Erasing fear from memory
Soeter, M.

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Chapter 5

Noradrenergic Enhancement of Associative Fear Memory in Humans

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Ample evidence in animals and humans supports the noradrenergic modulation in the formation of emotional memory. However, in humans the effects of stress on emotional memory are traditionally investigated by declarative memory tests (e.g., recall, recognition) for non-associative emotional stimuli (e.g., stories, pictures). Given that anxiety disorders are thought to originate from associative learning processes and are characterized by distressing emotional responses, the existing literature seems to be inconclusive for the understanding of these disorders. Here, we tested whether noradrenaline strengthens the formation of associative fear memory by using a differential fear conditioning procedure in humans. Stimulation of the noradrenergic system by the administration of yohimbine HCl (20 mg) during memory formation did not directly augment the differential startle fear response 48 hr later. Yet, the other retention tests uncovered that the administration of yohimbine HCl contrary to placebo pill extensively delayed the process of extinction learning and generated a superior recovery of fear (i.e., reinstatement and reacquisition). Conversely, the yohimbine HCl manipulation did not affect the skin conductance responding and the US expectancy ratings, emphasizing the concept of multiple memory systems. Our data demonstrate that increased noradrenaline release during or shortly after a stressful event strengthens the emotional expression of human associative fear memory (i.e., startle fear response). The present findings suggest that noradrenaline may play an important role in the etiology and maintenance of anxiety disorders.
Emotionally arousing experiences often attain a privileged status in memory (McGaugh, 2002; LaBar & Cabeza, 2006). Ample evidence in animals demonstrates that stress hormones released by arousing learning experiences activate noradrenergic mechanisms within the basolateral nucleus of the amygdala (BLA) (McGaugh & Roozendaal, 2009). This noradrenergic activation of the BLA in turn strengthens consolidation processes in brain regions such as the hippocampus (McGaugh, 2004). The involvement of noradrenergic hormones in human memory has been investigated by either blocking or activating noradrenergic receptors in the brain. That is, blockade of noradrenergic functioning with propranolol HCl selectively impairs memory performance for emotional-arousing material (Cahill et al., 1994; Hurlemann et al., 2005; van Stegeren, 1998, 2008). Conversely, post-learning administration of adrenergic drugs or hormones (e.g., yohimbine HCl) enhances memory consolidation of emotionally arousing information (Southwick et al., 2002; Cahill & Alkire, 2003). These findings thus support the noradrenergic modulation in the formation of human emotional memory (McGaugh, 2004). Even though these memory-enhancing effects of emotional arousal are extremely functional from an evolutionary perspective, the impact of emotion on memory can also have long-term detrimental consequences.

Research into the effects of stress on emotional memory is highly relevant for a better understanding of the etiology and maintenance of emotional disorders, such as anxiety disorders. However, in humans the effects of stress on memory are traditionally investigated for non-associative and distinct emotional stimuli (e.g., emotional stories, pictures) (Cahill et al., 1994; van Stegeren et al., 1998; Southwick et al., 2002; Cahill & Alkire, 2003; Hurlemann et al., 2005). Given that patients with anxiety disorders either fear stimuli that are intrinsically non-threatening or they persist in fear responding whilst the acute threat already disappeared (e.g., after traumatic experiences), the emotional memory literature seems to be inconclusive for the understanding of these disorders. Indeed, a valuable experimental model for the pathogenesis of anxiety disorders is that they originate from a learned association between a previously neutral event (Conditioned Stimulus, CS) (e.g., stranger) and an anticipated disaster (Unconditioned Stimulus, US) (e.g., physical attack). Remarkably, the effect of stress hormones such as noradrenaline on associative fear memory in humans currently remains unknown.
Another notable aspect of research into human emotional memory is that most studies did not assess the emotional response but the declarative memory for the emotional stimuli (Cahill et al., 1994; van Stegeren et al., 1998; Southwick et al., 2002; Cahill & Alkire, 2003; Hurlemann et al., 2005). However, not the factual recollection but the concomitant excessive emotional expression is the main problem in emotional disorders (Ehlers et al., 2004). In particular, hyper-noradrenergic activity in the wake of a life-threatening event may contribute to the ‘overconsolidation’ of memory for trauma, generating disturbing intrusive memories that are characteristic of posttraumatic stress disorder (PTSD) (Pitman & Delahanty, 2005; Glannon, 2006; Henry et al., 2007). In patients with PTSD, these involuntary traumatic memories may be experienced as reenactments of the original trauma (‘flashbacks’) and are associated with significant emotion and distress (DSM-IV-R, American Psychiatric Association, 2000). Thus, taken together, a crucial question is whether noradrenaline strengthens the emotional expression of associative fear memory.

