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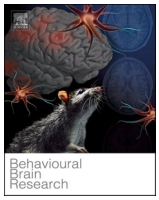

Research report

Some key parameters in contextual fear conditioning and extinction in adult rats

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ABSTRACT

Contextual fear conditioning is a behavioral paradigm used to assess hippocampal-dependent memory in experimental animals. Perception of the context depends on activation of a distinct population of neurons in the hippocampus and in hippocampal-related areas that process discrete aspects of context perception. In the absence of any putatively associated cue, the context becomes the salient element that may warn of an upcoming aversive event; and in particular conditions, animals generalize this warning to any new or similar context. In this study we evaluated the effects of the number of sessions, the number of unconditioned stimuli per acquisition session and the distribution of extinction sessions to assess fear acquisition and extinction and determine under which conditions generalization occurred in adult, male rats. We observed that the organization and spacing of sessions were relevant factors in the acquisition and extinction of contextual fear memories. Extinction occurred with significantly greater robustness when sessions were spread over two days. Furthermore, results indicated that exposure to a single 0.3 mA, 0.5 s footshock in two different sessions could produce context-specific fear, while more acquisition sessions or more footshocks within a single session produced a generalization of the fear response to a new context. Notably, when generalization occurred, successive re-exposure to the generalized context produced extinction in a similar way to the paired exposure. Together, the present findings identify clear procedural and behavioral parameters amenable to neural systems analysis of three clinically relevant outcomes of contextual fear conditioning, i.e., memory acquisition, storage and extinction.

1. Introduction

Context can be defined as a multisensory and diverse backdrop, and is considered stable, despite fluctuations in discrete elements (like objects, sounds and smells) [1]. Context fulfils a psychological function; it is essential for abstracting situation-informed meaning from the world. Furthermore, it assigns contingencies, spatial locations, necessary conditions and unusual circumstances to salient cues and memory traces [2].

The distinction between harmful, neutral, and beneficial stimuli allows organisms to adapt their behavior to environmental conditions [3]. In humans, the inability to adequately contextualize information can lead to various symptomatology [2,4–6], which is seen in multiple psychiatric disorders, such as schizophrenia, posttraumatic stress disorder (PTSD), depression, generalized anxiety disease (GAD) and drug addiction [2,7,8]. These anxiety, stress and trauma disorders are among the most common and debilitating mental illnesses [9–11]. Among these disorders, PTSD is likely the most representative pathology of contextual processing [2].

In many of these anxiety disorders, patients tend to overestimate the possibility of potential threats in their environment and involuntarily recall a traumatic memory triggered by a neutral environment that is

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similar to the environment in which the traumatic event occurred [12]. This is thought to be due to a process known as fear generalization, in which a fear response to a specific stimulus is extended to other similar stimuli. Thus, after an individual has learned to fear or feel anxious about a particular stimulus, that fear response may manifest to stimuli that share similar characteristics with the original stimulus [13,14]. This phenomenon can be useful in certain contexts, as it allows animals and humans to generalize cautious responses to potentially threatening stimuli, even if they are slightly different to the stimulus that originally triggered fear. However, excessive fear generalization in humans can lead to problems of irrational anxiety and phobias [15].

Contextual fear conditioning, (CxFC), a variation of classical Pavlovian conditioning is widely used to investigate the mechanisms of fear and its generalization, as the neural circuitry underlying these processes is similar in humans and rodents [16]. In a typical context conditioning protocol, a conditioned neutral stimulus (CS; a contextual setting) is paired with an unconditioned stimulus (US; footshock) eliciting a conditioned fear response (CR). In rats, the most commonly employed fear response is freezing, characterized by the total absence of movement except that necessary for breathing. Then, there is a gradual decrease in the magnitude and/or frequency of the CR after repeated exposure of the CS without the US. This process is known as extinction [17]. Extinction is not eradication of the memory, but rather a new learning that the CS no longer predicts the US [18–20].

One of the prominent therapeutic modalities in the field of clinical psychology, aimed at addressing anxiety and fear disorders, is re-exposure therapy [21–24]. This therapy is based on the behavioral principle of fear extinction [21]. A consistent problem in these therapies is the generalization of the original fear memory to neutral contexts/situations. Generalization may compromise therapeutic outcomes by manifesting itself in a wide range of situations, and consequently, limit the effectiveness of therapeutic exposure and fear extinction in long-term recovery [13,25]. Therefore, understanding the mechanisms underlying generalization is essential to optimize therapeutic interventions and mitigate the recurrence of symptoms. This requires a behavioral model that allows a straightforward assessment of fear conditioning to context and its generalization.

In this study we examined different models of contextual fear conditioning to test whether the intensity of the protocol used influenced fear extinction and generalization. Our results provide a new understanding of the generalization of short-term fear and should facilitate future research on the psychopathology of anxiety-related disorders.

2. Materials and methods

2.1. Animals

Adult Wistar rats were used (n = 70, 2–3 months-old, 200–512 g). Rats were housed under a 12:12 h light–dark cycle (2–3 male rats per cage) with access to food and water ad libitum. All studies were conducted during the light phase. All experimental procedures were approved by the Animal Welfare Ethics Committee of the Universitat Jaume I, Castellón (Spain) and developed in accordance with the European Community Council Directive (86/609/EEC; 2010/63/EU), Spanish directive BOE 34/11370/2013, and local directive DOGV 26/2010.

2.2. Apparatus

The conditioning apparatus used consisted of two fear chambers (28 × 21 × 26 cm; Model 80015, Lafayette Instruments, Lafayette, IN, USA) with an opening in the ceiling where a camera was placed to record behavior. The chambers were equipped with a stainless-steel, shock-delivery grid floor (0.9 cm inter-bar separation) and two dim lights. The chamber could be transformed into multiple contextual configurations, alternating between walls with a plastic-coated pattern of black and white vertical lines, clear plexiglass door and stainless-steel floor bars (context A), and walls with a plasticized pattern of colored figures, transparent plexiglass door and smooth plasticized floor (context B). Odors also differed between contexts, with green forest scented cleaner in context A and rose scented cleaner in context B (see Table 1).

The equipment was calibrated to deliver discharges with an amplitude of 0.3 mA and a duration of 0.5 s (Lafayette Instruments). The timing of shock administration was established prior to the behavioral sessions and was automatically administered through a shocker controlled by an Arduino card (Uno R3, Ivrea, Italy) using customized software (HackCS, Castellón, Spain). The apparatus was cleaned before and after each rat with 30% ethanol.

2.3. Behavioral protocols

Context A consisted of black and white vertical line visual cues, with a floor of stainless metal bars and a forest-scented cleaner as an olfactory cue. Context B was in the same experimental room, but the visual cues were replaced by walls with geometric figures, a black laminated sheet was placed on the floor to modify its texture and a rose-scented cleaner was added as an olfactory cue.

One day before commencing the behavioral tests, rats were handled for 10 min during the light cycle. Rats underwent training sessions the day after handling. Testing began 2 h after turning on the lights in the vivarium. Rats were transported to the conditioning room in their home cages and were returned to the vivarium immediately afterwards. Contextual adjustments were counterbalanced between rats.

2.3.1. Experiment 1. Fear conditioning extinction testing

Initially, we planned to assess acquisition and extinction by testing extinction in different day sessions vs extinction across different sessions on a single day (see Fig. 1A, B).

For extinction across two days (n = 13), the process was divided into 6 sessions of 10 min (2 acquisition and 4 extinction sessions) over 3 days.

