Preventing the return of fear memories with post-retrieval extinction: a human study using a burst of white noise as an aversive stimulus.

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Abstract

Standard extinction procedures seem to imply an inhibition of the fear response, but not a modification of the original fear memory trace, which remains intact (Bouton, 2002, 2004). Typically, the behavioural procedure used to modify this trace is the so-called *post-retrieval extinction*, consisting of fear memory reactivation followed by extinction applied within the reconsolidation window. However, the application of this technique yields mixed results, probably due to a series of boundary conditions that limit the effectiveness of post-retrieval extinction effects. In this study a number of potential, and hitherto unexplored, moderators of such effects are considered. Using an interval of 48 hours between extinction and re-extinction, the findings show a spontaneous recovery similar to that found in studies that use a 24 hour interval. Also, the use of intervals of 10 and 20 minutes between reactivation and extinction led to a similar fear return. Finally, the burst of white noise used as an unconditioned stimulus (US) here was shown to be as effective as the electric shock normally used in the study of fear memory reconsolidation. These findings suggest that post-retrieval extinction is an effective behavioural technique for modifying the original fear memory and for the elimination of the fear return.

Keywords: Reconsolidation, fear memory, post-retrieval extinction, return of fear, boundary conditions.

1. Introduction

It is traditionally held that fear memories, once consolidated into long term memory (LTM), are unalterable, stable and insensitive to disruption (Jacobs & Nadel, 1985; LeDoux, Romanski, & Xagoraris, 1989). Such memories have typically been studied using Pavlovian conditioning (Lang, Davis, & Öhman, 2000; Rosen & Schulkin, 1998; Wolpe, 1981) and extinction procedures (Foa, Franklin, & Moser, 2002; Rauch, Shin, & Phelps, 2006). In Pavlovian conditioning, a neutral stimulus that does not initially elicit a response (conditioned stimulus, CS) is repeatedly paired with an aversive stimulus (unconditioned stimulus, US) that itself elicits a fear response (unconditioned response, UR) without previous training. As a consequence of this pairing, the CS acquires the capacity to raise a fear response (conditioned response, CR; e.g. Domjan, 2005; Lovibond & Shanks, 2002). As for the extinction procedures, based on the repeated presentation of the CS without the US, these are intended to reduce the fear response (Foa et al., 2002; Myers & Davis, 2002; Rauch et al., 2006). Thus, the idea that the fear memories are persistent is maintained, despite resulting in loss of the fear response initially, fear can return (Miracle, Brace, Huyck, Singler, & Wellman, 2006). This return, after its apparent removal, might occur as a consequence of the presentation of the US (reinstatement), as a response to the CS in the same context after the simple passage of time (spontaneous recovery, SR), or by placing the subject in a context different from that which produced the reduction of the CR (renewal). The reason for its return might be that such extinction procedures appear to involve an inhibition of the fear response, but not the modification of the original fear memory trace, which remains intact (Bouton, 2002, 2004).

Recently, studies have been conducted based on interference in memory reconsolidation mechanisms as a new procedure to reduce the likelihood of fear return (Nader, Schafe, & LeDoux, 2000; Kindt, Soeter, & Vervliet, 2009; Monfils, Cowansage, Klann, & LeDoux, 2009; Schiller et al., 2010; Steinfurth et al., 2014; for reviews, see Lee, Nader, & Schiller, 2017; Schiller & Phelps, 2011). Memory reconsolidation is based on the reactivation of a memory consolidated in the LTM, the memory being returned to a state in which it is vulnerable and susceptible to modification or updating. Such reactivation is usually achieved through the presentation of a trial of the CS without the US, which generates a prediction error that may be necessary for the destabilization of the memory trace (Sevenster, Beckers, & Kindt, 2013; 2014). In this way, the CS serves as the fear memory reminder (e.g. Golkar, Bellander, Olsson, & Öhman, 2012; Schiller et al., 2010). There is evidence that, after reactivation, the memory is

susceptible to updating during a time interval, known as the reconsolidation window, which might last up to 6 hours (Agren et al., 2012a; Duvarci & Nader, 2004; Nader et al., 2000; Walker, Brakefield, Hobson & Stickgold, 2003). Finally, once this time period has passed, the labile memory trace is "restabilized" in process similar to consolidation (Dudai, 2004; Lee, 2009; Nader, 2015; Sara, 2000; Tronson &Taylor, 2007). It is known that the synthesis of new proteins in neurons is necessary for memory consolidation (Goelet, Castellucci, Schacher, & Kandel, 1986; Dudai, 2004). Likewise, it has been observed that, once reactivated, memories require de novo protein synthesis for reconsolidation (Nader et al., 2000). Blocking such synthesis, then, might produce a disruption or interference of the fear memory reconsolidation. This interference would be specific to the component of the memory that had been reactivated and would not affect other aspects of the original memory (Schiller et al., 2010). In this way one might, for example, eliminate the emotional component of a memory without altering its cognitive component (Soeter, & Kindt, 2010).

