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Abstract: Treatments currently licensed for Alzheimer's dementia target cholinergic brain systems. In vivo nicotinic receptor binding may provide an early marker of illness and treatment suitability. In this pilot, we examined nine patients with amnestic mild cognitive impairment (MCI) and ten age and education matched healthy volunteers with high resolution SPECT and the nicotinic receptor ligand 123I-5-IA-85380. Uptake data were analysed using voxel-based techniques for group comparisons and regression analyses with cognitive impairment as covariates. MCI patients had discrete reductions in uptake in medial temporal cortex. Correlations with cognitive impairment were found in left temporo-parietal areas (Addenbrooke's Cognitive Examination) and bilateral temporo-limbic areas (Reye Auditory Verbal Learning Test), and right parahippocampal gyrus (Reye Complex Figure Test) within the patient group. In vivo nicotinic receptor

binding appears to be sensitive to brain changes in MCI. Larger scale explorations of patients undergoing treatment will be necessary to evaluate its use in predicting or monitoring treatment response.

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Dear Dr Coleman,

Re: Neurobiology of Aging Ms. No.: NBA-08-166R1
"5-123I-A-85380 binding to the alpha4-beta2-nicotinic receptor in mild cognitive impairment"; by Emma Terrière, MBChB MRCPsych; Mary F Dempsey, PhD; Lucie L Herrmann, MA (Hons); Kevin M Tierney, MSc; Jane A Lonie, MSc; Ronan E O'Carroll, PhD; Sally Pimlott, PhD; David J Wyper, PhD; Karl Herholz, MD & Klaus Peter Ebmeier MD

Thank you for your letter of the 5th September and the short reviewers' comments. We have made the requested changes and include the detailed responses in the attachment "Responses to Reviewers".

Yours sincerely,

Klaus Ebmeier

(Attached: Responses to Reviewers)

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5-^{123}I-A-85380 binding to the α4-β2-nicotinic receptor in mild cognitive impairment

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ABSTRACT

Treatments currently licensed for Alzheimer's dementia target cholinergic brain systems. In vivo nicotinic receptor binding may provide an early marker of illness and treatment suitability. In this pilot, we examined nine patients with amnestic mild cognitive impairment (MCI) and ten age and education matched healthy volunteers with high resolution SPECT and the nicotinic receptor ligand ^{123}I-5-IA-85380. Uptake data were analysed using voxel-based techniques for group comparisons and regression analyses with cognitive impairment as covariates. MCI patients had discrete reductions in uptake in medial temporal cortex. Correlations with cognitive impairment were found in left temporo-parietal areas (Addenbrooke's Cognitive Examination) and bilateral temporo-limbic areas (Reye Auditory Verbal Learning Test), and right parahippocampal gyrus (Reye Complex Figure Test) within the patient group. In vivo nicotinic receptor binding appears to be sensitive to brain changes in MCI. Larger scale explorations of patients undergoing treatment will be necessary to evaluate its use in predicting or monitoring treatment response.

KEY WORDS

Alzheimer's disease; dementia; mild cognitive impairment, amnestic; nicotinic acetylcholine receptors; single photon emission tomography

1. Introduction

In addition to reduced levels of synaptic choline-acetyl transferase and acetylcholine, Alzheimer's dementia (AD) is also associated with changes in muscarinic and nicotinic acetyl-cholinergic receptors (nAChRs). Reduced activity of the α4β2 nicotinic receptor subtype has been consistently reported (C. Martin-Ruiz, et al., 2000), both in terms of reduced binding and subunit expression (Burghaus, et al., 2000, C. M. Martin-Ruiz, et al., 1999, Wevers, et al., 1999). The receptor may be lost early in the disease process. This indicates the likely importance of α4β2 nAChRs in the pathophysiology of AD and hence their potential as therapeutic targets. Demonstration of early changes may lead to advances in early diagnosis and the prediction of future decline.

Mild cognitive impairment (MCI) represents an attempt to define features of the dementias in their pre-clinical phases. The original criteria for MCI set out by Petersen (Petersen, et al., 2001) require that a person must present i) with a memory complaint, ii) show evidence of objective memory decline in relation to age and education, iii) demonstrate preservation of other areas of cognitive function and iv) activities of daily life and iv) not fulfill criteria for dementia. Recently developed research criteria supply a cut-off for prose recall as objective evidence of episodic memory impairment (Grundman, et al., 2004).

There is good evidence in vitro and in vivo, using positron emission tomography, that nicotinic receptors are reduced in Alzheimer's disease in proportion with cognitive impairment and other pathological changes (Court, et al., 2001, Nordberg, et al., 1997, Nordberg, et al., 1995, Nordberg and Winblad, 1986, Perry, et al., 2000, Sabbagh, et al., 1998, Sabbagh, et al., 2001). However, initial studies in MCI have been inconsistent (Sabbagh, et al., 2006, Sabri, et al., 2008).

Whilst SPECT with ^{99m}Tc-HMPAO- and other blood flow ligands can assist in the differential diagnosis of patients with dementia, it is less sensitive than clinical criteria in detecting AD (Dougall, et al., 2004). Attention has therefore turned to SPECT with radioligands for acetylcholine receptor subtypes. (S)-5-[123I]iodo-3-(2-azetidinylmethoxy)pyridine (5-IA-85380 or 5-IA) is a nicotine-based radioligand with high affinity for the $\alpha4\beta2$-receptor, to a lesser extent to the $\alpha7$-receptor (Mukhin, et al., 2000). High affinity 5-IA binding is seen in thalamus, striatum and temporal regions, with some moderate in vitro binding in white matter of cingulate, occipital, and temporal areas (Pimlott, et al., 2004). 5-IA SPECT is suitable for the quantification of brain nAChRs in healthy human volunteers (Mamede, et al., 2004). It is well tolerated and whole body distribution and dosing data have been reported (Fujita, et al., 2002, Vaupel, et al., 2003). Age and sex related changes of plasma-levels and receptor binding have been characterised (Cosgrove, et al., 2007, Mitsis, et al., 2008, Mitsis, et al., 2007). 5IA-SPECT abnormalities have been described in first studies of AD (O'Brien, et al., 2007, Terrière, et al., 2008). This initial study aims to estimate effect sizes of 5-IA uptake changes in mild cognitive impairment to explore the possibility of using nAChR binding in differentiating between pre-diagnostic groups.

2. Methods

2.1 Participants

We recruited ten patients with amnestic mild cognitive impairment (MCI) from the Edinburgh Neuropsychological Assessment Service for Older People (Lonie, et al., 2008) and ten healthy elderly control subjects over the age of 59 from amongst friends and spouses of patients matched for age, years of education, pre-morbid IQ and sex..

All participants gave written informed consent following a protocol approved by the Lothian Local Research Ethics Committee (LREC) and the Administration of Radioactive Substances Advisory Committee (ARSAC). Subjects were recompensed for travelling costs, but received no financial incentive to participate. Prior to study entry, all MCI patients completed the Addenbrooke's Cognitive Examination (ACE), a screening measure of global cognitive impairment that includes a score for the 30-item Mini-Mental State Examination (Mathuranath, et al., 2000), and the Geriatric Depression Scale (Yesavage, 1988). They underwent medical and psychiatric screening (including blood screen), as well as neuroimaging (x-ray computed tomography) to exclude vascular or other brain lesions. One patient did not receive a CT scan at assessment, as he did not generate any clinical indication for a scan during 2 years of 6-monthly follow-up (MMSE: 24 - 28 - 24 – 24 with a pre-morbid IQ of 120).

