Association between cytokine levels, verbal memory and hippocampus volume in psychotic disorders and healthy controls

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Abstract

Objective: We investigated whether elevated plasma levels of immune markers were associated with verbal memory and hippocampal subfield volumes in patients with severe mental illnesses and in healthy controls.

Method: 230 patients with a broad DSM-IV schizophrenia spectrum illness or bipolar disorder and 236 healthy controls were recruited. Memory was assessed using the Wechsler Memory Scale-Third Edition (WMS-III) Logical Memory immediate and delayed recall, and the California Verbal Learning Test summed recall over learning list (CVLT learning) and delayed free recall. We measured plasma levels of soluble tumor necrosis factor receptor 1 (sTNF-R1), interleukin-1 receptor antagonist, interleukin-6, von Willebrand factor, osteoprotegerin, high-sensitivity C-reactive protein and sCD40 Ligand. Hippocampal subfield estimates were obtained using FreeSurfer.

Results: We found a moderate negative association between sTNF-R1 and performance on verbal memory learning and recall tests as measured by the WMS-III Logical Memory after controlling for age, sex and diagnosis. We observed no interaction effect of diagnosis and sTNF-R1 on memory scores. We also found a nominally significant positive association between CVLT learning and hippocampal volumes.

Conclusions: The findings suggest a role for immune involvement in memory independent of severe mental disorders, and may support the "bigger is better" hypothesis of hippocampal subfield volumes.

Significant outcomes

1. Elevated plasma levels of sTNF-R1 are associated with impaired verbal memory in both patients with severe mental disorder and in healthy controls.

2. Increased hippocampal volumes are nominally associated with higher performances in verbal memory.

   We observed no interaction effect of diagnosis and sTNF-R1 on memory scores.

Limitations

1. MRI images were obtained on a 1.5 T scanner, which may have decreased sensitivity to disease-related biological variability.

2. The timing of blood sampling reduced the possibility of investigating state-related phenomenon.

3. Most patients were taking psychotropic medications, which may influence levels of inflammatory markers.

Introduction

Schizophrenia (SCZ) and bipolar disorder (BD) are severe mental disorders with an estimated heritability of approximately 70 – 80 % (1, 2). They rank among the most costly disorders causing long-term disability and are relatively common with a combined life time prevalence of approximately 3 %. Despite extensive research, the pathogenesis of these disorders remains largely unknown. A considerable genetic overlap exists between the two disorders which suggests common disease mechanisms (3, 4). Among other factors, the immune system has been implicated in the pathophysiology of SCZ and BD through findings from genome wide association studies for SCZ (5), as well as experimental studies and clinical studies of circulating levels of inflammatory markers in both disorders (6-11). However, the precise role of the immune system in the development of these disorders is yet to be unravelled.

Cognitive dysfunction is an important characteristic of severe mental disorders (12). Impaired verbal memory is a consistent finding in SCZ, and is also seen in patients with BD (13, 14). Several lines of evidence obtained mainly through animal studies have implicated the immune system in memory processes both under normal conditions and in impaired memory (15). One way to study enhanced inflammatory activity in humans is by measuring the plasma levels of immune markers that are not only expressed by immune cells, but also by cells of the central nervous system including neurons, astrocytes and microglia (16). We have previously shown increased levels of soluble TNF receptor 1 (sTNF-R1), which is a surrogate marker for the proinflammatory TNFα, in patients with severe mental disorders as well as other markers of immune activation including von Willebrand factor (vWF) (17), which reflects endothelial cell activation, and osteoprotegerin (OPG) (7), which indicates vascular inflammation. There is increased prevalence of cardiovascular diseases in both patients with SCZ and BD compared to healthy controls (18, 19). As endothelial cell activation is part of the pathological mechanisms in cardiovascular disease, and may contribute

to the disruption of the blood-brain-barrier, markers of vascular inflammation (e.g. OPG) and endothelial activation (e.g. vWF) could prove interesting markers to explore in patients with SCZ and BD. In fact, OPG has been shown to influence the development of ischemic stroke through modulation of neuroinflammation (20). We have also investigated other immune markers in relation to psychotic and affective symptoms such as IL-1 receptor antagonist (IL-1Ra), which is the surrogate marker of the pro-inflammatory cytokine interleukin-1β, interleukin-6 (IL-6) (21), sCD40 ligand (sCD40L), which reflects platelet-mediated inflammation, and high sensitivity C-reactive protein (hsCRP), which is a reliable down-stream marker of inflammation (6, 10).sCD40L is a reliable marker of platelet action in addition to interacting with endothelial cells and T cells, and has been associated with major depression (22). Several studies have investigated associations between verbal memory and the immune system (23, 24). However, such associations have not been previously explored in patients with severe mental disorders.

The hippocampal formation is a unique anatomical complex comprising several subfields, and plays an important role in verbal learning and memory (25). Reductions in hippocampal formation volumes are a consistent finding in SCZ, but can also be seen in patients with BD (26). Since immune processes seem to affect hippocampal function and neurogenesis (27), they may also be associated with changes in hippocampal volume. However, little is known about the association between immune-related markers and hippocampal structural properties in general, and associations between disease specific reductions in hippocampal volumes and plasma levels of immune markers have seldom been studied. Due to the heterogeneous functional and structural anatomy of the hippocampus, it has been hypothesized that subfield-specific measures of the hippocampal formation will prove more sensitive to disease effects than whole hippocampus volume measures (28). Indeed, distinct hippocampal subfield volume reductions with associations to cognitive performance have recently been reported in patients across the psychosis spectrum (29, 30), but if such associations are related to inflammatory markers is not known.

Aims of the study

We hypothesized that plasma levels of inflammatory markers would be negatively associated with performance on verbal memory tests in patients with severe mental disorders and in young healthy controls. Secondly, we hypothesized that these inflammatory markers would show a negative correlation with hippocampal volumes, indicating that high levels of inflammatory markers would be associated with reduced hippocampal subfield volumes. Lastly, in order to explore the potential neurocognitive relevance of any volumetric associations with cytokine levels, we also tested for associations between performance on verbal memory tests and hippocampal volumes probing the "bigger is better" hypothesis in hippocampal subfields in patients with severe mental disorder.

Material and methods

Study Design and Ethics

The Thematically Organized Psychosis (TOP) Study at the Oslo University Hospital and collaborating Hospitals across Norway, is a large ongoing study. The study sample for the present article consisted of patients and healthy control subjects included between May 2003 and September 2007. The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate, and the biobank is approved by the Norwegian Directorate of Health.

Participants

Patients: Patients were included in the TOP-Study if they had DSM-IV diagnoses of schizophrenia spectrum disorders or bipolar spectrum disorders, IQ > 70 and age between 18 and 65 years. For the present project we had the following additional inclusion criteria: having either Norwegian as a mother tongue or receiving all compulsory education in Norway, no coexisting neurological illness or autism spectrum disorder (ASD), no illnesses involving chronic inflammation and no head-injury with EEG, CT or MRI scan findings following the injury. Patients were excluded if they scored below 15 on the Californian Verbal Learning Test (CVLT) forced recognition (31), indicating

suboptimal motivation. In total 4 cases were excluded. The schizophrenia spectrum group (N = 109) comprised patients with schizophrenia (N = 86), schizophreniform disorder (N = 8) and schizoaffective disorder (N = 15). The bipolar spectrum disorder group (N = 117) included patients with bipolar I disorder (BD I, N = 68), bipolar II disorder (BD II, N = 42) and bipolar disorder not otherwise specified (BD NOS, N = 7).

