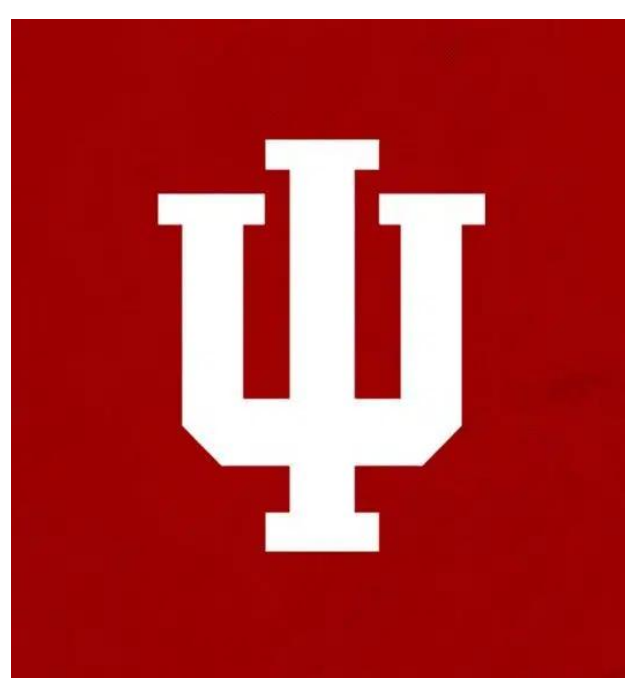




# Examining a role for unconditional stimulus deflation in delay fear conditioning.

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## Introduction

Extinction is the repeated presentation of an unreinforced conditional stimulus (CS) after it has come to predict an aversive unconditional stimulus (US). Extinction reliably reduces fear to the CS and results in a new CS-no US association. However, retrieval of the original CS-US association over the CS-no US association learned during extinction, can lead to a return, or relapse, of extinguished fear (Bouton, 2002).

Methods targeting the original fear memory could therefore be more effective at reducing fear long-term. US deflation is the repeated presentation of a previously-acquired CS-US pairing with a weaker version of the US to deflate the value of the representation and consequently elicit less of a response to the CS itself.

Prior work has found that weak shock exposures successfully reduce context fear (Bonanno et al., 2023; Brooks et al., 2025); however, it remains unclear if the weak shock will be effective in other types of associative learning (e.g., delay fear conditioning; DFC) or reduce relapse, like renewal or reinstatement.

Several accounts have found fear behavior to track closely with zif268 expression (Hoffman et al., 2015; Robinson et al., 2024) and increased phosphorylation of CREB is indicative of memory consolidation (Bourtchuladze et al., 1994). Therefore, using these two markers of neural activity, we examined if there were any differences in neural activity between groups following DFC using immunofluorescence.

Here, we aim to extend our previous results using the weak shock in a delay fear conditioning paradigm and examine its ability to prevent common forms of relapse.

## Methods

**Subjects.** Animals were age-matched (60 days) Long Evans male and female rats.

**Apparatus.** Behavioral procedures occurred in two sets of Colbourn conditioning chambers, each housed in its own sound-attenuating cubicle. Each set was in its own room in the lab.

