Anticholinergic drugs and forebrain MRI changes in cognitively normal and mildly impaired people

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Research

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Abstract

Background

Anticholinergic (AC) medication use is associated with cognitive decline and dementia, which may be related to an AC induced central hypocholinergic state, but the exact mechanisms remain to be understood. We aimed to further elucidate the putative link between AC drug prescription, cognition and structural and functional impairment of the forebrain cholinergic nucleus basalis of Meynert (NBM).

Methods

Cognitively normal (CN, n = 344) and mild cognitively impaired (MCI, n = 224) ADNI-3 participants who had undergone 3T-MRI were included. Structural (regional grey matter [GM] density) and functional NBM integrity (functional connectivity [FC]) were compared between those on anticholinergic medication for over one year (AC^+) and those without (AC^-) in each condition. AC burden was classes as mild, moderate or severe.

Results

31% of participants were AC^+ (≥ 1 mild AC drug) demonstrating lower NBM-GM density in CN ($CNAC^-$: 0.6 ± 0.03; $CNAC^+$: 0.6 ± 0.02; P < 0.001), and MCI subgroups ($MCIAC^-$: 0.6 ± 0.03; $MCIAC^+$: 0.6 ± 0.03; P < 0.001), but with a larger effect size in MCI. NBM-FC was lower in $CNAC^+$ vs. $CNAC^-$ (3.6 ± 0.5 vs. 3.9 ± 0.6; P = 0.001), and in MCI ($MCIAC^+$ 3.3 ± 0.2 vs. vs. $MCIAC^-$ 3.7 ± 0.5; P < 0.001) again with larger effect size in MCI. NBM-FC partially mediated the association between AC medication burden and cognition.

Conclusions

Our findings provide novel support for a detrimental effect of mild AC medication on the forebrain cholinergic system characterised as functional central hypocholinergic that partially mediated AC-related cognitive impairment. Moreover, structural tissue damage suggests neurodegeneration, and larger effect sizes in MCI point to enhanced susceptibility for AC medication in those at risk of dementia.

Background

Cognition is known to depend on modulation of cortical activity by cholinergic innervation from the nucleus basalis of Meynert (NBM) [1]. Cholinergic neurons of the NBM are particularly vulnerable to Alzheimer disease (AD) and other neurodegenerative disorders [2–4], and the subsequent loss of cholinergic innervation plays an important role in the development of cognitive impairment [5, 6]. Mechanistic evidence for AD as hypocholinergic syndrome comes from the therapeutic benefits of enhancing cholinergic neurotransmission by cholinesterase inhibitors (ChEIs) [7], with early beneficial results being reported from trials of deep brain stimulation of the NBM [8].

Further support of a hypocholinergic mechanism of cognitive decline comes from consistent observations of cognitive impairment after experimental exposure to anticholinergic (AC) drugs with some evidence that AC drugs may accelerate amyloid deposition [9, 10]. Several epidemiological studies reported AC-related increased risk of dementia [11, 12], which may account for 10% of dementia cases [12], equating to around 20,000 new cases of dementia per year in the UK alone. This has raised significant interest in possible deprescription trials [13] but there is limited mechanistic understanding of how AC drugs increase the risk of dementia in specific populations across the ageing-predementia continuum.

Neuroimaging studies allow to investigate the underpinnings of detrimental brain effects of centrally acting medication. MRI studies have demonstrated NBM atrophy across the ageing-MCI-AD continuum using a range of measurements [14–17] with predictive power for the transition to AD [18] and cognitive decline in PD [19]. An association of AC medication use with structural impairment of the NBM would provide suggestive evidence for cumulative tissue damage as a marker of accelerated neurodegeneration of the central cholinergic system. Functional markers of the forebrain cholinergic system, on the other hand, would be expected to more sensitively indicate inhibitory AC drug effects on the cholinergic circuit and to precede structural changes. NBM functional disconnection has been shown in preclinical states of AD [4, 20] in line with the expected hypocholinergic states due to NBM degeneration. Importantly, the combined use of functional and structural NBM markers can shed light onto the largely unexplored interplay between AC drugs and cognition in preclinical and prodromal AD.

The aim of this study is to investigate the link between AC medication use, cognitive decline and structural and functional integrity of the NBM in a cohort of elderly cognitively normal (CN) and MCI participants. We hypothesised that participants with AC medication use compared to those without show (i) NBM grey matter (GM) density loss as a marker of structural damage to NBM, and display (ii) disrupted functional cholinergic networks as marker of central hypocholinergic state. We also hypothesised (iii) that AC burden is associated with cognitive impairment and that

this association is partially mediated by NBM affection. We also report on secondary tests that include comparisons of AC medication effect size between MRI markers, and bias assessment by comparing clinical risk profiles and AD biomarkers between AC medication strata.

Methods

Participants

Data used in the preparation of this article were obtained from the ADNI database (http://adni.loni.usc.edu, details in supplementary material 1).

