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Volume and connectivity of the Ventral Tegmental Area are linked to neurocognitive signatures of Alzheimer's disease in humans

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Running Title

Ventral tegmentum in preclinical Alzheimer

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Abstract

Background: There is an urgent need to identify the earliest biological changes within the neuropathological cascade of Alzheimer's disease (AD) processes. Recent findings in a murine model of AD showed significant preclinical loss of dopaminergic neurons in the ventral tegmental area (VTA), accompanied by reduced hippocampal innervation and declining memory. It is unknown if these observations can be translated in humans.

Objective: We tested the hypothesis that VTA volume is associated with the typical clinical markers of AD in a cohort of patients and healthy controls.

Methods: Structural and resting state functional MRI scans, and neuropsychological scores were acquired for 51 healthy adults, 30 patients with a diagnosis of mild cognitive impairment, and 29 patients with a diagnosis of AD dementia. VTA volume was quantified together with other control nuclei. The association between nuclei volume, hippocampal size, memory performance, and linguistic-executive skills was tested. The effect of VTA functional connectivity was also tested.

Results: VTA size, but not of control nuclei, yielded a strong association with both hippocampal size and memory competence (but not linguistic-executive performance), and this was particularly strong in healthy adults. In addition, functional connectivity between the VTA and hippocampus was significantly associated with both markers of AD.

Conclusion: Diminished dopaminergic VTA activity may be crucial for the earliest pathological features of AD and might suggest new strategies for early treatment. Memory encoding processes may represent cognitive operations susceptible to VTA neurodegeneration.

Keywords (and MeSH Unique ID)

Alzheimer Disease (D000544); Dopaminergic Neurons (D059290); Gray Matter (D066128); Functional Neuroimaging (D059907); Neuroimaging (D059906); Early Diagnosis (D042241); Memory, (D008568); Cognitive Dysfunction/Mild Cognitive Impairment (D060825); Hippocampus (D006624); Tegmentum Mesencephali/Ventral Tegmental Nucleus (D013681)

INTRODUCTION

The epidemiological and economic burden of Alzheimer's disease (AD) increases [1], but the exact mechanisms by which the initial neuropathological changes are triggered are still elusive. The "classic" amyloid-β cascade hypothesis posits that it is the abnormal accumulation of this protein in parenchymal regions that induces all subsequent changes in neural structure and function seen in AD. This hypothesis is currently at the center of a scientific debate, with evidence in its support [2], and research conclusions, which, instead, do not sustain its claims [3]. A crucial aspect in the identification of the ontogenesis of the disease is certainly a progressive shift towards the characterization of the earliest preclinical stages of AD (i.e., when individuals are in their early adulthood, or even earlier). This has been pursued both to clarify the causing mechanisms, but also to find an early disease marker, that may be of assistance in the diagnostic process. Although focusing on the genetic form of AD is by far the most convenient approach to study preclinical AD in humans (young carriers of a mutation in one of the AD-related genetic loci will inevitably go on developing the disease), the study of sporadic preclinical AD (not necessarily bound to its genetic forms) is instead a much more effortful enterprise that demands large cohorts and long study durations. On this note, only a few studies have identified variables that could be exploitable in a clinical setting and, at the same time, shed light on the mechanism behind the neurotoxic cascade of AD. A prominent finding emerged from this type of research is that showing that an impoverishment of lexical-semantic abilities during early adulthood is a significant predictor of AD pathology at post-mortem [4]. A second finding has emerged from detailed histological analysis of brain tissue: non fibrillar precursors of abnormal TAU protein are detected in early adulthood (i.e., "pre-tangle" material) in brainstem nuclei, especially in the locus coeruleus [5]. In an attempt to identify a preclinical marker, we tested a hypothesis derived from the results of a study published very recently. In their manuscript, Nobili and

colleagues found that, in a mouse model of the disease, very early anatomical changes are present in a subcortical brain region rich in dopaminergic neurons, the ventral tegmental area (VTA), or ventral tegmentum [6]. Specifically, neuronal loss seems to be present in this area prior to any deposition of amyloid-β plaques. Moreover, this is accompanied by reduced dopaminergic innervation to the hippocampus, and decreased memory performance [6]. Although this was found in a group of transgenic mice carriers of an AD related genetic mutation, the principle that a dopaminergic process may be a prime mechanism that contributes to triggering the neurotoxic cascade would be a valid principle in any form of AD. On these grounds, we thus transposed and tested this hypothesis in a sample of humans.

We hypothesized that the size of the VTA, estimated with a volumetric index, obtained from magnetic resonance imaging, would be significantly associated with the size of the hippocampus and with performance on a test of episodic memory. We also tested whether VTA functional connectivity would co-vary with memory performance and hippocampal volume.

MATERIAL AND METHODS

Participants

A cohort of 110 individuals was included in this study. These had been recruited at the Royal Hallamshire Hospital (Sheffield, UK), as part of the EU-funded research initiative Virtual Physiological Human: DementiA Research Enabled by IT (www.vph-dare.eu/; see Acknowledgments section). Of those included in this cohort, fifty-one were heathy adults free from neurological symptoms or cognitive complaints. Other twenty-nine were patients

with a clinical diagnosis of mild/moderate AD dementia. The remaining thirty participants were patients with a diagnosis of mild cognitive impairment (MCI) of the single-domain amnestic type (n = 1), multiple-domain amnestic type (n = 12), single-domain non-amnestic type (n = 7), multiple-domain non-amnestic type (n = 10), that could not be accounted for by neurovascular, psychiatric, metabolic or traumatic reasons [7]. The clinical profile of these patients (detailed by a senior neurologist and a senior clinical neuropsychologist) was strongly indicative of underlying AD pathology as the main etiology causing their symptoms, and the diagnostic criteria for MCI due to AD were applied to classify each of these 30 patients as prodromal AD [8]. Specifically, all patients had been followed up clinically at regular intervals for at least two and a half years for the confirmation of the diagnosis.

