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Moderate Psychosocial Stress Appears Not to Impair Recall of Words Learned 4 Weeks Prior to Stress Exposure

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Recent studies in humans have reported that recall of previously learned material is especially sensitive to the disruptive effects of pharmacologically induced cortisol elevations. Whether similar effects occur after exposure to psychosocial stress remains to be shown. Moreover it is unknown whether stress before or after the initial learning interacts with the later effects of repeated stress on delayed recall (e.g. state-dependent learning).

Forty subjects participated in the present experiment. They learned a word list either one hour before or 10 min after exposure to a psychosocial laboratory stressor. Delayed recall was tested 4 weeks later, again either before or after stress. Salivary cortisol levels increased significantly in response to both stress exposures. Stress had no effects on the initial learning and also did not impair delayed recall. Moreover there was no evidence for state-dependent learning. The current data seem to be in conflict with previous studies demonstrating that delayed recall is especially sensitive to elevated cortisol levels. Several reasons for these discrepancies are discussed. Among them is the small sample size, the moderate cortisol increase in response to the second stress exposure but also the long recall delay, which might lead to memory traces less susceptible to stress.

Keywords: Cortisol; Delayed recall; Glucocorticoids; Hippocampus; Humans; Memory; Stress; Trier social stress test

INTRODUCTION

The secretion of glucocorticoids increases in response to a stressful event. In addition to their peripheral site of action these hormones also modulate brain functions. Among various other effects, a wealth of studies has shown that glucocorticoids modulate memory in animals and humans. Studies in rodents have revealed that glucocorticoids modulate memory in a time and task-dependent fashion (Lupien and McEwen, 1997; De Kloet *et al.*, 1999; Diamond *et al.*, 1999; Roozendaal, 2000). A recent publication by de Quervain *et al.* demonstrated for the first time in rats that stress or corticosterone treatment impaired 24 h delayed recall in the water maze (de Quervain *et al.*, 1998).

In humans glucocorticoid administration can interfere with performance in declarative memory as well as in working memory tasks (Wolkowitz *et al.*, 1990; Kirschbaum *et al.*, 1996; Lupien *et al.*, 1999; Newcomer *et al.*, 1999; Young *et al.*, 1999; de Quervain *et al.*, 2000). de Quervain *et al.* showed that glucocorticoids given one hour prior to recall testing impairs delayed recall of a word list learned 24 h earlier (de Quervain *et al.*, 2000). In line with these data is another recent study, in which cortisol administration impaired recall of a word list learned a little more than one hour prior to drug administration (Wolf *et al.*, in press). It was speculated that this effect might be mediated by cortisol effects on the hippocampus, even though other sites of action in the central nervous system are conceivable. Reduced recall performance was also noted in elderly subjects after exposure to psychosocial laboratory stressors (Lupien *et al.*, 1997; Wolf *et al.*, 1999). However, in these studies stress exposure started shortly after learning and recall was tested after a brief delay, thereby not allowing a strict separation between consolidation and recall.

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TABLE I Experimental conditions for the four groups

Group	*Treatment during initial learning*	*Treatment during delayed (4 weeks) recall*
1. (control/control), *n*=15 (F/M=4/11)	1 h prior to stress exposure (control condition)	1 h prior to stress exposure (control condition)
2. (stress/control), *n*=11 (F/M=5/6)	10 min after stress exposure (stress condition)	1 h prior to stress exposure (control condition)
3. (control/stress) *n*=7 (F/M=4/3)	1 h prior to stress exposure (control condition)	10 min after stress exposure (stress condition)
4. (stress/stress), *n*=7 (F/M=6/1)	10 min after stress exposure (stress condition)	10 min after stress exposure (stress condition)

The study reported here was designed to test whether stress in the human impairs recall delayed for 4 weeks. In addition, the possibility was explored that stress before the initial learning might modulate the effects of stress on delayed recall in the sense of state-dependent learning (Clark *et al.*, 1983; Schramke and Bauer, 1997). Therefore subjects learned the word list either before or after stress exposure and were asked to recall the list 4 weeks later, again either before or after stress exposure. Thus, four different recall conditions were investigated. The experiment was part of a larger study investigating endocrine effects of repeated stress. Initial results of the stress exposure on the learning of words with a larger pool of subjects are reported elsewhere (Wolf *et al.*, 2001). The present paper describes the results of the stress exposure on delayed recall testing in those subjects, who participated in the repeated stress memory part of the study.

