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Plasma concentrations of anti-inflammatory cytokine TGF-β are associated with hippocampal structure related to explicit memory performance in older adults

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Abstract

Human cognitive abilities, and particularly hippocampus-dependent memory performance typically decline with increasing age. Immunosenescence, the age-related disintegration of the immune system are increasingly coming into the focus of research as a considerable factor contributing to cognitive decline. In the present study, we investigated potential associations between plasma levels of pro- and anti-inflammatory cytokines and learning and memory performance as well as hippocampal anatomy in young and older adults. Plasma concentrations of the inflammation marker CRP as well as the pro-inflammatory cytokines IL-6 and TNF-α and the anti-inflammatory cytokine TGF-β_1 were measured in 142 healthy adults (57 young, 24.47 ± 4.48 years; 85 older, 63.66 ± 7.32 years) who performed tests of explicit memory (Verbal Learning and Memory Test, VLMT; Wechsler Memory Scale, Logical Memory, WMS) with an additional delayed recall test after 24 hours. Hippocampal volumetry and hippocampal subfield segmentation were performed using FreeSurfer, based on T1-weighted and high-resolution T2-weighted MR images. When investigating the relationship between memory performance, hippocampal structure, and plasma cytokine levels, we found that TGF-β1 concentrations were positively correlated with the volumes of the hippocampal CA4-dentate gyrus region in older adults. These volumes were in turn positively associated with better performance in the WMS, particularly in the delayed memory test. Our results support the notion that endogenous anti-inflammatory mechanisms may act as protective factor in neurocognitive aging.

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1. Introduction

While age-related structural and functional alterations of the hippocampus-dependent memory system are an established and well-replicated finding (Nyberg 2017; Gorbach et al. 2017), the underlying (patho-)physiological mechanisms are subject to ongoing investigation. In the past two decades, research has increasingly focused on age-related dysregulation of the immune system as a potential factor contributing to age-related cognitive decline and ultimately clinically relevant memory disorders (Brosseron et al. 2022). Although normal aging is not considered a disease (Rattan 2014), it is nevertheless accompanied by a chronic low-grade pro-inflammatory state, commonly termed "inflammaging" (Franceschi et al. 2000). Humans are exposed to an accumulating antigen load over their lifespans, which acts as a chronic stressor on the immune system and, possibly due to the phylogenetic longevity of humans beyond an evolutionarily intended level, promotes chronic asymptomatic inflammatory activity (De Martinis et al. 2005). During aging, a considerable proportion of cells, including, for example, epithelial cells and fibroblasts enter the state of senescence, which is characterized by a silenced cell cycle and a proinflammatory phenotype ("senescence-associated secretory phenotype", SASP). SASP is defined by senescence-induced increased secretion of proinflammatory cytokines, proteases, growth factors, chemokines, and matrix metalloproteases (Basisty et al. 2020) (see http://www.saspatlas.com/). The thus produced inflammation mediators in turn induce cellular senescence in adjacent cells as a paracrine bystander effect (Nelson et al. 2012; Acosta et al. 2013), resulting in a self-reinforcing process. Furthermore, declining performance of the adaptive immune system during aging is thought to drive increased activation of the innate immune system through increased release of pro-inflammatory cytokines as compensatory mechanism (Fülöp et al. 2017). However, the resulting pro-inflammatory state increases the risk for age-related diseases like cancer or cardiovascular disease and also for age-related cognitive impairment (Tracy 2003; Akbaraly et al. 2013). Notably, not all humans are affected by age-related cognitive impairment to the same degree. Some individuals show relatively preserved memory performance even in old age, a phenomenon often referred to as “successful aging” (Nyberg and Pudas 2019). Research in over-centenarians suggests that balanced anti-inflammatory activity in addition to the age-typical pro-inflammatory phenotype may act as a protective factor counteracting the negative effects of inflammaging (Franceschi 2007).

A prominent anti-inflammatory cytokine is the transforming growth factor β (TGF-β). The TGF-β protein exists in three different isoforms in mammals (TGF-β_1, TGF-β_2 and TGF-β_3), with TGF-β_1 being the most abundant isoform (Dobaczewski et al. 2011). In aging and in the

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context of cellular senescence, TGF-β_1 is secreted by cells in SASP state and contributes to the maintenance of this phenotype. TGF-β_1 also exerts pronounced anti-inflammatory and neuroprotective effects (Preller et al. 2007; Harder et al. 2014) and there is evidence that TGF-β_1 is also actively involved in plasticity processes related to learning and memory. For example, mice treated with TGF-β_1 before being exposed to amyloid-β exhibited less loss of synapses and relatively better-preserved memory (Diniz et al. 2017), and TGF-β_1 protects against amyloid-β-dependent neurodegeneration and release of pro-inflammatory cytokines by glia (Shen et al. 2014; Chen et al. 2015). Furthermore, TGF-β_1 can induce the regrowth of damaged neurons after axonal injury (Abe et al. 1996). TGF-β_1 appears to promote both synaptic plasticity and neurogenesis in hippocampal neurons. TGF-β_1 knock-out mice express lower levels of the synaptic marker synaptophysin and the plasticity-related protein laminin as well as lower synaptic density in hippocampal neurons (Brionne et al. 2003). In mice, inhibition of TGF-β_1 signaling pathways in the hippocampus leads to reduced expression of long-term potentiation (LTP) and impaired object recognition (Caraci et al. 2015), and further studies suggest that TGF-β_1 is involved in the expression of the late phase rather than the early phase of LTP (Mikheeva et al. 2019; Nenov et al. 2019; Caraci et al. 2015).

