Psychoneuroendocrinology 110 (2019) 104447

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Relationship between footshock intensity, post-training corticosterone release and contextual fear memory specificity over time

Moisés dos Santos Corrêa[a], Barbara dos Santos Vaz[a], Gabriel David Vieira Grisanti[a], Joselisa Péres Queiroz de Paiva[b], Paula Ayako Tiba[a], Raquel Vecchio Fornari[a,*]

[a] Centro de Matemática, Computação e Cognição (CMCC), Universidade Federal do ABC (UFABC), São Bernardo do Campo, SP, Brazil
[b] Brain Institute, Hospital Israelita Albert Einstein (HIAE), São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Glucocorticoids
Fear conditioning
Memory discrimination
Memory generalization
Stress
Systems consolidation

ABSTRACT

Overgeneralized fear has long been implicated in generalized anxiety and post-traumatic stress disorder, however, time-dependent mechanisms underlying memory retrieval are still not completely understood. Previous studies have revealed that stronger fear conditioning training protocols are associated with both increased post-training corticosterone (CORT) levels and fear responses at later retrieval tests. Here we used contextual fear conditioning (CFC) to investigate the relationship between post-training CORT levels and memory specificity in different retrieval timepoints. Wistar rats were exposed to CFC training with increasing footshock intensities (0.3, 0.6 or 1.0mA) and had their blood collected 30 min afterwards to measure post-training plasma CORT. After 2, 14 or 28 days, rats were tested for memory specificity either in the training or in the novel context. Regression analysis was used to verify linear and non-linear interactions between CORT levels and freezing. Higher footshock intensities increased post-training CORT levels and freezing times during tests in all timepoints. Moreover, stronger trainings elicited faster memory generalization, which was associated with higher CORT levels during memory consolidation. The 0.3mA training maintained memory specificity up to 28 days. Additionally, linear regressions suggest that the shift from specific to generalized memories is underway at 14 days after training. These results are consistent with the hypotheses that stronger training protocols elicit a faster generalization rate, and that this process is associated with increased post-training CORT release.

1. Introduction

The overgeneralization of fear is associated with generalized anxiety and post-traumatic stress disorder (Dymond et al., 2015; Lissek et al., 2014, 2011) and has recently become a target of intense investigation (Asok et al., 2019; Dymond et al., 2015; Laufer et al., 2016). Contextual fear conditioning (CFC) is a useful and widely recognized task to investigate contextual memory acquisition, consolidation and retrieval. The precision of the memory retrieval (i.e. memory specificity) changes over time and can be evaluated in proper designed CFC protocols, where trained animals are reexposed to either the training context or to a novel context that is slightly different from the original one (Bueno et al., 2017; Fanselow, 1980; Huckleberry et al., 2016; Pedraza et al., 2016; Poulos et al., 2016; Wiltgen et al., 2010; Wiltgen and Silva, 2007; Winocur et al., 2010; Xu and Südhof, 2013). Failure to discriminate between both contexts, as demonstrated by conditioned fear responses, provides a proxy of memory generalization (Hunsaker and Kesner, 2013, 2008; Kesner and Hunsaker, 2010; Rudy and O'Reilly, 1999). At short post-training intervals (1–7 days), fear memories are usually specific to the training context but later become generalized across different contexts (Biedenkapp and Rudy, 2007; de Oliveira Alvares et al., 2012; Haubrich et al., 2016; Wiltgen and Silva, 2007; Winocur et al., 2007). It is suggested that memory becomes more dependent of neocortical regions as time goes by, losing its dependence from the hippocampus. This reallocation is associated with a transformation in the strength and quality of memory, thus, context-dependent memories may transform to semantic-like memories, becoming less detailed and less vivid (i.e., generalized) (Asok et al., 2019; Frankland and Bontempi, 2005).

The consolidation of fear memories is modulated by glucocorticoids (GCs) — cortisol in humans and corticosterone (CORT) in rats (De Kloet et al., 1999; de Quervain et al., 2009; Finsterwald and Alberini, 2014; McGaugh and Roozendaal, 2002). Increasing the intensity of the CFC training protocol (i.e. current or number of footshocks) has been shown to elicit higher levels of post-training plasma CORT, which is associated with increased time spent on freezing in retrieval tests (Cordero et al.,

* Corresponding author.
E-mail address: raquel.fornari@ufabc.edu.br (R.V. Fornari).

https://doi.org/10.1016/j.psyneuen.2019.104447
Received 27 May 2019; Received in revised form 9 August 2019; Accepted 13 September 2019

1998; Marchand et al., 2007), suggesting that CORT enhances the consolidation of fear memory (Atsak et al., 2012; Cordero and Sandi, 1998; Reul and Kloet, 1985; Roozendaal et al., 2003). It is suggested that CORT acts on both mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in the hippocampus, amygdala and prefrontal cortex to enhance fear memory (Atsak et al., 2012; Cordero and Sandi, 1998; Reul and Kloet, 1985; Roozendaal et al., 2003). However, the role played by the MR and GR in the process of memory generalization remains unclear.

To date, few studies have explored the relationship between stress hormones and the specificity of contextual fear memories (Bueno et al., 2017; Pedraza et al., 2016). Post-training inhibition of plasma CORT levels with metyrapone has been shown to prolong memory specificity (Pedraza et al., 2016). Nevertheless, we have previously reported that the post-training administration of CORT did not affect memory specificity of a single-trial CFC task, in both recent and remote memory tests (Bueno et al., 2017). Hence, the association between footshock intensity and plasma CORT release during memory consolidation and their effect on fear memory specificity remains unclear, and we speculate that time-dependent memory generalization may be affected by the strength of the training protocol and later post-training release of CORT. Here, we investigated how increasing the intensity of footshocks affected post-training endogenous CORT and memory specificity at different retention intervals.

2. Methods

2.1. Subjects

Three-month old male Wistar rats, obtained from *Instituto Nacional de Farmacologia* (INFAR-UNIFESP (total n = 232; weighing between 275–385 g at time of training), were kept in controlled conditions of temperature (23 ± 2 °C) and light/darkness cycle of 12:12 h (light phase starting at 7am). Rats were housed in individual cages (20 cm × 16 cm × 18 cm) and provided with food and water *ad libitum*. The rats were adapted to the vivarium for at least one week before the beginning of the experiments. Training and testing were performed during the light phase of the cycle (between 10:00am-03:00pm), at the rat's nadir of the diurnal rhythm for CORT. All procedures were conducted according to the guidelines and standards of CONCEA - *Conselho Nacional de Controle de Experimentação Animal* (Brazilian Council of Animal Experimentation) and were previously approved by the Ethics Committee on Animal Use - UFABC (CEUA - protocol numbers 5676291015 and 7479070916). Each experiment was conducted with different groups of animals.

2.2. Apparatus

Behavioral experiments were conducted in two identical automated fear conditioning chambers (Med-Associates, Inc., St. Albans, VT), connected to a computer interface for video recording, analysis and measurement of the rat's freezing behavior in real time. The conditioning box (32 cm wide, 25 cm high and 25 cm deep, VFC-008) was surrounded by a sound attenuating chamber (63.5 cm × 35.5 cm × 76 cm, NIR-022SD) and illuminated by a LED light source (Med Associates NIR-100), which provided visible white light (450–650 nm) and invisible near-infrared light (NIR, 940 nm) spectra. A NIR video camera (VID-CAM-MONO-4 Fire Wire Video Camera) was attached to the front of the sound attenuating chamber, facing the transparent front wall of the conditioning chamber. The scrambled footshocks were administered through the grid floor of the cage (AC constant current), controlled by an Aversive Stimulator (ENV-414S). A general activity index was derived in real time from the video stream. A software (Video Freeze, Version 1.12.0.0, Med-Associates) performed real-time video recordings (30 frames per second) based on a threshold level (20 arbitrary units of movement), previously set and calibrated with the experimenters' freezing scores (r^2 = .997).