**Procedure.** Animals were handled 2 days prior to behavioral procedures.

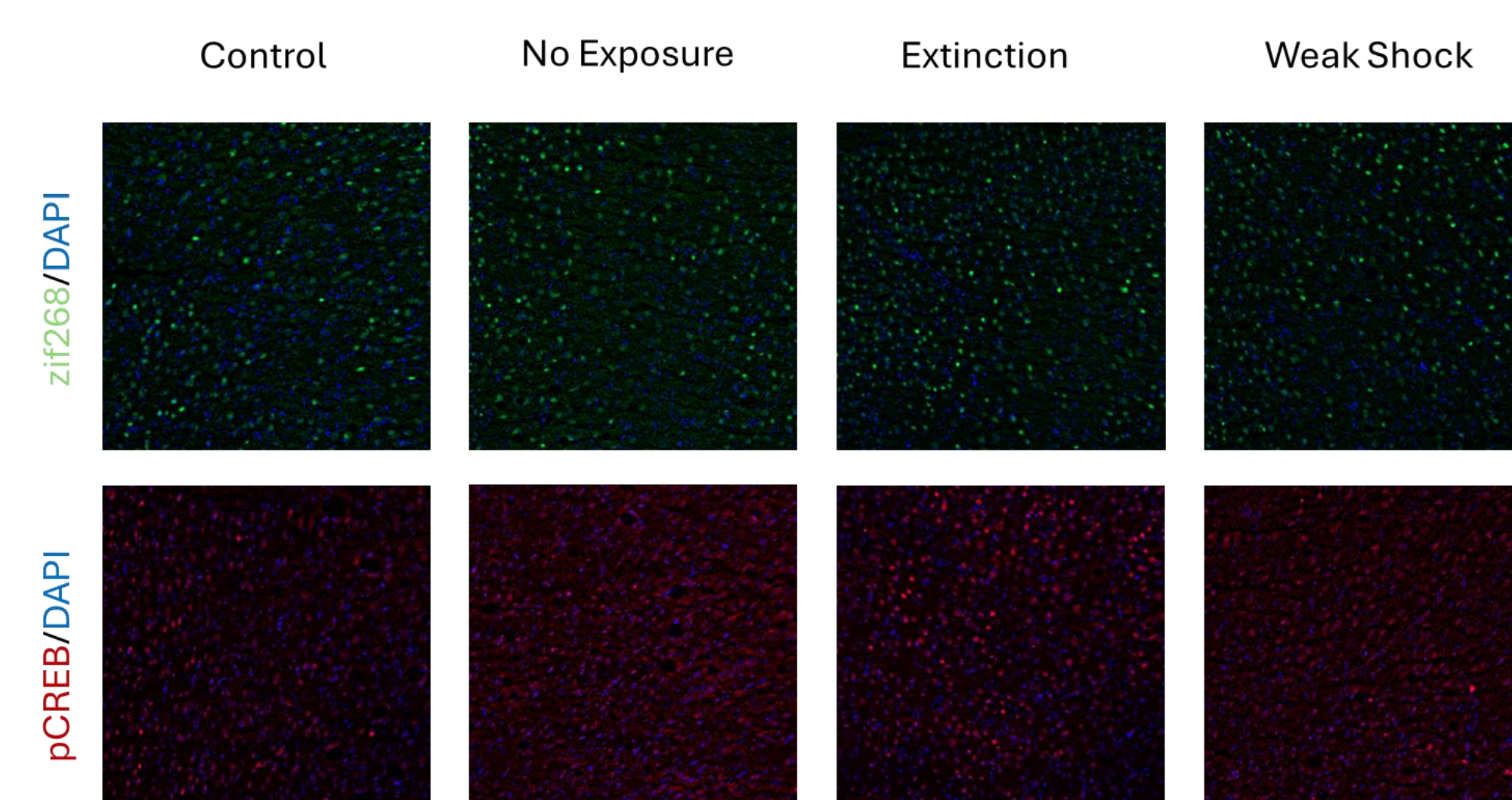
Delay fear conditioning: Following a 6-minute baseline period, animals were given 4 pairings of a 10 s white noise CS that ended with a 1-s 1.0mA footshock US. There was a 110-s ITI. Following the final foot shock, animals remained in the chamber for an additional 4 minutes before being removed and returning to their homecage.

Extinction or Deflation: The next day, animals received extinction with 10 CS-alone (white noise) presentations or deflation with 10 white noise CS-0.3mA weak footshock US pairings. The first CS occurred after 1 minute in the chamber. During this phase, each CS was 30 s and there was an ITI of 60 s between presentations. Animals were removed from the chamber 15-s following the final CS.

Test: During Test, freezing elicited by the presentation of the 10-s white noise CS was used to assess delay fear. The first CS was played after 1 minute in the chamber, and there was a 60-s ITI between CS presentations.

