

Cholinesterase inhibition modulates visual and attentional brain responses in Alzheimer's disease and health

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Visuo-attentional deficits occur early in Alzheimer's disease (AD) and are considered more responsive to pro-cholinergic therapy than characteristic memory disturbances. We hypothesised that neural responses in AD during visuo-attentional processing would be impaired relative to controls, yet partially susceptible to improvement with the cholinesterase inhibitor physostigmine. We studied 16 mild AD patients and 17 age-matched healthy controls, using fMRI-scanning to enable within-subject placebo-controlled comparisons of effects of physostigmine on stimulus- and attention- related brain activations, plus between-group comparisons for these. Subjects viewed face or building stimuli while performing a shallow judgement (colour of image) or a deep judgement (young/old age of depicted face or building). Behaviourally, AD subjects performed slower than controls in both tasks, while physostigmine benefited the patients for the more demanding age-judgement task. Stimulus-selective (face minus building, and vice versa) BOLD signals in precuneus and posterior parahippocampal cortex were attenuated in patients relative to controls, but increased following physostigmine. By contrast, face-selective responses in fusiform cortex were not impaired in AD and showed decreases following physostigmine for both groups. Task-dependent responses in right parietal and prefrontal cortices were diminished in AD but improved following physostigmine. A similar pattern of group and treatment effects was observed in two extrastriate cortical regions that showed physostigmine-induced enhancement of stimulus-selectivity for the deep versus shallow task. Finally, for the healthy group, physostigmine decreased stimulus and task-dependent effects, partly due to an exaggeration of selectivity during the shallow relative to deep task. The differences in brain activations between groups and treatments were not attributable merely to performance (reaction time) differences. Our results demonstrate that physostigmine can improve both stimulus- and attention-dependent responses in functionally affected extrastriate and frontoparietal regions in AD, while perturbing the normal pattern of responses in many of the same regions in healthy controls.

Keywords: fMRI; cholinergic; Alzheimer's disease; visual processing; attention

Abbreviations: AD = Alzheimer's disease; BOLD = blood oxygenation level dependent; pSTS = posterior superior temporal sulcus; SPM = statistical parametric maps

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Introduction

Understanding how neuromodulatory systems affects cognitive function is important for conditions such as Alzheimer's disease, cortical Lewy body disease, vascular dementia, and head injury (Tiraboschi *et al.*, 2000; Auld *et al.*, 2002; Wilkinson *et al.*, 2005; Conner *et al.*, 2005; Salmund *et al.*, 2005), in which cholinergic modulation has

been implicated. In Alzheimer's disease (AD), the association of acetylcholine deficiency with cognitive impairment is supported by at least three observations. First, cortical cholinergic neurons, along with medial temporal structures, are preferential victims of the degenerative process in AD (e.g. Mesulam, 2004a). Second, selective lesions of cholinergic neurons in experimental animals reproduce memory

and attentional deficits found in AD (e.g. Everitt and Robbins, 1997). Third, cholinesterase inhibitors can improve, or at least slow deterioration for some aspects of cognitive performance in AD (e.g. Rogers *et al.*, 1998). Linking these different strands of evidence more directly ideally requires a demonstration within the same patients that abnormal performance in AD and abnormal brain activations in AD can both be at least partially reversed by pharmacological manipulation of acetylcholine levels (while ensuring that differences in brain activations do not merely reflect changes in performance *per se*). From a clinical perspective, it is important to study how neuromodulatory drugs affect brain function as well as behaviour, as this may guide or predict treatment responses in individual patients or patient types (Matthews *et al.*, 2006).

Our group has previously investigated effects of cholinesterase inhibition on visual processing and selective attention, using fMRI in healthy young adults. As animal studies demonstrate a dependence of visuo-attentional processes on cortical cholinergic inputs (Sarter *et al.*, 2001), we originally anticipated that attention-related fMRI effects, expressed in parietal and visual cortices, might be enhanced by boosting acetylcholine via administration of physostigmine. To our surprise, across three different paradigms (Thiel *et al.*, 2002; Bentley *et al.*, 2003, 2004) we systematically found an opposite pattern in healthy adults. Parietal and visual areas differentially activated as a function of top-down factors typically showed *decreased* activation after physostigmine. One possible explanation is that physostigmine acts to increase stimulus-evoked activity, with this being most pronounced for task-irrelevant stimuli (thereby apparently reducing top-down effects), since attended task-relevant stimuli already typically activate sensory cortex at, or near, to maximum (*see* Thiel *et al.*, 2002; Bentley *et al.*, 2004). This possibility accords with neurobiological models predicting that acetylcholine favours bottom-up over top-down sensory processing (e.g. Yu and Dayan, 2005; Hasselmo and Giocomo, 2006). It also fits with data suggesting that excessive cholinergic stimulation may underlie increased processing of irrelevant stimuli (Thiel *et al.*, 2005), including in neuropsychiatric states (Bernston *et al.*, 1998; Sarter *et al.*, 2005b). We note that Furey *et al.* (2000) also demonstrated reduced task-related prefrontal activity with physostigmine in healthy adults, although that study reported enhanced activity in extrastriate cortex for the same treatment.

In AD, degeneration of cortical cholinergic neurons is an early pathological finding, whereas the intrinsic structure of early sensory cortices often appears relatively spared (Mesulam, 2004a). Animal studies indicate that cholinergic stimulation of normal sensory cortex can have facilitatory effects on stimulus-processing parameters such as selectivity and signal-to-noise ratio (e.g. Sato *et al.*, 1987, Murphy and Sillito, 1991); while cholinergic inputs to frontoparietal cortices provide a contribution to tasks requiring sustained or selective attention (Sarter *et al.*, 2001). Behavioural testing in mild-to-moderate AD patients has

identified deficits in both sensory processing (e.g. visual contrast sensitivity; *see* Cronin-Golomb *et al.*, 1991; Tippett *et al.*, 2003) and in selective attention (Perry *et al.*, 2000; Rizzo *et al.*, 2000; Baddeley *et al.*, 2001), that are associated with under-activity of visual and frontoparietal cortices (Buck *et al.*, 1997; Mentis *et al.*, 1998; Prvulovic *et al.*, 2002; Hao *et al.*, 2005). Moreover, attentional deficits in AD appear more responsive to cholinesterase inhibition than the well-known memory defects (Sahakian *et al.*, 1993; Lawrence and Sahakian, 1995). It thus appears reasonable to hypothesise that one factor underlying visual attention deficits in AD is reduced cholinergic modulation of visual and frontoparietal cortices (*see* Perry and Hodges, 1999), and that this would be expected to be partially reversible following cholinesterase inhibition (whether through direct or indirect actions).

Using fMRI, we tested this by examining visual processing (brain responses to buildings versus faces) and attentional affects (shallow or deep tasks on these visual stimuli) in both AD and healthy controls. The primary question we asked was whether administration of the cholinesterase inhibitor physostigmine could to some extent 'restore' stimulus- and task-dependent brain responses that were impaired in AD, and how this compared with drug effects for the healthy controls. We made the following predictions: First, stimulus-selectivity in extrastriate visual cortex will be decreased in AD relative to controls, yet ameliorated to some extent with physostigmine. Second, attention-dependent activations in frontoparietal cortex due to task will be attenuated in AD relative to controls, but again ameliorated by physostigmine. Third, attention-dependent modulation of extrastriate cortex (stimulus-selectivity compared between deep versus shallow tasks) will be decreased in AD relative to controls, but ameliorated by physostigmine. Finally, the effect of physostigmine on brain responses in controls will be opposite to that found in AD, given findings from earlier studies (Furey *et al.*, 2000; Bentley *et al.*, 2003, 2004; Thiel *et al.*, 2005), and the proposal (*see* above) that effects on healthy individuals are constrained by attended stimuli producing optimal or near maximal responses in the healthy brain. Since most of our expected activations were in visual extrastriate cortices it is unlikely that these could be affected by changes in performance (e.g. RT differences) alone (Honey *et al.*, 2000); however, where performance differences did occur we attempted to control for this through separate modelling of individual behavioural effects (Dannhauser *et al.*, 2005).

Methods

Subjects

Sixteen right-handed patients with newly-diagnosed AD and minimal-state (MMSE) scores of 20–26 were recruited from the Dementia Research Group, National Hospital for Neurology and Neurosurgery (London, UK) over a 15 month period. Seventeen right-handed healthy subjects, matched for age and sex, were

recruited over the same period. No subjects were active smokers. Characteristics of the two groups are listed in Table 1. Note that due to clinical constraints IQ was measured with the WAIS test in patients unlike the NART in controls. The WAIS-IQ verbal scores were closely correlated with the WAIS-IQ performance scores within the AD group ($r=0.89$; $P<0.001$) suggesting that both below-normal scores reflected some influence of dementia, rather than that lower verbal IQ in AD reflecting some other pre-morbid difference (e.g. in education between the two groups).

All subjects gave written informed consent in accord with local ethics. Patients fulfilled the following criteria: (i) probable AD according to international criteria (National Institute of Neurological and Communication Disorders/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) and the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV); (ii) a full neuropsychological, neurological and general clinical examination, as well as dementia-screening blood tests, chest x-ray, brain MRI, electroencephalography and cerebrospinal fluid examinations (where felt to be appropriate

Table 1 Characteristics of control and AD subjects ($\pm 95\%$ confidence intervals)

	Controls	AD
Number	17	16
Males	8	9
Age	64.9 (± 4.0)	66.4 (± 4.4)
Education (in years)	12.7 (± 0.8)	12.5 (± 0.9)
Baseline blood-pressure	129/75.8 ($\pm 90/4.9$)	135/82.4 ($\pm 6.5/3.5$)
MMSE	29.6 (± 0.2)	23.9 (± 1.2)*
Verbal IQ (WAIS)	n/a	94.2 (± 5.7)*
Performance IQ (WAIS)	n/a	92.7 (± 7.9)*
Verbal IQ (NART)	115 (± 1.1)	n/a
Performance IQ (NART)	115 (± 1.1)	n/a

IQ scores in controls are estimated from National Adult Reading Test (NFER-NELSON Publishing Co. Ltd., Berkshire, England, 2nd Edition, 1991).

