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### Erasing fear from memory

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## Chapter 3

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# Dissociating Response Systems: Erasing Fear from Memory

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In addition to the extensive evidence in animals, we previously showed that disrupting reconsolidation by noradrenergic blockade produced amnesia for the original fear response in *humans*. Interestingly, the declarative memory for the fear association remained intact. These results asked for a solid replication. Moreover, given the constructive nature of memories, the intact recollection of the fear association could eventually ‘rebuild’ the fear memory, resulting in the spontaneous recovery of the fear response. Yet, perseverance of the amnesic effects would have substantial clinical implications, as even the most effective treatments for psychiatric disorders display high percentages of relapse. Using a differential fear conditioning procedure in humans, we replicated our previous findings by showing that administering propranolol HCl (40 mg) prior to memory reactivation eliminated the startle fear response 24 hr later. But most importantly, this effect persisted at one-month follow-up. Notably, the propranolol HCl manipulation not only left the declarative memory for the acquired contingency untouched, but also skin conductance discrimination. In addition, a close association between declarative knowledge and skin conductance responses was found. These findings are in line with the supposed double dissociation of fear conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. They support the view that skin conductance conditioning primarily reflects contingency learning, whereas the startle response is a rather specific measure of fear. Furthermore, the results indicate the absence of a causal link between the actual knowledge of a fear association and its fear response, even though they often operate in parallel. Interventions targeting the amygdalar fear memory may be essential in specifically and persistently dampening the emotional impact of fear. From a clinical and ethical perspective, disrupting reconsolidation points to promising interventions persistently erasing fear responses from trauma memory without affecting the actual recollection.

Memories are fundamentally dynamic processes. They are constructive in nature and always changing (Nader, 2003). The phenomenon of reconsolidation, the stabilization of a memory after retrieval, enables the modification of memory representation (Nader et al., 2000). Abundant evidence in animals indicates that blockade of the reconsolidation process following memory reactivation, produces amnesia for the original learning (Nader et al., 2000). Recently, the study of reconsolidation blockade of emotional (fear) memory progressed from animals to humans (Brunet et al., 2008; Kindt et al., 2009). We demonstrated that oral administration of a  $\beta$ -adrenergic receptor antagonist (i.e., propranolol HCl) prior to reactivation of a fear memory resulted in amnesia of the fear memory expression in humans 24 hr later (Kindt et al., 2009). Interestingly, the propranolol HCl manipulation left the declarative memory for the learned fear association between the conditioned stimulus and its aversive consequence intact, but this knowledge no longer produced a fear response. This remarkable dissociation is clearly in line with the concept of multiple memory systems, involving a distinction between declarative memory (i.e., the conscious recollection of facts and events) and procedural memory, expressed through performance rather than recollection (Squire, 2004). While declarative memory is based on the functional integrity of the hippocampal complex (Squire et al., 2004), the acquisition and expression of a fear response requires intact amygdala functioning (LeDoux, 2000). Hence, the observed double dissociation of fear conditioning and declarative knowledge relative to the amygdala and hippocampus further highlights the independent function of these two memory systems (Phelps, 2004; LaBar & Cabeza, 2006).

Even though the amygdala and hippocampal complex can operate independently, they also interact in subtle but important ways (Phelps, 2004; LaBar & Cabeza, 2006). For instance, hippocampal-dependent declarative memories can lead to activation of the amygdala, mediating our emotional reactions (Phelps, 2004). Alternatively, disrupting the reconsolidation of the hippocampal memory trace or hippocampal-dependent extinction learning can produce amnesia for the amygdalar fear memory (LeDoux, 2000; Bouton, 2002). Since declarative memories are supported by the gradual formation of a more distributed memory network, complete or partial disruption of the hippocampal memory trace could eventually be 'reconstructed' (Nakazawa et al., 2002;

Amaral et al., 2008), reactivating the emotional (fear) memory accordingly. In a similar vein, extinction learning involves the formation of a new inhibiting hippocampal association that leaves the original fear memory unaffected (Bouton, 2002). Thus, the putative inhibitory role performed by the hippocampus could be essential in the spontaneous recovery of (fear) memory expression (i.e., transient amnesia). If disrupting reconsolidation of fear memory will be of value for clinical practice, *persistent* rather than transient amnesic effects are desired.

In this human fear conditioning study, we replicated and extended our previous study (Kindt et al., 2009) by testing whether *disrupting the reconsolidation process by noradrenergic blockade would persistently reduce the fear response from its memory*. In order to maximize the likelihood of fear memory expression, we included two well-established retrieval techniques, that is, the administration of *reminder shocks* on both day 3 and during follow-up, and a *long-term* test one month later. Participants were subjected to a differential fear conditioning procedure including different phases: *fear acquisition* (day 1), *memory reactivation* (day 2), *extinction* followed by a *reinstatement* procedure and a *test* phase (day 3), and a *follow-up session* including an additional extinction, reinstatement and test phase one month later (day 30). Fear conditioning typically involves the pairing of an initially neutral conditioned stimulus (CS<sup>+</sup>) with an intrinsically aversive unconditioned stimulus (US) (e.g., electric shock). 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. Stronger startle responses to the loud noise during the fear conditioned stimulus (CS<sup>+</sup>) as compared to the control stimulus (CS<sup>-</sup>) reflects the fearful state of the participant elicited by the feared stimulus (CS<sup>+</sup>). Potentiation of the startle blink response is only observed during aversive fear conditioning (Weike et al., 2007). Neurally, it reflects the influence of direct and indirect connections from the amygdala to the primary startle-reflex pathway in the brainstem (Davis & Whalen, 2001). *Declarative knowledge* of the fear association was measured through online US-expectancy ratings during each CS presentation within 5 s after stimulus onset. In addition, *skin conductance responses* were obtained as an objective measure of expectancy learning. Note, however, that SCR discrimination is not only observed for aversive but also for nonaversive conditioning. It primarily reflects the more

cognitive level of contingency learning (i.e., declarative knowledge) (Weike et al., 2007). Reconsolidation of the fear memory was manipulated by the systemic administration of propranolol HCl, double-blind placebo controlled. To determine whether the effect of propranolol required active retrieval of the fear memory, propranolol HCl was administered to another fear-conditioned group without reactivation of the memory.

We hypothesized that disrupting reconsolidation by noradrenergic blockade would result in the persistent weakening of the *startle fear response*, while leaving the *declarative memory* for the fear association intact. Given the close association between declarative knowledge and electrodermal activity (Hamm & Weike, 2005), we reasoned that  $\beta$ -adrenergic blockade during memory reactivation would not sort any effect on *skin conductance* conditioning. Salivary alpha amylase (sAA) and blood pressure levels were obtained to ensure the propranolol manipulation exerted its intended physiological effect. US evaluation and state anxiety were assessed to test whether the expected reduction in startle responses could be explained by any general effects of propranolol HCl on these variables.

## Materials and Methods

### Participants

Sixty undergraduate students (15 men, 45 women) from the University of Amsterdam ranging in the age of 18 to 46 years (mean  $\pm$  SD age, 20.4  $\pm$  3.8 years) participated in the study. All participants reported to be free from any current or previous medical or psychiatric condition that would contraindicate taking a single 40 mg oral dose of propranolol hydrochloride (i.e., pregnancy; seizure disorder; respiratory disorder; cardiovascular disease; diabetes; liver or kidney disorder; previous adverse reaction to a  $\beta$ -blocker; use of another  $\beta$ -blocker; use of medication that could involve potentially dangerous interactions with propranolol HCl; depression; or psychosis). To be eligible for participation, blood pressure had to be  $\geq$  90/60 mmHg during medical screening as well as before pill intake the following day. In order to eliminate individuals who might have difficulty with any temporary symptoms induced by propranolol HCl, an additional exclusion criterion contained a score  $\geq$  26 on the Anxiety Sensitivity

Index (ASI) (Peterson & Reiss, 1992). Three participants were excluded before data collection: respiratory disorder ( $n = 2$ ); depression ( $n = 1$ ).

Participants were randomly assigned to one of two conditions with the restriction that conditions were matched on Spider Phobic Questionnaire (SPQ) (Klorman et al., 1974) scores as close as possible; pill placebo ( $n = 20$ ; mean SPQ score  $\pm$  SD,  $6.9 \pm 4.7$ ) and propranolol HCl ( $n = 20$ ; mean SPQ score  $\pm$  SD,  $6.6 \pm 6.0$ ). For the additional control condition (i.e., propranolol no reactivation;  $n = 20$ ) a mean SPQ score of 6.9 (SD = 5.3) was obtained. Participants received either partial course credits or were paid a small amount (€ 49,-) for their participation in the experiment. The study was approved by the ethical committee of the University of Amsterdam and informed consent was obtained from all participants.

