Fear memory uncovered: Prediction error as the key to memory plasticity

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Chapter 4

Retrieval per se is not sufficient to trigger reconsolidation of human fear memory

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ABSTRACT – Ample evidence suggests that consolidated memories, upon their retrieval, enter a labile state, in which they might be susceptible to change. It has been proposed that memory labilization allows for the integration of relevant information in the established memory trace (memory updating). Memory labilization and reconsolidation do not necessarily occur when a memory is being reactivated, but only when there is something to be learned during memory retrieval (prediction error). Thus, updating of a fear memory trace should not occur under retrieval conditions in which the outcome is fully predictable (no prediction error). Here, we addressed this issue, using a human differential fear conditioning procedure, by eliminating the very possibility of reinforcement of the reminder cue. A previously established fear memory (picture-shock pairings) was reactivated with shock-electrodes attached (Propranolol group, \( n = 18 \)) or unattached (Propranolol No-Shock Expectation group, \( n = 19 \)). We additionally tested a placebo-control group with the shock-electrodes attached (Placebo group, \( n = 18 \)). Reconsolidation was not triggered when nothing could be learned during the reminder trial, as noradrenergic blockade did not affect expression of the fear memory 24 h later in the Propranolol No-Shock Expectation group. Only when the outcome of the retrieval cue was not fully predictable, propranolol, contrary to placebo, reduced the startle fear response and prevented the return of fear (reinstatement) the following day. In line with previous studies, skin conductance response and shock expectancies were not affected by propranolol. Remarkably, a double dissociation emerged between the emotional (startle response) and more cognitive expression (expectancies, SCR) of the fear memory. Our findings have important implications for reconsolidation blockade as treatment strategy for emotional disorders. First, fear reducing procedures that target the emotional component of fear memory do not necessarily affect the cognitive component and vice versa. Second, mere retrieval of the fear memory is not sufficient to induce its labilization and reconsolidation.
BACKGROUND

A dynamic balance between stability and plasticity of memory seems to be crucial for adaptation to an ever-changing environment. Stability of fear memory guarantees a fast response to threat and does not require continuous re-learning, whereas plasticity permits modification of an established memory trace, should conditions require such adaptation (Dudai, 2009). Plasticity of fear memory is also of clinical importance, as it provides a window of opportunity to target unwanted, excessive emotional memories such as those that underlie anxiety disorders (e.g., PTSD). While consolidation, the strengthening of a memory trace over time, serves the stability of memory (McGaugh, 1966), the process of reconsolidation, the protein-synthesis dependent restabilization of memory upon retrieval, allows for memory modification (Nader et al., 2000). Reconsolidation is typically demonstrated by the amnesic effects of protein synthesis inhibitors administered before or after memory reactivation (Nader et al., 2000; Pedreira, Pérez-Cuesta, & Maldonado, 2002; Sara, 2000). Recently, we demonstrated that administration of the β-adrenergic receptor antagonist propranolol before memory reactivation disrupted the emotional expression of fear memory (startle fear response) and prevented the return of fear 24 h later, while declarative memory remained intact (Kindt et al., 2009; Soeter & Kindt, 2010, 2011b).

If retrieval per se were sufficient to render a memory trace labile, memory would be hypermalleable. Indeed, it has been suggested that reconsolidation does not necessarily occur when a memory is being reactivated, but only when something can be learned during memory retrieval (memory updating) (Forcato, Argibay, Pedreira, & Maldonado, 2009; Forcato, Rodríguez, Pedreira, & Maldonado, 2010; Lee, 2009; Pedreira et al., 2004). A violation of expectation based on prior learning — the magnitude of the outcome or the outcome itself is not fully predicted (prediction error) — is thus a prerequisite for reconsolidation to take place. We hypothesize that when nothing can be learned during memory reactivation (no prediction error), there is no need for the memory to be updated and reconsolidation will not be triggered.

Here we examined in a differential fear conditioning study in humans whether reconsolidation of a previously established fear memory depends on the outcome of the reactivation trial. We applied a reactivation procedure similar to our previous studies (i.e., unreinforced presentation of the feared stimulus) (Kindt et al., 2009; Soeter & Kindt, 2010, 2011b), but discarded the reactivation trial of prediction error by excluding the very possibility of reinforcement. Briefly, on day 1,
fear acquisition was established with use of two spider pictures (Conditioned Stimuli; CSs). One of the pictures (CS1) was followed by an aversive electrical stimulation (Unconditioned Stimulus; US), while the other was not (CS2). On day 2, participants received propranolol before the fear memory was reactivated with a non-reinforced reminder presentation of the feared stimulus (CS1-R). During memory retrieval, shock electrodes were attached in one group (Propranolol group), but not in the other (Propranolol No-Shock Expectation). As such, presentation of the CS1 was supposed to elicit active anticipation of the US in the former group but not in the latter. To test whether the observed effect of the manipulation of shock expectation of the reminder trial was indeed related to a propranolol-induced disruption of reconsolidation, an additional group of participants received a placebo pill before memory reactivation. Similar to the Propranolol group, the shock electrodes were attached during the reminder presentation in the Placebo group. In all groups, expression of the fear memory was tested 24 h later (day 3). We hypothesize that a reactivation session that is devoid of prediction error (i.e., prior knowledge that the presentation of the feared stimulus (CS1-R) cannot be followed by shock) will fail to trigger reconsolidation. Noradrenergic blockade will disrupt reconsolidation of a previously acquired fear memory when learning can occur during reactivation. Therefore, we predict that in contrast to both the Propranolol No-SE and the Placebo group, administration of propranolol before memory reactivation will attenuate subsequent expression of the fear memory (startle fear response) in the Propranolol group. In line with our previous studies, declarative memory will remain unaffected (US-expectancies). Given the close association of the skin conductance response (SCR) with declarative knowledge (Hamm & Weike, 2005), noradrenergic blockade will not affect electrodermal responding either.

