Erasing fear from memory
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Chapter 7

Disrupting Reconsolidation: On the Verge of Clinical Application

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

Soeter, M., & Kindt, M. Disrupting Reconsolidation Targets the Subjective Feelings of Anxiety for an Imagined Threat Event.
Even though ‘disrupting reconsolidation’ may point to a promising strategy providing long-term cure for patients suffering from anxiety disorders, the fear reducing effects are thus far only demonstrated for ‘freezing reactions’ in rodents and ‘autonomic fear responding’ in humans. If ‘disrupting reconsolidation’ will be of value for clinical practice, it should also target the subjective feelings of anxiety. Using an instructed fear learning paradigm in humans, we here tested whether ‘disrupting reconsolidation’ would diminish the subjective feelings of anxiety for a noxious event (i.e., ‘electric stimulus’) that was anticipated but never actually experienced. Testing included different phases across three consecutive days each separated by 24 hr. Reconsolidation of the fear memory was manipulated by administering the β-adrenergic receptor antagonist propranolol HCl after reactivation of the memory (n = 12); double-blind placebo controlled (n = 12). Beta-adrenergic receptor blockade during reconsolidation strongly diminished the behavioral expression of the instructed fear memory (i.e., ‘startle fear responding’) as well as the subjective feelings of anxiety 24 hr later, yet without affecting both the ‘physiological’ and ‘cognitive’ component of the anticipation of threat (i.e., ‘skin conductance responding’, ‘US expectancy ratings’). Together, the present findings suggest that the various memory expressions of a single learned fear association do not necessarily undergo reconsolidation in harmony. Considering that patients with anxiety disorders (1) often fear objects and situations that they have never actually experienced, and (2) primarily suffer from the subjective feelings of anxiety, the present findings may have important ramifications for psychotherapy.
Recent insights into the neurobiological underpinnings of fear memory suggest novel strategies to improve therapeutic interventions for anxiety disorders (Nader, 2003). Specifically, a substantial body of animal and human research now indicates that a permanent reduction of fear may be realized through targeting the process of ‘reconsolidation’ (e.g., Nader et al., 2000; Kindt et al., 2009). This protein-synthesis dependent restabilization of a memory upon retrieval enables the modification of the original fear memory representation through either ‘pharmacological’ (Nader et al., 2000; Kindt et al., 2009) or ‘behavioral’ manipulations (Monfils et al., 2009; Schiller et al., 2010). Even though disrupting reconsolidation may thus point to a promising therapeutic tool providing long-term cure for patients suffering from anxiety disorders, the fear reducing effects are thus far only demonstrated for the behavioral expression of fear memories (e.g., freezing in rodents or autonomic fear responding in humans) (e.g., Nader et al., 2000; Brunet et al., 2008; Kindt et al., 2009; Monfils et al., 2009; Schiller et al., 2010; Soeter & Kindt, 2010, 2011). For the feasibility of reconsolidation in psychotherapy, disrupting reconsolidation should also diminish the subjective feelings of anxiety.

The phenomenon of ‘fear’ memory reconsolidation is traditionally investigated for a learned ‘association’ between a visual or auditory stimulus (i.e., Conditioned Stimulus, CS) (e.g., ‘pictures’, ‘tones’) and a noxious event (i.e., Unconditioned Stimulus, US) (e.g., ‘electric stimulus’). Pavlovian fear conditioning is in fact a valuable experimental model to test novel procedures for diminishing acquired fears and anxiety. Using such a differential fear conditioning paradigm in humans, we previously demonstrated that disrupting reconsolidation specifically targeted the startle fear responding without affecting the subjective feelings of distress (Soeter & Kindt, 2011). This finding that memories may undergo reconsolidation at one level, while leaving other aspects of the fear memory untouched, may be interpreted from a functional perspective on reconsolidation (Lee, 2009). That is, reconsolidation may be viewed as a fundamental process in the ongoing modification and storage of memories, which seems potentially adaptive in terms of maintaining a memory’s relevance in guiding future behavior (Dudai, 2006, 2009; Lee, 2009). Indeed, labilization and reconsolidation do not necessarily occur when a memory is being reactivated, but only when there is something to be learned (i.e., ‘informational value’) during memory retrieval.
(Pedreira et al., 2004; Forcato et al., 2009; Lee, 2009). A violation based upon prior learning is supposed to be a necessary condition for reconsolidation, meaning that the magnitude of the outcome or the outcome itself in not being fully predicted (i.e., ‘prediction error’) (Pedreira et al., 2004; Forcato et al., 2009; Lee, 2009). Traditional human fear conditioning paradigms seem not to be suitable for targeting the subjective component of fear memory given that the repeated pairing of the CS with the ‘experience’ of a relatively mild noxious event (e.g., ‘electric stimulus’) makes the aversiveness of the outcome very ‘predictable’ (Lee, 2009; Soeter & Kindt, 2011). In general, humans tend to fear objects and situations that either were so aversive that they fear the re-exposure or that they have never really experienced (Rachman, 1977). We hypothesized that disrupting reconsolidation may target the subjective feelings of anxiety when participants fear something that they never actually experienced.