Each participant completed a magnetic resonance imaging (MRI) protocol (see subsequent section) and an extensive battery of cognitive tests, to comply with study criteria and clinical profiling (illustrated in **Table 1**). Of these, two indices of cognitive competence were extracted from the battery of tests: the performance on the Prose Memory test (the average of the immediate and delayed recall scores, as well as immediate and delayed recall scores taken separately) as a measure of verbal episodic memory [9], and the performance on the Letter Fluency test as a measure of language and executive functioning not reliant on the hippocampus [10]. Raw scores on these two tests were converted into z scores based on the mean and standard deviation of the entire cohort, with the following formula $z_x = (x_i - \mu) / \delta$.. Mini Mental State Examination (MMSE) scores [11] were also extracted from each assessment.

-- Please add **Table 1** about here --

This study received ethical approval from the Yorkshire and Humber Regional Ethics Committee, Ref No: 12/YH/0474. Written informed consent was obtained from all participants prior to enrollment.

MRI Acquisition

Each participant underwent an MRI research protocol (Philips Achieva, 3 T) inclusive of anatomical and functional image sequences. Of these, T1-weighted and resting-state fMRI images were the acquisition types suitable to address the planned experimental question.

T1-weighted images were acquired with the following parameters: voxel size: 0.94 mm × 0.94 mm × 1.00 mm; repetition time: 8.2 s; echo delay time: 3.8 s; field of view: 256 mm; matrix size: 256 × 256 × 170.

Resting-state fMRI images were based on 125 volumes, acquired with the following specifications: TR 2.6 s, TE 35 ms, flip angle 90°, voxel dimensions 1.80 × 1.80 × 4.00 mm, field of view 230 mm, 35 slices per volume.

MRI Processing

Image processing was carried out with Matlab (Mathworks Inc., UK) and Statistic Parametric Mapping 12 (Wellcome Trust Centre for Neuroimaging, London, UK). The T1-weighted MRI sequence was processed with standard voxel-based morphometry [12]. Images were initially segmented to separate the maps of gray matter, white matter, and cerebrospinal fluid, were registered to the Montreal Neurological Institute anatomical template, and were smoothed with an 8 mm full-width at half maximum Gaussian kernel.

The volumes of the three tissue class maps in the native space were quantified using the "get_totals" Matlab function (http://www0.cs.ucl.ac.uk/staff/g.ridgway/vbm/get_totals.m). These were added up to obtain the total intracranial volume and, in turn, the global gray matter ratio.

Volumes of interest were drawn using the PickAtlas toolbox and the Brodmann's atlas [13]. The VTA was defined in the Montreal Neurological Institute space as a spherical volume of 3 mm radius centered at x = 0, y = -16, z = -7, as implemented in previous research [14, 15]. Additional regions were selected as methodological control (**Fig.1**). These were the red nucleus (RN), based on its proximity to the VTA, and the substantia nigra (SN), another region rich in dopaminergic neurons which had been found to not play any role in the pre-plaque stage in the study by Nobili and colleagues [6]. Mean gray matter signal intensity was then extracted from each volume of interest with MarsBaR [16], as done in previous research (e.g., [17]). Since regional volumes are influenced by head size, these were normalized to ratios of the brainstem. To do so, a brainstem mask was created using PickAtlas, and volumes were extracted using the "get_totals" script.

- Add **Fig.1** about here -

Hippocampal volumes were calculated using Similarity and Truth Estimation for Propagated Segmentations (STEPS), an automated procedure that segments the hippocampus from native-space anatomical images based on multiple templates (http://cmictig.cs.ucl.ac.uk/niftyweb/). STEPS outperforms other methodologies on the segmentation of the hippocampus, and generates results that closely resemble those of

manual segmentation [18]. **Fig.2** illustrates two examples of the use of this procedure. Each of the 110 outputs was visually inspected for quality control. The "get_totals" Matlab function was used to convert the output files into a volumetric index. This was partialized based on total intracranial volumes, and left and right hippocampal ratios were averaged to obtain a global hippocampal ratio.

- Add **Fig.2** about here -

Resting state T2* images, indexing the haemodynamic properties of the brain, were preprocessed following a standardized methodology, as described elsewhere (e.g., [19]). Briefly. raw images were initially slice-timed and realigned to even out temporal and spatial variability in the acquisition process. A normalization followed, during which scans were registered to the Montreal Neurological Institute space. A band-pass filter was then applied to retain the range of frequencies relevant for neural signal (0.01 – 0.1 Hz). Finally, filtered scans were smoothed with a 6 mm full-width at half-maximum Gaussian kernel. The hemodynamic timecourse within each voxel was modelled as a function of the signal of the VTA, regressing out the signal coming from white matter and cerebrospinal fluid, and in-scanner motion parameters.

Modelling

Nonparametric correlation models were run to test the association between each anatomical ratios (VTA ratio, RN ratio, and SN ratio) and the neurocognitive features of AD

(hippocampal ratio, memory performance, and, as control measure, linguistic-executive performance). The threshold for statistical significance of the *Spearman's rho* coefficients accounted for nine (3 nuclei × 3 models) independent correlations ($p < 0.005$). Since healthy controls were significantly more educated than patients, education-corrected *Spearmann's* rho coefficients were also calculated. MMSE scores were added as second covariate in the models testing the correlation between hippocampal ratio and the size ratio of the nuclei. Since a very strong correlation existed between MMSE and Prose Memory scores (rho = 0.788, p = 1.64e-24), the correlation models testing the association between memory performance and VTA ratio were not corrected for MMSE.

To reach a better understanding of the structural and functional relation between each nucleus and the rest of the brain, other analyses were run. First, the structural covariance of the VTA and the other nuclei was explored. This served to understand what pattern of regions tend to covariate in volumetric terms with each nucleus. Voxel-by-voxel regression models were carried out across the entire cohort, in which gray matter maps were modelled as a function of the size of each nucleus. The score on the Mini Mental State Examination was used as a correction factor for these analyses.