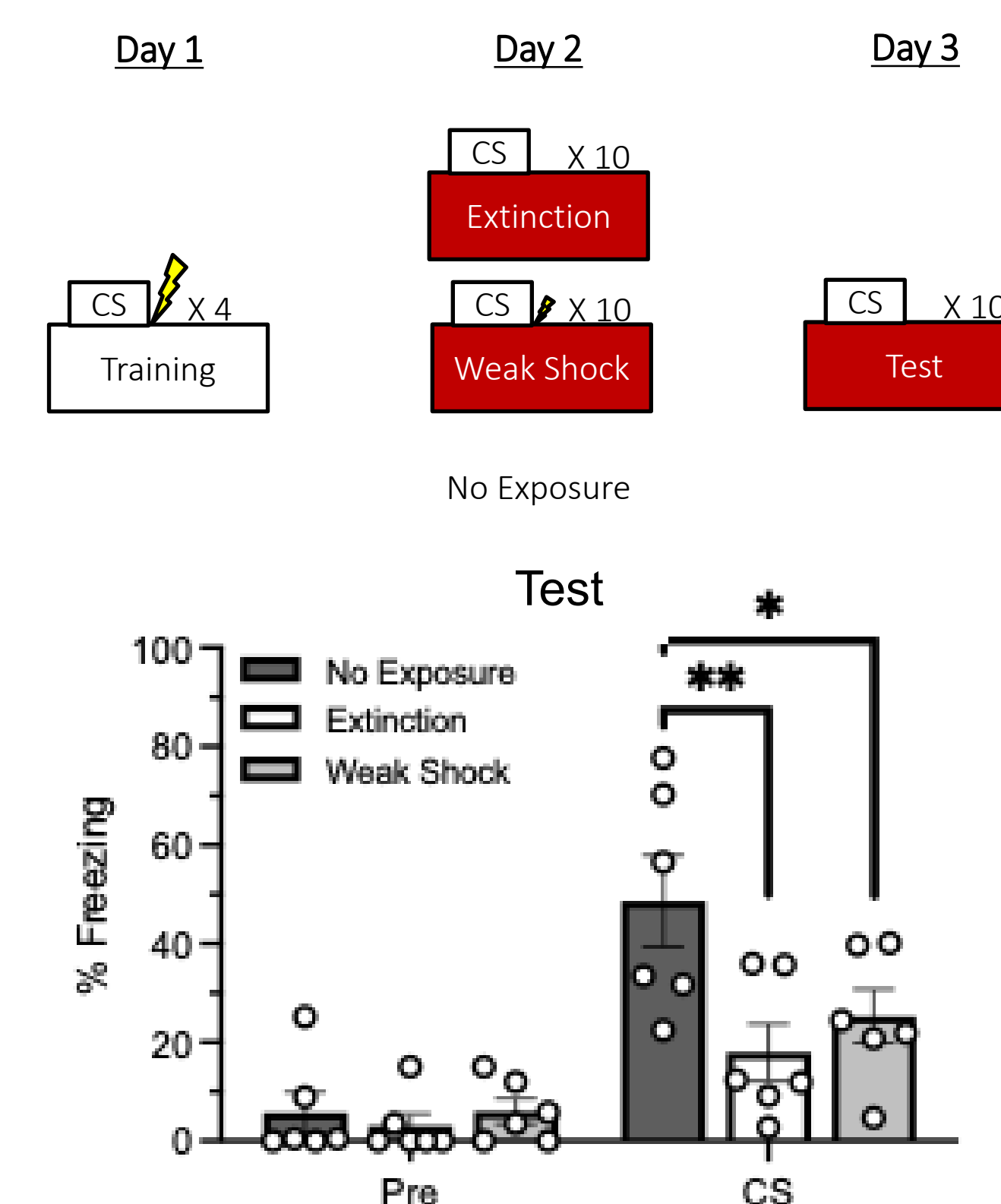
**Immunofluorescence.** Tissue collected 60 mins following testing in the initial experiment and was sliced, mounted, and analyzed using immunofluorescent probing for zif268 and pCREB.

**Data Analysis.** Freezing behavior was measured using FreezeFrame software. Zif268 and pCREB were normalized to DAPI staining present on the same section. Example images are depicted below.