Here, we addressed this issue using a differential fear conditioning procedure in humans. Testing included different phases across two days separated by 48 hr. During acquisition, one of two stimuli (CS1+) was repeatedly paired with an aversive electric stimulus (US), while the other stimulus (CS2-) was not (i.e., pictures of spiders; IAPS numbers 1200 - 1201) (Lang et al., 2005). The formation of fear memory was manipulated by the systemic administration of yohimbine HCl, a \( \alpha_2 \)-adrenergic antagonist known to stimulate central noradrenergic activity by blocking the \( \alpha_2 \)-adrenergic autoreceptor (Charney et al., 1987; Peskind et al., 1995). In order to reach peak plasma levels upon completion of the acquisition phase (\( T_{\text{max}} < 1 \); Grasing et al., 1996), participants received double-blind an oral dose of either 20 mg of yohimbine HCl or placebo pill 30 min prior to fear learning. To substantiate consolidation of the fear memory (Dudai, 2004), memory retention (CS1 vs. CS2) was tested 48 hr later. We further included an extinction procedure to investigate whether noradrenergic stimulation during memory formation would impair the ‘unlearning’ of fear-related behavior. Even though the extinction of fear (i.e., unpaired CS presentations) involves new learning that acts to inhibit or compete with the original fear association (e.g., Bouton, 1993), the net effect of the extinction procedure is not only established by the inhibitory learning but also by the strength of the original excitatory fear memory. Hence, a former strengthening of the fear association by noradrenergic stimulation may be
expressed in a resistance to fear extinction 48 hr later (Myers & Davis, 2002; Rodríguez Manzanares et al., 2005; Yamamoto et al., 2008). In addition to the extinction procedure two other retrieval techniques (i.e., reinstatement testing and reacquisition) were employed to test the noradrenaline-enhancing effects on the recovery of fear memory expression. The conditioned fear response 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 CS1+ as compared to the control stimulus (CS2) reflects the fearful state of the participant elicited by the feared CS. Startle potentiation is considered a reliable and specific index of fear (Hamm & Weike, 2005), directly connected with and modulated by the amygdala (Davis, 2006). Declarative knowledge of the fear association was measured through online US expectancy ratings during each CS presentation within 5 s after stimulus onset. We obtained skin conductance responses as an objective measure of US anticipation (Weike et al., 2007; Soeter & Kindt, 2010). In addition, salivary alpha amylase (sAA) and blood pressure levels were determined to ensure the yohimbine HCl manipulation exerted its intended physiological effect. We hypothesized that stimulation of the noradrenergic system during acquisition would strengthen the associative fear memory 48 hr later and thereby (1) augment the fear expression to the first extinction trials - (2) delay the process of extinction learning - and (3) generate a superior recovery of fear (i.e., reinstatement testing and reacquisition), relative to placebo pill.

**Materials and Methods**

**Participants**

Thirty undergraduate students (10 men, 20 women) from the University of Amsterdam ranging in the age of 18 to 29 years (mean ± SD age, 22.1 ± 3.0 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 20 mg oral dose of yohimbine hydrochloride (i.e., pregnancy; seizure disorder; respiratory disorder; cardiovascular disease; blood pressure ≥ 140/90; diabetes; liver or kidney disorder; use of medication that could involve potentially dangerous interactions with yohimbine HCl; depression; or psychosis). In order to eliminate individuals who might have difficulty with any temporary
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symptoms induced by the yohimbine HCl manipulation, an additional exclusion criterion contained a score ≥ 26 on the Anxiety Sensitivity Index (ASI) (Peterson & Reiss, 1992). 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; placebo pill (n = 15; mean STAI-T score ± SD, 37.3 ± 8.6; mean SPQ score ± SD, 9.4 ± 7.7; mean ASI score ± SD, 10.1 ± 3.6) and yohimbine HCl (n = 15; mean STAI-T score ± SD, 37.9 ± 8.6; mean SPQ score ± SD, 6.4 ± 4.3; mean ASI score ± SD, 9.5 ± 4.9). Participants received either partial course credits or were paid a small amount (€ 28,-) 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-in computer monitor. One of the slides (CS1+) was followed by an US (75 % of the presentations), while the other slide (CS2-) was not. Assignment of the slides as CS1+ and CS2- was counterbalanced across participants. Both the CS1 and CS2 stimuli were presented for 8 s. The startle probe was presented 7 s after CS onset and was followed by the US (CS1+) 500 ms later. An electric stimulus with duration of 2 ms, 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. 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). 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-6624). 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 (μS) was used. All other responses were scored as zero and remained in the analyses (Effting & Kindt, 2007). The raw SCR scores were log-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 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 instructed just to place the swab in their mouths for 3 min. 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.** In order to reach peak plasma levels upon completion of the acquisition phase ($T_{\text{max}} < 1$; Grasing et al., 1996), participants received double-blind an oral dose of either 20 mg of yohimbine HCl or placebo pill 30 min prior to acquisition. The dose of 20 mg of yohimbine HCl was based on previous findings showing successful increases in peripheral noradrenaline as well as sAA level in healthy participants (O’Carroll et al., 1999; van Stegeren et al., 2009). Yohimbine HCl (20 mg, Desma GmbH, Germany) and placebo (Albochin, Pharmachemie, The Netherlands) capsules were identical in appearance and blinded by a colleague independent of the study.

