Digging deeper in the differential effects of inflammatory and psychosocial stressors in remitted depression : effects on cognitive functioning

Digging deeper in the differential effects of inflammatory and psychosocial stressors in remitted depression: Effects on cognitive functioning

Peter Niemegeers [a,b]; Peter De Boer [a,c]; Jeroen Schuermans [a,d]; Glenn J.H. Dumont [a,e]; Violette Coppens [a,d]; K Spittaels [c], Stephan J. Claes [g]; Bernard G.C. Sabbe[a,d*], Manuel Morrens [a,d*]

a. Collaborative Antwerp Psychiatric Research Institute (CAPRI)
Faculty of Medicine and Health Sciences
University of Antwerp
Universiteitsplein 1
2610 Antwerp, Belgium
b. Department of psychiatry
ZNA Hospitals
Lange Beeldekensstraat 267
2060 Antwerp, Belgium
c. Janssen Research & Development, a division of Janssen Pharmaceutica N.V.
Turnhoutseweg 30
2340 Beerse, Belgium
d. Psychiatric Hospital Duffel
Stationsstraat 22C
2570 Duffel, Belgium
e. Leiden University Medical Centre
Albinusdreef 2
2333 ZA Leiden, The Netherlands
f. Antwerp University Hospital
Wilrijkstraat 10
2650 Edegem, Belgium
g. University Psychiatric Centre KU Leuven, campus Leuven
Herestraat 49
3000 Leuven, Belgium

* These authors contributed equally.

Corresponding author:
Peter Niemegeers
Collaborative Antwerp Psychiatric Research Institute (CAPRI)
Faculty of Medicine and Health Sciences
University of Antwerp – Campus "Drie Eiken"
Universiteitsplein 1
2610 Antwerp, Belgium
E-mail: peter.niemegeers@outlook.com

Contact information of the other authors:
Jeroen Schuermans: jeroen.schuermans@uantwerpen.be
Peter de Boer: pdeboer1@its.jnj.com
Glenn JH Dumont: glenn.dumont@gmail.com
Violette Coppens: violette.coppens@uantwerpen.be
Filip Van Den Eede: filip.van.den.eede@uza.be
Stephan J Claes: stephan.claes@uzleuven.be
Manuel Morrens: manuel.morrens@uantwerpen.be
Bernard GC Sabbe: bernard.sabbe@uantwerpen.be

Keywords: depression; recurrent depression; cognition; psychosocial stress; inflammation; typhoid vaccine; Trier Social Stress Test; vulnerability

Number of words in the abstract: 212
Number of words in the main text: 4227
Number of tables: 2
Number of figures: 0

Trial Registration: This study was registered in the ClinicalTrials.gov-database under the identifier *NCT01533285* (https://clinicaltrials.gov/ct2/show/NCT01533285) and the EudraCT-database under the identifier *2011-004898-80*.

Role of funding source: This work was supported with funding by Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Belgium.

Contribution of the individual authors

Peter Niemegeers helped with the design of the study, conducted the study, and prepared the manuscript.

Peter de Boer contributed with the design of the study and proofread the manuscript.

Jeroen Schuermans contributed in the preparation of the manuscript.

Glenn J.H. Dumont contributed in the conduction of the study and proofread the manuscript.

Violette Coppens helped in the preparation of the manuscript.

Filip Van Den Eede contributed in the recruitment of participants for the study.?

Stephan J Claes contributed in the design of the study, the conduction of the study, and proofread the manuscript. He was supervising coordinator of the study at the KU Leuven site.

Bernard GC Sabbe contributed in the design of the study, the conduction of the study, and proofread the manuscript. He was supervising coordinator of the study at the PZ Duffel site.

Manuel Morrens contributed to study design and had a main role in the preparation of the manuscript.

Declaration of interest

Peter Niemegeers received support from Janssen Pharmaceutica.

Peter de Boer

Jeroen Schuermans

Glenn J.H. Dumont

Violette Coppens

Filip Van Den Eede

Stephan J Claes

Bernard GC Sabbe

Manuel Morrens received support for research activities and scientific presentations from Janssen Pharmaceutica, Lundbeck and Astra Zeneca.

Abstract

Major Depressive Disorder (MDD) covers a wide spectrum of symptoms, including cognitive dysfunction, which can persist during remission. Both inflammatory states and psychosocial stress play a role in MDD pathogenesis. The effects of inflammatory (i.e. *Salmonella typhi* vaccine) and psychosocial stressor (i.e. Trier Social Stress Test), as well as their combination were investigated on cognition in women (aged 25-45 years, n = 21) with (partially remitted MDD) and healthy controls (n = 18) in a single-blind placebo-controlled study. In a crossover design, patients received on the first day one of the aforementioned interventions and on the other day a placebo, or vice versa, with a washout period of 7-14 days. Short-term and verbal memory, working memory, attention, verbal fluency, information processing speed, psychomotor function, and measures of attentional bias to emotions were measured. Exploratory analyses were performed to assess the effects of biomarkers of inflammation and the Hypothalamic-Pituitary-Adrenal axis on cognitive functioning. In patients, inflammatory stress decreased information processing and verbal memory, and increased working memory; after psychosocial stress, there was an increase in attention. There was also an increased negative attentional bias in patients after inflammatory stress. Neither stressor had any effect in controls. Patients were sensitive to the effects of inflammation and psychosocial stress on cognition, while controls were not.

Highlights

- Inflammatory stress decreases information processing speed and verbal memory in remitted MDD
- Inflammatory stress increased a negative attentional bias in remitted MDD
- Psychosocial stress increased attention in remitted MDD
- Patients are more sensitive to inflammatory and social stress, as no effects were found in controls

Main Text

1. Introduction

Major depressive disorder (MDD) is a complex and heterogeneous syndrome that covers a wide spectrum of symptoms, including cognitive dysfunction in two-thirds of depressed patients (Afridi et al., 2011). Cognitive deficits have typically been identified in the areas of executive functioning, visual learning and memory, attention, and psychomotor speed; and have been shown to influence psychosocial functioning (Rock et al., 2014). However, while during remission clinically relevant symptoms of low mood are absent, cognitive shortfall can persist in many patients who had cognitive impairments while being depressed (Reppermund et al., 2009; Rock et al., 2014). Moreover, each recurrent depressive episode induces an additional decline in global cognitive function (Kessing, 1998) and importantly, some measures (such as psychomotor speed and cognitive inhibition) are possibly irreversibly declined (Ardal and Hammar, 2011; Halvorsen et al., 2012).