Day 1 - Acquisition. In the first session, rats received an electric footshock after 4 min in the conditioning box. In the second session, 4 h later, the footshock was applied after 6 min in the conditioning boxes.

Days 2 and 3 - Extinction. On day 2, rats were returned to the conditioning box for extinction sessions 1 and 2, the delay between the two sessions was 4 h. During these sessions, rats were placed in the boxes with the same context, but without footshock. On day 3, the same procedure of day 2 was repeated for extinction sessions 3 and 4, with a 4 h delay between them.

For extinction within a day (n = 9), conditioning was divided into 7 sessions of 10 min (2 acquisition and 5 extinction sessions) over 3 days. All the sessions were conducted 4 h apart.

Day 1 - Acquisition. In the first session, rats received an electric footshock after 4 min in the conditioning box. In the second session, four h later, the footshock was applied after 6 min in the conditioning boxes.

Day 2 and 3 - Extinction. On day 2, rats were returned to the conditioning box for extinction sessions 1 to 4, 1 h apart, during which rats were placed in the boxes with the same context, but without receiving any footshock. On day 3, rats were returned to the conditioning box for the fifth extinction session.

2.3.2. Experiment 2. Fear conditioning acquisition testing

In this experiment, we aimed to determine which of the proposed

Table 1
Description of the two contexts apparatus.

	Context A	Context B
Odor	Forest scented cleaner	Rose scented cleaner
Wall Visual Cues	Black and white vertical lines	Colorful geometrical shapes
Floor	Stainless steel bars	Smooth plasticized

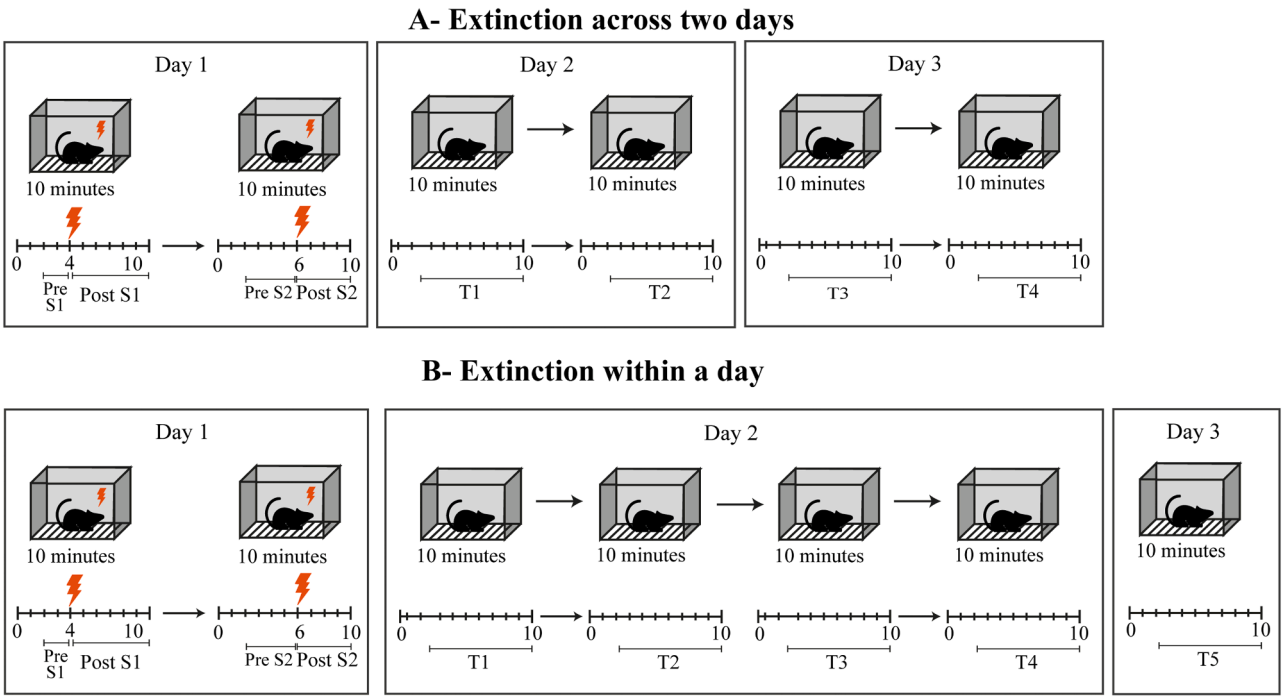


Fig. 1. Behavioral testing protocol used in Experiment 1. (A) Protocol of extinction across two days, rats received two acquisition trials on the same day 4 h apart, and the extinction in four trials during two days with two trials per day 4 h apart. (B) Protocol of extinction in a single day. Rats received two acquisition trials on the same day 4 h apart. The extinction was in four trials in a single day 1 h apart. An additional single extinction trial was tested next day. PreS1: time lapse before first shock; PostS1: time lapse after the first shock; PreS2: time before the first shock; PostS2: time after the second shock; T1: first extinction test; T2: second extinction test; T3: third extinction test; T4: fourth extinction test; T5: fifth extinction test.

conditioning protocols produced a more consistent pattern of fear conditioning acquisition without affecting fear generalization. In the three behavioral tests, the acquisition of the conditioning was performed in one context (conditioning context), while the extinction tests were completed either in the same context as the acquisition (conditioning context) or in another context (novel context). We performed protocols to study the effect of the number of footshocks per session and of the number of acquisition sessions (Fig. 2A).

Rats were divided into groups for use in three different protocols involving weak (n = 16), mild (n = 16) and strong conditioning (n = 16). The weak protocol was the same as used in Experiment 1 "extinction-across-two-days".

Day 1 - Acquisition. In the first session, rats received an electric footshock after 4 min in the conditioning box. In the second session, 4 h later, the footshock was applied after 6 min in the conditioning boxes.

Days 2 and 3 - Extinction. On day 2, rats were returned to the conditioning room for extinction sessions 1 and, four h later, session 2, in which rats were placed in the conditioning boxes, but without footshock. Half of the rats were placed within the conditioning context and the other half within the novel context. On day 3, the same procedure of day 2 was repeated for extinction sessions 3 and 4.

We contrasted the previous protocol with one in which the acquisition consisted of the same number of sessions, but with two footshocks per session (mild conditioning protocol; Fig. 2B)).

Day 1 - Acquisition. In the first session, rats received a footshock at 4 and 6 min in the conditioning box. In the second session, conducted 4 h later, the footshocks were applied at 3 and 7 min

Days 2 and 3 - Extinction. On day 2, rats were returned to the conditioning room for extinction sessions 1 and, four h later, session 2, in which the rats were placed in the conditioning boxes, but without footshock. Half of the rats were placed in the conditioning context and the other half in the novel context. On day 3, the same procedure as day 2 was repeated for extinction sessions 3 and 4.

To test the effect of the number of acquisition sessions, we added two acquisition sessions each containing a footshock (strong conditioning protocol; Fig. 2C). This protocol was completed over four days, and each day consisted of two sessions. All sessions in a single day were conducted 4 h apart.

Acquisition ran on days 1 and 2. In the first session, rats received a footshock after 4 min. In the second session, four h later, the footshock was applied at 6 min. The following day, the same process was repeated, changing the timing of the footshocks, in the first session of the day (session 3) the rats received the footshock at 3 min. In the second session (session 4) the footshock was applied at 7 min

Extinction was conducted on days 3 and 4 by exposing rats to the different contexts without footshocks. On day 3, rats were returned to the conditioning room for extinction sessions 1 and, 4 h later, session 2, in which rats were placed in the conditioning boxes, but without footshock. Half of the rats were placed in the conditioning context and the other half in the novel context. On day 4, the same procedure as day 3 was repeated for extinction sessions 3 and 4.