**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, evaluation of the US was measured on an 11-point rating scale ranging from -5 (unpleasant) to 5 (pleasant).
Experimental Procedure

Participants were subjected to a differential fear conditioning procedure including several phases across two days separated by 48 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 eight startle probes to reduce initial startle reactivity. Characteristics of the CSs, trial order, ITIs, and startle probes as well as instructions regarding US-expectancy measurement during the extinction phase (day 3) were similar to acquisition (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. Participants were interviewed regarding their health and any medical or psychiatric conditions that would contraindicate taking a single dose of 20 mg of yohimbine 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.

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’. To allow control over the potential effects of yohimbine HCl on the fear potentiated startle response (Davis et al., 1993), eight baseline startle probes (Noise Alone; NA) were presented before pill intake. Afterward, participants were detached from the experimental set-up and received double-blind an oral dose of either 20 mg of yohimbine HCl or placebo pill. The administration of yohimbine HCl and placebo pill was randomized across participant with the restriction that conditions were matched on STAT-T, SPQ, and ASI scores as close as possible. In order to reach peak plasma levels upon completion of the acquisition phase (Tmax < 1; Grasing et al., 1996), a resting period of 30 min was inserted. Participants were offered magazines to read. Prior to acquisition, participants were attached to the experimental apparatus and 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 most cases, while the other slide would never be followed by the US. They were told to learn to predict whether an electric stimulus would occur or not on basis of the two slides. Participants were required to rate the expectancy of the electric stimulus during the presentation of each slide by shifting a cursor on a continuous 11-point rating scale and push the left mouse button within 5 s following stimulus onset, that is, before administration of the startle probe.

In the **acquisition** phase, both the CS1 and 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 delay the onset of within-session extinction, only 75% of the presentations of the CS1+ were reinforced (LaBar et al., 1998). In addition, 8 baseline startle probes were presented alone (Noise Alone; NA) during the intertrial intervals (ITI). The ITIs varied between 15, 20, and 25 s with a mean of 20 s. Order of trial type and ITI were randomized within blocks.

Upon completion of the acquisition phase (i.e., approximately 60 min from arrival at the experimental site), the STAI-S was filled out and blood pressure as well as saliva samples were again collected. In addition, participants were asked to evaluate the pleasantness of the US. Furthermore, they were explicitly instructed to remember what they had learned during acquisition. 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.

**Extinction, Testing.** In order to substantiate consolidation of the fear memory (Dudai, 2004), a break of 48 hr was inserted after acquisition. 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 of spiders provided during acquisition would be presented. Moreover, participants were again required to rate the expectancy of the electric stimulus during the presentation of each slide. In the **extinction** phase, participants were exposed to both the CS1+ and CS2+ for 16 times without the US. Furthermore, 16 startle probes were presented alone (NA) during ITI. To avoid a ceiling effect in the return of fear in the placebo pill condition, participants received only one unsignaled US subsequent to extinction learning as opposed to the traditional procedure of three unsignaled shocks (Norrholm et al., 2006; Kindt et al., 2009;
Soeter & Kindt, 2010). The time between the last extinction trial and the reinstating US was 19 s. Following the unsignaled US, participants were again presented with 8 CS\textsuperscript{1}, CS\textsuperscript{2} and NA trials (i.e., reinstatement testing). The time between the reinstating US and reinstatement testing was 18 s. Next, reacquisition took place. That is, participants were exposed to another 5 CS\textsuperscript{1}, CS\textsuperscript{2} and NA trials. At the conclusion of the experiment, participants were asked to complete the STAIS and to judge the pleasantness of the US.