### **Apparatus and Materials**

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

**Fear Potentiated Startle.** The conditioned fear response (CR) was measured as potentiation of the eyeblink startle reflex to a loud noise by electromyography (EMG) of the right orbicularis oculi muscle. Startle potentiation taps directly into the amygdala and fear conditioning procedures yield highly reliable and robust startle potentiation (Davis, 2006). The loud noise (40 ms; 104 dB) was administered during each CS presentation and during intertrial intervals (NA: Noise Alone). Two 7 mm Ag/AgCl electrodes filled with

electrolyte gel were positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus. In order to maintain electrically identical paths and reduce common noise, the ground reference was placed  $\pm 3$  cm below the orbicularis oculi pars orbitalis on an electrically neutral site. All acoustic stimuli were delivered binaurally through headphones (Model MD-4600; Compact Disc Digital Audio, Monacor). The eyeblink EMG activity was measured using a bundled pair of electrode wires connected to a front-end amplifier with an input resistance of 10 M $\Omega$  and a bandwidth of DC-1500 Hz. To remove unwanted interference, a notch filter was set at 50 Hz. Integration was handled by a true-RMS converter (i.e., contour follower) with a time constant of 25 msec. The integrated EMG signal was sampled at 100 Hz. Peak amplitudes were identified over the period of 20 - 200 ms following startle probe onset. Note that in our previous study (Kindt et al., 2009) the magnitude of the fear potentiated startle was multiplied by factor two.

**Skin Conductance Response.** Electrodermal activity (SCR) was measured using an input device with a sine shaped excitation voltage ( $\pm .5$  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 second 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. A minimum response criterion of 0.02 micro Siemens ( $\mu$ S) 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.

**US Expectancy Measures.** Rated expectations of the US were measured online during CS presentation using a computer mouse on a continuous rating scale placed within reach of the preferred hand. The scale consisted of 11 points labeled from 'certainly no electric stimulus' (-5) through 'uncertain' (0) to 'certainly an electric stimulus' (5). The scale and the participant's rating were continuously presented at the bottom of the computer screen in order to encourage participants to focus their attention to the CSs. Participants were required to rate the expectancy of an electric stimulus during the presentation

of each slide by shifting the cursor on the scale and push the left mouse button within 5 s following stimulus onset, that is, before administration of the startle probe. Once the slides disappeared, the cursor automatically returned to the 'uncertain' position.

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

**Saliva Sampling.** The salivary enzyme  $\alpha$ -amylase (sAA) is supposed to be 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 instructed just to place the swab in their mouths for 3 min. After removal, the salivettes were stored at -25 °C. 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 (i.e., STAI-S and STAI-T) (Spielberger et al., 1970). The degree of spider fear was determined by the Spider Phobic Questionnaire (SPQ) (Klorman et al., 1974). The Anxiety Sensitivity Index (ASI) (Peterson & Reiss, 1992) was used to assess one's tendency to respond fearfully to anxiety-related symptoms. In addition, evaluation of the US was measured on a 11-point rating scale ranging from -5 (unpleasant) to 5 (pleasant).

### **Experimental Procedure**

The experiment consisted of different phases across three subsequent days each separated by 24 hr and a follow-up session one month later. During each session, participants sat behind a table with a computer monitor at a distance of 50 cm in a sound-attenuated room. Each phase began with a 1-min acclimation period consisting of 70 dB broadband noise, which continued throughout the session as background noise, followed by a habituation phase consisting of ten startle probes to reduce initial startle reactivity. Characteristics of the CSs, trial order, ITIs, and startle probes as well as the instructions regarding the US

expectancy measures during *memory reactivation* (day 2), *extinction-test* (day 3), and *follow-up* were similar to *acquisition* (day 1). Assignment of the slides as CS1<sup>+</sup> and CS2<sup>-</sup> was counterbalanced across participants.

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

After attachment of the startle, skin conductance and shock electrodes, the intensity of the US was determined. Starting at an intensity of 1 mA, the level of a 2-ms aversive electric stimulus delivered to the wrist of the non-preferred hand was gradually increased. The intensity of shock was individually set at a level defined by the participant as ‘uncomfortable, but not painful’ and remained set to this intensity throughout the following days. After US selection, participants were informed regarding the US-expectancy measures. They were instructed to look carefully at both slides, as an electric stimulus would follow one of the slides in general, while the other slide would never be followed by the US. They were told that they should learn to predict whether an electric stimulus would occur or not on the basis of the slides. Participants were required to rate the expectancy of the electric stimulus during the presentation of each slide by shifting a cursor on a continuous 11-point rating scale and push the left mouse button within 5 s following stimulus onset, that is, before administration of the startle probe.

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

At the conclusion of the acquisition phase, participants were asked to evaluate the pleasantness of the US. In addition, they were explicitly instructed

to remember what they had learned. These instructions were included to enhance retention of the CS-US contingency on the following days (Norrholm et al., 2006) and to prevent participants from erroneously expecting a different contingency scheme during subsequent testing. To facilitate salivary sampling, participants were instructed to refrain from exercise, caffeine, and alcohol during the 12 hr before the memory reactivation. Furthermore, they were asked to abstain from brushing their teeth for 1 h and avoid food intake, drinking any beverages other than water, and smoking for 2 h before the experiment.

**Memory Reactivation.** In order to substantiate consolidation of the fear memory, a break of 24 hr after acquisition was inserted. In view of the peak plasma concentration of propranolol (Gilman & Goodman, 1996), participants received double-blind an oral dose of either 40 mg of propranolol HCl or pill placebo 90 min prior to memory reactivation (CS1-R). Administration of propranolol HCl and pill placebo was randomized across participants with the restriction that conditions were matched on SPQ scores as close as possible. Before pill administration and upon completion of the experiment, participants filled out the STAI-S and blood pressure levels were obtained. In addition, at these time points, saliva samples were collected. To this end, the participants were instructed just to place the swab in their mouths for 3 min.

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

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

Instructions regarding the CSs only revealed that the same two images of spiders provided during acquisition would be presented. In the *extinction* phase, participants were exposed to both the CS1<sup>-</sup> and CS2<sup>-</sup> for 12 times without the US. Furthermore, 12 startle probes were presented alone (NA). After extinction, participants received three unsignaled USs. The time between the last extinction trial and the first reinstating US was 19 s. Following the three unsignaled USs, participants were again presented with one CS1<sup>-</sup>, CS2<sup>-</sup> and NA trial each (i.e., *reinstatement testing*). The time between the reinstating USs and reinstatement testing was 18 s. At the end of the experiment, participants completed the STAI and judged the pleasantness of the US.

**Follow Up.** To test for the persistence of the fear erasure, participants were retested one month later (see also Dębiec & LeDoux, 2004). The *follow-up* session paralleled the *extinction - reinstatement testing* session on day 3 apart from the number of CS1<sup>-</sup>, CS2<sup>-</sup>, and NA trials presented. Here, the experiment consisted of 8 *extinction* and 5 *reinstatement testing* trials of each type. In addition, at the end of the experiment, participants were asked which pill they thought they had been taken on day 2 (i.e., propranolol HCl or pill placebo).

### Statistical Analysis

The SPQ, STAI-T and ASI were analyzed using independent *t*-tests. To examine the effect of pill intake on the course of US evaluation and state anxiety, mixed ANOVA's with condition (propranolol vs. placebo; propranolol vs. propranolol no reactivation; placebo vs. propranolol no reactivation) as between-subjects factor and moment (day 1 vs. day 3; before vs. after pill intake) as within-subjects factor were performed. Salivary alpha amylase and systolic as well as diastolic blood pressure were subjected to a 2 (condition: propranolol vs. placebo; propranolol vs. propranolol no reactivation; placebo vs. propranolol no reactivation) x 2 (moment: before vs. after pill intake) mixed ANOVA. Paired *t*-tests within each condition were conducted to compare moment-to-moment alteration.