Controls: A group of healthy volunteers (N = 236), from the same catchment area, representative of the typical broad demography, age and sex distribution of the patient samples, was randomly selected from the National Population Registry (www.ssb.no) and contacted by a letter of invitation. All control subjects were screened for illness using the Primary Care Evaluation of Mental Disorders and interviewed about severe psychiatric disorders, drug abuse, and somatic disease. Exclusion criteria were any history of severe psychiatric disorders (major depression, bipolar disorders, and schizophrenia) in the control subjects or in any of their first-degree relatives, substance or alcohol abuse or dependency, and the previously described exclusion criteria for patients.

None of the patients or the controls had any acute infections at the time of blood sampling, as indicated by plasma levels of CRP below 20, and no signs of infection during the physical examination.

Of the 462 participants included in the present study (Main group) 224 participants also underwent MRI (MRI subgroup). The MRI subgroup included 111 healthy controls, in addition to patients diagnosed with schizophreniform disorder (N = 6), schizoaffective disorder (N = 4) and schizophrenia (N = 36), BD I (N = 36), BD II (N = 28), and BD NOS (N = 3) diagnoses. All patients were examined by a physician to assess current health and potential neurological signs.

Clinical Assessments

Diagnosis was obtained with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Clinical symptoms were evaluated using the Young Mania Rating Scale, Inventory of

Depressive Symptoms and the Positive and Negative Syndrome Scale, while functioning was measured using the Global Assessment of Functioning split version function and symptom scale. The clinical assessment team consisted of clinical psychologists or psychiatrists, who all were trained until satisfactory inter-rater reliability was obtained (12).

Memory Assessments

Verbal memory was assessed by trained clinical psychologists using the Wechsler Memory Scale-Third Edition (WMS-III) Logical Memory immediate (LM-learning) and delayed recall (LM-recall) (32), and the California Verbal Learning Test summed recall over learning list (CVLT-learning) and delayed free recall (CVLT-recall) (12, 31). Neurocognitive testing was routinely performed the same day following blood sampling, except for a subgroup (7.9%) where there was a mean interval between assessments of 8 days (median, IQR = 4 – 15). Forty two subjects have missing data regarding time of assessment.

Blood sampling

Blood sampling was performed primarily in the morning (average at 10 am) for patients, and in the afternoon for a majority of controls (average at 3 pm).

Assessment of immune markers: Plasma levels of sTNF-R1, OPG, IL1-Ra and IL-6 were measured using enzyme immunoassays (EIA) obtained from R&D systems (Minneapolis, MN). Plasma sCD40L level was analyzed using EIA obtained from Bender Medsystem (Vienna Austria), whereas CRP (high-sensitivity) and vWf levels were measured using EIA antibodies from DakoCytomation (Oslo, Norway). vWf levels are given as plasma concentration percent (%), where the standard curve is based on samples from healthy individuals and normal range is set to 70–130%. Intra- and interassay coefficients of variance were less than 10%.

Assessment of cortisol levels: Free cortisol levels were measured in urine (33) and saliva (34) obtained the same day as the blood samples in a subsample (n=83 and n=61 respectively) of

patients and controls. Both urine and salivary samples were immediately frozen after sampling for later analysis of urinary free cortisol by liquid chromatography tandem mass spectrometry and radioimmunoassay respectively. Cortisol was also measured in the same blood samples as the cytokines in the patient group (n=165). A detailed description of the measurement techniques can be found in the Supplement section.

MRI acquisition

Imaging data was obtained between august 2003 and December 2007 on a 1.5 Tesla Siemens Sonata scanner using an 8-channel head coil. The pulse sequence used for volumetric assessments was a sagittal T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) with the following parameters: time of repetition (TR)/echo time (TE)/inversion time (TI) = 2730ms/3.93ms/1000ms, flip angle (FA) = 7°, field of view (FOV) = 240mm, acquisition matrix = 256x192, voxel size = 1.33x0.94x1 mm3, and 160 slices. The sequence was repeated and the two runs were combined during postprocessing in order to increase signal-to-noise ratio. Patients and healthy controls were scanned consecutively.

MRI processing and analysis

FreeSurfer (http://surfer.nmr.mgh.harvard.edu) was used to obtain estimates of the hippocampal subfield volumes, total hippocampal formation volume, and intracranial volume (ICV) (35). The processing includes motion correction and averaging (36) of the two T1 weighted volumes, removal of non-brain tissue using a hybrid watershed/surface deformation procedure (37), automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures by combining information on image intensity, probabilistic atlas location, and the local spatial relationships between structures to automatically assign a neuroanatomical label to each voxel in the MRI volume (38, 39). The MRI processing procedures were fully automated without manual editing. All segmented scans were visually inspected following standard procedures.

The novel approach for hippocampal subfield segmentation is based on a Bayesian modelling approach and manual delineations of each hippocampal subfield (40). The subfield volumes obtained have been compared to manual hippocampal subfield tracings, and shown to be most reliable for the larger subfields including CA2/3, CA4/DG, and subiculum, with acceptable reliability for the CA1, presubiculum, and fimbria (40).

Statistical analysis

All statistical analyses were performed using the SPSS software package for Windows, version 22.0.

Data normality was assessed using the Kolmogorov-Smirnov test. Differences in demographic data between groups were investigated using the chi-square test for categorical variables, the Kruskal-Wallis test for continuous variables, and the Mann-Whitney U test for post hoc analyses. Cytokine levels and scores on the CVLT learning and recall tests showed skewed distributions, and were investigated using the Kruskal-Wallis test and the Mann-Whitney U test for post hoc analyses. As hippocampal volumes and LM learning and recall showed normal distributions, we used one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis to examine differences between groups. We used linear regression models to investigate associations and to control for confounders. sTNF-R1, IL-1Ra, vWF and OPG were log-transformed, and sCD40L was square-root transformed prior to the linear regression analyses. IL-6 and hsCRP remained highly skewed after attempts at transformation, and were thus not transformed. Interaction effects were investigated using general linear models.

We used raw data in all our analyses regarding verbal memory. The CVLT-learning and recall variables also showed skewed distributions, which remained skewed after logarithmic transformation. However, the distribution of the standardized residuals in the general linear model showed normal distributions for analyses including the CVLT-learning, but not for the CVLT-recall.

We used linear regression models to investigate associations between cytokines and cortisol levels. Due to the diurnal variation in cortisol levels we controlled for the time of sampling. For

urine samples we also controlled for the differences in urine concentration by adding urine-creatinine levels as a covariate (41).

We corrected for multiple testing. Given the mixture of hypothesis based and explorative analyses, and the use of a range of non-independent measures a common significance threshold estimated using Bonferroni procedure across all tests would be overly conservative (42). Therefore, in order to decrease the probability of Type II errors we calculated separate alpha thresholds for each of the main analyses. Due to the strong a priori hypotheses for the role of sTNF-R1, IL1-Ra and IL-6 in verbal memory, the significance threshold was set at (3 cytokine variables x 4 verbal memory variables = 12 tests) $p < 0.004$. The associations between performance on verbal memory and hippocampal volumes have been much investigated. The significance threshold for findings in these analyses was set at (4 verbal memory variables x 6 hippocampal volume variables = 24 tests) $p < 0.002$. We used an explorative approach to investigate associations between cytokines and hippocampal volumes, and between the remaining cytokines and verbal memory. The significance threshold is therefore set at (7 cytokine variables x 6 hippocampal volume variables + 4 cytokine variables x 4 verbal memory variables = 58 tests) $p < 9 \times 10^{-4}$.

Due to differences in the time elapsed between blood sampling and neuropsychological testing, and missing data we repeated our analyses for the subgroup of subjects where both procedures were performed on the same day, and compared results with the main findings. We also controlled for the differences in elapsed time to MRI scanning for the MRI subgroup by covarying for time interval in the statistical models.

Results

The immune marker, memory test and hippocampal volume results from partly overlapping samples have been reported previously in separate analyses (12, 17, 26).