**Statistical Analysis**

Startle responses, electrodermal activity and US-expectancy ratings were analyzed by means of a mixed analysis of variance for repeated measures (ANOVA) with condition (yohimbine vs. placebo pill) as between-subjects factor and stimulus (CS\textsuperscript{1} vs. CS\textsuperscript{2}) and trial (i.e., stimulus presentation) as within-subjects factors. Planned comparisons were performed for each condition separately. The first two as well as the last two trials of each stimulus type (CS\textsuperscript{1} vs. CS\textsuperscript{2}) were averaged and compared over testing phases respectively. To determine the speed of extinction learning, a 2 (condition: yohimbine vs. placebo pill) x 2 (stimulus: CS\textsuperscript{1} vs. CS\textsuperscript{2}) x 8 (trials: averaging over each two consecutive extinction trials) mixed ANOVA was performed. Missing data points were excluded from the analyses. Significance was set at $P < 0.05$.

**Results**

The yohimbine and placebo pill condition did not differ in terms of reported spider fear [$t_{28} < 1.31$], trait anxiety [$t_{28} < 1$], anxiety sensitivity [$t_{28} < 1$], and shock intensity [$t_{28} < 1.19$] (see Table 5.1). Selected shock intensities ranged from 4 to 52 mA with a mean of 18.00 mA (SD = 10.50). We observed no differences between conditions to the degree participants experienced the US [$t_{28} < 1$]. Moreover, no differential effect of condition on the course of US evaluation was found [day 1 vs. day 3; moment x condition, $F_{1,28} < 1$]. The yohimbine manipulation did also not affect the reported state anxiety that was assessed before and after pill intake [moment x condition, $F_{1,28} < 1$]. However, a significant difference on the course of the reported state anxiety was observed between conditions [day 1 vs. day 3; moment x condition, $F_{1,28} = 4.24$, $P < 0.05$, $\eta_p^2 = .13$]. That is, the reported state anxiety significantly decreased from 37.9 (SD = 8.6) to 34.3 (SD = 8.9) in the
placebo pill condition \([t_{14} = 2.79, P < 0.05, \text{two-tailed}]\), but remained stable in the yohimbine treated group \([t_{14} < 1]\).

**Table 5.1.** Mean values (SD) of the reported spider fear, trait anxiety, anxiety sensitivity, shock intensity and US evaluation for the Placebo Pill and Yohimbine HCl group.

<table>
<thead>
<tr>
<th></th>
<th>Placebo Pill</th>
<th>Yohimbine HCl</th>
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</thead>
<tbody>
<tr>
<td><strong>Spider Fear</strong></td>
<td>9.40 (7.72)</td>
<td>6.40 (4.34)</td>
</tr>
<tr>
<td><strong>Trait Anxiety</strong></td>
<td>37.33 (8.55)</td>
<td>37.93 (8.59)</td>
</tr>
<tr>
<td><strong>Anxiety Sensitivity</strong></td>
<td>10.07 (3.60)</td>
<td>9.53 (4.88)</td>
</tr>
<tr>
<td><strong>Shock (US) (mA)</strong></td>
<td>20.27 (11.56)</td>
<td>15.73 (9.15)</td>
</tr>
<tr>
<td><strong>US Evaluation</strong></td>
<td>-2.93 (0.96)</td>
<td>-3.13 (1.13)</td>
</tr>
</tbody>
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**Manipulation Check Yohimbine HCl**