MCI patients met the original criteria developed by Petersen and colleagues (Petersen, et al., 1999): three had verbal amnestic MCI, two visual, two verbal and visual memory impairment, and two had combined verbal amnesia and executive problems. We found that using more than one neuropsychological test increased sensitivity to detect episodic memory impairment, so we did not use the newer Petersen

(Grundman, et al., 2004) criteria that define objective memory impairment by > 1.5 S.D. deficits on a prose recall test. There is indeed evidence to suggest that the use of a single episodic memory measure can result in arriving at an unreliable diagnosis of MCI (Brooks, et al., 2008, Lonie, et al., 2008). We thus include a few patients who performed only at one standard deviation below control means in some tests. This more mildly impaired group (see below) was, if anything, less likely to show brain abnormalities.

Subjects who took concomitant psychotropic medication (over the counter preparations including gingko biloba and vitamin E were permitted), those with a history of thyroid disorder, or a history of smoking within the last two years were excluded. After also excluding one patient with a SPECT scan that failed quality assurance criteria, the imaged group consisted of four males and five females with a mean age of 73.9 years (range 65 to 86 years) and a mean MMSE score of 28.9 (range 28-30). We also recruited a total of ten healthy controls (6 males and 4 females) with a mean age of 73.9 years (range 65 to 80 years) and a mean MMSE score was 29.8 (range 29 to 30).

2.2 Neuropsychological Testing

A neuropsychological test battery was given to MCI subjects as well as healthy controls. It consisted of tests assessing the primary domains of verbal (RAVLT = Rey Auditory Verbal Learning Test, (Rey, 1964)) and visual episodic memory (RCF = Rey Complex Figure (Rey, 1941)), processing speed (Trail Making Test Part A (Reitan, 1985)), and speeded divided attention (Trail Making Test Part B (Reitan, 1985)). Z-values (Table 2) were computed by subtracting the matched-control group mean from individual patient scores and dividing the result by the control group standard deviation.

2.3 Synthesis of 5-IA

5-[^{123}I]-A85380 was produced via electrophilic iodo-destannylation of the corresponding stannyl precursor, 5-SnBu3-A85380. The radiolabelling was performed as described previously (Terrière, et al., 2008). The 5-[^{123}I]-A85380 was formulated as 150 MBq in 5 ml 0.9 % saline for intravenous injection containing up to 1.5 mg ascorbic acid and filtered through a 0.22 μm filter. The 5-[^{123}I]-A85380 was produced with an isolated radiochemical yield of 71.6% ± 8.1 (n = 15), a specific activity of 477.8 GBq/μmol ± 140.7 (n = 14) and had a radiochemical purity of >98 %.

2.4 Scanning procedure

Subjects were treated with oral potassium iodate from the day before to the day after the scan date (170mg, 170mg and 85mg respectively). 150MBq (±10%) of 5-[^{123}I]-A85380 (5-IA) were given by intravenous injection over 30 seconds. To sample early tracer uptake affected by regional cerebral blood flow (rCBF), the first five control subjects were immediately scanned with a single-slice, high-sensitivity, twelve-detector head scanner with an in-slice and z-axis resolution of 8.5 mm full width half-maximum (FWHM) using the intermediate 572-hole collimators (Neuro 900, Strichman Medical Equipment Inc., Boston, MA). Data were acquired in axial 'slices' using spacing of 12mm, each acquired over 120 sec. All participants were scanned after four hours for specific binding close to equilibrium of 5-IA, using 7mm slice spacing, each slice acquired over 210 seconds.

2.5 Image Analysis

Image data acquisition and analysis: Images were acquired and reconstructed in 3D (96x96 matrix with 7mm slice thickness) Neurofocus software version 3.39 (NeuroPhysics Corporation, Shirley MA, USA). They were checked for artefacts and

exported in Analyze 7.5 format (Mayo Foundation, Rochester MN, USA) for VBA (128x128 matrix with 5mm slice thickness). Image quality was unacceptable in one image due to patient movement (MCI study) and this was excluded from analysis. Analysis for regionally specific effects of 5-IA distribution was done with SPM2 (Wellcome Department of Imaging Neuroscience, University College London, 2003) in conjunction with MATLAB version 7.2 (The MathWorks, Inc. Natick MA, USA). In order to optimise spatial normalisation, we created a 5-IA template in the following manner: The five 5-IA SPECT control images acquired within ten minutes post-injection (with uptake patterns similar to perfusion scans) were co-registered with those acquired four hours post-injection for the same five control subjects, using rigid body transformations. Early 5-IA scans were then spatially transformed into MNI-space using the perfusion template supplied with SPM2. Following this, the corresponding five late scans were transformed into MNI space using the parameters derived from the individual early scans. The standard 5-IA template was created from averaging and 8 mm smoothing the spatially transformed late scans (Terrière, et al., 2008). SPM2 spatial normalisation was employed (12-parameter affine followed by 16 iterations of non-linear transformations) to match each subject's 5-IA image to the new 5-IA template (Figure 1).

Statistical analysis: Image intensity was normalised between subjects using proportional scaling to a reference region to prevent inter-subject variability masking any regional changes. No area of non-specific uptake has yet been identified in 5-IA studies. In this study therefore, three different regions were selected as potential reference regions for voxel based analysis: 1) the cerebellum, was selected for two reasons. It is sufficiently large to be less liable to partial volume effects experienced by smaller regions. It is also considered to be without major pathological involvement

in Alzheimer's disease (Elser, et al., 1996, Pickut, et al., 1999, Soonawala, et al., 2002); 2) the corpus callosum (CC), which has previously been said to exhibit only non-specific uptake (Staley, et al., 2005); 3) SPM2's default: global mean activity within the boundaries of 90% maximum activity. The cerebellar binary mask was created and used as described previously (Soonawala, et al., 2002), the corpus callosum mask by using the segmentation tool in Analyze and the single subject T1 weighted MRI supplied with SPM. Masks were made smaller than the structure of interest to reduce partial volume effects at the edges, i.e. 2.3 cm^3 and 3.7 cm^3, respectively. Each image was scaled appropriately, producing two sets of scaled images (to cerebellum and corpus callosum). All images were finally smoothed with a 12-mm FWHM 3D Gaussian filter and a 90% threshold was applied which appeared to remove most of the regions containing little or no specific 5-IA uptake in both sets of scaled data.

Group differences in 5-IA uptake between MCI and control subjects were assessed using t-tests. As it was likely that the MCI group would consist of some patients who will go on to develop Alzheimer's disease and others who will not dement in the future, correlation analyses were run within the MCI group with ACE, RAVLT, and RCF scores, after removing age as a nuisance variable. As variability of these cognitive tests was limited amongst controls compared with the patients (see Table 1), our power to detect such associations within the control group would have been small, and so we did not examine these. Data scaled to global brain uptake, cerebellar uptake and corpus callosum reference regions were analysed separately. Effects were considered if uncorrected $p<0.001$. If possible, significance levels were also given after correction for volume examined and data smoothness ("corrected").

3. Results

3.1 Subject data

Table 1 illustrates the demographic and clinical characteristics of the study.

