Erasing fear from memory
Soeter, M.

Citation for published version (APA):
Soeter, A. C. (2012). Erasing fear from memory Amsterdam
Chapter 2

Beyond Extinction: Erasing Human Fear Responses and Preventing the Return of Fear

This chapter is based on the article that is published as:

Animal studies have shown that fear memories can change when recalled, a process referred to as reconsolidation. We found that oral administration of the β-adrenergic receptor antagonist propranolol HCl before memory reactivation in humans erased the behavioral expression of the fear memory 24 hr later and prevented the return of fear. Disrupting the reconsolidation of fear memory opens up new avenues for providing long-term cure for patients with emotional disorders.
Since the dawn of psychology at the end of the nineteenth century, psychologists and psychiatrists have tried with dozens of pharmacological and psychological treatments to change undesired emotional memory. However, even the most effective treatments only eliminate fearful responding, leaving the original fear memory intact (Bouton, 2002), as is substantiated by the high percentages of relapse after apparently successful treatment (Craske, 1999). Once emotional memory is established, it appears to last forever. From an evolutionary perspective, it is extremely functional to never forget the most significant events in life. However, the putative indelibility of emotional memory can also be harmful and maladaptive, such as in some trauma victims who suffer from dreadful memories and anxiety. If emotional memory could be weakened or even erased, then we might be able to eliminate the root of many psychiatric disorders, such as post-traumatic stress disorder. Recently, it was rediscovered that fear memory in animals is not necessarily permanent, but can change when retrieved (Nader et al., 2000; Dudai, 2006; Tronson & Taylor, 2007). The reactivation of a consolidated (fear) memory can return it to a labile, supposedly protein synthesis-dependent state, a process referred to as reconsolidation (Nader et al., 2000). Reconsolidation of fear memory can be influenced by neurobiological manipulations during or shortly after the reactivation period (Tronson & Taylor, 2007). These manipulations are thought to alter protein synthesis directly (Nader et al., 2000) or by interacting with the release of neurotransmitters (e.g., norepinephrine) within the amygdala (McGaugh, 2004; Canal & Gold, 2007). At the behavioral level, this may lead to changes in later expressions of that fear memory. In particular, infusion of a β-adrenergic blocker (i.e., propranolol HCl) into the amygdala of rats shortly after the reactivation period of a previously acquired fear association impaired the fear expression on a long-term test. Apparently, propranolol HCl disrupts the reconsolidation of reactivated fear memories (Dębiec & LeDoux, 2004). Animal and human studies have shown that adrenal stress hormones activate adrenergic receptors in the amygdala and that the basolateral amygdala is essential for fear memory (McGaugh, 2004; van Stegeren et al., 2005).

In this human study we tested the hypotheses that the fear response can be weakened by disrupting the reconsolidation process and that disrupting the reconsolidation of the fear memory will prevent the return of fear. To test these hypotheses, we used a differential fear-conditioning procedure with fear-relevant
stimuli. Testing included different phases across three days: *fear acquisition* (day 1), *memory reactivation* (day 2) and *extinction* followed by a *reinstatement* procedure and a *test* phase (day 3). The conditioned fear response was measured as potentiation of the *eyeblink startle reflex* to a loud noise (40 ms; 104 dB) by electromyography (EMG) of the right orbicularis oculi muscle. Stronger startle responses to the loud noise during the fear conditioned stimulus (CS1+) as compared to the control stimulus (CS2-) reflects the fearful state of the participant elicited by the feared stimulus (CS1+). Startle potentiation taps directly into the amygdala and fear conditioning procedures yield highly reliable and robust startle potentiation (Davis, 2006). In addition, declarative knowledge of the contingency between the conditioned stimulus and the unconditioned stimulus was measured through online shock expectancy ratings during each CS presentation. Reconsolidation of fear memory was manipulated by the administration of propranolol HCl (40 mg, n = 20), randomized and double-blind placebo controlled (n = 20). In order to test whether the effect of propranolol HCl requires active retrieval of the fear memory, propranolol HCl (40 mg) was administered to a control condition (n = 20) without reactivation of the memory.

