Is it possible to modify fear memories in humans with extinction training within a single day?

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Abstract

Extinction procedures have been used widely in the study of fear memories, and different positions have been adopted regarding the efficacy of such procedures and the mechanisms involved. It has been argued that extinction may interfere with the consolidation of the fear memory if the procedure is applied with the appropriate timing after acquisition. However, the opposite position is also held, that is, that the extinction does not achieve an elimination of the fear response. The aim of the present study is to test the short-term effects of immediate extinction in fear reduction when this extinction is preceded by a retrieval trial. For this, a procedure similar to that employed by Schiller et al. (2010) was used, but in a single day and with white noise as an aversive unconditioned stimulus. The results indicate that a CS+ single retrieval trial before the extinction procedure after acquisition was more effective in fear reduction than standard immediate extinction.

Keywords: fear memory, extinction, skin conductance response (SCR).

1. Introduction

We all possess an evolutionary mechanism that triggers a fight or flight response in the organism when faced with danger (e.g. Lee, 2009; Pedreira, Cuesta, & Maldonado, 2002; Tooby & Cosmides, 1990). However, if such responses occur persistently in reaction to events that do not involve any real danger, they can lead to the formation of maladaptive fear memories (Öhman & Mineka, 2001). These have their origin in a kind of fear learning where initially neutral events are associated with negative events (Lang, Davis, & Öhman, 2000; Maren, 2001). This association or fear learning at the root of fear memories occurs through so-called Pavlovian conditioning. In such conditioning, a stimulus that initially does not elicit an activation response (conditioned stimulus, CS) is conditioned through repeated pairing with the stimulus that itself elicits a high activation response without previous training (unconditioned stimulus, US) (Domjan, 2005; Lovibond & Shanks, 2002).

One of the main objectives of research in the study of fear memories has been extinction procedures (Hermans, Craske, Mineka, & Lovibond, 2006; Milad & Quirk, 2012; Quirk et al., 2010). These are used to reduce the activation response, called the fear response. Such procedures are based on the repeated presentation of the CS without the US, with the aim of eliminating the conditioned response (CR) elicited by the CS. Hence, during the extinction a new association, inhibitory in nature, might take place between the CS and the absence of the US (Bouton, 1993; LeDoux, 1989), which would compete against the fear memory but would not eliminate it (Bouton, 2002, 2004). Such a fear CR could therefore reappear over the course of time (spontaneous recovery), in different contexts (renewal), or on the presentation of the US (reinstatement) (Bouton, 2002; for a more recent review, see Vervliet, Baeyens, Van den Bergh, & Hermans, 2013).

The possibility of the reappearance of the fear CR has led to the development of studies focusing on the modification, through a change at the synaptic level, of the fear memory which is the consequence of the original association between the CS and the US (Agren et al., 2012; Nader, Schafe, & LeDoux, 2000). For this, some authors suggest the possibility of interfering in the consolidation of fear memories (Myers, Ressler, & Davis, 2006; for a review see Quirk et al., 2010). During their formation, such memories are labile until they become established in a stable way in the long term memory (LTM) through the mechanism of consolidation already mentioned (Dudai, 2004). In order to interfere in the consolidation of memories, various techniques have been employed. For example, pharmacological blockade has been used (Schafe et al., 2000), above all with adrenergic β-blockers, as a means of interfering with the

consolidation of the fear memory in LTM (Cahill, Prins, Weber, & McGaugh, 1994; van Stegeren, Everaerd, Cahill, McGaugh, & Gooren 1998). On the other hand, behavioral techniques have also been used, notably the above mentioned extinction procedure, but applied within the time window in which the memory is in formation.