In recent years, preclinical and clinical findings have emerged suggesting the involvement of the immune system in the pathophysiology of depression. In MDD patients, increased levels of peripheral inflammatory markers such as Interleukin-6 (IL-6) and Tumor Necrosis Factor-Alpha (TNF-α) have been found compared to healthy controls (Liu et al., 2017). Moreover, MDD is a common side effect of immune stimulating treatments such interferon-α (IFN-α), with up to 40% developing MDD (Schafer et al., 2007). Additionally, chronic inflammatory diseases (*e.g.,* rheumatoid arthritis, inflammatory bowel disease, and others) have a high comorbidity of depression (Ambriz Murillo et al., 2015; D'Mello and Swain, 2017; Pryce and Fontana, 2017). Indeed, several studies suggest that induction of an inflammatory response can trigger depressive symptoms. As such, several intervention studies with healthy subjects demonstrated increases in depressive symptomatology upon administration of endotoxin (Brydon et al., 2009; DellaGioia and Hannestad, 2010; Eisenberger et al., 2010; Eisenberger et al., 2009; Reichenberg et al., 2001; Strike et al., 2004) or the *Salmonella typhi* vaccine (Wright et al., 2005). Interestingly, immune stimulating treatments are associated with cognitive decline as well, as reviewed in Wichers and Maes (2002).

It has been shown that, in addition to inflammatory stimuli, prolonged psychological stress can also result in depressive symptoms (Anisman and Merali, 2003; Kendler et al., 1999). Interestingly, the detrimental effects of psychological stress on mood are suggested to be at least partially mediated through immunologic alterations (Anisman and Merali, 2003; Glaser and Kiecolt-Glaser, 2005). One potential mechanism underlying this phenomenon is stress-induced modulation of the hypothalamic-pituitary-adrenal (HPA) axis (Marques et al., 2009). Several components of this stress response pathway are known to enhance inflammatory stimuli (Sorrells et al., 2009). For instance, Corticotropin Releasing Hormone (CRH) stimulates the production of pro-inflammatory cytokines IL-6 and TNF-α (Angioni et al., 1993; Kato et al., 2013) and exposure to glucocorticoids sensitizes the neuro-inflammatory response to endotoxins (Frank et al., 2010). Lastly, peripheral blood mononuclear cells of patients with a depressive syndrome display reduced glucocorticoid receptor functioning in vitro (Pariante, 2004).

However, how inflammatory mediators and psychological stress interact to develop depressive episodes and cognitive deficits is not well understood. Moreover, it remains to be elucidated which specific cognitive domains are most sensitive to the effects of these stressors in subjects vulnerable for depressive disorders. Therefore, we investigated how experimental inflammatory and psychological challenges alone and in combination affect cognitive performance and inflammatory-related markers in remitted MDD patients and healthy individuals.

2. Methods

2.1 Participants

Twenty-one women with (partially) remitted moderate to severe recurrent MDD and 18 female controls aged 25–45 years were recruited. Inclusion criteria for both groups were a score below 15 on the Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979); being medically stable based on vital signs, clinical examination, and clinical laboratory tests (blood and urine sample); and having a body mass index (BMI) between 18–30 kg/m^2. Exclusion criteria were a current DSM-IV axis 1 diagnosis other than MDD, including substance abuse or dependence within the past 6 months (excluding nicotine and caffeine), treatment with more than one antidepressant or with drugs that compromise the immune system, acute suicidal behavior, a relevant medical history of disorders associated with increased inflammation, prior exposure to the psychosocial stress test, exposure to severe psychosocial stress within the past 6 months, and the administration of a typhoid vaccination within the last 5 years.

2.2 Study design

We conducted a two-way, randomized, single-blind, placebo-controlled crossover study to assess the effects of an inflammatory stressor, a psychosocial stressor, and their combination on cognition and their relationship with markers of the HPA-axis and inflammation. This study was part of a larger study design, the results of which can be found along a more detailed explanation of the study design in Niemegeers et al. (2016).

The study consisted of an eligibility screening examination, a run-in visit, two single-blinded intervention periods separated by a wash-out of 7-14 days, and a follow-up phone call 7-14 days after the last intervention day. Before the first intervention period, the Childhood Trauma Questionnaire (CTQ), a retrospective questionnaire of childhood trauma, was administered (Bernstein and Fink, 1997). On the run-in visit, the cognitive test battery was administered to obtain a baseline measurement.

The participants were randomized by the sponsor using a computer-generated randomization sequence to receive on an intervention (the inflammatory stressor, the psychosocial stressor, or the combination of both) or placebo. The *Salmonella typhi* vaccine was used as an inflammatory stressor and the Trier Social Stress Test (TSST) was used as a psychosocial stressor. The TSST was performed as described in Kirschbaum et al. (1993).

At the start of each intervention period, an alcohol breath test, a urine drug and a pregnancy screening test were performed. In addition, the MADRS was administered by trained blinded staff. If applicable, the TSST was administered at 12:00h. At 12:20h, either the typhoid vaccine (0.5 mL containing 25 µg *Salmonella typhi* capsular polysaccharide; Typhim® Vi, Sanofi Pasteur MSD, Diegem, Belgium) or a placebo (0.5 mL NaCl 0.9%), both transferred to a similar looking syringe, were injected. Throughout the testing periods, vital signs were monitored regularly. The participant left the study center after the last post-dose measurements.

2.3 Assessments

2.3.1 Cognition

The cognitive test battery was directed at the following cognitive domains: short-term and working memory, verbal fluency, verbal memory, sustained attention, psychomotor function, cognitive processing, and emotional interference on cognitive processing. The cognitive test battery consisted of two blocks, administered 3 and 4 h after the study drug administration, respectively.

2.4.2.1 Block 1

The *Digit Span Forward and Backward* are tests of short-term and working memory, respectively (Wechsler, 1997). The subject is asked to repeat an increasing sequence of numbers either forward or backward. The outcome measure is the number of correctly repeated sequences.

In the *Controlled Oral Word Association Test (COWAT)*, the participant is asked to sum up as many words of a certain category (two trials) or with the same starting letter (three trials) in one minute. The outcome measure was the sum of the scores on the five trials (Lezak et al., 2004).