Second, maps of VTA functional connectivity were analysed. This was done as a function of the normalized hippocampal ratio and memory performance, in the entire cohort and within each diagnostic group. Age, education levels, and gray matter ratios were included as covariates in each model. Scores on the Mini Mental State Examination were added as further covariate in the model run in the whole cohort.

RESULTS

Correlation models

The three groups are characterized in **Table 1**. No significant difference was found among the three groups in the size of the VTA ratio, RN ratio, or SN ratio. In the entire cohort, the hippocampal ratio was significantly associated with the VTA ratio ($rho_{(n = 110)}$ = 0.482, p = 9.88e-08, **Fig.3a**; education and MMSE-corrected $rho_{(df = 106)}$ = 0.427, p = 4.00e-06). This was replicated only in the groups of healthy controls ($rho_{(n = 51)}$ = 0.586, p = 6.00e-06, **Fig 4a**; education and MMSE-corrected $rho_{(df = 47)}$ = 0.415, p = 0.003). In the entire cohort the association between the VTA ratio and the memory index was significant ($rho_{(n = 110)}$ = 0.290, p = 0.002, **Fig.3c**; education-corrected $rho_{(df = 107)}$ = 0.291, p = 0.002), while neither the other ratios nor the performance on the Letter Fluency test showed significant associations (**Fig.3b, d, f**). Focusing on each diagnostic group, no significant associations were found in the two patient groups (**Fig.4b-c, e-f**). Similarly, no effect emerged after the MCI group was separated into amnestic and non-amnestic patients. In the group of healthy adults, memory scores (but not Letter Fluency scores) correlated with the SN ratio ($rho_{(n = 51)}$ = 0.428, p = 0.002; education-corrected $rho_{(df = 48)}$ = 0.380, p = 0.007), but the association with the VTA ratio was far stronger ($rho_{(n = 51)}$ = 0.495, p = 2.25e-04, **Fig.4g**; education-corrected $rho_{(df = 48)}$ = 0.474, p = 0.001). To characterize the role of encoding and retrieval mechanisms in this pattern of findings, the analyses were then re-run separately for z scores derived separately for immediate and delayed recall. The only associations which survived the $p < 0.005$ statistical threshold were those between immediate recall and VTA ratio in the entire cohort ($rho_{(n = 110)}$ = 0.294, p = 0.002, **Fig.3e**; education-corrected $rho_{(df = 107)}$ = 0.296, p = 0.002) and in the group of healthy controls ($rho_{(n = 51)}$ = 0.483, p = 3.33e-04, **Fig, 4j**;

education-corrected $rho_{(df = 48)} = 0.460$, $p = 0.001$). The association between VTA ratio and delayed recall only approached statistical significance.

- Add **Fig.3** and **4** about here –

Structural covariance of the VTA

The structural covariance of the VTA extended to hippocampus, insula, and medial prefrontal cortex. The structural covariance of RN and SN was instead regionally confined to the nuclei themselves (**Fig.5**).

- Add **Fig.5** about here –

Functional connectivity of the VTA

In the whole cohort hippocampal volume (**Fig.6a**) and memory performance (**Fig.6b**) were associated with the functional connectivity between the VTA and the left hippocampus. Memory performance was also associated with the functional connectivity between the VTA and the medial prefrontal cortex. The association was very similar when immediate and delayed recall were used as predictors.

Albeit the analysis of the subgroup of healthy controls revealed a set of trends qualitatively similar to those of the global analyses, the findings emerging from the analyses limited to each diagnostic group did not reach any statistical significance.

- Add **Fig.6** about here –

DISCUSSION

The findings of this study provide confirmatory evidence from humans in support of a significant role of the VTA in the preclinical phase of the sporadic form of AD, specifically in predicting variability of the typical neurocognitive features of the disease, i.e., hippocampal size and memory ability. Strong correlations were found in the group of healthy individuals, but not in MCI or AD dementia patients. This is in line with the evidence of VTA neuronal loss occurring very early along the disease timeline. In fact, it is expected that only in an asymptomatic population there will be sufficient variability to enable the significant associations to emerge. No similar association was found between the SN ratio and the hippocampus, indicating that it is not a generic volumetric decrease of dopaminergic nuclei associated with hippocampal reduction but, rather, a specific involvement of the VTA in the preclinical stage of AD, as originally found in a murine model [6]. This confirmatory evidence of a selective involvement of the VTA is particularly important because research strives for the detection of a preclinical marker of sporadic AD and novel paradigms of investigation are needed [20].

To contextualize the findings of this study in a manner that can be functionally relevant (i.e., what exact cognitive function is sustained by the VTA and, thus, could be potentially exploited for a preclinical diagnosis of AD), it is informative to address first the anatomy of this region and the connections it forms. The VTA is a group of heterogeneous mesencephalic nuclei located in the midbrain, 163 mm^3 large in humans, and counting about

690,000 neurons [21]. The major component (≈ 55%) are dopaminergic cells [22], while the remaining part is mainly composed by GABAergic neurons that serve as regulatory inhibitory control [23]. The dopaminergic neurons of the VTA project directly to a series of regions, including the nucleus accumbens, amygdala, hippocampus, and the medial prefrontal cortex [24]. These connections are at the basis of the role of the VTA as a circuital hub in support of a number of functions, e.g., reward mechanisms [25] and emotional processing [26], and in behavioral and psychiatric disorders in which deficits of these functions are a central trait, such as in schizophrenia [27] or craving behavior [28]. In AD, however, it is the depletion of dopaminergic innervation to the hippocampus that is the major pathological change [6]. This finds confirmation in the results of this study. In fact, the anatomical variability of this regions was found to be profoundly linked to distinctive features of AD widely and routinely implemented in clinical settings, i.e., the smaller the VTA, the worse these indices.