Among the studies that have sought to interfere in the consolidation of fear memories, Myers et al. (2006) stands out. The authors here sought to interfere in the memory consolidation window using an extinction procedure applied shortly after the fear acquisition. This procedure, known as *immediate extinction*, had the aim of preventing the consolidation of the fear memory. In particular, a greater reduction in spontaneous recovery, renewal and reinstatement was achieved using immediate extinction than *delayed extinction*, which involves a longer delay (24-72h) between acquisition and extinction. The authors suggested that immediate extinction had interfered in the formation of the fear memory. Other studies have obtained similar results in humans (Golkar & Öhman, 2012; Norrholm et al., 2008) and in rats (Johnson, Escobar, & Kimble, 2010). On the contrary, other studies have suggested that immediate extinction implies a greater fear return than using a delayed extinction (Álvarez, Johnson, & Grillon, 2007; Chang & Maren, 2009; Huff, Hernandez, Blanding, & LaBar, 2009; Maren & Chang, 2006; Schiller et al., 2008; Woods & Bouton, 2008).

The use of procedures of immediate extinction are generally seen as justified in the study of the possible effects of interrupting the consolidation of original fear memories, assuming that such extinction would take place in a period in which the processes of synaptic/cellular consolidation are ongoing (see Lonsdorf et al., 2017, for a recent review of methodological considerations in fear conditioning research). In fact, the fear reduction obtained with this extinction procedure, using long-term tests (e.g. Myers et al., 2006), has been interpreted in terms of the disruption of memory consolidation. If the manipulation of the immediate extinction affects the consolidation, fear memory would be maintained in short-term tests, given that the consolidation processes would be ongoing while memories are tested, and the reduction in fear would only be achieved in long-term tests. This is exactly what has been observed in some experiments with rats (e. g., Bourtchuladze et al., 1998; Schafe & LeDoux, 2000). However, in a study using rats by Ponnusamy et al. (2016) a reduction in fear in the short-term was also found using immediate extinction. This is of particular interest because, as the authors suggest, it leads to the possibility that the expression of fear, more than the consolidation, was affected by extinction.

Hence, given that in studies with humans tests of fear recovery have always been over the long-term (see Lonsdorf et al., 2017), we ask whether it would also be possible to obtain a reduction of fear with immediate extinction training in human subjects in short-term tests.

Moreover, the effects of an immediate extinction preceded by the exposure to reminder cues that allow for the reactivation of the fear memory, making it more malleable and consequently more susceptible to being modified, have, to the best of our knowledge, not yet been evaluated (see Gisquet-Verrier & Riccio, 2012, for a review of memory reactivation effects). Therefore, in the present study we compare the short-term effects of standard immediate extinction versus immediate extinction preceded by a retrieval trial. If the immediate extinction in humans is sufficient for the reduction of fear, as found in the study with rats by Ponnusamy et al. (2016), then one would expect a reduction in fear in both conditions. If in addition to this the reactivation of the fear memory is also necessary, then its reduction would only be observed when the immediate extinction is preceded by a retrieval trial. To this end, a procedure similar to that used in Schiller et al. (2010) was adopted. Essentially, its procedure consisted of three phases: a first phase of differential Pavlovian conditioning in which an electric shock was used as a US; after 24 hours, a second phase of retrieval (reactivation)+extinction; finally, 24 hours after the previous one, a phase of re-extinction (see Schiller et al., 2010, for more details). However, whereas in the aforementioned work the phases were carried out on different days, in our study all phases were performed in a single day. Also, we used a burst of white noise as US instead of an electric shock. The experiment began in both groups with a fear acquisition phase, using a differential Pavlovian conditioning procedure. Thus, a stimulus (CS+) was presented which was associated with the US, and another stimulus (CS-) was always associated with the absence of the US. In the retrieval group, a CS+ retrieval trial was presented 5 minutes after the acquisition phase, followed by a 10 minutes rest. Following that, participants carried out the extinction phase, and 5 minutes later the re-extinction phase. In the no-retrieval group, the extinction phase began 5 minutes after the end of acquisition, and, as in retrieval group, the re-extinction phase began 5 minutes later. The whole procedure was conducted in one day because the present research focuses on testing the short-term effects of fear reactivation, whereas Schiller et al. (2010) looked at interference in the mechanism of reconsolidation. These authors suggested that the process of reconsolidation is based on the reactivation of a stable and consolidated memory by making it return to a state susceptible to being modified. In addition, reconsolidation is also based on a return to a fixed and stable state of such memories in the LTM once modified (e.g. Agren et al., 2012; Agren, Björkstrand, & Fredrikson, 2017; Björkstrand et al., 2015; Björkstrand et al., 2016, 2017; Dudai, 2004; Johnson & Casey, 2015; Lee, 2009; Nader et al., 2000; Oyarzún et al., 2012; Sara, 2000; Steinfurth et al., 2014; Tronson & Taylor, 2007). However, findings from other studies point in the opposite direction (Golkar, Bellander, Olsson, & Öhman, 2012; Kindt & Soeter, 2013; Klucken et al., 2016; Meir-Drexler et al., 2014; Shiban, Brütting, Pauli, &