The *Continuous Performance Test* (CPT) is a measure of sustained attention, where stimuli (either numbers or shapes) were shown on a screen. When two identical stimuli were presented in a row, the subject had to respond. The main outcome measure of the CPT is *dprime* (*d'*), a measure of attentional capacity (Cornblatt et al., 1988; Niemegeers et al., 2014). A secondary outcome measure is the reaction time to hits, which can be interpreted as a measure processing speed (Morrens et al., 2007).

2.4.2.2 Block 2

The *Hopkins Verbal Learning Test* (HVLT) is a test of verbal memory, consisting of a list of 12 words that should be memorized. Twenty minutes after three consecutive learning trials, the participant was asked to repeat the list. The number of correctly remembered words is the main outcome measure (Brandt and Benedict, 2001).

The *Symbol Digit Substitution Test* (SDST) is a measure of information-processing speed (Niemegeers et al., 2014; Wechsler, 1981). In this test, performed on a digitizing tablet, a series of symbols should be decoded as fast as possible, within a 90-s time limit, using a list of corresponding digit-symbol pairs (Morrens et al., 2006). Outcome measures are the number of correctly substituted digits, the matching time (i.e., time to find the corresponding digit) and the mean writing time (i.e., time to write the digit).

In the *Line Copying Test* (LCT), a straight line should be copied as quickly as possible from a computer screen to a form, which is placed on a digitizing tablet (Docx et al., 2012; Morrens et al., 2008; Niemegeers et al., 2014). The outcome measures are initiation time (i.e., the time to initiate the drawing) and movement time (i.e., the time to draw the line).

In the *Emotional Stroop*, the subject should name the color of the ink with which certain words are written as quickly as possible. Contrasting to the standard Stroop task, the words are either positive, neutral, or negative words. It has been well reported that depressed individuals show a negative bias towards negative stimuli (Clark et al., 2009; Roiser et al., 2012). The Emotional Stroop has been previously used to measure attentional bias (Dresler et al., 2009; Peckham et al., 2010). Ten negative, neutral and positive words were selected using a procedure based on Dresler et al. (2009). In a pilot study, 10 healthy volunteers were asked to rate the valence of a list of 300 words on a seven-point scale. The ten most negative words were selected, to which the ten most neutral and ten most positive words were matched taking word's frequency, function, length, and number of syllables into account, using data from the SUBTLEX-NL database (Keuleers et al., 2010). Ten rows of 10 words in the colors red, yellow, blue or green were printed on white paper. As such, there were 3 cards (a neutral, negative, and positive one). A practice card with only colors was administered first. The cards were presented in a randomized order and the subject had to name the colors as quickly as possible. The outcome measure is the time needed to read the whole card.

2.3.2 Biological markers

Pro-inflammatory markers (*i.e.*, interferon-γ [IFN-γ], TNF-α, and IL-6) and markers of the HPA-axis (*i.e.,* adrenocorticotropic hormone [ACTH] and cortisol) were measured before each testing block (*i.e.,* at 3h and 4h post-intervention). Cortisol and ACTH were analyzed using a Siemens® IMMULITE 2000 Immunoassay System at PRA International, Zuidlaren, the Netherlands, with following detection ranges: ACTH, 1.1 to 278 pmol/L; and cortisol, 28 to 1380 nmol/L. Inflammatory markers were analyzed at Janssen Biobank, Beerse, Belgium, using quantitative electrochemiluminescence immunoassays, namely the Meso Scale Discovery® V-PLEX Proinflammatory Panel 1 (human) kits, with following detection ranges: IFN-γ, 0.2-0.9 to 1060-1320 ng/L; TNF-α, 0.06-0.3 to 320-352 ng/L; and IL-6, 0.07-0.3 to 743-833 ng/L.

2.4 Statistics

Differences in demographics and baseline (i.e., cognitive performance on the run-in visit) between patients and controls were examined with an unpaired *Student's t*-test or the *Wilcoxon-Mann-Whitney*-test for non-normal data. The *chi-square* test was used for non-continuous variables.

The effect of the intervention was estimated using a linear mixed model. Both cohorts were analyzed separately. Dropouts were included in the analysis. The mixed model included as fixed effects administration of the typhoid vaccine (yes or no), TSST (yes or no), and the vaccine × TSST interaction. Baseline score of the run-in visit, treatment period, study center, body mass index, age, CTQ, pre-dose MADRS score, antidepressant use, and the number of education years were also included as fixed effects; subject was included as a random effect. A secondary analysis was performed to test any difference between groups. The same procedure was used in this secondary analysis, except that group (control or patient group) was also added as fixed effect with all the appropriate interactions. When a significant interaction was found, between-group comparisons were performed (e.g., between placebo and the three different treatments) using Bonferroni-correction. The estimated difference (β) between placebo and the intervention is reported with the 95% confidence interval (CI).

Exploratory analyses were performed to assess whether the effects of biomarker levels on cognitive performance were significantly different between the two groups. The Area Under the Curve (AUC) was calculated from the start of the measurements (pre-dose) until the time of the test (3h or 4h post-intervention). A linear mixed model was performed with the biomarker × group interaction as fixed effect, as well as biomarker, group, pre-dose biomarker concentration, baseline score of the run-in visit, treatment period, study center, body mass index, age, CTQ, pre-dose MADRS score, antidepressant use, and the number of education years. Subject was again included as a random effect. Only the biomarker × group interaction was examined. The estimated coefficient (β) is reported with the standard error and a t-test is done to examine if it significantly differs from zero.

3. Results

The baseline demographic characteristics and the results of the cognitive tests on baseline are summarized in **Table 1**.

3.1 Effect of the interventions

The effects of the interventions on cognition are summarized in **Table 2**. In controls, none of the interventions had any significant effect.

No effects were observed on short-term memory (measured in the Digit Span Forward). After the vaccine, an improvement of working memory (Digit Span Backward) was observed. The group × vaccine interaction was significant ($p = 0.046$).

While there was no effect on main outcome measure of the attention task (i.e., the Continuous Performance Task) in patients after vaccine, their reaction time worsened after the vaccine. Conversely, after the TSST, both attention and reaction time improved in patients. There were no significant group × intervention interactions.

