Behavioural Brain Research 441 (2023) 114285

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

The effectiveness of extinction training in male rats: Temporal considerations and brain mechanisms

Pedro Correia [a,b,d,1], Kornél Demeter [a,c,1], János Varga [a], Eszter Urbán [a], Bibiána Török [a,b,d], Diána Balázsfi [a], Nikoletta Bakos [a], József Haller [a], Dóra Zelena [a,d,*]

[a] Department of Behavioral Neurobiology, Institute of Experimental Medicine, Budapest, Hungary
[b] Janos Szentagothai School of Neurosciences, Semmelweis University, Budapest, Hungary
[c] Behavioral Studies Unit, Institute of Experimental Medicine, Budapest, Hungary
[d] Institute of Physiology, Medical School, University of Pécs, Centre for Neuroscience, Szentágothai Research Centre, Pécs, Hungary

ARTICLE INFO

Keywords:
PTSD
Extinction training
Freezing
Hormones
Prefrontal cortex
Amygdala
Hippocampus
c-Fos

ABSTRACT

The extinction of conditioned fear is frequently used in laboratories as a model for human exposure therapy and is crucial for studies of posttraumatic stress disorder (PTSD). However, the efficacy of specific protocols can vary greatly, and the underlying brain mechanisms are not sufficiently clarified. To address this issue, variable starting time (one or twenty-eight days after fear conditioning) and extinction protocols were used, and the efficacy and durability of fear extinction were also studied. Changes in the behavior, stress hormone levels and neuronal activation patterns of stressed rats were analyzed. Conditioned fear was rapidly and efficiently extinguished by all the protocols investigated. However, when these extinction protocols were initiated one day after fear training, conditioned fear relapsed spontaneously four weeks later. In contrast, when extinction trials were started 28 days after conditioning, no relapse occurred. Hormone measurements taken by the end of extinction trials indicated that adrenocorticotropin, but not corticosterone responses reflected behavioral extinction without any sign of relapse. The last extinction training increased the activation of the medial prefrontal cortex and decreased the activation of the central and medial amygdala when extinction began one day after fear conditioning. By contrast, the activation of the basolateral amygdala and the entire hippocampus decreased by the last training session when extinction started 28 days after fear conditioning. Our findings show that extinction training can extinguish remote fear memories more effectively than recent ones, and that the brain mechanisms underlying remote and recent fear memory extinction differ. Laboratory models should also focus on a later time point to increase their translational value.

1. Introduction

Memories of frightening traumatic events can persist for a lifetime. Fear memory resistance to extinction can result in fear-related disorders such as anxiety and post-traumatic stress disorder (PTSD) [22,61], which prevalence is increasing worldwide. Except for certain cases (e.g. soldiers) [65], life-threatening events are unpredictable, therefore the preventive treatment to avoid the development of PTSD should start after the triggering incident. The most recommended treatment for PTSD is psychotherapy, which might focus on the memory of the traumatic event or its meaning during an exposure therapy [3,14,18,20]. The laboratory model of this therapy is called fear extinction [31,40,69]. For each trial, a conditioned stimulus (CS), such as a tone, is combined with a negative unconditioned stimulus (US), such as electric foot-shock. After the trials, the CS evokes fear responses such as freezing. Once learned, however, the animal is repeatedly presented to the CS in the absence of the US which induces the conditioned fear responses to extinguish [36]. The conditioned fear memory is not erased by extinction [44]; the "bad" fearful memory co-exists with the new "safe" memory learned through fear extinction [29,38,6,70]. The same cue is used to retrieve both memories, and it is unclear which memory will be retrieved in any given situation [49].

* Correspondence to: Laboratory of Behavioural and Stress Studies, Institute of Physiology, Medical School, University of Pécs, Szigeti str. 12, Pécs H-7624, Hungary.
E-mail address: dora.zelena@aok.pte.hu (D. Zelena).
[1] These authors contributed equally.

https://doi.org/10.1016/j.bbr.2023.114285
Received 21 September 2022; Received in revised form 12 December 2022; Accepted 3 January 2023
Available online 4 January 2023

The goal of exposure therapy is to assist in breaking the cycle of fear and avoidance and prevent relapse. To achieve this result with animal models, various protocols are used, ranging from less intensive, albeit prolonged [41,54] to rather intensive extinction trainings [58,69]. A systematic comparison of such protocols and the durability of extinction following them is still lacking. Moreover, many researchers focus on fear extinction in rodents shortly after conditioning training (starting 1–72 h after fear conditioning) [43,61]. However, research indicates that the level of fear at the time of intervention is critical to the effectiveness of extinction [35]. Another study found that immediate extinction cannot prevent the recovery of an extinguished fear response, and that the degree of fear expressed during treatment is more important to its long-term efficacy than the acquisition-extinction interval [27]. While those studies were limited to 24 h after extinction training, our study aimed to extend this time frame to several weeks. In fact, symptoms of PTSD appear in human patients 2–6 months after trauma [1]. Thus, treatment usually begins long after trauma exposure. Laboratory models should also focus on a later time point to increase their translational value. Indeed, our previous research confirmed that a radically different neural activation pattern occurred 1 day (recent) than 28 days (remote) after fear conditioning, despite the same behavioral response [62].

The medial prefrontal cortex (mPFC) [22,24,42], amygdala [21,32,34] and hippocampus (HC) [12,4] are all heavily involved in the extinction of conditioned fear and may undergo structural and functional changes [31,52,55]. HC–cortical networks are responsible for the context (as neutral conditioned stimulus) representation, whereas the basolateral amygdala (BLA) processes the pairing of the conditioned stimulus (e.g. context) with the unconditioned stimulus (e.g. electric shock) [16,46]. Furthermore, because the mPFC is thought to be responsible for the consolidation of extinction learning, its activation may prevent fear memory relapse [37].

Our aim was to investigate the efficacy of fear extinction training 1 or 28 days after fear conditioning, as well as the associated neural and hormonal changes. We measured the freezing behavior of rats to assess the efficacy of three different extinction protocols based on their starting point (1 day vs 28 days) after fear induction. As fear can easily reappear even after successful extinction [23,30], the durability of fear extinction was also investigated four weeks later. Repeated blood sampling was conducted to analyze the adaptation of the stress hormone response (adrenocorticotropin (ACTH) and corticosterone). Furthermore, in the three previously mentioned brain regions (mPFC, amygdala and HC), we compared context-induced neuronal activation by c-Fos immunohistochemistry with and without extinction training [13].

2. Materials and Methods

2.1. Animals

Subjects were male Wistar rats (n = 140, Charles River, Hungary) weighing approximately 300 g at the start of the experiments. Food and water were available ad libitum, while temperature and relative humidity were kept at 22 ± 2 °C and 60 ± 10%, respectively. Rats were maintained in a reversed light cycle of 12 h with lights off at 9:00 h. Acclimatization to the day–night schedule lasted 2 weeks. Rats were isolated 3 days before the fear conditioning, and thereafter housed individually in Techniplast 1291 H Eurostandard Type III H cages (425 ×266 × 185 mm).

Experiments were carried out in accordance with the European Communities Council Directive recommendations for the care and use of laboratory animals (2010/63/EU) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine.

2.2. Shock exposure

Electric foot-shocks were administered at Day 0 (D0) as described in earlier studies [62]. Between 10 and 12 h a.m., in a separate, quiet room with day-light illumination, 3 mA electric shocks were administered via the metal grid floor of a Plexiglas cage (30 ×30×30 cm). For 5 min, two shock trains were delivered every minute (i.e., each subject received 10 shocks). Each shock train lasted 1 s and was made up of 0.01 s shocks separated by 0.02 s breaks. Control rats were placed in a similar box for 5 min, but no shocks were administered. Between shock sessions the box was cleaned with soapy water and then with tap water.

2.3. Extinction protocols

During extinction rats (n = 10 animals per protocol) were placed in the shocking apparatus but were not subjected to foot-shocks. We used three different extinction protocols, beginning on day 1 (D1, 24 h after fear conditioning) or day 28 (D28) following the foot-shock exposure. The extinction protocol and further investigations are depicted on Fig. 1. (Exp.1).

Protocol 1 (7 ×1): Animals were reintroduced into the shocking chamber for 5 min once daily for 7 days without receiving shocks, and then at day 28 (D28) or day 56 (D56) (e.g., 3 weeks after the extinction protocol was terminated) to study possible relapse.

Protocol 2 (3 ×5): Next, we used a more intensive protocol to test whether the intensity of the extinction training influences the durability of extinction. The 5 min reintroduction occurred 5 times per day, 3 days in a row, and once at D28/D56 (to test for relapse). Within a day, there was a 60-minute gap between two replacements.