Mühlberger, 2015; see Kredlow, Unger, & Otto, 2016 for a recent metaanalysis of reconsolidation updating).

The current study aims to test the short-term effects of fear reactivation in humans using an immediate extinction procedure. . Specifically, we hypothesize that in the group with retrieval+rest prior to extinction, the response to the CS+ will be similar to the response to the CS- at the start of the re-extinction, which would be an indicator of fear reduction. However, we expect that in the no-retrieval group (with standard immediate extinction) the response to the CS+ will be greater than in the case of the CS-, which would indicate that there is no reduction of fear.

2. Material and Methods

2.1 Participants

The initial sample for the experiment consisted of 66 Psychology and Neuroscience students (55 women and 11 men), mean age 20.22 (SD=2.18), who participated voluntarily in the experiment in return for course credits. Participants were randomly assigned to one of two groups (retrieval group, no-retrieval group). After applying the acquisition and extinction criteria (see Measurements and analysis), the sample was reduced from 34 to 16 participants in retrieval group (14 women and 2 men), and in no-retrieval group from 32 to 15 participants (12 women and 3 men). Each participant signed an informed consent form and was told that they could leave the experiment at any time.

2.2 Stimuli and apparatus

The CSs consisted of geometric figures presented at the center of a computer screen (LCD monitor, 22´) on a black background. As a CS+, a yellow square of 10x10 cm was used and was presented as associated with the US. As a CS-, a blue square with the same characteristics was used, this always associated with the absence of the US. The duration of both CSs was 4 seconds. As a US, a 0.5 s burst of white noise with an intensity of 105 dB (A) was used, administered through Senheisser HZR 62 headphones. The US began 0.5 s before the completion of the CS+, so that they ended simultaneously.

A *Biopac MP150-WSW* was used for the registration of electrodermal activity. The skin conductance response (SCR) was registered with the module GSR-100C, using 0.6mm Ag/AgCl

electrodes filled with isotonic contact gel (Gel 101, Isotonic Recording Electrode Gel from BIOPAC Systems, Inc.). A constant voltage of 0.5V was applied between the electrodes placed in the middle phalanges of the index and middle fingers of the participant's non-dominant hand. For the measurement of the SCR, the AcqKnowledge 3.9.1 (BIOPAC Systems, Inc., Goleta, California) was used. Finally, we employed the "SuperLab 4.0" program (Cedrus Corporation, 2008) for the programming and presentation of stimuli sequences.

2.3 Procedure

After signing a consent form, participants sat facing a computer screen, through which both the instructions and the visual stimuli were presented. After placing the electrodes, instructions were presented on the screen indicating that attention should be paid to the visual and acoustic stimuli given.