Until recently, most studies looking at the interference of fear memory reconsolidation were based on the use of drugs, especially in animals (e.g. Debiec & LeDoux, 2004; Duvarci, Nader, & LeDoux, 2005; Liu et al., 2014; Lee, Di Ciano, Thomas, & Everitt, 2005; Lee, Milton, & Everitt, 2006; Przybyslawski, Roullet, & Sara, 1999), and to a lesser degree with human subjects (e.g. Brunet et al., 2008; Kindt et al., 2009; Sevenster et al., 2013). Very rarely have studies with human subjects used behavioural techniques instead of drugs (e.g. Agren et al., 2012a; Agren, Björkstrand, & Fredrikson, 2017; Klucken et al., 2016; Schiller et al., 2010; Thompson & Lipp, 2017), and in such work the behavioural approach commonly used consists of a memory reactivation followed by extinction applied within the reconsolidation window, this known as post-retrieval extinction (Monfils et al., 2009; see Kredlow, Unger, & Otto, 2016 for meta-analysis of retrieval-extinction procedures). Through this procedure, it is assumed that the original fear memory trace is modified (Agren et al., 2012a; Agren, Furmark, Eriksson, & Fredrikson, 2012b; Tronson, Wiseman, Olausson, & Taylor, 2006; Tronson & Taylor, 2007). However, by using a standard extinction procedure, that is, without prior reactivation, this trace is not modified (Alberini, 2005; Bouton, 2002, 2004; Merlo, Milton, Goozée, Theobald, & Everitt, 2014). A seminal work on this research line is that of Schiller et al. (2010), whose procedure is the one usually employed in that field of study. This procedure involves an initial phase of Pavlovian conditioning followed, 24 hours later, by a post-retrieval extinction procedure, and finally, on the last day, an evaluation of the fear return through a re-extinction phase.

Findings from the different studies using this procedure have been mixed. In some studies it was observed that, when the extinction was carried out within the reconsolidation window after prior reactivation, the likelihood of fear return was less than when a standard extinction was used (e.g. Agren et al., 2012a; Agren et al., 2017; Björkstrand et al., 2015; Björkstrand et al., 2016, 2017; Johnson & Casey, 2015; Oyarzún et al., 2012; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Schiller et al., 2010; Steinfurth et al., 2014, Warren et al., 2014). However, other findings point in the opposite direction; that is, the likelihood of fear return was found to be similar with both types of extinction (e.g. Golkar et al., 2012; Kindt & Soeter, 2013; Klucken et al., 2016; Meir-Drexler et al., 2014; Shiban, Brütting, Pauli, & Mühlberger, 2015; Soeter & Kindt, 2011, experiment II).

It is possible that such inconsistencies are due to the so-called boundary conditions (Besnard, Caboche, & Laroche, 2012), that is, the conditions that limit the effectiveness of the post-retrieval extinction procedure and the reconsolidation interference mechanisms in general (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Nader & Hardt, 2009). Such conditions include, for example, the type of CS used, the duration of the US, differences in inter-trial interval (ITI), the number of acquisition trials, and the different strategies of analysis (see Klucken et al., 2016). These boundary conditions were studied in a meta-analysis of fear memory reconsolidation by Kredlow, Unger, & Otto (2016), who noted some of the moderators that could be influencing the post-retrieval extinction procedure effects in humans, these including memory strength, the nature of the fear learning, and the use of expectancy ratings. However, they also claimed that other moderators might exist, the investigation of which would be of interest, since in this way some of the boundary conditions for the post-retrieval extinction effects might be clarified. For example, in terms of timing procedures, they observed that in all the studies covered in their meta-analysis (up to 2014) intervals of 10 minutes were used between retrieval and extinction in the reactivation group. In addition, in the majority of studies the interval between extinction and the test of fear return was 24 hours. As a result, they conclude that in the studies reviewed there is not enough variation of time between extinction and test, or in the interval between retrieval and extinction, for these time intervals to be analysed as moderators of post-retrieval extinction effects. They also note that in the vast majority of studies of the interference of fear memory reconsolidation, an electric shock was used as an aversive US, and it has therefore not been possible to examine the type of US as a moderator. These suggestions remain valid since, to our knowledge, studies subsequent to Kredlow and colleagues´ meta-analysis have not

investigated such moderators (Agren et al., 2017; Björkstrand et al., 2015; Björkstrand et al., 2016, 2017; Klucken et al., 2016; Shiban et al., 2015; Thompson & Lipp, 2017).

