Fading memories : the impact of stress hormones on the retrieval of emotional memories

Tollenaar, M.S.

Chapter 4

Immediate and prolonged effects of cortisol, but not propranolol, on memory retrieval in healthy young men

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M.S. Tollenaar, B.M. Elzinga, Ph. Spinhoven, & W.T.A.M. Everaerd

Summary

While acute cortisol administration has been found to impair retrieval of emotional memories in healthy subjects, the duration of this memory impairment is still unknown. Propranolol, on the other hand, may impair the reconsolidation of emotional memories during reactivation, although human studies examining such effects are scarce. The present investigation was therefore undertaken to examine the immediate and prolonged effects of a single administered dose of cortisol or propranolol on memory retrieval in a double-blind placebo controlled design. Eighty-five healthy male participants were asked to retrieve previously learned emotional and neutral information after ingestion of 35 mg cortisol, 80 mg propranolol or placebo. After a washout period of one week, recall was again tested. Memory retrieval of neutral and emotional information was impaired by a single dose of cortisol compared to placebo. The memory impairment due to cortisol remained, even after a washout period of 1 week. No immediate or prolonged effects of propranolol on memory retrieval were found, despite significant reductions in sympathetic arousal. These results lend support to the hypothesis that cortisol is able to attenuate (emotional) memory recall in men over longer time spans and may therefore augment the treatment of disorders like post-traumatic stress disorder and phobias, but do not clarify the mechanism(s) through which propranolol exerts its therapeutic effects.

Introduction

Stress hormones like (nor)adrenaline (NA) and cortisol have since long been found to influence memory processes (Cahill et al., 1994; Lupien & McEwen, 1997; Wolf, 2008). Brain areas that are thought to mediate memory processes, like the hippocampus, the prefrontal cortex and for emotional memory, the amygdala, are highly occupied with both adrenergic and glucocorticoid receptors (de Kloet et al., 1998; Ramos & Arnsten, 2007). Cortisol is released by the adrenal cortex and can cross the blood-brain barrier (BBB) while NA is released both peripherally by the adrenal medulla and within the brain as a neurotransmitter (van Stegeren, et al., 2007; Wolf, 2008). The interaction between memory processes and stress hormones has been an area of interest in the last two decades (Cahill et al., 2003; de Kloet et al., 1999; Joels et al., 2006; Lupien & Lepage, 2001; Wolf, 2008).

It has been found that the effects of human stress hormones on memory are dependent on the memory stage that is studied (Roozendaal, 2002). Encoding and consolidation phases of memory in humans are found to be enhanced by increased cortisol and NA levels (Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Cahill & Alkire, 2003; O'Carroll et al., 1999) and impaired by beta-adrenergic blockers like propranolol that cross the BBB (Cahill et al., 1994; van Stegeren et al., 1998). Retrieval, on the other hand, is found to be impaired by increased cortisol levels (de Quervain et al., 2000; Het, Ramlow & Wolf, 2005). Furthermore, this impairment seems to be dependent on the activity of the adrenergic system (Kuhlmann & Wolf, 2006b; Roozendaal, Hahn et al., 2004; Tollenaar et al., 2008a / Chapter 2). There are no reports of increased levels of NA leading to impairment in memory retrieval in humans (see Chamberlain et al., 2006, for an overview of studies on NA and memory). A single human study, whereby NA levels were manipulated by blockade with propranolol (40 mg) and memory retrieval was measured, has been reported and did not find an effect on memory retrieval (de Quervain et al., 2007). More detailed studies investigating the effects of different doses of propranolol on memory retrieval are needed to clarify this relation.

Recent animal studies have suggested that when memories are retrieved, they are consolidated again after a labile period during which the reactivated memories are prone to change. This process is often referred to as reconsolidation (Debiec et al., 2006; Nader et al., 2000; Przybyslawski & Sara, 1997). Post-retrieval administration of propranolol has been found to disrupt spatial memory and inhibitory avoidance learning in rodents (Przybyslawski et al., 1999), as well as auditory fear conditioning (Debiec & Ledoux, 2004), and both findings have been explained in terms of impaired reconsolidation processes. Tronel and Alberini (2007) have recently shown that reconsolidation might also be dependent on the glucocorticoid system, as they found that a glucocorticoid receptor antagonist can disrupt conditioned fear in rats after reactivation of an inhibitory avoidance memory. In line with that, Maroun and Akirav (2007) have found an impairing effect of stress on reconsolidation in rats, which was reversed by a glucocorticoid receptor antagonist. However, cortisol may also impair memory after reactivation by enhancing extinction rather than reducing reconsolidation (Abrari et al., 2008; Cai et al., 2006). In the present study we will

therefore merely refer to reconsolidation as the post-retrieval stage during which memories might be prone to change.

While reconsolidation of fear related memories has most often been studied in animals, human declarative memories may also become labile during reactivation (Hupbach et al., 2007; Walker et al., 2003). Human studies on reconsolidation and the effects of cortisol and NA on this process are scarce. In a previously reported study, we examined the effects of elevated stress hormones on post-retrieval processes in humans (Tollenaar et al., 2008b / Chapter 3). In line with animal studies (Maroun & Akirav, 2007), a post-retrieval decline in memory performance was observed when memories were reactivated during stress (5 weeks after encoding). However, whether cortisol or other stress hormones were active in this process remains unclear. The effect of blocking adrenergic activity during memory reactivation has recently been studied in humans by Miller et al. (2004) and Brunet et al. (2008). Miller and colleagues reported that fear conditioning was reduced when a conditioned cue was reactivated and followed by NA beta-blockade. In addition, Brunet and colleagues found that post-retrieval propranolol reduced psycho-physiological responding to mental imagery of a past traumatic event in post-traumatic stress disorder (PTSD). However, the effects of propranolol on human declarative memory reconsolidation still remain to be elucidated.