2.3.3. *Data analyses*

Videos obtained in each session were analyzed in their entirety and divided into 2 min blocks using Any-Maze 7 software (Stoelting, Kiel, WI, USA). Freezing was automatically detected by the system and represented as a percentage of total time analyzed, and freezing was registered when the period of absolute immobility and curved position was > 2 s. Measures obtained were plotted as percentages in a database of the statistical program GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA). Normality Shapiro-Wilk and Kolmogorov-Smirnov tests were applied to assess the normal distribution of data in each sample. Two-way ANOVAs were performed, followed by Bonferroni and Tukey post-hoc analyses. The α value was set at 0.05 for all analyses. A context discrimination ratio was calculated as $(Freezing_{Context\ A} - Freezing_{Context\ B}) / (Freezing_{Context\ A} + Freezing_{Context\ B})$.

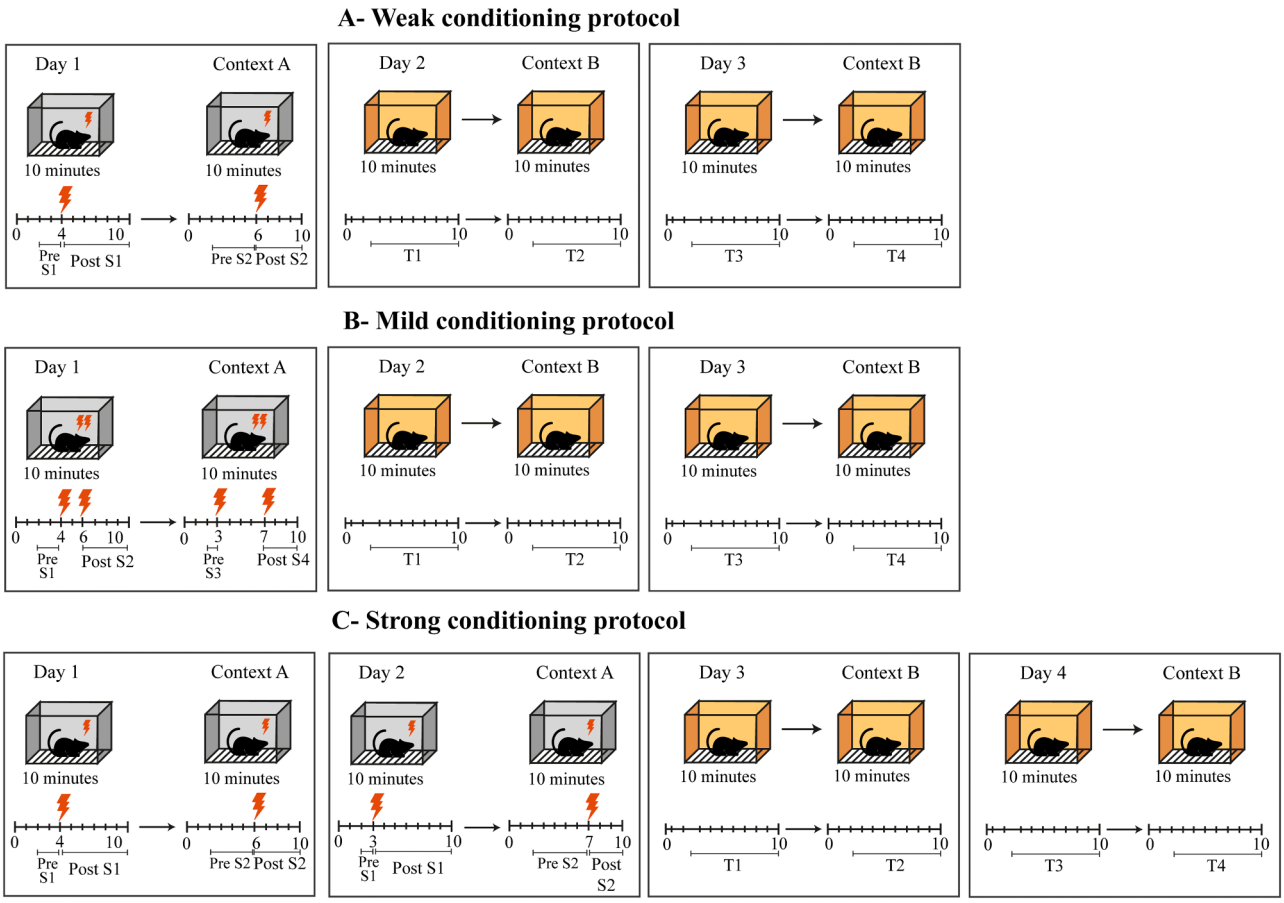
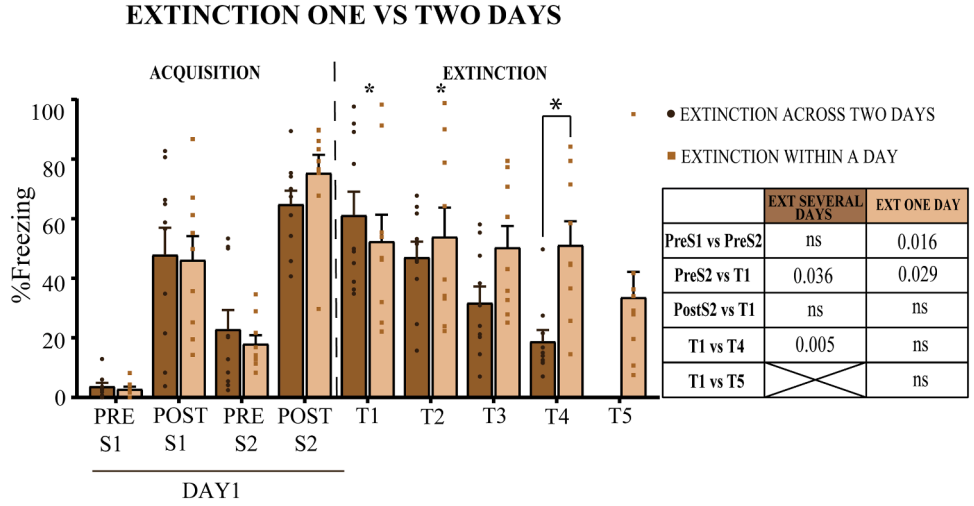


Fig. 2. Behavioral testing protocols used in Experiment 2. A. Regular protocol of extinction across two days, rats received two acquisition trials on the same day 4 h apart. Extinction was conducted in four trials during two days with two trials per day 4 h apart. B. Mild conditioning protocol in which rats received two footshocks in each of the two acquisition trials on the same day, 4 h apart. Extinction was conducted in four trials during two days in two trials per day 4 h apart. C. Strong conditioning protocol in which rats received four acquisition trials over two days, 4 h apart for acquisition trials the same day. Extinction was conducted in four trials during two days with two trials per day 4 h apart. PreS1: time previous to first shock; PostS1: time after the first shock; PreS2: time before the first shock; PostS2: time after the second shock; PreS3: time prior to third shock; PostS4: time after fourth shock; T1: first extinction test; T2: second extinction test; T3: third extinction test; T4: fourth extinction test.