Here, we addressed this issue by using an instructed fear learning paradigm in which the aversiveness of the US is imagined and thereby ‘unpredictable’ as the US is anticipated but never really felt. Testing included different phases across three consecutive days each separated by 24 hr. Prior to fear acquisition (day1), the participants were instructed which out of two fear-relevant stimuli (CS1) would at times be followed by a very unpleasant electric stimulus (US) delivered to the wrist of the unpreferred hand (see Fig. 7.1). The US was never administered. After the reactivation of the CS1 stimulus (day2), the participants received double blind an oral dose of placebo pill or 40 mg of propranolol HCl, a β-adrenergic receptor antagonist known to disrupt reconsolidation (Dębiec & LeDoux, 2004; Kindt et al., 2009; Soeter & Kindt, 2010, 2011). The retention of the memory (CS1 vs. CS2) was tested 24 hr later (day3). Given that a context switch offers a potent means to trigger the original fear memory (Bouton, 2002), we presented renewal stimuli (CS1R, CS2R) to assess the generalization of fear reduction across contexts. The fear response was measured as potentiation of the eyeblink startle reflex to a loud noise by electromyography of the right orbicularis oculi muscle. Startle potentiation is considered a reliable and specific index of fear (Hamm & Weike, 2005), which is directly connected with and modulated by the amygdala (Davis, 2006). The subjective feelings of distress (i.e., anxiety, tension or nervousness) were measured through online ratings during each stimulus presentation (Fig. 7.2). We further obtained skin conductance responding and retrospective US expectancy ratings to assess the physiological and subjective level of threat anticipation,
respectively (Weike et al., 2007). Salivary alpha amylase and blood pressure levels were determined to ensure the drug manipulation exerted its intended physiological effect (van Stegeren et al., 2006). We hypothesized that targeting the reconsolidation of the instructed fear memory would allow for the updating and hence weakening of the behavioral expression of fear (i.e., startle fear responding) as well as the subjective feelings of anxiety. In line with our previous findings (Kindt et al., 2009; Soeter & Kindt, 2010, 2011), we did - on the other hand - not expect any effects of the propranolol HCl manipulation on skin conductance discrimination and the US expectancy ratings given that there was nothing to be learned about the ‘contingency’ at the time of memory retrieval.

Materials and Methods

Participants
Twenty-four undergraduate students (3 men, 21 women) from the University of Amsterdam ranging in the age of 18 to 30 years (mean ± SD age, 20.9 ± 3.5 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 dose of propranolol HCl (i.e., pregnancy - seizure disorder - respiratory disorder - cardiovascular disease - blood pressure ≤ 90/60 - diabetes - liver or kidney disorder - depression - 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 score ≥ 26 on the Anxiety Sensitivity Index (ASI) (Peterson & Reiss, 1992). The participants were randomly assigned to one of two conditions with the restriction that conditions were matched on Trait Anxiety (STAI-T) (Spielberger et al., 1970), Spider Phobic Questionnaire (SPQ) (Klorman et al., 1974), and ASI scores as close as possible (see Table 7.1). Participants received either partial course credits or were paid a small amount (€ 35,-) for their participation in the experiment. The ethical committee of the University of Amsterdam approved the study and informed consent was obtained from all participants.

Apparatus and Materials
Stimuli. In order to strengthen the fear association during acquisition, two fear relevant stimuli of different stimulus categories served as CSs (i.e., spider -
Table 7.1. Mean values (SD) of the reported spider fear, trait anxiety, and anxiety sensitivity for the Propranolol HCl and Placebo Pill condition.