Knowledge on the impact of (stress) hormones on human memory retrieval and reconsolidation is of therapeutic interest, since reducing the recall and/or experience of (intrusive) emotional memories might be of use in augmenting treatments for stress-related disorders like PTSD. Several studies have examined the utility of cortisol and a beta-adrenergic blocker (propranolol) in the treatment of PTSD. These studies have shown promising results, with reductions in re-experiencing and chronic stress symptoms after cortisol administration (Aerni, et al., 2004; Weis, et al., 2006) and reduced physiological reactivity after propranolol treatment (Brunet et al., 2008; Pitman, et al., 2002; Vaiva, et al., 2003). Phobic fears and mood responses to stress also seem to be reduced by cortisol administration (Het & Wolf, 2007; Soravia, et al., 2006).

To gain more insight into the effects of cortisol and propranolol on memory retrieval and reconsolidation, the present study investigated the effects of 35 mg hydrocortisone and 80 mg propranolol on memory retrieval and post-retrieval processes in healthy young men. By testing memory retrieval both during elevated cortisol or lowered NA levels and 1 week later (after clearance of the drug), the immediate treatment effects of cortisol and propranolol on memory retrieval were investigated, as well as whether these effects were prolonged up to 1 week later. We expected impairing effects of cortisol on memory retrieval, both immediate and prolonged. We had no expectations on the immediate effects of propranolol on memory retrieval, but did expect an impairing effect on reconsolidation, reflected in retrieval impairments one week after treatment.

Methods

Participants

Eighty-five Dutch male students were recruited through advertisements at colleges and the University of Leiden. Only men were selected because of possible confounding effects of menstrual cycle and contraceptive pills on the relation of cortisol and propranolol treatment with memory (Cahill & van Stegeren, 2003; Kuhlmann & Wolf, 2005). Participants were screened before inclusion. Inclusion criteria were: no reported history of disease or psychiatric problems, no current use of prescribed medication including corticosteroid containing ointments, no chronic disease requiring medical attention including diabetes, allergies and asthma, no use of psychotropic drugs, alcohol intake under 20 glasses per week, smoking less than 10 cigarettes per day, age between 18 and 35 years, an estimated Body Mass Index (BMI) between 19 and 26 and blood pressure levels over 100/70 mmHg. Before participation, written informed consent was obtained and after participation participants were rewarded with either course credits or a monetary compensation (40 Euros). The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Centre.

To minimize influences on baseline cortisol levels, participants were instructed to refrain from drinking any sweet or caffeinated drinks and eating heavy meals on the morning of the second (treatment) session. Furthermore, they were instructed not to eat or drink anything but water, and not to smoke an hour before the second session would start.

Of the 85 recruited participants, 2 men were excluded after the first session due to low blood pressure. Two participants were ill during one of the sessions and one person dropped out after the first session. We excluded one more participant due to problems with his Dutch written language. Hence, 79 participants completed the study. Participants were randomly assigned to one of three experimental groups in a double blind between subjects design (placebo: N = 27, cortisol: N = 26, propranolol: N= 26). Dependent on group, 35 mg hydrocortisone, 80 mg propranolol or a placebo was administered orally, in identical capsules.

Table 4.1 shows the demographic variables of the participants per group. No differences between groups were found for BMI, anxiety (STAI-trait) and general psychopathology (Symptoms Checklist, SCL-90). Age was significantly lower in the placebo group compared to the cortisol group ($t(32) = 2.42$, $p < .05$) and depression scores on the Beck Depression Inventory (BDI-II) were marginally higher in the control group compared to both the cortisol ($t(46) = 1.83$, $p = .07$) and propranolol group ($t(50) = 1.95$, $p = .06$).

Table 4.1. Demographic variables (mean ± SD).

	Placebo (N = 27)	Cortisol (N = 26)	Propranolol (N = 26)
Age	19.51 (1.37)[a]	21.35 (3.61)[a]	20.62 (2.16)
BMI	22.07 (2.35)	22.40 (1.98)	21.69 (2.07)
Depression (BDI-II)	6.59 (4.39)[b]	4.69 (3.04)[b]	4.44 (3.48)[b]
Anxiety (STAI trait)	33.74 (9.08)	33.73 (9.08)	31.38 (6.97)
Psychopathology (SCL-90)	28.19 (24.83)	28.73 (23.23)	28.00 (20.25)

Notes: BMI = Body Mass Index; BDI-II = Beck Depression Inventory II; SCL-90 = Symptom Checklist-90; [a] Significant difference in age between the placebo and cortisol group (p < .05); [b] Marginally significant difference in depression scores between the placebo group and the cortisol and propranolol group (p < .10)