Demographics and clinical characteristics

The socio-demographic and clinical characteristics of the participants are presented in Table 1 for the Main group and in Supplementary Table 1 for the MRI subgroup.

There was no significant difference in demographic and clinical characteristics of the MRI subgroup (N = 224) and the Main group (N = 462), however, the two groups slightly differed in terms of intra group *post hoc* analyses of ethnicity, self-reported head injury, age, alcohol use, years of education and YMRS scores (see Table 1 and Supplementary Table 1).

Verbal memory and immune markers

Levels of immune markers, scores on verbal memory and hippocampal volumes for the current sample are summarized in Supplementary tables 2 and 3. We have previously reported increased sTNF-R1 and vWF in the Main group and the MRI subgroup mirrored these results showing significantly higher plasma levels of sTNF-R1 and vWF compared to the healthy controls. There were no significant differences between the Main group and the MRI subgroup in plasma levels of immune markers and performances on verbal memory tests.

We found moderate but significant negative associations between sTNF-R1 and LM-learning (β = -0.16, $p = 4 \times 10^{-4}$) and LM-recall (β = -0.15, p = 0.001) after controlling for age, sex and diagnosis (Fig. 1, Table 2). Both findings remained significant after correcting for multiple testing. We observed no interaction effect of diagnosis and sTNF-R1 on memory scores, indicating that the associations between sTNF-R1 and verbal memory were not significantly different between groups. We also found significant negative associations between vWF and CVLT learning and recall (β = -15, p = 0.005), however, these findings did not remain significant after controlling for age, sex and diagnosis, or when only controlling for age and sex, and correcting for multiple testing.

There were no significant associations between the other cytokines and verbal memory after controlling for age, sex and diagnosis (Supplementary Table 4).

Verbal memory and hippocampal volumes

The associations between CVLT recall and subiculum have been recently investigated in a partially overlapping sample (29).

Both CVLT learning and CVLT recall were moderately positively associated with the total hippocampal formation volume and hippocampal subfield volumes (β = 0.22 - 0.22 and p = 0.006 – 0.003, respectively), except for CA1 and CVLT learning, after controlling for age, sex, estimated intracranial volume and diagnosis (Fig. 2, Table 2 and Supplementary Table 5). However, these associations did not remain significant after correction for multiple testing. There were no significant interaction effects between hippocampal volumes and diagnosis on verbal memory scores, indicating similar associations between hippocampal volumes and verbal memory in patients and controls.

LM-learning and LM-recall were not associated with hippocampal volumes in our analyses.

Hippocampal volumes and cytokines

There were no significant associations between cytokines and hippocampal subfields after controlling for age, sex, diagnosis and estimated intracranial volume (Supplementary Table 6). However, we found a trend (p = 0.09) towards a negative association (β = - 0.10) between OPG and the volume of the total hippocampal formation after controlling for age, sex, estimated intracranial volume and diagnosis (Table 2).

Cortisol

There was a borderline significant association between urine free cortisol and sCD40L levels, but we found no significant associations between cortisol levels measured in saliva and

blood and any cytokines in the subsamples (N = 61-165). There was a nominally significant association between blood cortisol levels and hippocampal volumes after controlling for age, sex, intracranial volume and time of sampling in the patient subsample (N = 85). These associations did not remain significant after correcting for multiple testing. We added cortisol levels as confounder in the analysis of this subsample, but this did not affect the main findings.

Diurnal variation

Due to differences in blood sampling procedure for patients and controls we examined the associations between cytokine levels and the time of blood sampling using Spearman's rank correlation (Supplement table 7). In order to avoid possible multicolinearity with illness related variables, this analysis was performed in the subset of controls who had the time of blood sampling recorded (N = 174). We found a significant correlation for OPG (r = - 0.15, p = 0.047) and a trend-level association for IL-1Ra (r = 0.13, p = 0.08). There were no significant or trend-level associations for the other cytokines.

Discussion

The main finding of the present study was a moderate negative association between sTNF-R1, a marker of TNF activity, and performance on verbal memory learning and recall tests as measured by the WMS-III logical memory in both patients with schizophrenia spectrum and bipolar spectrum disorders and in healthy controls. This association was similar in all three groups. We also found a nominally significant positive association between estimates of hippocampal volumes and verbal memory performance assessed using the CVLT test that did not differ significantly in patients and controls. These findings may indicate a role for TNF related pathway involvement in memory processes independent of the pathological mechanisms underlying severe mental disorders.

There are several lines of evidence suggesting that inflammatory and immune-mediated mechanisms could interfere with memory performance. Although normal levels of TNFα do not seem to be involved in long term potentiation (LTP) induction or maintenance (15), TNFα at pathophysiological concentrations inhibit LTP in the dentate gyrus and CA1 regions of the hippocampus (43), thus negatively influencing memory processes. Our findings of negative associations between sTNF-R1 and performances on the WMS-III logical memory tests were independent of diagnosis, and we found no significant interaction effects of diagnosis and sTNF-R1 on verbal memory scores. This suggests that the potential cytokine-mediated regulation of verbal memory is not limited to patients with SCZ and BD where a cytokine imbalance is likely, but may be a "naturally" occurring mechanism, that doesn't depend on pathological plasma levels of TNFα in humans. Another putative mechanism by which TNFα may influence memory is through its ability to regulate levels of major histocompatibility complex (MHC) class I proteins in the brain (44). There is now growing evidence suggesting that MHC class I proteins play a crucial role in synaptic plasticity and hippocampal function (45). They are critical for Hebbian synaptic plasticity (the theory suggesting that long-term potentiation and long-term depression are the underlying mechanisms for memory consolidation (27)) and have been proposed to limit synaptic strength and alter the trafficking and function of NMDA and AMPA receptors (46, 47).

One could argue that plasma levels of cytokines are less relevant for the brain. However, circulating cytokines bypass the blood-brain-barrier (BBB) by activation of vagal afferents (48) and endothelial cells in the BBB can be activated by circulating cytokines, which in turn produce and secrete cytokines into the brain parenchyma (49). In addition, cytokines can directly reach the brain by leakage through the BBB or by active transport through the BBB (48). It is therefore possible that the cytokines influencing cognitive functions in the brain are the result of the complex interplay between central and peripheral cytokines (50). It is important to denote that sTNF-R1 doesn't reflect the actual immunoactivity of TNFα but rather the activity in TNF signaling.

We found no significant associations between IL-1Ra, IL-6, hsCRP, OPG, vWf and verbal memory after controlling for age, sex and multiple testing. Although our analyses investigating the relationship between cytokine levels and time of blood sampling revealed only one significant association (OPG) in our control sample, diurnal variations have been shown to influence cytokine levels (51). Therefore, we cannot exclude the possibility that our negative findings might in part be due to the differences in blood sampling procedures. Further, our findings may also be affected by storage from blood sampling to cytokine measures potentially leading to the degeneration of cytokines. However, the problem was limited by primarily analyzing stable markers, and using same procedure for cases and controls who were recruited to the project in parallel during the same time period.

We found a trend toward negative associations between sTNF-R1 and hippocampal volumes, indicating that increased activity in the TNF system might be associated with smaller hippocampal volumes. Although we have no clear explanation for this association, experimental data suggest that inflammatory cytokines like TNF could inhibit adult neurogenesis in rats by inhibiting new neuron survival and proliferation (52, 53). Further, systemic administration of TNF has been shown to attenuate hippocampal growth (54). We have previously found that patients have higher levels of sTNF-R1 (17), suggesting increased activation of the TNF system, and that patients have smaller hippocampal volumes (26). Based on these findings one could hypothesize that whereas there is an inverse correlation between hippocampal volumes and sTNF-R1 in our sample, increased activation of the TNF system, as in the patient group, could potentially contribute to a decrease in hippocampal volumes.

