Psychological Medicine (2015), **45**, 1799–1810. © Cambridge University Press 2014
doi:10.1017/S0033291714002876

ORIGINAL ARTICLE

Joint effects of gray matter atrophy and altered functional connectivity on cognitive deficits in amnestic mild cognitive impairment patients

C. Xie[1,2]*, F. Bai[1,2], B. Yuan[1,2], H. Yu[1], Y. Shi[2], Y. Yuan[3], D. Wu[1], Z.-S. Zhang[1,2] and Z.-J. Zhang[1,2]*

[1] *Department of Neurology, Affiliated Zhongda Hospital of Southeast University, Nanjing, People's Republic of China*
[2] *Institute of Neuropsychiatry of Southeast University, Nanjing, People's Republic of China*
[3] *Department of Psychology, Affiliated Zhongda Hospital of Southeast University, Nanjing, People's Republic of China*

Background. Gray matter (GM) atrophy and disrupted intrinsic functional connectivity (IFC) are often present in patients with amnestic mild cognitive impairment (aMCI), which shows high risk of developing into Alzheimer's disease. Little is known, however, about the relationship between GM atrophy and altered IFC, and whether they are related to cognitive decline.

Method. A total of 30 aMCI and 26 cognitively normal (CN) subjects were recruited for this study. Optimized voxel-based morphometric and resting-state functional connectivity magnetic resonance imaging approaches were performed to measure the GM volumes (GMVs) and atrophy-related IFC, respectively. Multivariate linear regression analysis was used to examine the effects of GM atrophy and IFC on cognitive performance across subjects, after controlling for the effects of age, education, gender and group.

Results. Compared with CN subjects, aMCI subjects showed significantly reduced GMVs and decreased IFC in the frontal-parietal and medial temporal lobe systems. Multivariate regression analysis further demonstrated that the GMVs and decreased IFC simultaneously affected the cognitive function. Specifically, GMVs were positively correlated with cognitive performances, including global cognition and episodic memory, and showed a strong trend in correlation between GMVs and non-episodic memory, whilst IFC was positively correlated with the above three cognitive measures, across all subjects. In addition, significant correlation was found between GMVs and altered IFC strength across all subjects.

Conclusions. Our findings demonstrated that GMVs and IFC jointly contribute to cognitive performance, and combining quantitative information about GMVs and the strength of functional connectivity may serve as an indicator of cognitive deficits in non-demented elderly.

Received 5 October 2013; Revised 9 November 2014; Accepted 10 November 2014; First published online 16 December 2014

Key words: Amnestic mild cognitive impairment, functional MRI, gray matter atrophy, intrinsic functional connectivity.

Introduction

Amnestic mild cognitive impairment (aMCI) is considered as the middle stage of the Alzheimer's disease (AD) spectrum (Kong *et al.* 2013) and, when present, significantly increases the subsequent risk of developing AD (Gauthier *et al.* 2006). Structural imaging studies using volume, shape or cortical thickness measures have demonstrated clear differences between normal subjects and those with mild cognitive impairment (MCI) (for reviews, see Smith, 2010, 2012). Prominent gray matter (GM) volume reductions in aMCI patients may occur in the medial temporal lobes and thalamus (Bell-McGinty *et al.* 2005; Whitwell *et al.* 2007*a*, *b*; Karas *et al.* 2008; Balthazar *et al.* 2009; Morbelli *et al.* 2010; Venneri *et al.* 2011; Serra *et al.* 2013), extending into more posterior regions of the temporal lobe, parietal lobe, posterior cingulate and part of the occipital lobe, as well as substantial involvement of the frontal lobes (Bell-McGinty *et al.* 2005; Whitwell *et al.* 2007*b*). Interestingly, reductions in hippocampal volume and more widespread cortical atrophy in aMCI patients are similar to AD, and associated with a greater risk of conversion of aMCI to AD (Hamalainen *et al.* 2007; Ferreira *et al.* 2011). Moreover, intermediate amounts of AD-related neuropathological findings, including amyloid deposition and neurofibrillary tangles, were also found in the mesial temporal and neocortical structures in aMCI patients relative to age-matched normal adults and patients with AD (Bourgeat *et al.* 2010; Markesbery, 2010). These findings strongly suggest that the GM atrophy observed in

* Address for correspondence: C. Xie, Ph.D., M.D., Department of Neurology, Affiliated Zhongda Hospital of Southeast University, no. 87 DingJiaQiao Road, Nanjing 210009, People's Republic of China.
(Email: drchmxie@gmail.com) [C.X.]
(Email: janemengzhang@vip.163.com) [Z.-J.Z.]

aMCI substantially increases the risk of aMCI progression to AD.

Recently, functional magnetic resonance image (fMRI) task-specific studies have shown abnormal hippocampal activation during episodic memory performance in aMCI (Dickerson *et al.* 2005; Bai *et al.* 2009*b*; Machulda *et al.* 2009). Moreover, greater hippocampal activation predicts cognitive decline in persons with aMCI (Miller *et al.* 2008). These studies have identified task-related brain network activation and substantially advance our understanding of the regions involved in the performance of a given task, performance being dependent on the difficulty of the task, subject's compliance, and so on. To overcome the task-dependent fMRI pitfalls, the resting-state functional connectivity MRI (R-fMRI) approach has been employed to detect the relationship between different regions; R-fMRI measures temporal correlations between the spontaneous blood oxygenation level-dependent (BOLD) signals in different brain regions, and provides new information into how structurally segregated and functionally specialized brain regions are interconnected. Abnormal resting-state functional connectivity has been found in aMCI in several networks, including in frontal–parietal (Rombouts *et al.* 2005; Qi *et al.* 2010), posterior cingulate cortex (PCC)–temporal lobe (Bai *et al.* 2009*a*), hippocampus–PCC (Sorg *et al.* 2007) and insula–prefrontal (Xie *et al.* 2012) networks in aMCI subjects. These investigations suggest that cognitive impairment may result from the disruption of functional integrity in the distributed brain networks in aMCI subjects at the systems level (Palop *et al.* 2006). Taken together, volumetric MRI and fMRI studies have separately revealed the potential contribution of GM volumes and distributed networks, which both collect information from multiple domain-specific brain systems, to higher-order cognitive processing in the human brain (Buckner *et al.* 2009).

