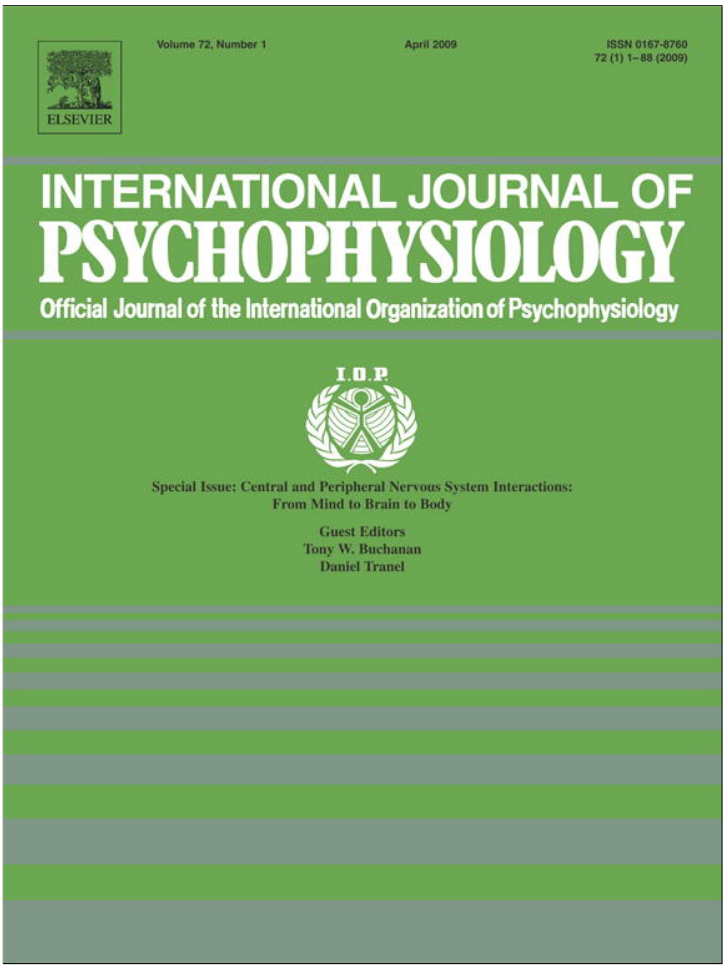

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Effects of cortisol on emotional but not on neutral memory are correlated with peripheral glucocorticoid sensitivity of inflammatory cytokine production

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ABSTRACT

Cortisol responses to stress have important physiological effects on several target tissues throughout the body, including the central nervous system and the immune system. The ability of target tissues to receive cortisol signals has been shown to vary between individuals and over time. Conflicting data exist on whether different target tissues' glucocorticoid (GC) sensitivity is related. In a double-blind, placebo-controlled design, $n=19$ participants ($n=15$ men, $n=4$ women) received an oral dose of 30 mg of cortisol and placebo in randomized order. Memory retrieval of previously learned neutral and emotional words was tested after cortisol or placebo application. Peripheral GC sensitivity was tested by measuring in-vitro stimulated production of interleukin-6 (IL-6) in whole blood before and after cortisol vs. placebo application. Cortisol treatment reduced retrieval of neutral and emotional words (marginally significant at $p=0.07$), and significantly reduced stimulated IL-6 production ($p<0.001$). Relative suppression of IL-6 production was associated with impairment of memory retrieval of emotional ($r=0.48$; $p=0.039$), but not neutral words ($r=-0.17$; $p=0.48$). In summary, results show an association of peripheral glucocorticoid sensitivity with emotional, but not neutral, memory retrieval. Given that these findings can be extended to clinical populations, the association of peripheral glucocorticoid sensitivity with emotional memory retrieval might have important implications for understanding and treatment of stress-related disorders.

1. Introduction

Glucocorticoids (GCs) are important not only as stress hormones but also in the regulation of non-stressed functioning of the organism. Cortisol released by the HPA axis impacts the central nervous system as well as the periphery of the body (e.g. Sapolsky et al., 2000). Recent evidence shows that there is a significant degree of variation in the effectiveness of glucocorticoid signaling between individuals or within individuals over time. However, little is known about the association of glucocorticoid sensitivity of different target tissues within the same individual. We don't know for example if glucocorticoid responsive tissues in the central nervous system are equally receptive for the glucocorticoid signal as tissues in the periphery. Because of its potential use in understanding and treating specific psychiatric disorders, the aim of the present study is to investigate the association of central and peripheral glucocorticoid sensitivity.