Memory and attention tasks

To measure memory retrieval a word task adapted from Smeets et al. (2006) and Hermans and De Houwer (1994) was employed. Thirty emotional and 30 neutral words were selected that were matched on familiarity and word length. Fifteen words from each category were used for the retrieval task and the other words for a recognition task. During encoding of the words in the first session, words were randomly presented on a 17 inch computer screen for 4 seconds (word height: 13 mm, distance to screen: 60 cm). After presentation of each word, participants rated the word on two standardized, 5-point Likert scales on arousal (emotionality) and valence from the Self-Assessment Manikin (SAM: Bradley & Lang, 1994). A higher score on the arousal scale indicates higher emotionality and on the valence scale more negative emotions. After presentation of the words, a surprise memory task was given in which participants had to write down as many words as they could remember within 4 min (free recall). Then the same words were presented a second time, but with the deliberate instruction to remember as many words as possible. Words were presented for 5 s with 2 s intervals in between. A free recall test was again administered afterwards. These two trials served as the encoding/learning trials. During the second session, free recall of the words was again tested (in written form) with a maximum time of 4 min, followed by a cued recall task in which the first letters of each word were given and participants were asked to write down as many words as they could remember in 5 min. The third session consisted of a last free recall task, followed by a recognition task in which the old words were mixed with (15 neutral and 15 negative) new words and displayed on a computer screen. Participants were required to make a forced classification of words as old or new.

To obtain an estimate of verbal working memory, the digit span forward and backward from the WAIS were administered (WAIS, 1970; WAIS-III, 1997). Two versions of each task were randomly varied between the first and second session. In the forward condition, participants had to recall strings of numbers ranging from 4 to 8 in length. In the backward condition, participants had recall strings of numbers in a backward fashion.

To get an estimate of attention, the Sustained Attention to Response Task (SART) was administered, measuring vigilance (Manly, Robertson, Galloway & Hawkins, 1999). In this task, digits between 1 and 9 were presented for 250 ms in one of five randomly assigned font sizes with an inter-stimulus interval of 900 ms. Participants were asked to press a key (as fast as possible) in response to the digits except for the number 3. Misses and errors of commission were added to calculate an overall error score. Additional tasks were administered during the study that will be described in future reports.

Physiological and subjective measures

Saliva samples were obtained using Salivettes (Sarstedt, Germany) to measure unbound cortisol and alpha-amylase levels. Alpha-amylase has been shown to be an estimate of adrenergic activity (Nater et al., 2006; Rohleder et al., 2004) and is sensitive to beta-blockage by propranolol (van Stegeren et al., 2005). Saliva samples were stored at -20 °C prior to analyses. The saliva samples were analyzed by the Kirschbaum lab, Technical University of Dresden (see Rohleder et al., 2006).

Heart rate and blood pressure were measured to asses adrenergic functioning using an automatic upper arm blood pressure monitor (OMRON, M6). In addition to each physiological recording, participants were given a questionnaire with 7 questions on subjective experiences like anxiety, mood and motivation. Answers were given on Visual Analogue Scales (VAS) of 100 mm in length, leading to a score from 0 to 100 on each scale.

Questionnaires

The BDI-II (Beck, Steer, & Brown, 1996; van der Does, 2002) was administered to assess depressive feelings in the past 2 weeks, a Dutch version of the STAI-trait (Spielberger, 1983) to measure the level of generalized anxiety and the SCL-90 (Arrindell & Ettema, 1986) to assess psychological symptoms and psychopathology during the last week.

Procedure

Participants came to a lab at the Faculty of Social and Behavioral Sciences in Leiden for 3 sessions. The interval between each session was 1 week (see Figure 4.1a for an overview of the 3 test sessions). On the first session screening measurements of blood pressure and heart rate were taken after 3 rest periods of 4 min. During this first session, words were encoded for the retrieval task and baseline working memory performance was measured. At the start of the second session, after a 4 min rest period (given before each physiological measurement), baseline measurements of heart rate and blood pressure were assessed and baseline saliva samples obtained. Participant then ingested a capsule containing placebo, 35 mg hydrocortisone or 80 mg propranolol. During the next 75 min, participants completed several computer based questionnaires and were instructed to remain in the lab and read (reading material was provided). At t = 75 min after ingestion, participants heart rate and blood pressure were again assessed and saliva measurements obtained. Memory was then tested, including working memory. Physiology was measured again at 110 min after treatment after which an attention task was given. At 135 min after treatment, the last

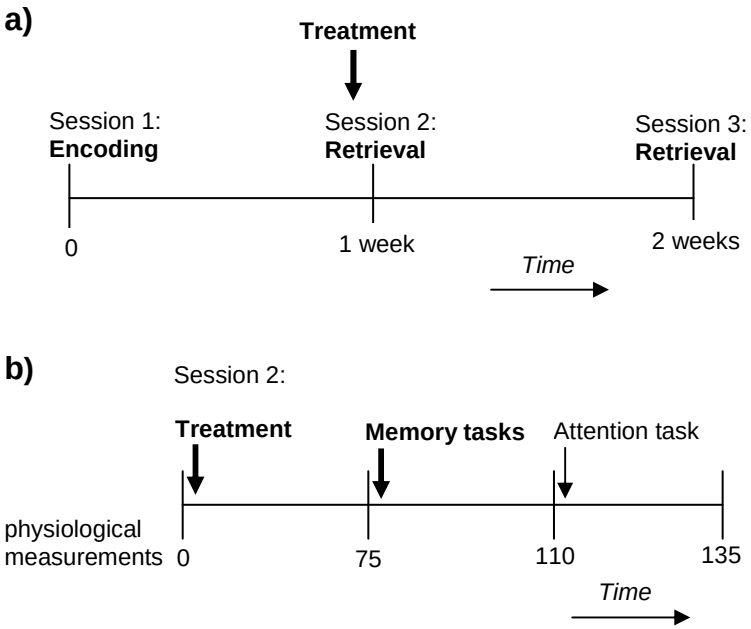


Figure 4.1. a) Schematic overview of the 3 sessions. b) Schematic overview of the second session. Treatment consisted of either cortisol (35 mg), propranolol (80 mg) or a placebo. The memory tasks were a free recall, cued recall and working memory task.

physiological measurements were taken as well as an interview on side effects and expectations of the memory task (see Figure 4.1b for an overview of session 2). In the third session, memory was tested again followed by an exit interview (including expectancies of the last memory task and an awareness check for treatment) as well as a debriefing concerning the goals of the study.

