Cholinergic challenge in Alzheimer patients and mild cognitive impairment differentially affects hippocampal activation—a pharmacological fMRI study

Rutger Goekoop, Philip Scheltens, Frederik Barkhof and Serge A. R. B. Rombouts

Department of Neurology/Alzheimer Center, Department of Physics and Medical Technology and Department of Radiology, VU University Medical Center, Amsterdam, The Netherlands

Correspondence to: Rutger Goekoop, MD, Department of Neurology, De Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands
E-mail: R.Goekoop@dds.nl

Pharmacological functional MRI (phMRI) examines the impact of pharmacologically induced neurochemical changes on brain function at a system level. The current phMRI study directly compared effects of cholinergic stimulation on brain function between patients with Alzheimer’s disease and mild cognitive impairment, a disease stage preceding the development of Alzheimer’s disease. Brain function during recognition of (un)familiar information was examined for changes after exposure to galantamine, a cholinesterase inhibitor used for treating memory deficits in Alzheimer’s disease. Alzheimer patients [n = 18; age 74.5 years ± 8.2; Mini-Mental State Examination (MMSE) 22.5 ± 2.4] and patients with mild cognitive impairment [n = 28; mean age 73.6 ± 7.5; MMSE 27.0 ± 1.2] were scanned during face recognition under three different conditions: at baseline, and after acute (single dose) and prolonged exposure (5 days) to galantamine. Functional data were analysed in an event-related fashion. In both groups, acute exposure produced strong increases in brain activation (Z > 3.1). Prolonged exposure produced less strong effects that mainly involved decreases in activation (Z > 3.1). In mild cognitive impairment, acute exposure increased activation in posterior cingulate, left inferior parietal, and anterior temporal lobe. Prolonged exposure decreased activation in similar posterior cingulate areas, and in bilateral prefrontal areas. Effects were stronger for positive (‘familiar’) than for negative (‘unfamiliar’) decisions, indicating that the effect was specific to memory retrieval. In Alzheimer patients, acute exposure increased activation bilaterally in hippocampal areas, whereas prolonged exposure decreased activation in these areas. Effects were more pronounced for negative than for positive decisions, suggesting a preferential effect on memory encoding. Unique profiles of signal reactivity were found in a number of areas, including left inferior parietal lobe and left hippocampus proper. The reactivity of posterior cingulate and hippocampal structures to cholinergic challenge suggests a key role of the cholinergic system in the functional processes that lead to Alzheimer’s disease. The differential response to cholinergic challenge in mild cognitive impairment and Alzheimer patients may reflect a difference in the functional status of the cholinergic system between both groups, which is in line with recent results showing a differential clinical response to cholinergic treatment.

Keywords: fMRI; mild cognitive impairment; Alzheimer’s disease; galantamine; challenge study

Abbreviations: MMSE = Mini-Mental State Examination; phMRI = pharmacological functional magnetic resonance imaging/pharmacological fMRI; TN = true negative/correct rejection; TP = true positive/correct hit

Introduction

Alzheimer’s disease is characterized by a progressive neurodegenerative process that initially affects only the entorhinal cortex and hippocampus in the medial temporal lobe, but gradually spreads outward to affect the entire cortical mantle in more advanced stages of disease (Braak et al., 1999). Loss of hippocampal function is considered a primary factor in...
causing memory problems in Alzheimer’s disease. Additionally, a cholinergic deficit is found that may contribute significantly to memory problems (Bartus, 2000; Mesulam, 2004). Apart from symptoms of amnesia, low acetylcholine levels have been associated with a variety of other clinical manifestations, including impaired verbal fluency and neuropsychiatric symptoms (Assal and Cummings, 2002). Current therapies against Alzheimer’s disease largely aim at restoring low acetylcholine levels with pharmacological agents (Lanctot et al., 2003; Trinh et al., 2003). Although the role of a cholinergic deficit in Alzheimer’s disease has been well established, the extent to which cholinergic function is impaired in Alzheimer’s disease, along with the time of onset of this impairment, are still subjects of debate. In vivo measurements of cholinergic receptor expression at different stages of Alzheimer’s disease using PET have shown receptor abnormalities that change with disease progression (Nordberg, 2001). Post-mortem studies have shown decreased levels of molecular markers of cholinergic function in advanced stages of Alzheimer’s disease, but not in mild cognitive impairment, a disease stage preceding the development of Alzheimer’s disease. Instead, such markers may be upregulated in MCI, possibly to compensate for incipient neurofunctional defects (DeKosky et al., 2002). This suggests that cholinergic system function is relatively spared in early stages of Alzheimer’s disease, and is gradually affected in more advanced stages of disease. Despite these findings, however, the question remains whether the alterations observed at the molecular level indeed affect brain function at a system level. Evidence for a difference in cholinergic system function between mild cognitive impairment and Alzheimer patients may be relevant for subsequent dosage and timing of pharmacological treatment (Ackerman et al., 2000; Freo et al., 2002; Thiel, 2003; Goekoop et al., 2004; Saykin et al., 2004).

Pharmacological functional MRI (pharmacological fMRI or phMRI) is a technique that is used to study the impact of pharmacologically induced neurochemical changes on brain function at a system level (Honey and Bullmore, 2004). Since many psychopharmacological substances target specific neurotransmitter systems, phMRI may be used to study neurotransmitter system function in both healthy subjects and patients. Effects of neurotransmitter depletion or overexpression on brain function and behaviour have been studied in healthy controls to model disease mechanisms (e.g. Sperling et al., 2002; Thiel, 2003). Additionally, the therapeutic mechanism of a number of compounds has been studied in patients by examining changes in brain function after pharmacological treatment. Effects of pharmacological substances on brain function have been found to be region-specific, process-specific and even genome-specific, and may vary with age and cognitive capacity (Honey and Bullmore, 2004). Recent results show that phMRI may successfully predict clinical response to pharmacological treatment in patients with major depression, by correlating initial changes in brain function to clinical outcome after treatment (Fu et al., 2004). Based on such findings, it has been suggested that the cortical response to pharmacological challenge is also disease-specific, i.e. reflects the functional status of the neurotransmitter system under investigation (Thiel, 2003; Honey and Bullmore, 2004; Fu et al., 2004; Goekoop et al., 2004; Saykin et al., 2004). We therefore used phMRI to compare changes in brain function as a result of cholinergic stimulation between patients with mild cognitive impairment and Alzheimer’s disease. In two previous phMRI studies, we examined cholinergic system reactivity in both groups separately while varying a number of parameters, including memory tasks and exposure durations (Goekoop et al., 2004, 2005). Short periods of exposure to the cholinesterase inhibitor galantamine affected brain activation during encoding of unfamiliar information in different brain areas in both patient groups, suggesting a differential involvement of the cholinergic system. Treatment effects were very small, however. When compared statistically, no significant differential response to cholinergic challenge was observed between patients with mild cognitive impairment and Alzheimer’s disease (Goekoop et al., submitted for publication).

