ARTICLE

Alzheimer's disease pathology: pathways between central norepinephrine activity, memory, and neuropsychiatric symptoms

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Abstract
The locus coeruleus (LC) supplies norepinephrine to the brain, is one of the first sites of tau deposition in Alzheimer's disease (AD) and modulates a variety of behaviors and cognitive functions. Transgenic mouse models showed that norepinephrine dysregulation after LC lesions exacerbates inflammatory responses, blood–brain barrier leakage (BBB), and cognitive deficits. Here, we investigated relationships between central norepinephrine metabolism, tau and beta-amyloid (Aβ), inflammation, BBB-dysfunction, neuropsychiatric problems, and memory in-vivo in a memory clinic population (total $n = 111$, 60 subjective cognitive decline, 36 mild cognitively impaired, and 19 AD dementia). Cerebrospinal fluid (CSF) and blood samples were collected and analyzed for 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), CSF/plasma albumin ratio (Q-alb), Aβ, phosphorylated tau, and interleukins. The verbal word learning task and the neuropsychiatric inventory assessed memory functioning and neuropsychiatric symptoms. Structural equation models tested the relationships between all fluid markers, cognition and behavior, corrected for age, education, sex, and clinical dementia rating score. Our results showed that neuropsychiatric symptoms show strong links to both MHPG and p-tau, whereas memory deficits are linked to MHPG via a combination of p-tau and inflammation-driven amyloidosis (30–35% indirect effect contribution). These results suggest that the LC-norepinephrine may be pivotal to understand links between AD pathology and behavioral and cognitive deficits in AD.

Introduction

The two main neuropathological hallmarks of Alzheimer's disease (AD) are accumulations of the extracellular

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amyloid-beta (Aβ) and intracellular tau proteins [1, 2]. Disease models suggest a serial model of causality, in which Aβ elevations drive tau pathology and possibly other pathological events [3]. However, a certain amount of tau pathology may precede the presence of detectable Aβ [2, 4], suggesting a complex interplay between pathologies with potentially another common upstream driver.

Autopsy studies showed that the locus coeruleus (LC), a tiny nucleus in the brainstem and the major site for norepinephrine synthesis, is one of the first sites affected by tau pathology [2, 5, 6]. Loss of LC neurons and lower norepinephrine in cortical regions at autopsy correlated with the severity and duration of dementia [7–9]. Dysregulation of the norepinephrine-LC system has been associated with AD-related cognitive deficits and neuropsychiatric symptoms, including depression, anxiety, psychosis, and sleeping disorders [10–12].

The mechanisms underlying LC's involvement in the neuropathology of AD have been carefully examined in animal studies and point to norepinephrine dysregulation as the culprit [13]. Norepinephrine influences microglial functions; it curbs production of proinflammatory molecules

and promotes production of anti-inflammatory molecules [14]. Destruction of LC neurons has been associated with increased tau phosphorylation [15], and seems to spur inflammatory responses [14, 16, 17], which in turn aggravates Aβ accumulation through reduced clearance of Aβ [18]. In addition, norepinephrine depletion induced by lesioning the LC was associated with blood–brain-barrier (BBB) dysfunction, which in turn may be reinforced by inflammatory responses [19, 20]. These observations suggest that an affected LC-system and associated alterations in norepinephrine form a quadruple threat for AD pathogenesis: it exacerbates tau pathology, changes inflammatory responses, leads to BBB disruption and impairs Aβ degradation, which ultimately may lead to the clinical and cognitive symptoms of AD [21].

However, the norepinephrine system has a remarkable compensatory capacity, that, in animal studies, is often neurochemically suppressed or absent due to neuronal destruction. Reductions in norepinephrine-producing neurons are associated with subsequent increases in norepinephrine metabolism or turnover, as measured with the intraneuronal metabolite of norepinephrine, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) in cerebrospinal fluid (CSF) [22–26]. Loss of neurons in the LC is inversely correlated with norepinephrine metabolism [22]. Postmortem increases in CSF-MHPG correlated with antemortem worse clinical severity [24]. While animal and pharmacological human studies showed that the LC norepinephrine-system modulates cognitive functions and behaviors [27, 28], evidence for a role of the norepinephrine-system in the pathophysiology and behavior and cognition in AD remains equivocal due to small sample sizes or not taking into account important confounders, such as age, sex, or other biomarkers.

We aimed to investigate the complex relationships between central norepinephrine metabolism, tau and Aβ, inflammation, BBB-dysfunction memory, and neuropsychiatric problems in-vivo in a memory clinic population with the goal to understand whether central norepinephrine function might be the driver of downstream events in the pathophysiological cascade of AD. Ultimately, these findings will contribute to our understanding of disease mechanisms of AD and facilitate the exploration of pharmacological interventions targeting the norepinephrine system.

