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Neurobiology of Aging 29 (2008) 1204–1217

NEUROBIOLOGY OF AGING

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PET imaging of the *in vivo* brain acetylcholinesterase activity and nicotine binding in galantamine-treated patients with AD

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Received 24 October 2006; received in revised form 18 January 2007; accepted 14 February 2007
Available online 26 March 2007

Abstract

The effect of galantamine treatment on cortical acetylcholinesterase (AChE) activity and nicotinic receptor binding was investigated by positron emission tomography (PET) in 18 patients with mild Alzheimer's disease (AD) in relation to galantamine concentration and the patients' cognitive performances. The first 3 months of the study was of a randomized double-blind placebo-controlled design, during which 12 patients received galantamine (16–24 mg/day) and 6 patients the placebo, and this was followed by 9 months' galantamine treatment in all patients. The patients underwent PET examinations to measure cortical AChE activity (^{11}C-PMP) and ^{11}C-nicotine binding. Neuropsychological tests were performed throughout the study. Inhibition (30–40%) of cortical AChE activity was observed after 3 weeks to 12 months of galantamine treatment. No significant change in mean cortical ^{11}C-nicotine binding was observed during the study. ^{11}C-Nicotine binding, however, positively correlated with plasma galantamine concentration. Both the changes of AChE activity and ^{11}C-nicotine binding correlated positively with the results of a cognitive test of attention. In conclusion, galantamine caused sustained AChE inhibition for up to 12 months. At the individual level, the *in vivo* cortical AChE inhibition and ^{11}C-nicotine binding were associated with changes in the attention domain of cognition rather than episodic memory.

Keywords: Alzheimer's disease; Acetylcholinesterase; ^{11}C-Nicotine binding; Positron emission tomography (PET); Cerebrospinal fluid; Plasma; Cognitive function; Attention; Galantamine

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia. Cholinesterase inhibitors (ChEIs) are clinically used for symptomatic treatment in AD. Donepezil (Whitehead et al., 2004; Winblad et al., 2001), rivastigmine (Rosler et al., 1999), and galantamine (Raskind et al., 2000, 2004; Tariot et al., 2000; Wilcock et al., 2003), have shown both short- and long-term benefits as regards functional, behavioral and cognitive measurements.

Positron emission tomography (PET) has successfully been used for measuring functional activities of the brain such as glucose metabolism, regional blood flow, neurotransmitter receptor distribution and acetylcholinesterase (AChE) activity. The activity of AChE *in vivo* has been mapped in the human brain by PET, using two different acetylcholine analog tracers, namely *N*-[^{11}C]-methyl-piperidine-4-yl-propionate (^{11}C-PMP) and

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doi:10.1016/j.neurobiolaging.2007.02.020

N-[^{11}C]-methyl-piperidine-4-yl-acetate (^{11}C-MP4A) (Iyo et al., 1997; Koeppe et al., 1999; Kuhl et al., 1999). PET evaluations using ^{11}C-PMP following 8–12 weeks of donepezil treatment at 10 mg/day show 19–27% inhibition of cortical AChE activity in AD patients (Bohnen et al., 2005b; Kuhl et al., 2000), but 29–39% cortical AChE inhibition is observed after donepezil at 3–5 mg/day (Kaasinen et al., 2002; Shinotoh et al., 2001) or rivastigmine at 9 mg/day (Kaasinen et al., 2002) using the other PET tracer (^{11}C-MP4A).

^{11}C-Nicotine was the first PET ligand applied in monkeys and humans for visualizing nicotinic acetylcholine receptors (nAChRs) in the brain. An earlier PET study has shown lower binding of ^{11}C-nicotine in the brains of AD patients compared with control subjects, probably as a result of loss of high and low affinity nicotinic receptor sites (Nordberg et al., 1990). In addition, reduction of cortical ^{11}C-nicotine binding correlates with cognitive impairment in AD patients (Nordberg et al., 1995). Following treatment with ChEIs such as tacrine, an increase in ^{11}C-nicotine binding has been observed in cortical regions of the brains of AD patients (Nordberg et al., 1992, 1997, 1998). However, the major drawbacks of ^{11}C-nicotine as a PET tracer are that nicotine is a non-selective agonist of nAChR subtypes, it has short-term receptor interaction and its distribution strongly depends on cerebral blood flow (Maziere and Delforge, 1995). The latter, however, can be overcome by applying dual tracer methodology (Lundqvist et al., 1998). Therefore, in several studies, we have used a kinetic model to account for cerebral blood flow (Nordberg et al., 1995, 1997, 1998). In this model, ^{11}C-nicotine binding is expressed as a rate constant (k_2^*) which is independent of cerebral blood flow, as confirmed in a study on monkeys (Lundqvist et al., 1998). In this paradigm, a low k_2^* rate constant corresponds to a high ^{11}C-nicotine binding level in the brain. A significant increase in k_2^* values has been observed in the temporal and frontal cortex and hippocampus of patients with AD compared with age-matched healthy controls (Nordberg et al., 1995, 1997).

Although α4 nAChRs dominate in human brain, ^{11}C-nicotine also has high affinity to other nAChR subtypes. Several different compounds have therefore been developed and tested in humans in order to specifically label the α4 nAChRs in the brain by PET, e.g. 2- and 6-[^{18}F] fluoro-A-85380 (Ding et al., 2004; Gallezot et al., 2005; Nordberg, 2006). *In vitro* binding studies with these ligands indicate very high affinity for α4β2 nAChR subunits (Gundisch et al., 2005), although affinity for the α6β2 nAChR subtype could not be excluded (Mogg et al., 2004). Nevertheless, the major drawback with the 2- and the 6-[^{18}F] fluoro-A-85380 tracers is the considerably longer scanning time (7–8 h) than with ^{11}C-nicotine (1 h or less).

Galantamine is a moderate and competitive reversible ChEI (Bores et al., 1996). A few ChEIs, e.g. galantamine and physostigmine, are able to interact with nAChRs directly, as allosterically potentiating ligands (APLs), which seem to act by both slowing down receptor desensitization as well as by sensitizing the nAChRs and hence increasing the probability of channel opening induced by acetylcholine or nicotinic agonists (Maelicke et al., 2001; Samochocki et al., 2000). Several studies in transgenic mice over-expressing human AChE show an increase in cortical nAChRs. These findings are interesting because they suggest that in these animals the high AChE activity which inevitably will shorten the action of ACh at the synaptic cleft, is compensated for by an increase in nAChRs (Mousavi et al., 2004; Svedberg et al., 2002, 2003).

While several clinical studies in AD patients have shown the long-term efficacy of galantamine (Raskind et al., 2000, 2004), there are no reports regarding the effect of galantamine on cortical AChE inhibition and ^{11}C-nicotine binding in AD patients. Hence, the primary aim of the present study was to measure the effects of short-term (3 months) and long-term (9–12 months) treatment with galantamine at 16 or 24 mg daily on cortical AChE activity using ^{11}C-PMP, and on nAChRs by using (*S*)-[^{11}C]-methyl nicotine (^{11}C-nicotine) in the brains of 18 patients with mild AD. The secondary objectives were to measure galantamine concentrations in CSF and plasma as well as to evaluate performances of the patients in different neuropsychological tests in relation to the PET parameters.

2. Methods

2.1. Study design

This was a two-phase study lasting 12 months involving 18 subjects with mild AD (Fig. 1 illustrates the study design). The first phase of the study was a 3-month, double-blind, placebo-controlled, randomized period. The subjects were grouped according to the treatment they received, placebo group ($n = 6$) and galantamine group ($n = 12$). The second phase was a 9-month, open-label extension phase, during which subjects in the placebo group received galantamine treatment using a flexible dosing regimen and subjects in the galantamine group remained on the galantamine.

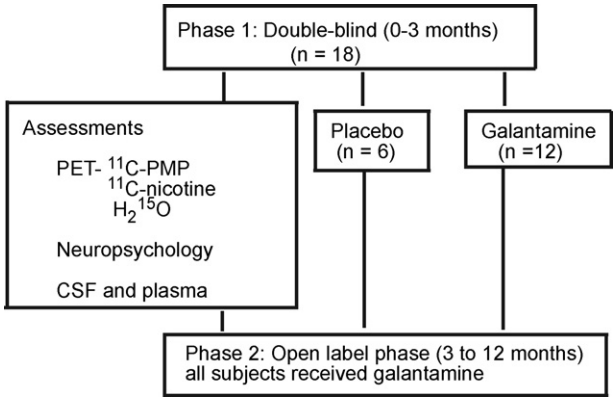

Fig. 1. Schematic presentation of the study design.

During the dose-escalation periods, weeks 1–6 in the double-blind phase for the galantamine group and weeks 14–19 (comparable to weeks 1–6 in the galantamine group) in the open-label phase for the placebo group, who converted to active treatment, subjects received 4 mg b.i.d. for the first week, 8 mg b.i.d. for the next 4 weeks and 12 mg b.i.d. in the 6th week. If the patients could not tolerate the higher dose of 24 mg daily owing to side-effects such as nausea, vomiting or diarrhea, the dose was reduced at the end of the 6th week to 16 mg daily.

2.2. Patients

Eighteen subjects with a diagnosis of mild AD (MMSE score ≥ 21) were recruited from the Department of Geriatric Medicine, Karolinska University Hospital Huddinge and Danderyd Hospital, Stockholm, Sweden. All subjects were referred because of a memory problem for assessment of dementia and underwent a thorough clinical investigation including medical history, cognitive (MMSE score), physical and neurological examination, laboratory blood tests, apolipoprotein E (ApoE) genotyping, psychometric investigation, lumbar puncture and magnetic resonance imaging/computed tomography scans. The diagnosis of AD was made by exclusion of other dementia diseases, in accordance with criteria from the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984).

All patients and their responsible caregivers provided written informed consent to participate in the study and it was conducted according to the declaration of Helsinki and subsequent revisions and was approved by the Ethics Committee of Karolinska University Hospital Huddinge and the Faculty of Medicine and Isotope Committee of Uppsala University, Sweden.