MATERIALS AND METHODS

Participants
Sixty (19 male; 41 female) healthy undergraduate students participated in the study, ranging in age between 18 and 30 years, with a mean age of 21.08 years (SD = 2.61). All participants were free from any condition contraindicative to the administration of 40 mg propranolol (see Soeter & Kindt, 2010). Participants received either partial course credit or a small amount of money (€ 35,-) for their participation. All participants gave informed consent and were notified that they could withdraw from participation at any time. The study had full ethical approval.
Participants were assigned to the Propranolol group (n = 20, 7 male), the Propranolol No-Shock Expectation (No-SE) group (n = 20, 6 male) or the Placebo group (n = 20, 5 male) with the restriction that groups were matched on gender and Spider Phobia Questionnaire (SPQ) scores.

**Apparatus**

**Stimuli.** The conditioned stimuli (CS) consisted of 2 different images depicting spiders (IAPS, nr 1200; 1201). Electrical stimulation was delivered through a pair of Ag electrodes of 20 by 25mm with a fixed inter-electrode mid-distance of 45 mm. Shock deliverance was controlled by a Digitimer DS7A constant current stimulator (Hertfordshire, UK). Between the electrodes and the skin a conductive gel (Signa, Parker) was applied.

**Fear potentiated startle (FPS).** Startle response was measured through electromyography (EMG) of the right orbicularis oculi muscle. Two 5-mm Ag/AgCl electrodes filled with a conductive gel (Signa, Parker) were positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus, respectively; a ground electrode was placed on the forehead, 1 cm below hairline (Blumenthal et al., 2005). Acoustic stimuli were presented binaurally through headphones (Sennheiser, model HD 25-1 II). The EMG signal was sampled at 1000 Hz and amplified in two stages. The input stage had an input resistance of 10 MOhm, a frequency response of DC-1500Hz and an amplification factor of 200. A 50Hz notch filter was used to reduce interference of the mains noise. The second stage amplified the signal with a variable amplification factor of 0-100 x and integrated the signal. The raw EMG data were band-pass filtered (28-500Hz, Butterworth, 4\textsuperscript{th} order (Blumenthal et al., 2005)) to obtain the cleanest possible data without affecting response amplitude. Peak blink amplitude was determined in a 30-150 ms interval following probe onset.

**Skin conductance response (SCR).** Electrodermal activity was measured using an input device with a sine-shaped excitation voltage (7.5 V) of 50 Hz, which was derived from the mains frequency. Two Ag/AgCl electrodes of 20 by 16 mm were attached with adhesive tape to the medial phalanges of the first and third fingers of the non-preferred hand. The signal from the input device was led through a signal-conditioning amplifier and the analogue output was digitized at 100 Hz by a 16-bit AD-converter (National Instruments, NI-6224). Startle response and electrodermal activity were recorded with the software program VSSRP98 v6.0. Electrodermal responding to the CS was calculated by subtracting the
baseline (2 s before stimulus onset) from the maximum score during the 0 to 7 s window after CS onset. This is a well-established approach of examining electrodermal reactivity and has been used extensively in human psychophysiological research (Milad et al., 2005; Orr et al., 2000; Pineles et al., 2009).

**Subjective distress ratings.** Subjective distress was measured online during each image presentation, on an 11-point scale ranging from ‘not distressed at all’ (0) to ‘very distressed’ (10). The scale was placed at the bottom of the screen below the CS picture. Participants rated distress levels by shifting the cursor on the scale with use of the mouse and confirmed their ratings by pushing the left mouse button within 7 s following stimulus onset.

**US-expectancy ratings.** Participants were asked to complete a graph representing the evolution of their US-expectancies during the experiment. US expectancy was depicted on the Y-axis ranging from ‘at that moment, I very strongly expected a shock’ (5), through ‘I didn’t know what to expect’ (0) to ‘at that moment, I very strongly expected no shock’ (-5). On the X-axis the different experimental phases were depicted (Vervliet et al., 2005).

**Drug treatment.** Propranolol HCl (40 mg) and placebo pills were prepared by a pharmacy (Huygens Apotheek, Voorburg, The Netherlands). We measured blood pressure with a cuff attached to the right upper arm, using an electronic sphygmomanometer (Omron, model HEM-780-D).

**Subjective assessments.** The Spider Phobia Questionnaire (SPQ; Klorman, Weerts, Hastings, Melamed, & Lang, 1974) was used to assess the degree of spider fear. In addition, the Anxiety Sensitivity Index (ASI; Peterson & Reiss, 1992) was taken to assess a subject’s tendency to respond anxiously to the temporary symptoms of the use of propranolol. State and trait anxiety were measured with the State and Trait Anxiety Inventory (STAI-S/STAI-T; Spielberger, Gorsuch, & Lushene, 1970) to assess the influence of propranolol on state anxiety and general level of anxiety, respectively. Evaluation of the US was assessed on an 11-point scale ranging from ‘unpleasant’ (-5) to ‘pleasant’ (5) to investigate the effect of pill administration on the course of US-evaluation.

**Procedure**

The experiment consisted of three testing sessions on consecutive days. Each testing session started with ten startle habituation trials to stabilize baseline startle reactivity. To assess baseline startle responding during the experimental phases
startle probes alone (Noise Alone; NA) were presented in addition to the CS presentations. Throughout all the experimental phases participants rated their subjective distress during each CS presentation. Testing procedures were adapted from Kindt et al. (2009). For a schematic representation of the experimental design see Fig. 1.

**Fear conditioning.** All participants underwent fear conditioning on day 1. The CSs consisted of 2 different images depicting spiders (IAPS, nr 1200; 1201). One of the spider pictures (CS1) was paired with a mild shock to the wrist (US, determined individually to be ‘uncomfortable though not painful’) on 75% of the trials, whereas the other spider picture was never paired with a shock (CS2). Assignment of the pictures as CS1 or CS2 was counterbalanced across participants. Both CSs were presented 8 times for 8 s. Startle probe was delivered 7 s after CS onset, followed by the US 500 ms later. The US consisted of an electrical stimulus (2 ms). Intertrial intervals (ITI) varied from 15 s to 25 s with an average of 20 s. All participants were instructed that one of the pictures was followed by a shock on most trials, while the other picture was never followed by a shock.