<table>
<thead>
<tr>
<th></th>
<th>Propranolol HCl</th>
<th>Placebo Pill</th>
<th>t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spider Fear</td>
<td>7.0 (6.6)</td>
<td>7.7 (6.8)</td>
<td>(t_{22} &lt; 1)</td>
</tr>
<tr>
<td>Trait Anxiety</td>
<td>32.9 (5.9)</td>
<td>34.0 (8.8)</td>
<td>(t_{22} &lt; 1)</td>
</tr>
<tr>
<td>Anxiety Sensitivity</td>
<td>9.6 (5.7)</td>
<td>10.2 (5.5)</td>
<td>(t_{22} &lt; 1)</td>
</tr>
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</table>

gun; IAPS numbers 1201 - 6200; see Fig. 7.1) (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-in computer monitor. Assignment of the slides as CS1 and CS2 was counterbalanced across participants. The renewal stimuli (CS1\(_r\) and CS2\(_r\)) (Fig. 7.1) were comprised of the original stimuli with an alteration in background color. All stimuli were presented for 8 s - the startle probe was presented 7 s after CS onset.

**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. 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; a ground reference was placed on the forehead (Blumenthal et al., 2005). 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 (contour follower) with a time constant of 25 msec. The integrated EMG signal was sampled at 1000 Hz. Peak amplitudes were identified over the period of 50 - 100 ms following probe onset.
Skin Conductance Response. Electrodermal activity (SCR) was measured using an input device with a sine shaped excitation voltage (± .5 V) of 50 Hz, derived from the mains frequency. The input device was connected to two Ag/AgCl electrodes of 20 by 16 mm. The electrodes were attached 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). Skin conductance responses elicited by the CS were determined by taking the average baseline (i.e., 2 s before CS onset) to peak difference within the 1 to 7 s window following stimulus onset. Although many studies examining skin conductance reactivity have either used the first interval response (FIR) or second interval response (SIR), the present scoring method allowed for the detection of the maximal increase in skin conductance levels at any point during the 7 s presentation. This method makes no assumptions about where a response is likely to occur within the CS-US interval. This eliminates the risk of underestimating a
larger response when the onset or peak of the response occurs near a previously established boundary between the FIR and SIR or when the latency of the peak response shifts over trials (Pineles et al., 2009). This approach has been used in previous human psychophysiological research, which has supported its validity (Orr et al., 2000; Milad et al., 2005; Pineles et al., 2009). A minimum response criterion of 0.02 micro Siemens (µS) was used. All other responses were scored as zero and remained in the analyses (Effting & Kindt, 2007). The raw SCR scores were square root transformed to normalize distributions.

**Subjective Distress Ratings.** In order to focus the participants’ attention on their bodily sensations of distress, a body chart was presented during CS presentation. The subjective distress (i.e., anxiety, tension or nervousness) was measured using a computer mouse on a visual analogue scale placed within reach of the preferred hand. The scale consisted of a vertical colored bar with endpoints ‘not at all’ and ‘very’ (Fig. 7.2). The participants were required to rate their subjective distress during each slide by shifting the cursor on the scale and push the left mouse button within 5 s following stimulus onset (i.e., before presentation of the startle probe).

![Fig. 7.2. The online subjective distress ratings.](image-url)
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.

Saliva Sampling. The salivary enzyme α-amylase (sAA) is a reliable indicator of noradrenergic activation (van Stegeren et al., 2006). Levels were assessed out of unstimulated saliva samples obtained using regular cotton Salivette sampling devices (Sarstedt, Nümbrecht, Germany) without chemical stimulants. Subjects were asked just to place the swab in their mouths for a 3 min period. After removal, the salivettes were stored at -25 °C. To facilitate salivary sampling, participants were instructed to refrain from exercise, caffeine, and alcohol during the 12 hr before each session. Also, they were instructed to abstain from brushing their teeth for 1 hr and avoid food intake, drinking any beverages other than water, and smoking for 2 hr before each session. Upon completion of the study, the samples were sent to Groningen for biochemical analysis (Universitair Medisch Centrum, Groningen, The Netherlands).

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 (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. In addition, the expectancy of the US was measured retrospectively on a continuous rating scale consisting of 11 points labeled from ‘certainly no electric stimulus’ (-5) through ‘uncertain’ (0) to ‘certainly an electric stimulus’ (5).

Experimental Procedure

Participants were subjected to an instructed differential fear conditioning procedure including several 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 session 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 subjective distress ratings during memory reactivation (i.e., day 2) and extinction, test (i.e., day 3) were similar to acquisition (i.e., day 1). Assignment of the slides as CS1 and CS2 was counterbalanced across participants.

**Acquisition.** Details of the various study procedures were explained in full and possible questions were answered. The participants were interviewed regarding their health and any medical or psychiatric conditions that would contraindicate taking a single 40 mg dose 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 were administered. Furthermore, saliva samples were collected. To this end, participants were instructed just to place the swab in their mouths for 3 min.