Startle responses, electrodermal activity and US expectancy ratings were analyzed by means of a mixed analysis of variance for repeated measures (ANOVA) with condition (propranolol vs. placebo; propranolol vs. propranolol no reactivation; placebo vs. propranolol no reactivation) as between-subjects factor and stimulus (CS1 vs. CS2; CS1 vs. NA) and trial (i.e., stimulus presentation) as

within-subjects factors. Planned comparisons were performed for each condition separately. Acquisition was assessed by comparing the differential response (CS1 vs. CS2) to the first acquisition trials (trial 1-2) with the last trials of acquisition (trial 7-8). Responding during memory reactivation was analyzed by comparing the differential response (CS1-R vs. NA) during the reactivation trial. To examine consolidation of the startle fear response, we compared differential responding (CS1 vs. NA) to the last trials of acquisition (trial 1-2) with reactivation (CS1-R vs. NA). The alteration in differential responding (CS1 vs. CS2) from acquisition to extinction was assessed by comparing the last acquisition trials (trial 7-8) with the first trials of extinction (trial 1-2). To test for extinction learning, differential responding (CS1 vs. CS2) to the first trials of extinction (trial 1-2) was compared with the last extinction trials (trial 11-12). In addition, to determine the *speed* of extinction learning, we performed a 2 (condition: propranolol vs. placebo) x 2 (stimulus: CS1 vs. CS2) x 6 (trial: averaging over each two consecutive extinction trials) mixed ANOVA. The reinstatement effect on day 3 was assessed by comparing the differential response (CS1 vs. CS2) to the last trials of extinction (trial 11-12) with the test trial after the reminder shocks. To test for the persistence of the fear erasure one month later, the differential response (CS1 vs. CS2) to both the test trial after the reminder shocks (day 3) and the last trials of extinction (trial 11-12; day 3) was compared with the first trials at test (trial 1-2; day 30). For the analysis of the startle response to NA, mixed ANOVA's with condition (propranolol vs. placebo; propranolol vs. propranolol no reactivation; placebo vs. propranolol no reactivation) as between-subjects factor and trial (trial 1-2 vs. the last two trials of each phase of the experiment separately) as within-subjects factor were performed. Correlations between (differential) startle responding and both trait anxiety and sAA percent change were computed using Pearson correlation analysis. Missing data points were excluded from the analyses. Significance was set at  $P < 0.05$ .

## Results

The propranolol, placebo and propranolol no reactivation condition did not differ in terms of reported spider fear [ $t_{s_{38}} < 1$ ], trait anxiety [ $t_{s_{38}} < 1.33$ ] and anxiety sensitivity [ $t_{s_{38}} < 1.29$ ]. Selected shock intensities ranged from 4 to 65

mA with a mean of 15.52 mA (SD = 11.00). Although individual variation in shock sensitivity resulted in the rather low intensity in the placebo condition as opposed to both the propranolol groups (see **Table 3.1**), no differences in shock intensity were observed between conditions [ $t_{s_{38}} < 1.44$ ]. Importantly, there were also no differences between conditions to the degree participants experienced the US [ $t_{s_{38}} < -1.45$ ]. Furthermore, no differential effect of propranolol intake on the course of US evaluation and state anxiety between the propranolol, placebo and propranolol no reactivation group was found [moment x condition,  $F_{s_{1,38}} < 1$ ]. Consistent with other studies (Grillon et al., 2004), propranolol HCl did also not affect the reported state anxiety that was assessed before and after pill intake on day 2 [moment x condition,  $F_{s_{1,38}} < 1.67$ ].

**Table 3.1.** Mean values (SD) of the intensity of the unconditioned stimulus, reported spider fear, trait anxiety, US evaluation, and Anxiety Sensitivity for the experimental groups.

	<b>Placebo Pill Reactivation</b>	<b>Propranolol HCl Reactivation</b>	<b>Propranolol no Reactivation</b>
Shock (US) (mA)	12.70 (7.23)	15.95 (9.99)	17.90 (14.45)
Spider Fear	6.90 (4.75)	6.65 (6.02)	6.90 (5.23)
Trait Anxiety	33.70 (6.19)	33.20 (7.98)	36.25 (6.74)
US Evaluation	-3.15 (1.09)	-2.96 (0.99)	-2.65 (1.09)
Anxiety Sensitivity	8.95 (3.69)	7.70 (4.51)	9.45 (4.07)

### Manipulation Check Propranolol HCl

Analysis of the effect of propranolol on blood pressure in the propranolol and propranolol no reactivation condition revealed the expected decrease in both systolic [moment x condition,  $F_{1,38} = 32.32$ ,  $P < 0.001$ ,  $\eta_p^2 = .46$ ;  $F_{1,38} = 30.67$ ,  $P < 0.001$ ,  $\eta_p^2 = .45$ , respectively] and diastolic blood pressure [moment x condition,  $F_{1,38} = 12.28$ ,  $P < 0.01$ ,  $\eta_p^2 = .24$ ;  $F_{1,38} = 5.29$ ,  $P < 0.05$ ,  $\eta_p^2 = .12$ , respectively] in comparison to placebo. No differences in decrease of systolic and diastolic blood pressure between the propranolol no reactivation and propranolol condition were observed [moment x condition,  $F_{5,138} < 1.06$ ], indicating that both propranolol conditions exerted a similar physiological effect. Further analysis of blood pressure showed that, in the propranolol condition, the systolic blood pressure significantly decreased from 128.1 mmHg (SD = 11.8) to 110.55 mmHg (SD = 8.1) [ $t_{19} = 8.98$ ,  $P < 0.001$ , two-tailed] and the diastolic blood pressure from 72.2 mmHg (SD = 9.1) to 67.2 mmHg (SD = 8.2) after pill intake [ $t_{19} = 5.50$ ,  $P < 0.001$ , two-tailed]. In the placebo condition, the systolic blood pressure significantly decreased from 120.7 mmHg (SD = 13.4) to 116.6 mmHg (SD = 12.6) after pill intake [ $t_{19} = 3.17$ ,  $P = 0.01$ , two-tailed], but we observed no decrease in diastolic blood pressure [ $t_{19} < 1$ ]. In addition, in the propranolol no reactivation condition, both the systolic and diastolic blood pressure significantly decreased from 127.3 mmHg (SD = 10.1) to 112.3 mmHg (SD = 8.5) [ $t_{19} = 10.30$ ,  $P < 0.001$ , two-tailed] and from 70.8 mmHg (SD = 8.1) to 66.15 mmHg (SD = 6.5) [ $t_{19} = 2.85$ ,  $P = 0.01$ , two-tailed] after pill intake, respectively.

Analysis of the effect of propranolol HCl on sAA levels in the propranolol and propranolol no reactivation condition demonstrated the expected decrease in amylase level in comparison to placebo [moment x condition,  $F_{1,30} = 4.78$ ,  $P < 0.05$ ,  $\eta_p^2 = .14$ ;  $F_{1,29} = 4.75$ ,  $P < 0.05$ ,  $\eta_p^2 = .14$ , respectively]. No difference in decrease of sAA level between the propranolol no reactivation and propranolol condition was found [moment x condition,  $F_{1,35} < 1$ ]. Consistent with other studies (van Stegeren et al., 2006, 2008), the amylase levels in the propranolol and propranolol no reactivation condition significantly decreased from 62.4 U/ml (SD = 46.8) to 31.1 U/ml (SD = 21.0) [ $t_{18} = 3.42$ ,  $P < 0.01$ , two-tailed] and 80.5 U/ml (SD = 68.5) to 40.8 U/ml (SD = 29.5) [ $t_{17} = 2.92$ ,  $P = 0.01$ , two-tailed], respectively, whereas the sAA levels remained stable in the placebo condition [ $t_{12} < 1$ ].

### Fear Potentiated Startle Response

**Propranolol vs. Placebo.** Analysis of variance showed fear conditioning on day 1 by a significant increase of the differential startle response (CS1 vs. CS2) from trial 1-2 to trial 7-8 [stimulus x trial,  $F_{1,38} = 26.18$ ,  $P < 0.001$ ,  $\eta_p^2 = .41$ ; **Fig. 3.1**]. No difference in fear learning between the propranolol and placebo condition was observed [stimulus x trial x condition,  $F_{1,38} < 1$ ]. Since the reactivation session on day 2 serves as both a retrieval cue and an initial test of memory strength (Tronson & Taylor, 2007), we were able to assess the nonspecific effects of propranolol on the retrieval of the previously acquired fear association. Although the two groups expressed comparable response levels to the reactivated stimulus [CS1-R;  $t_{38} < 1$ ], the differential startle response during memory reactivation (CS1-R vs. NA) was significantly reduced in the placebo as opposed to the propranolol group [stimulus x condition;  $F_{1,37} = 5.57$ ,  $P < 0.05$ ,  $\eta_p^2 = .13$ ]. However, the absence of a significant decrease in differential startle response (CS1 vs. NA) from the last two acquisition trials to reactivation between the propranolol and placebo condition, indicates that the conditioned fear was equally well consolidated [stimulus x trial x condition,  $F_{1,37} < 1.61$ ]. In addition, we observed no correlation between the differential fear response during reactivation (CS1 vs. NA) and the percent change in sAA levels before and after propranolol administration [ $r = .27$ ,  $P = 0.28$ ] in the propranolol reactivation group. These data demonstrate that propranolol did not directly affect the expression of the fear memory. Propranolol did also not reduce the startle response per se, as no effects were found on habituation while the drug was on board [main effect of condition; trial x condition,  $F_{5,30} < 1.76$ ].