Analysis of the effect of the yohimbine HCl manipulation on blood pressure revealed the expected increase in both systolic and diastolic blood pressure in comparison to placebo pill [day 1; moment x condition, \(F_{1,28} = 25.32, P < 0.001, \eta^2_p = .48, F_{1,28} = 9.53, P < 0.01, \eta^2_p = .25\), respectively]. Further analysis showed that, in the yohimbine condition, the systolic blood pressure significantly increased from 119.5 mmHg (SD = 10.9) to 129.9 mmHg (SD = 12.7) \([t_{14} = -5.53, P < 0.001, \text{two-tailed}]\) and the diastolic blood pressure from 73.5 mmHg (SD = 8.0) to 77.4 mmHg (SD = 9.4) after pill intake [day 1; \(t_{14} = -2.18, P < 0.05, \text{two-tailed}\)]. The increase in systolic as well as diastolic blood pressure by the yohimbine HCl manipulation did not differ between the sexes (5 men, 10 women) [day 1; moment x gender, \(F_{5,13} < 1\)]. In the placebo pill condition, both the systolic and diastolic blood pressure
significantly decreased from 127.7 mmHg (SD = 20.1) to 120.9 mmHg (SD = 12.8) [day 1; \( t_{14} = 2.38, P < 0.05 \), two-tailed] and from 74.2 mmHg (SD = 8.5) to 69.5 mmHg (SD = 9.7) [day 1; \( t_{14} = 2.19, P < 0.05 \), two-tailed] after pill intake, respectively. Additional analyses of the blood pressure before pill intake and upon arriving at the experimental site 48 hr later revealed no differential effect of pill administration on the course of both the systolic and diastolic blood pressure [day 1 vs. day 3; moment x condition, \( F_{5,28} < 1.17 \), respectively].

Analysis of the effect of yohimbine on sAA levels also demonstrated the expected increase in amylase level in comparison to placebo pill [day 1; moment x condition, \( F_{1,27} = 13.26, P = 0.001, \eta_p^2 = .33 \)]. Consistent with other studies (van Stegeren et al., 2009), the amylase levels in the yohimbine condition significantly increased from 73.1 U/ml (SD = 33.8) to 141.1 U/ml (SD = 89.3) [day 1; \( t_{13} = -4.17, P = 0.001 \), two-tailed] after pill intake, whereas the sAA levels remained stable in the placebo pill group [day 1; \( t_{14} < 1 \)]. The increase in salivary alpha amylase by the yohimbine HCl manipulation did also not differ between the sexes [day 1; moment x gender, \( F_{5,12} < 1 \)]. We further observed no differential effect of pill administration on the salivary alpha amylase levels before pill intake and upon arriving at the experimental site 48 hr later [day 1 vs. day 3; moment x condition, \( F_{1,27} < 1 \)].

**Fear Potentiated Startle Response**

Analysis of variance showed fear conditioning on day 1 by a significant increase of the differential startle response (CS1 vs. CS2) during acquisition [stimulus x trial, \( F_{1,28} = 75.47, P < 0.001, \eta_p^2 = .73 \); Fig. 5.1]. We observed no difference in fear learning between the placebo pill and yohimbine condition [stimulus x trial x condition, \( F_{1,28} < 1 \)], indicating that the pharmacological manipulation did not directly enhance the memory of the fear association (i.e., encoding). Yohimbine did also not affect the startle response per se, as we found no effects on habituation following pill administration [time x trial x condition, \( F_{7,19} < 1.04 \)].

Contrary to our expectations, the administration of yohimbine HCl relative to placebo pill did not result in the augmentation of the differential startle response (CS1 vs. CS2) from the last acquisition trials to the first trials of extinction learning [day 1 vs. day 3; stimulus x trial x condition, \( F_{1,28} < 2.01 \); Fig. 5.1]. However, in both the placebo pill and yohimbine condition, the startle fear response (CS1 vs. CS2)
remained stable 48 hr later [day 1 vs. day 3; stimulus x trial, $F_{1,28} < 1$], which may indicate a ceiling effect in fear acquisition. Moreover, we observed no difference in extinction learning between conditions [stimulus x trial x condition, $F_{1,28} < 2.08$]. That is, the extinction procedure yielded a significant decrease of the differential startle response (CS1 vs. CS2) from the first two extinction trials to the last two trials of extinction learning in the placebo pill as well as the yohimbine treated group [stimulus x trial, $F_{1,28} = 63.21, P < 0.001, \eta^2_p = .69$]. On the other hand, a significant difference in the speed of the extinction learning process was observed between conditions [stimulus x trial x condition, $F_{7,22} = 6.23, P < 0.001, \eta^2_p = .67$]. Bonferroni-adjusted pairwise comparisons indeed showed that the differential startle response (CS1 vs. CS2) only reached significance on the first trials of extinction learning (trial 1-2) in the placebo pill group [$p < 0.001$; Fig. 5.1 A]. Conversely, the differential startle response (CS1 vs. CS2) remained significant up to trial 11-12 of extinction learning in the yohimbine condition [all $ps \leq 0.001$; Fig. 5.1 B], indicating that the $\alpha_2$-adrenergic drug strongly delayed the extinction learning process.