* $P<0.01$ between-group difference.

for diagnosis), with all these examinations and tests in keeping with a sole diagnosis of AD; (iii) no major visuo-spatial or visuo-perceptual impairment or severe apraxia apparent clinically; (iv) no coexistent significant central nervous system disease, e.g. epilepsy, movement disorder, head injury, drug or alcohol abuse; (v) they were receiving no psychoactive drugs clinically, including no cholinesterase inhibitors, *N*-methyl-D-aspartate antagonist, or antidepressants.

All patients were started on therapeutic oral cholinesterase inhibitor following the second experimental session (see below), and were followed up for a minimum of one year to ensure that no other features developed that would suggest an alternative cause for dementia other than AD.

Task design

On each of two sessions (placebo/physostigmine), subjects performed two tasks [Colour (C) or Age (A) judgements; Fig. 1] separated into blocks of 48 trials each, and repeated once (i.e. there were two blocks per task per session) in one of the following orders: CACA, ACAC, CAAC or ACCA. Task order was counterbalanced across subjects, but repeated across sessions within subjects, while treatment order (placebo in first session, physostigmine in second, or vice versa) was also counterbalanced across subjects. The two sessions were separated in time by 1–2 weeks. Both tasks comprised serial presentation of single faces or single buildings (randomly intermingled in an event-related fashion) with no image being repeated across sessions. The images for both tasks were presented in isoluminant red or green monochrome. The 'shallow' task of judging colours simply required an indication as to whether an image was red or green; the 'deeper' age task required a judgment as to whether the particular face or building shown in any single image was old or young (the latter choice denoting 'modern' in the case of buildings). The stimulus set comprised an equal number of 'young' (individuals aged 21–35) and 'old' faces (individuals aged over 65), as well as an equal number of modern (e.g. office-blocks) and old buildings (e.g. castles). We excluded faces and buildings that were famous or were depicted from a non-canonical view, and faces with overtly emotional expressions. The particular

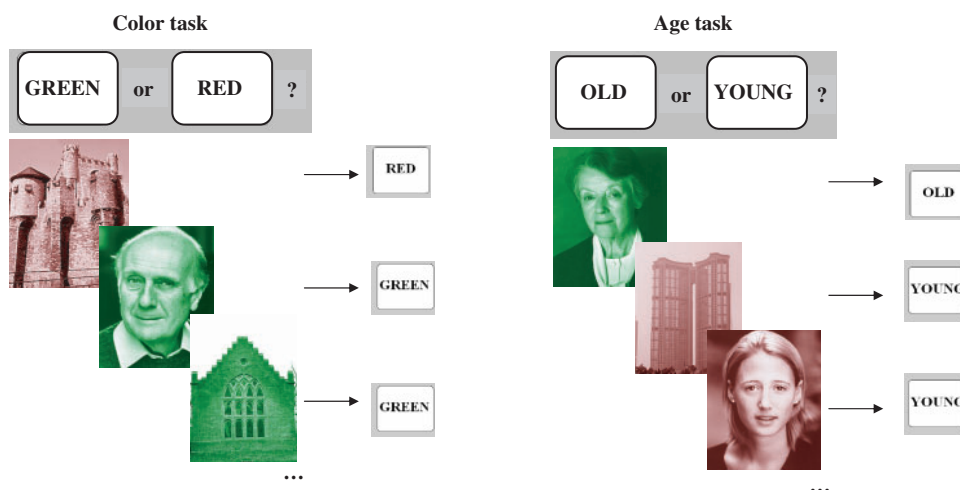


Fig. 1 In the scanner, subjects performed one of two tasks in block-fashion: Colour task: subjects were prompted as to whether the image was red or green; Age task: subjects were prompted as to whether the depicted object was old or young/modern. Face and building-stimuli occurred with equal frequency in each task. Subjects were reminded of the key-press meanings prior to each stimulus.

stimuli comprising any session were counterbalanced across subjects for task, treatment and group.

Responses were recorded by one of two possible button-presses made with the right-hand. The SOA was 4.05 s (between onsets of successive images), with each image being presented for 1 s. A reminder of the button meanings for that block preceded each image. Subjects were taught and practiced the tasks with repeating stimuli 60 min prior to scanner entry (at each session) for as long it took them to achieve a stable performance. A short practice run was also performed before each block in the scanner. Images were presented at central fixation and subtended 5° vertically and 3° horizontally. Subjects were fitted with appropriate MRI-compatible refractive lenses where required to correct their visual acuity (i.e. for individuals who would normally wear spectacles). Eye position was monitored with an infra-red eye tracker (ASL Model 540, Applied Science Group Co., Bedford, MA; refresh rate = 60 Hz) in 16 control and 11 AD subjects during scanning. Saccade frequency was 0.8% in controls and 1% in patients. There were no interactions of eye-movement with stimulus-type, task, treatment or group, so eye position is not considered further.

Treatment

A double-blind placebo-controlled drug administration technique was used. Each subject received an intravenous cannula into the left cubital fossa and an infusion of either physostigmine or saline, depending on session. In the drug-session, subjects first received 0.2 mg intravenous glycopyrrolate (peripheral muscarinic receptor antagonist) before being administered an infusion of physostigmine at a rate of 1 mg/h. Testing took place at 25 min from the start of the infusion. In the placebo-session, an equivalent volume of saline was administered in all steps. We employed a lower dosage of physostigmine relative to our previous studies (Bentley *et al.*, 2003, 2004) that had used subjects aged between 20 and 30 since a pilot study showed an unacceptably high level of adverse effects (predominantly nausea and vomiting in 4/6 subjects) in the age-range of the present study. The dosage and timing schedule of physostigmine that we used was based upon previous studies in which performance improvements were observed over a range of tasks in AD (Mohs and Davis, 1982; Christie *et al.*, 1981; Muramoto *et al.*, 1984; Asthana *et al.*, 1995). Blood pressure was checked before and after scanning, whilst pulse-oximetry was performed continuously. Subjects were given a questionnaire before and after scanning that allowed a ranked measurement (0–6 scale) of seven recognized adverse reactions to physostigmine and glycopyrrolate, as well as visual analogue scales for alertness and physical wellbeing.

Image acquisition

Data were collected on a 1.5T MRI scanner (Siemens, Erlangen, Germany) using gradient echo T2*-weighted echo-planar images, with blood oxygenation level dependent (BOLD) contrast. Volumes consisted of 39 horizontal slices through the whole brain, each 2 mm thick with a 1 mm gap between slices. In-plane resolution was 3 × 3 mm. The effective repetition time (TR) was 3.51 s (note that this is a non-integer multiple of the trial rate, since the SOA between successive stimulus onsets was 4.05 s). Each block entailed 63 volumes being acquired, with the task only beginning after the sixth volume to allow for T1 equilibration effects. Imaging data were pre-processed and analysed using SPM2 (Wellcome Department of Imaging Neuroscience, London;

<http://www.fil.ion.ucl.ac.uk/spm>). Pre-processing consisted of determining and applying rigid affine transformations to the image series to realign the scans (Friston *et al.*, 1995a), normalization (Friston *et al.*, 1995a) to a standard EPI template in MNI space and smoothing with a 3D 8 mm Gaussian kernel to account for residual inter-subject anatomical differences, in accord with the standard SPM approach.

Statistics

Data were analysed with a general linear model for a mixed blocked (task, treatment) and event-related (stimulus type) design (SPM2; Wellcome Department of Cognitive Neurology, London, UK; Friston *et al.*, 1995b) using a random-effects analysis to assess reliability across subjects. Data were globally scaled and high-passed filtered at 1/256 Hz. Events were modelled by delta functions convolved with a synthetic hemodynamic response function (Friston *et al.*, 1998); temporal derivatives of these functions were modelled separately for completeness (Friston *et al.*, 1998). Within-subject conditions of interest were stimulus-type, task and treatment. Stimuli in different scanning-blocks were modelled separately to enable estimation of session effects. 6D head movement parameters derived from image-realignment were included within the model as confounding covariates.

Activity differences between conditions of interest (stimulus-type, task and their interaction) were estimated for each subject and treatment (yielding subject-specific parameter estimates at a first-level of analysis), before being submitted *t*-tests and generation of statistical parametric maps (SPMs) and a second level of analysis, across subjects within a particular group, or between groups. We first report effects for stimulus-selectivity, task and task by stimulus interactions in control subjects in the drug-free state where voxels are significant at $P < 0.05$, corrected (false-discovery rate) based upon a visual cortex mask for stimulus-dependent effects, or on the whole-brain volume for any task effects. This visual cortex mask was constructed manually using MRICRO software (www.mricro.com) and the combined-group mean EPI image so as to encompass the entire occipital, temporal and parietal lobes but excluding somatosensory and auditory cortices. This mask encompassed regions of activation from our previous study employing similar stimulus classes (Bentley *et al.*, 2003). The interaction of task × stimulus was constrained by further masking with simple effects of stimulus-selectivity at each task level (thresholded at $P < 0.01$, uncorrected), to isolate any task effects upon stimulus-selective regions. In the task analysis, the threshold was dropped to $P < 0.001$, uncorrected, to explore any effects in prefrontal cortex (an *a priori* region of interest—see also Furey *et al.*, 2000—that did not reach significance at the conservative whole-brain-corrected level here). Having identified regions showing primary effects (stimulus, task and stimulus × task) in drug-free controls, we then interrogated these same areas (thresholded at $P < 0.01$, uncorrected) for drug effects and/or differences between group for those effects; assessing treatment in each group separately, and a treatment-by-group interaction (reported at $P < 0.001$, uncorrected). For completeness, we also report regions that showed *enhanced* stimulus and/or task-effects in AD relative to controls, at $P < 0.001$ uncorrected. Group-effects were overlaid on mean-normalized functional images of the appropriate group(s) to enable anatomical localization.