In human participants, the VTA-hippocampus interplay is normally visible via the analysis of functional connectivity [29, 30]. Based on this, if preclinical AD caused neuronal loss in the VTA, the VTA-hippocampus functional pathways should suffer considerably and would be quantifiable via measures of hemodynamic connectivity. This would also be in line with the early histological conceptualizations of AD, described as a syndrome that isolates the hippocampal formation computationally [31]. Our findings confirmed this hypothesis, since the functional connectivity between the VTA and the left hippocampus was associated with both hippocampal size and memory performance. It has to be acknowledged that we could not replicate this finding in the group of healthy controls, but, in all likelihood, this was due to a marked decrease in statistical power consequential to the reduction of sample size. Memory performance was also associated with the functional connectivity between the VTA and the medial prefrontal cortex. The medial prefrontal cortex is one of the regions that

receives dopaminergic innervation from the VTA [24]. This is also consistent with the role of this structure in long-term memory processes [32].

To put these findings even more in context, it is important to review the recent literature on VTA research. As outlined in the following section, not only does the evidence collected in recent studies support our findings, but it also suggests that the computational role of the VTA-hippocampus pathway appears to be particularly relevant for pathological and clinical processes of early-stage AD. On one hand, in fact, findings indicate that, in the initial stages of disease, neuronal loss in subcortical nuclei is at least as intense as in the mediotemporal complex [33]. This may account for the significant structural covariance we found between the VTA and the hippocampal formation (**Fig.4**). On the other hand, convergent evidence associated with the role of the VTA-hippocampus loop is suggestive of a specific cognitive component that could be the key aspect for a preclinical diagnosis. The computational role of the VTA-hippocampus pathway, in fact, seems to be particularly relevant for material that is associated with a degree of novelty [34]. Specifically, evidence has shown that the VTA-hippocampus interaction is somehow involved in the encoding phase of memory [35]. The encoding phase of mnestic processes consists of the exposure to and acquisition of new stimuli [36]. Obviously, it is widely established that the role of declarative memory impairment is central in AD when memory is intended as a global function (without separating the mechanisms of encoding, retrieval, and storage). However the identification of a memory-related mechanism of clinical relevance during the preclinical stage is a hard task, as this stage is asymptomatic. Longitudinal evidence indicates that, among healthy adults, measures of episodic memory are the best predictors of subsequent conversion to the early symptomatic stage of AD [37]. The simple retrospective use of neuropsychological tests, however, does not allow a clear separation between encoding and retrieval efficiency. It is generally established, however, that measures of immediate recall of a short story (which

constitute the Prose Memory test) rely more on encoding than retrieval, as opposed to measures of delayed recall that rely more on retrieval. We thus explored the association between VTA ratio and immediate and delayed recall performance, separately. The findings indicate that immediate recall was the only score significantly associated with the VTA. This corroborates the role of encoding as pivotal cognitive indicator of early VTA disruption. The neural system supporting memory encoding has been characterized with fMRI paradigms, since these allow a temporal separation of the distinct memory phases. Under normal conditions, the hippocampal and perirhinal cortices play a major role during episodic encoding [38, 39]. A recent fMRI study characterized the profile of AD pathology associated with abnormal encoding (de-activation preceding hits and increased activation preceding false alarms) in healthy elderly adults, potentially presymptomatic AD individuals. Findings indicated that abnormal encoding was associated with the presence of a Braak stage I/II, as measured with neuromolecular TAU imaging [40], indicating that the encoding stage is particularly informative. In this scenario, if perirhinal and hippocampal activation supports encoding, the connectivity between these areas and the VTA has been found to support the consolidation of encoded material [41].

To draw a parallel with the aforementioned description of the role of the VTA in episodic encoding, the role of the VTA in semantic encoding would also be relevant, as semantic material can also be characterized by a degree of novelty, if the experimental paradigm is appropriately designed. With remarkable convergence, the VTA was found to be the center of repeated semantic encoding for novel material [42]. This is unmistakable evidence that a paradigm of episodic memory encoding and semantic memory encoding might be the main neuropsychological candidate for the detection of VTA suffering. The specific involvement of the encoding and consolidation of episodic and semantic material during the preclinical stage of AD is interlaced with two unconfutable facts that help corroborate the role of this

specific cognitive function and, in turn, the VTA as prime suspect of preclinical AD. Firstly, patients with AD show particular difficulties with episodic material encoded in recent, rather than remote past [43, 44], as to draw a trait of gradual failure in day-to-day encoding abilities. Secondly, the concept of semantic encoding for novel material resembles profoundly the operations requested by educational and academic activities. In other words, it is through education that a major amount of novel semantic knowledge gets encoded. Albeit novel semantics is encoded and stored throughout the entire life, this process arguably becomes less and less engaged during the course of adulthood. On this note, if the preclinical stage of AD consists of a reduction of semantic encoding abilities, then it is unsurprising that low levels of education are one of the crucial risk factors for developing the disease. This may shed new light on the role of education levels as integral part of the concept of cognitive reserve, a well-established protecting factor for the onset of AD symptoms [45].

Although this multidimensional set of findings converges towards a robust association between the clinical markers of AD and the size of the VTA, it has to be acknowledged that no significant differences were found between the VTA ratio of healthy controls and patients. This may be due to a multitude of reasons. Firstly, since neuronal loss in the VTA is a presymptomatic occurrence, the variability in VTA size should be informative only in the group of healthy controls. Secondly, when genetics is not characterized, groups of asymptomatic controls are necessarily heterogeneous, as they may include healthy adults as well as presymptomatic individuals. As a consequence, the volumetric properties of the VTA may reflect either a nucleus that had not been subjected to any significant neuronal loss, or, viceversa, a degenerated nucleus that has lost a considerable number of neurons in comparison with its premorbid status. On these grounds, a cross-sectional between groups comparison of VTA size yields limitations and would not be a valid source of information. Longitudinal studies would provide complementary insight, and would also shed light on the

connection between reduced dopaminergic input to the mediotemporal regions and possible enhanced susceptibility of these regions to the deposition of the distinctive peptidic hallmarks.