In the CNS, GCs exert negative feedback action on the pituitary and the hypothalamus (Dallman et al., 1987). In addition, GCs also act on a range of other brain structures, which are involved in HPA control, but are also crucially important for learning and memory (Gold and Chrousos, 2002; McEwen, 2002; de Kloet et al., 2005; Roozendaal et al., 2006; Wolf, 2006). In this context the hippocampus, the amygdala but also medial prefrontal regions have received the most attention. With respect to memory, GCs facilitate memory consolidation, which leads to an enhanced storage of stressful episodes (Oitzl et al., 1997; Sandi et al., 1997; Joels et al., 2006; Roozendaal et al., 2006). The size of this effect is influenced by multiple variables such as magnitude of the GC increase, coactivation of the (nor)adrenergic system, subjective arousal, but also trait-like variables like gender, age, genetic background, and concentration of local enzymes involved in GC metabolism (Abercrombie et al., 2006; de Kloet et al., 2002; Herbert et al., 2006; Holmes et al., 2003; Roozendaal et al., 2006). In contrast, other aspects of memory are functioning less efficient after stress exposure or after GC administration. Among those is memory retrieval. This has been shown repeatedly in rodents (de Quervain et al., 1998; Roozendaal et al., 2004; Diamond et al., 2006), and humans (de Quervain et al., 2000; de Quervain et al., 2003; Wolf et al., 2004). In humans the negative effect of cortisol on memory retrieval are especially pronounced for emotionally arousing material (e.g. Kuhlmann et al., 2005a; Kuhlmann et al., 2005b; Buchanan et al., 2006). The arousal induced by the testing context is another variable known to modulate GC effects (Okuda et al., 2004; Kuhlmann and Wolf, 2006; Tops et al., 2006). Even if those situational factors are controlled, a substantial amount of interindividual variance in

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the size of the GC effects on memory retrieval remains. Differences in GC sensitivity in addition to, or caused by the potential mediators mentioned above most likely contribute to the variance observed in these behavioral data. In humans, central GC effects have been indirectly assessed either by measuring the impact of GCs on cognitive function, for example learning and memory (e.g. Het et al., 2005; Lupien et al., 2005), or with neuroendocrine test paradigms, such as the dexamethasone suppression test (DST; The APA Task Force on Laboratory Tests in Psychiatry, 1987).

An important target tissue for GCs in the periphery is the immune system, where GCs have rather complex effects. While they have initially been used to suppress immune responses (Hench et al., 1949), more recent evidence gathered over the last decade(s) suggests that short-term increases in the physiological range can also stimulate immune functioning, while long-term increases or pharmacological concentrations suppress most functions (e.g. Dhabhar and McEwen, 1999; Sapolsky et al., 2000). Glucocorticoid sensitivity can be assessed by co-incubation of mitogen-stimulated whole blood or cell cultures in-vitro with different concentrations of glucocorticoids and measuring the relative suppression of stimulated cytokine production. We and others have shown that GC sensitivity of the inflammatory response is subject to inter- and intra-individual variation and responds to acute psychosocial stress and exercise (DeRijk et al., 1996; Rohleder et al., 2001; Rohleder et al., 2002; Rohleder et al., 2003a; Rohleder et al., 2003b; Rohleder et al., 2004). Long-term changes have also been documented in populations suffering from chronic stress (Miller et al., 2002) or vital exhaustion (Wirtz et al., 2003).

It has been speculated that central and peripheral GC sensitivity might be related, but this issue remains controversial. Earlier work from our group showed that GC sensitivity as measured by cortisol response to the DST was unrelated to peripheral GC sensitivity of mitogen-stimulated cytokine production in healthy young participants (Ebrecht et al., 2000). In contrast to that, Yehuda et al. have demonstrated a substantial association of the cortisol response to the DST with GC sensitivity of lysozyme activity in PBMCs in healthy participants (Yehuda et al., 2003).