Exposure to the reminder shock significantly increased the differential startle response (CS1 vs. CS2) from the last trials of extinction learning to the first trials at test in both the placebo pill and yohimbine condition [stimulus x trial, $F_{1,14} = 12.39, P < 0.01, \eta^2_p = .47$; $F_{1,14} = 26.42, P < 0.001, \eta^2_p = .65$, respectively]. However, we observed a superior reinstatement effect in the yohimbine treated group [stimulus x trial x condition, $F_{1,28} = 9.90, P < 0.01, \eta^2_p = .26$; Fig. 5.1]. Consequently, a significant difference in re-extinction learning was observed between conditions [stimulus x trial x condition, $F_{1,28} = 12.21, P < 0.01, \eta^2_p = .30$]. Yet, in both the yohimbine and placebo pill group, we observed a significant decrease of the startle response (CS1 vs. CS2) from the first re-extinction trials to the last trials of re-extinction learning [stimulus x trial, $F_{1,14} = 34.42, P < 0.001, \eta^2_p = .71$; stimulus x trial, $F_{1,14} = 30.18, P < 0.001, \eta^2_p = .68$, respectively; Fig. 5.1].

The administration of yohimbine contrary to placebo pill also resulted in a superior recovery of fear (CS1 vs. CS2) during the first trials of reacquisition learning [stimulus x trial x condition, $F_{1,27} = 6.55, P < 0.05, \eta^2_p = .20$]. That is, we observed a significant increase of the differential startle response from the last trials of re-extinction learning to the first trials of reacquisition in the yohimbine condition [$F_{1,13} = 6.47, P < 0.05, \eta^2_p = .33$; Fig. 5.1 B], but not in the placebo pill group [$F_{1,14} < 1$; Fig. 5.1 A].
Noradrenergic Enhancement of Associative Fear Memory

Analysis of the startle response to noise alone (NA) unveiled no significant differences between the yohimbine and placebo pill condition during acquisition [main effect of pill, $F_{1,28} < 2.69$; Fig. 5.1], nor during extinction learning, reinstatement testing and reacquisition [main effect of pill, $F_{1,27} < 1$].

**Fig. 5.1.** Yohimbine HCl delays the process of extinction and generates a superior recovery of fear (i.e., reinstatement, reacquisition). Mean startle potentiation to the feared stimulus (CS1), the control stimulus (CS2) and noise alone (NA) trials during acquisition, extinction, testing and reacquisition for the (A) Placebo Pill and (B) Yohimbine HCl Group. Error bars represent SEM.

**Skin Conductance Response**

Overall analysis of electrodermal responding showed no fear conditioning on day 1 [stimulus x trial, $F_{1,28} < 1.61$]. Therefore, only subjects showing successful levels of fear acquisition (i.e., mean trial 7-8 CS1 > CS2) were included in the
analyses. *Eight* subjects were eliminated. That is, *five* subjects from the placebo pill condition and *three* subjects from the yohimbine HCl group.

**Fig. 5.2.** Mean skin conductance responding to the feared stimulus (CS1) and the control stimulus (CS2) during acquisition, extinction, testing and reacquisition for the (A) Placebo Pill and (B) Yohimbine HCl Group. Error bars represent SEM.

Subsequent analysis of variance revealed a significant increase (CS1 vs. CS2) in electrodermal activity during acquisition (day 1) \[\text{stimulus} \times \text{trial}, F_{1,20} = 15.67, P = 0.001, \eta_p^2 = .44; \text{stimulus} \times \text{trial} \times \text{condition}, F_{1,20} < 1; \text{Fig. 5.2}\]. Moreover, in both the placebo pill and yohimbine condition, the differential skin conductance response (CS1 vs. CS2) obtained during acquisition remained stable 48 hr later \[\text{stimulus} \times \text{trial} \times \text{condition}, F_{1,20} < 1\].

The extinction procedure (day 3) yielded a significant decrease in electrodermal responding (CS1 vs. CS2) from the first extinction trials to the last
4 trials of extinction learning in the placebo pill as well as yohimbine HCl group [stimulus x trial, $F_{1,20} = 6.21$, $P < 0.05$, $\eta_p^2 = .24$; stimulus x trial x condition, $F_{1,20} < 1.22$; Fig. 5.2]. We further observed no difference in the course of the extinction learning process between the placebo pill and yohimbine condition [stimulus x trial x condition, $F_{(7,14)} < 1$].