The dopaminergic nature of the neural pathways involved in preclinical AD is suggestive of potential intervention routes. These, however, should be designed to target the system with appropriate timing, i.e., when the disease is at the preclinical stage. Vice versa, dopaminergic therapies introduced at the dementia stage are not expected to be effective. Proof of this is the unsatisfactory outcome of dopaminergic trials for the treatment of AD in the form of seligiline [46]. Other monoamine oxidase inhibitor B molecules have been objet of research interest for AD for the regulation of dopaminergic activity, alone and in combination with the conventional cholinergic approach [47-49]. More investigations are needed to study these early changes more in detail, and more clinical studies based on a dopaminergic framework of AD are warranted.

In conclusion, the pre-plaque VTA neuronal loss seen in a rodent model of AD [6] finds here confirmatory support in a human cohort. Today, novel approaches to study the preclinical biological changes of AD are urgently needed [20]. The VTA and the VTA-hippocampal loop are hereby outlined and confirmed as potential preclinical markers of AD that deserve to be investigated more in detail. Clinical focus on memory encoding might provide a neuropsychological measure of assistance. In addition, the dopaminergic nature of this circuit might be suggestive of novel and effective early therapeutic avenues.

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Conflict of Interest/Disclosure Statement

The authors have no conflict of interest to report.

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Tables

Table 1. Variables included in this study as distributed across the different diagnostic cohorts

Variable	Healthy (n = 51)	MCI (n = 30)	AD Dementia (n = 29)	Group Differences (p)*	Bonferroni-Corrected Post Hoc Significance
Demographic Characteristics					
Age (years)	61.96 (16.38)	64.67 (10.12)	63.97 (9.52)	0.638	N/A
Education (years)	14.88 (3.18)	12.90 (2.99)	12.00 (2.34)	< 0.001	Healthy > MCI/AD
Gender Ratio (f/m)	34/17	15/15	9/20	0.008	Healthy ≠ AD
Mini Mental State Examination	28.24 (1.79)	25.63 (2.16)	19.24 (3.18)	< 0.001	Healthy > MCI > AD
Cognitive Indices					
Prose Memory – Average Recall (z score)	0.84 (0.42)	-0.28 (0.67)	-1.19 (0.51)	< 0.001	Healthy > MCI > AD

Prose Memory – Immediate Recall (z-score)	0.82 (0.58)	-0.29 (0.60)	-1.14 (0.50)	< 0.001	Healthy > MCI > AD
Prose Memory – Delayed Recall (z-score)	0.82 (0.34)	0.26 (0.77)	-1.18 (0.57)	< 0.001	Healthy > MCI > AD
Letter Fluency (z-score)	0.66 (0.79)	-0.39 (0.77)	-0.75 (0.79)	< 0.001	Healthy > MCI/AD
		Neuroanatomical Indices			
VTA Ratio	5.26e-03 (7.19e-04)	5.23e-03 (6.65e-04)	4.92e-03 (7.36e-05)	0.107	N/A
RN Ratio	3.38e-03 (4.72e-04)	3.47e-03 (3.91e-04)	3.30e-03 (4.43e-04)	0.317	N/A
SN Ratio	1.55e-03 (2.13e-04)	1.51e-03 (2.09e-04)	1.45e-03 (2.13e-04)	0.129	N/A
Gray Matter Ratio	0.44 (0.06)	0.43 (0.05)	0.37 (0.05)	< 0.001	Healthy/MCI > AD
Hippocampal Ratio (STEPS)	1.77e-03 (2.25e-04)	1.70e-03 (2.66e-04)	1.46e-03 (3.49e-04)	< 0.001	Healthy/MCI > AD

*One-way ANOVA and chi-square tests were used. MCI: Mild Cognitive Impairment. Aside from Gender Ratio, means and standard deviations are indicated for each variable. Ratio indices of each sub-cortical nucleus was calculated based on the volume of brainstem space. Hippocampal ratios reported here are based on the use of the STEPS procedure

Artwork

Fig.1: The regions included in this study identified by masks superimposed to the MNI anatomical template. The RN, SN and VTA are shown in red, green and blue, respectively. Each axial slice (Montreal Neurological Institute coordinates from the top left: z = -12, -10, -8, -6, clockwise) is identified on the orthogonal view by the shade of yellow/orange.

Fig.2: Two examples of hippocampal segmentation using STEPS. Hippocampal volumes were calculated based on the T1-weighted image in its native space. These two examples show the segmentation of the left hippocampus of a patient with AD dementia (A) and a healthy control (B). Slices are shown with and without the hippocampal overlay.

Fig.3: The linear association models carried out in the entire cohort: between the hippocampal ratio and VTA ratio (**a**), between the hippocampal ratio and SN ratio (**b**), between the scores on the Prose Memory test (average of immediate and delayed recall) and VTA ratio (**c**), between the scores on the Prose Memory test (average of immediate and delayed recall) and SN ratio (**d**), between immediate recall scores and VTA ratio (**e**), and between the scores on the Letter Fluency test and VTA ratio (**f**). Although the figure illustrates linear associations, nonlinear associations were run as part of the methodology. Ratios were scaled up (multiplied by 10^3). Models testing the association between RN ratio and clinical indices of AD are not shown.

