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Brief-Stress Cue Alleviates Forgetting the Stress-Induced Impairment of Extinction Learning in Rats

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Introduction

Extinction is a procedure where cues (CS) that have been previously paired with a biologically relevant reinforcer (UCS) are presented without the reinforcement. Although there is a reduction in response (CR), it is well established that extinction is a process involving new learning rather than "unlearning" or a breakdown of the original association[1]. Moreover, research has shown that memory for extinction is susceptible to disruption and that amnesia for extinction shares similar characteristics to amnesia for original acquisition memories[2].

Stress has also been shown to impair extinction learning. In addition, the memory for stress is susceptible to retrograde amnesia[3]. That the memory for stress is susceptible to disruption led to the current studies to determine whether reexposure to the amnestic cycloheximide (Experiment 1) and a brief stress session (Experiment 2) would serve as a reminder that would alleviate the stress-induced impairment of extinction learning.

Experiment 1

Subjects. Thirty-six female Long-Evans rats, approximately 100 days of age, were used as subjects.

Apparatus & Context. Training, extinction, and testing were conducted in a black-white shuttle box with grid floor that was located in a quiet, well-lit room. The shuttle box was divided into two compartments (one white, one black) by a guillotine door.

Procedure.
Stress – Four groups of rats were subjected to a single stress session consisting of a 1-hour restraint in a plastic restraint cone (DecapiCone, Braintree Scientific). Two groups received an injection (1mg/kg, i.p.) of cycloheximide (CHX; protein synthesis inhibitor – amnesia treatment) immediately after stress to assess retrograde amnesia for stress. The other two groups received a control injection of saline. Forty-eight hours after stress, all animals received punishment training (see Table 1).

Training – Punishment training consisted of placing the rat in the white side of the shuttle box with the door closed. After a brief period, the door was opened allowing the rat to cross to the black side. Upon entering the black side the door was automatically closed and one inescapable footshock (1 sec, 0.8 mA) was delivered. The animal was removed following the footshock.

Twenty minutes before training one group from each condition (CHX and saline) received an injection of CHX and the other received an injection of saline. The reexposure to CHX before training was to assess reactivation of the stress memory in a state dependent manner.

Extinction – Twenty-four hours after training, all animals received a single extinction session. Extinction consisted of placing the rat in the white compartment of a white/black chamber for 15 seconds followed by a 60 second fear probe trial. After the probe trial, the animal was placed in the black compartment for 10 minutes. The door separating the two compartments remained closed so the rat could not cross into the white side.

Test – Testing for passive avoidance (fear of black compartment) was conducted 24 hours after extinction training. Testing consisted of placing the rat in the safe (white) side and opening the sliding door. The door remained open for 5 minutes or until the animal crossed. The latency to cross to the black compartment was recorded as the dependent measure.

Table 1. Experimental design.

Group	Stress	Injection	*-48 hr-*	Injection	Training	*-24 hr-*	Extinction	*-24 hr-*	Test
Sal/Sal	Yes	Saline		Saline	Yes		Yes		Yes
CHX/Sal	Yes	CHX		Saline	Yes		Yes		Yes
CHX/CHX	Yes	CHX		CHX	Yes		Yes		Yes
Sal/CHX	Yes	Saline		CHX	Yes		Yes		Yes

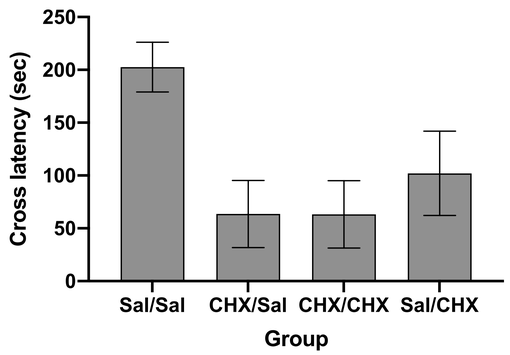

Figure 1. Mean (± *SEM*) cross through latencies.

Results & Discussion.
The rats in all four groups exhibited short cross-latencies at training. An ANOVA revealed no differences among groups, F (3, 32) = .534, p = .663.

The ANOVA for the extinction cross latencies revealed a significant difference, F (3, 32) = 7.82, p < .001. Post hoc tests confirmed a significant difference between the groups that received CHX prior to conditioning from those that received saline before conditioning, suggesting CHX induced anterograde amnesia for fear conditioning (data not shown).