Protocol 3 (1 ×5): Observing extinction after 6 repetitions in the previous trial (3 ×5), our next question was whether further confirmatory reinstatement is required for durability. Thus, in this protocol the reintroduction was done 5 times for 5 min on the first day of the extinction procedure, once the next day, and once on D28/D56 (relapse). Within a day, there was a 60-minute gap between two replacements.

Protocol 1 was used in a separate set of animals for stress hormone measurement and c-Fos immunohistochemistry (IHC) investigation.

2.4. Behavioral analysis

Behavior was video-recorded, and later scored by an experimenter blind to the treatment groups using computer-based event recorder software (H77, Budapest, Hungary). Freezing behavior (no active movements except for breathing) was expressed as percentage of the total time of the test (5 min). Fear relapse was measured by the difference of the time percentage of freezing between the last day of extinction and 28 days after the extinction began (D28/D56).

2.5. Hormone measurements

In a separate set of animal (n = 40, ten in each experimental group) blood samples were collected from the tail vein during Protocol 1 right at the end of the appropriate reintroduction (Fig. 1, Exp.2). One day before the foot-shock, basal levels were measured. Additional blood samples were collected immediately following the foot-shock, on the first and last days of the extinction, and 28 days after the protocol began as a relapse sample.

Blood samples were cooled on ice and after centrifugation (3000 rpm/min for 20 min) at 4 °C the serum was stored at − 20 °C for later hormone measurement. Plasma ACTH and corticosterone was measured by radioimmunoassay (RIA) in 50 or 10 µl unextracted plasma, respectively, as described earlier using specific antisera developed in our Institute [68]. The intra-assay coefficients of variation were 4.7 or 12.3%, respectively. Samples from one experiment were measured in one RIA.

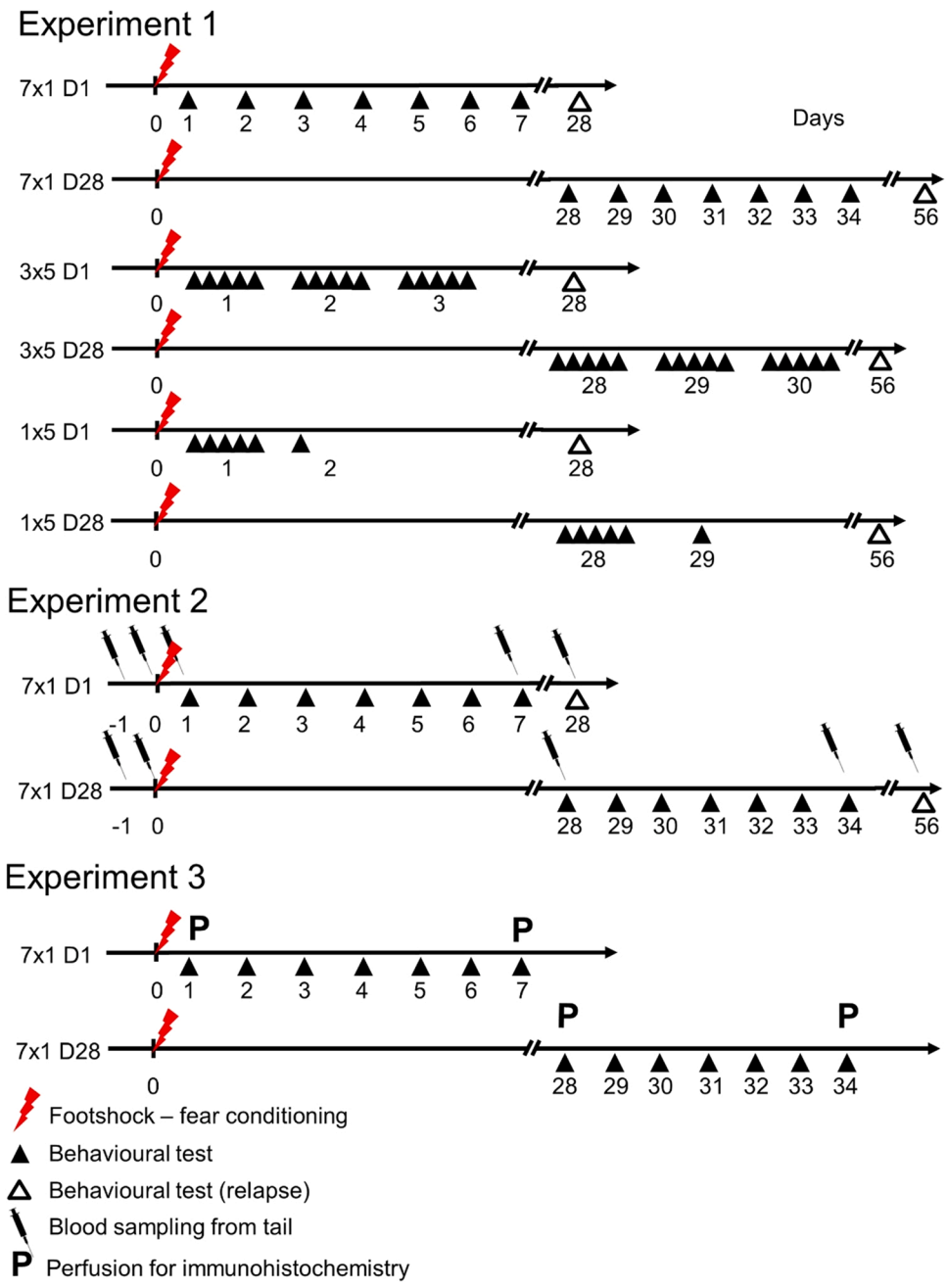

Fig. 1. Schedule of experiments. On Day 0, we performed the foot-shock (red flash) in each experiment; on all other days the animals were returned to the same chamber without further foot-shock. Black triangles indicate the introduction of animals into the shocking chamber and the recording of their behavior during the extinction protocols. Syringes represent blood sampling from tail in Experiment 2. On Day − 1, the basal hormone levels were measured. P (perfusion) denotes the day when the transcardial perfusion for immunohistochemistry was performed in Experiment 3.

2.6. *Immunohistochemistry*

In another set of rats (n = 5 in each experimental groups) neuronal activation patterns were studied by c-Fos immunohistochemistry on perfused brains collected 90 min after a single replacement at D1 or D28 or after the last extinction training day of Protocol 1 [62] (Fig. 1, Exp.3). Half of the animals were shocked while the other half were non-shocked controls (but were placed in the shocking chamber) with a single replacement into the context or after extinction (grouping variables were (each with two options, -totaling 2×2x2 =8 groups): foot-shock, starting day of extinction and the fact of extinction).

Animals were anaesthetized by the mixture of Ketamine (75 mg/kg), Xylazin (15 mg/kg) and Pipolphen (7.5 mg/kg) intraperitoneally and perfused through the ascending aorta with 150 ml ice-cold 0.1 M phosphate-buffered saline (PBS) followed by 300 ml 4% paraformaldehyde (PFA) in 0.1 M PBS. The brains were removed, overnight post-fixed in PFA solution and cryoprotected by 20% sucrose in PBS at 4 °C. Six series of 30 μm frozen sections were cut in the frontal plane on a sliding microtome. Floating sections were permeabilized by 0.5% Triton X-100 for 30 min. Endogenous peroxidase activity was blocked by 0.5% H_2O_2 for 30 min. Nonspecific antibody binding was prevented by 2% bovine serum albumin blocking solution for 30 min. The c-Fos protein was labeled for 48 h with a rabbit polyclonal antibody (1:5000, Santa Cruz Biotechnology, USA, sc-52). Primary antibodies were detected by biotinylated anti-rabbit goat serum (1:500) and avidin–biotin complex (1:1000, Vectastain ABC Kit, Vector Laboratories, USA) diluted in 0.05 M Tris buffer (pH 7.6) (Sigma-Aldrich). The peroxidase reaction was developed in the presence of 3,3′-diaminobenzidine tetrahydrochloride hydrate (DAB, Fluka, 0.2 mg/ml), nickel–ammonium sulfate (0.1%) and H_2O_2 (0.003%) dissolved in 0.05 M Tris buffer. The sections were mounted on glass slides in chrome-gelatin solution (0.5% gelatin, 0.05% chrome(III)-sulfate). Dried

sections were incubated for 3 min in mixture of Xylene isomers and covered by a DPX mounting medium (Sigma). Section planes were standardized according to the atlas of Paxinos and Watson [45]. For quantifying c-Fos activation, microscopic images were digitized by an OLYMPUS CCD camera (2040 × 1536 pixels/image) using a 20x objective, and stained particles were counted by means of the Scion-Image software developed at the National Institutes of Health, USA. Uniform thresholds were used, the size of stained particles was set between 20 and 200 pixels. At each level, the c-Fos signal was counted bilaterally in three sections that were 180 μm apart, and their average was considered.