MATERIALS AND METHODS

Subjects

Participants in a larger study, which investigated the endocrine effects of repeated stress exposure were also tested for memory performance, depending on availability of additional testing personnel. Only subjects who showed an initial free cortisol stress response (net increase) larger than 2.5 nmol/l during the first stress session were considered for the repeated stress paradigm. Smokers, subjects suffering from hormonal dysregulation, atopic, psychosomatic or psychiatric disease were excluded. All participants reported they were free of medication. All subjects received detailed information about the study and provided written consent. All participants underwent a brief medical examination, which consisted of a clinical interview, heart and lung check-up, testing blood pressure assessment, and blood counts. Psychiatric screening consisted of a clinical interview as well as a German depression questionnaire (Hautzinger and Bailer, 1993). The study protocol was approved by the ethics committee of the University of Trier.

Forty young healthy university students (18 women and 22 men) participated in the delayed recall resting study reported here. None of the women used oral contraceptives and all women were tested in the late luteal phase (days 21–25) of their menstrual cycle as self-reported. The luteal phase was chosen since during this phase psychosocial stress-induced free cortisol levels do not differ between men and women (see Kirschbaum *et al.*, 1999).

Experimental Protocol

Subjects were exposed to a psychosocial stressor twice, with a 4-week interval between the two sessions. After waking up at 7:00 a.m. (participants received a wake-up call by the experimenter), subjects were tested between 10:00 and 12:00 a.m. During the first session subjects learned a word list 1 h before or 10 min after stress exposure. This list had to be recalled during the second session 4 weeks later, again either one hour before stress exposure (control condition, see Table I) or 10 min after stress exposure (stress condition). Out of the total of 40 subjects, 14 participants were exposed to a psychosocial stressor before recall testing (9 women and 5 men), while the others served as a control group. The latter group was exposed to the laboratory stressor one hour after recall testing. The two groups did not differ significantly in age (stress group 24.2 ± 1.0 years (mean ± S.E.); control group 24.8 ± 1.0 years) and body mass index (stress group 22.6 ± 0.4 kg/m^2; control group 22.1 ± 0.4 kg/m^2). In addition the two groups were further divided according to when they learned the list initially (see Table I). Half of the group learned the list one hour prior to their first stress exposure (at a time when consolidation is well-established and pharmacological manipulations are less effective), while the other half learned the list 10 min after stress exposure, at a time when cortisol levels reach their peak. It has to be noted that the four groups did differ in sample size as well as distribution of men and women.

Memory Testing

A word list containing 25 words was presented to the subjects on paper with the instruction to learn the words by reading them aloud at a speed of one word every three seconds. The words were taken from a pool of German words, all of which were of high concreteness (e.g. typewriter, rainbow) and 10–15 letters in length. After the learning phase a 25 s distractor task was presented (reading loud colour words) in order to prevent the subject from using silent rehearsal strategies. Immediately after the distractor task free recall of the words was tested. This procedure was repeated three times to assure proper learning and to avoid a floor effect at the 4-week delayed recall. At the second session delayed free recall was tested. The delayed recall was scored as the percentage of