Figure 1 shows the mean cross latencies for all four groups at test. The ANOVA performed on the testing cross latencies revealed a significant difference among the groups, F (3, 32) = 4.14, p = .014.

Tukey's post-hoc tests revealed a significant difference between the groups that received CHX after stress (CHX/Sal and CHX/CHX) compared to the saline only (Sal/Sal) group. Thus, the restraint stress impaired extinction learning and CHX induced amnesia for stress.

These results are difficult to interpret because the animals that received CHX prior to training crossed during the extinction probe trial, suggesting no fear to the black compartment. Thus, the short cross latencies at test may not be due to the extinction of fear but rather anterograde amnesia for training. Both explanations would produce these results. Moreover, the reexposed group (CHX/CHX) did not show increased latencies, demonstrating that the second dose did not act as a reminder for the stress.

Experiment 2 was designed to test whether reexposure to the stress (i.e., brief stress) following retrograde amnesia for stress would act as a reminder and alleviate the stress-induced impairment of extinction.

Experiment 2

Subjects. Thirty-six female Long-Evans rats, approximately 90 days of age, were used as subjects.

Apparatus & Context. Training, extinction, and testing were conducted in the same chambers as described in Experiment 1.

Procedure.
Stress – Three groups were subjected to restraint stress as described in Experiment 1. A Brief Stress control group did not receive stress. One of the three stress groups (Stress) received an injection of saline immediately following stress. The other two stress groups received injections of CHX immediately after stress. The Brief Stress control group received a CHX injection in their home cage (see Table 2).

Training – Forty-eight hours after stress, all rats received punishment training as described in Experiment 1. Twenty minutes before conditioning, one of the CHX groups (Reexpose) and the Brief Stress control group received a brief stress exposure (5 minutes restrained) to assess reactivation of the stress.

Extinction – Twenty-four hours after training, extinction was conducted as described in Experiment 1. (No animals crossed during the extinction probe trial.)

Test – Twenty-four hours after extinction, all animals were tested as described in Experiment 1.

Table 2. Experimental design.

Group	Stress	Injection	*-48 hr-*	Stress	Training	*-24 hr-*	Extinction	*-24 hr-*	Test
Stress	Yes	Saline		No	Yes		Yes		Yes
RA/Stress	Yes	CHX		No	Yes		Yes		Yes
Reexpose	Yes	CHX		5 min	Yes		Yes		Yes
Brief Stress	No	CHX		5 min	Yes		Yes		Yes

Results & Discussion.
All four groups exhibited short cross-latencies at training. An ANOVA revealed no significant differences among groups, F (3, 32) = .609, p = .614.

Figure 2 shows the mean cross latencies of all four groups at test. An ANOVA revealed that there was an overall difference among the groups, F (3, 32) = 5.89, p = .003.

Tukey's post-hoc tests confirmed a significant difference between the RA/Stress group and the Stress group, demonstrating forgetting of the stress effects on extinction. Importantly, the Reexpose group was also found to be significantly different from the RA/Stress and Brief Stress groups, suggesting that the brief stress reminder before training reactivated the stress memory. Thus, the restraint stress impaired extinction learning and the reexposure to stress alleviated the forgetting.

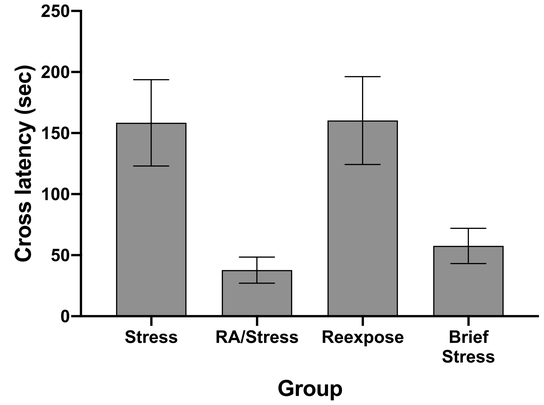

Figure 2. Mean (± *SEM*) cross through latencies.

Conclusions

Experiment 1 failed to demonstrate that reexposure to the amnestic agent prior to training would alleviate retrograde amnesia for the stress-impairment of extinction learning.

Although, these findings provide evidence that amnesia for the restraint stress can be obtained using the protein synthesis inhibitor CHX; however, because the animals crossed during extinction it appears that anterograde amnesia for fear conditioning was observed.

Experiment 2 results also show that memories for restraint stress are susceptible to disruption similar to other memories. In addition, the results demonstrate that the forgotten stress memories can be reactivated, which can again lead to the impairment of extinction learning.

References

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