The following brain regions were investigated: cingulate cortex area 1 (Cg1), prelimbic cortex (PrL), infralimbic cortex (IL) and dorsal peduncular cortex (DP) from the mPFC, the basolateral (BLA), central (CeA) and medial (MeA) part of the amygdala and area 1–3 of cornu ammonis (CA1–3) from the dorsal HC (Fig. 5). In all experimental setup control group meant non-shocked animals placed into the shocking chamber without shock.

For better understanding of the mechanism behind the extinction of fear memory we illustrate the differences of the percentage of changes of the number of c-Fos immunopositive cells compared to the average of the respective non-shocked control animals (i.e., D1 extinction group

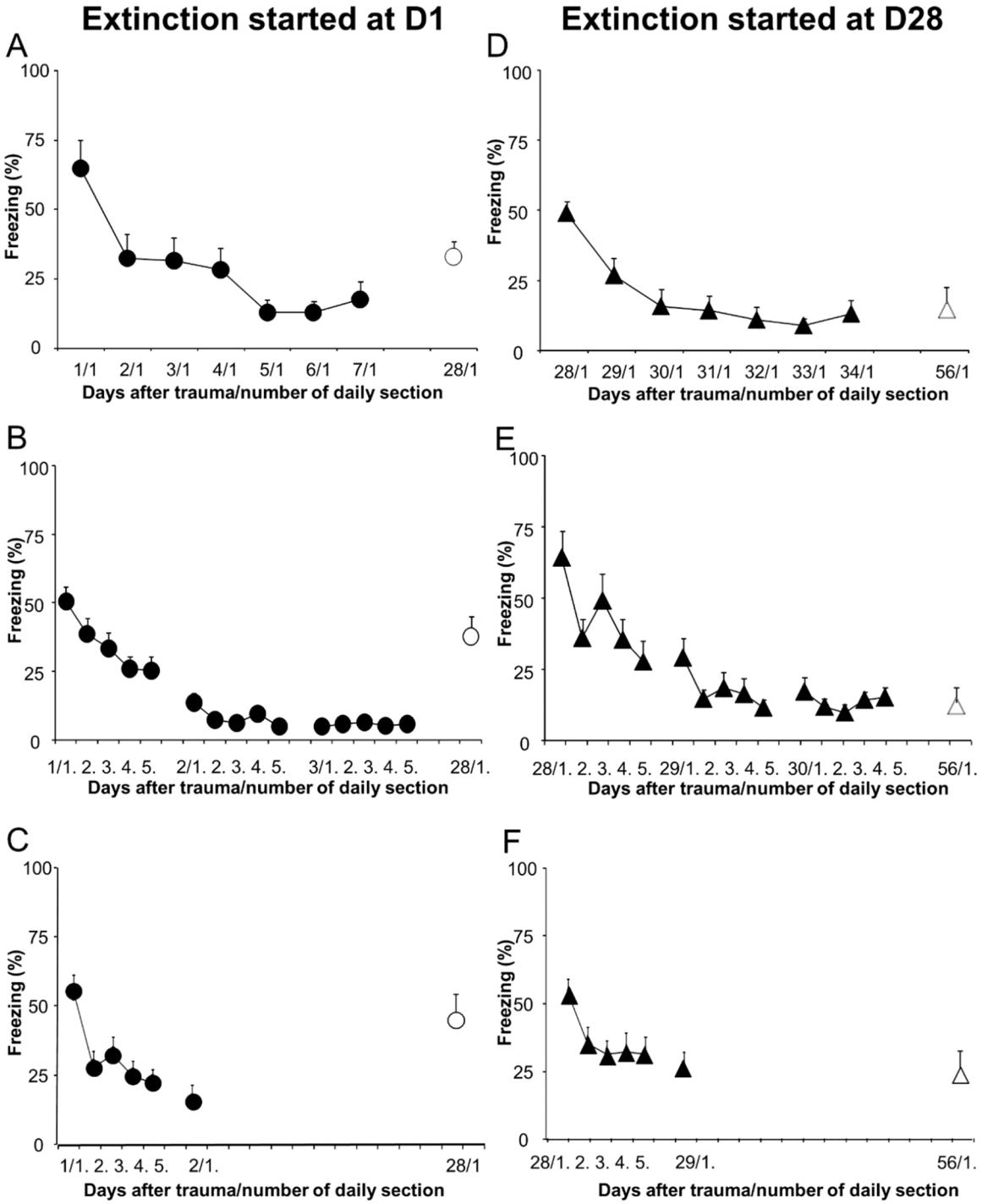

Fig. 2. Expression of fear response (freezing) during extinction. A-C represent the time spent freezing when the extinction started 24 h (D1) after fear conditioning (recent fear memory protocols). D-F represent figures on remote fear response (e.g., extinction started on day 28 after fear conditioning). n = 10/group.

with fear conditioning was compared to D1 extinction group without fear conditioning using previous cage-mates which were tested on the very same days).

$$\left(\left(\frac{c - Fosimmunopositivecellsinshockedanimal}{averageofc - Fosimmunopositivecellsinallnon - shockedanimals}\right) * 100\right) - 100$$

2.7. Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using the STATISTICA 13.3 software package (TIBCO, USA). Two-way (factors protocol and starting date of extinction), three-way (factors foot-shock, starting day of extinction and the fact of extinction), repeated measure (factor foot-shock, repeated factor time) ANOVA or general linear model (factors foot-shock and time of extinction, repeated factors days, time points) were conducted. In case the main effect of ANOVA was significant multiple pairwise comparisons were made by the Newman Keuls post hoc analysis. In case of changes in freezing (relapse on Fig. 3) the data were additionally tested by single sample t-test against 0 representing no relapse. Data were expressed as mean±SEM and the level of significance was set at $p < 0.05$.

3. Results

3.1. Experiment 1: Comparison of the effectiveness and durability of fear extinction protocols

The freezing time was significantly reduced at the end of all three extinction protocols (Fig. 1) when compared to the first reintroduction to the fear context (Fig. 2; 7 ×1 protocol, effect of days: $F_{(6,108)}$=34.459, $p < 0.01$; 3 × 5 protocol, effect of days: $F_{(2,36)}$=88.488, $p < 0.01$; effect of time within the day: $F_{(4,72)}$=15.446, $p < 0.01$; interaction between day and time: $F_{(8,144)}$=7.371, $p < 0.01$; 1 × 5 protocol, effect of time: $F_{(5,80)}$=20.001, $p < 0.01$). Indeed, in all cases, the animals showed reduced freezing already during the second reintroduction, which was further reduced on days 5 and 6 (7 ×1 protocol) or until the seventh occasion (2. day 2. replacement) (3 ×5 protocol). Based on these findings, we terminated the extinction after the sixth occasion (the freezing was at its lowest at this point) and examined the extinction's durability in this case as well (Fig. 2 **C,F**).

All in all, all extinction protocols effectively diminished freezing in trauma context (even when comparing the first and last trial for all protocol, time: $F_{(1,52)}$= 234.053, $p < 0.01$). The effectiveness of extinction was not different whether we started the protocol one or 28 days after fear conditioning. Moreover, there was no significant difference between the different experimental protocols as well ($F_{(5,52)}$= 1.403, $p = 0.239$). The only detectable difference was that - starting 28 days after the trauma - the 3 × 5 extinction protocol was more effective than the 1 × 5 one (protocol x time: $F_{(5,52)}$= 4.711, $p < 0.01$; 3 × 5 vs 1 × 5: $p = 0.015$).

When extinction was started at the following day after fear conditioning, spontaneous relapse of fear was observed on D28 independently from the protocol (Fig. 3, significant effects by single sample t-test). However, when extinction was started on D28, the effect was more durable (no significant effects by single sample t-test; two-way ANOVA, starting date of extinction: $F_{(1,52)}$=27.641, $p < 0.01$).

The manifestation of fear was similar as described above during Exp.2 and 3 (Suppl. Figs. 1 and 2). During Exp.2 we even counted the number of faeces boli left in the shocking chamber after reintroduction and discovered a positive correlation between fear manifestation (freezing) and the number of faeces boli (Suppl. Fig. 1C, D).