In order to obtain a baseline measurement of both physiological and subjective responding, the shock electrodes remained detached during the first part of the acquisition phase. Subsequent to the attachment of the startle and skin conductance electrodes, the participants were informed regarding the CSs. They were told that one picture of a spider and one picture of a gun would be presented on the computer screen and that no shocks could be delivered yet. Moreover, they were instructed to focus their attention during each CS presentation on their bodily sensations and to subsequently rate their level of distress (i.e., anxiety, tension or nervousness) by shifting a cursor on a colored bar and push the left mouse button within 5 s following stimulus onset. The CS1 and CS2 as well as the NA (i.e., baseline startle probe) were presented only once during this initial part of the acquisition phase.

Prior to the actual fear learning, the shock electrodes were attached and the electric stimulus was described as causing a brief and localized but very unpleasant sensation (Grillon & Davis, 1997). Furthermore, the participants were now instructed that the electric shock would follow the spider picture in some of the cases, whereas the picture of the gun would never be followed by the US (i.e., counterbalanced fashion). The participants were again required to rate their level of distress during each CS presentation. To disentangle subjective distress from expectancy of the US, it was explained that the mere expectation of the electric stimulus could, but did not necessarily had to cause feelings of distress. In the acquisition phase, the CS1 and CS2 were presented for three times. In addition, three startle probes were presented alone (NA). Order of trial type was
randomized within blocks (i.e., CS1, CS2 and NA). Intertrial intervals (ITI) varied between 15, 20 and 25 s with a mean of 20 s.

At the conclusion of the acquisition phase, the participants completed the STAI-S and were asked about the number of shocks they had received. It was made clear that the possibility of the non-occurrence of the electric stimulus existed. The participants were explicitly instructed to remember what they had learned during acquisition; the probability of receiving the electric stimulus the following days markedly increased in case no shocks were delivered yet.

**Memory Reactivation.** In order to substantiate consolidation of the fear memory, a break of 24 hr was inserted after acquisition. Subsequent to the attachment of the electrodes, the participants were again instructed that the spider picture would be followed by the electric stimulus in some of the cases, whereas the picture of the gun would never be followed by the US (i.e., counterbalanced fashion). They were further told that the probability of receiving the electric stimulus was markedly increased in case no shocks were delivered the previous day (i.e., day 1). In the memory reactivation phase, a single CS1-R was presented followed by a NA startle probe.

The participants received double-blind an oral dose of 40 mg of propranolol HCl or placebo pill subsequent to the reactivation of the memory. The administration of drug was randomized across participants with the restriction that conditions were matched on STAI-T, SPQ and ASI scores as close as possible (Table 7.1). In view of the peak plasma levels of propranolol HCl (Gilman & Goodman, 1996), a resting period of 90 min was inserted following the pill administration. The STAI-S was filled out both before and upon completion of the experiment (i.e., after the resting period). In addition, at these time points, blood pressure and saliva samples were collected.

**Extinction, Testing.** Upon arriving at the experimental site, blood pressure and saliva samples were collected. In addition, the STAI-S was completed. Instructions regarding the CSs only revealed that the same two pictures provided during acquisition would be presented. In the extinction phase, participants were exposed to the CS1 and CS2 for 12 times. Furthermore, 12 startle probes were presented alone (NA). The participants were subsequently presented with the renewal stimuli (CS1r, CS2r) as well as a NA startle probe. At the conclusion of the experiment, the participants completed the STAI-S and were asked to indicate for
each phase (i.e., beginning vs. end) of the experiment to what extent they had expected the US after each of the CSs.

**Statistical Analysis**

Startle responses, electrodermal activity, subjective distress and US expectancy ratings were analyzed by means of a mixed analysis of variance (ANOVA) for repeated measures with condition (i.e., propranolol HCl vs. placebo pill) as between-subjects factor and stimulus (i.e., CS1 vs. CS2) and trial (i.e., stimulus presentation) as within-subjects factors. The differential response (CS1 vs. CS2) was compared over testing phases respectively (first trial vs. last trial). Planned comparisons were performed for each condition separately. Missing data points were excluded from the analyses. Outliers were replaced by linear trend at point for each phase and stimulus type independently. Significance was set at $P < 0.05$.