** Reactivation.** On day 2, participants were randomly assigned to the Propranolol group or the Propranolol No-Shock Expectation (No-SE) group. Assignment to the additional Placebo group was not random. Participants in the Propranolol and the Propranolol No-SE group received a propranolol pill (40 mg), while participants in the Placebo group received a placebo pill (blind). To take advantage of the peak plasma levels of propranolol (Gilman & Goodman, 1996), the memory was reactivated 90 minutes after pill intake. We previously showed that propranolol did not affect the startle fear expression during memory retrieval, suggesting that it affected the process of reconsolidation (Kindt et al., 2009; Soeter
& Kindt, 2010). After attachment of the SCR and EMG electrodes, all participants were told that the same pictures of spiders would be presented again. Participants in both the Propranolol group and the Placebo group got the US-electrodes attached to the wrist and were instructed to remember what they had previously learned (i.e., CS-US contingencies). Crucially, participants in the Propranolol No-SE group were explicitly instructed that the CS1 would not be followed by shock. Additionally, the US-electrodes was not attached in this group of participants to ensure they believed the US not to come. By this means the reactivation trial was discarded of prediction error. Participants in all groups were presented with a single unreinforced CS1 presentation of 8 s. State anxiety and US-evaluation were measured before and after pill administration.

**Extinction training and reinstatement test.** One day later, on day 3, the procedure was similar for the three groups. Participants were instructed that the same two pictures of spiders would be presented again. The instructions did not reveal anything regarding the occurrence of the US. During extinction, participants were presented with 16 unreinforced CS1 and CS2 trials. After extinction learning, three unsignaled reminder shocks were administered to the wrist. Participants were presented with five unreinforced CS1 and CS2 trials to test reinstatement of fear. At the end of the experiment participants retrospectively rated their US-expectancies.

**Data Analysis**
For both startle and SCR data outliers were defined for each day separately (Z > 3) and replaced by linear trend at point. To reduce variability of startle response, electrodermal activity and distress ratings data were averaged in blocks of two trials, with the exception of the reactivation trial and the first reinstatement test trial. Startle responses, US-expectancy ratings, electrodermal activity and distress ratings were then subjected to a mixed analysis of variance for repeated measures (ANOVA) with group (Propranolol vs. Propranolol No-SE; Propranolol vs. Placebo) as between-subjects factor and stimulus (CS1 vs. CS2; CS1 vs. NA) and time (stimulus presentation) as within-subjects factors.

Startle response to NA and habituation trials were analysed with use of mixed repeated measures ANOVAs with group (Propranolol vs. Propranolol No-SE; Propranolol vs. Placebo) as between-subjects factor and trial (stimulus presentation averaged over two trials or single trials) as within-subjects factors. Planned comparisons were performed for each group separately. SPQ, ASI and
STAI-T scores were subjected to ANOVAs with group (Propranolol vs. Propranolol No-SE; Propranolol vs. Placebo) as between-subjects factor. Mixed ANOVAs with group (Propranolol vs. Propranolol No-SE; Propranolol vs. Placebo) as between-subjects factor and moment (day 1 vs. day 3; before vs. after pill administration) as within-subjects factor were used to analyse the effect of pill administration on the course of blood pressure, US-evaluation and STAI-S scores. Moment-to-moment differences were analysed with repeated measures (ANOVA) with stimulus (CS1 vs. CS2; CS1 vs. NA) and trial (i.e., stimulus presentation) as within-subjects factors. A Greenhouse-Geisser procedure was used in case of violation of the sphericity assumption in ANOVAs. The alpha level was set at .05 for all statistical analyses. Five participants were left out for the analysis (Propranolol group, n = 18; Propranolol No-SE group, n = 19; Placebo group, n = 18), because of a failure to demonstrate successful fear acquisition. Non-successful fear conditioning was defined as greater NA than CS1 startle response during the second half of acquisition (trials 5 to 8). One subject (Propranolol No-SE group) was excluded from analysis of the day 2 startle response and skin conductance response data, due to a physiological data registering problem on day 2.

RESULTS

Questionnaires, Evaluations and Blood Pressure

The Propranolol group did not differ in reported spider fear, anxiety sensitivity or trait anxiety from the Propranolol No-SE group and the Placebo group (Fs < 1.05). The individually set shock intensity ranged from 4 to 55 mA (M = 16.5, SD = 10.08). Comparing the groups with respect to US-intensity revealed higher shock intensities for the Propranolol group (M = 18.83, SD = 8.7) compared to the Propranolol No-SE group (M = 12.53, SD = 6.95) (F(1, 36) = 5.97, p < .05) but not compared to the Placebo group (M = 18.22, SD = 12.76) (F(1, 35) < 1) (Table 1). Note that higher US-intensities do not necessarily result in a stronger CS-US association. Still, if stronger fear memory would have been established in the Propranolol group, it should, if anything, be more difficult to weaken the subsequent fear expression in this group. Importantly, there were no differences in the evaluation of the US between the Propranolol group and the Propranolol No-SE and Placebo groups on either day 1 or day3 (Fs < 1.50), indicating that participants experienced the US similarly. In addition, no differences between the Propranolol group and the Propranolol No-SE and Placebo groups were observed in either the change of US-evaluation from day 1 to day 3 (Table 2) or change in state anxiety (Table 3) before and after pill
Table 1. Mean values (SD) of the US-intensity, US-evaluation, reported spider fear (FSQ), anxiety sensitivity (ASI), and trait anxiety (STAI-T) for the Propranolol No-Shock Expectation (n = 19), Propranolol (n = 18), and the Placebo group (n = 18).