**Materials and Methods**

**Participants**

Sixty undergraduate students (17 men, 43 women) from the University of Amsterdam ranging in the age of 18 to 28 (mean ± SD age, 20.70 ± 4.1 years) participated in the study. All participants were assessed to be free from any current or previous medical or psychiatric condition that would contraindicate taking a single 40 mg oral dose of propranolol HCl (i.e., pregnancy, seizure disorder, respiratory disorder, cardiovascular disease, BP < 90/60, diabetes, liver or kidney disorder, depression, and psychosis). In order to eliminate individuals who might have difficulty with any temporary symptoms induced by the propranolol HCl manipulation, an additional exclusion criterion contained a high score (i.e., index above 26) on the ASI (Peterson & Reiss, 1992). Participants were randomly assigned to one of two conditions with the restriction that conditions were matched on SPQ (Klorman et al., 1974) scores as close as possible; pill placebo (n = 20; mean SPQ score ± SD, 6.10 ± 3.9) and propranolol HCl (n = 20; mean SPQ score ± SD 9.10 ± 6.3). For the additional control condition (i.e., propranolol no
reactivation; \( n = 20 \) a mean SPQ score of 8.05 (SD = 5.1) was obtained. The participants received either partial course credits or were paid a small amount (€ 35,-) for their participation in the experiment. The study was approved by the ethical committee of the University of Amsterdam and informed consent was obtained from all participants.

**Apparatus and Materials**

**Stimuli.** In order to strengthen the fear association during acquisition, fear relevant stimuli served as CSs (i.e., pictures of spiders; IAPS numbers 1200 - 1201) (Lang et al., 2005). The slides were 200 mm high and 270 mm wide and were presented in the middle of a black screen on a 19-inch computer monitor. One of the slides (CS1\(^+\)) was followed by an US (75 % of the presentations), while the other slide (CS2\(^-\)) was not. Assignment of the slides as CS1\(^+\) and CS2\(^-\) was counterbalanced across participants. Both the CS1 and CS2 stimuli were presented for 8 s. The startle probe was presented 7 s after CS onset and was followed by the US (CS1\(^+\)) 500 ms later. An electric stimulus with duration of 2 msec, delivered to the wrist of the non-preferred hand, served as US. Delivery of the electric stimulus was controlled by a Digitimer DS7A constant current stimulator (Hertfordshire, UK) via a pair of Ag electrodes of 20 by 25 mm with a fixed inter-electrode mid-distance of 45 mm. A conductive gel (Signa, Parker) was applied between the electrodes and the skin.

**Fear Potentiated Startle.** The conditioned fear response (CR) was measured as potentiation of the eyeblink startle reflex to a loud noise by electromyography (EMG) of the right orbicularis oculi muscle. Startle potentiation taps directly into the amygdala and fear conditioning procedures yield highly reliable and robust startle potentiation (Davis, 2006). The loud noise (40 ms; 104 dB) was administered during each CS presentation and during intertrial intervals (NA: Noise Alone). Two 7 mm Ag/AgCl electrodes filled with electrolyte gel were positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus. In order to maintain electrically identical paths and reduce common noise, the ground reference was placed ± 3 cm below the orbicularis oculi pars orbitalis on an electrically neutral site. All acoustic stimuli were delivered binaurally through headphones (Model MD-4600; Compact Disc Digital Audio, Monacor). The eyeblink EMG activity was measured using a bundled pair of electrode wires connected to a front-end amplifier with an input resistance of 10 MΩ and a bandwidth of DC-1500
Hz. To remove unwanted interference, a notch filter was set at 50 Hz. Integration was handled by a true-RMS converter (i.e., contour follower) with a time constant of 25 msec. The integrated EMG signal was sampled at 100 Hz. Peak amplitudes were identified over the period of 20 - 200 ms following startle probe onset.

**US Expectancy Measures.** Rated expectations of the US were measured online during CS presentation using a computer mouse on a continuous rating scale placed within reach of the preferred hand. The scale consisted of 11 points labeled from ‘certainly no electric stimulus’ (-5) through ‘uncertain’ (0) to ‘certainly an electric stimulus’ (5). The scale and the participant’s rating were continuously presented at the bottom of the computer screen in order to encourage participants to focus their attention to the CSs. Participants were required to rate the expectancy of an electric stimulus during the presentation of each slide by shifting the cursor on the scale and push the left mouse button within 5 s following stimulus onset, that is, before administration of the startle probe. Once the slides disappeared, the cursor automatically returned to the ‘uncertain’ position.