3.2 Cognitive Testing

Table 2 demonstrates the results of neuropsychological testing. As anticipated, MCI patients generally performed worse than controls, in particular in the MMSE and in measures of episodic verbal and visuo-spatial memory.

3.3 Group differences in 5IA uptake

After global normalisation, there were significant reductions in medial temporal lobe uptake, particularly right (maximum [20, -22, -26], $Z=3.40$, $p<0.001$, uncorrected) and in right middle frontal gyrus (maximum [50, 26, 26], $Z=3.32$, $p<0.001$, uncorrected) in the MCI patients compared with the controls (Figure 2). Using cerebellar normalisation or corpus callosum normalisation, there were no differences between MCI patients and controls.

3.4 Regression with cognitive impairment

We examined the relationship between cognitive performance and globally normalised 5-IA uptake after removing age-effects (Mitsis, et al., 2008). The maximum regression coefficient with *Addenbrooke's Cognitive Examination* total score (Mathuranath, et al., 2000; see Figure 3a) extended from the left supramarginal gyrus [-46, -42, 32] to left superior temporal gyrus and left post-central gyrus (voxel maximum: $Z=5.16$, $p<0.019$, corrected; cluster-level 5.73 cm^3, $p<0.001$, corrected). A second cluster of effects was in the right inferior parietal lobule ([34, -48, 36]: voxel maximum: $Z=4.09$, $p<0.001$, uncorrected; cluster-level 0.97 cm^3, $p<0.042$, corrected), and a third in the posterior lobe of the right cerebellum ([44, -64, -26]: voxel

maximum: Z=4.03, p<0.001, uncorrected; cluster-level 3.73 cm^3, p<0.001, corrected). The maximum regression coefficient with the *Rey Auditory Verbal Learning Test*, total score (Rey, 1964; see Figure 3b) was in left lingual gyrus ([−18 −42 −2]: voxel maximum: Z=4.54, p<0.001, uncorrected; cluster-level 1.01 cm^3, p<0.04, corrected), as well as the right hippocampus ([32, -40, 0]; voxel maximum: Z= 4.22, p<0.001, uncorrected; cluster-level 0.92 cm^3, p<0.004, uncorrected), and in left claustrum ([-34, -4, -10]; Z=3.56, p< 0.001, uncorrected; cluster-level 0.34 cm^3, p<0.06, uncorrected). The maximum regression coefficient with the *Rey Complex Figure delayed recall* (Rey, 1941; see Figure 3c) was found in right parahippocampal gyrus ([34, -30, -14], Z=3.58, p<0.001, uncorrected; cluster-level 0.29 cm^3, p<0.1, uncorrected).

4. Discussion

Our primary question was whether mild cognitive impairment is associated with abnormal brain uptake of 5-IA. After global normalisation, there was a small medial temporal reduction in the MCI group (Figure 2). This would appear plausible, as the MCI patients were all classified as amnestic. The extent to which this reduction is influenced by (para-)hippocampal atrophy would require a study with co-registration to MRI. We felt that an additional within-group correlation with cognitive function would be informative, as we expected that within the MCI group some patients were likely to be similar to the healthy volunteers while others were more likely to develop dementia in the near future. Current ACE, RAVLT, or RCF scores are of course not validated predictors of outcome, but a first approximation to current cognitive (dys-) function. A regression with ACE, RAVLT and RCF yielded significant associations within temporal-parietal cortex, emphasising the relevance of cognitive impairment in general and verbal/visual episodic memory impairment in particular in this group.

Several novel image analysis steps were introduced with this study. First, a 5-IA template was created to optimise spatial normalisation. Second, objectively identified cerebellum and corpus callosum regions were used as reference regions to scale data. There are recognised limitations of using such reference regions. Preclinical studies in nonhuman primates have indicated that there is no grey matter region with only non-specific binding for 5-IA. The cerebellum demonstrated at least 35% displacement of 5-IA uptake by nicotine or the nicotinic agonist cystine in nonhuman primates. This finding has recently been confirmed in human subjects, where up to 70% of 5-IA cerebellar uptake was displaced by nicotine delivered from smoking two consecutive cigarettes (Staley, et al., 2005). A second study of quantification of human nicotinic acetylcholine receptors with 5-IA SPECT also concluded that the cerebellum was an

inappropriate reference region and the authors indicated that there was no other receptor-poor region that is appropriate as a reference region for SPECT (Mamede, et al., 2004). Atrophy affecting tissues both with and without nicotinic receptors may explain greater group differences after cerebellar normalisation: relative absence of cerebellar atrophy in early Alzheimer's disease and MCI will highlight any relative atrophy or perfusion changes in cerebral cortex (Soonawala, et al., 2002). While it is likely that there is reduction of cell elements containing nicotinic receptors, this is possibly not a specific reduction of such cells. Such partial volume effects are less likely if whole brain ratios are used, where all atrophic regions are both in the denominator and the numerator. Consequently, the whole brain ratio results were the most conservative. Although white matter clearly accumulated the tracer, at least two studies suggest that the corpus callosum does not (Ding, et al., 2004, Staley, et al., 2005). While further work is required to confirm this, using callosum as a reference region would greatly simplify quantification of 5-IA uptake. However, as demonstrated by our results, its relatively small size and low activity counts could introduce significant noise during quantification. As our (most conservative) results after whole brain normalisation show significant diagnostic group differences and correlations with cognitive impairment can be measured with 5-IA SPECT, this imaging modality may be helpful in further exploring brain changes in MCI.

While imaging markers of amyloid accumulation may turn out to be the most sensitive method of detecting patients with a high potential to develop Alzheimer's disease, nicotinic binding may prove to be useful in both detecting early changes and following functional deterioration and possible interaction with drugs acting on the acetylcholine system. Further larger and longitudinal studies will be necessary to examine this question.

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Table 1: Mean scores (standard deviations) and p-values for patients included in comparisons.

	Diagnostic Group				T-Test (p)
	Healthy Controls (n=10, 6 ♂)†		Mild Cognitive Impairment (n=9, 4 ♂)†		
	Mean	SD	Mean	SD	
Age	**73.9**	**6.8**	**73.9**	**5.6**	**0.997**
Estimated IQ (NART*)	**122.0**	**1.9**	**116.9**	**6.5**	**0.12**
Years of Education	**15.4**	**3.5**	**14.3**	**3.7**	**0.53**
Addenbrooke's Cognitive Examination	**95.9**	**2.4**	**91.1**	**5.2**	**0.03**
Mini-Mental State Examination	**29.9**	**0.3**	**28.9**	**0.6**	**0.000**

*NART – National Adult Reading Test (Nelson and Willison, 1991), available only for 5 controls.

†Chi-square=0.048 (continuity correction), p=0.827

Table 2: Z-values of neuropsychological test scores for MCI patients – means and 95%-confidence intervals (note that trails tests are scored as time to completion, i.e. larger values are more abnormal) - Computed as average difference between patient values and control mean divided by control standard deviation.