In contrast to the considerable body of literature on the role of TGF-β_1 in the cellular mechanisms of learning and memory in rodents, little is known about the potential role of TGF-β_1 in human neural plasticity and memory. There is, however, evidence for a protective role of TGF-β_1 in neuroinflammatory and neurodegenerative diseases, particularly in Alzheimer's disease and multiple sclerosis (MS) (Diniz et al. 2017; Martínez-Canabal 2015).

Based on the well-documented role of TGF-β_1 in hippocampal plasticity and memory in rodents and its neuroprotective effects in human diseases, we aimed to assess to what extent TGF-β_1 concentrations are related to hippocampal structure and to learning and memory performance in healthy humans, with a focus on effects on older adults. To this end, we conducted well-established neuropsychological tests of explicit memory (Verbal Learning and Memory Test, VLMT; Wechsler Memory Scale, WMS) and performed automated hippocampal segmentation and volumetry of hippocampal subfields (Quattrini et al. 2020) using magnetic resonance imaging (MRI) in a previously described cohort of young and older adults (Soch et al. 2021b; Soch et al. 2021a). We hypothesized that higher TGF-β_1 plasma concentrations would be associated with larger hippocampal volumes and better memory performance, particularly in older adults. Since hippocampal neurogenesis occurs primarily in the subgranular zone (SGZ) of the dentate gyrus and the functionally closely related CA4 region (Bond et al. 2021), we also hypothesized that those subregions of the hippocampus would be most likely affected by TGF-

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β_1 concentrations. According to previous studies showing that the volumes of the input regions of the hippocampus (i.e., dentate gyrus [DG] and the CA4 and CA3 regions) are positively correlated with learning performance in memory tests like the California Verbal Learning Test (CVLT) and the WMS (Mueller et al. 2011; Aslaksen et al. 2018; Travis et al. 2014), we further hypothesized that larger volumes of the CA4 region and DG would correlate with better memory performance in the VLMT and WMS.

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2. Materials and Methods

2.1 Study Cohort

2.1.1 Recruitment

Participants were recruited via local press in Magdeburg, the online presence (www.lin-magdeburg.de) and social media of the Leibniz Institute for Neurobiology, as well as flyers in shopping centers and at Otto von Guericke University events. Individuals interested in participating provided information about potential contraindications for participation via telephone interview before they were invited to the study. All procedures were approved by the Ethics Committee of the Medical Faculty of the Otto von Guericke University Magdeburg and conducted according to their guidelines (ethics approval number 33/15). All subjects provided written informed consent to participate in accordance with the Declaration of Helsinki (World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects 2013).

2.1.2 Participants

In the present study, we investigated participants from a previously described cohort consisting of neurologically and psychiatrically healthy young and older adults (Table 1) who underwent multimodal phenotyping, including neuropsychology, MRI, and investigation of blood-based biomarkers as part of the *Autonomy in Old Age* research alliance. A detailed characterization of the cohort and description of testing procedures has been reported previously (Soch et al. 2021b; Soch et al. 2021a; Richter et al. 2022). All participants were right-handed according to self-report. The Mini-International Neuropsychiatric Interview (M.I.N.I.; (Sheehan et al. 1998); German version by (Ackenheil et al. 1999)) was used to exclude present or past psychiatric disorders. Further contraindications for participation included alcohol or drug abuse, the use of neurological or psychiatric medication, and major psychosis (schizophrenia, bipolar disorder) in a first-degree relative. For the purpose of the present study, additional contraindications were chronic infectious, autoimmune or other inflammatory diseases (e.g., Crohn's disease, rheumatic diseases, Hashimoto's thyroiditis, or celiac disease) as well as regular use of immunomodulatory and anti-inflammatory medication. After excluding participants with missing data, a total of 142 participants (57 young, 85 older) were available for data analysis.

2.2. Cognitive testing

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2.2.1 General procedure of testing

Testing took place on two consecutive days, if possible. After signing the consent and privacy forms, participants completed health and MRI questionnaires, followed by a health questionnaire and the M.I.N.I. (see above). Participants older than 50 years additionally underwent the Mini Mental State Examination (MMSE) (Folstein et al. 1975) to rule out possible dementia. Afterwards, all participants completed the Multivocabulary Intelligence Test (MWT-B) (Lehrl 2005). This was followed by the actual computer-based cognitive test battery, which included a German version of the Verbal Learning and Memory Test (VLMT, see 2.2.2) (Helmstaedter 2001) and a German auditory version of the Wechsler Memory Scale (WMS) for logical memory (see 2.2.3) (Härting et al. 2000). For a comprehensive description of the test battery see (Richter et al. 2022).

