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Lipopolysaccharide-induced experimental immune activation does not impair memory functions in humans

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ABSTRACT

Systemic immune activation occurring together with release of peripheral cytokines can affect behavior and the functioning of the central nervous system (CNS). However, it remains unknown whether and to what extent cognitive functions like memory and attention are affected during transient immune activation. We employed a human endotoxemia model and standardized neuropsychological tests to assess the cognitive effects of an experimental inflammation in two groups of 12 healthy young men before and after intravenous injection of lipopolysaccharide (LPS, *Escherichia coli*, 0.4 ng/kg) or physiological saline. Endotoxin administration caused a profound transient physiological response with elevations in body temperature, number of circulating neutrophils, and increases in plasma cytokine levels [interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-α], and concentrations of norepinephrine, ACTH and cortisol. However, these changes in immune and neuroendocrine parameters were not associated with alterations of memory performance, selective attention or executive functions.

1. Introduction

Inflammatory processes are characterized by the release of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α and anti-inflammatory cytokines like IL-10 and endogenous IL-1 receptor antagonist (IL-1ra) (Feghali & Wright, 1997). These cytokines not only control local inflammatory processes in peripheral tissues and mediate immune responses but also affect central nervous system (CNS) activity. They coordinate physiological responses such as fever, activate the hypothalamic–pituitary–adrenal (HPA) axis, and induce behavioral changes like psychomotor slowing, social withdrawal, anhedonia and disturbed sleep architecture, which are adaptive responses collectively termed "sickness behavior" (Conti, Tabarean, Andrei, & Bartfai, 2004; Dantzer, 2001; Mullington et al., 2000; Turnbull & Rivier, 1995).

Circulating cytokines can signal the brain through a number of routes including the activation of vagal afferent fibers projecting to the nucleus of the solitary tract and higher viscerosensory centers, cytokine-specific transport molecules expressed on the brain endothelium, and circumventricular organs lacking the blood–brain barrier (BBB) (Besedovsky & del Rey, 1996; McAfoose & Baune, 2009; Quan, 2008; Seruga, Zhang, Bernstein, & Tannock, 2008). Upon reaching the brain, cytokine signals can be amplified through a central cytokine network that has profound effects on neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and behavior (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Hayley, Poulter, Merali, & Anisman, 2005; Raison, Capuron, & Miller, 2006).

Studies suggest that systemic low-grade inflammation may be involved in the pathology of neuropsychiatric diseases such as depression and schizophrenia (Drexhage et al., 2010; Meyer et al., 2009, 2008; Miller, Maletic, & Raison, 2009). However, since the results from clinical samples are restricted, a more controlled experimental approach is required in order to understand the possible functional relationship between systemic inflammation and cognitive performance. Thus, more recent experimental approaches induced transient inflammatory responses in healthy humans

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through vaccination and injection of endotoxins (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008; Dowlati et al., 2010; Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009; Harrison et al., 2009; Krabbe et al., 2005; Reichenberg et al., 2001).

Administering the bacterial endotoxin lipopolysaccharide (LPS), a complex glycolipid found in the outer membrane of all Gram-negative bacteria, induces an increase in body temperature, malaise, and increased production and secretion of cortisol and cytokines, particularly TNF-α, IL-6, IL-1 and IL-1ra (Bahador & Cross, 2007). Furthermore, experimental induction of a low-grade transient inflammatory response with LPS intensified anxiety, depression or negative mood in otherwise healthy male subjects (Reichenberg et al., 2001; Wright, Strike, Brydon, & Steptoe, 2005). However, only a few studies analyzed psychomotor, cognitive and memory performance, with inconsistent results (Brydon et al., 2008; Cohen et al., 2003; Krabbe et al., 2005; Reichenberg et al., 2001).

In this study, we induced a low-grade inflammatory response in healthy volunteers by administering LPS, and analyzed memory and cognitive performance before and after the injection of LPS or saline. We also determined plasma levels of pro- and anti-inflammatory cytokines as well as cortisol, ACTH, norepinephrine and prolactin concentrations to analyze the effects of immune activation on neuroendocrine parameters and thereby identify the possible immune-driven modulators of cognitive performance.

2. Materials and methods

2.1. Ethical approval

The study was approved by the local ethics committee of the University of Duisburg-Essen and follows the rules stated in the Declaration of Helsinki.

