Cognitive correlates of α4β2 nicotinic acetylcholine receptors in mild Alzheimer's dementia

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Supplementary material

Supplementary results

α4β2-nAChR availability in Alzheimer's dementia compared with healthy controls

VOI analysis of GMD-mask-weighted V_T data (V_T[GMDW])

VOI analysis identified that in Alzheimer's dementia, compared with healthy controls, similar to V_T- and V_S-VOI analyses, there was lower V_T[GMDW] in cortical and subcortical brain regions reaching significance within four of the five a-priori selected brain regions, such as the hippocampus as part of the mesial temporal cortex (-7%; P=0.002; $P<0.05_{corrected}$), basal forebrain (-8%; P=0.008; $P<0.05_{corrected}$), frontal cortex (-4%; P=0.019; $P<0.05_{uncorrected}$) and mean cortex (-4%; P=0.021; $P<0.05_{uncorrected}$), remaining significant following correction for multiple testing within the hippocampus and basal forebrain.

For post-hoc selected brain regions, in Alzheimer's dementia, there was significantly lower V_T[GMDW] within the thalamus (-9%; P=0.002; $P<0.05_{corrected}$), remaining significant following correction for multiple comparisons. In Alzheimer's dementia, lower V_T[GMDW] was present within the lateral temporal cortex (-3%; P=0.047; $P<0.05_{uncorrected}$), anterior cingulate cortex (-2%; P=0.023; $P<0.05_{uncorrected}$), and cerebellar cortex (-6%; P=0.031; $P<0.05_{uncorrected}$), although those differences did not remain significant following correction for multiple comparisons (*Supplementary Table 4*).

VOI analysis of PVE-corrected V_T data (V_T[PVEC])

Following partial volume effect (PVE) correction, there was significantly lower V_T[PVEC] in patients with Alzheimer's dementia as compared to healthy controls, similar to analyses of V_T, V_S, and V_T[GMDW] in four of the five a-priori selected brain regions. V_T[PVEC] was lower within the hippocampus as part of the mesial temporal cortex (-11%; P=0.003; $P<0.05_{corrected}$), basal forebrain (-13%; P=0.009; $P<0.05_{corrected}$), mean cortex (-4%; P=0.031; $P<0.05_{uncorrected}$), frontal cortex (-4%; P=0.034; $P<0.05_{\textit{uncorrected}}$), remaining significant following correction for multiple testing within the hippocampus and basal forebrain.

Furthermore, for post-hoc selected brain regions, in Alzheimer's dementia, there was significantly lower V_T[PVEC] within anterior cingulate cortex (-4%; P=0.013; $P<0.05_{uncorrected}$), pons (-7%; P=0.042; $P<0.05_{uncorrected}$), and cerebellar cortex (-6%; P=0.026; $P<0.05_{uncorrected}$), though those differences did not remain significant after correction for multiple comparisons (*Supplementary Table 5*).

Given the smaller variance (*Table 2 and Supplementary Table 5*) and that similar findings were produced using V_T[PVEC] and V_T data, the primary outcome measure used for all PET analyses was the V_T data (Villemagne *et al.*, 2013).

Relationship between α4β2-nAChR availability in a-priori defined brain regions and cognition in Alzheimer's dementia as assessed by VOI analysis

V_S

In Alzheimer's dementia, a significant positive correlation between episodic memory and V_S within a-priori defined brain regions was restricted to the frontal cortex (r=0.57; *P*=0.044; $P<0.05_{uncorrected}$). Further, a positive significant correlation between executive function/working memory and V_T within the parietal cortex was found (r=0.62; *P*=0.021; $P<0.05_{uncorrected}$). There were no significant correlations between attention, visuospatial function or language and V_S within any of the a-priori selected cortical regions.

Thus, compared with the correlation results of the V_T data, correlations between V_S and cognitive function were less significant and not significant following correction for multiple comparisons. A possible explanation for this is that the corpus callosum, which is used to calculate the Vs, has a small volume with the disadvantage of a higher noise part.