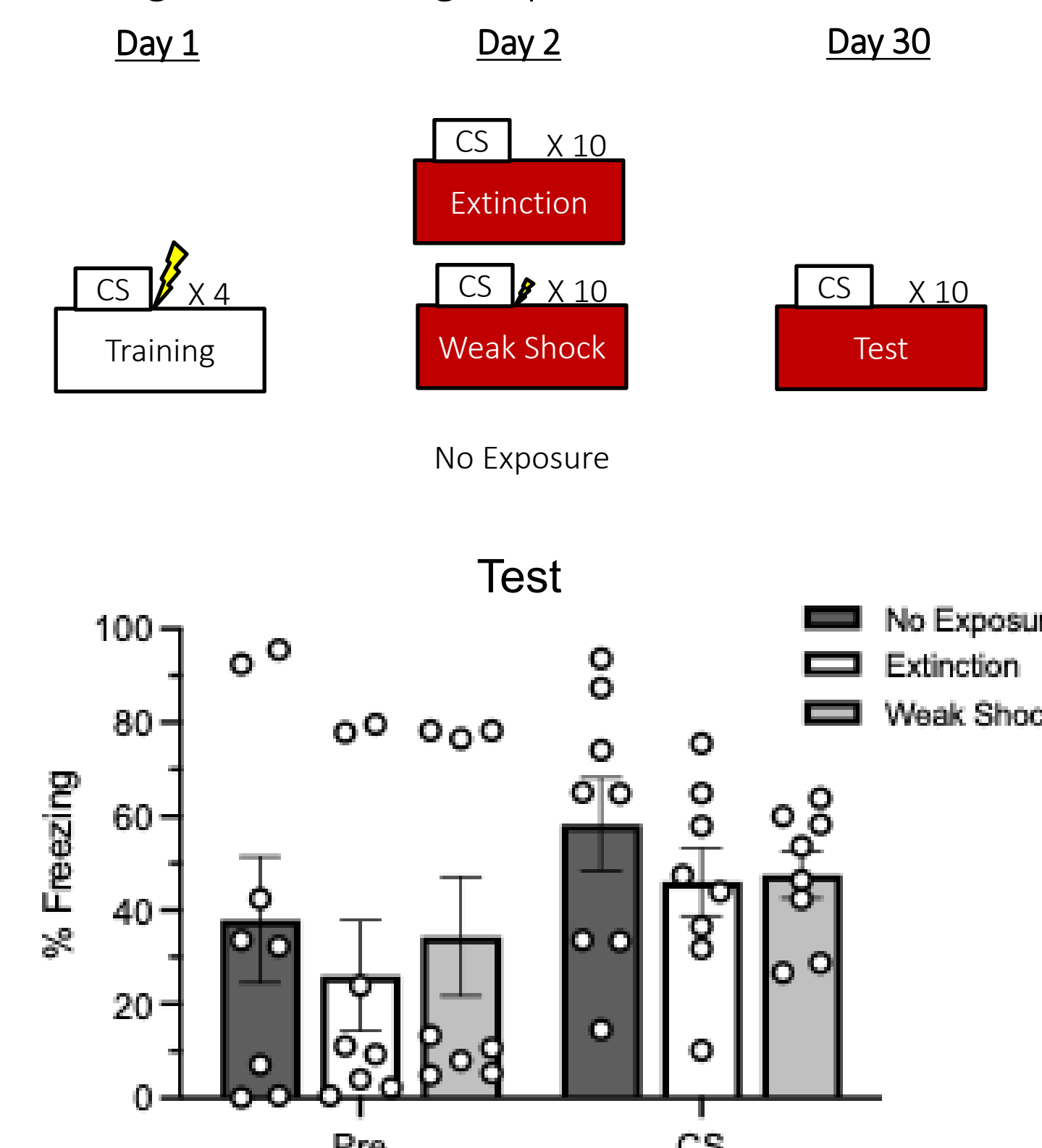


## Results

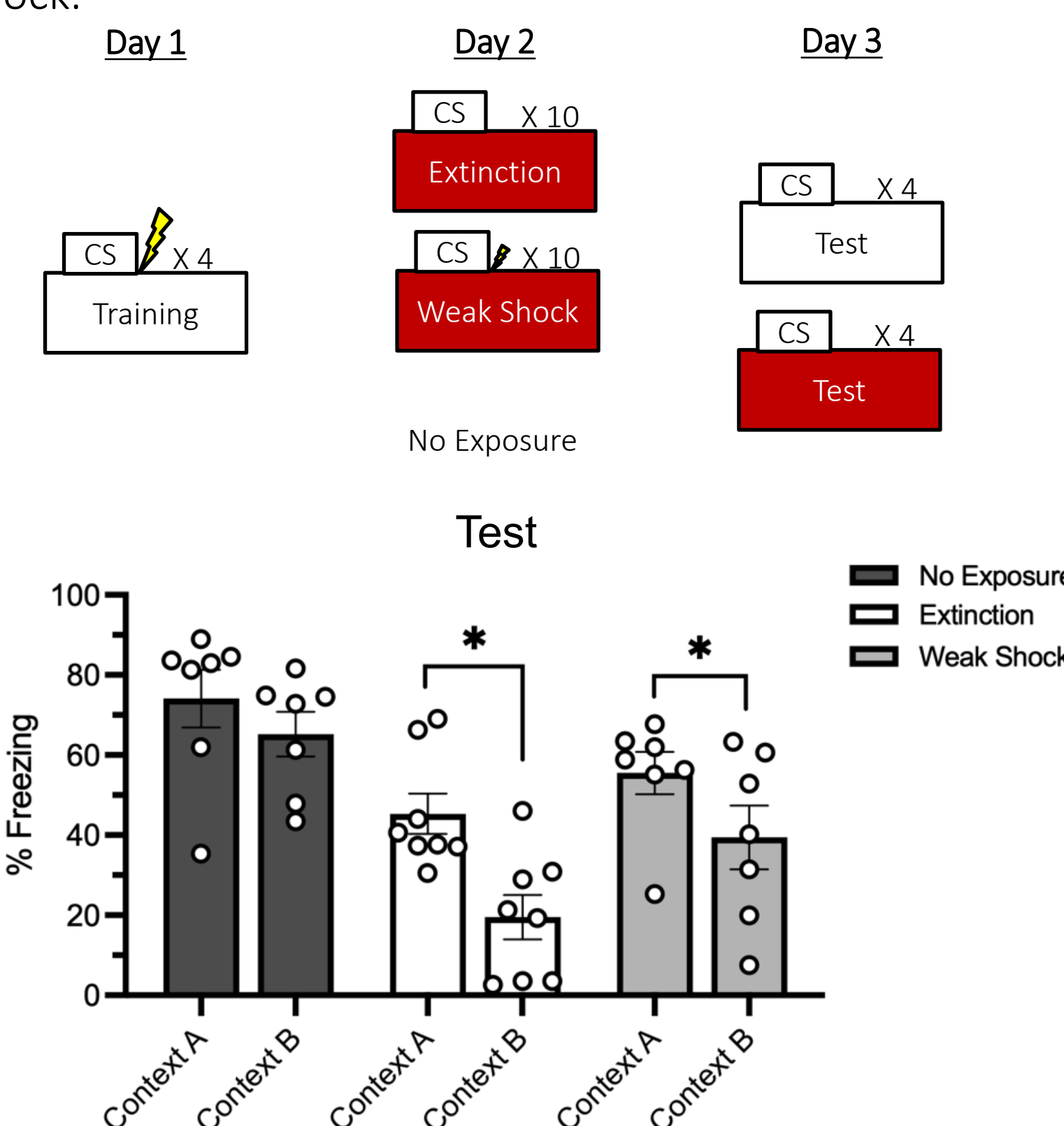
**Short-term Test.** The weak shock and extinction resulted in reduced freezing to the CS following DFC.