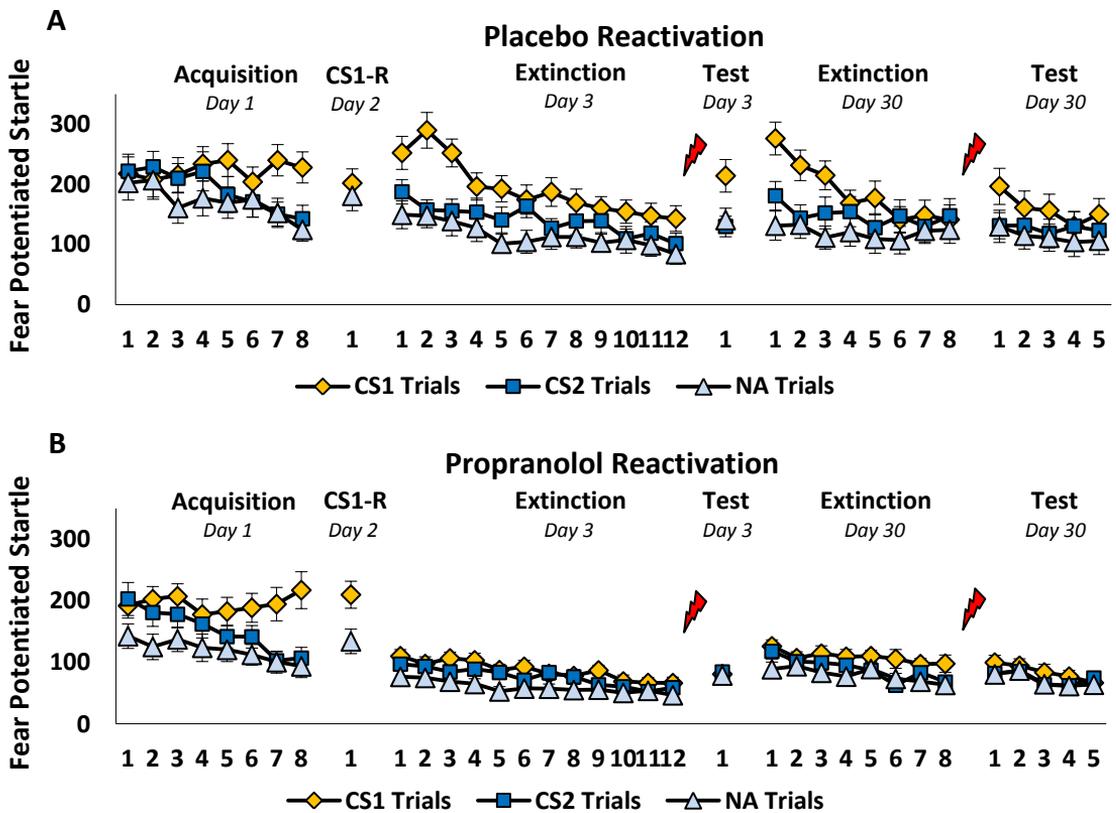
The administration of propranolol contrary to pill placebo significantly decreased the differential startle response 48 hr later (**Fig. 3.1**), that is, from acquisition (trial 7-8) to extinction (trial 1-2) [stimulus x trial x condition,  $F_{1,38} = 9.11$ ,  $P < 0.01$ ,  $\eta_p^2 = .19$ ]. As predicted, propranolol strongly reduced the differential startle response [stimulus x trial,  $F_{1,19} = 14.23$ ,  $P = 0.001$ ,  $\eta_p^2 = .43$ ], whereas the fear response remained stable in the pill placebo condition [stimulus x trial,  $F_{1,19} < 1$ ]. In the propranolol condition, the conditioned fear response was not only reduced but even eliminated, as we no longer observed a differential startle response [CS1 vs. CS2; extinction trial 1-2;  $t_{19} < 1.21$ ]. In contrast, the fear response remained significant in the placebo condition [CS1 vs. CS2; extinction trial 1-2;  $t_{19} = 6.34$ ,  $P < 0.001$ , two-tailed].

Given that the startle response (CS1 vs. CS2) was already eliminated in the propranolol condition, the two groups differed over the course of extinction learning from trial 1-2 to trial 11-12 on day 3 [stimulus x trial x condition,  $F_{1,38} = 15.53$ ,  $P < 0.001$ ,  $\eta_p^2 = .29$ ]. There was no differential change of the startle response in the propranolol condition [stimulus x trial,  $F_{1,19} < 1$ ], whereas the startle response significantly decreased in the placebo group [stimulus x trial,  $F_{1,19} = 17.73$ ,  $P < 0.001$ ,  $\eta_p^2 = .48$ ; **Fig. 3.1**].

The *reminder shocks* also generated the predicted effect between the propranolol and placebo condition for the differential startle response from the last extinction trials (trial 11-12) to the test trial on day 3 [stimulus x trial x condition,  $F_{1,38} = 5.84$ ,  $P < 0.05$ ,  $\eta_p^2 = .13$ ]. In the propranolol condition, the reinstatement effect was not only absent [stimulus x trial,  $F_{1,19} < 2.1$ ; **Fig. 3.1 B**], but the reminder shocks did also not reveal any differential fear response to test [ $t_{19} < 1$ ]. Conversely, the startle response (CS1 vs. CS2) in the placebo condition approached significance [stimulus x trial,  $F_{1,19} = 4.00$ ,  $P = 0.06$ ,  $\eta_p^2 = .17$ ; **Fig. 3.1 A**]. However, for both the feared CS1 and the control CS2 a significant reinstatement effect was observed in the placebo group [main effect of trial,  $F_{1,19} = 11.05$ ,  $P < 0.01$ ,  $\eta_p^2 = .37$ ]. This observation may indicate a generalization of the previously acquired fear to the control stimulus (Dirikx et al., 2007). Previously, we found that this generalization of fear after reinstatement was explained by trait anxiety (Kindt et al., 2009). Again, a positive correlation emerged between trait anxiety and the startle response to the control stimulus (CS2) after reinstatement in the placebo group [ $r = .49$ ,  $P < 0.05$ , two-tailed]. Re-analyzing the reinstatement effect with trait anxiety as covariate produced a significant differential return of fear (CS1 vs. CS2) in the placebo condition [stimulus x trial,  $F_{1,18} = 7.74$ ,  $P < 0.04$ ,  $\eta_p^2 = .30$ ; **Fig. 3.1 A**].

At *follow-up*, we found a significant increase of the differential startle response from the last extinction trials (trial 11-12; day 3) to the first test trials (trial 1-2; day 30) in the placebo as opposed to the propranolol group [stimulus x trial x condition,  $F_{1,38} = 6.78$ ,  $P < 0.05$ ,  $\eta_p^2 = .15$ ]. *Planned* comparisons indeed showed that the differential startle response remained stable in the propranolol condition [stimulus x trial,  $F_{1,19} < 1$ ; **Fig. 3.1 B**], whereas it resurfaced in the placebo group [stimulus x trial,  $F_{1,19} = 6.21$ ,  $P < 0.05$ ,  $\eta_p^2 = .25$ ; **Fig. 3.1 A**]. Once more, no differential startle response was observed in the propranolol condition [follow-up trial 1-2;  $t_{19} < 1.45$ ], whereas a significant startle response appeared

in the placebo group [CS1 vs. CS2;  $t_{19} = 5.07$ ,  $P < 0.001$ , two-tailed]. Moreover, no difference was found between the propranolol and placebo condition for the startle response (CS1 vs. CS2) from the one test trial after the reminder shocks (day 3) to the first follow-up trials (trial 1-2; day 30) [stimulus x trial x condition,  $F_{1,38} < 1$ ]. Hence, the disparity in fear reinstatement on day 3 between the two conditions was not affected by simply the passage of time.



**Fig. 3.1.** Propranolol HCl persistently disrupts the reconsolidation of fear memory. Mean startle potentiation to the feared stimulus (CS1), the control stimulus (CS2) and noise alone (NA) trials during acquisition, memory reactivation, extinction, testing and follow up for the (A) Placebo Reactivation and (B) Propranolol Reactivation Group. Error bars represent SEM.

Consequently, the two groups differed over the course of extinction learning during follow-up [stimulus x trial x condition,  $F_{1,38} = 13.85$ ,  $P = 0.001$ ,  $\eta_p^2 = .27$ ]. Whereas extinction training significantly reduced the startle response (CS1 vs. CS2) in the placebo condition [stimulus x trial,  $F_{1,19} = 11.47$ ,  $P < 0.01$ ,  $\eta_p^2 = .38$ ; **Fig. 3.1 A**], we observed no differential change of the startle response in the propranolol group [stimulus x trial,  $F_{1,19} < 2.45$ ; **Fig. 3.1 B**]. In addition, exposure to the reminder shocks following extinction, again reinstated the expression of the fear memory in the placebo condition contrary to the propranolol group [stimulus x trial x condition,  $F_{1,38} = 5.23$ ,  $P < 0.05$ ,  $\eta_p^2 = .12$ ]. Analysis of the differential startle response from the last extinction trials (trial 6-8) to the first test trials during follow-up (trial 1-2) showed a significant reinstatement effect in the placebo condition [stimulus x trial,  $F_{1,19} = 4.87$ ,  $P < 0.05$ ,  $\eta_p^2 = .20$ ], but not in the propranolol group [stimulus x trial,  $F_{1,19} < 1$ ]. Once again, the reminder shocks did not uncover any differential fear response to the first follow-up trials (trial 1-2) in the propranolol condition [ $t_{19} < 1.7$ ].