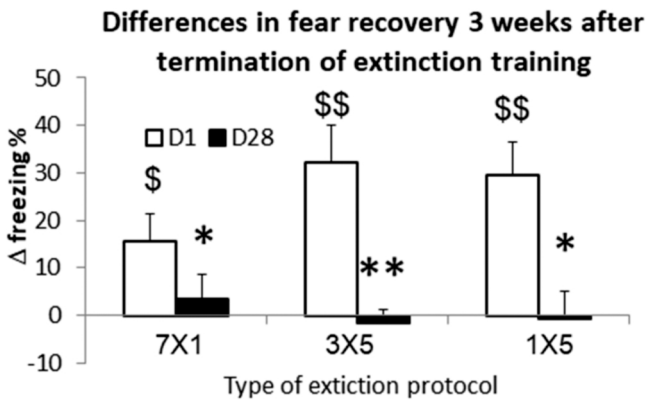

Fig. 3. Relapse of fear response. When animals were reintroduced to the shocking cage 28 days (D1 protocol) or 56 days (D28 protocol) after shock, freezing increased in the D1 series but not in the D28 series. The data represent the difference in freezing between D28/56 and the last occasion of extinction. Single sample t test; D1(7 ×1): $t_{(9)}$= 2.595, $p = 0.029$; D28(7 ×1): $t_{(9)}$= 0.712, $p = 0.494$; D1(3 ×5): $t_{(9)}$= 4.074, $p = 0.003$; D28(3 ×5): $t_{(9)}$= −0.565, $p = 0.585$; D1(1 ×5): $t_{(9)}$= 4.243, $p = 0.004$; D28(1 ×5): $t_{(9)}$= −0.107, $p = 0.917$; n = 10/group *$p < 0.05$, * *$p < 0.01$ vs D1 by two-way ANOVA; \$$p < 0.05$, \$\$$p < 0.01$ vs 0 by single sample t-test.

3.2. Experiment 2: ACTH and Corticosterone levels on recent and remote fear extinction protocols

ACTH plasma concentration increased significantly after the foot-shock (effect of foot-shock: $F_{(1,96)}$= 31.952, $p < 0.01$), remained high during the first reintroduction and then decreased 7 days later (days: $F_{(4,96)}$= 6.412, $p < 0.01$; shock x day: $F_{(4,96)}$= 6.049, $p < 0.01$) independently from the start of the extinction protocol (Fig. 4A,B). When we returned the animals to the shocking chamber (context) 21 days after the extinction training ended, we found no hormonal relapse.

Corticosterone levels were higher in fear conditioning groups (effect of foot-shock: $F_{(1,141)}$= 5.391, $p < 0.05$) and increased in the days following the foot-shock (effect of day: $F_{(4,141)}$= 4.665, $p < 0.01$; Fig. 4C,D). However, we were unable to detect any extinction-like changes or differences in the timing of the two extinctions (D1 vs D28). Accordingly, no relapses were detected.

3.3. Experiment 3: Measurement of context-induced c-Fos activation in brain areas related to PTSD

The statistical data of neuronal activation is presented in Table 1. Previous aversive event affected (mostly reduced) the fear conditioning box-induced neuronal activation in a limited part of the mPFC (Cg1 and IL), BLA, and all three examined HC regions (Table 1. column Trauma; Fig. 5).

The starting day of extinction, on the other hand, had a significant effect in all studied brain areas (Table 1. column Start day), with higher levels in D28 groups compared to D1 groups. Extinction training decreased context-induced activation of MeA neurons while increased it in all three HC regions (Table 1. column Extinction). The effect of fear conditioning was influenced by the starting point of the extinction protocol in PrL, IL, BLA, CeA and CA3 regions (Table 1. column Tr x Day). The extinction training significantly affected the effect of fear conditioning in IL and whole HC (Table 1. column Tr x Ext). The starting point of the protocol influenced the effectiveness of extinction training in PrL, the entire amygdala, and HC (Table 1. column Day x Ext). Indeed, the extinction training starting on D1 or D28 modified the effect of fear conditioning on complementary brain areas. When started on D1, extinction training enhanced the fear conditioning-induced activation in the mPFC (more specifically in IL and DP), while in CeA and MeA reduced it (Table 2., interactions). On the other hand, when extinction

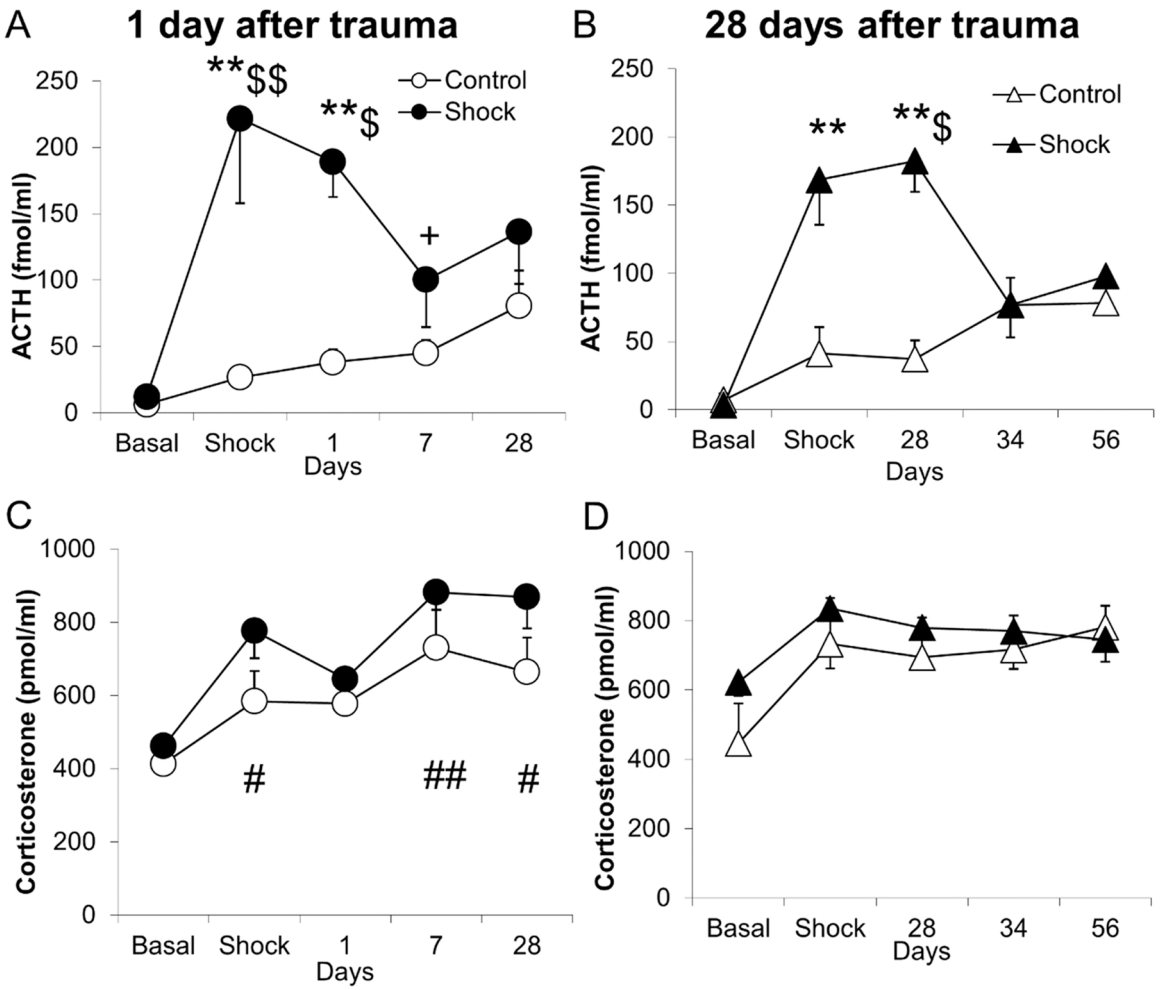

Fig. 4. Hormone levels during extinction. ACTH (A, B) and corticosterone (C, D) levels were measured at the end of a 5 min exposure to context (or at rest, Basal levels) using extinction protocol 1. (7 ×1) started 1 (A, C) or 28 (B-D) days after unconditioned stimulus (foot-shock). Shocked animals had higher hormone levels regardless of when the extinction began. ACTH levels decreased during extinction, but corticosterone levels did not. Relapse was not present in any cases. n = 10/group; * *p < 0.01 vs basal level; +p < 0.05 vs. D0 (foot-shock); $p < 0.05, $$p < 0.01 vs Control, non-shocked; #p < 0.05, ##p < 0.01 vs basal level in all cases.