Fig.4: The linear association models between the VTA ratio and hippocampal ratio in the group of healthy controls (**a**), MCI patients (**b**), and patients with dementia (**c**). This is

followed by the linear association between the SN ratio and hippocampal ratio in the group of healthy controls (**d**), MCI patients (**e**), and patients with dementia (**f**). Immediately below, the linear association between the VTA ratio and scores on the Prose Memory test (average of immediate and delayed recall) in the group of healthy controls (**g**), MCI patients (**h**), and patients with dementia (**i**), and, specifically, between immediate recall and VTA ratio in the group of healthy controls (**j**), MCI patients (**k**), and patients with dementia (**l**). The linear association between the VTA ratio and scores on the Letter Fluency test are shown at the bottom in the group of healthy controls (**m**), MCI patients (**n**), and patients with dementia (**o**). Although the figure illustrates linear associations, nonlinear associations were run as part of the methodology. Ratios were scaled up (multiplied by 10^3). Models testing the association between RN ratio and clinical indices of AD are not shown.

Fig.5: Structural covariance of the VTA (blue, Montreal Neurological Institute coordinates: y = -26, x = 0), SN (green, Montreal Neurological Institute coordinates: y = -10, x = 4) and RN (orange, Montreal Neurological Institute coordinates y = -20, x = 4). These findings survive a Family Wise Error corrected $p < 0.001$.

Fig.6: Functional connectivity of the VTA as a function of hippocampal volume (**a**. Montreal Neurological Institute coordinates: z = -22, x = -29, y = -6) and memory performance (**b**. Montreal Neurological Institute coordinates: z = 9, x = -23, y = -26). These findings are significant with an uncorrected $p < 0.01$.