Data analysis

The effects of the treatment (placebo vs. cortisol vs. propranolol) on physiological and subjective measures were analyzed using repeated measure (RM-) ANOVAs with time as within subject and group as between subject variable, followed by Student Newman Keuls (SNK) *post hoc* tests. A χ^2 test was used to analyze side effects and treatments awareness in the three groups. Memory retrieval over the three sessions was analyzed using a RM-ANOVA with session and emotion as within subject and group as between subject variable. The percentages correct recall on session 2 and 3 were also analyzed using RM-ANOVAs with emotion as within subject and group as between subject factor. Additional analyses were conducted using univariate ANOVAs or simple *t*-tests. Greenhouse-Geisser corrected *p* values were used when indicated by violated Sphericity. Analyses were performed with SPSS 14.0 (SPSS, Chicago, IL). The criterion for statistical significance was $p < 0.05$.

Results

Effect of treatments on physiology

Cortisol measures

Table 4.2 shows the salivary cortisol levels in the three groups in nmol/L. For the RM-ANOVA, log values of cortisol were calculated to account for non-normality. One participant in the propranolol group was excluded from the analyses due to a missing sample. A significant group by time interaction was found ($F(3, 107) = 30.16$, $p < .001$). As expected, Student Newman Keuls (SNK) *post hoc* analyses revealed that cortisol levels were significantly increased in the cortisol group compared to both the placebo and propranolol group at t = 75, 110 and 135 min (all *p*s < .01), while not differing from the other 2 groups at baseline, t = 0 min ($p > .50$). In addition, a group by time interaction was also found between the placebo and propranolol group ($F(2, 87) = 10.26$, $p < .001$). The propranolol group showed increased cortisol levels compared to placebo at t = 110 and t = 135 (both *p*s < .01).

Table 4.2. Free salivary cortisol in nmol/L (± SEM) in each treatment group.

Group	Time			
	t = 0	*t* = 75	*t* = 110	*t* = 135
Placebo	9.01 (0.69)	5.02 (17.20)	4.57 (8.85)	4.95 (5.45)
Cortisol	7.47 (0.71)	206.61 (17.53) [a]	134.79 (9.01) [a]	99.37 (5.55) [a]
Propranolol	7.98 (0.72)	5.84 (17.88)	8.26 (9.19) [b]	9.81 (5.66) [b]

Notes: [a] Significant increase in cortisol levels in the cortisol group vs. the placebo and propranolol group (p < .001). [b] Significant increase in cortisol levels in the propranolol group vs. the placebo group (p < .001).

Adrenergic measures

Figure 4.2a-d shows the changes in alpha-amylase (AA), heart rate (HR), systolic (SBP) and diastolic blood pressure (DBP) in all groups before (*t* = 0) and at three time points after treatment (*t* = 75, 110 and 135 min) on session 2.

For the RM-ANOVA, log values of AA were calculated to account for non-normality. Four participants were excluded from the analyses due to missing AA samples (*n* = 2 from the propranolol, *n* = 1 from the cortisol and *n* = 1 from the placebo group). A significant interaction between group and time was found for AA levels ($F(4, 160) = 5.33$, $p < .001$). SNK *post hoc* tests revealed that AA levels were marginally lower in the propranolol group compared to both the placebo and cortisol group at *t* = 75 ($p = .09$) and 110 min ($p = .06$) and significantly lower at 135 min ($p < .02$), while not differing from the other 2 groups at baseline, *t* = 0 min ($p > .20$).

A significant group by time interaction was also found for HR ($F(4, 134) = 6.03$, $p < .001$). SNK *post hoc* analyses revealed that HR levels were significantly decreased in the propranolol group compared to both the placebo and cortisol group at t = 75, 110 and 135 min (all *ps* < .01), while not differing from the other 2 groups at baseline, t = 0 min ($p > .80$).