The three phases of the experiment were conducted in a single day, with an interval of around 5 minutes between each phase. In order to separate the phases as far as possible, a message was presented at the end of each phase on the computer screen, indicating to the participant that the phase had ended. In addition, another message was shown at the beginning of the next phase, indicating to the participant that from that moment on he/she had to pay attention once more to the stimuli that would appear on the screen. These phases were as follows (see Figure 1):

(Insert Figure 1)

2.3.1 Acquisition

During this phase a classical differential conditioning procedure was used. Thus, the participants received 6 presentations CS+/US, 10 CS+/noUS and 10 CS-/noUS, in a randomized order, with the restriction that no more than two identical trials appeared in sequence. In this way, the CS+ was paired with the US on a 38% partial reinforcement schedule and the CS- was never paired with the US. In this way we maintain the same reinforcement schedule as that used by Schiller et al. (2010). Also, as in that study, the SCRs elicited by the CS+ in the CS+/noUS trials and those found for the CS- will be analysed (see the Results section, below). The interval between trials (ITI) was randomized to 10-12s, and this ITI was maintained in the following phases. Throughout the whole ITI, a white fixation cross was presented at the center

of the screen in both groups, in this and the remaining phases, as in Schiller et al. (2010). This cross was 2x2 cm high and wide, with each line 3 mm thick.

2.3.2 Retrieval

Once the previous phase had finished, the retrieval phase began, in which each participant of retrieval group received an isolated presentation of the CS+ without US. No-retrieval group did not receive this CS+ presentation, but went directly from the acquisition phase to the extinction phase.

2.3.3 Extinction

In retrieval group, after the retrieval phase, participants rested for a 10 minute interval in which they were shown a cartoon video. After the video, 10 trials of CS+/noUS and 11 of CS- were presented. In the case of no-retrieval group, after the acquisition, each participant was subjected directly to a standard extinction (11 +CS/noUS and 11 CS-), without the presentation of an isolated CS+ trial. Again, the sequence of trials was randomized for each subject in both groups, with the restriction that no more than two CS+ or CS- could appear in sequence.

2.*3.4 Re-extinction*

In this phase the participants of both groups received 11 presentations of the CS+ and 11 presentations of the CS-, always without US. As usual, the stimuli were randomized for each subject, with the restriction that no more than two identical stimuli could appear in sequence.

2.4 *Measurements and analysis*

CR was defined as a SCR with the greatest amplitude in the range between 1 and 4.5s after the presentation of the CS. A minimum value of 0.01 micro-Siemens (μS) was used for the measurement of responses. As a prior step in the analysis, the raw SCRs (see Appendix) were transformed logarithmically to improve their statistical properties, normalizing the distribution of the responses (Venables & Christie, 1980). As usual, the value 1 was added to all measurements of amplitude so as to avoid a value of log(0), as well as the log of amplitudes lower than 1 μS. As a consequence, data appear as log(1+SCRamp). Also, range correction (Lykken, 1972) was used, dividing the SCRs of each subject by their response of greatest amplitude. In this way, the results vary between 0 and 1, and these were multiplied by 1000 in order to avoid excessively small calculations.

Using a procedure similar to Schiller et al. (2010), for the final analysis we took into consideration only those participants who conformed to the following criteria. First, that the

mean SCR to the CS+ presented without US had to be greater than the mean SCR to the CS- in the late phase of acquisition, that is, the last 5 trials for each CS (20 participants who did not meet this criterion were excluded). Second, that this differential mean had to be equal or greater than 0.1 μS (15 participants did not meet this criterion). Third, that a participant's response had to be considered extinguished, that is, their mean differential response in late extinction phase had to be less than 0.1 μS (no participants were excluded on the grounds of this criterion).

Finally, the terms early and late phase were used in the following way: in the acquisition phase, early phase consisted of the first 5 trials, and late phase the final 5. In the extinction phase, early phase also consisted of the first 5 trials, and late phase the final 5. In the re-extinction, early phase referred to the first 5 trials, whereas late phase to the last 5 trials. It should be noted that the same number of trials as in Schiller et al. (2010) were used in all phases.

3. Results

For the analysis of the data we used an analysis of variance (ANOVA) and Bonferroni corrections in the follow-up analysis, in order to avoid Type 1 error. For the analysis of the main effects and interactions, a 0.05 alpha level was considered.

