doi:10.1093/brain/awp176 Brain 2009: 132; 2356–2371 | 2356

BRAIN
A JOURNAL OF NEUROLOGY

Modulation of fusiform cortex activity by cholinesterase inhibition predicts effects on subsequent memory

P. Bentley,[1,2] J. Driver[1,3] and R. J. Dolan[1]

1 Wellcome Centre for Neuroimaging at UCL, University College London, London WC1N 3BG, UK
2 Deparment of Clinical Neuroscience, Charing Cross Hospital, Imperial College London, London, W6 8RF, UK
3 UCL Institute of Cognitive Neuroscience, University College London, London WC1N 3AR, UK

Correspondence to: Paul Bentley,
Department of Clinical Neuroscience,
Charing Cross Hospital,
Imperial College London,
Fulham Palace Road,
London,
W6 8RF, UK
E-mail: p.bentley@imperial.ac.uk

Cholinergic influences on memory are likely to be expressed at several processing stages, including via well-recognized effects of acetylcholine on stimulus processing during encoding. Since previous studies have shown that cholinesterase inhibition enhances visual extrastriate cortex activity during stimulus encoding, especially under attention-demanding tasks, we tested whether this effect correlates with improved subsequent memory. In a within-subject physostigmine versus placebo design, we measured brain activity with functional magnetic resonance imaging while healthy and mild Alzheimer's disease subjects performed superficial and deep encoding tasks on face (and building) visual stimuli. We explored regions in which physostigmine modulation of face-selective neural responses correlated with physostigmine effects on subsequent recognition performance. In healthy subjects physostigmine led to enhanced later recognition for deep- versus superficially-encoded faces, which correlated across subjects with a physostigmine-induced enhancement of face-selective responses in right fusiform cortex during deep- versus superficial-encoding tasks. In contrast, the Alzheimer's disease group showed neither a depth of processing effect nor restoration of this with physostigmine. Instead, patients showed a task-independent improvement in confident memory with physostigmine, an effect that correlated with enhancements in face-selective (but task-independent) responses in bilateral fusiform cortices. Our results indicate that one mechanism by which cholinesterase inhibitors can improve memory is by enhancing extrastriate cortex stimulus selectivity at encoding, in a manner that for healthy people but not in Alzheimer's disease is dependent upon depth of processing.

Keywords: fMRI; cholinergic; Alzheimer's disease; physostigmine; memory

Introduction

Among its numerous cognitive impacts, the basal forebrain—neocortical cholinergic system exerts important influences on sensory processing (Everitt and Robbins, 1997; Sarter *et al*., 2005). For example, acetylcholine release in sensory cortices enhances stimulus-evoked responses (Sato *et al*., 1987); modifies stimulus selectivity (Sillito and Kemp, 1983), and alters the configurations of sensory representation maps (Weinberger, 2007). Indeed, the ability of acetylcholine to influence plasticity mechanisms within

Received December 5, 2008. Revised May 13, 2009. Accepted May 22, 2009. Advance Access publication July 15, 2009

sensory cortices during stimulus encoding—in addition to its separate actions on the hippocampus—has been proposed to contribute to the well-established effects of acetylcholine on memory (Kirkwood *et al*., 1999; Boroojerdi *et al*., 2001; Gu, 2003; Hasselmo and McGaughy, 2004; Schon *et al*., 2005). The present study was designed to test this hypothesis by examining whether effects of cholinesterase inhibitors on processing in higher sensory cortex processing, for healthy subjects and in mild Alzheimer's disease (Furey *et al*., 2000; Rombouts *et al*., 2002), may be directly related to its effects on subsequent memory (Davis *et al*., 1978; Davis and Mohs, 1982).

Previous functional imaging studies using visual paradigms have shown that pro-cholinergic drugs increase stimulus-driven extrastriate visual cortex responses in a task-dependent fashion (Furey *et al*., 2000; Lawrence *et al*., 2002; Bentley *et al*., 2003, 2004). In a similar way, we note from psychopharmacological studies that the pro-mnemonic effects of cholinergic-enhancing drugs are also related to encoding task, with a greater memory improvement noted for stimuli that have undergone 'deep' relative to 'shallow' processing (Rusted and Warburton, 1992; Warburton *et al*., 2001; Fitzgerald *et al*., 2008). In other words, cholinergic manipulation interacts with the well-recognized depth-of-processing effect on memory (Craik and Tulving, 1975; Baddeley, 1990). Here, we sought to bridge these two effects, by testing whether cholinergic enhancement of task-dependent activity in visual extrastriate cortex relates to the impact on subsequent memory. We predicted that the cholinergic enhancer physostigmine would increase memory selectively for deeply relative to shallowly encoded faces, and, critically, that this would correlate with the degree to which physostigmine enhances face-selective fusiform cortex activity during the deep- relative to shallow-encoding task.

A further question we addressed was whether effects of cholinesterase inhibition on the relationship between face encoding and subsequent recognition differ between healthy older subjects and patients with Alzheimer's disease. Previous studies in Alzheimer's disease have shown impaired extrastriate visual cortex activation during memory tasks, associated with poor subsequent recall (Machulda *et al*., 2003; Grön and Riepe, 2004; Golby *et al*., 2005; Rombouts *et al*., 2005); while cholinesterase inhibition may reverse impairments in sensory cortex activity (Rombouts *et al*., 2002; Kircher *et al*., 2005; Grön *et al*., 2006). No studies, however, have shown or assessed any direct relationship between enhanced extrastriate cortex activity following cholinesterase inhibitor treatment in Alzheimer's disease and improved subsequent recognition. Furthermore, it remains unknown whether impairments in depth of processing (Bird and Luszcz, 1991; Beauregard *et al*., 2001) or task modulation of sensory cortex activity (Mandzia *et al*., 2004; Gazzaley and D'Esposito, 2007) seen in Alzheimer's disease and ageing, are reversible with pro-cholinergic treatments. Since both pathological (Mesulam, 2004) and pharmacological (Lawrence and Sahakian, 1995) studies have suggested that cholinergic deficits or manipulation produce more impact upon attentional than memory processes, and given that stimulus depth-of-processing effects may partly depend upon attentional processes (Baddeley, 1990), we tested whether effects of cholinesterase inhibition on memory in Alzheimer's disease are dependent upon encoding task.

Finally, given the likely importance of sensory–frontoparietal–hippocampal cortex interactions in memory and depth-of-processing (Celone *et al*., 2006; Rissman *et al*., 2008), we tested in both healthy and Alzheimer's disease groups the relationship between activity in fusiform cortex and that in wider brain regions, and the effects of cholinergic manipulation on such co-variations between areas. An earlier paper (Bentley *et al*., 2008) had studied effects of physostigmine on face- and task-selective responses employing a similar paradigm, but not considering subsequent memory effects.

Methods

Subjects

Eighteen right-handed healthy older subjects (mean age 64.8 ± 4.2; hereon referred to as 'healthy subjects') participated, plus 13 right-handed patients with newly diagnosed Alzheimer's disease [Mini Mental State Examination (MMSE) of 20–26; mean age 64.8 ± 4.4], who were recruited from the Dementia Research Group, National Hospital for Neurology and Neurosurgery (London, UK) over a 16-month period. No subjects were active smokers. Summary characteristics of the two groups are listed in Table 1. We used the Revised National Adult Reading Test (NART-R) test to assess IQ in healthy subjects as previous studies have shown that its score correlates robustly with verbal and performance IQ scores from the Revised Wechsler Adult Intelligence Scale (WAIS-R) (e.g. Schretlen *et al*., 2005) that Alzheimer's disease subjects underwent as part of their clinical management.

All subjects gave written informed consent. The inclusion criteria for patients were probable Alzheimer's disease 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 (DSMIV). Exclusion criteria for patients were (i) if an alternative or additional diagnosis contributing to cognitive symptoms was considered possible; this was assessed following a full neuropsychological, neurological and general clinical examination, as well as dementia-screening blood tests, chest X-ray, brain MRI, electroencephalography and cerebrospinal fluid

Table 1 Summary characteristics of healthy elderly and Alzheimer's disease subjects (±95% CIs)

	Healthy	Alzheimer's disease
Number	18	13
Age	10	9
Males	64.8 (±4.2)	64.8 (±4.4)
Education	12.4 (±0.9)	12.9 (±1.0)
Hypertension	4	5
Baseline blood-pressure	128/75 (±10/6.2)	138/84 (±7.3/4.5)
MMSE	29.4 (±0.4)	23.6 (±1.3)*
Verbal IQ	114 (±2.4)	94.2 (±7.0)*
Performance IQ	114 (±2.3)	94.2 (±9.8)*

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.

examinations (where felt to be appropriate for diagnosis); (ii) mild cognitive impairment; (iii) major visuospatial or visuo-perceptual impairment or severe apraxia; (iv) coexistent significant central nervous system disease, e.g. no epilepsy, movement disorder, head injury, drug nor alcohol abuse; and (v) receiving psychoactive drugs, including cholinesterase inhibitors, *N*-methyl-D-aspartate antagonist, or anti-depressants. Patients or healthy subjects found to have significant lesions on brain MRI (other than Alzheimer's disease-associated changes in the case of the Alzheimer's disease group) such as ischaemic changes were excluded.

All patients were started on therapeutic oral cholinesterase inhibitor following the second experimental session, and were followed up for a minimum of 1 year to ensure that no other features developed that would suggest an alternative cause for dementia other than Alzheimer's disease.