So far, most phMRI studies of cholinergic stimulation in patients examined brain function during encoding and working memory performance. Since the effects of pharmacological challenge may be process-specific (Honey and Bullmore, 2004), cholinergic stimulation may differentially affect encoding and recognition stages of memory performance. For this reason, we used phMRI to compare effects of cholinergic stimulation on brain function associated with recognition of previously stored information between patients with mild cognitive impairment and Alzheimer’s disease. The current study involved the same design and patient groups as reported in our previous studies (Goekoop et al., 2004, 2005). A face-recognition task was used in which the familiarity of subjects was tested with items that had been presented a few minutes before, during encoding. As a cholinergic stimulator we used galantamine, which is a cholinesterase inhibitor, with an additional sensitizing effect on nicotinic receptors, that has known therapeutic efficacy in Alzheimer patients (Raskind, 2003). Effects were examined after both acute (single dose) and prolonged (5 days) galantamine exposure. We hypothesized that cholinergic challenge would differentially affect brain function in patients with mild cognitive impairment and Alzheimer’s disease during face recognition. If so, this would provide in vivo evidence for a differential involvement of the cholinergic system in different stages of disease, with consequences for brain function at a system level.

Materials and methods

Study design

Patients were screened for participation in a randomized study design with patients serving as their own controls. fMRI was performed at baseline (baseline, no medication), after oral intake of a single dose of galantamine with water (acute) and after prolonged exposure to galantamine (prolonged). Thus, three scanning sessions were performed in each patient, each of which corresponded to a
different medication regime. Scanning sessions for different regimes were exactly 1 week apart and occurred at the same hour of day for each patient. Acute intake involved oral ingestion of 8 mg galantamine with water. Prolonged exposure involved a 120 h period (5 days) of galantamine intake, spread over 6 weekdays, during which period steady state plasma levels were reached, i.e. 4 mg galantamine (first dose, evening of day 1), 4 mg galantamine b.i.d. (mornings and evenings; 4 consecutive days), 4 mg galantamine (final dose, morning of day 6). At this rate, steady state plasma levels are reached within 2–3 days in healthy controls (with minimum serum galantamine concentrations 10.6 ± 4.0 ng/ml and maximum levels 30.7 ± 6.2 ng/ml (Mannens et al., 2002; Zhao et al., 2002). Baseline, acute and prolonged regimes were randomized across scanning sessions to prevent between-session (e.g. learning) effects from interfering with possible effects of medication. To avoid carry-over effects between the regimes, periods of acute and prolonged intake were separated by a washout period of at least 2 days of zero galantamine intake, which is more than six times the half-life of galantamine (7.4 h) (Mannens et al., 2002; Zhao et al., 2002). Scanning sessions were performed 3 h after acute (1 × 8 mg) and 9 h after prolonged (2 doses of 4 mg each day; 5 days) exposure. Dosage and timing of sessions was such that, on average, galantamine plasma levels after acute and prolonged exposure could be considered equal at the time of scanning. This was done in order to facilitate comparisons between treatment effects produced by different exposure durations.

Subject recruitment

The study had approval of the review board of the committee of medical ethics of the VU University Medical Center in Amsterdam, The Netherlands. Thirty (30) elderly patients with mild cognitive impairment, 9 male, 21 female, aged 73.6; ±7.7 (range 54–89 years) were recruited from the Alzheimer Center at the VU Medical Center, Amsterdam, The Netherlands. Patients with mild cognitive impairment were diagnosed using Petersen’s criteria for amnestic mild cognitive impairment, i.e. a slowly progressive memory decline without the involvement of another domain of cognitive function, that did not interfere significantly with activities of daily living (Petersen et al., 2001). For further details, see Goekoop et al. (2004). Additionally, twenty (20) age matched patients with Alzheimer’s disease, 11 male, 9 female, aged 74.5 ± 8.2 (range 55–83 years), were recruited in a similar fashion. Alzheimer patients were diagnosed using the NINCDS-ADRDA criteria for Alzheimer’s disease (McKhann et al., 1984). For further details, see Goekoop et al. (submitted for publication). All patients provided informed consent according to the Declaration of Helsinki under supervision of a lawful caretaker during a screening visit in which the procedure was explained and contraindications were checked. Apart from neuropsychological assessment during clinical investigation, all patients underwent additional Mini-Mental State Examination (MMSE) (Folstein et al., 1975), CDR (Morris, 1997) and NYU-paragraph recall tests, which were used for cognitive profiling. Formal education was determined on a discrete scale with three levels (1 = low, 2 = middle, 3 = high). Patients were excluded if they had any significant medical, neurological or psychiatric illness (other than mild cognitive impairment or Alzheimer’s disease), or if they were taking medication or other substances that are known to influence cerebral function, including antidepressants and cholinesterase inhibitors. Patients were excluded if their history showed excessive nicotine or alcohol intake (>0.5 packs of cigarettes, >4 glasses of an alcoholic substance a day), a severe allergy to pharmacological substances or their constitutive compounds, or the use of any experimental medication within 3 months prior to enrolment in the trial. Exclusion criteria to MRI involved the presence of a pacemaker, metallic implants in high-risk areas (i.e. vessel clips) and a history of claustrophobia.

fMRI

Data acquisition

Imaging was carried out on a 1.5 T Sonata scanner (Siemens, Erlangen, Germany), using a standard circularly polarized head coil with foam padding to restrict head motion. For fMRI, an echo planar imaging sequence was used (echo time 60 ms, flip angle 90°, matrix 64 × 64, field of view 192 × 192 mm), to obtain 21 transverse slices (thickness 5 mm, interslice-gap 1 mm). Task stimuli were projected on a screen located at the head end of the scanner table via an LCD projector located outside the scanner room. Subjects viewed the screen through a mirror located on the head coil. In each hand, subjects held an fMRI compatible response-box through which they were able to react to task stimuli by pressing the left or right button using their index-fingers. A T₁-weighted structural MRI-scan was obtained of each subject (MPRAGE; inversion time: 300 ms, TR = 15 ms; TE = 7 ms; flip angle = 8°; 160 coronal slices, 1 × 1 × 1.5 mm voxels).

Memory-task: face recognition

A face recognition task was administered immediately after a face encoding task. The encoding task involved the presentation of four blocks of unfamiliar faces (24 in total) alternating with blocks of fixation (Goekoop et al., 2004, 2005). During face recognition, 24 faces were presented sequentially in random order on a black background, of which 12 had been shown during encoding and 12 were new. Each face was presented for a duration of 3 s, and was followed by a white fixation-cross presented for 3 s on a black background. Patients were instructed to indicate whether a presented face had been shown previously by pressing one of two buttons. Written instructions ‘Seen previously?’, ‘– Yes’ and ‘– No’ also appeared alongside the pictures. Response types (familiar, unfamiliar, none) and response latencies to individual stimuli were recorded.

Three different but comparable versions of each paradigm were constructed and randomized across the scanning sessions (baseline, acute and prolonged regimes). All paradigms were practised using dummy tasks to ensure that patients mastered the general procedure of task performance before scanning. One day before the start of the first session, a home visit was scheduled during which all memory tasks were practised on a laptop computer. Five minutes before the onset of the first measurements, the face encoding paradigm was practised again, while patients were in the scanner. During the first 10.5 s of each task, patients saw a circle indicating time left before the onset of the first condition. Total time for one scanning session including instructions of memory tasks was ~1 h.