There was a vaccine × TSST interaction on Verbal Fluency (measured in the Controlled Oral Word Association Task), with a non-significant lowering after both the vaccine ($\beta \pm 95\%$ CI = -1.90 ± 9.06) and the TSST ($\beta \pm 95\%$ CI = -9.26 ± 9.47), and a non-significant increase after the combination intervention ($\beta \pm 95\%$ CI = 10.30 ± 10.18). While the group × vaccine × TSST interaction did not reach significance ($p = 0.078$), there was a significant group × TSST interaction ($p = 0.039$). Examination of the separate groups showed a significant increase in score in patients after the TSST ($\beta \pm 95\%$ CI = 8.44 ± 7.32, $p = 0.050$) and a non-significant decrease in controls ($\beta \pm 95\%$ CI = -2.57 ± 7.41). While this is a seemingly conflicting result, the increase after TSST in this analysis can be explained by the increase after the combination treatment in patients.

After the vaccine, a decrease in verbal memory was observed in patients, but not in controls. Patients reacted significantly different on the vaccine than controls ($p = 0.045$). The TSST had no effect.

Information processing (the number correct answers on the SDST) decreased in patients after the vaccination, though this effect was not seen in the matching time. The TSST had no effects and patients did not react significantly different on the intervention compared to controls. Neither the vaccine or the TSST had any effect on psychomotor function (namely the LCT and the writing time of the SDST).

After the vaccine, there was an increase in time to read the colors of negative words in the Emotional Stroop, suggesting an increased interference of negative words after inflammatory stress. The group × vaccine interaction did not reach significance though ($p = 0.089$). No significant effects were seen in the neutral and positive words. While no significant reaction of the TSST were seen in both groups, there was a significant group × TSST interaction ($p = 0.026$) in the time to read the colors of negative words, reflecting a non-significant decrease in time in patients and a non-significant increase in controls.

3.2 Effect of biomarker levels on cognition

There were no significant biomarker × group interactions for Digit Span (forward and backward), CPT, COWAT, and HVLT.

There was a ACTH AUC × group interaction ($p = 0.009$) for the matching time of the SDST, with a significant relation between ACTH AUC in patients ($\beta \pm SE = 0.28 \pm 0.09$, $p = 0.005$) but not in controls ($\beta \pm SE = 0.28 \pm 0.09$, p = not significant [n.s.]). There were no significant interactions for the other biomarkers and the matching time. No biomarker × group interactions were observed in the other outcome variables of the SDST (number correct and writing time).

Although there was an ACTH AUC × group interaction for the initiation time of the LCT ($p = 0.046$), the estimated coefficients did not differ significantly from zero (patients, $\beta \pm SE = 0.06 \pm 0.05$, p = n.s.; controls, $\beta \pm SE = -0.12 \pm 0.09$, p = n.s.). A similar pattern is seen for the TNF-α × group interaction ($p = 0.041$), with none of the estimated coefficients significantly differing from zero (patients, $\beta \pm SE = 0.00 \pm 0.28$, p = n.s.; controls, $\beta \pm SE = -0.34 \pm 0.29$, p = n.s.). No interactions were observed for the other three biomarkers, neither was an interaction between any biomarker and the movement time of the LCT observed.

The interaction between IL-6 AUC and group was significant for the reading time of the negative words of the Emotional Stroop ($p = 0.047$). While the estimated coefficient was not significant in controls ($\beta \pm SE = -0.005 \pm 0.005$, p = n.s.), it was for patients ($\beta \pm SE = 0.007 \pm 0.003$, $p = 0.013$), suggesting and increased reading time with increasing IL-6 AUC. No interactions were found for the other biomarkers. No interaction between group and any biomarker was found for the reading time of the positive and neutral words.

4. Discussion

Numerous preclinical and clinical studies point towards the involvement of inflammatory mechanisms in the pathophysiology of depression (Liu et al., 2017). Besides affective symptoms, chronic inflammation is also associated with cognitive symptoms such as impaired memory, decreased motivation and confusion (Dantzer, 2009). This is in line with the findings of the current study, which shows a decrease in information processing and verbal memory following the administration of the typhoid vaccine, in remitted patients but not in healthy controls. Conversely, an increase in working memory was also observed in the patient group after the vaccine. As remitted patients had comparable cognitive scores compared to controls, these results suggest that an induced pro-inflammatory state affects cognitive functions in patients that had previously suffered a depressive episode, but not in their healthy peers.

Previous studies suggest that healthy subjects who received a therapeutic dose of pro-inflammatory cytokines such as IFN-α or TNFα, reported neuropsychiatric side effects including impaired thought processing next to depressed mood (Licinio et al., 1998). Cancer and Hepatitis C patients receiving IFN-α therapy develop not only depression as a side effect but also cognitive dysfunction (Capuron and Miller, 2004; Scheibel et al., 2004). Although we did not find these cognitive alterations in our control sample, they were clearly observable in subjects who previously suffered a depressive episode currently in remission. The changes in verbal memory, attention and information speed in the remitted patients in our study are in line with the findings in healthy controls of Krabbe et al. (2005) and Bollen et al. (2017) although these alterations were not always found (Bollen et al., 2017).

A possible mechanism through which inflammatory stress alters cognition is through the *kynurenine-pathway*. Tryptophan is metabolized to serotonin and kynurenine (KYN). KYN is further metabolized through two different pathways to quinolinic acid (QUIN) and kynurenic acid (KYNA). QUIN shows neurotoxic effects by increasing oxidative stress (through its metabolite 3-hydroxykynurenine) and by being an NMDA-receptor-agonist. KYNA, on the other hand, is an antagonist of both the NMDA-

receptor and the α-7 nicotinic acetylcholine receptor (α7nAchR), both known to play an important role in the regulation of cognitive functioning. Pro-inflammatory cytokines, such as IFN-γ, stimulate a shift of tryptophan towards this pathway, with increased concentration of QUIN and KYNA, and decreased concentrations of serotonin. For a thorough review of the interaction between inflammation, the kynurenine-pathway, and cognition, we refer to Allison and Ditor (2014).

The finding that working memory was increased is of unclear significance. This effect was also observed by Cohen et al. (2003) and (Ofek et al., 2007). These authors suggest that a subgroup of patients react on inflammatory stress with reductions of the acetylcholine degrading enzyme Acetylcholine Esterase (AchE), reflecting an increase in acetylcholine associated with increased condition. At the other hand, a subgroup also had increased concentration of AchE, with reduced cognition. As such, this suggest that there are different types of response to inflammation, reflecting also different responses on several subdomains of cognition.