2.2.2 Verbal Learning and Memory Test (VLMT)

The VLMT includes two lists of 15 semantically unrelated words, a study list and a distracter list (Helmstaedter 2001). The experiment was divided into a learning phase and a recall phase. In the learning phase, the words of the first list were presented consecutively visually. After the presentation of all words participants were asked to write down each word they could remember. This procedure was repeated five times in succession. Next, a second list was presented once, followed by a written recall. This list served as a distractor list and was followed by the recall phase, in which the words from the first list were to be written down. Further recall phases followed after 30 minutes and after 24 hours.

2.2.3 Wechsler Memory Scale (WMS): Logical Memory

The subscale *Logical Memory* of the WMS was implemented as a slightly modified, auditory version (Härting et al. 2000). The test persons listened to two short stories over headphones, which they were asked to write down immediately after listening. Participants were then informed that they would be asked to recall the stories again later. Recall tests took place 30 minutes and 24 hours later. The recalled stories were rated by two independent experimenters according to an evaluation sheet with 25 items (i.e., details from the stories), and thus a maximum of 25 points could be obtained per story and recall delay.

2.3 Determination of TGF-β plasma levels

2.3.1 Sample collection, processing and storage

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Venous blood samples were collected from all participants by healthcare professionals after informed consent, including two plasma tubes with sodium citrate as anticoagulant. Platelet-poor citrate plasma was prepared using a standardized two-step separation method (Reinhold et al. 1997). Citrate plasma samples was stored as aliquots in Eppendorf tubes at -80 °C until measurement. Whenever the interval between the two test days was seven days or more, a blood sample was taken for both test days.

2.3.2 Enzyme-linked immunosorbent assay (ELISA)

Plasma concentrations of TGF-β_1 were determined via enzyme-linked immunosorbent assay (ELISA). Additional ELISA measurement were carried out to determine plasma levels of C-reactive protein (CRP) and the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Commercially available Quantikine® ELISA kits (R&D Systems Inc., Minneapolis, MN, USA) were used for this purpose. For IL-6 and TNF-α, the high-sensitivity (HS) variant was used, as low plasma concentrations of these cytokines were expected in healthy individuals, and the HS variant is characterized by a lower minimum detection dose (MDD).

2.4. Magnetic resonance imaging

2.4.1. MRI data acquisition

MRI data were collected using two MRI systems (3 Tesla Verio-Syngo MR system, Siemens Medical Systems, Erlangen, Germany and 3 Tesla Skyra Fit MR system). For volumetric analyses, a T1-weighted 3D Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) image was acquired (TR = 2.5 s, TE = 4.37 ms, flip angle = 7°, 192 sagittal slices, in-plane resolution = 256 x 256, isotropic voxel size = 1 mm^3. In addition, high-resolution coronal T2-weighted images were acquired using a protocol optimized for MTL volumetric analyses (TR = 3.5 s, TE = 353 ms, 64 coronal slices orthogonal to the hippocampal axis, in-plane resolution = 384 x 384, voxel size = 0.5 x 0.5 x 1.5mm^3) (Richter et al. 2022).

2.4.2. Volumetric measurement of hippocampal subfields

Automated volumetric analysis of the individual participants' hippocampi and their subfields was performed using FreeSurfer 6.0 (Fischl 2012) and the module for segmentation of hippocampal subfields, which is based on both *in* vivo scans of human participants and *ex vivo* scans of hippocampi from autopsy specimens (Iglesias et al. 2015). Previous analyses have confirmed the robustness of this protocol across different MRI scanners (Quattrini et al. 2020).

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In addition to the T1-weighted MPRAGE images, high-resolution coronal T2-weighted images (see 2.4.1) were used to improve segmentation accuracy (Dounavi et al. 2020).

2.5. Statistical analysis

Statistical analysis was performed using Matlab R2018b (Mathworks, Natick, MA) and R, version 4.0.4 (https://www.r-project.org/) with RStudio version 1.4.1103 (RStudio Team, 2021), employing the R packages psych (Revelle 2022) and sjPlot (Lüdecke 2022). To investigate a potential direct association of pro- and anti-inflammatory cytokines with learning and memory performance in the VLMT and in the WMS Logical Memory subscale, multiple linear regressions were computed with the concentrations of TGF-β_1, CRP, IL-6, and TNF-α as well as age and gender as independent variables. An additional independent variable was the infection history of the subjects (i.e., self-report of "cold, flu, urinary tract infection or similar" or vaccination) within four weeks prior to testing (hence referred to as "immune event history"). The dependent variables were learning and memory performance and delayed recall in the VLMT and the WMS. The measure of learning performance for the VLMT was the sum of remembered items from learning sessions 1-5. For the WMS, the sum of the remembered items from both stories in the learning session served as measure of learning performance. Delayed recall performance was quantified as the number of recalled words or story items in the 24-hour recall of the VLMT and WMS, respectively.

Statistical analysis of the relationship between TGF-β plasma concentrations and MRI data (i.e., hippocampal volumes and individual hippocampal subfields) was performed analogously to the behavioral data analysis. Multiple linear regressions were calculated with the concentrations of TGF-β, IL-6, TNF-α, and CRP as well as age, sex, and immune event history as independent variables and the volumes of the hippocampus or hippocampal subregions (DG, CA) as dependent variables, separately for each hemisphere. A correction for the false discovery rate (FDR) was applied for the number of regression analyses (N = 6). To assess for a potential association between hippocampal structure and memory performance, Pearson's correlations were computed between the volumes of subregions that showed a robust association with cytokine plasma concentrations ($p < .05$, FDR-corrected) and performance in the VLMT (learning, delayed recall) and in the WMS (immediate and delayed recall).