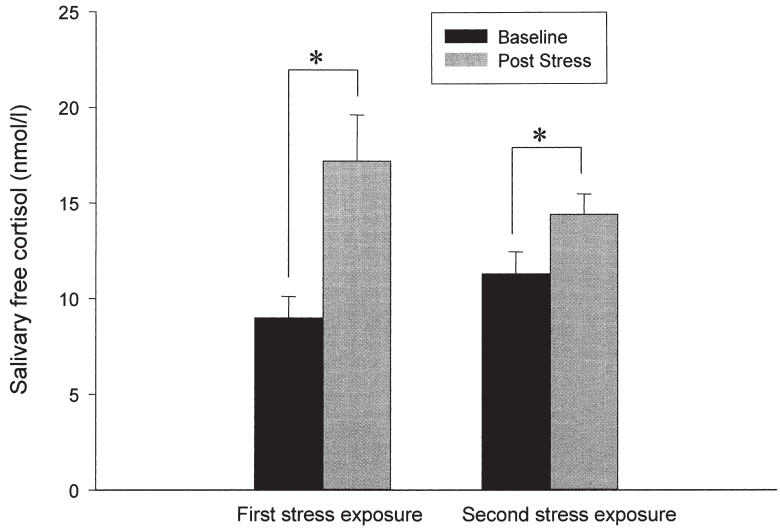

FIGURE 1 Salivary free cortisol response to the Trier social stress test in those subjects having to recall the word list after stress exposure (groups 3 and 4; n=14). The first stress exposure was 4 weeks prior to the second stress exposure. While cortisol concentrations increased significantly at both sessions the increase was less pronounced at the second session (significant stress by session interaction). *$p < 0.05$ compared to pre-stress cortisol concentrations.

correctly recalled items from the last learning trial. Subjects were not told at the first session that delayed recall would be tested again 4 weeks later in order to prevent the subjects from preparing for the delayed recall session at home (e.g. writing down the words and restudying them shortly before coming to the laboratory again).

Psychosocial Stress

The Trier social stress test (TSST) was employed for induction of psychosocial stress (Kirschbaum *et al.*, 1993). This laboratory stressor consists of a free speech and a mental arithmetic task in front of an audience. Including an introduction and a preparation phase the total procedure takes approximately 15 min. The TSST has been repeatedly shown to substantially increase cortisol secretion. Salivary samples for the assessment of free salivary cortisol were collected immediately before onset of the stress sessions as well as 10 min after cessation of stress, when cortisol levels peak and list learning (first session) or delayed recall testing (second session) started.

Saliva Sampling and Free Cortisol Analysis

Saliva was collected by the subjects using Salivette (Sarstedt, Rommelsdorf, Germany) collection devices. The devices were stored at -20°C until biochemical analysis. Free salivary cortisol was determined by a time-resolved immunoassay with time-resolved fluorometric detection (Dressendorfer *et al.*, 1992).

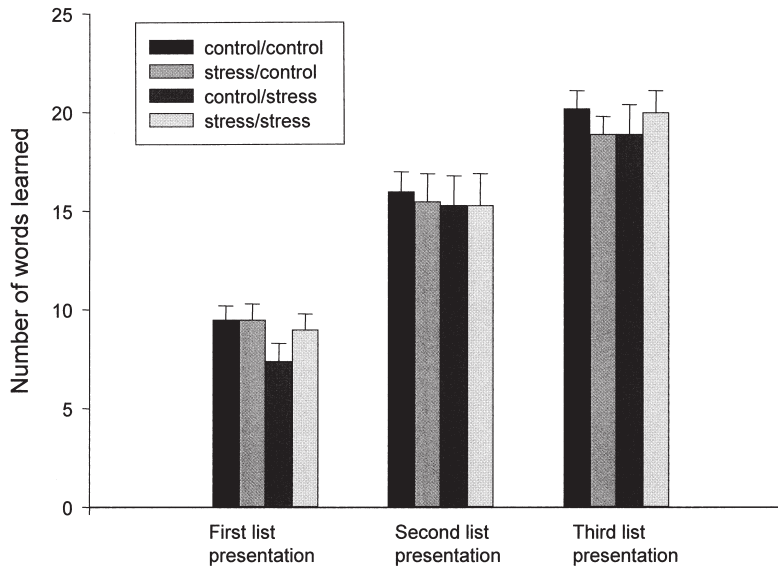

FIGURE 2 Initial word list learning by the four experimental groups. Subjects read the list three times with recall being tested each time after a brief distractor task. Stress did not influence learning of the word list.