Materials and methods

Participants

One-hundred and fifteen patients were recruited from the memory clinic of the Maastricht University Medical Center (MUMC), diagnosed with subjective cognitive decline (SCD, $n = 60$), mild cognitive impairment (MCI, $n = 36$) or probable AD dementia ($n = 19$). Diagnoses were made by experienced physicians based on the core clinical criteria for MCI [29] and AD dementia [30]. Criteria for SCD included self-reported presence of subjective cognitive complaints and endorsing the question "Do you think your memory is becoming worse" [31]. SCD patients did not have impairments on cognitive tests (defined as a score below -1.5 SD of the age-, sex-, and education-adjusted mean). Exclusion criteria were: major neurological disease, clinical diagnosis of other neurodegenerative disorders (e.g., frontotemporal dementia), recent transient ischemic attack or cerebrovascular accident (<2 years), history of psychiatric disorders, and alcohol or drug abuse (Supp-Methods). All patients provided informed consent and the study protocols were approved by the Medical Ethics Committee of the MUMC.

CSF and blood analyses

CSF was collected via a lumbar puncture in the L3 to L5 vertebral interspaces, centrifuged, aliquoted, and stored at -80 °C in polypropylene tubes. Biochemical analysis of CSF Aβ$_{1-42}$ and p-tau$_{181p}$, (Innotest ELISA, Innogenetics, Ghent, Belgium) was done following standardized protocol and blinded to diagnostic information [32]. We focused on p-tau as it is more closely related to AD pathology than t-tau [33], and correlated with t-tau ($r = 0.92$, this data). Both CSF Aβ$_{1-42}$ and p-tau$_{181p}$ were treated as continuous measures as cumulative evidence indicates that normal CSF levels can predict future clinical progression and do not exclude incident AD [34]. MHPG concentrations are independent of the CSF fraction and we followed previously reported methods [35].

APOE genotyping was determined on genomic DNA using polymerase chain reaction. In addition, serum analyses of interleukin (IL)-1β, IL-6, IL12p70 (BD cytometric bead array, BD-biosciences, NJ, USA) were performed and the CSF/plasma albumin ratio (Q-albumin ratio (Q-alb)) was determined using nephelometry. Q-alb is a well-established measure of BBB-dysfunction. However, it may not purely reflect BBB permeability, but also the blood–CSF-barrier (BCSFB) at the choroid plexus [36]. Both the BBB and BCSFB falter in AD [37]. Quantification of Q-alb may be influenced by an attenuation of the turnover rate of CSF during aging, therefore we adjusted all analyses for age.

Data for Q-alb was incomplete for three patients (2 SCD and 1 MCI) and one MCI patient did not have Aβ and p-Tau values. The final sample for analyses was 111 participants (Table 1, Supp-Methods).

Table 1 Overview of the sample

$N = 111$	Mean (sd) or n (%)	Range
Age (years)	63.01 (9.15)	38–89
Females (n, %)	32 (28.83%)	
Education (level)	3.88 (1.95)	1–8
APOE-E4 carrier (n, %)	53 (47.75%)	
MMSE (score)[a]	27.53 (2.54)	16–30
CDR-score (n, %)	0: $n = 8$ (7.21%)	
	0.5: $n = 93$ (83.78%)	
	1: $n = 10$ (9.01%)	
Fluency animals (score)	19.3 (6.12)	7–36
Fluency professions (score)	14.68 (5.48)	2–31
LDST 90 s (score)[b]	36.99 (11.86)	9–62
WLT learning (score)	36.26 (11.65)	11–66
WLT delayed recall (score)	6.35 (3.90)	0–15
NPI Total score	13.71 (14.02)	0–84
CSF Aβ_{1-42} (ng/L)	929.18 (362.85)	140–1898
CSF p-Tau$_{181p}$ (ng/L)	62.87 (33.77)	22–188
CSF-MHPG (nM/L)	38.28 (8.98)	21–62

Note: Education level (eight levels), this coding system is based on Verhage (1964) and the Standard Classification of Education of the Dutch Central Bureau of Statistics (CBS, 2014). It is equivalent to the International Standard Classification of Education (UNESCO, 1997)

[a]MMSE score for one patient was missing

[b]LDST scores for five patients were missing

Neuropsychological assessment

The Clinical Dementia Rating (CDR) scale [38] and Neuropsychiatric inventory (NPI) [39] were administered by a trained professional. The CDR resulted in a global score of 0 ("healthy"), 0.5 ("mild cognitive impairment"), 1 ("mild dementia"), 2 ("moderate dementia) or 3 ("severe dementia"). The NPI detects and quantifies psychiatric symptoms and the total score is the sum of 12 domain scores, that each consists of the product of the severity (1 to 3) and frequency score (1 to 4).