<table>
<thead>
<tr>
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<th>Propranolol No-Shock Expectation</th>
<th>Propranolol</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>US-intensity (mA)</td>
<td>12.5 (6.9)</td>
<td>18.8 (8.7)</td>
<td>18.2 (12.8)</td>
</tr>
<tr>
<td>US-evaluation day 1</td>
<td>-2.9 (.7)</td>
<td>-2.8 (1.0)</td>
<td>-2.7 (1.0)</td>
</tr>
<tr>
<td>US-evaluation day 3</td>
<td>-1.9 (1.5)</td>
<td>-2.0 (1.0)</td>
<td>-2.4 (.8)</td>
</tr>
<tr>
<td>Spider fear (SPQ)</td>
<td>4.6 (3.6)</td>
<td>5.8 (4.4)</td>
<td>6.0 (4.9)</td>
</tr>
<tr>
<td>ASI</td>
<td>9.2 (4.5)</td>
<td>10.7 (6.0)</td>
<td>8.9 (6.0)</td>
</tr>
<tr>
<td>STAI-T</td>
<td>32.8 (7.8)</td>
<td>35.4 (7.2)</td>
<td>35.1 (8.0)</td>
</tr>
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</table>

administration (time x group; Fs < 2.49). Finally, analysis revealed no differences in the course of systolic and diastolic blood pressure before and after pill intake between the Propranolol and the Propranolol No-SE groups (time x group; F(1, 35) < 1). We observed near significant differences between the Propranolol and the Placebo group in the course of systolic blood pressure (time x group; F(1, 34) = 2.57, p = .112, η² = .07) and diastolic blood pressure (time x group; F(1, 34) = 5.32, p = .071, η² = .09) before and after pill intake. Systolic blood pressure decreased from the first to the second measurement in the Propranolol group (main effect of time; F(1, 17) = 29.10, p < .001, η² = .63), in the Propranolol No-SE group (main effect of time; F(1, 18) = 29.10, p < .001, η² = .63), but also in the Placebo group (main effect of time; F(1, 17) = 10.55, p < .01, η² = .38). The course of diastolic blood pressure showed a significant decrease in the Propranolol group (main effect of time; F(1, 17) = 10.92, p < .01, η² = .39) and the Propranolol No-SE group (main effect of time; F(1, 18) = 10.83, p < .01, η² = .38). Diastolic blood pressure did not decrease in the Placebo group (main effect of time; F(1, 17) < 1). Finally, no differences between the Propranolol group and the Propranolol No-SE and Placebo groups were observed in the change in state anxiety before and after pill administration (time x group; Fs < 2.49). (Table 2).
Table 2. Mean values (SD) of the systolic blood pressure (BP), diastolic blood pressure and state anxiety before (t=0) and 90 min after (t=1) pill administration for the Propranolol No-Shock Expectation (n = 19), Propranolol (n = 18), and the Placebo group (n = 18).

<table>
<thead>
<tr>
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<th>Propranolol No-Shock Expectation</th>
<th>Propranolol</th>
<th>Placebo</th>
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<tbody>
<tr>
<td></td>
<td>t=0</td>
<td>t=1</td>
<td>t=0</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>115.7 (13.3)</td>
<td>119.4</td>
<td>117.1</td>
</tr>
<tr>
<td></td>
<td>(13.3)</td>
<td>(14.4)</td>
<td>(11.3)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>69.8 (5.8)</td>
<td>70.1</td>
<td>72.7</td>
</tr>
<tr>
<td></td>
<td>(7.3)</td>
<td>(7.3)</td>
<td>(7.3)</td>
</tr>
<tr>
<td>BP</td>
<td>31.2 (6.8)</td>
<td>32.4</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>(6.5)</td>
<td>(5.6)</td>
<td>(7.2)</td>
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**Propranolol vs. Propranolol No-Shock Expectation (No-SE)**

Fear potentiated startle. There was successful fear conditioning on day 1, as indicated by a gradual increase (trials 1-2 to 7-8) in differentiation between CS1 and CS2 (stimulus x time; \(F_{(2.77, 97.17)} = 7.00, p < .001, \eta^2_p = .17\) [Fig. 2A, B]. This pattern did not differ between groups (stimulus x a differential startle response, as was indicated by more time x group; \(F_{(2.77, 97.17)} < 1\)). Analysis of the startle data at day 2 revealed responding to the reactivated feared stimulus (CS1-R) than to the noise alone (NA-R) [main effect of stimulus; \(F_{(1, 35)} = 8.51, p < .01, \eta^2_p = .20\)]. Differential responding during reactivation did not differ between the two groups (stimulus x group; \(F_{(1, 35)} < 1\)). Hence, startle potentiation to the feared stimulus was higher compared to the noise alone in both the Propranolol group (main effect of stimulus; \(F_{(1, 17)} = 4.32, p < .05, \eta^2_p = .19\)) and the Propranolol No-SE group (main effect of stimulus; \(F_{(1, 18)} = 6.59, p < .05, \eta^2_p = .27\)). The fear potentiated startle response thus seems resistant to higher-order cognition (i.e., knowledge that the feared stimulus will not be followed by the shock), since the expression of the previously acquired fear association was not affected by the absence of US-expectation. The groups did not differ in the general startle response (NA) during habituation and over the course of habituation (main effect of group; time x group, \(F_{(1, 34)} < 1\) on day 2, indicating that the absence of the US-electrodes during reactivation did not affect baseline startle responding either (for a more elaborate discussion on fear potentiated startle on day 2, see supplement).
As expected, analysis of startle fear response on day 3 revealed a difference in extinction learning (trials 1-2 to 15-16); there was larger differential responding (CS1 vs. CS2) in the Propranolol No-SE group compared to the Propranolol group (stimulus x group; $F_{(1, 35)} = 10.17, p < .01, \eta^2_g = .23$) (Fig. 2A,B). There was no reduction in differential fear responding in the Propranolol No-SE group throughout the course of extinction (stimulus x time; $F_{(4.28, 77.06)} < 1$). Higher
startle response to the feared stimulus (CS1) compared to the non-feared stimulus (CS2) was present not only during trials 1-2 of extinction (main effect of stimulus; \( F_{1, 18} = 18.69, p < .001, \eta^2_p = .51 \)) but also at the end of extinction (trials 15-16) (main effect of stimulus; \( F_{1, 18} = 7.89, p < .05, \eta^2_p = .31 \)). We did not observe an immediate reduction in differential startle fear responding at the beginning of extinction (trials 1-2) in the Propranolol group (main effect of stimulus; \( F_{1, 17} = 15.05, p = .001, \eta^2_p = .47 \)). Thus, startle responses were significantly potentiated when probed during the first trials (1-2) of the CS1 relative to the CS2 in the Propranolol group. This potentiation, however, was no longer observed during the remaining trials of extinction (trials 3-4; trials 15-16; main effect of stimulus; \( F_{1, 17} < 2.20 \); stimulus x time; \( F_{3.74, 63.66} < 1.10 \)). Analysis of reinstatement testing showed an effect of group in differential responding during the first test trial (stimulus x group; \( F_{1, 35} = 8.22, p < .01, \eta^2_p = .19 \)). Responses to the feared stimulus (CS1) were higher than startle response to the safe stimulus (CS2) in the Propranolol No-SE group (main effect of stimulus; \( F_{1, 18} = 9.96, p < .01, \eta^2_p = .36 \)), while there was a lack of differential responding (CS1 vs. CS2) in the Propranolol group (main effect of stimulus; \( F_{1, 17} < 1 \)).