**Blood Pressure.** Blood pressure was measured using an electronic sphygmomanometer (OMRON M4-I, Healthcare Europe BV, Hoofddorp, The Netherlands), with a cuff applied around the right upper arm.

**Pharmacological Treatment.** Propranolol HCl (40 mg) and placebo pills were prepared and blinded by the pharmacy (Huygens Apotheek, Voorburg, The Netherlands).

**Subjective Assessments.** State and trait anxiety were assessed with the State and Trait Anxiety Inventory (i.e., STAI-S and STAI-T) (Spielberger et al., 1970). The degree of spider fear was determined by the Spider Phobic Questionnaire (SPQ) (Klorman et al., 1974). The Anxiety Sensitivity Index (ASI) (Peterson & Reiss, 1992) was used to assess one’s tendency to respond fearfully to anxiety-related symptoms.

**Experimental Procedure**

The experiment consisted of different phases across three subsequent days each separated by 24 hr. During each session, participants sat behind a table with a computer monitor at a distance of 50 cm in a sound-attenuated room. Each phase began with a 1-min acclimation period consisting of 70 dB broadband noise, which continued throughout the session as background noise, followed by a habituation phase consisting of ten startle probes to reduce initial startle
reactivity. Characteristics of the CSs, trial order, ITIs, and startle probes as well as the instructions regarding the US expectancy measures during memory reactivation (day 2) and extinction-test (day 3) were similar to acquisition (day 1). Assignment of the slide as CS1+ and CS2− was counterbalanced across participants.

**Acquisition.** Details of the various study procedures were explained in full and possible questions were answered. Participants were interviewed regarding their health and any medical or psychiatric conditions that would contraindicate taking a single dose of 40 mg of propranolol HCl. In addition, blood pressure was measured. Once a participant was medically cleared, written informed consent was obtained and the ASI, SPQ, and STAI-S were administered.

After attachment of the EMG and shock electrodes, the intensity of the US was determined by gradually increasing the level of a 2-ms aversive electric stimulus delivered to the wrist of the non-preferred hand. The intensity of shock was individually set at a level defined by the participant as ‘uncomfortable, but not painful’. After US selection, the participants were informed regarding the US expectancy measures. They were instructed to look carefully at both slides, as an electric stimulus would follow one of the slides in general, while the other slide would never be followed by the US. They were told that they should learn to predict whether an electric stimulus would occur or not on basis of the two slides. Participants were required to rate the expectancy of the electric stimulus during the presentation of each slide by shifting a cursor on a continuous 11-point rating scale and push the left mouse button within 5 s following stimulus onset, that is, before administration of the startle probe.

In the acquisition phase, both the CS1 and CS2 were presented 8 times for 8 s. The startle probe was presented 7 s after CS onset and was followed by the US 500 ms later. In order to prevent that the memory reactivation trial on day 2 would result in extinction learning, only 75 % of the presentations of the CS1 were reinforced (LaBar et al., 1998). Furthermore, 8 startle probes were presented alone (Noise Alone; NA). Intertrial intervals (ITI) varied between 15, 20, and 25 s with a mean of 20 s. Order of trial and ITI were quasi-random, with the restriction that no more than two consecutive trials or ITIs were of the same type.

At the conclusion of the acquisition phase, participants were explicitly instructed to remember what they had learned. These instructions were included to enhance retention of the CS-US contingency on the following days (Norrholm et
and to prevent participants from erroneously expecting a different contingency scheme during subsequent testing.

**Memory Reactivation.** In order to substantiate consolidation of the fear memory, a break of 24 hr after acquisition was inserted. In view of the peak plasma concentration of propranolol HCl (Gilman & Goodman, 1996), participants were given double-blind an oral dose of 40 mg propranolol HCl or pill placebo 90 minutes before memory reactivation. Administration of propranolol HCl and pill placebo was randomized across participants with the restriction that conditions were matched on SPQ scores as close as possible. Before pill administration and upon completion of the experiment, participants filled out the STAI-S and blood pressure levels were obtained.