3.1 *Acquisition*

In the acquisition phase we used a mixed ANOVA, Stimulus (CS+, CS-) x Group (retrieval group, no-retrieval group) x Time (early and late phase). In the Stimulus factor, we only used those CS+ presented without the US. This analysis showed a significant Stimulus effect ($F_{1,29}$= 65.59, p< .01, η^2_{part}= .69), Stimulus x Time effect ($F_{1,29}$= 78.49, p< .01, η^2_{part}= .73), but not for the Stimulus x Group x Time interaction ($F_{1,29}$= 1.51, p> .05, η^2_{part}= .05). The follow-up Bonferroni tests showed that responses to the CS+ (M= 285.73; SD= 169.37) were similar to responses to the CS- (M= 271.04; SD= 183.63) in the early phase of acquisition (see figure 2 and 3, panel A). In the late phase of acquisition, responses to the CS+ (M= 454.83; SD= 180.26) were significantly greater (p< .01) than to the CS- (M= 79.55; SD= 111.53). These findings show that there were differences between early and late phase of acquisition, suggesting that successful acquisition of the CR to the CS+ occurred, regardless of the group.

3.2 Retrieval / Extinction

In the extinction phase we used a mixed ANOVA, Stimulus (CS+, CS-) x Group (retrieval group, no-retrieval group) x Time (late phase of acquisition, and late phase of extinction). In the retrieval group, the retrieval trial was analyzed as an extinction trial in order to achieve the same number of trials as in the no-retrieval group (see Figure 1). A significant Stimulus effect ($F_{1,29}$= 82.43, p< .01, η^2_{part}= .74), Time effect ($F_{1,29}$= 67.13, p< .01, η^2_{part}= .70) and Stimulus x Time interaction ($F_{1,29}$= 124.50, p< .01, η^2_{part}= .81) were observed here, but not for the Stimulus x Group x Time interaction ($F_{1,29}$= 3.51, p> .05, η^2_{part}= .11). The Bonferroni tests showed that, in the late extinction phase, responses to the CS+ (M= 96.87; SD= 122.72) were significantly lower (p< .01) than the SCRs to the CS+ during the late phase of acquisition. The SCRs to the CS- in the late extinction phase (M= 86.88; SD= 144.40) were similar to responses to the CS- in the late acquisition phase (see figure 2 and 3, panel A and B). Indeed, both the ANOVA and the Bonferroni tests indicate that the response to the CS+ was extinguished in both groups.

3.3 Re-extinction

In order to compare the responses to the CSs in the early re-extinction phase versus late extinction phase, a mixed ANOVA Stimulus (CS+, CS-) x Group (retrieval group, no-retrieval group) x Time (late phase of extinction and early phase of re-extinction) was used. This analysis indicated a significant effect of Stimulus ($F_{1,29}$= 10.39, p< .01, η^2_{part}= .26), Time effect ($F_{1,29}$= 29.95, p< .01, η^2_{part}= .51), Stimulus x Time interaction ($F_{1,29}$= 9.13, p< .01, η^2_{part}= .24) and a Stimulus x Group x Time interaction ($F_{1,29}$= 5.97, p< .02, η^2_{part}= .17).

The post-hoc tests showed that, in the late phase of extinction, responses to the CS+ and to the CS- were similar in the two groups. In the early phase of re-extinction, the no-retrieval group showed significantly higher SCRs to the CS+ (M= 340.96; SD= 305.41) than to the CS- (M= 124.98; SD= 193.58). The retrieval group, in the same phase, showed no differences between responses to the CS+ (M= 216.12; SD= 295.72) and the SCRs to the CS- (M= 154.82; SD= 187.43).

The comparison of responses to the CSs in the early re-extinction phase versus late extinction phase showed, for the retrieval group, that the SCRs to the CS+ in early re-extinction were significantly higher (p< .01) than in late extinction. In this group, the analysis also showed that the responses to the CS-, in early re-extinction, were higher (p<. 02) than in late extinction. The no-retrieval group, in turn, showed responses to the CS+ in the early re-extinction phase that were significantly higher (p< .01) than responses to the CS+ in the late extinction phase, whereas responses to the CS- were similar in both phases.