The training context (context A) was characterized by a grid floor composed of 20 stainless steel rods (diameter: 4.8 mm), top and front walls made of transparent polycarbonate, a back wall made of white acrylic, stainless-steel sidewalls and drop pan. The light in the conditioning box remained on and a background noise was emitted during the training and test sessions. Context A was cleaned with alcohol 10% before and after each session.

The novel context (Context B) consisted of the conditioning chamber and stainless-steel drop pan personalized with a grid floor (20 interleaved rods of either 4.8 or 9.5 mm of diameter) and white Plexiglas curved sidewalls extending across the back wall. The light in the box remained turned off and no white noise was emitted during the test. Context B was cleaned with a 5% acetic acid solution before and after each session. The dissimilarities between Contexts A and B were chosen according to previous studies that related tactile and olfactory stimuli as the most salient ones in discriminatory fear conditioning tasks (Fanselow, 1980; Huckleberry et al., 2016).

2.3. Behavioral procedures

Fig. 1 shows an overview of the experimental procedure. In all experiments, rats were randomly assigned to one of four experimental groups, balanced for mean body weight. Prior to the CFC training, rats were handled for 3 days, for 3 min each, in a room adjacent to the experimentation room. Rats were habituated to the adjacent room for at least 90 min before both training and testing

Two groups of animals were used as controls for this experimental design: the home-cage and the 0.0 mA. Rats in the home-cage group were subjected to handling, stayed in the adjacent room with the other animals, had their blood sample collected but did not undergo training. Animals in the 0.0 mA group did not receive any footshocks during the training session. On Day 1 (training session), rats (except the ones in the home-cage group) were individually transported from the adjacent room to the conditioning room and placed in context A. After 2 min of free exploration, three footshocks (0.3, 0.6 or 1.0 mA, according to their assigned group, 1 s each) were delivered with intervals of 30 s between them. One minute after the third shock, the animal was removed from the apparatus and returned to its home-cage and transported back to the adjoining room. Recent or remote memory tests were performed 2, 14 or 28 days after training. For each shock intensity group, half of the animals was exposed to context A and the other half to context B. Each

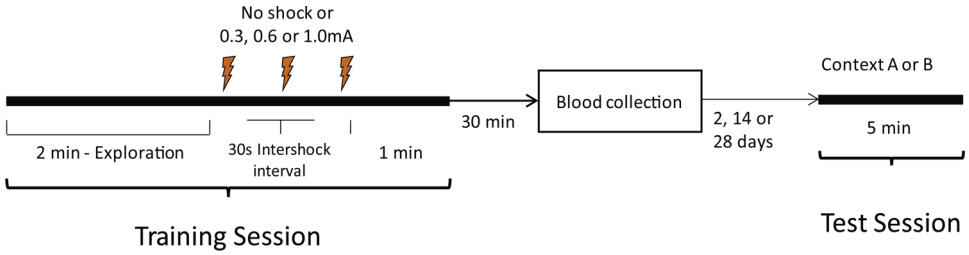

Fig. 1. Schematic representation of the experimental design.

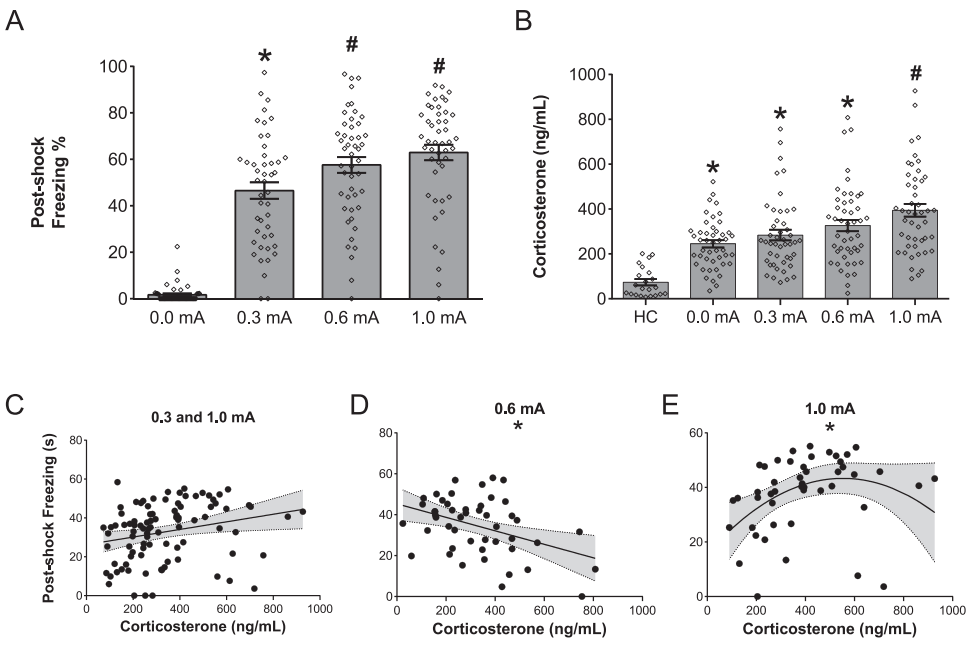

Fig. 2. Footshock intensity during CFC training is associated with post-footshock freezing levels and post-training plasma CORT. *(A) Freezing time percentage (mean ± S.E.M) during the last minute of the CFC training session.* N(0.0 mA) = 46, N (0.3 mA) = 46, N(0.6 mA) = 48, N (1.0 mA) = 47, symbols show data from each rat. *(*) $p < 0.01$ when compared to the 0.0 mA group. (#) $p < 0.05$ when compared to the 0.3 and 0.0 mA groups. (B) Post-training Corticosterone levels (mean ± S.E.M). (*) $p < 0.0001$ when compared to the Home-cage group. (#) $p < 0.01$ when compared to the 0.0 and 0.3 mA groups.* N(HC) = 22, N (0.0 mA) = 46, N(0.3 mA) = 46, N (0.6 mA) = 48, N(1.0 mA) = 47, symbols show data from each rat. *(C, D, E) Correlation between post-footshock freezing time (s) and post-training plasma CORT (ng/mL) of trained rats (0.3, 0.6 and 1.0 mA groups). Animals trained with 0.6 mA footshocks show a significant negative correlation ($r = -.35$, $p = 0.02$) whereas animals from group 1.0 mA show a significant inverted-U curve relationship* ($F_{(1,44)} = 5.00$, $p = 0.03$, $r^2 = 0.15$).

test lasted 5 min and no shock was delivered.

2.4. Blood collection and plasma corticosterone quantification

Thirty minutes after the training – interval in which CORT reaches its peak release after a stressful event (Vahl et al., 2005) – 500 µL of blood was collected from each rat through the tail-clip method (Kim et al., 2018). We first sprayed the rat's tail with lidocaine 2% (anesthetic) to clip its most distal portion (roughly 0.5 mm). The tail was carefully manipulated by the experimenter so that droplets of blood accumulated at its tip and were collected using EDTA-covered tubes. The tail tip was then treated with a balm of ketoprofen (anti-inflammatory and analgesic) and rifampicin (antibiotic).