2.3. Drug concentration measurements

A 10-ml blood sample was collected at baseline, and at 3 weeks, and 3 and 12 months, 2 h after morning intake of the drug. A 12-ml CSF sample was collected at baseline and at months 3 and 12. Plasma and CSF samples were assayed for galantamine concentrations using a validated liquid chromatography method with tandem mass spectrometric detection (LC–MS/MS).

2.4. PET methods

Positron emission tomography studies were performed for all participants at the PET-Center, Uppsala Academical Hospital, Uppsala University, at baseline, and at 3 weeks, and 3 and 12 months of the study.

The tracers, [^{15}O] water, (*S*)-[^{11}C]-methyl nicotine (^{11}C-nicotine), and [^{11}C]-methylpiperidine-4-yl-propionate (^{11}C-PMP) were synthesized according to a standard manufacturing procedure based on the methods described by Maziere et al. (1976) (^{11}C-nicotine) and Snyder et al. (1998) (^{11}C-PMP) for each tracer and local QC procedures. [^{15}O] water was produced by a catalyzed reaction between $^{15}O_2$ and H_2.

Tomography was performed with one of two Siemens ECAT HR$^+$ cameras with an axial field of view of 155 mm, providing 63 contiguous 2.46 mm slices with 5.6 mm transaxial and 5.4 mm axial resolution. The orbitomeatal line was used to center the subjects so that the first slice corresponded to the lowest level of the cerebellum.

Tracers were given as intravenous bolus injections. With PET the time course of regional radioactivity concentration was measured in the brain. The following tracer doses and predetermined scanning protocols were used: 22 MBq of [^{15}O] water per kilogram of body weight (MBq/kg) (frames of 17×5 and 2×20 s over 125 s in 2D-mode); approximately 5 MBq of [^{11}C] nicotine/kg (frames of 20×6, 6×30 and 1×120 s over 7 min in 3D-mode); and finally 250 MBq of [^{11}C] PMP (frames of 4×30, 3×60, 2×150, 2×300 and 4×600 s over 60 min in 3D-mode). The time intervals between tracer injections were at least 2 h between [^{11}C] nicotine and [^{11}C] PMP and at least 20 min between [^{15}O] water and [^{11}C] nicotine, i.e. physical decay of radioactivity allowed for 6–10 half-lives. Accordingly, minor radioactivity, which can be neglected in the models used to evaluate the [^{11}C] nicotine and [^{11}C] PMP scans, remained at the times of injection of [^{11}C] nicotine and [^{11}C] PMP.

Attenuation correction was based on a 10-min windowed transmission scan with rotating ^{68}Ge rod sources before administration of the tracer. The emission data were normalized, corrected for random coincidences and dead time, and for scatter using the method published by Watson et al. (1997). Images were reconstructed with the standard software supplied with the scanner (ECAT 7.1 CTI PET systems, Knoxville, TN), using Fourier rebinning followed by 2D filtered back projection applying a 5 mm Hanning filter. In a subsequent step, image data were converted from Siemens/CTI format to Scanditronix/General Electric format with software developed in-house. The 63 slices were resampled to 30 slices with a slice thickness of 5 mm, since the local procedures for PET image analysis utilize tools based on the image analysis software IDA from Scanditronix/General Electric and the image realignment program runs with the Scanditronix/General electric image format. A computerized reorientation procedure was used to align consecutive PET studies for accurate intra- and interindividual comparisons, and to correct for movements within the PET scans (Anderson, 1995).

Continuous blood sampling with on-line registration of radioactivity in blood from the arteria radialis, report rate of 1 sample/s, was used during the [^{15}O] water and [^{11}C] nicotine scans. Blood sampling was started simultaneously with tracer injection; 3 ml/min were withdrawn over 145 and 300 s during the [^{15}O] water and the [^{11}C] nicotine scans, respectively. Arterial samples (4 ml/sample) were

withdrawn at 6 and 7 min after [^{11}C] nicotine injection, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 10, 15, 20, 35, 45 and 60 min after [^{11}C] PMP injection. Radioactivity in whole blood and, for [^{11}C] PMP, in whole blood and plasma was measured.

The extent of [^{11}C] PMP metabolism in blood plasma was determined in all plasma samples by means of a solid phase extraction method. Arterial blood was collected in ice-chilled heparinized test tubes containing 50 μl of a 4 mM physostigmine ethanol solution. The blood was centrifuged at 3000 × *g* and 4 °C for 2 min, whereupon plasma (200 μl) was mixed with 10 mM sodium borate buffer, pH 10 (300 μl). SEP-PAK 200 mg C18 SPE columns were pretreated with 40 μl triethylamine, and conditioned with ethanol (1 ml) followed by 10 mM sodium borate (1 ml). The samples were applied to the columns, followed by washing with 10% ethanol/90% 10 mM sodium borate buffer pH 10 (2.9 ml). The non-metabolized [^{11}C] PMP was eluted with ethanol (2.9 ml). Both the initial buffer (wash) and the ethanol ([^{11}C] PMP) fractions were collected and assayed for ^{11}C radioactivity. The proportion of intact [^{11}C] PMP was determined as the percentage in the ethanol eluate over the sum of radioactivity in the collected fractions.

2.4.1. Analysis of PET data

Regions of interest (ROIs) were drawn on a [^{11}C] nicotine image where the brain anatomy was seen most clearly. Most of the ROIs were delineated in several consecutive slices and were linked, i.e. summed up to a volume of interest (VOI). The defined regions were checked to ensure that they were inside the realigned volume for every PET scan. The regions delineated were putamen, thalamus, parietotemporal cortex and the whole brain at the level of the basal ganglia in one slice per region; pons, sensorimotor cortex, primary visual cortex, cerebellum and frontal association cortex were drawn in two consecutive slices; frontal cortex and anterior cingulate cortex in four consecutive slices; and the parietal cortex in six consecutive slices. The regions for the temporal cortex were drawn in six consecutive coronal slices, and the medial temporal lobe in two coronal slices, with a slice thickness of 8 mm. The VOIs were subsequently used to generate time-activity (TACT) data or for measurements of regional cerebral blood flow (rCBF). In this paper we focused the data analysis on those ROIs that are considered to be most critical in AD, i.e. frontal cortex, anterior cingulate cortex, frontal association cortex, parietal cortex, parietotemporal cortex, temporal cortex and medial temporal lobe of both hemispheres. For presentation of PET data the regions were grouped into four major areas: (i) frontal cortex (including the ROIs of frontal, anterior cingulate and frontal association cortices), (ii) parietal cortex, (iii) parietotemporal cortex and (iv) temporal cortex (including the ROIs of temporal and medial temporal lobe). The average cortical was calculated as a composite of the above four regions. The mean values for left and right hemispheres were used.

2.4.2. k_3 model for AChE activity

The kinetic model used for [^{11}C] PMP was developed by Koeppe et al. (1999). The model consists of two tissue compartments representing authentic tracer in tissue and the trapped metabolic product of hydrolysis by AChE. The model has three rate constants: k_1 and k_2 for transport in and out across the BBB, and k_3, which is assumed to be proportional to the concentration of AChE and therefore is the parameter of primary interest. Regional k_3 values were obtained by fitting the model to the regional time-activity data, using standard iterative algorithms. The radioactivity from authentic (non-metabolized) tracer in arterial plasma was used as input function. Using this model, maps of the rate constant k_3 can also be constructed using a modification of the linear algorithm originally developed by Blomqvist (1984). Although the regional k_3 values obtained by this method agree well with the corresponding k_3 values obtained by means of the iterative algorithm, the maps are here only used as illustrations. According to Koeppe et al. (1999) k_3 as an index of AChE activity is less reliable in regions with high AChE activity such as the cerebellum and basal ganglia. The parametric map of k_3 (Fig. 3) has been selected to show cortical areas where k_3 has been found to be a reliable index of AChE activity. During AChE activity measurement two patients' data at 3 weeks (one in the placebo group and one in the galantamine group) and three patients' data at 12 months were excluded as a result of technical problems. For simplicity in the current study, percentage changes in the k_3 value were used, defined by the following formula: $100 - \%k_3 = 100 - (k_{3(f)}/k_{3(b)} \times 100)$, f and b indicating k_3 values at the times of follow-up and at baseline, respectively.

2.4.3. k_2^* model for ^{11}C-nicotine binding

A dual tracer model, with administration of [^{15}O] water and [^{11}C] nicotine in close succession, was used to assess nicotine binding. The parameter k_2 for [^{11}C] nicotine is highly flow-dependent, and a flow-compensated parameter (k_2^*) was calculated as k_2 for nicotine divided by regional cerebral blood (rCBF) (Lundqvist et al., 1998). In this model, a low k_2^* value indicated more ^{11}C-nicotine binding. The TACT data for VOIs, and the blood TACT data were the input functions in the two-compartment model used to calculate k_2 for [^{11}C] nicotine. Five parameters were included in the model: k_1 = rate constant of radioactivity transport from blood to tissue, k_2 = rate constant of radioactivity transport from tissue to blood, t_d = time delay in the input function due to the transport time in the arterial catheter, k (min^{-1}) = blood dispersion constant and ε = blood volume. During the modeling, k_1 and k_2 were fitted independently. Radioactivity in the whole brain was fitted with the dispersion parameter free. Dispersion for the whole brain region was subsequently used as a fixed constant to model k_2 for the other VOIs. Data from the first 5 min of the investigation were used for the calculation of k_2. A full description of the compartment model has been reported previously (Lundqvist et al., 1998).

The rCBF flow was measured from parametric blood flow images generated according to a method described previously (Herscovitch et al., 1983; Raichle et al., 1983). The integration time interval used was 0–60 s after the bolus appeared in the brain; blood density was set at 1.05 g/cm^3, and the distribution volume was set at 0.95. In the present study, percentage changes in k_2^* were used, defined according to the following formula: $100 - \%k_2^* = 100 - (k_{2(f)}^*/k_{2(b)}^* \times 100)$, f and b indicating the k_2^* values at the times of follow-up and at baseline, respectively.