Recently, multimodal neuroimaging techniques have been engaged in exploring the associations among GM atrophy, intrinsic connectivity in distributed networks coordinated in large-scale brain systems, and cognitive performance. For example, GM atrophy is associated with altered intrinsic connectivity in various types of dementias (Seeley *et al.* 2009), heroin addicts (Yuan *et al.* 2010) but not in schizophrenia (Lui *et al.* 2009). Moreover, GM atrophy and intrinsic connectivity are partially correlated with behavioral performance in these studies, whereas other fMRI studies in aMCI subjects removed the confounding effects of brain atrophy on the functional connectivity changes (Sorg *et al.* 2007; Bai *et al.* 2009*b*). Currently, it is still unclear as to whether the changes in brain anatomy, particularly in the atrophic regions, affect the alterations of intrinsic connectivity, and how GM atrophy and intrinsic connectivity affect cognitive performance, in non-demented elderly. Thus, we propose a network-based hypothesis that the reduced GM volumes seen in critical brain structures and atrophy-related intrinsic connectivity can jointly affect cognitive deficits, and GM volumes will significantly influence the altered intrinsic connectivity in aMCI subjects.

In the current study, therefore, we use the R-fMRI approach to test the following network-based hypotheses: in aMCI subjects, (i) reduced GM volumes will be seen in critical brain structures; (ii) GM atrophy-related intrinsic connectivity will predict cognitive deficits; and (iii) reduced GM volumes will be significantly associated with altered intrinsic connectivity.

Method

Participants

A total of 33 aMCI patients and 30 cognitively normal (CN) healthy subjects were recruited to this study. All subjects were enrolled through community health-screening activities and newspaper advertisements (all were Chinese Han and right handed) in Nanjing, China.

Protocol was approved by the Research Ethics Committee of the Affiliated Zhongda Hospital, Southeast University, and written informed consent was obtained from all participants. All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Three aMCI and four CN subjects were excluded, because of excessive motion during fMRI runs (i.e. exceeding more than 1 mm translational movement or more than 1° rotational movement) or incomplete image scans, yielding a total of 30 aMCI and 26 controls for this analysis.

All subjects underwent comprehensive neuropsychological assessments, including the Auditory Verbal Memory Test-Delayed Recall (AVMT-DR) and the Rey–Osterrieth Complex Figure Test-Immediate Recall (ROCFT-IR), Trail Making Tests A and B, Digit Span Test, Symbol Digit Modalities Test, and the Clock Drawing Test, which covered memory, executive function, perceptual speed, and visual–spatial domains. All aMCI patients met the diagnostic criteria described previously (Petersen *et al.* 1999; Petersen, 2004; Bai *et al.* 2009*a*; Liu *et al.* 2013). Exclusion criteria were: regular use of drugs or medications; history of neurological or psychiatric illness; and contraindications for MRI scanning. Control subjects were required to have a Clinical Dementia Rating of 0, a Mini Mental State Examination (MMSE) score $\geqslant$26, and an

https://doi.org/10.1017/S0033291714002876 Published online by Cambridge University Press

AVMT-DR score >4 for those with 8 or more years of education. The inclusion and exclusion assessments were performed by an experienced neuropsychiatrist, who administered a structured interview to subjects and their informants.

MRI protocol

Imaging was performed using a General Electric 1.5 Tesla scanner (USA) with a homogeneous birdcage head coil. High-resolution spoiled gradient-recalled echo (SPGR) three-dimensional axial images were acquired for anatomical reference. The parameters were: repetition time (TR) = 9.9 ms, echo time (TE) = 2.1 ms, flip angle (FA) = 15°, acquisition matrix = 256 × 192, field of view (FOV) = 240 × 240 mm, thickness = 1.0 mm, gap = 0 mm, number of excitations (NEX) = 1.0. Axial resting-state (no cognitive tasks were performed, eyes closed, and ears occluded) functional connectivity fMRI (R-fMRI) datasets were obtained in 7 min and 6 s with a gradient-recalled echo-planar imaging (GRE-EPI) pulse sequence, and in total 142 volumes were acquired. The R-fMRI imaging parameters were: TR = 3000 ms, TE = 40 ms, FA = 90°, acquisition matrix = 64 × 64, FOV = 240 × 240 mm, thickness = 4.0 mm, gap = 0 mm, NEX = 1.0.

