



## STRESS- AND TREATMENT-INDUCED ELEVATIONS OF CORTISOL LEVELS ASSOCIATED WITH IMPAIRED DECLARATIVE MEMORY IN HEALTHY ADULTS

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### SUMMARY

Two studies investigated the association between cortisol levels and memory performance in healthy adults. In a first study, 13 subjects were exposed to a brief psychosocial laboratory stress ("Trier Social Stress Test") with a subsequent test of declarative memory performance. Results indicated a significant negative relationship between stress-induced cortisol levels and performance in the memory task, i.e. subjects with high cortisol response to the stressor showed poorer memory performance. In a second experiment it was investigated if cortisol alone, i.e. independent of psychological stress, would also impair memory function. In this study, 40 healthy subjects received either 10 mg cortisol or placebo orally. One hour later they were tested for procedural and declarative memory and spatial thinking. Subjects who received cortisol showed impaired performance in the declarative memory and spatial thinking tasks but not in the procedural memory task. From these results we conclude that in healthy adults elevated free cortisol levels are associated with impaired memory function.

*Key Words:* cortisol, saliva, memory, hippocampus

Several lines of evidence suggest that elevated glucocorticoid levels can impair cognitive functions. In clinical studies, patients with a pathologically increased activity of the hypothalamus-pituitary adrenal (HPA) axis have been reported to show deficits in attention, concentration, and memory. Whelan et al. (1) could show that approximately two out of three Morbus Cushing patients (increased cortisol production) display significant cognitive performance deficits in a neuropsychological test battery. Similar results were obtained in semi-structured interviews, with concentration and memory problems in 66-83% of the Cushing patients investigated by Starkman and Schteingart (2).

Patients with major depression also appear to show elevated cortisol levels concomitant with impaired cognitive performance. Rubinow and coworkers (3) found a significant correlation between cortisol levels and the number of errors in the Halstead Category Test. Interestingly, cortisol nonsuppressors in the dexamethasone suppression test showed more pronounced cognitive impairment than cortisol suppressors, which may indicate a more direct association between glucocorticoid levels and specific cognitive functions (for review of the clinical studies see 4).

While these data provide correlational evidence for an association between cortisol and some cognitive functions such as memory, they do not allow a causal interpretation of this relationship. A more direct approach was used by Wolkowitz et al. (5). In two studies they tested the effects of synthetic steroids with glucocorticoid activity (dexamethasone and prednisolone, respectively) on memory processes and reported that after pharmacological treatment subjects appear to direct more attention to irrelevant stimuli leading to an increase in errors of omission without an increase of errors in commission. This observation is consonant with findings of reduced evoked potentials to relevant but not to irrelevant stimuli (6) as well as impaired selective attention (7) following acute cortisol infusions. Furthermore, two studies with healthy volunteers observed no or mixed results when cortisol-treated subjects were tested in an immediate word recall task (8,9).

Although this literature suggests that cortisol can impair memory functions, only one study with human subjects provided evidence for a more precise identification of the CNS structures involved and the specific type of memory processes affected by pharmacological treatment with glucocorticoids. Newcomer et al. (10) reported that four-day but not one day, treatment with dexamethasone selectively impaired performance in a declarative memory task (paragraph recall test) whereas other cognitive performances were unaffected. In contrast to the so called procedural (or implicit) memory, declarative (or explicit) memory appears to require processing by the hippocampus (11,12).

Results from animal studies provide additional support for the pivotal role of the hippocampus in glucocorticoid-induced impairment of declarative memory. In two physiological models of memory, primed burst (PB) and long-term potentiation (LTP), corticosterone has been shown to affect PB and LTP in hippocampal neurons in an inverted U-shaped manner (13,14). It should be noted that the CA1 and CA3 fields contain an abundant number of both type I (mineralocorticoid) as well as type II (glucocorticoid) intracellular receptors which bind cortisol with different affinities. While the majority of receptors are occupied by cortisol released during the circadian rhythm, type II receptors appear to be the prime binding structures for cortisol after stress exposure or after pharmacological application of corticosteroids (15).