(Insert Figure 2)

(Insert Figure 3)

4. Discussion

The aim of the present study was to analyze how a retrieval trial prior to extinction training, using a single-day procedure, affects the fear return in the short-term in humans. The results showed that, in both groups, fear conditioning was obtained in the acquisition phase. Once the extinction phase had ended, neither group showed fear response to the CS+. Finally, in the early re-extinction phase the fear return was evaluated with the aim of confirming the predictions of our hypothesis.

The results partially support our hypothesis, given that in the early re-extinction phase the response to the CS+ was similar to the response to the CS- in the group that underwent retrieval+rest before extinction. Also, the response to the CS+ was higher than to the CS- in the no-retrieval group. However, the complementary analyses carried out to confirm the results by analyzing the responses to the CSs in late extinction versus early re-extinction were not in the expected direction. Thus, in both groups the response to the CS+ was greater in early re-extinction than in late extinction, despite this outcome having been expected only in the no-retrieval group. In addition, the response to the CS- in early re-extinction was greater than in late extinction in the retrieval group. There was no difference in responses to the CS- in the no-retrieval group, whereas we might have expected this in both groups.

Such findings provide more information on how the timing used in immediate extinction might have an effect on fear return. Previous studies have achieved varying results. One group of studies found that the immediate extinction achieved a greater reduction in the fear return than delayed extinction (Golkar & Öhman, 2012; Johnson et al., 2010; Myers et al., 2006; Norrholm et al., 2008). However, results from another group of studies suggested otherwise, that is, that immediate extinction implied a greater fear return than delayed extinction (Alvarez et al., 2007; Chang & Maren, 2009; Huff et al., 2009; Maren & Chang, 2006; Schiller et al., 2008; Woods & Bouton, 2008). These latter findings have been the more frequent (for a review, see Maren, 2014). However, they should be taken with caution, since the two groups of studies differ in terms of population, procedure, measures or timing, among other parameters.

Thus, in both groups of studies, results from experiments with animals and with humans can be compared. For example, Myers et al. (2006) and Johnson et al. (2010) used rats, while Alvarez et al. (2007), Huff et al. (2009) and Schiller et al. (2008) used humans. However, the sequences of learning necessary to establish a solid contingency relationship between CS and US differ between animals and humans (Clark & Squire, 1998; Davey, 1992; Norrholm et al., 2008). Even when the same population is used, some studies differ as to the rate of reinforcement of CS+/US. Hence, in the fear acquisition phase, with rats, Myers et al. (2006) used 15 light-shock pairings, while Maren and Chang (2006) used 5 tone-footshock trials. Moreover, the two groups of studies differ in terms of behavioral and psychophysiological measures used, with measures employed including expectancy ratings and eyeblink startle response using electromiography (EMG) (Alvarez et al., 2007; Golkar & Öhman, 2012; Norrholm et al., 2008), conditioned freezing (Chang & Maren, 2009), fear potentiated-startle (Myers et al., 2006), SCR (Huff et al., 2009; Schiller et al., 2008), among others (see Johnson et al., 2010). Finally, one of the main differences concerns the timing used, given that different time intervals between acquisition and extinction have been used to refer to the same type of immediate extinction, ranging from 10-12 seconds (Schiller et al., 2008) to 1 day (Rescorla, 2004). In short, all these differences might serve to explain the diversity of results here.

So, our results are intended to bring some clarity to this area. In our experiment, we employed as a reference the procedure used in Schiller et al. (2010). This gave us greater comparability and replicability, by maintaining the conditions that allowed proper acquisition and extinction in human fear memory to develop, despite the conceptual differences between the two studies. Also, the results showed that immediate extinction preceded by one retrieval trial (reactivation) was more effective in fear reduction in the short-term than standard immediate extinction, which suggests that fear memory was more malleable in the former case. This is of potential interest for applications aimed at preventing the return of fear. However, our results should be viewed with caution, and certain limitations of the present study should be borne in mind. Thus, the absence of observed differences in CS+ vs. CS- in the retrieval group in the early re-extinction phase could be due to an increase in the response to the CS- in this group compared to that in the non-retrieval group. In addition it would be convenient to counterbalance the colours of the CS, given that in this study we have not done this. Moreover, it would also be useful to add a control group as a means of evaluating the possible effect of the rest period between acquisition and extinction. Finally, a larger final sample would also be advantageous as a means of increasing the power of the study.