The cognitive assessment included the Mini-Mental State Examination (MMSE), categorical verbal fluency task [40], letter-digit substitution test (LDST) [41], and the verbal word learning task (WLT) [42]. WLT scores of the total learning and delayed recall were z-transformed and averaged.

Statistical analyses

Statistical analyses were conducted in R version 3.4.2. No statistical methods were used to predetermine the sample size. Analyses were performed in the total sample to obtain a broad range in pathology and cognitive performance. Demographics are reported using mean, standard deviation, and range. Zero-order correlations between all measures were examined with Pearson's correlations for continuous variables and point-biserial correlations for dichotomous variables.

To test relationships between CSF and blood markers and memory, we constructed several competing hypothesis-driven structural equation models (SEM). No adjustment for multiple comparisons was performed. A latent variable was constructed for the inflammation markers using confirmatory factor analyses using maximum likelihood estimation. As inflammation consisted of three interleukin markers only one factor was tested. All paths in the models were corrected for age and sex, and regressions involving memory were also corrected for education. In addition, to correct for the imbalance in sample size representing varying levels of clinical impairment, we added CDR-score as covariate. The first model, the null-model, assumed that Aβ, p-tau, inflammation, and Q-alb are independent mediators of the relationship between MHPG and memory. The second model was based on the literature and assumes a synergistic relationship between MHPG and inflammation on Aβ and on Q-alb. In addition, we also added a relationship between Aβ and p-tau based on current disease models that indicate that amyloidosis facilitates tau. The third model was a carefully pruned model based on the previous statistical results. Variables that were close to significance ($p < 0.3$) or had an impact on the model statistics despite being non-significant were not removed. To ensure that clinically normal individuals were not influencing model statistics, we ran the pruned model in patients with CDR scores ≥ 0.5 and in patients > 50 years.

In the final step, we investigated if MHPG is linked to NPI via our measured markers. First, we performed a backward linear regression with NPI total score as outcome variable and all markers and covariates as predictors. Variable selection was done based on the Akaike Information Criterion, but covariates were retained, irrespective of their contribution. The remaining regression model determined the paths added to the model. Models fit was considered acceptable at χ^2/df < 2 [43], a comparative fit index (CFI) of >0.90 [44], root-mean-square error of approximation (RMSEA) of < 0.08 [45] and standardized root-mean square approximation residual (SRMR) < 0.08. The standard errors of the estimates were bias-corrected and accelerated bootstrapped over 10,000 replicates. We reported standardized beta coefficients, 95% confidence intervals and two-sided p-values ($\alpha = 0.05$).