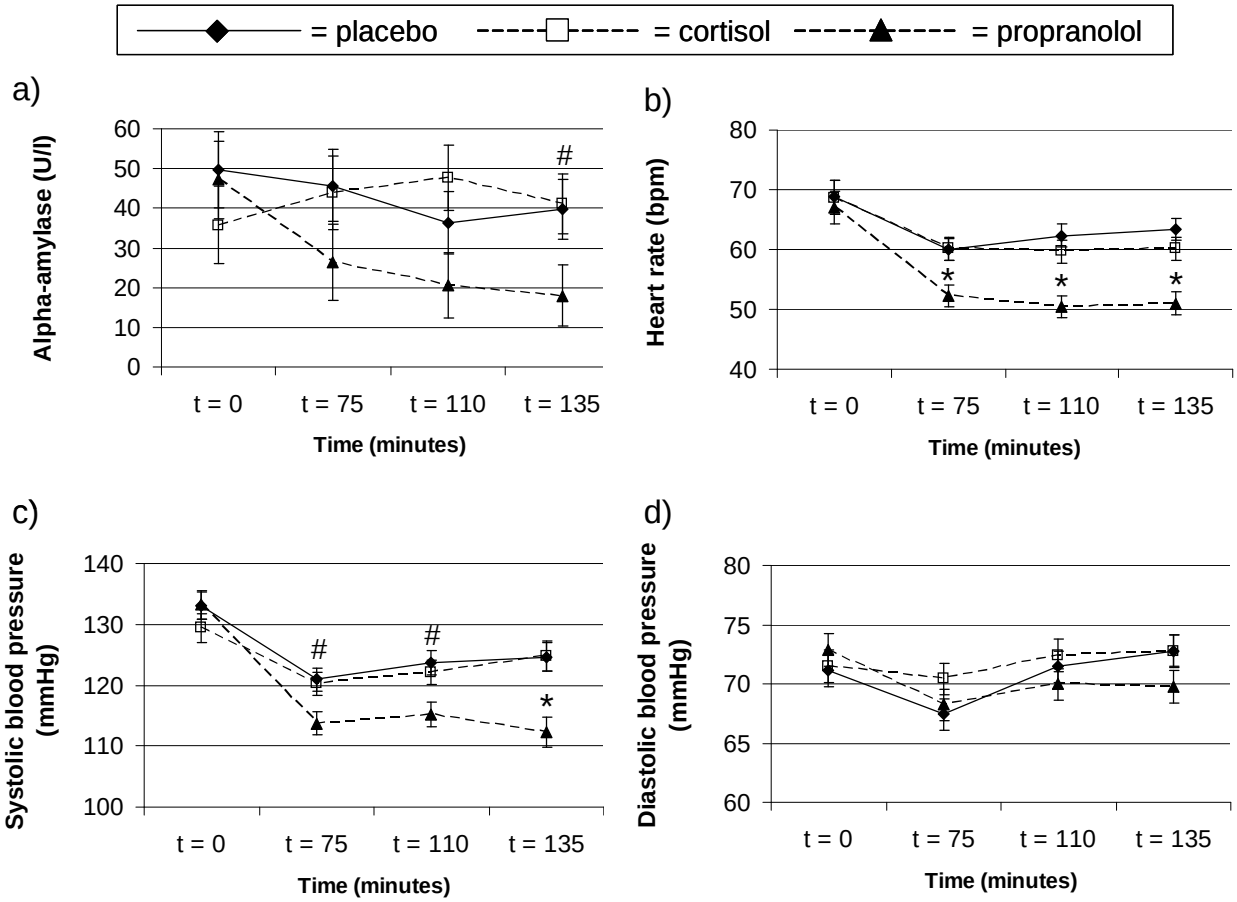


Figure 4.2. Physiological measures of adrenergic activation on session 2, before treatment (*t* = 0) and after treatment (*t* = 75, 110 and 135 min). a) Alpha-amylase. b) Heart rate. c) Systolic blood pressure. d) Diastolic blood pressure. Mean levels ± SEM are displayed.

Notes: U/L = Units per Liter; bpm = beats per minute; mmHg = millimeter of Mercury * *= significant difference in the propranolol versus the placebo and cortisol group (p < .01)* # *= significant difference in the propranolol versus the placebo and cortisol group (p < .05).*

Similar results were found for SBP. The group by time interaction was significant ($F(4, 171) = 9.41$, $p < .001$) and SNK *post hoc* analyses revealed that SBP levels were significantly decreased in the propranolol group compared to both the placebo and cortisol group at t = 75 ($p < .03$), 110 ($p < .02$) and 135 min ($p < .01$), while not differing from the other 2 groups at baseline, $t = 0$ min ($p > .40$). Even though an interaction was found for group by time for DBP as well ($F(5, 180) = 2.91$, $p < .05$), SNK *post hoc* tests revealed no significantly lower DBP at any of the time point in the propranolol group versus the other groups (all *p*s > .10).

Subjective measures

No effects of treatment over time were found on subjective feelings of tension, insecurity, irritation, motivation, mood and tiredness (all *p*s > .10). We did find an

interaction effect of group with time on anxiety ($F(5, 186) = 2.30$, $p < .05$). The propranolol group showed a trend towards lower anxiety at the end of the 2nd session compared to the placebo group ($t(46) = 1.74$, $p = .09$).

Side-effects and awareness check

After the treatment session participants were asked to report any side-effects or strange feelings. Feelings that were reported included: tiredness, tense feeling, cold hands, headache, light nausea and concentration problems. However, each of these reported feelings were evenly distributed across the 3 treatment groups (Pearson's χ^2 (6) = 5.35, $p = .50$). Furthermore, in the exit interview, participants were asked to speculate which treatment they received to check for awareness of treatment. Answers were categorized as placebo, cortisol, propranolol, no idea or simply a medicine. Participants did not guess which treatment they received (Pearson χ^2 (8) = 6.96, $p = .54$).

Memory performance

Arousal and valence ratings

On average, negative words were rated as significantly more emotional (mean = 2.76, SD = 0.74) than neutral words (mean = 1.70, SD = 0.55, $t(78) = 16.24$, $p < .001$). Negative words were also rated as significantly more negatively valenced (mean = 3.79, SD = 0.53) than neutral words (mean = 2.72, SD = 0.38, $t(78) = 22.25$, $p < .001$). No differences in ratings were found between the three groups (all ps > .10).

Memory performance

Table 4.3 shows performance on the memory tasks on sessions 1, 2 and 3. For session 1 recall performance on the last learning trial is shown. For session 2 data on the free recall and the cued recall task are shown and for session 3 performance on the free recall and the recognition task are shown.