2.5. Neuropsychological tests

To evaluate the effect of galantamine treatment on cognitive function, neuropsychological assessments were performed. Global cognition function was estimated by means of MMSE (Folstein et al., 1975) at baseline, and at 3 and 12 months of the study, and Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) (Mohs et al., 1983) was used at baseline, at 3 weeks, and at 3 and 12 months. The expanded ADAS-cog scale with 13 items (ADAS-cog/13), score range 0–85, was used in this study. A higher ADAS score indicates a greater degree of cognitive impairment.

Neuropsychological tests of episodic memory, visuospatial ability and attention were carried out at baseline (pre-drug treatment) and during treatment at weeks 3 and months 3, 6, 9 and 12.

Episodic memory was evaluated using two measures from the Stockholm Gerontology Research Center test (Backman and Forsell, 1994) of memory for words: (1) the number of correct responses in free recall of words (word recall); and (2) the d-prime value (an integration of correct responses and false alarms following decision theory) in recognition of words (word recognition-d). It is believed that these measures of memory are related to medial temporal brain activity in particular (Cabeza and Nyberg, 2000).

Attention was assessed using two measures: (1) the number of correct responses in the Digit-Symbol response (Attention-DS) test from the revised Wechsler Adult Intelligence Scale (Wechsler, 1981); and (2) the time needed to complete Trail Making Test A (TMT-A) (Lezak, 1995). These measures are thought to be associated with the frontal and parietal network of brain activity (Cabeza and Nyberg, 2000).

Visuospatial ability was judged on the basis of the number of correct responses in drawing and recognition of clock time (Luria, 1966). These tests were chosen to reflect parietal lobe function (Cahn-Weiner et al., 1999).

2.6. Statistical analysis

Data are expressed as mean values and standard error of the mean (S.E.M.). The equivalence of groups in demographic variables was checked by means of one-way ANOVA. In the double-blind phase, the effects of treatment were analyzed by means of two-way repeated measures ANOVA of the raw data. The between-group factor was the treatment group and the within-group factor was each follow-up interval and baseline. In the open-label phase, the possible change over time was analyzed by means of within-group repeated measures ANOVA. Significant ($p < 0.05$) main effect analyses of variance were followed by Bonferroni-corrected *post hoc* tests that tested the significance of results at each time point compared with baseline and other time points. Two-tailed Pearson's correlation coefficients and (non-parametric) Spearman's Rank correlations were used in correlation analysis, which was then visualized graphically using simple regression plots.

3. Results

3.1. Demographic data

The demographic characteristics of the patients are presented in Table 1. Although the patients in the randomized placebo group were younger and showed shorter duration of the disease, higher MMSE scores and lower ADAS-cog/13 scores at baseline compared with the galantamine group, the demographic characteristics (age, gender, level of education, duration of disease, MMSE score and ADAS-cog/13 score at baseline) did not show any significant difference between the groups (all $p > 0.10$ except for ADAS-cog/13, $p = 0.09$).

3.2. Patient dropout

Of the 18 subjects who entered, 14 completed the 12-month PET study. During the double-blind phase (day 23), one subject (from the galantamine group) was withdrawn as a result of second-degree antrioventricular block. The event was considered possibly related to the study drug. Of the 17 subjects who entered the open-label phase, three patients were withdrawn: one from the *Pla/Gal* group owing to chronic lymphocytic leukemia (this event was considered unrelated to galantamine), one patient because of cardiac arrhythmia and syncope (which was considered possibly related to galantamine), and one patient as a result of non-compliance. In addition, at 12 months, only 13 patients

Table 1
Demographic data; placebo- and galantamine-treated groups

	Placebo	Galantamine	Total subjects
Total subjects	6	12	18
Male/female	3/3	7/5	10/8
Age (years)	65.8 ± 3.7	70.9 ± 2.7	69.2 ± 2.2
Education (years)	12.8 ± 0.9	10.9 ± 1.1	11.4 ± 0.9
Duration of disease (years)	2.2 ± 0.7	5.1 ± 1.1	4.1 ± 0.8
ApoE 4 carriers (+/−)	3/2	7/3	10/5
MMSE at baseline	27.3 ± 0.8	25.6 ± 1.0	26.2 ± 0.7
ADAS-cog/13 at baseline	19.0 ± 1.1	28.0 ± 3.4	25 ± 2.5

Data are presented as mean ± S.E. ApoE = apolipoprotein E; MMSE = Mini Mental State Examination; ADAS-cog/13 = Alzheimer's Disease Assessment Scale-cognitive subscale.

completed the neuropsychological tests, since 1 of them could not undergo the neuropsychological evaluation as a result of myocardial infarction (the relationship of this event to galantamine treatment was considered doubtful).

3.3. Galantamine concentrations

The plasma galantamine concentration was 56 ± 5 ng/ml ($n = 12$) after 3 weeks, 77 ± 12 ng/ml ($n = 11$) after 3 months and 73 ± 7 ng/ml ($n = 14$) after 12 months of treatment. In the CSF, galantamine concentrations were 71 ± 12 ng/ml ($n = 8$) at 3 months and 43 ± 6 ng/ml ($n = 8$) at 12 months. The galantamine concentration in the plasma did not differ from that in the CSF at 3 months, while in the CSF it was significantly lower ($p < 0.01$) than in the plasma at 12 months. A significant positive correlation was observed between plasma and CSF galantamine concentrations at 3 months (Fig. 2, $r = 0.96$, $p < 0.0001$, $n = 8$), while no significant correlation ($p > 0.10$) was found after 12 months of treatment.

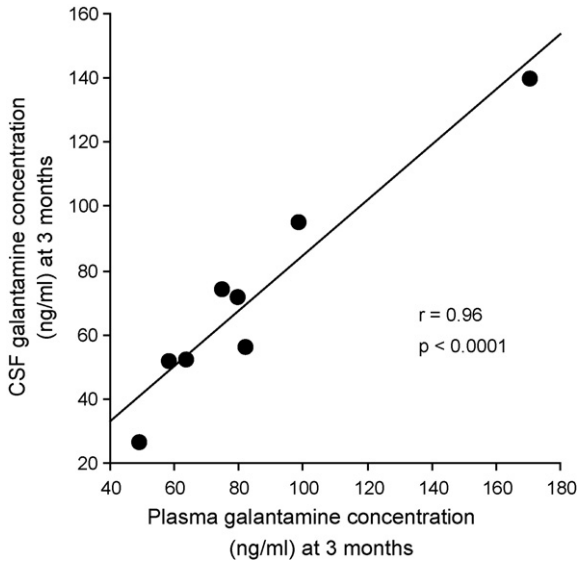

Fig. 2. Positive correlation between plasma and CSF galantamine concentrations after 3 months.

3.4. Changes in cortical AChE activity (k_3)

We aggregated the data from the right and left hemispheres of the studied brain regions, as there were no statistically significant differences.

No significant difference was observed in AChE activity (k_3) at baseline between the placebo and galantamine groups in any of the cortical regions studied (all $p > 0.10$, Table 2).

The average cortical AChE activity in the double-blind phase was assessed by two-way repeated measures ANOVA, which showed that the main effect of the group was not significant ($F = 3.22$, d.f. = 1, $p = 0.09$), but the time effect was significant ($F = 13.57$, d.f. = 2, $p < 0.0001$). In addition, the interaction of group and time was significant ($F = 3.57$, d.f. = 2, $p < 0.04$). In the galantamine group, the average cortical AChE inhibition was 37 ± 5% ($p < 0.0001$) at 3 weeks and 36 ± 5% ($p < 0.0001$) at 3 months compared with baseline activity. Fig. 3 illustrates cortical AChE inhibition in a patient with mild AD after 3 months of galantamine treatment, compared with baseline. In the double-blind phase, after 3 weeks and after 3 months of galantamine treatment there was significant inhibition of AChE activity in all four cortical regions (Table 2). In contrast, the placebo group did not show any significant inhibition at 3 weeks or at 3 months compared with baseline activity ($p > 0.10$, Table 2).

In the open-label phase, when all patients had received galantamine for 9–12 months, the average cortical AChE inhibition was 41 ± 6% (time effect, $F = 36.88$, d.f. = 1, $p < 0.0001$) compared with baseline activity. The inhibition levels of AChE activity in all four cortical regions are shown in Table 2.

Table 2
Cortical AChE activity measured using *N*-[^{11}C]-methyl-piperidine-4-yl-propionate (^{11}C-PMP) in patients with mild AD following placebo or galantamine treatment

Brain regions	Double-blind phase						Open-label phase	
	Placebo			Galantamine			Baseline ($n = 11$)	9–12 months ($n = 11$)
	Baseline ($n = 5$)	3 weeks ($n = 5$)	3 months ($n = 5$)	Baseline ($n = 10$)	3 weeks ($n = 10$)	3 months ($n = 10$)		
Average cortical	0.0270 ± 0.0014	11 ± 10	12 ± 7	0.0278 ± 0.0020	37 ± 5[b]	36 ± 5[b]	0.0280 ± 0.0018	41 ± 6[b]
Frontal cortex	0.0244 ± 0.0011	11 ± 10	9 ± 7	0.0262 ± 0.0020	36 ± 5[b]	34 ± 6[a]	0.0261 ± 0.0018	40 ± 7[a]
Parietal cortex	0.0231 ± 0.0014	14 ± 10	11 ± 8	0.0254 ± 0.0020	37 ± 5[b]	37 ± 6[b]	0.0253 ± 0.0018	45 ± 6[b]
Parietotemporal cortex	0.0253 ± 0.0020	12 ± 10	13 ± 7	0.0264 ± 0.0023	36 ± 5[b]	34 ± 6[a]	0.0266 ± 0.0021	40 ± 7[a]
Temporal cortex	0.0352 ± 0.0020	9 ± 11	12 ± 7	0.0333 ± 0.0018	37 ± 4[b]	38 ± 5[b]	0.0340 ± 0.0016	38 ± 5[b]

Data are expressed as mean ± S.E. Post-treatment k_3 expressed as percentage decrease from baseline (pretreatment) value by using the following formula: $100 - \%k_3 = 100 - (k_{3(f)}/k_{3(b)} \times 100)$, where f and b indicate k_3 values at the times of follow-up and at baseline, respectively. [a]$p < 0.001$, [b]$p < 0.0001$ indicate differences compared with baseline. In the double-blind phase, p was adjusted by Bonferroni correction for two groups and three time points. The PET data were grouped into four major brain regions: (i) frontal (including the ROIs of frontal, anterior cingulate and frontal association cortices), (ii) parietal, (iii) parietotemporal and (iv) temporal (including the ROIs of temporal and medial temporal lobe). The average cortical AChE activity was calculated as a composite of the above four regions.