Analysis of behavioural data

Differences between demographic values and neuropsychological test scores were analysed with SPSS 11.5, using chi-square analysis for discrete parameters (gender, education level, CDR scores), and a multivariate analysis of variance (ANOVA) for continuous parameters (age, MMSE, NYU-paragraph scores). Overall accuracy scores and mean reaction times during recognition task performance were calculated for each patient. This was done separately for performance under each scanning session/exposure duration (i.e. baseline, acute, prolonged). Overall accuracy scores were calculated
by subtracting false answers [false rejections (FN) + false recogni-
tions (FP)] from correct answers [correct rejections (TN) + correct
recognitions (TP)], and dividing the result by the total number of
items (24 if no misses). Accuracy scores thus varied from $-1$ (100% in-
correct) to 1 (100% correct), with 0 indicating chance level (50% cor-
correct, 50% incorrect). For analysis of treatment effects, a mixed
effects ANOVA was performed in which accuracy scores and
response latencies were entered as dependent variables, with med-
ication ‘regime’ (3 levels: baseline, acute, prolonged), ‘group’ (2
levels: mild cognitive impairment and Alzheimer’s disease), ‘test
version’ (3 levels: 1, 2, 3) and ‘scan order’ (3 levels: 1, 2, 3) as
fixed factors, and education level (3 levels: low, medium, high) and
gender (2 levels: male and female) as covariates. ‘Scan order’ was
specified as the repeated factor, to account for the effect of taking
repeated measures from the same subject. Given the large number
of possible interactions between these terms, Akaike’s information
criterion (AIC) was used to calculate an optimal model-structure,
which at an AIC value of $-17.0$ was found to contain only two-way
interactions between all terms. Non-significant interactions were
eliminated from the analysis in a stepwise process (two steps).
Effects of the interaction regime x group were then considered
representative of a differential effect of galantamine treatment
on behaviour in both groups ($P < 0.05$, Bonferroni corrected for
multiple comparisons).

Analysis of functional neuroimaging data

Functional datasets were analysed using FSL (Smith et al., 2004).
The first five volumes of each dataset were discarded to account for
T1-saturation effects. At first level (individuals), the following pre-
processing was applied: non-brain removal, slice-timing correction
using Fourier-space time-series phase-shifting, motion correction
and spatial smoothing using a Gaussian kernel of FWHM 8 mm,
mean-based intensity normalization of all volumes by the same factor
and high (0.02 Hz) and low pass temporal filtering (Jenkinson
et al., 2001). Registration of functional neuroimages to high
resolution and/or standard images was carried out using an inter-
modal registration tool based on the correlation ratio (Jenkinson
and Smith, 2001). After pre-processing, the following statistics was
applied on a voxelwise basis on each time series, using local auto-
correlation correction (Woolrich et al., 2001): signal change during
face recognition was modelled in an event-related fashion, using
separate regressors for TP, TN, FP and FN response types (see
above). Type and onset time of the events were determined by
post hoc sorting, based on the responses given by the individual
subjects. Signal variance during fixation (X condition) was not mod-
eled, to prevent overspecification of the model. Thus, a unique
model of signal response was obtained for each individual patient,
containing a single regressor for each response type and their tem-
poral derivative, which was convolved with a gamma function to
model the haemodynamic response. Model fitting generated whole
brain native space images of parameter estimates for each condition,
representing average signal change during face recognition versus
fixation (X), along with corresponding variance images. To reduce
the size of the analysis, only effects on brain activation during correct
responses (i.e. true positive and true negative responses) were further
considered. Thus, the following lower-level contrasts were generated:
TP (versus X), TN (versus X), and TP < TN. TP and TN versus low-
level fixation (X) contrasts examine general aspects of recognition
memory performance, which are biased with respect to successful
retrieval processes (TP > X) and encoding processes (TN > X),
respectively (Buckner et al., 2001). TP > TN contrasts specifically
examine brain areas where signal intensity during successful retrieval
of familiar information was significantly stronger than signal intensi-
during successful rejection/encoding of new information. Areas of
significant signal differences may therefore represent areas associated
with ‘successful retrieval’. Conversely, the reverse contrast TN > TP
examined brain function related to ‘encoding during attempted
retrieval’. Brain areas that show significant effects for these contrasts
may differ from those involved in either TP or TN (versus X)
decisions, thus providing additional information concerning specific
subcomponent processes during retrieval (Daselaar et al., 2003). The
corresponding functional images were resampled to a size of 2 × 2 × 2 mm
in standard space and fed into a group-level statistical analysis to
examine activation patterns for differences between treatment
durations (baseline, acute and prolonged exposure).

At group level, average activation maps (‘main effects’) were
computed for all lower-level contrasts (see above). This was done
for all regime types in both patient groups in a mixed effects higher
level analysis (Woolrich et al., 2004), using clusters determined by
$Z > 2.3$ and a corrected cluster significance threshold of $P = 0.05$
(Worsley et al., 1992; Friston et al., 1994; Forman et al., 1995).
Effects at baseline were then tested for significant changes after
acute or prolonged exposure (‘treatment effects’). Treatment
effects were calculated for each group separately, and for their between-
group comparison. This was performed in a single group-level
analysis using a mixed effects ‘Triple T-test’ model (http://www.fmr.
ibox.ac.uk/fsl/feat5/index.html), which examined lower-level
contrast maps of both groups for effects of treatment, scanning
order, test version, and the act of taking repeated measurements
from single subjects (Woolrich et al., 2004; Goekoop et al., 2004,
2005). Separate variances were assumed for both patient groups.
Treatment effects were examined at a voxel threshold for significant
brain activation determined by $Z > 3.1$ (i.e. $P = 0.001$), and a
minimal cluster size of 160 mm$^3$. An F-test was used to test for
any effect of cholinergic challenge (either acute or prolonged). If
present, specific contributions of acute and prolonged exposure
were analysed using pairwise comparisons (T-tests). All group anal-
yses were performed in a common reference space (standard space)
(Talairach and Tournoux, 1988). For display purposes, treatment
effects pertaining to a specific group were rendered on mean anatom-
ical brain volumes corresponding to that group in standard space.
Results of comparisons of treatment effects between groups were
rendered on a mean anatomical brain volume of all patients in
standard space.

Results

Demographics and results of
cognitive profiling

Table 1 shows the results of demographic and cognitive pro-
file in mild cognitive impairment and Alzheimer’s disease
patient groups. Where possible, statistics are given to indicate
the significance of a difference between the groups.