Future studies might continue to expand the timing used between acquisition and extinction. In this way, it would be possible to explore the question of the optimum moment after the

acquisition phase to apply the extinction, and to ask what is the most effective extinction procedure in the prevention of the fear return. Also, it would be useful to continue evaluating how this technique would affect long-term spontaneous recovery. In our experiment no long-term test was carried out, and this might indeed be done in future studies. Finally, in future research the interference in reconsolidation could be evaluated, increasing the interval between phases. In our own laboratory we are currently carrying out a study along these lines with human subjects.

Figures

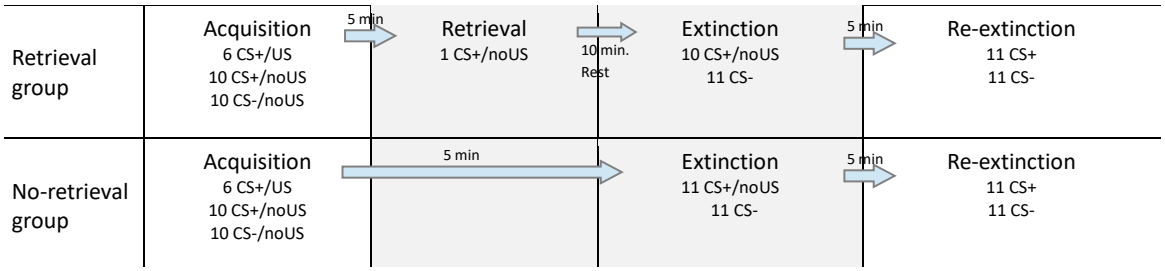

Fig. 1. Experimental phases and groups.

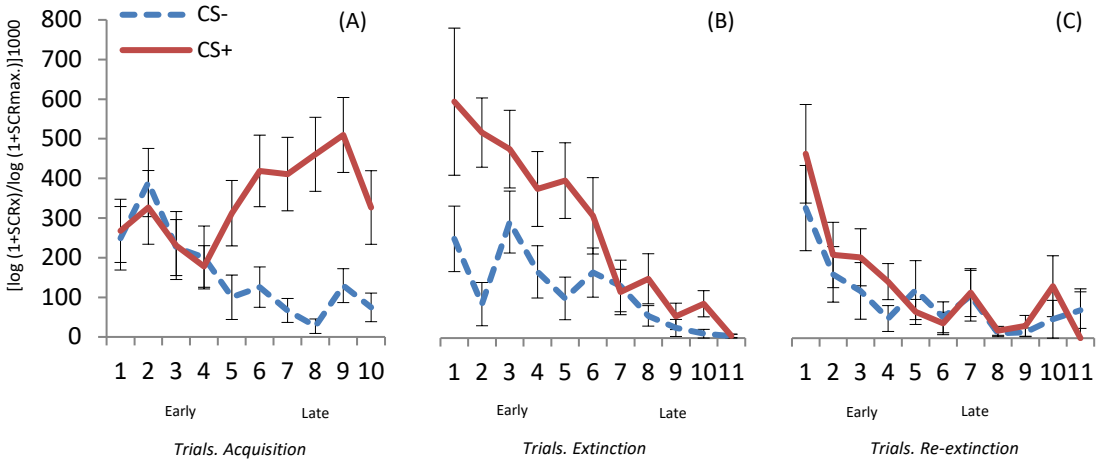

Fig.2. Retrieval group corrected responses to the CSs during all the experiment phases. (A) Acquisition phase (Early phase, 1-5 trials; Late phase, 6-10). (B) Extinction phase (Early phase, trials 1-5; Late phase, trial 7-11). (C) Re-extinction phase (Early phase, trials 1-5; Late phase, trials 7-11).

