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The integrity of the cholinergic system determines memory performance in healthy elderly

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ABSTRACT

The cholinergic system plays a central role in episodic memory-related processes in health and disease. Cerebral acetylcholinesterase (AChE) activity, a measure of the integrity of the cholinergic system, can be assessed in vivo using positron emission tomography (PET) and [^{11}C]N-methyl-4-piperidyl acetate (MP4A). A close relationship between the kinetic constant k3 of MP4A and mnestic functions has been demonstrated for patients suffering from amnestic mild cognitive impairment and Alzheimer's disease. Under the hypothesis that AChE activity and memory are intimately linked in older age, we obtained MP4A-PET and structural magnetic resonance images as well as neuropsychological data from fourteen healthy older adults. Multiple regression analysis revealed that AChE activity in areas previously implicated in mnestic functions predicted episodic memory performance irrespective of cortical atrophy. Data suggest that in older adults the integrity of the cholinergic system underlies inter-individual variability in memory function.

Introduction

Aging is associated with a decline of memory performance, although great inter-individual variability is observed (Hedden and Gabrieli, 2004). In today's aging societies it has become critically important to differentiate normal aging-related changes from neurodegenerative processes. Increasing efforts are currently undertaken worldwide to detect these processes prior to irreversible neuronal damage using non-invasive cerebral imaging (Herholz et al., 2002; Klunk et al., 2004; Maruyama et al., 2013). It is well established that in Alzheimer's disease (AD), neurodegeneration especially affects cholinergic neurons (Mesulam, 2004; Schliebs and Arendt, 2006). However, to which degree the integrity of the cholinergic system accounts for inter-individual variability in memory performance of healthy older adults remains to be investigated.

The function of the cholinergic system can be assessed in vivo by measuring the activity of acetylcholinesterase (AChE) using positron emission tomography (PET) and the radioactively tagged enzyme substrate [^{11}C]N-methyl-4-piperidyl acetate (MP4A). Using this approach, a cholinergic deficit in AD and its prodromal stage, i.e., mild cognitive impairment, has been demonstrated in vivo (Herholz et al., 2005; Shinotoh et al., 2000). AChE activity has also been shown to correlate with measures of cognitive and mnestic functions across patients and controls (Haense et al., 2012; Marcone et al., 2012). Although the latter correlation could not be demonstrated within the subgroup of cognitively normal individuals, a close relationship between the cholinergic system and memory performance in healthy individuals is highly plausible in view of indirect evidence provided by pharmacological studies (Kukolja et al., 2009; White and Levin, 2004; Wink et al., 2006). Accordingly, we here investigated cerebral cholinergic activity in memory relevant brain areas in cognitively normal elderly subjects. We hypothesized that the integrity of the cholinergic system accounts for inter-individual variability in mnestic performance.

Methods

Subjects

Fourteen healthy older adults (9 male, aged 53 to 77 years, mean 63.71 years +/− 6.99 years) without history of neurological or psychiatric disease and without cholinergic, anticholinergic or other psychoactive drugs were recruited from the community. The level of education ranged from 11 to 20 years of formal education and training (mean 15.03 years, +/− 3.29 years). Occupational level was assessed using a six level scale (Garibotto et al., 2013) and ranged from 3 (housewife)

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to 6 (senior civil servant or management, senior academic position, self-employed with high degree of responsibility (mean 4.21 +/− 0.80)). All subjects underwent a physical and neurological examination by a trained neurologist as well as a comprehensive neuropsychological test battery to exclude cognitive deficits or depression: MMSE (German version by (Kessler et al., 1990), Beck's Depression Inventory V (Schmitt et al., 2006), Bayer Activities of Daily Life (Hindmarch et al., 1998), Trail-Making-Test (Berres et al., 2000; Reitan, 1956), and Brief Test of Attention (Schretlen et al., 1996). Verbal episodic memory was assessed using the Verbal Learning and Memory Test (VLMT), the German equivalent of the Rey Auditory Learning Test (Helmstaedter et al., 2001; Rey, 1964). This test consists of i) five trials during which a fifteen-word list is learned, ii) an interference trial, iii) an immediate free recall and iv) a delayed free recall after 30 min. The performance on the delayed recall is the parameter most sensitive to deficits in episodic memory (Helmstaedter et al., 2001). Additional information about memory retention, consolidation and resilience to interference is provided by the number of words forgotten during the delay period (words reproduced on the last learning trial minus words at delayed recall) (Chang et al., 2010; Helmstaedter et al., 2001; Ptok et al., 2005). All subjects performed within normal range in all neuropsychological tests (see Table 1 for summary statistics). In the present sample no significant correlations between neuropsychological performance and educational or occupational level were observed. Therefore these measures were not included in further analyses. The study was approved by the ethics committee of the medical faculty of the University of Cologne and written informed consent was obtained from all participants prior to the study.