**Results**

**Manipulation Check Propranolol HCl**

Analysis of the effect of the propranolol HCl manipulation on blood pressure and sAA level during memory reactivation (i.e., day 2) revealed the expected decrease in both systolic and diastolic blood pressure [moment x condition, $F_{1,22} = 38.76, P < 0.001, \eta_p^2 = .64; F_{1,22} = 5.74, P < 0.05, \eta_p^2 = .21$, respectively] as well as salivary alpha amylase [moment x condition, $F_{1,19} = 5.11, P < 0.05, \eta_p^2 = .21$] (van Stegeren et al., 2006) in comparison to placebo pill, indicating that the drug manipulation exerted its intended physiological effect (Table 7.2). However, in the propranolol HCl group, the blood pressure and salivary alpha amylase again returned to baseline levels at retention testing (i.e., day 3) given that we observed no differential effect of pill administration on the course of the systolic and diastolic BP [day 1 vs. day 3; moment x condition, $F_{5,22} < 1$] as well as sAA level [day 1 vs. day 3; moment x condition, $F_{1,16} < 1$].

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 during memory reactivation (i.e., day 2) [moment x condition, $F_{1,22} < 1$]. We also observed no differential effect of pill administration on the
reported state anxiety that was assessed before acquisition and upon arriving at the experimental site 48 hr later [day 1 vs. day 3; moment x condition, $F_{1,22} < 1$].

Table 7.2. Mean values (SD) of the systolic and diastolic blood pressure in mmHg and amylase level in U/ml pre and post pill intake during memory reactivation for the Propranolol HCl and Placebo Pill group.

<table>
<thead>
<tr>
<th>Memory Reactivation</th>
<th>Pre Pill Intake</th>
<th>Post Pill Intake</th>
<th>$t$ Test</th>
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<tbody>
<tr>
<td><strong>Propranolol HCl Condition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>135.5 (SD = 12.3)</td>
<td>113.2 (SD = 8.3)</td>
<td>$t_{11} = 9.37, P &lt; 0.001$</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>72.3 (SD = 10.6)</td>
<td>66.2 (SD = 7.6)</td>
<td>$t_{11} = 2.78, P &lt; 0.05$</td>
</tr>
<tr>
<td>sAA Level</td>
<td>67.8 (SD = 66.6)</td>
<td>28.1 (SD = 38.4)</td>
<td>$t_{10} = 2.69, P &lt; 0.05$</td>
</tr>
</tbody>
</table>

| **Placebo Pill Condition** | | | |
| Systolic BP           | 121.8 (SD = 10.9) | 117.8 (SD = 9.2) | $t_{11} = 2.32, P < 0.05$ |
| Diastolic BP          | 68.6 (SD = 9.4) | 68.3 (SD = 7.7) | $t_{11} < 1$ |
| sAA Level             | 70.6 (SD = 53.6) | 74.4 (SD = 35.1) | $t_{9} < 1$ |

**Fear Potentiated Startle Response**

The analysis of variance showed fear learning (i.e., day 1) in both the propranolol HCl and placebo pill condition by a significant increase of the differential startle fear response (CS1 vs. CS2) from baseline to the last trial of acquisition [stimulus x trial, $F_{1,22} = 32.36, P < 0.001, \eta^2_p = .60$; stimulus x trial x condition, $F_{1,22} < 1$; Fig. 7.3 A and B]. We observed no difference in responding
(CS1 vs. CS2) at baseline testing (i.e., shock electrodes detached) [stimulus, stimulus x condition, $F_{1,22} < 1$]. Moreover, the two groups expressed comparable levels of differential startle potentiation (CS1-R vs. NA) during memory reactivation (day 2) [stimulus x condition, $F_{1,22} < 1$]. The absence of a significant change in startle fear responding (CS1 vs. NA) from the last trial of acquisition to memory reactivation [stimulus x trial x condition, $F_{1,22} < 1$] further demonstrates that the acquired fear memory was equally well consolidated in the two groups. However, the administration of propranolol HCl contrary to placebo pill significantly decreased the differential startle response from the last trial of acquisition to the first extinction trial 48 hr later (i.e., day 3) [stimulus x trial x condition, $F_{1,22} = 6.04, P < 0.05, \eta^2 = .22$; Fig. 7.3 A and B]. Planned comparisons indeed showed that the startle fear response (CS1 vs. CS2) remained stable in the placebo pill condition [stimulus x trial, $F_{1,11} < 1$], whereas the propranolol HCl manipulation strongly reduced the emotional expression of the fear memory (CS1 vs. CS2) [stimulus x trial, $F_{1,11} = 18.53, P = 0.001, \eta^2 = .63$]. This reduction in startle fear responding could not be explained by a general fear reducing effect of the drug manipulation seeing that the two groups showed a similar level of startle potentiation during habituation (i.e., day 3) [condition, $F_{1,22} < 1$; Fig. 7.3 C]. Given that the differential startle response was in fact already eliminated following the propranolol HCl manipulation [i.e., first extinction trial, $t_{11} < 1$], the two groups differed over the course of the extinction learning process [stimulus x trial x condition, $F_{1,22} = 15.73, P = 0.001, \eta^2 = .22$]. That is, the startle fear response significantly decreased from the first trial of extinction to the last extinction trial in the placebo pill condition [stimulus x trial, $F_{1,11} = 24.61, P = 0.001, \eta^2 = .69$], but we did not observe any differential change in startle fear responding in the propranolol HCl group [stimulus x trial, $F_{1,11} < 1$]. Furthermore, the renewal test also unveiled a significant difference between the two conditions [CS1R vs. CS2R; stimulus x condition, $F_{1,22} = 11.87, P < 0.01, \eta^2 = .35$]. Contrary to the recovery of fear in the placebo pill group [CS1R vs. CS2R; $t_{11} = 3.65, P < 0.01$, two-tailed; Fig. 7.3 B], the renewal test did not uncover any startle fear response in the propranolol HCl condition [CS1R vs. CS2R; $t_{11} < 1$; Fig. 7.3 A], indicating that the drug manipulation affected the context.