	Mean Z-Score (Cohen's d)	**95% CI**
Addenbrooke's Cognitive Examination	**-0.94**	-1.85 to -0.02
Mini Mental State Examination	**-1.52**	-2.57 to -0.46
Rey Complex Figure Immediate Recall	**-0.88**	-1.62 to -0.14
Rey Complex Figure Delayed Recall	**-0.81**	-1.51 to -0.10
Trails A	-0.23	-0.64 to 0.19
Trails B	0.83	-0.20 to 1.86
Auditory Verbal Learning Test (Total)	-0.54	-1.10 to 0.02
Auditory Verbal Learning Test (Delayed Recall)	-0.31	-0.84 to 0.22

Figure Legends:

Figure 1:

5IA Template co-registered with T1-weighted MRI image

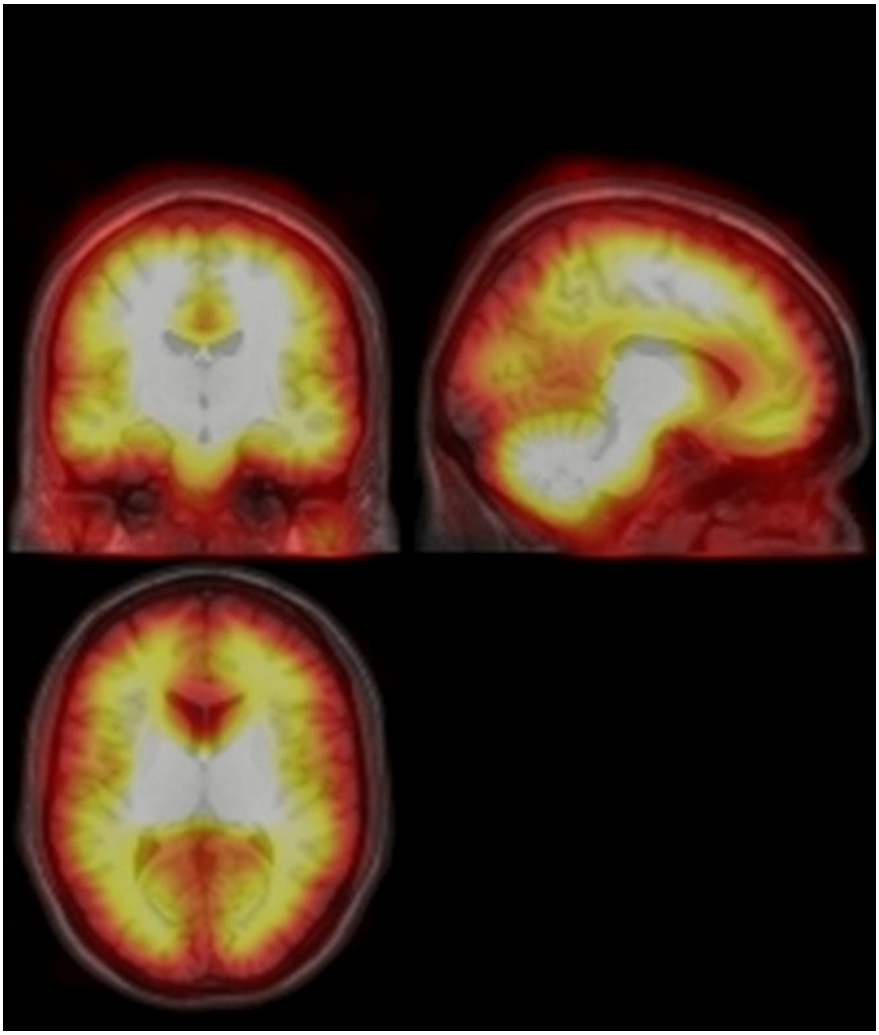
Figure 2:

Group contrast giving relative reductions in 5IA uptake in MCI patients after global normalisation (presented at height threshold of T=2.57, p<0.01). Only right parahippocampus [22, -22, -26] and right middle frontal gyrus [50, 26, 26] achieved criterion of uncorrected voxel-level p<0.001.

Figure 3:

Correlation of 5IA uptake with A) ACE total score; B) AVLT total score; C) RCF delayed score within the MCI group (all presented at height threshold of T=5.21, p<0.001)

Figure 1:



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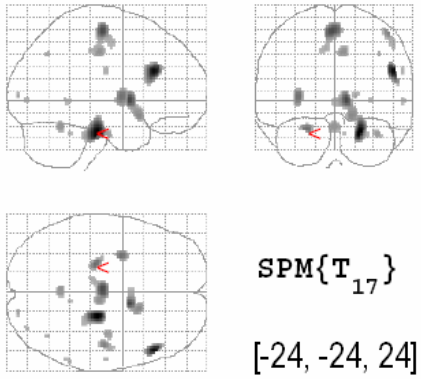
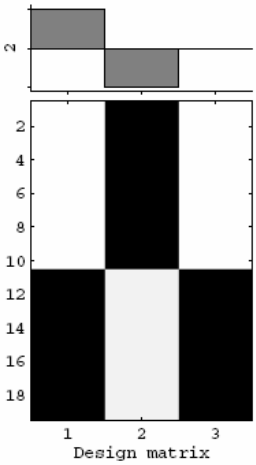


SPM{T_{17}}

[-24, -24, 24]



Fitted activity (response) Group effect in left parahippocampal gyrus

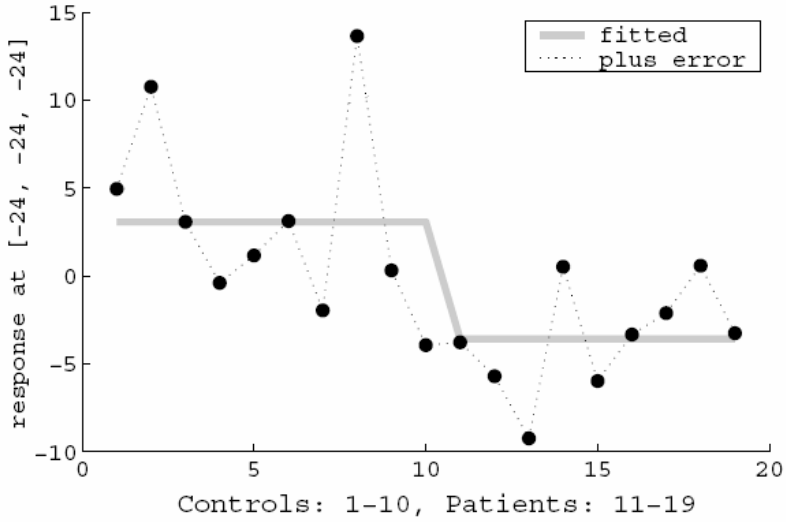


Figure 3:

A)

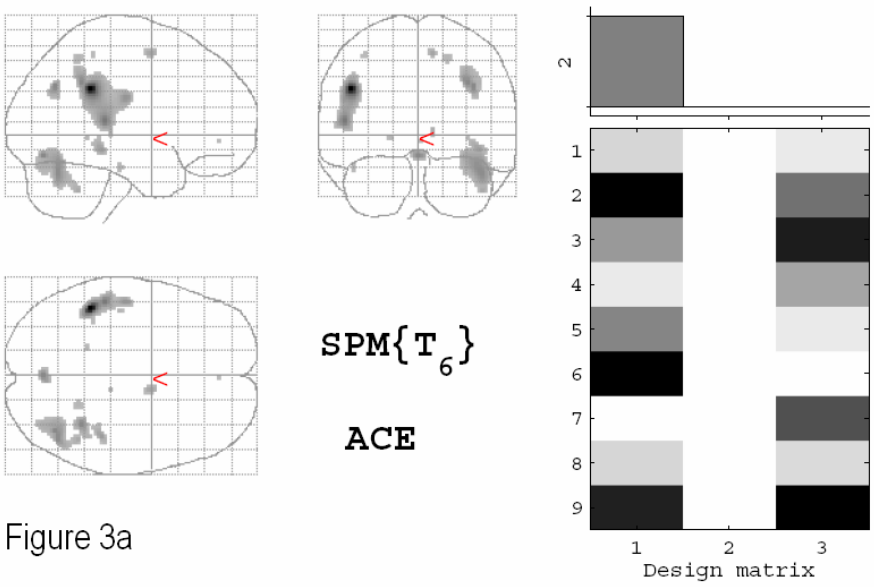


Figure 3a

B)