Patient compliance and discontinuation

Data from 28 patients with mild cognitive impairment (25
complete datasets including scans at baseline, and after acute
and prolonged exposure, and 3 incomplete datasets) and 18
Alzheimer patients (17 complete datasets, 1 incomplete
dataset) was used in the current study (see also Table 1).
Table 1 Demographics and results of cognitive profiling of mild cognitive impairment (MCI) and Alzheimer (AD) patients participating in this study

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Measure</th>
<th>MCI</th>
<th>AD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean</td>
<td>73.8</td>
<td>74.5</td>
<td>[F(1,44) = 0.181, P = 0.67]</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>7.7</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>8</td>
<td>11</td>
<td>(χ² = 13.2, df = 1, P = 0.0003)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>20</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td>Low</td>
<td>3</td>
<td>7</td>
<td>(χ² = 15.3, df = 2, P = 0.0005)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Handedness</td>
<td>Left</td>
<td>3</td>
<td>1</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>25</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>Yes</td>
<td>3</td>
<td>1</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>25</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Neuropsychosocial examination (NPE)</td>
<td>MMSE Mean</td>
<td>27</td>
<td>22.5</td>
<td>[F(1,44) = 70.4, P = 0.0001]</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.2</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CDR Mean</td>
<td>0.5</td>
<td>1.6</td>
<td>(χ² = 46.0, df = 2, P = 0.0001)</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>None</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NYU (delayed) Mean</td>
<td>3.2</td>
<td>0</td>
<td>[F(1,44) = 16.3, 16.3, P = 0.0002]</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.9</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

MMSE, mini-mental state examination; CDR, clinical dementia rating scale; NYU (delayed), New York University paragraph (delayed) recall test; P-values, Chi-square or F-values and df are supplied to indicate the significance of the difference between both patient groups. NP, not provided, given small number of cases.

Compliance was good, as assessed by pill-counts and the caretaker’s comments. For further details, see Goekoop et al. (2004, 2005).

**Task performance**

Table 2 lists numbers and percentages of response types (i.e. TP, TN, FP and FN responses) for mild cognitive impairment and Alzheimer groups, specified by regime type. Table 3 shows means and standard errors of overall accuracy and latency scores during face recognition for each patient group and regime type separately. Information is provided regarding the significance of (differential) effects of galantamine challenge in both groups.

Overall recognition accuracy scores were above chance levels in both patient groups (Table 3). Accuracy scores in the Alzheimer group were significantly lower than those in the mild cognitive impairment group [F(1,46.8) = 31.4, P = 0.0001]. When both groups were pooled, recognition accuracy was 0.30 (SE 0.35) at baseline, 0.40 (SE 0.037) after acute intake, and 0.36 (SE 0.035) after prolonged exposure. A trend was found for increased recognition accuracy scores after galantamine treatment [F(2,78.4) = 2.6, P = 0.08]. This effect was largely due to an effect of acute galantamine intake (SE 0.042, P = 0.080) (Table 3). No significant effects of galantamine challenge were found on recognition accuracy in each of the groups separately (Table 3) (Goekoop et al., 2004, 2005). No significant effect was observed for the interaction regime × group, indicating that galantamine challenge did not differentially affect recognition accuracy between patient groups [F(2,83.7) = 2.0, P = 0.14] (Table 3).

Latency scores did not differ significantly between mild cognitive impairment and Alzheimer patients [F(1,45.9) = 0.123, P = 0.72]. When both groups were pooled, response latency was 2.28 s (SE 0.068 s) at baseline, 2.25 s (SE 0.070 s) after acute intake, and 2.31 s (SE 0.069 s) after prolonged exposure. No effect of galantamine intake was found on response latency when groups were pooled [F(2,70.4) = 0.46, P = 0.61], or in both groups separately (Table 3) (Goekoop et al., 2004, 2005). No significant effect was observed for the interaction regime × group, indicating that galantamine challenge did not differentially affect response latency in both patient groups [F(2,75.5) = 0.37, P = 0.69] (Table 3).

**fMRI analyses**

**Main effects: mild cognitive impairment and Alzheimer patients**

Table 1 lists numbers and percentages of correct and incorrect responses in both patient groups (Table 1). Mild cognitive impairment and Alzheimer patients activated roughly the same structures during recognition task performance. At baseline (no treatment), main effects during correct (TP and TN) responses in both groups involved activation of ventral and dorsal occipital (visual) areas, bilateral inferior parietal, (para)hippocampal, superior temporal and prefrontal areas and the lateral sulci (Fig. 1A, B, E and F). Activation patterns of TP and TN decisions differed significantly in both groups (Fig. 1C and G). A baseline TP > TN contrast averaged over all subjects showed activation bilaterally in primary visual cortex, anterior and posterior cingulate cortex,
inferior parietal lobes and anterior temporal lobe, whereas unilateral activation was observed in right motor cortex, right basal ganglia, left cerebellum, and left inferior, middle and superior frontal cortices. The lateralization of activation in right motor-related areas likely represents increased motor activity of the left hand and fingers during TP decisions when compared to TN decisions. In contrast, only left motor cortex showed increased activation in group-level TN > TP contrasts (Fig. 1D and H).

**Treatment effects: mild cognitive impairment**

When compared with baseline, acute exposure to galantamine increased brain activation in a large number of

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**Table 3** Results of statistical analysis of task performance data (recognition accuracy and response latency) specified by patient group and regime type

<table>
<thead>
<tr>
<th>Group</th>
<th>Regime</th>
<th>Nr sessions</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Total responses</th>
<th>Total forgot</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCI</td>
<td>Baseline</td>
<td>28</td>
<td>221</td>
<td>233</td>
<td>97</td>
<td>112</td>
<td>663</td>
<td>9</td>
<td>672</td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>26</td>
<td>218</td>
<td>251</td>
<td>59</td>
<td>94</td>
<td>622</td>
<td>2</td>
<td>624</td>
</tr>
<tr>
<td></td>
<td>Prolonged</td>
<td>27</td>
<td>226</td>
<td>239</td>
<td>71</td>
<td>87</td>
<td>623</td>
<td>25</td>
<td>648</td>
</tr>
<tr>
<td>AD</td>
<td>Baseline</td>
<td>18</td>
<td>98</td>
<td>142</td>
<td>66</td>
<td>108</td>
<td>414</td>
<td>18</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>18</td>
<td>118</td>
<td>135</td>
<td>79</td>
<td>95</td>
<td>427</td>
<td>5</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>Prolonged</td>
<td>18</td>
<td>115</td>
<td>150</td>
<td>55</td>
<td>92</td>
<td>412</td>
<td>20</td>
<td>432</td>
</tr>
</tbody>
</table>

Results are shown for each group, regime type and response type separately. MCI, mild cognitive impairment; AD, Alzheimer’s disease; Nr sessions, total number of scanning sessions performed to obtain the reported number of response types; TP, correct hits (true positives); TN, correct rejections (true negatives); FP, false hits (false positives); FN, false rejections (false negatives); Total responses, total number of responses given (i.e. TP + TN + FP + FN); Total forgot, total number of cases in which presentation of a face was not followed by a key-press; Total, total number of presented items. Percentages of individual response types (%TP, %TN, %FP, %FN) are calculated with respect to the total number of responses given (% Total responses). Percentages of forgotten items (% forgot) are calculated with respect to the total number of presented items (% total).