The analysis of the startle fear response to habituation (i.e., prior to acquisition, memory reactivation and extinction learning) unveiled no significant differences between the propranolol HCl and placebo pill condition [condition,
Fig. 7.3. The erasure of the *startle fear response* by the *propranolol HCl* manipulation. Mean startle potentiation to the fear conditioned stimulus (CS1), the control stimulus (CS2) and noise alone (NA) trials during acquisition, memory reactivation, extinction and renewal for the (A) Propranolol HCl and (B) Placebo Pill condition. Mean startle potentiation to the habituation trials (C) prior to acquisition, memory reactivation and extinction for the Propranolol HCl and Placebo Pill condition. Error bars represent SEM. BS = baseline (i.e., electrodes detached).
We also observed no differences between the two groups in startle fear responding to noise alone during acquisition [condition, $F_{1,22} < 1$] and memory reactivation [$t_{22} < 1$]. However, the startle responding to NA relative to the control stimulus (i.e., NA vs. CS2) was significantly augmented during acquisition [stimulus, $F_{1,22} = 30.66, P < 0.001, \eta^2_p = .58$; stimulus x condition, $F_{1,22} < 1.19$], indicating that the instructed fear learning paradigm in which the participants anticipated an ‘imaginary’ aversive event induced some contextual fear (Grillon & Ameli, 1998).

Given that the startle responding to NA in the propranolol HCl condition contrary to the placebo pill group was significantly reduced during extinction learning [condition, $F_{1,22} = 11.09, P < 0.01, \eta^2_p = .34$] and renewal testing [$t_{22} = 2.44, P < 0.05$, two-tailed], it may be suggested that the fear reducing effects following the propranolol HCl manipulation generalized to the specific fear learning context.

**Online Subjective Distress Ratings**

The analysis of the distress ratings revealed a comparable responding (CS1 vs. CS2) at baseline testing [stimulus, stimulus x condition, $F_{5,122} < 1$] as well as a significant increase in subjective distress during fear acquisition in both the propranolol HCl and placebo pill condition [CS1 vs. CS2; stimulus x trial, $F_{1,22} = 42.13, P < 0.001, \eta^2_p = .61$; stimulus x trial x condition, $F_{1,22} < 1$; Fig. 7.4]. However, closely resembling the startle fear response results, we observed a significant difference between the two groups in the subjective distress ratings from the last trial of acquisition to the first extinction trial 48 hr later (day 3) [stimulus x trial x condition, $F_{1,22} = 9.39, P < 0.01, \eta^2_p = .30$; Fig. 7.4]. That is, the distress ratings remained stable in the placebo pill condition [stimulus x trial, $F_{1,11} < 1$], whereas we observed a significant decrease in subjective distress in the propranolol HCl group [stimulus x trial, $F_{1,11} = 20.89, P = 0.001, \eta^2_p = .66$]. Consequently, the two groups differed over the course of the extinction learning process [stimulus x trial x condition, $F_{1,22} = 4.34, P < 0.05, \eta^2_p = .17$]. The distress ratings significantly decreased from the first trial of extinction to the last extinction trial in the placebo pill condition [stimulus x trial, $F_{1,11} = 8.62, P < 0.05, \eta^2_p = .44$], but we did not observe any differential change in the propranolol HCl group [stimulus x trial, $F_{1,11} < 2.44$]. Moreover, the renewal test also generated a significant difference between the two groups [CS1R vs. CS2R; stimulus x condition, $F_{1,22} = 5.43, P < 0.05, \eta^2_p = .20$], again mirroring the startle fear response data. Though we observed a significant renewal of the distress ratings in the placebo pill condition [CS1R vs.
On the Verge of Clinical Application

CS2$_R$; $t_{11} = 3.24, P < 0.01$, two-tailed; Fig. 7.4 B], the fear renewal was absent in the propranolol HCl group [CS1$_R$ vs. CS2$_R$; $t_{11} < 1.07$; Fig. 7.4 A].