The inflammatory stressor leads to an increase in reading time of the colors of negative words in the Emotional Stroop test. This effect was not observed in the neutral and positive words. This suggests that there is an increased negative attentional bias in patients following inflammatory stress. While the response was not significantly different from controls, there was a trend. Moreover, there was a correlation between IL-6 and the time to read, only in patients, in which the patients did differ significantly from controls. This is in line with a previous study, which saw a correlation between inflammation and negative emotional bias in breast cancer survivors (Boyle et al., 2017). It should be noted that the analyses of biomarkers and cognition were mainly exploratory, and replication is necessary. Previous studies showed effects of inflammation on social/emotional processing (namely: reduced perception of emotions, increased avoidance of punishment/loss experiences, and increased social disconnectedness) (Bollen et al., 2017). As depression is associated with negative emotional bias (Baert et al., 2010), which is reversible by antidepressants (Godlewska et al., 2016), the observed effect of inflammation on negative attentional bias further confirms inflammatory processes play a significant part in the pathogenesis of MDD.

In our study, the TSST was associated with increased attention in patients, but not in controls. In previous studies on healthy controls, the effects of psychosocial stress on cognition are contradictory, with some studies (Nater et al., 2007; Smeets et al., 2006), but not all (Kuhlmann et al., 2005; Oei et al., 2006), reporting increased performance. In previous studies cortisol responses to the TSST (and as such, increased HPA axis activation) have indeed been associated with improvements in cognition (Nater et al., 2007; Plieger et al., 2017). In the study of Plieger et al. (2017) it is suggested that acute stress enhances selective attention as cognitive resources are limited and used optimally in stress situation. Indeed, acute psychosocial stress increased the attention in our sample. Interestingly, ACTH was negatively correlated to information processing, a more complex cognitive task, which may be in line with the notion of stress optimizing limited cognitive resources.

It should also be noted that the acute psychosocial stressor reduces pro-inflammatory cytokines in this study (Niemegeers et al., 2016). As such, this improvement may be due to a reduction of inflammation. The effects of psychosocial stress can also be interpreted as an arousal effect, leading to increased vigor and thus increasing performance on cognitive tasks. Indeed, the TSST increased wakefulness in another study (Kudielka et al., 2004), though this effect was not seen in the present study (Niemegeers et al., 2016).

As mentioned before, it has been shown that psychological stress results in negative mood symptoms in depressed patients (Hammen et al., 2009) as well as in healthy humans (Steptoe et al., 2007). Interestingly however, psychological stress exacerbated the deterioration of mood following administration of typhoid vaccine in healthy subjects, which suggests that inflammatory stress and psychological stress may have an accumulating effect on mood and possibly cognitive symptoms (Wright et al., 2005). In line with these findings, we investigated whether the combination of psychological and inflammatory stressors may have an accumulating effect on cognitive functioning. Although we demonstrated some mild effect of the combination of both stressors on verbal learning and memory which was not seen by either stressor alone, none of the other cognitive domains seemed

to be affected by the combined stressors. Our findings thus partly argue against the hypothesis of accumulating effects of both stressors on cognitive functions in remitted subjects.

Our study has several limitations. The main limitation is the relatively small sample size. Although some of the mentioned effects are in line with previous observations in a healthy population in the literature, we observed them only in the patient group. This may reflect that the study is possibly underpowered to find small effects in the control population, but it does suggest that the patient group is more sensitive to the effects of inflammatory and psychosocial stress, compared to controls. Another confounding factor is that the majority of the patients took antidepressants, which are known to normalize pro-inflammatory states and reduce negative attentional bias (Godlewska et al., 2016; Kenis and Maes, 2002). Additionally, the effects of the interventions were only examined in a short time frame (6 hours), potentially masking other and stronger effects later in time. Although a correlation between biomarkers and cognitive measures was found, it does not provide conclusive evidence that these biomarkers mediate direct changes in cognition. As expression levels of cytokine are highly correlated, it remains to be determined what their specific roles are.

In conclusion, patients were sensitive to the effect of both inflammatory and psychosocial stress. Inflammatory stress reduced information processing speed and verbal memory, but increased working memory. It increased a negative attentional bias. Psychosocial stress increased on the other hand attention. Controls were not sensitive to the effects of both stressors.

Acknowledgements

We thank Silke Apers, Dr. Titia Hompens, Els Huyghe, Joke Lauwers, Mona Laureys, Sieglinde Meganck, Dr. Tim Moons, and Sara Vermeylen for their help in conducting the study. We are grateful to Magdaleen De Breuckere, Dr. Carmen De Grave, Dr. Iris De Mulder, Dr. Esther Maris, Dr. Charel Quisenaerts, and Dr. Jos Verbelen for their help in the recruitment of participants. We also thank Iris De Mulder, Katrien Enzlin, Jasper Truyens, and Seline Van Den Ameele for repeatedly making time to play the critical audience in the Trier Social Stress Test. We are also grateful to the Clinical Pharmacological Unit of Merksem (Antwerp), Belgium, and the team from Janssen Pharmaceutica for their practical and technical support. And last but not least, we would like to thank the participants of this study.

References

Afridi, M.I., Hina, M., Qureshi, I.S., Hussain, M., 2011. Cognitive disturbance comparison among drug-naive depressed cases and healthy controls. Journal of the College of Physicians and Surgeons--Pakistan : JCPSP 21, 351-355.

Allison, D.J., Ditor, D.S., 2014. The common inflammatory etiology of depression and cognitive impairment: a therapeutic target. J Neuroinflammation 11, 151.

Ambriz Murillo, Y., Menor Almagro, R., Campos-Gonzalez, I.D., Cardiel, M.H., 2015. Health related quality of life in rheumatoid arthritis, osteoarthritis, diabetes mellitus, end stage renal disease and geriatric subjects. Experience from a General Hospital in Mexico. Reumatologia clinica 11, 68-72.

Angioni, S., Petraglia, F., Gallinelli, A., Cossarizza, A., Franceschi, C., Muscettola, M., Genazzani, A.D., Surico, N., Genazzani, A.R., 1993. Corticotropin-releasing hormone modulates cytokines release in cultured human peripheral blood mononuclear cells. Life sciences 53, 1735-1742.

Anisman, H., Merali, Z., 2003. Cytokines, stress and depressive illness: brain-immune interactions. Annals of medicine 35, 2-11.

