Cued Fear Conditioning: Minimizing 'Contextual Leftover'-Freezing by Maximizing Changes of Context

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Introduction

Rodent fear conditioning is a relatively simple, cognitive-based paradigm extensively used to study the neurobiological mechanisms underpinning associative and emotional learning and memory. In the cued fear conditioning, animals learn to predict aversive events by associating a neutral stimulus (Conditional stimulus, CS: white noise) with an aversive stimulus (Unconditional stimulus, US: electrical foot shock). This results in the expression of fear responses when later re-exposed to the original neutral stimulus in a different context (recall). The dependent measure used as a read-out for memory function is a characteristic freezing response to the CS in rodents. This response is defined as the absence of any movement, except that required for breathing.

Ideally, rodents exposed to such novel context would not display a freezing response in the absence of the noise (CS). However, in our original setup, a certain amount of freezing was still observed prior to exposure to the noise during the recall session, which indicated that the context differences were not optimal. This 'contextual leftover'-freezing possibly could compromise drug effects because the window between baseline and CS-response becomes smaller.

The objective of this study was to eliminate factors contributing to this 'contextual leftover'-freezing response during recall.

Methods

Subjects

Male C57Bl/6 mice (22-35 gram at test) were obtained from Janvier (France). Mice were single housed on arrival and allowed 5 days of acclimatization before starting the experiment. The Institutional Ethical Committee on Animal Experimentation approved the experimental protocols, in compliance with Belgian law (Royal Decree on the protection of laboratory animals dd. April 6, 2010) and the facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Apparatus

Fear conditioning studies were performed in mouse modular test chambers (MED Associates, USA) placed in ventilated, illuminated cabinets. The modular test chamber was equipped with a speaker controlled by a white noise amplifier. Each chamber was equipped with a top-view video camera.

Conditioning. A white PVC test cubicle (13×13×13cm, Context A) was placed in the center of the test chambers. The foot shock was delivered through stainless steel floor grids which were calibrated for each cage to ensure appropriate shock intensity. All programming, timing, and shock presentations were computer controlled (Med-PC IV software). Freezing responses were determined using an automated video acquisition based system (Activity detection module, EthoVision XT9®, Noldus, The Netherlands).

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Recall. For the auditory CS test, contextual cues were changed. In the standard setup (Context B) the aluminum wall panels, door and back panel, were replaced by black and white striped PVC panels. The grid floor was covered with an opaque grey plastic plate and 1 ml of an aromatic substance (vanilla) was added to the waste pan. It appeared that these changes to the context were insufficient (figure 1): the test box cover was identical, the aluminum columns between the wall plates were still visible, the white PVC-plate on the bottom didn't hide the grid floor completely, and the box shape and illumination remained the same.

Criteria for the new context development therefore where: different shape and color, different lighting, absence of odor usage and optimal environmental conditions for automated video analysis.

In the modified context C, the grid floor was removed and a new insert (grey PVC cylinder, Ø 12 cm, H=13 cm) was placed on top of a light box made of black infra-red transparent PVC and equipped with near infra-red LED's. Four visual light LED's were integrated in the cylinder. The insert was covered by an infra-red transparent lid.

Procedure

On the conditioning day, mice were placed in the test chamber (context A) and after 2 minutes of free exploration, a 75 dB white noise serving as the conditioned stimulus (CS) was presented for 30 sec. Then a foot shock (0.70 mA, 1 sec) was applied, which served as the unconditioned stimulus (US). This presentation of CS/US pairings was repeated at, 240 and 360 seconds after the start of the conditioning phase. The mouse was removed 90 seconds after the last pairing and returned to its home cage.

Twenty-four hours later, each mouse was returned to the test chamber in which the environmental and contextual cues were changed to context B or C. The mice were placed in the box and freezing behaviour was determined for 2 minutes in the absence of the auditory CS.

Results

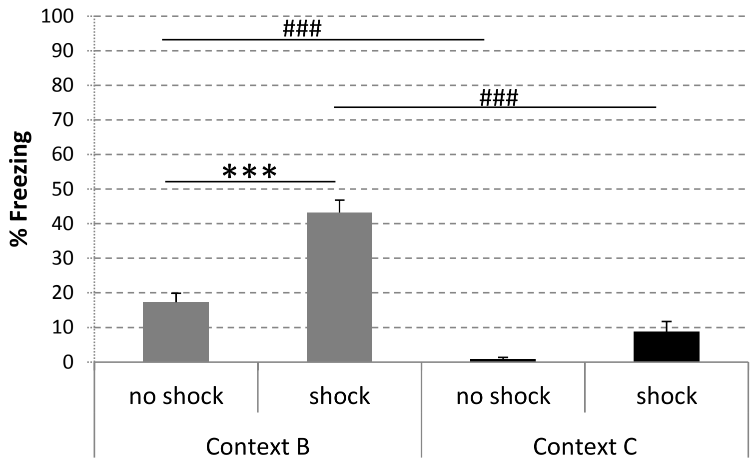

Figure 1. Percent of time freezing ± SEM during the first 2 min (pre-cue) in a novel context at recall. Context B: n=8 and Context C: n=12 per group (see text for details).

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One-way ANOVA with context as a factor showed a significant difference between contexts ($P < 0.0001$).

The effect of context was shock-dependent (2-way ANOVA, context x shock: $P=0.0012$). Shocked mice in the modified context C showed lower % freezing than those in the standard context B ($P<0.0001$ – Bonferonni-adjusted).

Discussion

In our standard setup (context B), we observed a substantial amount of freezing during the baseline period prior to exposure to the tone during recall. We hypothesized that this 'contextual leftover'-freezing could possibly compromise compound effects because of a smaller window between freezing elicited by the CS vs. baseline.

The effort to enlarge the difference between the conditioning context and the test context resulted in a much smaller 'contextual leftover'-freezing and a larger difference compared to the CS induced freezing. It also resulted in an improved environment for automated video analysis using EthoVision XT9®, in terms of image contrast, reflection, illumination, etc...

In our opinion, it will be very hard to further decrease the 'contextual leftover'-freezing, because the influence of other factors, such as the experimenter, handling, lab environmental conditions, is very difficult or even impossible to exclude.