Consequently, the present study aims to investigate the effect of these moderators in the disruption of fear memory reconsolidation in humans. First, spontaneous recovery 48 hours after the extinction phase will be measured, in order to observe whether the reduction in the CR achieved at this stage is maintained in the re-extinction phase. In this way we will be able to confirm whether the same effects obtained in previous studies employing a 24 hour interval are maintained (e.g. Agren et al., 2017; Schiller et. al., 2010). Second, in addition to a control group (without reactivation) two experimental groups will be used in which we will apply extinction within the reconsolidation window, 10 or 20 minutes after reactivation. This will allow us to explore the possible effect here in spontaneous recovery, using a greater time interval between retrieval and extinction than used in previous studies (e.g. Agren et al., 2017; Johnson & Casey, 2015; Schiller et al., 2010; Shiban et al., 2015; Soeter & Kindt, 2011; Thompson & Lipp, 2017; Warren et al., 2014). Finally, a burst of white noise was used as a US, instead of the electric shock habitually employed (e.g. Golkar et al., 2012; Klucken et al., 2016; Schiller et al., 2010; Soeter & Kindt, 2011; Thompson & Lipp, 2017). In this way, it is intended to verify the possible generalization of the results of these works using a US frequently used in human classical conditioning (Boucsein, 2012). This US has a similar intensity to the burst of white noise typically used to elicit and measure fear-potentiated startle. However, in this case the duration of the US is much shorter (from 40 ms to 500 ms; e.g. Norrholm et al., 2014) than that used in Pavlovian conditioning.

Other potential boundary conditions exist which might also explain some of the conflicting findings in the literature. These include the fear measure used and the reinforcement rate during acquisition of CR. With respect to the fear measure, the physiological indices most often used have been the SCR (e.g. Schiller et al., 2010; Oyarzun et al., 2012) and the fear-potentiated startle (e.g. Norrholm et al., 2011; Warren et al., 2014), and to a lesser extent heart rate and pupillary response, among others, as well as US-expectancy measures (e.g. Warren et al., 2014; see Lonsdorf et al., 2017, for a recent review). For the reinforcement rate, there are clear differences between the various studies conducted, from 38% (e.g. Schiller et al., 2010; Oyarzun et al., 2012) to 100% (Agren et al., 2012a; Warren et al., 2014), including 50%, 75% and 80% (see the meta-analysis by Kredlow et al., 2016, for more information on the boundary conditions).

In summary, the current study aims to further examine the effects of post-reactivation extinction during the reconsolidation window as a behavioural method of interference in the context of a paradigm of human fear memory, and to clarify some of the boundary conditions of such effects. We hypothesize that spontaneous recovery tested 48 hours after extinction will be similar to that obtained with an interval of 24 hours. In addition, we hypothesize that in the re-extinction phase there will be no differences in the response to the CSs (CS+, and CS-) between the groups of 10 and 20 minutes after reactivation. Moreover, we assume that responses to the CS+ will be lower in the reactivation groups than in the control group. Finally, we expect that the use of a burst of white noise will have the same effectiveness as an aversive US as the electric shock most commonly employed in previous work.

2. Material and Methods

2.1 Participants

The sample for the experiment consisted of 93 Psychology students (65 women and 28 men), mean age 20.94 (SD=2.94), who participated voluntarily in the experiment after signing an informed consent form approved by the Ethical Committee of the University. They received course credits for their participation in the experiment. After applying the acquisition and extinction criteria, the initial sample was reduced by 35 participants. The remaining participants were randomly assigned to each of the three groups (reactivation-10, reactivation-20, no-reactivation). The reactivation-10 group comprised 19 participants (13 women and 6 men), the reactivation-20 group, 20 participants (13 women and 7 men) and the no-reactivation group, 19 participants (15 women and 4 men).

2.2 Stimuli and apparatus

The US consisted of a 0.5s burst of white noise with an intensity of 105 dB(A). This was administered through Senheisser HZR 62 headphones. Two different coloured squares were used as CSs. A blue square of 10x10 cm was used as a CS-, that is, it was never paired with the US. A yellow square with the same characteristics was used as a CS+ and was paired in 38% of the trials with the US. The CSs were presented at the centre of a computer screen (LCD monitor, 22”) on a black background. The duration of both CSs was 4 seconds, and the US began 3.5s after the start of the CS+, so that they ended simultaneously.

We used SuperLab 4.0 software (Cedrus Corporation, 2008) for the programming and presentation of stimuli sequences. Furthermore, skin conductance response (SCR) was used as a fear response measure. This is the most frequently used measure in human subjects, both in experiments on the lines of the present one and in Pavlovian conditioning in general (Boucsein, 2012; Dawson, Schell, & Fillion, 2007). The SCR was registered with the module GSR-100C, coupled to a *Biopac MP150-WSW* system. For the registration of the SCR, 6 mm Ag/AgCl electrodes were used. The electrodes were placed in the middle phalanges of the index and middle fingers of the participant's non-dominant hand and filled with isotonic contact gel (Gel 101, Isotonic Recording Electrode Gel from BIOPAC Systems, Inc.). Finally, for the measurement of the SCR, AcqKnowledge 3.9.1 (BIOPAC Systems, Inc., Goleta, California) was used.

2.3 Procedure

After signing a consent form, the participant entered an acoustically isolated chamber. Both stimuli and instructions were presented on a computer screen located approximately 70 cm away. Temperature was maintained at between 20 and 25°C. A webcam located on the screen was used to monitor participants from the space used by the experimenter. After the placement of electrodes and headphones, participants were informed through the computer screen that attention should be paid to the visual and acoustic stimuli that would be presented. Finally, the experiment began when electrodermal activity had stabilised.