Extrapolation of these data to healthy human subjects (along with results from (10) suggests that cortisol affects especially those memory functions which are dependent on hippocampal activity. While this has been shown to some extent in a longer-term treatment study with a synthetic glucocorticoid (10), we attempted to reveal similar memory impairment effects of acute elevations of cortisol in healthy human subjects. In two studies we thus investigated (a) the effect of stress-induced cortisol increases on a word-list learning task, and (b) the effect of an acute oral treatment with 10 mg cortisol on word learning and spatial thinking performance.

## Materials and Methods

### **Study 1:**

#### Subjects and Stress Procedure

Five female and eight male paid students participated in the first study. They had to refrain from strenuous physical exercise, psychological stress, larger meals, drinks with low pH, and cigarette smoking for at least one hour prior to the experiment. After a brief introduction to the study (10 minutes), the subjects were exposed to the "Trier Social Stress Test" (16) which mainly consists of a public speaking task (5 minutes) and a subsequent mental arithmetic task (5 minutes) in front of an audience.

In more than a dozen independent studies, the TSST has proven to faithfully induce moderate psychosocial stress in a variety of subject populations and numerous psychological, endocrine and cardiovascular stress indices. All experiments were performed during late afternoon hours to

control for possible circadian rhythm effects.

### Declarative Memory Task

Ten minutes after cessation of stress, the subjects were presented a list of 24 nouns with the instruction to learn this list of words during the next five minutes. After a distraction task (5 minutes) they were asked to recall and write down all words beginning with the letters "Mo" of the previous list. The list consisted of 10 words beginning with those letters. No time limit was set for completion of this task. This type of memory task was described by Eichenbaum and Otto (11) as a typical example for a test of declarative memory.

### Saliva Sampling and Biochemical Analysis of Cortisol

The subjects collected saliva samples with the Salivette sampling device (Sarstedt Inc., Rommelsdorf, Germany) 5 minutes before and 10 minutes after the TSST. Samples were kept at -20°C until analysis. After thawing the samples were spun at 3000 rpm for 5 minutes, and 50 µl of the clear supernatant was removed for duplicate cortisol analysis employing an immunoassay with a biotin-cortisol tracer, streptavidin-europium label, and a time-resolved fluorescence detection system (DELFLIA) (17). Intraassay variation of this assay is less than 8%, interassay variation below 10%, respectively.

### Statistical Analysis

Student's t-test was used to test the effect of the psychosocial stress on cortisol levels and Pearson correlation was computed for the increase in cortisol and the number of correctly recalled words. Mann-Whitney U-test was computed to examine sex differences in cortisol response to the stressor.

## **Study 2:**

### Subjects and Pharmacological Treatment

Forty male volunteers with a mean age of  $24.7 \pm 2.7$  (SD) years participated in the second study. They had to follow the same procedural restrictions as the subjects in Study 1. They were randomly assigned to either of two experimental groups, those receiving cortisol or placebo, respectively. After written consent was obtained, the subjects received 10 mg hydrocortisone acetate (Hydrocortison, Hoechst®, Frankfurt/Main, Germany) or placebo single-blind orally. In a pretest it was established that this dose would yield salivary cortisol levels in the upper concentration range obtained after more intense physical or psychological stress (30-60 nmol/l) one hour after ingestion. Therefore, 60 minutes after cortisol or placebo ingestion subjects began to work on the memory tasks (see below). Memory was tested between 10 am and 1 pm.

### Memory Tasks

In the second study subjects received all test instructions in written form to reduce the likelihood of an investigator-induced manipulation of task performance. Here, the number and content of the cognitive tasks were extended in order to possibly enable a differentiation between hippocampus-mediated *declarative* and non hippocampus-mediated *procedural* memory. Declarative memory was assessed with a cued recall test and two mental rotation tasks. Procedural memory was tested with a wordstem priming task. Priming is intact in amnesic patients suffering from hippocampal lesions and therefore seems not to depend on this structure (11,12).