**Retrospective US-expectancy ratings.** We observed acquisition, extinction and reinstatement of US-expectancy in both groups as was evidenced by a significant increase in differential US-expectancy (CS1 vs. CS2) from the beginning to the end of acquisition (stimulus x time; \( F_{1, 35} = 336.36, p < .001, \eta^2_p = .91 \)), a decrease in differential US-expectancy from the beginning to the end of extinction learning (stimulus x time; \( F_{1, 35} = 62.28, p < .001, \eta^2_p = .64 \)) and an increase in differential US-expectancy from end of Figure 3. Mean retrospective expectancy of the unconditioned stimulus (US) during acquisition, reactivation, extinction and reinstatement test for (A), the Propranolol No-SE (n = 19) (B), Propranolol (n = 18) and (A), the Placebo (n = 18) conditions. Error bars represent SEM.
extinction to test (stimulus x time; \( F_{(1, 35)} = 11.56, p < .01, \eta^2_p = .25 \}) (Fig. 3A,B), respectively. There were no differences between the groups (stimulus x time x group; \( F_{(1, 35)} < 1 \), except for the predicted difference in US-expectancy ratings during reactivation from the end of acquisition to the reactivation trial (time x group; \( F_{(1, 35)} = 110.71, p < .001, \eta^2_p = .76 \)). As may be expected from our manipulation, CS1 ratings were significantly reduced from the end of acquisition to the reactivation trial (CS1-R) in the Propranolol No-SE group (main effect of time; \( F_{(1, 18)} = 125.04, p < .001, \eta^2_p = .87 \), whereas ratings remained stable in the Propranolol group (main effect of time; \( F_{(1, 17)} < 1 \)).

An intriguing finding is the double dissociation between the startle fear response and US-expectancy ratings during reactivation (day 2) and reinstatement test (day 3). Participants in the Propranolol No-SE group did not expect the occurrence of the US (Fig. 3A) during reactivation (CS1-R), whereas the startle potentiation persisted in response to the feared stimulus at the reactivation trial (Fig. 2A). In contrast, participants in the Propranolol group expected the occurrence of the US at reinstatement testing (Fig. 3B), while the startle response to the feared stimulus was absent at the reinstatement test trial (Fig. 2B).

**Skin conductance response (SCR).** An increase in differential electrodermal activity showed acquisition of SCR on day 1 (trials 1-2 to 7-8) (stimulus x time; \( F_{(3, 114)} = 4.83, p < .01, \eta^2_p = .11 \)). This pattern did not differ between groups (stimulus x time x group; \( F_{(3, 114)} < 1.54 \)). However, the groups differed on general skin conductance levels (main effect of group; \( F_{(1, 35)} = 4.20, p < .05, \eta^2_p = .11 \)). To correct for differences between groups in general levels of electrodermal responding, skin conductance responses (SCR) were standardized using within-participants Z-scores. Again we observed acquisition of SCR on day 1 (trials 1-2 to 7-8) (stimulus x time; \( F_{(3, 105)} = 5.32, p < .01, \eta^2_p = .13 \)) (Fig. 4A,B), in absence of a group difference (stimulus x time x group; \( F_{(3, 105)} < 1.91 \)) or difference in general skin conductance levels (main effect of group; \( F_{(1, 35)} < 1 \)). Notably, there was a significant difference in SCR from the end of acquisition (trials 7-8) to reactivation between the two groups (time x group; \( F_{(1, 35)} = 4.92, p < .05, \eta^2_p = .12 \)). In line with the US-expectancy ratings, the electrodermal responding to the feared stimulus (CS1) decreased from the end of acquisition (trials 7-8) to the reactivation trial (CS1-R) in the Propranolol No-SE group (main effect of stimulus; \( F_{(1, 18)} = 5.15, p < .05, \eta^2_p = .22 \)), while responses to the feared stimulus remained stable in the Propranolol group (main effect of stimulus; \( F_{(1, 17)} < 1.06 \)).
Extinction learning on day 3 (trials 1-2 to 15-16) generated a significant decrease in differential responding (stimulus x time; $F_{(4,62, 161.44)} = 2.37, p < .05, \eta^2_p = .06$) and no differences between groups were observed (stimulus x time x group; $F_{(4,62, 161.44)} < 1$). The three reminder shocks yielded a reinstatement of differential SCR at the test trial (main effect of stimulus; $F_{(1, 35)} = 10.58, p < .01, \eta^2_p = .23$) (Fig. 4A,B).

Figure 4. Mean skin conductance responses to the CS1 and CS2 during acquisition, reactivation, extinction and reinstatement test for (A), the Propranolol No-SE ($n = 19$) (B), Propranolol ($n = 18$) and (A), the Placebo ($n = 18$) conditions. Error bars represent SEM.
Similar to the expectancy data, we observed no differences in reinstatement effect for the two groups (stimulus x time x group; $F_{(1, 38)} < 1$). In line with our previous findings (Soeter and Kindt, 2010; 2011), skin conductance responding closely corresponded to the observed pattern of US-expectancy ratings.