The experiment consisted of four phases: acquisition, reactivation (retrieval), extinction and re-extinction (see figure 1). All phases were conducted on different days. On the first day the acquisition phase took place. 24 hours later the reactivation and extinction phases were carried out, and the re-extinction took place 48 hours after the extinction phase. In all these phases, the sequence of trials was randomized for each participant, with the restriction that no more than two CS+ or CS- could appear in sequence.

Insert Figure 1

2.3.1 Acquisition

In this phase a Pavlovian differential conditioning procedure was used. Thus, participants received 10 CS-/noUS presentations, 10 CS+/noUS and 6 CS+/US. Following a procedure similar to Schiller et al. (2010), the CS+ was paired with the US using a 38% reinforcement schedule, and the CS- was never paired with the US. The interval between trials (ITI) was randomized to 10-12s, and it was the same in all subsequent phases.

2.3.2 Reactivation

24 hours after the acquisition phase, the reactivation phase began. Each participant in the reactivation groups received an isolated presentation of the CS+ without US. Those in the no-reactivation group did not receive this CS+ presentation and went directly to the extinction phase.

2.3.3 Extinction

Following the previous phase, participants in the reactivation groups rested by watching a cartoon video for a specific interval: those in the reactivation-10 group rested for 10 minutes, and the reactivation-20 group for 20 minutes. After the rest period, 10 trials of CS+/noUS and 11 of CS- were presented. In the case of the no-reactivation group, 24 hours after the acquisition each participant went directly to the extinction phase, in which 11 CS+/noUS and 11 CS- were presented.

2.3.4 Re-extinction

This phase started 48 hours after the extinction. Participants in both groups received 11 presentations of the CS+ and 11 of the CS-, always without US.

2.4 Measurements and analysis

CR was defined as a SCR with the greatest amplitude in the interval between seconds 1 and 4.5 after the presentation of the CS. For the measurement of responses a minimum value of 0.01 micro-Siemens (μS) was used. Once the SCR had been measured, and prior to analysis, we normalize the distribution of responses using logarithmic transformation in order to improve their statistical properties (Venables & Christie, 1980). A value of 1 was added to all measures of amplitude to avoid log (0) or logarithms with amplitudes below 1 μS. Hence we obtained a log (1+SCRamp) to which we could subsequently apply a range correction (Lykken, 1972). With this correction the responses of each participant were divided by her/his response of greatest amplitude, and thus the results obtained were between the values of 0 and 1. Finally, values were multiplied by 1000, so as to avoid working with very small values.

A criterion similar to that used in Schiller et al. (2010) was used for the selection of participants. Thus, the mean SCR to the CS+ had to be greater than the mean SCR to the CS- in the last 5 trials in acquisition phase. In addition, this differential mean had to be equal to or greater than 0.1 μS. Finally, the differential mean response of a participant in late extinction had to be less than 0.1 μS.

3. Results

SPSS 20 software (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses. For all stages we used a three-way ANOVA (Stimulus x Time x Group) and Bonferroni corrections for the follow-up analysis in order to avoid the accumulation of Type 1 error. For the analysis of the main effects and interactions a 0.05 alpha level was used.

3.1 Acquisition

In the acquisition phase we carried out a mixed three-way ANOVA, Stimulus (CS+, CS-) x Time (early and late phase) x Group (reactivation-10, reactivation-20, no-reactivation). For the Time factor, 'early' refers to the first half of the trials of the acquisition phase, and 'late' to the second half. This analysis only revealed a significant effect of the Stimulus ($F_{1,55}$= 220.75, p< .001, η^2_{part}= .80) and of the interaction of Stimulus x Time ($F_{1,55}$= 198.54, p< .001, η^2_{part}= .78).

In the reactivation-10 group the follow-up Bonferroni analysis showed that in the early phase there were no statistically significant differences between the SCR to the CS+ (M= 241.96, SD= 116.42) and to the CS- (M= 234.61, SD= 143.64). In the late phase, the SCR to the CS+ (M= 490.43, SD= 179.19) was significantly greater than the response to the CS- (M= 35.89, SD= 75.23) (see Fig. 2, panel A).

In the reactivation-20 group post-hoc tests showed no significant differences, in the early phase, between the responses to the CS+ (M= 249.22, SD= 170.99) and to the CS- (M= 257.75, SD= 165.21). In the late phase the response to the CS+ (M= 566.73, SD= 170.06) was significantly greater than the SCR to the CS- (M= 51.64, SD= 72.74), as seen in fig. 3 (panel A).

Finally, in the no-reactivation group post-hoc tests showed significant differences (p= .03) in the early phase between responses to the CS+ (M= 348.81, SD= 170.72) and to the CS- (M= 257.38, SD= 147.60). In the late phase, the SCR to the CS+ (M= 539.47, SD= 173.00) was significantly greater than that to the CS- (M= 69.24, SD= 109.43) (Fig. 4, panel A). In no group was the factor Time significant (p> .05).

Insert Figure 2

Insert Figure 3

Insert Figure 4

3.2 Reactivation/Extinction

In the reactivation groups (10 and 20 minutes) the reactivation trial was analyzed as an extinction trial in order to achieve the same number of trials as in the no-reactivation group (see Figure 1).