A total of 417 CN and 332 MCI participants' (details in supplementary material 1 and supplementary table 1) T1-weighted MRI and resting-state (rfMRI) data were downloaded from ADNI3 (2019.10.26). Information of age, sex, medical history and education years was retrieved from the latest available dataset in ADNI documents. For cognition, we chose the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) [21] and Trail making test B providing comprehensive assessment of cognitive functions including memory and executive function that are linked with cholinergic function [22].

Anticholinergic medication exposure

Information of medications with potential anticholinergic effect was manually extracted from the ADNI concomitant medication file, and drugs were classified according to the most commonly used anticholinergic burden (ACB) scale [23]. An AC^+ participant was defined as anyone taking any AC medication for at least one year [24] until enrolled in the ADNI study. AC^- status was defined as no documented exposure to any AC drug for any duration. The total AC burden score (TACB) was the sum of ACB scores (low [ACB = 1], medium [ACB = 2] or high [ACB = 3]) of all medication prescribed to each participant. We excluded participants who took antiparkinson medication with anticholinergic effects to avoid inclusion of prodromal PD pathology with known NBM degeneration [19]. Participants who took cholinesterase inhibitors (ChEIs) were also excluded from this study.

MRI data

3T Sagittal T1-weighted images and axial rsfMRI data were downloaded. Details about sequences, quality assessment and preprocessing protocol were provided in Supplementary material 2. Figure 1 showed the flowchart of participant selection.

To reduce risk of comorbidity bias in the AC medication use cases, we undertook manual sample matching at subgroup level to identify the equivalent number of AC^+ to AC^- matched for age, sex, APOE ε4 and amyloid status in CN and MCI subcohorts (Table 1). The remainder subsample of rsfMRI participants not included in the matched AC^+ and AC^- test sample (130 CN and 32 MCI participants, no AC^+) provided an independent discovery sample to reconstruct the NBM FC network template.

Table 1
Demographic, clinical and cognitive information in the matched sample (age- ,sex-, APOE ε4-and amyloid status- matched)

Characteristic	Cognitively normal (n = 196)				MCI (n = 160)				P[c]
	Total (n = 196)	AC⁻participants (n = 98)	AC⁺participants (n = 98)	P[a]	Total (n = 160)	AC⁻participants (n = 80)	AC⁺participants (n = 80)	P[b]	
Age, mean (SD),years	74.4 (9.1)	73.6 (8.4)	75.1 (7.9)	0.240	74.3 (7.9)	74.1 (7.9)	74.6 (8.2)	0.687	0.987
Female, n (%)	121 (61.7)	60 (61.2)	61 (62.2)	0.883	83 (51.9)	41 (51.2)	42 (52.5)	0.874	0.068
Education, mean (SD), years	16.6 (2.4)	16.9 (2.4)	16.3 (2.4)	0.051	16.6 (2.4)	16.7 (2.4)	16.5 (2.4)	0.647	0.867
ADAS-Cog, mean (SD)	13.4 (4.9)	12.2 (4.1)	14.4 (5.3)	0.004*	15.9 (5.9)	15.1 (5.3)	16.7 (6.5)	0.173	< 0.001*
Trail making test B, mean (SD), seconds	80.8 (40.5)	79.2 (40.7)	82.3 (40.5)	0.616	96.5 (55.8)	91.9 (49.4)	101.1 (61.3)	0.331	0.005*
APOE ε4 carriers, n (%)	35 (26.9)	20 (29.0)	15 (24.6)	0.692	29 (29.0)	13 (27.7)	16 (30.2)	0.828	0.768
Amyloid positive, n (%)	50 (41.3)	27 (43.5)	23 (39.0)	0.712	36 (44.4)	17 (43.6)	19 (45.2)	0.881	0.666
Vascular risk, CMC score, mean(SD)	1.13 (1.1)	0.86 (0.88)	1.41 (1.16)	< 0.001*	1.3 (0.9)	1.2 (0.9)	1.4 (0.9)	0.377	0.140
Total ACB score, median (range)	-	-	1 (1–7)	-	-	-	1 (1–7)	-	0.483
Psychiatric	56 (28.6)	22 (22.4)	34 (34.7)	0.081	62 (39.5)	25 (31.6)	37 (47.4)	0.051	0.032*
Neurologic (other than cognitive disorder)	76 (38.8)	28 (28.6)	48 (49.0)	0.005*	85 (54.1)	39 (49.4)	46 (59.0)	0.263	0.005*
Head, Eyes, Ears, Nose, Throat	132 (67.3)	61 (62.2)	71 (72.4)	0.170	102 (65.0)	49 (62.0)	53 (67.9)	0.504	0.652
Cardiovascular	135 (68.9)	56 (57.1)	79 (80.6)	0.001*	113 (72.0)	52 (65.8)	61 (78.2)	0.110	0.559
Respiratory	47 (24.0)	17 (17.3)	30 (30.6)	0.044*	51 (32.5)	19 (24.1)	32 (41.0)	0.027*	0.094
Hepatic	6 (3.1)	3 (3.1)	3 (3.1)	1.000	6 (3.8)	2 (2.5)	4 (5.1)	0.442	0.772
Dermatologic-Connective Tissue	74 (37.8)	38 (38.8)	36 (36.7)	0.883	54 (34.4)	27 (34.2)	27 (34.6)	0.954	0.578
Musculoskeletal	154 (78.6)	74 (75.5)	80 (81.6)	0.384	114 (72.6)	57 (72.2)	57 (73.1)	0.897	0.211