Reinstatement testing as well as reacquisition learning no longer resulted in a (differential) return of fear in both conditions (CS1; CS1 vs. CS2) [main effect of trial, $F_{5,120} < 1$; stimulus x trial, $F_{5,120} < 1$; stimulus x trial x condition, $F_{5,120} < 2.19$; Fig. 5.2]. Note that analyses over the entire sample also revealed no differences between the placebo pill and yohimbine HCl group [CS1 vs. CS2; stimulus x trial x condition, $F_{5,28} < 1.65$].

**US Expectancy Ratings**

Analysis of the US expectancy data revealed a significant differential increase (CS1 vs. CS2) in expectancy ratings during acquisition (day 1) [stimulus x trial, $F_{1,28} = 270.47$, $P < 0.001$, $\eta_p^2 = .91$; stimulus x trial x condition, $F_{1,28} < 1$; Fig. 5.3]. In both the placebo pill and yohimbine group, the expectancy ratings (CS1 vs. CS2) significantly decreased from the last acquisition trials to the first extinction trials 48 hr later [stimulus x trial, $F_{1,28} = 13.17$, $P = 0.001$, $\eta_p^2 = .32$; Fig. 5.3]. However, the differential expectancy (CS1 vs. CS2) remained significant to the first trials of extinction learning in the placebo pill [$t_{14} = 6.85$, $P < .0001$, two-tailed] as well as the yohimbine condition [$t_{14} = 9.71$, $P < .0001$, two-tailed].

Analysis of variance showed extinction learning on day 3 by a significant differential decrease in expectancy ratings from the first extinction trials to the last trials of extinction learning [stimulus x trial, $F_{1,28} = 58.23$, $P < 0.001$, $\eta_p^2 = .68$; stimulus x trial x condition, $F_{1,28} < 2.42$; Fig. 5.3]. Moreover, we observed no differences in the speed of the extinction learning process between the placebo pill and yohimbine condition [stimulus x trial x condition, $F_{7,22} < 1$].

Analysis of the reinstatement effect showed a significant increase in expectancy ratings from the last extinction trials to the first test trials following the reminder shock in the placebo pill as well as yohimbine HCl group [stimulus x trial x condition, $F_{1,28} < 1$; stimulus x trial, $F_{1,28} = 5.82$, $P < 0.05$, $\eta_p^2 = .17$; Fig. 5.3]. In both the conditions, the expectancy ratings (CS1 vs. CS2) again re-extinguished over the course of reinstatement testing [stimulus x trial x condition, $F_{1,28} < 1$; stimulus x trial, $F_{1,28} = 14.66$, $P = 0.001$, $\eta_p^2 = .34$]. Furthermore, we observed a significant
differential increase in expectancy ratings (CS1 vs. CS2) in the placebo pill and the yohimbine condition during reacquisition [stimulus x trial x condition, $F_{1,28} < 1$; stimulus x trial, $F_{1,28} = 102.75$, $P < 0.001$, $\eta^2_p = .91$].

**Fig. 5.3.** Mean US expectancy ratings to the feared stimulus (CS1) and the control stimulus (CS2) during acquisition, extinction, testing and reacquisition for the (A) Placebo Pill and (B) Yohimbine HCl Group. Error bars represent SEM.

**Discussion**

The present findings demonstrate that stimulation of the noradrenergic system during memory formation strengthened the emotional expression (i.e., *startle fear response*) of the associative fear memory 48 hr later. That is, the administration of yohimbine HCl contrary to placebo pill extensively delayed the process of extinction and generated a superior recovery of fear (i.e., *reinstatement*
testing and reacquisition). The competition between the original excitatory fear association and the newly formed inhibitory memory trace determines the behavioral outcome of extinction learning. Given that yohimbine HCl was administered during fear conditioning (i.e., 48 hr prior to fear extinction), the noradrenergic manipulation apparently delayed the process of extinction by strengthening the original excitatory fear association. Yet, the yohimbine HCl manipulation did not directly augment the differential startle fear response 48 hr after learning. This may be due to a ceiling effect in startle fear conditioning given that the acquired fear response remained stable from acquisition to retention testing in both the placebo pill and yohimbine HCl condition. Even though administering pills prior to acquisition does not discard the possibility that the memory-enhancing effects can be attributed to actions on encoding processes (McGaugh & Roozendaal, 2009), the peak plasma concentrations were attained after learning was completed. Together with evidence showing similar memory-enhancing effects by either pre or posttraining drug administration (McGaugh & Roozendaal, 2009), the present findings suggest that the drug-induced enhancement of the startle fear response was also due to influences on memory consolidation.