---

**Table 2** Numbers and percentages of hits and misses during face recognition

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<th>Group</th>
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<th>Nr sessions</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Total responses</th>
<th>Total forgot</th>
<th>Total</th>
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<td>15.1</td>
<td>100 (622 responses)</td>
<td>0.3</td>
<td>100 (624 responses)</td>
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<td>38.4</td>
<td>11.4</td>
<td>14.0</td>
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<td>26.1</td>
<td>100 (414 responses)</td>
<td>4.2</td>
<td>100 (432 responses)</td>
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<td>22.3</td>
<td>100 (412 responses)</td>
<td>4.6</td>
<td>100 (432 responses)</td>
</tr>
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</table>

Results are shown for each group, regime type and response type separately. MCI, mild cognitive impairment; AD, Alzheimer’s disease; Nr sessions, total number of scanning sessions performed to obtain the reported number of response types; TP, correct hits (true positives); TN, correct rejections (true negatives); FP, false hits (false positives); FN, false rejections (false negatives); Total responses, total number of responses given (i.e. TP + TN + FP + FN); Total forgot, total number of cases in which presentation of a face was not followed by a key-press; Total, total number of presented items. Percentages of individual response types (%TP, %TN, %FP, %FN) are calculated with respect to the total number of responses given (% Total responses). Percentages of forgotten items (% forgot) are calculated with respect to the total number of presented items (% total).
structures, including (left) posterior cingulate cortex, left anterior temporal lobe, left superior parietal lobe, left superior temporal cortex, right frontal lobe and cerebellum. These effects were mainly due to activation changes during true positive (TP) decisions (Fig. 2A and B; Table 4), ($Z > 3.1$). No significant decreases were observed after acute galantamine intake. Prolonged exposure produced no significant increases in brain activation ($Z > 3.1$; data not shown). Instead, decreases in brain activation were found in (left) posterior cingulate, right middle frontal and bilateral superior prefrontal cortex. Again, these effects were mainly due to activation changes during true positive (TP) decisions (Fig. 2C and D; Table 4), ($Z > 3.1$). When acute and prolonged effects of galantamine intake were compared (acute <> prolonged), the differences were significant ($Z > 3.1$). Increases after acute intake and decreases in similar areas after prolonged exposure summed during these comparisons in areas of significant overlap (i.e. posterior cingulate cortex), producing stronger effects (data not shown). Significant treatment effects were found on a contrast describing TP > TN (but not TN > TP) activation differences. Acute galantamine intake produced strong increases in right visual cortex, left parahippocampal cortex, right inferior prefrontal and medial prefrontal cortex (Fig. 2E, Table 4) ($Z > 3.1$). Prolonged intake of galantamine decreased brain activation in right caudate nucleus and left putamen (Fig. 2F, Table 4). Differences between acute and prolonged exposure were significant ($Z > 3.1$; data not shown).

Fig. 1 Axial slices showing main effects during face recognition task performance of mild cognitive impairment (MCI) and Alzheimer’s disease (AD) patient groups, for which effects of galantamine treatment were examined. Effects are rendered on their respective mean anatomical brain volumes (average T1-weighted brains of MCI or AD patients: Mean_anat_MCI, Mean_anat_AD). Left in the image is left in the brain. Effects are cluster corrected using $Z = 2.3$ and $P < 0.05$. Colour scale extends from $Z = 2.3$ (orange) to $Z = 10.5$ (yellow). (A) MCI; true positive items (TP); (B) MCI; true negative items (TN); (C) MCI; true positive > true negative items (TP > TN); (D) MCI; true negative items > true positive items (TN > TP). See also Materials and methods. (E–H) Same contrasts, involving AD patients. See text for further details.
Treatment effects: Alzheimer patients

When compared with baseline, acute exposure to galantamine increased brain activation in a limited number of structures, including the vermis of the cerebellum, right inferior temporal gyrus and the parahippocampal areas (bilaterally). Effects of response type were substantial. When TN items were considered separately, additional treatment effects were found in the body of the left hippocampus (Fig. 3A and B; Table 5) ($Z > 3.1$). No significant decreases in brain activation were observed after acute galantamine intake. Prolonged exposure produced no significant increases in activation ($Z > 3.1$; data not shown). Instead, a substantial decrease in brain activation was found in right (para)hippocampal cortex. Again, these effects were mainly due to the activation changes during true negative (TN) decisions (Fig. 3C and D; Table 5) ($Z > 3.1$). When acute and prolonged effects of galantamine intake were compared, the differences were significant ($Z > 3.1$). Increases after acute intake and decreases in similar areas after prolonged exposure showed a significant overlap in right (para)hippocampal cortex, producing stronger effects when compared (data not shown). No significant treatment effects were found on contrasts describing TP $<>$ TN activation differences.

Treatment effects: mild cognitive impairment versus Alzheimer patients

Visual inspection of treatment effects in both patient groups showed both differences and similarities in reactivity to galantamine challenge (Figs 2 and 3). In both groups, maximum reactivity to galantamine challenge occurred after acute exposure. Effects of acute exposure involved increases in activation only. Additionally, both groups showed decreases in brain activation after prolonged exposure, which occurred in brain areas similar to those showing increases in activation after acute exposure. Effects of prolonged exposure were of smaller magnitude than effects of acute exposure. Both groups differed in the spatial extent, amplitude and location of treatment effects. The mild cognitive impairment group showed a larger number of activation changes than the Alzheimer group, which were spread across a larger number of brain structures. Activation changes in the mild cognitive impairment group involved cortical (posterior cingulate, prefrontal, lateral temporal) and subcortical areas, but not the hippocampal areas. In contrast, activation changes in the Alzheimer group mainly involved hippocampal areas.

A direct statistical comparison between treatment effects of mild cognitive impairment and Alzheimer patients showed...
Table 4 Volume, Z-scores and coordinates of peak voxels of local maxima for effects of galantamine challenge on brain activation patterns of patients with mild cognitive impairment during face recognition

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<th>Nr. Vox</th>
<th>Z-score</th>
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<th>y</th>
<th>z</th>
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<td>3.82</td>
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<td>–38</td>
<td>–14</td>
<td>Left</td>
<td>Parahippocampal gyrus</td>
</tr>
</tbody>
</table>

Nr. Vox, total number of voxels (volume) of local maximum (voxel size 2 × 2 × 2 mm; effects at Z = 3.1). Z, Z-score of peak voxel. x, y, z, coordinates of peak voxel; Left/Right, left or right hemisphere. TP items, acute intake (increases). Only effects with volumes >400 mm³ (50 voxels) are listed. TN items, acute intake (increases). TP items, prolonged intake (decreases). TN items, prolonged intake (decreases). TP > TN items, acute intake (increases). TP > TN items, prolonged intake (decreases). See text and Fig. 2 for further details.
significant differences in treatment response between both groups. With respect to acute effects of galantamine intake, the mild cognitive impairment group produced significantly more activation changes than Alzheimer patients in a number of areas, including the left posterior parietal cortex (Fig. 4A; Table 6) ($Z > 3.1$). These effects were found for TP decisions only. Conversely, enhancement of hippocampal activation during TN decisions was significantly stronger in the Alzheimer group than in the mild cognitive impairment group (Fig. 4B; Table 6) ($Z > 3.1$). No significant
between-group differences were found in the magnitude of treatment effects described by TP <> TN activation differences. Additionally, no differences were found with respect to effects of prolonged galantamine intake ($Z > 3.1$). Thus, significant differences were found in the reactivity of patients with mild cognitive impairment and patients with Alzheimer’s disease to cholinergic challenge. Plots of per cent signal change (relative to global mean signal intensity) in peak voxels of local maxima of treatment effects illustrate the significance of the differences in intensity changes between both groups (Fig. 4).