The blood samples were centrifuged at 2300 rpm for 20 min at 4 °C. The extracted plasma was kept at −20 °C until determination of hormone concentrations. Plasma CORT was measured using ELISA (enzyme-linked immunosorbent assay), which allows detection of specific antibodies in blood plasma using the Corticosterone Enzyme Immunoassay Kit (Arbor Assays LLC, MI, USA). This kit is supplied with clear plastic microtiter plates coated with donkey anti-sheep IgG, sheep polyclonal antibody specific for CORT, a vial of CORT at 100,000 pg/mL to be used as a standard, and the CORT-peroxidase conjugate. The ELISA procedure was conducted according to the manufacturer's instructions. All samples were analyzed in duplicates. Later optical densities from the samples were analyzed using the Epoch Spectrophotometer system (BioTek Instruments, Inc.). CORT sample concentrations were calculated using the Arbor Assays DetectX® Corticosterone (OD) software. According to the protocol in the ELISA kit, the sensitivity of the essay was determined as 18.6 pg/mL, the intra-assay coefficient of variation was in average less than 10% and the inter-assay coefficient of variation was less than 15%. ELISA standard curves were also adequate ($r^2 = 0.955–0.995$).

2.5. Statistical analysis

The conditioned fear response to context was quantified as the percent time the animal spent freezing in context A, whereas the percent time of freezing in context B during the recall test was considered as a measure of generalization. Behavioral results are expressed as the group mean percent freezing time ± standard error of the mean (S.E.M). Post-training plasma CORT levels are expressed as the group mean ± S.E.M.

To satisfy the requirements for the use of the ANOVA, the total freezing times and plasma CORT levels were transformed using the square root transform function (Lix et al., 1996; Tukey, 1957) to improve data homogeneity and normality, as ascertained by Kolmogorov-Smirnov and Levene tests. The Grubbs test for outliers was used to determine extreme outliers. The transformed data was then analyzed with either a one or two-ways ANOVAs. The Student-Newmann-Keuls (SNK) *post hoc* test was further used to identify significant differences when applicable. Significance was set at $p < 0.05$. For the replication experiment, the total freezing times and plasma CORT levels were analyzed using a Student *t*-test for 2 independent samples, comparing means between no-shock and 0.6 mA groups (CORT) or between 0.6 mA groups exposed to A or B (Behavior). The effect sizes (Cohen's d or ω^2) were reported when the parametric test was found significant ("d" or "ω^2" values above 0.8 and 0.14, respectively, are considered large effects; values between 0.5 and 0.8 ("d") or 0.06 and 0.14 ("ω^2") are considered moderate; and below 0.5 or 0.06, small). Significance was set at $p < 0.05$.

We analyzed the correlation between post-shock freezing times and plasma CORT levels using Spearman product-moment correlational coefficients. Associations between post-training plasma CORT and total freezing time during the test sessions were also analyzed. Significance was set at $p < 0.05$. Linear and quadratic regression algorithms were also tested for the training and test data. We chose *a priori* to only test the hierarchical regression for the groups that showed significant Spearman correlations between the variables. The quadratic function ($Y' = a + b_1X_1 + b_2X_1^2$) is a second order polynomial regression representing the inverted U-shape model, which describes a parabola where Y' is the expected CORT level and X_1 is the observed freezing time. Higher order regressions have not been tested. Regressions coefficients of the quadratic models were considered only if the regression analysis of variance (ANOVA) was significant at $p < 0.05$. The model which explained most of the variance (change in $r^2 > 0.07$) is given in the figures and was performed according to previous studies in the literature that investigate the relationship between memory performance and endogenous CORT levels (Lubec and Korz, 2016; McCullough et al., 2015).

3. Results

3.1. CFC training and post-training plasma corticosterone levels

During training, animals that were exposed to more intense footshocks displayed more freezing on the last minute of the session (after the footshocks, Fig. 2A). This result was confirmed by a 1-way ANOVA that indicated a significant group effect [$F_{(3, 183)} = 151.18$, $p < 0.01$, $\omega^2 = 0.71$, Fig. 2(A)]. The SNK *post-hoc* tests indicate that all trained groups showed greater freezing levels when compared to the no-shock group ($p < 0.01$). In addition, the 0.3 mA group expressed less freezing compared to the animals in groups 0.6 ($p < 0.05$) and 1.0 mA ($p < 0.01$).

Fig. 2(B) shows the post-training CORT levels. One-way ANOVA showed a significant effect [$F_{(4, 204)} = 26.56$, $p < 0.01$, $\omega^2 = 0.33$]. The *post-hoc* tests indicated that all groups exposed to the conditioning chamber showed higher plasma CORT levels when compared to the homecage group ($p < 0.01$). Moreover, the 1.0 mA group had higher CORT levels compared to the 0.3 ($p < 0.01$) and no-shock ($p < 0.01$) groups, and the 0.6 mA group was not significantly different from the no-shock ($p = 0.09$), 0.3 mA ($p = 0.26$) or 1.0 mA groups ($p = 0.07$).

In order to verify a possible relationship between behavioral and physiological responses to the footshocks, the post-footshocks freezing time and post-training plasma CORT levels from each animal were analyzed for bivariate correlations using the Spearman test. The analysis showed a negative correlation when using the data from the 0.6 mA group [$r(46) = -.35$, $p = 0.02$, $r^2 = 0.12$], whereas data from the 1.0mA group showed a positive correlation [$r(45) = .41$, $p = 0.00$, $r^2 = 0.17$]. When tested together, data from the 0.3mA and 1.0mA groups showed a positive correlation [$r(91) = .34$, $p = 0.01$, $r^2 = 0.11$]. Table 1 shows the correlation results. In each set, the Grubbs test revealed no outlier data for either freezing time or post-training plasma CORT. The analysis revealed a significant linear relationship between post-shocks freezing and plasma CORT in the 0.6 mA group [$F_{(1,45)} = 2.23$, $p = 0.14$, $r^2 = 0.17$] that fits the data better than the quadratic model [$r^2 = 0.21$, change in $r^2 = 0.04$]. For the 1.0 mA group, on the other hand, the analysis revealed a significant quadratic relationship between the post-training plasma CORT levels and post-shocks freezing time [$F_{(1,44)} = 5.00$, $p = 0.03$, $r^2 = 0.15$] that fits the data significantly better than the linear model [$r^2 = 0.06$, change in $r^2 = 0.09$]. Finally, for the combination of the 0.3 and 1.0 mA groups, the analysis revealed a weak linear relationship [$F_{(1,90)} = 3.40$, $p = 0.07$, $r^2 = 0.06$] that fits the data better than the quadratic model [$r^2 = 0.09$, change in $r^2 = 0.03$]. Fig. 2(C, D, E) shows the linear and quadratic fits for these groups.

In summary, stronger footshock intensities elicit higher post-shock freezing times and higher post-training plasma CORT levels. In addition, post-shock freezing times and post-training CORT, which are markers of behavioral and physiological responses to the CFC training, are correlated but do not necessarily follow a positive, linear association.

Table 1
Spearman correlations between post-shock freezing times and post-training CORT levels according to footshock intensity.

Group	Correlation	p-value	N
Trained rats (0.3, 0.6, 1.0)	0.12	0.16	141
0.3 and 1.0	**0.34**	**0**	**93**
0.3 and 0.6	−0.1	0.32	94
0.6 and 1.0	−0.08	0.47	95
0.3	0.09	0.57	46
0.6	**−0.35**	**0.02**	**48**
1.0	**0.41**	**0**	**47**

Note. Data in bold are significant Spearman correlations.