In posttraumatic stress disorder (PTSD), alterations have been reported in central and peripheral GC sensitivity. Although results are not consistent, a large number of studies revealed a group of PTSD patients with a pattern of reduced basal cortisol levels, (e.g. Yehuda et al., 1995a,b; Yehuda et al., 1996; Rohleder et al., 2004; Wessa et al., 2006), increased cortisol suppression in response to the DST (e.g. Stein et al., 1997), and greater GC sensitivity of peripheral immune cells (Yehuda et al., 2004; Rohleder et al., 2004). Some studies also investigated central GC sensitivity by assessing the effects of glucocorticoids on learning and memory. Two studies reported stronger negative effects of cortisol on hippocampal dependent declarative memory (Grossman et al., 2006) or hippocampal dependent trace conditioning (Vythilingam et al., 2006) in PTSD patients, suggesting higher central GC sensitivity in PTSD. In contrast to that, Bremner et al. reported blunted effects of prolonged dexamethasone treatment on declarative memory in PTSD (Bremner et al., 2004). While these data clearly show higher GC sensitivity in the CNS and in the periphery in PTSD, heterogeneous findings exist with respect to the question if these increases are correlated. Only in one study an association between peripheral (suppression of glucocorticoid receptors) and central GC sensitivity (response to the 0.5 mg DST) was found (Yehuda et al., 1995a,b).

In light of these scarce data on association of central and peripheral GC effects, we set out in the present study to address this question in healthy young participants. We decided to assess the effect of a single dose of oral cortisol on memory retrieval as an example for GC effects on a highly relevant area of cognitive functioning. We decided to assess peripheral GC sensitivity by measuring the effect of the same oral cortisol dose on mitogen-stimulated production of the pro-inflammatory cytokine interleukin-6 in-vitro, because inflammation and its control by endogenous factors are emerging as important determinants for somatic health. In contrast to previous studies, this direct assessment of oral cortisol effects on stimulated cytokine production, instead of interpreting the effects of co-incubation with glucocorticoids in culture, was used to achieve better comparability with assessment of GC effects on memory. We hypothesized that cortisol would impair memory retrieval and suppress pro-inflammatory cytokine production, and we aimed to investigate the association of GC effects on these parameters.

2. Materials and methods

2.1. Sample

We recruited a total of $n=23$ healthy young women and men, four of which were later excluded due to problems during blood draw or laboratory procedures. The remaining sample of $n=19$ had a mean age of 27.1 years (SD=4.03; range=21 to 35) and a mean body mass index (BMI) of 22.8 kg/m^2 (SD=2.2; range=18 to 26). Four participants were women and 15 were men, and five participants reported to be habitual smokers. The female participants were part of a larger study on the acute effects of cortisol on memory retrieval (Kuhlmann et al., 2005a). All participants were Caucasian, and none of the participants reported any acute or chronic diseases or taking any medication. None of the women used hormonal contraceptives. The study protocol was approved by the local ethics committee and all participants gave written informed consent.

2.2. Procedure

The effects of oral cortisol were tested in a double-blind, cross-over, placebo-controlled experiment with randomized treatment order. Participants received either three pills containing 10 mg hydrocortisone (Hoechst, Germany) or three similar looking placebo pills. The current dose (30 mg) was chosen to be similar to previous studies showing impairing effects of cortisol on retrieval (de Quervain et al., 2000; Wolf et al., 2001). Participants were recruited through advertisements at the University of Düsseldorf and invited to the laboratory on two days with a four-week interval. Female participants were invited during the first half of their menstrual cycle to control influences of gonadal steroids on memory performance and immune measures. Upon arrival at the laboratory between 10:00 and 11:00 h participants were asked to learn a list of 15 neutral and 15 negative words (see below), after which they were allowed to leave the laboratory until the second part of the experiment began. Participants returned to the laboratory between 15:00 and 16:00 h and were instructed to refrain from smoking, eating, and drinking anything but water 30 min before their return to the laboratory. Participants provided a baseline saliva sample for assessment of baseline cortisol, after which they received an indwelling catheter into an antecubital vein of the non-dominant arm. A first blood sample was immediately taken for measurement of cytokine production. After that participants provided a second saliva sample before they received either hydrocortisone (30 mg) or placebo orally. Further saliva samples were collected 15, 30, 45, 60, 90, and 120 min after treatment, further blood samples were collected 60 and 90 min after treatment. Memory retrieval was tested 60 min after treatment as described below.

2.3. Memory testing

A detailed description of the memory test used can be found in our previous publication (Kuhlmann et al., 2005a). In brief, a word list (with two parallel versions available) containing 15 negative (e.g. pain, explosion, prison) and 15 neutral words (e.g. street, blouse, stone) was used. There were no differences between neutral and negative words or between the two lists with respect to word frequency or word length.