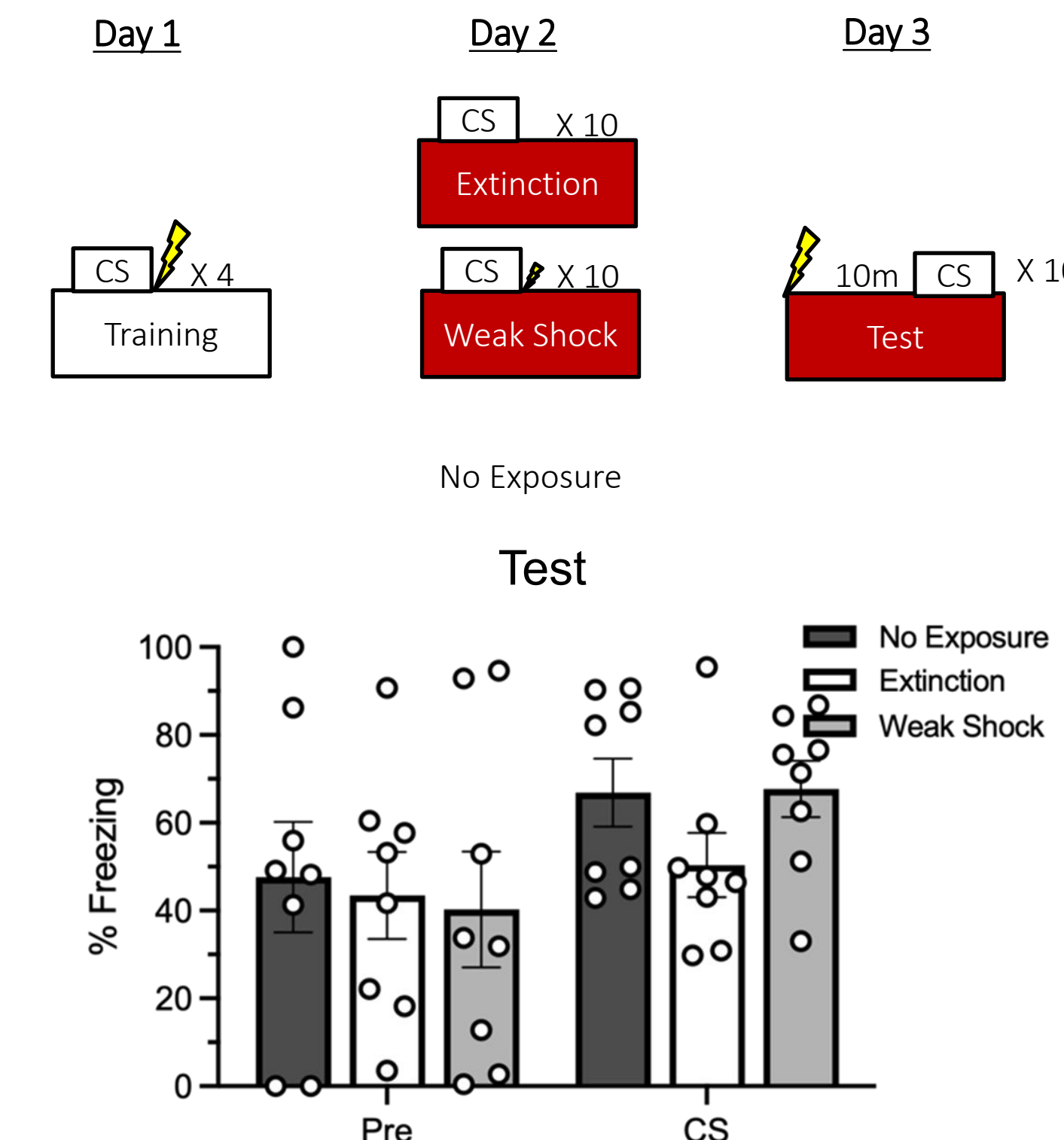
**Long-term Test.** The weak shock group's CS freezing did not differ from their pre-CS levels, nor from the CS freezing of the other groups.