Voxel-based morphometry (VBM) analysis

Optimized VBM, combined with Statistical Parametric Mapping (SPM8) software (http://www.fil.ion.ucl.ac.uk/spm) on a Matlab 7.5 (MathWorks, http://www.mathworks.com/) platform were utilized to perform GM volume analysis (Duyn & Moonen, 1993). First, the study group-specific template was created by averaging the high-resolution T1-weighted images for all participants after spatial normalization. Second, two-step segmentation of the brain tissues was done using the analysis of likelihood of each voxel of the brain image to be classified as GM, white matter or cerebrospinal fluid (CSF). Third, spatial normalization for the GM volume of all participants was conducted based on study group-specific GM template and the absolute GM volume modulated for each subject. Fourth, all segmented GM images were smoothed with an 8-mm Gaussian kernel and transformed using a *logit* transformation $[logit(n) = 0.5\ \ln(n/[1-n])]$ to make the data more normally distributed. Finally, these data were transformed to Montreal Neurological Institute (MNI) space. To find brain regions with a robust group difference, a two-sample voxelwise t test was performed, and the significance level was set at $p < 0.0001$ (corrected for multiple comparisons with family-wise error). Several regions of interests (ROIs) reflecting significant clusters of GM atrophy found in aMCI subjects compared with control subjects were selected as seed regions for the functional connectivity analysis and these ROIs volumes were also extracted from all subjects using masks generated from the group comparison.

Functional connectivity analysis

R-fMRI data analysis was carried out using Analysis of Functional NeuroImages (AFNI) software (http://afni.nimh.nih.gov/afni). Briefly, motion correction was performed by volume registration on the R-fMRI data (3dvolreg). Then, detrending was carried out to remove Legendre polynomials (3dDetrend). Possible contamination from the signals in white matter and CSF, and six-motion vectors were regressed out from the whole brain (3dDeconvolve), and the global signal was also regressed out (Chen *et al.* 2012). Then, a band-pass filter was applied to keep only low-frequency fluctuations within the frequency range of 0.015 and 0.1 Hz.

The time course of each subject's ROI from the functional EPI images was extracted. The spatial resolutions in the SPGR (1 × 1 × 1 mm) and EPI images (3.75 × 3.75 × 4 mm) were different; therefore, only those voxels in the EPI images that contained at least 50% of the individual ROI voxels masked on the three-dimensional SPGR images were included in the voxel time course analysis (Xu *et al.* 2008; Xie *et al.* 2011). Then, the averaged time course of each ROI, as the seed time course, was correlated with the time courses of all brain voxels using Pearson cross-correlation. Next, we applied the Pearson correlation coefficients (r) to a Fisher z transform analysis, which yielded variants of approximately normal distribution $[m = 0.5\ \ln(1+r)/(1-r)]$ (Zar, 1996). Finally, the data were spatially normalized to the Talairach template image, resampled to 2-mm isotropic voxels, and smoothed with a Gaussian kernel (6 mm full width half maximum), using AFNI software.

Statistical analysis

Behavioral data

The composite scores for cognition were developed in a two-step process (Xie *et al.* 2012). First, the composite scores for different cognitive domains were created: z scores for each test for each subject were converted from the raw scores with reference to the means and standard deviations of all the subjects. We then calculated composite scores by averaging the z scores of individual tests: Episodic memory (two tests including AVMT-DR and ROCFT-IR) and non-episodic memory (five tests, executive function including Trail Making Tests A and B, working memory including the Digit Span Test, perceptual speed including the Symbol Digit Modalities Test, and visual–spatial function

https://doi.org/10.1017/S0033291714002876 Published online by Cambridge University Press

including the Clock Drawing Test). The global cognitive function score was formed by averaging the z scores of all seven tests. These composite scores were compared between aMCI and CN subjects by t tests. The MMSE was performed for description and diagnostic classification, but not used in the composite cognitive measures. These composite scores increase power by reducing random variability, floor and ceiling effects, and the number of correlated data in analyses (Schneider *et al.* 2007), and are widely used (Ringman *et al.* 2005; Schneider *et al.* 2007; Wilson *et al.* 2010).

Group-level functional connectivity analysis

Individual connectivity maps of ROIs for each subject were analysed with a random-effects one-sample t test to identify voxels. This showed a significant positive or negative correlation with the seed time series, and the pattern of each ROI network for CN and aMCI groups was obtained, respectively ($p < 0.01$, corrected with AlphaSim, cluster size >450 mm^3). To find brain regions with a significant group difference, a voxelwise two-sample t test for each network was performed at the threshold of $p < 0.01$ (corrected with AlphaSim, cluster size >450 mm^3). Then, the average strength of functional connectivity in each cluster was extracted from each subject using masks generated from the group comparison in the distributed network, and then underwent the following regression analysis using commercially available statistical software (SPSS 16.0, USA).

Joint effects of GM atrophy and altered intrinsic connectivity on behavioral performance

To investigate the effects of GM atrophy and altered intrinsic connectivity on cognitive performance, a multivariate linear regression analysis was employed as below:

$$Beh = \beta_0 + \beta_1 \times GMV + \beta_2 \times Network_{FC} + \beta_3 \times AGE + \beta_4 \times Edu + \beta_5 \times Gen + \beta_6 \times Group + \in$$

where *Beh* is the composite scores of global cognition, episodic memory, and non-episodic memory in each subject, *GMV* is the total *GM* volumes of eight atrophic regions and $Network_{FC}$ is the averaged strength of functional connectivity in all eight functional networks for individual subject (see description below). β_0 is the intercept of straight line fitting in the model; β_1 and β_2 are the effects of *GMV* and $Network_{FC}$ on the cognitive performance, respectively; β_3, β_4, β_5, and β_6 are the effects of age, education, gender and group in the above model; $\in$ is the error of this model.

To increase the statistical power, the raw GM volumes of each ROI and the functional connectivity strength in the distributed network for each subject were converted to z scores according to the mean and standard deviation for all participants, respectively, and then, the total z scores of GM volumes for all ROIs were summed to yield the composite measures of the GM volumes, and the z scores of functional connectivity strength in all networks were averaged to obtain composite measures of functional connectivity for each subject, separately. These are similar to the behavioral data processing. Then, we performed the above multivariate linear regression model using these variables after controlling for the effects of age, education, gender and group.