Analysis of the startle response to Noise Alone (NA) unveiled a marginally significant difference between the propranolol and placebo condition during acquisition [main effect of pill,  $F_{1,38} < 3.36$ ,  $P = 0.075$ ,  $\eta_p^2 = .08$ ], with NA levels somewhat higher in the placebo condition [trial 1-2:  $M = 204.6$ ,  $SD = 129.5$ ; trial 7-8:  $M = 138.4$ ,  $SD = 94.5$ ] as opposed to the propranolol group [trial 1-2:  $M = 133.9$ ,  $SD = 88.1$ ; trial 7-8:  $M = 97.0$ ,  $SD = 78.6$ ]. This effect could mainly be ascribed to a difference in response levels during the first acquisition trials [trial 1-2;  $t_{38} = 2.02$ ,  $P = 0.05$ , two-tailed], as group differences were no longer present by the end of acquisition [trial 7-8;  $t_{38} < -1.5$ ]. In addition, the two groups expressed comparable levels of responding to the one NA trial during reactivation [ $t_{37} < 1.46$ ]. In contrast to the placebo condition, however, startle responses to the NA trials in the propranolol group were reduced during extinction training [trial 1-2 vs. trial 11-12; main effect of pill,  $F_{1,38} = 8.96$ ,  $P < 0.01$ ,  $\eta_p^2 = .19$ ], reinstatement testing [ $t_{38} = 2.87$ ,  $P < 0.01$ , two-tailed], and follow-up extinction [trial 1-2 vs. trial 7-8; main effect of pill,  $F_{1,38} = 5.18$ ,  $P < 0.05$ ,  $\eta_p^2 = .12$ ]. Analysis of the startle response to the follow-up NA trials after reinstatement approached significance [trials 1-2 vs. trial 4-5; main effect of pill,  $F_{1,38} < 3.05$ ,  $P = 0.089$ ,  $\eta_p^2 = .07$ ]. Although these findings suggest a trend towards a difference in startle responding between the propranolol and placebo

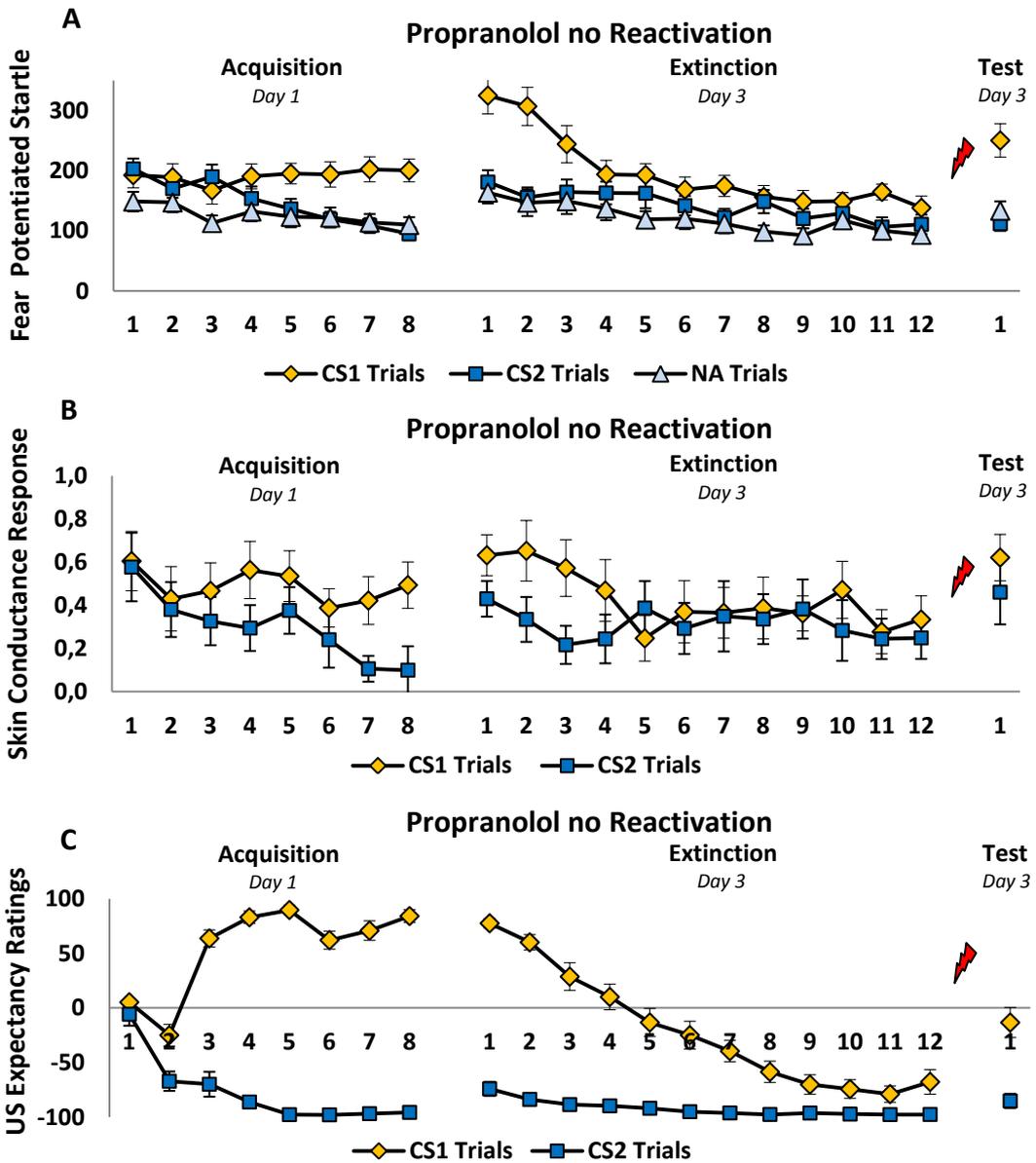
condition, they may also indicate that the fear erasure effect generalized to the context.

**Propranolol No Reactivation.** Analysis of the startle response (CS1 vs. CS2) on day 1 showed no differences in fear learning between the propranolol no reactivation and both the propranolol and placebo condition from trial 1-2 to trial 7-8 [stimulus x trial x condition,  $F_{1,38} < 1$ ; **Fig. 3.1**; **Fig. 3.2 A**].

Similar to the placebo condition, the differential startle response in the propranolol no reactivation condition remained stable from the last acquisition trials (trial 7-8) on day 1 to the first extinction trials (trial 1-2) on day 3 [stimulus x trial x condition,  $F_{1,38} < 1.50$ ; **Fig. 3.1 A**; **Fig. 3.2 A**]. Hence, we observed a normal fear response in the propranolol no reactivation group 48 hr after acquisition. Moreover, the reduction of the conditioned startle response in the propranolol condition 24 hr after reactivation differed significantly from the propranolol no reactivation group [stimulus x trial x condition,  $F_{1,38} = 20.94$ ,  $P < 0.001$ ,  $\eta_p^2 = .36$ ; **Fig. 3.1 B**; **Fig. 3.2 A**]. In contrast to the propranolol condition, the differential startle response in the propranolol no reactivation condition even increased from day 1 to day 3 [stimulus x trial,  $F_{1,19} = 6.76$ ,  $P < 0.05$ ,  $\eta_p^2 = .26$ ]. Together, these findings indicate that the decrease of the fear response in the propranolol condition was dependent on the active retrieval of the fear memory.

Analysis of the startle response (CS1 vs. CS2) on day 3 showed no difference in extinction learning between the propranolol no reactivation and the placebo condition from trial 1-2 to trial 11-12 [stimulus x trial x condition,  $F_{1,38} < 3.03$ ]. In addition, the course of extinction learning between the propranolol no reactivation and propranolol condition differed significantly [stimulus x trial x condition,  $F_{1,38} = 28.96$ ,  $P < 0.001$ ,  $\eta_p^2 = .46$ ; **Fig. 3.1 B**; **Fig. 3.2 A**]. The extinction training significantly reduced the startle response (CS1 vs. CS2) in the propranolol no reactivation condition [stimulus x trial,  $F_{1,19} = 31.91$ ,  $P < 0.001$ ,  $\eta_p^2 = .63$ ], whereas we observed no differential change of the startle response in the propranolol group [stimulus x trial,  $F_{1,19} < 1$ ].