The word list was presented to the participants on a piece of paper with the instruction to memorize them. They were given 2 min to learn the list with immediate free recall being tested. This procedure was repeated once resulting in two learning trials. In the afternoon (5 h after initial learning, 1 h after oral cortisol or placebo treatment)

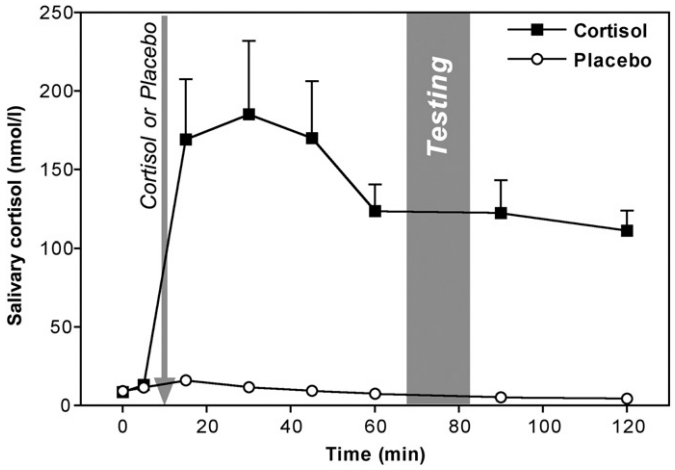

Fig. 1. Salivary free cortisol concentrations after oral hydrocortisone (30 mg) or placebo application (figure shows means ± SEM).

free recall of the words was tested. In order to account for possible within and between participant variance in initial learning, free recall performance in the afternoon was expressed as the percentage of words remembered in relation to the second (and last) learning trial in the morning (see Kuhlmann et al., 2005a; Kuhlmann et al., 2005b; Buchanan et al., 2006).

2.4. Cortisol measurement

Cortisol was measured in saliva collected repeatedly throughout the experiment using salivettes (Sarstedt, Nümbrecht, Germany). Salivary cortisol has been shown to reflect the unbound fraction of cortisol in plasma, thereby providing an estimate of the biologically active fraction of cortisol, and is therefore considered an appropriate measure of cortisol effects in the organism (Kirschbaum and Hellhammer, 1994). Salivettes were stored at −20 °C until completion of the study, thawed, and centrifuged at 1200 g and 4 °C for 5 min. The resulting saliva was analyzed using a commercial chemiluminescence immuno assay (CLIA; IBL-Hamburg, Hamburg, Germany) with a lower detection limit of 0.41 nmol/l. Intra- and inter-assay coefficients were below 10%.

2.5. Inflammatory cytokine production

Blood for assessment of inflammatory cytokine production was collected at three time points throughout the experiment using heparinized syringes with a volume of 5 ml (Braun, Melsungen, Germany). Heparinized whole blood was diluted 10:1 with saline and incubated with the bacterial endotoxin lipopolysaccharide (LPS, *E. coli*, Sigma, Deisenhofen, Germany) in a final concentration of 30 ng/ml on sterile 24-well cell culture plates (Greiner, Nürtingen, Germany). After 6 h incubation at 37 °C and 5% CO_2, plates were centrifuged for 10 min at 2000 g and 4 °C and the plasma supernatant was collected and stored at −80 °C until analysis after completion of the study. Concentrations of the inflammatory cytokine IL-6 were measured using a commercial ELISA (BD Pharmingen, San Diego, CA, USA). The detection limit of the ELISA was 4.7 pg/ml, and intra- and inter-assay coefficients were below 10%.

