

Cerebral acetylcholinesterase activity is not decreased in MS patients with cognitive impairment

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Jere R Virta¹, Sari Laatu^{2,3}, Riitta Parkkola^{1,4}, Vesa Oikonen¹,
Juha O Rinne¹ and Juhani Ruutiainen²

Abstract

Background: Neuropsychological studies have extensively described the presence of cognitive dysfunction in MS patients. One possible pharmacological treatment of the impairment could be based on acetylcholinesterase inhibitors (AChEIs), which have shown efficacy in alleviating cognitive impairment in many other disorders. The findings on the efficacy of AChEI medication in MS associated cognitive symptoms are preliminary and no studies concerning cerebral acetylcholinesterase (AChE) activity in these patients have been published.

Objective: The objective of the study was to examine cerebral AChE activity in cognitively deteriorated MS patients. Cerebral AChE activity of 10 MS patients with secondary progressive disease and marked cognitive impairment, and 10 healthy controls, was studied with positron emission tomography using tracer ¹¹C-MP4A.

Methods: The cognitive profile of the patients was assessed with CERAD (Consortium to Establish a Registry for Alzheimer's Disease).

Results: No differences in cortical AChE activity between MS patients and controls were seen.

Conclusions: In the patient group regional AChE activities had inverse correlations with Word learning and MMSE (Mini-Mental State Examination) scores. In the group of cognitively deteriorated MS patients no change in cerebral AChE activity, compared with controls, was observed, but within the patient group more pronounced cognitive symptoms were associated with higher cerebral AChE activity.

Keywords

acetylcholinesterase, cognitive impairment, multiple sclerosis, PET

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Introduction

Cognitive impairment occurs in about half of patients diagnosed with MS.¹ Most commonly it manifests as deficits in memory, attention, learning, conceptual reasoning, executive functions and information processing speed. Also psychiatric disorders such as depression, anxiety, sleep disturbance, irritability and apathy are frequent in MS.² While evidence suggests that there may be some benefit from psychological counselling, cognitive training and disease modifying therapies, there certainly remain unmet needs for therapy. One often suggested treatment could be based on the use of acetylcholinesterase inhibitors (AChEIs), which have also efficacy in many other diseases with cognitive and psychiatric deficits, such as Alzheimer's disease,

Parkinson's disease, dementia with Lewy bodies, vascular dementia and traumatic brain injury.^{3,4}

The axons of cholinergic neurons from the basal forebrain project throughout the cortex by periventricular pathways that are typically damaged in MS and cerebral spinal fluid markers indicate decreased cholinergic activity in MS.^{4,5} Data regarding the

¹Turku PET Centre, University of Turku, Finland.

²Masku Neurological Rehabilitation Centre, Masku, Finland.

³Outpatient Ward of Neuropsychiatry, University Hospital of Turku, Finland.

⁴Department of Radiology, University Hospital of Turku, Finland.

Corresponding author:

Jere Virta, Turku PET Centre, University of Turku, PO Box 52, FIN-20521, Turku, Finland
Email: jere.virta@utu.fi

status of the central cholinergic system in MS are limited and no explanation of the underlying mechanism can be inferred from the previous studies. A positron emission tomography (PET) study of acetylcholinesterase (AChE) activity would assist in determining whether a loss of central nervous system cholinergic function exists in patients with MS. With PET imaging cortical glucose hypometabolism has been demonstrated in hippocampi bilaterally and in the left thalamus in patients with MS and cognitive deficits, as well as reduced metabolism in bilateral prefrontal, inferior parietal cortices and basal ganglia in patients with MS and signs of frontal dysfunction.⁶⁻⁸

The objective of the present study was to examine the activity of the AChE in MS patients showing marked cognitive impairment. Cerebral AChE activity was studied with PET tracer ¹¹C-methyl-4-piperidyl propionate (¹¹C-MP4A), an acetylcholine analog that, in the human brain, is selectively metabolized by AChE and thus the rate of tracer accumulation in brain tissue represents the local AChE activity.⁹ The method has been used in research of neurodegenerative diseases, and it has revealed reduced AChE activity, for example, in Alzheimer's disease,^{10,11} dementia of Parkinson's disease,^{12,13} Lewy body dementia¹⁴ and age-associated periventricular leukoaraiosis.¹⁵

Methods

Subjects

The study was approved by the Ethical Committee of the South-Western Finland Health Care District. All the subjects gave their written informed consent for participation in the study.