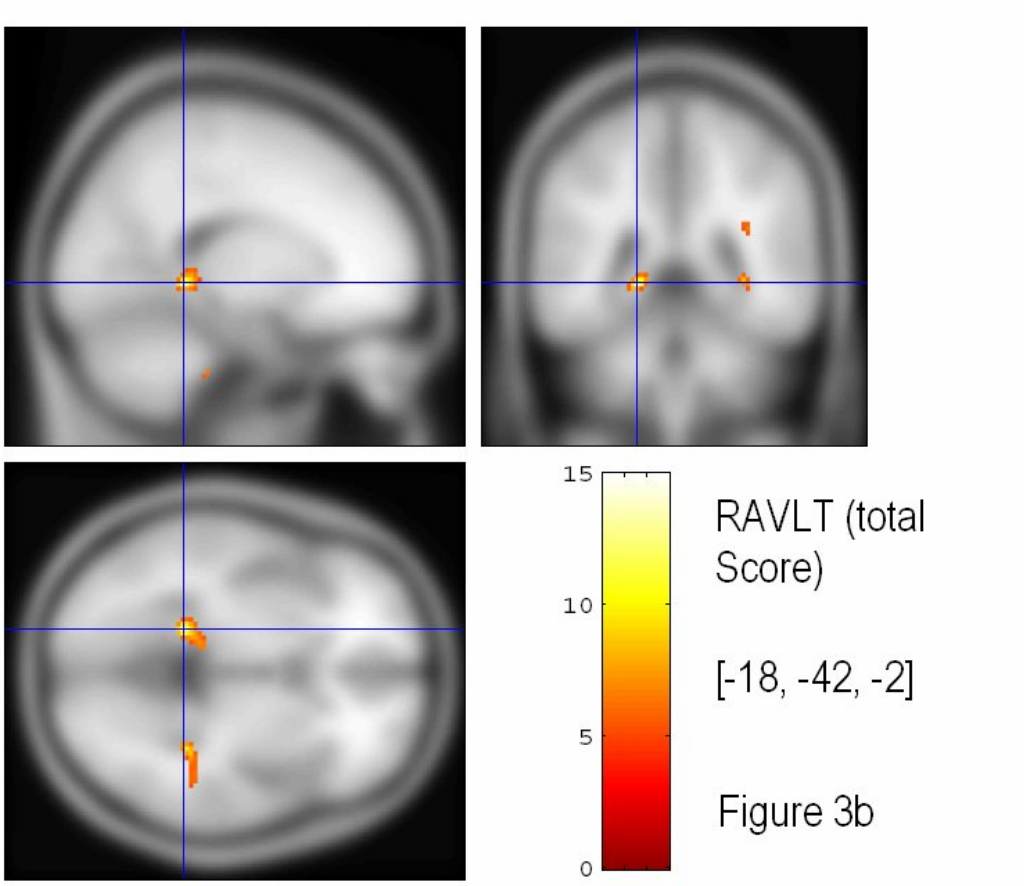


Figure 3b

C)

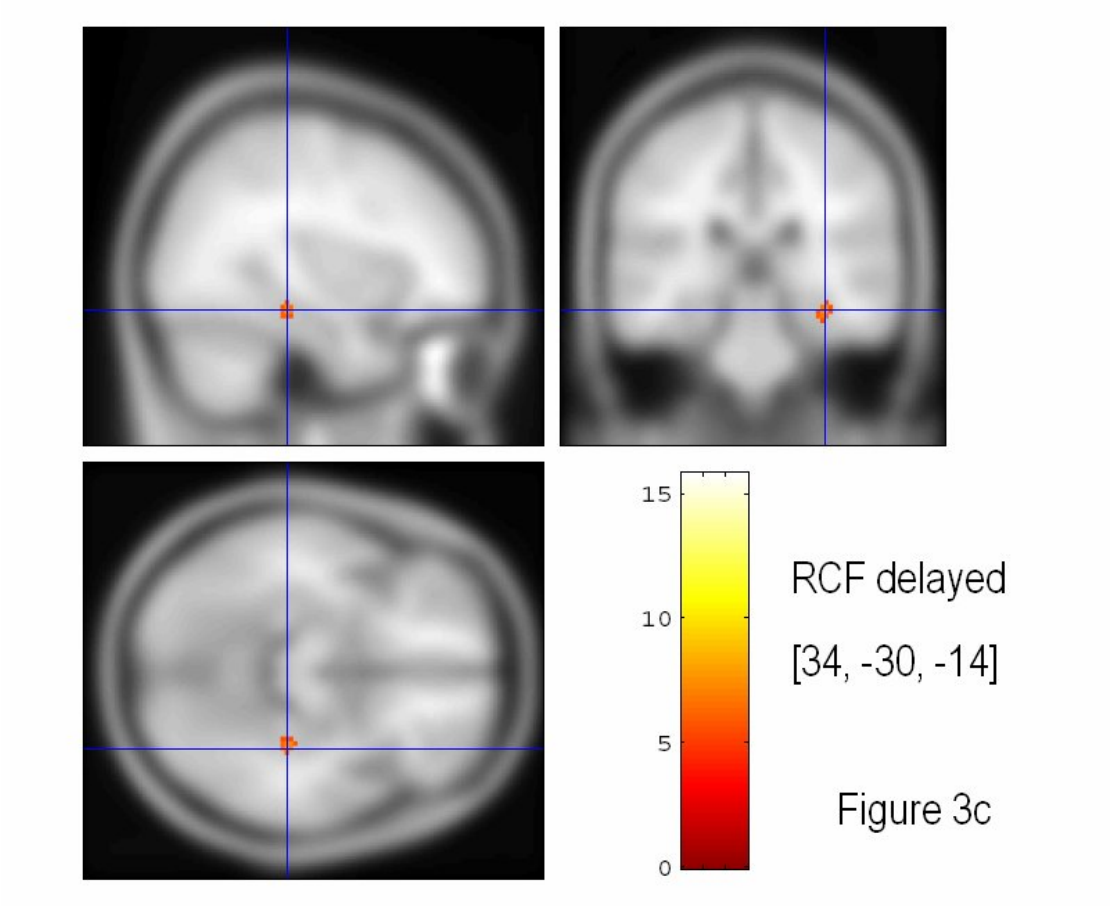


Figure 3c

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08 September 2008

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USA

Responses to Reviewer 2:

1. Abstract page 2: The total for MCI patients is 9 and not 10 as indicated.

   The number has been changed

2. Page 5, section 2.1: use acronym first time for Addenbrooke's Cognitive Estimation and provide a one sentence description (or if it is a global measure such as the CDR, then state that), since comparisons were made and are shown in Table 2.

   Acronym has been introduced and the test has been described briefly, as requested.