Results

Sample characteristics

Mean age of the sample was 63.01 ± 9.15 years (range: 38–89). It should be noted that seven participants were
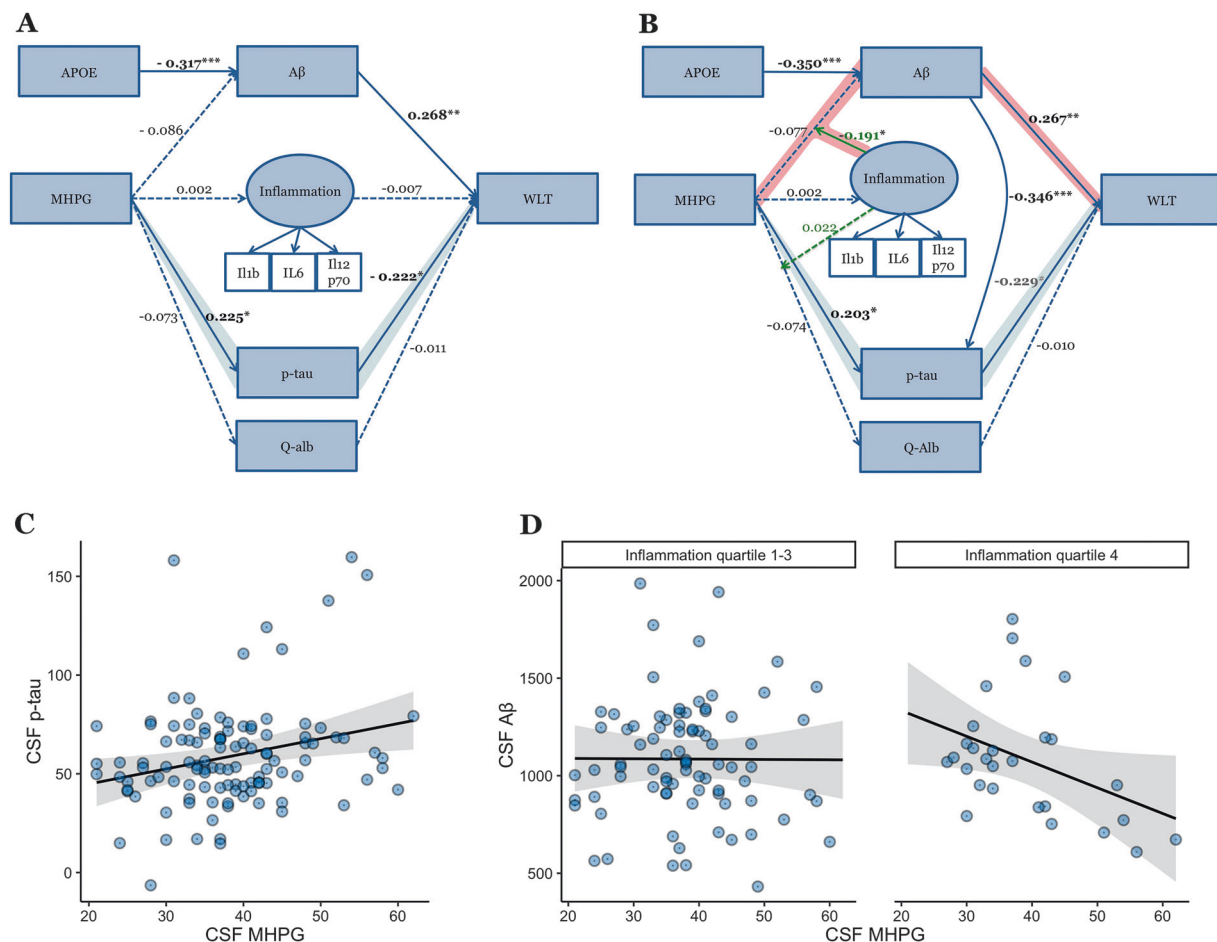
Fig. 1 Relationships between MHPG, biomarkers, and memory. Graphical representation of the null (**a**) and theoretical model (**b**). Non-significant paths are indicated as dotted lines, significant coefficients (and #: $p < 0.10$) are indicated in solid lines. Green lines show interaction effects (MHPG by inflammation). Latent variables are indicated in oval structures. The numbers indicate the standardized beta coefficients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Red and blue shading marks indirect paths from MHPG to WLT (red via inflammation-related amyloidosis and blue via p-tau). MHPG shows a positive relationship with p-tau (**c**) and a positive relationship with Aβ under high levels of inflammation (**d**). The plots depict the estimated marginal means effect, corrected for the covariates. Shaded regions show 95% confidence intervals

younger than 50 years, and five out of these 7 were APOE-ε4 carrier. There were more males than females and almost half of the sample carried at least one APOE-ε4 allele. Distributions of the cognitive scores are provided in Table 1. Eight of the 111 individuals were assessed as clinically normal (CDR = 0). NPI-domains with the highest proportion of individuals endorsing the items: irritability ($n = 60$), depression ($n = 49$), night-time behaviors ($n = 39$) and anxiety ($n = 31$). The lowest rate of endorsement was observed for hallucinations ($n = 4$) and delusions ($n = 6$).

Zero-order correlations (S-Table 1) showed that higher age was associated with abnormal Aβ, p-tau, and with higher MHPG, IL-6, lower MMSE, and WLT performance. Females and ε4-carriers have lower Aβ, greater p-tau and lower Q-alb levels compared to males or non-ε4-carriers. Males and females did not differ in MHPG. Higher p-tau was associated with higher MHPG, worse MMSE and WLT performance, higher NPI scores and higher IL-6 and IL12p70. Il-12p70 was also related to higher MHPG and total NPI scores.

Biomarkers mediating the relationship between MHPG and memory

The latent variable "inflammation" (factor loading weights between parentheses) was constructed with the z-score transformation of IL1β (0.040), IL-6 (0.793) and IL12p70 (0.158). The null SEM-model (Fig. 1a) indicated a direct link between MHPG and p-tau ($\beta = 0.225$, Wald $z = 2.38$, $p = 0.017$, 95% CI = [0.035, 0.436]), but this model was not a good fit to the data. There was no effect of MHPG on Q-alb ($\beta = -0.073$, Wald $z = -0.76$, $p = 0.45$, 95% CI = [−0.262, 0.231]) or on inflammation ($\beta = 0.002$, Wald $z = 0.02$, $p = 0.98$, 95% CI = [−0.253, 0.209]).