2.2. Subjects

Twenty-four male subjects (mean age, 24.9 ± 5.0 years; range 18–38 years; mean body mass index (BMI), 22.9 ± 3.2; range, 18–30) participated in the study. They were randomly allocated either to the experimental group receiving LPS or to a control group receiving physiological saline injection. Before the detailed screening, the subjects were informed about the study design, and were only enrolled in the experiment after written informed consent had been obtained. All of the volunteers underwent a physical and psychiatric assessment. The physical examination included a complete blood cell count, liver enzymes, renal parameters, electrolytes, coagulation factors and C-reactive protein (CRP). Moreover, an interview was conducted to exclude the presence or history of any physical or psychiatric disorder. The groups did not differ in age, years of education or body weight, nor in any of the physical or psychological screening parameters, and there was no detectable influence of these parameters on any of the outcome measures.

Subjects and investigators where blinded with respect to the study condition with the exception of the attending physician who injected LPS or placebo but was not involved in the neuropsychological assessments or any of the data analyses.

2.3. Experimental design

The double-blind, randomized controlled trial was conducted at the Department of Trauma Surgery of the University Hospital Essen. An intravenous cannula was inserted into an antecubital forearm vein for intermittent blood sampling and drug injection. Between 9 and 10 a.m., each subject was injected intravenously with LPS (0.4 ng of *Escherichia coli* endotoxin per kilogram of body weight) or with the same volume of saline. The endotoxin (serotype O113:H10, Lot G3E069, United States Pharmacopeia, Rockville, Maryland, USA) had been prepared for use in humans. It had been dissolved in sterile water, filtered through a 0.2-μm membrane, and subjected to a microbial safety testing routine approved by the German Federal Agency for Sera and Vaccines (Paul Ehrlich Institute, Langen, Germany). The LPS solution was stored in endotoxin-free borosilicate tubes at −20 °C.

During the experimental session, the subjects performed neuropsychological tests three times, at baseline and starting at 1.5 and 3 h post-injection. Blood samples were drawn in heparinized and EDTA-treated tubes at baseline, and 1, 1.5, 2, 3, 4, and 6 h post-injection. Plasma for the measurement of cytokine, cortisol, norepinephrine, ACTH, and prolactin levels was separated by centrifugation and stored at −80 °C until analysis. Temperature, heart rate and blood pressure were analyzed immediately prior to the blood collections using an ear thermometer and a blood pressure cuff. Saliva for cortisol analysis was collected at baseline and 3 and 6 h post-injection. At the end of each experimental day, subjects underwent a physical examination before being discharged from the clinical research unit, and they were invited for thorough follow-up examinations 24 h and 1 week after the experimental session.

2.4. Blood cell counts

White blood cell differential counts were obtained from EDTA-treated blood samples using an automated hematology analyzer (KX-21N, Sysmex Deutschland GmbH, Norderstedt, Germany).

2.5. Cytokine and hormone determinations

Concentrations of cytokines in the plasma were quantified using multiplexed bead-based assays (Bio-Plex Cytokine Assays, Bio-Rad Laboratories GmbH, Munich, Germany). Samples were prepared according to the manufacturer's instructions and were analyzed on a triple-laser FACSCanto II flow cytometer using FACS-Diva software (BD Immunocytometry Systems, Heidelberg, Germany). Absolute cytokine levels were calculated based on the mean fluorescence intensity of cytokine standard dilutions with a 4 Parameter Logistics (4PL) curve model using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). The detection limit of the assays was 0.2 pg/ml for IL-6, 0.4 pg/ml for IL-10, and 3 pg/ml for TNF-α.

Norepinephrine concentrations were determined by HPLC as previously described (del Rey et al., 2008). Plasma levels of cortisol, ACTH, and prolactin and salivary levels of cortisol were determined using enzyme-linked immunosorbent assays (ELISA; IBL International, Hamburg, Germany) according to the test protocol of the manufacturer, and were analyzed on a Fluostar OPTIMA Microplate Reader (BMG Labtech, Offenbach, Germany). The detection limits were 0.46 pg/ml (ACTH), 0.35 ng/ml (prolactin), and 0.3 ng/ml (cortisol), respectively.

