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Research article

Understanding the contributions of visual stimuli to contextual fear conditioning: A proof-of-concept study using LCD screens

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HIGHLIGHTS

- Features of a context may differentially contribute to the CS-US representation in contextual fear conditioning.
- Visual information from an environment may statistically contribute to contextual fear learning.
- LCD monitors can be readily incorporated to current fear conditioning protocols in order to precisely control visual stimuli of contexts.

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ABSTRACT

The precise contribution of visual information to contextual fear learning and discrimination has remained elusive. To better understand this contribution, we coupled the context pre-exposure facilitation effect (CPFE) fear conditioning paradigm with presentations of distinct visual scenes displayed on 4 LCD screens surrounding a conditioning chamber. Adult male Long-Evans rats received non-reinforced context pre-exposure on Day 1, an immediate 1.5 mA foot shock on Day 2, and a non-reinforced context test on Day 3. Rats were pre-exposed to either digital Context (dCtx) A, dCtx B, a distinct Ctx C, or no context on Day 1. Digital context A and B were identical except for the visual image displayed on the LCD screens. Immediate shock and retention testing occurred in dCtx A. Rats pre-exposed dCtx A showed the CPFE with significantly higher levels of freezing compared to controls. Rats pre-exposed to Context B failed to show the CPFE, with freezing that did not highly differ from controls. These results suggest that visual information contributes to contextual fear learning and that visual components of the context can be manipulated via LCD screens. Our approach offers a simple modification to contextual fear conditioning paradigms whereby the visual features of a context can be manipulated to better understand the factors that contribute to contextual fear discrimination and generalization.

1. Introduction

In fear conditioning, manipulating contextual cues is important for elucidating how learning is either "context-specific" or "context-independent" given that distinct, although possibly overlapping [6,29], neural systems support different types of conditioned fear [13,20]. "Context," in fear conditioning, is defined as the multimodal sensory experience, including temporal and spatial factors, encountered concurrently during a conditioning trial (for reviews see [17,31,36]; the context is usually the conditioning chamber, an enclosed box with visual, tactile, olfactory, and auditory cues that are incidentally encountered and associated with an aversive stimulus (e.g., foot shock). Following conditioning, rodents typically exhibit freezing (a species-specific defensive reaction; [2,3]) when placed back into the context where the foot shock occurred, but not in a novel context, suggesting that the defensive reaction is context specific. However, the features that make one context distinct from another are unclear. Typically, researchers manipulate multiple sensory features (e.g., odors, lighting, spatial layout, etc.) of a context to make one context distinct from another. Yet, our understanding of how the independent sensory features of a context differentially contribute to the CS-US representation (i.e.,

Abbreviations: CPFE, context pre-exposure facilitation effect; LCD, liquid-crystal display; dCtx, digital context; AC, alternating current; CS, conditional stimulus; US, unconditional stimulus; Pre, pre-exposure; No Pre, no pre-exposure; ANOVA, analysis of variance.

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is one feature more prominent than another?) or what specific elements are most important in distinguishing between contexts is limited [10].

Rodents are able to utilize visual information presented on LCD screens to perform a variety of behavioral tasks such as navigating a virtual maze or making appropriate behavioral choices [21,38,39]. Additionally, presenting looming or sweeping visual stimuli on a ceiling-mounted LCD screen – to simulate predatory threats – can control rodent flight or freezing behavior [8]. Incorporating LCD monitors into contextual fear conditioning paradigms may offer a means to systematically control visual elements of a context during aversive learning.

We used a variant of contextual fear conditioning known as the context pre-exposure facilitation effect (CPFE) paradigm. The CPFE paradigm separates incidental contextual learning from context-shock associative learning. The CPFE relies on the immediate shock deficit – a phenomenon where animals that are not given enough time to learn about the context prior to receiving a shock (e.g., <10-s [9]) fail to exhibit conditioned freezing [5,11]. However, pre-exposure to the conditioning context on the day prior to receiving an immediate shock is sufficient to overcome this deficit [11]. More importantly, the CPFE is only evident when context pre-exposure occurs to the training context and not a distinct context [33]. While the CPFE paradigm has been useful in understanding how gross incidental contextual learning is processed distinctly from fear-learning [23,24], the contribution of visual features to contextual learning remains unclear.

In the present study, we investigated how altering visual information in the CPFE affects contextual fear-learning. We restricted changes in visual features to incidental contextual learning during context pre-exposure. We placed four LCD monitors around a clear chamber (three on the sides and one on top); each displayed one of two images on all screens during the pre-exposure phase, with all other context features (i.e., tactile, spatial, olfactory, and auditory components) held constant between the two groups. During immediate-shock training and testing, only one of the two visual scenes was displayed on the monitors. We hypothesized that rats pre-exposed to the testing context would show the CPFE whereas those pre-exposed to the alternate visual context would not. We also included two control groups as CS and US associative-learning controls, one of which was pre-exposed to a distinct context (different visual, spatial, auditory, and olfactory components) and one that received no pre-exposure.

