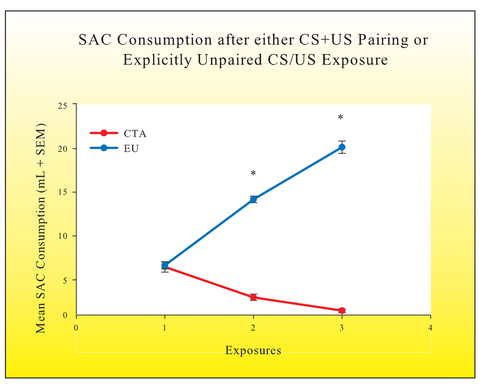
Spontaneous Recovery (SR)

- After reaching asymptotic extinction, animals were daily given two 30 min presentations of water for 29 days.
- On day 30 following asymptotic extinction, animals were re-exposed to SAC for 30 min.

Results

CTA Acquisition

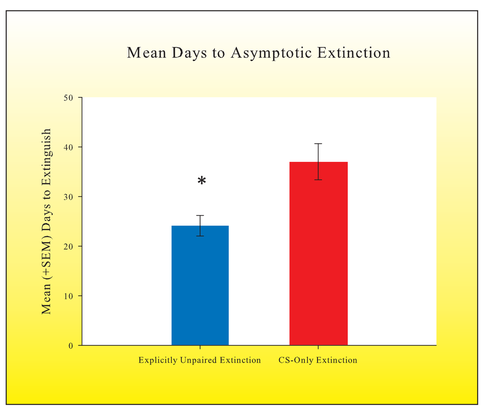
SAC Consumption After Either CS+US Pairing or Explicitly Unpaired CS/US Exposure:
The CTA group showed a significant decrease in the amount of saccharin (SAC) consumed over the three exposures. The Explicitly Unpaired (EU) group showed a significant increase in SAC consumption over the three CS/US exposures. This indicates that that CTA groups had acquired the CTA, whereas the EU (conditioning control groups) did not acquire a CTA. The SAC consumption of EU and CTA rats was the same on Exposure Day 1 but significantly different on exposure days 2 and 3. * = $p < 0.001$ (Bonferroni corrected *t*-tests)



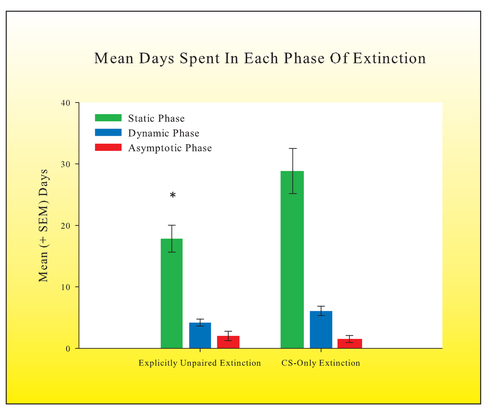
A repeated measures ANOVA [Treatment (CTA or EU) x CS Exposure Day] revealed a significant main effect for Exposure Day [$F(1,70) = 80.982$; $p < 0.0001$] and Treatment [$F(1,70) = 413.25$; $p < 0.001$]. There was also a significant interaction [$F(1,70) = 381.743$; $p < 0.0001$].

CTA Extinction

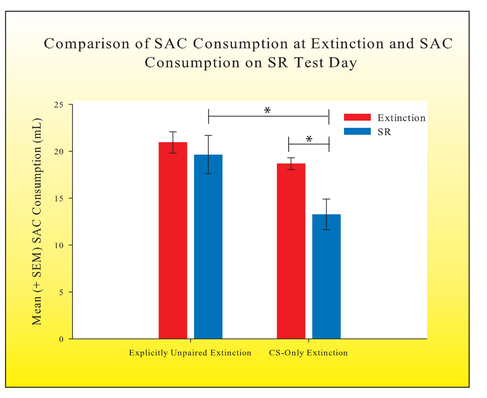
Mean Days To Extinguish a CTA:
The explicitly unpaired extinction group took significantly fewer days to extinguish the learned fear than the CS-only extinction group. * = Significantly different from the CS-Only Extinction Group: [$t(21) = 3.00$; $p = 0.007$]



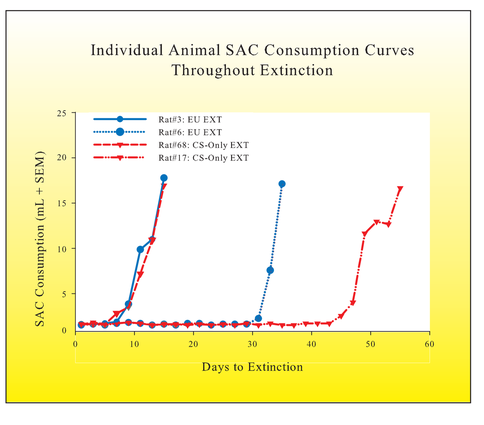
Mean Days In Each Phase Of Extinction:
Nolan et al. (1997) identified three phases of extinction: static, dynamic and asymptotic. Rats experiencing the explicitly unpaired extinction procedure (EU-EXT) spent significantly fewer days in the static phase (SAC reacceptance < 10% of baseline) than the CS-only extinction group. The EU-EXT group and CS-only extinction group spent about the same number of days in the dynamic phase (SAC reacceptance ≥ 10 % but < 80% of baseline) and also the asymptotic phase (SAC reacceptance ≥ 80% of baseline). * = Significantly Different from CS-Only Extinction Group: [$t(21) = 2.52$; $p = 0.02$].



SAC Consumption on the Day of Asymptotic Extinction and Spontaneous Recovery Test:
The explicitly unpaired extinction group (EU-EXT) drank nearly the same amount of SAC on the day of extinction as they did on the 30-Day SR test day. The CS-only extinction group drank significantly more SAC on the day of extinction than on the day of the SR test [$t(5) = 2.72$; $p = 0.042$]. Likewise, SAC drinking at the SR test was significantly less in the CS-only rats than in the EU-EXT rats [$t(9) = 2.47$; $p = 0.036$]. This indicates that the CS-only extinction animals had a SR of the fear, but the EU-EXT group did not.



Individual Animal SAC Consumption Curves throughout Extinction:
The extinction curves of the slowest animal to extinguish and fastest animal to extinguish from each of the two main experimental groups (EU-EXT = Red; CS-Only Extinction = Blue). Rats in the EU-EXT group exhibited a smaller range of days to extinguish than did the CS-Only Extinction group.



Summary and Conclusions

- Extinction learning that employed the EU-EXT procedure of disassociating the CS and US produced more rapid extinction of a CTA and also inhibited spontaneous recovery of this defensive reaction to a learned fear.
- The use of EU-EXT procedures seems to produce consistent results across two pre-clinical paradigms that have employed experimental animals: CER (Thomas et al., 2005) and CTA (current study).
- Following further pre-clinical testing, health care providers treating disorders where fear is prominent may wish to consider how EU-EXT procedures may facilitate this therapy (Holmes et al., 2007; Basoglu et al., 2007).

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