Figure 1

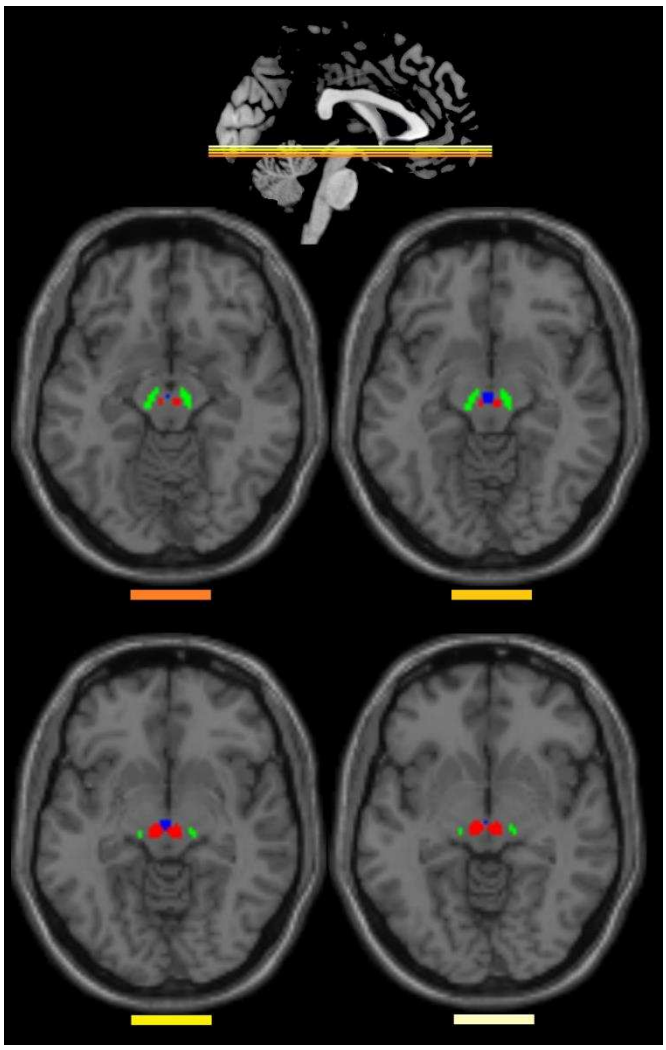

Figure 2

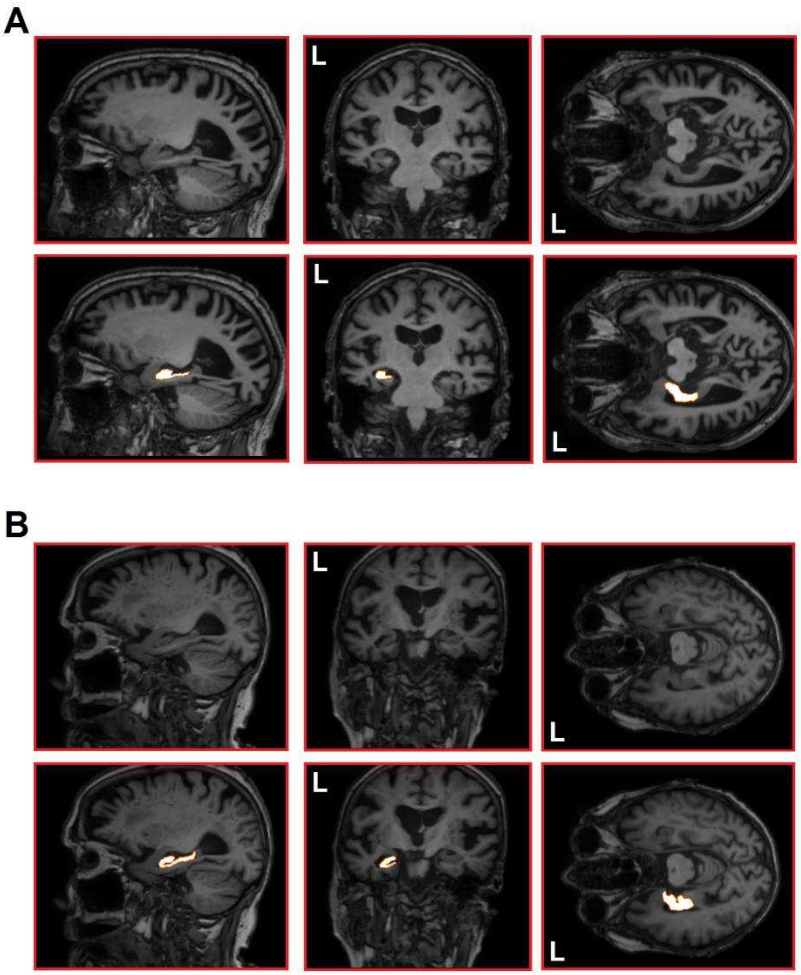

Figure 3

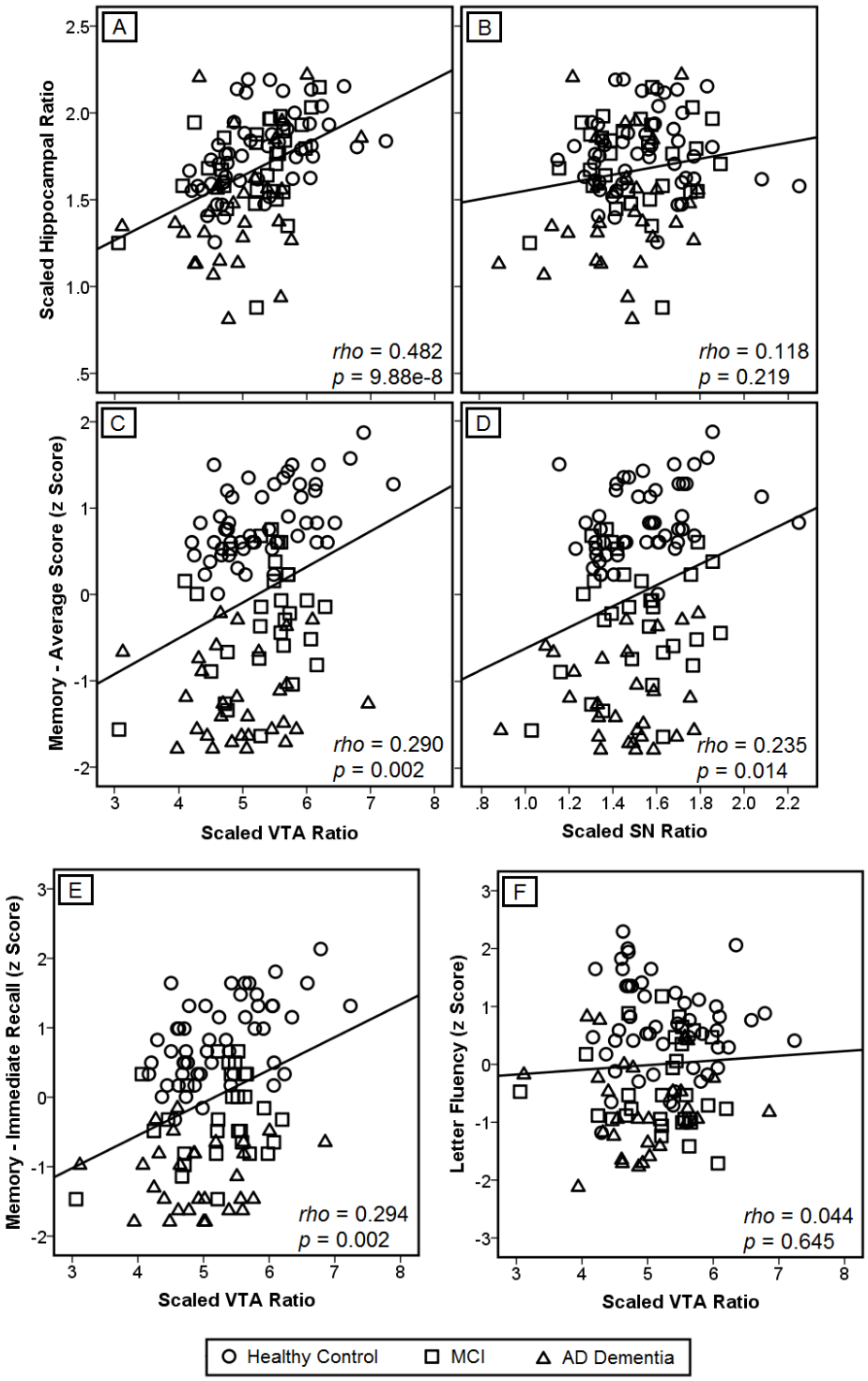
A
B
C
D
E
F
Scaled Hippocampal Ratio
Memory - Average Score (z Score)
Memory - Immediate Recall (z Score)
Letter Fluency (z Score)
Scaled VTA Ratio
Scaled SN Ratio
rho = 0.482
p = 9.88e-8
rho = 0.118
p = 0.219
rho = 0.290
p = 0.002
rho = 0.235
p = 0.014
rho = 0.294
p = 0.002
rho = 0.044
p = 0.645
Healthy Control
MCI
AD Dementia