2.6. Neuropsychological tests

2.6.1. Revised Wechsler Memory Scale

For the measurement of memory performance, 3 h after the injection, a German version of the revised Wechsler Memory Scale (WMS-R) was applied (Klaiberg, 2002; Wechsler, 1987). The WMS-R consists of different tasks testing the free and serial immediate and delayed recall of sequences and items that include numbers, words, stories, figures and positions. The test is guided by an investigator, takes about 1 h in total, and consists of 13 subtests: 1. information and orientation; 2. mental control; 3. figural mem-

ory; 4. logical memory I; 5. visual pair recognition I; 6. verbal pair recognition I; 7. visual repetition I; 8. digit span; 9. block span; 10. logical memory II; 11. visual pair recognition II; 12. verbal pair recognition II; and 13. visual repetition II. In subtests 1–9, the content is recalled immediately after acquisition, while subtests 10–13 involve a delayed recall of the content of subtests 4–7. From these subtests, five indices are calculated: 1. global memory score; 2. attention; 3. verbal memory; 4. visual memory; and 5. delayed reproduction. All of the indices have a mean of 100 and a standard deviation of 15.

2.6.2. Color word stroop task

Fifteen minutes before and 1.5 h after the injection, the participants performed the color word Stroop task (Bäumler, 1985; Stroop, 1935). This is a well-established task for assessing high-demand cognitive processing, including attention and executive control processes, via the effects of stimulus conflict on psychomotor responses. The task requires the participant to name the ink color of word stimuli. The target color-words are presented in a randomly ordered list and participants are instructed to name as rapidly as possible the color of the target word. Thus, the participant has to overcome distraction from the meaning of the target word. From the time needed for a list of 72 items and that for similar lists consisting of color-words written in black or just colors in the form of blocks, a concentrative resistance score was calculated according to the test manual.

2.7. Statistics

Statistical analyses were performed using SPSS 16 (SPSS Inc., Chicago, IL). Absolute changes in the parameters following endotoxin administration were analyzed using repeated measures analysis of variance (ANOVA) with 'group' as between-subjects factor (LPS/placebo) and 'time' as repeated measures within-subject factor. In case of a significant time × group interaction, Bonferroni-corrected post hoc tests were computed to assess mean differences between the control and endotoxin groups for specific time points. To compare the endotoxin and control groups in the WMS-R, an unpaired *t*-test with Welch's correction was used. Correlations were calculated using Pearson's correlation coefficient. In all of the tests, $p < 0.05$ was considered significant.

3. Results

Endotoxin administration transiently increased body temperature compared to the control group ($F = 10.90$, $p < 0.001$) with a maximum of 37.4 °C ± 0.10 vs. 36.7 °C ± 0.09 at 4 h after injection (Fig. 1A), and caused a vague feeling of getting sick without the appearance of distinct symptoms in most of the subjects. The LPS-induced immune activation was also characterized by a rapid and profound increase in the number of circulating neutrophils, peaking 3 h after LPS injection and returning to baseline values within 24 h after endotoxin administration ($F = 26.58$, $p < 0.001$; Fig. 1B).

In parallel to increases in body temperature and neutrophil counts, LPS application significantly increased plasma concentrations of the pro-inflammatory cytokines TNF-α ($F = 7.93$, $p < 0.001$) and IL-6 ($F = 10.28$, $p < 0.001$) relative to the control group, with the most pronounced increases 1.5–3 h after endotoxin administration (Fig. 1C and D). Both cytokine concentrations returned to their baseline levels within 3–4 h after injection. In addition, the anti-inflammatory cytokine IL-10 showed an up to