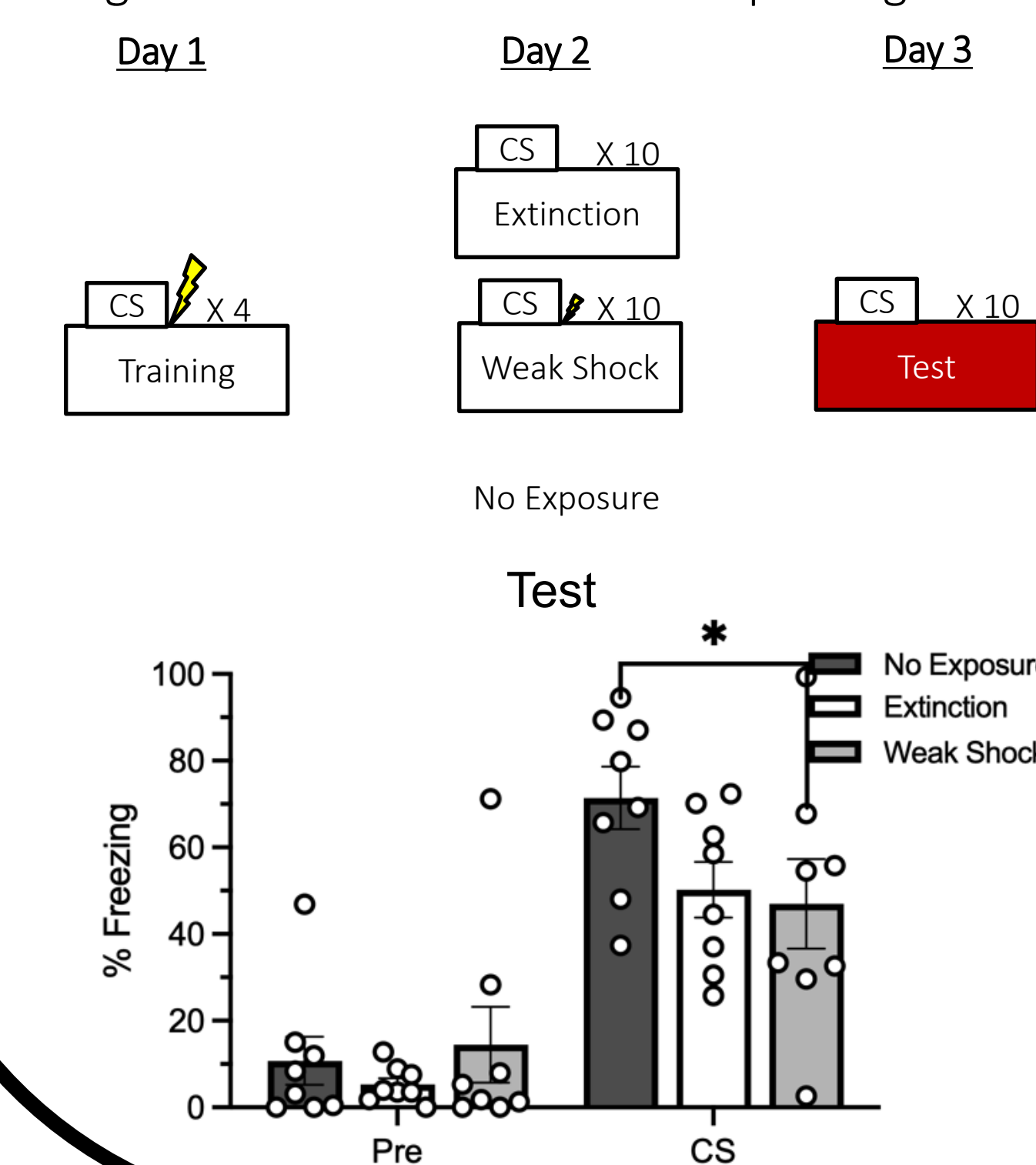
**ABA Renewal.** The CS elicited more freezing in Context A than in Context B following both extinction and weak shock.