Table 1
c-Fos activation, results of three-way ANOVA.

Brain area	Trauma	Start day	Extinction	Tr x Day	Tr x Ext	Day x Ext	Tr x Day X Ext
Cg1	6.50	56.75					
PrL		145.92		4.77		13.52	
IL	6.88	122.69		5.93	4.94		
DP		134.25					
BLA	5.97	213.33		10.44		41.17	5.64
CeA		593.62		25.39		18.66	7.04
MeA		677.71	5.58			8.12	
CA1	9.41	12.31	7.78		3.97	5.35	
CA2	28.80	38.60	9.86		9.51	15.84	
CA3	32.41	14.24	17.64	6.19	21.53	17.07	

The $F_{(1,29)}$ values are given in the Table. Note, only significant changes are included, in case of non-significant changes the squares are left empty.

training started on D28, it reduced context-induced neuronal activation in BLA and all three regions of HC (Table 3. interactions).

4. Discussion

The main findings of this study were that (1) all protocols effectively reduced freezing as a sign of fear. (2) However, spontaneous relapse of fear was detected in protocols when the fear extinction was commenced on the day after fear conditioning, but not when the extinction training started at later time point (e.g. 28 days after fear conditioning). (3) ACTH levels measured at specific time points after a 5 min context exposure showed extinction without relapse; however, corticosterone levels were only affected by fear conditioning and no extinction could be detected. (4) When comparing recent and remote fear extinction training, distinct reminder-induced activation patterns of different brain areas were observed. When extinction began one day after fear conditioning, mPFC, CeA, and MeA were more involved, whereas BLA and HC were more affected by later extinction training.

PTSD patients are constantly re-experiencing the traumatic event through flashbacks trigged by environmental cues [67]. To avoid this disorder, therapeutic techniques must be carried out as efficiently as possible. In our hands all of the protocols we used diminished the subjects' freezing behavior, suggesting the erasure of their fearful memories. However, the durability of the extinction might be crucial. Fear extinction research suggested that slowing or impeding fear relapse may improve the long-term outcome of fear extinction [28]. When extinction training began 28 days after fear conditioning in Experiment 1, no significant spontaneous relapse of fear memory was detected compared to recent extinction protocols. The relapse was more pronounced when the extinction was more intense over a shorter time-period (3 ×5 or 1 ×5 protocols), so we chose the less intensive protocol (7 ×1) for future studies. Our findings were somewhat unexpected, implying that the

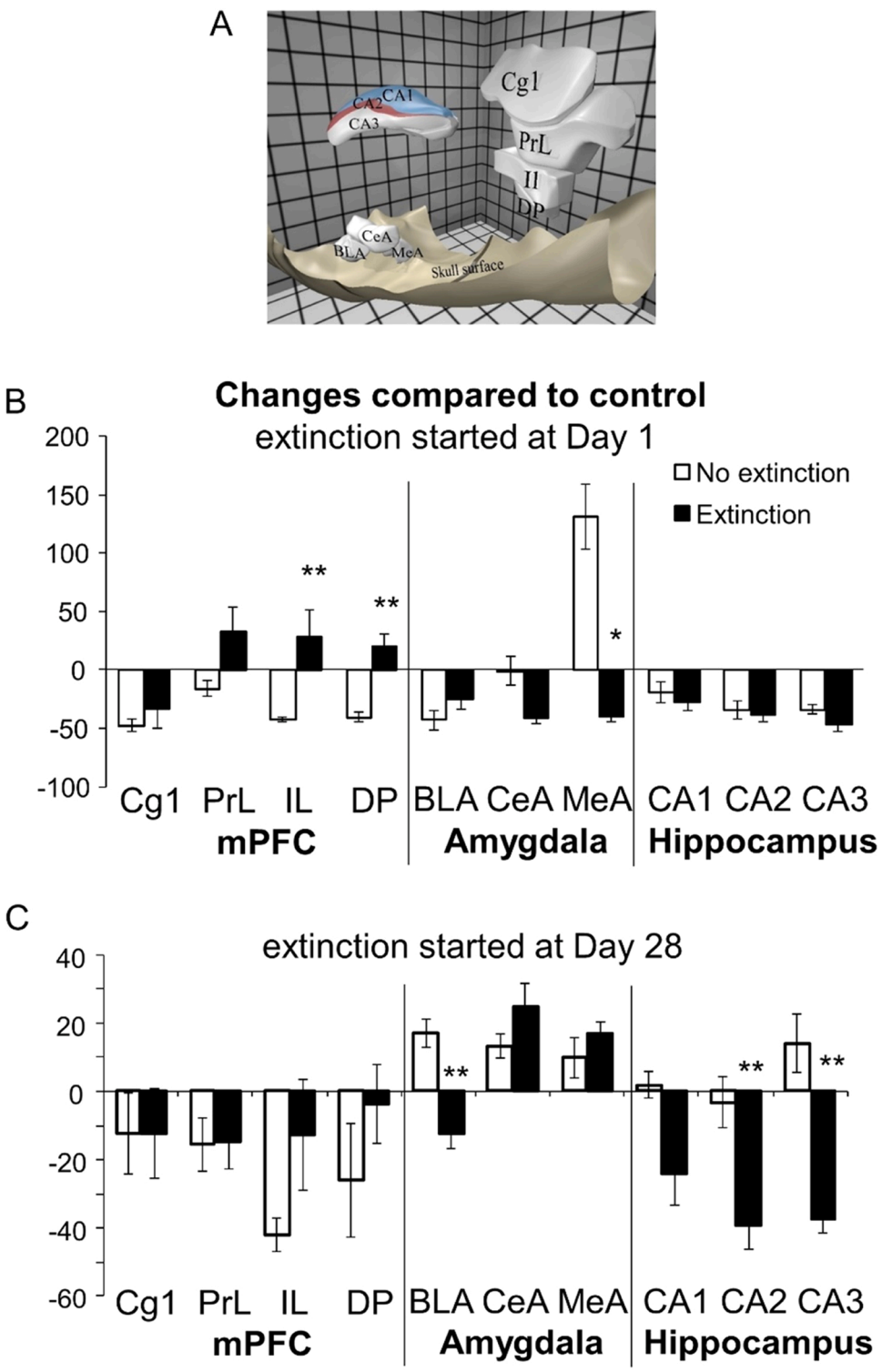

Fig. 5. c-Fos activation on different brain areas in comparison to non-traumatized groups. (A) Representation of the examined brain areas. Extinction initiated at D1 (B) increased context-induced neuronal activation in some prefrontal cortical regions while decreasing it in the amygdala and hippocampus, whilst extinction initiated at 28 days (C) after fear conditioning only reduced it in the hippocampus. Abbreviations: Parts of the medial prefrontal cortex: Cg1: cingulate cortex 1; PrL: prelimbic cortex; IL: infralimbic cortex; DP: dorsal peduncular cortex; CA1–3: cornu ammonis, different parts of the hippocampus; BLA: basolateral amygdala, CeA: central amygdala, MeA: medial amygdala, different parts of amygdala. n = 5/group * $p < 0.05$, ** $p < 0.01$ effect of extinction.

effect of immediate intervention was not as long-lasting as remote extinction therapy. However, a previous study already suggested that immediate extinction cannot prevent the recovery of an extinguished fear response [27]. Nevertheless, these findings, may aid in understanding the ineffectiveness of some early therapeutic interventions, such as Critical Incident Stress Management [17,66]. More research on the topic is required to fully elaborate this hypothesis.

Studies investigating victims of traumatic events have demonstrated the importance of stress hormone levels in PTSD [50,60]. ACTH has long been recognized as a modulator of fear extinction. Administration of this hormone can impair extinction of conditioned avoidance behavior [63], while its antagonizing can facilitate fear extinction [15]. These data suggested that ACTH might contribute even to relapse. In our hands, at the end of a contextual reminder, the ACTH levels were higher during and one day after the electric foot-shock-induced trauma than in the control group. The fear conditioning box-induced level of this hormone had decreased significantly by the end of the fear extinction training and had remained low three weeks later, indicating no relapse. Since,

Table 2
c-Fos activation to trauma-context, when the extinction training started on D1. Results of two-way ANOVA.