V_T[GMDW]

In Alzheimer's dementia, there were significant positive associations between episodic memory and V_T[GMDW] within a-priori defined brain regions such as the frontal (r=0.71; *P*=0.011; $P<0.05_{corrected}$), mesial temporal (r=0.62; *P*=0.027; $P<0.05_{uncorrected}$) and parietal cortices (r=0.64; *P*=0.024; $P<0.05_{uncorrected}$). These findings remained significant following correction for multiple testing within the frontal cortex. There was no significant relationship between memory and V_T[GMDW] within the basal forebrain (r=0.35; *P*=0.164). Executive function/working memory and V_T[GMDW] showed a significant positive correlation within the parietal cortex (r=0.79; *P*=0.003; $P<0.05_{corrected}$), although a significant correlation was not found within the frontal cortex (r=0.52; *P*=0.063). There were no significant correlations between attention, visuospatial function or language and V_T[GMDW] within any of the a-priori selected cortical regions. Thus, findings of the correlation analyses between V_T[GMDW] and cognitive partial functions in Alzheimer's dementia show similarities to findings obtained by correlation analyses of V_T, although following correction for multiple comparisons, correlations remain only significant between episodic memory and V_T[GMDW] within the frontal cortex and executive function / working memory and V_T[GMDW] within the parietal cortex.

V_T[PVEC]

The findings of correlation analyses between V_T[PVEC] and cognitive partial functions in Alzheimer's dementia were similar to the findings obtained by correlation analysis of V_T:

In Alzheimer's dementia, there were significant positive associations between episodic memory and V_T[PVEC] within a-priori defined brain regions such as the frontal (r=0.79; P=0.003; $P<0.05_{corrected}$), mesial temporal (r=0.67; P=0.017; $P<0.05_{uncorrected}$) and parietal cortices (r=0.65; P=0.021; $P<0.05_{uncorrected}$), remaining significant following correction for multiple testing within the frontal cortex. There was no significant relationship between memory and V_T[PVEC] within the basal forebrain (r=0.20; P=0.286).

Executive function/working memory and V_T[PVEC] demonstrated a significant positive correlation within the frontal cortex (r=0.59; P=0.037; $P<0.05_{uncorrected}$) and parietal cortex (r=0.83; P=0.002; $P<0.05_{corrected}$). There were no significant correlations between attention, visuospatial function or language and V_T[PVEC] within any of the a-priori selected cortical regions. Thus, findings of the correlation analyses between V_T[PVEC] and cognitive partial functions in Alzheimer's dementia show similarities to findings obtained by correlation analyses of V_T, however, after correction for multiple comparisons, correlations remain only significant between episodic memory and V_T[PVEC] within the frontal cortex and executive function / working memory and V_T[PVEC] within the parietal cortex.

Relationship between α4β2-nAChR availability within subregions of the mesial temporal cortex and episodic memory in Alzheimer's dementia as assessed by post hoc explorative VOI-analysis

V_T

Between episodic memory and V_T there was a trend for a positive correlation within the hippocampus (r=0.52; *P*=0.06) and a significant, positive correlation within the amygdala (r=0.67; *P*=0.018).

V_S

Between episodic memory and V_S there was no significant correlation within the hippocampus (r=0.31; *P*=0.188) and a significant, positive correlation within the amygdala (r=0.80; *P*=0.003).

V_T[GMDW]

Between episodic memory and V_T[GMDW] there was a trend for a positive correlation within the hippocampus (r=0.50; *P*=0.07) and a significant, positive correlation within the amygdala (r=0.58; *P*=0.038).

V_T[PVEC]

Between episodic memory and V_T[PVEC] there was a trend for a positive correlation within the hippocampus (r=0.46; *P*=0.09) and a significant, positive correlation within the amygdala (r=0.65; *P*=0.022).

To summarize, post-hoc VOI-based correlation analysis between episodic memory and PET data within subregions of the mesial temporal cortex showed a trend for a positive correlation within the hippocampus (V_T, V_T[GMDW], V_T[PVEC]), and a significant, positive correlation within the amygdala for all aforementioned PET parameters. This relationship between episodic memory and α4β2-nAChR availability within the amygdala is interesting, however not so surprising as 1) there is a close bidirectional interaction between the amygdala and hippocampus, 2) episodic memory has been to linked to the frontal cortex, hippocampus and amygdala, as shown by functional MRI studies, and 3) as previously shown, direct electrical stimulation of the amygdala increases declarative memory in humans without emotional contribution (Yancey and Phelps 2001; Phelps 2004; Inman *et al.*, 2018). Further prospective

α4β2-nAChR PET investigations of subregions of the mesial temporal cortex in Alzheimer's dementia are needed for verification of these findings.