A mixed three-way ANOVA (Stimulus x Time x Group) was carried out. The levels of the factors Stimulus and Group were similar to those of the acquisition phase. In the factor Time the mean

response to the CSs during the late acquisition phase was compared (last 5 trials of acquisition) to the response to the CSs in the last trial of extinction, following the procedure used by Schiller et al. (2010). This analysis only showed a significant effect of the Stimulus ($F_{1,55}$= 263.21, p< .001, η^2_{part}= .83), Time ($F_{1,55}$= 150.42, p< .001, η^2_{part}= .73) and the interaction Stimulus x Time ($F_{1,55}$= 292.21, p< .001, η^2_{part}= .84)

In the reactivation-10 group the *a posteriori* tests showed that the response in the late phase of acquisition to the CS+ was significantly greater than in the last trial of extinction (M= 49.95, SD= 116.72). No statistically significant differences were found between the response to the CS- in the late phase of acquisition and to the SCR to the CS- in the last trial of extinction (M= 33.66, SD= 131.24) (see Fig. 2, panel A, late acquisition phase, and panel B, last trial of extinction).

In the reactivation-20 group post-hoc tests showed that the SCR in the late phase of acquisition to the CS+ was significantly greater than in the last trial of extinction (M= 9.47, SD= 23.04). No differences were found between the SCR to the CS- in the late phase of acquisition and to the SCR to the CS- in the last trial of extinction (M= 56.94, SD= 155.78) (see Fig. 3, panels A and B)

Finally, in the no-reactivation group Bonferroni tests showed that the SCR in the late phase of acquisition to the CS+ was significantly greater than the SCR to the CS+ in the last extinction trial (M= 39.57, SD= 118.53). By contrast, there were no differences between the response to the CS- in the late phase of acquisition and the response to the CS- in the last extinction trial (M= 73.97, SD= 232.53) (Fig. 4, panels A and B).

3.3 Re-extinction

Finally, in this phase a mixed ANOVA (Stimulus x Time x Group) was carried out. The factor Stimulus was considered in the same way as in the previous phases. For the factor Time a comparison was made of the first 4 trials of this phase with the subsequent 4 (early and late phase, respectively) following the same criteria as Schiller et al. (2010). This analysis showed significant effects of the Stimulus ($F_{1,55}$= 17.08, p< .001, η^2_{part}= .24), Time ($F_{1,55}$= 88.59, p< .001, η^2_{part}= .62), the interaction Stimulus x Time ($F_{1,55}$= 13.60, p= .001, η^2_{part}= .20), the interaction

Stimulus x Group ($F_{2,110}$= 8.63, p= .001, η^2_{part}= .24), and the interaction Stimulus x Time x Group ($F_{2,110}$= 12.95, p< .001, η^2_{part}= .32).

The Stimulus x Time ANOVAs conducted in each group showed that in the reactivation-10 group a significant effect was only obtained in Time ($F_{1,19}$= 14.00, p< .01, η^2_{part}= .42). Post-hoc tests showed that, in the early phase of re-extinction, there were no differences between the response to the CS+ (M= 175.17, SD= 183.20) and to the SCR to the CS- (M= 199.01, SD= 170.37). In the late phase of re-extinction, there were also no differences between the SCR to the CS+ (M= 67.46, SD= 102.86) and the response to the CS- (M= 51.73, SD= 101.41) (see Fig. 2, panel C).

In the reactivation-20 group, the analysis only showed a significant effect for Time ($F_{1,18}$= 27.59, p< .01, η^2_{part}= .61). As in the reactivation-10 group, Bonferroni tests showed that, in the early phase of re-extinction, there were no difference between the SCR to the CS+ (M= 312.18, SD= 286.61) and the SCR to the CS- (M= 225.26, SD= 181.15). In the late phase of re-extinction, the response to the CS+ (M= 119.24, SD= 151.47) also did not differ from the response to the CS- (M= 83.44, SD= 138.87), as can be seen in Figure 3 (panel C).

Finally, in the no-reactivation group, a significant effect was found for Stimulus ($F_{1,18}$= 36.03, p< .01, η^2_{part}= .67), for Time ($F_{1,18}$= 56.46, p< .01, η^2_{part}= .76) and for the interaction Stimulus x Time ($F_{1,18}$= 49.50, p< .01, η^2_{part}= .73). Differently from other groups, post-hoc tests showed that in the early phase of re-extinction, the response to the CS+ (M= 493.19, SD= 213.67) was significantly greater than the response to the CS- (M= 97.28, SD= 124.33), indicating a spontaneous recovery of the response. In the late phase of re-extinction, no differences were found between the response to the CS+ (M= 71.80, SD= 102.42) and to the CS- (M= 48.28, SD= 69.28) (see Fig. 4, panel C).