2.6. Statistical analyses

The distributions of all variables were evaluated prior to analyses. Because cortisol and IL-6 levels were skewed, they were subjected to log-10 transformations. To test for effects of sequence, univariate ANOVAs with the factor sequence were run. There were no significant effects of sequence on cytokine production (effect of sequence on IL-6 suppression after placebo: $F=1.1$; $p=0.32$; after cortisol: $F=2.6$; $p=0.16$), and no effects of sequence on memory retrieval (effect of sequence on retrieval of all words after placebo: $F=0.1$; $p=0.75$; after cortisol: $F=0.93$; $p=0.35$. Effect of sequence on retrieval of neutral words after placebo: $F=0.14$; $p=0.71$; after cortisol: $F=0.61$; $p=0.45$. Effect of sequence on retrieval of emotional words after placebo: $F=0.04$; $p=0.84$; after cortisol: $F=0.75$; $p=0.40$). The effects of cortisol vs. placebo application on salivary cortisol and IL-6 production were then analyzed with repeated measures ANCOVAs with the factors time (8 levels for cortisol; 3 levels for IL-6) and treatment (cortisol vs. placebo), and the covariate sex. For assessment of memory performance, the percentage of recalled words was calculated revealing percentages of total, neutral, and negative words. To test the effect of cortisol vs. placebo application on memory retrieval, we used a repeated measures ANOVA with the factors treatment (cortisol vs. placebo), arousal (neutral vs. negative), and the covariate sex. Omega squared (ω^2) is reported as a measure of effect size for all ANCOVA results. To analyze associations between cortisol impact on memory performance and inflammatory cytokine production, we calculated additional delta scores by subtracting decrease of cytokine production from pre to post treatment (60 min), percent recall of all words, neutral words, and negative words after cortisol treatment from the same variables after placebo treatment. All delta scores were normally distributed. Bivariate Pearson correlations between the resulting delta scores were calculated to test for interrelations of cortisol effects. All analyses were performed using SPSS version 13 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Effect of hydrocortisone vs. placebo application on salivary cortisol levels

Hydrocortisone application induced significant increases in salivary free cortisol, while no changes were found after placebo treatment. Repeated measures ANCOVA revealed a significant treatment by time interaction ($F[2.86,48.64]=19.63$; $p<0.001$; $\omega^2=0.30$), while no effect of sex could be detected (all Fs<1). Cortisol concentrations differed significantly at the time of memory testing (before testing at 60 min: $F[1,18]=177.36$; $p<0.001$; $\omega^2=0.82$; after testing at 90 min: $F[1,18]=431.34$; $p<0.001$; $\omega^2=0.92$; see Fig. 1).

3.2. Effect of hydrocortisone vs. placebo application on in-vitro inflammatory activity

Fig. 2 shows the impact of hydrocortisone vs. placebo application on LPS-stimulated production of the inflammatory cytokine IL-6 in whole blood. Repeated measures ANOVA revealed a significant treatment by time interaction ($F[1.46,24.81]=4.93$; $p<0.024$; $\omega^2=0.06$), indicating

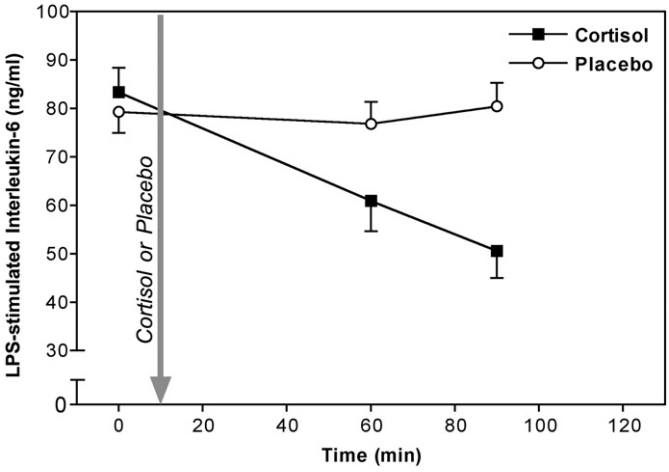

Fig. 2. LPS-stimulated production of interleukin-6 (IL-6) in whole blood after hydrocortisone or placebo application (figure shows means ± SEM).

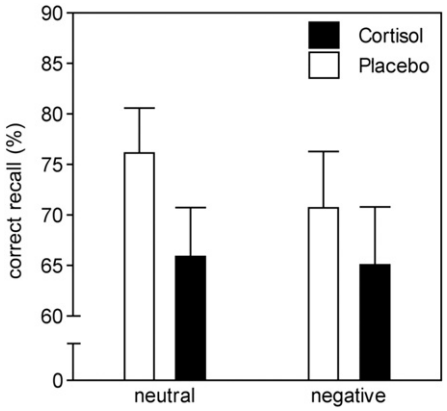

Fig. 3. Memory retrieval after oral cortisol vs. placebo application (figure shows adjusted means ± SEM).

suppression of inflammatory cytokine production after hydrocortisone application. Sex did not affect cytokine production (all *F*s<1).