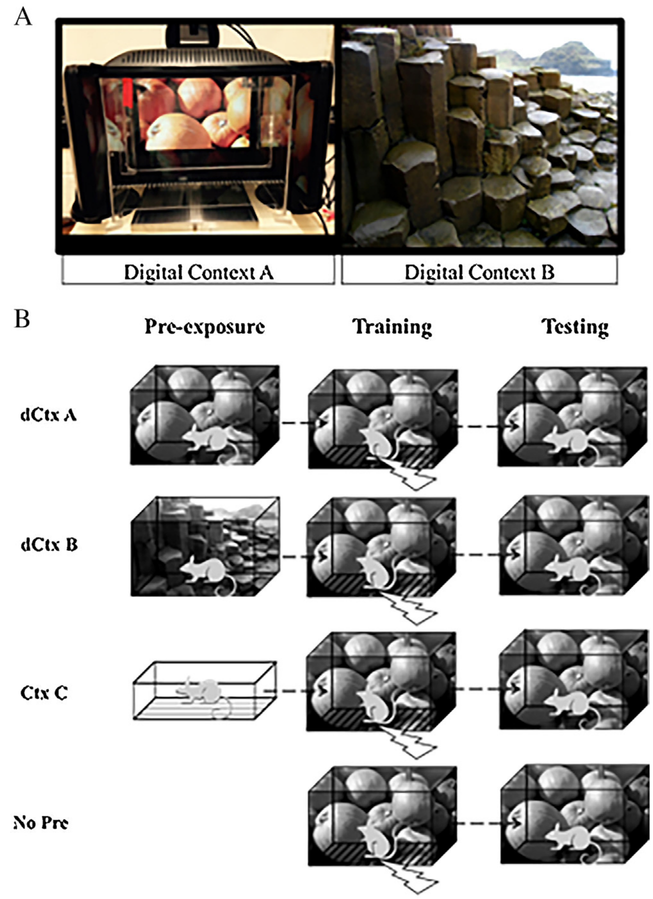

Fig. 1. Visual Images and CPFE Behavioral Design. (A) Image of the conditioning apparatus with LCD screens and the visual images used for digital context (dCtx) A (left panel) and dCtx B (right panel). dCtx A and dCtx B were identical except for either pumpkins or a rock formation displayed on the LCD screens. Ctx C was distinct with regard to spatial, tactile, visual, olfactory, and auditory characteristics. No Pre animals were not pre-exposed to any context.
(B) The CPFE fear conditioning experiments were run over three days. On day 1, rats were pre-exposed to dCtx A, dCtx B, Ctx C, or remained in their home cages (No Pre). On day 2, all groups were given immediate shock training in dCtx A. On day 3, all groups were tested for conditioned freezing in dCtx A.

2. Methods

2.1. Subjects

Forty adult male Long Evans rats 8–9 weeks of age were used. Thirty-two were purchased from Harlan breeders (Indianapolis, IN) and eight were bred in house (University of Delaware). All rats were housed in the animal colony at the University of Delaware. Pairs of rats were housed in opaque polypropylene cages (45 × 24 × 21 cm) with standard bedding and free access to food and water. Rats were maintained on a 12:12 h light/dark cycle with lights on at 7:00a.m. Testing occurred during the light phase (12:00P.M.–5:00P.M.). All animals were treated in accordance with NIH guidelines for the care and use of laboratory animals.

2.2. Apparatus

The conditioning chamber was made out of clear Plexiglas (40 × 22 × 24 cm); one of the four walls could be opened to allow for animal placement. The chamber floor consisted of 40 grid bars (0.4 cm in diameter) that ran parallel to the shorter wall of the chamber. The grid bars were connected to a shock generator (Med Associates; ENV 410B) that delivered an alternating current foot shock US. Four LCD monitors (Dell, Plano, TX) were placed flush to three of the external walls of the chamber with one monitor acted as the ceiling. All monitors projected the same image concurrently and were connected to a Dell computer. The monitors projected one of two images (found on an internet search without copyright attribution; Fig. 1A). The first image consisted of multiple pumpkins with dominant ovoid contours and light and dark coloring throughout. The second image consisted of rock formations with dominant linear contours featuring darker coloring toward one side and lighter coloring towards the other side. The lighting levels between the two images were adjusted via the LCD monitors so that their total luminance (~200–225 lx) was equivalent. Image presentation was controlled by custom software (available at: https://sites.google.com/site/aaasok/programmed-software). The conditioning chamber was cleaned with a 70% ethanol solution prior to animal placement. When the monitors projected the pumpkin image, the chamber was determined to be in the Context A configuration; projection of the rock formation produced the Context B configuration. With the exception of the projected images, there were no other differences between Context A and B.