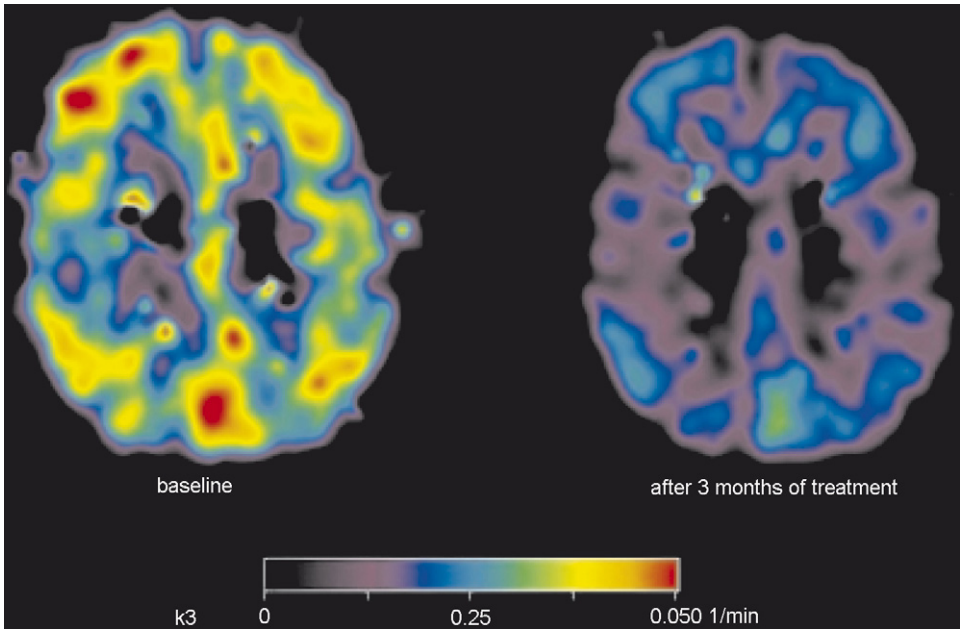

Fig. 3. Parametric map illustrating regional cortical AChE activity (k_3) before and after 3 months of galantamine treatment in a patient with mild AD measured by using *N*-[^{11}C]-methyl-piperidine-4-yl-propionate (^{11}C-PMP). 46% cortical AChE inhibition was found in this patient at 3 months compared with baseline.

No significant correlations were observed between AChE inhibition and galantamine concentrations in CSF and plasma ($p > 0.10$) (data not shown).

3.5. Changes in cortical ^{11}C-nicotine binding (k_2^*)

In the double-blind phase, no difference in average cortical ^{11}C-nicotine binding was observed between the groups as assessed by two-way ANOVA. The main effects (group and time) and their interaction were not significant ($p > 0.10$). No significant changes in ^{11}C-nicotine binding were observed in any of the cortical regions after 3 weeks and after 3 months of treatment compared with baseline in either group (Table 3).

Cortical ^{11}C-nicotine binding did not significantly differ from the baseline values after 9–12 months of galantamine treatment (Table 3).

Nonetheless, a positive correlation was observed between changes in the average cortical k_2^* values and plasma galantamine concentrations at 3 weeks ($r = 0.80$, $p < 0.006$, $n = 12$), at 3 months ($r = 0.65$, $p < 0.04$, $n = 11$) and at 12 months ($r = 0.46$, $p < 0.10$, $n = 14$). Positive although statistically non-significant correlation was observed between CSF galantamine concentrations and changes in k_2^* at 3 months ($r = 0.39$, $p > 0.10$, $n = 8$) or at 12 months ($r = 0.52$, $p > 0.10$, $n = 8$). The percentage changes of cortical k_2^* values and galantamine concentrations in plasma at three time points are plotted in Fig. 4. The analysis indicates that patients with higher

Table 3
^{11}C-Nicotine binding (k_2^*) adjusted by regional cerebral blood flow in placebo- and galantamine-treated patients with mild AD

Brain regions	Double-blind phase						Open-label phase	
	Placebo			Galantamine			Baseline ($n = 14$)	9–12 months ($n = 14$)
	Baseline ($n = 6$)	3 weeks ($n = 6$)	3 months ($n = 6$)	Baseline ($n = 11$)	3 weeks ($n = 11$)	3 months ($n = 11$)		
Average cortical	0.315 ± 0.020	12 ± 3	6 ± 6	0.310 ± 0.012	1 ± 9	9 ± 6	0.300 ± 0.010	−1 ± 6
Frontal cortex	0.348 ± 0.015	13 ± 5	11 ± 5	0.327 ± 0.014	2 ± 7	9 ± 4	0.321 ± 0.010	−1 ± 6
Parietal cortex	0.326 ± 0.024	16 ± 6	4 ± 6	0.331 ± 0.012	6 ± 9	11 ± 5	0.319 ± 0.011	5 ± 6
Parietotemporal cortex	0.277 ± 0.021	7 ± 7	6 ± 8	0.282 ± 0.013	0.3 ± 11	6 ± 9	0.269 ± 0.010	−5 ± 8
Temporal cortex	0.309 ± 0.026	7 ± 5	0.3 ± 9	0.300 ± 0.017	−8 ± 12	6 ± 8	0.291 ± 0.014	−7 ± 8

Data are expressed as mean ± S.E. Post-treatment ^{11}C-nicotine binding was expressed as percentage increase from baseline (pretreatment) value by using the following formula: $100 - \%k_2^* = 100 - (k^*_{2(f)}/k^*_{2(b)} \times 100)$, where *f* and *b* indicate the k_2^* values at the time of follow-up and baseline, respectively. The PET data were grouped into four major brain regions: (i) frontal (including the ROIs of frontal, anterior cingulate and frontal association cortices), (ii) parietal, (iii) parietotemporal and (iv) temporal (including the ROIs of temporal and medial temporal lobe). The average cortical ^{11}C-nicotine binding level was calculated as a composite of the above four regions.

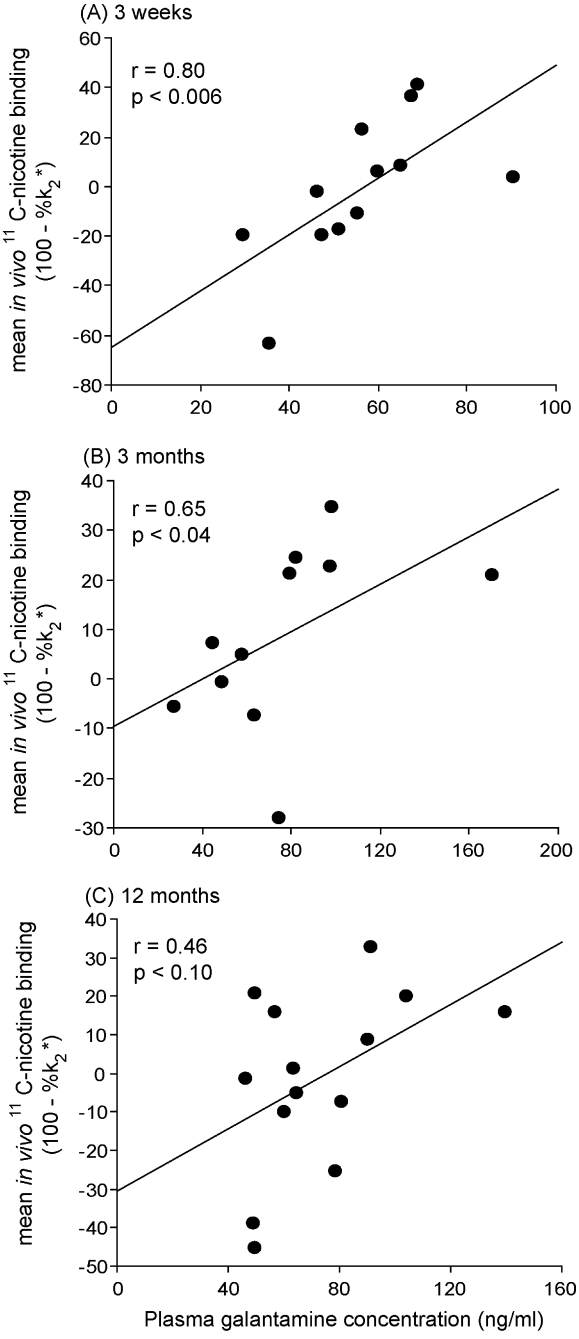

Fig. 4. Positive correlations between mean cortical ^{11}C-nicotine binding (k_2^*) and plasma galantamine concentrations after 3 weeks (A), 3 months (B) and 12 months (C) of the study. The k_2^* values are expressed as percentages of individual baseline values. A positive k_2^* values indicate more ^{11}C-nicotine binding.

plasma galantamine concentrations had increased cortical ^{11}C-nicotine binding.