a:Group comparison using T test between AC⁻ CNs and AC⁺ CNs

b:Group comparison using T test between AC⁻ MCI participants and AC⁺ MCI participants

c:Group comparison using T test between CNs and MCI participants

d:160 CNs and 120 MCI participants had ADAS-cog score information

e:130 CNs and 100 MCI participants had genotype information

f:121 CNs and 81 MCI participants had amyloid PET information

*: Significance level at P < 0.05

Characteristic	Cognitively normal (n = 196)				MCI (n = 160)				P^c
	Total (n = 196)	AC^-participants (n = 98)	AC^+participants (n = 98)	P^a	Total (n = 160)	AC^-participants (n = 80)	AC^+participants (n = 80)	P^b	
Endocrine-Metabolic	107 (54.6)	52 (53.1)	55 (56.1)	0.774	90 (57.3)	45 (57.0)	45 (57.7)	0.926	0.666
Gastrointestinal	111 (56.6)	48 (49.0)	63 (64.3)	0.043*	89 (56.7)	37 (46.8)	52 (66.7)	0.016*	0.992
Hematopoietic-Lymphatic	22 (11.2)	13 (13.3)	9 (9.2)	0.498	22 (14.0)	15 (19.0)	7 (9.0)	0.106	0.517
Renal-Genitourinary	103 (52.6)	51 (52.0)	52 (53.1)	0.886	64 (40.8)	32 (40.5)	32 (41.0)	0.947	0.032*
Allergies or Drug Sensitivities	77 (39.3)	33 (33.7)	44 (44.9)	0.143	59 (37.6)	26 (32.9)	33 (42.3)	0.251	0.826
Smoking, Alcohol Use, and/or Drug Use	18 (9.2)	6 (6.1)	12 (12.2)	0.215	24 (15.3)	8 (10.1)	16 (20.5)	0.080	0.098
Malignancy	44 (22.4)	18 (18.4)	26 (26.5)	0.231	20 (12.7)	11 (13.9)	9 (11.5)	0.812	0.026
a:Group comparison using T test between AC^- CNs and AC^+ CNs									
b:Group comparison using T test between AC^- MCI participants and AC^+ MCI participants									
c:Group comparison using T test between CNs and MCI participants									
d:160 CNs and 120 MCI participants had ADAS-cog score information									
e:130 CNs and 100 MCI participants had genotype information									
f:121 CNs and 81 MCI participants had amyloid PET information									
*: Significance level at $P < 0.05$									

For associations between structural NBM metrics and AD risk markers, cognitive performance and comorbidities the full sample (n = 587) was used, whereas for functional NBM metrics we only used the matched sample (n = 319).

NBM MRI metrics

The mean NBM-GM density of each participant was estimated using an existing probabilistic anatomical map of Ch4 [25] available in SPM 12 Anatomy Toolbox [26] (supplementary Fig. 1) and established approach using FSL tools (details in supplementary material 3).

To address the lack of a NBM network template and to overcome limitations of manual seed extraction [27] of the complex anatomical shape of NBM, we reconstructed an NBM-FC network template using the NBM template as seed in the independent discovery sample. Details of NBM seed-based FC analysis are provided in Supplementary material 3. As marker of the functional integrity of the NBM network we chose individual FC metrics derived as mean Z scores from individual NBM seed to NBM network maps using a priori defined seed and network templates.

Bias assessment tests

We computed a score for chronic metabolic conditions (CMC) to reflect the systemic vascular health and dichotomised participants using the median score of 2 [28]. Participants were designated as APOE ε4 carriers if they had one or two copies of allele 4, and as non-carriers if they had no allele 4 in their genotype. Participants with positive amyloid pathology were classified according to the semi-quantitative Amyloid-β1–42 peptide (Aβ) positron emission tomography (PET) results which retrieved from the latest available dataset in ADNI documents (https://adni.loni.usc.edu).

We also extracted hippocampus volume and precuneus GM volumes as established MRI markers of AD pathology from latest ADNI documents which were analysed by using the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/). To control for non-specific, such as vascular effects of anticholinergic medication on FC metrics, we also compared FC changes in the visual cortex between AC strata.

Statistical analysis

The statistical analyses were conducted using SPSS (version 21, Chicago). One-way analysis of variance and χ^2 test were used to compare demographics, cognitive performance, AD risk markers and CMC risk between AC^+ and AC^- in CN and MCI, and between CN and MCI with a significance level was set at $P < 0.0056$. For exploratory bias assessment between all other comorbidity comparisons, $P < 0.05$ was reported.