We note that in so far as many of the expected brain activations are in visual extrastriate cortex our interpretation of activation differences between treatments or groups is relatively immune

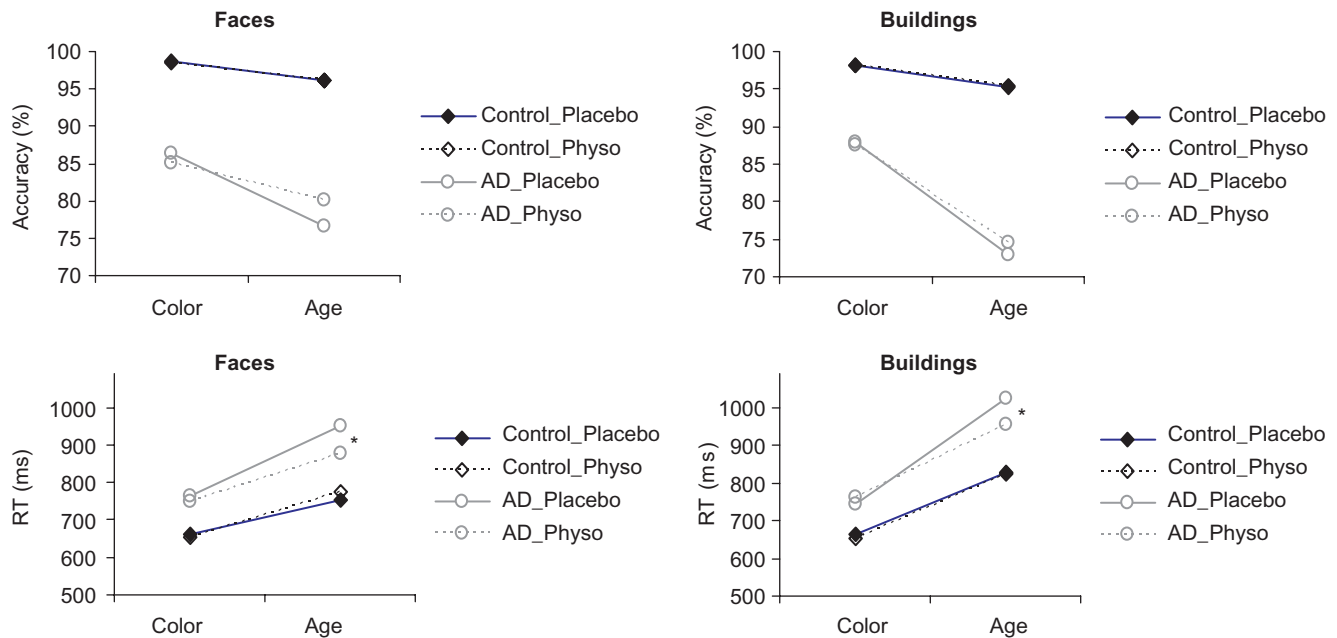


Fig. 2 RT and accuracy responses separated by stimulus-type and task for each combination of group and treatment. *Denotes significant task \times treatment interactions for the AD group ($P < 0.05$).

to confounding explanations in terms of uninteresting differences in performance (e.g. reaction time). Nevertheless, in order to reduce the risk of performance confound of BOLD effects (that would be mostly relevant in frontoparietal regions; see Honey *et al.*, 2000), we repeated the random-effects analyses whilst including individual RTs (or drug-induced RT differences, as appropriate) as a separate regressor in an ANCOVA design (Dannhauser *et al.*, 2005) for those contrasts where a behavioural effect was found (namely between groups for task-independent effects, and between treatments for task-dependent effects in AD).

Results

Behavioural

RT and accuracy were submitted to between-subject (controls *versus* AD) repeated-measures ANOVAs with factors of stimulus (building, face), task (Colour, Age), and treatment (placebo, physostigmine); see Fig. 2. For both RT and accuracy, there were main effects of task [$F(1,31) > 24$, $P < 0.01$], group [$F(1,31) > 4$, $P < 0.05$] as well as a task by group interaction for accuracy [$F(1,31) = 9$, $P < 0.01$] reflecting a greater performance cost within AD relative to control subjects for the Age task relative to the Colour task [task effect in AD: $F(1,15) = 16$, $P < 0.01$; in controls: $F(1,16) = 8$, $P < 0.05$]. The equivalent interaction for RT showed a non-significant trend in the same direction [$F(1,31) = 2$, $P = 0.13$].

The effect of treatment (physostigmine) was evident in a significant interaction of treatment \times group \times task [$F(1,31) = 9$, $P < 0.01$] for RT. Hence, whilst there was no treatment effect on performance in controls, physostigmine in AD shortened RTs for the more demanding Age task [$F(1,15) = 14$, $P < 0.01$] but not for the less demanding Colour task [$F(1,15) = 0$, ns]; $F(1,31) = 10$, $P < 0.01$, for the

treatment \times task interaction. This effect was also present when face and house stimuli were analysed separately ($P < 0.05$ for each stimulus-class; there was no stimulus \times treatment \times group \times task \times stimulus interaction) suggesting that the drug benefit specific to AD for the more demanding task applied for both faces and buildings, even though Age judgements were more difficult for buildings than faces across all subjects {task \times stimulus interaction [$F(1,31) > 4$, $P < 0.05$ for both accuracy and RT]}.

Session effects

Estimates of the mean BOLD signal across session were obtained both for the whole-brain (global) and in specific regions described below as showing stimulus and/or task effects in healthy controls. Neither global nor regional session BOLD estimates were influenced by group or treatment, and there was no interaction between these factors ($P > 0.05$).

There were no effects of drug, time-point, or group, or interactions between these factors on blood-pressure ($P > 0.05$). The only physical side-effects reported after the physostigmine (with glycopyrrolate) session, documented in more than one subject, were nausea (controls: four subjects; AD: four subjects; median severity 1.5/7 within these subjects) and dry mouth (controls: eight; AD: seven; median severity 3/7). Subjective scores of alertness and physical wellbeing both showed an interaction of time-point with treatment ($P < 0.01$) reflecting mean reductions over time by 0.14 and 0.15, respectively (on a scale of 0–1) under physostigmine, compared to 0.05 and 0.03, respectively under placebo. However, there was neither effect of

group nor interaction of group with treatment and time ($P > 0.1$) for either measure. We note that the frequency and type of side-effects associated with the physostigmine session are similar to those reported in our previous studies (Bentley *et al.*, 2003, 2004).

Stimulus-selective regions revealed by fMRI

We next identified regions of extrastriate cortex selective in their response to faces minus buildings, or to buildings minus faces. The main effects of stimulus type in controls under placebo are listed in Table 2 (first column; see also Figs 3A, 3G, 5A) and include regions found in previous studies for corresponding contrasts of faces minus 'houses' (instead of versus buildings more generally, as here), such as right fusiform cortex, and likewise for the reverse comparison (see also Bentley *et al.*, 2003). In AD a similar set of areas were activated (see Figs 3B, 3H, 5B), but a direct comparison of stimulus-selectivity between groups in the drug-free state revealed a subset of these regions for which selectivity for either class of stimuli was reduced in AD relative to controls, but not vice versa (Table 2, second column; Figs 3E, I). In order to control for any performance differences in behavioural latency between groups, we repeated the group \times stimulus contrasts but now including individual reaction time, RT, as a covariate (and thus regressing out separately RT differences *per se*): this did not significantly alter the fMRI results: Z -scores changed only slightly from 4.19 to 3.88 (precuneus) and 3.94 to 3.52 (parahippocampal cortex). Thus the fMRI results did not trivially reflect differences in reaction times.

In controls, physostigmine reduced both face- and building-selectivity in many of the regions that had been identified in controls under placebo (third column; Figs 3C, J). In AD (fourth column), physostigmine modulated stimulus-selectivity in one or other of two ways that reflected whether or not there had been a difference in stimulus-selectivity between AD and controls in the drug-free state. In right fusiform cortex, the region showing the strongest face-selectivity in untreated normals, and also where there was no difference between groups in stimulus-selectivity ($P > 0.1$; peak coordinate in AD being 40, 54, -24 ; $Z = 4.26$), physostigmine in AD resulted in a similar *decrease* of stimulus-selectivity to that observed in controls (Fig. 3D, M—first graph). By contrast, in precuneus (face-selective), and right posterior parahippocampal cortex (building-selective), where untreated AD showed reduced selectivity relative to untreated controls, physostigmine resulted instead in an *increased* selectivity in AD (Figs 3F, K, M—second and third graphs), ameliorating this relative abnormality. Consequently, the latter two regions responded to physostigmine in an opposite manner when comparing controls and AD, as demonstrated by the group \times treatment \times stimulus-selectivity interactions for them (Table 2, final column; Fig. 3L).

Task effects independent of stimulus type revealed by fMRI

The contrast of the more demanding Age-task minus the less demanding Colour-task in controls for the drug-free state yielded strong activation within right posterior parietal cortex (Table 3—first column; Fig. 4A). At a less conservative statistical threshold ($P < 0.001$, uncorrected for whole-brain) there were also activations of right dorsolateral, left inferior and inferomedial prefrontal cortices; there was no effect of stimulus-type in these areas ($P > 0.5$). In AD, the Age-task minus Colour-task contrast highlighted bilateral posterior parietal cortices (46, -56 , 52; -40 , -70 , 42; $Z > 4.07$; Fig. 4B). However, right parietal, left prefrontal and superomedial prefrontal cortex were less activated by this contrast in AD than in controls (group \times task interaction under placebo; second column; Fig. 4C). There were no regions for which task effects were greater in AD than controls in the placebo state.

Physostigmine in controls resulted in reduced task effects (for Age minus Colour) in both right parietal and left prefrontal cortex (treatment \times task interaction; third column; Fig. 4D). Simple-effects analysis revealed that this reflected both a drug-induced increase for the Colour task and a decrease for the Age task relative to placebo ($P < 0.05$ for both). Thus, in controls physostigmine rendered the two different tasks more similar, in terms of right parietal and prefrontal activity levels. Importantly, when administered to AD patients, physostigmine had the *opposite* effect: task-dependent activations now increased in right parietal cortex, and also in superomedial prefrontal cortex (treatment \times task interaction; fourth column; Fig. 4E; there was a trend for the same effect in left inferior prefrontal cortex at $P = 0.006$, uncorrected). But this opposite effect was due exclusively to effects during the Age task, i.e. to physostigmine-induced *increases* in activity ($P < 0.05$) in the more demanding Age task, for AD patients. Consequently, regions showing decreases in task effects when comparing AD with controls in the drug-free state were the same areas that showed enhancements in task-related activity following physostigmine in AD. The difference in response to physostigmine between groups as a function of task demand was confirmed in a significant group \times treatment \times task interaction (fifth column; Fig. 4F).

These 3-way interactions in both prefrontal and parietal cortices were not significantly altered when individual RT differences (between tasks and treatments) were modelled as a nuisance variable in an analysis of covariance: Z -scores reduced only slightly, from 4.94 to 4.40 (parietal) and 3.76 to 3.40 (prefrontal cortex). The influence of RT difference between task and treatment (as seen in performance for AD), localized to very different brain areas: right inferior frontal and left middle temporal gyri ($P < 0.0001$, uncorrected). Thus our critical fMRI results cannot be reduced merely to changes in RT performance.