*Exposure to Word List.* First, all subjects received a list of 26 nouns with the instruction to rate the nouns according to their melodious sounds on a five-point scale ('studied' list). No time limit was set for the ratings. At this point, subjects did not know that they participated in an experiment

investigating memory performance. This test was considered a test of incidental learning. There were two parallel forms of the wordlist consisting of different nouns. The second list was used to determine if priming had occurred (see below). Exposure to either list was balanced between the two experimental groups.

*Spatial thinking Test - 1 'Park'*. Next, the volunteers received the instructions for the two spatial thinking tests. In the first test they were instructed to carefully read a short description of a walk through a park. In this description the subjects were 'guided' along a path with several 'attractions', e.g., specific trees, flowers and animals which were situated either on the right or left side of the path. Additionally, the stroller 'saw' three bifurcations where they either 'turned' left or right or 'kept going' straight on. The subjects were given three minutes to memorize the description. Thereafter they returned the description to the investigator and received a second instruction sheet. For testing purposes they had to imagine that the stroller decided to turn around and walk his way back to the entrance of the park. In a multiple-choice test they had to choose the correct paths (at bifurcations) or describe whether an attraction was located on the left or right side of the path on the way out. There was a total of 18 multiple-choice questions.

*Spatial thinking Test - 2 'Barn'*. The second memory task was a variation of a test developed by Franklin et al. (18). In the adapted version, the subjects had to imagine standing on a timber in a barn with a total of ten objects being placed above, below, in front of, behind, to the left or to the right of the observer. The subjects were given two minutes to memorize the position of each object relative to the subject's location. Then the subjects were asked to 'rotate' mentally by 90 degrees to the right and write down the 'new location' of the objects relative to the observer. The test was evaluated with respect to incorrect positioning of the 10 critical object locations.

*Procedural Memory Test (Priming)*. After they finished the second spatial thinking task, subjects were tested for procedural memory. For this purpose, subjects received a list containing 52 two-letter wordstems. These were derived from the two parallel word lists subjects had to rate for melodious sound at the beginning of the experimental session. Thus each subject had been prior exposed to half of the words whereas the other half was novel to them. Subjects were asked to complete those letters to the first noun which came to their mind. Priming is demonstrated if the probability of the completion of the previously presented words ('old') is increased in comparison to completion for the nonstudied words from the second list ('novel'); (19).

*Declarative Memory Test (Cued-Recall)*. Subsequently, the subjects received a list of wordstems with the first two letters of those 26 nouns they had rated earlier with the instruction to complete the wordstems to the exact nouns they had rated earlier. No time limit was set for completion of the task. Cued recall was intended to assess declarative memory.

Subjects needed about 30 minutes for completion of all memory tasks.

### Measurement of Cortisol Levels

In order to assure that the administration of cortisol induced a significantly higher cortisol level, saliva samples were obtained before ingestion of cortisol or placebo, immediately before and after completion of the memory tests. Sample processing and biochemical analyses were identical to Study 1.

## Results

### **Study 1:**

Fig. 1 shows the increase in salivary cortisol levels in response to the TSST. Mean cortisol levels rose significantly from  $8.46 \pm 1.02$  (SE) nmol/l to  $17.65 \pm 2.17$  nmol/l ( $t_{12}=3.98$ ,  $p=0.0018$ ). Nine out of 13 subjects showed an elevation of more than 2.5 nmol/l, i.e., a clear-cut cortisol

response to the psychosocial stressor. As observed in other studies (20), cortisol responses in men were again more pronounced than in women ( $p=0.028$ ). The correlation coefficient between the cortisol response (difference sample 2 - sample 1) and the number of correctly recalled words was  $r = -.70$  ( $p=0.007$ ). Although women tended to recall more words than men, this difference was not statistically significant. Figure 2 shows the scattergram for this correlation.

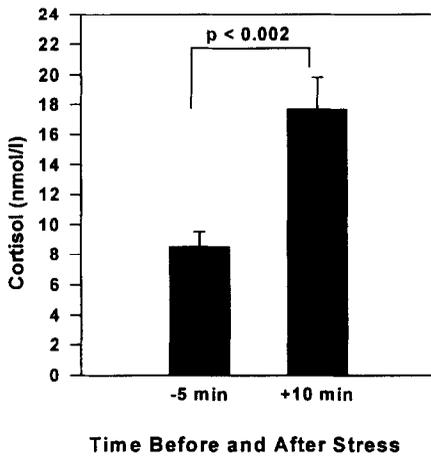


Fig. 1

Mean cortisol levels (+SE) before and after psychosocial stress (Study 1).