After electrode attachment, participants were told that the same two slides of spiders would be presented. They were asked to look carefully at both slides and to remember what they had learned during acquisition. Further instructions regarding the US expectancy measures were similar to day 1. Reconsolidation of fear memory can be separately manipulated from extinction by a single as opposed to repeated unreinforced CS presentations (Doyère et al., 2007). In the *memory reactivation* phase, a single unreinforced CS1-R was presented for 8 s, followed by a startle probe presented alone. The procedure for the propranolol no reactivation condition paralleled the above, excepts for excluding memory reactivation and the electrode attachment.

**Extinction, Testing.** In view of the elimination half-life (Gilman & Goodman, 1996) and the possible effects of propranolol HCl on the startle fear response (Davis et al., 1993), *extinction - reinstatement* testing took place 24 hr after drug intake, allowing the drug to wash out before testing. Therefore, we could test the specific effect of propranolol HCl on the subsequent fear responding.

Instructions regarding the CSs only revealed that the same two pictures of spiders provided during acquisition would be presented. In the *extinction* phase, the participants were exposed to both the CS1` and CS2` for 10 times without the US. Furthermore, 10 startle probes were presented alone (NA). After the extinction procedure, participants received three unsignaled USs. The time between the last extinction trial and the first reinstating US was 19 s. Following the unsignaled USs, participants were presented with another 5 CS1`, CS2`, and NA trials (*reinstatement testing*). The time between the reinstating USs and
reinstatement testing was 18 s. At the end of the experiment, participants completed the STAI-T.

**Statistical analyses**

Startle responses and US expectancy ratings were analyzed by means of a mixed analysis of variance for repeated measures (ANOVA) with condition (propranolol HCl vs. pill placebo or propranolol HCl vs. propranolol no reactivation or pill placebo vs. propranolol no reactivation) as between-subjects factor and stimulus (CS1 vs. CS2) and trial (i.e., stimulus presentation) as within-subjects factors. Planned comparisons were performed for each condition separately. The first three as well as the last three trials of each stimulus type (CS1 vs. CS2) were averaged and compared over testing phases respectively. Missing data point were excluded from the analyses. Significance was set at $P < 0.05$.

**Results**

The propranolol HCl and pill placebo condition did not differ in terms of reported spider fear [$t_{38} < -1.7$], trait anxiety [$t_{38} < 1$] and shock intensity [$t_{38} < 1$]. We also observed no differences in reported spider fear between the propranolol no reactivation and the other two conditions [$t_{38} < 1$]. However, comparison of trait anxiety showed a marginally significant difference between the propranolol no reactivation (mean ± SD = 37.65 ± 4.05) and the pill placebo condition (mean ± SD = 32.15 ± 3.92) [$t_{38} = -1.99, P = .055, \text{two-tailed}$]. The difference between the propranolol no reactivation and propranolol condition (mean ± SD = 32.95 ± 5.07) approached significance [$t_{38} = -1.85, P = .072, \text{two-tailed}$]. Furthermore, the intensity of shock was significantly lower in the propranolol no reactivation (mean ± SD = 11.45 ± 3.88) as compared to the placebo condition (mean ± SD = 16.00 ± 4.71) [$t_{38} = 2.08, P < .05, \text{two-tailed}$] and compared to the propranolol condition the effect approached significance (mean ± SD = 14.10 ± 3.25) [$t_{38} = -1.74, P = .091, \text{two-tailed}$]. The differences in trait anxiety and US intensity will be discussed in the analyses of the startle fear response and US expectancy ratings. Consistent with other studies (Grillon et al., 2004), the propranolol HCl manipulation did not affect the reported state anxiety that was assessed before and after pill intake on day 2 [moment x condition, $F_{5,38} < 1.4$].
Manipulation Check Propranolol HCl