We did not find significant negative associations between plasma levels of cytokines and hippocampal volumes after controlling for age, sex, diagnosis and estimated intracranial volume. This may be due to the reduced statistical power as our MRI sample size was half the size of the Main sample. We did, however, observe a trend level negative association between OPG and

hippocampal volumes. The potential role of OPG in hippocampal volume reduction has not yet been investigated, and its association to severe mental disorders has not been firmly established. Further studies with larger sample sizes are necessary to confirm these results.

We also found a moderate positive association between hippocampal subfield volumes and performance on the CVLT learning after controlling for confounders, which, however, did not remain significant after correction for multiple testing. Several studies have also found positive associations between performances on CVLT learning and hippocampal volumes (55, 56), as well as on distinct hippocampal subfield volumes (30), supporting the validity of our findings. We did not find any interaction effect between diagnosis and hippocampal subfield volumes on memory performances suggesting that bigger hippocampal subfield volumes may modestly reflect higher performance on CVLT learning both in patients with SCZ and BD, as well as in healthy adults.

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Declaration of interest

OAA received speaker's honorarium from GSK, Lundbeck, Otsuka. The other authors have nothing to declare.

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Table 1. Demographic and clinical characteristics of participants in the Main group (N = 462).

Parameters	Bipolar Disorder (N = 117)	Schizophrenia (N = 109)	Healthy Controls (N = 236)	Post Hoc Analysis
Male sex, N (%)	46 (39.3)	59 (54.1)	104 (44.1)	NS
Ethnicity (European)[a]	106 (90.6)	97 (89.0)	234 (99.6)	CTR >SCZ, BD
Tobacco (users)[b]	62 (53.4)	58 (53.2)	32 (20.4)	SCZ, BD > CTR
Cannabis (users)[c]	11 (9.4)	16 (14.8)	1 (0.4)	SCZ, BD > CTR
Head injury[d]	8 (6.8)	4 (3.7)	2 (0.9)	BD > CTR
Medication:				
Antipsychotics	53 (45.3)	95 (87.2)	-	SCZ > BD
Lithium	15 (12.8)	1 (0.9)	-	BD > SCZ
Antidepressants	46 (39.3)	32 (29.4)	-	NS
Mood stabilizers	65 (55.6)	18 (16.5)	-	BD > SCZ
Hypnotics	14 (12.9)	10 (9.2)	-	NS
Age (years), median (IQR)	32 (21)	30 (13)	35 (17)	CTR > SCZ
Alcohol (IU)[e]	4 (16)	0 (8)	6 (13)	CTR, BD > SCZ
Education (years)	14 (3)	12 (4)	15 (4)	CTR > BD >SCZ
IQ [f]	109 (16)	103 (25)	114 (11)	CTR > BD >SCZ
Sampling – Testing (days)[g]	0 (0)	0 (7)	0 (0 – 0)	SCZ > BD, CTR
Body Mass Index[h]	25.2 (5.9)	25.6 (6.5)	-	NS
PANSS total score[i]	45 (11)	58 (21)	-	SCZ > BD
YMRS total score [j]	2 (5)	5 (9)	-	SCZ > BD
IDS total score[k]	13 (18)	11 (16)	-	NS
GAF-F [l]	57.5 (20)	42 (14)	-	BD > SCZ
GAF-S [m]	60 (13)	40 (16)	-	BD > SCZ

Missing: [a] N = 1, [b] N = 80, [c] N = 1, [d] N = 2, [e] N = 29, [f] N = 3, [g] N = 42, [h] N = 3, [i] N = 2, [j] N = 2, [k] N = 34, [l] N = 1, [m]N = 1

Abbreviations: CTR = Controls; BD = Bipolar Disorder Spectrum; SCZ = Schizophrenia Spectrum; NS = Non-Significant; IU = International Units two weeks prior to inclusion in the study; IQ = Wechsler Abbreviated Scale of Intelligence; PANSS=Positive and Negative Syndrome Scale; YMRS=Young Mania Rating Scale; IDS=Inventory of Depressive Symptoms; GAF-F = Global Assessment of Functioning - Function Scale; GAF-S = Global Assessment of Functioning - Symptom Scale.

Categorical data are given as percent, while continuous data are given as median with interquartile range. Post Hoc Analysis is performed using Pearson Chi-square for categorical data, and Kruskal-Wallis and Mann-Whitney tests for continuous data.

Table 2. Significant associations between cytokines, verbal memory and hippocampal subfield volumes investigated by linear regression analyses.

Parameters	Parameters	Total population (N = 462)		Patients (N = 226)		Controls (N = 236)	
Memory test:	*Cytokines:*	Uni.	Adj.[1]	Uni.	Adj.[2]	Uni.	Adj.[2]
LM learning	sTNF-R1	**-.20*** **	**-.16*** **	**-.15***	**-.17***	**-.18****	**-.18****
LM recall	sTNF-R1	**-.19*** **	**-.15****	**-.14***	**-.15***	**-.17***	**-.17****
CVLT learning	vWF	**-.15****	-.08	**-.15***	-.13	-.08	-.04
CVLT recall	vWF	**-.14****	-.07	-.12	-.08	-.08	-.05
		(N = 224)		(N = 113)		(N = 111)	
Hippocampus:	*Cytokines:*	Uni.	Adj.[3]	Uni.	Adj.[4]	Uni.	Adj.[4]
Total hippocampus	OPG	**-.16***	-.10	-.24	-.19*	-.04	.06
Memory test:	*Hippocampus:*	Uni.	Adj.[3]	Uni.	Adj.[4]	Uni.	Adj.[4]
CVLT learning	Subiculum	**.14***	**.22****	**.20***	**.29****	-.05	.11
CVLT recall	Subiculum	.11	**.21****	.13	**.25***	-.02	.16

* p < 0.05 level (2-tailed) ** p < 0.01 level (2-tailed) *** p < 0.001 level (2-tailed)

Univariate association (= Uni.) and Adjusted association (=Adj.) are given as standardized beta.

[1] Adjusted for age, sex and diagnosis; [2] Adjusted for age and sex; [3] Adjusted for age, sex, estimated intracranial volume and diagnosis; [4] Adjusted for age, sex and estimated intracranial volume.

Abbr.: LM = Logical Memory subtest of the Wechsler Memory Scale III; CVLT = California Verbal Learning Test; sTNF-R1 = soluble Tumor Necrosis Factor Receptor 1; vWF = von Willebrand Factor; DG = Dentate Gyrus; OPG = Osteoprotegerin.

Supplementary Table 1. Demographic and clinical characteristics of participants in the MRI subgroup (N = 224).