Supplementary references

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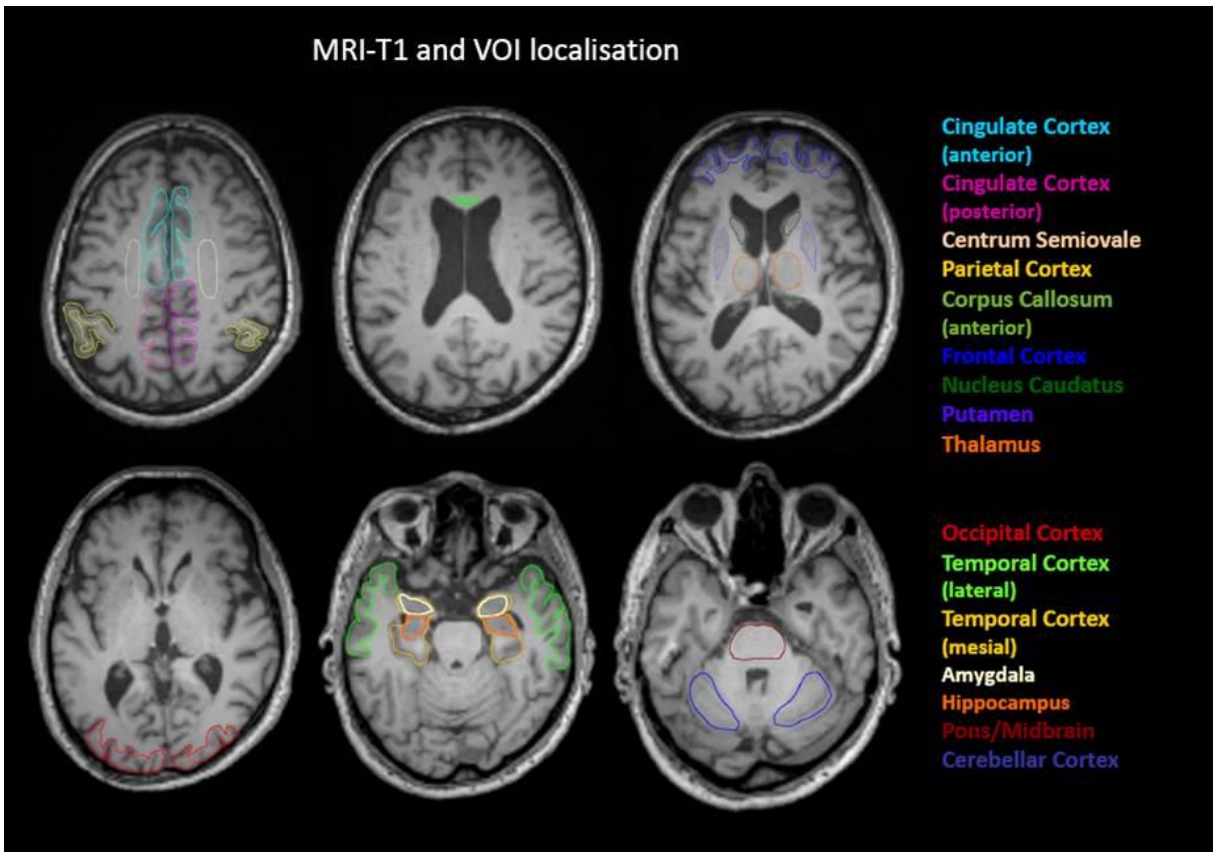
Supplementary Figure



Supplementary Figure. Localizations of individually drawn regions of interest for VOI analysis.

The localizations of individually drawn regions of interest for VOI analysis within the MRI of one study subject are shown.