Analysis of the effect of propranolol HCl on blood pressure in the propranolol and propranolol no reactivation condition revealed the expected decrease in both systolic [moment x condition, $F_{1,38} = 6.10, P < 0.05, \eta_p^2 = .14$; $F_{1,38} = 9.47, P < 0.01, \eta_p^2 = .20$, respectively] and diastolic blood pressure [moment x condition, $F_{1,38} = 5.25, P < 0.05, \eta_p^2 = .12$; $F_{1,38} < 4.51, P < 0.05, \eta_p^2 = .09$, respectively] in comparison to pill placebo. No differences in decrease of both the systolic and diastolic blood pressure were observed between the propranolol and propranolol no reactivation condition [$F_{1,38} < 1.7$], indicating that both propranolol conditions exerted a similar physiological effect. Further analysis of blood pressure showed that, in the propranolol condition, the systolic blood pressure significantly decreased from 127.95 mmHg (SD = 9.9) to 113.50 mmHg (SD = 8.4) [$t_{19} = 7.50, P < 0.001$, two-tailed] and the diastolic blood pressure from 77.15 mmHg (SD = 6.5) to 70.35 mmHg (SD = 4.9) after pill intake [$t_{19} = 4.29, P < 0.001$, two-tailed]. In the placebo condition, we observed no decrease of either systolic or diastolic blood pressure [$t_{19} < 1.54$; $t_{19} < 1.15$, respectively]. In addition, in the propranolol no reactivation condition, both the systolic and diastolic blood pressure significantly decreased from 124.60 mmHg (SD = 11.3) to 107.95 mmHg (SD = 9.4) [$t_{19} = 8.99, P < 0.001$, two-tailed] and from 72.55 mmHg (SD = 5.3) 68.40 mmHg (SD = 4.9) [$t_{19} = 3.20, P = 0.05$, two-tailed] after pill intake, respectively.

Fear Potentiated Startle Response

Propranolol vs. Placebo. Analysis of variance showed fear conditioning on day 1 [stimulus x trial, $F_{1,38} = 46.91, P < 0.001, \eta_p^2 = .55$] (Fig. 2.1). We observed no difference in fear learning between the propranolol and placebo group [stimulus x trial x condition, $F_{1,38} < 1.37$]. On day 2, the two groups expressed comparable levels of startle responding during the fear memory reactivation (CS1-R) [$t_{38} < 1$]. In addition, the conditioned fear memory was equally well consolidated in the two groups, as is indicated by both the absence of a significant main effect of trial from the last three acquisition trials (CS1’) to the reactivation trial (CS1-R) [$F_{1,38} < 1$], and the absence of a trial x condition interaction effect [$F_{1,38} < 1$]. These data demonstrate that propranolol did not directly affect the expression of the fear memory. Propranolol did also not reduce the startle response per se, as we found no effects of propranolol HCl on the habituation trials [main effect of condition and trial x condition interaction, $F_{5,1,35} < 1$].
However, as can be seen in Figure 2.1, the administration of propranolol significantly decreased the differential startle response 48 hr later, that is, from acquisition (trial 6-8, day 1) to extinction (trial 1-3, day 3), whereas the differential startle response remained stable in the placebo condition [stimulus x trial x condition, $F_{1,38} = 17.17$, $P < 0.001$, $\eta_p^2 = .31$]. Post hoc comparisons indeed showed that propranolol strongly reduced the expression of fear memory [stimulus x trial, $F_{1,19} = 25.47$, $P < 0.001$, $\eta_p^2 = .57$], while the differential startle response remained stable in the pill placebo condition [stimulus x trial, $F_{1,19} < 1$]. In the propranolol condition, the conditioned fear response was not only reduced, but even eliminated since we no longer observed the differential startle response (CS1 versus CS2) (extinction trial 1-3, day 3) [$t_{19} < 1.22$]. In contrast, the differential startle response remained significant in the placebo condition [$t_{19} = 5.26$, $P < 0.001$, two-tailed].

Given that the differential startle response was already eliminated in the propranolol condition, the two groups differed over the course of extinction training on day 3 [stimulus x trial x condition, $F_{1,38} = 5.38$, $P < 0.05$, $\eta_p^2 = .12$]. Post hoc comparisons showed a significant decrease of the differential startle response in the placebo condition [stimulus x trial, $F_{1,19} = 11.31$, $P < 0.005$, $\eta_p^2 = .37$], but no change of the differential startle response in the propranolol condition [stimulus x trial, $F_{1,19} < 1$] (Fig. 2.1). At the end of extinction (trial 8-10), the differential startle response was still lower in the propranolol condition than in the placebo condition [stimulus x condition, $F_{1,38} = 7.94$, $P < 0.01$, $\eta_p^2 = .17$].