3.3. Effect of hydrocortisone vs. placebo application on delayed recall

The effect of hydrocortisone vs. placebo application on recall of previously learned words is shown in Fig. 3. ANCOVA with the factors treatment (cortisol vs. placebo), arousal (negative vs. neutral), and the covariate sex revealed a marginally significant main effect of treatment on delayed recall ($F[1,17]=3.52$; $p=0.078$; $\omega^2=0.03$), but no interaction effect of arousal and treatment on memory performance ($F[1,17]=0.30$; $p=0.59$) and no effects of sex ($F<1$). Adding cortisol level at time of memory testing as an additional covariate did not change the overall results (main effect of treatment: $F[1,16]=3.06$; $p=0.099$).[1]

3.4. Associations of cortisol effects on memory and inflammatory cytokine production

To test for associations between central nervous system and peripheral effects of hydrocortisone vs. placebo treatment, bivariate Pearson correlations were calculated between the change scores in memory recall and inflammatory cytokine production. There was no significant association between changes in cytokine production and overall memory recall ($r=0.17$; $p=0.48$). When testing for associations between cortisol effects on delayed recall of emotionally negative vs. neutral words and on cytokine production, analyses revealed a significant correlation between cortisol effects on cytokine production and on recall of emotionally negative words ($r=0.48$; $p=0.039$; see Fig. 4). Controlling for sequence revealed a partial correlation of $r=0.49$; $p=0.039$, and controlling for sex revealed a partial correlation of $r=0.44$; $p=0.07$.[2] Recall of neutral words was not associated with cytokine production ($r=-0.17$; $p=0.48$; partial correlation controlling for sequence: $r=-0.23$; $p=0.36$). Salivary cortisol concentration at the time of testing was weakly associated with cortisol suppression of IL-6 production ($r=-0.34$; $p=0.16$), while no relationship was found with cortisol effects on memory retrieval (all *r*s<0.30; *p*s>0.20).

[1] All analyses were repeated after excluding all women. The effect of cortisol on biological parameters (i.e. increase of salivary cortisol and cortisol effect on stimulated cytokine production) remained largely unchanged (data not shown). While the main effect of treatment on delayed recall ($F=0.22$; n.s.) was abolished, a trend towards an arousal effect emerged ($F=3.32$; p=0.09), and the treatment by arousal interaction was changed ($F=2.41$; $p=0.14$).

[2] This correlation was not caused by the outlier visible in Fig. 4A, as elimination of this outlier strengthened the correlation between cortisol effects on emotional memory and on cytokine production ($r=0.54$; $p=0.027$). Excluding women attenuated the correlation between cortisol effects emotional memory and on cytokine to $r=0.38$; $p=0.17$.

4. Discussion

The results of the present study showed that oral hydrocortisone application induces a marked suppression of in-vitro stimulated production of the inflammatory cytokine IL-6. Secondly, cortisol reduced memory retrieval of previously learned words. This effect however constituted only a non-significant trend ($p=0.07$), which is in contrast to previous findings from us and others, where with a similar sample size robust impairing effects of cortisol administration have been found (de Quervain et al., 2000; de Quervain et al., 2003; Wolf et al., 2001; Kuhlmann et al., 2005a; Kuhlmann and Wolf, 2005). Finally, we showed that immune effects of hydrocortisone were associated with cortisol effects on memory retrieval of emotionally negative words. These three major findings will be discussed in the following.

Our finding of inhibition of in-vitro stimulated production by oral glucocorticoid application is in line with previous results. Sauer et al. (1996) reported that an oral dose of 100 mg hydrocortisone suppressed mitogen-stimulated production of IL-1 receptor antagonist (IL-1ra) in cultured monocytes. Oral dexamethasone application was reported to decrease plasma IL-6 and TNF-alpha in patients with major depression (Schuld et al., 2001). Our study is, however, the first to document this effect in this specific setting, i.e. using a lower dose of oral hydrocortisone in a group of healthy participants, and showing inhibition of LPS-stimulated in-vitro production of IL-6.

Effects of cortisol on memory retrieval only revealed a statistical trend in the present sample. This most likely reflects the somewhat larger variance in the data. Whether this is secondary to fact that blood samples were taken throughout the study is unknown. Furthermore, in this study the effects of cortisol on memory retrieval were not influenced significantly by the arousal of the words (neutral vs. negative). Our group has previously reported that cortisol has a stronger negative impact on the retrieval of arousing material (Kuhlmann et al., 2005a; Kuhlmann et al., 2005b). This effect has been replicated by others (Buchanan et al., 2006; de Quervain et al., 2007). It has been argued that this effect reflects the interaction of adrenergic activation in the basolateral amygdala with the GC effects in the amygdala and the hippocampus (see Roozendaal et al., 2006). However, impairing effects of cortisol on memory retrieval have also been observed in studies using only relatively neutral words (Wolf et al., 2001). When discussing this issue, it has to be emphasized that the required heightened noradrenergic arousal can also be induced through the test environment (Okuda et al., 2004; Kuhlmann and Wolf, 2006; Tops et al., 2006). Here the blood sampling employed in the present study could have made the difference. It might have resulted in increased arousal independently of the testing material. This effect might have overruled the probably more subtle effects of the differences in emotional arousal induced by the different words.