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3. Results

3.1. Association of TGF-β plasma concentrations with hippocampal structure

As TGF-β has previously been described to affect hippocampal neurogenesis, which occurs primarily in the SGZ, we hypothesized the subregions of the hippocampus most likely to be affected by TGF-β_1 plasma concentrations would be the DG and the CA4 region. To investigate potential associations between plasma concentrations of TGF-β1 or other cytokines (IL-6, TNF-α) and hippocampal structures, we computed multiple linear regressions with the volumes of the entire hippocampus and the DG and CA4 regions as the dependent variable, separately for each hemisphere. Immune event history, age, and gender were included as additional independent variables.

TGF-β1 plasma concentrations were significantly positively correlated with the volumes of the CA4 and DG subregions as well as the volumes of the entire hippocampus in both hemispheres (all $p < .050$, FDR-corrected) in older adults. Other factors influencing the volumes of these regions in older adults were age and gender (all $p < .032$). Table 2 displays the adjusted regression coefficients and significance levels for all immune markers and anatomical regions, and Figure 1 depicts the correlations between TGF-β1 plasma concentrations and hippocampal volumes. However, no effects of TGF-β1 plasma concentrations, age or gender could be observed in young adults (all $p > .060$, uncorrected), suggesting that the positive relationship between TGF-β1 plasma levels and the hippocampal structure was restricted to older adults.

3.2. Correlations of hippocampal subfield volumes with memory performance

To assess a potential relationship between the volumes of the hippocampus and its subregions affected by immune markers with memory performance, Pearson's correlations between volumes and memory performance in the VLMT and WMS were computed for all regions showing a robust relationship (FDR-corrected $p < .05$) with at least one immune marker. As no robust associations were found in young adults, these analyses were restricted to the older participants in our cohort. We observed a positive correlation of the WMS Logical Memory scale with the volume of the right DG ($p = .025$, two-tailed) and, as a trend, also of left DG and the CA4 region bilaterally as well as the volume of the whole right hippocampus (all $p < .05$, one-tailed; see Figure 2). Moreover, significant positive correlations with memory performance in 24-hour delayed recall test of the WMS were observed for the volumes of the dentate gyrus

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bilaterally and the right CA4 region (all $p < .05$, two-tailed) and, as a trend, also for the left CA4 region ($p = .042$, two-tailed). On the other hand, for the VLMT, no significant correlations with the volumes of any of the hippocampal regions investigated were detected (all $p > .096$).

3.3. Age, TGF-β plasma concentrations and memory performance

In both the learning and the 24 h delayed recall phases of the VLMT, older adults performed significantly worse than young adults (linear regression, complete sample, effect of age; learning: standardized $\beta = -0.70$, confidence interval [CI] = [-0.83 -0.57], $p < .001$; delayed recall: standardized $\beta = -0.66$, confidence interval [CI] = [-0.80 -0.53], $p < .001$). Within the group of older adults, linear regression analysis revealed significant effects of age and gender on learning performance (age: standardized $\beta = -0.34$, CI = [-0.56 -0.13], $p = .002$; gender: standardized $\beta = 0.44$, CI = [0.02 0.86], $p = .038$) and delayed recall (age: standardized $\beta = -0.31$, CI = [-0.53 -0.09], $p = .006$; gender: standardized $\beta = 0.47$, CI = [0.05 0.89], $p = .028$). We found, however, no effect of TGF-β1 plasma concentrations on learning or delayed recall performance (linear regression, older adults; learning: standardized $\beta = -0.00$, CI = [-0.35 0.06], $p = .157$; delayed recall: standardized $\beta = 0.00$, CI = [-0.30 0.10], $p = .328$). There were also no effects of plasma levels of other cytokines or CRP nor of immune event history on either learning or delayed recall performance in the VLMT (all $p > .217$).

In the Logical Memory subscale of the WMS, older adults also showed worse performance than young adults in both immediate and delayed recall (linear regression, complete sample, effect of age; immediate recall: standardized $\beta = -0.46$, confidence interval [CI] = [-0.63 -0.30], $p < .001$; delayed recall: standardized $\beta = -0.48$, confidence interval [CI] = [-0.64 -0.31], $p < .001$). When restricting the analysis to older adults, we also observed significant effects of age on memory performance (immediate recall: standardized $\beta = -0.38$, CI = [-0.60 -0.17], $p < .001$; delayed recall: standardized $\beta = -0.28$, CI = [-0.51 -0.06], $p = .014$). As with the VLMT, however, no effect of TGF-β1 plasma concentrations on memory performance was found (linear regression, older adults; immediate recall: standardized $\beta = -0.00$, CI = [-0.35 0.06], $p = .157$; delayed recall: standardized $\beta = 0.00$, CI = [-0.30 0.10], $p = .328$). There was also no significant statistical association between plasma levels of other cytokines or CRP or of immune event history and either immediate or delayed recall performance in the WMS, nor an effect of gender (all $p > .165$).