	EXT SEVERAL DAYS	EXT ONE DAY
PreS1 vs PreS2	ns	0.016
PreS2 vs T1	0.036	0.029
PostS2 vs T1	ns	ns
T1 vs T4	0.005	ns
T1 vs T5		ns

Fig. 3. Comparison of freezing levels observed during the "extinction-across-two-days" and "extinction-within-a-day" protocols. The table documents the intra-group comparisons in each conditioning session. Error bars indicate standard error of the mean (SEM). * $p < 0.05$; ns, non-significant. PreS1: time lapse before the first shock; PostS1: time after the first shock; PreS2: time before the first shock; PostS2: time after the second shock; T1: first extinction test; T2: second extinction test; T3: third extinction test; T4: fourth extinction test; T5: fifth extinction test.

3. Results

3.1. Experiment 1. Extinction requires consolidation

Both groups passed the normality tests (Shapiro-Wilk test, extinction across two days, $p = 0.85$; extinction within a day, $p = 0.374$; and Kolmogorov-Smirnov test, $p > 0.1$ for both groups).

The main effect of the protocol was not significant $F(1, 19) = 1.260$, $p = 0.276$. Post-hoc Bonferroni test analysis indicated that in the last extinction session (T4) rats subjected to the extinction across two days protocol displayed significantly less freezing than rats subjected to the extinction within a day protocol ($p = 0.029$). No other comparisons between protocols were significant (Fig. 3).

We compared the different groups using two-way repeated measures ANOVA with one group variable (protocol used) and one repeated measure (conditioning sessions). The main effect observed was associated with the conditioning session, $F(7, 133) = 36.50$, $p < 0.001$, and the two-way interaction, conditioning sessions × protocol, $F(7, 133) = 4.286$, $p < 0.001$.

Acquisition of conditioned responses was examined by measuring freezing in sessions Pre S1, Pre S2, Post S2 and T1. On day 1 in the first conditioning session, rats in both groups displayed exploratory movements for most of the session before the footshock (Pre S1). On the same day, in the second conditioning session, rats still displayed little freezing less than 30% freezing during the 6 min prior to the footshock; (Pre S2), but did display freezing after the footshock (Post S2). Extinction was tested on days 2 and 3, in sessions T1–T4. No extinction took place in the "extinction-in-one-day" protocol (Fig. 3). A Tukey post-hoc test indicated that in the extinction-across-two-days protocol there was a similar amount of freezing prior to the first and second footshock (Pre S1 and Pre S2, $p = 0.169$). Before the footshock during the second conditioning session (Pre S2) rats froze for significantly less time than during the first extinction session (T1; $p = 0.036$), reflecting the acquisition of fear conditioning. Lastly, rats exhibited significantly less freezing during the last extinction session (T4) than during T1 ($p = 0.005$), reflecting the extinction of conditioned fear (see Table 1 in Fig. 3).

Examination of the extinction-in-one-day protocol, revealed that rats displayed significantly higher freezing levels during Pre S2 than during Pre S1 ($p = 0.016$; Fig. 3). A significant increase in freezing levels was also observed between the Pre S2 and T1 sessions ($p = 0.029$), indicating the acquisition of fear conditioning. No significant differences in freezing levels were observed between the T1 and T4 sessions in the extinction-in-one-day protocol, indicating that extinction of conditioned fear had not occurred in this group during a single day ($p > 0.999$). There were also no significant differences between T1 and T5 ($p = 0.405$; Fig. 3A). These data were in contrast with the extinction observed after the second day, during which a progressive and significant decrease in freezing occurred between sessions T1 and T4 (Fig. 3).

3.2. Experiment 2. Fear conditioning acquisition testing

All groups passed the Shapiro-Wilk and Kolmogorov-Smirnov normality tests (Shapiro-Wilk test: weak protocol, conditioning context $p = 0.983$, novel context $p = 0.383$; mild protocol: conditioning context $p = 0.337$, novel context $p = 0.699$; strong protocol: conditioning context $p = 0.165$, novel context $p = 0.0584$; and Kolmogorov-Smirnov test, $p > 0.100$ for all groups).

3.2.1. Weak conditioning protocol

We used two-way repeated measures ANOVA for between group comparisons, with one group variable (extinction in the conditioning or the novel context) and one repeated measure (conditioning sessions). The main effects of the conditioning sessions, $F(7, 98) = 27.22$, $p < 0.001$, and context group $F(1, 13) = 7.665$, $p = 0.016$, were all significant, as was the interaction - conditioning session × context group $F(7, 91) = 2.420$, $p = 0.026$. Post-hoc analysis with a Bonferroni test indicated that in the first extinction session (T1), rats in the novel context exhibited significantly less freezing than rats in the conditioning context ($p = 0.049$). The same pattern was observed in the second re-exposure session (T2, $p = 0.017$). No other comparisons between groups were significant (Fig. 4A).

Extinction was tested on days 2 and 3, in sessions T1-T4 (Fig. 4A), and the freezing displayed by rats in the conditioning context group decreased gradually after each re-exposure. A Bonferroni post-hoc test indicated that rats undertook significantly less freezing during the last extinction session (T4) than in T1 ($p = 0.0323$), which reflected an extinction of the contextual conditioned fear. In contrast, rats in the novel context group displayed significantly more freezing during Post S2 than during T1 ($p = 0.002$), which indicated an ability to discriminate the novel and non-hazardous context, freezing levels were the same throughout all extinction sessions, with no significant differences between T1 and T4 ($p > 0.99$; see Table 1 in Fig. 4A).

3.2.2. Mild conditioning protocol

In this protocol rats underwent two acquisition sessions on the same day and received two footshocks in each session (Fig. 2). We made between groups comparisons using two-way repeated measures ANOVA with one grouping variable (extinction in the conditioning context or novel context) and one repeated measure (conditioning sessions) and tested the freezing levels in both groups (same and different context) in each session. Only the main effects of the conditioning sessions, $F(7, 98) = 34.20$, $p < 0.001$ was significant. Neither the main effects of the context group $F(1, 15) = 1.756$, $p = 0.205$ nor the interaction conditioning session × context group effects $F(7, 105) = 0.6633$, $p = 0.703$ was significant. Rats in both groups displayed the same behavioral pattern in all sessions, as demonstrated by the lack of significant differences in the post-hoc analyses (Pre S1, $p = 0.773$; Post S2 and Pre S3, $p > 0.99$; Post S4, $p = 0.838$; T1, T2, T3 and T4, $p > 0.99$) (Fig. 4B). Thus, this protocol resulted in a generalization process in which the discriminative capacity to differentiate between the conditioning or non-conditioning contexts was impaired.

Acquisition of context fear conditioning was examined by measuring freezing in sessions Pre S1, Pre S3, Post S4 and T1. In Pre S1, rats displayed mainly exploratory-like behavior, after receiving the two footshocks in the first acquisition session, in Pre S3 rats exhibited higher freezing levels, but these did not reach statistical significance compared to Pre S1 ($p = 0.072$). In the conditioning context group, a decrease in freezing levels was observed after footshock between Post S2 and Post S4 sessions ($p = 0.015$), indicating a deteriorated response of rats to footshock with the addition of new US. However, relative to Pre S3, rats had statistically higher freezing levels during the first extinction session, T1 ($p = 0.008$), indicating the acquisition of fear conditioning. Moreover, during T1 rats displayed higher freezing levels than during Post S4 ($p = 0.004$). Lastly, during T4, rats displayed significantly less freezing than during T1 ($p = 0.031$), manifesting the extinction of conditioned fear. Freezing levels decreased after each extinction session, reaching statistical significance between T1 and T4 ($p = 0.021$), clearly reflecting a process of context-conditioned fear extinction.