Linear regression was used to identify demographic and clinical factors that were significantly associated with NBM structural and functional imaging metrics in the $CNAC^-$. Factors that were significantly correlated with NBM structural or functional imaging metrics were controlled in further analyses.

Primary outcomes: One-way ANCOVA was used separately in CN and MCI matched samples to compare NBM-GM densities and NBM-FC between AC^- and AC^+, controlled for identified demographic and clinical factors. The Benjamini–Hochberg procedure was applied to correct for the false discovery rate (FDR) [29].

We assessed the association between TACB and cognition as well as the association of NBM metrics and cognition followed by mediation analysis using the PROCESS v3.1 macro (http://www.processmacro.org/index.html) for SPSS 21. The significance of indirect effects was tested using bootstrapping with 5000 replications. Mediation was accepted as having occurred if the indirect effect (x*y) was statistically significant.

ANOVA tests were used to compare NBM-GM density and FC between MCI and CN to confirm the expected sensitivity of the NBM imaging metrics to at-risk or prodromal AD. To explore preferential AC effects, we computed effect sizes and 95% confidence intervals (CI) in the various contrasts. We chose Cohen's f to reflect the effect size with $f \geq 0.4$ considered a large, $0.25 \leq f < 0.4$ medium and $f < 0.25$ small effect size [30].

We ran ANOVA tests to compare hippocampal GM, precuneus GM, visual FC network strength and comorbidities between the respective AC medication strata.

Results

A total of 568 participants (mean age ± SD, 73.5 ± 7.8; age range: 55–97; 301 women [53.0%]) were included in this study (details of whole cohort in the Supplementary Table 1). 178 (31%) participants were classed as AC^+ with the majority taking one mild AC drug (TACB range:1–7, median: 1). The number of participants who had low, medium or high TACB score was not significantly different between $CNAC^+$ and $MCIAC^+$ ($P = 0.655$).

Clinical, cognitive and demographic findings

The matched test sample included 356 participants (mean age ± SD, 74.3 ± 8.0; 204 women [57.3%], supplementary Table 1). $CNAC^+$ compared to $CNAC^-$ showed lower ADAS-Cog score (14.4 ± 5.3 vs. 12.2 ± 4.1, $P = 0.004$), but did not differ in demographic factors, with a trend of fewer education years in $CNAC^+$. No significant differences were seen for cognitive and demographic factors between $MCIAC^-$ and $MCIAC^+$.

Anticholinergic medication and NBM MRI metrics

To explore relevant covariates of no interest for NBM-GM density, we assessed their association between demographics factors in the full 246 CNAC- participants with T1 data. No significant correlation was found between NBM-GM density and age ($P = 0.487$). Yet, based on prior reports on NBM atrophy over the age span [31], we chose to control for age in the analyses of NBM-GM density.

A medium effect of AC medication use was observed on NBM in CN with lower NBM-GM density in AC^+ compared to AC^- (0.59 ± 0.02 vs. 0.61 ± 0.03; Cohen's $f = 0.28$ [95%CI:0.14–0.43], $P < 0.001$; corrected $P < 0.05$; Fig. 2A). NBM-GM density was also reduced in $MCIAC^+$ compared to $MCIAC^-$ with a large effect size (0.56 ± 0.03 vs. 0.59 ± 0.03, Cohen's $f = 0.42$ [95%CI:0.26–0.59], $P < 0.001$; corrected $P < 0.05$; Fig. 2A). There was no correlation between NBM-GM density and TACB in $CNAC^+$ and $MCIAC^+$.

NBM functional network was reconstructed in the independent discovery sample (n = 162). NBM was functionally connected with bilateral frontal cortex, anterior cingulate cortex, bilateral insula, bilateral thalamus, bilateral hippocampus, posterior cingulate cortex (PCC) and bilateral lateral occipital cortex (Fig. 3, Supplementary Table 2) which was similar to the NBM functional maps identified in 33 CN from ADNI-2 in our previous study [27] but with additional network areas in the PCC and bilateral thalami.

Education was significantly but weakly correlated with NBM-FC Z score ($r = 0.191$, $P = 0.001$), and was hence controlled for in NBM-FC tests in addition to age due to previous reports on age-related changes of NBM-FC [32].

There was a significant, but small effect of AC medication use on NBM-FC with lower NBM-FC observed in $CNAC^+$ compared to $CNAC^-$ (3.6 ± 0.5 vs. 3.9 ± 0.6; Cohen's $f = 0.24$ [95%CI:0.10–0.39], $P = 0.001$; corrected $P < 0.05$, Table 2; Fig. 2B). $MCIAC^+$ vs. $MCIAC^-$ also had lower NBM FC with large effect (3.29 ± 0.20 vs. 3.66 ± 0.46; Cohen's $f = 0.49$ [95%CI:0.30–0.68], $P < 0.001$; corrected $P < 0.05$, Table 2; Fig. 2B). There was a trend correlation between NBM-FC and TACB in $CNAC^+$ ($r=-0.200$, $P = 0.054$) and $MCIAC^+$ ($r=-0.252$, $P = 0.059$).