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4. Discussion

Our results suggest that plasma concentrations of the anti-inflammatory cytokine TGF-β_1 are associated with hippocampal CA4 and dentate gyrus volumes in older adults. The volumes of these hippocampal regions were in turn associated with immediate and particularly delayed recall performance in the WMS Logical Memory scale in the older participants.

4.1. A potentially protective role for TGF-β_1 in the aging hippocampus

While young and older participants did not *per se* show age-related differences in TGF-β_1 plasma concentrations (see Table 1), specifically in older adults, higher concentrations of TGF-β1 were associated with hippocampal structure and particularly the volumes of the hippocampal CA4 and DG subregions. Previous rodent studies and investigations in human neurodevelopmental disorders have pointed to a role for TGF-β signaling in hippocampal development and maintenance. (Johnson et al. 2020; Stegeman et al. 2013; Oishi et al. 2016). In adult mice, TGF-β has can promote hippocampal long-term potentiation (LTP), particularly the transition from early to late-phase LTP (Caraci et al. 2015; Nenov et al. 2019), as well as adult neurogenesis in the DG (Gradari et al. 2021). Given the age-dependence of the association between TGF-β_1 plasma concentrations and hippocampal structure in the present study, one possibility could be that effects of impaired TGF-β signaling during development may be compensated for during young adulthood, but, during aging, the capacity for compensation decreases. On the other hand, it must be noted that TGF-β_1 plasma concentrations were measured at or around the time of testing in our study, and we cannot make statements about long-term availability of TGF-β_1.

Therefore, in our view, a more likely or perhaps additional explanation for the age-dependence of the observed association could be related to the prominent role of TGF-β_1 signaling in CNS, but also systemic inflammation. It has been recognized for over two decades that microglia and astrocytes in particular play a central role as mediators between peripheral, low-grade inflammation and the cellular mechanisms of learning and memory (Yirmiya and Goshen 2011). They also express increased pro- and anti-inflammatory cytokines in aging and respond to peripheral inflammatory processes (Sierra et al. 2007). For example, microglia secrete the anti-inflammatory cytokine IL-10 in response to an inflammatory stimulus, which in turn induces astrocytes to produce TGF-β, resulting in an inhibition of the microglial inflammatory response (Norden et al. 2014). However, aging astrocytes frequently show a blunted response

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to IL-10, resulting in lower levels of TGF-β_1 expression (Norden et al. 2016) and, consequently to a shift toward a pro-inflammatory state in microglia (Diniz et al. 2017), which has a negative impact on neural plasticity (Kempermann and Neumann 2003; Yirmiya and Goshen 2011). While this could in principle explain a relationship between higher TGF-β_1 plasma levels and larger volumes of the hippocampal CA4 region and DG, an explanation primarily based on neurogenesis may be questionable, as recent investigations cast doubt on the robustness of findings on adult neurogenesis in humans (Sorrells et al. 2021). Nevertheless, the role of chronic inflammation in synaptic dysfunction and loss and ultimately neurodegeneration is a well-documented finding (Yirmiya and Goshen 2011; Daulatzai 2014), and increased TGF-β_1 levels have been found in studies of pharmacological neuroprotection (Hosseini et al. 2018; Wiciński et al. 2018). Furthermore, TGF-β_1 also promotes the outgrowth of dendrites (Battista et al. 2006; Luo et al. 2016; Mathieu et al. 2010).

One limitation of the present study is that we could measure TGF-β_1 concentrations only in plasma but not, for example, in CSF. There is surprisingly little available literature on the relationship between CSF and plasma concentrations of TGF-β_1. Previous studies that included measures from both CSF and blood have often treated those measures separately and not reported the correlations between them (Iłzecka et al. 2002; Liu et al. 2022). One study in patients with Alzheimer's disease explicitly mentioned an absence of such correlations (Motta et al. 2007), but in that study, peripheral blood levels of TGF-β_1 were measured in serum and were thus most likely largely attributable to TGF-β_1 released from degranulated platelets (Grainger et al. 2000; Iłzecka et al. 2002). In the present study, we used a protocol that avoids contamination from platelet-derived TGF-β_1 (Reinhold et al. 1997), but a potential relationship with CSF TGF-β_1 remains nevertheless elusive. Furthermore, it must be noted that TGF-β_1 is not able to penetrate the intact blood-brain barrier (BBB) (Kastin et al. 2003). On the other hand, BBB integrity declines with age, even in healthy individuals. The hippocampus and in particular the CA1 region, but also the DG seem to be particularly affected by age-related BBB permeability, while the BBB remains comparably intact in neocortical areas (Montagne et al. 2015). Furthermore, increased BBB permeability in aging elicits increased activation of TGF-β signaling pathways and increased expression of TGF-β_1 in the hippocampus (Senatorov et al. 2019). Additionally, TGF-β1 is involved in angiogenesis and BBB assembly (Diniz et al. 2017) and might increase reactively in response to BBB damage.