Exposure to the aversive stimulus (US) following extinction has been shown to reinstate the expression of the original fear memory in animals (Bouton, 2002) and humans (Norrholm et al., 2006). Evidence for a reinstatement effect is indicated by an increase of the differential startle response from the last extinction trials (trial 8-10) to the first test trial. Comparison of the reinstatement effect between the propranolol and placebo condition showed significantly more reinstatement in the placebo condition [stimulus x trial x condition, $F_{1,37} = 8.72$, $P < 0.01$, $\eta_p^2 = .19$]. Figure 2.1 indeed shows a significant reinstatement effect in the placebo condition [stimulus x trial, $F_{1,18} = 10.33$, $P < 0.01$, $\eta_p^2 = .37$], but not in the propranolol condition [stimulus x trial, $F_{1,19} < 1$]. The reinstatement procedure did even not reveal any differential startle response to the first test trial in the propranolol group [$t_{19} < 1$].
Fig. 2.1. Mean startle potentiation to the feared stimulus (CS1), the control stimulus (CS2) and noise alone (NA) trials during acquisition, memory reactivation, extinction and test for the (A) Placebo Reactivation, (B) Propranolol Reactivation, and (C) Propranolol no Reactivation Group. Error bars represent SEM.
Analysis of the startle response to Noise Alone trials (NA) unveiled neither a significant difference between the propranolol and placebo condition in the acquisition phase [main effect of pill, $F_{1,38} < 1$], nor to the one NA trial during memory reactivation [$t_{38} < 1$]. However, during extinction the startle response to the NA trials was slightly attenuated in the propranolol condition compared to the placebo condition though not significant [main effect of pill, $F_{1,38} = 3.11, P < 0.09, \eta_p^2 = .08$]. Moreover, the response to the first NA trial during test (i.e., after reinstatement) was reduced in the propranolol condition compared to the placebo condition [$t_{36} = 2.06, P < 0.05$, two-tailed], suggesting that the fear erasure effect at test (day 3) generalized to the context.

**Propranolol no Reactivation.** Trait anxiety and shock intensity differed between the propranolol no reactivation condition and both other conditions. In order to control for the possible effects of these variables on the startle response, we calculated Pearson correlations for both the whole sample and for the separate conditions. Only one significant correlation appeared between trait anxiety and the startle response to the control stimulus (CS2) after reinstatement in the propranolol no reactivation condition [$r = 0.49, P < 0.05$, two-tailed]. Therefore, only the analysis of the reinstatement effect of the differential startle response (CS1 vs. CS2) included trait anxiety as a covariate. Note that the positive correlation between trait anxiety and startle response to the control stimulus (CS2) is in line with other human fear conditioning studies (Grillon & Ameli, 2001; Grillon, 2002).

Analysis of the differential startle response (CS1 vs. CS2) on day 1 showed no difference in fear learning from trial 1-3 to trial 6-8 between the propranolol no reactivation and placebo condition [$F_{1,38} < 1$], but a marginally significant difference was observed between the propranolol no reactivation and propranolol condition [$F_{1,38} = 3.87, P = 0.056, \eta_p^2 = .09$]. Further analysis showed a significant increase of the differential startle response during acquisition in both the propranolol no reactivation [$F_{1,19} = 13.50, P < 0.01, \eta_p^2 = .42$] and the propranolol condition [$F_{1,19} = 28.01, P < 0.001, \eta_p^2 = .60$]. Note that the superior acquisition observed in the propranolol condition in comparison to the propranolol no reactivation condition (Fig. 2.1) works against the hypothesis that administration of propranolol combined with active retrieval of the fear memory would reveal less fear responses at test.
Similar to the placebo condition, the differential startle response remained stable from the last acquisition trials (trial 6-8) on day 1 to the first extinction trials (trial 1-3) on day 3 \([F_{1,38} < 1]\) (Fig. 2.1). Hence, we observed a normal fear response in the propranolol no reactivation condition 48 hr after acquisition (day 3). Moreover, the reduction of the conditioned startle response in the propranolol condition 24 hr after reactivation differed significantly from the propranolol no reactivation condition \([F_{1,38} = 29.02, P < 0.001, \eta^2_p = .43]\) (Fig. 2.1). In contrast to the propranolol condition, the differential startle response in the propranolol no reactivation condition even slightly increased from day 1 to day 3 \([F_{1,19} = 3.65, P = 0.07, \eta^2_p = .16]\). Thus, the decrease of the fear response in the propranolol condition is dependent on the active retrieval of the fear memory.