Parameters	Bipolar Disorder (N = 67)	Schizophrenia (N = 46)	Healthy Controls (N = 111)	Post Hoc Analysis
Male sex, N (%)	29 (43.3)	25 (54.3)	49 (44.1)	NS
Ethnicity (European)[a]	64 (95.5)	39 (84.8)	109 (99.1)	CTR > SCZ
Tobacco (users)[b]	39 (58.2)	22 (47.8)	16 (20.3)	SCZ, BD > CTR
Cannabis (users)[c]	9 (13.4)	6 (13.3)	1 (0.9)	SCZ, BD > CTR
Head injury[d]	5 (7.5)	1 (2.2)	1 (0.9)	BD > CTR
Medication:				
Antipsychotics	31 (46.3)	39 (84.8)	-	SCZ > BD
Lithium	7 (10.4)	0 (0)	-	BD > SCZ
Antidepressants	26 (38.8)	16 (34.8)	-	NS
Mood stabilizers	37 (55.2)	6 (13.0)	-	BD > SCZ
Hypnotics	8 (11.9)	4 (8.7)	-	NS
Age (years)	31 (14)	30 (10)	36 (17)	CTR > SCZ
Alcohol (IU)[e]	4 (18)	1,5 (9)	6 (13)	CTR > SCZ
Education (years)	14 (3)	12 (3)	15 (11)	CTR, BD >SCZ
IQ	111 (12)	105 (25)	116 (11)	CTR > BD >SCZ
Sampling – Testing (days) [f]	0 (0)	4 (7)	0 (0)	SCZ > BD, CTR
Sampling – Scanning (days) [g]	263.5 (560)	74 (388)	406 (893)	CTR > BD > SCZ
Body Mass Index	24.7 (4.9)	25.2 (5.8)	-	NS
PANSS total score [h]	45 (11)	55 (27)	-	SCZ > BD
YMRS total score [i]	1 (4.3)	3 (8)	-	NS
IDS total score [j]	14.5 (19)	9 (15)	-	NS
GAF-F	59 (20)	42 (13)	-	BD > SCZ
GAF-S	61 (11)	40.5 (16)	-	BD > SCZ

Missing: [a] N = 1; [b] N = 32; [c] N = 1; [d] N = 1; [e] N = 13, [f] N = 20, [g] N = 15, [h] N = 3, [i] N = 2, [j] N = 24

Abbreviations: IQR = Interquartile Range; CTR = Controls; BD = Bipolar Disorder Spectrum; SCZ = Schizophrenia Spectrum; NS = Non-Significant; IU = International Units two weeks prior to inclusion in the study; IQ = Wechsler Abbreviated Scale of Intelligence; PANSS=Positive and Negative Syndrome Scale; YMRS=Young Mania Rating Scale; IDS=Inventory of Depressive Symptoms; GAF-F = Global Assessment of Functioning - Function Scale; GAF-S = Global Assessment of Functioning - Symptom Scale.

Categorical data are given as percent, while continuous data are given as median with interquartile range. Post Hoc Analysis is performed using Pearson Chi-square for categorical data, and Kruskal-Wallis and Mann-Whitney tests for continuous data.

Supplementary Table 2. Immune and verbal memory characteristics for the Main group (N = 462).

Parameters	Bipolar Disorder (N = 117)	Schizophrenia (N = 109)	Healthy Controls (N = 236)	Post Hoc Analysis
Cytokines				
sTNF-R1	1.00 (0.27)	1.04 (0.44)	0.91 (0.33)	SCZ, BD > CTR
IL1-RA	0.28 (0.58)	0.39 (0.78)	0.34 (0.61)	NS
hsCRP	0.35 (0.76)	0.32 (0.84)	0.30 (0.97)	NS
vWF	98(70)	93 (60)	77 (57)	SCZ, BD > CTR
OPG	2.65 (1.18)	2.47 (0.85)	2.42 (1.06)	NS
IL-6	0.19 (0.30)	0.17 (0.37)	0.17 (0.25)	NS
sCD40L	1.42 (1.92)	1.73 (1.98)	1.65 (1.76)	NS
Verbal Memory Tests				
CVLT learning	57 (17)	49 (13)	59 (13)	CTR, BD > SCZ
CVLT recall	14 (4)	11 (5)	14 (3)	CTR, BD > SCZ
LM learning	25.16 (6.66)	21.63 (6.93)	26.95 (6.18)	CTR > BD > SCZ
LM recall	22.02 (7.34)	17.87 (7.30)	24.16 (6.87)	CTR > BD > SCZ

Abbreviations: CTR = Controls; BD = Bipolar Disorder Spectrum; SCZ = Schizophrenia Spectrum; NS = Non-Significant; sTNF-R1 = Soluble Tumor Necrosis Factor Receptor 1; IL1-RA = Interleukin 1 Receptor antagonist; hsCRP = High Sensitivity C-Reactive Protein; vWF = von Willebrand Factor; OPG = Osteoprotegerin; IL-6 = Interleukin 6; sCD40L = CD40 Ligand; CVLT = California Verbal Learning Test; SD = Standard Deviation; LM = Logical Memory subtest of the Wechsler Memory Scale III.

Cytokines and CVLT tests are given as median with interquartile range. LM test are given as mean and standard deviation. Post Hoc analysis is performed using Kruskal-Wallis and Mann-Whitney tests for cytokines and CVLT tests, and ANOVA, Tukey for LM tests.

Supplementary Table 3. Immune, verbal memory and hippocampal volume characteristics for the MRI subgroup (N = 224).

Parameters	Bipolar Disorder (N = 67)	Schizophrenia (N = 46)	Healthy Controls (N = 111)	Post Hoc Analysis
Cytokines				
sTNF-R1	1.00 (0.27)	1.09 (0.48)	0.87 (0.28)	SCZ, BD > CTR
IL1-RA	0.27 (0.52)	0.42 (0.88)	0.37 (0.72)	NS
hsCRP	0.35 (0.73)	0.27 (0.64)	0.27 (0.76)	NS
vWF	90 (68)	99.5 (51)	79 (50)	SCZ, BD > CTR
OPG	2.38 (0.98)	2.42 (0.87)	2.37 (0.97)	NS
IL-6	0.15 (0.20)	0.16 (0.20)	0.19 (0.27)	NS
sCD40L	1.37 (1.73)	1.96 (2.00)	1.68 (1.79)	NS
Verbal Memory Tests				
CVLT learning	58 (17)	48.5 (12)	59 (12)	CTR, BD > SCZ
CVLT recall	14 (4)	11 (4)	14 (4)	CTR, BD > SCZ
LM learning	25.84 (7.02)	22.37 (6.78)	28.30 (5.99)	CTR > BD > SCZ
LM recall	22.70 (7.82)	18.61 (6.74)	25.36 (6.45)	CTR > BD > SCZ
MRI Volumes (cm^3)				
Hippocampal formation	8.15 (0.88)	7.94 (0.89)	8.36 (0.74)	CTR > SCZ
CA1	0.66 (0.10)	0.65 (0.09)	0.67 (0.07)	NS
CA2/3	2.00 (0.27)	1.94 (0.28)	2.07 (0.23)	CTR > SCZ
CA4/DG	1.12 (0.15)	1.08 (0.15)	1.16 (0.12)	CTR > SCZ
Presubiculum	0.91 (0.12)	0.85 (0.10)	0.91 (0.10)	CTR, BD > SCZ
Subiculum	1.28 (0.17)	1.24 (0.14)	1.31 (0.13)	CTR > SCZ
ETIV	1601.67 (164.56)	1600.32 (160.33)	1590.30 (162.91)	NS

Abbreviations: CTR = Controls; BD = Bipolar Disorder Spectrum; SCZ = Schizophrenia Spectrum; NS = Non-Significant; sTNF-R1 = Soluble Tumor Necrosis Factor Receptor 1; IL1-RA = Interleukin 1 Receptor antagonist; hsCRP = High Sensitivity C-Reactive Protein; vWF = von Willebrand Factor; OPG = Osteoprotegerin; IL-6 = Interleukin 6; sCD40L = CD40 Ligand; CVLT = California Verbal Learning Test; SD = Standard Deviation; LM = Logical Memory subtest of the Wechsler Memory Scale III; ETIV = Estimated Total Intracranial Volume.

Cytokines and CVLT tests are given as median with interquartile range. LM tests and hippocampal volumes are given as mean and standard deviation. Post Hoc analysis is performed using Kruskal-Wallis and Mann-Whitney tests for cytokines and CVLT tests, and ANOVA, Tukey for LM tests and hippocampal volumes.

Supplementary Table 4. Linear regression models investigating the associations between verbal memory and cytokines in whole sample (N = 462) after controlling for age, sex and diagnosis.