Both Aβ ($\beta = 0.268$, Wald $z = 3.35$, $p = 0.001$, 95% CI = [0.118,0.437]) and p-tau ($\beta = -0.222$, Wald $z = -2.49$, $p = 0.013$, 95% CI = [−0.418, −0.049]) were associated with memory performance. About 68.92% of the relationship between MHPG and memory occurred through p-tau (Fig. 1a, blue shade), but MHPG had no direct relationship with memory ($\beta = 0.019$, Wald $z = -0.23$, $p = 0.82$, 95% CI = [−0.175, 0.196]).

The second model, based on literature (Fig. 1b), confirmed the association between MHPG and p-tau, even when controlled for Aβ ($\beta = 0.203$, Wald $z = 2.39$, $p = 0.017$, 95% CI = [0.041, 0.375], Fig. 1c). Furthermore, greater MHPG in combination with elevated inflammation was associated with lower Aβ ($\beta = -0.191$, Wald $z = -2.19$, $p = 0.029$, 95% CI = [−0.440, −0.023], Fig. 1b, red shade; Fig. 1d). This model also showed that Aβ ($\beta = 0.267$, Wald $z = 3.09$, $p = 0.002$, 95% CI = [0.100, 0.443]) shares memory-related variance with p-tau: the effect of p-tau on memory became borderline significant: $\beta = -0.224$, Wald $z = -1.92$, $p = 0.055$, 95% CI = [−0.474, −0.013]. A dominance analyses showed that Aβ and p-tau share 4% memory-related variance, leaving ~12% of the 45.6% total explained variance of the model to either uniquely Aβ (6.9%) or p-tau (5.3%). MHPG has no direct relationship with memory ($\beta = 0.019$, Wald $z = -0.24$, $p = 0.81$, 95% CI = [−0.143, −0.202]). There was no interaction between MHPG and inflammation on Q-alb ($\beta = 0.022$, Wald $z = -0.29$, $p = 0.77$, 95% CI = [−0.112, 0.188]). The model fit was optimal. Results remained similar for the pruned model in the clinically impaired individuals ($n = 103$) and in individuals >50 years ($n = 104$, S-Fig. 1). Model fits are reported in Table 2.

We examined the product of path coefficients of the theoretical model to determine the relative contributions of individual pathways linking MHPG with memory (S-Table 2 and Fig. 2), including pathways contingent on inflammation. The largest simple indirect effect between MHPG and memory was through p-tau (14%). The effect of MHPG on memory was substantially larger when contributions of p-tau where combined with the conditional effect of inflammation on Aβ (30%). A comparable proportion could be attributed to Aβ in combination with greater levels of inflammation (35%). The contribution of Q-alb, direct or contingent on inflammation, to the relationship of MHPG on memory was minimal.

AD-biomarkers as mediator between MHPG and neuropsychiatric symptoms

The remaining model from the backward regression ($R^2 = 0.22$) included MHPG ($\beta = 0.27$, $t = 2.91$, $p = 0.004$, 95% CI = [0.089, 0.458]), p-tau ($\beta = 0.0.33$, $t = 3.11$, $p = 0.002$, 95% CI = [0.123, 0.546]) and APOE ($\beta = -0.18$, $t = -1.94$, $p = 0.055$, 95% CI = [−0.357, 0.002]). APOE-ε4-carriers had lower NPI scores than non-carriers. This was added into our pruned SEM-model (Fig. 3). Relationships between MHPG, Aβ, inflammation, p-tau, and memory remained the same. There is a relationship between p-tau and NPI ($\beta = 0.321$, Wald $z = 3.34$, $p = 0.001$, 95% CI = [0.146, 0.496]), independent of the association between MHPG and NPI ($\beta = 0.268$, Wald $z = 2.02$, $p = 0.043$, 95% CI = [0.038, 0.498], Fig. 3a, b, orange shade). Model fit parameters indicate that the model is acceptable (Table 2). P-tau mediated the relationship between MHPG and NPI ($\beta = 0.065$, Wald $z = 2.05$, $p = 0.04$, 95% CI = [0.004, 0.127]).

Posthoc, we examined which NPI-domains contributed to the model, by including a confirmatory factor analyses

Table 2 Model fit indices for the investigated models

Model	χ^2	CFI	RMSEA	SRMR
Null-model	36.96 (df: 15) χ^2/df = 2.46	0.882	0.115 pClose = 0.014	0.059
Theory-model	21.06 (df: 18) χ^2/df = 1.17	0.984	0.039 pClose = 0.563	0.044
Pruned in CDR ≥ 0.5	14.65 (df: 9) χ^2/df = 1.63	0.967	0.078 pClose = 0.232	0.036
Pruned with NPI	21.08 (df:12) χ^2/df = 1.76	0.963	0.083 pClose = 0.166	0.041
Pruned with NPI-domains (CFA)	76.36 (df = 47) χ^2/df = 1.62	0.944	0.075 pClose = 0.095	0.070