3. Page 9 and Page 10, section 3.4: Correlational analyses were conducted between MCI 5IA uptake and cognitive data (ACE, RAVLT, and RCF scores only; Trails omitted). Were similar analyses conducted for healthy controls to provide further evidence that the findings are specific to MCI only? If healthy data was analysed, show data or provide a sentence with the outcome.

   We did *not* conduct these analyses for two reasons: one, the variability of these tests amongst controls was small compared with the patient group (see Table 1). This would have reduced our power to detect any association. I did a quick SPM analysis, which confirms this suspicion (no effects of ACE controlled for Age within the control group). Two, any variability within the patient group was assumed to consist of the physiological and measurement inter-subject variability also found within controls plus any variable putative pathological changes associated with cognitive impairment, and would have consequently to be interpreted differently.

4. The authors' comments in response to concerns regarding age and sex effects are adequate.

   Thank you!

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*** Verification**

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27 March 2008

Paul D. Coleman
Editor-in-Chief
Neurobiology of Aging

Dear Dr Coleman,

RE: 5-123I-A-85380 binding to the α4-β2-nicotinic receptor in mild cognitive impairment

None of the authors has

1. (a) any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work.

1. (b) Authors' institutions have no contracts relating to this research through which they or any other organization may stand to gain financially now or in the future.

1. (c) There are no other agreements of authors or their institutions that could be seen as involving a financial interest in this work.

2. Financial support related to the manuscript being submitted was from: the Gordon Small Charitable Trust and the European Commission Network of Excellence ''Diagnostic Molecular Imaging'' (FP6-LIFESCIHEALTH Project Reference: 512146).

3. The data contained in the manuscript being submitted have not been previously published, have not been submitted elsewhere and will not be submitted elsewhere while under consideration at Neurobiology of Aging.

4. Appropriate approval and procedures concerning human subjects were received from the Administration of Radioactive Substances Advisory Committee at the UK Department of Health (ARSAC) and the local Research of Ethics Subcommittee.

5. All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

With best wishes,

Klaus Ebmeier

Prof KP Ebmeier, Chair of Old Age Psychiatry,
E-mail: klaus.ebmeier@psych.ox.ac.uk

Figure 1

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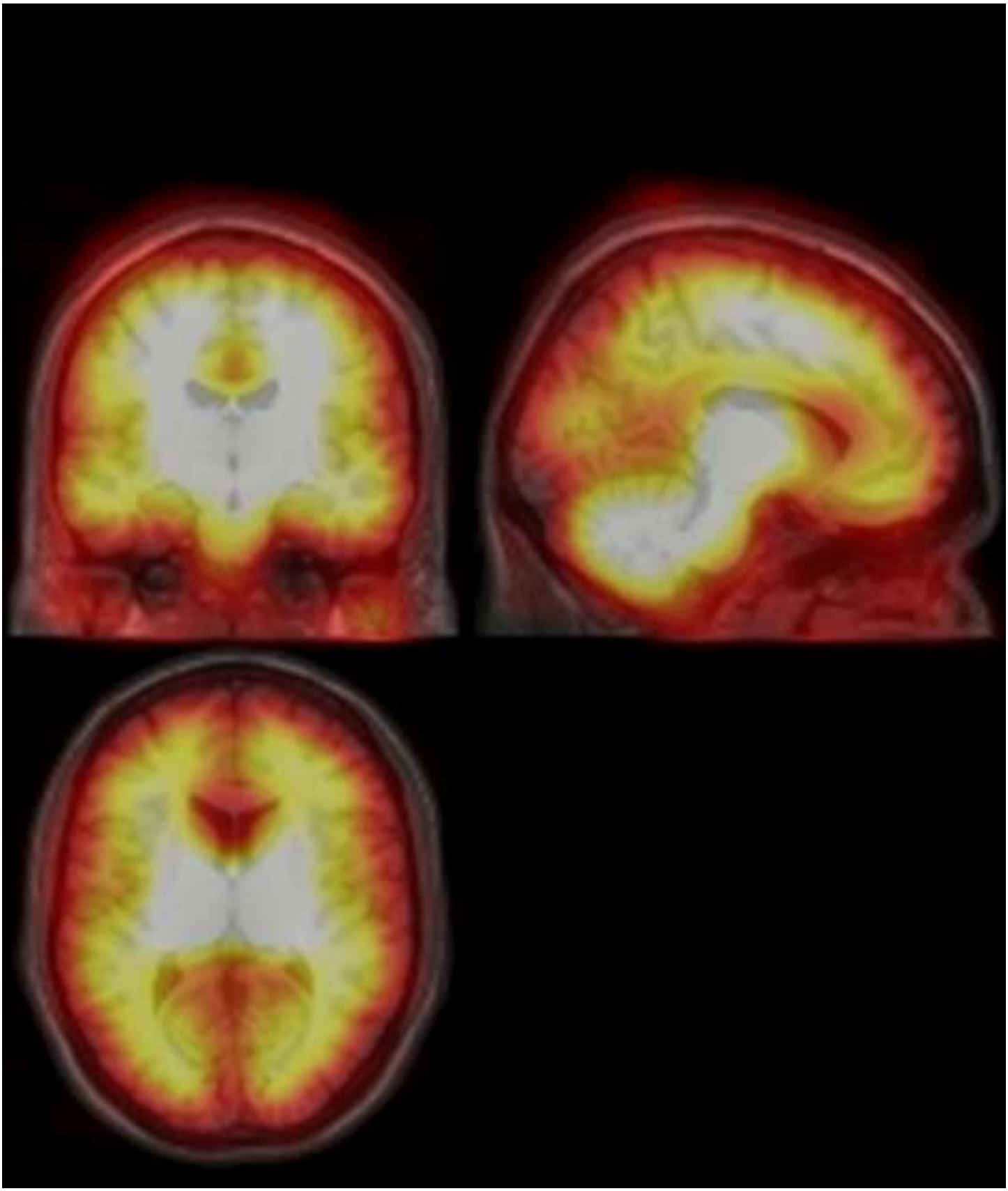

Figure 2

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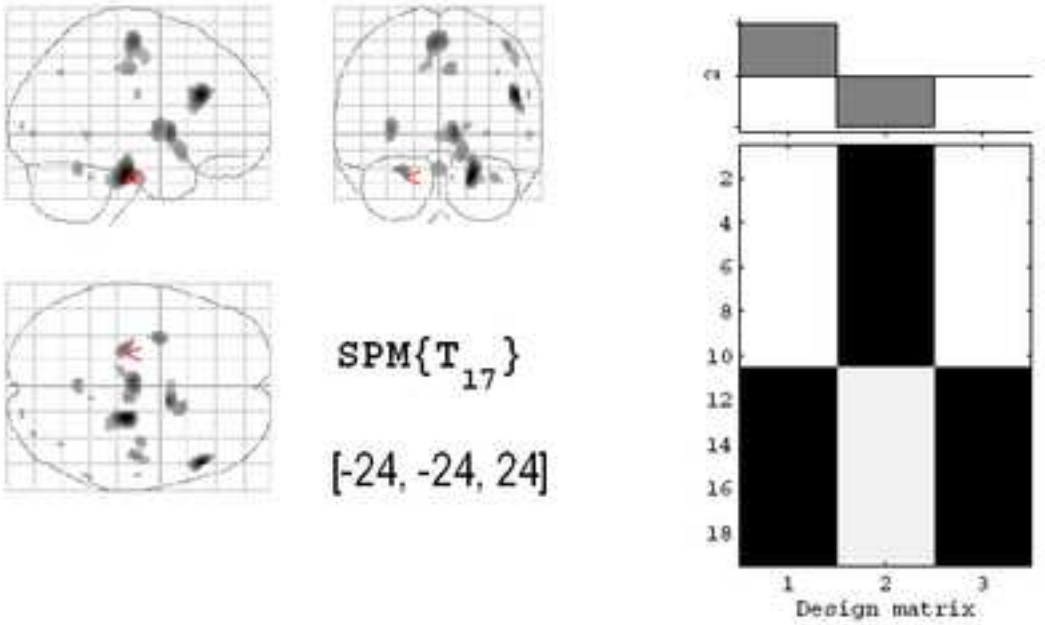