The inclusion criteria for the MS patients were: age between 18 and 70 years, a definite diagnosis of MS,¹⁶ relapsing–remitting or secondary progressive course of MS, MMSE (Mini-Mental State Examination) performance related to mild or moderate dementia (scores 18–26), and at least one score below the cut point in the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) neuropsychological battery subtests. The exclusion criteria were: primary progressive course of MS, the use of cholinergic medication or a change in the dosage of benzodiazepine, antidepressive, antispastic medication or mild anticholinergic medication (for overactive bladder) during the preceding month, and any medical, psychological or other reason indicating that the patient would not be able to complete the study protocol. The exclusion criteria for the healthy

controls were: abnormal performance on the MMSE test or any medication or severe disease that could influence cognitive performance.

Ten healthy controls and 10 MS patients with a secondary progressive disease and marked cognitive impairment were recruited for the study. All the voluntary MS patients were clients of the Masku Neurological Rehabilitation Centre. The disability of the patients was evaluated by the Expanded Disability Status Scale (EDSS). The overall cognitive performance was evaluated with the CERAD and NPI (Neuropsychiatric Inventory) neuropsychological test batteries.

Scanning protocol

The PET imaging was performed with a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, WI). The radiochemical synthesis and quality control of ¹¹C-MP4A have been described in detail elsewhere.¹⁷ At the start of the scan ¹¹C-MP4A was injected in an antecubital vein as a constant bolus during 80 s, and the radioactivity was measured in a consecutive series of 22 frames (1 × 30 s, 4 × 15 s, 5 × 30 s, 2 × 60 s, 2 × 120 s, 6 × 300 s, 2 × 600 s) with a total scan duration of 60 min.

All patients and controls were scanned with a Philips Gyroscan Intera 1.5T CV Nova Dual scanner (Philips, Best, The Netherlands). Axial T2 and coronal FLAIR sequences were obtained to visualize MS lesion load and in controls to exclude any brain pathology. Whole brain T1-weighted 3D T1 data set was acquired in transverse plane.

Image preprocessing

PET and MR images were processed with in-house software (<http://www.turkupetcentre.net>) and Statistical Parametric Mapping version 2 (SPM2) (Wellcome Department of Cognitive Neurology, London, UK; <http://www.fil.ion.ucl.ac.uk/spm>) running on Matlab 6.5 (The MathWorks, Natick, MA, USA). The images were movement corrected and normalized to a common stereotactic space as described in detail earlier.^{18,19} In brief, first the individual MRIs were co-registered to summated ¹¹C-MP4A images and then the MRIs were normalized to T1-weighted MRI template in the MNI Space. Using the acquired transformation parameters, ¹¹C-MP4A summated images were normalized and the mean of these normalized images was averaged with its mirror copy (left–right flipped image). This symmetrical mean ¹¹C-MP4A summated image was smoothed using an 8 mm Gaussian kernel,

to create a ligand-specific template. Individual dynamic PET images were spatially normalized to the ligand-specific template with parameters estimated from summated ^{11}C -MP4A images in native space.

MRI images co-registered to the summated PET image in native space were also written in the MNI space with the parameters estimated from the individual's summated PET image to the ligand-specific template. A set of regions of interest (ROIs) was defined on the average of these normalized MRI images using Imadeus software (version 1.50, Forima Inc., Turku, Finland), and the location of ROIs visually checked to match each normalized PET image. ROIs were delineated bilaterally on anterior cingulate (AC), lateral frontal cortex (LFC), medial temporal lobe (MTL), lateral temporal cortex (LTC) and occipital cortex (OCC), parietal cortex (PAR) and posterior cingulate (PC). The ROIs were then transferred to the corresponding planes in the normalized dynamic PET images and time-activity curves (TACs) for ROIs calculated.

All MR images of both patients and controls were visually evaluated. The coronal FLAIR images were analysed to assess the MS lesion load of the patients with a semi-automated thresholding technique using GE AW1.0 software (GE Medical Imaging Systems, Milwaukee, WI, USA). The MR images of the patients and controls were scored regarding central, cortical, posterior fossa and hippocampal atrophy. A score of 0 means no atrophy and a score of 4 severe atrophy.²⁰

Modelling of tracer kinetics

AChE activity was calculated applying the transport-limited reference tissue model for irreversible uptake, in which the k_3 rate constant represents the hydrolysis rate of the tracer in tissue and thus the local AChE activity. This model is based on the reference tissue compartment model, but here it is assumed that in reference tissue tracer metabolism is fast and thus tracer uptake is limited only by transport into tissue.^{21,22} Putamen has high AChE activity resulting in fast tracer metabolism, and thus was used as reference tissue. The model was solved for ROI-TACs to calculate regional AChE activity and for each image pixel separately to produce parametric images representing AChE activity.