Supplementary Tables

Supplementary Table 1. Clinical characteristics and MRI measures in Alzheimer's dementia and healthy controls: comparison of means (SD).

	Alzheimer's dementia (n = 14)	**Healthy controls (n = 15)**	***P*-value**
Age	75.1 (6.2)	71.3 (4.7)	0.071
Education	13.1 (2.2)	13.8 (1.5)	0.378
Sex (Female/Male)[a]	10 / 4	7 / 8	0.264
MMSE	24.0 (2.6)	28.4 (0.9)	**<0.001**
GDS	7.4 (3.6)	3.3 (2.2)	**<0.001**
Scheltens-Score	1.4 (1.0)	0.7 (0.5)	**0.025**
Fazekas-PVH	1.3 (0.8)	0.8 (0.7)	0.074
Fazekas-DWMH	1.3 (0.7)	0.8 (0.6)	0.053

Abbreviations: DWMH = deep white matter hyperintensities; GDS = Geriatric Depression Scale; MMSE = Mini-Mental-State-Examination; PVH = periventricular white matter hyperintensities; SD = standard deviation.

Unpaired two-tailed t-test for the comparison between Alzheimer's dementia and healthy controls; significance at $P<0.05$ (bold).

[a]Fisher's exact test; significance at $P<0.05$ uncorrected.

Supplementary Table 2. Cognitive test results in Alzheimer's dementia patients and healthy controls

	[a]Z-scores				
	Alzheimer's dementia	**Healthy controls**	**t/F**	**df/N**	***P value***
[b]Attention					
AKT-G	0.54 (1.10)	1.47 (0.99)	-2.30	25	**0.030**
Digit span forward	-0.68 (0.74)	-0.01 (0.70)	-1.92	18	0.071
Executive function/working memory					
TMT-A	-1.13 (1.35)	0.18 (1.17)	-2.76	26	**0.010**
TMT-B	-0.66 (1.08)	0.55 (1.00)	-2.58	20	**0.018**
TMT-B/A	-0.20 (1.08)	0.41 (0.90)	-1.39	20	0.180
Digit span backward	-0.51 (0.77)	0.24 (1.08)	-1.53	18	0.143
Letter fluency	-1.49 (1.14)	0.73 (0.66)	-6.47	27	**<0.001**
Language					
Category fluency	-0.52 (0.98)	0.66 (1.14)	-2.98	27	**0.006**
BNT	-0.69 (1.30)	0.45 (0.74)	-2.93	27	**0.007**
Visuospatial abilities					
CERAD visuoconstruction copy	-1.93 (1.54)	-0.61 (0.95)	-2.80	27	**0.009**
CERAD visuoconstruction recall	-2.17 (0.95)	0.22 (0.98)	-6.63	27	**<0.001**
Episodic memory					
CERAD wordlist immediate recall	-2.88 (1.47)	0.45 (0.72)	-7.83	27	**<0.001**
CERAD wordlist delayed recall	-2.14 (0.76)	0.39 (1.11)	-7.10	27	**<0.001**
CERAD wordlist savings score	-1.14 (1.72)	0.15 (1.20)	-2.31	26	**0.029**
CERAD visuoconstruction savings score	-1.44 (1.14)	0.43 (0.87)	-4.95	27	**<0.001**
WMS Logical Memory I	-2.56 (1.55)	0.31 (1.19)	-4.53	18	**<0.001**
WMS Logical Memory II	-3.19 (0.86)	0.43 (0.94)	-8.03	18	**<0.001**

Abbreviations: AK-T = Alters-Konzentrations-Test; BNT = Boston Naming Test; CERAD = The Consortium to Establish a Registry for Alzheimer's dementia; TMT = Trail Making Test; WMS = Wechsler Memory Scale.

[a]Z-scores are given as mean and standard deviation (in brackets).

[b]ANCOVA was carried out for variable to account for years of education as covariate.

Unpaired two-tailed t-test for the comparison between Alzheimer's dementia and healthy controls; significance at $P<0.05$ (bold).

Supplementary Table 3. VOI analysis of V_S (specific binding part of the distribution volume) with the corpus callosum as pseudo-reference region within the brain of Alzheimer's dementia and healthy controls.