Figure 4

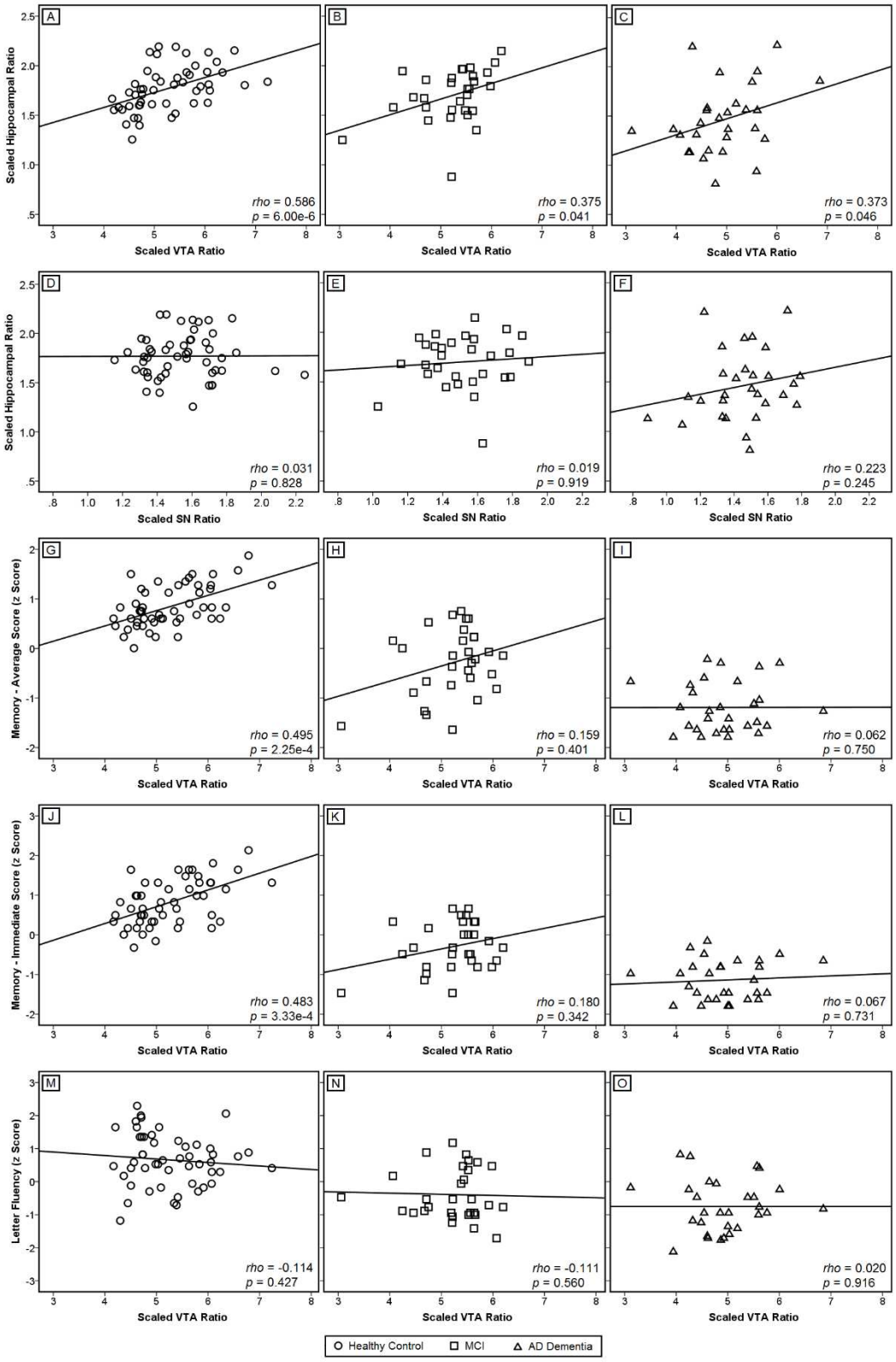
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
Scaled Hippocampal Ratio
Memory - Average Score (z Score)
Memory - Immediate Score (z Score)
Letter Fluency (z Score)
Scaled VTA Ratio
Scaled SN Ratio
rho = 0.586
p = 6.00e-6
rho = 0.375
p = 0.041
rho = 0.373
p = 0.046
rho = 0.031
p = 0.828
rho = 0.019
p = 0.919
rho = 0.223
p = 0.245
rho = 0.495
p = 2.25e-4
rho = 0.159
p = 0.401
rho = 0.062
p = 0.750
rho = 0.483
p = 3.33e-4
rho = 0.180
p = 0.342
rho = 0.067
p = 0.731
rho = -0.114
p = 0.427
rho = -0.111
p = 0.560
rho = 0.020
p = 0.916
Healthy Control
MCI
AD Dementia

Figure 5

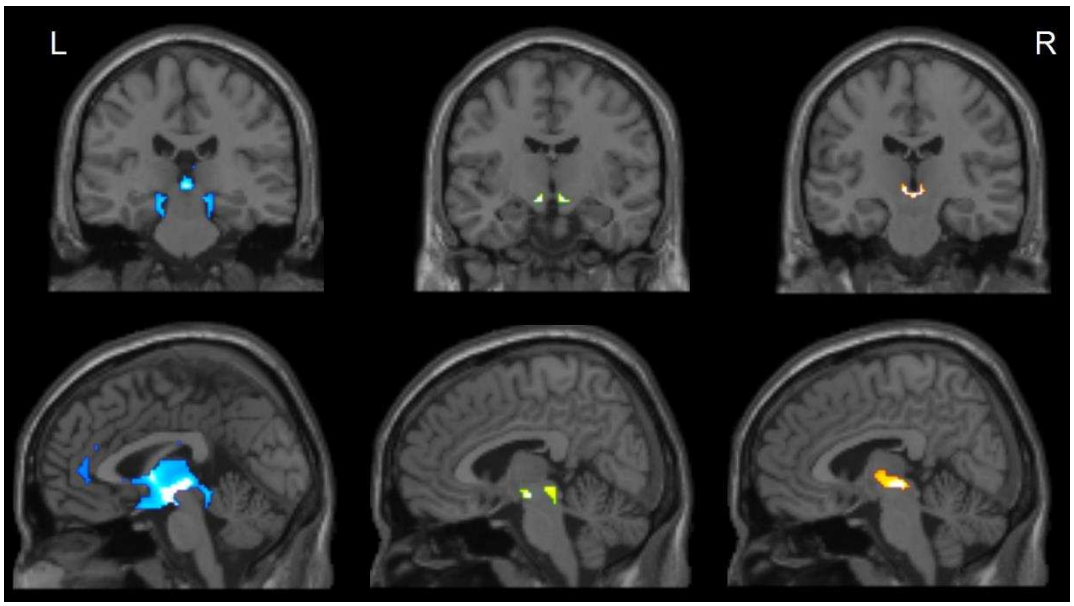

Figure 6