4.2. Hippocampal subregion structural integrity and memory performance in old age

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While we observed no direct effect TGF-β_1 plasma concentrations on memory performance, we found that the volumes of the hippocampal regions shown to be affected by TGF-β_1 levels were associated with immediate and particularly delayed recall performance in the WMS Logical Memory scale in the older participants. Previous volumetric segmentation of hippocampal subregions and their association with mnestic abilities have revealed that the volumes of the input regions of the hippocampus (i.e., the DG and the CA4 and CA3 regions) are positively correlated with learning performance in well-established memory tests (Mueller et al. 2011; Aslaksen et al. 2018; Travis et al. 2014). On the other hand, the volumes of hippocampal output regions (i.e., CA1 and subiculum), are more related to delayed memory retrieval (Mueller et al. 2011; Aslaksen et al. 2018). This distinction is further supported by a functional MRI (fMRI) study on memory-related activations of hippocampal subregions (Eldridge et al. 2005). Nevertheless, caution is warranted, as other works have also yielded relationships between CA1 volume and learning performance as well as dentate gyrus and CA4 volumes with performance in delayed retrieval (Aslaksen et al. 2018). Along the same line, a high-resolution fMRI study of successful encoding of information showed that encoding success correlated with activity in the CA1 region and subiculum, while activity in the dentate gyrus (DG), CA2 and CA3 was related to stimulus novelty (Maass et al. 2014). Evidence from rodent studies suggests that the volume of the CA4 region is associated with better spatial memory (Schwegler et al. 1990; Crusio and Schwegler 2005). To interpret these findings, one should keep in mind that "CA4" likely constitutes a misnomer, as, unlike CA1, CA3 and the small C2 region, CA4 mainly contains non-pyramidal cells and is functionally closely related to the adjacent DG (Amaral 1978; Amaral et al. 2007). As such, the region labeled "CA4" in the FreeSurfer-based segmentation of the hippocampus also contains the mossy fibers that originate from neurons of the DG, which was itself also associated with better memory performance in the older group, compatible with findings from previous studies (Zheng et al. 2018; Broadhouse et al. 2019; Kern et al. 2021; Wan et al. 2020). A larger volume in these regions might, for example, have resulted both from more neurons in the DG due to better-preserved structural integrity and possibly neurogenesis as well as from a larger number of dendrites, possibly due to the role of TGF-β_1 in promoting the outgrowth of dendrites (Battista et al. 2006; Luo et al. 2016; Mathieu et al. 2010).

4.3. Clinical implications

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Our results suggest that plasma TGF-β_1 levels are associated with a better-preserved hippocampal structure in older adults, which in turn is predictive for greater episodic memory performance. Notably, our current data are based on physiological variability of TGF-β_1 plasma levels, and potential effects of raising the concentrations to supra-physiological levels, for example, by pharmacological intervention, warrant further investigation. Increased TGF-β_1 plasma levels have, for example, been found in patients with MS and are further increased by immunomodulatory treatment with interferon-β1b (Nicoletti et al. 1998), suggesting that the treatment might augment an already active endogenous anti-inflammatory mechanism. Little is known, however, about potential cognitive effects of elevated TGF-β_1 levels. Notably, CSF TGF-β_1 concentrations are increased in Alzheimer's disease (Swardfager et al. 2010), but their relationship with cognitive performance depends on the disease stage, with both unaffected and severely affected individuals showing a positive correlation with cognitive performance (MMSE), whereas mildly to moderately affected patients exhibit a negative relationship (Motta et al. 2007). Future research should further elucidate the relationship between TGF-β_1 levels and brain, particularly hippocampal, integrity in clinical populations to clarify, for example, its role in inflammatory processes related to neurodegeneration in Alzheimer's disease (Brosseron et al. 2022).

4.5. Conclusions

Our results suggest that TGF-β_1 plasma levels are associated with better structural preservation of the hippocampus in older adults, which is predictive for better episodic memory performance. The present data highlight the role of anti-inflammatory mechanisms as potential protective factors in neurocognitive aging.

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5. Notes

5.1. Acknowledgments

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5.2. Data Availability Statement

Access to de-identified raw data and R scripts used for data analysis will be provided by the authors upon reasonable request.