Statistical analyses

The statistical analyses of regional AChE activity values, CERAD results and parameters visually

obtained from MRI images were performed with the SPSS for Windows software, release 13.0 (SPSS Inc., Chicago, IL, USA). The normality of the AChE activity in ROIs was characterized by the Shapiro–Wilks test. The difference of regional (ROI-wise) AChE activity between patients and controls was explored with one-way ANOVA. Correlations of AChE activity with subjects' age in both groups, and for patients also with EDSS, CERAD, lesion load and regional atrophy scores were explored by Pearson's tests, or if the normality assumption was not met, with Spearman's correlation test. Sex differences in AChE activity were tested with the Mann–Whitney U test in each group.

The statistical analyses of voxel-wise AChE activity were performed with SPM2. Prior to statistical analysis, the parametric PET images were smoothed with a 14mm FWHM Gaussian kernel. A SPM t -test was performed between the MS and the control group; pixels with cluster-level p -value corrected for multiple comparisons below 0.05 were regarded as significant.

Results

The demographic and clinical characteristics of the studied patients are described in Table 1. All patients had a history of MS for more than nine years and most of them were severely disabled both in ambulation and cognition. In both patient and control groups the female/male ratio was 7/3. The mean age of controls was 54 years (range 38–64) and of patients 56 years (49–63), the difference not being significant ($p=0.513$). Controls had normal brain MRI. All patients had severe MS lesion load in their brain (average 67.6ml, range 20.2–133.1ml). Based on the atrophy scores, MS patients had significant central, cortical, posterior fossa and hippocampal atrophy (compared with controls, tested with Mann–Whitney U test; see Table 1).

Regional AChE activity in both MS patient and control groups in all ROIs complied with normal distribution (Shapiro–Wilk statistic $p>0.05$). In the patient group the CERAD subtest scores complied with normal distribution, except for Constructional praxis ($p=0.045$) and Clock drawing ($p=0.005$) subtests. No significant group difference in AChE activity was observed in any ROI. The AChE activities, group differences and the statistical power of the null hypothesis are tabulated in Table 2. The largest group difference was observed in anterior cingulate; in the MS group the AChE activity was $6.7 \pm 14.5\%$ higher than in the control group. The statistical power for the null hypothesis

Table 1. The demographic and clinical characteristics of the studied patients. The *p* values for grey matter atrophy scores are from patients versus controls comparison with *t*-test

Variable	
Sex, <i>n</i> : female/male	7/3
Age, years: mean (range)	56 (49–63)
Education, years: mean (range)	13 (8 – 20)
MMSE, test points: mean (range)	23.2 (18–26)
CERAD, <i>n</i> scores below cut point: mean (range)	3.1 (1–6)
NPI, <i>n</i> per category	Aberrant motor behaviour 2 Agitation 7 Anxiety 7 Apathy 7 Appetite 6 Delusions 3 Depression 8 Disinhibition 5 Hallucinations 2 Euphoria 1 Irritability 9 Sleep disturbance 5
Grey matter atrophy score, mean $\pm$ SD, <i>p</i>	
Central atrophy	1.80 $\pm$ 1.14, <i>p</i> = 0.001
Cortical atrophy	1.30 $\pm$ 0.95, <i>p</i> = 0.003
Posterior fossa atrophy	1.80 $\pm$ 0.92, <i>p</i> = 0.001
Hippocampal atrophy	1.50 $\pm$ 0.71, <i>p</i> = 0.001
EDSS, mean (range)	6.5 (4–8.5)
Disease duration, years from diagnosis: mean, (range)	25.8 (10–30)