3.2. CFC specificity tests

Two days after training, each group had some of the animals exposed to the training context (Context A) and the others exposed to a novel context (Context B, Fig. 3A). The Grubbs test did not find any outliers across groups. The 2-way ANOVA (Footshock × Context) showed a significant effect for Footshock [F (3, 55) = 38.35, $p < 0.01$, $\omega^2 = 0.56$], Context [F (1, 55) = 16,04, $p < 0.01$, $\omega^2 = 0.08$] and the interaction between Footshock and Context [F (3, 55) = 3.99, $p < 0.01$, $\omega^2 = 0.05$]. The *post-hoc* test revealed that all groups of trained animals displayed higher freezing times when compared to the no-shock control group (0.0 mA), in both contexts ($p < 0.05$). For animals tested in context A, the 0.3 mA group presented lower freezing times than the 0.6 mA and 1.0 mA groups ($p < 0.01$), whereas there was no significant difference between these last two groups ($p = 0.50$). For animals tested in context B, there was no significant difference in freezing times between the different trained groups ($p > 0.1$). The SNK test also showed that non-trained animals and the 0.3 mA groups tested in context A had freezing times similar to their counterparts tested in context B ($p = 0.82$ and $p = 0.37$, respectively), whereas animals from the 0.6 and 1.0 mA groups exposed to context A showed higher freezing times compared to their counterparts exposed to context B ($p < 0.01$).

Another set of animals was tested 14 days after training (Fig. 3B). At this time-point, the two-way ANOVA showed a significant effect of Footshock [$F_{(3, 56)} = 51.42$, $p < 0.01$, $\omega^2 = 0.61$], Context [$F_{(1, 56)} = 24.13$, $p < 0.01$, $\omega^2 = 0.11$], and interaction between Footshock and Context [$F_{(3, 56)} = 2.92$, $p = 0.04$, $\omega^2 = 0.02$]. For animals tested in context A, the post-hoc test revealed that all trained animals had higher freezing times when compared to the no-shock group ($p < 0.01$). Moreover, the trained groups showed a training intensity-response curve, where animals trained with 1.0 mA had higher freezing than those trained with 0.6 mA ($p < 0.05$) and 0.3 mA ($p < 0.01$) and rats trained with 0.6 mA had higher freezing compared to the 0.3mA group ($p < 0.05$). Among animals exposed to context B, only those trained with 0.6 or 1.0 mA had higher freezing than the non-trained animals ($p < 0.01$). The SNK test also showed that animals in groups 0.0 and 0.6mA presented similar freezing times in either contexts (A or B, $p = 0.64$ and $p = 0.14$, respectively), whereas animals from the 0.3 and 1.0mA groups, exposed to context A had higher freezing time compared to their counterparts exposed to context B ($p < 0.01$).

The last set of animals was tested 28 days after training (Fig. 3C). The two-way ANOVA showed a significant effect of Footshock [$F_{(3, 52)} = 76.62$, $p < 0.01$, $\omega^2 = 0.76$], Context [$F_{(1, 52)} = 6.21$, $p = 0.02$, $\omega^2 = 0.02$], and interaction between Footshock and Context [$F_{(3, 52)} = 3.36$, $p = 0.03$, $\omega^2 = 0.02$]. The post-hoc test showed that, for animals tested in context A, all trained animals had higher freezing when compared to the no-shock group ($p < 0.01$) and the trained groups also displayed an intensity-response curve, where the 1.0mA group showed higher freezing time than the other two groups ($p < 0.05$), although the 0.6mA group did not differ from group 0.3mA ($p = 0.42$). For animals exposed to context B, only those trained with 0.6 or 1.0 mA showed higher freezing times than the no-shock group ($p < 0.01$). The SNK test also showed that animals trained with 0.6 or 1.0 mA presented similar freezing times in both contexts ($p = 0.36$ and $p = 0.46$, respectively), whereas the 0.3mA group exposed to context A showed higher freezing time compared to its counterpart exposed to context B ($p < 0.01$).

The post-training plasma CORT levels and freezing times during CFC test from each animal were analyzed for bivariate correlations using the Spearman test. All groups were tested together or separately, in each time point and context to which animals were exposed to (Table 2). When analyzing data from animals tested in context B, 14 days after training, there is a positive correlation between CORT and freezing times [$r(23) = .50$, $p = 0.01$, $r^2 = 0.25$]. As performed earlier with the training data, we analyzed non-linear interactions for the test