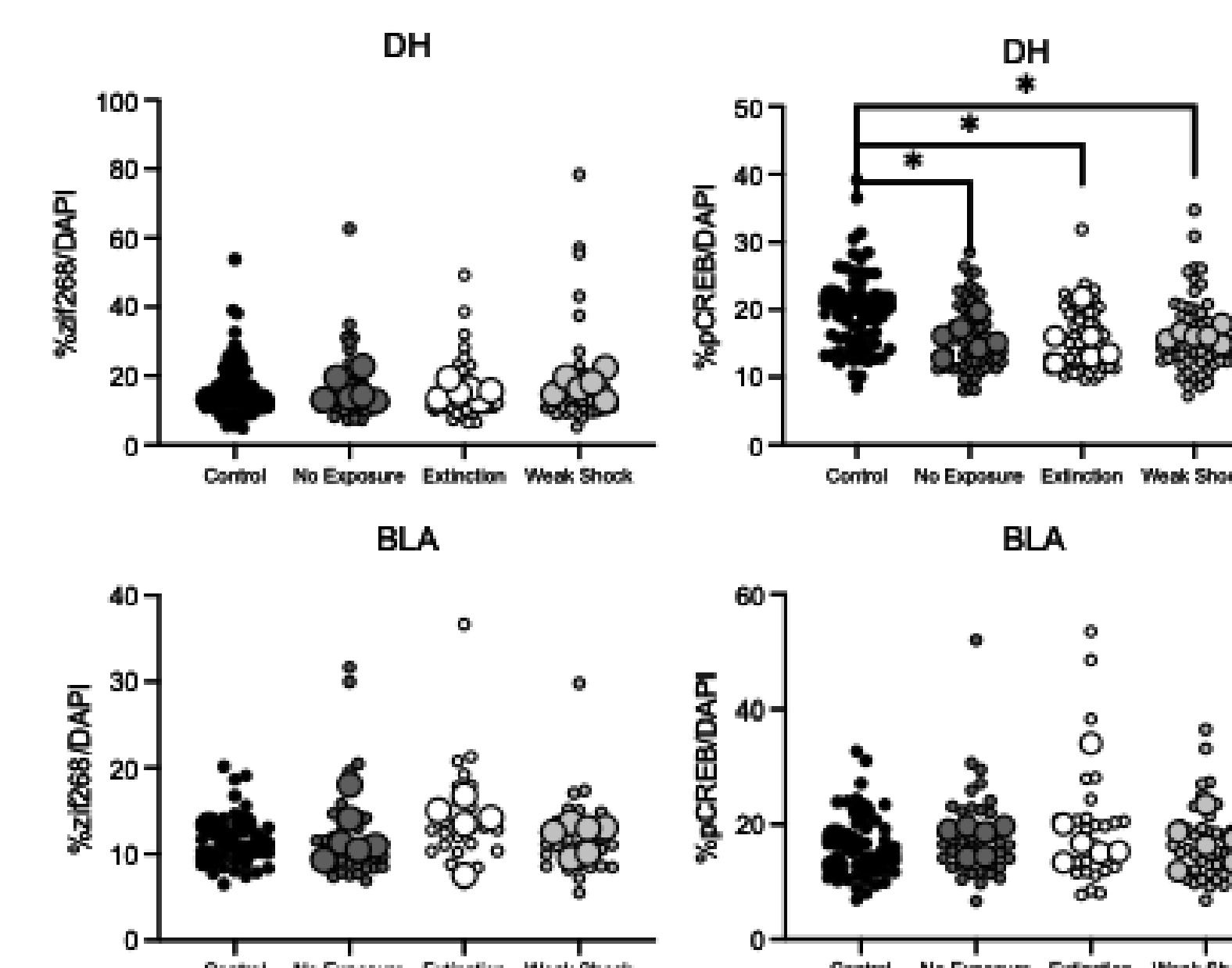
**Reinstatement.** There were no group differences following reinstatement of DFC.