Analysis of the reinstatement effect showed no difference between the propranolol no reactivation and placebo condition for the startle response (CS1 vs. CS2) from the last extinction trials (trial 11-12) to the one test trial after reinstatement [stimulus x trial x condition,  $F_{1,38} < 1.78$ ; **Fig. 3.1 A**; **Fig. 3.2 A**]. Conversely, comparison of the reinstatement effect between the propranolol no



**Fig. 3.2.** Omission of memory reactivation after propranolol intake yields normal fear responses. Mean startle potentiation to the CS1, the CS2 and NA trials (A) and mean skin conductance responses (B) as well as mean expectancy scores (C) to the CS1 and CS2 trials during acquisition, extinction and testing for the Propranolol no Reactivation Group. Error bars represent SEM.

reactivation and propranolol condition revealed the expected difference in fear reinstatement [stimulus x trial x condition,  $F_{1,38} = 16.97$ ,  $P < 0.001$ ,  $\eta_p^2 = .31$ ; **Fig. 3.1 B**; **Fig. 3.2 A**], indicating that the absence of fear reinstatement in the propranolol condition was dependent on the active retrieval of the fear memory. Analysis of the fear reinstatement in the propranolol no reactivation condition showed a significant return of the fear response [stimulus x trial,  $F_{1,19} = 14.92$ ,  $P = 0.001$ ,  $\eta_p^2 = .44$ ]. Interestingly, no difference was found in startle response (CS1 vs. CS2) from the last acquisition trials (trial 7-8) to the one test trial after reinstatement between the propranolol no reactivation and placebo condition [stimulus x trial x condition,  $F_{1,38} < 1.53$ ; **Fig. 3.1 A**; **Fig. 3.2 A**], demonstrating a complete post-extinction recovery of fear. In sum, both the oral administration of propranolol and the reactivation of the fear memory seemed to be necessary for the observed erasure of the fear response.

Analysis of the startle response to the Noise Alone (NA) trials produced no significant differences between the propranolol no reactivation and placebo condition during acquisition and extinction [main effect of pill,  $F_{5,138} < 2.20$ ,  $P_s > 0.15$ ], nor to the one NA trial after reinstatement [ $t_{38} < 1$ ]. Also, we observed no difference in startle responses to the NA trials between the propranolol no reactivation and propranolol condition during acquisition [main effect of pill,  $F_{1,38} < 1$ ]. However, the startle response to the NA trials was reduced in the propranolol condition in contrast to the propranolol no reactivation condition during both extinction [main effect of pill,  $F_{1,38} = 16.99$ ,  $P < 0.001$ ,  $\eta_p^2 = .31$ ] and after reinstatement [ $t_{38} = -3.07$ ,  $P < 0.01$ , two-tailed; **Fig. 3.1**; **Fig. 3.2 A**].

### Skin Conductance Response

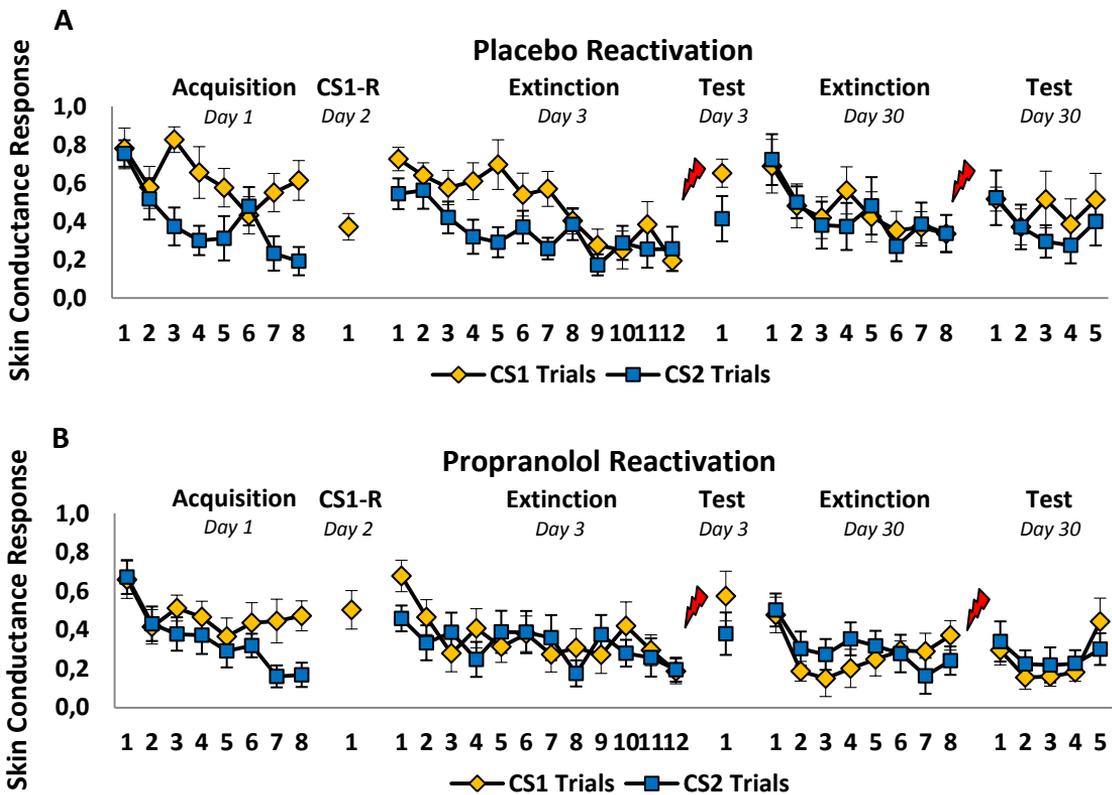
Overall analysis of electrodermal responding showed no fear conditioning on day 1 [stimulus x trial,  $F_{1,57} < 1.82$ ]. When fear responses are not successfully acquired, one cannot assess the return of fear. Therefore, only subject showing successful levels of fear acquisition (i.e., mean trial 7-8 CS1 > CS2) were included in the analyses<sup>1</sup>. A total of 21 subjects were eliminated, that is, *6 subjects* from

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<sup>1</sup> For the startle response data, excluding subjects showing unsuccessful levels of fear acquisition ( $n = 3$ ) revealed no differences in results. Therefore, the analyses were carried out over the entire sample.

the propranolol condition, 6 subjects from the placebo condition and 9 subjects from the propranolol no reactivation group.

**Propranolol vs. Placebo.** Analysis of variance showed fear conditioning on day 1 by a significant differential increase (CS1 vs. CS2) in electrodermal activity from trial 1-2 to trial 7-8 [stimulus x trial,  $F_{1,26} = 22.30$ ,  $P < 0.001$ ,  $\eta_p^2 = .46$ ; **Fig. 3.3**]. No difference between the propranolol and placebo condition was found [stimulus x trial x condition,  $F_{1,26} < 1$ ]. Furthermore, the two groups showed comparable levels of electrodermal responding during the fear memory reactivation on day 2 [CS1-R;  $t_{26} < 1.08$ ].



**Fig. 3.3.** Mean skin conductance responding to the CS1 and CS2 trials during acquisition, memory reactivation, extinction, testing and follow up for the (A) Placebo Reactivation and (B) Propranolol Reactivation Group. Error bars represent SEM.

In both the propranolol and placebo condition, the skin conductance response (CS1 vs. CS2) significantly decreased from the last acquisition trials (trial 7-8) on day 1 to the first extinction trials (trial 1-2) on day 3 [stimulus x trial,  $F_{1,26} = 8.08$ ,  $P < 0.01$ ,  $\eta_p^2 = .24$ ; **Fig. 3.3**]. However, the differential skin conductance response (extinction trial 1-2) remained significant in the propranolol condition [ $t_{13} = 3.01$ ,  $P < 0.01$ , two-tailed] as well as in the placebo group [ $t_{13} = 2.39$ ,  $P < 0.05$ , two-tailed]. Hence, the acquisition of electrodermal responding seemed to be maintained 48 hr later.

Analysis of the differential skin conductance response (CS1 vs. CS2) on day 3 showed no difference in extinction learning between the propranolol and placebo group [stimulus x trial x condition,  $F_{1,26} < 1$ ]. In both the propranolol and placebo condition, the extinction procedure yielded a significant decrease in SCR from trial 1-2 to trial 11-12 [stimulus x trial,  $F_{1,26} = 4.44$ ,  $P < 0.05$ ,  $\eta_p^2 = .15$ ]. However, a significant difference in *speed* of extinction learning was observed between the propranolol and placebo group [stimulus x trial x condition,  $F_{5,22} = 3.02$ ,  $P < 0.05$ ,  $\eta_p^2 = .41$ ; **Fig. 3.3**], revealing faster extinction learning in the propranolol as opposed to the placebo condition.