Contrary to our expectations, the yohimbine HCl manipulation did not affect the skin conductance responding. Perhaps the current measurement of electrodermal activity was too insensitive to obtain any condition effect since we had to exclude several participants to even observe fear acquisition. Alternatively, the electrodermal conditioning may primarily reflect the more cognitive level of contingency learning (Weike et al., 2007; Soeter & Kindt, 2010; but see Schultz & Helmstetter, 2010), which may be less susceptible to the noradrenergic manipulation. germane to this argument is that yohimbine HCl did also not interfere with the US expectancy ratings (i.e., declarative knowledge). Our observation that the declarative memory remained untouched is at odd with human studies showing memory-enhancing effects of noradrenaline on emotionally arousing information (Southwick et al., 2002; Cahill & Alkire, 2003). The discrepancy between findings could be explained by a number of methodological differences, such as the nature and mode of learning (differential fear conditioning vs. emotional stories or pictures). That is, the differential fear conditioning paradigm (CS1 vs. CS2) as well as the measure of declarative memory performance (concurrent expectancy ratings vs. delayed recall or recognition of
pictures) may be too simple to observe any effects on declarative knowledge. Clearly, concurrent 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). The time elapsed between learning and retention testing (48 hr vs. 7 days) may also have been responsible for the discrepancy between findings. The consolidation process is thought to require an extended period to be completed (Dudai, 2004). The modulatory influence of emotional arousal via the amygdala on the hippocampal dependent declarative memory may therefore have been observed with time (e.g., 7 days), as the gradual process of consolidation proceeds (Hamann, 2001). Overall, the present constellation of findings again emphasizes the concept of multiple memory systems (Squire, 2004), involving a distinction between declarative (i.e., US expectancy ratings) and procedural (i.e., startle fear response) memory. The selective augmentation of the startle fear response is in line with our previous observations of a reduction in startle fear responding without affecting declarative knowledge (Kindt et al., 2009; Soeter & Kindt, 2010).

The current findings complement the existing human emotional memory literature by showing that increased noradrenaline release during or shortly after a stressful event strengthens associative fear memory. Our data indicate that the noradrenaline level in response to a stressor not necessarily intensifies the initial emotional expression but may impair the later ‘unlearning’ of fear-related behaviors. The excessive release of noradrenaline may further increase the likelihood of relapse when re-exposed to the original stressor (i.e., reinstatement, reacquisition). Several prospective studies of PTSD have shown that initial fear responding in the aftermath of a traumatic event is very common and a poor indicator of symptom development or PTSD diagnosis (e.g., Rothbaum et al., 1992, Rothbaum & Foa, 1993; Riggs et al., 1995; Murray et al., 2002). In contrast, the impairment of extinction learning determines the maintenance and consequently the etiology of chronic PTSD. It may be hypothesized that the level of noradrenaline during or shortly after a traumatic experience is a vulnerability factor in the development of PTSD - even though this is not necessarily reflected in immediate stronger fear symptoms. Indeed, abnormal noradrenergic functioning has been related to intrusive memories in PTSD (Southwick et al., 1993). The present findings further suggest that the level of noradrenaline in response to a traumatic event may limit the beneficial outcomes of the current treatment of
choice for PTSD (i.e., exposure therapy), which relies on extinction-based mechanisms (Rothbaum & Foa, 2002; Rothbaum & Davis, 2003). Given that the β-adrenergic receptor antagonist propranolol HCl selectively reduces memory consolidation for emotionally arousing information, beta-blockers are currently being evaluated as potential agents for secondary prevention of PTSD (Pitman et al., 2002; Vaiva et al., 2003; LaBar & Cabeza, 2006; but see Stein et al., 2007).

However, since only a small proportion of individuals exposed to severe stressors develop PTSD (Breslau, 2009), the ethics of this approach are still subject of some debate. Recent findings demonstrate that disrupting reconsolidation results in a persistent weakening of the emotional expression of an already-established fear memory (Kindt et al., 2009; Soeter & Kindt, 2010). Yet, further research in humans is required to examine whether strong associative fear memory also impedes the disruption of reconsolidation just like extinction learning.

References