Table 2
Structural and functional measurements of imaging markers

	Cognitively Normal				MCI				P[a]
	Whole cohort (n = 196)	**AC- participants (n = 98)**	**AC + participants (n = 98)**	**P[a]**	**Whole cohort (n = 160)**	**AC- participants (n = 80)**	**AC + participants (n = 80)**	**P[a]**	
NBM GM density, mean voxel value (SD)	0.58 (0.03)	0.61 (0.03)	0.59 (0.02)	< 0.001*	0.60 (0.03)	0.59 (0.03)	0.56 (0.03)	< 0.001*	< 0.001*
	Whole cohort (n = 145)	**AC- participants (n = 78)**	**AC + participants (n = 67)**	P[a]	**Whole cohort (n = 130)**	**AC- participants (n = 69)**	**AC + participants (n = 61)**	P[a]	P[a]
Whole hippocampal GM volume[d], mean (SD), cm^3	7.3 (0.9)	7.4 (0.8)	7.2 (0.9)	0.070	7.10 (0.95)	7.2 (0.91)	6.9 (1.00)	0.273	< 0.001*
Whole precuneus GM volume, mean (SD)[e], cm^3	7.1 (0.9)	17.1 (2.4)	17.0 (2.4)	0.894	17.4 (2.3)	17.5 (2.3)	17.2 (2.3)	0.528	0.354
	Whole cohort (n = 193)	**AC- participants (n = 97)**	**AC + participants (n = 96)**	P[b]	**Whole cohort (n = 126)**	**AC- participants (n = 67)**	**AC + participants (n = 59)**	P[b]	P[b]
NBM-NBM network functional connectivity, mean (SD), Z score[c]	3.7 (0.5)	3.9 (0.6)	3.6 (0.5)	0.001*	3.48 (0.4)	3.66 (0.46)	3.29 (0.20)	< 0.001*	< 0.001*
Visual cortex functional connectivity, mean (SD), Z score	5.1 (0.8)	4.9 (0.8)	5.1 (0.8)	0.254	4.9 (0.8)	4.8 (0.8)	5.0 (0.8)	0.388	0.188
a:Group comparison using ANOVA test controlled for age									
b: Group comparison using ANOVA test controlled for age and education									
*: Significant level at P < 0.05									

Secondary tests revealed the reduction of NBM MRI metrics in MCI, and AC ssubtrata compared to CN and substrata (s. supplementary material 4).

NBM imaging metrics mediates the relationship between AC medication burden and cognition

All analyses were performed in the AC^+ sample. TACB was weakly correlated with ADAS-Cog (r = 0.168, P = 0.005; Fig. 2C). No significant correlation was identified between NBM-GM density and ADAS-Cog (P = 0.645), TACB (P = 0.450). ADAS-Cog was weakly negatively correlated with NBM-FC (r=-0.249, P = 0.005; Fig. 2D). Hence, only NBM-FC was tested as potential mediator of the association between TACB and ADAS-Cog. NBM-FC modestly (indirect effect:0.30 [95%CI: 0.16–0.49]) mediated the association between TACB and ADAS-Cog.

Bias assessment of AC medication effects

No significant effect of AC medication was seen for CN or MCI strata on amyloid or APOE ε4 carriers status or on hippocampal or precuneus GM volumes. There was also no difference seen in visual cortex FC between subgroups (details in supplementary material 5). $CNAC^+$ vs. $CNAC^-$ had higher vascular risk profile (CMC score: 1.41 ± 1.16 vs. 1.13 ± 1.1, P < 0.001), with no difference seen between $MCIAC^+$ and $MCIAC^-$. As expected, the range of indications for AC medication prescriptions was reflected in higher reports of neurological, cardiovascular, respiratory and gastrointestinal disorders.

Discussion

We provide the first evidence of structural and functional impairment of the NBM, a key cholinergic forebrain hub, linked to anticholinergic prescription in the ADNI3 cohort highlighting a possible mechanism of the reported elevated risk of dementia linked to AC medication. Importantly, the detrimental central cholinergic effects were demonstrated in both cognitive healthy and mild cognitively impaired participants with a prescription history of at least one year of drugs with mainly mild anticholinergic burden. AC medication strata were matched for APOE ε4 carrier

or amyloid status and did not differ in established MRI AD markers arguing against bias effect from preclinical AD and for a specific central cholinergic effect.

We found NBM-GM density loss associated with use of the mild-moderate AC medication that was carefully matched on demographics, ApoE and amyloid markers. This is the first NBM morphometric study on AC medication effects, but findings are generally in line with two previous MRI studies in CN on anticholinergic medication that reported increased brain atrophy and temporal cortical thinning [33, 34]. It has been suggested that global brain atrophy in AC medication may indirectly arise from affected central cholinergic pathways that may render the brain more vulnerable to stress-related neurotoxicity. In our study, there was only a trend reduction of hippocampal GM volume, but no change was seen in precuneus GM volume, which would favour an increased cholinergic rather than general brain vulnerability. Selective vulnerability of the basal forebrain cholinergic neurons, and in particular those in Ch4 (NBM), to oxidative stress is well established [35]. Beyond oxidative stress, basal forebrain cholinergic neurons are also strongly dependent on target-derived nerve growth factor for preservation of cholinergic phenotype which led to the notion of retrograde NBM atrophy in AD [36], but links between NGF and long-term anticholinergic medication remain to be investigated.