Brain area	Trauma	Extinction	Tr x Ext
Cg1	9.36	6.30	
PrL		6.86	
IL		10.93	4.80
DP		20.56	8.89
BLA	10.46	17.34	
CeA	14.35	31.77	13.60
MeA			26.90
CA1	6.09	10.73	
CA2	15.77	23.61	
CA3	20.14	20.88	

The $F_{(1,13)}$ values are given in the Table. Note, only significant changes are included, in case of non-significant changes the squares are left empty.

Table 3
c-Fos activation to trauma-context, when the extinction training started on D28. Results of two-way ANOVA.

Brain area	Trauma	Extinction	Tr x Ext
Cg1			
PrL	6.09	7.00	
IL	8.81		
DP			
BLA		24.13	14.51
CeA	15.03		
MeA		8.49	
CA1			4.40
CA2	13.47		10.19
CA3	9.08		29.09

The F(1,16) values are given in the Table. Note, only significant changes are included, in case of non-significant changes the squares are left empty.

contrary to freezing, the ACTH concentrations were unaffected by the start of the extinction protocol (D1 vs D28), this hormone does not appear to be a good predictor of later relapse and does not appear to contribute to it.

On the other hand, a growing body of evidence suggests that glucocorticoids positively correlate with fear extinction success by influencing fear memory formation and consolidation. [7]. Therefore, it was surprising that – although fear conditioning increased context-related corticosterone levels - no significant decrease of corticosterone was found after fear extinction. It is important to consider that corticosterone secretion shows ultradian rhythms [59], while we were concentrating on a single time point. Moreover, it follows ACTH changes with a considerable delay, thus, the chosen timepoint (right at the end of a contextual reminder, 5 min stimulation) may not be appropriate for its study. Therefore, further studies on the connections between corticosterone fluctuation and fear extinction are required to discern the reason behind our results. Nonetheless, it is clear that acutely detectable corticosterone levels are not the primary determinant of the animal's behavior during extinction, but they may have long-term consequences (i.e. affecting the behavior on the next day).

Our group earlier investigated the effect of previous fear conditioning on context-induced neuronal activation [62]. In contrast to previous results in mPFC now we have seen reduced neuronal activation both at D1 and D28, while previously at D28 an increased activation was detected (see Fig. 5, white columns). In terms of the BLA, we confirmed previously detected reduction at D1 and enhancement at D28. We also replicated the findings in HC, with decreased activation at D1 and a non-significant increase in activation at D28 following trauma.

The timing of extinction modulated mPFC activity differently, indicating its importance in the process (Exp.3, Fig. 5). In general, context-induced c-Fos activation was stimulated by extinction at D1 only, IL and DP regions being the most sensitive ones. These findings are consistent with current knowledge of fear extinction circuitry, in which the IL subregion can enhance extinction by inhibiting the amygdala [9].

Recent research found that a thalamo-amydalar pathway is implicated in remote, but not recent memory formation [56]. In contrast, orexin A, through regulation of the activity of the amygdala, could influence the retention of recent fear memory extinction, without affecting remote fear extinction [54]. In line with the divergent role of amygdala in recent and remote fear memory formation our c-Fos expression analysis showed a reduced activation after extinction with the D1 protocol. Interestingly, when the extinction training started 28 days after fear conditioning, it induced an activation of CeA and MeA compared to D1, with significant reduction in BLA compared to the non-extinction group. We might assume that these shifts contribute to the durability of the extinction. Indeed, previous studies has shown that manipulating the BLA can lead to a decreased fear relapse [48].

It has also been demonstrated that HC participates in fear extinction [11,12,2,19]. When we used a single acute reminder, HC showed reduced activation only 1 but not 28 days after fear conditioning supporting changes in neuronal network activity. On the contrary, the extinction-induced impairment was only visible with the D28 protocol. This is in line with previous studies indicating that HC regions are associated with relapse of contextual fear memory after fear extinction [57]. Moreover, we can interpret the low c-fos levels as a sign that this brain area is less sensitive to environmental challenges. It might be considered as a reflection of isolation, which is an important symptom of PTSD [64].

5. Limitations

Our study has certain limitations. To begin with, we only used male rats. Although masculinity is inconclusively associated with PTSD, in fact, there is a well-established 2:1 gender prevalence ratio favouring women [10,25,26]. Thus, females are more likely to require treatment by exposure therapy than male. Moreover, the underlying brain mechanisms might be also sex-dependent [33,39]. Thus, the impact of the examined parameters on females remains to be elucidated.

In addition, we kept the time between the exposure training and the examination of the relapse constant instead of focusing on the time from the trauma exposure. We cannot entirely close out the possibility that 56 days after trauma the D1 group would also show extinction. However, in previous studies both rats [47] and mice [8] showed progressive increase rather than spontaneous decrease in fear response over time (e.g. comparing 1-month with 2-month after fear conditioning). Moreover, relapse in rats was suggested to be influenced more by the extinction-test interval than the acquisition-extinction interval [27]. Indeed, in another rat study when extinction was started after conditioning the freezing gradually "recovered", which can be explained by the independent storage of conditioning and extinction memory of fear [51]. However, in support for the effectiveness of the early intervention we have to admit that our D1 extinction started 24 h after fear conditioning, which might be already out of the window of memory consolidation, another probably optimal timepoint for exposure therapy [53,5].

We must admit that in our present set of data at D28 the control group have higher levels than at D1, which was previously detected in some, but not all, brain regions. We took extra precautions to ensure that the data could be compared, such as processing brains from all groups on the same day and using the same conditions and frame for immunohistochemistry and analysis (all compared slides were processed simultaneously). The inconsistencies can be explained by individual variations and further studies should focus on confirming the results.

6. Conclusions

Surprisingly, remote fear memories were more susceptible to extinction than recent ones. Differences in reminder-induced brain activation patterns suggest network reorganization over time which may open a time-window for neuronal plasticity and enables successful fear

extinction. As remote extinction protocol resembles human situations more closely (e.g., treatment normally start after appearance of the symptoms) we suggest that preclinical studies should focus on this later time point, particularly when looking for pharmacological treatment or its combination with exposure therapy.

CRediT authorship contribution statement

Pedro Correia: Formal analysis, Writing – original draft, Visualization. **Kornél Demeter:** Formal analysis, Investigation, Writing – review & editing, Visualization. **János Varga:** Formal analysis, Investigation, Writing – review & editing. **Eszter Urbán**: Formal analysis, Investigation. **Bibiána Török:** Formal analysis, Investigation, Writing – review & editing. **Diána Balázsfi**: Formal analysis, Investigation. **József Haller**: Conceptualization, Writing – review & editing, Supervision. **Dóra Zelena**: Conceptualization, Resources, Writing – original draft, Supervision, Project administration.

Data Availability

Data will be made available on request.

Acknowledgement

This study was supported by the National Research Development and Innovation Office of Hungary (grant numbers K141934, K138763, and K120311), by the Thematic Excellence Program 2021 Health Subprogram of the Ministry for Innovation and Technology in Hungary, within the framework of the TKP2021-EGA-16 project of the Pécs of University as well as by NAP3 of Hungarian Academy of Sciences. The agencies had no further role in study design, and in the collection, analysis, or interpretation of the data. We are thankful to Éva Dobozi for her help with the radioimmunoassay and the István Ábrahám Nano-Bio-Imaging Centrum for kindly providing microscopy support.