Design of paradigm

On each of two sessions (placebo or physostigmine), subjects performed two tasks of varied processing depth. For the shallow task they judged the Colour (C) of colour-washed red or green faces or building stimuli. For the deep task they judged instead the Age (A; young/old) of comparable face or building stimuli. The two tasks were separated into blocks of 48 trials each, and repeated once each session (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 presentations of different single faces or buildings (randomly intermingled in an event-related fashion) with no image being repeated across sessions. The images for both tasks were presented in red or green monochrome. The 'shallow' Colour task required reporting (by one of the two possible button presses) whether an image was red or green; the 'deeper' Age task required a judgement of whether the particular face or building currently shown was old or young (the latter choice denoting 'modern' in the case of buildings), again by either of the same two possible button presses The stimulus set comprised an equal number of 'young' (individuals aged 21–35 years) and 'old' faces (individuals aged over 65 years), 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 depicted from a non-canonical viewpoint, as well as any faces with overtly emotional expressions. The particular stimuli for any session were counterbalanced across subjects for task, treatment and group. Subjects were informed that a recognition test of faces would be carried out after scanning but were instructed simply to perform their best on the within-scanner Colour or Age tasks, rather than trying specifically to memorize items.

Responses were recorded as one of two possible button presses made with the right hand in one of the control subjects these button-press data were lost for technical reasons. The onset asynchrony between successive stimuli was 4.05 s, with each stimulus presented for 1 s. A reminder of the button meanings (and thus task) for that block preceded each image. Subjects practiced the tasks with repeating stimuli 60 min before scanner entry (at each session), until they achieved accurate performance. A short practice run (without scanning) was also performed before each block in the scanner. Stimuli were presented at central fixation and subtended $\sim 5^\circ$ vertically and $\sim 3^\circ$ horizontally. Subjects wore appropriate MRI-compatible refractive lenses if required to correct their visual acuity (i.e. for individuals who would normally wear spectacles). Eye position was monitored during scanning and task performance, with a remote infra-red eye tracker (ASL Model 540, Applied Science Group Co., Bedford, MA, USA; refresh rate = 60 Hz) for 16 control and 11 Alzheimer's disease subjects. Saccades arose on only 0.8% of trials in controls and only 1% in patients. Moreover, there were no interactions of saccade-rate with stimulus-type, task, treatment or group, so eye position was not considered further.

Recognition memory for exposed faces (versus foils) was tested 10 min following the end of the encoding. Subjects were removed from the scanner for testing and sat in front of a laptop computer. Test stimuli were presented singly, and together consisted of the 96 faces that had appeared during the encoding task (presented in the same colour used for either the Colour- or Age-task during exposure), randomly intermixed with 96 foils (equally divided into red and green) that were also presented singly. Thus each trial comprised either a previously shown face or a foil face. The recognition probe stimuli subtended $\sim 7 \times \sim 4^\circ$ visual angle. Subjects were prompted on the screen to say whether they had seen each face or not during the encoding phase, and whether they were confident or not of this judgement. Subjects' verbal responses were recorded by an examiner blind to the test stimuli. Recognition accuracy was scored using a discrimination index (DI) calculated as: p(hit)-p(false alarm) (Snodgrass and Corwin, 1988).

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 that reduces side-effects such as nausea and bradycardia) before being administered an infusion of physostigmine at a rate of 1 mg/h. In the placebo-session, an equivalent volume of saline was administered at all steps. We employed a lower dosage of physostigmine relative to our previous studies of younger normals (Bentley *et al*., 2003, 2004), that had used subjects aged between 20 and 30, since a pilot study showed a higher level of side-effects (predominantly nausea and vomiting in 4/6 subjects) in the age range of the present study. The dosage and timing schedule of physostigmine used was based on previous studies in which performance improvements were observed over a range of tasks in patients with Alzheimer's disease (Christie *et al*., 1981; Davis and Mohs, 1982; Muramoto *et al*., 1984; Asthana *et al*., 1995). The encoding task took place 25 min from the start of the infusion. The infusion was continued until the end of the encoding phase, (i.e. $\sim$45 min from the start of the infusion), but then terminated to minimize drug side-effects and permit subject mobility. The recognition task took place 10 min after termination. Since previous data (Christie *et al*., 1981; Muramoto *et al*., 1984; Asthana *et al*., 1995) indicate a pharmacodynamic half-life for intravenous physostigmine of $\sim$60 min, there will have been significant cholinesterase inhibition during both encoding and recognition phases here.

Blood pressure was checked before and after scanning, and 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 well-being.

fMRI image acquisition

Functional magnetic resonance imaging (fMRI) data were collected during the encoding tasks on a 1.5 T MRI scanner (Siemens, Erlangen, Germany) using gradient echo T_2*-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 (field-of-view, $192 \times 192\,mm^2$; matrix size 64×64). In-plane resolution was 3×3 mm with effective repetition time (TR) 3.51 s; echo time (TE) 50 ms and flip angle 90°. For each block 63 volumes were acquired, with the task only beginning after the sixth volume to allow for T1 equilibration effects.

Image pre-processing

Imaging data were pre-processed and analysed using Statistical Parametric Mapping-2 (SPM2) software (Wellcome Centre for Neuroimaging at UCL; http://www.fil.ion.ucl.ac.uk/spm). This consisted of determining and applying rigid affine transformations to the image series to realign the scans with respect to the first scan (Friston *et al*., 1995). Scans were then normalized to a standard echo-planar imaging (EPI) template (Montreal Neurological Institute) with a resampled voxel size of $3 \times 3 \times 3$ mm (Friston *et al*., 1995), and smoothed using a Gaussian kernel with a full width at half maximum (FWHM) of 8 mm. The same template was used for healthy subjects and Alzheimer's disease in order to allow for unbiased between-group comparison.

Statistics—Behaviour

Behavioural data were analysed with Statistical Package for the Social Sciences (SPSS) software (v16.0). DI scores were entered into mixed analysis of variance (ANOVAs), with task (shallow or deep), treatment (physostigmine or placebo) and recognition confidence (confident or not) as repeated-measure factors, and group (healthy or Alzheimer's disease) as a non-repeat factor. For completeness, performance during initial encoding [Reaction Time (RT) and accuracy for Colour or Age tasks] underwent comparable ANOVAs with the same factors. Treatment order (physostigmine given in first or second session) produced neither main effects nor interactions with other factors, so was not considered further.

Statistics—fMRI

Imaging data were analysed with a general linear model for combined blocked (here, Colour- or Age-task at encoding) and event-related (here, face or building stimuli in a randomly intermingled sequence within each block) factors, using SPM2 with a random-effects approach. Data were globally scaled so as to remove the possibility that between-treatment or between-group effects were caused by any differences in baseline BOLD values, and high-passed filtered at 1/256 Hz. Events were modelled by delta functions convolved with a synthetic haemodynamic 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 any session effects. Six-dimensional head movement parameters derived from image realignment were included within the model as confounding covariates of no interest.

For each of 31 subjects, BOLD differences were estimated for the following contrasts of interest: (i) face selectivity under placebo, i.e. face > building; (ii) physostigmine-induced enhancement of face selectivity, i.e. two-way interaction of treatment × stimulus [physostigmine (face > building)] > [placebo (face > building)]; (iii) task modulation of face selectivity under either treatment, i.e. two-way interaction of stimulus × task under placebo, or physostigmine [age (face > building)] > [colour(face > building)] and (iv) physostigmine-induced enhancement of task modulation of face selectivity, i.e. three-way interaction of treatment × task × stimulus, {physostigmine[age(face > building)] > [colour(face > building)]} > {placebo[age (face > building)] > [colour (face > building)]}.

We next calculated depth-of-processing effects on later behavioural recognition scores for each subject (i.e. DI for deep- minus shallow-encoded faces) under placebo, and the change in this score when comparing physostigmine with placebo. These values for each subject were then correlated respectively with each subject's own BOLD-derived measure of the task × stimulus (under placebo) [contrast (iii), above] and treatment × task × stimulus [contrast (iv), above] interactions, separately for the two groups. Since the Alzheimer's disease group showed a treatment effect on memory that was independent of task, we also correlated subjects' treatment effects on recognition score (i.e. DI for all faces) with subjects' treatment × stimulus BOLD effect [contrast (ii), above], separately for healthy and Alzheimer's disease subjects. Group comparisons of correlation coefficients were performed at the peak estimates for each group using Fisher's *Z*-test (i.e. for balance, we compared between groups the strongest correlations found within each group, rather than the strongest within one against an unselected score for the other). We were guided by behavioural effects of drug on recognition at the group level in deciding whether to use all recognition responses, or instead just confident recognition responses, as the covariate with BOLD activity during the encoding phase. In order to facilitate interpretation of interactions, we limited the search volume to those regions also showing a main effect of face selectivity in the appropriate subject group under placebo (thresholded at $P < 0.001$, uncorrected).

In a separate model, for each subject incorporating the same factors as before (stimulus, task, treatment), we re-classified face stimuli according to whether they were later recalled confidently, recalled non-confidently or forgotten. In this way, we could identify any areas that showed heightened BOLD responses at initial exposure for faces that were later recognized or forgotten, i.e. a 'subsequent-memory' analysis (Rugg *et al*., 2002). This was performed for all recognized faces in healthy subjects, but with a focus on confidently recalled faces in Alzheimer's disease patients, given the specific physostigmine effect that we found on later recognition confidence for this patient group (see below). Interactions of a subsequent-memory effect with task, treatment and group were also performed within those regions also showing a main effect of subsequent memory (thresholded at $P < 0.001$, uncorrected).