Table 2 Effect of stimulus-type on extrastriate cortex

	Control				Control > AD				Control: Physo > Placebo				AD: Physo > Placebo				(AD > Control) × (Physo > Placebo)			
	x	y	z	Z-score	x	y	z	Z-score	x	y	z	Z-score	x	y	z	Z-score	x	y	z	Z-score
Faces > Buildings																				
R fusiform cortex	42	-50	-22	4.93					46	-48	-18	-3.95	40	-54	-24	-3.74				
R STS/occipital junction	58	-12	-14	4.04	60	-12	-16	3.74												
	46	-18	-20	4.09																
	56	-38	0	3.64																
	62	-54	10	4.00																
	48	-70	0	4.03																
L STS/occipital junction	-54	-66	14	3.92					-48	-66	14	-3.54								
Precuneus/post. cingulate	-6	-56	38	4.45	-8	-50	40	4.19	-14	-56	40	-3.31	-10	-48	30	4.05	14	-46	18	3.32
	6	-48	28	4.34													-12	-48	32	3.12
Buildings > Faces																				
L sup.lat. occipital cortex	-30	-92	12	5.71	-22	-90	12	4.00	-18	-100	22	-3.79					-28	-90	8	3.87
R sup.lat. occipital cortex	32	-82	18	5.56	28	-98	8	3.10												
	34	-96	10	5.12	30	-90	12	3.68												
R parahippocampal cortex	20	-74	-18	6.01	24	-32	-26	4.05	24	-32	-26	-3.74					26	-32	-26	3.35
					20	-74	-16	3.94	18	-76	-16	-4.11	28	-68	-16	3.63	30	-72	-18	4.23
L parahippocampal cortex	-26	-72	-16	5.33	-24	-74	-16	3.99												
					-24	-42	-20	3.62												
R retrosplenial cortex	14	-54	6	5.24																
L retrosplenial cortex	-12	-56	8	4.80	-12	-52	4	3.90												

First column lists effects in controls under placebo; second column lists differences in stimulus-selectivity between controls and AD under placebo. Remaining columns lists regions showing modulation of stimulus-selectivity by physostigmine relative to placebo in controls, AD, and the difference between groups in their response to physostigmine. Interactions with group and treatment are confined to regions showing effects in controls in drug-free state.

Regions listed under control are significant at $P < 0.05$, corrected; interactions within these regions are thresholded at $P < 0.001$, uncorrected. Negative Z-values denote drug effect in opposite direction to that stated (i.e. placebo > physostigmine). STS = superior temporal sulcus.

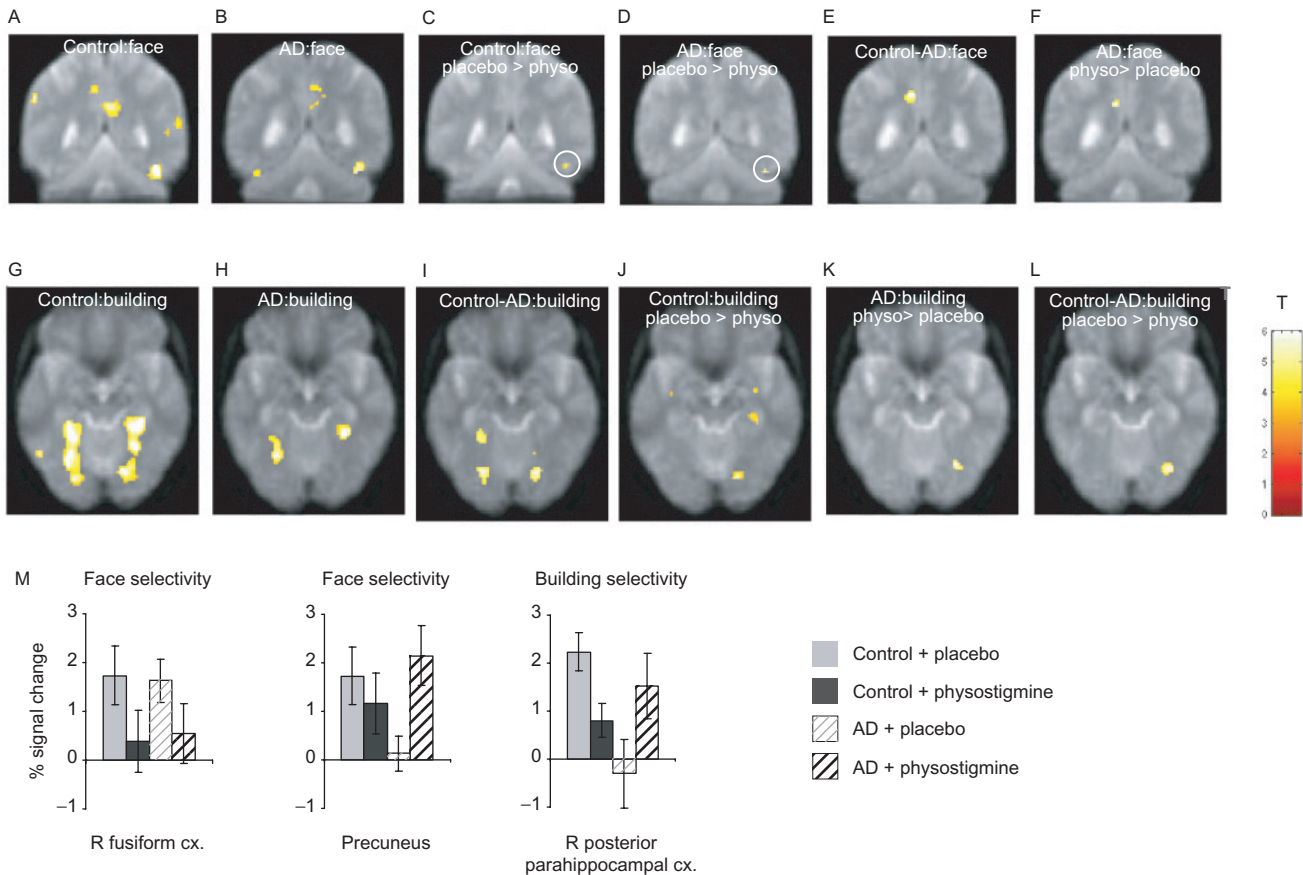


Fig. 3 (A, B)—Main-effect of face > building in controls (A) and AD (B) on placebo at the level of mid-fusiform cortex and precuneus ($y = -50$). (C, D)—Interaction of face-selectivity \times treatment in controls (C) and AD (D) demonstrating *reduced* selectivity in right fusiform cortex with physostigmine in both groups ($y = -50$ and -54 , respectively). There was no between-group difference in face-selectivity or in the interaction of selectivity \times treatment in the right fusiform cortex ($P > 0.1$). (E) interaction of face-selectivity \times group (on placebo) demonstrating reduction of selectivity in AD relative to controls in precuneus. (F) interaction of face-selectivity \times treatment in AD demonstrating *increased* selectivity in precuneus with physostigmine relative to placebo. Main-effect of building > face in controls (G), AD (H) and the difference between them (I), on placebo, at the level of parahippocampal cortices ($z = -16$), demonstrating reduction of selectivity in AD relative to controls in posterior parahippocampal cortex. (J, K, L) Interaction of building-selectivity \times treatment in controls (J) and AD (K) demonstrating that physostigmine induces a *reduction* of selectivity in controls (J) but an *increase* in selectivity in AD (K) in right posterior parahippocampal cortex. L depicts the interaction of building-selectivity \times treatment \times group. (M) Plots of %-signal change for face > building contrast in right fusiform cortex and precuneus, and for building > face contrast in right posterior parahippocampal cortex, under each combination of treatment and group. Coordinates plotted are those at the maxima of selectivity \times treatment interaction in controls (first graph); and selectivity \times treatment in AD (second and third graphs). Activations are thresholded at $P < 0.001$, uncorrected, and are superimposed on the mean normalized EPI of controls or patients as appropriate (group interactions are overlaid on patients' mean).

Task \times stimulus-selectivity interactions revealed by fMRI

We next tested for brain regions where stimulus-selectivity was modified by task. In the control group, under placebo, face-selectivity was enhanced for the Age- *versus* Colour-task in right posterior superior temporal sulcus (pSTS), while building-selectivity was increased in left posterior occipital cortex for the same comparison (Table 3—first column; Fig. 5C). There were no regions in which stimulus-selectivity was greater with Colour than Age. In AD, the effect of task on selectivity in these two regions was less than that in controls (second column; Fig. 5D, E), due predominantly to *diminutions* of stimulus-selectivity for

the Age task in particular for both regions ($P < 0.05$), although right pSTS also showed an additional AD-associated increase in selectivity with the colour task (again $P < 0.05$).

Physostigmine in controls attenuated the effect of task on stimulus-selectivity in the same two regions (third column; Fig. 5F), due to relative increase in selectivity for the Colour task ($P < 0.05$) rather than exclusive decrease in selectivity with the Age task ($P > 0.1$). By contrast, in the AD group, physostigmine increased stimulus-selectivity in both areas when comparing Age to Colour tasks (fourth column; Fig. 5G), effectively restoring a similar relationship between task and stimulus selectivity for these regions as observed in controls in the drug-free state. This drug effect on AD

Table 3 Effects of task independent of stimulus-type (first row section) and task on stimulus-selectivity effects (second row section)

	Control				Control > AD				Control: Physo > Placebo				AD: Physo > Placebo				(AD > Control) × (Physo > Placebo)			
	x	y	z	Z-score	x	y	z	Z-score	x	y	z	Z-score	x	y	z	Z-score	x	y	z	Z-score
Task (independent of stimulus)																				
R posterior parietal cortex	52	−58	48	5.48	36	−50	60	3.14												
	46	−42	58	4.82	44	−42	60	4.81	56	−34	56	−4.78	42	−42	60	3.76	44	−42	60	4.94
	38	−54	62	3.87	58	−32	50	3.27	32	−60	62	−4.33					32	−58	62	3.42
R dorsolateral PFC	44	12	54	3.58																
L inferior PFC	−54	20	16	3.47	−54	16	14	3.40	−54	20	16	−4.00	−58	18	12	2.50*	−58	20	14	3.76
Task × (Faces > Buildings)																				
R posterior STS	60	−64	12	4.80	56	−68	12	5.23	56	−66	10	−3.46	56	−70	14	3.24	58	−66	12	4.29
	54	−58	8	3.62																
	48	−54	12	3.43																
Task × (Buildings > Faces)																				
L lateral occipital cortex	−24	−100	2	5.01	−24	−98	2	4.43	−28	−102	4	−3.46	−26	−100	0	3.88	−26	−100	2	4.64
	−36	−94	6	3.80																

Task effects were observed for Age > Colour, but not vice versa for both stimulus-dependent and stimulus-independent effects. First column lists effects in controls under placebo; second column lists task-effect differences between controls and AD under placebo (i.e. group × task × stimulus and group × task interactions). Third and fourth columns list task-effects showing modulation by physostigmine relative to placebo in each group (i.e. treatment × task × stimulus and treatment × task interactions); fifth column lists the between-group comparison of these treatment effects. Interactions with group and treatment are confined to regions showing effects in controls in drug-free state.