Analysis of extinction learning showed no difference of the startle response (CS1 vs. CS2) from trial 1-3 to trial 8-10 between the propranolol no reactivation and the placebo condition \([F_{1,38} < 1]\). In addition, the course of extinction between the propranolol no reactivation and the propranolol condition differed significantly \([F_{1,38} = 13.46, P < 0.01, \eta^2_p = .26]\) (Fig. 2.1). The extinction training significantly reduced the differential startle response in the propranolol no reactivation condition \([F_{1,19} = 35.40, P < 0.001, \eta^2_p = .65]\), whereas we observed no differential change of the startle response in the propranolol condition \([F_{1,19} < 1]\).

Analysis of the reinstatement effect with trait anxiety as covariate showed no difference between the propranolol no reactivation and placebo condition for the differential startle response (CS1 vs. CS2) from the last extinction trials (trial 8-10) to the first reinstatement trial \([F_{1,36} < 1.2]\). Hence, the fear reinstatement was not affected by the administration of propranolol HCl without active retrieval of the fear memory. Comparison of the reinstatement effect between the propranolol no reactivation and propranolol condition did not reveal the expected difference \([F_{1,36} < 2.0]\). However, as can be seen in Figure 2.1, not only the startle response to the feared CS1 but also to the control CS2 increased after the US only trials. Analysis of the reinstatement effect within the propranolol no reactivation condition showed a significant increase of the startle response (i.e., reinstatement effect for both CS1 and CS2) from the last extinction trials (trial 8-10) to the first reinstatement trial \([F_{1,19} = 7.40, P < 0.05, \eta^2_p = .28]\), but no increase of the differential startle response (CS1 vs. CS2) \([F_{1,19} < 1.7]\). The observation that the return of fear after reinstatement is not only observed for the feared stimulus (CS1), but also for the control stimulus (CS2), indicates a generalization of the previously acquired fear to
the safety signal in the propranolol condition without reactivation. This generalization effect has also been observed in other studies on fear reinstatement in humans (Dirikx et al., 2004, 2007). Since the generalization of fear was only observed in the propranolol no reactivation condition, further analysis of the fear reinstatement comprised the startle response to the feared CS1. Comparison of the propranolol no reactivation and propranolol condition revealed a significant difference of the fear reinstatement to the CS1 \(F_{1,37} = 4.46, P < 0.05, \eta_p^2 = .11\), indicating that the absence of fear reinstatement in the propranolol condition was dependent on the active retrieval of the fear memory (Fig. 2.1). Analysis of the fear reinstatement in the propranolol no reactivation condition indeed showed a significant return of fear to the feared stimulus (CS1) \(F_{1,19} = 8.40, P < 0.01, \eta_p^2 = .31\). Interestingly, the placebo and propranolol no reactivation condition both revealed a complete post-extinction recovery of the fear response, as is indicated by no difference in startle responding to the last acquisition trial (CS1) and the first reinstatement trial \(t_{36} < 1.4\) (Fig. 2.1). In sum, both the oral administration of propranolol HCl and the reactivation of the fear memory seemed to be necessary for the observed eradication of the fear response.

Analysis of the startle response to the Noise Alone trials (NA) unveiled no significant differences between the propranolol no reactivation and placebo condition during acquisition and extinction [main effect of pill, \(F_{5,38} < 1.5\], nor to the first NA trial after reinstatement \(t_{36} < -1.1\]. Also, we observed no differences in startle responding to the NA trials between the propranolol no reactivation and propranolol condition during acquisition [main effect of pill, \(F_{1,38} < 2.2\]. However, similarly to the differences between the propranolol and placebo condition, the startle responses to the NA trials were lower in the propranolol condition than in the propranolol no reactivation condition during extinction [main effect of pill, \(F_{1,38} = 8.35, P < 0.01, \eta_p^2 = .18\] and after reinstatement \(t_{38} = -2.96, P < 0.01, \) two-tailed]. Again, this suggests that the amnesic effect of propranolol HCl not only disrupted the reconsolidation of the previously learned fear association but also its context.