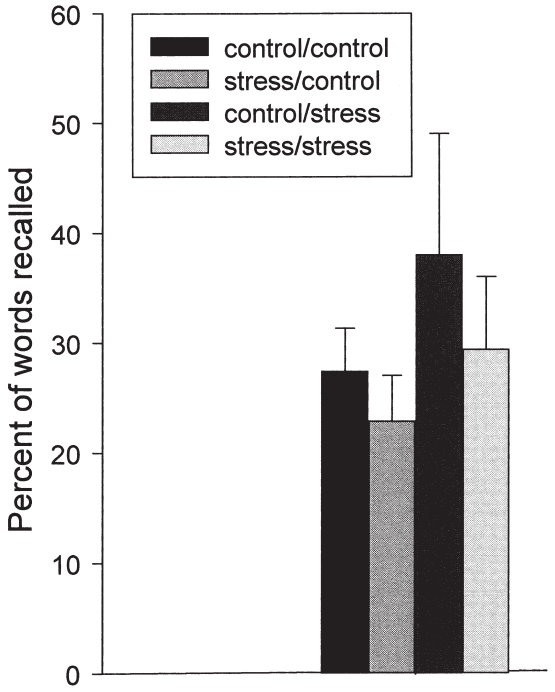

FIGURE 3 Delayed recall (4 weeks later) in the four experimental groups. Test scores are expressed as percent of words recalled from the last learning trial. Subjects stressed prior to delayed recall testing (groups 3 and 4) did not show evidence of impaired delayed recall. Moreover no evidence for state-dependent learning was observed.

Statistical Analysis

The cortisol response to the stressor was analysed by ANOVA with the two within factors "session" (first and second stress exposure) and "stress" (pre and post). For analysis of memory performance, tested first was whether the four groups did not differ in their initial learning. ANOVA with the between factor "group" and the within factor "learning trial" (1–3) was used. Afterwards the delayed recall performance was analysed first with a *t*-test for independent factors with the grouping variable "stress" versus "control" during recall. As a next step the condition during learning was also taken into account by using an ANOVA with the four groups as between group factor. Finally the relationship between the cortisol response (absolute levels and the increase [delta]) and delayed performance within the "stressed recall group" was computed using Pearson's correlation. Identical results as presented below were obtained when all analyses were performed using nonparametric tests (data not shown).

RESULTS

Cortisol Reaction to the Stressor

ANOVA with the cortisol data from the stressed recall group indicated a significant main effect of stress exposure ($F(1,13)=20.1$, $p < 0.001$) and no main effect of session ($p > 0.20$). In addition a significant stress by session interaction was observed ($F(1,13)=5.9$, $p < 0.05$), demonstrating habituation in response to the repeated stress exposure (Fig. 1).

Stress Effects on Initial Learning

Subjects from the four groups did not differ in their initial learning of the lists (Fig. 2). The ANOVA indicated neither a group main effect nor a group by trial interaction (both $F < 1$). As expected a strong learning trial main effect was observed ($F(2,72)=343.1$, $p < 0.001$).

Stress Effects on Delayed Recall

Subjects stressed before the 4-week delayed recall testing (groups 3 and 4) did not show impaired performance, when compared to the unstressed control groups (1 and 2). The percentage (mean ± S.E.M.) of words recalled was 33.8 ± 6.3 for the stressed group versus 25.6 ± 2.8 for the control group; $t(1,38)= -1.4$, $p=0.17$). Similar non-significant results were obtained when the four groups were analysed using an ANOVA ($F < 1$; Fig. 3).