Regions listed under control are significant at $P < 0.05$, corrected, except for PFC regions that were significant at $P < 0.001$ uncorrected (*a priori* region of interest); interactions within these regions are thresholded at $P < 0.001$, uncorrected, except for for which $*P = 0.006$, uncorrected. Negative Z-values denote drug effect in opposite direction to that stated (i.e. placebo > physostigmine). PFC = prefrontal cortex.

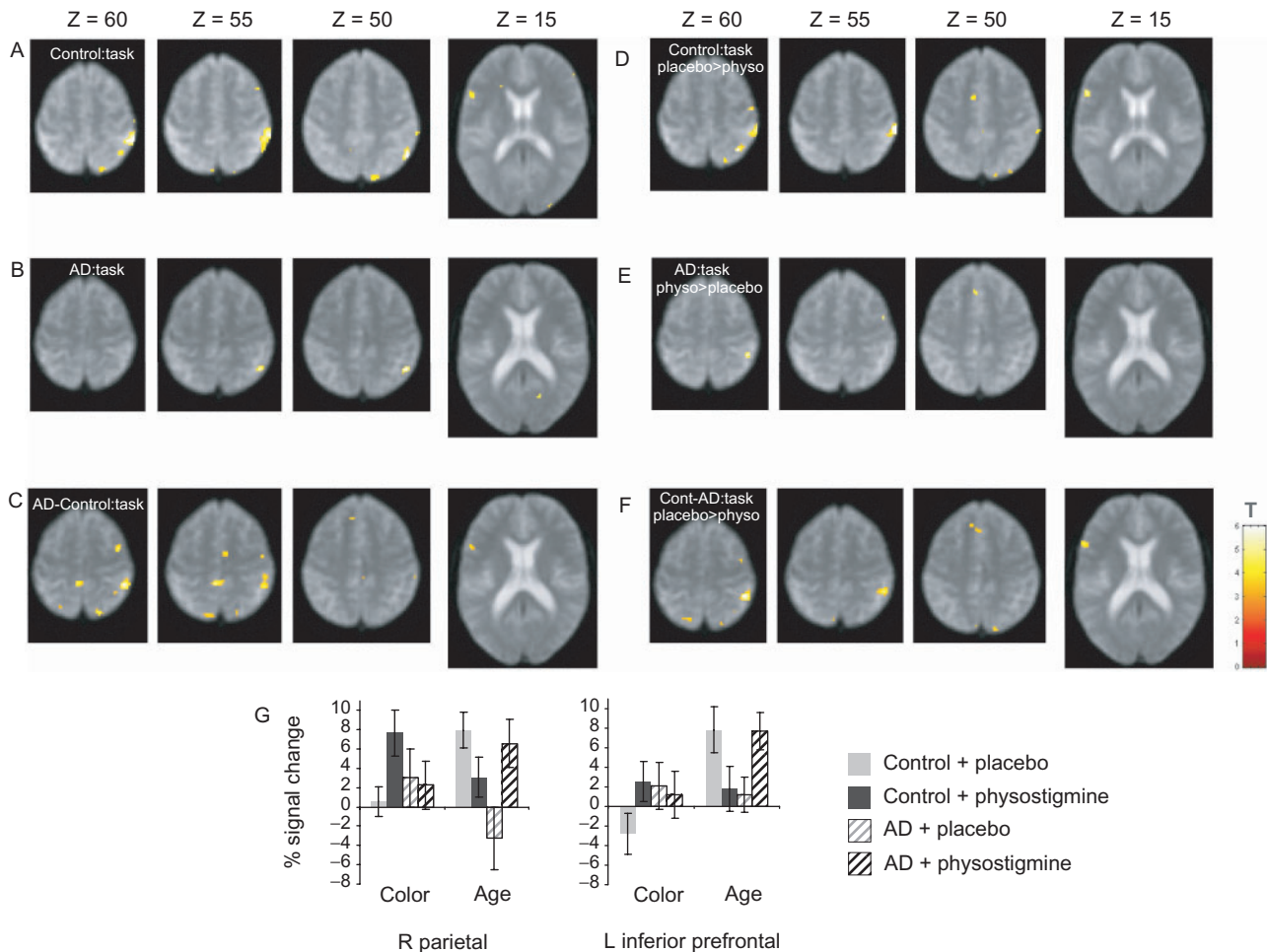


Fig. 4 (A, B, C) Main-effect of task (Age > Colour) in controls (A), AD (B), and the difference between them (C), on placebo. There were no interactions with stimulus-type in regions shown ($P > 0.1$). (D, E, F) Interaction of task \times treatment in controls (D), AD (E) and the difference between them (F): regions shown are those in which the task-effect is decreased by physostigmine relative to placebo in controls (D) but increased by physostigmine relative to placebo in AD (E). (G) Plots of %-signal change for Colour and Age tasks, for each treatment and group at the maxima for the 3-way interaction (from F). Activations are thresholded at $P < 0.001$, uncorrected, and are superimposed on the mean normalized EPI of controls or patients as appropriate (group interactions are overlaid on patients' mean).

reflected an increase in stimulus-selectivity for the Age tasks in both regions ($P < 0.05$), together with a decrease in selectivity for the Colour tasks in right pSTS ($P < 0.05$). The effect of physostigmine on the task \times stimulus-selectivity interaction was therefore opposite between controls and AD, manifest as a strong group \times treatment \times task \times stimulus interaction in both regions ($P < 0.0001$, uncorrected; Fig. 5H; covarying out any RT differences between task and treatment again did not significantly affect results: Z-scores reduced only slightly, from 4.29 to 4.22 (posterior-STs) and 4.64 to 4.31 (LOC). A subject-based correlation analysis of the task \times stimulus \times treatment interaction in the above two extrastriate regions, with the task \times treatment interaction identified in right parietal cortex, in AD, identified a significant correlation with the right pSTS ($r = 0.50$, $P < 0.05$); but not left posterior occipital region ($r = -0.05$).

The AD group also showed distinct patterns of stimulus-selectivity \times task interactions compared to controls when untreated. Left lateral occipital cortex showed enhanced face-selectivity under Age-versus Colour-tasks (-38 , -74 , 8 ; $Z = 4.93$; $P < 0.05$, corrected; Fig. 5I); while right superior occipital cortex showed enhanced building-selectivity under Age versus Colour (36 , -86 , 18 ; $Z = 3.90$; $P < 0.0001$, uncorrected). The former region differed significantly from controls who did not demonstrate task-modulation of selectivity in this area (group \times task \times stimulus interaction: $Z = 5.79$; $P < 0.05$ corrected). When physostigmine was administered to AD this region lost its task-dependency (treatment \times task \times stimulus interaction: $Z = 3.01$; $P = 0.001$ uncorrected), reverting to the control pattern. Controls were uninfluenced by physostigmine in this area (group \times treatment \times task \times stimulus interaction: $Z = 3.87$; $P < 0.001$ uncorrected; Fig. 5J; third graph).