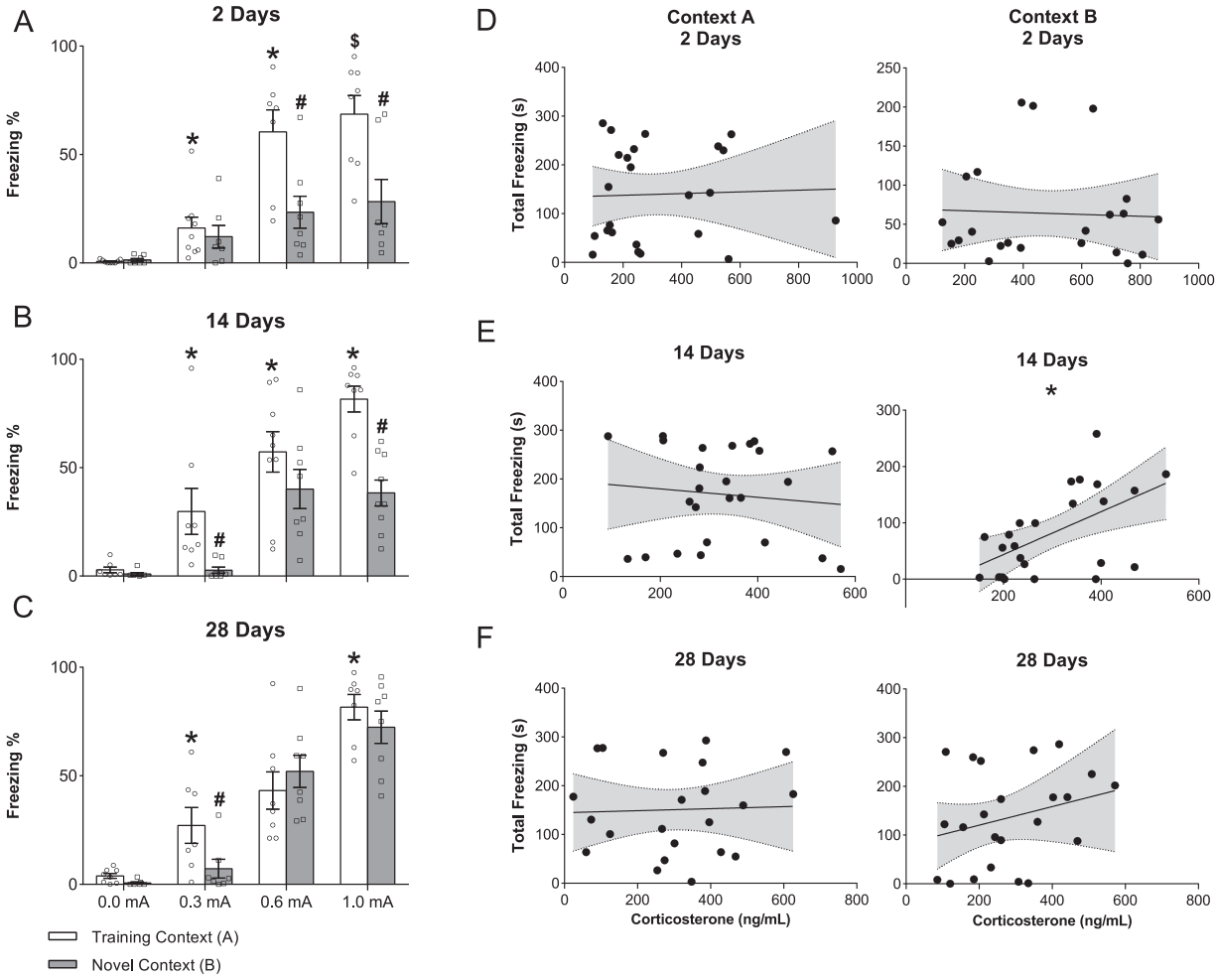

Fig. 3. Memory retrieval tests at different timepoints. *(A, B, C) Freezing time percentage (mean ± S.E.M) in context A or B during retrieval tests performed at 2, 14 or 28 days after training, respectively. (*) $p < 0.05$ when compared to rats trained in all lower footshock intensities and no shock. (#) $p < 0.05$ when compared to the group trained with the same footshock intensity but tested in context A. ($) $p < 0.05$ when compared to the no shock and 0.3 mA groups exposed to context A. (D, E, F) Correlations between post-training plasma CORT (ng/mL) and total freezing time (s) of all trained rats (0.3, 0.6 and 1.0 mA groups) tested in context A or B at 2, 14 or 28 days after training, respectively. A significant positive correlation was found only between animals that were exposed to the Context B14 days after training ($r = .5$, $p = 0.01$). The number of animals are as follow, 2 days (A):* N(0.0/A) = 9, N(0.0/B) = 8, N(0.3/A) = 9, N(0.3/B) = 7, N(0.6/A) = 7, N(0.6/B) = 8, N(1.0/A) = 8, N(1.0/B) = 7; 14 days (B): N(0.0/A) = 7, N(0.0/B) = 7, N(0.3/A) = 8, N(0.3/B) = 8, N(0.6/A) = 9, N(0.6/B) = 8, N(1.0/A) = 8, N(1.0/B) = 9; 28 days (C): N(0.0/A) = 8, N(0.0/B) = 7, N(0.3/A) = 7, N(0.3/B) = 7, N(0.6/A) = 8, N(0.6/B) = 8, N(1.0/A) = 7, N(1.0/B) = 8. *Symbols show data from each rat.*

data using the same hierarchical regression to evaluate whether data followed a linear or quadratic fit. In this set of animals, the Grubbs test for outliers revealed one outlier for CORT [$G = 2.88$, $p < 0.05$], which was removed from the hierarchical analysis. This analysis revealed a linear relationship between total freezing time and post-training plasma CORT for the 14 days, Context B group [$F_{(}1,21_{)} = 0.56$, $p = 0.46$, $r^2 = 0.30$] that fit the data significantly better than the quadratic model [$r^2 = 0.32$, change in $r^2 = 0.02$]. Fig. 3(D, E, F) shows the correlation and linear fits for all trained groups exposed to context A or B.

In brief, animals trained with a low CFC training intensity (0.3 mA) and tested 14 or 28 days after training, appear to show, at the group level, a discriminative fear response to the training context. On the other hand, animals submitted to higher training intensities (0.6 and 1.0 mA) seem to present a generalized fear response after 28 days. Post-training CORT levels are linearly associated with freezing times showed by the animals exposed to context B at the 14 day interval, suggesting that generalized freezing behavior may be associated with higher CORT levels released during memory consolidation.

3.3. Replication of the 0.6 mA group (moderate CFC)

Due to the large variability of the 0.6 mA groups exposed to context A or B at 14 days post-training, this experiment was replicated with a new set of animals (N = 23), tested 14 days later, when half was exposed to context A, and the other half to context B. A no-shock group of 9 animals was used as a control for the post-training plasma CORT levels. The Student *t*-test showed a significant increase in plasma CORT levels for the 0.6 mA group [$t(30) = -2.39$, $p < 0.05$, $d = -0.94$]. Moreover, animals trained with 0.6 mA and tested in context A presented higher freezing times than their counterparts tested in context B [$t(21) = 6.04$, $p < 0.01$, $d = 2.47$), Table 3].

4. Discussion

Our findings indicate that the intensity of the footshock in the CFC training is associated with the rate of time-dependent generalization of fear memories. This effect may be due, although not entirely dependent, to post-training plasma CORT (Kaouane et al., 2012; Pedraza et al., 2016). The results from the training sessions partially replicate those from Cordero et al. (1998), which suggested a linear relationship

Table 2
Spearman correlations according to shock intensity, timepoint and test context.

Timepoint	Group	Context A			Context B		
		Correlation	p-value	N	Correlation	p-value	N
2 days	Trained rats (0.3, 0.6, 1.0)	0.07	0.76	24	−0.05	0.83	22
	0.3 and 1.0	0.22	0.4	17	−0.06	0.84	14
	0.3 and 0.6	−0.24	0.38	16	−0.17	0.55	15
	0.6 and 1.0	−0.21	0.45	15	−0.05	−0.86	15
	0.3	−0.42	0.27	9	−0.46	0.29	7
	0.6	−0.25	0.59	7	−0.05	0.91	8
	1.0	−0.55	0.16	8	−0.18	0.7	7
14 days	Trained rats (0.3, 0.6, 1.0)	−0.09	0.67	25	**0.5**	**0.01**	**25**
	0.3 and 1.0	−0.19	0.48	16	**0.5**	**0.04**	**17**
	0.3 and 0.6	−0.09	0.74	17	0.4	0.13	16
	0.6 and 1.0	−0.12	0.65	17	0.47	0.06	17
	0.3	−0.33	0.42	8	0.05	0.91	8
	0.6	0.07	0.87	9	0.19	0.65	8
	1.0	−0.6	0.12	8	0.53	0.14	9
28 days	Trained rats (0.3, 0.6, 1.0)	0.06	0.81	22	0.28	0.2	23
	0.3 and 1.0	0.13	0.65	14	0.36	0.19	15
	0.3 and 0.6	−0.09	0.75	15	0.34	0.22	15
	0.6 and 1.0	0.08	0.78	15	0.06	0.24	16
	0.3	0.32	0.48	7	0.13	0.79	7
	0.6	−0.25	0.55	8	−0.05	0.91	9
	1.0	0.14	0.76	7	0.45	0.26	8

Note. Data in bold are significant Spearman correlations.

Table 3
Post-training plasma CORT levels of the replication experiment.

Group	Corticosterone ± S.E.M (ng/mL)	N
0.0 mA	128.5 ± 21.24	9
0.6 mA	232.2 ± 29.64*	24
Freezing times during the retrieval tests at 14 days after the training		
Group	Freezing time ± S.E.M (%)	N
0.6 mA Context A	69.41 ± 4.08	12
0.6 mA Context B	29.97 ± 5.10*	12

Note. (*) $p < 0.05$ when compared to the control groups.

between post-training plasma CORT levels and post-shock freezing times during CFC training (Cordero et al., 1998). Our data, however, only points to a linear relationship between CORT and freezing if we ignore the animals that underwent a moderate intensity training (0.6 mA group). Indeed, when we restrict our analysis to individual subsets (0.6 or 1.0 mA), the relationship found between post-shock freezing times and plasma CORT follows different trendlines (i.e. negative linear and inverted u-curve, respectively). Our evidence of a non-linear interaction between these variables is strengthened by the study of Marchand et al. (2007), whose results did not corroborate a positive linear correlation between post-shock freezing levels during the training session and the post-training plasma CORT levels (Marchand et al., 2007). We should note, however, that their study did not contemplate the analysis of quadratic interactions between the variables. Furthermore, our results show that, on average, rats that released moderate amounts of CORT displayed higher freezing times during the last minute of the CFC training, whereas lower or higher CORT levels were associated to lower post-shock freezing times. Hence, it is possible to speculate that the relationship between CORT and CFC training intensity follows a more complex trend than a simple linear relationship.