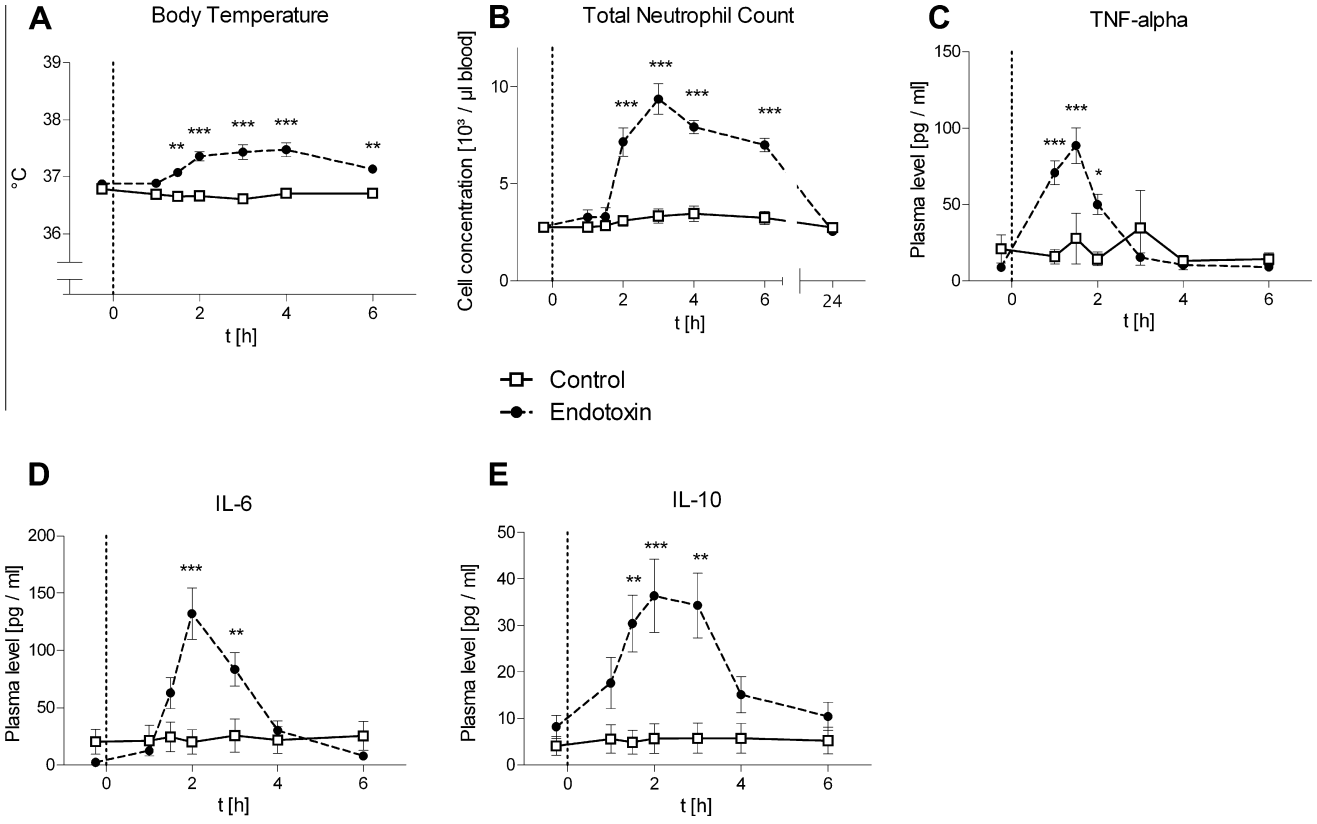

Fig. 1. Body temperature, numbers of neutrophil granulocytes and plasma cytokine concentrations in healthy male subjects following the administration of 0.4 ng/kg *E. coli* endotoxin ($n = 12$) or physiological saline ($n = 12$). Data are presented as means ± SEM. Significant differences between the saline and endotoxin groups: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Bonferroni post hoc test).