Analysis of the reinstatement effect on day 3 showed no differential increase (CS1 vs. CS2) in electrodermal activity from the last extinction trials (trial 11-12) to test [stimulus x trial,  $F_{1,26} < 2.16$ ]. However, for both the feared CS1 and the control CS2 the skin conductance response increased after the *reminder shocks* [main effect of trial,  $F_{1,26} = 24.67$ ,  $P < 0.001$ ,  $\eta_p^2 = .49$ ], demonstrating a generalization of the previously acquired fear to the control stimulus (Dirikx et al., 2007). This generalization of fear could not be explained by trait anxiety. Therefore, further analyses of the reinstatement effect only comprised the skin conductance response to the feared CS1. Re-analyzing the reinstatement effect produced a significant return of fear [stimulus x trial,  $F_{1,26} = 17.32$ ,  $P < 0.001$ ,  $\eta_p^2 = .40$ ; **Fig. 3.3**]. No difference in reinstatement between the propranolol and placebo condition was found [stimulus x trial x condition,  $F_{1,26} < 1$ ].

At *follow-up*, the differential skin conductance response (CS1 vs. CS2) no longer reached significance [trial 1-2;  $t_{s_{14}} < 1$ ; **Fig. 3.3**]. Also, no further significant differences between the propranolol and placebo condition were observed. Therefore, the follow-up data will not be presented.

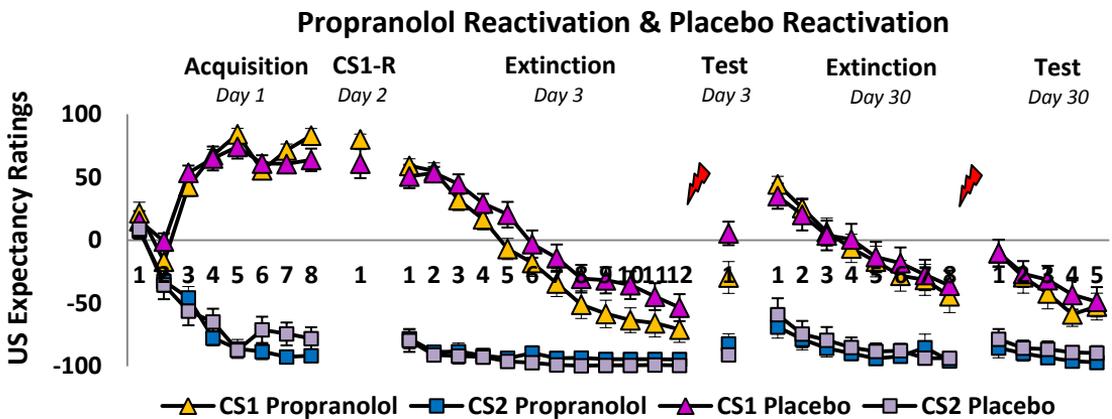
**Propranolol No Reactivation.** No differences between the propranolol no reactivation and both the propranolol and placebo condition were observed during acquisition, extinction, and reinstatement testing (CS1) [stimulus x trial x condition,  $F_{s_{1,23}} < 1.94$ ; **Fig. 3.2 B**; **Fig. 3.3**]. Separate analysis for the propranolol no reactivation condition indeed showed fear conditioning on day 1 (CS1 vs. CS2) [stimulus x trial,  $F_{1,10} = 4.99$ ,  $P < 0.05$ ,  $\eta_p^2 = .33$ ]. In addition, the differential skin conductance response remained stable 48 hr after acquisition [stimulus x trial,  $F_{1,10} < 2.33$ ]. Furthermore, analysis of variance showed extinction learning on day 3 [stimulus x trial,  $F_{1,10} = 5.03$ ,  $P < 0.05$ ,  $\eta_p^2 = .34$ ] as well as a significant return of fear following the reminder shocks (CS1) [stimulus x trial,  $F_{1,10} = 4.97$ ,  $P = 0.05$ ,  $\eta_p^2 = .33$ ].

### US Expectancy Ratings

**Propranolol vs. Placebo.** Analysis of the US expectancy data on day 1 (CS1 vs. CS2; **Fig. 3.4**) showed a significant difference between the propranolol and placebo condition from the first acquisition trials (trial 1-2) to the last acquisition trials (trial 7-8) [stimulus x trial x condition,  $F_{1,38} = 5.47$ ,  $P < 0.05$ ,  $\eta_p^2 = .13$ ]. However, in both the propranolol and placebo group a significant differential increase (CS1 vs. CS2) in US expectancy was found [stimulus x trial,  $F_{1,19} = 202.59$ ,  $P < 0.001$ ,  $\eta_p^2 = .91$ ;  $F_{1,19} = 109.36$ ,  $P < 0.001$ ,  $\eta_p^2 = .85$ , respectively]. Note that the superior acquisition observed in the propranolol condition (i.e., contingency ratings corresponding to the actual reinforcement scheme) works against the hypothesis that the administration of propranolol combined with the active retrieval of the fear memory would reveal less fear responses at test. Moreover, the two groups did not differ in their expectancy rating during the fear memory reactivation on day 2 [CS1-R;  $t_{38} < 1.57$ ]. Furthermore, in both the propranolol and placebo condition, the increase in US expectancy (CS1 vs. CS2) obtained during acquisition (trial 7-8) remained stable 48 hr later (extinction trial 1-2) [stimulus x trial,  $F_{1,38} < 2.43$ ].

Analysis of variance showed extinction learning on day 3 by a significant differential decrease in US expectancy from trial 1-2 to trial 11-12 [stimulus x trial,  $F_{1,38} = 140.50$ ,  $P < 0.001$ ,  $\eta_p^2 = .79$ ]. No difference in extinction learning between the propranolol and placebo condition was found [stimulus x trial x condition,  $F_{1,38} < 2.53$ ]. Furthermore, we observed a marginally significant difference in the *speed* of extinction learning between the propranolol and

placebo group [stimulus x trial x condition,  $F_{5,34} = 2.31$ ,  $P = 0.065$ ,  $\eta_p^2 = .25$ ], closely resembling the skin conductance data. The analyses of the skin conductance response, however, only included subjects showing successful levels of fear acquisition. Re-analyzing the *speed* of extinction learning in the corresponding subjects revealed a highly similar result, that is, significantly faster extinction learning in the propranolol condition as opposed to the placebo group [stimulus x trial x condition,  $F_{5,22} = 2.82$ ,  $P < 0.05$ ,  $\eta_p^2 = .39$ ], perhaps resulting from a difference in response tendency between the two conditions. Note that the placebo group also revealed less extreme US expectancy ratings during acquisition.



**Fig. 3.4.** Mean expectancy scores of the US to the CS1 and CS2 trials during acquisition, memory reactivation, extinction, testing and follow up for the Placebo Reactivation and Propranolol Reactivation Group. Error bars represent SEM.

Analysis of the reinstatement effect on day 3 showed a significant increase in US expectancy from the last extinction trials (trial 11-12) to the test trial following the reminder shocks [stimulus x trial,  $F_{1,38} = 24.55$ ,  $P < 0.001$ ,  $\eta_p^2 = .39$ ]. No difference in fear reinstatement between the propranolol and placebo group was found [stimulus x trial x condition,  $F_{1,38} < 1.81$ ].

At *follow-up*, no differences in extinction learning and fear reinstatement between the propranolol and placebo group were found [stimulus x trial x condition,  $F_{5,1,38} < 1.99$ ]. In both the propranolol and placebo condition, we

observed a significant decrease in US expectancy during extinction [stimulus x trial,  $F_{1,38} = 27.77$ ,  $P < 0.001$ ,  $\eta_p^2 = .42$ ]. In addition, we found a significant reinstatement effect in the propranolol condition [stimulus x trial,  $F_{1,18} = 6.89$ ,  $P < 0.05$ ,  $\eta_p^2 = .27$ ], but not in the placebo group [stimulus x trial,  $F_{1,19} < 1$ ]. In the placebo condition, however, a significant reinstatement effect for both the feared CS1 and the control CS2 was found [main effect of trial,  $F_{1,19} = 6.60$ ,  $P < 0.05$ ,  $\eta_p^2 = .26$ ].