Face-selective regions were initially identified by performing a one-sample *t*-test in healthy or Alzheimer's disease subjects separately to generate corresponding statistical parametric maps (SPMs), thresholded at $P < 0.05$, corrected for whole-brain volume (false-discovery rate). Behavioural–BOLD correlations and subsequent memory effects were first explored within 8 mm (i.e. the smoothing kernel) of the fusiform peaks of face selectivity (as identified initially without considering behaviour) for each group. We then explored face-selective regions of interest more widely—namely fusiform gyri and superior temporal sulci (Haxby *et al*., 2000)—that were defined functionally from the face > building statistical parametric map contrast in the corresponding subject group under placebo, itself thresholded at $P < 0.001$, uncorrected (Worsley *et al*., 1996). The medial temporal lobes were also interrogated as regions of interest given their central

role in episodic memory (Rugg *et al*., 2002), and were defined anatomically here (see Rorden and Brett, 2000). We used a conventional statistical threshold of $P<0.001$ (uncorrected) within these regions of interest. The rest of the brain was also examined for these correlations and contrasts, but for those areas we applied a threshold of $P<0.05$ (false-discovery rate; corrected for whole brain). Group effects were overlaid on mean normalized T1 structural images of the appropriate group(s) to enable anatomical localization.

In order to ascertain whether those regions implicated in differences for behavioural–BOLD correlations between healthy subjects and Alzheimer's disease groups also differed in grey matter volume, we analysed T_1-structural images with voxel-based morphometry using SPM5 software (see Mechelli *et al*., 2005). Essentially, this process involves segmenting volumes to extract grey matter; normalizing to an asymmetric T_1-weighted template in Montreal Neurological Institute (MNI) stereotactic space; modulating for total volume changes; smoothing (by 8-mm kernel), for each subject's scan, before applying a two-sample *t*-test to compare healthy subjects with those with Alzheimer's disease.

Finally, we tested for relationships between right fusiform effects of task × stimulus and stimulus and inter-regional co-variation with wider brain regions showing task effects and subsequent-memory effects, respectively. For the first of these connectivity analyses, we first identified regions showing a task effect (Age > Colour) under placebo over all healthy subjects (and separately for Alzheimer's disease), thresholded at $P<0.001$ uncorrected, and smoothed with an 8-mm kernel. Within this predetermined area, we then tested for subregions in which this task effect for individual subjects co-varied with task modulation of face-selective fusiform activity [contrast (iii), above] sampled from the peak of the pharmacological behavioural–BOLD correlation, separately for subject group and treatment. We then compared differences in correlation coefficients between treatments for each group. Similarly, we tested for regions that showed co-variation of a subsequent-memory effect (see above) with face-selective fusiform activity [contrast (i), above] also sampled from the peak of the pharmacological behavioural–BOLD correlations. These results are reported at $P<0.001$ uncorrected, within regions showing a main effect of task or in medial temporal lobe regions of interest (no other brain areas exhibited these correlations when thresholding at $P<0.05$, whole-brain corrected).

The influence of physostigmine on group effects of stimulus selectivity and task modulation independent of subsequent recognition scores are reported in an earlier paper (Bentley *et al*., 2008).

Results

Session effects

We obtained estimates of the mean BOLD signal per session for the whole brain (global) and in functionally defined (face > house) face-selective extrastriate cortical regions. Importantly, neither global (whole-brain) nor regional (face-selective areas) session BOLD estimates were influenced by group or treatment overall, and there was no significant interaction between these factors. This means that the specific results reported later below cannot be a trivial outcome of any non-specific drug or group influences on whole-brain or face-selective BOLD signals. The only side-effects reported in the treatment group in more than one subject were nausea and dry mouth. Blood pressure was unaffected. Subjective scores of alertness and physical wellbeing reduced between beginning and end of session somewhat more for physostigmine than placebo (time × treatment interaction $P<0.05$). There was no effect or interaction concerning group (healthy or Alzheimer's disease) for any of these measures (all $P>0.1$).

Behavioural

The expected difference in attentional demand for deep- versus shallow-encoding tasks was found in control and Alzheimer's disease groups, expressed as slower RTs and decreased accuracy for the Age- versus Colour task ($P<0.01$ for each measure and group). Alzheimer's disease subjects performed worse than controls for both tasks in RT and accuracy [both $F(1,28)>4$, both $P<0.05$]. A task × group interaction arose for accuracy, due to Alzheimer's disease patients showing a greater difference between the two tasks than controls [$F(1,28)=5.5$, $P<0.05$]. Physostigmine led to faster RTs selectively in Alzheimer's disease but not healthy subjects, during the Age but not the Colour task [$F(1,28) = 9.0$, $P < 0.01$].

Recognition memory performance is shown in Fig. 2, separately for all responses and for just confident responses. Healthy subjects demonstrated superior memory to Alzheimer's disease patients (main effect of group) [$F(1,29)=5.4$, $P<0.05$]; dividing up recognition score by encoding task identified a selective group difference for Age-encoded [$t(29)=3.0$; $P<0.01$], but not Colour-encoded faces ($P=0.13$). Furthermore, healthy subjects showed a strong benefit in memory when comparing Age- with Colour-encoding tasks [$F(1,17)=14.2$; $P<0.01$; also significant at $P<0.05$ under each treatment], whereas there was no such effect in Alzheimer's disease patients [$F(1,12)=0.3$, NS; no task effect under either treatment] leading to a significant task × group interaction for recognition memory scores [$F(1,29)=4.4$, $P<0.05$]. Among Alzheimer's disease subjects, there was a trend for a correlation between MMSE scores and recognition memory of deep- versus shallow-encoded faces [$r(12)=5.3$; $P=0.06$]. There was also a confidence × task × group interaction [$F(29,1)=4.9$; $P<0.05$], that reflected healthy subjects showing a task effect for confident ($P<0.01$), but not un-confident judgements, while Alzheimer's disease subjects showed no task effect for either (Fig. 1).

Physostigmine had distinct influences on the impact of encoding task upon memory for healthy subjects versus Alzheimer's disease patients, leading to a three-way group × task × treatment interaction [$F(1,29)=4.5$, $P<0.05$]. In healthy subjects, physostigmine increased the difference in memory between the two types of encoding-task, relative to placebo, specifically enhancing the depth-of-processing effect [$F(17,1)=4.7$, $P<0.05$]. This effect occurred regardless of recognition confidence. In contrast, in Alzheimer's disease patients, there was no effect ($P>0.1$) of physostigmine on task-dependent memory, relative to placebo, i.e. no tendency for it to restore the depth-of-processing effect found in healthy subjects. However, when analyzing only those recognition judgements that Alzheimer's disease patients rated with confidence (see Fig. 2, rightmost graph), we found that physostigmine exerted a beneficial effect on their memory [$F(12,1)=5.2$; $P<0.05$], although this was equivalent for faces

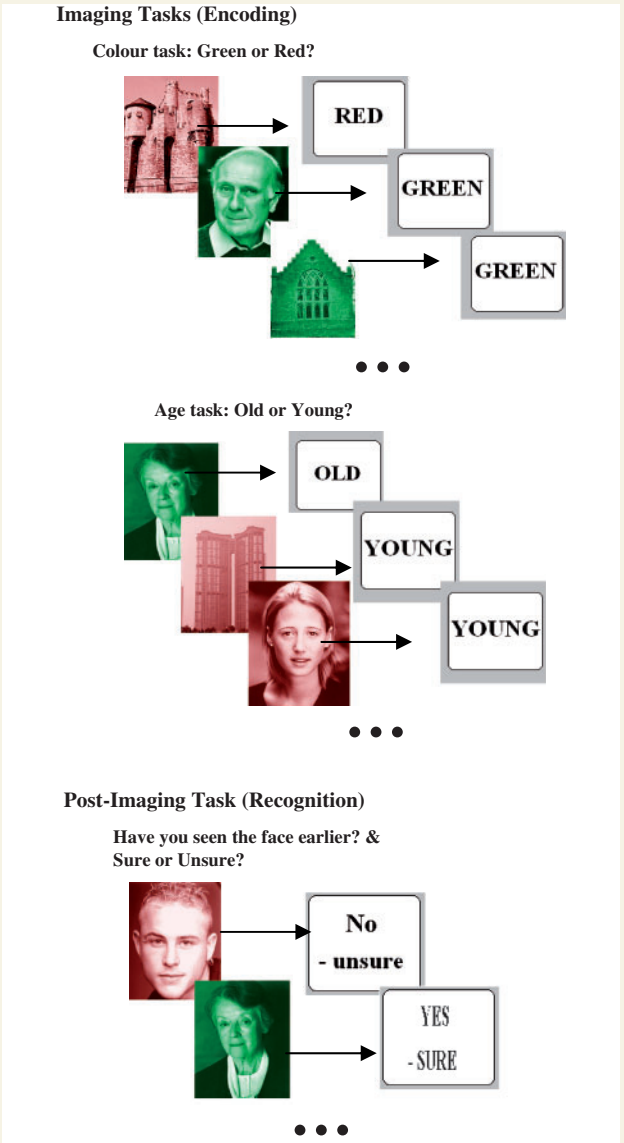

Figure 1 Schematics of tasks performed during *f*MRI scanning (encoding) and afterwards (recognition task).

encoded during the Age- and Colour-task (i.e. there was no task × treatment interaction for the Alzheimer's disease group, $P > 0.1$).

fMRI: Face-selectivity, subsequent memory and depth of processing

Extrastriate cortical regions showing higher BOLD-signals for face than building stimuli in healthy subjects were most apparent in right fusiform cortex (Fig. 3A; Table 2). In Alzheimer's disease patients, the same contrast showed activation of bilateral fusiform cortices (Fig. 4A; Table 2), with no significant group differences in face selectivity (i.e. no interaction of face > building with group, all $P > 0.1$) for fusiform cortex in either hemisphere. Effects of task, treatment and group on face-selective responses that do not take into account individuals' subsequent recognition performance have been reported previously (Bentley *et al*., 2008).