**Propranolol vs. Placebo**

The startle fear response disappeared quickly and did not return after a well-known recovery method (reinstatement) in the Propranolol group. However, there were some savings of the fear memory at the beginning of day 3 during the extinction training. The non-reinforced reactivation trial may already have elicited some extinction learning, whereas in the Propranolol No-SE group the participants did not learn anything during memory reactivation. To ascertain that the weakened startle response on day 3 could be attributed to disruption of the reconsolidation process, we tested an additional Placebo group.

**Fear potentiated startle.** We observed successful fear learning on day 1, evidenced by an increase (trials 1-2 to 7-8) in differential (CS1 vs. CS2) startle responding (stimulus x time; $F_{(3.77, 97.17)} = 7.00, p = .001, \eta^2_p = .17$) (Fig. 2B,C). The Placebo group did not differ in fear learning from the Propranolol group (stimulus x time x group; $F_{(3, 102)} < 1$). Analysis of startle fear response on day 2 revealed more startle responding to the reactivated feared stimulus (CS1-R) than to the noise alone (main effect of type; $F_{(1, 34)} = 10.59, p < .01, \eta^2_p = .24$), which did not differ between the groups (stimulus x group; $F_{(1, 34)} < 1$). Hence, noradrenergic blockade did not dampen potentiation of the startle response during memory reactivation. The groups did also not differ in the general startle response (NA) during habituation and over the course of habituation on day 2 (main effect of group; time x group, $F_{(1, 34)} < 1.53$). Thus, propranolol administered before memory reactivation did not affect baseline startle responding either.

In line with our prediction, the Placebo and Propranolol group differed over the course of extinction learning (trials 1-2 to 15-16) (time x group; $F_{(4.70, 170.16)} = 4.20, p < .01, \eta^2_p = .11$) (Fig. 2B,C). In contrast to the Propranolol group (see section 3.2.1), extinction training induced a significant reduction in differential startle responding in the Placebo group (stimulus x time; $F_{(7, 119)} = 2.28, p < .05, \eta^2_p = .12$). Indeed, higher startle response to the feared stimulus (CS1) compared to the non-feared stimulus (CS2) was present at the beginning of extinction (trials 1-2) (main
effect of stimulus; $F_{(1, 17)} = 14.00, p < .01, \eta^2_p = .45$), while differential responding was absent at the end of extinction (trials 15-16) (main effect of stimulus; $F_{(1, 17)} < 1$).

We observed a difference between groups in differential startle response at reinstatement test (stimulus x group; $F_{(1, 34)} = 5.78, p < .05, \eta^2_p = .15$) (Fig. 2B,C). Contrary to the Propranolol group (see section 3.2.1), the fear response recovered in the Placebo group as is indicated by more startle potentiation to the feared stimulus (CS1) than to the safe stimulus (CS2) at test (main effect of stimulus; $F_{(1, 17)} = 6.13, p < .05, \eta^2_p = .27$).

**Retrospective US-expectancy ratings.** We observed acquisition (stimulus x time; $F_{(1, 38)} = 303.45, p < .001, \eta^2_p = .89$), extinction (stimulus x time; $F_{(1, 34)} = 69.11, p < .001, \eta^2_p = .67$) and reinstatement (stimulus x time; $F_{(1, 38)} = 7.18, p < .01, \eta^2_p = .17$) of differential US-expectancy ratings (Fig. 3B,C). Ratings to the feared stimulus (CS1) remained stable from the end of acquisition to the reactivation trial (main effect of stimulus; $F_{(1, 34)} < 1$). Administration of propranolol contrary to placebo did not affect US-expectancy ratings. That is, analysis of variance revealed no differences between the groups (time x group; stimulus x time x group; $F_{(1, 34)} < 1.58$).

**Skin conductance response.** Electrodermal conditioning on day 1 was evidenced by an increase (trials 1-2 to 7-8) in differential responding (CS1 vs. CS2) (stimulus x time; $F_{(3, 102)} = 5.09, p < .01, \eta^2_p = .13$) (Fig. 4B,C). Response to the reactivation trial (CS1-R) remained stable relative to trials 7-8 of acquisition (main effect of time; $F_{(1, 34)} < 1.43$). Extinction training (trials 1-2 to 15-16) did not induce the expected decrease in differential electrodermal responding (stimulus x time; $F_{(1, 34)} < 1.20$). However, while differential electrodermal responding to the feared stimulus (CS1) was significantly higher than responding to the safe stimulus (CS2) at the beginning of extinction (trials 1-2) (main effect of stimulus; $F_{(1, 34)} = 7.34, p < .01, \eta^2_p = .18$), differential responding was absent at the end of extinction (trials 15-16) (main effect of stimulus; $F_{(1, 34)} < 1$) (Fig. 4B,C). Analysis of reinstatement testing revealed more electrodermal responding to the feared stimulus (CS1) compared to the safe stimulus (CS2) at the test trial (main effect of stimulus; $F_{(1, 34)} = 5.44, p < .05, \eta^2_p = .14$) (Fig. 4B,C). We observed no differences between the Placebo and the Propranolol group (stimulus x time x group; stimulus x group; $F_{s} < 1$). In sum, noradrenergic blockade did not affect the conditioned electrodermal responding. Online subjective distress ratings closely mirrored the US-expectancy and the SCR data (see supplementary data).
DISCUSSION

The present findings show that reconsolidation of a human fear memory is not triggered when nothing can be learned from the memory retrieval session. That is, administration of the β-adrenergic receptor antagonist propranolol prior to reactivation did not affect the startle fear response 24 h later, when the outcome of the reminder trial was perfectly predictable (no prediction error). Noradrenergic blockade did reduce the startle fear response on day 3, when there was something to be learned during the reactivation session (prediction error). Although there were some savings of the originally acquired fear association at initial testing on day 3, the fear response was absent after the first two trials of extinction and did not recover after the reminder shocks.