In addition, another mixed ANOVA (Stimulus x Time x Group) was carried out, comparing the response to stimuli in the latest trial of extinction with the SCR to stimuli in the first trial of re-extinction. This analysis revealed a significant effect of the Stimulus ($F_{1,55}$= 6.02, p= .02, η^2_{part}= .10), Time ($F_{1,55}$= 66.71, p< .001, η^2_{part}= .55), the interaction Stimulus x Time ($F_{1,55}$= 8.77, p< .01, η^2_{part}= .14), the interaction Stimulus x Group ($F_{2,110}$= 7.37, p=.001, η^2_{part}= .21) and the interaction Stimulus x Time x Group ($F_{2,110}$= 9.02, p< .001, η^2_{part}= .25).

As previously done, Stimulus x Time ANOVAs were conducted for each group. In the reactivation-10 group, the only significant effect found was that of the Stimulus ($F_{1,19}$= 16.04, p< .01, η^2_{part}= .46), not of Time or the interaction Stimulus x Time. *A posteriori* tests showed that the response to the CS+ in the last trial of extinction (M= 49.95, SD= 116.72) was statistically lesser than in the first trial of re-extinction (M= 295.62, SD= 397.34). The response to the CS- (M= 33.66, SD= 131.24) in the last trial of extinction was significantly lesser than in the first trial of re-extinction (M= 443.64, SD= 462.79) (see Fig. 2, panels B and C).

In the reactivation-20 group, a significant effect was only found for Time ($F_{1,18}$= 27.73, p< .01, η^2_{part}= .61), not for Stimulus or interaction Stimulus x Time. Post-hoc tests showed that the SCR to the CS+ (M= 9.48, SD= 23.04) in the last extinction trial was statistically lesser than the response to the CS+ (M= 450.40, SD= 434.46) in the first re-extinction trial. The SCR to the CS- (M= 56.94, SD= 155.78) in the last extinction trial was statistically lesser than the SCR to the CS- (M= 270.29, SD= 313.84) in the first re-extinction trial, as can be seen in Figure 3 (panels B and C).

Finally, in the no-reactivation group we observed a significant effect for the Stimulus ($F_{1,18}$= 20.70, p< .01, η^2_{part}= .54), for Time ($F_{1,18}$= 27.59, p< .01, η^2_{part}= .61) and for the interaction Stimulus x Time ($F_{1,18}$= 31.22, p< .01, η^2_{part}= .63). Post-hoc tests showed that, in the last extinction trial, the SCR to the CS+ (M= 39.57, SD= 118.53) was significantly lesser than the response to the CS+ (M= 678.45, SD= 395.04) in the first re-extinction trial. However, there were no significant differences between the response to the CS- (M= 73.97, SD= 232.53) in the last extinction trial and the response to the CS- (M= 158.06, SD= 276.95) in the first re-extinction trial (Fig. 4, panels B and C).

4. Discussion

Previous work (e.g. Agren et al., 2012a; Schiller et al., 2010; Thompson & Lipp, 2017) found that the use of an extinction procedure after the previous reactivation of a fear memory could disrupt the reconsolidation of the memory and prevent fear return. However, in other studies (e.g. Golkar et al., 2012; Kindt & Soeter, 2013; Klucken et al., 2016) no evidence of such an effect was found. As we noted above, such inconsistent findings may be due to the existence of boundary conditions or methodological differences across works (Klucken et al., 2016; Schroyens, Beckers, & Kindt, 2017).

Bearing in mind these discrepancies, and also the suggestions arising from the meta-analysis of Kredlow et al. (2016), in this paper we have set out to examine how certain moderators of a post-retrieval extinction procedure may affect the disrupting of human fear memory reconsolidation. To do this we adopted a procedure similar to that used in Schiller et al. (2010), in order to keep all other parameters constant and to control for variables that might affect the results, and that these were thus comparable. In our study the following were kept the same: CSs and number of presentations, a 38% rate of reinforcement of the CS+/US, the instructions given to participants, and the acquisition and extinction criteria used for data analysis. As already mentioned, the experiment began with an acquisition phase in which a CS was associated with a US, and a CS was associated with the absence of the US. Subsequently the sample was divided into three groups: two experimental groups (extinction 10 and 20 min. after reactivation) and a control group (extinction, without previous reactivation). Finally, the phase of re-extinction began 48 hours after the extinction phase, and the spontaneous recovery was measured.

The results showed that, in the acquisition phase, conditioning was obtained in the three groups, as expected. In all groups, conditioning was evident in the late phase, as shown by the fact that response to the CS+ was significantly greater than to the CS-. As for the reactivation/extinction phase, the results for all groups showed that the response to the CS+ in late acquisition phase was greater than in the last extinction trial, whereas no such difference was found to the CS-, suggesting that the response was extinguished in the three groups at the end of the extinction phase. This finding points in the same direction as that obtained by Schiller et al. (2010). Finally, in the re-extinction phase, the results showed a significant interaction of Stimulus x Time only in the control group (no-reactivation). *A posteriori* tests showed that this interaction was probably due to a spontaneous recovery of the response in the early phase in this group, which was not observed in the experimental groups. Again, this is similar to the findings of Schiller et al. (2010). However, the results of our comparison between the last extinction trial and the first re-extinction trial are not in keeping with the findings of that study. It is probable, then, that our results here confirm the suggestion in Klucken et al. (2016, p. 119) that different strategies used to analyse the extent of fear return can lead to inconsistent results.