Table 4.3. Memory performance (Mean ± SD) on session 1, 2 and 3 in number of words correctly recalled.

Group	Word valence	Session 1	Session 2		Session 3	
		recall	recall	cued recall	recall	recognition[a]
Placebo	Neu	8.63 (2.17)	4.70 (1.56)	6.11 (2.33)	5.22 (2.08)	10.52 (2.23)
	Emo	10.11 (2.31)	6.07 (2.73)	7.15 (2.43)	6.37 (2.39)	10.67 (2.63)
Cortisol	Neu	9.27 (1.85)	4.27 (2.18)	5.69 (2.29)	4.77 (1.80)	10.42 (2.89)
	Emol	10.42 (2.14)	5.46 (2.10)	6.58 (2.27)	5.42 (1.88)	9.31 (2.94)
Propranolol	Neu	9.35 (2.12)	5.54 (2.20)	6.42 (2.69)	5.85 (2.41)	11.73 (2.16)
	Emo	11.04 (1.64)	6.81 (2.12)	7.88 (1.75)	6.85 (1.99)	10.54 (3.09)

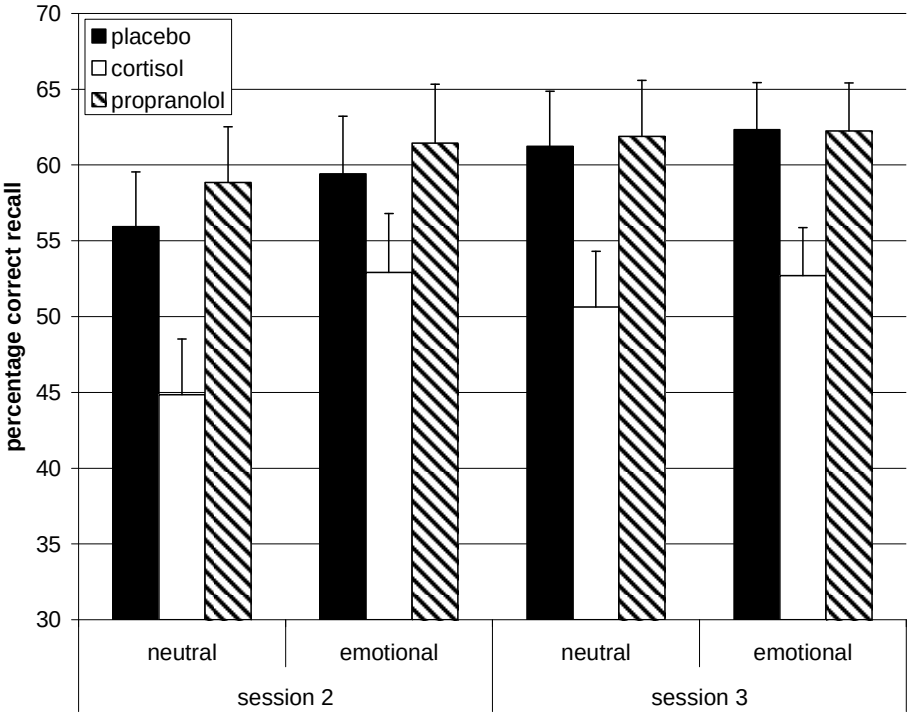
Notes: [a] *Recognition scores were calculated by subtracting the falsely recognized items from the number of correctly recognized items; neu = neutral; emo = emotional.*

In the RM-ANOVA used to test for effects of treatment on memory retrieval the last recall trial from session 1 and the free recall trials from sessions 2 and 3 were analyzed. The RM-ANOVA with session (1 vs. 2 vs. 3) and emotion (neutral vs. emotional) as within-subject variables, and group as between-subject variable was calculated for retrieval performance. A significant main effect of session was found ($F(2, 122) = 537.49$, $p < .001$), showing a decrease in performance from session 1 to 2 ($F(1, 76) = 648.80$, $p < .001$) and a slight increase in performance from session 2 to 3 ($F(1, 76) = 6.59$, $p < .02$) for all groups. This increase in recall performance from the second to third week may be due to the cued recall task that was performed after the free recall task of the second session. Furthermore, a main effect of emotion was found ($F(1, 76) = 26.83$, $p < .001$) reflecting a higher recall of emotional vs. neutral words.

No main effect of group was found. In line with our expectations, a significant group by session interaction was found ($F(3, 122) = 3.62$, $p = .013$). With planned comparison analyses for the cortisol and propranolol group separately, this interaction appeared to be significant between the placebo and cortisol group ($F(2, 77) = 6.00$, $p < .01$), but not between the placebo and propranolol group ($F(2, 89) = 0.35$, $p = .68$). To clarify in which phase the interaction effects for cortisol were apparent, separate RM-ANOVAs were conducted on session 1 vs. session 2 and session 1 vs. session 3. In the first RM-ANOVA with session 1 and session 2, and emotion as within-subject factors, and cortisol vs. placebo as between-subject factor, it was shown that memory performance in the cortisol group decreased significantly more from session 1 to session 2 than in the placebo group (time x group interaction: $F(1, 51) = 5.35$, $p = .025$), while performance on session 1 did not differ between these groups ($F(1, 51) = 1.08$, $p = .30$). This interaction was also found in the RM-ANOVA with session 1 and 3 as within-subject variable (time x group interaction: $F(1, 51) = 8.81$, $p < .01$), indicating a higher decrease in memory retrieval from session 1 to 3 in the cortisol group compared to the placebo group.