We next investigated the relationship between face-selective fusiform cortex activations during encoding with memory performance post-scanning. Specifically, we tested: (i) whether the strength of fusiform responses to faces was associated with subsequent successful recognition, and (ii) whether task modulation of face-selective responses in this region was associated with task-dependent recognition scores, i.e. the depth of processing memory effect. For the first question, we compared responses with faces that were later correctly recognized to those which were incorrectly rejected later as foils. This 'subsequent memory' contrast in healthy subjects under placebo showed higher BOLD for faces later recognized than forgotten in anterior right fusiform cortex (Fig. 3B; Table 2). The right hippocampus, as an *a priori* anatomical region of interest (Rugg *et al*., 2002), also showed this subsequent memory effect at a lower statistical threshold (28, −4, −24; $Z = 2.10$; $P < 0.05$, uncorrected). In Alzheimer's disease subjects under placebo, there was no such subsequent-memory effect in fusiform cortex for either hemisphere, leading to a between-group difference for this in right fusiform cortex (44, −38, −18; $Z = 3.95$; $P < 0.001$, uncorrected). However, on comparing faces later recognized confidently by Alzheimer's disease patients to those forgotten by them (for which a drug effect

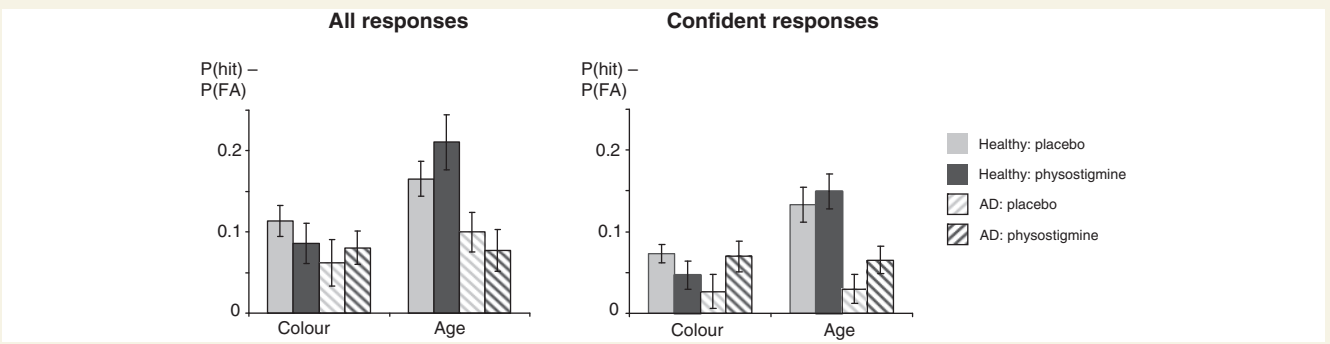

Figure 2 Behavioural recognition results for each group. Discrimination indices [*p*(hit)-*p*(false alarm)] plotted separately for faces that had earlier been encoded during 'shallow' Colour task, or encoded during 'deep' Age task, under placebo or physostigmine, in control or Alzheimer's disease subjects. The left graph scores all recognition responses as hits, while the right graph scores only confident recognition judgements as hits.

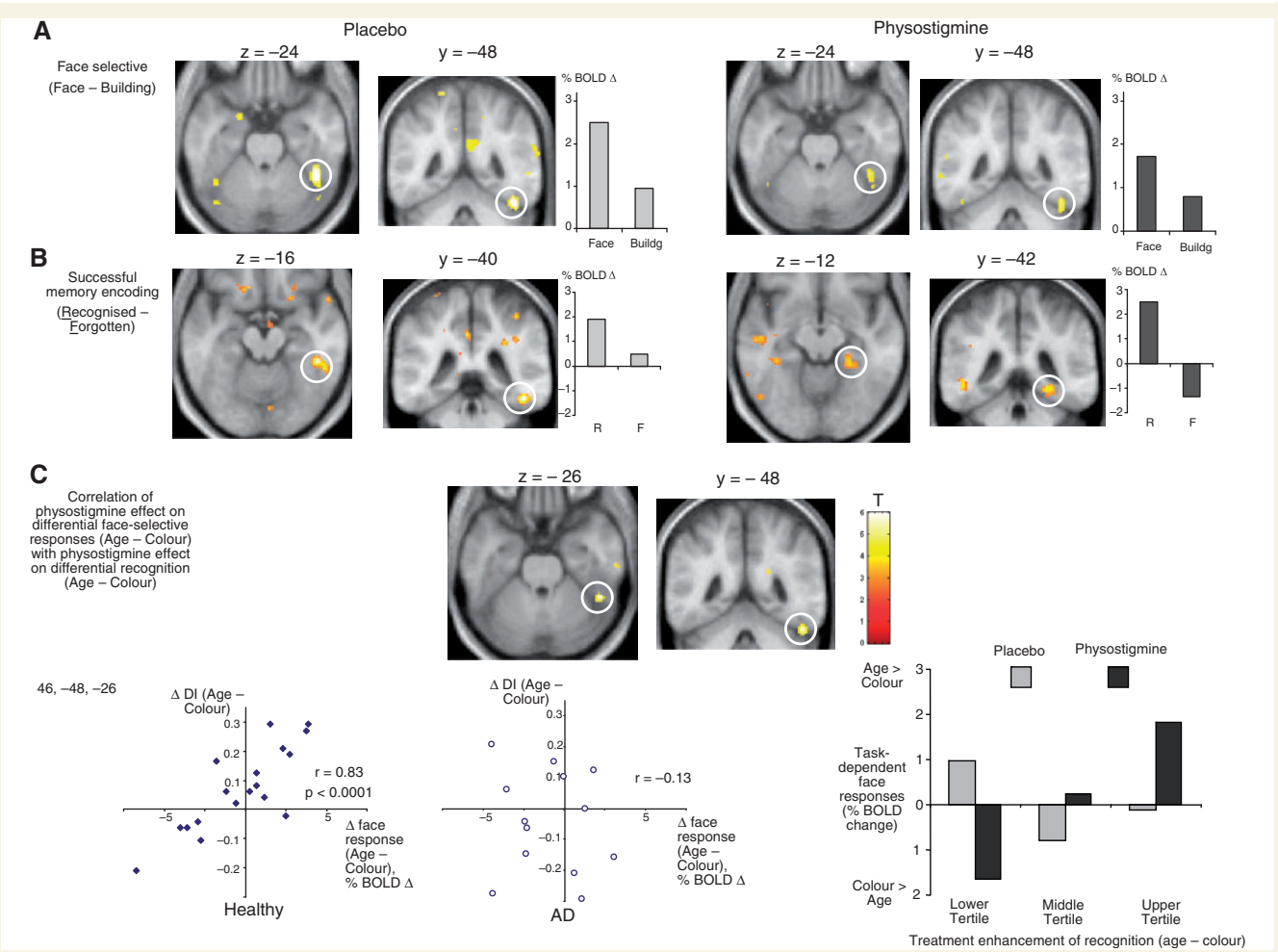

Figure 3 BOLD responses obtained from fMRI scanning during encoding in healthy control participants. (**A**) Face-selective responses (faces > houses) regardless of task during encoding, under placebo, and physostigmine, show strongest activation in right mid-fusiform gyrus. (**B**) Regions where higher BOLD signals during face encoding (independent of task or drug) predict subsequent recognition (i.e. faces reclassified as later recognized or forgotten), under placebo and physotigmine. (**C**) Regions where physostigmine-induced enhancements of task modulation at encoding (i.e. face-selective BOLD responses for deep minus shallow task) correlate with physostigmine-induced enhancements of depth-of-processing effect on later recognition (i.e. discrimination indices for deeply- minus shallowly encoded faces), across healthy participants. Graphs show individual subject scatter plots for this relationship in right fusiform cortex, which was significant in healthy subjects (scatter plot shown at left, with diamond symbols for each healthy participant), but not for the Alzheimer patients (scatter plot shown centrallym with open-circle symbols for each Alzheimer patient). The BOLD–behavioural relation found for healthy controls in right fusiform cortex can also be seen (right bar graph) by dividing subjects into tertile subgroups according to the degree that physostigmine increased memory for Age-encoded relative to Colour-encoded faces. The extent to which physostigmine increased face-selective responses during encoding, specifically for the Age relative to Colour tasks, mirrored the degree to which physostigmine-induced enhancements in the depth-of-processing effect for subsequent memory. SPM contrasts shown are thresholded for display purposes at $P < 0.001$ uncorrected, in A and C, or $P < 0.01$, uncorrected, in **B**, and overlaid on mean T_1-weighted MRI of the healthy subjects. Per cent BOLD signal changes for the conditions making up each contrast are plotted for the peaks in each circled cluster.

had been observed behaviourally—see above), a subsequent-memory effect did emerge for this patient group under placebo in left fusiform cortex, within 8 mm of the local peak of face selectivity for the Alzheimer's disease group (Fig. 4B). The Alzheimer's disease group also showed a subsequent-memory effect in the left hippocampus (−18, −16, −8; $Z = 3.35$; $P < 0.001$, uncorrected) but only under physostigmine. Apart from the right fusiform cortex region mentioned showing a greater subsequent-memory effect for healthy subjects than Alzheimer's disease, there were no other interactions of subsequent memory with task, treatment or group (thresholded at $P < 0.001$, uncorrected in regions of interest; $P < 0.05$ corrected in other brain regions).