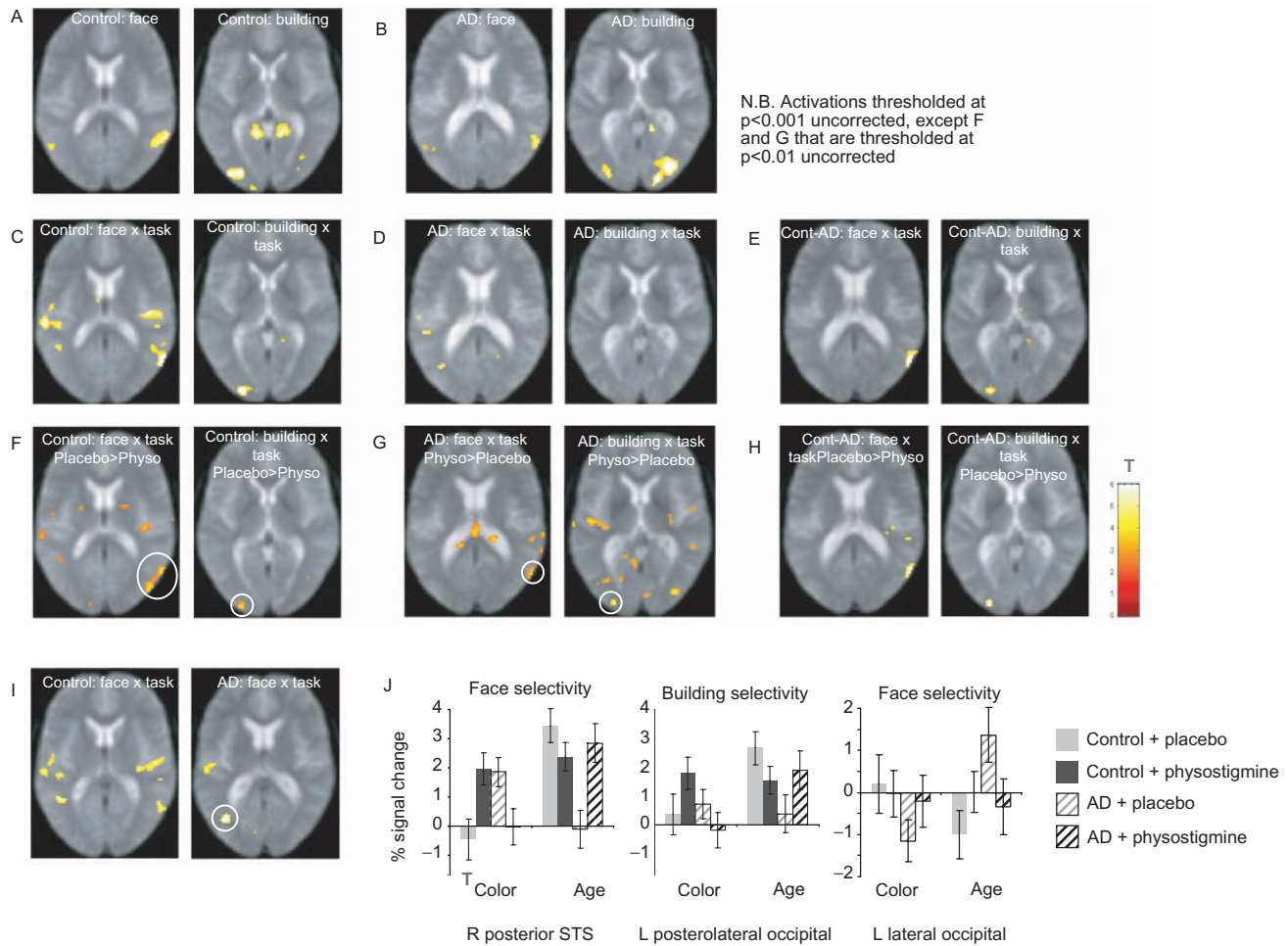


Fig. 5 (A, B) Main-effect of face > building (first slice) and building > face (second slice) stimuli in control (A) and AD (B) subjects on placebo. The slices chosen include regions which additionally show interactions with task, treatment and group as illustrated below. Regions shown for face-selectivity are bilateral posterior STS ($z = +12$); and for building-selectivity are lateral occipital and retrosplenial cortices ($z = +2$). (C, D, E) Interaction of stimulus-selectivity \times task in controls (C), AD (D) and the difference between them (E) on placebo: regions shown are those in which Age relative to Colour task results in greater face-versus-building (first slice) and building-versus-face (second slice) responses. (F, G, H) Interaction of stimulus-selectivity \times task \times treatment in controls (F), AD (G) and the difference between them (H): circled regions are those in which task-enhancements of face- and building- selectivity are decreased by physostigmine relative to placebo in controls (F) but increased by physostigmine relative to placebo in AD (G). (I) Interaction of stimulus-selectivity (face > building) \times task in controls (first slice) and AD (second slice); region circled shows greater task task-modulation of stimulus-selectivity in AD relative to controls, that itself is cholinergic dependent ($z = +8$; see text). (J) Plots of %-signal change for face > building (first and third graphs) and building > face (second graph) contrasts, under each task, treatment and group at the maxima for the 4-way interaction (from H; first two graphs) and at the maximum task \times stimulus interaction in AD (from I; third graph). Activations are thresholded at $P < 0.001$, uncorrected, except for F and G that are thresholded at $P < 0.01$, uncorrected, and are superimposed on the mean normalized EPI of controls or patients as appropriate (group interactions are overlaid on patients' mean).

Discussion

We examined how stimulus-selectivity and attention-related brain activations differ between AD and healthy controls, and whether these differences are susceptible to modulation by physostigmine. We found that (i): AD patients showed impaired stimulus-selectivity in extrastriate visual cortices that was partially reversed with physostigmine in precuneus and parahippocampal cortex. Right fusiform cortex, by contrast, showed an equivalent level of face-selectivity in AD as in controls, and was negatively modulated by physostigmine in a manner that matched controls;

(ii): AD subjects, relative to controls, were more impaired in performance of the Age than Colour discrimination task, which corresponded to reduced task-dependent activity in right parietal and prefrontal cortices. Physostigmine resulted in a task-specific improvement in performance and an increase in task-related activity for right parietal cortex (as well a trend for this in left prefrontal cortex). Similarly, the normal pattern of task-dependent modulation of stimulus-selectivity (i.e. greater for Age than Colour tasks) was also reduced in AD in two extrastriate regions, yet partially restored with physostigmine; while (iii): controls

showed negative effects of physostigmine on brain activations that, in the case of task-influences, were partly due to augmented activity levels during the less-demanding task.

Stimulus-selectivity

Psychophysical and functional imaging studies in mild-to-moderate AD have demonstrated defects in both early and late stages of visual processing (Cronin-Golomb *et al.*, 1991; Pietrini *et al.*, 2000; Prvulovic *et al.*, 2002; Tippett *et al.*, 2003). Considering that early sensory cortices are relatively spared from degeneration until the disease becomes advanced, one possible (though not necessarily exclusive) explanation for this impaired performance is a deficiency of cholinergic input from basal forebrain to sensory regions (Mesulam, 2004a). In invasive animal work, stimulus-selective responses of occipital neurons have been shown to be influenced either positively or negatively by cholinergic enhancers or antagonists, respectively (e.g. Sato *et al.*, 1987, Murphy and Sillito, 1991), that may reflect the role of acetylcholine in promoting visual-feature detection or signal-to-noise ratios in sensory processing (Hasselmo and Giocomo, 2006). We had predicted that AD patients may show an impaired level of stimulus-selectivity that would partly be correctible with physostigmine. We tested this using a robust fMRI measure of category-specific brain responses, concerning higher-order visual processing in extrastriate cortex, that may be more likely to detect disparities between AD and controls than when using very simple visual stimuli (Mentis *et al.*, 1998; Dannhauser *et al.*, 2005). Our results show that functional stimulus-selectivity of extrastriate cortical regions is indeed diminished in AD relative to controls. In two of the affected areas—precuneus and parahippocampal cortex—physostigmine increased and thus to some extent restored stimulus-selectivity in AD. This may be consistent with cholinergic deficiency being, at least in part, responsible for some of the visual processing deficits reported in AD, although it should be noted that drugs like physostigmine may have both direct and indirect actions.

Whereas superior occipital, precuneus and parahippocampal cortices showed impaired stimulus-selectivity in AD relative to controls, activation in right fusiform cortex—the region showing the strongest face-selective responses, was unaffected by disease. This finding is consistent both with previous functional imaging studies in AD that demonstrate a relatively greater attenuation of activations in dorsal parieto-occipital (Prvulovic *et al.*, 2002) and medial parietal (Bradley *et al.*, 2002) than temporo-occipital areas; and also with an association of AD with atrophy in medial more than lateral temporal structures (Fox *et al.*, 2001). Our finding that functionally-impaired posterior parahippocampal and precuneus regions showed stimulus-selectivity increases with physostigmine, while functionally-intact fusiform cortex showed the control pattern of a decrease, may reflect a region-specific loss of functional cholinergic cortical inputs in

AD (Geula and Mesulam, 1989, Geula and Mesulam, 1996) and/or regional variations in cortical AD neuropathology (Arnold *et al.*, 1991). The pattern of neurofibrillary tangle distribution found in AD—significantly worse in inferior temporal regions than medial parietal and peristriate regions—does not exactly correlate with the detrimental BOLD pattern and responsiveness to physostigmine that we observed, suggesting that regional differences in cholinergic (as well as non-cholinergic) inputs to these cortical regions in AD may also contribute for our findings. The current resolution of the most comprehensive topographical map of cholinergic fibre-degeneration published in AD (Geula and Mesulam, 1996) does not allow as yet exact cross-referencing with the specific areas of activation that we found. We note that precuneus was also the region showing the strongest enhancement following treatment with another cholinesterase inhibitor, galantamine, in a visual working memory task in patients with mild cognitive impairment (MCI) in a recent fMRI study by another group (Goekoop *et al.*, 2004).

Attention: frontoparietal effects

Whilst amnesia is the hallmark of AD, attentional impairments are now well described even in early stages of the disease (Perry *et al.*, 2000; Baddeley *et al.*, 2001; Levinoff *et al.*, 2005). Furthermore, whereas the memory impairments in AD seem to derive largely from selective atrophy of medial temporal structures (Fox *et al.*, 2001), the attentional defects of AD most likely reflect a deficiency of input—both cortico-cortical and cholinergic—to areas that are relatively intact structurally (Perry and Hodges, 1999). This seems consistent with observations that cholinesterase inhibitors can improve attention more than memory scores in AD (Sahakian *et al.*, 1987, 1988, 1993; Lawrence and Sahakian, 1995; Blin *et al.*, 1998; Foldi *et al.*, 2005); and that lesions to basal forebrain cholinergic neurons can induce deficits in visual-attention more than memory tasks (Everitt and Robbins, 1997; Kirkby and Higgins, 1998) that may be reversed with cholinesterase inhibition (Balducci *et al.*, 2003). One of the principal aims of our study was to test whether AD-associated impairments in attention, at the levels of both behavioural manifestations and fMRI activations, can be influenced by physostigmine. A key finding was that AD patients showed relatively greater impairment in both performance and also frontoparietal activations during a more attention-demanding task (the deeper ‘Age’ judgement), than for a less demanding task (the more shallow ‘Colour’ judgement). Both these abnormalities—behavioural and task-related BOLD activations—were significantly attenuated following administration of physostigmine. The fMRI results could not be trivially reduced to confounds of performance change *per se*, as shown when covarying out effects associated with RT differences. These results clearly show for the first time that attentional abnormalities in early AD

can be modulated by cholinesterase inhibition in a manner that relates to levels of activity in frontoparietal cortex.

The strongest task-related activation in our design was seen in right parietal cortex, a region well known to show impaired activation in AD during attentional paradigms (Nestor *et al.*, 1991; Buck *et al.*, 1997; Prvulovic *et al.*, 2002; Hao *et al.*, 2005). We expected this region to show particular sensitivity to physostigmine given a wealth of cholinergic animal studies, largely using visuo-spatial paradigms, which show a critical dependency of attention on cholinergic inputs to parietal cortex (Sarter *et al.*, 2001). As well as replicating previous findings of impaired task-related attention in right parietal cortex for AD, we now show for the first time that physostigmine can restore a normal pattern of task-dependent parietal activation.

A similar, albeit less strong, pattern of treatment-dependent modulation of task-dependent activity was found in pre-frontal cortex. Recent fMRI studies in mild AD / MCI have also shown hypoactivation of left pre-frontal regions during attentional demands, such as divided attention (Dannhauser *et al.*, 2005), visual search (Hao *et al.*, 2005) and working memory tasks (Saykin *et al.*, 2004), the latter of which similarly found reversibility following cholinesterase inhibition.