Table 2. Cortical AChE activity in MS patients and healthy controls

	$k3_{MS}$	$k3_{cont}$	Δ	Power
Anterior cingulate	0.095 $\pm$ 0.013	0.089 $\pm$ 0.010	6.7 $\pm$ 14.5%	0.80
Lateral frontal cortex	0.076 $\pm$ 0.008	0.076 $\pm$ 0.008	0.3 $\pm$ 10.2%	0.95
Medial temporal lobe	0.076 $\pm$ 0.007	0.076 $\pm$ 0.008	0.4 $\pm$ 8.9%	0.95
Lateral temporal cortex	0.075 $\pm$ 0.009	0.075 $\pm$ 0.008	0.4 $\pm$ 11.6%	0.95
Occipital cortex	0.066 $\pm$ 0.007	0.069 $\pm$ 0.008	–3.7 $\pm$ 10.0%	0.89
Posterior cingulate	0.065 $\pm$ 0.005	0.067 $\pm$ 0.008	–2.1 $\pm$ 7.9%	0.93
Parietal cortex	0.069 $\pm$ 0.005	0.070 $\pm$ 0.008	–1.6 $\pm$ 7.6%	0.93

$k3_{MS}$ is average $\pm$ SD AChE activity ($k3$ rate constant, L/min) in the patient group, $k3_{cont}$ the average AChE activity in the control group, Δ is the average $\pm$ SD difference between the groups, and the power the observed statistical power for hypothesis $k3_{MS} = k3_{cont}$.

of no group differences in AChE activity was $\geq 80\%$ in all ROIs. Assuming the within-group variances of the sample in the present study, group differences larger than 9.1% would have been significant in PAR and larger than 12.1% significant in AC, the group difference threshold for significance in other ROIs being between these two. The highest coefficient of variation was observed in AC in the MS group, 13.6%, and the lowest in PC

in MS group, 8.0%. The inference of pixel-wise statistical analysis complied with the region-wise analyses; in the SPM *t*-test between the MS patient and control group no significant differences were observed.

There was no sex difference in AChE activity and the activity did not correlate with age in either groups. In the MS group AChE activity did not correlate with disease duration, EDDS, lesion load