We also calculated the percentages correct recall on sessions 2 and 3 with respect to the last learning trial on session 1 (see Figure 4.3). RM-ANOVAs for the percentages correct recall on both session 2 and session 3 with emotion as within-subject factor, and cortisol vs. placebo as between-subject factor, showed that the cortisol group remembered significantly less from the last learning trial than the control group in both session 2 ($F(1, 51) = 4.17$, $p = .046$) and session 3 ($F(1, 51) = 6.60$, $p = .013$). No interaction effects with emotion were found (all ps $> .40$), indicating that the immediate and prolonged effects of cortisol on memory retrieval were similar for both neutral and emotional memory retrieval. The propranolol group did not remember less from the last learning trial than the control group in either session 2 ($F(1, 51) = 0.37$, $p = .92$) or session 3 ($F(1, 51) = .512$, $p = .48$).

To examine the change in retrieval performance from session 2 to session 3, an additional RM-ANOVA with session 2 and session 3, and emotion as within-subject factors, and group (cortisol vs. propranolol vs. placebo) as between-subjects factor was performed. No interaction between group and time was found ($F(2, 76) = 0.45$, $p = .64$), indicating that memory after treatment changed in a similar way in each group. In addition, when the percentage correct recall on session 3 was calculated with respect to session 2 and compared between groups in an ANOVA, no



Figuur 4.3. Percentage correct recall (Mean ± SEM) on session 2 and 3 with respect to the last learning trial on session 1. The cortisol group showed a lower memory performance on both session 2 and 3 compared to the placebo and propranolol groups (*p*s < .05).

effect of group was found either ($F(2, 76) = 0.41$, $p = .67$). To test whether there was a change in the reactivated items from session 2 to session 3, we also calculated the percentage correct recall on session 3 with respect to session 2 including only the words from session 3 that were also recalled on session 2. However, no further decline was found in recall of words that were reactivated during treatment ($F(2, 76) = .87$, $p = .42$.). No main or interaction effects of emotion were found either (*p*s > .65).

When conducting separate ANOVAs on the absolute scores on each of the three sessions, no differences in recall performance were found for any of the sessions between the placebo and the treatment groups (all *p*s > .10). Also, no effect of group was found on cued recall or recognition scores (all *p*s > .10).

In both sessions 2 and 3 participants were asked whether they expected a memory test. In session 2, more participants expected a memory task in the propranolol group ($F(2, 76) = 3.09$, $p = .05$). Furthermore, as mentioned in the methods section, age and depression scores differed in the control group compared to the other 2 groups. Therefore, these 3 variables were subsequently entered in the above analyses as covariates. Controlling for these possible confounding variables did not affect the main interaction between group (placebo, cortisol and propranolol) and session (sessions 1, 2, and 3) ($F(4, 144) = 3.57$, $p = .014$).

Working memory and attention

A RM-ANOVA with session (1 vs. 2) and order (forward vs. backward) as within-subject variable and group as between-subject variable was performed for the working memory scores on the digit span. Performance increased from session 1 to 2 ($F(1, 76) = 37.17$, $p < .001$) and performance on digits forward was higher than digits backward ($F(1, 76) = 19.46$, $p < .001$). No effects of group were found however (all ps > .15). Moreover, an ANOVA also failed to reveal significant effects of group on errors in the sustained attention task ($F(2, 76) = 1.51$, $p = .23$).

Discussion

In this study we found evidence that the retrieval impairments that have been observed as a result of cortisol administration are still observable after a wash out period of 1 week. These immediate and prolonged impairments in memory retrieval were found for the retrieval of both neutral and emotional words. These results are in line with earlier studies showing impairing effects of acute cortisol administration on memory retrieval (de Quervain et al., 2000; Het et al., 2005). They also relate to an earlier study by our group (Tollenaar et al., 2008b / Chapter 3) in which we found that stress impairs long-term memory retrieval when memories are reactivated during stress. While in the previous study stress was found to further diminish long-term memory retrieval when memory was reactivated during stress, in the present study we did not find a further decrease in memory performance after cortisol treatment. The persistence of the retrieval impairment in the cortisol group might be due to a lowered rehearsal during treatment and hence a lower re-encoding of the learned material or to the effects of cortisol on post-retrieval (reconsolidation) processes. The latter is less likely as there was no further decline in the retrieval of reactivated information, but from these data it cannot be concluded which of these two processes were involved in the memory impairments 1 week after treatment. The differences found between the long-term effects of exogenous cortisol administration and stress-induced endogenous cortisol increases may be related to the additional physiological and psychological responses that arise during stress. Furthermore, to examine whether reconsolidation specifically is affected by cortisol, future investigations should increase cortisol levels *after* memory reactivation to separate the effects on retrieval and reconsolidation, and compare the effects of cortisol not only to a placebo group, but also to a group in which cortisol is administered without reactivation, to rule out non-specific effects of cortisol on long-term memory.