**Fig. 7.4.** The erasure of the subjective feelings of distress by the propranolol HCl manipulation. Mean subjective distress ratings to the CS1 and CS2 trials during acquisition, memory reactivation, extinction and renewal for the (A) Propranolol HCl and (B) Placebo Pill condition. Error bars represent SEM. BS = baseline (i.e., electrodes detached).

**Skin Conductance Response**

Overall analysis of electrodermal responding revealed no fear conditioning during acquisition (CS1 vs. CS2) [stimulus x trial, $F_{1,22} < 2.51$]. When fear responses are not successfully acquired, one cannot assess the return of fear. Therefore, only subjects showing successful levels of fear acquisition (i.e., trial 3 CS1 > CS2) were included in the analyses. Two subjects were eliminated. That is, one subject from the propranolol HCl condition and one subject from the placebo pill group.
Subsequent analyses of variance showed no effect of the drug manipulation on skin conductance discrimination (CS1 vs. CS2) \([\text{stimulus x trial x condition}, F_{1,20} < 1.48]\). In both the propranolol HCl and placebo pill group, we observed a significant increase in electrodermal activity during acquisition \([\text{CS1 vs. CS2; stimulus x trial } F_{1,20} = 6.20, P < 0.05, \eta_p^2 = .24; \text{Fig. 7.5}]\), a significant decrease in electrodermal responding during extinction learning \([\text{CS1 vs. CS2; stimulus x trial } F_{1,20} = 6.53, P < 0.05, \eta_p^2 = .25]\), and a significant renewal effect \([\text{CS1}_R \text{ vs. CS2}_R; \text{stimulus, } F_{1,20} = 5.73, P < 0.05, \eta_p^2 = .22]\).

**Fig. 7.5.** Mean skin conductance responding to the CS1 and CS2 trials during acquisition, memory reactivation, extinction and renewal for the (A) Propranolol HCl and (B) Placebo Pill condition. Error bars represent SEM. BS = baseline (i.e., electrodes detached).
Retrospective US Expectancy Ratings

The analyses of variance revealed no effect of the drug manipulation on retrospective US expectancy (CS1 vs. CS2) [stimulus x trial x condition, $F_{5,122} < 1$]. In both the propranolol HCl and placebo pill condition, we observed a significant increase in expectancy ratings during acquisition [CS1 vs. CS2; stimulus x trial, $F_{1,22} = 410.88$, $P < 0.001$, $\eta^2_p = .95$; Fig. 7.6], a differential decrease in US expectancy during extinction learning [CS1 vs. CS2; stimulus x trial, $F_{1,22} = 83.66$, $P < 0.001$, $\eta^2_p = .79$], and a significant renewal effect [CS1 vs. CS2; stimulus, $F_{1,22} = 123.22$, $P < 0.001$, $\eta^2_p = .85$], indicating that the threat generalized across contexts.

**Fig. 7.6.** Mean retrospective US expectancy scores to the CS1 and CS2 trials during acquisition, memory reactivation, extinction and renewal for the (A) Propranolol HCl and (B) Placebo Pill condition. Error bars represent SEM.
Discussion