**Discussion**

The current study examined patients with mild cognitive impairment and Alzheimer’s disease for a differential response to cholinergic stimulation with the cholinesterase inhibitor galantamine by using phMRI with a face recognition task. Differences were found in the reactivity of patients with mild cognitive impairment and patients with Alzheimer’s disease to cholinergic challenge. Plots of per cent signal change (relative to global mean signal intensity) in peak voxels of local maxima of treatment effects illustrate the significance of the differences in intensity changes between both groups (Fig. 4).
task. To our knowledge, this is the first event-related phMRI study to directly compare the effects of pharmacological intervention on brain activation during delayed recognition between patient groups. In two previous phMRI studies, we examined effects of cholinergic challenge in mild cognitive impairment and Alzheimer patients separately, while they engaged in encoding and working memory tasks (Goekoop et al., 2004, 2005). The reactivity of mild cognitive impairment and Alzheimer patients to acute and prolonged galantamine exposures was examined using identical procedures as described in the current study. Overall, treatment effects were small in both spatial extent and magnitude. Patients with mild cognitive impairment responded to prolonged galantamine exposure only (both tasks), whereas Alzheimer patients responded to acute galantamine challenge only (encoding only). Such differences in reactivity to cholinergic stimulation may be due to a number of confounding factors (Goekoop et al., submitted for publication), including a preference of galantamine for certain mental processes, and a difference in the functional status of the cholinergic system between these groups (i.e. process-specificity and disease-specificity of galantamine treatment; see below). When treatment effects during encoding were compared statistically between both groups, however, no significant differential response to cholinergic challenge was observed between mild cognitive impairment and Alzheimer patients (Goekoop et al., submitted for publication).

Process-specificity

The current study extended our previous research by reporting effects of cholinergic challenge in the same patient groups during face recognition. Although identical procedures were followed, a larger number of stronger effects was found in both groups than observed for face encoding and working memory performance, supporting previous findings that effects of cholinergic enhancement are process-specific (Thiel, 2003; Honey and Bullmore, 2004). However, direct comparisons between treatment effects across different memory tasks were not made, since these tasks were too dissimilar (i.e. block versus event-related designs, lexical versus visuospatial processing) to allow meaningful comparisons of cholinergic reactivity across different memory domains. We therefore report our findings of the effects of cholinergic challenge separately for all memory tasks. Nevertheless, we were able to examine the specificity of galantamine challenge with respect to encoding and retrieval processes by studying brain function during retrieval alone. Since our face recognition task contained both novel and familiar items, both encoding and retrieval processes occurred during task performance (Buckner et al., 2001). By examining brain function during correct hits (TP items) and correct rejections (TN items), brain regions that are involved in (successful) retrieval and encoding during attempted retrieval may be studied separately (see Materials and methods). Although encoding processes during attempted retrieval may differ slightly from encoding during attempted encoding (Rombouts et al., 2001; Rugg et al., 2002), this methodology allows examination of process-specificity of pharmacological compounds with respect to encoding and retrieval processes within the same scanning session, which avoids some of the potential confounds that may be introduced by across-task or across-session comparisons of treatment effects (e.g. task design and relative timing of scanning sessions). If confirmed, a preferential targeting of memory retrieval processes rather than memory encoding by galantamine may have some clinical significance. For instance, the maximum benefit of cholinergic therapy with galantamine may be limited by existing pathology affecting the initial encoding of information. Future studies of drug design and development may therefore benefit from information provided by phMRI studies, by studying effects of pharmacological treatment in relation to specific neural processes. Such studies may help to increase efficacy and reduce side effects of novel psychopharmacological compounds.

Mild cognitive impairment

In patients with mild cognitive impairment, acute galantamine challenge enhanced brain activation in posterior cingulate cortex, anterior temporal lobe, lateral temporal, parietal and prefrontal areas, basal ganglia and medial septal areas (Fig. 2, Table 3), which are all known to depend on cholinergic innervation (Selden et al., 1998). Effects were stronger during familiar (TP) than for unfamiliar (TN) decisions, suggesting a preferential effect on brain activation during retrieval of familiar information. This was confirmed by subsequent analyses examining effects of galantamine challenge on brain activation during TP > TN decisions. Effects were found for TP > TN decisions in medial and right prefrontal cortex, visual cortex and left parahippocampal area, which are part of a well-described encoding-retrieval network (Simons and Spiers, 2003). Galantamine intake may therefore have affected successful retrieval in these patients. No effects were found for TN > TP decisions, suggesting that galantamine challenge in mild cognitive impairment did not specifically affect the encoding of new pictures during attempted retrieval.

Posterior cingulate areas showed widespread increases in activation for TP items (Fig. 2A). In healthy controls, posterior cingulate cortex has been implicated in visuospatial attention (Small et al., 2003) and episodic memory performance (Cabeza and Nyberg, 2000), whereas anterior and posterior temporal multimodal association cortex and inferior parietal cortex may play important roles in episodic memory performance (Frankland and Bontempi, 2005). Hypofunction of posterior cingulate cortex has been consistently shown to represent the first neurofunctional alterations in early Alzheimer’s disease (see below). Thus, enhanced brain activation in these areas by galantamine intake may signify an enhancement of visuospatial attention and episodic memory. However, the observed effects of treatment may also reflect more complex alterations in memory-related processes (see below).
Alzheimer’s disease

In Alzheimer patients, acute galantamine intake increased brain activation mainly in (para)hippocampal areas (bilaterally), visual cortex, left fusiform gyrus and cerebellar vermis (Fig. 3, Table 5). Effects were dependent on response type, since most significant effects of galantamine intake occurred during TN decisions (Fig. 3B). Although no significant effects were found on brain activation reflected by TN > TP comparisons (which may be more specific to encoding processes than TN > X comparisons), this suggests a preferential effect of galantamine intake on brain activation involving encoding during attempted retrieval. Cholinergic modulation of brain activation in visual areas and fusiform cortex has been observed previously during face encoding tasks and may involve alterations in neural processing requirements that are specific to perception and processing of facial stimuli (Sperling et al., 2001; Rombouts et al., 2002). The increase in hippocampal activation after galantamine intake in these patients is remarkable, since hippocampal atrophy is considered the primary deficit in Alzheimer’s disease, which is mainly responsible for the observed symptoms of memory impairment (Braak et al., 1999). Previous fMRI studies have found decreased hippocampal activation in Alzheimer patients when compared with healthy subjects (Rombouts et al., 2000; Remy et al., 2005). To our knowledge, this is the first pHMRI study to demonstrate an enhancement of hippocampal function in Alzheimer patients after cholinergic stimulation. Such signal changes may be related to the extent of clinical improvement after cholinergic therapy. Future clinical studies may therefore require to focus on changes in hippocampal function after cholinergic challenge during recognition memory performance, in order to predict treatment response and long-term clinical outcome in Alzheimer patients.