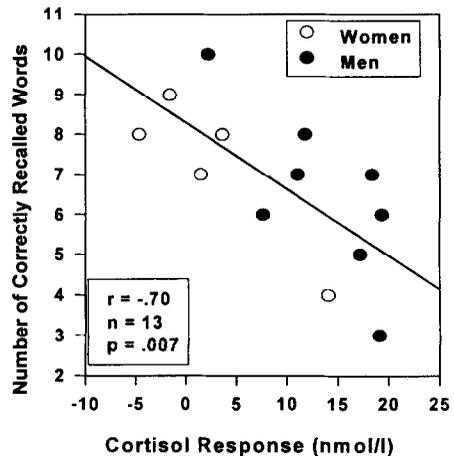


Fig. 2

Scattergram of the correlation between cortisol responses and memory performance (Study 1).

**Study 2:**

Cortisol levels did not differ between the two experimental groups at baseline ( $t_{37} = 1.10$ ,  $p > .20$ ) but were elevated one hour after application of cortisol as well as after completion of the memory tasks ( $64.2 \pm 9.8$  and  $52.4 \pm 9.6$  vs.  $9.3 \pm 0.7$  and  $8.7 \pm 0.7$  nmol/l; means  $\pm$  SE; both group comparisons  $p < .001$ ) validating the efficacy of the pharmacological treatment. One subject of the cortisol group had to be excluded from further evaluation, since he failed to show an elevation of the steroid level after cortisol administration due to reasons unknown.

Testing whether priming had occurred in the procedural memory task, the number of words from the studied list was compared to the number of 'novel' words, i.e. words which were part of the control list. A paired t-test revealed that the two letters were much more likely completed to one of the 'old' words than to 'novel' words indicating a strong priming effect (old:  $2.0 \pm 0.2$ , novel:  $0.8 \pm 0.1$ ;  $t_{38} = 5.75$ ,  $p < .0001$ ). Next, group comparisons were computed for memory performance. While there was no treatment effect for procedural memory performance ( $t_{37} < 1$ ), the placebo treated group recalled significantly more words from the studied list under the declarative memory instructions than did the cortisol group ( $t_{37} = 2.27$ ,  $p =$

.015; Fig.3). Likewise, the cortisol group made almost twice as many errors in locating the objects 'in the barn' ( $t_{37} = 1.71, p < .05$ ) and tended to make more errors in the 'park' task ( $t_{37} = 1.50, p = .08$ ; Fig. 4).

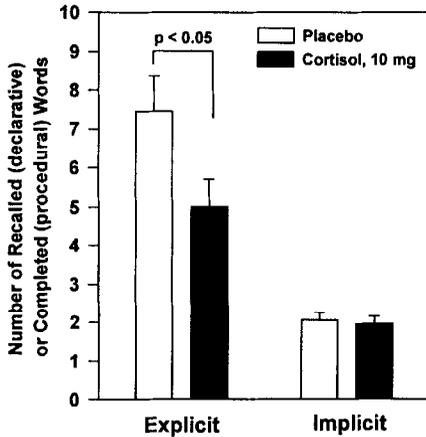


Fig. 3

Declarative and procedural memory performance in placebo and cortisol-treated subjects (means +SE; Study 2).

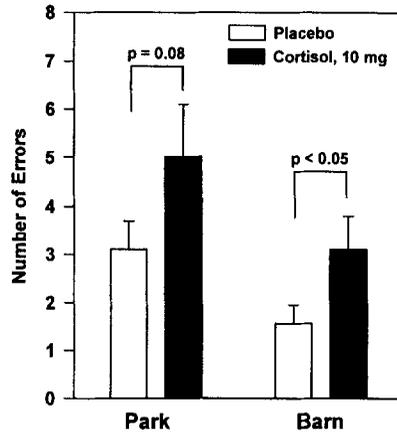


Fig. 4

Performance in two spatial memory tests in placebo and cortisol-treated subjects (means +SE; Study 2).