**Propranolol No Reactivation.** Analysis of the US expectancy data on day 1 (**Fig. 3.2 C**; **Fig. 3.4**) showed a superior acquisition in the propranolol no reactivation condition contrary to the placebo group [stimulus x trial x condition,  $F_{1,38} = 4.33$ ,  $P < 0.05$ ,  $\eta_p^2 = .10$ ]. However, in both the propranolol no reactivation and placebo condition, a significant increase (CS1 vs. CS2) in US expectancy was found [stimulus x trial,  $F_{1,19} = 345.44$ ,  $P < 0.001$ ,  $\eta_p^2 = .94$ ;  $F_{1,19} = 69.30$ ,  $P < 0.001$ ,  $\eta_p^2 = .79$ , respectively]. Similar to the placebo condition, the differential increase in US expectancy remained stable from the last acquisition trials (trial 7-8) on day 1 to the first extinction trials (trial 1-2) 48 hr later [stimulus x trial x condition,  $F_{1,38} < 2.39$ ]. The propranolol no reactivation condition, however, showed significantly stronger extinction learning in contrast to the placebo group [stimulus x trial x condition,  $F_{1,38} = 5.09$ ,  $P < 0.05$ ,  $\eta_p^2 = .12$ ]. Yet again, in both the propranolol no reactivation and placebo group a significant decrease in US expectancy was found [stimulus x trial,  $F_{1,19} = 123.24$ ,  $P < 0.001$ ,  $\eta_p^2 = .87$ ;  $F_{1,19} = 56.79$ ,  $P < 0.001$ ,  $\eta_p^2 = .75$ , respectively]. In addition, no difference in fear reinstatement between the two groups was observed [stimulus x trial x condition,  $F_{1,38} < 1$ ]. Separate analyses for the propranolol no reactivation condition indeed showed a significant differential reinstatement effect from the last extinction trials (trial 11-12) to the one test trial after reinstatement [stimulus x trial,  $F_{1,19} = 13.76$ ,  $P = 0.001$ ,  $\eta_p^2 = .42$ ]. Moreover, no differences between the propranolol no reactivation and propranolol condition during acquisition, extinction, and fear reinstatement were found [stimulus x trial x condition,  $F_{1,38} < 1.50$ ].

## Discussion

Supporting our previous findings (Kindt et al., 2009), oral administration of the  $\beta$ -adrenergic receptor antagonists propranolol HCl prior to reactivation of a

fear memory resulted in the erasure of the startle fear response 24 hr later, irrespective of the intact recollection of the fear association. But most importantly, this effect persisted at one-month follow-up. Beta-adrenergic blockade during memory reactivation seemed to be highly effective in eliminating the fear expression, as both retrieval techniques employed (i.e., *reminder shocks* and *long-term testing*) failed to uncover any startle fear response. This contrasts sharply with the placebo condition in which reinstatement as well as one-month follow-up testing revealed a post-extinction fear response. Notably, the startle potentiation remained stable during memory reactivation, suggesting that the erasure of the startle fear response cannot be explained by a nonspecific dampening effect of the  $\beta$ -adrenergic blocker. Human studies showing no effects of propranolol HCl on startle reactivity (Grillon et al., 2004) further support this notion. Even though administering pills prior to reactivation does not provide direct evidence of interference with the process of reconsolidation, the fear reducing effects were only observed during the post-reactivation tests either one day or one month later in the propranolol reactivation condition and not in the propranolol *no* reactivation group. The reduction in startle responses could not be explained by any general effects of the propranolol HCl manipulation on state anxiety and US evaluation. Combined with the findings that blood pressure and alpha amylase levels were similarly reduced in both propranolol conditions, these results suggest that the drug manipulation specifically affected the molecular processes mediating reconsolidation (Nader et al., 2000).

As expected, and in contrast to the startle fear response,  $\beta$ -adrenergic blockade during memory reactivation did not sort any effect on skin conductance conditioning. This result is in line with numerous data showing a clear dissociation between conditioned startle potentiation and electrodermal activity (see Hamm & Weike, 2005). Electrodermal conditioning seems to primarily reflect the cognitive level of contingency learning (i.e., declarative memory) (Weike et al., 2007), based on the functional integrity of the hippocampal complex (Squire et al., 2004). In line with other studies on human fear conditioning (Hamm & Vaitl, 1996; Weike et al., 2007), a close association between electrodermal activity and contingency learning was found. Conversely, human startle potentiation is considered to be a reliable and specific index of fear (Hamm & Weike, 2005) and is directly connected with, and modulated by,

the amygdala (Davis, 2006). Propranolol HCl is supposed to specifically act on the  $\beta$ -adrenergic receptors in the basolateral amygdala (McGaugh, 2004; Hurlmann et al., 2010). Although the current study only allows for speculation on the effects of propranolol HCl in the amygdala, functional magnetic resonance imaging (fMRI) has demonstrated selectively disturbed amygdala activation following propranolol intake in humans (van Stegeren et al., 2005). In addition, animal studies show that the *systemic* effects of propranolol on reconsolidation are achieved by targeting the amygdala (Dębiec & LeDoux, 2004). In rats it is demonstrated that disruption of fear memory reconsolidation is correlated with a reduction of synaptic potentiation in the lateral amygdala selective to the reactivated fear memory (Doyère et al., 2007). The  $\beta$ -adrenergic receptor is one of the three major neurotransmitter receptors that are positively coupled to the cAMP/PKA pathway, which can lead to de novo protein synthesis necessary for LTP (Huang & Kandel, 2007). Hence, it may be suggested that  $\beta$ -adrenergic blockade during reconsolidation selectively disrupts the protein synthesis of the amygdalar fear memory resulting in the *persistent* erasure of the fear potentiated startle response, while leaving the hippocampal recollection of the fear memory untouched. In fact, the vast majority of reconsolidation studies involving the amygdala have yielded persistent amnesia effects (Amaral et al., 2008). Conversely, most studies using either extinction or reconsolidation blockade targeting the hippocampus only show transient amnesia (Bouton, 2002; Amaral et al., 2008; but see Lee et al., 2004).

Recently, however, it was demonstrated in rats that extinction training may also lead to permanent amnesia when presented *within* the reconsolidation window, thereby causing the destabilization of the fear memory trace in the lateral amygdala (Monfils et al., 2009). When presented outside the reconsolidation window, extinction training only temporarily inhibited the activation of the initial fear memory trace. Following these findings, the supposed updating of fear memory with non-fearful information provided during reconsolidation was also demonstrated in humans (Schiller et al., 2010). It should be noted, however, that conditioned fear was measured by the skin conductance response. As mentioned before, electrodermal activity does not specifically index fear but rather reflects the more cognitive level of contingency learning. Thus, it remains to be seen whether behavioral interventions such as extinction training within the reconsolidation window will be as effective in

erasing human fear responses as pharmacological interventions targeting the amygdala during memory reactivation.

Studying human fear conditioning allows for the independent evaluation of both declarative knowledge and the fear response. In most fear conditioning studies, the conscious anticipation of an aversive stimulus (US) is associated with an increase in startle potentiation (Grillon & Davis, 1995; Lovibond & Shanks, 2002). However, there are also several observations showing that unawareness of a CS-US contingency does not preclude a startle fear response (Weike et al., 2007). This indicates that the anticipation of an aversive stimulus is not a necessary condition to observe fear potentiated startle responses. Our findings suggest that the anticipation of an US is also not a sufficient condition to generate a fear potentiated startle response. As such, our results challenge the cognitive account on the close association between declarative knowledge and fear conditioning (Lovibond, 2004).

The propranolol HCl manipulation left the declarative memory for the learned fear association intact. This finding is at odds with the literature showing selectively reduced memory for emotional events when encoding takes place following propranolol HCl administration (Cahill et al., 1994; van Stegeren et al., 1998, 2005). Note, however, that in these studies memory encoding as opposed to reconsolidation was the process under investigation. The discrepancy between findings could also be explained by a number of other methodological differences, such as the nature and mode of learning (i.e., differential fear conditioning vs. emotional stories or pictures) and the measure of declarative memory performance employed (i.e., concurrent US expectancy ratings vs. delayed recall and recognition of pictures). Clearly, concurrent US expectancy ratings direct the attention towards the CS-US relation, thereby affecting the very entity they are designed to measure (Baeyens et al., 1990; Lovibond & Shanks, 2002). Thus, the current US-expectancy ratings may be limited in detecting the effects of propranolol on declarative knowledge. In addition, the US expectancy ratings are possibly too straightforward to be affected by the neurobiological manipulation.

In considering clinical implications, several issues need to be addressed. First, it should be demonstrated that disrupting reconsolidation not only erases the startle fear response, but also diminishes subjective experienced fear. Furthermore, as reconsolidation blockade has only been observed in animals

(Nader et al., 2000) and healthy participants (Kindt et al., 2009), we do not know whether the current procedure will be as effective in patient populations. Apparently, there are a number of experimental conditions under which reconsolidation does not seem to occur, such as the strength of training and memory age (Suzuki et al., 2004). A crucial question is whether strong trauma memories in PTSD patients will also be sensitive to the blockade of reconsolidation. For now, preliminary evidence in trauma patients is promising; showing reduced trauma-relevant physiological responding following post-retrieval propranolol HCl intake (Brunet et al., 2008). Acknowledging that disrupting reconsolidation is only a proof of principle, at least, we may conclude that it clearly outperformed traditional extinction learning. Procedures that persistently weaken fear responses without causing universal amnesia alleviate the ethical concerns regarding memory erasure, while pointing to new treatments providing *long-term* cure for patients suffering from emotional disorders such as posttraumatic stress disorder, phobias, and drug addiction.

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## Chapter 3

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