3.6. Changes in global cognition

Fig. 5 illustrates the mean change of ADAS-cog/13 score over time. The mean score did not significantly differ at baseline between the placebo and the galantamine group

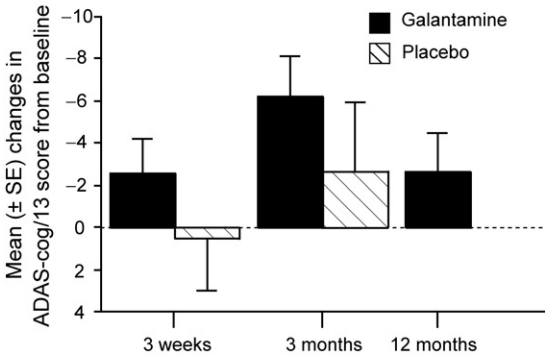

Fig. 5. Mean (±S.E.) change from baseline in global cognitive performance of the patients in ADAS-Cog tests over time showing a trend in improvement in the galantamine-treated subjects. A reduction in ADAS-cog/13 scores indicates more improvement.

(Table 1). In the double-blind phase, two-way repeated measures ANOVA of ADAS-cog data showed that the main effect of group was not significant ($p > 0.10$), but time was significant ($p = 0.02$), and the interaction of group and time was not significant ($p > 0.10$). There was a trend towards improvement in the galantamine group (-6.2 ± 1.9; $n = 11$) that was not found in the placebo group (-2.7 ± 3.2; $n = 6$) at 3 months compared with baseline.

After 9–12 months of galantamine treatment, the mean ADAS-cog/13 score was unchanged compared with baseline (-2.6 ± 1.8; $p > 0.10$, $n = 13$).

As regards MMSE scores in the double-blind phase, no significant effect was found as regards group, time, or group and time interaction (all $p > 0.10$). No considerable declines in mean MMSE sum scores were observed in either the placebo group (-1.2 ± 0.6; $n = 6$) or the galantamine group (-0.55 ± 0.7; $n = 11$) after 3 months of treatment compared with baseline. The MMSE scores showed no significant decline (-1.4 ± 0.7; $p > 0.05$, $n = 13$) compared with baseline after 9–12 months of galantamine treatment.

3.7. Changes in neuropsychological performances

In order to make comparisons between various neuropsychological tests, raw scores were z-transformed by using reference data from the Geriatric Clinic, Karolinska University Hospital Huddinge, Stockholm, Sweden (I. Bergman, unpublished data). The outcome of the neuropsychological tests was calculated as mean composite z-score changes from baseline in the following six subtests: Word Recall, Word Recognition-d, Digit-Symbol, TMT-A (time), Clock Drawing and Clock Recognition (Fig. 6).

In the double-blind phase, two-way ANOVA of the composite z-scores yielded a significant group by time interaction ($F = 4.43$, d.f. $= 2$, $p = 0.02$), but the main effects of group ($p > 0.10$) and time ($p = 0.06$) were not significant. According to pair-wise comparisons, no significant improvement was observed after 3 weeks of treatment in either group

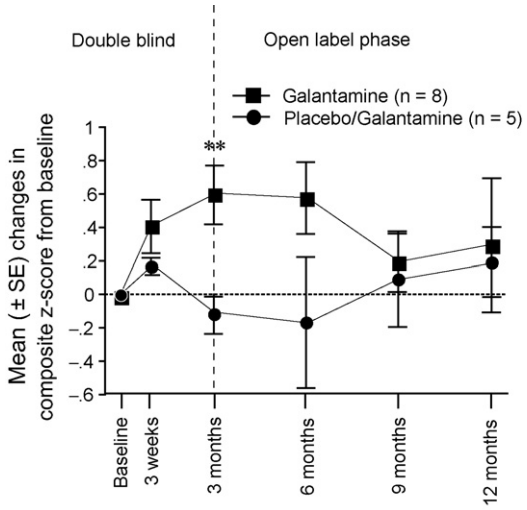

Fig. 6. Neuropsychological test performances summarized by z-transformation of the raw scores in six subtests in the placebo- and galantamine-treated groups. The values are expressed as mean ± S.E. $^{**}p = 0.01$, indicates a significant change compared with baseline at the specified treatment intervals. Squares = galantamine group and circles = placebo/galantamine group.

compared with baseline. However, at 3 months a significant improvement was observed in the galantamine-treated patients (3 months versus baseline, $p = 0.01$) and the placebo group showed a non-significant change.

In the open-label phase, there was no significant effect of time ($p > 0.10$) in the galantamine-treated group. However, a trend towards improvement was observed at 6 months (z-score = 0.6 ± 0.2) and stabilization was found at 12 months (z-score = 0.3 ± 0.4) compared with baseline (Fig. 6).

The placebo/galantamine group exhibited a non-significant time effect ($p > 0.10$) in mean z-score of the six composite neuropsychological tests following the open-label phase (Fig. 6).

3.8. Correlation between PET parameters and neuropsychological test data

We would like to emphasize that the observations in this section should be interpreted with caution and only as a pattern of findings, because a large number of correlation analyses were performed between the changes in the six neuropsychological subtests and the changes in AChE activity and ^{11}C-nicotine binding in 14 cortical brain regions at different follow-up intervals, among a relatively small number of subjects.

3.8.1. After 3 weeks

The changes in AChE activity positively correlated with the patient's performance in the Attention-DS test in the following brain regions: the left anterior cingulate (Fig. 7A, $r = 0.55$, $p < 0.04$, $n = 15$), the right anterior cingulate ($r = 0.52$, $p < 0.05$), the left parietal ($r = 0.54$, $p < 0.04$) and the right parietal cortices ($r = 0.56$, $p < 0.04$).

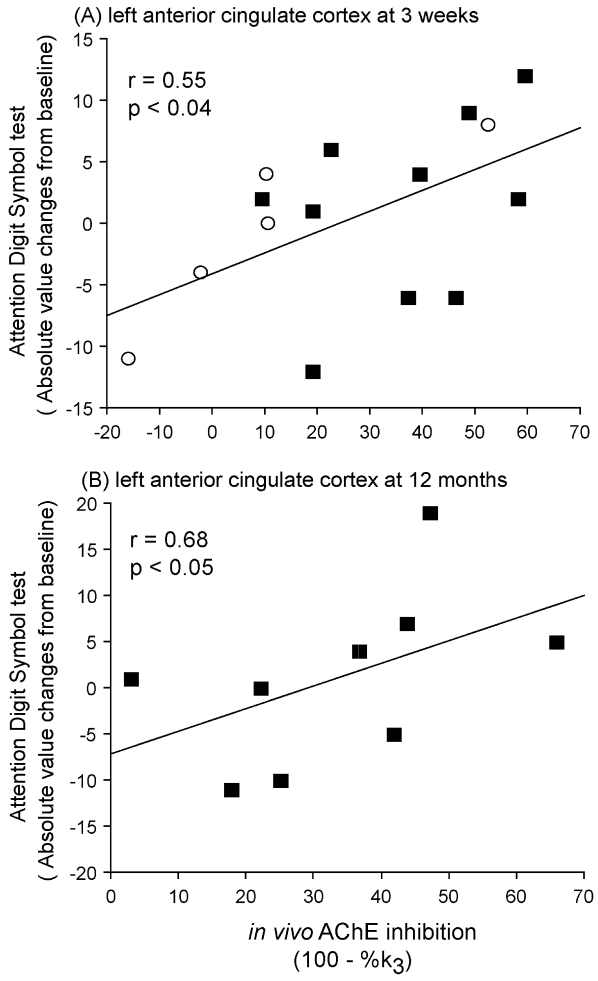

Fig. 7. Positive correlations between changes in cortical AChE activity (k_3) in the left anterior cingulate cortex and changes in the results of the Digit-Symbol test of attention at 3 weeks (A) and at 12 months (B). All values are changes from individual baseline values. Open circles = placebo group and filled squares = galantamine group.

No significant correlation was observed between the changes in ^{11}C-nicotine binding and the patient's performance in the Attention-DS test or the other subtests at this follow-up point.

3.8.2. After 3 months

No significant correlation was observed between the changes in AChE activity and the patient's performance in the Attention-DS test. At this follow-up point, however, the changes in AChE activity positively correlated with the patient's performance in the clock drawing test (visuospatial ability) in the following brain regions: the right anterior cingulate ($r = 0.55$, $p < 0.03$, $n = 16$), the left frontal association ($r = 0.52$, $p < 0.04$), the right frontal association ($r = 0.52$, $p < 0.04$), the left frontal ($r = 0.52$, $p < 0.04$), the left medial temporal ($r = 0.51$, $p < 0.05$), the left parietal ($r = 0.52$, $p < 0.04$), the left parietotemporal ($r = 0.51$, $p < 0.05$) and the left temporal cortices ($r = 0.54$, $p < 0.03$).

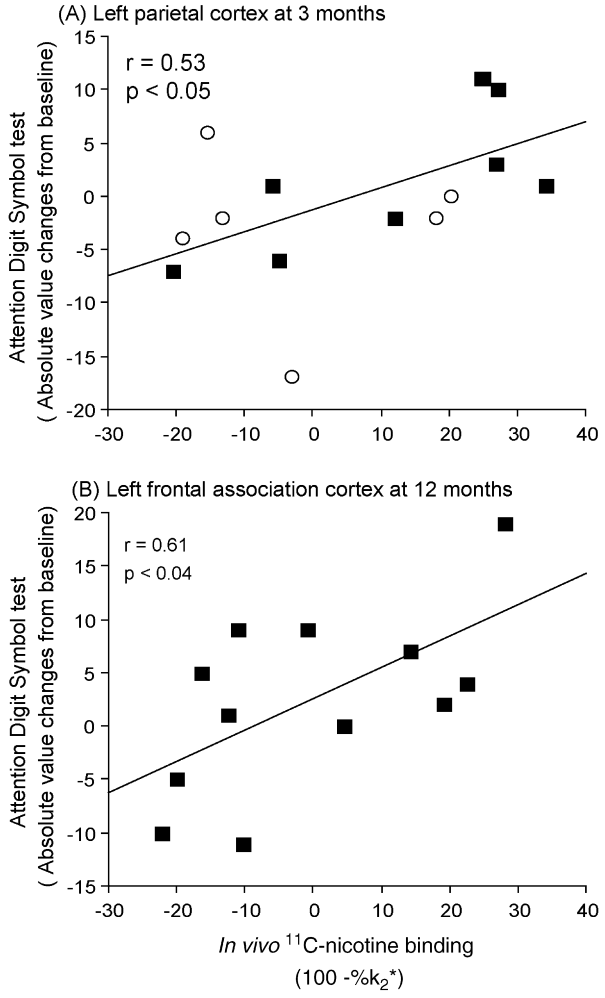

Fig. 8. Positive correlations between changes in cortical ^{11}C-nicotine binding (k_2^*) in the left parietal and left frontal association cortices and changes in Digit-Symbol test performance after 3 months (A) and 12 months (B). All values are changes from individual baseline values. Open circles = placebo group and filled squares = galantamine group.