3.2.3. Strong conditioning protocol

Rats in this protocol underwent a total of four acquisition sessions across two days, receiving a single footshock in each session (Fig. 2). Group comparisons were performed using two-way repeated measures ANOVA with one group variable (extinction in the conditioning context or novel context) and one repeated measure (conditioning sessions) and compared the freezing levels exhibited by both groups (in the same and different context) in each session. The main effect of the conditioning sessions, $F(7,98) = 44.90$, $p < 0.001$ was significant, whereas the main effect of the context group $F(1, 17) = 0.2355$, $p = 0.634$ and the interaction (conditioning session × context group) effects $F(11, 187) = 0.3796$, $p = 0.963$ were not significant. Rats in both groups displayed the same behavioral pattern in all sessions, as demonstrated by the lack

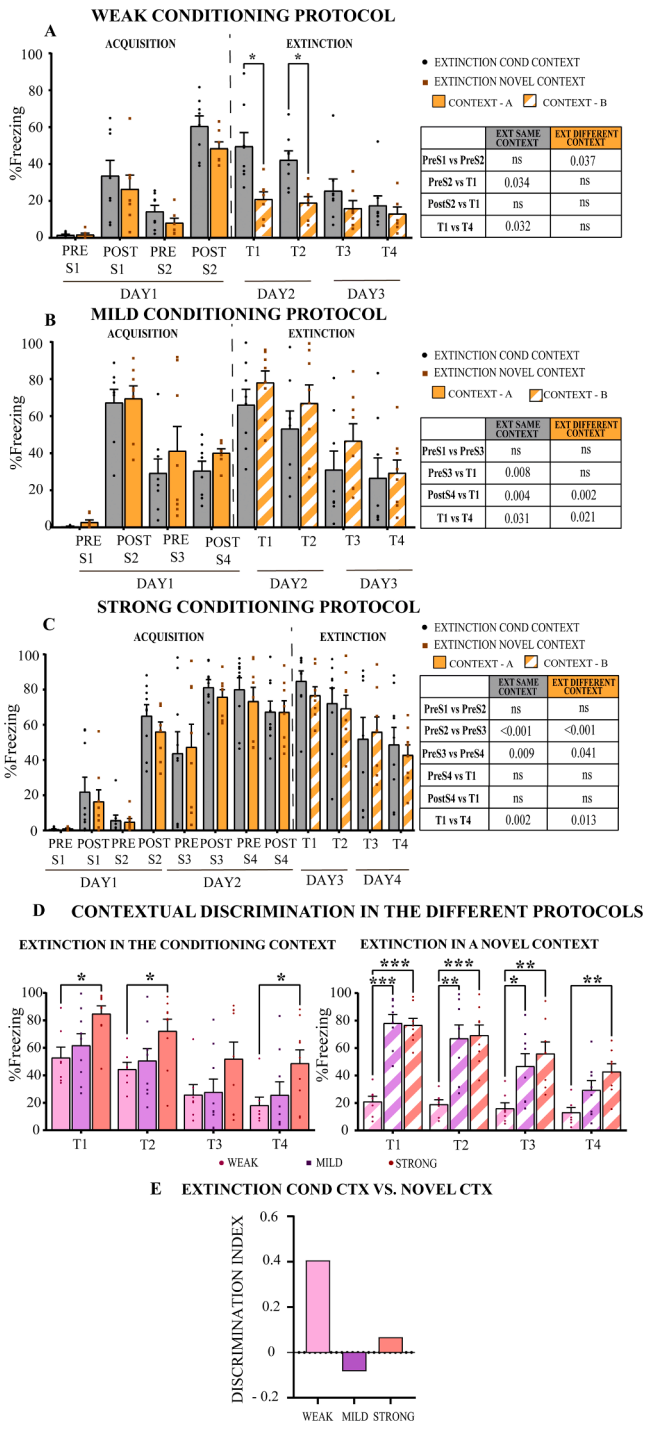


Fig. 4. Comparison of freezing levels in rats that underwent extinction in the conditioning context (Ext same context, plotted in grey) or a novel context (Ext different context, plotted in orange). (A) Protocol "one footshock - two acquisition sessions", (B) Protocol "two footshocks - two acquisition sessions", (C) Protocol "one footshock - four acquisition sessions", and (D) Analysis of the context discrimination in the different protocols described in Experiment 2. (E) Discrimination ratio between extinction in the conditioning context versus the novel context. Tables illustrate the within-group comparisons for each conditioning session. Error bars indicate standard error of the mean (SEM). * $P < 0.05$; ** $P < 0.01$ *** $P < 0.001$, ns, non-significant. PreS1: time previous to first shock; PostS1: time after the first shock; PreS2: time before the first shock; PostS2: time after the second shock; PreS3: time prior to third shock; PostS4: time after fourth shock; T1: first extinction test; T2: second extinction test; T3: third extinction test; T4: fourth extinction test.

of significant differences in post-hoc analyses ($p > 0.99$ for all comparisons; Fig. 4C).

Acquisition of context fear conditioning was examined by measuring freezing in sessions Pre S1, Pre S2, Pre S3, Pre S4, Post S4 and T1. A Bonferroni post-hoc test indicated that for both groups (conditioning and novel context), rats displayed a similar amount of freezing in Pre S1 and Pre S2 ($p > 0.99$ for both groups). After the two footshocks received in the two first acquisition sessions, in Pre S3, rats displayed significantly higher freezing levels than in Pre S2 ($p < 0.001$, for both groups). Moreover, freezing levels were augmented significantly after the third acquisition session, with significant differences between Pre S3 and Pre S4 (conditioning context, $p = 0.009$; novel context, $p = 0.041$). No significant differences were observed between Post S4 and T1 ($p > 0.99$ for both groups). Significant differences were observed in T1 and T4 in the conditioning context ($p = 0.002$) and in the novel context ($p = 0.013$); indicating that an extinction process was occurring in a similar way to that in generalized contexts (Fig. 4C) [26].

3.2.4. *Contextual discrimination in the different protocols*

We analyzed the effects of the different protocols on the discriminative process when rats were tested in the conditioning context or the novel context (Fig. 4D). Within the conditioning context group, post-hoc analyses indicated significant differences between the weak and strong protocols in extinction sessions T1, T2 and T4 ($p = 0.016$, $p = 0.04$ and $p = 0.047$, respectively), indicating the effect of increased conditioning sessions on freezing levels and the extinction pattern.

It is noteworthy, however, that in the novel context group, rats in the weak protocol displayed significantly less freezing than rats in the mild and strong protocols ($p < 0.001$, for both comparisons), indicating the ability of the weak protocol to produce context-specific fear conditioning. This same pattern was repeated in T2 (weak vs mild, $p = 0.004$ and weak vs strong, $p < 0.001$) and in T3 (weak vs mild, $p = 0.031$ and weak vs strong, $p < 0.005$). Finally, in T4, significant differences were only observed between the weak and strong protocols ($p = 0.003$).

The freezing discrimination index provides a measure of how the two groups of rats react to the different contexts. A positive value indicates that rats undertake more freezing when tested in the conditioning context, while a negative value indicates the opposite. A value close to 0 indicates that there is no substantial difference in freezing behavior between the two contexts. Rats in the weak protocol make a clear differentiation between contexts, with more freezing in the conditioning context (index value 0.4), while rats in the mild and strong conditioning protocols had values close to 0, indicating little differentiation between the two contexts and context fear generalization (Fig. 4E).