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Figures

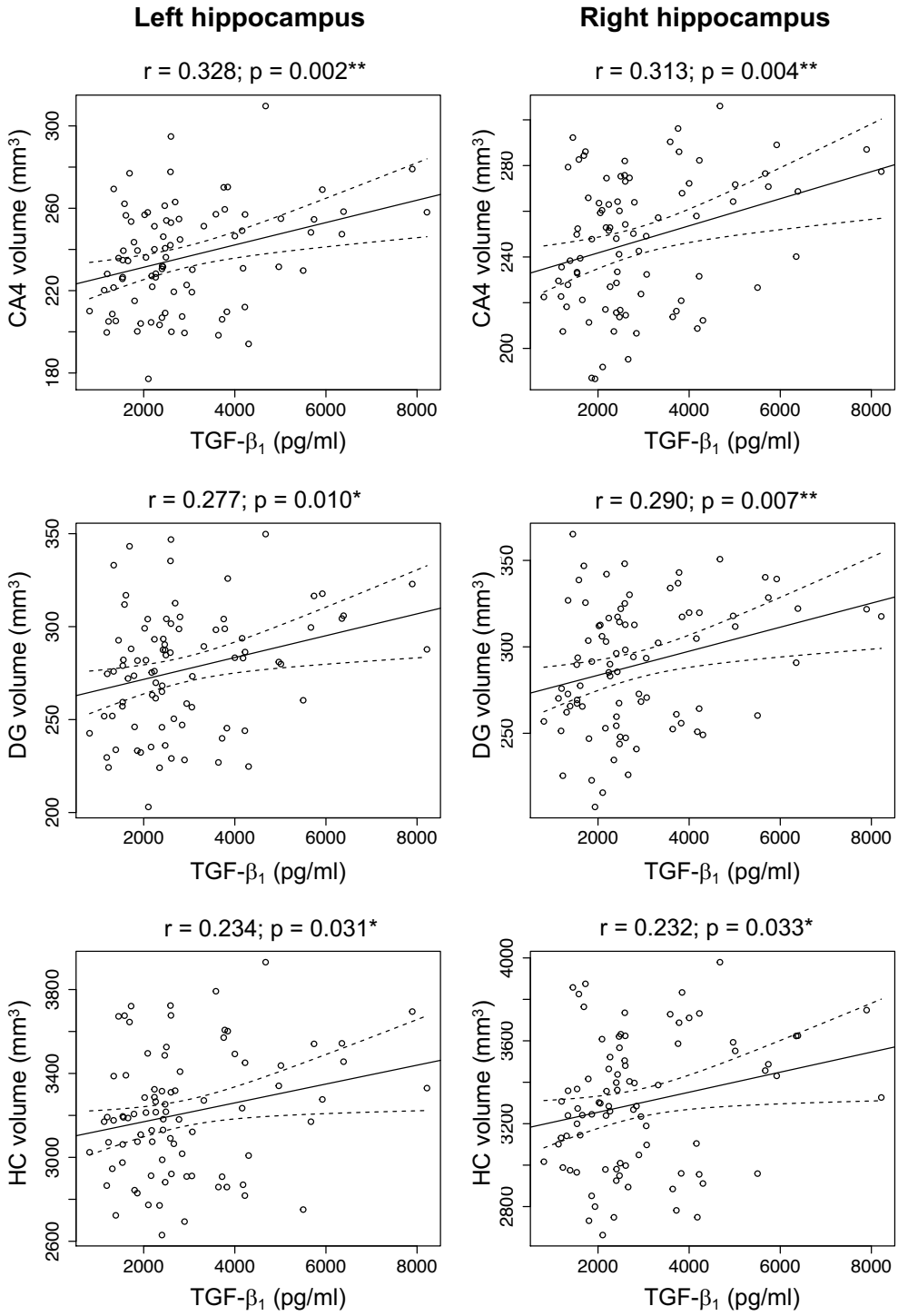

Figure 1: Correlation of TGF-β plasma concentrations with hippocampal subfield volumes. Pearson's correlations are shown for the dentate gyrus (DG), CA4 and whole hippocampus, separated by hemisphere. * $p < .05$, two-tailed; ** $p < .01$, two-tailed.

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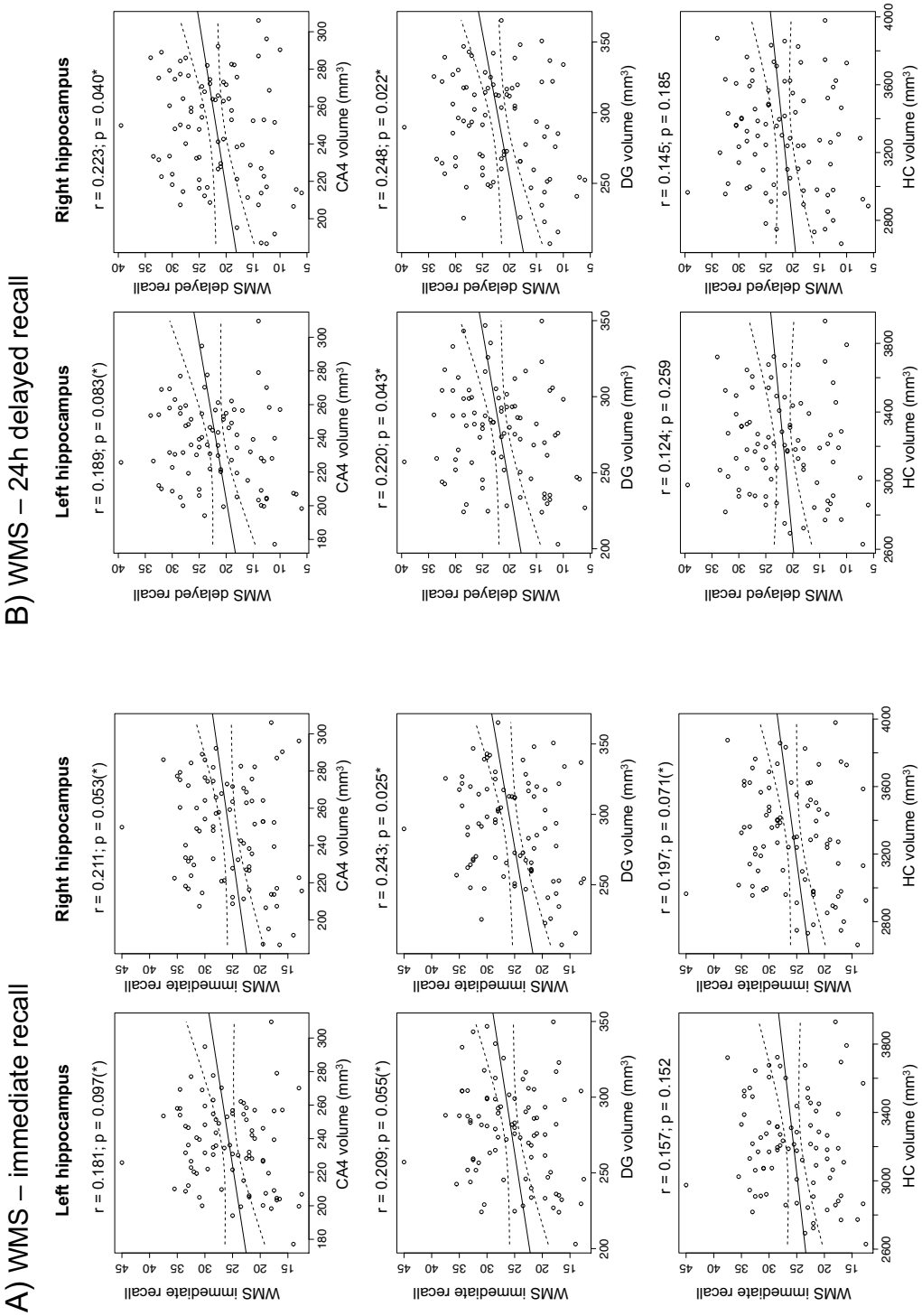