In addition, at this follow-up point, the changes in ^{11}C-nicotine binding positively correlated with changes in the Attention-DS test scores as regards the left anterior cingulate ($r = 0.52$, $p < 0.05$, $n = 15$), the right frontal association ($r = 0.60$, $p < 0.02$) and the left parietal cortices (Fig. 8A, $r = 0.53$, $p < 0.05$).

3.8.3. After 9–12 months

After 9–12 months of galantamine treatment in all patients, AChE inhibition positively correlated with changes in the Attention-DS test score as regards the following brain regions: the left anterior cingulate (Fig. 7B, $r = 0.68$, $p < 0.05$, $n = 9$), the left frontal association ($r = 0.70$, $p < 0.04$), the left frontal ($r = 0.68$, $p < 0.05$), the left parietotemporal ($r = 0.72$, $p < 0.03$) and the right parietotemporal cortices ($r = 0.68$, $p < 0.05$).

Interestingly, at this follow-up point, changes in ^{11}C-nicotine binding showed positive correlations with changes in the Attention-DS test scores as regards the following brain regions: the left frontal association (Fig. 8B, $r = 0.61$, $p < 0.04$, $n = 12$) and the left frontal cortices ($r = 0.57$, $p = 0.05$).

It should be noted that no significant correlations between changes in cortical AChE activity or ^{11}C-nicotine binding versus changes in the neuropsychology subtest results addressing episodic memory function were observed throughout the study.

4. Discussion

In the present study we took advantage of multiple tracer PET assessment to investigate directly the relationships between biological factors such as brain AChE activity and ^{11}C-nicotine binding with drug concentrations and cognitive function of patients with AD at regular follow-up intervals following galantamine or placebo treatment in a double-blind randomized study followed by an open-label phase.

We found that cortical AChE activity was inhibited by 30–40% following galantamine treatment for 3 weeks to 12 months. This cortical level of *in vivo* AChE inhibition was highly consistent and correlated well with 30–36% inhibition of a synaptic AChE variant, which was assessed in the CSF of the same patients (Darreh-Shori et al., 2008).

Intriguingly, although galantamine is a moderate inhibitor of AChE (Bores et al., 1996), the level of cortical AChE inhibition achieved by means of galantamine in the current study seems to be larger than the 19–27% level of cortical AChE inhibition reported by others in AD patients following 8–12 weeks of donepezil treatment at 10 mg/day using the same PET tracer (^{11}C-PMP) (Bohnen et al., 2005b; Kuhl et al., 2000), but in line with the 29–39% cortical AChE inhibition observed after donepezil at 3–5 mg/day (Kaasinen et al., 2002; Shinotoh et al., 2001), or rivastigmine at 9 mg/day (Kaasinen et al., 2002), using a different PET tracer (^{11}C-MP4A). The most plausible explanation may be deduced from the observation that the concentration of galantamine was comparable in plasma and CSF, indicating good penetration into the brain, whereas it has been shown that donepezil concentrations in CSF are about one-tenth of plasma levels (Darreh-Shori et al., 2006).

We found that galantamine uniformly inhibited AChE activity across the cerebral cortex, whereas it has been reported that both rivastigmine and donepezil induce stronger inhibition of AChE activity in the frontal cortex of AD patients than in other cortical regions (Kaasinen et al., 2002). A possible explanation might be that the patients in the study by Kaasinen et al. (2002) were more progressed in their disease (i.e. lower MMSE scores) compared with the patients in our study.

In the present study we found an average 11% non-significant decline in the cortical AChE activity in the placebo-treated patients after 3 weeks and after 3 months. This reduction is most likely to be the result of an outlier

effect and the small number of patients ($n = 5$) in the placebo group, but such a deviation was not observed in the galantamine group. One patient in the placebo group showed low k_3 activity at 3 weeks and a second patient showed relatively low k_3 activity at 3 months. Owing to the small number of patients we decided not to exclude these two patients from the study. However, the average changes in cortical AChE activity were 1% ($n = 4$) at 3 weeks and 6% ($n = 4$) at 3 months after exclusion of the outliers.

We found no significant changes in mean cortical ^{11}C-nicotine binding following both short-term and long-term galantamine treatment compared with baseline. This observation is consistent with the findings in another galantamine study, in which no significant changes in cortical nicotinic binding sites are observed using a selective α4β2 nAChR PET tracer, [^{18}F] fluoro-A-85380, in 10 patients with mild AD following 8 weeks of galantamine treatment (Ellis et al., 2006). In contrast, tacrine and rivastigmine seems to cause an up-regulation of cortical ^{11}C-nicotine binding in the brains of AD patients after 3 months of treatment, which overlaps the clinical improvement (Nordberg et al., 1992, 1997, 1998; Kadir et al., 2007). This restoration of cortical nicotinic receptors following use of the above ChEIs may be due to secondary stimulation of nicotinic receptors caused by AChE inhibition and hence increased amounts of ACh in the synaptic cleft, which in turn may lead to desensitization and finally up-regulation of nicotinic receptors to compensate for and maintain adequate signaling. In an *in vitro* study however, it has been shown that galantamine, tacrine and donepezil up-regulates the nAChRs in the α4 nAChRs' expressing M10 cell cultures (Svensson and Nordberg, 1997). The lack of a significant increase in ^{11}C-nicotine binding following galantamine treatment may hence suggest a different underlying mechanism of action *in vivo* for galantamine compared with other ChEIs. A plausible mechanistic explanation may be inferred from allosteric interaction of galantamine with nAChRs. The results of experimental studies suggest that galantamine may directly bind to and act as an allosterically potentiating ligand (APL) (Maelicke et al., 2001), which may both sensitize nAChRs and slow down receptor desensitization (Geerts et al., 2002; Maelicke et al., 2001; Woodruff-Pak et al., 2002). Considering this possible APL activity of galantamine on nAChRs, together with its inhibitory action on AChE, leading to persistence of ACh at the synapses, we might intuitively expect a small decrease or maintenance of the *in vivo* nicotine binding sites, as observed in the present study. This effect on nAChRs might be dose-dependent and an increase in ^{11}C-nicotine binding might be observed at higher galantamine concentrations and levels of AChE inhibition.

We observed positive correlations between cortical ^{11}C-nicotine binding and plasma but not CSF galantamine concentrations following both short- and long-term treatment. This finding suggests that the patients with the highest plasma galantamine concentrations also showed the highest increases in cortical ^{11}C-nicotine binding. The lack of correlation with galantamine concentrations in CSF is probably because fewer CSF samples were available compared with the number of plasma samples, which reflects the invasive nature of CSF sampling.

Interestingly, both cortical AChE inhibition and the changes in ^{11}C-nicotine binding positively correlated with the changes in the neuropsychological subtests involving attention rather than episodic memory. Furthermore, inhibition of the synaptic AChE variant by galantamine in the CSF of the current AD patients has been shown to induce changes in the expression pattern of AChE splice variants in CSF which correlates with the patients performance in the neuropsychological subtests of both visuospatial ability and attention (Darreh-Shori et al., 2008). Experimental lesions in the basal forebrain of monkeys disrupts attention rather than memory (Voytko et al., 1994), which supports the findings in several studies that activation of the central cholinergic system by different ChEIs improves the attention domain of cognition (Alhainen et al., 1993; Almkvist et al., 2001; Bohnen et al., 2005b; Darreh-Shori et al., 2002; Levin and Rezvani, 2002; Sahakian et al., 1993; Vellas et al., 2005). In a recent study, we have also shown that cortical ^{11}C-nicotine binding significantly correlates with the results of cognitive tests of attention but again not of episodic memory in a group of untreated mild AD patients (Kadir et al., 2006). Similarly, basal cortical AChE activity has been shown to correlate with attention in a group of untreated AD patients and healthy controls (Bohnen et al., 2005a).

Attention may reflect the function of frontal and parietal cortices in the brain (Cabeza and Nyberg, 2000), supported by the findings in the present study, as the changes in the AChE activity and ^{11}C-nicotine binding in the above areas correlated with the attention test scores.

In this study we summarized the performance of the AD patients in six subsets of neuropsychological tests by *z*-transformation of the data. The galantamine-treated AD patients showed an improvement compared with their baseline levels for up to 6 months. After 9–12 months, the performance of the patients in the neuropsychological tests was comparable to the baseline levels, which might indicate stabilization of the disease, since our earlier observation in untreated AD patients has shown significant deterioration in performance in similar neuropsychological tests after 12 months (Almkvist et al., 2004). Although not significant (probably due to lack of power), the placebo/galantamine group showed some improvement after 3 months of treatment in the open-label phase.

Global cognition was assessed by two tests, namely MMSE and ADAS-Cog. However, this study was designed as a PET investigation and therefore included a small group of patients with mild AD. This feature renders the power of this study inadequate to address clinical conclusions on data addressing changes in the global cognition of the patients. Furthermore, previous clinical studies with adequate statistical power have demonstrated that galantamine treatment confers a significant benefit on cognitive, functional and behavioral symptoms in patients with mild to moderate AD

compared with placebo (Raskind et al., 2000, 2004; Tariot et al., 2000), which is in agreement with the results of numerous clinical trials with other ChEIs (Ringman and Cummings, 2006).