4. Discussion

The major goal of the present study was to develop a robust behavioral protocol for the study of the acquisition, retrieval, and extinction of contextual fear conditioning in rats. Therefore, we tested three acquisition protocols and two extinction protocols, and under our experimental conditions, the protocol that produced the best acquisition of context-specific fear conditioning *and* the best extinction was the "weak" or "extinction-across-two-days" protocol (Fig. 4). Furthermore, we demonstrated that an increase in the number of sessions and/or footshocks produced an increase in freezing levels, and a generalization to the original conditioning context.

We also observed that re-exposure to the aversive, conditioning context in an interrupted manner on the same day did not produce a reduction in freezing levels (Fig. 3). However, when the same four re-exposure sessions were performed on two separate days and separated by 4 h, a decrease in freezing extinction levels was observed. This effect may be the result of the participation of several neural systems. If contextual conditioning and extinction are at least in part analogous to cued conditioning and extinction, then acquisition and extinction depend on neural activity in the lateral amygdala, while retrieval of

extinction memories involves the infralimbic prefrontal cortex [27,28]. However, in contextual fear memory, the hippocampus has a central role in perception and the amygdala is central to the acquisition of the fear memory [29,30]. In contrast, it is unclear whether a particular anatomical structure stores extinction memories or whether it is the coherence between retrosplenial cortex, hippocampus and amygdala activity that supports this function [31–33]. Nonetheless, the reconsolidation of memories requires a time-restricted synthesis of new proteins in relevant brain circuits [34–36].

Additionally, the data obtained in our optimal "extinction-across-two-days" protocol, follow the Rescorla-Wagner learning model [26] (Fig. 5), in which both the acquisition and extinction of learning, in this case context fear conditioning, follow an asymptotic curve, based on stimulus unexpectedness ($\Delta V = (\lambda\text{-})$). α is a constant in that A αA VT refers to the association ability of a CS to an US. When calculating the value of α for our data, despite theoretically being a constant, we observed that for acquisition α had a value of 0.4, while for extinction, it had a value of 0.24. This α variation could be related to the use of a context as a CS instead of a discrete stimulus. The most relevant aspect of this model is the prediction error ($\lambda - V$). The term λ corresponds to the maximum associative strength of a T US, and it is 100 if the US is present, and 0 if the US is not present. Our data display an asymptotic growth and decay of learning (Fig. 5, graph), as theorized in the Rescorla-Wagner model [26].

However, when we increased the number of footshocks per acquisition session ("mild protocol") or increased the number of acquisition sessions ("strong protocol"), we observed an increase in freezing levels when rats were exposed to a novel unpaired context, with no differences between the freezing displayed in the conditioning context and this novel context, as a generalization process had occurred (Fig. 4). These results are consistent with previous research demonstrating that when fear was tested in a novel context, the one and two footshock groups failed to freeze, whereas the 5-footshock group displayed a time-dependent generalization of contextual memory [37]. Although this earlier study investigated how modifying the intensity of the implemented protocol affected the generalization of fear to the context, it focused on the incubation process of fear memory. Their research demonstrated how fear memories generalize over time, involving processes of forgetting specific contextual cues, becoming more vague and generalized memories. However, our study focused on investigating the intensity of the behavioral protocol required to allow the study of short-term generalization, without involving memory processes.

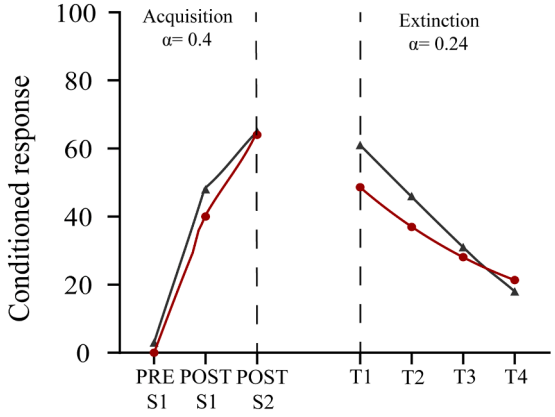
In the weak conditioning protocol, we observed that the modification of visual, olfactory, and tactile parameters, without the modification of spatial parameters, was sufficient for rats to recognize the new context as non-threatening (Fig. 4). This is in line with a previous study in mice that observed that the modification of these parameters produced generalization just after the consolidation of memory [12]. However, this study did not consider how generalized memories behave during an extinction process. Moreover, it was based on pre-exposure to the conditioning context. The present study revealed how generalized false memories can also be extinguished by re-exposure, which is of considerable therapeutic value in clinical psychology. In addition, in the present research we did not use pre-exposure to the conditioned context, since it was observed previously that pre-exposure to certain visual cues reduced their efficacy as feedback stimuli, decreasing the ability of rats to avoid a shock in a passive avoidance test [38], which when extrapolated to Pavlovian conditioning could affect the ability of rats to respond to different contexts during the extinction process, leading to biased results.

Results obtained using the "mild" and "strong" protocols present similarities with the symptomatology described in humans with disorders such as GAD and PTSD, such as the generalization of fear to novel contexts [39–41].

It is noteworthy that, according to our results, the generalized fear to an unpaired context followed the same scheme of extinction as the originally paired context, when the rats were re-exposed to this unpaired context without US. Studies with rats have revealed that extinction of the generalized contextual fear conditioning depends on the infralimbic cortex [42]. However, the best way to perform extinction needs to be considered. In many studies, extinction is obtained by exposing rats for 15 min or more to the conditioned context, but without the US [37,42]. In our studies, we observed that even four re-exposures in a single day were not sufficient to produce extinction, but when the four re-exposure trials extended across two days, a strong and specific extinction was observed.

Another aspect that could be considered is the possible effect of circadian activity on the CxFC process. Some studies have reported a dependency of the process on hippocampal expression of circadian-related genes such as Period genes [43]. Circadian expression of endogenous corticoids may also have an impact on contextual fear extinction [44]. However, the effect of circadian activity should be considered as another aspect that is independent of the features that configure the context [45]. In fact, it has been reported that conditioning of nocturnal rodents during the inactive (light) phase improved acquisition, but not extinction [46,47]. Thus, when performing a CxFC protocol, care could be taken to complete the different steps within the same circadian phase. However, variations in different step time points within the inactive or active phases, apparently does not produce an impact on fear memory in either mice [48] or rats [49].

The reliability of these protocols suggests they represent good animal models for studying how a mental burden can become a generalization perception that requires the mobilization of additional mental resources to face the problem. For example, the generalized perception could



	Rescorla Wagner	CxFC
PRE S1	0	3
POST S1	40	48
POST S2	64	65
T1	48.64	61
T2	36.97	46
T3	28.09	31
T4	21.35	18

Fig. 5. Rescorla-Wagner simulation. PreS1: time previous to first shock; PostS1: time after the first shock; PostS2: time after the second shock; T1: first extinction test; T2: second extinction test; T3: third extinction test; T4: fourth extinction test.

become pathological and represent a model of GAD or PTSD. Further studies are needed to determine the anatomical and biochemical elements involved in the transition between specific contextual conditioning to generalized fear to any context, and the persistence of contextual fear in conditions in which contextual fear should be extinguished [20,39,50].

Finally, one limitation of the present work is that we only used male rats, thus parameters may need to be adapted when studying females.