First, our results confirmed the first of our hypotheses, in that by using an interval of 48 hours between extinction and re-extinction we obtained similar results in the spontaneous recovery

to those of other studies which used a 24-hour interval (e.g. Schiller et al., 2010; Thompson & Lipp, 2017). Despite the fact that a greater interval between extinction and the fear return test means that the memory has had more time to reconsolidate, this does not seem to involve differences in the disrupting of the fear memory reconsolidation.

Second, our results confirmed the second hypothesis, given that in the re-extinction phase no differences were found in responses to the CSs between the experimental groups of reactivation-10 and reactivation-20. In addition, these groups showed a lower response to the CS+ than the observed in control group. This suggests that the use of different time intervals between reactivation and extinction, within the reconsolidation window, does not have different effects on the fear return. In this sense, it would be interesting to investigate the delay between reactivation and extinction as a possible moderator of post-retrieval extinction procedure effects.

Finally, we were able to confirm our hypotheses that through use of a burst of white noise as a US, results are in keeping with the majority of previous studies using electric shock as a US (see Kredlow et al., 2016). One of the few studies in which a shock was not used as a US was that of Oyarzún et al. (2012), in which aversive sounds were used. However, the aversiveness of this US was based more on its unpleasant emotional valence than on its intensity. To the best of our knowledge, our study is the first to use a white noise as US (instead of the usual shock), the effectiveness of which has already been seen in many previous studies on human electrodermal conditioning (e.g. Boucsein, 2012). In future research it would be interesting to study how the type of US and its salience might affect the disrupting of fear memory reconsolidation (Shiban et al., 2015; Thompson & Lipp, 2017).

In conclusion, the timing between extinction and the fear return test, the delay between the retrieval and extinction, and the type of US in post-retrieval extinction procedures, might all serve to clarify some of the boundary conditions of the interference in fear memory reconsolidation. A clearer picture of these conditions would make it possible to obtain more effectively the behavioural memory updating effect. In future research, in addition to the previously mentioned potential boundary conditions (the fear measure and the reinforcement rate), other less explored conditions might be considered, such as the effect of context, which in the present study was the same in all phases. The ability to update memories after retrieval has tremendous clinical potential in the treatment of emotional disorders, including anxiety disorders, post-traumatic stress disorder (PTSD), and addiction (Auber et al., 2013; Beckers &

Kindt, 2017; Kredlow et al., 2016; Schiller, 2014; Shiban et al., 2015; Xue et al., 2012). Indeed, some authors (Ecker, Ticic, & Hulley, 2013) claim that the post-retrieval extinction procedure seems to be the only behavioural technique able to modify the original fear memory, as well as being the most effective in the elimination of the fear return. However, we concur with other authors (e.g. Beckers & Kindt, 2017; Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Kredlow et al., 2016) that more research is needed into the appropriate transfer of this technique to the area of clinical intervention.

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Figures

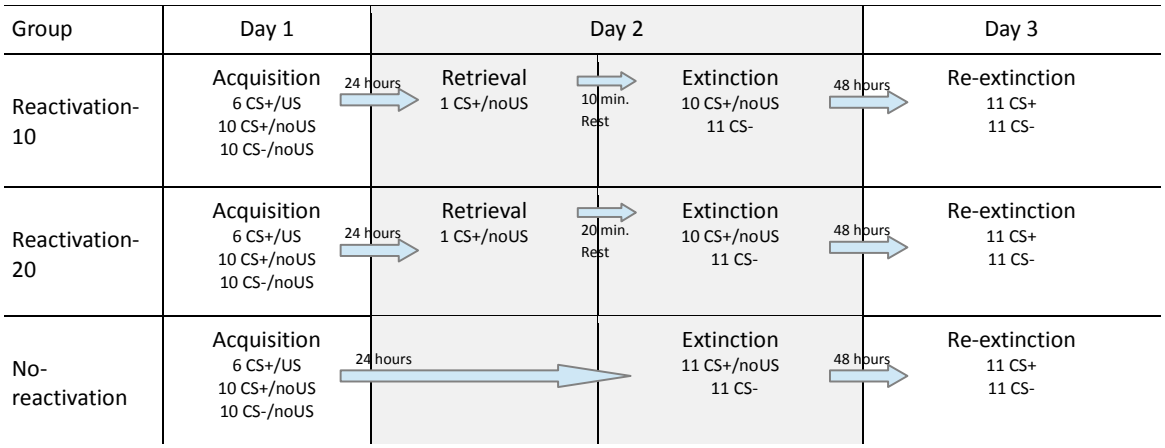

Fig 1. Groups and experimental phases.