Few previous studies have explored the possibility that effects of GCs in the periphery and might be related to GC effects on CNS functioning (Ebrecht et al., 2000; Yehuda et al., 2003). To the best of our knowledge none of these studies has tested for associations between the cognitive (central) effects of a single cortisol administration and the effect of cortisol on the immune system (periphery). Our preliminary findings suggest that participants who are sensitive for acute GC increases in the periphery also show the strongest effect of the GC on memory. In this data set the association was only observed with emotionally arousing words, but not with neutral words. Reasons for this specificity remain to be explored. A highly speculative explanation could entail the idea that effects on emotional memory retrieval might involve to a larger extend GRs in the amygdala (in contrast to GRs in the hippocampus). However more trivial methodological issues (small sample size and its associated lack of power) cannot be ruled out. It remains to be determined which specific mechanisms are responsible for the memory impairment observed after peripheral application of cortisol. Since it cannot be excluded that GC effects outside the central nervous system, for example on the endocrine and the immune system, are involved in mediating the memory impairment, it is unclear whether the memory effect reported

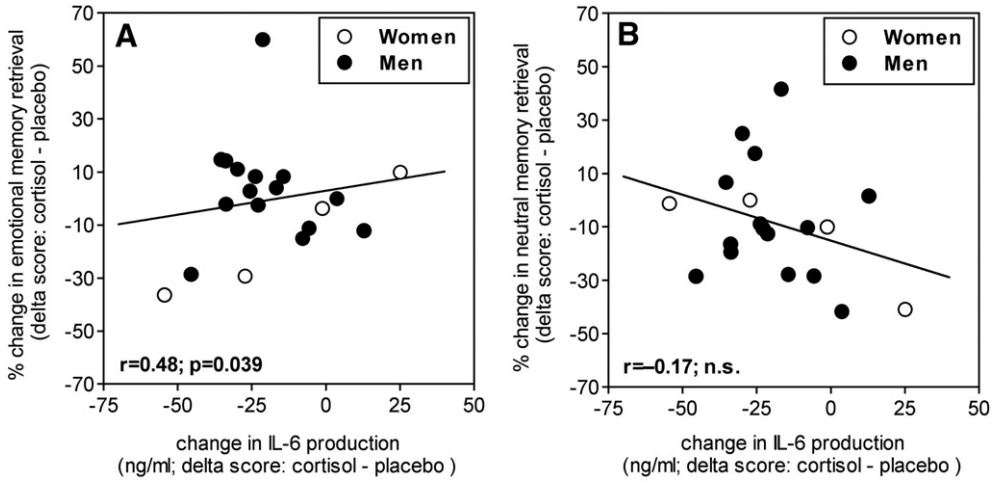

Fig. 4. Scatterplots showing the association of cortisol effects on inflammatory cytokine production with cortisol effects on retrieval of (A) emotionally negative and (B) neutral words. Delta scores are presented on the *y*-axes (memory retrieval after cortisol minus retrieval after placebo) and on the *x*-axes (cytokine production after cortisol minus cytokine production after placebo). Negative deltas indicate poorer retrieval or lower cytokine production after cortisol treatment.

here truly represents central nervous system glucocorticoid sensitivity. Animal studies using intra hippocampal injections of GR agonists (Roozendaal et al., 2004), as well as human neuroimaging studies showing specific activity reductions in the medial temporal lobe after peripheral GC treatment (de Quervain et al., 2003), suggest that the effects of peripherally administered GCs on human memory retrieval are caused by their central action on medial temporal lobe neurons. However this cannot be determined within the present study design.