We report reduced NBM-GM density in MCI compared to CN which is well in line with early cholinergic failure in MCI and previous studies demonstrating NBM atrophy [37]. Importantly, we observed NBM-GM density loss in MCI with AC medication use compared to those without, providing the first evidence of accelerated NBM neuronal tissue injury associated with anticholinergic medication despite the absence of differences in the severity of cognitive impairment. These findings are further in support of a putative neurotrophic role of cholinergic tone orchestrated through the emerging understanding of modulated gene expression, translation and cellular signalling cascades [38].

We demonstrate the detrimental effects of AC medication use on the NBM cholinergic network in keeping with the hypothesised central hypocholinergic state. The NBM-FC map was generated in an independent subsample of older participants using a Ch4/NBM template seed to increase anatomical specificity over expert manual seeds [27] which allowed robust reconstruction of the medial and lateral cholinergic pathways [39] similar as previously reported in a younger sample [32]. NBM-FC was significantly reduced in CN and MCI AC^+ compared to AC^-. In parallel, we confirm previous reports that AC medication use is associated with impaired global cognitive performance in CN [12, 24, 34], but surprisingly not in MCI. We further showed that cholinergic network functional integrity partially mediated the association between AC medication use and global cognitive performance. These findings provide a potential biological basis for the impaired cognition associated with AC medication use through the functional changes of the cholinergic network.

The effect size of AC medication on NBM imaging metrics was large in MCI and small-medium in CN suggesting a preferential vulnerability to AC in the at-risk population. This interpretation is further supported by the observed significant anticorrelation between NBM-FC and ACB burden in $MCIAC^+$ with only a trend association between NBM-FC and ACB burden in $CNAC^+$. Also, cognition was weakly correlated with NBM-FC only in the MCI cohort. It is conceivable that MCI status and AC medication have superadditive effects on NBM impairment. In keeping with previous studies, we show significant cognitive cohort effects on the NBM imaging metrics with GM loss in MCI vs CN [40] and NBM-FC reduction in MCI vs CN [20], and additionally report that the effect sizes of MCI status for both metrics were small in AC^-, but large in AC^+. Taken together this suggests a more complex multifactorial interplay which could lead to increased vulnerability of the central cholinergic pathways to AC medication in MCI with some pre-existing NBM disruption.

Several limitations were noted in this study. First, according to the ADNI3 protocol, medication use was based on self-report which may lead to underreporting of AC medication use. There is also no accurate information of the duration of AC medication available in the ADNI3 study. Future studies using medical/prescription records would be needed to further characterise the specific medication effects. Second, we excluded participants using ChEI so cannot comment on the degree to which AC related NBM effects may be reversible. Third, while the groups were well matched for demographics, AD risk and showed no differences in AD general MRI markers due to the cross-sectional nature we cannot exclude biases from comorbidities and as expected by indications for commonly prescribed AC medication, we found a higher vascular risk factor score in $CNAC^+$ vs $CNAC^-$ and in general somewhat higher frequencies of cardiovascular, neurological, respiratory and gastrointestinal comorbidities in AC^+ subgroups.

Conclusion

Our study provides new evidence of the detrimental effect of even mild-moderate AC medication use on NBM-GM density and functional network in CN and MCI. The link between NBM-GM density loss and AC medication suggests accelerated NBM degeneration as putative mechanism of the elevated risk of dementia. Impaired NBM-FC in AC^+ provides support for an AC medication induced central hypocholinergic state which partially mediated the association between anticholinergic burden and cognitive impairment. Moreover, a pattern of nominally stronger AC effects on NBM-GM and FC changes in MCI point to a possible increased vulnerability of the central cholinergic pathways to anticholinergic medication in pre-existing early cholinergic failure. Last, our findings highlight the suitability of the reported NBM imaging metrics as markers of central cholinergic failure for future mechanistic studies to inform on trials that may co-prescribe ChEI or de-prescribe AC in the most at risk population to prevent dementia.