### US Expectancy Ratings

**Propranolol vs. Placebo.** We found no effects of the propranolol HCl manipulation on the US expectancy ratings [stimulus x trial x condition, \(F_{5,38} < 1\] (Fig. 2.2). In both the propranolol and placebo condition, we observed a significant
Fig. 2.2. Mean US expectancy ratings to the feared stimulus (CS1) and the control stimulus (CS2) during acquisition, memory reactivation, extinction and test for the (A) Placebo Reactivation, (B) Propranolol Reactivation, and (C) Propranolol no Reactivation Group. Error bars represent SEM.
differential increase in US expectancy (CS1 vs. CS2) during acquisition [stimulus x trial, $F_{1,38} = 190.92, P < 0.001, \eta_p^2 = .83$], a significant decrease in US expectancy during extinction [stimulus x trial, $F_{1,38} = 111.78, P < 0.001, \eta_p^2 = .75$] and a significant reinstatement effect [stimulus x trial, $F_{1,38} = 23.04, P < 0.001, \eta_p^2 = .38$] (Fig. 2.2).

**Propranolol no Reactivation.** Analysis of the US expectancy data revealed no differences in acquisition, extinction and fear reinstatement between the propranolol no reactivation condition and the other two conditions [stimulus x trial x condition, $F_{5,38} < 2.4$] (Fig. 2.2). Separate analyses for the propranolol no reactivation condition showed a significant acquisition effect [$F_{1,19} = 116.95, P < 0.001, \eta_p^2 = .86$] and a significant extinction effect [$F_{1,19} = 48.61, P < 0.001, \eta_p^2 = .72$]. In line with the startle responses, we observed a reinstatement effect for both the feared stimulus (CS1) and the control stimulus (CS2) [$F_{1,19} = 10.03, P < 0.01, \eta_p^2 = .35$], but no differential fear reinstatement effect [$F_{1,19} < 1$]. Analysis of the US expectancy to the feared CS1 stimulus alone also showed a significant reinstatement effect [$F_{1,19} = 8.73, P < 0.01, \eta_p^2 = .32$].

**Discussion**

In sum, oral administration of the β-adrenergic receptor antagonist propranolol HCl before reactivation of a fear memory resulted in a substantial weakening of the fear response. We used fear-relevant stimuli (i.e., pictures of spiders) because these are especially resistant to extinction following fear conditioning (Mineka & Öhman, 2002). Even more notable is our finding that one reactivation trial combined with the administration of propranolol HCl completely eliminated the behavioral expression of the fear memory 24 hours later. Second, our finding that a well-established retrieval technique for fear memories (i.e., reinstatement) failed to uncover any fear response suggests that the fear memory may either be erased (i.e., storage theory) or may be unavailable as a result of retrieval failure (i.e., retrieval theory) (Tronson & Taylor, 2007). Note that no behavioral procedure is currently available that differentiates between these two views of amnesia (Nader & Wang, 2006). Notably, the propranolol HCl manipulation left the declarative memory for the acquired contingency between the conditioned and unconditioned stimulus intact, but this knowledge no longer produced emotional effects. Our finding that propranolol eliminated the fear
response, without affecting declarative memory, is consistent with the observed double dissociation of fear conditioning and declarative knowledge relative to the amygdala and hippocampus in humans (Phelps, 2004). Propranolol selectively acts on the β-adrenergic receptors in the amygdala during emotional information processing in animals and humans (McGaugh, 2004; van Stegeren et al., 2005). It may be hypothesized that beta-adrenergic blockade during reconsolidation selectively disrupts the protein synthesis of the amygdalar fear memory, resulting in deconsolidation of the fear memory trace, while leaving the declarative memory in the hippocampus untouched.

Our findings are consistent with a recent preliminary study of patients with post-traumatic stress disorder in which post-retrieval propranolol HCl seemed to reduce subsequent physiological responding to traumatic memory (Brunet et al., 2008). Together, these results strongly suggest that β-adrenergic receptors are critically involved in the reconsolidation process of conditioned fear memories in humans. It is clear that β-adrenergic blockade during reconsolidation outperformed the traditional extinction procedure. But most importantly, and in contrast to the traditional extinction procedure, disrupting reconsolidation of fear memory prevented the return of fear. Millions of people suffer from emotional disorders and the relapse of fear, even after successful treatment. Our findings may have important implications for the understanding and treatment of persistent and self-perpetuating memories in patients suffering from emotional disorders.

References