As mentioned above, the present study has several limitations. An important limitation is that relatively large numbers of statistical analyses were performed on the neuropsychological test results in relation to the changes in cortical AChE activity and ^{11}C-nicotine binding, which were not corrected for multiple comparisons and hence may render some of the results liable to be obtained by chance. For this reason, result patterns rather than isolated findings were taken into consideration. Another limitation of the study is the relatively small number of subjects and the dropout during the study period. These features make it necessary to be cautious when interpreting the data, since small sample size may result in a lack of statistical power, although this might make the significant results even more interesting.

In summary, we showed for the first time that galantamine treatment (16–24 mg/day) causes 30–40% cortical AChE inhibition after 3 weeks to 12 months treatment. No significant change in the mean cortical ^{11}C-nicotine binding was observed during the study. The ^{11}C-nicotine binding, however, positively correlated with plasma galantamine concentration. The changes in cortical AChE activity and ^{11}C-nicotine binding were mainly associated with the attention domain of cognition in AD patients rather than episodic memory.

Disclosure

This study was partially financed by Janssen-Cilag, USA. The authors A. Kadir, O. Almkvist, T. Darreh-Shori, A. Wall, M. Grut, B. Strangberg, A. Ringheim, G. Blomquist and B. Långström have reported no conflicts of interest. The author B. Eriksson is a current employee of Janssen-Cilag, Sweden. A. Nordberg has received honoraria from Janssen-Cilag for giving lectures at scientific meetings as well as for participation in advisory board meetings concerning nicotinic receptors and the treatment of AD.

Acknowledgements

This research was sponsored by the Medical Research Council (project no. 05817), Stiftelsen for Gamla Tjänarinnor, KI foundations, Stohne's foundation, and Janssen-Cilag (Sweden and USA).

References

Alhainen, K., Helkala, E.L., Riekkinen, P., 1993. Psychometric discrimination of tetrahydroaminoacridine responders in Alzheimer patients. Dementia 4 (1), 54–58.

Almkvist, O., Darreh-Shori, T., Stefanova, E., Spiegel, R., Nordberg, A., 2004. Preserved cognitive function after 12 months of treatment with rivastigmine in mild Alzheimer's disease in comparison with untreated AD and MCI patients. Eur. J. Neurol. 11 (4), 253–261.

Almkvist, O., Jelic, V., Amberla, K., Hellstrom-Lindahl, E., Meurling, L., Nordberg, A., 2001. Responder characteristics to a single oral dose of cholinesterase inhibitor: a double-blind placebo-controlled study with tacrine in Alzheimer patients. Dement. Geriatr. Cogn. Disord. 12 (1), 22–32.

Anderson, J., 1995. A rapid and accurate method to realign PET-scans utilizing image edge information. J. Nucl. Med. 36, 657–669.

Backman, L., Forsell, Y., 1994. Episodic memory functioning in a community-based sample of old adults with major depression: utilization of cognitive support. J. Abnorm. Psychol. 103 (2), 361–370.

Blomqvist, G., 1984. On the construction of functional maps in positron emission tomography. J. Cereb. Blood Flow Metab. 4 (4), 629–632.

Bohnen, N.I., Kaufer, D.I., Hendrickson, R., Ivanco, L.S., Lopresti, B., Davis, J.G., Constantine, G., Mathis, C.A., Moore, R.Y., DeKosky, S.T., 2005a. Cognitive correlates of alterations in acetylcholinesterase in Alzheimer's disease. Neurosci. Lett. 380 (1–2), 127–132.

Bohnen, N.I., Kaufer, D.I., Hendrickson, R., Ivanco, L.S., Lopresti, B.J., Koeppe, R.A., Meltzer, C.C., Constantine, G., Davis, J.G., Mathis, C.A., Dekosky, S.T., Moore, R.Y., 2005b. Degree of inhibition of cortical acetylcholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry 76 (3), 315–319.

Bores, G.M., Huger, F.P., Petko, W., Mutlib, A.E., Camacho, F., Rush, D.K., Selk, D.E., Wolf, V., Kosley Jr., R.W., Davis, L., Vargas, H.M., 1996. Pharmacological evaluation of novel Alzheimer's disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. J. Pharmacol. Exp. Ther. 277 (2), 728–738.

Cabeza, R., Nyberg, L., 2000. Imaging cognition. II. An empirical review of 275 PET and fMRI studies. J. Cogn. Neurosci. 12 (1), 1–47.

Cahn-Weiner, D.A., Sullivan, E.V., Shear, P.K., Fama, R., Lim, K.O., Yesavage, J.A., Tinklenberg, J.R., Pfefferbaum, A., 1999. Brain structural and cognitive correlates of clock drawing performance in Alzheimer's disease. J. Int. Neuropsychol. Soc. 5 (6), 502–509.

Darreh-Shori, T., Almkvist, O., Guan, Z.Z., Garlind, A., Strandberg, B., Svensson, A.L., Soreq, H., Hellstrom-Lindahl, E., Nordberg, A., 2002. Sustained cholinesterase inhibition in AD patients receiving rivastigmine for 12 months. Neurology 59 (4), 563–572.

Darreh-Shori, T., Kadir, A., Almkvist, O., Grut, M., Wall, A., Blomquist, G., Eriksson, B., Langstrom, B., Nordberg, A., 2008. Inhibition of acetylcholinesterase in CSF versus brain assessed by (11)C-PMP PET in AD patients treated with galantamine. Neurobiol Aging. 29, 168–184.

Darreh-Shori, T., Meurling, L., Pettersson, T., Hugosson, K., Hellstrom-Lindahl, E., Andreasen, N., Minthon, L., Nordberg, A., 2006b. Changes in the activity and protein levels of CSF acetylcholinesterases in relation to cognitive function of patients with mild Alzheimer's disease following chronic donepezil treatment. J. Neural. Transm. 113 (11), 1791–1801.

Ding, Y.S., Fowler, J.S., Logan, J., Wang, G.J., Telang, F., Garza, V., Biegon, A., Pareto, D., Rooney, W., Shea, C., Alexoff, D., Volkow, N.D., Vocci, F., 2004. 6-[18F]Fluoro-A-85380, a new PET tracer for the nicotinic acetylcholine receptor: studies in the human brain and in vivo demonstration of specific binding in white matter. Synapse 53 (3), 184–189.

Ellis, J.R., Villemagne, V.L., Nathan, P.J., Mulligan, R.S., Gong, S.J., Smith, C.L., Tochon-Danguy, H., Savage, G.R., Rowe, C.C., 2006. Nicotinic receptor upregulation and the cognitive effects of galantamine in Alzheimer's disease: a human in vivo study using [18F]F-A-85380 PET. Alzheimers Dement. 2 (3), S353–S354.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12 (3), 189–198.

Gallezot, J.D., Bottlaender, M., Gregoire, M.C., Roumenov, D., Deverre, J.R., Coulon, C., Ottaviani, M., Dolle, F., Syrota, A., Valette, H., 2005.

In vivo imaging of human cerebral nicotinic acetylcholine receptors with 2-18F-fluoro-A-85380 and PET. J. Nucl. Med. 46 (2), 240–247.

Geerts, H., Finkel, L., Carr, R., Spiros, A., 2002. Nicotinic receptor modulation: advantages for successful Alzheimer's disease therapy. J. Neural. Transm. Suppl. (62), 203–216.

Gundisch, D., Koren, A.O., Horti, A.G., Pavlova, O.A., Kimes, A.S., Mukhin, A.G., London, E.D., 2005. In vitro characterization of 6-[18F]fluoro-A-85380, a high-affinity ligand for alpha4beta2* nicotinic acetylcholine receptors. Synapse 55 (2), 89–97.

Herscovitch, P., Markham, J., Raichle, M.E., 1983. Brain blood flow measured with intravenous H2(15)O. I. Theory and error analysis. J. Nucl. Med. 24 (9), 782–789.

Iyo, M., Namba, H., Fukushi, K., Shinotoh, H., Nagatsuka, S., Suhara, T., Sudo, Y., Suzuki, K., Irie, T., 1997. Measurement of acetylcholinesterase by positron emission tomography in the brains of healthy controls and patients with Alzheimer's disease. Lancet 349 (9068), 1805–1809.

Kaasinen, V., Nagren, K., Jarvenpaa, T., Roivainen, A., Yu, M., Oikonen, V., Kurki, T., Rinne, J.O., 2002. Regional effects of donepezil and rivastigmine on cortical acetylcholinesterase activity in Alzheimer's disease. J. Clin. Psychopharmacol. 22 (6), 615–620.

Kadir, A., Almkvist, O., Wall, A., Langstrom, B., Nordberg, A., 2006. PET imaging of cortical ^{11}C-nicotine binding correlates with the cognitive function of attention in Alzheimer's disease. Psychopharmacology 188 (4), 509–520.

Koeppe, R.A., Frey, K.A., Snyder, S.E., Meyer, P., Kilbourn, M.R., Kuhl, D.E., 1999. Kinetic modeling of *N*-[^{11}C]methylpiperidin-4-yl propionate: alternatives for analysis of an irreversible positron emission tomography trace for measurement of acetylcholinesterase activity in human brain. J. Cereb. Blood Flow Metab. 19 (10), 1150–1163.

Kuhl, D.E., Koeppe, R.A., Minoshima, S., Snyder, S.E., Ficaro, E.P., Foster, N.L., Frey, K.A., Kilbourn, M.R., 1999. In vivo mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease. Neurology 52 (4), 691–699.

Kuhl, D.E., Minoshima, S., Frey, K.A., Foster, N.L., Kilbourn, M.R., Koeppe, R.A., 2000. Limited donepezil inhibition of acetylcholinesterase measured with positron emission tomography in living Alzheimer cerebral cortex. Ann. Neurol. 48 (3), 391–395.

Kadir, A., Darreh-Shori, T., Almkvist, O., Wall, A., Långström, B., Nordberg, A., 2007. Changes in brain ^{11}C-nicotine binding sites in patients with mild Alzheimer's disease following rivastigmine treatment as assessed by PET. Psychopharmacology, doi:10.1007/s00213-007-0725-z.

Levin, E.D., Rezvani, A.H., 2002. Nicotinic treatment for cognitive dysfunction. Curr. Drug Targets CNS Neurol. Disord. 1 (4), 423–431.