V_S	**Alzheimer's dementia (n=14)**		**Healthy controls (n=15)**				
	Mean	**SD**	**Mean**	**SD**	**Change of AD compared with HC (%)**	***t/F***	***P value***
[a]**Mean cortex**	2.88	0.76	3.34	0.60	-14	4.79	**0.009**
[a]**Frontal cortex**	2.96	0.89	3.42	0.65	-13	4.14	**0.016**
Lateral temporal cortex	2.80	0.81	3.10	0.60	-10	3.33	0.036
Mesial temporal cortex	2.78	0.80	3.52	0.63	-21	6.04	0.003
[a]**Hippocampus**	3.13	0.97	4.09	0.78	-23	6.82	**0.002**
Amygdala	3.32	0.68	3.76	0.82	-12	1.97	0.144
[a]**Parietal cortex**	2.97	0.80	3.30	0.71	-10	2.87	0.057
Occipital cortex	2.24	0.80	2.18	0.63	3	2.91	0.054
[a]**Basal forebrain**	1.48	1.22	2.24	0.82	-34	4.00	**0.019**
Anterior cingulate cortex	3.10	0.67	3.39	0.64	-8	3.25	0.039
Posterior cingulate cortex	3.25	0.49	3.43	0.64	-5	1.46	0.249
Caudate nucleus	4.14	1.25	4.38	0.88	-5	0.26	0.852
Putamen	5.70	1.27	5.55	0.78	3	0.40	0.752
Thalamus	17.29	3.11	19.61	2.98	-12	3.88	0.021
Pons/Midbrain	4.54	1.03	5.28	1.10	-14	2.14	0.120
Cerebellar cortex	6.09	1.17	6.86	0.88	-11	1.99	0.141
White matter-centrum semiovale	4.20	1.32	4.26	0.84	-2	3.49	0.030

Abbreviations: AD = Alzheimer's dementia; FDR = False discovery rate; HC = healthy controls; V_S = specific binding part of the distribution volume; VOI = volume of interest.

[a]a-priori selected brain region with known Alzheimer's dementia pathology.

ANCOVA for the comparison of V_S between Alzheimer's dementia and healthy controls (adjusted for age and sex), within five a-priori defined cortical brain regions, such as mean cortex, frontal, mesial temporal [hippocampus], parietal cortices, and basal forebrain (in bold); significance at $P<0.05_{corrected}$ (false discovery rate correction according to Benjamini-Hochberg; in bold). Furthermore exploratory post-hoc analysis for eleven additional brain regions, significance at $P<0.005_{uncorrected}$ ($P<0.05_{corrected}$; FDR-correction).

Supplementary Table 4. Individual VOI analysis of the GMD-mask weighted V_T values ($V_{T[GMDW]}$) in Alzheimer's dementia and healthy controls.

$V_{T[GMDW]}$	**Alzheimer's dementia (n=14)**		**Healthy controls (n=15)**				
	Mean	**SD**	**Mean**	**SD**	**Change of AD compared with HC (%)**	***t/F***	***P-value***
[a]Mean cortex	8.07	1.00	8.41	0.46	-4	3.87	0.021
[a]Frontal cortex	8.11	1.05	8.49	0.54	-4	3.98	0.019
Lateral temporal cortex	8.03	0.96	8.24	0.38	-3	3.05	0.047
Mesial temporal cortex	8.03	1.10	8.62	0.54	-7	4.61	0.011
[a]Hippocampus	8.46	1.22	9.08	0.57	-7	6.29	**0.002**
Amygdala	8.53	1.19	8.74	0.69	-2	0.92	0.444
[a]Parietal cortex	8.10	1.09	8.27	0.57	-2	2.15	0.119
Occipital cortex	7.51	0.85	7.46	0.34	1	0.99	0.414
[a]Basal forebrain	6.95	1.29	7.52	0.98	-8	4.97	**0.008**
Anterior cingulate cortex	8.35	1.01	8.51	0.69	-2	3.78	0.023
Posterior cingulate cortex	8.41	0.97	8.53	0.54	-1	1.05	0.388
Caudate nucleus	8.85	1.30	9.32	0.81	-5	1.13	0.356
Putamen	10.49	1.21	10.55	0.92	-1	1.39	0.270
Thalamus	20.77	3.74	22.90	2.86	-9	6.65	**0.002**
Pons/Midbrain	7.86	1.13	8.44	1.31	-7	1.45	0.253
Cerebellar cortex	10.91	1.49	11.56	0.85	-6	3.46	0.031