Memory Test	Cytokine	Age	Sex (M)	Group (P)	Cytokine	Full Model	
		t	t	t	t	F	R^2
LM learning	**sTNF-R1**	**0.01**	**-1.77**	**-5.06*** **	**-3.57*** **	**12.22**	**.10**
	IL1-RA	-0.17	-1.68	-5.59***	-1.26	9.22	.08
	CRP	-0.11	-1.68	-5.79***	1.08	9.57	.08
	vWF	-0.24	-1.74	-5.71***	0.89	9.01	.07
	OPG	-0.20	-1.61	-5.65***	0.45	8.85	.07
	IL-6	-0.14	-1.69	-5.64***	-0.28	8.81	.07
	sCD40L	-0.17	-1.69	-5.63***	-0.32	8.82	.07
LM recall	**sTNF-R1**	**-0.82**	**-2.52***	**-5.64*** **	**-3.29** **	**14.21**	**.11**
	IL1-RA	-0.98	-2.43*	-6.13***	-1.38	11.76	.09
	CRP	-1.01	-2.32*	-6.28***	1.71	12.04	.10
	vWF	-1.00	-2.46*	-6.19***	0.52	11.32	.09
	OPG	-1.02	-2.32*	-6.22***	0.66	11.36	.09
	IL-6	-0.95	-2.44*	-6.17***	0.00	11.24	.09
	sCD40L	-1.00	-2.43*	-6.18***	-0.69	11.37	.09
CVLT learning	sTNF-R1	-2.87**	-5.61***	-5.97***	-1.04	19.44	.15
	IL1-RA	-2.95**	-5.59***	-6.16***	-1.33	19.64	.15
	CRP	-2.93**	-5.55***	-6.23***	0.46	19.19	.14
	vWF	-2.76**	-5.52***	-5.93***	-1.37	19.67	.15
	OPG	-2.92**	-5.50***	-6.20***	0.19	19.14	.14
	IL-6	-2.92**	-5.61***	-6.25***	0.80	19.32	.15
	sCD40L	-2.93**	-5.59***	-6.21***	-0.30	19.15	.14
CVLT recall	sTNF-R1	-3.48***	-5.59***	-5.13***	-0.64	17.43	.13
	IL1-RA	-3.53***	-5.58***	-5.27***	-0.82	17.50	.13
	CRP	-3.52***	-5.55***	-5.31***	0.26	17.33	.13
	vWF	-3.30**	-5.49***	-4.95***	-1.50	17.75	.13
	OPG	-3.45***	-5.56***	-5.28***	-0.17	17.32	.13
	IL-6	-3.51***	-5.60***	-5.45***	0.90	17.54	.13
	sCD40L	-3.55***	-5.58***	-5.30***	-0.68	17.44	.13

* $p < 0.05$ level (2-tailed) ** $p < 0.01$ level (2-tailed) *** $p < 0.001$ level (2-tailed)
Abbreviations: M = male; P = patients; LM = Logical Memory subtest of the Wechsler Memory Scale III; CVLT = California Verbal Learning Test; sTNF-R1 = Soluble Tumor Necrosis Factor Receptor 1; IL1-RA = Interleukin 1 Receptor antagonist; hsCRP = High Sensitivity C-Reactive Protein; vWF = von Willebrand Factor; OPG = Osteoprotegerin; IL-6 = Interleukin 6; sCD40L = CD40 Ligand.

Supplementary Table 5. Linear regression analyses of associations between verbal memory and hippocampal volumes in subsample (N = 224) after controlling for age, sex, estimated total intracranial volume and diagnosis (patient or control).

Verbal memory Test	MRI volume	Age t	Sex (M) t	ETIV t	Group (P) t	MRI volume t	Full Model F	Full Model R^2
LM learning	Hippocampus	-1.51	-0.02	0.30	-4.35***	0.48	4.58	.10
	CA1	-1.63	-0.05	0.42	-4.53***	0.42	4.57	.10
	CA2/3	-1.56	-0.07	0.21	-4.29***	0.77	4.66	.10
	CA4/DG	-1.60	-0.06	0.19	-4.26***	0.92	4.72	.10
	Presubiculum	-1.61	-0.03	0.51	-4.56***	0.06	4.53	.09
	Subiculum	-1.58	-0.03	0.24	-4.37***	0.73	4.65	.10
LM recall	Hippocampus	-1.09	-0.20	-0.40	-4.20***	1.49	5.05	.10
	CA1	-1.39	-0.27	0.14	-4.60***	0.57	4.63	.10
	CA2/3	-1.30	-0.29	-0.07	-4.35***	0.90	4.74	.10
	CA4/DG	-1.34	-0.29	-0.12	-4.30***	1.13	4.84	.10
	Presubiculum	-1.39	-0.29	-0.03	-4.55***	0.78	4.69	.10
	Subiculum	-1.32	-0.25	-0.09	-4.42***	1.00	4.77	.10
CVLT learning	**Hippocampus**	-0.55	-1.93	-1.79	-2.89**	**2.78****	6.27	.13
	CA1	-1.13	-2.08*	-1.09	-3.43**	1.78	5.26	.11
	CA2/3	-0.86	-2.16*	-1.70	-2.89**	**2.87****	6.37	.13
	CA4/DG	-0.99	-2.11*	-1.55	-2.93**	**2.72****	6.19	.12
	Presubiculum	-1.11	-2.11*	-1.37	-3.35**	**2.05***	5.49	.11
	Subiculum	-0.92	-2.03*	-1.75	-3.02**	**3.04****	6.60	.13
CVLT recall	**Hippocampus**	-1.54	-1.89	-2.37*	-2.78**	**2.71****	7.20	.14
	CA1	-2.14*	-2.08*	-1.90	-3.25**	**2.23***	6.66	.13
	CA2/3	-1.85	-2.12*	-2.35*	-2.76**	**2.89****	7.42	.15
	CA4/DG	-1.99*	-2.07*	-2.20*	-2.80**	**2.72****	7.20	.14
	Presubiculum	-2.11*	-2.08*	-2.06*	-3.20**	**2.19***	6.62	.13
	Subiculum	-1.92	-1.99*	-2.34*	-2.91**	**2.91****	7.45	.15

* $p < 0.05$ level (2-tailed) ** $p < 0.01$ level (2-tailed) *** $p < 0.001$ level (2-tailed)
Abbreviations: M = male; ETIV = estimated total intracranial volume; P = patients; LM = Logical Memory subtest of the Wechsler Memory Scale III; CVLT = California Verbal Learning Test

Supplementary Table 6. Linear regression analyses investigating associations between hippocampal subvolumes and cytokines in MRI subgroup (N = 224) after controlling for age, sex, estimated total intracranial volume and diagnosis (patient or control).