Figure 2: Correlation of hippocampal subfield volumes with memory performance (WMS Logical Memory). Pearson's correlations are shown for the dentate gyrus (DG), CA4 and whole hippocampus, separated by hemisphere. A) WMS immediate recall. B) WMS 24 h delayed recall. * $p < .05$, two-tailed; (*) $p < .05$, one-tailed.

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Tables

Table 1: Demographics and cytokine plasma concentrations

	whole cohort	≥ 50 yrs	18-35 yrs
Size of cohort	n = 142	n = 85	n = 57
Age distribution	47.93 ± 20.29	63.66 ± 7.32	24.47 ± 4.48
Sex distribution (f/m)	87/55	53/32	34/23
IL-6 [pg/ml]	**1.24** ± 0.92	**1.50** ± 0.99	**0.85** ± 0.64
TNF-α [pg/ml]	**0.34** ± 0.19	**0.39** ± 0.15	**0.27** ± 0.21
TGF-β_1 [pg/ml]	**3059.04** ± 1645.62	**2897.37** ± 1517.86	**3300.14** ± 1806.62
CRP [ng/ml]	2**539.63** ± 2572.32	**2622.00** ± 2202.47	**2416.8** ± 3059.14

Sex (female/male) and age as well as cytokine and CRP concentrations (mean +/- standard deviation) are shown.

Table 2: Immune markers and hippocampal subfield volumes

	whole HC		**CA4**		**DG**	
	left	**right**	**left**	**right**	**left**	**right**
TGF-β						
std. β	***0.43***	***0.22***	***0.32***	***0.32***	***0.27***	***0.29***
CI	***[0.11 0.76]***	***[0.03 0.40]***	***[0.13 0.51]***	***[0.13 0.50]***	***[0.07 0.46]***	***[0.10 0.48]***
p	***.025*****	***.023*****	***.001*****	***.001*****	***.008*****	***.003*****
IL-6						
std. β	0.06	0.08	0.13	0.18	0.16	0.18
CI	[-0.16 0.29]	[-0.13 0.29]	[-0.09 - 0.35]	[-0.04 0.40]	[-0.06 0.39]	[-0.04 0.40]
p	.566	.465	.242	.099	.154	.105
TNF-α						
std. β	0.00	-0.11	-0.00	-0.08	-0.02	-0.07
CI	[-0.21 0.21]	[-0.32 0.09]	[-0.21 0.21]	[-0.28 0.13]	[-0.24 0.19]	[-0.28 0.14]
p	.999	.285	.994	.466	.833	.505
CRP						
std. β	0.21	***0.22***	0.07	-0.02	0.06	-0.03
CI	[-0.01 0.43]	***[0.01 0.44]***	[-0.15 0.29]	[-0.24 0.19]	[-0.17 0.28]	[-0.25 0.19]
p	.066	***.039****	.504	.843	.340	.793
immune event history						
std. β	-0.47	-0.47	-0.36	***-0.56***	-0.26	-0.47
CI	[-0.99 0.06]	[-0.97 0.03]	[-0.88 0.15]	***[-1.07 -0.06]***	[-0.78 0.27]	[-0.98 0.04]
p	.080	.063	.160	***.029****	.340	.072

Results of multiple regression analyses are shown. std. β: standardized regression coefficients. CI: confidence interval. * p < .05, uncorrected; ** p < .05, FDR-corrected.

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