Lezak, M., 1995. Neuropsychological Assessment, 3rd ed. Oxford University Press, New York.

Lundqvist, H., Nordberg, A., Hartvig, P., Langstrom, B., 1998. (*S*)-(−)-[^{11}C]Nicotine binding assessed by PET: a dual tracer model evaluated in the rhesus monkey brain. Alzheimer Dis. Assoc. Disord. 12 (3), 238–246.

Luria, A., 1966. Higher Cortical Functions in Man. Basic Books, New York.

Maelicke, A., Samochocki, M., Jostock, R., Fehrenbacher, A., Ludwig, J., Albuquerque, E.X., Zerlin, M., 2001. Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. Biol. Psychiatry 49 (3), 279–288.

Maziere, M., Comar, D., Maranzo, C., Berger, G., 1976. Nicotine-^{11}C: synthesis and distribution kinetics in animals. Eur. J. Nucl. Med. 1, 255–258.

Maziere, M., Delforge, J., 1995. PET imaging [^{11}C]nicotine: historical aspects. In: Domino, E. (Ed.), Brain Imaging of Nicotine and Tobacco Smoking. NPP Books, Ann Arbor, pp. 13–28.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34 (7), 939–944.

Mogg, A.J., Jones, F.A., Pullar, I.A., Sharples, C.G., Wonnacott, S., 2004. Functional responses and subunit composition of presynaptic nicotinic receptor subtypes explored using the novel agonist 5-iodo-A-85380. Neuropharmacology 47 (6), 848–859.

Mohs, R.C., Rosen, W.G., Davis, K.L., 1983. The Alzheimer's disease assessment scale: an instrument for assessing treatment efficacy. Psychopharmacol. Bull. 19 (3), 448–450.

Mousavi, M., Bednar, I., Nordberg, A., 2004. Selective changes in expression of different nicotinic receptor subtypes in brain and adrenal glands of mice carrying human mutated gene for APP or over-expressing human acetylcholinestrase. Int. J. Dev. Neurosci. 22 (7), 545–549.

Nordberg, A., 2006. Visualization of nicotinic and muscarinic receptors in brain by positron emission tomography. In: Giacobini, E., Pepeu, G. (Eds.), The Brain Cholinergic System in Health and Disease. Informa Healthcare, London, pp. 181–190.

Nordberg, A., Amberla, K., Shigeta, M., Lundqvist, H., Viitanen, M., Hellstrom-Lindahl, E., Johansson, M., Andersson, J., Hartvig, P., Lilja, A., Langstrom, B., Winblad, B., 1998. Long-term tacrine treatment in three mild Alzheimer patients: effects on nicotinic receptors, cerebral blood flow, glucose metabolism, EEG, and cognitive abilities. Alzheimer Dis. Assoc. Disord. 12 (3), 228–237.

Nordberg, A., Hartvig, P., Lilja, A., Viitanen, M., Amberla, K., Lundqvist, H., Andersson, Y., Ulin, J., Winblad, B., Langstrom, B., 1990. Decreased uptake and binding of ^{11}C-nicotine in brain of Alzheimer patients as visualized by positron emission tomography. J. Neural. Transm. Park Dis. Dement. Sect. 2 (3), 215–224.

Nordberg, A., Lilja, A., Lundqvist, H., Hartvig, P., Amberla, K., Viitanen, M., Warpman, U., Johansson, M., Hellstrom-Lindahl, E., Bjurling, P., et al., 1992. Tacrine restores cholinergic nicotinic receptors and glucose metabolism in Alzheimer patients as visualized by positron emission tomography. Neurobiol. Aging 13 (6), 747–758.

Nordberg, A., Lundqvist, H., Hartvig, P., Andersson, J., Johansson, M., Hellstrom-Lindahi, E., Langstrom, B., 1997. Imaging of nicotinic and muscarinic receptors in Alzheimer's disease: effect of tacrine treatment. Dement. Geriatr. Cogn. Disord. 8 (2), 78–84.

Nordberg, A., Lundqvist, H., Hartvig, P., Lilja, A., Langstrom, B., 1995. Kinetic analysis of regional (*S*)(−)^{11}C-nicotine binding in normal and Alzheimer brains—in vivo assessment using positron emission tomography. Alzheimer Dis. Assoc. Disord. 9 (1), 21–27.

Raichle, M.E., Martin, W.R., Herscovitch, P., Mintun, M.A., Markham, J., 1983. Brain blood flow measured with intravenous H2(15)O. II. Implementation and validation. J. Nucl. Med. 24 (9), 790–798.

Raskind, M.A., Peskind, E.R., Truyen, L., Kershaw, P., Damaraju, C.V., 2004. The cognitive benefits of galantamine are sustained for at least 36 months: a long-term extension trial. Arch. Neurol. 61 (2), 252–256.

Raskind, M.A., Peskind, E.R., Wessel, T., Yuan, W., 2000. Galantamine in AD: a 6-month randomized, placebo-controlled trial with a 6-month extension. The Galantamine USA-1 Study Group. Neurology 54 (12), 2261–2268.

Ringman, J.M., Cummings, J.L., 2006. Current and emerging pharmacological treatment options for dementia. Behav. Neurol. 17 (1), 5–16.

Rosler, M., Anand, R., Cicin-Sain, A., Gauthier, S., Agid, Y., Dal-Bianco, P., Stahelin, H.B., Hartman, R., Gharabawi, M., 1999. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. BMJ 318 (7184), 633–638.

Sahakian, B.J., Owen, A.M., Morant, N.J., Eagger, S.A., Boddington, S., Crayton, L., Crockford, H.A., Crooks, M., Hill, K., Levy, R., 1993. Further analysis of the cognitive effects of tetrahydroaminoacridine (THA) in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB. Psychopharmacology (Berlin) 110 (4), 395–401.

Samochocki, M., Zerlin, M., Jostock, R., Groot Kormelink, P.J., Luyten, W.H., Albuquerque, E.X., Maelicke, A., 2000. Galantamine is an allosterically potentiating ligand of the human alpha4/beta2 nAChR. Acta Neurol. Scand. Suppl. 176, 68–73.

Shinotoh, H., Aotsuka, A., Fukushi, K., Nagatsuka, S., Tanaka, N., Ota, T., Tanada, S., Irie, T., 2001. Effect of donepezil on brain acetylcholinesterase activity in patients with AD measured by PET. Neurology 56 (3), 408–410.

Snyder, S.E., Tluczek, L., Jewett, D.M., Nguyen, T.B., Kuhl, D.E., Kilbourn, M.R., 1998. Synthesis of 1-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]PMP) for in vivo measurements of acetylcholinesterase activity. Nucl. Med. Biol. 25 (8), 751–754.

Svedberg, M.M., Svensson, A.L., Bednar, I., Nordberg, A., 2003. Neuronal nicotinic and muscarinic receptor subtypes at different ages of transgenic mice overexpressing human acetylcholinesterase. Neurosci. Lett. 340 (2), 148–152.

Svedberg, M.M., Svensson, A.L., Johnson, M., Lee, M., Cohen, O., Court, J., Soreq, H., Perry, E., Nordberg, A., 2002. Upregulation of neuronal nicotinic receptor subunits alpha4, beta2, and alpha7 in transgenic mice overexpressing human acetylcholinesterase. J. Mol. Neurosci. 18 (3), 211–222.

Svensson, A.L., Nordberg, A., 1997. Interaction of tacrine, galanthamine, NXX-066, and E2020 with neuronal a4β2 nicotinic receptors expressed in fibroblast cells. In: Iqbal, K., Winblad, B., Nishimura, T., Takeda, M., Wisniewski, H.M. (Eds.), Alzheimer's Disease Biology, Diagnosis and Therapeutics. John Whiley & Sons, Chichester, pp. 751–756.

Tariot, P.N., Solomon, P.R., Morris, J.C., Kershaw, P., Lilienfeld, S., Ding, C., 2000. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. Neurology 54 (12), 2269–2276.

Vellas, B., Cunha, L., Gertz, H.J., De Deyn, P.P., Wesnes, K., Hammond, G., Schwalen, S., 2005. Early onset effects of galantamine treatment on attention in patients with Alzheimer's disease. Curr. Med. Res. Opin. 21 (9), 1423–1429.

Voytko, M.L., Olton, D.S., Richardson, R.T., Gorman, L.K., Tobin, J.R., Price, D.L., 1994. Basal forebrain lesions in monkeys disrupt attention but not learning and memory. J. Neurosci. 14 (1), 167–186.

Watson, C.C.N.D., Casey, M.E., et al., 1997. Evaluation of simulation based scatter correction for 3D PET cardiac imaging. IEEE Trans. Nucl. Sci. 21, 136–144.

Wechsler, D., 1981. Wechsler Adult Intelligence Scale—Revised Manual. Psychological Corporation, New York.

Whitehead, A., Perdomo, C., Pratt, R.D., Birks, J., Wilcock, G.K., Evans, J.G., 2004. Donepezil for the symptomatic treatment of patients with mild to moderate Alzheimer's disease: a meta-analysis of individual patient data from randomised controlled trials. Int. J. Geriatr. Psychiatry 19 (7), 624–633.

Wilcock, G., Howe, I., Coles, H., Lilienfeld, S., Truyen, L., Zhu, Y., Bullock, R., Kershaw, P., 2003. A long-term comparison of galantamine and donepezil in the treatment of Alzheimer's disease. Drugs Aging 20 (10), 777–789.

Winblad, B., Engedal, K., Soininen, H., Verhey, F., Waldemar, G., Wimo, A., Wetterholm, A.L., Zhang, R., Haglund, A., Subbiah, P., 2001. A 1-year, randomized, placebo-controlled study of donepezil in patients with mild to moderate AD. Neurology 57 (3), 489–495.

Woodruff-Pak, D.S., Lander, C., Geerts, H., 2002. Nicotinic cholinergic modulation: galantamine as a prototype. CNS Drug Rev. 8 (4), 405–426.