Results

Subject characterization

Demographic information and clinical evaluations for all participants are shown in Table 1. No significant differences in age or gender were noted between the aMCI and CN groups. In examining both groups, significant cognitive deficits were found in the aMCI group.

Reduced GM volume in aMCI group

Brain regions with significant GM atrophy included the bilateral precuneus and insula, and the left postcentral gyrus (PostCG), medial frontal gyrus (MeFG), middle frontal gyrus (MFG) and hippocampus, as shown in Fig. 1 (detailed information for these eight regions are described in online Supplementary Table S1).

Resting-state functional connectivity patterns in CN and aMCI subjects

Resting-state functional connectivity maps of each atrophy-related region in the CN and aMCI groups ($p < 0.01$, with cluster size >450 mm^3) are briefly illustrated in Fig. 2. Generally, each network is composed of a positive network and anticorrelated (negative) network, and the strongest connectivity of each network was adjacent to its seed region. Non-adjacent regions also showed significant connectivity associated with each seed ROI. Detailed information for each network pattern is described in online Supplementary Table S2.

Altered functional connectivity in distinct networks in aMCI group

We examined the group differences of connectivity specificity in each network. As shown in online Supplementary Tables S3 and S4, significantly decreased functional connectivity in eight distributed

https://doi.org/10.1017/S0033291714002876 Published online by Cambridge University Press

Table 1. *Demographic information and psychometric data on the cognitive tests and composite measures*

Characteristic	aMCI ($n = 30$)	CN ($n = 26$)	p
Gender, *n*			N.S.[a]
Female	11	12	
Male	19	14	
Age, years	72.57 (4.83)	70.31 (4.76)	0.085
Education, years	14.27 (3.23)	14.27 (3.23)	0.402
MMSE	27.10 (1.58)	28.15 (1.29)	0.009[b]
Global cognitive score, *z* score	−0.19 (0.35)	0.30 (0.22)	<0.001[b]
Episodic memory	−0.58 (0.71)	0.67 (0.32)	<0.001[b]
AVMT-DR	−0.84 (0.41)	0.96 (0.45)	<0.001[b]
ROCFT-IR	−0.33 (1.23)	0.38 (0.41)	0.008[b]
Non-episodic memory	−0.03 (0.37)	0.16 (0.27)	0.040
Trail Making Test-A	0.34 (1.00)	−0.39 (0.86)	0.005[b]
Trail Making Test-B	0.37 (1.13)	−0.43 (0.61)	0.002[b]
Symbol Digit Modalities Test	−0.45 (0.87)	0.52 (0.90)	<0.001[b]
Clock Drawing Test	−0.01 (1.00)	0.64 (0.43)	0.004[b]
Digit Span Test	−0.39 (0.92)	0.45 (0.90)	0.001[b]

Data are given as mean (standard deviation) unless otherwise indicated.

aMCI, Amnesic mild cognitive impairment; CN, cognitively normal; N.S., non-significant; MMSE, Mini-Mental State Examination; AVMT-DR, Auditory Verbal Memory Test-Delayed Recall; ROCFT-IR, Rey–Osterrieth Complex Figure Test-Immediate Recall.

[a] p Value was obtained by χ^2 test.

[b] p Values were obtained by two-sample t tests (two-tailed).

networks was observed in the aMCI cohort. Briefly, these brain regions were found in: (1) the left hippocampus network, primarily including the bilateral cuneus, right middle occipital gyrus, precuneus and left inferior frontal gyrus (IFG); (2) the left MeFG network, primarily involved in the left lingual gyrus (LG) and anterior temporal pole (aTP) and right precuneus; (3) the left MFG network, mainly encompassing the left PCC, right inferior parietal cortex (IPC), precuneus, IFG and fusiform area; (4) the left PostCG network, including the right superior temporal gyrus (STG) and PCC; (5) the left precuneus network, particularly in the right precuneus and STG, left SFG and LG; (6) the left insula network, mainly located in the left IPC, dorsolateral prefrontal cortex (DLPFC), pre-somatomotor area, MeFG and aTP; (7) the right precuneus network, especially in the left anterior cingulate cortex, IFG and MeFG; and (8) the right insula network, primarily localized in the right middle cingulate cortex, aTP, left PostCG and IFG. We did not find significantly increased functional connectivity of individual networks in the aMCI subjects compared with CN subjects, after correction for multiple comparisons. Representative connectivity changes for the distributed functional networks are shown in Fig. 3.

Relationship of behavioral performance, GM atrophy and altered intrinsic connectivity

A multivariate linear regression model was established ($R^2 = 0.24$, $p = 1.79 \times 10^{-3}$). Further, we also identified that GM volumes were positively correlated with global cognition ($R^2 = 0.13$, $p < 0.0006$), episodic memory ($R^2 = 0.09$, $p < 0.01$) and with non-episodic memory ($R^2 = 0.06$, $p < 0.07$); intrinsic connectivity strength was positively correlated with global cognition ($R^2 = 0.26$, $p < 0.0001$), episodic memory ($R^2 = 0.18$, $p < 0.001$) and non-episodic memory ($R^2 = 0.14$, $p < 0.004$), after controlling for the effects of the age, education, gender and group, as described in Fig. 4. These different R^2 values suggest the distinct contribution of GM atrophy and intrinsic functional connectivity to cognitive impairment in aMCI patients.