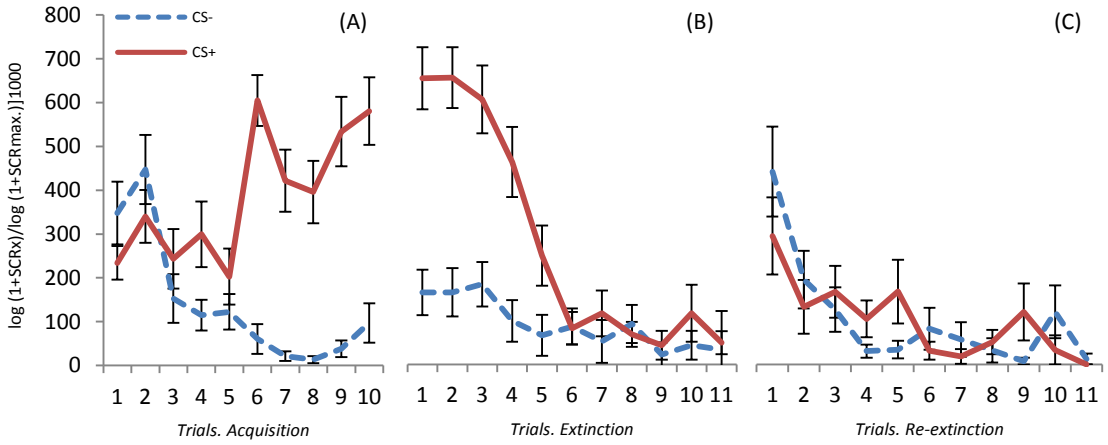

Fig 2. Reactivation-10 group corrected responses to the CSs during all the experiment phases. (A) Acquisition phase, (B) Extinction phase, (C) Re-extinction phase.

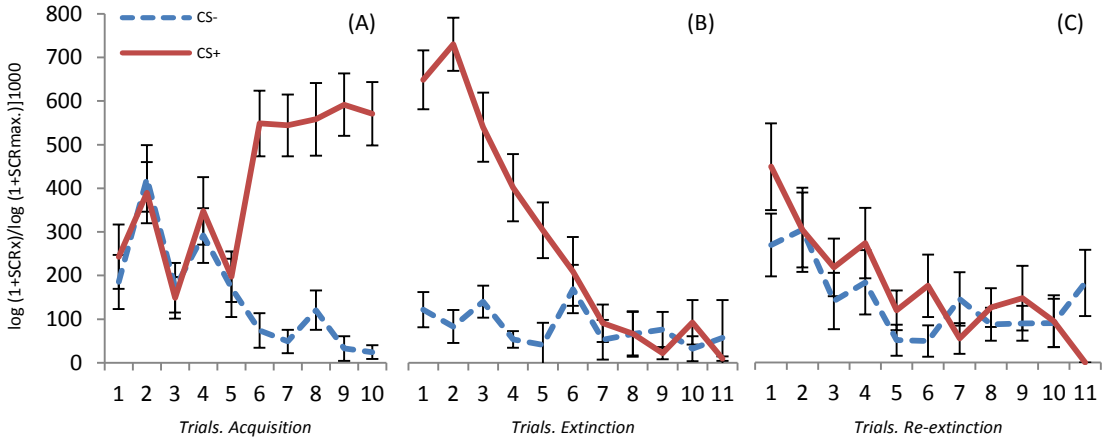

Fig 3. Reactivation-20 group corrected responses to the CSs during all the experiment phases. (A) Acquisition phase, (B) Extinction phase, (C) Re-extinction phase.

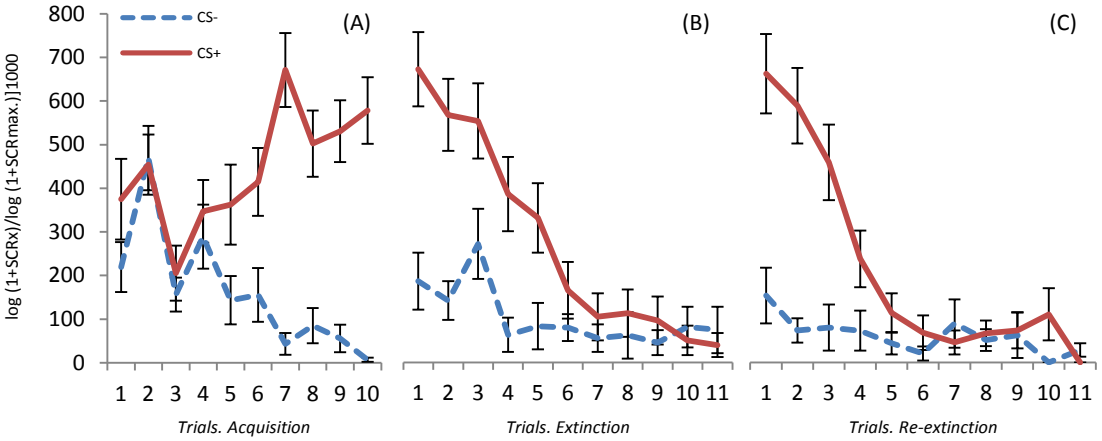

Fig 4. No-reactivation group corrected responses to the CSs during all the experiment phases. (A) Acquisition phase, (B) Extinction phase, (C) Re-extinction phase.