MRI volumes	Cytokine	Age	Sex (M)	ETIV	Group (P)	Cytokine	Full Model	
		t	t	t	t	t	F	R^2
Hippocampus	sTNF-R1	-2.58*	-0.51	7.90***	-3.75***	-0.50	22.35	.34
	IL1-RA	-2.59*	-0.41	7.65***	-3.92***	0.40	22.32	.34
	IL-6	-2.60*	-0.46	7.84***	-3.95***	0.36	22.32	.34
	vWF	-2.49*	-0.43	7.88***	-3.69***	-0.86	22.50	.34
	OPG	-2.55*	-0.51	7.79***	-3.94***	-1.73	23.18	.35
	hsCRP	-2.64**	-0.51	7.91***	-3.90***	-0.58	22.38	.34
	sCD40L	-2.78**	-0.70	8.09***	-4.05***	-1.58	23.03	.35
CA1	sTNF-R1	0.79	0.67	5.20***	-1.80	-0.69	10.72	.20
	IL1-RA	0.83	0.91	4.77***	-1.96	1.52	11.18	.20
	IL-6	0.76	0.75	5.11***	2.00*	0.43	10.65	.20
	vWF	0.71	0.74	5.16***	-1.98*	0.19	10.61	.20
	OPG	0.80	0.72	5.07***	-1.97*	-1.15	10.93	.20
	CRP	0.74	0.76	5.14***	-1.98*	0.14	10.61	.20
	sCD40L	0.62	0.56	5.29***	-2.06*	-1.17	10.94	.20
CA2/3	sTNF-R1	-0.88	0.57	7.04***	-3.47**	-1.51	20.26	.32
	IL1-RA	-0.99	0.73	6.74***	-3.84***	0.03	19.60	.31
	IL-6	-0.96	0.74	6.81***	-3.89***	0.78	19.77	.31
	vWF	-0.89	0.76	6.87***	-3.64***	-0.70	19.74	.31
	OPG	-0.94	0.70	6.79***	-3.84***	-1.14	19.97	.31
	CRP	-0.96	0.81	6.83***	-3.89***	0.89	19.83	.31
	sCD40L	-1.16	0.48	7.10***	-3.97***	-1.64	20.38	.32
CA4/DG	sTNF-R1	-0.22	0.38	6.33***	-3.46***	-1.40	16.42	.27
	IL1-RA	-0.32	0.53	6.08***	-3.81***	-0.04	15.88	.27
	IL-6	-0.29	0.54	6.13***	-3.84***	0.68	16.01	.27
	vWF	-0.20	0.57	6.18***	-3.56***	-0.92	16.12	.27
	OPG	-0.26	0.50	6.10***	-3.80***	-1.31	16.35	.27
	CRP	-0.30	0.58	6.16***	-3.83***	0.53	15.96	.27
	sCD40L	-0.48	0.30	6.39***	-3.92***	-1.51	16.51	.28
Presubiculum	sTNF-R1	0.58	0.73	7.13***	-2.12*	-0.59	18.96	.30
	IL1-RA	0.48	0.69	7.15***	-2.31*	-0.91	19.10	.31
	IL-6	0.53	0.80	7.08***	-2.28*	-0.01	18.86	.30
	vWF	0.62	0.82	7.09***	-2.11*	-0.68	18.99	.30
	OPG	0.56	0.79	7.05***	-2.28*	-0.46	18.92	.30
	CRP	0.50	0.70	7.17***	-2.24*	-1.09	19.20	.31
	sCD40L	0.44	0.65	7.16***	-2.35*	-0.92	19.10	.31
Subiculum	sTNF-R1	-0.58	-0.06	6.77***	-2.90**	-1.07	16.22	.27
	IL1-RA	-0.63	0.11	6.45***	-3.17**	0.48	15.97	.27
	IL-6	-0.63	0.06	6.62***	-3.22**	0.70	16.04	.27
	vWF	-0.54	0.09	6.66***	-2.96**	-0.85	16.10	.27
	OPG	-0.59	0.02	6.59***	-3.18**	-1.29	16.36	.27
	CRP	-0.66	0.03	6.68***	-3.16**	-0.28	15.93	.27
	sCD40L	-0.84	-0.22	6.94***	-3.32**	-1.83	16.82	.28

* $p < 0.05$ level (2-tailed) ** $p < 0.01$ level (2-tailed) *** $p < 0.001$ level (2-tailed)

Abbreviations: M = male; ETIV = estimated total intracranial volume; P = patients; LM = Logical Memory subtest of the Wechsler Memory Scale III; CVLT = California Verbal Learning Test; sTNF-R1 = Soluble Tumor Necrosis Factor Receptor 1; IL1-RA = Interleukin 1 Receptor antagonist; hsCRP = High Sensitivity C-Reactive Protein; vWF = von Willebrand Factor; OPG = Osteoprotegerin; IL-6 = Interleukin 6; sCD40L = CD40 Ligand.

Supplementary table 7. Spearman's rank correlation between cytokines and time of blood sampling for the control group (N = 174)

cytokines	Blood sampling	
	r	*p*
sTNF-R1	.02	.76
IL-1Ra	**.13**	**.08**
IL-6	-.08	.31
CRP	-.09	.24
vWF	.05	.53
OPG	**-.15***	**.047**
sCD40L	-.02	.77

*$p < 0.05$

sTNF-R1 = Soluble Tumor Necrosis Factor Receptor 1; IL1-RA = Interleukin 1 Receptor antagonist; hsCRP = High Sensitivity C-Reactive Protein; vWF = von Willebrand Factor; OPG = Osteoprotegerin; IL-6 = Interleukin 6; sCD40L = CD40 Ligand

Supplement information

Blood sampling procedure

Blood samples were drawn into EDTA tubes, stored at room-temperature for 45 minutes and placed in refrigerator at 4^{o}C. They were then transported to the Biobank, where 2 x 9 ml EDTA tubes were centrifuged at 1800 *g* for 15 minutes, plasma was collected and stored at -80^{o}C in multiple aliquots. Samples were not thawed prior to the analysis of cytokines.

Cytokine detectability

We had the following detection limits for our cytokines: sTNF-R1: 0.38 ng/ml; IL-1Ra: 0.02 ng/ml; IL-6: 0.10 ng/ml; hsCRP: 0.10 mg/l; vWf: 15%; OPG: 0.33 ng/ml; sCD40L: 0.10 ng/ml. 36 % of the sample had an IL-6 value below the detection limit, 29 % had hsCRP levels, 5 % had IL-1Ra levels and 3 % had sCD40L levels below the detection limit. All measured values were above the detection limit for sTNF-R1, vWf and OPG.

Cortisol measurements

Urine: 200 µL of urine was diluted 1:1 with the internal standard, deuterated cortisol-d4 (4-Pregnen-11β,17α,21-triol-3,20-dione-9,11,12,12-d_4, 97.5 atom % D) (C/D/N Isotopes Inc, Quebec, Canada) in 1% formic acid. A 20-µL sample was injected into an Oasis hydrophilic-lipophilic-balanced (HLB) column, 2.1 x 20 mm, 25 µm (Waters Corporation, Milford, Massachusetts), for sample cleanup at a flow rate of 2,500 µL/min. Washing steps included 0.5 minute with 0.1% formic acid, 0.5 minute with 40:4:56 MeOH:N with 40:4:56 MeOH:NH4O with 40:4:56 MeOH:NH_4OH:H_2O (v/v/v), and 1.0 minute with 0.1% formic acid. After 2.0 minutes, a valve directed the flow from a secondary pump through the extraction column in backflush mode and eluted retained substances to the analytic column (Sunfire 2.1 x 100 mm, 3.5 µm) (Waters Corporation, Milford, Massachusetts). This secondary pump was running a gradient from 10% to 50% acetonitrile with 0.1% formic acid from t = 2 to t = 10 minutes. Flow rate was 300 µL/min,

and column temperature was 20^{o}C. The API-4000 Q-Trap operated in ESI positive mode. Ion source conditions were as follows: curtain gas, 30 psig; CAD, medium; ionspray voltage, 5,500 V; temperature, 550^{o}C; gas 1, 50 psig; gas 2, 50 psig; entrance potential, 10 V; and resolution set to *unit*. The between-day precision was below 5.3% and accuracy ranged from 99% to 108%. There were no interfering substances.

Saliva: The Salivette (Sarstedt AG& Co, Nümbrecht, Germany) was used for sampling. Saliva were immediately frozen for later analysis of free cortisol, which was performed using a radioimmunoassay from Siemens Healthcare Diagnostics (Los Angeles, CA, USA) at the Hormone Laboratory at Oslo University Hospital, Aker (inter assay CV% = 11 at 7 nmol/L). Cortisol levels below the reference limit (< 3.5 nmol/L) were treated as missing in the analyses.