Ardal, G., Hammar, A., 2011. Is impairment in cognitive inhibition in the acute phase of major depression irreversible? Results from a 10-year follow-up study. Psychology and psychotherapy 84, 141-150.

Baert, S., De Raedt, R., Koster, E.H.W., 2010. Depression-related attentional bias: The influence of symptom severity and symptom specificity. Cognition and Emotion 24, 1044-1052.

Bernstein, D.P., Fink, L., 1997. Childhood trauma questionnaire: a retrospective self-report. Pearson, San Antonio, TX.

Bollen, J., Trick, L., Llewellyn, D., Dickens, C., 2017. The effects of acute inflammation on cognitive functioning and emotional processing in humans: A systematic review of experimental studies. J Psychosom Res 94, 47-55.

Boyle, C.C., Ganz, P.A., Van Dyk, K.M., Bower, J.E., 2017. Inflammation and attentional bias in breast cancer survivors. Brain Behav Immun 66, 85-88.

Brandt, J., Benedict, R., 2001. Hopkins Verbal Learning Test-Revised: Professional Manual. PAR, Inc., Lutz, FL.

Brydon, L., Walker, C., Wawrzyniak, A., Whitehead, D., Okamura, H., Yajima, J., Tsuda, A., Steptoe, A., 2009. Synergistic effects of psychological and immune stressors on inflammatory cytokine and sickness responses in humans. Brain, behavior, and immunity 23, 217-224.

Capuron, L., Miller, A.H., 2004. Cytokines and psychopathology: lessons from interferon-alpha. Biological psychiatry 56, 819-824.

Clark, L., Chamberlain, S.R., Sahakian, B.J., 2009. Neurocognitive mechanisms in depression: implications for treatment. Annu Rev Neurosci 32, 57-74.

Cohen, O., Reichenberg, A., Perry, C., Ginzberg, D., Pollmacher, T., Soreq, H., Yirmiya, R., 2003. Endotoxin-induced changes in human working and declarative memory associate with cleavage of plasma "readthrough" acetylcholinesterase. J Mol Neurosci 21, 199-212.

Cornblatt, B.A., Risch, N.J., Faris, G., Friedman, D., Erlenmeyer-Kimling, L., 1988. The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. Psychiatry research 26, 223-238.

D'Mello, C., Swain, M.G., 2017. Immune-to-Brain Communication Pathways in Inflammation-Associated Sickness and Depression. Current topics in behavioral neurosciences 31, 73-94.

Dantzer, R., 2009. Cytokine, sickness behavior, and depression. Immunology and allergy clinics of North America 29, 247-264.

DellaGioia, N., Hannestad, J., 2010. A critical review of human endotoxin administration as an experimental paradigm of depression. Neuroscience and biobehavioral reviews 34, 130-143.

Docx, L., Morrens, M., Bervoets, C., Hulstijn, W., Fransen, E., De Hert, M., Baeken, C., Audenaert, K., Sabbe, B., 2012. Parsing the components of the psychomotor syndrome in schizophrenia. Acta psychiatrica Scandinavica 126, 256-265.
Dresler, T., Meriau, K., Heekeren, H.R., van der Meer, E., 2009. Emotional Stroop task: effect of word arousal and subject anxiety on emotional interference. Psychological research 73, 364-371.
Eisenberger, N.I., Inagaki, T.K., Mashal, N.M., Irwin, M.R., 2010. Inflammation and social experience: an inflammatory challenge induces feelings of social disconnection in addition to depressed mood. Brain, behavior, and immunity 24, 558-563.
Eisenberger, N.I., Inagaki, T.K., Rameson, L.T., Mashal, N.M., Irwin, M.R., 2009. An fMRI study of cytokine-induced depressed mood and social pain: the role of sex differences. NeuroImage 47, 881-890.
Frank, M.G., Miguel, Z.D., Watkins, L.R., Maier, S.F., 2010. Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to E. coli lipopolysaccharide. Brain, behavior, and immunity 24, 19-30.
Glaser, R., Kiecolt-Glaser, J.K., 2005. Stress-induced immune dysfunction: implications for health. Nature reviews. Immunology 5, 243-251.
Godlewska, B.R., Browning, M., Norbury, R., Cowen, P.J., Harmer, C.J., 2016. Early changes in emotional processing as a marker of clinical response to SSRI treatment in depression. Translational Psychiatry 6, e957.
Halvorsen, M., Hoifodt, R.S., Myrbakk, I.N., Wang, C.E., Sundet, K., Eisemann, M., Waterloo, K., 2012. Cognitive function in unipolar major depression: a comparison of currently depressed, previously depressed, and never depressed individuals. Journal of clinical and experimental neuropsychology 34, 782-790.
Hammen, C., Kim, E.Y., Eberhart, N.K., Brennan, P.A., 2009. Chronic and acute stress and the prediction of major depression in women. Depression and anxiety 26, 718-723.
Kato, T.A., Hayakawa, K., Monji, A., Kanba, S., 2013. Missing and Possible Link between Neuroendocrine Factors, Neuropsychiatric Disorders, and Microglia. Front Integr Neurosci 7, 53.
Kendler, K.S., Karkowski, L.M., Prescott, C.A., 1999. Causal relationship between stressful life events and the onset of major depression. The American journal of psychiatry 156, 837-841.
Kenis, G., Maes, M., 2002. Effects of antidepressants on the production of cytokines. Int J Neuropsychopharmacol 5, 401-412.
Kessing, L.V., 1998. Cognitive impairment in the euthymic phase of affective disorder. Psychol Med 28, 1027-1038.
Keuleers, E., Brysbaert, M., New, B., 2010. SUBTLEX-NL: a new measure for Dutch word frequency based on film subtitles. Behavior research methods 42, 643-650.
Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76-81.
Krabbe, K.S., Reichenberg, A., Yirmiya, R., Smed, A., Pedersen, B.K., Bruunsgaard, H., 2005. Low-dose endotoxemia and human neuropsychological functions. Brain Behav Immun 19, 453-460.
Kudielka, B.M., Schommer, N.C., Hellhammer, D.H., Kirschbaum, C., 2004. Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. Psychoneuroendocrinology 29, 983-992.
Kuhlmann, S., Piel, M., Wolf, O.T., 2005. Impaired memory retrieval after psychosocial stress in healthy young men. The Journal of neuroscience : the official journal of the Society for Neuroscience 25, 2977-2982.
Lezak, M.D., Howieson, D.B., Loring, D.W., 2004. Neuropsychological Assessment, 4th ed. Oxford University Press, New York, NY.
Licinio, J., Kling, M.A., Hauser, P., 1998. Cytokines and brain function: relevance to interferon-alpha-induced mood and cognitive changes. Seminars in oncology 25, 30-38.