References

[1] American, Psychiatric, & Association. (2013). Diagnostic and Statistical Manual of Mental Disorders (DSM-5®).
[2] M.C. Anderson, S.B. Floresco, Prefrontal-hippocampal interactions supporting the extinction of emotional memories: the retrieval stopping model, Neuropsychopharmacology 47 (1) (2022) 180–195, https://doi.org/10.1038/s41386-021-01131-1.
[3] Association, A.P. (2017). Clinical Practice Guideline for the Treatment of Posttraumatic Stress Disorder (PTSD) in Adults.
[4] X.L. Bian, C. Qin, C.Y. Cai, Y. Zhou, Y. Tao, Y.H. Lin, D.Y. Zhu, Anterior cingulate cortex to ventral hippocampus circuit mediates contextual fear generalization, J. Neurosci. 39 (29) (2019) 5728–5739, https://doi.org/10.1523/JNEUROSCI.2739-18.2019.
[5] J. Bjorkstrand, T. Agren, F. Ahs, A. Frick, E.M. Larsson, O. Hjorth, M. Fredrikson, Think twice, it's all right: long lasting effects of disrupted reconsolidation on brain and behavior in human long-term fear, Behav. Brain Res. 324 (2017) 125–129, https://doi.org/10.1016/j.bbr.2017.02.016.
[6] D.W. Bloodgood, J.A. Sugam, A. Holmes, T.L. Kash, Fear extinction requires infralimbic cortex projections to the basolateral amygdala, Transl. Psychiatry 8 (1) (2018) 60, https://doi.org/10.1038/s41398-018-0106-x.
[7] J. Blundell, C.A. Blaiss, D.C. Lagace, A.J. Eisch, C.M. Powell, Block of glucocorticoid synthesis during re-activation inhibits extinction of an established fear memory, Neurobiol. Learn Mem. 95 (4) (2011) 453–460, https://doi.org/10.1016/j.nlm.2011.02.006.
[8] A. Cardenas, M. Blanca, E. Dimitrov, Persistent pain intensifies recall of consolidated fear memories, Neurobiol. Stress 10 (2019), 100163, https://doi.org/10.1016/j.ynstr.2019.100163.
[9] J.H. Cho, K. Deisseroth, V.Y. Bolshakov, Synaptic encoding of fear extinction in mPFC-amygdala circuits, Neuron 80 (6) (2013) 1491–1507, https://doi.org/10.1016/j.neuron.2013.09.025.
[10] D.M. Christiansen, E.T. Berke, Gender- and sex-based contributors to sex differences in PTSD, Curr. Psychiatry Rep. 22 (4) (2020) 19, https://doi.org/10.1007/s11920-020-1140-y.
[11] C.D. Conrad, J.E. LeDoux, A.M. Magarinos, B.S. McEwen, Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy, Behav. Neurosci. 113 (5) (1999) 902–913, https://doi.org/10.1037//0735-7044.113.5.902.
[12] K.A. Corcoran, S. Maren, Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction, J. Neurosci. 21 (5) (2001) 1720–1726.
[13] Curzon, P., Rustay, N.R., & Browman, K.E. (2009). Cued and Contextual Fear Conditioning for Rodents. In J. J. Buccafusco (Ed.), Methods of Behavior Analysis in Neuroscience (2nd ed.). Boca Raton (FL).
[14] K. Cusack, D.E. Jonas, C.A. Forneris, C. Wines, J. Sonis, J.C. Middleton, B. N. Gaynes, Psychological treatments for adults with posttraumatic stress disorder: a systematic review and meta-analysis, Clin. Psychol. Rev. 43 (2016) 128–141, https://doi.org/10.1016/j.cpr.2015.10.003.
[15] D. De Wied, The influence of the posterior and intermediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats, Int J. Neuropharmacol. 4 (1965) 157–167, https://doi.org/10.1016/0028-3908(65)90005-5.
[16] J. Debiec, L. Diaz-Mataix, D.E. Bush, V. Doyere, J.E. Ledoux, The amygdala encodes specific sensory features of an aversive reinforcer, Nat. Neurosci. 13 (5) (2010) 536–537, https://doi.org/10.1038/nn.2520.
[17] A.A. van Emmerik, J.H. Kamphuis, A.M. Hulsbosch, P.M. Emmelkamp, Single session debriefing after psychological trauma: a meta-analysis, Lancet 360 (9335) (2002) 766–771, https://doi.org/10.1016/S0140-6736(02)09897-5.
[18] A.A. Feduccia, M.C. Mithoefer, MDMA-assisted psychotherapy for PTSD: are memory reconsolidation and fear extinction underlying mechanisms, Prog. Neuropsychopharmacol. Biol. Psychiatry, (2018) 221–228, https://doi.org/10.1016/j.pnpbp.2018.03.003, 84(Pt A).
[19] A. Fischer, F. Sananbenesi, C. Schrick, J. Spiess, J. Radulovic, Distinct roles of hippocampal de novo protein synthesis and actin rearrangement in extinction of contextual fear, J. Neurosci. 24 (8) (2004) 1962–1966, https://doi.org/10.1523/JNEUROSCI.5112-03.2004.
[20] C. Furini, J. Myskiw, I. Izquierdo, The learning of fear extinction, Neurosci. Biobehav Rev. 47 (2014) 670–683, https://doi.org/10.1016/j.neubiorev.2014.10.016.
[21] G.D. Gale, S.G. Anagnostaras, B.P. Godsil, S. Mitchell, T. Nozawa, J.R. Sage, M. S. Fanselow, Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats, J. Neurosci. 24 (15) (2004) 3810–3815, https://doi.org/10.1523/JNEUROSCI.4100-03.2004.
[22] T.F. Giustino, S. Maren, The role of the medial prefrontal cortex in the conditioning and extinction of fear, Front. Behav. Neurosci. 9 (298) (2015), https://doi.org/10.3389/fnbeh.2015.00298.
[23] S. Huang, Y. Zhou, F. Wu, C. Shi, H. Yan, L. Chen, Y. Luo, Berberine facilitates extinction and prevents the return of fear, Front Pharm. 12 (2021), 748995, https://doi.org/10.3389/fphar.2021.748995.
[24] S. Hugues, R. Garcia, Reorganization of learning-associated prefrontal synaptic plasticity between the recall of recent and remote fear extinction memory, Learn Mem. 14 (8) (2007) 520–524, https://doi.org/10.1101/lm.625407.
[25] H. Javidi, M. Yadollahie, Post-traumatic stress disorder, Int. J. Occup. Environ. Med. 3 (1) (2012) 2–9.
[26] D.G. Kilpatrick, H.S. Resnick, M.E. Milanak, M.W. Miller, K.M. Keyes, M. J. Friedman, National estimates of exposure to traumatic events and PTSD prevalence using DSM-IV and DSM-5 criteria, J. Trauma Stress 26 (5) (2013) 537–547, https://doi.org/10.1002/jts.21848.
[27] J.H. Kim, R. Richardson, Expression of renewal is dependent on the extinction-test interval rather than the acquisition-extinction interval, Behav. Neurosci. 123 (3) (2009) 641–649, https://doi.org/10.1037/a0015237.
[28] G. King, B.M. Graham, R. Richardson, Effects of d-cycloserine on individual differences in relapse of fear, Prog. Neuropsychopharmacol. Biol. Psychiatry 84 (Pt A) (2018) 115–121, https://doi.org/10.1016/j.pnpbp.2018.02.005.
[29] A.F. Lacagnina, E.T. Brockway, C.R. Crovetti, F. Shue, M.J. McCarty, K.P. Sattler, M.R. Drew, Distinct hippocampal engrams control extinction and relapse of fear memory, Nat. Neurosci. 22 (5) (2019) 753–761, https://doi.org/10.1038/s41593-019-0361-z.
[30] S.J. Li, L.X. Zhang, G.J. Zou, M.H. Ma, S.F. Zhou, X.Y. Lu, C.Q. Li, Infralimbic YTHDF1 is necessary for the beneficial effects of acute mild exercise on auditory fear extinction retention, Cereb. Cortex (2022), https://doi.org/10.1093/cercor/bhac174.
[31] N.W. Lingawi, V. Laurent, R.F. Westbrook, N.M. Holmes, The role of the basolateral amygdala and infralimbic cortex in (re)learning extinction, Psychopharmacol. (Berl.) 236 (1) (2019) 303–312, https://doi.org/10.1007/s00213-018-4957-x.
[32] J. Liu, M.S. Totty, L. Melissari, H. Bayer, S. Maren, Convergent coding of recent and remote fear memory in the basolateral amygdala, Biol. Psychiatry 91 (9) (2022) 832–840, https://doi.org/10.1016/j.biopsych.2021.12.018.
[33] T.A. Lovick, H. Zangrossi Jr., Effect of estrous cycle on behavior of females in rodent tests of anxiety, Front Psychiatry 12 (2021), 711065, https://doi.org/10.3389/fpsyt.2021.711065.
[34] S. Maren, G.J. Quirk, Neuronal signalling of fear memory, Nat. Rev. Neurosci. 5 (11) (2004) 844–852, https://doi.org/10.1038/nrn1535.
[35] S. Maren, C.H. Chang, Recent fear is resistant to extinction, Proc. Natl. Acad. Sci. USA 103 (47) (2006) 18020–18025, https://doi.org/10.1073/pnas.0608398103.
[36] S. Maren, A. Holmes, Stress and fear extinction, Neuropsychopharmacology 41 (1) (2016) 58–79, https://doi.org/10.1038/npp.2015.180.
[37] M.R. Milad, G.J. Quirk, Neurons in medial prefrontal cortex signal memory for fear extinction, Nature 420 (6911) (2002) 70–74, https://doi.org/10.1038/nature01138.
[38] M.R. Milad, S.L. Rauch, R.K. Pitman, G.J. Quirk, Fear extinction in rats: implications for human brain imaging and anxiety disorders, Biol. Psychol. 73 (1) (2006) 61–71, https://doi.org/10.1016/j.biopsycho.2006.01.008.
[39] M. Morena, A.S. Nastase, A. Santori, B.F. Cravatt, R.M. Shansky, M.N. Hill, Sex-dependent effects of endocannabinoid modulation of conditioned fear extinction in rats, Br. J. Pharm. 178 (4) (2021) 983–996, https://doi.org/10.1111/bph.15341.