Serum: Serum cortisol levels were measured using luminoimmunoassay (Diagnostic Products Corporation, Los Angeles, California) at the Hormone Laboratory, Department of Endocrinology, Aker University Hospital.

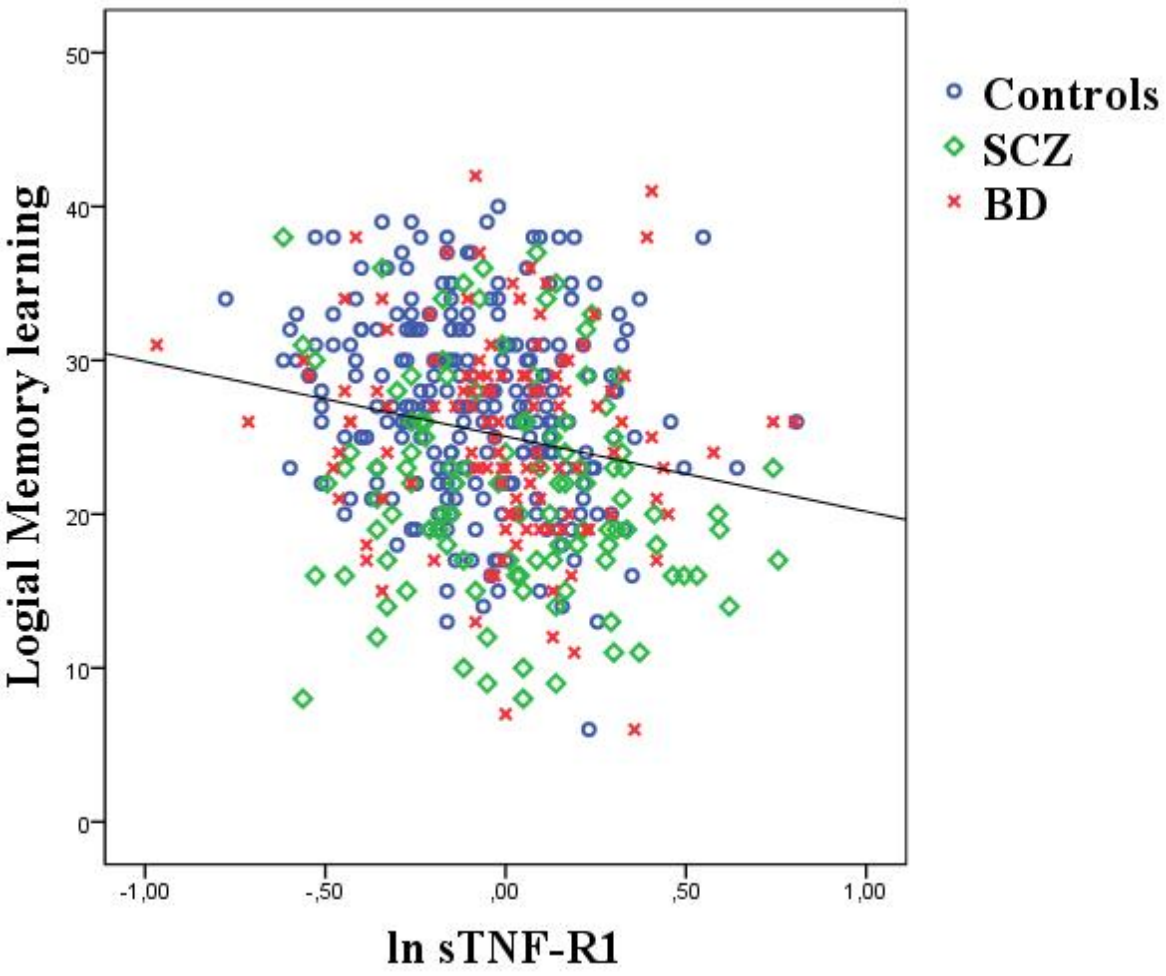

Figure 1. Scatterplot for WMS-III logical memory learning and plasma levels of sTNF-R1 in the Main sample (N = 462).

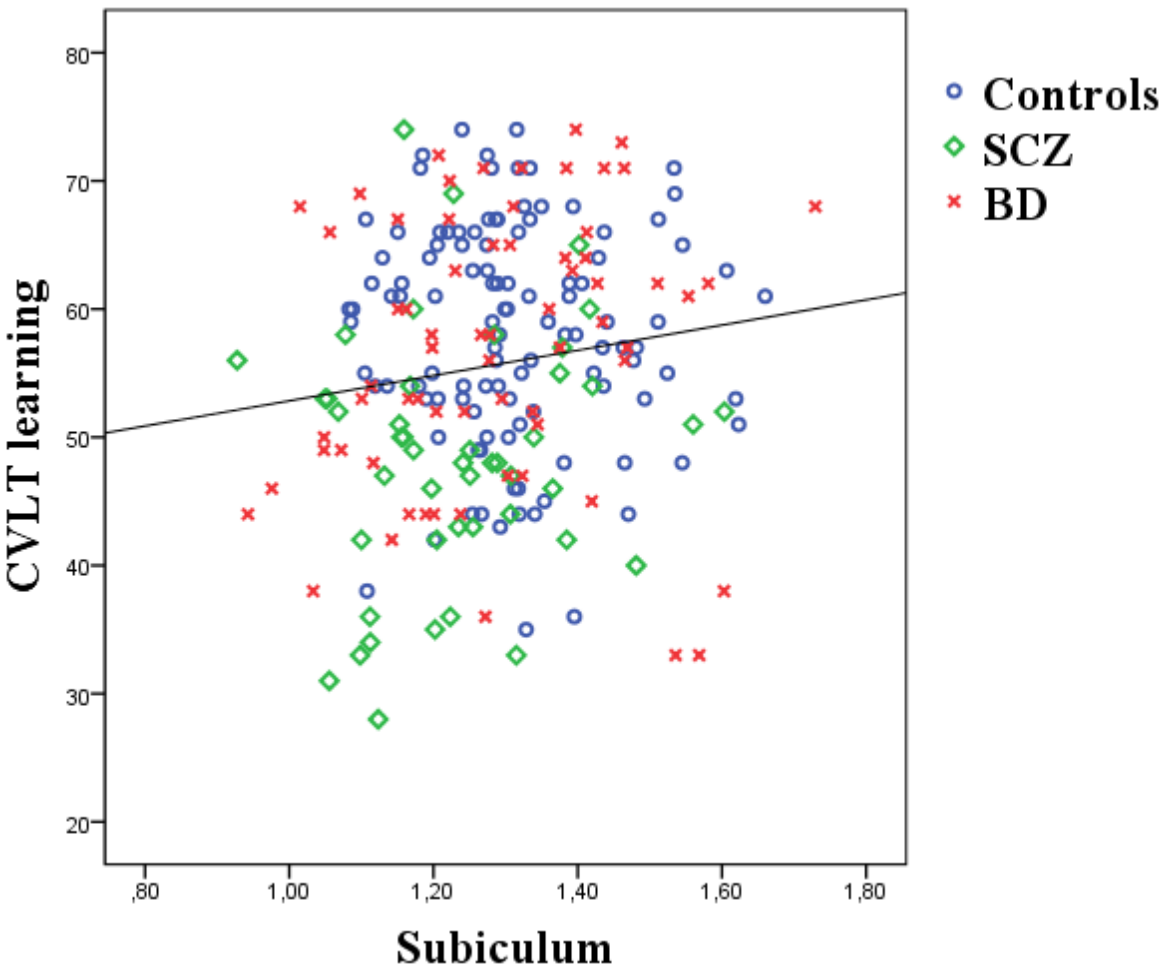

Figure 2. Scatterplot for CVLT learning and Subiculum volume (cm^3) in the MRI group (N = 224).