Finally, the possibility was explored that the stress-induced cortisol increase might be associated with delayed recall performance within the stressed group. Therefore correlation of the cortisol increase (post-stress minus baseline levels) with the delayed recall performance was tested; however no significant correlation was observed ($r=0.34$, $n=14$, $p=0.23$).

DISCUSSION

In the present study psychosocial stress did not influence the initial learning of a word list, which is in line with several recent stress and pharmacological experiments in rats and humans (de Quervain *et al.*, 1998; Lupien *et al.*, 1999; Newcomer *et al.*, 1999; de Quervain *et al.*, 2000). A second exposure to psychosocial stress 10 min prior to recall testing did not impair 4 week delayed recall of a word-list. Moreover the timing of the first stress exposure one hour before or 10 min after the initial learning had no effect on the delayed recall 4 weeks later. Therefore evidence for state-dependent learning was not observed in the current study, even though past experiments observed state-dependent learning with mild exercise stress (Clark *et al.*, 1983; Schramke and Bauer, 1997).

The cortisol increase in response to the stressor was not negatively associated with the delayed recall, which is in contrast to the negative correlation between the free cortisol response and word learning observed in an earlier study from this laboratory (Kirschbaum *et al.*, 1996). Recent studies in rats have demonstrated that stress, as well as corticosterone treatment, impairs 24 h delayed recall in the water maze (de Quervain *et al.*, 1998). Similarly, in humans glucocorticoid administration was found to impair recall of words learned 24 h (de Quervain *et al.*, 2000) or 75 min (Wolf *et al.*, in press) earlier. When reporting non-significant results the issues of test power

and type II error have to be discussed. The present experiment employed for testing the main hypothesis 14 subjects in the stressed delayed recall group and 26 subjects as unstressed controls. The sample size per group is therefore larger than the one of previous pharmacological studies reporting specific negative effects of cortisol on delayed recall (de Quervain *et al.*, 2000; Wolf *et al.*, in press). Moreover the impairing effects of cortisol on delayed recall appear to be rather large (de Quervain *et al.*, 2000), suggesting that the sample size of the present study was sufficient. However, the further distribution of the subjects into four subgroups with cell sizes ranging from 7 to 15 and uneven distribution of men and women is certainly problematic.

In the present study, subjects were exposed to the stressor twice with a 4-week break in between. While cortisol levels increased by 90% in response to the first stress exposure, they only increased by 27% in response to the second stress exposure 4 weeks later, typical for the habituation occurring with this paradigm (Kirschbaum *et al.*, 1995). While this increase was still significant, the resulting peak cortisol levels might have been too low to produce recall impairment. Indeed, the cortisol levels induced pharmacologically by de Quervain *et al.* (2000) were three to fourfold higher than those induced with the repeated stress paradigm. It is conceivable that a stronger stress-induced cortisol increase, as observed in response to the first stress exposure would have resulted in a recall impairment. Another possible explanation for the absence of a recall impairment could be the delay period used in the present study (4 weeks), which is much longer than those used previously (de Quervain *et al.*, 2000; Wolf *et al.*, in press). One could speculate that items remembered over such a long period are less susceptible to the impairing effects of stress. Indeed there is evidence from studies in the nonhuman primate that the hippocampus, which might be mediating the cortisol effects on delayed recall, becomes less important for a successful recall the longer the period between the initial learning and the delayed recall test (Zola-Morgan and Squire, 1990).

In sum, the present study did not detect an impairing effect of moderate psychosocial stress on the delayed recall of words learned 4 weeks earlier. Whether the absence of an impairing stress effect is related to the moderate cortisol increase and/or the long recall delay cannot be answered with the current data. Due to the small sample size and the uneven distribution of women and men in the four groups these data should be viewed as preliminary until replicated in a larger sample. Future studies, which should mimic the experiments by de Quervain *et al.* (2000) more closely by using a single stress exposure after a 24 h delay, will be necessary to test whether psychosocial stress impairs delayed recall in humans. Moreover, additional experiments should address the issue of a possible time limited "sensitive period" during which such a negative effect on recall can occur.

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