5. Conclusions

In these studies, we identified some key parameters for the acquisition, extinction, and maintenance of contextual fear memories in adult male rats. From the behavioral analysis of six contextual fear conditioning protocols, it was concluded that the organization and spacing of the sessions are relevant factors for the acquisition, retrieval, and extinction of contextual fear memories. Our results indicated that extinction of fear of context occurred with greater statistical robustness when extinction sessions were completed over two days. Furthermore, our results indicated that the exposure to single footshocks of 0.3 mA for 0.5 s in two different acquisition sessions can produce context-specific conditioned fear. Increasing the number of footshocks or the number of acquisition trials resulted in a generalization process. Acquired generalized responses to unpaired contexts can be extinguished in the same way as regularly acquired contextual memories by re-exposure trials to this context without US.

Further examination of new protocols and contextual configurations in male and female experimental animals is needed to better understand the neural circuits involved in context processing in normal subjects and to identify abnormalities that may accompany various psychiatric disorders [2,3].

CRediT authorship contribution statement

Mónica Navarro-Sanchez: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Isis Gil-Miravet:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Daniel Montero-Caballero:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Esther Castillo-Gomez:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Andrew L. Gundlach:** Writing – review & editing, Writing – original draft, Visualization. **Francisco E. Olucha-Bordonau:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Declaration of generative AI in scientific writing

The authors declare no generative AI was used in the scientific writing of this paper.

References

[1] N. Chaaya, A.R. Battle, L.R. Johnson, An update on contextual fear memory mechanisms: Transition between amygdala and hippocampus, Neurosci. Biobehav. Rev. 92 (2018) 43–54, https://doi.org/10.1016/j.neubiorev.2018.05.013.
[2] S. Maren, K.L. Phan, I. Liberzon, The contextual brain: implications for fear conditioning, extinction and psychopathology, Nat. Rev. Neurosci. 14 (2013) 417–428, https://doi.org/10.1038/nrn3492.
[3] S. Sangha, M.M. Diehl, H.C. Bergstrom, M.R. Drew, Know safety, no fear, Neurosci. Biobehav. Rev. 108 (2020) 218–230, https://doi.org/10.1016/J.NEUBIOREV.2019.11.006.
[4] D.E. Glenn, V.B. Risbrough, A.N. Simmons, D.T. Acheson, D.M. Stout, The Future of Contextual Fear Learning for PTSD Research: A Methodological Review of Neuroimaging Studies, Curr. Top Behav. Neurosci. 38 (2018) 207–228, https://doi.org/10.1007/7854_2017_30. PMID: 29063483.
[5] J.E. Dunsmoor, A.R. Otto, E.A. Phelps, Stress promotes generalization of older but not recent threat memories, Proc. Natl. Acad. Sci. USA 114 (2017) 9218–9223, https://doi.org/10.1073/PNAS.1704428114.
[6] A.S. Russo, R.G. Parsons, Behavioral expression of contextual fear in male and female rats, Front. Behav. Neurosci. 15 (2021) 671017, https://doi.org/10.3389/fnbeh.2021.671017.
[7] R. Andero, K.J. Ressler, Fear extinction and BDNF: translating animal models of PTSD to the clinic, Genes Brain Behav. 11 (2012) 503–512, https://doi.org/10.1111/j.1601-183X.2012.00801.x.
[8] L. Luyten, D. Vansteenwegen, K.V. Kuyck, B. Nuttin, Towards chronic contextual conditioning in rats, Acta Neurobiol. Exp. (Wars.) 71 (2011) 331–338.
[9] B. Bandelow, S. Michaelis, Epidemiology of anxiety disorders in the 21st century, Dialogues Clin. Neurosci. 17 (2015) 327–335, https://doi.org/10.31887/dcns.2015.17.3/bbandelow.
[10] C.A. Essau, P.M. Lewinsohn, J.X. Lim, M. ho, R. Ho, P. Rohde, Incidence, recurrence and comorbidity of anxiety disorders in four major developmental stages, J. Affect Disord. 228 (2018) 248–253, https://doi.org/10.1016/J.JAD.2017.12.014.
[11] D.J. Stein, K.M. Scott, P. de Jonge, R.C. Kessler, Epidemiology of anxiety disorders: from surveys to nosology and back, Dialogues Clin. Neurosci. 19 (2017) 127–136, https://doi.org/10.31887/DCNS.2017.19.2/DSTEIN.
[12] J. Yu, T. Naoi, M. Sakaguchi, Fear generalization immediately after contextual fear memory consolidation in mice, Biochem. Biophys. Res. Commun. 558 (2021) 102–106, https://doi.org/10.1016/j.bbrc.2021.04.072.
[13] S. Lissek, S. Rabin, R.E. Heller, D. Lukenbaugh, M. Geraci, D.S. Pine, C. Grillon, Overgeneralization of conditioned fear as a pathogenic marker of panic disorder, Am. J. Psychiatry 167 (2010) 47–55, https://doi.org/10.1176/appi.ajp.2009.09030410.
[14] A.L. McGlade, T.D. Zbozinek, M. Treanor, M.G. Craske, Pilot for novel context generalization paradigm, J. Behav. Ther. Exp. Psychiatry 62 (2019) 49–56, https://doi.org/10.1016/j.jbtep.2018.08.009.
[15] M.G. Craske, K. Kircanski, M. Zelikowsky, J. Mystkowski, N. Chowdhury, A. Baker, Optimizing inhibitory learning during exposure therapy, Behav. Res. Ther. 46 (2008) 5–27, https://doi.org/10.1016/j.brat.2007.10.003.
[16] J.J. Kim, M.W. Jung, Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review, Neurosci. Biobehav. Rev. 30 (2006) 188–202, https://doi.org/10.1016/j.neubiorev.2005.06.005.
[17] J.E. LeDoux, Coming to terms with fear, Proc. Natl. Acad. Sci. USA 111 (2014) 2871–2878, https://doi.org/10.1073/pnas.1400335111.
[18] S.E. Cooper, J.E. Dunsmoor, Fear conditioning and extinction in obsessive-compulsive disorder: a systematic review, Neurosci. Biobehav. Rev. 129 (2021) 75–94, https://doi.org/10.1016/J.NEUBIOREV.2021.07.026.
[19] N.V. Luchkina, V.Y. Bolshakov, Mechanisms of fear learning and extinction: synaptic plasticity–fear memory connection, Psychopharmacology 236 (2018) 163–182, https://doi.org/10.1007/S00213-018-5104-4.
[20] S. Maren, A. Holmes, Stress and fear extinction,, Neuropsychopharmacology 41 (2016) 58–79, https://doi.org/10.1038/npp.2015.180.
[21] C.P. McLean, H.C. Levy, M.L. Miller, D.F. Tolin, Exposure therapy for PTSD: a meta-analysis, Clin. Psychol. Rev. 91 (2022) 102115, https://doi.org/10.1016/j.cpr.2021.102115.
[22] J.B. Williamson, M.S. Jaffee, R.E. Jorge, Posttraumatic stress disorder and anxiety-related conditions, Continuum (Minne Minn.) 27 (2021) 1738–1763.
[23] Y. Yabuki, K. Fukunaga, Clinical therapeutic strategy and neuronal mechanism underlying post-traumatic stress disorder (PTSD), Int. J. Mol. Sci. 20 (2019) 3614, https://doi.org/10.3390/ijms20153614.
[24] S. Kida, Reconsolidation/destabilization, extinction and forgetting of fear memory as therapeutic targets for PTSD, Psychopharmacology (Berl.) 236 (2019) 49–57, https://doi.org/10.1007/s00213-018-5086-2.
[25] L. Tuominen, S.N. DeCross, E. Boeke, C.M. Cassidy, O. Freudenreich, A.K. Shinn, R. B.H. Tootell, D.J. Holt, Neural abnormalities in fear generalization in