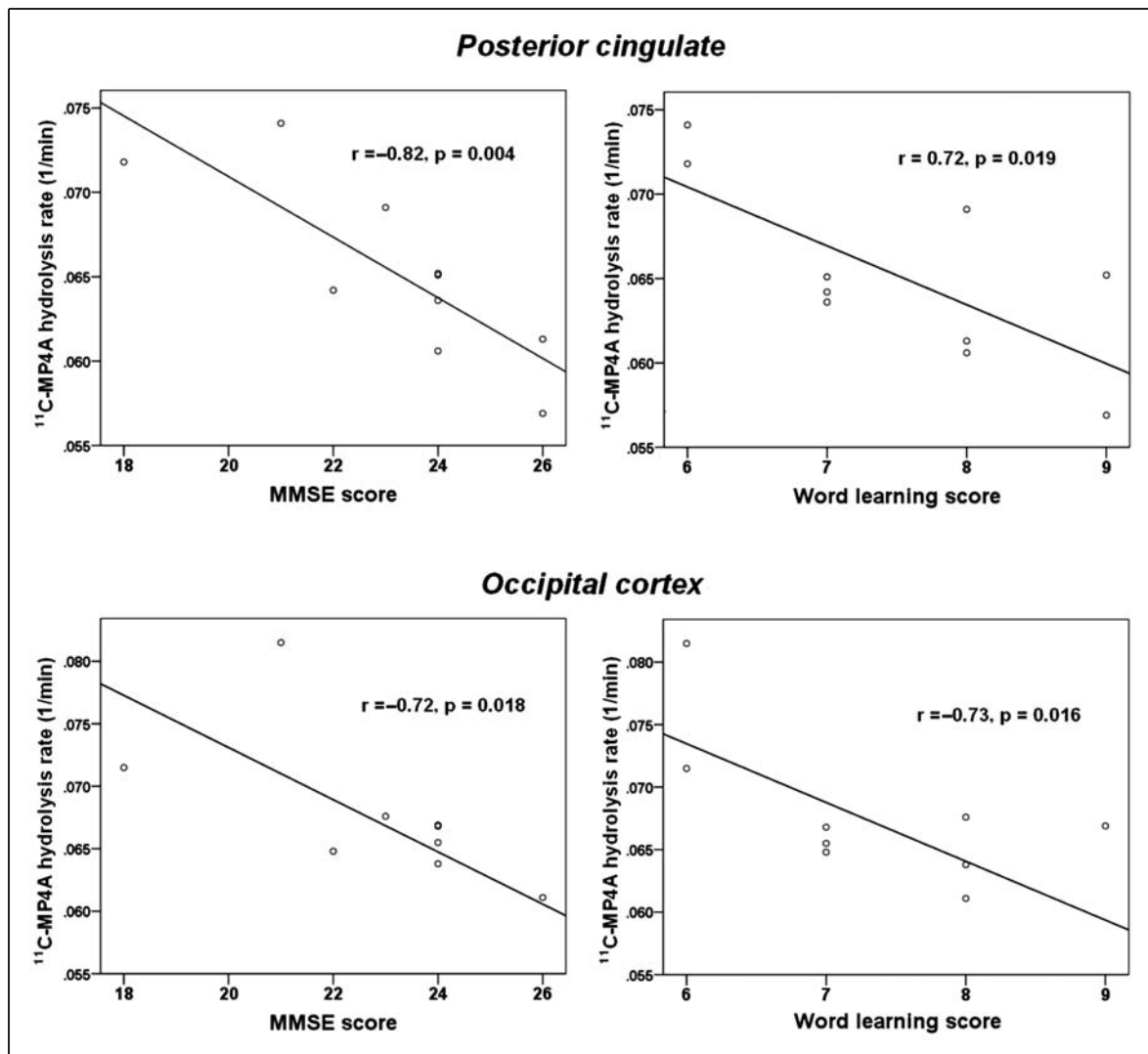


Figure 1. Correlation of regional AChE activity (k3 rate constant, L/min) in posterior cingulate and occipital cortex with MMSE and Word learning subtest scores of the CERAD battery. Correlations were tested with Pearson's test.

or brain atrophy. In the MS group significant correlations were observed between the CERAD sub-scores and AChE activity: MMSE and Word learning scores had significant inverse correlations with AChE activity in PC (MMSE $r = -0.82$, $p = 0.004$; Word learning $r = -0.72$, $p = 0.019$) and OCC (MMSE $r = -0.72$, $p = 0.018$; Word learning $r = -0.73$, $p = 0.016$). The relationships of AChE activity in OCC and PC to MMSE and Word learning scores are illustrated in Figure 1. Other scores of the CERAD battery had no significant correlations with cortical AChE activity.

Discussion

The intention of our study was to investigate whether there are changes in cerebral AChE activity

in cognitively impaired MS patients. In line with evidence on the failure of the cholinergic system in MS,^{4,5} we anticipated a low AChE activity in MS brains to be observed, as has previously been observed, for example, in Alzheimer's disease.¹¹ Yet no difference between MS patients and healthy controls was observed; the statistical power of the null hypothesis of no group difference was high in all ROIs. Within-group variation was small, and thus a group difference of 10% or more would have been detectable with our method. For comparison, in Alzheimer's disease the cortical AChE activity as measured by $^{11}\text{C-MP4A}$ -PET is decreased by 20–30%.^{10,11}

While there were no group differences in cortical AChE activity between patients and controls, within the patient group inverse correlations were observed

between the CERAD scores and cortical AChE activity: increased AChE activity in occipital cortex and posterior cingulate was associated with lower scores in MMSE and Word learning tests. Word learning tests in particular are sensitive in detecting cognitive impairment associated with MS²³ and the test scores correlate with the magnitude of white matter volume and lesion changes typical for MS.²⁴ In previous studies cortical AChE activity measured by ¹¹C-MP4A PET has been observed to correlate with neuropsychological test scores in Alzheimer's disease¹⁸ and dementia of Parkinson's disease,²⁵ but in both conditions more pronounced symptoms were associated with decreased cortical AChE activity. To our knowledge, more pronounced cognitive symptoms have not previously been associated with increased cortical AChE activity measured with PET in any condition.

There are a few shortcomings in our study. First, our results are based on a small convenience sample of patients and healthy controls. Yet the statistical power of our finding is good. Second, we tested a homogenous patient group with a marked cognitive impairment and no inference based on our study can be made on the possible changes of cerebral AChE activity in MS patients with milder cognitive symptoms or without cognitive symptoms. Third, we do not know if the patients or controls in our sample would have benefited from treatment with AChE inhibitors. If the drug response in our group had been tested and analysed in conjunction with the PET results, the study could have provided more information on the mechanisms of possible effects of AChE inhibitors in cognitive impairment associated with MS. The pathophysiology of cognitive impairment in MS might be heterogeneous and the respondents for AChEI treatment might form a subgroup of patients who would also show altered cerebral AChE activity. With these limitations borne in mind, our results indicate that MS with cognitive impairment is not associated with changes in cerebral AChE activity, at least not on a group level. If cerebral AChE activity is taken as a marker of the integrity of cholinergic pathways, our results suggest that there is no significant dysfunction of cholinergic system in MS with cognitive impairment.