Attention: extrastriate effects of task on stimulus selectivity

A putative role of the cortical cholinergic system is in regulating a balance between executive-attentional top-down control of processing on the one hand, and bottom-up, stimulus-driven processing on the other (Sarter *et al.*, 2005a; Yu and Dayan, 2005). Cholinergic inputs to frontoparietal cortex are necessary for selective visual attention (Sarter *et al.*, 2005a) that involves a preferential facilitation of task-relevant stimulus-encoding. Since frontoparietal activity in AD is impaired during attentional tasks (see above), we predicted a 'knock-on' detrimental effect in the attentional-modulation of extrastriate cortex; furthermore, we predicted that this would also be sensitive to physostigmine. To test this we chose two visual tasks that differed in a top-down manner for the required level of processing (shallow *versus* deep, for the colour-*versus* age-tasks, respectively), while keeping the bottom-up stimulus inputs (faces or building) equivalent for the two tasks. Controls showed face-selectivity that was modulated by task (stronger for the deeper task) in right pSTS; while right fusiform cortex was unaffected by task—in broad agreement with the distinct roles ascribed to different face-sensitive regions of extrastriate cortex (Haxby *et al.*, 2000). Building-selectivity was modulated by task in early visual regions (approximately V2/3) that encode for features such as orientations and angles, and are often activated by houses *versus* faces (e.g. see Bentley *et al.*, 2003) in addition to more anterior regions.

A crucial finding was our observation that physostigmine in AD enhanced the degree to which stimulus-selectivity

was favoured by the Age- relative to the Colour-task within the same regions (right pSTS and left posterior occipital cortices) that showed impaired levels of selectivity in untreated AS. Hence, the action of cholinesterase inhibition within these extrastriate regions was neither upon overall baseline activity there, nor on the main-effect of stimulus type, but specifically upon top-down influences upon stimulus selectivity (i.e. at the interface of top-down and bottom-up processing). This might reflect the diffuse innervation pattern of cortical cholinergic neurones (Sarter *et al.*, 2001), which can lead to cholinergic dependence in both higher-level (e.g. frontoparietal) and lower-level (e.g. visual) areas. The drug- and group-dependent profiles of task-related activity in frontoparietal regions were similar to that seen in these extrastriate visual regions. Moreover, the response of one extrastriate region (pSTS) correlated in its response profile with that for right parietal cortex (across AD subjects).

Effects of physostigmine in controls

A striking aspect of our results was the finding that the influence of physostigmine on stimulus-selectivity and/or task-related responses was often opposite between AD and controls. Thus, physostigmine impaired stimulus-related and task-related activity in controls, but restored a more normal pattern for this in AD subjects. However, the reduction of attentional effects by physostigmine in controls was predominantly due to an enhancement of stimulus-selectivity during the *low-attention* task; whereas AD patients showed impaired attention-dependent neural responses, and a partial restoration of these effects with physostigmine, primarily during the *high-attention* task. Combining both results may suggest that a normal level of acetylcholine is required both for frontoparietal and sensory cortex facilitation specifically during attention-demanding conditions; whereas excessive acetylcholine enhances the same functional responses during low-attention conditions that do not normally engage such areas. The latter finding would be in keeping with previous studies from our group showing physostigmine-induced reductions in top-down sensory modulation in normals (Bentley *et al.*, 2003; Thiel *et al.*, 2002; Bentley *et al.*, 2004; see also Sahakian, 1988; Thiel *et al.*, 2005), due primarily to excessive cortical activation during task-irrelevant conditions. The observation that either deficient or excessive levels of a neuromodulator may cause detrimental pattern of neural processing is not unexpected, considering that similar effects have often been described with other neuromodulators e.g. the 'inverted-U' function for prefrontal dopaminergic levels affecting working memory performance (Williams and Castner, 2006). Furthermore, our results in controls provide support for models of anxiety (Bernston *et al.*, 1998) and psychosis (Sarter *et al.*, 2005b) that envisage heightened cortical acetylcholine levels being responsible for the finding of abnormally exaggerated processing of stimuli within such psychiatric states.

Non-specific effects of physostigmine and limitations

Pharmacological fMRI studies must always consider (Blin *et al.*, 1998; Tsukada *et al.*, 2004) the possible impact of drug treatment on metabolism, blood-flow and neurovascular coupling, and how this might impact differentially on two distinct groups, as for the AD patients and healthy controls here. But in this respect it is noteworthy that here we found baseline BOLD levels did not differ between treatments or between groups at the level of whole brain, nor within the regions that exhibited task \times group and/or treatment interactions. Furthermore, the profile of drug effects on event-related BOLD activity that we found, including the pattern of ‘cross-over’ effects—where drug enhanced activity during one condition but decreased it during another in the same voxel—implies a role for the cognitive factors of interest. We can also discount explanations in terms of nonspecific drug-induced effects on alertness or side-effects, since both groups were affected equally along these dimensions, in contrast to the effects of interest that were often conceptually opposite between groups.

Physostigmine acts on cholinergic pathways throughout the brain, including the basal forebrain–neocortex connection as well as within the thalamus, striatum and brainstem (including effects on other neuromodulatory nuclei; Mesulam, 2004b). Although our whole-brain imaging results demonstrate pharmacological modulation of stimulus and task-specific brain activity within cerebral cortex, we cannot discern the extent to which this is caused by *direct* modulation of the basal forebrain–neocortical system, rather than via *indirect* pharmacological influences (e.g. acting through subcortical pathways). However, we suggest that our results are most likely to be accounted for in terms of a direct, rather than indirect, action of physostigmine, because: (i) the basal forebrain is by far the most affected cholinergic structure in mild-to-moderate AD (Mesulam, 2004a) and would therefore be expected to provide some anatomical basis for a normalization of cerebral activity following cholinergic enhancement, and (ii) the cortical-cholinergic system has been shown in animal studies to modulate *selective* cortical responses to complex stimuli or task instructions (as we have observed here), as opposed to *non-selective* alerting-arousal responses that are influenced more by subcortical cholinergic structures (Sarter *et al.*, 2001). Since our AD group were mild in severity it is likely that there would have been sufficient residual cortical cholinergic neurons for physostigmine to have had a cholinergic-enhancing action (Geula and Mesulam, 1996), although we note that rises in cortical acetylcholine following cholinesterase inhibition have only been directly demonstrated in animal models of dementia (e.g. Tsukada *et al.*, 2004).

Finally, we suggest that our results may sidestep several potential confounding factors that often unavoidably affect clinical fMRI studies. First, while it is likely that cerebral

atrophy in our AD group (appreciable on comparing the mean T_2^* images between groups in our figures) can explain some of the hypoactivations observed (e.g. Teipel *et al.*, 2007), our finding that physostigmine was able partially to overcome this deficit, suggests that the abnormal activations were often functional rather than purely structural, in keeping with the known cortical cholinergic deficiency in AD (Geula and Mesulam, 1996). To the extent that physostigmine-induced reversibility was incomplete, and that many impaired cortical responses in AD that did *not* significantly improve following physostigmine, it is possible that this arose from significant atrophy in these regions. Second, since our study concentrated on physostigmine effects on stimulus and attentional processing within extrastriate cortex—i.e. sensory areas—it seems unlikely that the differences between groups and/or treatments reflect trivial non-specific differences in performance (e.g. motor-related, or RT dependent). Where we did find a treatment effect in parietal cortex—namely, heightened activation following physostigmine—this was associated with *shorter* RTs in AD, which is the opposite effect to what would be expected if RT difference was the only cause for this (as parietal activation is associated with *longer* RTs *per se*: Honey *et al.*, 2000). There was also no difference in eye movements between groups or treatments that could account for our results. Finally, group and treatment effects in frontoparietal areas, as well as extrastriate visual cortices, remained significant even when partialling out any drug effects on reaction time *per se* (see also Honey *et al.*, 2000; Dannhauser *et al.*, 2005).

Conclusions

We show that mild Alzheimer disease patients have impairments in both visual- and attentional-related cortical activations that in a number of regions are partially reversible with physostigmine. The results provide new evidence for an association between the recognized central cholinergic deficiency of AD; dysfunctional cortical processing, and certain aspects of cognitive impairment seen in AD. Furthermore, our ability to demonstrate responsiveness of functionally-impaired brain activations to an acute drug challenge raises the clinical possibility of using pharmacological fMRI to select patients or disease subtypes for particular treatments (Matthews *et al.*, 2006). Finally, we show that physostigmine administration to healthy controls may itself disturb visual-attentional processing, in a differential manner to that observed in AD.

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Conflict of interest statement. None declared.