Further, partial correlation analysis identified that GM volumes were significantly correlated with intrinsic connectivity strength after controlling for the effects of the above covariance ($p < 0.025$).

Discussion

In this study, we demonstrated reduced GM volumes and altered functional connectivity of GM atrophy-related networks in the aMCI cohort relative to CN subjects, and, importantly, GM atrophy and these disrupted functional connectivities jointly affected cognitive performance in these non-demented elderly. In addition, we also found that GM volumes potentially influenced functional connectivity strength across all subjects.

We first identified that the GM atrophic regions were mainly located in the frontal–parietal system, bilateral insula and left hippocampus. This is consistent with previous studies reporting that these regions were typically involved in AD-related pathology (Karas *et al.* 2004; Gili *et al.* 2011). Importantly, these brain areas are widely recognized to mediate episodic memory encoding, and are densely connected to other neocortical regions (Brambati *et al.* 2009; Schmidt-Wilcke *et al.* 2009). Therefore, regional abnormalities, such as hippocampal atrophy, as frequently seen in aMCI and AD subjects, may lead to a dysfunction in information processing related to episodic and semantic memory (Schmidt-Wilcke *et al.* 2009; Joubert *et al.*

https://doi.org/10.1017/S0033291714002876 Published online by Cambridge University Press

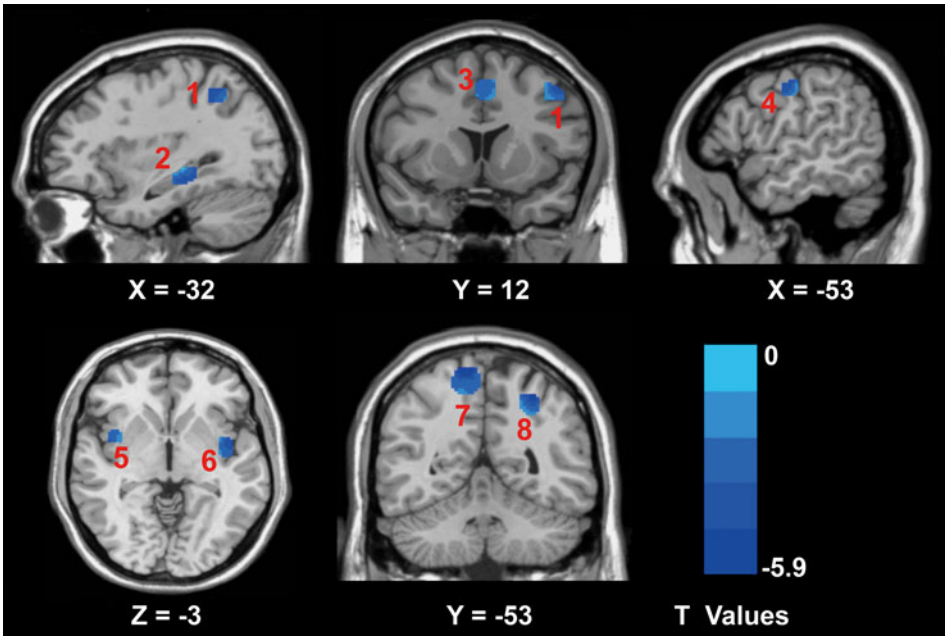

Fig. 1. Group comparison identifying atrophic gray matter (GM) regions. The results display significant GM volume reduction in: 1, the left postcentral gyrus; 2, left hippocampus; 3, left medial frontal gyrus; 4, left middle frontal gyrus; 5, right precuneus; 6, left precuneus; 7, left insula; and 8, right insula on representative sections of the template brain. Blue color indicates decreased GM volumes in the amnestic mild cognitive impairment patients compared with the cognitively normal subjects. The color bar is presented with *T* values.

2010). Recently, a meta-analysis of VBM studies in the aMCI subjects showed that significant GM volume reduction, especially in the left hippocampus and parahippocampus, serves as a good biomarker for predicting the conversion from aMCI to AD (Ferreira *et al.* 2011). It is also intriguing that widespread cortical areas, as well as the medial temporal lobe, are affected in the early stages of AD. For example, atrophy in the precuneus, extending to more diffuse medial and lateral posterior parietal regions, as well as extensively accelerated atrophy in frontal regions were identified in very mild stages of AD (Buckner *et al.* 2005). In addition, insula atrophy has often been reported in the previous studies that focused on aMCI subjects and AD patients, suggesting that insula atrophy may be a biomarker to differentiate progressive MCI and stable MCI in the early stages of AD, and to monitor the progression of aMCI conversion to AD (Spulber *et al.* 2012). The consistency of these observations indicates that GM atrophy in the above regions in aMCI subjects is associated with a high risk of subsequent progression to AD.

Next, significantly decreased functional connectivity of eight GM atrophy-related networks were found in aMCI subjects relative to the CN subjects (online Supplementary Tables S3 and S4). Briefly, in the CN subjects, these eight ROI networks, as expected, presented similar patterns to previous findings (Cavanna, 2006; Bai *et al.* 2009*b*; Xie *et al.* 2012). However, in the aMCI subjects, the disrupted intrinsic connectivity of each network was primarily located in the frontal–parietal system, temporal and occipital lobes, and subcortical regions. Recently, network analyses of anatomical and functional connectivity in non-human and human primates have indicated that some brain areas, known as hubs, are highly interconnected and functionally specialized (Bullmore & Sporns, 2009). These hubs, often including the DLPFC, MeFG, PCC and pMTG, play an essential role in the integration of diverse information, balancing the signal transfer among multiple, specific neural pathways in order to perform high-order cognitive process (Sporns *et al.* 2007; Hagmann *et al.* 2008; Buckner *et al.* 2009). Damage to these hubs can disrupt the resting-state default mode network activity, which as a core network has been suggested to play a pivotal role in cognitive processes, including mind wandering, goal-directed behavior and self-preference (Dosenbach *et al.* 2007; Fox & Raichle, 2007; Mason *et al.* 2007). Also, destruction of the precuneus and insula networks perhaps leads to dysfunctional attention and episodic memory impairment (Cavanna, 2006; Dorfel *et al.* 2009; Xie *et al.* 2012), which present as core features characterizing aMCI patients. Moreover, disruption of these delicately balanced regulations in the functional integrity among various neural networks, but not

https://doi.org/10.1017/S0033291714002876 Published online by Cambridge University Press