Region-, process- and disease-specificity

Convergent evidence from functional studies including PET, SPECT and fMRI shows that resting state hypometabolism or hypofunction in limbic areas is central to pathology in both very early Alzheimer’s disease (i.e. mild cognitive impairment) and mild-to-moderate Alzheimer’s disease (Matsuda, 2001; Nestor et al., 2003; Greicius et al., 2004; Rombouts et al., 2005). In the mild cognitive impairment stage, mainly posterior cingulate structures are affected, although sensitive region-of-interest techniques have detected hypometabolism in thalamus and hippocampus as well (Nestor et al., 2003). In more advanced stages of Alzheimer’s disease, the same limbic network is affected more extensively, and may show additional hypometabolism in hippocampal, temporoparietal and frontal association cortices (Nestor et al., 2003). Although the earliest structural changes appear in the medial temporal lobe [entorhinal cortex and hippocampus (Braak et al., 1999)], functional changes first appear in posterior cingulate areas (Chetelat et al., 2003). This has led to the hypothesis that functional changes in posterior cingulate areas represent secondary effects of structural changes in hippocampal areas (Matsuda, 2001). Indeed, hippocampal and posterior cingulate areas are strongly connected through limbic structures such as the thalamus and mammillary bodies (Nestor et al., 2003). In a recent study in patients with mild cognitive impairment, posterior cingulate hypometabolism was related specifically to (deficits in) retrieval memory performance, whereas hippocampal hypometabolism was related to (deficits in) memory encoding (Chetelat et al., 2003). Hypometabolism in Alzheimer’s disease therefore seems to be region-specific (posterior cingulate versus hippocampus), disease-stage specific (mild cognitive impairment versus Alzheimer’s disease) and process-specific (recognition versus encoding). These findings show a strong similarity with the results from the current challenge study, which show enhancement of posterior cingulate activation in mild cognitive impairment related to retrieval and increases in hippocampal activation in Alzheimer’s disease that are likely to be related to encoding (see above). Although a definite link between resting state limbic hypofunction and the reactivity of posterior cingulate and hippocampal structures to cholinergic challenge could not be made in the current (task-related) study, our results show that a cholinergic factor may be relevant to hypofunction in posterior cingulate and hippocampal areas as observed in early Alzheimer’s disease.

The nature of this possible cholinergic influence and its time of onset remain unclear from the present data. Hypometabolism in posterior cingulate (mild cognitive impairment) and hippocampal areas (Alzheimer’s disease) may simply reflect a hypofunction of the cholinergic system, which would argue for an early involvement of cholinergic system dysfunction in Alzheimer’s disease. According to this view, the selective enhancement of hippocampal and posterior cingulate areas after galantamine challenge may reflect enhanced sensitivity of these structures to cholinergic stimulation as a result of cholinergic denervation. Both the hippocampus proper and the posterior cingulate gyrus are densely innervated by cholinergic fibres originating from separate branches (Ch2 and Ch4, respectively) of the basal forebrain cholinergic system (Selden et al., 1998). Selective denervation of (cholinergic) hippocampal afferents, as observed in Alzheimer’s disease, may alter both nicotinic and muscarinic receptor expression (Nordberg, 2001), producing increased receptor sensitization (Erb et al., 2001; Svedberg et al., 2002) and an increased response of brain structures to cholinergic challenge (Goekoop et al., submitted for publication).

However, our current results also fit a different scenario, where posterior cingulate cortex hypometabolism and recognition deficits (as a result of early medial temporal atrophy) are kept partially in check by an intact cholinergic system. Hippocampal hypometabolism and encoding deficits (as observed in later stages of Alzheimer’s disease) would then reflect a definite failure of cholinergic system compensation. According to this view, increased activation in posterior cingulate and hippocampal structures after cholinergic stimulation may reflect a partial remission from a hypofunctional...
state of these structures, and cholinergic therapy may simply be an add-on to natural cholinergic system compensation. It is likely that a combination of both scenarios, i.e. receptor hypersensitivity and partial compensation, may best explain the observed results. Future studies may require to combine molecular imaging techniques (e.g. PET) and phMRI in order to relate cholinergic receptor status to signal changes in specific brain structures and corresponding clinical phenotypes.

**Mild cognitive impairment and Alzheimer's disease: similarities in cholinergic system reactivity**

When signal changes in mild cognitive impairment and Alzheimer patients were visually compared, both groups showed signal increases after acute intake of galantamine and signal decreases after prolonged exposure (Fig. 4, Table 6). In some cases, increases and decreases in brain activation as observed for different exposure durations involved similar brain areas (Fig. 2A, C; 2B, D; Fig. 3A, C; 3B, D). The reason for this signal behaviour is unclear, but may involve effects of nicotinic and muscarinic receptor sensitization after galantamine treatment. A strong response to acute galantamine challenge may reflect (nicotinic) receptor sensitization under normal or hypocholinergic circumstances, as it may occur in mild cognitive impairment or Alzheimer’s disease. In contrast, decreased levels of brain activation when compared with baseline may represent (muscarinic) receptor desensitization due to prolonged exposure to galantamine (Volkow et al., 2001; Quick and Lester, 2002). Similar effects of receptor (de)sensitization have been suggested to underlie differential responses in smokers versus non-smokers (Ernst et al., 2001) and the modest effects of long-term treatment with cholinesterase inhibitors (Geerts et al., 2002). This mechanism seems to fit most of our results, including our previous findings in Alzheimer patients during face encoding, where effects were observed after acute intake, but not after prolonged intake (Goekoop et al., submitted for publication). However, it does not fit our previous findings in patients with mild cognitive impairment during encoding, where effects were only observed after prolonged exposure (Goekoop et al., 2004). The origin of this ‘signal deviance’ in mild cognitive impairment remains unknown, but can be explained by the actions of several factors. Patients with mild cognitive impairment responded selectively to prolonged exposure (encoding) and to acute galantamine challenge (recognition). A difference in plasma concentrations between acute and prolonged regime types is therefore unlikely to fully explain the observed differences (see also Materials and methods), since such a factor is likely to exert a comparable influence on brain function during encoding and recognition. Since patients with mild cognitive impairment responded both to prolonged and acute exposures to galantamine challenge, a disease-specific factor (e.g. the absence of cholinergic receptor sensitization in mild cognitive impairment) is not likely to give a complete account of these signal changes. Since data-analyses were identical for both patient groups, a combination of disease-specific (MCI versus Alzheimer’s disease) and process-specific (encoding versus retrieval) factors seems to offer the best explanation for signal reactivity in the mild cognitive impairment group with respect to different exposure durations.

Other studies have shown increases in brain activation after weeks of treatment with different memory tasks and cholinomimetic substances in healthy controls or patients (Rombouts et al., 2002; Parry et al., 2003; Thiel, 2003; Bentley et al., 2004; Saykin et al., 2004). Since effects of cholinergic therapy may take 6–12 weeks to reach their maximum (Scarpini et al., 2003), short-term effects of cholinergic treatment may represent a transitory state between immediate effects and therapeutic effects of a pharmacological substance, be process-specific, compound-specific, disease-specific, or may involve some other phenomenon. Future phMRI studies may require to investigate more memory tasks, pharmacological substances and timepoints after treatment in order to solve these issues.