References

- Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW. The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb Cortex* 1991; 1: 103–16.
- Asthana S, Raffaele KC, Berardi A, Greig NH, Haxby JV, Schapiro MB, et al. Treatment of Alzheimer disease by continuous intravenous infusion of physostigmine. *Alzheimer Dis Assoc Disord* 1995; 9: 223–32.
- Auld DS, Kornecook TJ, Bastianetto S, Quirion R. Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. *Prog Neurobiol* 2002; 68: 209–45.
- Baddeley AD, Baddeley HA, Bucks RS, Wilcock GK. Attentional control in Alzheimer's disease. *Brain* 2001; 124: 1492–508.
- Balducci C, Nurra M, Pietropoli A, Samanin R, Carli M. Reversal of visual attention dysfunction after AMPA lesions of the nucleus basalis magnocellularis (NBM) by the cholinesterase inhibitor donepezil and by a 5-HT1A receptor antagonist WAY 100635. *Psychopharmacology (Berl)* 2003; 167: 28–36.
- Bentley P, Husain M, Dolan RJ. Effects of cholinergic enhancement on visual stimulation, spatial attention, and spatial working memory. *Neuron* 2004; 41: 969–82.
- Bentley P, Vuilleumier P, Thiel CM, Driver J, Dolan RJ. Cholinergic enhancement modulates neural correlates of selective attention and emotional processing. *Neuroimage* 2003; 20: 58–70.
- Berntson GG, Sarter M, Cacioppo JT. Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. *Behav Brain Res* 1998; 94: 225–48.
- Blin J, Ivanoiu A, De Volder A, Michel C, Bol A, Verellen C, et al. Physostigmine results in an increased decrement in brain glucose consumption in Alzheimer's disease. *Psychopharmacology (Berl)* 1998; 136: 256–63.
- Bradley KM, O'Sullivan VT, Soper ND, Nagy Z, King EM, Smith AD, et al. Cerebral perfusion SPET correlated with Braak pathological stage in Alzheimer's disease. *Brain* 2002; 125: 1772–81.
- Buck BH, Black SE, Behrmann M, Caldwell C, Bronskill MJ. Spatial- and object-based attentional deficits in Alzheimer's disease: relationship to HMPAO-SPECT measures of parietal perfusion. *Brain* 1997; 120: 1229–44.
- Christie JE, Shering A, Ferguson J, Glen AI. Physostigmine and arecoline: effects of intravenous infusions in Alzheimer presenile dementia. *Br J Psychiatry* 1981; 138: 46–50.
- Conner JM, Chiba AA, Tuszynski MH. The basal forebrain cholinergic system is essential for cortical plasticity and functional recovery following brain injury. *Neuron* 2005; 46: 173–9.
- Cronin-Golomb A, Corkin S, Rizzo JF, Cohen J, Growdon JH, Banks KS. Visual dysfunction in AD: relation to normal aging. *Ann Neurol* 1991; 29: 41–52.
- Dannhauser TM, Walker Z, Stevens T, Lee L, Seal M, Shergill SS. The functional anatomy of divided attention in amnesic mild cognitive impairment. *Brain* 2005; 128: 1418–27.
- Everitt BJ, Robbins TW. Central cholinergic systems and cognition. *Annu Rev Psychol* 1997; 48: 649–84.
- Foldi NS, White RE, Schaefer LA. Detecting effects of donepezil on visual selective attention using signal detection parameters in Alzheimer's disease. *Int J Geriatr Psychiatry* 2005; 20: 485–8.
- Fox NC, Crum WR, Scahill RI, Stevens JM, Janssen JC, Rossor MN. Imaging of onset and progression of Alzheimer's disease with voxel-compression mapping of serial magnetic resonance images. *Lancet* 2001; 358: 201–5.
- Friston K, Ashburner J, Frith AD, Poline JB, Heather JD, Frackowiak RSJ. Spatial registration and normalization of images. *Hum Brain Mapp* 1995a; 2: 165–89.
- Friston K, Holmes AP, Worsley K, Poline JB, Frith C, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. *Hum Brain Mapp* 1995b; 2: 189–210.
- Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg MD, Turner R. Event-related fMRI: characterizing differential responses. *Neuroimage* 1998; 1: 30–40.
- Furey ML, Pietrini P, Haxby JV. Cholinergic enhancement and increased selectivity of perceptual processing during working memory. *Science* 2000; 290: 2315–9.
- Geula C, Mesulam MM. Cortical cholinergic fibers in aging and Alzheimer's disease: a morphometric study. *Neuroscience* 1989; 33: 469–81.
- Geula C, Mesulam MM. Systematic regional variations in the loss of cortical cholinergic fibers in Alzheimer's disease. *Cerebral Cortex* 1996; 6: 165–77.
- Goekoop R, Rombouts SARB, Jonker C, Hibbel A, Knol DL, Truyen L, et al. Challenging the cholinergic system in mild cognitive impairment: a pharmacological fMRI study. *Neuroimage* 2004; 23: 1450–9.
- Hao J, Li K, Li K, Zhang D, Wang W, Yang Y, et al. Visual attention deficits in Alzheimer's disease: an fMRI study. *Neurosci Lett* 2005; 385: 18–23.
- Hasselmo ME, Giocomo LM. Cholinergic modulation of cortical function. *J Mol Neurosci* 2006; 30: 133–6.
- Haxby JV, Hoffman EA, Ida Gobbini M. The distributed human neural system for face perception. *Trends Cogn Sci* 2000; 4: 223–33.
- Honey GD, Bullmore ET, Sharma T. Prolonged reaction time to a verbal working memory task predicts increased power of posterior parietal cortical activation. *Neuroimage* 2000; 12: 495–503.
- Kirkby DL, Higgins GA. Characterization of perforant path lesions in rodent models of memory and attention. *Eur J Neurosci* 1998; 10: 823–38.
- Lawrence AD, Sahakian BJ. Alzheimer disease, attention, and the cholinergic system. *Alzheimer Dis Assoc Disord* 1995; 9 Suppl 2: S43–9.
- Levinoff EJ, Saumier D, Chertkow H. Focused attention deficits in patients with Alzheimer's disease and mild cognitive impairment. *Brain Cogn* 2005; 57: 127–30.
- Matthews PM, Honey GD, Bullmore ET. Applications of fMRI in translational medicine and clinical practice. *Nat Rev Neurosci* 2006; 7: 732–44.
- Mentis MJ, Alexander GE, Krasuski J, Pietrini P, Furey ML, Schapiro MB, et al. Increasing required neural response to expose abnormal brain function in mild versus moderate or severe Alzheimer's disease: PET study using parametric visual stimulation. *Am J Psychiatry* 1998; 155: 785–94.
- Mesulam M. The Cholinergic Lesion of Alzheimer's Disease: Pivotal Factor or Side Show?: a review. *Learn Mem* 2004a; 11: 43–9.
- Mesulam M. The cholinergic innervation of the human cerebral cortex. *Prog Brain Res* 2004b; 145: 67–78.
- Mohs RC, Davis KL. A signal detectability analysis of the effect of physostigmine on memory in patients with Alzheimer's disease. *Neurobiol Aging* 1982; 3: 105–10.
- Murphy PC, Sillito AM. Cholinergic enhancement of direction selectivity in the visual cortex of the cat. *Neuroscience* 1991; 40: 13–20.
- Nestor PG, Parasuraman R, Haxby JV, Grady CL. Divided attention and metabolic brain dysfunction in mild dementia of the Alzheimer's type. *Neuropsychologia* 1991; 29: 379–87.
- Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease. A critical review. *Brain* 1999; 122: 383–404.
- Perry RJ, Watson P, Hodges JR. The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: relationship to episodic and semantic memory impairment. *Neuropsychologia* 2000; 38: 252–71.
- Pietrini P, Alexander GE, Furey ML, Dani A, Mentis MJ, Horwitz B, et al. Cerebral metabolic response to passive audiovisual stimulation in

- patients with Alzheimer's disease and healthy volunteers assessed by PET. *J Nucl Med* 2000; 41: 575–83.
- Prvulovic D, Hubl D, Sack AT, Melillo L, Maurer K, Frolich L, et al. Functional imaging of visuospatial processing in Alzheimer's disease. *Neuroimage* 2002; 17: 1403–14.
- Rizzo M, Anderson SW, Dawson J, Nawrot M. Vision and cognition in Alzheimer's disease. *Neuropsychologia* 2000; 38: 1157–69.
- Rogers SL, Farlow MR, Doody RS, Mohs R, Friedhoff LT. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Donepezil Study Group. *Neurology* 1998; 50: 136–45.
- Sahakian BJ. Cholinergic drugs and human cognitive performance. In: Iversen LL, Iversen SD, Snyder SH, editors. *Handbook of Psychopharmacology of the Aging Nervous System*. London: Plenum Press; 1988. p. 393–423.
- Sahakian B, Joyce E, Lishman WA. Cholinergic effects on constructional abilities and on mnemonic processes: a case report. *Psychol Med* 1987; 17: 329–33.
- Sahakian BJ, Owen AM, Morant NJ, Eagger SA, Boddington S, Crayton L, et al. Further analysis of the cognitive effects of tetrahydroaminoacridine (THA) in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB. *Psychopharmacology (Berl)* 1993; 110: 395–401.
- Salmond CH, Chatfield DA, Menon DK, Pickard JD, Sahakian BJ. Cognitive sequelae of head injury: involvement of basal forebrain and associated structures. *Brain* 2005; 128: 189–200.
- Sarter M, Givens B, Bruno JP. The cognitive neuroscience of sustained attention: where top-down meets bottom-up. *Brain Res Brain Res Rev* 2001; 35: 146–60.
- Sarter M, Hasselmo ME, Bruno JP, Givens B. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Brain Res Rev* 2005a; 48: 98–111.
- Sarter M, Nelson CL, Bruno JP. Cortical cholinergic transmission and cortical information processing in schizophrenia. *Schizophr Bull* 2005b; 31: 117–38.
- Sato H, Hata Y, Masui H, Tsumoto T. A functional role of cholinergic innervation to neurons in the cat visual cortex. *J Neurophysiol* 1987; 58: 765–80.
- Saykin AJ, Wishart HA, Rabin LA, Flashman LA, McHugh TL, Mamourian AC, et al. Cholinergic enhancement of frontal lobe activity in mild cognitive impairment. *Brain* 2004; 127: 1574–83.
- Teipel SJ, Bokde AL, Born C, Meindl T, Reiser M, Möller HJ, et al. Morphological substrate of face matching in healthy ageing and mild cognitive impairment: a combined MRI-fMRI study. *Brain* 2007; 130: 1745–58.
- Thiel CM, Bentley P, Dolan RJ. Effects of cholinergic enhancement on conditioning-related responses in human auditory cortex. *Eur J Neurosci* 2002; 16: 2199–206.
- Thiel CM, Zilles K, Fink GR. Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. *Neuropsychopharmacology* 2005; 30: 810–20.
- Tippett LJ, Blackwood K, Farah MJ. Visual object and face processing in mild-to-moderate Alzheimer's disease: from segmentation to imagination. *Neuropsychologia* 2003; 41: 453–68.
- Tiraboschi P, Hansen LA, Alford M, Sabbagh MN, Schoos B, Masliah E, et al. Cholinergic dysfunction in diseases with Lewy bodies. *Neurology* 2000; 54: 407–11.
- Tsukada H, Nishiyama S, Fukumoto D, Ohba H, Sato K, Kakiuchi T. Effects of acute acetylcholinesterase inhibition on the cerebral cholinergic neuronal system and cognitive function: functional imaging of the conscious monkey brain using animal PET in combination with microdialysis. *Synapse* 2004; 52: 1–10.
- Wilkinson DG, Doody RS, Black SE, Salloway SP, Schindler RJ. Donepezil in vascular dementia: combined analysis of two large-scale clinical trials. *Dement Geriatr Cogn Disord* 2005; 20: 338–44.
- Williams GV, Castner SA. Under the curve: critical issues for elucidating D1 receptor function in working memory. *Neuroscience* 2006; 139: 263–76.
- Yu AJ, Dayan P. Uncertainty, neuromodulation, and attention. *Neuron* 2005; 46: 681–92.