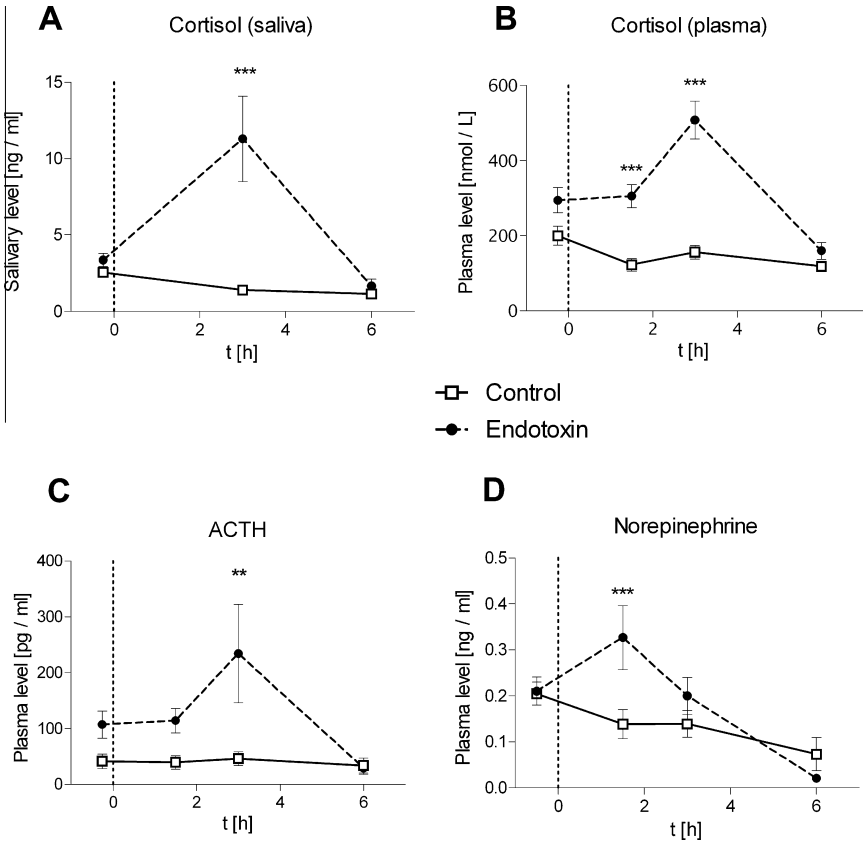

Fig. 2. Salivary and plasma concentrations of cortisol as well as plasma level of ACTH and norepinephrine in healthy male subjects following administration of 0.4 ng/kg *E. coli* endotoxin ($n = 12$) or saline ($n = 12$). Data are presented as means ± SEM. Significant differences between the saline and endotoxin groups: ** $p < 0.01$, *** $p < 0.001$ (Bonferroni post hoc test).

15-fold significant increase 2–3 h after endotoxin injection ($F = 2.70$, $p = 0.026$; Fig. 1E).

As it has been demonstrated that the innate immune response generated by endotoxin application not only increases cytokine release but also activates the hypothalamus–pituitary–adrenal axis, we analyzed plasma and saliva cortisol and plasma ACTH levels. The neuroendocrine response to the LPS injection was reflected by a marked increase in the level of free cortisol in the saliva 3 h after injection ($F = 10.70$, $p < 0.001$; Fig. 2A) and total cortisol in plasma peaking 3 h after injection ($F = 12.12$, $p < 0.001$, Fig. 2B). The LPS-induced activation of the HPA-axis was further documented by a significant elevation in the ACTH plasma levels 3 h after injection ($F = 3.23$, $p = 0.028$; Fig. 2C).

Norepinephrine plays a pivotal role as hormone, neurotransmitter and modulator of immune functions (Besedovsky & del Rey, 1996). Therefore, we analyzed the levels of circulating norepinephrine and observed a significant increase shortly after the LPS injection, indicating an activation of the sympathetic nervous system, followed by a profound decline ($F = 3.70$, $p = 0.017$; Fig. 2D).

Plasma prolactin levels were analyzed before and 3 h after the LPS administration. However, prolactin levels remained unaffected by the endotoxin treatment ($F = 1.87$, $p = 0.186$; Fig. 3).

The neuropsychological performance in subjects who received LPS or a vehicle injection was analyzed with the Wechsler Memory Scale-R (WMS-R), which measures verbal and non-verbal short- and long-term memory using five different subscales. The test was done 3 h after LPS injection. Endotoxin treatment with the subsequent increase in the levels of cytokines, norepinephrine, ACTH and cortisol did not affect the overall mean performance (global memory score, $p = 0.987$) nor did it affect the four WMS-R subscales analyzing performance in attention ($p = 0.930$), verbal memory ($p = 0.599$), visual memory ($p = 0.585$), and delayed reproduction ($p = 0.970$; Fig. 4A). In addition to the WMS-R, the Stroop color test was perfomed before and 1.5 h after LPS injection to test for cognitive processing. The data showed no significant difference between the two treatment groups (vehicle vs. control) nor between the time points before and 1.5 h after endotoxin administration ($F = 0.17$, $p = 0.688$), indicating that the LPS-induced increases in cytokine and neuroendocrine parameters did not affect memory functions, attention or cognitive processing.