Liu, C.S., Adibfar, A., Herrmann, N., Gallagher, D., Lanctot, K.L., 2017. Evidence for Inflammation-Associated Depression. Current topics in behavioral neurosciences 31, 3-30.
Marques, A.H., Silverman, M.N., Sternberg, E.M., 2009. Glucocorticoid dysregulations and their clinical correlates. From receptors to therapeutics. Annals of the New York Academy of Sciences 1179, 1-18.
Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. Br J Psychiatry 134, 382-389.
Morrens, M., Hulstijn, W., Lewi, P., Sabbe, B., 2008. Bleuler revisited: psychomotor slowing in schizophrenia as part of a catatonic symptom cluster. Psychiatry Res 161, 121-125.
Morrens, M., Hulstijn, W., Sabbe, B., 2007. Psychomotor slowing in schizophrenia. Schizophr Bull 33, 1038-1053.
Morrens, M., Hulstijn, W., Van Hecke, J., Peuskens, J., Sabbe, B.G., 2006. Sensorimotor and cognitive slowing in schizophrenia as measured by the Symbol Digit Substitution Test. Journal of psychiatric research 40, 200-206.
Nater, U.M., Moor, C., Okere, U., Stallkamp, R., Martin, M., Ehlert, U., Kliegel, M., 2007. Performance on a declarative memory task is better in high than low cortisol responders to psychosocial stress. Psychoneuroendocrinology 32, 758-763.
Niemegeers, P., De Boer, P., Dumont, G.J.H., Van Den Eede, F., Fransen, E., Claes, S.J., Morrens, M., Sabbe, B.G.C., 2016. Differential Effects of Inflammatory and Psychosocial Stress on Mood, Hypothalamic-Pituitary-Adrenal Axis, and Inflammation in Remitted Depression. Neuropsychobiology 74, 150-158.
Niemegeers, P., Dumont, G.J., Quisenaerts, C., Morrens, M., Boonzaier, J., Fransen, E., de Bruijn, E.R., Hulstijn, W., Sabbe, B.G., 2014. The effects of nicotine on cognition are dependent on baseline performance. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 24, 1015-1023.
Oei, N.Y., Everaerd, W.T., Elzinga, B.M., van Well, S., Bermond, B., 2006. Psychosocial stress impairs working memory at high loads: an association with cortisol levels and memory retrieval. Stress 9, 133-141.
Ofek, K., Krabbe, K.S., Evron, T., Debecco, M., Nielsen, A.R., Brunnsgaad, H., Yirmiya, R., Soreq, H., Pedersen, B.K., 2007. Cholinergic status modulations in human volunteers under acute inflammation. J Mol Med (Berl) 85, 1239-1251.
Pariante, C.M., 2004. Glucocorticoid receptor function in vitro in patients with major depression. Stress (Amsterdam, Netherlands) 7, 209-219.
Peckham, A.D., McHugh, R.K., Otto, M.W., 2010. A meta-analysis of the magnitude of biased attention in depression. Depress Anxiety 27, 1135-1142.
Plieger, T., Felten, A., Diks, E., Tepel, J., Mies, M., Reuter, M., 2017. The impact of acute stress on cognitive functioning: a matter of cognitive demands? Cogn Neuropsychiatry 22, 69-82.
Pryce, C.R., Fontana, A., 2017. Depression in Autoimmune Diseases. Current topics in behavioral neurosciences 31, 139-154.
Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A., Pollmacher, T., 2001. Cytokine-associated emotional and cognitive disturbances in humans. Archives of general psychiatry 58, 445-452.
Reppermund, S., Ising, M., Lucae, S., Zihl, J., 2009. Cognitive impairment in unipolar depression is persistent and non-specific: further evidence for the final common pathway disorder hypothesis. Psychol Med 39, 603-614.
Rock, P.L., Roiser, J.P., Riedel, W.J., Blackwell, A.D., 2014. Cognitive impairment in depression: a systematic review and meta-analysis. Psychol Med 44, 2029-2040.
Roiser, J.P., Elliott, R., Sahakian, B.J., 2012. Cognitive mechanisms of treatment in depression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 37, 117-136.
Schafer, A., Wittchen, H.U., Seufert, J., Kraus, M.R., 2007. Methodological approaches in the assessment of interferon-alfa-induced depression in patients with chronic hepatitis C - a critical review. International journal of methods in psychiatric research 16, 186-201.

Scheibel, R.S., Valentine, A.D., O'Brien, S., Meyers, C.A., 2004. Cognitive dysfunction and depression during treatment with interferon-alpha and chemotherapy. The Journal of neuropsychiatry and clinical neurosciences 16, 185-191.
Smeets, T., Jelicic, M., Merckelbach, H., Peters, M., Fett, A., Taverniers, J., Henquet, C., Dautzenberg, J., 2006. Enhanced memory performance on an internal-internal source monitoring test following acute psychosocial stress. Behavioral neuroscience 120, 1204-1210.
Sorrells, S.F., Caso, J.R., Munhoz, C.D., Sapolsky, R.M., 2009. The stressed CNS: when glucocorticoids aggravate inflammation. Neuron 64, 33-39.
Steptoe, A., Hamer, M., Chida, Y., 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. Brain, behavior, and immunity 21, 901-912.
Strike, P.C., Wardle, J., Steptoe, A., 2004. Mild acute inflammatory stimulation induces transient negative mood. Journal of psychosomatic research 57, 189-194.
Wechsler, D., 1981. Manual for the Wechsler Adult Intelligence Scale-Revised. The Psychological Corporation, San Antonio.
Wechsler, D., 1997. Wechsler Adult Intelligence Scale-III. The Psychological Corporation, San Antonio.
Wichers, M., Maes, M., 2002. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. The international journal of neuropsychopharmacology 5, 375-388.
Wright, C.E., Strike, P.C., Brydon, L., Steptoe, A., 2005. Acute inflammation and negative mood: mediation by cytokine activation. Brain, behavior, and immunity 19, 345-350.