In contrast to the impairing effects of cortisol, we found no immediate effect of propranolol on memory retrieval. So far, only one other study reported on the effects of propranolol on memory retrieval (de Quervain et al., 2007). In this study, propranolol did not reduce retrieval either. Furthermore, we found no indications for effects of propranolol on post-retrieval processes. That is, performance 1 week after treatment was still comparable to placebo. This is in contrast with our expectations based upon studies in which propranolol was found to affect post-retrieval processes like reconsolidation (Debiec & Ledoux, 2004; Miller et al., 2004; Przybyslawski et al., 1999). However, these studies used mostly fear conditioning paradigms, which are

not directly comparable to our declarative memory task. While fear conditioning is concerned with implicit learning, generally involves higher levels of fear, and is found to be mediated by the amygdala (Debiec & LeDoux, 2006), memory retrieval is thought to be primarily mediated by the hippocampus and prefrontal regions (Squire et al., 2004; Takashima et al., 2006), although the amygdala has also been implicated in emotional memory retrieval (Dolcos et al., 2005). A reason that might therefore explain our non-results is that propranolol may be more involved in (amygdala related) physiological and anxiety reducing mechanisms, as in fear conditioning. Although we did find a slight decrease in anxiety in the propranolol group at the end of the treatment session, this effect did not remain 1 week after treatment. Propranolol may potentially only affect declarative memory reconsolidation when related emotions and physiological responses are very strong, as in PTSD (Orr et al., 2002). That is, propranolol has been found to affect declarative memory *consolidation* in humans (Cahill et al., 1994; van Stegeren, Rohleder et al., 1998), but in those studies picture tasks were used that might have elicited more emotional arousal than our word task. Another reason that could explain the non-results, is that the administered dose of propranolol was too low. This is not a likely explanation however, since the expected physiological effects of propranolol administration were clearly observed. There was a very significant decrease in adrenergic activity measured with heart rate and blood pressure (although only on systolic blood pressure, as previously reported by Maheu et al. (2005) and van Stegeren, Rohleder et al. (2005)), but also with alpha-amylase. Alpha-amylase measured from saliva seems to be a valid and non invasive measure of adrenergic activity and is sensitive to beta-adrenergic blockade (Nater et al., 2006; van Stegeren et al., 2005). Besides the fact that propranolol induced the expected physiological effects, we have administered a dose of propranolol (80mg) that was twice as high as in the study by de Quervain et al. (2007). Taken together, even though we did not find evidence for effects of propranolol on reconsolidation, this study does not rule out that propranolol might potentially play a role in reconsolidation in humans. Reconsolidation in humans is still a relatively unstudied area and future studies using different memory paradigms will have to elucidate whether propranolol can affect post-retrieval memory processes.

Interestingly, propranolol also led to a moderate, but significant, increase in cortisol levels, which was previously reported by Maheu et al. (2004) as well. Apparently, this increase did not impair memory recall. The increase might not have been sufficiently large to cause any effects (less than 2 nmol/L cortisol), but the fact that noradrenergic activation was blocked by propranolol might have prevented cortisol increases from any impairing effects on memory as well (see also de Quervain et al., 2007).

Overall, the present findings suggest that reactivation of memories when cortisol levels are high may lead to long-term memory attenuation. This is highly relevant for the treatment of post-traumatic stress disorder (PTSD), in which a lasting diminished recall of trauma-related memories might be beneficial (de Quervain, 2007). Moreover, these findings are in accordance with clinical observations of prolonged beneficial effects of glucocorticoids in PTSD and phobias (de Quervain & Margraf, 2008). However, our findings suggest that cortisol may impact both emotional and neutral memory retrieval. The role of emotionality and valence in the

effect of cortisol on memory retrieval in humans is still unclear. That is, several other studies have found effects of cortisol on retrieval of neutral memories as well (Buss et al., 2004; de Quervain et al., 2000), while other studies found effects of cortisol primarily on emotional memory retrieval (Domes et al., 2004; Kuhlmann, Kirschbaum et al., 2005). The effects of endogenous increases of cortisol on memory retrieval seem to be dependent on the emotionality of the material or on an arousing context (Kuhlmann, Piel et al., 2005; Tollenaar et al., 2008a / Chapter 2), while high exogenous doses of cortisol might affect memory retrieval as long as the subject has a normal level of sympathetic arousal (de Quervain et al., 2007; Kuhlmann & Wolf, 2006b). With regard to the clinical setting, the impairing effects of cortisol on the retrieval of neutral information may be a potential negative side effect, and merit special attention in future clinical trials.

There are some limitations to our current investigation that merit consideration. The effects we found of cortisol on memory retrieval were all within-subject effects. They were expressed in an interaction between group and session and present in the percentage correct recall with respect to the individual last learning trial. No effects were found on absolute memory scores when the sessions were analyzed separately. Other studies have reported within-subject effects of cortisol on memory retrieval as well (Buss et al., 2004; Kuhlmann, Piel et al., 2005; Kuhlmann, Kirschbaum et al., 2005), suggesting that these effects, while subtle, are consistent. Second, the present study only included men, while disorders related to stress and memory problems like PTSD and depression are highly prevalent in women. Future studies should examine whether similar results are found in females, while taking into account hormonal fluctuations due to menstrual cycle and birth control agents. Another point is that our control group differed from the drug groups on depression and age scores despite randomization. In the control group, two participants were over 30 years leading to a higher mean. However, including both age and depression scores as covariates in the analyses did not change our results. Furthermore, the fact that the impairments in memory were prolonged up to 1 week does not necessarily mean that the memory traces are impaired indefinitely. A longer follow-up is needed to indicate whether these effects are persistent or temporary. Of interest is also whether memory losses can be restored with cues or in a different context (Bouton, 2002). Moreover, future studies should investigate the effects of multiple reactivations under treatment, as animal research has indicated this might strengthen the effects (Cai et al., 2006).

In summary, these results lend support to the hypothesis that cortisol might aid in the treatment of disorders like PTSD and phobias by diminishing (emotional) memory recall over extended time spans, but does not clarify the mechanisms through which propranolol exerts its therapeutic effects.