RMSEA root-mean square error of approximation, *CFI* comparative Fit index, *pCLOSE* p-value of close fit, *SRMR* standardized root-mean square approximation residual, *CFA* confirmatory factor analyses, *CDR* clinical dementia rating, *NPI* neuropsychiatric inventory

Criteria for a well fitting model are: χ^2/df < 2, CFI > 0.90, RMSEA < 0.08 accompanied by a non-significant pCLOSE and SRMR < 0.08
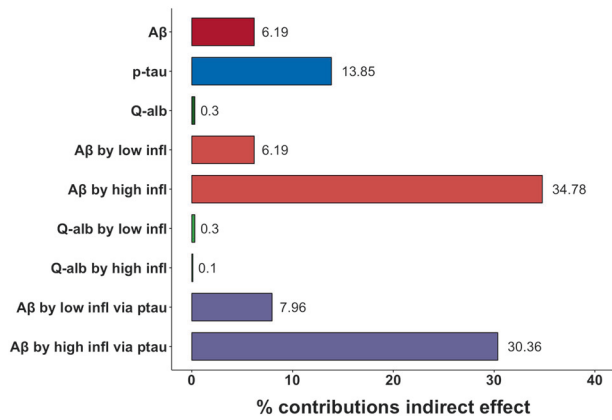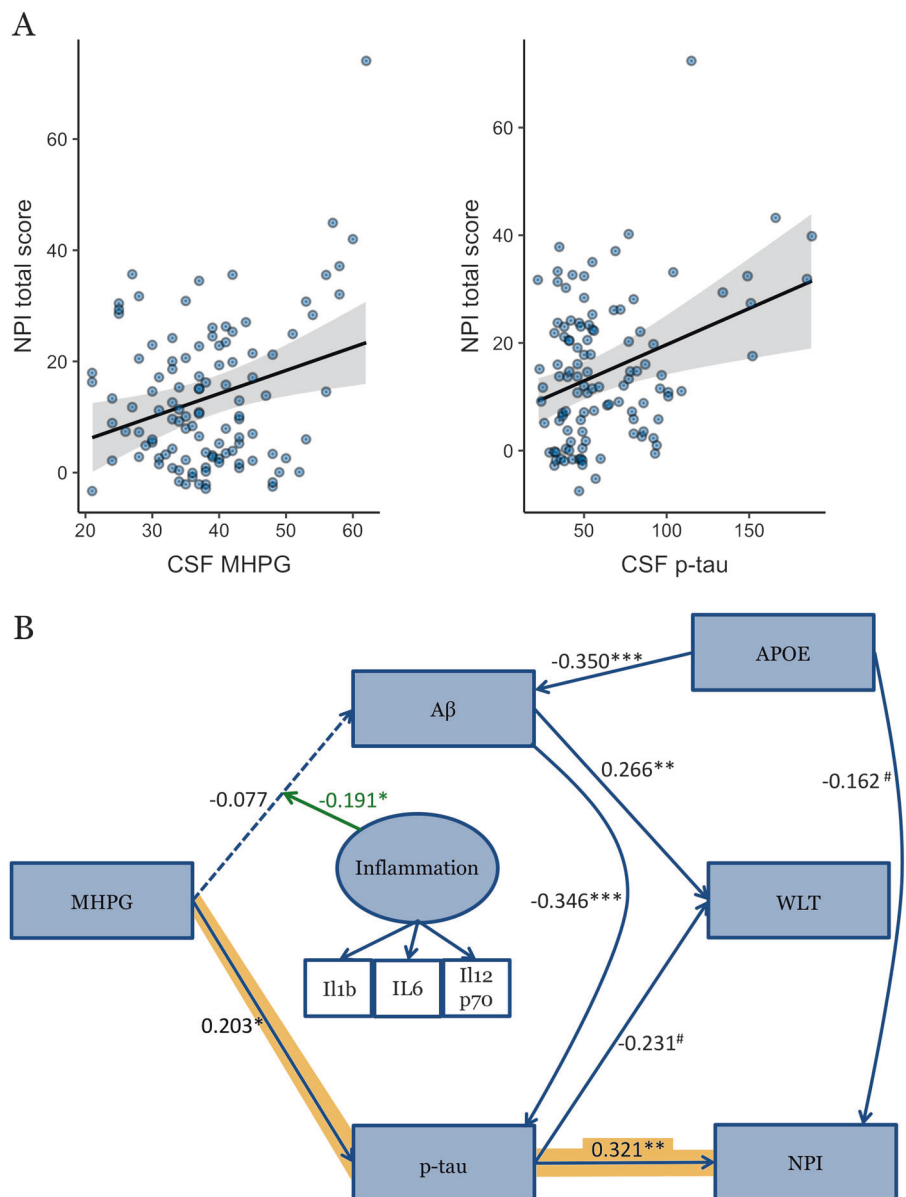
Fig. 2 Percent contributions of indirect effect of MHPG through the markers on memory performance. infl = inflammation