Table 1: Baseline demographic and clinical characteristics

	Mean (SD), unless specified otherwise		
Measure	**Patients (n = 21)**	**Controls (n = 18)**	**p-value**
Demographics			
Age	33.9 (7.02)	32.7 (6.65)	*n.s.*
BMI	24.0 (2.80)	22.4 (3.18)	*n.s.*
Education years	15.2 (2.40)	15.7 (2.97)	*n.s.*
Right-handedness (%)	85.0%	82.4%	*n.s.*
Antidepressant use (%)	66.7%	0.0%	< .001
Ethnicity (%):			*n.s.*
European descent	95.2%	88.9%	
Maghrebi descent	0.0%	11.1%	
African descent	4.8%	0.0%	
Clinical characteristics			
MADRS	6.38 (5.29)	0.64 (1.07)	< .001
Cognitive measures			
Digit Span:			
Forward	8.83 (1.948)	9.10 (2.189)	*n.s.*
Backward	6.72 (1.527)	6.76 (1.700)	*n.s.*
CPT:			
dprime	2.04 (0.862)	2.21 (0.794)	*n.s.*
Reaction Time (ms)	662 (85.3)	658 (78.4)	*n.s.*
COWAT			
HVLT	10.18 (1.704)	10.14 (1.493)	*n.s.*
SDST:			
Nr. Correct	63.53 (10.625)	61.81 (7.467)	*n.s.*
Matching Time (ms)	914 (216.1)	965 (171.6)	*n.s.*
Writing Time (ms)	471 (113.8)	491 (117.7)	*n.s.*
LCT:			
Initiation Time (ms)	712 (134.1)	746 (131.5)	*n.s.*
Movement Time (ms)	268 (73.3)	297 (164.7)	*n.s.*
Emotional Stroop:			
Neutral Words (s)	69.5 (10.26)	69.0 (14.53)	*n.s.*
Negative Words (s)	68.6 (10.82)	70.3 (14.66)	*n.s.*
Positive Words (s)	73.3 (11.49)	71.4 (16.92)	*n.s.*

BMI: body mass index; COWAT: Controlled Oral Word Association Test; CPT: Continuous Performance Test; HVLT: Hopkins Verbal Learning Test; LCT: Line Copying Test; MADRS: Montgomery-Åsberg Depression Rating Scale; *n.s.*: not significant; SDST: Symbol Digit Substitution Test.

Table 2: Results of the interventions

	Patiënt					Control				
	Vaccine		TSST		Vaccine × TSST	Vaccine		TSST		Vaccine × TSST
Measure	**β ± CI**	***p***	**β ± CI**	***p***	***p***	**β ± CI**	***p***	**β ± CI**	***p***	***p***
Digit Span										
Forward	-0.39 ± 0.90	*n.s.*	0.69 ± 0.90	*n.s.*	*n.s.*	0.60 ± 1.42	*n.s.*	0.69 ± 1.45	*n.s.*	*n.s.*
Backward	1.21 ± 0.96	0.016	-0.88 ± 0.95	0.069	*n.s.*	-0.40 ± 1.22	*n.s.*	0.65 ± 1.25	*n.s.*	*n.s.*
CPT										
dprime	-0.04 ± 0.41	*n.s.*	0.41 ± 0.41	0.049	*n.s.*	0.03 ± 0.46	*n.s.*	0.04 ± 0.48	*n.s.*	*n.s.*
reaction time	25.3 ± 21.8	0.026	-22.0 ± 10.2	0.049	*n.s.*	22.1 ± 34.0	*n.s.*	7.3 ± 34.5	*n.s.*	*n.s.*
COWAT	*	*	*	*	0.015	2.84 ± 8.01	*n.s.*	-4.15 ± 8.35	*n.s.*	*n.s.*
HVLT	-0.77 ± 0.77	0.049	0.08 ± 0.77	*n.s.*	*n.s.*	0.62 ± 1.18	*n.s.*	0.41 ± 1.23	*n.s.*	*n.s.*
SDST										
Nr. Correct	-3.02 ± 2.90	0.042	1.13 ± 2.87	*n.s.*	*n.s.*	0.50 ± 5.05	*n.s.*	-0.87 ± 5.07	*n.s.*	*n.s.*
Matching Time (ms)	53.8 ± 90.5	*n.s.*	17.4 ± 89.7	*n.s.*	*n.s.*	-28.3 ± 112.6	*n.s.*	-13.4 ± 119.0	*n.s.*	*n.s.*
Writing Time (ms)	22.3 ± 30.6	*n.s.*	-11.8 ± 30.9	*n.s.*	*n.s.*	-6.0 ± 49.5	*n.s.*	0.6 ± 51.1	*n.s.*	*n.s.*
LCT										
Initiation Time (ms)	15.9 ± 45.8	*n.s.*	-5.9 ± 46.0	*n.s.*	*n.s.*	13.7 ± 43.4	*n.s.*	-7.2 ± 44.8	*n.s.*	*n.s.*
Movement Time (ms)	-7.9 ± 40.4	*n.s.*	5.7 ± 40.9	*n.s.*	*n.s.*	-4.5 ± 39.5	*n.s.*	-8.4 ± 42.1	*n.s.*	*n.s.*
Emotional Stroop										
Neutral (s)	3.23 ± 5.52	*n.s.*	-2.82 ± 5.52	*n.s.*	*n.s.*	0.71 ± 3.84	*n.s.*	-3.01 ± 3.98	*n.s.*	*n.s.*
Negative (s)	5.51 ± 4.71	0.025	-3.47 ± 4.67	*n.s.*	*n.s.*	-0.32 ± 3.71	*n.s.*	3.56 ± 3.81	*n.s.*	*n.s.*
Positive (s)	0.01 ± 6.95	*n.s.*	-0.4 ± 6.93	*n.s.*	*n.s.*	-0.04 ± 5.73	*n.s.*	1.10 ± 5.91	*n.s.*	*n.s.*

*: if the vaccine × TSST interaction is significant, results of the single treatment groups have to be analyzed separately and are reported in the results section of the article

COWAT: Controlled Oral Word Association Test; CPT: Continuous Performance Test; HVLT: Hopkins Verbal Learning Test; LCT: Line Copying Test; *n.s.*: not significant; SDST: Symbol Digit Substitution Test.