In order to analyze the interrelations between the studied neuropsychological parameters and the levels of peripheral hormones and cytokines, we conducted correlation analyses and observed a pronounced correlation in the LPS group between the WMS-R

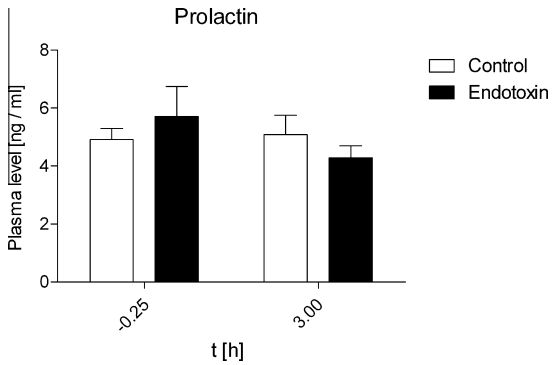

Fig. 3. Plasma concentration of prolactin in healthy male subjects following administration of 0.4 ng/kg *E. coli* endotoxin ($n = 12$) or saline ($n = 12$). Data are presented as means ± SEM.

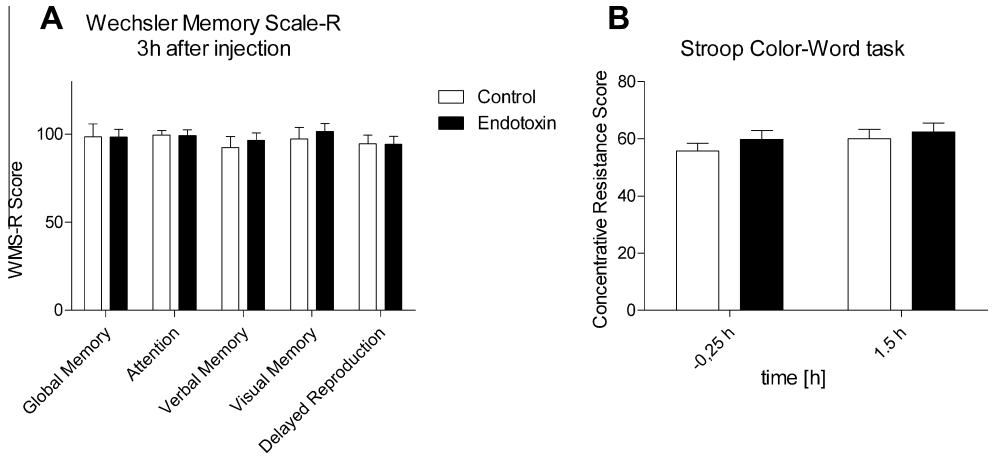

Fig. 4. Results of the revised Wechsler Memory Scale (WMS-R) for global memory and the four subscales (attention, verbal memory, visual memory and delayed reproduction), and the concentrative resistance scores calculated from the Stroop color word task following administration of 0.4 ng/kg *E. coli* endotoxin ($n = 12$) or saline ($n = 12$). Data are presented as means ± SEM.

attention subscale scores and the plasma concentration of prolactin (Pearson $r = 0.904$; $p < 0.001$; Fig. 5). This correlation was not observed in the control group (Pearson $r = -0.438$; $p = 0.278$, data not shown). We did not find any other significant correlations between neuropsychological performance and hormone or cytokine concentrations in the peripheral blood, neither in the LPS nor in the control group.

4. Discussion

A number of studies in experimental animals together with observations in humans suggest that there is a cytokine-mediated decrease in memory performance and cognitive functions as a consequence of acute peripheral inflammatory processes (Aubert, Vega, Dantzer, & Goodall, 1995; Gibertini, 1998; Pugh et al., 1998). However, only a small number of studies employed experimental models of acute systemic inflammation to address this issue in humans, with inconsistent results (Brydon et al., 2008; Cohen et al., 2003; Krabbe et al., 2005; Reichenberg et al., 2001). In this study, we employed a human endotoxemia model in healthy subjects and analyzed their cognitive performance during the peak of the innate immune response. Despite the pronounced increase in body temperature, and the pro- and anti-inflammatory cytokine, total cortisol, ACTH and norepinephrine levels in the plasma and free cortisol levels in the saliva, no changes in memory performance, attention and executive control processes were observed during the inflammatory response.