Declarations

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Figures

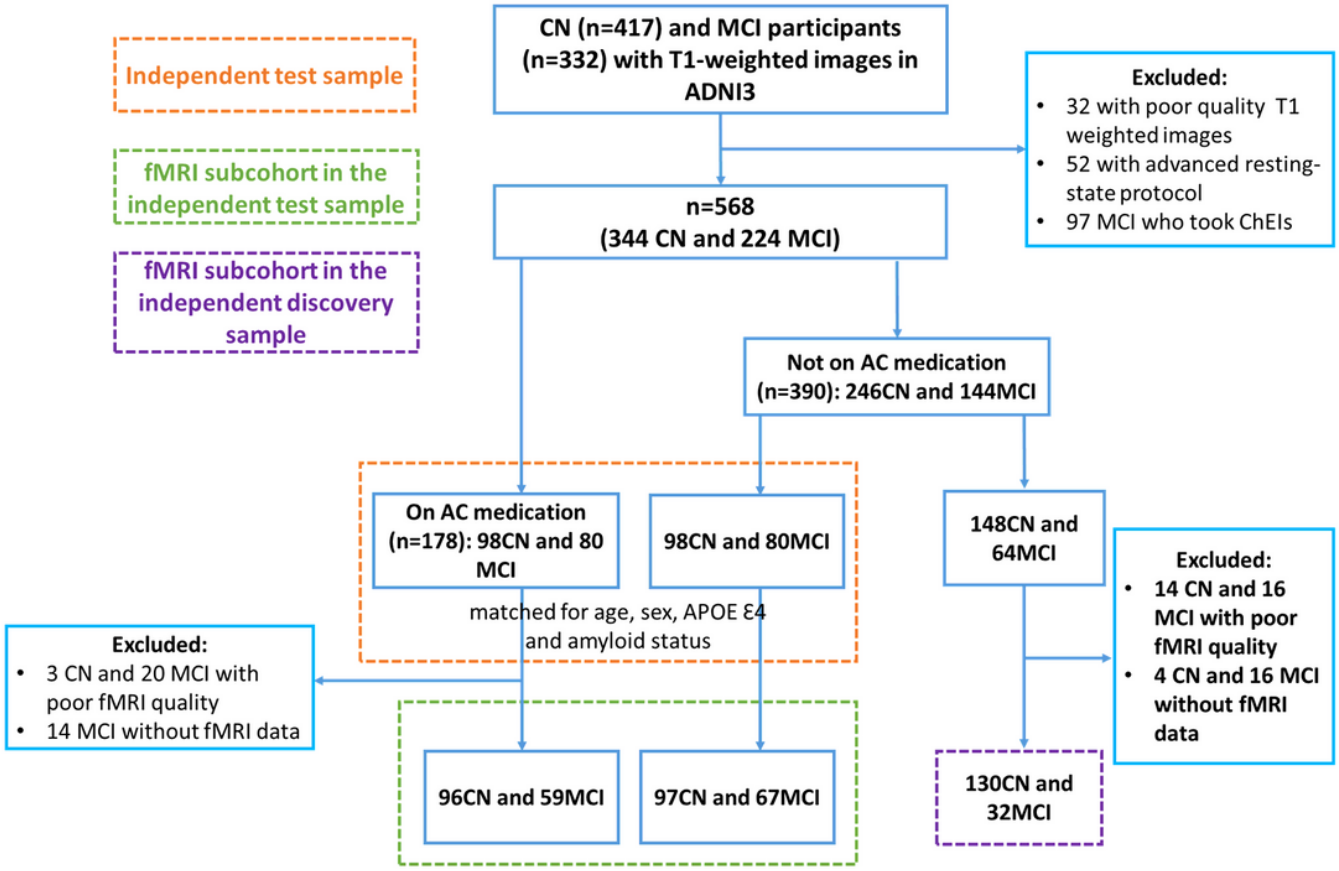

Figure 1

Flowchart shows participant selection. AC:anticholinergic medication, CN:Cognitively normal, MCI:mild cognitive impairment, fMRI:task-free functional MRI.