For the second question, we examined whether the behavioural improvement in recognition for faces encoded deeply (Age task) relative to faces encoded shallowly (Colour task) found in healthy

Table 2 Co-ordinates in fusiform cortex showing maxima of face-selective and subsequent-memory effects

	Placebo	Z	P	Physostigmine	Z	P
Face-selective effects (face–building)						
Healthy	42, −48, −24	4.92	0.001	42, −48, −22	3.82	<0.05
Alzheimer's disease	44, −52, −30	5.06	<0.01	44, −52, −28	3.75	<0.0001*
	−38, −56, −22	4.68	<0.05	−40, −48, −22	4.14	<0.0001*
Subsequent-memory effects (recognized–forgotten)						
Healthy	44, −40, −18	4.28	<0.0001*	−36, −34, −16	3.25	<0.001*
	50, −46, −14	3.68	<0.001*	24, − 42, −10	3.17	<0.001*
Alzheimer's disease[a]	−44, −56, −24	3.91	<0.0001*	34, −44, −16	3.45	<0.001*
	−42, −36, −14	3.53	<0.001*	−28, −50, −20	2.89	<0.01*

a The Alzheimer's disease group only showed subsequent memory effects using the contrast of confidently recognized–forgotten faces (for which the healthy group did not show effects).
*Significance values are corrected for whole-brain volume (false-discovery rate) except that are reported uncorrected for completeness.

subjects would correlate in a subject-by-subject manner with task modulation of face-selective responses in the extrastriate cortex at encoding. Right fusiform cortex, at the peak of face selectivity identified above in a behaviour-independent manner for healthy subjects under placebo, showed a correlation between task modulation of BOLD signal at exposure, and the behavioural depth-of-processing effect on later recognition [$r(30) = 0.49$; $Z = 2.82$, $P < 0.01$], with no difference between patients and controls ($P > 0.1$). Left fusiform cortex did not show a significant correlation in either group. We note that the extrastriate regions showing the strongest effects for this correlation in healthy subjects were in bilateral superior temporal sulci [60, −38, −2; $r(17) = 0.86$, $Z = 4.52$; and −44, −48, −8; $r(17) = 0.80$, $Z = 3.93$; both $P < 0.0001$, uncorrected]. For these superior temporal regions, Alzheimer's disease subjects failed to show positive correlations [$r(12) = -0.25$ and −0.12, NS] leading to between-group differences in this respect ($Z = 2.56$ or 1.65, $P < 0.05$ or $P < 0.1$, respectively).

Summarizing this section, we found that fusiform cortices in both healthy and Alzheimer's disease groups showed activations that were (i) greater for faces than buildings; (ii) greater for faces subsequently remembered than forgotten; and (iii) greater for faces shown during the deep, relative to the shallow, encoding task in subjects showing a greater depth-of-processing subsequent memory effect.

fMRI: Cholinergic modulation of task-dependent encoding in health

Our principle hypothesis was that physostigmine-induced enhancement of extrastriate visual cortex activations during encoding would relate systematically to effects of physostigmine on subsequent recognition performance. Since in healthy subjects, the behavioural effect of physostigmine on recognition was dependent upon encoding task (i.e. greater improvement for deeply- than shallowly encoded faces), we assessed whether this effect related to physostigmine-induced enhancements of face responses during the deep- relative to the shallow-encoding tasks. As predicted, we found in healthy controls a correlation of exactly this type, i.e. higher subject-by-subject recognition for deeply studied, relative to superficially studied, faces under physostigmine, associated with higher face-selective BOLD responses during deep versus superficial encoding tasks, under physostigmine in right mid-fusiform cortex [peak at 46, −48, −26, this being within 8mm of the peak for face selectivity reported above in healthy subjects; $r(17) = 0.79$; $Z = 4.22$; $P < 0.0001$, uncorrected; Fig. 3C]. The impact of this relationship can also be seen by ordering healthy subjects into tertile subgroups, according to the degree to which physostigmine increased memory of deep- relative to shallow-encoded faces, i.e. Physostigmine [DI (Age) > DI (Colour)] > Placebo[DI (Age) > DI (Colour)] (see Fig. 3C). While all three subgroups showed positive face-selective responses at this fusiform peak under both placebo and drug, the relative strength by which face-selective fusiform responses were increased by physostigmine during Age versus Colour tasks paralleled the drug's enhancement of memory for faces presented during the Age relative to Colour tasks. There were no other face-selective regions showing this BOLD-behavioural correlation ($P > 0.05$).

The equivalent correlation analysis for Alzheimer's disease subjects showed no such relationship at the right fusiform peak identified above [$r(12) = -0.13$, NS], leading to a reliable between-group difference there in this respect [$Z(12) = 3.71$, $P < 0.01$]. The Alzheimer's disease group did not show such a correlation in any other face-selective area, whether using all recognition responses or only those judged as being confident. A voxel-based morphometric analysis showed that there was no significant structural difference ($P > 0.05$ uncorrected) in grey matter density at this right fusiform peak between groups.

fMRI: Cholinergic modulation of task-independent encoding in Alzheimer's disease

Since the behavioural influence of physostigmine in the Alzheimer's disease group had arisen specifically for confident recognition of faces, regardless of encoding task (see above), we next examined in this patient group whether physostigmine-induced enhancements of face-selective BOLD signals (at exposure) correlated with physostigmine induced increases in

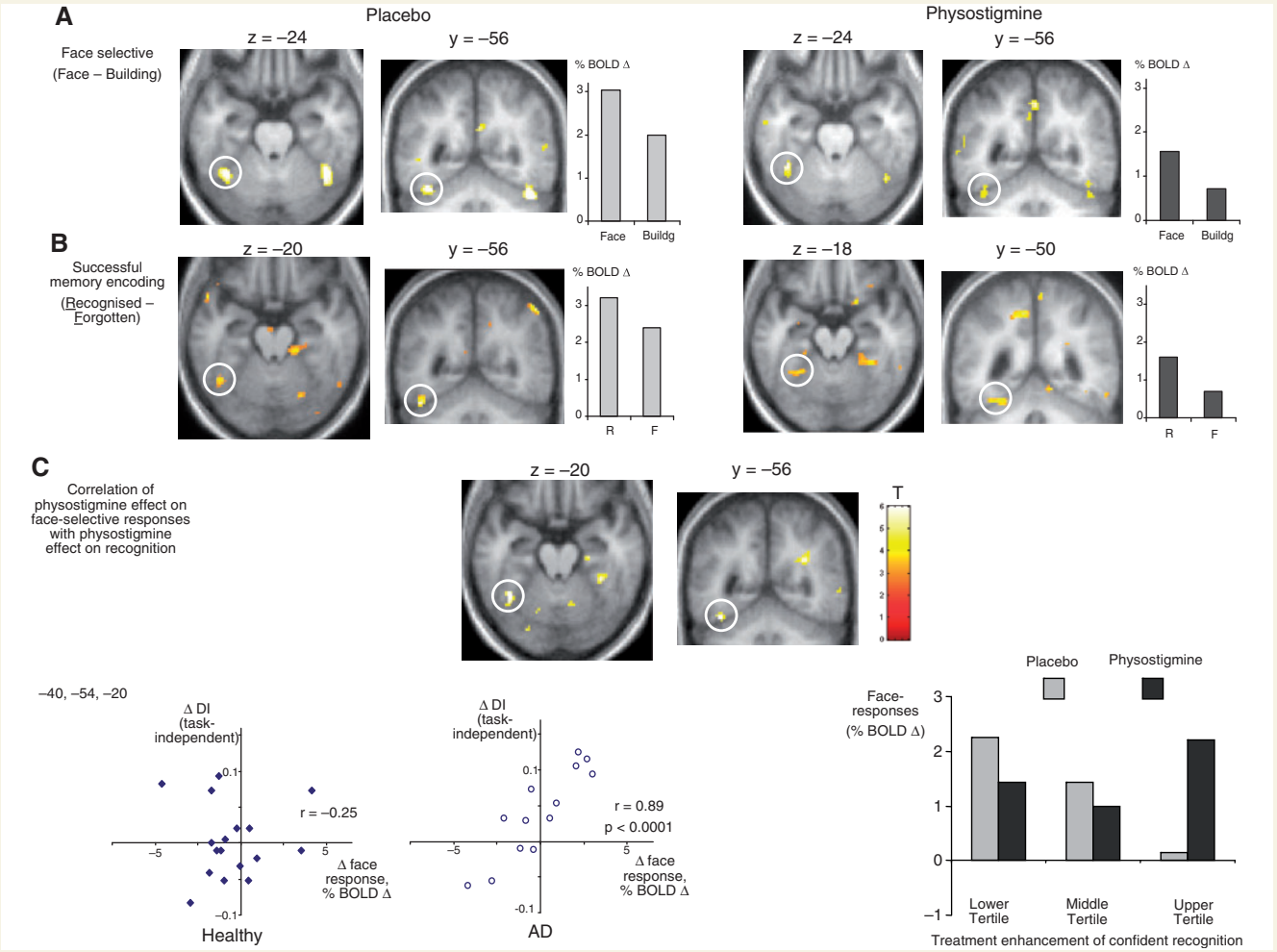

Figure 4 BOLD responses obtained from fMRI scanning during encoding in Alzheimer patients. (**A**) Face-selective responses (faces > houses) regardless of task during encoding, under placebo and physotigmine, show strongest activations in bilateral mid-fusiform gyri. (**B**) Regions where heightened BOLD signal during face encoding (independent of task or drug) predict subsequent confident recognition in Alzheimer's disease patients under placebo and drug. (**C**) Regions where physostigmine-induced enhancements of face-selective BOLD responses (independent of task) correlate with physostigmine-induced enhancements of confident recognition performance, across Alzheimer's disease patients. Regions showing a significant BOLD-behaviour relation of this specific type included middle left fusiform, anterior right fusiform and right hippocampal cortex. Graphs show subject-by-subject scatter plots for this relationship in left fusiform gyrus, separately for controls (scatter plot shown at left with diamond symbols for each healthy participant, no significant relationship) and for Alzheimer patients (scatter plot shown centrally, with open-circle symbols for each patient, illustrating the significant relationship found only for this pathological group). The rightmost bar graph further illustrates the relation in left fusiform cortex by dividing patients into three tertile subgroups, ordered by the effect of physostigmine on confident recognition. The upper-tertile subgroup shows the strongest impact of physostigmine on left fusiform at encoding. SPM contrasts shown are thresholded for display purposes at $P < 0.001$ uncorrected, in **A** and **C**, or $P < 0.01$, uncorrected, in **B**, and overlaid on mean T_1-weighted MRI of the Alzheimer's disease patients. *Per cent* signal changes of the conditions making up each contrast are plotted for the peaks in each circled cluster.