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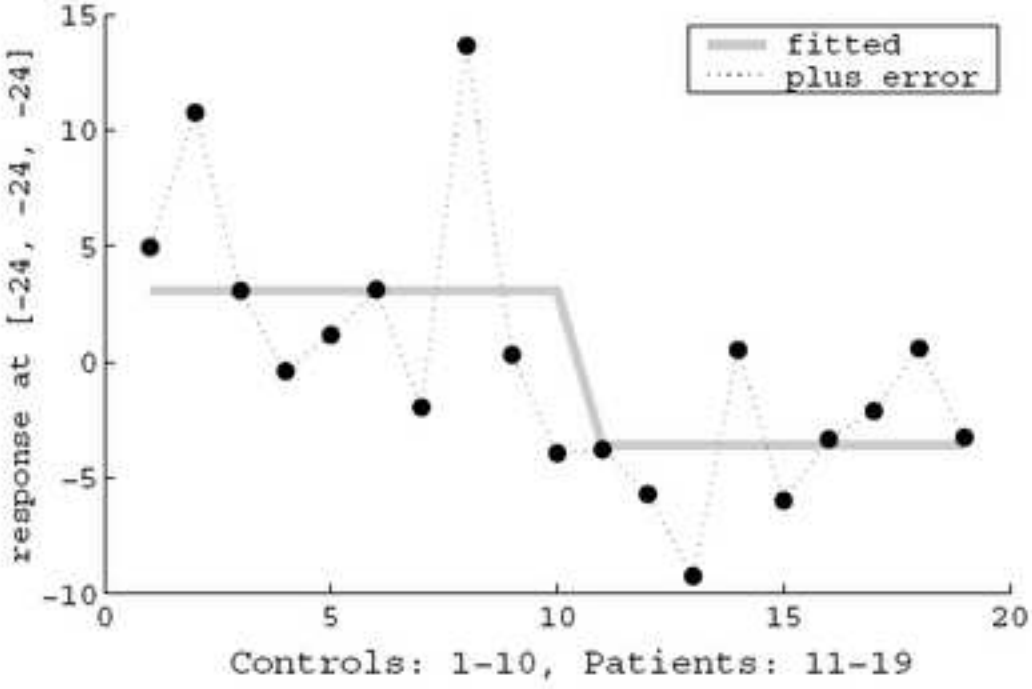

Figure 3a

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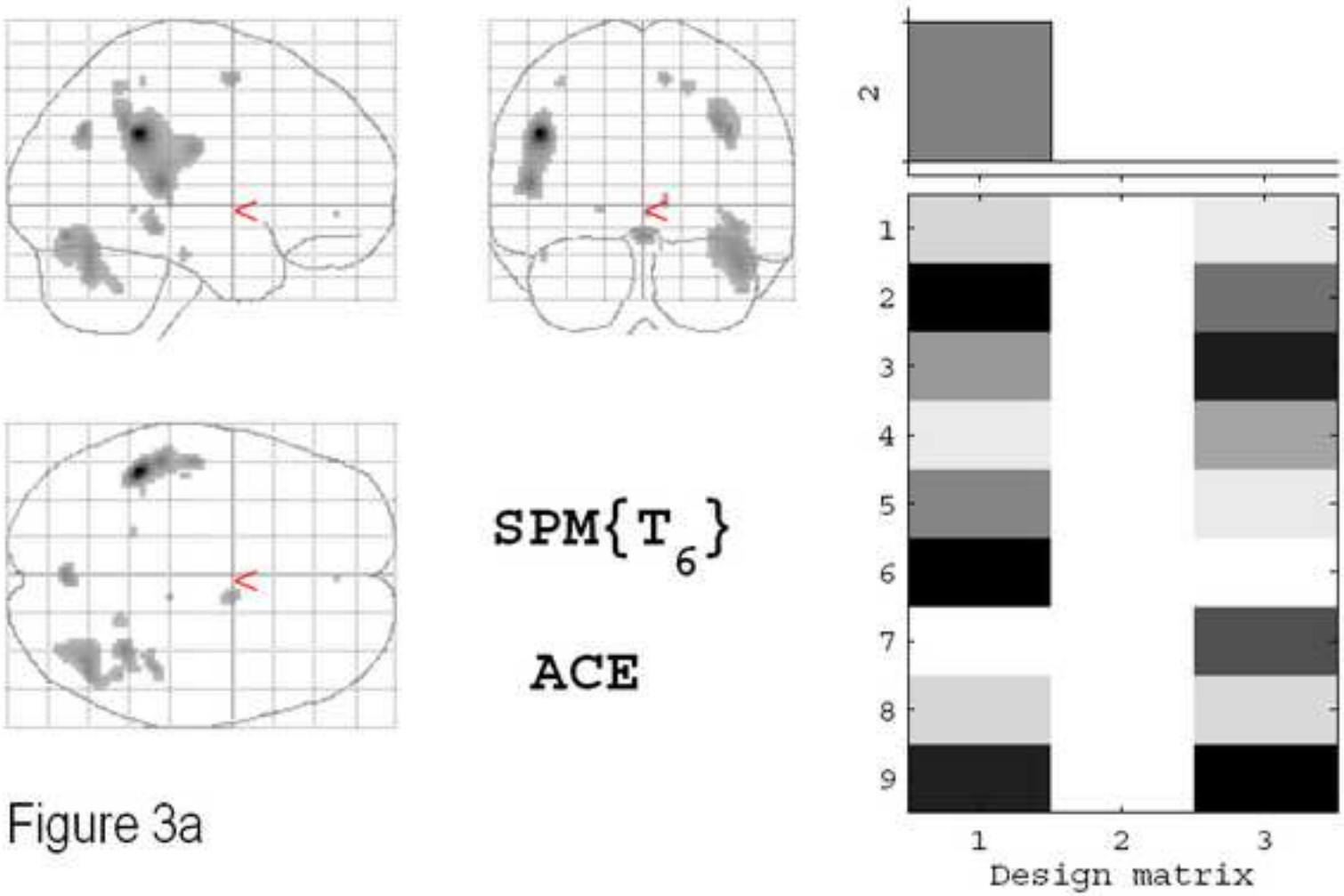