with NPI score as a latent variable consisting of the 12 domains product scores. Domains with a factor loading of <0.5 (standardized) or non-significance were removed. Our final latent variable (loadings between parentheses) consisted of the delusions (0.556), anxiety/agitation (0.904), elation/euphoria (0.896) and disinhibition (0.740) domains. The model fit was reasonable (Table 2).

Discussion

Accumulation of Aβ and tau are the defining neuropathological hallmarks of AD [1, 2]. Yet, the mechanisms underlying the initiation of pathology are not well understood. As the first pathological changes emerge decades

Fig. 3 Relationships between MHPG, biomarkers, and neuropsychiatric symptoms. MHPG and p-tau show a positive relationship with NPI total score (**a**). The association is the estimated marginal means effect, corrected for the covariates. Shaded regions show 95% confidence intervals. Graphical representation of adding NPI total score into the pruned model (**b**) based on results of the backward linear regression. Non-significant coefficients are indicated as dotted lines, significant coefficients (and #: $p < 0.10$) are indicated in solid lines. Green lines show interaction effects (MHPG by inflammation). Latent variables are indicated in oval structures. The numbers indicate the standardized beta coefficients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Orange shading marks indirect path from MHPG to NPI via p-tau

prior to the first clinical symptoms [4], ascertaining the role of processes related to the possible initial site of pathology, the norepinephrine-LC system, will be critical to inform disease models as well as therapeutic avenues. Guided by animal studies, we investigated relationships between in-vivo norepinephrine metabolites, AD pathology, inflammation, BBB-dysfunction on the one hand and memory deficits and neuropsychiatric symptoms on the other in memory clinic patients. Our results provide evidence that greater levels of the norepinephrine metabolite MHPG, were associated with higher p-tau, independently of amyloidosis. Furthermore, we showed that greater levels of MHPG were associated with memory deficits via tau and inflammation-associated amyloidosis pathology. Neuropsychiatric symptoms were directly associated with MHPG, and this relationship was partially mediated by tau pathology. The fact that memory and neuropsychiatric symptoms are related to MHPG via partially overlapping AD-pathology pathways, and that norepinephrine was related to both AD-related pathology and symptomatology suggests that the norepinephrine system holds promise as a target for early intervention.

Prior postmortem and antemortem CSF studies reported that greater MHPG levels are associated with greater age or disease stage, even in the presence of conspicuous neuronal loss in the LC [22, 24, 46–48]. It has been hypothesized that increases in MHPG reflect compensatory mechanisms of surviving neurons [22, 49]. Possible mechanisms attributed to this compensation are neuronal plasticity prompting hyperinnervation of target brain regions, increased norepinephrine synthesis and firing rate of damaged neurons or a decreased ability of presynaptic α_2-receptors inhibiting norepinephrine activity [23, 50].

We found that higher MHPG was associated with older age, higher p-tau and NPI scores. Phosphorylation of tau may be more closely related to increases in MHPG than norepinephrine. Transgenic AD mice with COMT-deletion showed less widespread tau phosphorylation than AD mice, suggesting that O-methylation into MHPG is necessary to facilitate tau spread [51]. While the Oikawa study, reporting that LC destruction was associated with increased tau, may seem contradictory, it is worth nothing that the LC was only partially destructed (54% norepinephrine loss) [15]. This may not have been sufficient to lower the MHPG response.

In our data, MHPG had a closer relationship with neuropsychiatric symptoms than with cognition. Higher levels of MHPG are related to arousal dysregulation. Administration of foot shocks increased MHPG in rats [52]. Infant rhesus monkeys who were repeatedly separated from their mother or peers exhibited increased MHPG levels, which remained high over the 2-year follow-up period [53]. This increase in MHPG was associated with more fearful and less explorative behavior at novel stimuli, suggesting a link between MHPG and behavioral symptoms. In humans, dysregulation of norepinephrine has been associated with increased agitation and anxiety [10, 11]. Autopsy studies in AD reported correlations between MHPG or tau and neuropsychiatric domains overlapping with our results (affective disturbances, hallucinations, and aggression), but not always in the same direction [54–56]. More dynamic behavior in MHPG might be observed in populations with a higher neuropsychiatric burden compared to our relatively low endorsing sample.