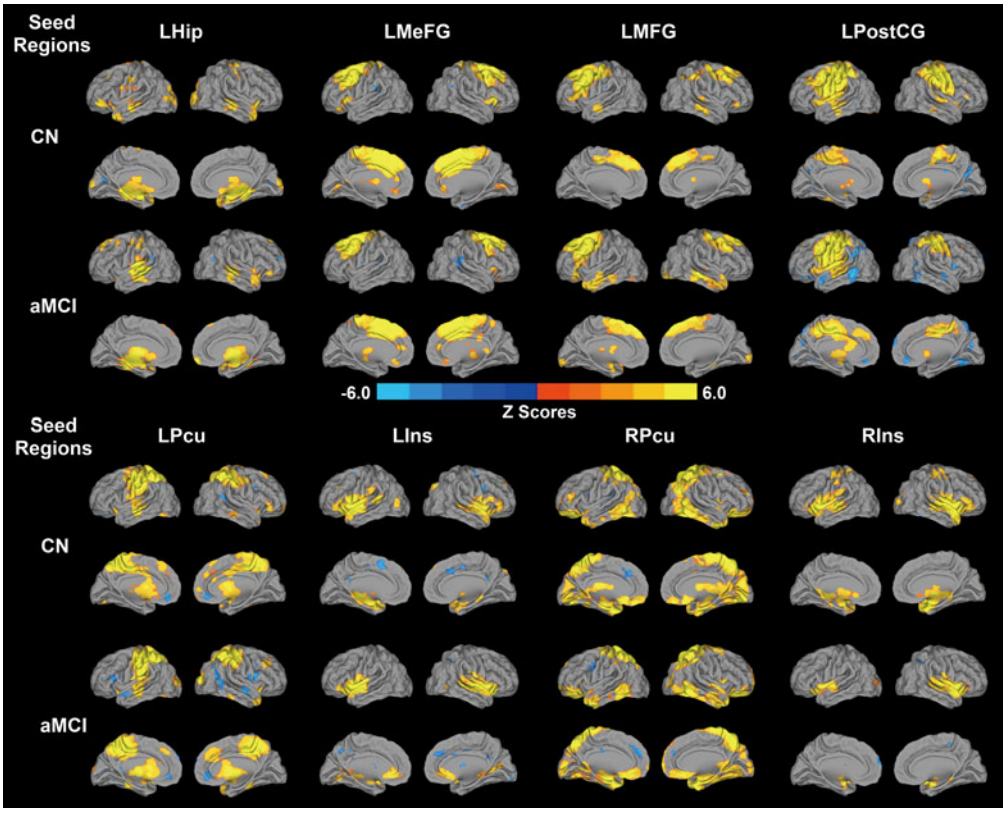

Fig. 2. Resting-state functional connectivity maps between each gray matter atrophy-related region of interest and the rest of the brain in cognitively normal (CN) and amnestic mild cognitive impairment (aMCI) subjects ($p_{corrected} < 0.01$). These maps show the functional connectivity pattern of each network between CN and aMCI subjects. Bright color indicates voxels with significant positive connectivity and blue color indicates voxels with negative (anticorrelation) connectivity with the seed region. The color bar is presented with *z* scores. LHip, Left hippocampus; LMeFG, left medial frontal gyrus; LMFG, left middle frontal gyrus; LPostCG, left postcentral gyrus; LPcu, left precuneus; LIns, left insula; RPcu, right precuneus; RIns, right insula.

structural damage, increases vulnerability to AD pathology (Buckner *et al.* 2009). This evidence, at least in part, supports the notion that AD, and possibly aMCI, are disconnection syndromes (Greicius *et al.* 2004; Delbeuck *et al.* 2007). Taken together, these disrupted connectivities in aMCI patients may reflect the decoupling of functional brain networks underlying interoception, memory processing, appropriate emotional and cognitive responses, and probably represent the potential atrophy-related network organization among individuals with aMCI.

It is widely accepted that high-order cognitive processing results from the dynamic interaction of distributed brain areas operating at the large-scale neural-network level instead of isolated brain regions (Pessoa, 2008; Mesulam, 2009). Currently, the fact that GM volumes and intrinsic connectivity of specific neural networks are functionally linked to distinctive behavioral modulation indicates that intactness in the cognitive functions requires the integrity of the complex neural networks and stability in the GM volumes in discrete anatomic brain regions at the system level. Understanding the underlying mechanism of this fact is critical for gaining new insight into the neural basis of the conversion of aMCI to AD. First, accumulating evidence has suggested that separate physiological processes are associated with distinct cognitive performances (Andrews-Hanna *et al.* 2007). Previous studies have reported that GM atrophy or dysfunctional connectivity are associated with the decline in cognition in aMCI patients (Laird *et al.* 2009; Wang *et al.* 2010), further indicating that coherent mental representation generated through reorganization of neural networks to improve the ability to perform cognitively demanding tasks are seen in patients with MCI and AD (Agosta *et al.* 2010). Second, we also found that overall connectivity strength in aMCI patients was lower than that in CN subjects (see Fig. 4), suggesting the functional decoupling of information in aMCI patients. Interesting, our findings identified a distinct contribution of GM atrophy and intrinsic functional connectivity to cognitive

https://doi.org/10.1017/S0033291714002876 Published online by Cambridge University Press