later confident recognition, regardless of task. This analysis revealed such a positive BOLD–behaviour correlation for the patient group in left fusiform cortex [peak at −40, −54, −20; $r(12) = 0.89$; $Z = 4.44$; $P < 0.0001$, uncorrected] within 8 mm of the left fusiform peak effect of face selectivity already described above for the Alzheimer's disease group (Fig. 4B, circled); as well as in right fusiform cortex, [34, −40, −24; $r(12) = 0.89$; $Z = 4.06$; $P < 0.001$, uncorrected], and posterior hippocampus [24, −24, −20; $r(12) = 0.81$; $Z = 3.50$; $P < 0.001$, uncorrected]. The impact of this relationship in left fusiform cortex can also be appreciated by dividing up patients into three ordered tertile subgroups, according to the degree to which physostigmine increased confident face recognition; see Fig. 4C. Physostigmine increased face responses selectively in the subgroup showing the greatest drug-induced enhancement of subsequent memory.

The equivalent analysis for healthy subjects found no reliable correlation of physostigmine modulation of face-selective BOLD responses with physostigmine modulation of later confident recognition, regardless of task, in any region (all $r \leqslant 0.104$, NS). This led to reliable between-group differences between all the brain regions showing a significant brain–behaviour correlation of this type for the Alzheimer's disease patients (as listed above) but

not for the healthy participants (all $Z \geqslant 2.56$, all $P \leqslant 0.01$). Voxel-based morphometric comparison of grey-matter density between groups showed no significant structural differences at any of these these voxels ($P > 0.05$, uncorrected).

There were no correlations between drug modulation of task independent face selectivity and subsequent recognition for healthy subjects if using all recognition judgements, rather than just confident responses.

fMRI: Cholinergic modulation of fusiform–parietal and fusiform–hippocampal functional coupling

Finally, we probed for remote brain regions whose task-related, or memory-related, activity may co-vary (in a subject-by-subject manner) with the relevant fusiform activations described above as showing BOLD–behavioural correlations (i.e. at 46, −48, −26 for healthy subjects; plus at −40, −54, −20 and 34, −40, −24 for Alzheimer's disease). We also assessed whether physostigmine might impact on any such inter-regional relationships. In healthy subjects, the main effect of task (Age versus Colour) under placebo activated right superior parietal cortex (peak: 48, −42, 58; $Z = 5.46$; $P < 0.001$, corrected; Fig. 5A); no other regions were significant after whole-brain correction. We found that the task effect within this right parietal region also correlated with the task modulation of face-selective responses in right fusiform cortex under both the placebo (66, −36, 40; $Z = 3.37$; $P < 0.001$, uncorrected) and physostigmine (38, −40, 56; $Z = 3.49$; $P < 0.001$, uncorrected). Comparing each of these two parietal peaks with the equivalent two voxels under the alternative treatment showed a significant between-treatment difference only for the latter peak, i.e. at 38, −40, 56 there was a greater correlation coefficient under physostigmine than under placebo [$Z(17) = 1.96$, $P < 0.05$; Fig. 5B]. In Alzheimer's disease, task-related regions beyond fusiform cortex did not show correlations with task modulation of face-selective fusiform cortex under either treatment.

We also investigated any association of fusiform face-selective responses with regions showing a subsequent-memory effect (i.e. higher responses for faces during encoding that were subsequently recognized relative to those forgotten). This showed that healthy subjects showing greater face-selective responses at the right fusiform peak (46, −48, −26) also showed a greater subsequent memory effect in bilateral amygdala (36, 6, −42, $Z = 3.42$; −30, −4, −24; $Z = 3.31$; $P < 0.001$, uncorrected) under placebo, and in right hippocampus (24, −8, −16; $Z = 3.85$; $P < 0.0001$, uncorrected) under physostigmine. The latter region also showed a greater correlation coefficient under physostigmine than under placebo [$Z(17) = 2.07$; $P < 0.05$; Fig. 5C]. In Alzheimer's disease, correlations were found between left fusiform face-selective responses and a subsequent-memory effect in right amygdala (24, 2, −36; $Z = 3.62$; $P < 0.0001$, uncorrected) under placebo, and in extensive regions of bilateral hippocampus—amygdala under physostigmine (−26, −12, −18; $Z = 4.03$; 26, −8, −14; $Z = 3.83$; $P < 0.0001$, uncorrected; Fig. 5D; note that confident responses only were included, in line with the preceding results for the Alzheimer's disease group). Each of these Alzheimer's disease fusiform—medial temporal correlations as specified were greater than under the alternative treatment [all $Z(12) > 2.08$; $P < 0.05$]. Face-selective activations in right fusiform cortex did not show correlations with subsequent memory responses in any brain region in Alzheimer's disease under either treatment.

Discussion

Cholinesterase inhibitors are one of the most widely used symptomatic treatments for dementia (Gruber-Baldini *et al*., 2007), but the physiological basis for their performance benefits are unclear. We show here for the first time a direct relationship between the behavioural and neural effects of a single challenge with a cholinesterase inhibitor in both health and dementia. The principal findings are (i) the cholinesterase inhibitor physostigmine produced small overall improvements in face-recognition memory, that in healthy subjects but not Alzheimer's disease were dependent upon encoding task; (ii) in healthy subjects, the degree to which physostigmine improved the memory of faces studied deeply (relative to those studied shallowly) correlated with the degree to which physostigmine enhanced face-selective fusiform cortex activity during the deep (relative to the shallow)-encoding task; (iii) in Alzheimer's disease, improvements in confidently judged face recognition caused by physostigmine correlated with drug-induced enhancements of fusiform face-selective responses during encoding, that unlike the case for healthy subjects, were independent of encoding task; and (iv) the fusiform cortex regions showing these neural–behavioural correlations also showed increases in their functional coupling with parietal and hippocampal regions following physostigmine. We discuss the results of the healthy and Alzheimer's disease groups in turn.

Healthy subjects

A recent integrative model of memory suggests that the physiological actions of acetylcholine on both sensory and entorhinal cortices enable the cortical dynamics necessary for new memory formation (Hasselmo, 2006). For example, acetylcholine increases both sensitivity and specificity of stimulus-evoked visual cortical responses (Sato *et al*., 1987; Murphy and Sillito, 1991), while suppressing feedback connections to the same areas (Kimura *et al*., 1999), thereby potentiating the formation of novel input associations (Hasselmo and McGaughy, 2004). Additionally, plastic changes in the response pattern of sensory cortices to specific stimuli (e.g. as seen with fear conditioning) are dependent on cholinergic inputs from basal forebrain to sensory cortices (Gu, 2003; Weinberger, 2007). In the current study, we sought to bridge the neurophysiological actions of acetylcholine on sensory cortices with the well-recognized influences of cholinergic-enhancing drugs on memory performance (Grön *et al*., 2005) through the use of functional imaging.