An alternative explanation for reduced startle responding on day 3 in the Propranolol group is that it reflects a facilitation of extinction learning rather than a propranolol-induced disruption of reconsolidation of the original memory. Extinction learning may have been initiated during reactivation when the feared stimulus was not followed by the expected shock. This interpretation is, however, unlikely since the non-reinforced reminder trial did not result in diminished startle responding on day 3 in the Placebo group. Also, as propranolol has been shown to impede extinction learning (Mueller, Porter, & Quirk, 2008; Ouyang & Thomas, 2005), its impairment during the retrieval session should have resulted in enhanced rather than diminished fear responding on day 3. Contrary to our previous studies, differential startle responding was still present at the beginning of extinction in the Propranolol group, indicating that memory of the original fear association was not fully disrupted. An explanation in terms of propranolol effects on the course of systolic blood pressure could possibly account for this finding. Propranolol intake resulted only in near significant decreases in systolic and diastolic blood pressure compared to placebo. This contrasts with previous studies that observed a drop in either systolic and diastolic blood pressure (Kindt et al., 2009; Soeter & Kindt, 2010) or only systolic blood pressure (Tollenaar, Elzinga, Spinhoven, & Everaerd, 2009; van Stegeren, Rohleder, Everaerd, & Wolf, 2006). The finding that propranolol did not exert its normal physiological effect suggests that noradrenergic blockade was not optimal. Consequently, reconsolidation of the fear memory may not have been fully disrupted. Higher dosage of propranolol or a dosage adjusted to bodyweight could possibly enhance propranolol efficacy.

We used fear-relevant stimuli (spider pictures) to establish associative fear memory. As fear associations are much stronger for fear-relevant stimuli than
neutral stimuli (Lang, Bradley, & Cuthbert, 2008), and most anxiety disorders are
developed for these categories of stimuli, we are specifically interested in
targeting strong fear memory. Nonetheless, fear conditioning studies using these
stimuli have been criticized. Fear-relevant stimuli are supposed to have an innate
pre-potency for eliciting fear responses (Lovibond et al., 1994). According to this
hypothesis, enhanced physiological responding (startle response) to the CS1 could
just be the result of sensitization by the aversive stimulus (US) instead of associative
learning. Subsequently, noradrenergic blockade during reactivation could have
desensitized the fear response, instead of targeting the association itself. However,
our results clearly refute this alternative hypothesis by demonstrating that solely the
presentation of the feared stimulus (CS1) after propranolol intake was not sufficient
to reduce the fear response 24 h later. Note that, ideally, a reconsolidation-
blocking agent would be administered immediately after reactivation of the
memory to exclude possible interference with memory retrieval. In view of the
peak levels (Gilman & Goodman, 1996), propranolol was given before memory
reactivation. The finding that the expression of the fear response was not affected
when propranolol was on board, suggests that noradrenergic blockade did not
interfere with memory retrieval but rather with reconsolidation of the memory.

Furthermore, we demonstrate an exciting double dissociation between
the emotional and cognitive components of the fear memory. First, the startle
response remained intact in participants who did not expect the shock (US) during
memory reactivation. Second, in line with our previous studies, disrupting
reconsolidation reduced the startle fear response while declarative memory was
not affected (Kindt et al., 2009; Soeter & Kindt, 2010). Thus, in addition to targeting
the fear response and leaving declarative knowledge intact, contingency
knowledge can be manipulated separately from the automatic startle fear
response. These findings stress the involvement of multiple and dissociable memory
systems in fear learning. Acquisition and expression of conditioned fear both
depend on amygdala functioning, while memory for the association between the
conditioned stimulus and its aversive consequence relies on the hippocampus
(Hunsaker & Kesner, 2013). Here we provide evidence that the startle response —
as it is a fear-specific, amygdala-initiated response — is resistant to hippocampal-
mediated knowledge about CS-US contingencies. Furthermore, our finding that
startle potentiation remained intact in the Propranolol No-SE group demonstrates
that memory expression can also dissociate from mechanisms that induce
labilization of the memory trace. This is in line with a study by Mamou, Gamache,
ABSENCE OF PREDICTION ERROR

and Nader (2006) showing a double dissociation between the mechanisms mediating memory labilization and the mechanisms that underlie the behavioural expression of memory.

Electrodermal responses were not affected by reconsolidation blockade, but showed a pattern very similar to US-expectancy (see also Soeter & Kindt, 2010; 2011). Electrodermal activity is supposed to reflect the more cognitive level of associative learning and shows a close association with declarative knowledge (Weike et al., 2007). Our finding that propranolol did not affect the cognitive component of the fear memory does not imply that reconsolidation cannot be triggered at other levels of memory representation. Readjusting fear learning to the current changes in the environment might occur under different conditions than adjustment of contingency learning. Stated differently, amygdala-based plasticity and hippocampus-based plasticity might call for specific reactivation conditions.

The present study sheds light on the retrieval-labilization-restabilization sequence involved in reconsolidation. Memory reactivation, either with or without anticipation of the shock (US), did not affect the emotional expression of the fear memory (startle response). Thus, retrieval per se seems not to be sufficient to trigger reconsolidation. This confirms previous findings in aversive learning in the crab Chasmagnathus that reconsolidation does not start at CS onset but is triggered by CS offset (Pedreira et al., 2004), but only when termination of the reminder cue co-occurs with a violation based upon previous learning (prediction error). We argue that the prediction error of a reminder session is defined by the interaction between the available information and the learning history. This is in line with general learning models of classical conditioning (Hull, 1943; Rescorla & Wagner, 1972). According to these models, not the mere co-occurrence of CS and US, but the discrepancy between what has already been learned and what can be learned determines the degree of learning on each trial. The associative strength between CS and US reaches asymptotic levels after multiple conditioning trials. In case of asymptotic levels of learning, an additional learning trial (CS-US) does not affect memory, since the memory cannot gain in strength. For reconsolidation, both reinforced and non-reinforced reactivation trials are capable of destabilizing memory (Duvarci & Nader, 2004; Lee, 2008). Again, the learning history may define whether (non)reinforcement acts as a boundary condition for reconsolidation.