Here, we demonstrate that β-adrenergic receptor blockade during reconsolidation strongly attenuated the behavioral expression of the instructed fear memory (i.e., startle fear responding) as well as the subjective feelings of anxiety. The propranolol HCl drug seemed to be highly effective in weakening the fearful aspects of the memory 24 hr later as one of the hallmark recovery phenomena (i.e., ‘renewal’ testing) failed to uncover any fear responding (i.e., startle fear responding, subjective distress ratings). This contrasted sharply with the placebo pill condition in which ‘renewal’ testing unveiled a post-extinction return of fear (Fig. 7.3 and 7.4). Notably, the startle fear responding to the context (i.e., noise alone trials) relative to the control stimulus (i.e., CS2 trials) was augmented during fear acquisition in both conditions (i.e., day 1), indicating that the instructed fear learning paradigm in which the participants anticipated on an ‘imaginary’ aversive event induced some contextual fear (Grillon & Ameli, 1998). Interestingly, the startle fear responding to the ‘noise alone’ trials during subsequent retention testing (i.e., day 3) was reduced in the propranolol HCl condition but not in the placebo pill group. Hence, it may be suggested that the fear reducing effects following the propranolol HCl drug also generalized to the specific fear learning context. Contrary to the startle fear responding and subjective feelings of anxiety, the β-blocker during reconsolidation neither affected the skin conductance discrimination nor the US expectancy ratings. In line with our preceding studies (Kindt, et al., 2009; Soeter & Kindt, 2010, 2011), this observation again demonstrates that the ‘updating’ of the anticipation of threat (i.e., skin conductance responding, US expectancy ratings) calls for different reactivation conditions (Kindt et al., 2009; Lee, 2009; Soeter & Kindt, 2010, 2011). Apparently, the various memory expressions of a single learned fear association do not necessarily undergo reconsolidation in harmony. While the emotional and cognitive memory system are interrelated, they can indeed also operate independently (Phelps, 2004; LaBar & Cabeza, 2006). For instance, a purely cognitive intervention may change the irrational beliefs of patients with anxiety disorders (e.g., overestimation of danger), while leaving the emotional component of the fear memory untouched. On the other hand, we here show that the behavioral expression and subjective feelings of anxiety can be modified without affecting the ‘physiological’ as well as ‘cognitive’ component of the anticipation of
threat (i.e., skin conductance responding, US expectancy ratings, respectively). Evidently, when anxious individuals suffer from irrational beliefs such as an overestimation of danger, interventions should also target the more cognitive aspects of a fear memory.

Our findings suggest that the present fear reducing effects are not the result of a general nonspecific dampening effect of the propranolol HCl drug, even though small doses of propranolol HCl can have anxiolytic effects (Granville-Grossman & Turner, 1966; Wheatley, 1969; Tyrer & Lader, 1974). First, since the two groups showed a similar level of startle potentiation during habituation prior to retention testing (i.e., day 3) (Fig. 7.3 C), the reduction in startle fear responding could not be explained by a general effect of the propranolol HCl drug on the startle responding itself. This is in line with other human studies showing no effects of propranolol HCl on startle reactivity (Grillon et al., 2004). Second, the fear reducing effects could also not be explained by any anxiolytic properties of the \( \beta \)-adrenergic drug on state anxiety (i.e., STAI-S). Moreover, the blood pressure and salivary alpha amylase returned to baseline levels 24 hr after propranolol HCl intake (i.e., day 2), indicating that the drug was washed out before retention testing (i.e., day 3). Combined with our earlier findings that propranolol HCl does not affect fear responding in the absence of the memory retrieval (Kindt et al., 2009; Soeter & Kindt, 2010), the alternative explanation for the fear reducing effects seems to be highly improbable. Rather, the administration of the \( \beta \)-adrenergic drug after memory retrieval appears to have weakened the underlying fear association (i.e., CS1-US), thereby attenuating the behavioral expression as well as subjective feelings of anxiety 24 hr later.

Contrary to the traditional studies on ‘fear’ memory reconsolidation (e.g., Nader et al., 2000; Kindt et al., 2009; Monfils et al., 2009; Schiller et al., 2010), we here established associative ‘fear’ memory through ‘verbal communication’ (i.e., instructed fear learning) whereby the aversive event was only ‘imagined’ instead of really experienced (i.e., Pavlovian fear conditioning). Even though the neural mechanisms underlying Pavlovian fear conditioning are proposed to differ from those underlying instructed fear learning (Olsson & Phelps, 2007), the present findings demonstrate that imagined aversive events also undergo reconsolidation. Considering that humans often obtain their fears through ‘observational learning’ and ‘language’ (i.e., without directly experiencing the aversive event itself) (Rachman, 1977), the propranolol HCl drug as add on memory retrieval may have
potentially important implications for the treatment of anxiety disorders. Preliminary evidence in trauma patients already showed reduced physiological responding following post-retrieval propranolol HCl intake (Brunet et al., 2008). Here, we demonstrate that targeting the process of reconsolidation by a single dose of propranolol HCl also diminishes the subjective feelings of anxiety, which is particularly clinically relevant. Given that experimental research is restrictive by its very nature, research in patients is required to uncover the optimal and boundary conditions for reconsolidation in clinical practice. For now - at least - we may conclude that disrupting reconsolidation is within reach of clinical applicability.

References