Abbreviations: AD = Alzheimer's dementia; FDR = False discovery rate; GMD = grey matter density; HC = healthy controls; $V_{T[GMDW]}$ = grey matter density mask weighted distribution volume; VOI = volume of interest.

[a]a-priori selected brain region with known Alzheimer's dementia pathology.

ANCOVA for the comparison of $V_{T[GMDW]}$ between Alzheimer's dementia and healthy controls (adjusted for age and sex), within five a-priori defined cortical brain regions, such as mean cortex, frontal, mesial temporal [hippocampus], parietal cortices, and basal forebrain (in bold); significance at $P<0.05_{corrected}$ (false discovery rate

correction according to Benjamini-Hochberg; in bold). Further, exploratory post-hoc analysis for ten additional brain regions, significance at $P<0.005_{uncorrected}$ ($P<0.05_{corrected}$; FDR-correction).

Supplementary Table 5. Individual VOI analysis of the PVE-corrected V_T values ($V_{T[PVEC]}$) in Alzheimer's dementia and healthy controls.

$V_{T[PVEC]}$	**Alzheimer's dementia (n=14)**		**Healthy controls (n=15)**				
	Mean	**SD**	**Mean**	**SD**	**Change of AD compared with HC (%)**	***t/F***	***P-value***
[a]Mean cortex	9.65	1.16	10.04	0.59	-4	3.49	0.031
[a]Frontal cortex	9.77	1.40	10.23	0.72	-4	3.38	0.034
Lateral temporal cortex	9.77	1.12	9.96	0.57	-2	2.38	0.094
Mesial temporal cortex	9.34	1.22	10.11	0.68	-8	4.17	0.016
[a]Hippocampus	9.33	1.43	10.53	1.01	-11	6.06	**0.003**
Amygdala	10.00	1.42	10.33	1.24	-3	0.25	0.864
[a]Parietal cortex	9.82	1.28	10.11	0.76	-3	1.62	0.209
Occipital cortex	9.03	0.97	8.77	0.56	3	1.08	0.375
[a]Basal forebrain	7.77	2.73	8.92	2.01	-13	4.79	**0.009**
Anterior cingulate cortex	10.56	1.10	11.01	1.10	-4	4.36	0.013
Posterior cingulate cortex	10.37	1.13	10.61	0.86	-2	0.88	0.467
Caudate nucleus	13.76	2.40	13.65	1.60	1	0.50	0.685
Putamen	12.17	1.84	11.90	1.33	2	0.42	0.743
Thalamus	28.71	4.30	30.94	4.49	-7	2.61	0.074
Pons/Midbrain	11.53	1.47	12.37	1.10	-7	3.16	0.042
Cerebellar cortex	13.24	1.78	14.04	1.11	-6	3.67	0.026
White matter centrum semiovale	13.70	3.86	13.14	2.49	4	0.37	0.773

Abbreviations: AD = Alzheimer's dementia; FDR = False discovery rate; HC = healthy controls; PVEC = partial volume effect correction; $V_{T[PVEC]}$ = partial volume effect-corrected distribution volume; VOI = volume of interest.

[a]a-priori selected brain region with known Alzheimer's dementia pathology.

ANCOVA for the comparison of $V_{T[PVEC]}$ between Alzheimer's dementia and healthy controls (adjusted for age and sex) within five a-priori defined cortical brain regions, such as mean cortex, frontal, mesial temporal [hippocampus], parietal cortices, and basal forebrain (in bold); significance at $P<0.05_{corrected}$ (false discovery rate correction according to Benjamini-Hochberg; in bold). Further, exploratory post-hoc analysis for eleven additional brain regions, significance at $P<0.005_{uncorrected}$ ($P<0.05_{corrected}$; FDR-correction).