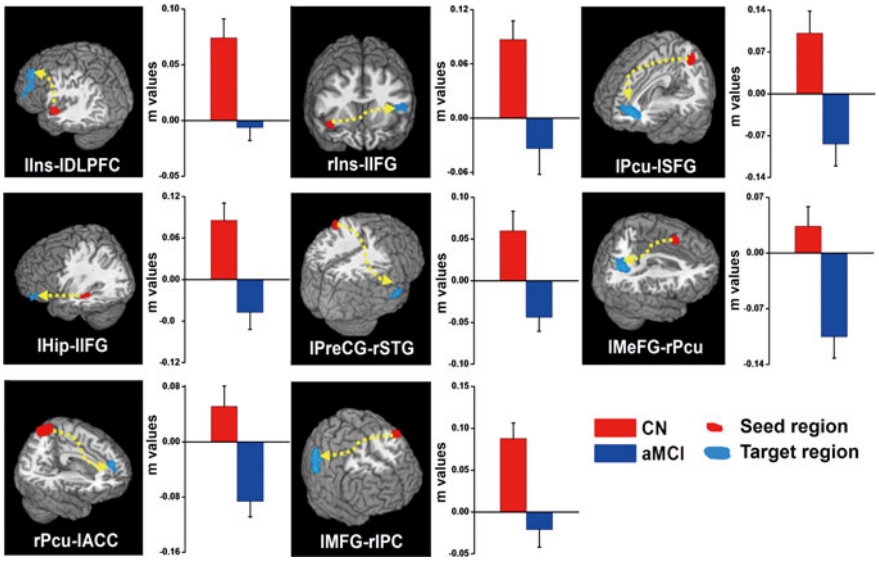

Fig. 3. Representative regions of significantly altered functional connectivity in distinct networks between cognitively normal (CN) and amnestic mild cognitive impairment (aMCI) subjects. The results illustrate decreased functional connectivity strength (*m* value) in the distinctive network in aMCI subjects, as opposed to that in CN subjects. In each panel, the left brain image shows functional connectivity from the seed region (red color) to the target region (blue color). The right image shows the numerical presentation of the functional connectivity strength in the target region connected to the seed region in the CN group (red color) and the aMCI group (blue color). Values are means, with standard deviations represented by vertical bars. lIns, Left insula; lDLPFC, left dorsolateral prefrontal cortex; lHip, left hippocampus; lIFG, left inferior frontal gyrus; rPcu, right precuneus; lACC, left anterior cingulate cortex; rIns, right insula; lPreCG, left precentral gyrus; rSTG, right superior temporal gyrus; lMFG, left middle frontal gyrus; rIPC, right inferior parietal gyrus; lPcu, left precuneus; lSFG, left superior frontal gyrus; lMeFG, left medial frontal gyrus.

impairment in aMCI patients; that is, the regional GM atrophy was significantly associated with poorer cognitive performance, including global cognition, episodic memory, and even non-episodic memory, and separately accounted for about 13, 9 and 6% of the variance in cognitive performance (Ersche *et al.* 2012). By contrast, the disrupted intrinsic connectivity can account for 26, 18 and 14% of the variance of poorer cognitive performance, as shown in Fig. 4, indicating the greater contribution of intrinsic connectivity over regional GM volumes to cognitive performance.

Additionally, we found that GM atrophy was significantly correlated with intrinsic connectivity, indicating that functional integrity in multiple distributed neural networks is selectively and vulnerably disrupted by structural damage. This will extend our understanding of fundamental cognitive process in the human brain with memory disorders, such as aMCI and AD. In addition, these atrophic regions are also key components of the higher-order cognitive networks; therefore, GM atrophy in these regions may result in the disruption of distributed cognitive networks, and thus, detrimentally affect information processing, especially for episodic memory, in aMCI subjects (Balthazar *et al.* 2010).

Our study is not without limitations. First, we predefined the atrophic areas as seed regions and proved the behavioral significance of the GM volumes and intrinsic connectivity. This may not reflect the significance of whole-brain GM atrophy and altered functional networks. Future studies are needed to investigate the functional role of the large-scale, distributed, functional networks, including the whole-brain regions in aMCI subjects. Second, anatomical connectivity studies with diffusion tensor imaging (DTI) have highlighted an important contribution of white matter fiber in the functional integrity of structural networks, so tracing white matter fibers based on structural connectivity analysis using the DTI approach to investigate the functional integrity of the neural network should also be a focus for future studies. Third, the combination of R-fMRI and specific cognitive tasks, which can reflect the subtle decline of cognition, can detect the association between cognitive performance and the neural network reorganization underlying the different pathological state. This is essential in determining the putative etiologic roles of functional network abnormalities in subjects with aMCI. Additionally, we focused on addressing the potential contribution of combining GM atrophy and altered functional networks to cognitive function in the

https://doi.org/10.1017/S0033291714002876 Published online by Cambridge University Press