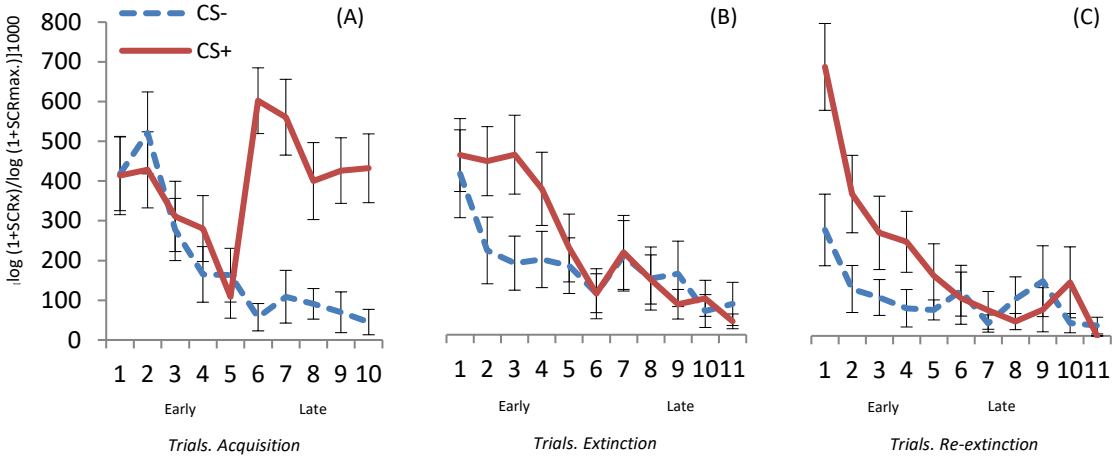

Fig.3. No-retrieval group corrected responses to the CSs during all the experiment phases. (A) Acquisition phase (Early phase, 1-5 trials; Late phase, 6-10). (B) Extinction phase (Early phase, trials 1-5; Late phase, trial 7-11). (C) Re-extinction phase (Early phase, trials 1-5; Late phase, trials 7-11).

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Appendix

Acquisition		1	2	3	4	5	6	7	8	9	10	
Retrieval group	CS+	0.29	0.32	0.15	0.21	0.25	0.32	0.43	0.49	0.39	0.43	
	CS-	0.23	0.40	0.14	0.10	0.20	0.11	0.04	0.02	0.07	0.11	
No-retrieval group	CS+	0.31	0.29	0.25	0.25	0.13	0.40	0.37	0.30	0.36	0.25	
	CS-	0.32	0.37	0.18	0.19	0.14	0.07	0.13	0.07	0.08	0.05	

Extinction		1	2	3	4	5	6	7	8	9	10	11
Retrieval group	CS+	0.31	0.37	0.38	0.54	0.32	0.21	0.09	0.13	0.04	0.07	0.01
	CS-	0.43	0.09	0.32	0.15	0.09	0.17	0.11	0.04	0.02	0.01	0.00
No-retrieval group	CS+	0.40	0.37	0.42	0.29	0.17	0.13	0.28	0.11	0.04	0.08	0.04
	CS-	0.43	0.25	0.19	0.14	0.12	0.12	0.26	0.08	0.17	0.03	0.14

Re-extinction		1	2	3	4	5	6	7	8	9	10	11
Retrieval group	CS+	0.39	0.32	0.29	0.12	0.07	0.03	0.16	0.01	0.05	0.06	0.00
	CS-	0.23	0.08	0.14	0.07	0.13	0.02	0.05	0.01	0.02	0.10	0.07
No-retrieval group	CS+	0.56	0.35	0.36	0.25	0.29	0.21	0.08	0.03	0.11	0.08	0.00
	CS-	0.25	0.15	0.08	0.04	0.04	0.20	0.01	0.07	0.08	0.03	0.02

Raw (un-transformed) SCRs (μS) during the experiment phases in both groups.