**AAB Renewal.** The weak shock resulted in reduced freezing to the CS in an AAB renewal paradigm.



**Immunofluorescence.** There were no differences of zif268 or pCREB expression between experimental groups in any regions.



## Conclusions

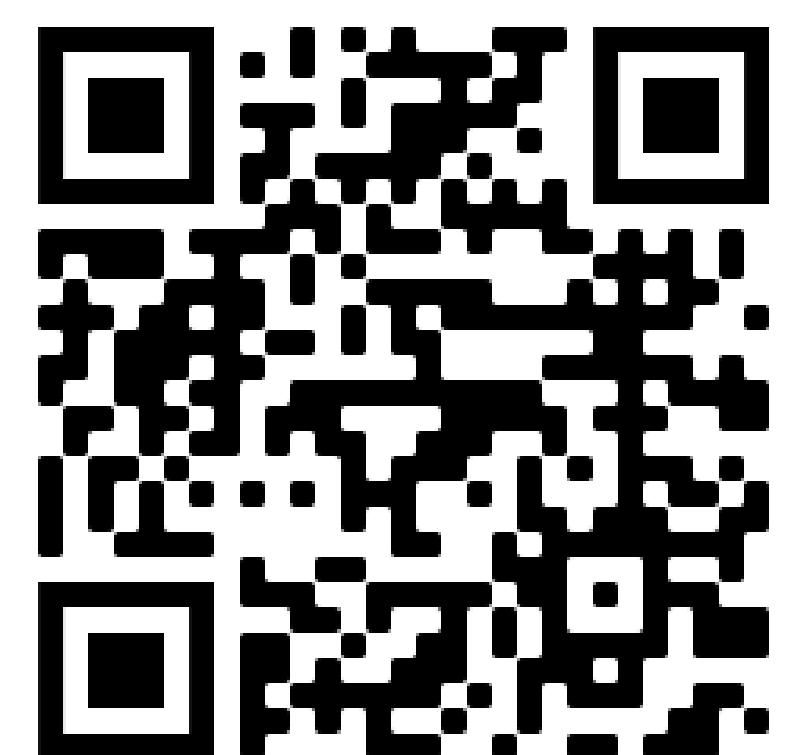
Weak shock effectively reduced delay fear, but this seemed to dissipate after four weeks at a long-term test.

Contrary to our hypothesis, weak shock did not reduce ABA renewal or reinstatement.

Kennedy et al. (2024) determined that a contextual match between acquisition and weak shock is needed to observe relapse-preventing by the weak shock as introducing a novel context engages extinction-like processes through enhanced prediction error. We therefore investigated the efficacy of the weak shock following AAB renewal. Here, the weak shock, but not extinction, was able to prevent a renewal of delay fear in context B. This suggests that the weak shock may prevent relapse effects in a context-dependent manner.

There were no differences in zif268 expression between any groups following DFC. Expression of pCREB was only different between groups in the DH, where all experimental groups had decreased expression relative to naive controls. It is possible that our 10-CS test elicits extinction-like processes, washing out neural effects (as was observed in Trask et al., 2025).

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