In sum, our data indicate that the updating of human fear memory does not take place when there is nothing to be learned during memory retrieval.
Furthermore, while the emotional and cognitive expression of fear memory usually converge in fear conditioning studies, we uncovered a double dissociation between these different response systems. The present findings provide insight into the necessary and boundary conditions for memory reactivation to transform our fear memories from a fixed state to one that is amenable to change. Such an understanding is critical if we are to consider reconsolidation blockade as a novel therapeutic strategy for treating people suffering from emotional disorders.
Supplementary Material

RESULTS

Propranolol vs. Propranolol No-Shock Expectation (No-SE)

Fear potentiated startle memory reactivation. One could argue that the differential startle response during reactivation (stronger response to CS1-R than NA) resulted from dishabituation. Given that the startle reflex habituates after 10 NA trials, the presentation of a fear-relevant picture in itself could have potentiated the response, irrespective of the picture’s association to the US. If so, the startle responses to the firstly presented CS2 on day 3 should also yield such a dishabituation effect (CS2 was also a fear-relevant stimulus). Yet, the startle response to the CS2 during trials 1-2 of extinction was not increased and similar to the NA (main effect of stimulus; \( F(1, 35) < 1 \); no differences between groups: stimulus x group; \( F(1, 35) < 1 \), irrespective of whether extinction started with CS1 or CS2 (stimulus x order; \( F(1, 35) < 1 \)). Thus, the presentation of a CS2 did not induce potentiation of the startle response upon its first presentation after habituation. Accordingly, startle potentiation to the fear-relevant picture during reactivation (CS1-R) cannot simply be attributed to dishabituation of the startle response either.

Online Distress Ratings
Participants (\( n = 2 \); Propranolol No-SE group) that reported not to experience distress during acquisition were excluded from analysis (scores of 0 on trials 7-8 of acquisition on both CS1 and CS2). Additionally, due to technical problems we excluded one participant in the Propranolol group from analysis of acquisition distress ratings.

Propranolol vs. Propranolol No-Shock Expectation (No-SE). There was acquisition of distress, as evidenced by a significant increase in differential distress ratings (CS1 vs. CS2) on day 1 (trials 1-2 to 7-8) (stimulus x time; \( F(1.72, 54.82) = 51.83, \ p < .001, \eta_p^2 = .62 \) [Fig. S1A,B]). A differential decrease in distress ratings was observed during extinction learning on day 3 (trials 1-2 to 15-16) (stimulus x time; \( F(1, 32) = 31.02; \ p < .001, \eta_p^2 = .49 \)). Furthermore, we observed a reinstatement effect from the end of extinction (trials 15-16) to the test trial (stimulus x time; \( F(1.72, 55.39) = 24.09, \ p < .001, \eta_p^2 = .43 \) [Fig. S1A,B]). Importantly, there were no differences between groups (\( F(1, 32) < 1 \)), except for the predicted decrease in distress ratings in the
Propranolol No-SE group from end of acquisition (trials 7-8) to reactivation, while responses in the Propranolol group remained stable (time x group; \( F_{1(1, 32)} = 5.18, p < .05, \eta^2_p = .14 \)). Thus, reactivation of the fear memory after administration of propranolol with the US-electrodes either attached or unattached does not affect subjective distress ratings 24 h later. Instead ratings closely mirror the US-expectancy and SCR data.

**Figure S1.** Mean distress ratings to the CS1 and CS2 during acquisition, reactivation, extinction and reinstatement test for (A), the Propranolol No-SE (\( n = 19 \)) (B), Propranolol (\( n = 18 \)) and (A), the Placebo (\( n = 18 \)) conditions. Error bars represent SEM.
Propranolol vs. Placebo. We observed acquisition of distress demonstrated by a significant increase in differential distress ratings (CS1 vs. CS2) on day 1 (trials 1-2 to 7-8) (stimulus x time; F(1.78, 60.65) = 37.18, p < .001, η²p = .52) (Fig. S1B,C). Responses to the feared stimulus (CS1) remained stable from the last trials (7-8) of acquisition to the reactivation trial (CS1-R) (main effect of time; F(1, 34) < 2.04) and did not differ between the groups (time x group; F(1, 34) < 1). Administration of propranolol before memory reactivation did not affect distress ratings. The groups did differ in the course of extinction learning (trials 1-2 to 15-16) (stimulus x time x group; F(1.94, 66.07) = 2.62, p < .01, η²p = .14). Differential ratings did not significantly extinguish in the Placebo group (stimulus x time; F(2,13,34,26) = 2.77, p = .07), while extinction training induced a significant reduction of differential ratings in the Propranolol group (stimulus x time; F(1,75, 29.69) = 12.32, p < .001, η²p = .42). The group interaction with extinction learning (trials 1-2 to 15-16) could be due to differences in distress learning on day 1 (trials 1-2 to 7-8) (stimulus x time x group; F(1.75, 60.65) = 3.98, p = .028, η²p = .11). While differential responding was similar on trials 1-2 of acquisition (stimulus x group; F(1, 34) < 1), differential ratings on trials 7-8 of acquisition were greater in the Propranolol group than in the Placebo group (stimulus x group; F(1, 34) = 4.71, p = .037, η²p = .12). Poor acquisition learning could have resulted in a failure to extinguish subjective distress. Finally, the unpredicted shocks generated an increase in differential ratings from the end of extinction (trials 15-16) to the test trial (stimulus x time; F(1, 34) = 9.77, p = .004, η²p = .22), which did not differ between groups (stimulus x time x group; F(1, 34) < 1) (Fig. S1B,C).

In sum, propranolol did not affect distress ratings. When the US-electrodes were not attached during day 2, the memory reactivation distress ratings were decreased on-line but were unaffected 24 h later at test. Interpretation of Placebo results is problematic due to a weak acquisition. Absence of a propranolol effect could be attributed to the experimental paradigm used in the present study. That is, similar to contingency learning, the reactivation conditions may not have been optimal to trigger reconsolidation of distress memory. An alternative proposition is that subjective ratings might not be suitable to assess the level of distress that participants experience. Subjective distress ratings as used here are straightforward and, therefore, reflect a more contingency-like learning than actual experienced distress. Startle and subjective ratings may reflect distinct aspects of anxiety, with “startle assessing primitive-defensive-reflex systems and verbal report assessing elaborative cognitive systems” (Grillon et al., 2009; p. 902).
In any case, subjective distress measures as applied in the present study are, unfortunately, not apt to investigate the subjective distress component.