The design of our study married together two previous sets of observations. First, both our group (e.g. Bentley *et al*., 2004) and

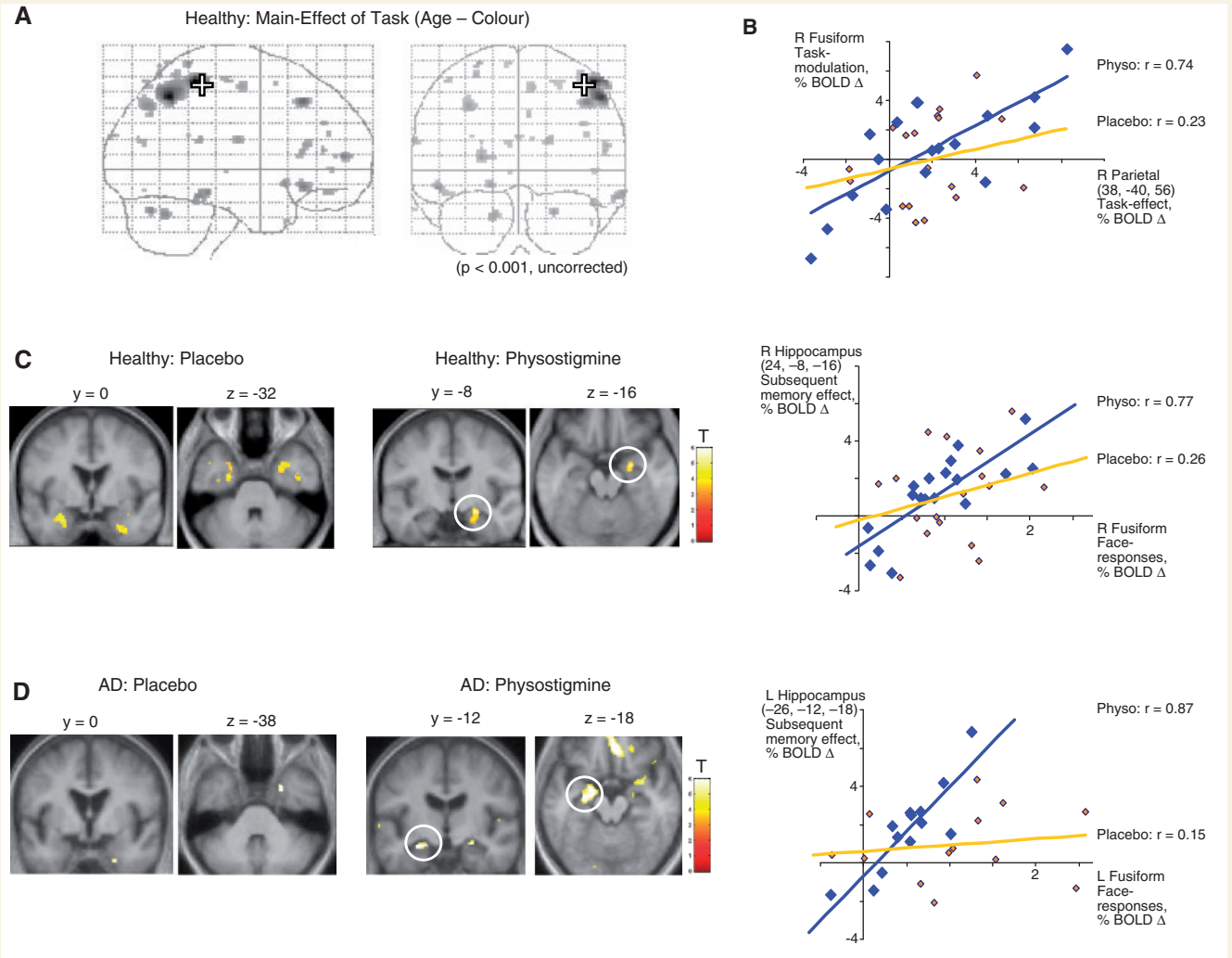

Figure 5 Regions showing main effect of Age > Colour task (**A**) or correlations of task effects (**B**), or subsequent-memory effects (**C** and **D**), with task modulation and face-selective responses of fusiform cortex, respectively. (**A**) SPM depicting regions in the whole brain showing a main effect of task (shown at $P<0.001$ uncorrected, depicted as a maximum-intensity projection), within which were found subregions where that effect correlated on a subject-by-subject basis with task modulation of face-selective responses in right fusiform cortex (at peak of treatment effect: 46, −48, −26) under both placebo and drug conditions. The cross indicates the voxel showing the greatest fusiform–parietal co-variation under physostigmine, at which there was a significantly greater correlation coefficient than under placebo as shown (**B**) in the scatter plot; (**C**) Medial temporal regions in which a subsequent memory effect (i.e. recognized versus forgotten faces) correlated with face-selective responses in right fusiform cortex (peak: 46, −48, −26) under placebo and physostigmine in healthy subjects; scatter plot at right depicts fusiform–hippocampal covariance for the hippocampal site showing the greatest difference in correlation coefficients between treatments ($P<0.05$); (**D**). As for **C**, except now in Alzheimer's disease subjects, with correlations of medial temporal regions' subsequent memory effect (for confident judgements) with face-selective responses in left fusiform cortex (at peak of treatment effect: −40, −54, −20) under placebo and physostigmine. Scatter plot at right depicts fusiform–hippocampal covariance at a hippocampal region showing greater correlation coefficient under physostigmine than placebo ($P<0.05$).

others (e.g. Furey *et al*., 2000; Lawrence *et al*., 2002) have shown that pro-cholinergic drugs can increase visual-evoked responses in visual extrastriate cortex, with this effect appearing to be greater for stimuli that are attended than for those that are incidental to the task. For example, in an earlier paper (Bentley *et al*., 2003), we found in healthy subjects that physostigmine increased fusiform cortex responses for faces that were task relevant, rather than those that were task irrelevant, thereby enhancing the usual pattern by which task demands, independent of stimulus changes, can modify sensory cortex activity (Vuilleumier *et al*., 2001). Second, psychophysical studies in humans suggest that nicotine or cholinesterase inhibitors enhance memory through effects during the encoding phase when stimuli are first presented (Ghoneim and Mewaldt, 1977; Rusted and Warburton, 1992; Wetherell, 1992), rather than during consolidation or recall, when they may exert a negative effect instead (Edginton and

Rusted, 2003; Gais and Born, 2004). Moreover, the pro-mnemonic actions of these drugs are experienced more for stimuli that are presented during deep, than shallow, encoding tasks (Warburton *et al*., 2001; Fitzgerald *et al*., 2008)—thereby mirroring the pattern of extrastriate cortex modulation found in functional imaging studies. Consequently, we predicted that physostigmine would increase memory more for faces studied during a deep task (of judging age) than during a shallow task in which the particular facial characteristics were incidental to the task (of ascertaining picture colour). Critically, we hypothesized that this behavioural effect (measured at later recognition) would correlate with enhancements in face-selective activity of fusiform cortex (measured during initial encoding), which should also be more pronounced during the Age than the Colour tasks. As Fig. 3C illustrates, such a BOLD–behavioural correlation was found to occur very close to the peak of face-selective responses in right fusiform cortex. In other words, in those subjects for whom physostigmine improved memory more for faces studied deeply than shallowly, physostigmine was also found to increase fusiform face responsiveness during the encoding phase when the faces were first presented, more during the deep than the shallow task.

Several features of our results suggest that the observed pharmacological modulation of fusiform cortex was instrumental to the drug's effects on subsequent memory performance. First, although our conclusion rests in part on a brain–behaviour correlation, it should be noted that this relationship was directional in time, i.e. physostigmine enhancement of face responses during encoding predicted later effects on memory. Since the behavioural performance of healthy subjects during the encoding tasks was unaltered by physostigmine our results at that time are unconfounded by performance considerations. Furthermore, although physostigmine would have been present during both encoding and recognition phases, the pharmacological effect observed here in healthy subjects occurred as an interaction with task that differed only during encoding. Second, both the data from our subjects in the placebo condition, and those from several previous studies (Grady *et al*., 1998; Bernstein *et al*., 2002; Otten *et al*., 2002; Mandzia *et al*., 2004) show that task modulation of face-selective responses of fusiform cortex during encoding correlates with a subsequent depth-of-processing (i.e. encoding task-dependent) effect on memory. Third, a separate 'subsequent-memory' analysis of the same subjects showed that faces later recognized, as compared with faces subsequently forgotten, elicited higher activity in right fusiform cortex during the encoding task (now independent of task or treatment)—again indicating the crucial role of fusiform activity at encoding for subsequent face memory. Previous (but non-drug) studies have analogously observed a subsequent memory effect to visual stimuli in fusiform cortex (Wagner *et al*., 1998; Kirchhoff *et al*., 2000; Golby *et al*., 2001; Sperling *et al*., 2003; Dickerson *et al*., 2007; Kircher *et al*., 2007). Fourth, we found a correlation of face-selective activity in right fusiform cortex with a subsequent-memory effect in hippocampal/amygdala regions that was enhanced under physostigmine specifically in right hippocampus (Fig. 5C). Thus, the observed effects of drug on memory here may arise from a combination of enhanced fusiform responses, specific to the encoding task and increases in functional connectivity between sensory cortex and the medial temporal cortices that are thought to be critical for memory formation (Rissman *et al*., 2008).

It is important to distinguish physostigmine-induced response increases in fusiform cortex shown here that are task dependent (and which mirror subjects' greater depth of processing memory effects), from physostigmine induced decreases in fusiform activity that are task independent (as reported in Bentley *et al*., 2008, and which did not take into account subsequent memory effects). This combination of findings seems consistent with previous fMRI studies showing that, on the one hand, physostigmine increases visual cortex BOLD activity selectively during encoding (Furey *et al*., 2000) or high-attention tasks (Bentley *et al*., 2003, 2004); but, on the other hand, that the same treatment causes decreases, or no change, in activity in the same regions during low attention (Bentley *et al*., 2004) or passive viewing tasks (Furey *et al*., 2000; Silver *et al*., 2008). This profile of functional imaging results parallels observations made using more basic neurophysiological techniques, namely direct acetylcholine application to visual cortex decreases the net stimulus-driven field potential of cortical columns (Kimura *et al*., 1999) due to suppressed intracortical signalling (Levy *et al*., 2006), but while increasing activity selectively in visual cortical units coding for task-relevant properties (Herrero *et al*., 2008).