We also found that increasing training intensity elicits higher freezing times during retrieval tests when animals are re-exposed to the training context even at remote timepoints, which corroborate the findings from previous studies (Cordero et al., 1998; Kaouane et al., 2012; Pedraza et al., 2016; Poulos et al., 2016). Norepinephrine (Atucha et al., 2017; Gazarini et al., 2015, 2013; Gold and Van Buskirk, 1976, 1975) and CORT (Abrari et al., 2009; Kaouane et al., 2012) have been assumed to enhance memory consolidation, following an inverted U-curve dose-effect relation. It is possible, therefore, that stronger CFC protocols induce stronger activation from the hypothalamus-pituitary-adrenal (HPA) axis and noradrenergic system. Moreover, our data show that the temporal rate of generalization can be modulated just by increasing footshock intensity during training, without changing the number of footshocks. This result is in line with the study of Pedraza and colleagues (2016), who reported that training protocols with more and stronger footshocks lead to early memory generalization. Curiously, in our study, the two subsets of rats submitted to the lowest intensity training protocol (0.3 mA) presented similar freezing times (albeit significantly different from the no-shock groups) in contexts A or B, when tested 2 days after training. This could reflect a sensitization to all novel situations due to the recentness of the training session, instead of an error of retrieval (Richardson, 2000). This alternative explanation to recent memory generalization is supported by the fear responses of rats trained with 0.3 mA footshocks and tested after 14 or 28 days, showing group-level discrimination between contexts A and B. Moreover, rats exposed to context B at the remote timepoints presented similar freezing times to the no-shock groups tested in the same context.

It is worth noting that the only significant linear relationship between freezing times and post-training plasma CORT occurred at 14 days (post-training) in animals exposed to context B. Some studies have shown that, after high intensity CFC training, animals tend to generalize across distinct contexts when tested between 15 and 28 days after training (Pedraza et al., 2016; Poulos et al., 2016; Wiltgen and Silva, 2007). Thus, our results suggest that the process of time-dependent generalization of fear in novel contexts may begin or be underway around this time point, even though we can only infer that for the group and not at the individual level. Moreover, this generalization process seems to be linearly correlated to the post-training plasma CORT levels (i.e. lower CORT levels elicit low freezing times in context B, whereas higher CORT levels elicit higher freezing times).

According to the systems consolidation theory, memories change dependence from hippocampus to neocortical areas overtime, which is usually associated with time-dependent generalization (Biedenkapp and Rudy, 2007; Frankland, 2004; Wang et al., 2009; Wiltgen et al., 2010; Wiltgen and Silva, 2007). The rate by which this transfer occurs is thought to be modulated by the intensity of the training protocol and possibly, according to our results, by the levels of plasma CORT released afterwards. It is important to point out, however, that this is a correlational study, hence different cognitive and molecular

mechanisms - other than post-training CORT release - may also be involved in the modulation of fear memory generalization. Other neurotransmitters, such as norepinephrine and endocannabinoids, may also play a role in this process (Atsak et al., 2015; Atucha et al., 2017; Gazarini et al., 2013), as well as other mechanisms involving the hippocampus (Restivo et al., 2009; Revest et al., 2010), amygdala (Dunsmoor et al., 2011; Ghosh and Chattarji, 2015), and the prefrontal cortex (Matos et al., 2019; Xu and Südhof, 2013). Besides, adult neurogenesis in the dentate gyrus has been associated with precise memories (Besnard and Sahay, 2016). Lastly, appraisal of salient stimuli (Menon and Uddin, 2010; Uddin, 2014) could also be relevant for the consolidation of a precise contextual memory trace. Nonetheless, there is evidence that CORT also modulates many of these mechanisms (Abrari et al., 2009; Asok et al., 2019; Finsterwald and Alberini, 2014; Fornari et al., 2012; Kerr et al., 1994; LaBar and Cabeza, 2006). An evidence that CORT may have a role in modulating the rate of time-dependent generalization was suggested by Pedraza et al. (2016) who have observed prolonged contextual fear memory specificity after inhibiting the glucocorticoid synthesis with metyrapone during a strong CFC training. Therefore, it is possible that the modulatory role of CORT and/or other mechanisms in memory generalization requires simultaneous but different processes in the prefrontal cortex, hippocampus, amygdala and other brain regions (i.e. complementary learning systems — McClelland et al., 1995; Norman, 2010; O'Reilly et al., 2014; O'Reilly and Norman, 2002).

Many previous studies have successfully tested memory discrimination by presenting different groups of rats to either context A or B (Baldi et al., 2004; Biedenkapp and Rudy, 2007; Haubrich et al., 2016; Pedraza et al., 2016; Poulos et al., 2016). A possible limitation for this protocol, however, is that rats were never tested in both contexts. Presenting animals to both contexts in a counterbalanced order could be a more direct way to evaluate contextual memory specificity (Bueno et al., 2017; Huckleberry et al., 2016; Poulos et al., 2016; Wang et al., 2009; Wiltgen and Silva, 2007). Nevertheless, our protocol was chosen to avoid any possible influence of memory reactivation induced by reexposure to the training context.

In conclusion, our study sheds light on a possible role for CORT in modulating time-dependent memory generalization, probably associated with the process of systems consolidation. Plasma CORT levels and freezing time elicited by the CFC training seem to follow a more complex relationship than the previously thought linear correlation. In addition, our findings support the hypothesis that the CFC training intensity modulates the rate of time-dependent generalization, with higher intensities eliciting faster generalization and lower intensities prolonging memory specificity. Finally, the interval between 14 and 28 days after training may be a tipping time period for memory transformation modulated by post-training plasma CORT levels, being the shift from specific to generalized memories around 14 days after training.

Author statement

MdSC helped conceptualizing, curating data, doing formal analysis, doing experiments, planning methodology and writing the original draft. BdSV and GDVG helped doing experiments and reviewing the manuscript. JPQP helped doing formal analysis and reviewing the manuscript. PAT and RVF helped conceptualizing, acquiring funds, planning methodology, administrating and supervising the project and reviewing the manuscript. The authors would like to thank the members of the Memory and Learning research group at UFABC (MANAs) and the reviewers for the comments and suggestions that contributed to improve several aspects of this study.

Funding

This work was financially supported by grants # 2011/10062-8(PAT), # 2015/26983-6(MdSC), # 2017/03820-0(RVF), São Paulo Research Foundation (FAPESP). Students MdSC, GDVG and BdSV were partially funded by grants from *Universidade Federal do ABC*.

Declaration of Competing Interest

The authors report no conflicts of interest in this work.

References

Abrari, K., Rashidy-Pour, A., Semnanian, S., Fathollahi, Y., 2009. Post-training administration of corticosterone enhances consolidation of contextual fear memory and hippocampal long-term potentiation in rats. Neurobiol. Learn. Mem. 91, 260–265. https://doi.org/10.1016/j.nlm.2008.10.008.

Asok, A., Kandel, E.R., Rayman, J.B., 2019. The neurobiology of fear generalization. Front. Behav. Neurosci. 12, 1–15. https://doi.org/10.3389/fnbeh.2018.00329.

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., Fornari, R.V., Roozendaal, B., 2015. Endocannabinoid signaling within the basolateral amygdala integrates multiple stress hormone effects on memory consolidation. Neuropsychopharmacology 40, 1485–1494. https://doi.org/10.1038/npp.2014.334.

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., McGaugh, J.L., Roozendaal, B., 2012. Glucocorticoids interact with the hippocampal endocannabinoid system in impairing retrieval of contextual fear memory. Proc. Natl. Acad. Sci. 109, 3504–3509. https://doi.org/10.1073/pnas.1200742109.

Atucha, E., Vukojevic, V., Fornari, R.V., Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M.W., Papassotiropoulos, A., McGaugh, J.L., de Quervain, D.J.-F., Roozendaal, B., 2017. Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory. Proc. Natl. Acad. Sci., 201710819. https://doi.org/10.1073/pnas.1710819114.

Baldi, E., Lorenzini, C.A., Bucherelli, C., 2004. Footshock intensity and generalization in contextual and auditory-cued fear conditioning in the rat. Neurobiol. Learn. Mem. 81, 162–166. https://doi.org/10.1016/j.nlm.2004.02.004.