MR and PET acquisition

MP4A was synthesized as previously described in Herholz et al., 2000 and Haense et al., 2012, with minor modifications. Between 233 and 586 MBq of MP4A was injected intravenously as bolus. Scanning was performed using an ECAT HRRT scanner (CPS Innovations, Knoxville TN, USA) using the protocol described by Haense et al. (2012). High-resolution T1-weighted images were acquired using a 3 T Trio scanner (Siemens, Erlangen, Germany). Sequence parameters were as follows: MDEFT3D; time of repetition 1930 ms; time to inversion 650 ms; echo time 5.8 ms; flip angle 18°; 128 sagittal slices; resolution, $1.0 \times 1.0 \times 1.25$ mm^3.

PET processing

AChE activity was assessed by quantifying the hydrolysis rate of MP4A, k3, at the voxel level. Images were processed as follows: (1) Rigid-body co-registration of the sum of the first 10 min of the PET-scan to the T1-image; (2) rigid-body co-registration of all consecutive frames to the co-registered first 10-minute frame; (3) filtering of all individual frames with a Gaussian kernel (12 mm FWHM) with restriction of the kernel to avoid smoothing across high gradients of signal contrast; (4) segmentation and parcellation of the T1-image using FreeSurfer (Dale et al., 1999; Fischl et al., 2002, 2004); (5) extraction of the kinetic reference curve from a putaminal region of interest (ROI) generated from the T1-image using FreeSurfer (Fischl et al., 2002); (6) nonlinear least squares fitting of tracer kinetics at the voxel level using a three parameter compartmental model and computation of maps of the tracer-hydrolysis rate k3 (Herholz et al., 2000; Zundorf et al., 2002) as implemented in the software package VINCI (version 4.20, Max-Planck Institute for Neurological Research, Cologne, Germany); (7) estimation of nonlinear normalization parameters to standard Montreal Neurological Institute space for the T1-images using FMRIB's Nonlinear Image Registration Tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT); (8) application of these normalization parameters to the parametric k3-images that were previously registered to the T1-images; (9) spatial smoothing of the normalized k3-images using a Gaussian kernel of 8 mm (FWHM) to account for anatomical variations as well as registration and normalization errors (Chételat et al., 2008). All subsequent statistical analyses at the region of interest and whole brain level were constrained to gray matter, excluding the cerebellum and the basal ganglia, since k3-estimation in these structures is often unreliable (Herholz et al., 2001, 2004).

Table 1
Neuropsychological test results.

Neuropsychological test	Mean	SD	Cut-off/normal range
VLMT[a] final learning trial	62.33	23.63	
VLMT[a] delayed recall	54.57	23.96	
MMSE[b]	29.42	0.64	27–30
BDI V[c]	17	12.66	<35
BTA[d]	71.3	14.35	
B-ADL[e]	1.55	0.59	<2
TMT[f] A	53.64	27.76	
TMT[f] B	66.32	19.73	

The results for the VLMT, BTA and TMT are presented as percentiles of the age-matched normative samples.
[a] VLMT: verbal learning and memory test.
[b] MMSE: Mini-Mental State Examination.
[c] BDI V: Beck's Depression Inventory V.
[d] BTA: Brief Test of Attention.
[e] B-ADL: Bayer Activities of Daily Life.
[f] TMT: Trail-Making-Test.

Voxel-based morphometry

Voxel-wise gray matter volume maps were generated using voxel-based morphometry (VBM) as implemented in the software FSL-VBM (Douaud et al., 2007; Good et al., 2001; Smith et al., 2004); http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM). Since the resulting modulated gray matter images differed from the k3-images in original spatial resolution, they were smoothed with an isotropic Gaussian kernel of 10.6 mm (FWHM) to obtain the same final resolution in both modalities (Chételat et al., 2008; Richardson et al., 1997).

Statistical analyses

Kolmogorov–Smirnov tests were performed to assess the distribution of demographics and neuropsychological test scores. All measures were normally distributed, with the exception of the MMSE, the final learning trial, and the number of words forgotten during the delay period of the verbal learning and memory test. The deviation from normal distribution is attributable to ceiling effects commonly observed for these measures (Helmstaedter et al., 2001). Correlations between measures of mnestic function, k3 of MP4A and gray matter volume were assessed at the voxel level. To account for sample size and the distribution of the data, a non-parametric permutation approach as implemented in FSL v5.0 (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki) was chosen. Episodic memory was operationalized as performance on the delayed recall and words forgotten during the delay period of the VLMT. The design matrices included a k3 and gray matter volume map for each subject, the respective neuropsychological measure as well as gender as covariates. Analyses were constrained to ROIs representing cortical regions known to be involved in mnestic function, i.e., the medial temporal lobe (MTL), the posterior cingulate cortex (PCC) and the lateral frontal lobes. The MTL was selected based on its central role in episodic memory, primarily encoding and retrieval (Cabeza and Nyberg, 2000; Davachi, 2006; Eichenbaum, 2004; Fink et al., 1996; Kukolja et al., 2009; Squire and Wixted, 2011; Wixted and Squire, 2011) and responsiveness of local neuronal activity to cholinergic modulation (Goekoop et al., 2006; Kukolja et al., 2009; Sperling et al., 2002; Wink et al., 2006). The PCC was chosen as a ROI since it is regarded as a central hub in episodic memory consolidation and retrieval (Cavanna and Trimble, 2006; Huijbers et al., 2012; Nielsen et al., 2005; Staresina