[40] F.G. Morrison, K.J. Ressler, From the neurobiology of extinction to improved clinical treatments, Depress Anxiety 31 (4) (2014) 279–290, https://doi.org/10.1002/da.22214.
[41] L.J. Noble, I.J. Gonzalez, V.B. Meruva, K.A. Callahan, B.D. Belfort, K. R. Ramanathan, C.K. McIntyre, Effects of vagus nerve stimulation on extinction of conditioned fear and post-traumatic stress disorder symptoms in rats, Transl. Psychiatry 7 (8) (2017), e1217, https://doi.org/10.1038/tp.2017.191.
[42] S. Pan, S.R. Mayoral, H.S. Choi, J.R. Chan, M.A. Kheirbek, Preservation of a remote fear memory requires new myelin formation, Nat. Neurosci. 23 (4) (2020) 487–499, https://doi.org/10.1038/s41593-019-0582-1.
[43] D. Paredes, D.A. Morilak, A rodent model of exposure therapy: the use of fear extinction as a therapeutic intervention for PTSD, Front Behav. Neurosci. 13 (2019) 46, https://doi.org/10.3389/fnbeh.2019.00046.
[44] P.I. Pavlov, Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex, Ann. Neurosci. 17 (3) (2010) 136–141, https://doi.org/10.5214/ans.0972-7531.1017309.
[45] Paxinos, G., & Watson, C. (1998). A stereotaxic atlas of the rat brain. New York: Academic.
[46] R.G. Phillips, J.E. LeDoux, Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, Behav. Neurosci. 106 (2) (1992) 274–285.
[47] C.L. Pickens, S.A. Golden, T. Adams-Deutsch, S.G. Nair, Y. Shaham, Long-lasting incubation of conditioned fear in rats, Biol. Psychiatry 65 (10) (2009) 881–886, https://doi.org/10.1016/j.biopsych.2008.12.010.
[48] A. Pignataro, R. Pagano, G. Guarneri, S. Middei, M. Ammassari-Teule, Extracellular matrix controls neuronal features that mediate the persistence of fear, Brain Struct. Funct. 222 (9) (2017) 3889–3898, https://doi.org/10.1007/s00429-017-1440-0.
[49] R. Ponnusamy, I. Zhuravka, A.M. Poulos, J. Shobe, M. Merjanian, J. Huang, M. S. Fanselow, Retrieval and reconsolidation accounts of fear extinction, Front Behav. Neurosci. 10 (2016) 89, https://doi.org/10.3389/fnbeh.2016.00089.
[50] D.J. de Quervain, J. Margraf, Glucocorticoids for the treatment of post-traumatic stress disorder and phobias: a novel therapeutic approach, Eur. J. Pharm. 583 (2–3) (2008) 365–371, https://doi.org/10.1016/j.ejphar.2007.11.068.
[51] G.J. Quirk, Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery, Learn Mem. 9 (6) (2002) 402–407, https://doi.org/10.1101/lm.49602.
[52] G.J. Quirk, D. Mueller, Neural mechanisms of extinction learning and retrieval, Neuropsychopharmacology 33 (1) (2008) 56–72, https://doi.org/10.1038/sj.npp.1301555.
[53] D. Schiller, C.M. Raio, E.A. Phelps, Extinction training during the reconsolidation window prevents recovery of fear, J. Vis. Exp. 66 (2012), e3893, https://doi.org/10.3791/3893.
[54] L. Shi, W. Chen, J. Deng, S. Chen, Y. Han, M.Z. Khan, J. Shi, Orexin A differentially influences the extinction retention of recent and remote fear memory, Front Neurosci. 12 (2018) 295, https://doi.org/10.3389/fnins.2018.00295.
[55] 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 (2) (2011) 529–538, https://doi.org/10.1038/npp.2010.184.
[56] B.A. Silva, S. Astori, A.M. Burns, H. Heiser, L. van den Heuvel, G. Santoni, J. Graff, A thalamo-amygdalar circuit underlying the extinction of remote fear memories, Nat. Neurosci. 24 (7) (2021) 964–974, https://doi.org/10.1038/s41593-021-00856-y.
[57] F. Sotres-Bayon, C.K. Cain, J.E. LeDoux, Brain mechanisms of fear extinction: historical perspectives on the contribution of prefrontal cortex, Biol. Psychiatry 60 (4) (2006) 329–336, https://doi.org/10.1016/j.biopsych.2005.10.012.
[58] R.R. Souza, N.M. Robertson, C.K. McIntyre, R.L. Rennaker, S.A. Hays, M.P. Kilgard, Vagus nerve stimulation enhances fear extinction as an inverted-U function of stimulation intensity, Exp. Neurol. 341 (2021), 113718, https://doi.org/10.1016/j.expneurol.2021.113718.
[59] F. Spiga, J.J. Walker, J.R. Terry, S.L. Lightman, HPA axis-rhythms, Compr. Physiol. 4 (3) (2014) 1273–1298, https://doi.org/10.1002/cphy.c140003.
[60] P.R. Szeszko, A. Lehrner, R. Yehuda, Glucocorticoids and hippocampal structure and function in PTSD, Harv. Rev. Psychiatry 26 (3) (2018) 142–157, 10.1097/HRP.0000000000000188.
[61] B. Torok, E. Sipos, N. Pivac, D. Zelena, Modelling posttraumatic stress disorders in animals, Prog. Neuropsychopharmacol. Biol. Psychiatry 90 (2019) 117–133, https://doi.org/10.1016/j.pnpbp.2018.11.013.
[62] A. Tulogdi, P. Soros, M. Toth, R. Nagy, L. Biro, M. Aliczki, J. Haller, Temporal changes in c-Fos activation patterns induced by conditioned fear, Brain Res. Bull. 88 (4) (2012) 359–370, https://doi.org/10.1016/j.brainresbull.2012.04.001.
[63] T.B. v Wimersma Greidanus, D. de Wied, Effects of systemic and intracerebral administration of two opposite acting ACTH-related peptides on extinction of conditioned avoidance behavior, Neuroendocrinology 7 (5) (1971) 291–301, https://doi.org/10.1159/000121977.
[64] I.I. Vlachos, C. Papageorgiou, M. Margariti, Neurobiological trajectories involving social isolation in PTSD: a systematic review, Brain Sci. 10 (3) (2020), https://doi.org/10.3390/brainsci10030173.
[65] I. Wald, E. Fruchter, K. Ginat, E. Stolin, D. Dagan, P.D. Bliese, Y. Bar-Haim, Selective prevention of combat-related post-traumatic stress disorder using attention bias modification training: a randomized controlled trial, Psychol. Med. (2016) 1–10, https://doi.org/10.1017/S0033291716000945.
[66] U. Wesemann, M. Mahnke, S. Polk, A. Buhler, G. Willmund, Impact of crisis intervention on the mental health status of emergency responders following the Berlin Terrorist attack in 2016, Disaster Med. Public Health Prep. 14 (2) (2020) 168–172, https://doi.org/10.1017/dmp.2019.60.
[67] R. Yehuda, Biology of posttraumatic stress disorder, J. Clin. Psychiatry 62 (Suppl 17) (2001) 41–46.
[68] D. Zelena, B. Stocker, I. Barna, Z.E. Toth, G.B. Makara, Vasopressin deficiency diminishes acute and long-term consequences of maternal deprivation in male rat pups, Psychoneuroendocrinology 51 (2015) 378–391, https://doi.org/10.1016/j.psyneuen.2014.10.018.
[69] D. Zelena, E. Mikics, D. Balazsfi, J. Varga, B. Klausz, E. Urban, J. Haller, Enduring abolishment of remote but not recent expression of conditioned fear by the blockade of calcium-permeable AMPA receptors before extinction training, Psychopharmacol. (Berl.) 233 (11) (2016) 2065–2076, https://doi.org/10.1007/s00213-016-4255-4.
[70] X. Zhang, J. Kim, S. Tonegawa, Amygdala reward neurons form and store fear extinction memory, Neuron 105 (6) (2020) 1077–1093, https://doi.org/10.1016/j.neuron.2019.12.025, e1077.