Besnard, A., Sahay, A., 2016. Adult hippocampal neurogenesis, fear generalization, and stress. Neuropsychopharmacology 41, 24–44. https://doi.org/10.1038/npp.2015.167.

Biedenkapp, J.C., Rudy, J.W., 2007. Context preexposure prevents forgetting of a contextual fear memory: implication for regional changes in brain activation patterns associated with recent and remote memory tests. Learn. Mem. 14, 200–203. https://doi.org/10.1101/lm.499407.

Bueno, A.P.A., de Paiva, J.P.Q., Corrêa, M., dos, S., Tiba, P.A., Fornari, R.V., 2017. Corticosterone administration after a single-trial contextual fear conditioning does not influence the strength and specificity of recent and remote memory in rats. Physiol. Behav. 171, 175–180. https://doi.org/10.1016/j.physbeh.2017.01.011.

Cordero, M.I., Merino, J.J., Sandi, C., 1998. Correlational relationship between shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear conditioning. Behav. Neurosci. 112, 885–891. https://doi.org/10.1037/0735-7044.112.4.885.

Cordero, M.I., Sandi, C., 1998. A role for brain glucocorticoid receptors in contextual fear conditioning: dependence upon training intensity. Brain Res. 786, 11–17 https://doi.org/S0006-8993(97)01420-0 [pii].

De Kloet, E.R., Oitzl, M.S., Joëls, M., 1999. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 22, 422–426. https://doi.org/10.1016/S0166-2236(99)01438-1.

de Oliveira Alvares, L., Einarsson, E.Ö., Santana, F., Crestani, A.P., Haubrich, J., Cassini, L.F., Nader, K., Quillfeldt, J.A., 2012. Periodically reactivated context memory retains its precision and dependence on the hippocampus. Hippocampus 22, 1092–1095. https://doi.org/10.1002/hipo.20983.

de Quervain, D.J.F., Aerni, A., Schelling, G., Roozendaal, B., 2009. Glucocorticoids and the regulation of memory in health and disease. Front. Neuroendocrinol. 30, 358–370. https://doi.org/10.1016/j.yfrne.2009.03.002.

Dunsmoor, J.E., Prince, S.E., Murty, V.P., Kragel, P.A., LaBar, K.S., 2011. Neurobehavioral mechanisms of human fear generalization. Neuroimage 55, 1878–1888. https://doi.org/10.1016/j.neuroimage.2011.01.041.

Dymond, S., Dunsmoor, J.E., Vervliet, B., Roche, B., Hermans, D., 2015. Fear generalization in humans: systematic review and implications for anxiety disorder research. Behav. Ther. 46, 561–582. https://doi.org/10.1016/j.beth.2014.10.001.

Fanselow, M.S., 1980. Conditional and unconditional components of post-shock freezing. Pavlov. J. Biol. Sci. 15, 177–182. https://doi.org/10.1007/BF03001163.

Finsterwald, C., Alberini, C.M., 2014. Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: from adaptive responses to psychopathologies. Neurobiol. Learn. Mem. 112, 17–29. https://doi.org/10.1016/j.nlm.2013.09.017.

Fornari, R.V., Wichmann, R., Atucha, E., Desprez, T., Eggens-Meijer, E., Roozendaal, B., 2012. Involvement of the insular cortex in regulating glucocorticoid effects on memory consolidation of inhibitory avoidance training. Front. Behav. Neurosci. 6, 10. https://doi.org/10.3389/fnbeh.2012.00010.

Frankland, P.W., 2004. The involvement of the anterior cingulate cortex in remote contextual fear memory. Science (80-) 304, 881–883. https://doi.org/10.1126/science.1094804.

Frankland, P.W., Bontempi, B., 2005. The organization of recent and remote memories. Nat. Rev. Neurosci. 6, 119–130. https://doi.org/10.1038/nrn1607.

Gazarini, L., Stern, Ca.J., Piornedo, R.R., Takahashi, R.N., Bertoglio, L.J., 2015. PTSD-like memory generated through enhanced noradrenergic activity is mitigated by a dual step pharmacological intervention targeting its reconsolidation. Int. J. Neuropsychopharmacol. 18https://doi.org/10.1093/ijnp/pyu026. pyu026–pyu026.

Gazarini, L., Stern, Ca J., Carobrez, A.P., Bertoglio, L.J., 2013. Enhanced noradrenergic activity potentiates fear memory consolidation and reconsolidation by differentially recruiting α1- and β-adrenergic receptors. Learn. Mem. 20, 210–219. https://doi.org/10.1101/lm.030007.112.

Ghosh, S., Chattarji, S., 2015. Neuronal encoding of the switch from specific to generalized fear. Nat. Neurosci. 18, 112–120. https://doi.org/10.1038/nn.3888.

Gold, P.E., Van Buskirk, R., 1976. Effects of posttrial hormone injections on memory processes. Horm. Behav. 7, 509–517. https://doi.org/10.1016/0018-506X(76)90021-0.

Gold, P.E., Van Buskirk, R.B., 1975. Facilitation of time-dependent memory processes with posttrial epinephrine injections. Behav. Biol. 13, 145–153. https://doi.org/10.1016/S0091-6773(75)91784-8.

Haubrich, J., Cassini, L.F., Diehl, F., Santana, F., Fürstenau de Oliveira, L., de Oliveira Alvares, L., Quillfeldt, J.A., 2016. Novel learning accelerates systems consolidation of a contextual fear memory. Hippocampus 26, 924–932. https://doi.org/10.1002/hipo.22575.

Huckleberry, K.A., Ferguson, L.B., Drew, M.R., 2016. Behavioral mechanisms of context fear generalization in mice. Learn. Mem. 23, 703–709. https://doi.org/10.1101/lm.042374.116.

Hunsaker, M.R., Kesner, R.P., 2013. The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. Neurosci. Biobehav. Rev. 37, 36–58. https://doi.org/10.1016/j.neubiorev.2012.09.014.

Hunsaker, M.R., Kesner, R.P., 2008. Dissociations across the dorsal-ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear. Neurobiol. Learn. Mem. 89, 61–69. https://doi.org/10.1016/j.nlm.2007.08.016.

Kaouane, N., Porte, Y., Vallée, M., Brayda-Bruno, L., Mons, N., Calandreau, L., Marighetto, A., Piazza, P.V., Desmedt, A., 2012. Glucocorticoids can induce PTSD-like memory impairments in mice. Science 335, 1510–1513. https://doi.org/10.1126/science.1207615.

Kerr, D.S., Huggett, a M., Abraham, W.C., 1994. Modulation of hippocampal long-term potentiation and long-term depression by corticosteroid receptor activation. Psychobiology 22, 123–133.

Kesner, R.P., Hunsaker, M.R., 2010. The temporal attributes of episodic memory. Behav. Brain Res. 215, 299–309. https://doi.org/10.1016/j.bbr.2009.12.029.

Kim, S., Foong, D., Cooper, M.S., Seibel, M.J., Zhou, H., 2018. Comparison of blood sampling methods for plasma corticosterone measurements in mice associated with minimal stress-related artefacts. Steroids 135, 69–72. https://doi.org/10.1016/j.steroids.2018.03.004.

LaBar, K.S., Cabeza, R., 2006. Cognitive neuroscience of emotional memory. Nat. Rev. Neurosci. 7, 54–64. https://doi.org/10.1038/nrn1825.

Laufer, O., Israeli, D., Paz, R., 2016. Behavioral and neural mechanisms of over-generalization in anxiety. Curr. Biol. 26, 713–722. https://doi.org/10.1016/j.cub.2016.01.023.