A likely source for task driven as opposed to stimulus-driven activation changes in sensory cortex would seem to be frontoparietal regions within the so-called dorsal attention network (Kastner *et al*., 1999). Hence, one possible explanation for the depth-of-processing memory effect is an enhancement of resource allocation through attentional mechanisms (Baddeley, 1990; Chun and Turk-Browne, 2007). Given that attention is critically dependent on cholinergic innervation to frontoparietal cortices (Sarter *et al*., 2005), we explored the possibility that the modulation of task effects by physostigmine in fusiform cortex (seen here as correlating with drug effects on subsequent memory) may reflect an impact of the drug on functional coupling between fusiform cortex and regions traditionally associated with attention. The main effect of task in our study (i.e. Age > Colour task) activated right parietal cortex most strongly (Fig. 5A). We found that this task effect in parietal cortex correlated across subjects with task modulation of face-selective right fusiform cortex under both placebo and physostigmine, supporting the idea of a functional connection between these regions. The strength of this relationship was greater under physostigmine than placebo, suggesting that cholinergic modulation of task responses in fusiform cortex, along with associated depth of processing subsequent memory effects, may involve cholinergic modulation of influences from regions such as parietal cortex that can exert top–down influences on sensory cortices. We note that drug-induced changes in the correlation coefficients for subject-by-subject effect sizes in fusiform and parietal cortex are distinct from drug effects on mean task-related parietal activity (which is depressed by the drug overall: see Bentley *et al*., 2008). Similar physostigmine-induced reductions in task-related activity in frontoparietal cortices, associated with performance improvements, have been reported before (Furey *et al*., 2000; Bentley *et al*., 2004) and may reflect either a reduced demand for resource allocation in

the face of enhanced sensory processing (Furey *et al*., 2000) or improved parietal–sensory coupling as suggested here.

Alzheimer's disease

Cholinesterase inhibitors enable modest improvements in memory performance in Alzheimer's disease (Almkvist *et al*., 2004), although whether these occur primarily through direct effects on memory processes (Grön *et al*., 2005, 2006), or via indirect actions on executive—attentional processes (Alhainen *et al*., 1993; Lawrence and Sahakian, 1995) is unclear. In our study, we were able to address this issue at both behavioural and neural levels by testing for interactions between drug-induced memory enhancement and the encoding task. Contrary to what might be expected from a purely attentional account, we found in the Alzheimer's disease group that physostigmine-induced memory improvement was both independent of encoding task, and did not correlate with task modulations of face-selective extrastriate cortex. Instead we found that physostigmine-induced improvement in recognition performance correlated with enhancement of face selectivity in left fusiform cortex, that was also independent of encoding task. Importantly, therefore, we show that both behavioural and physiological consequences of cholinesterase inhibition may differ between healthy subjects and dementia patients.

In contrast to healthy subjects, Alzheimer's disease subjects did not benefit from a depth-of-encoding manipulation in their subsequent recognition performance (as also shown behaviourally in Bird and Luszcz, 1991; Beauregard *et al*., 2001). In our situation, this was not due merely to Alzheimer's disease patients failing to follow task instructions, because Alzheimer's disease patients actually showed a greater performance difference between tasks during encoding than healthy subjects. A possible neurophysiological basis for this lack of depth-of-processing in Alzheimer's disease may lie in impaired top–down modulation of sensory cortices by frontoparietal regions (Walla *et al*., 2005; Gazzelley and D'Esposito, 2007). We found some support for this from our data in two respects: first, we found that healthy subjects showed correlations between depth-of-processing memory effects and task modulation of face-selective cortices (in superior temporal sulci) that were reduced in the Alzheimer's disease group. Second, we also found some correlations between task modulation of face-selective fusiform cortex and task effects in right parietal cortex in healthy subjects that were absent in dementia patients. A similar pattern of correlations arising between encoding-related activity and subsequent recognition in healthy subjects, but not in mild cognitive impairment patients, has recently been reported (Mandzia *et al*., 2009). Although in the latter study the main between-group differences arose in parahippocampal and hippocampal regions, the contrasts in Mandzia *et al*. (2009) were based upon stimulus-related activations, as opposed to task-related modulations as we report here which more closely reflect the depth-of-processing effect.

In an earlier report with a similar study design (Bentley *et al*., 2008), but analysing responses without taking into account subsequent memory performance, we reported that physostigmine partially reversed Alzheimer's disease-associated deficits in task-related frontoparietal activity, that was associated with a lesser performance impairment during the encoding task (of visual discrimination). However, we now show, by directly correlating task-related responses with effects on subsequent memory, that even under circumstances where physostigmine enhances frontoparietal task-related activity—as we previously observed (Bentley *et al*., 2008)—this may be insufficient to restore a depth-of-processing effect on subsequent memory. Two previous fMRI studies in mild cognitive impairment patients have similarly shown enhancements of task-related frontoparietal activity following cholinesterase inhibitor therapy that were associated with improvements in working memory/attention, but not in episodic memory (Goekoop *et al*., 2004; Saykin *et al*., 2004). Taken together, these observations argue for the existence of dissociable effects for cholinesterase inhibitors on episodic memory versus attention (Sahakian *et al*., 1993; Lindner *et al*., 2006), that parallel dissociable pathological correlates of episodic memory and attention impairments in Alzheimer's disease (Perry and Hodges, 1999; Perry *et al*., 2000; Buckner, 2004). One possible reason for the pharmacological/functional dissociation observed is that memory, and especially depth of processing memory effects, rely on frontoparietal–extrastriate–hippocampal functional connections (Grady *et al*., 2001; Bokde *et al*., 2006; Celone *et al*., 2006), whose impairments' in dementia may be less reversible by physostigmine than strength of activation for each of these regions considered in isolation. Our finding that physostigmine did not impact on fusiform–parietal functional coupling in Alzheimer's disease subjects, unlike in healthy subjects, seems broadly consistent with this.

Although physostigmine did not influence depth-of-processing recognition-memory effects in our Alzheimer's disease patients, the drug did exert a significant benefit in (confident) recognition that was independent of encoding task (Fig. 4C). Moreover, this behavioural memory effect of the drug in Alzheimer's disease correlated with physostigmine-induced enhancements of bilateral face-selective fusiform cortices at initial encoding, but did so regardless of encoding task. Left fusiform cortex also showed a subsequent memory effect for faces in Alzheimer's disease, suggesting that enhancement of activity in this region by physostigmine was related to subsequent recognition in these patients. This aspect of our results suggest that Alzheimer's disease-associated impairments in fusiform cortex activity (see also Machulda *et al*., 2003; Grön and Riepe, 2004; Golby *et al*., 2005; Rombouts *et al*., 2005) may not only be reversible with cholinergic enhancement (Rombouts *et al*., 2002; Kircher *et al*., 2005), but that a functional consequence of this can be a proportionate improvement in subsequent recognition memory. The fact that, unlike healthy subjects, the effects of physostigmine on encoding-related activity in Alzheimer's disease patients was independent of task also seems consistent with reports that cholinesterase inhibition may modulate sensory cortices in Alzheimer's disease under both low- and high-attention conditions (Rombouts *et al*., 2002; Teipel *et al*., 2006). Our findings also complement studies showing that cholinergic antagonism in healthy subjects impairs both encoding-related activity in fusiform cortices, and recognition performance (Rosier *et al*., 1999; Sperling *et al*., 2002; Thiel *et al*., 2002; Schon *et al*., 2005).

We found that Alzheimer's disease patients only showed a treatment effect on memory when selectively analysing confident judgements. To the extent that confident judgements can be thought of as indexing hippocampus-based recollection memory, as opposed to familiarity (Wais, 2008; Hudon *et al*., 2009), this behavioural result complements studies showing that Alzheimer's disease memory impairment is relatively specific for the former type of memory process (Dalla Barba, 1997; Rauchs *et al*., 2007). Indeed, we found that the drug-induced (confident) memory improvement in Alzheimer's disease correlated with activation enhancement, not only in fusiform cortex but also in hippocampus (Fig. 4C), as well as increasing functional coupling between these two regions (Fig. 5D). These findings complement a recent study showing that scopolamine reduces perirhinal activations specifically during contextual recollection, rather than for familiarity judgements (Bozzali *et al*., 2006), as well as supporting behavioural evidence suggesting a specificity of cholinergic actions for explicit relative to implicit memory (Kopelman and Corn, 1988; Knopman, 1991).

Conclusions

The current study unifies three previous sets of results: first, for behavioural studies showing that the memory-enhancing effects of pro-cholinergic drugs interact with encoding task (Warburton *et al*., 2001; Fitzgerald *et al*., 2008); second, functional imaging studies showing that cholinergic-enhancing drugs increase visual extrastriate cortex activity in a task-dependent pattern (Furey *et al*., 2000; Lawrence *et al*., 2002; Bentley *et al*., 2003, 2004); and third, a range of studies showing that cholinergic antagonism of higher sensory cortices (as well as perirhinal–entorhinal cortices) correlates with impaired encoding (Kirkwood *et al*., 1999; Boroojerdi *et al*., 2001; Sperling *et al*., 2002; Schon *et al*., 2005; Dotigny *et al*., 2008). Here, we show that the improvement in face-recognition memory induced by a cholinesterase inhibitor challenge directly correlates with drug induced increases in visual extrastriate cortex activity during encoding, that in healthy subjects, but not Alzheimer's disease, are task dependent. As well as lending further support to theoretical models that integrate cholinergic actions on sensory, attentional and memory processes (Sarter *et al*., 2003; Hasselmo and McGaughy, 2004), the BOLD–behavioural relations that we present here support aspirations to apply functional imaging technology to predict treatment responses in patients in future (Matthews *et al*., 2006).

Acknowledgements

We thank Prof M.N. Rossor of the Dementia Research Group, UCL Institute of Neurology, for help with patient recruitment.

Funding

Wellcome Trust (to R.J.D. and J.D.); MRC grant (to J.D., who also held a Royal Society Leverhulme Trust Senior Research Fellowship).

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