**Mild cognitive impairment and Alzheimer’s disease: differences in cholinergic system reactivity**

When compared statistically, patients with mild cognitive impairment and Alzheimer’s disease differed significantly with respect to their reactivity to cholinergic challenge (Fig. 4, Table 6). Left hippocampal activation was significantly more enhanced in Alzheimer patients than in patients with mild cognitive impairment (Fig. 4B, Table 6), whereas left anterior and posterior temporal activation was more enhanced in patients with mild cognitive impairment than in Alzheimer patients (Fig. 4A, Table 6). Plots of per cent signal change showed unique profiles of cholinergic reactivity in both patient groups for a number of brain areas (Fig. 4). Overall, these comparisons show that mild cognitive impairment and mild-to-moderate Alzheimer patients respond uniquely to cholinergic challenge, suggesting that cholinergic system function is differentially affected in earlier and later stages of Alzheimer’s disease. This is in line with previous findings from post-mortem studies, showing characteristic cholinergic changes in mild cognitive impairment and Alzheimer patients at the molecular level (DeKosky et al., 2002). The current study shows that such neurochemical alterations may eventually affect brain function in living subjects. No significant difference was found in posterior cingulate reactivity to cholinergic challenge between mild cognitive impairment and Alzheimer patients. This may reflect a lack of power, but may also point to comparable reactivity between both patient groups (i.e. because of smaller group size, posterior cingulate reactivity to galantamine challenge may have remained in a subthreshold level in Alzheimer patients). If a cholinergic component is indeed relevant to hippocampal and posterior cingulate hypofunction, comparable reactivity of posterior cingulate structures to cholinergic challenge
would fit previous findings of comparable rates of hypometabolism in posterior cingulate structures in mild cognitive impairment and Alzheimer patients (Nestor et al., 2003). In contrast, the differential response in hippocampal areas may reflect more severe cholinergic system impairment in later stages of Alzheimer’s disease, which is not present in patients with mild cognitive impairment. Since no differential response was found between mild cognitive impairment and Alzheimer patients on measures of task performance (Table 3), the relationship of the observed effects of treatment with changes at a behavioural level are unclear from the current study. This may reflect small sample size and short exposure durations rather than a true absence of such effects, since galantamine treatment has a well-documented effect on memory retrieval (Raskind and Truyen, 2002). Additionally, a trend was found for significant increases in recognition accuracy of galantamine intake across subjects ($P = 0.08$), suggesting that galantamine intake did have an effect on recognition accuracy in the current study.

**Cholinergic system reactivity: possible clinical relevance**

A differential response to cholinergic challenge in patients with mild cognitive impairment and Alzheimer’s disease may be clinically relevant. Previous phMRI studies have shown that both brain activation at baseline and initial effects of pharmacological treatment may successfully predict long-term treatment response and clinical outcome in patients with major depression (Davidson et al., 2003; Fu et al., 2004). Similarly, differences in cholinergic system reactivity as observed in the current study may to some degree reflect the clinical status of patients with memory complaints (Goekoop et al., 2004). Interestingly, recent results from clinical trials show that mild cognitive impairment and Alzheimer patients respond differentially to long-term treatment with cholinesterase inhibitors. Treatment with a number of different cholinergic agonists improves memory performance in Alzheimer patients (Lancot et al., 2003), but seems to have only limited effects in patients with mild cognitive impairment (Ihl, 2003; Salloway et al., 2004). Such differences in treatment response may reflect underlying differences in the functional status of the cholinergic system as suggested by the current study. Follow-up of mild cognitive impairment and Alzheimer patients should provide a measure of disease progression, which may be correlated with the observed effects of galantamine challenge on brain function in order to examine the predictive value of these effects in terms of disease outcome and response to treatment. phMRI may prove to be a valuable tool in clinical studies, since it offers non-invasive assessment of neurotransmitter deficits, and provides additional information concerning effects at system level that may not be found with any other technique (Sarter et al., 1996). However, the eventual clinical significance of phMRI studies depends strongly on the outcome of within-subject test–retest reliability studies (Saykin et al., 2004). Although treatment strategies can be reconsidered based on results produced at group level, much will depend on the ability of clinicians to demonstrate incipient neurotransmitter system dysfunction in individual patients.

**General considerations**

With respect to underlying neural processes, effects of pharmacological challenge as measured by fMRI should be interpreted with some caution. fMRI examines changes in blood oxygenation level dependent (BOLD) signal intensity, which is an indirect measure of neural activity (Matthews and Jezzard, 2004). BOLD signal changes represent changes in blood flow, volume and oxygenation as a result of metabolic changes, which are secondary to changes in neural activity. Such effects may reflect changes in both neural excitation and inhibition (Matthews and Jezzard, 2004). Future studies may therefore require to combine electrophysiological techniques (such as EEG) with phMRI in order to assess the nature of neural changes underlying the BOLD response. An important additional consideration for all phMRI studies is that pharmacological intervention, including cholinergic stimulation (Tsukada et al., 2000), may affect vascular tissue as well as neural tissue (Honey and Bullmore, 2004). However, such objections are likely to be less of an obstacle to clinical studies than to fundamental studies of cholinergic system function, since reactivity of both vascular and neural tissues to cholinergic challenge may contain information regarding the functional status of the cholinergic system in disease.

The current study had some limitations. Differences in cholinergic system function between patients with mild cognitive impairment and Alzheimer’s disease were examined in a cross-sectional manner. Although time-consuming, future phMRI studies may require to examine the development of a cholinergic deficit in time by using longitudinal study designs. Comparisons between patient groups in the current study may have suffered from bias introduced by group differences in sex and education levels (Table 1). Although little is known about the effects of gender on brain function, recent studies show that such effects may be significant (Lee et al., 2002; Cahill, 2003). Future phMRI studies may therefore require to balance male–female ratios to avoid effects of this possible confounder. Additionally, this study was not placebo controlled. Placebo effects may therefore have confounded some of the effects of treatment reported in both groups separately, although no effects were found at a behavioural level. If present, however, placebo effects are likely to have cancelled out in between-group comparisons.

**Conclusions**

Galantamine challenge affected brain function in posterior cingulate (mild cognitive impairment) and hippocampal areas (Alzheimer’s disease), suggesting a key role of the cholinergic system in the functional processes that lead to Alzheimer’s disease. A differential response to cholinergic challenge was found in mild cognitive impairment and Alzheimer patients, suggesting a difference in the functional
status of the cholinergic system in earlier and later stages of a disease. phMRI challenge tests may prove to be a valuable instrument to examine the functional status of central neurotransmitter systems in a disease. Such studies may help to assess neurotransmitter system pathology, monitor disease progression and predict response to pharmacological therapy. In addition, phMRI may be used to develop new drugs that target specific aspects of mental performance, such as encoding and retrieval processes.

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