Figure 3a

Figure 3b

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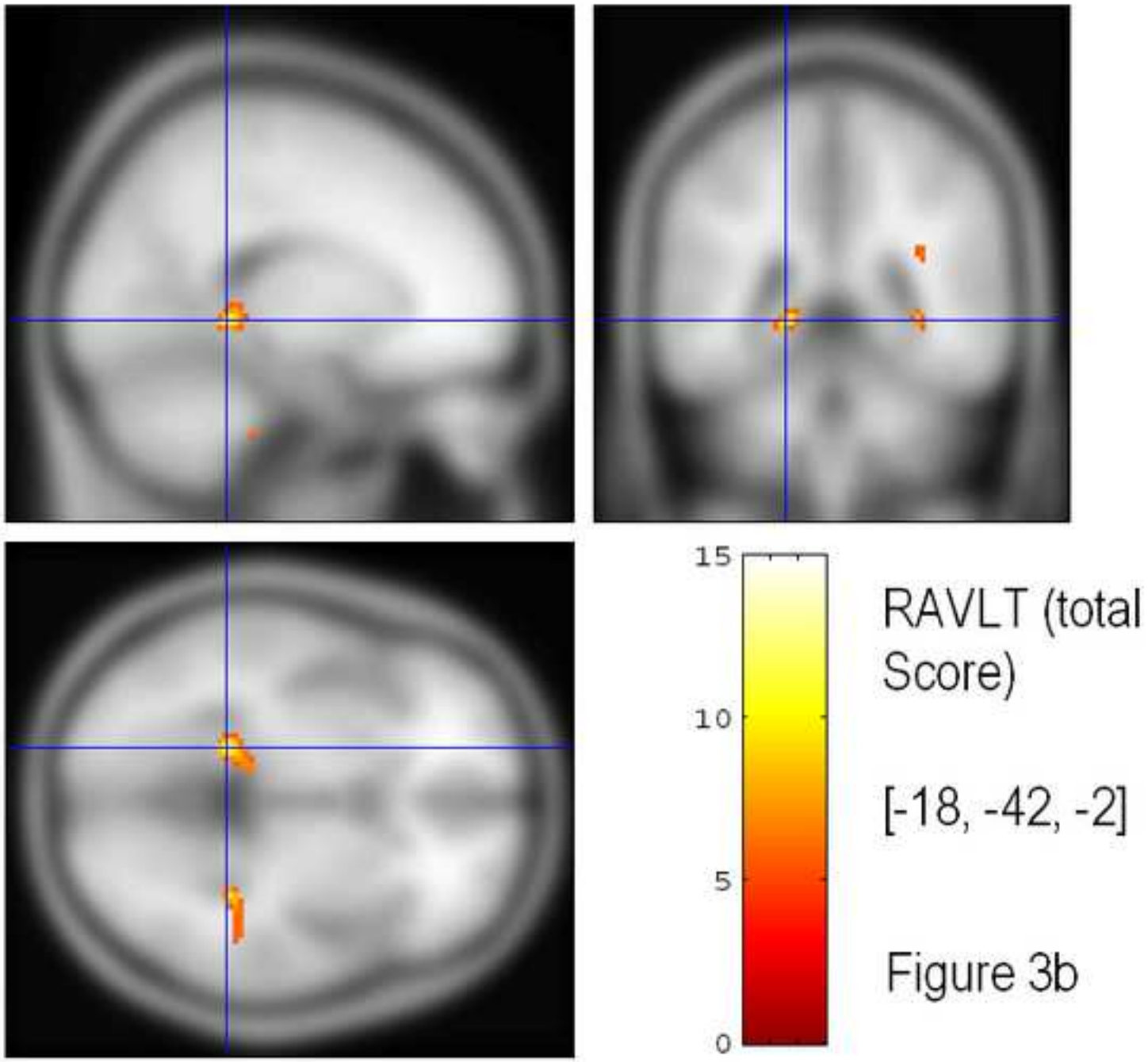

Figure 3c

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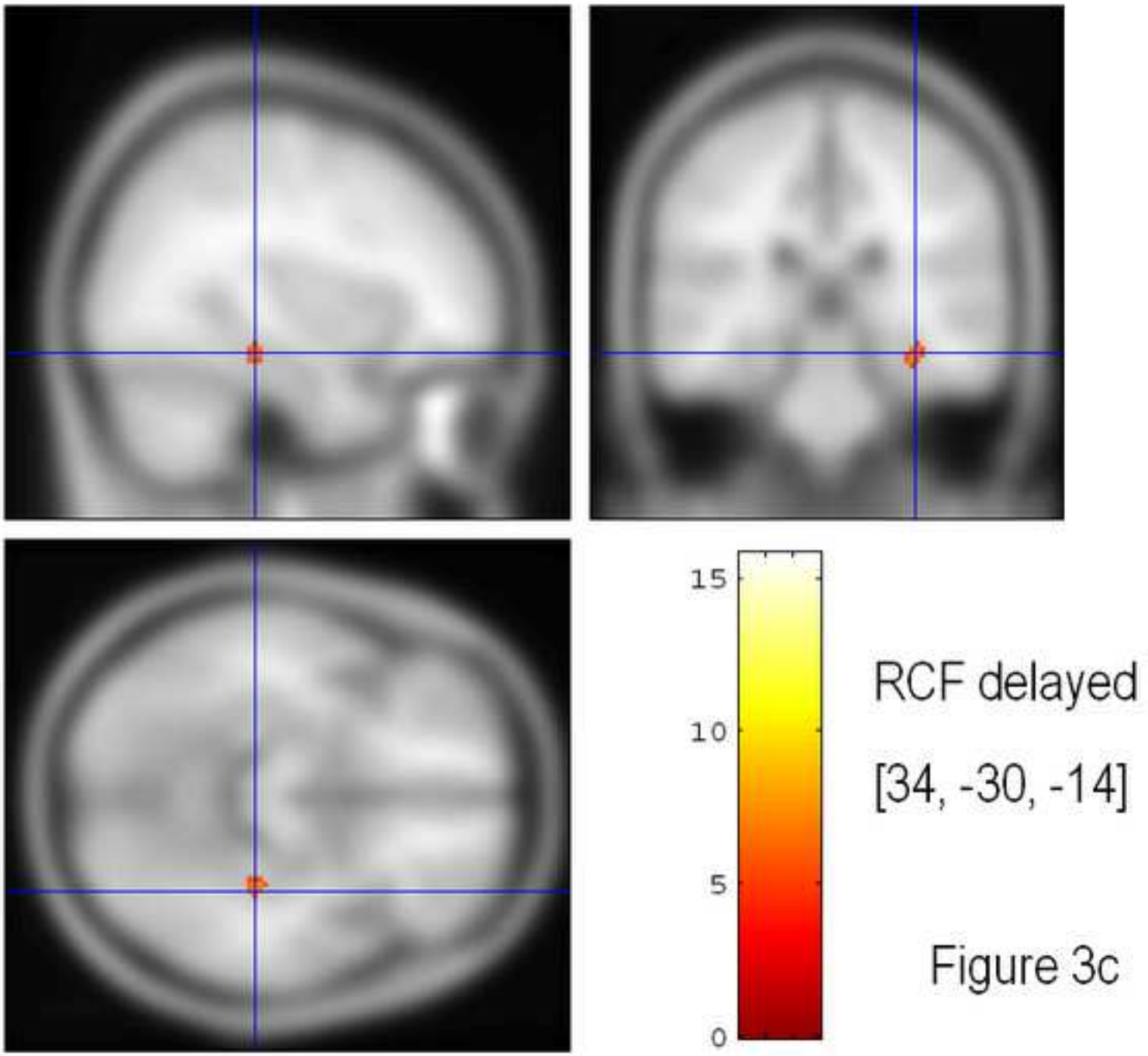

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