schizophrenia and associations with negative symptoms, Biol. Psychiatry Cogn. Neurosci. Neuroimaging 6 (2021) 1165–1175, https://doi.org/10.1016/j.bpsc.2021.01.006.
[26] R.A. Rescorla, A.R. Wagner, A Theory of Pavlovian Conditioning: Variations in the Effectiveness of Reinforcement and Nonreinforcement, Clasical Conditioning II: Current Research and Theory. (1972).
[27] D. Sierra-Mercado, N. Padilla-Coreano, G.J. Quirk, Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear, Neuropsychopharmacology 36 (2011) 529–538, https://doi.org/10.1038/NPP.2010.184.
[28] G.J. Quirk, G.K. Russo, J.L. Barron, K. Lebron, The role of ventromedial prefrontal cortex in the recovery of extinguished fear, J. Neurosci. 20 (2000) 6225–6231, https://doi.org/10.1523/jneurosci.20-16-06225.2000.
[29] R.G. Phillips, J.E. LeDoux, Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, Behav. Neurosci. 106 (1992) 274–285, https://doi.org/10.1037//0735-7044.106.2.274.
[30] W.B. Kim, J.H. Cho, Encoding of contextual fear memory in hippocampal-amygdala circuit, Nat. Commun. 11 (2020) 1382, https://doi.org/10.1038/S41467-020-15121-2.
[31] J. Radulovic, N.C. Tronson, Molecular specificity of multiple hippocampal processes governing fear extinction, Rev. Neurosci. 21 (2010) 1–17, https://doi.org/10.1515/REVNEURO.2010.21.1.1.
[32] K.A. Corcoran, B.J. Frick, J. Radulovic, L.M. Kay, Analysis of coherent activity between retrosplenial cortex, hippocampus, thalamus, and anterior cingulate cortex during retrieval of recent and remote context fear memory, Neurobiol. Learn. Mem. 127 (2016) 93–101, https://doi.org/10.1016/J.NLM.2015.11.019.
[33] T.P. Todd, M.Y. Jiang, N.E. DeAngeli, D.J. Bucci, Intact renewal after extinction of conditioned suppression with lesions of either the retrosplenial cortex or dorsal hippocampus, Behav. Brain Res. 320 (2017) 143–153, https://doi.org/10.1016/J.BBR.2016.11.033.
[34] K. Nader, G.E. Schafe, J.E. Le Doux, Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval, Nature 406 (2000) 722–726, https://doi.org/10.1038/35021052.
[35] G.E. Schafe, K. Nader, H.T. Blair, J.E. LeDoux, Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective, Trends Neurosci. 24 (2001) 540–546, https://doi.org/10.1016/S0166-2236(00)01969-X.
[36] S. Duvarci, K. Nader, Characterization of fear memory reconsolidation, J. Neurosci. 24 (2004) 9269–9275, https://doi.org/10.1523/JNEUROSCI.2971-04.2004.
[37] A.M. Poulos, N. Mehta, B. Lu, D. Amir, B. Livingston, A. Santarelli, I. Zhuravka, M. S. Fanselow, Conditioning- and time-dependent increases in context fear and generalization, Learn. Mem. 23 (2016) 379–385, https://doi.org/10.1101/lm.041400.115.
[38] E.J. Callen, Context preexposure influences the effectiveness of feedback stimuli in avoidance learning, Behav. Process. 66 (2004) 35–42, https://doi.org/10.1016/j.beproc.2003.12.002.
[39] A. Rougemont-Bücking, C. Linnman, T.A. Zeffiro, M.A. Zeidan, K. Lebron-Milad, J. Rodriguez-Romaguera, S.L. Rauch, R.K. Pitman, M.R. Milad, Altered processing of contextual information during fear extinction in PTSD: an fMRI study, CNS Neurosci. Ther. 17 (2011) 227–236, https://doi.org/10.1111/J.1755-5949.2010.00152.X.
[40] M.B. VanElzakker, M. Kathryn Dahlgren, F. Caroline Davis, S. Dubois, L.M. Shin, From Pavlov to PTSD: the extinction of conditioned fear in rodents, humans, and anxiety disorders, Neurobiol. Learn Mem. 113 (2014) 3–18, https://doi.org/10.1016/J.NLM.2013.11.014.
[41] L. Luyten, D.Vansteenwegen Debora, K. van Kuyck, D. Deckers, B. Nuttin, Optimization of a contextual conditioning protocol for rats using combined measurements of startle amplitude and freezing: the effects of shock intensity and different types of conditioning, J. Neurosci. Methods 194 (2011) 305–311, https://doi.org/10.1016/J.JNEUMETH.2010.11.005.
[42] H. Bayer, L.J. Bertoglio, Infralimbic cortex controls fear memory generalization and susceptibility to extinction during consolidation, Sci. Rep. 10 (2020) 15827, https://doi.org/10.1038/s41598-020-72856-0.
[43] M.W. Urban, C. Lo, K.K. Bodinayake, C.A. Brunswick, S. Murakami, A.C. Heimann, J.L. Kwapis, The circadian clock gene Per1 modulates context fear memory formation within the retrosplenial cortex in a sex-specific manner, Neurobiol. Learn. Mem. 185 (2021) 107535, https://doi.org/10.1016/j.nlm.2021.107535.
[44] E.R. Woodruff, B.N. Greenwood, L.E. Chun, S. Fardi, L.R. Hinds, R.L. Spencer, Adrenal-dependent diurnal modulation of conditioned fear extinction learning, Behav. Brain Res. 286 (2015) 249–255, https://doi.org/10.1016/j.bbr.2015.03.006.
[45] A. Albrecht, O. Stork, Circadian rhythms in fear conditioning: an overview of behavioral, brain system, and molecular interactions, Neural Plast 2017 (2017) 3750307, https://doi.org/10.1155/2017/3750307.
[46] D. Chaudhury, C.S. Colwell, Circadian modulation of learning and memory in fear-conditioned mice, Behav. Brain Res. 133 (2002) 95–108, https://doi.org/10.1016/S0166-4328(01)00471-5.
[47] R. Hagewoud, S.N. Whitcomb, A.N. Heeringa, R. Havekes, J.M. Koolhaas, P. Meerlo, A time for learning and a time for sleep: the effect of sleep deprivation on contextual fear conditioning at different times of the day, Sleep 33 (2010) 1315–1322, https://doi.org/10.1093/SLEEP/33.10.1315.
[48] A. Albrecht, M. Thiere, J.R. Bergado-Acosta, J. Poranzke, B. Müller, O. Stork, Circadian modulation of anxiety: a role for somatostatin in the amygdala, PLoS One 8 (2013) e84668, https://doi.org/10.1371/JOURNAL.PONE.0084668.
[49] J.W. Rudy, C.R. Pugh, Time of conditioning selectively influences contextual fear conditioning: further support for a multiple-memory systems view of fear conditioning, J. Exp. Psychol. Anim. Behav. Process 24 (1998) 316–324, https://doi.org/10.1037//0097-7403.24.3.316.
[50] M.G. Craske, D. Hermans, B. Vervliet, State-of-the-art and future directions for extinction as a translational model for fear and anxiety, Philos. Trans. R. Soc. Lond. B Biol. Sci. 373 (2018) 20180432, https://doi.org/10.1098/RSTB.2017.0025.