Functional magnetic resonance imaging (fMRI) studies have shown that cortical activation in MS patients is stronger and more widespread than that in healthy controls during cognitive tasks.^{26,27} The phenomenon has been interpreted to imply neural plasticity compensating for the neural damage due to the MS pathology. In one fMRI study an acute administration of rivastigmine normalized the increased activation seen in the brain of MS patients

with cognitive symptoms during a counting Stroop task,²⁸ which could indicate that the abnormal activation was compensatory to dysfunction of cholinergic pathways. This conclusion seems to be in contradiction with our results. No studies on the effects of rivastigmine on the cognitive functions of healthy controls have been published, but acute administration of similar AChE inhibitor donepezil has been observed to enhance the function of various cognitive domains also in healthy controls.²⁹ Thus it is possible that even if rivastigmine changed the brain activation profile of MS patients, the cholinergic pathways of the MS patients were nevertheless intact. In the fMRI study no effect of rivastigmine on the fMRI activation profile was seen in healthy controls, but only the brain regions where MS patients had abnormal activation were analysed.

Long-term treatment with AChE inhibitors donepezil or rivastigmine has been beneficial for the MS patients in a few small samples,^{30–32} but negative findings have also been reported.^{33,34} It has been suggested that AChEI drugs have functions independent of their ability to inhibit AChE, such as anti-inflammatory properties,³⁵ protection against oxidative stress³⁶ and improving the cerebrovascular regulation.³⁷ If there is no significant dysfunction of cholinergic system in MS even when marked cognitive impairment is present, the possible benefit of AChEI medication in the MS associated cognitive impairment might be due to these other mechanisms of action.

Even if there are no changes in cerebral AChE activity in MS associated cognitive impairment at group level compared with controls, the correlations between cortical AChE activity and CERAD scores suggest that changes in AChE activity might, nevertheless, have a role in the pathophysiology of the cognitive impairment. In Alzheimer's disease ¹¹C-MP4A PET shows clear reduction in cerebral AChE activity, this probably being related to degeneration of cholinergic pathways. But in vitro studies have demonstrated that in Alzheimer's disease there actually is an increase in AChE of glial origin in close vicinity to amyloid plaques and neurofibrillary tangles, the main neuropathological changes of the disease.³⁸ The different molecular form of AChE cannot be studied separately with ¹¹C-MP4A PET, which reflects only the total hydrolysis rate of the AChE-specific radiotracer.

Probably in MS the role of gliosis and neuroinflammation is more pronounced compared with neurodegeneration or neuronal damage than in Alzheimer's disease. Thus our finding of no group-level changes in AChE activity could be due to increase in glial AChE activity balancing the decrease of neuronal AChE, and the

inverse correlations between AChE activity and neuropsychological test scores reflecting more pronounced glial reaction in the patients with more severe cognitive symptoms. This explanation would be in line with the drug response of AChEIs in cognitive impairment of MS: the medication would counteract the decreased amount of acetylcholine both due to degeneration of cholinergic pathways and increased hydrolysis by glial AChE, and might also attenuate the inflammatory processes. Interestingly, butyrylcholinesterase, an enzyme structurally and functionally similar to AChE, but found predominantly in glial cells, is increased within MS white matter lesions.³⁹

Decreased cerebral AChE activity has been associated with many disorders leading to impairment cognitive functions and in which AChE inhibitors are a viable option for symptomatic treatment, but our study suggests that cognitive impairment associated with MS is an exception. To our knowledge there are no other disorders in which clinical benefit from AChE inhibitor treatment and constancy of cerebral AChE activity are both reported. In addition, in no other condition have increases in cerebral AChE activity been associated with more pronounced cognitive symptoms. If these findings are replicated and further explained by other studies, it could provide new insights to the functions of AChE inhibitor pharmaceuticals and to the pathophysiology of cognitive impairment in MS and related conditions.

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Conflict of interest statement

J.R. Virta is involved in contract research with Orion-Pharma Ltd, Wyeth Ltd and Turku Imanet Ltd. J.O. Rinne is involved in contract research with Orion-Pharma Ltd. Elan Ltd, Wyeth Ltd, Bristol-Myers-Squibb Ltd. and Turku Imanet Ltd. Other authors report no disclosures.

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