Intravenous administration of LPS is a robust and well-tested model for examinations of the innate immune response in humans

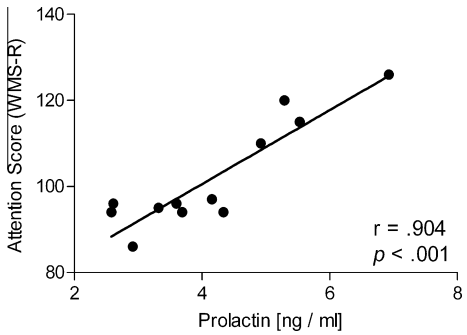

Fig. 5. Correlation analysis between plasma prolactin concentration 3 h after endotoxin administration and the attention subscale score of the revised Wechsler Memory Scale (WMS-R) 3 h after endotoxin administration ($n = 12$).

(Bahador & Cross, 2007). In the experiment described here, we observed pronounced increases in plasma levels of the pro-inflammatory cytokines IL-6 and TNF-α and the anti-inflammatory cytokine IL-10 during the first 3 h after LPS administration. These increases in cytokine levels were accompanied by an increased number of circulating neutrophil granulocytes, which are known to be an essential part of the innate immune system and one of first-responders of inflammatory cells. In parallel, body temperature and plasma levels of ACTH and cortisol were significantly elevated, indicating their reception and a reaction by the CNS to the LPS-induced immune activation. This data is in line with the findings of Reichenberg et al. (2001), who reported on the effects of peripheral immune activation on neuropsychological performance, employing a similar model of immune stimulation. By contrast, we did not observe any effects of the low-grade inflammatory response on memory performance, attention, or executive functions in our study. One possible reason for this discrepancy might be the use of different immune stimuli, since Reichenberg et al. (2001) used LPS derived from another species (*Salmonella abortus equi*). It is known that the host response is affected by the type, preparation and batch of LPS (Asai, Makimura, Kawabata, & Ogawa, 2007; Bahador & Cross, 2007). In addition, the dosage of LPS might be crucial; in that study, the subjects received 0.8 ng LPS/kg body weight, compared to 0.4 ng in our experiment. It might be possible that significant effects on higher brain functions do not appear before a certain threshold of immune activation is reached. Indeed, another study employing a lower dose (0.2 ng/kg) of *E. coli* LPS could not reproduce the effects on memory and cognition (Krabbe et al., 2005). We compared the data of our study with those from Krabbe et al. (2005) and Reichenberg et al. (2001) in order to find possible dose–response relationships for body temperature, cell count and hormone and cytokine levels across these studies, which could provide a hint towards a threshold to be reached to impact cognitive functions. Whereas Reichenberg et al. (2001) observed an even less pronounced increase in body temperature, employing a dosage of 0.8 ng LPS/kg, the peak levels of TNF-α, IL-6, and plasma cortisol are comparable to those observed in the current study. Remarkably, the relative changes in TNF-α and IL-6 levels in the study of Krabbe et al. (2005) induced by a dosage of 0.2 ng/kg were by the power of 10 smaller then in our and Reichenberg's study. These findings do not support the hypothesis that IL-6 and TNF-α are mainly responsible for the changes in neuropsychological parameters after LPS administration. In contrast to cytokine levels and body temperature, the increase in circulating neutrophils numbers is comparable between the study of Krabbe et al. (2005)

and the elevation reported in our study. Unfortunately Reichenberg et al. (2001) do not provide neutrophil or other leukocyte counts.

A strong correlation between the circulating levels of IL-6 and the reaction time in a computerized version of the Stroop color word task was reported when subjects received an intramuscular injection of typhoid vaccine. However, there was no significant effect of vaccination on the subject's reaction time (Brydon et al., 2008).

In this study, cognitive performance was analyzed in parallel with previous studies at two time points, within the time window of the most pronounced release of cytokines and endocrine variables. However, it is possible that the neuropsychological effects occur delayed or even independently from the cytokine concentrations in the circulation. This is supported by observations reporting a performance-enhancing effect on working memory 1–9 h after LPS (*E. coli* LPS; 0.8 ng/kg) administration (Cohen et al., 2003). Furthermore it needs to be pointed out, that at 3 h after injection, the time point, when the WMS-R was performed, the levels of TNF-α and norepinephrine already decreased. However, this observation cannot explain the discrepancy between the study of Reichenberg et al. (2001) and the current one, since Reichenberg observed memory impairments up to 9 h after the injection of LPS, when TNF-α and most likely also norepinephrine had turned back to baseline. In addition, it needs to be mentioned that the current study design with a sample size of 12 might have prevented the detection of small effects due to a substantial inter-individual variation. The WMS-R assesses parameters such as global memory score, attention, verbal memory, visual memory and delayed reproduction and the Stroop task assesses high-demand cognitive processing, including attentional and executive control processes, none of which were affected by the transient inflammatory process. However, there was no baseline assessment for the WMS-R and the tests we employed in this study had not the capability to effectively distinguish between short-, medium-, and long-term memory or specific impacts on encoding, consolidation, and recall of memory contents.