The partial mediation of p-tau on MHPG and NPI is consistent with a recent autopsy study reporting higher odds of developing agitation, anxiety, appetite dysfunction, depression, and sleep disturbances during Braak stage I-II [57]. At this stage tau pathology is mainly detected in the isodendritic core regions and transentorhinal cortex. Consistent with our findings, these relationships were stronger for tau than for Aβ.

MHPG has been related to cognition, but so far no study has related this to AD pathology. In normal older individuals, higher MHPG levels were associated with worse visuoperceptual performance, and in AD patients higher MHPG predicted clinical progression [24, 46, 58]. Autopsy studies related lower LC neuronal count to lower antemortem episodic memory and working memory performance [59, 60]. As these correlations were stronger for higher neuropathological burden, it indicates that cognitive deficits related to LC degeneration increase as a function of AD pathology. We observed larger contributions on the MHPG-memory relationship from a combination of tau and inflammation-based Aβ pathology compared to only tau pathology, which is consistent with the notion that cognitive deficits are noticeable when pathology has spread beyond the initial stages and frank Aβ pathology is present [61]. Even though, we were not able to separately examine unimpaired individuals, we observed a zero-order correlation between MHPG and memory in the complete sample ($n = 115$, $r = -0.203$, $p = 0.03$), suggesting that in larger sample sizes, MHPG may track with subtle memory deficits, possibly prior to accumulation of pathology.

Despite evidence from animal studies, we did not observe a relationship between MHPG, inflammation and BBB. Inflammatory responses and changes in Aβ clearance were observed in rats after destruction of LC neurons, especially when this was associated with a substantial decrease in norepinephrine [14, 16]. Experimentally rescuing norepinephrine in transgenic APP mice exhibiting LC degeneration partially restored microglial functioning [16, 62, 63], suggesting involvement of non-norepinephrine pathways, including the modulation of neurotrophic support, such as BDNF [64, 65]. As we observed synergistic relationship between MHPG and inflammation on Aβ, we speculate that higher MHPG may be an indication of

global norepinephrine dysregulation. These findings confirm that inflammation is part and parcel of AD's pathophysiology [66].

We were not able to confirm that inflammation exacerbates the negative effect of norepinephrine on BBB-dysfunction [19, 20]. Monoamines are assumed to regulate the intracerebral microvascular tone, but these effect sizes may be small. Higher levels of monoamines can open the BBB-barrier transiently, but most likely indirectly via acute and manifest rises in blood pressure [67]. Manipulations to induce hypertension in animal studies are performed at levels that are not comparable to the progressive natural blood pressure increases in older individuals.

There are limitations. First, the relationship between tau pathology and hyperexcitability of LC neurons may be a vicious cycle [21, 68]. Our analyses concerned cross-sectional observational data that do not allow any causal inferences. Second, CSF markers are sensitive to variation due to pre-analytical factors, such as tube type or time to freezing, or analytical factors such as lack of calibration across assays complicating comparisons across laboratories. We used the same assay for all patients of the same center using state of the art standardized biobanking procedures. One limitation inherent to CSF markers, including neurotransmitter metabolites, is that changes cannot be localized to specific brain regions [69]. Developments in PET-tracers such as ^{11}C-MeNER [70] opens up exciting opportunities to investigate anatomical localization, especially when combined with novel methods to image the LC at high resolution [71]. Third, animal studies reported a sex dimorphism on the relationship between norepinephrine and tau phosphorylation [15, 51], consistent with a female predisposition for norepinephrine-related disorders [72]. The large proportion of males in our sample precludes examining sex-differences, but this will be an important future research avenue. Fourth, age, height, and BBB-leakage may confound CSF monoamines metabolites [73]. We corrected all analyses for age and sex, as women tend to be shorter than men. There was no relationship between MHPG and Q-alb. Furthermore, four AD patients were using SSRI's, which could have influenced MHPG levels [74]. Finally, our sample size did not allow us to assess these relationships in different diagnostic groups. Therefore, we corrected for CDR-score and reran the analyses in clinically impaired individuals. Studies with larger sample sizes and longitudinal data can examine relationships along all disease stages and changes over time.

In conclusion, the relationship between memory or neuropsychiatric symptoms and MHPG is mediated by partially overlapping AD-pathology pathways. Neuropsychiatric symptoms show links to both MHPG and p-tau, independent of Aβ, whereas memory deficits are linked to MHPG via a combination of p-tau and inflammation-driven amyloidosis.

These observations place the LC-norepinephrine at the intersection of behavioral and cognitive deficits in AD and suggest that pharmacological interventions bolstering the integrity of this system early in the disease may be promising to combat further progression.

Data availability

Supplementary information is available at MP's website. Data used in the current study is available from the corresponding author on reasonable request and in accordance with the EU legislation on the general data protection regulation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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