The present finding of an association between GC effects on CNS function and peripheral measures of GC sensitivity would fit to clinical observations derived from patients with PTSD. These patients as a group show enhanced peripheral GC sensitivity (Yehuda et al., 2004; Rohleder et al., 2004), increased number of GRs on lymphocytes (Yehuda et al., 1991; Yehuda et al., 1993), a super suppression of cortisol after dexamethasone administration (Stein et al., 1997), as well as exaggerated changes in memory performance in response to acute GC treatment (Grossman et al., 2006; Vythilingam et al., 2006). Furthermore, the results of the present study are in agreement with some (Yehuda et al., 2003), but not all studies (Ebrecht et al., 2000) investigating the association of cortisol suppression in the DST with peripheral GC sensitivity. It has been questioned, however, whether the DST represents a good test of central GC sensitivity, because it is unclear if dexamethasone penetrates the central nervous system (De Kloet, 1997). Therefore, by using a memory test instead of the DST, we believe that the present study uses a more appropriate test of central GC sensitivity.

A prerequisite for finding a relation of peripheral GC sensitivity and cortisol effects on a cognitive parameter such as memory performance is relative stability of both measures. Huizenga et al. (1998) argue that GC sensitivity is a rather stable phenomenon, which is in part determined by genetic variants of the glucocorticoid receptor. On the other hand, GC sensitivity of immune cells has been shown to respond to short-term stress and exercise (DeRijk et al., 1996; Rohleder et al., 2003a), and to be down regulated in individuals suffering from chronic stress (Miller et al., 2002) or vital exhaustion (Wirtz et al., 2003). Furthermore, as noted above, changes in GC sensitivity also occur in some psychiatric conditions, most notably but not restricted to PTSD. Therefore, GC sensitivity might have a long-term/trait-like component, which could be determined in part by GR polymorphisms. But GC sensitivity is also subject to regulation by short term or acute influences, and it seems further to respond to longer-term changes, such as alterations in diurnal rhythms of stress hormone levels. While many of the molecular influences on GC sensitivity appear to be tissue specific, it might be speculated that the modulators named above, i.e. GR variants and acute and chronic stress hormone concentrations, affect all tissues in a similar fashion, which might explain the association of central and peripheral GC sensitivity found here.

The present results have to be interpreted in the light of several limitations. First, the sample size is rather small, even though this is not untypical for studies in this area. Thus missing effects could be related to a lack of power, while existing effects could be caused by the effects of outliers. However, elimination of outliers did increase instead of weakening the correlation between peripheral and central GC effects. Another consequence of the small sample size might be our finding that although no differences were detected between cortisol effects on retrieval of neutral vs. emotional words, we did find a significant association with peripheral cortisol effects only for emotional words. Secondly, the gender distribution is uneven with only four women being included in the final data set. One consequence is that gender effects cannot be reliably tested, and our findings of no gender differences in our outcome measures should not be interpreted due to a lack of power. Another consequence is that women are also unevenly distributed between the two randomized sequence conditions, which makes it impossible to reliably test and exclude order effects in women. Another more general limitation might be that GCs have multiple effects in the periphery, not only in the immune system. Similarly the central effects of GCs are not restricted to memory retrieval, but are also present for memory consolidation, working memory, fear conditioning, etc. Thus future studies with a larger sample size might consider testing the potential specificity of the effects reported in the present study. Also one avenue for future research could be the combination of peripheral GC measures with central GC measures derived from neuroimaging (de Leon et al., 1997; de Quervain et al., 2003; Stark et al., 2006). The present study only tested healthy young participants, so that it remains to be shown whether these associations change in the context of aging or diseases. It is imaginable that patient groups with specific somatic diseases (e.g. atopic dermatitis; (Buske-Kirschbaum et al., 2002) or certain psychiatric patients (e.g. dementia patients; de Leon et al., 1997; de Quervain et al., 2004) display specific changes in GC sensitivity in the vulnerable target tissue only.

In conclusion, we were able to show here that oral glucocorticoid application tends to impair memory retrieval and suppresses mitogen-stimulated production of the inflammatory cytokine IL-6. Our results further show that impairment of emotional memory retrieval is correlated with suppression of cytokine production. Although it remains to be determined whether the observed memory impairment specifically tests central GC sensitivity, and given that our findings can be replicated in a less restricted sample of healthy people of other age ranges and in clinical groups such as patients with PTSD or major depression, this

association might have important implications for treatment of the latter disorders. Cortisol treatment for example has been proposed for ameliorating the intensity of intrusive memories in PTSD (Aerni et al., 2004), while it might also be useful in preventing low-grade systemic inflammation, which in turn is discussed as pathophysiological mechanism in somatic symptoms in PTSD (Rohleder and Karl, 2006).

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