We did not observe significant correlations between cytokine, cortisol, ACTH or norepinephrine concentrations and neuropsychological performance parameters. However, we found a profound correlation between performance on the attention subscale of the WMS-R and circulating prolactin levels (Fig. 5). The prolactin system is strongly connected to the dopaminergic system and exhibits actions on a number of physiological and psychological processes (Ben-Jonathan & Hnasko, 2001; Grattan & Kokay, 2008; Sobrinho, 1993). The hypothesis that the immune system communicates with the brain to some extent via the prolactinergic/dopaminergic system is supported by the findings of an fMRI study demonstrating altered substantia nigra activity after experimental immune activation in healthy human subjects (Brydon et al., 2008). In addition, it was demonstrated that brain dopamine levels and prolactin secretion are modulated by peripheral cytokines and vice versa in rodents (De Laurentiis et al., 2002; Engler et al., 2009).

The increased secretion of cortisol and ACTH, with peak levels comparable to those observed during acute psychosocial stress (Fries, Hellhammer, & Hellhammer, 2006), indicate HPA-axis activation and a central stress response. Elevated cortisol concentrations can affect memory and other cognitive functions through glucocorticoid receptors in the hippocampus: cortisol impairs memory retrieval but also can enhance memory encoding (de Quervain, Aerni, Schelling, & Roozendaal, 2009; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Wolf, 2009). Since the neuropsychological tests employed were not capable to distinguish between these stages, a possible explanation for the absence of changes in memory performance could be that beneficial effects and impairments caused by cortisol compensated each other in this study. Another reason why the cortisol increase did not affect neuropsychological performance in this study might be that in the former studies, the cortisol increases were either caused by psychosocial stress, which could have more pronounced effects on neuropsychological performance than immunogenic stress, or by the application of exogenous cortisol leading to higher salivary concentrations of cortisol than those observed here (Kirschbaum et al., 1996). In this study, norepinephrine levels significantly increased in the early phase of the LPS response with a time course and peak levels similar to those observed during acute stress (Childs & de Wit, 2009; Wirtz, Ehlert, Bartschi, Redwine, & von Kanel, 2009), indicating an inflammation-induced activation of the sympathetic nervous system. In experimental animals, norepinephrine administration enhanced memory for emotional events and antagonizing beta-adrenoceptors impaired emotion-related declarative memory in humans (Cahill, Prins, Weber, & McGaugh, 1994; McGaugh, 1989; Rasch et al., 2009; Scullion, Kendall, Sunter, Marsden, & Pardon, 2009; Segal & Cahill, 2009). These neuroendocrine alterations together with the observation of enhanced working memory after LPS application (Cohen et al., 2003) support the idea of a broad network of multiple endocrine and immune-borne factors which affect the CNS in a complex and differential way instead of in a simple cause-and-effect chain from inflammation to impaired memory. This hypothesis should be tested in further experiments.

In summary, LPS administration in healthy male subjects induced a transient inflammatory response characterized by pronounced humoral and cellular immune changes, neuroendocrine activation and increased body temperature However, the changes in cytokine concentrations and endocrine variables did not affect cognitive or memory performance in healthy humans. Nonetheless, the strong correlation between attention and peripheral plasma concentration of prolactin suggests a link between the inflammatory response and neuropsychological performance via the prolactinergic/dopaminergic system. Future studies should use alternating dosages of LPS, and involve an extended time frame for cognitive assessment particularly focusing on the role and interaction of specific cytokines and/or neuroendocrine variables on cognitive performance. The effects of transient inflammatory responses on neuropsychological performance and on the role of peripheral cytokines in neuropsychiatric disorders in general have to be analyzed.

5. Conflicts of interest

The authors declare that they have no financial conflicts of interest.

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