Lissek, S., Bradford, D.E., Alvarez, R.P., Burton, P., Espensen-Sturges, T., Reynolds, R.C., Grillon, C., 2014. Neural substrates of classically conditioned fear-generalization in humans: a parametric fMRI study. Soc. Cogn. Affect. Neurosci. 9, 1134–1142. https://doi.org/10.1093/scan/nst096.

Lissek, S., Rabin, S., Heller, R.E., Lukenbaugh, D., Geraci, M., Pine, D.S., Grillon, C., 2011. Overgeneralization of conditioned fear as a pathogenetic marker of panic disorder. Am. J. Psychiatry 167, 47–55. https://doi.org/10.1176/appi.ajp.2009.09030410.Overgeneralization.

Lix, L.M., Keselman, J.C., Keselman, H.J., 1996. Consequences of assumption violations revisited: a quantitative review of alternatives to the one-way analysis of variance F test. Rev. Educ. Res. 66, 579–619. https://doi.org/10.3102/00346543066004579.

Lubec, G., Korz, V., 2016. Concerted gene expression of hippocampal steroid receptors during spatial learning in male Wistar rats: a correlation analysis. Front. Behav. Neurosci. 10, 94. https://doi.org/10.3389/fnbeh.2016.00094.

Marchand, A.R., Barbelivien, A., Seillier, A., Herbeaux, K., Sarrieau, A., Majchrzak, M., 2007. Contribution of corticosterone to cued versus contextual fear in rats. Behav. Brain Res. 183, 101–110. https://doi.org/10.1016/j.bbr.2007.05.034.

Matos, M.R., Visser, E., Kramvis, I., van der Loo, R.J., Gebuis, T., Zalm, R., Rao-Ruiz, P., Mansvelder, H.D., Smit, A.B., van den Oever, M.C., 2019. Memory strength gates the involvement of a CREB-dependent cortical fear engram in remote memory. Nat. Commun. 10, 1–11. https://doi.org/10.1038/s41467-019-10266-1.

McClelland, J.L., McNaughton, B.L., O'Reilly, R.C., 1995. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol. Rev. 102, 419–457.

McCullough, A.M., Ritchey, M., Ranganath, C., Yonelinas, A., 2015. Differential effects of stress-induced cortisol responses on recollection and familiarity-based recognition memory. Neurobiol. Learn. Mem. 123, 1–10. https://doi.org/10.1016/j.nlm.2015.04.007.

McGaugh, J.L., Roozendaal, B., 2002. Role of adrenal stress hormones in forming lasting memories in the brain. Curr. Opin. Neurobiol. 12, 205–210. https://doi.org/10.1016/S0959-4388(02)00306-9.

Menon, V., Uddin, L.Q., 2010. Saliency, switching, attention and control: a network model of insula function. Brain Struct. Funct. 214, 655–667. https://doi.org/10.1007/s00429-010-0262-0.

Norman, K.A., 2010. How hippocampus and cortex contribute to recognition memory: revisiting the complementary learning systems model. Hippocampus 20, 1217–1227. https://doi.org/10.1002/hipo.20855.

O'Reilly, R.C., Bhattacharyya, R., Howard, M.D., Ketz, N., 2014. Complementary learning systems. Cogn. Sci. 38, 1229–1248. https://doi.org/10.1111/j.1551-6709.2011.01214.x.

O'Reilly, R.C., Norman, K.A., 2002. Hippocampal and neocortical contributions to memory: advances in the complementary learning systems framework. Trends Cogn. Sci. 6, 505–510. https://doi.org/10.1016/S1364-6613(02)02005-3.

Pedraza, L.K., Sierra, R.O., Boos, F.Z., Haubrich, J., Quillfeldt, J.A., de Oliveira Alvares, L., 2016. The dynamic nature of systems consolidation: stress during learning as a switch guiding the rate of the hippocampal dependency and memory quality. Hippocampus 26, 362–371. https://doi.org/10.1002/hipo.22527.

Poulos, A.M., Mehta, N., Lu, B., Amir, D., Livingston, B., Santarelli, A., Zhuravka, I., Fanselow, M.S., 2016. Conditioning- and time-dependent increases in context fear and generalization. Learn. Mem. 23, 379–385. https://doi.org/10.1101/lm.041400.115.

Restivo, L., Vetere, G., Bontempi, B., Ammassari-Teule, M., 2009. The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. J. Neurosci. 29, 8206–8214. https://doi.org/10.1523/JNEUROSCI.0966-09.2009.

Reul, J.M.H.M., De Kloet, E.R., 1985. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117, 2505–2511. https://doi.org/10.1210/endo-117-6-2505.

Revest, J.-M., Kaouane, N., Mondin, M., Le Roux, a, Rougé-Pont, F., Vallée, M., Barik, J., Tronche, F., Desmedt, a, Piazza, P.V., 2010. The enhancement of stress-related memory by glucocorticoids depends on synapsin-Ia/Ib. Mol. Psychiatry 15 (1125), 1140–1151. https://doi.org/10.1038/mp.2010.40.

Richardson, R., 2000. Shock sensitization of startle: learned or unlearned fear? Behav. Brain Res. 110, 109–117. https://doi.org/10.1016/S0166-4328(99)00189-8.

Roozendaal, B., Griffith, Q.K., Buranday, J., De Quervain, D.J.-F., McGaugh, J.L., 2003. The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: dependence on the basolateral amygdala. Proc. Natl. Acad. Sci. U. S. A. 100, 1328–1333. https://doi.org/10.1073/pnas.0337480100.

Rudy, J.W., O'Reilly, R.C., 1999. Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. Behav. Neurosci. 113, 867–880. https://doi.org/10.1037/0735-7044.113.5.867.

Tukey, J.W., 1957. On the comparative anatomy of transformations. Ann. Math. Stat. 28, 602–632.

Uddin, L.Q., 2014. Salience processing and insular cortical function and dysfunction. Nat. Rev. Neurosci. 16, 55–61. https://doi.org/10.1038/nrn3857.

Vahl, T.P., Ulrich-Lai, Y.M., Ostrander, M.M., Dolgas, C.M., Elfers, E.E., Seeley, R.J., D'Alessio, D.A., Herman, J.P., 2005. Comparative analysis of ACTH and corticosterone sampling methods in rats. Am. J. Physiol. Endocrinol. Metab. 289, E823–E828. https://doi.org/10.1152/ajpendo.00122.2005.

Wang, S.-H., Teixeira, C.M., Wheeler, A.L., Frankland, P.W., 2009. The precision of remote context memories does not require the hippocampus. Nat. Neurosci. 12, 253–255. https://doi.org/10.1038/nn.2263.

Wiltgen, B.J., Silva, A.J., 2007. Memory for context becomes less specific with time. Learn. Mem. 14, 313–317. https://doi.org/10.1101/lm.430907.

Wiltgen, B.J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M.G., Parivash, S.N., Li, W., Silva, A.J., 2010. The hippocampus plays a selective role in the retrieval of detailed contextual memories. Curr. Biol. 20, 1336–1344. https://doi.org/10.1016/j.cub.2010.06.068.

Winocur, G., Moscovitch, M., Bontempi, B., 2010. Memory formation and long-term retention in humans and animals: convergence towards a transformation account of hippocampal-neocortical interactions. Neuropsychologia 48, 2339–2356. https://doi.org/10.1016/j.neuropsychologia.2010.04.016.

Winocur, G., Moscovitch, M., Sekeres, M., 2007. Memory consolidation or transformation: context manipulation and hippocampal representations of memory. Nat. Neurosci. 10, 555–557. https://doi.org/10.1038/nn1880.

Xu, W., Südhof, T.C., 2013. A neural circuit for memory specificity and generalization. Science 339, 1290–1295. https://doi.org/10.1126/science.1229534.