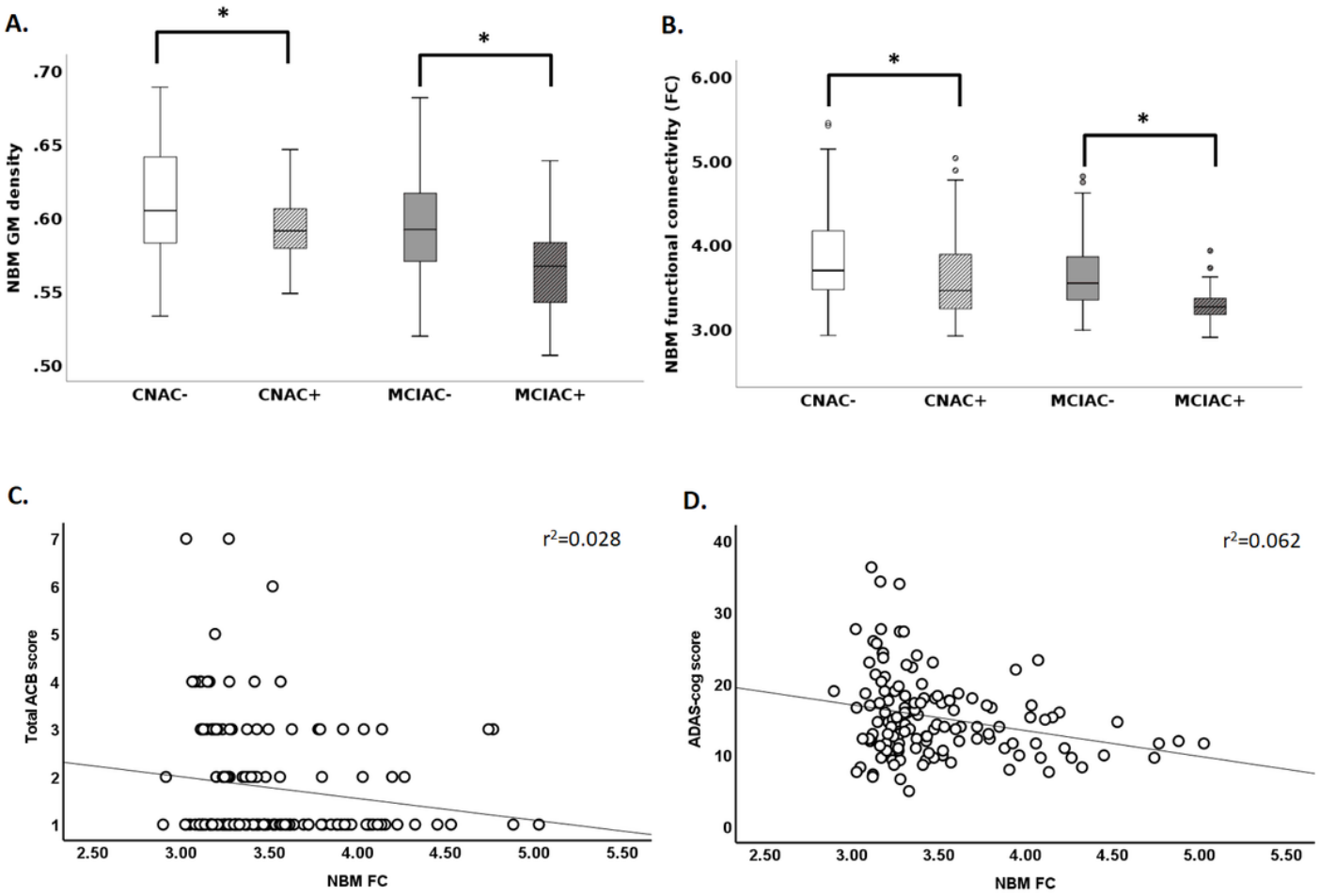

Figure 2

Anticholinergic drug use and NBM imaging metrics. Bar charts show the group differences of (A) NBM GM density and (B) NBM FC between CNAC-, CNAC+, MCIAC- and MCIAC+ participants. Box plots displaying the value range of 25% to 75% and the median values. (C) Scatterplots show the significant correlation between NBM functional connectivity (FC) and total ACB score. (D) Scatterplots show the significant correlation between NBM FC and ADAS-Cog score. Significance level at P<0.05. Asterisk (*) represents P<0.05.

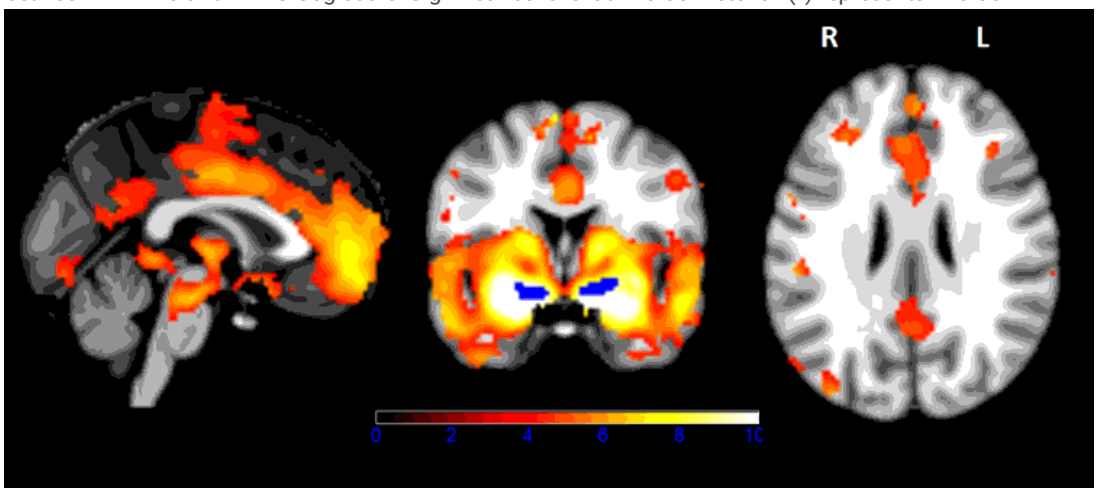

Figure 3

NBM functional network reconstructed as seed-based functional connectivity map of the NBM derived from the independent discovery subsample (n=162). All results were masked by gray matter template obtained from Montreal Neurological Institute 152 standard-space T1-weighted average

image (corrected P<0.05).

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