Discussion

The results of the present two studies support findings from animal studies and clinical observations suggesting that cortisol impairs simple and more complex forms of declarative memory in healthy human subjects. The net increase of cortisol induced by psychological stress in the first study was inversely correlated with the subjects' ability to memorize words learned after exposure to the stress. While a myriad of neurotransmitters, metabolic factors, and systemic hormones other than cortisol could have accounted for this effect, the magnitude of the correlation ( $r = .70$ ; explained variance = 49%) was surprising. In a more direct approach in the second study, we obtained evidence that shows that the amount of bioavailable cortisol indeed is an important factor responsible for modulation of declarative memory processes. In absence of aversive stimulation an elevation of free cortisol levels by synthetic cortisol resulted in a comparable memory-impairing effect like the one observed in the first study. Complementing findings by Newcomer and coworkers (10) we found that a single low dose administration of cortisol acutely impairs declarative memory performance in two independent tests. It should be noted that the free cortisol levels obtained after ingestion of 10 mg of cortisol orally represent the upper physiological limit for this hormone in man which is usually not observed under moderate psychological stress in the laboratory (as seen in Study 1). However,

exposure to more severe forms of stress have been found to induce similar peak free cortisol (21,22).

Our results are in contrast to two previous studies which tested the effects of cortisol on immediate recall. While Beckwith et al. (8) found mixed effects depending on the dose of cortisol used and the level of practice with both increases and decreases in memory performance, Fehm-Wolfsdorf and coworkers (9) failed to observe a significant effect of 50 mg cortisol on an immediate recall test. One possible explanation for the discrepant results is that our memory tasks addressed different CNS mechanisms since both Beckwith et al. and Fehm-Wolfsdorf et al. focused more on short-term memory processes which appear to be rather independent of the hippocampus (11,12).

From experimental studies in animals it is known that acute stress can interfere with memory processes (e.g. 23,24). The present data underscore the potential relevance of cortisol secretion following acute stress for hippocampus-mediated declarative memory in humans. Likewise, a prolonged elevation of cortisol levels as a result of chronic stress, psychiatric disease, steroid treatment or aging should also be associated with lowered declarative memory performance. In fact, numerous results in the literature support this hypothesis. In patients with Cushing's disease (2,4,25), major depression (3,5,26) or Alzheimer's disease (27) an enhanced HPA activity as well as various neuropsychological deficits including memory impairment could be demonstrated. Although it might be tempting to speculate that memory problems result -in part- from elevated glucocorticoid levels, these diseases are characterized by too many other physiological and psychological alterations to allow a causal interpretation. However in line with this argument are results obtained in healthy elderly individuals. Subjects with increasing adrenocortical activity over a period of three years were shown to perform poorer on declarative memory and selective attention tests than subjects with decreasing or stable cortisol levels (28).

Several lines of research suggest that the memory-impairing effect of glucocorticoids could be mediated by hippocampal neurons. While a number of studies showed an association between chronically elevated HPA axis activity and impaired memory performance in rats (e.g. 29,30), it is also conceivable that cortisol produces its effect within shorter time periods. Studies on the feedback action of glucocorticoids (31,32) suggest that cortisol can interact with CNS receptors within less than an hour. Moreover, Filippi and coworkers (33) reported an impairment of *in vivo* LTP after corticosterone treatment as soon as two minutes after drug injection, which is too fast to consider a receptor mediated effect, so non-genomic membrane effects of cortisol also have to be considered. The effect on hippocampus-dependent memory function could probably be 'relayed' by Type II receptors. While occupation of Type I receptors (basal cortisol levels) appears to improve memory, occupation of Type II receptors following stress or exogenous steroid administration inhibits long-term potentiation (34) (for an in-depth review of this topic see (35)).

In summary, the present results support data from animal studies and clinical observations suggesting that not only chronic but also acute elevations of cortisol levels can impair memory performance in human subjects. It appears that this effect is specific for declarative memory functions whereas at least some procedural memory functions as assessed by verbal perceptual tests seem to be spared.

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