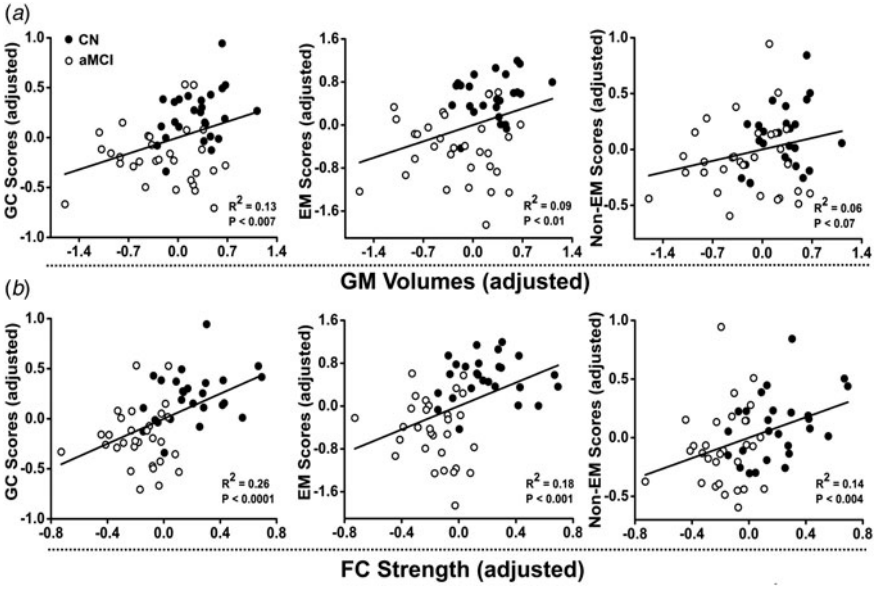

Fig. 4. Joint effects of gray matter (GM) volumes and intrinsic functional connectivity (FC) strength on cognitive performance across all subjects: cognitively normal (CN) and amnestic mild cognitive impairment (aMCI) subjects. Multivariate linear regression analysis demonstrated that both GM volumes in atrophic regions and FC strength contribute to cognitive performance. (*a*) GM volumes in atrophic regions are significantly correlated with global cognition (GC) ($R^2 = 0.13$, $p < 0.007$) and episodic memory (EM) ($R^2 = 0.09$, $p < 0.01$). There is a trend in correlation with non-EM ($R^2 = 0.06$, $p < 0.07$). (*b*) Intrinsic FC strength is significantly correlated with GC ($R^2 = 0.26$, $p < 0.0001$), EM ($R^2 = 0.18$, $p < 0.001$) and non-EM ($R^2 = 0.14$, $p < 0.004$).

current study, and did not consider the individual GM atrophic region's or network's influence. This may limit our understanding of the contribution of each region or network to cognition in the early stage of the AD process.

Conclusions

In summary, this study demonstrated that GM atrophy can significantly affect intrinsic connectivity, and that GM atrophy and altered functional connectivity jointly affect cognitive deficits, indicating that combining quantitative information about GM volumes and the strength of functional connectivity may successfully predict cognitive performance in non-demented elderly.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291714002876

Acknowledgements

C.X. receives research funding support from the National Natural Science Foundation of China (81171323, 91332118); F.B. receives support from the National Natural Science Foundation of China (81201080, 91332104). H.Y. was supported by the National Natural Science Foundation of China (81070877); Y.S. was funded by the National Natural Science Foundation of China (81171021); Y.Y. receives research funding support from the National Natural Science Foundation of China (30970814, 81071101), the Nature Science Foundation of Jiangsu Province (BK2009050) and the Key Program of Medical Development of Nanjing (ZKX07018); D.W., Z.-S.Z. and B.Y. do not report disclosures; Z.-J.Z. receives research support from the National Basic Research Program of China (973 Program) (2007CB512308), the National Hi-Tech Research and Development Program of China (863 Program) (2008AA02Z413), National Science and Technology Major Projects for 'Major New Drugs Innovation and Development' (2012ZX09506-001-009) and the National Natural Science Foundation of China (30770779, 30825014, 30971016, 81061120529).

The authors thank Dr Gavin Reynolds for editorial assistance and Mr B. Douglas Ward (Biophysics, Medical College of Wisconsin, WI 53226, USA) for his contribution to the statistical analysis.

Declaration of Interest

All authors have made substantial intellectual contribution to this paper in one or more of the following areas that included design or conceptualization of the study, analysis or interpretation of the data, or drafting

or revising the manuscript. C.X. conducted the statistical analysis. All authors gave final approval of the manuscript. No authors of this paper have any possible conflict of interest, financial or otherwise, related directly or indirectly to this work.

References

Agosta F, Rocca MA, Pagani E, Absinta M, Magnani G, Marcone A, Falautano M, Comi G, Gorno-Tempini ML, Filippi M (2010). Sensorimotor network rewiring in mild cognitive impairment and Alzheimer's disease. *Human Brain Mapping* **31**, 515–525.

Andrews-Hanna JR, Snyder AZ, Vincent JL, Lustig C, Head D, Raichle ME, Buckner RL (2007). Disruption of large-scale brain systems in advanced aging. *Neuron* **56**, 924–935.

Bai F, Watson DR, Yu H, Shi Y, Yuan Y, Zhang Z (2009*a*). Abnormal resting-state functional connectivity of posterior cingulate cortex in amnestic type mild cognitive impairment. *Brain Research* **1302**, 167–174.

Bai F, Zhang Z, Watson DR, Yu H, Shi Y, Yuan Y, Zang Y, Zhu C, Qian Y (2009*b*). Abnormal functional connectivity of hippocampus during episodic memory retrieval processing network in amnestic mild cognitive impairment. *Biological Psychiatry* **65**, 951–958.

Balthazar ML, Yasuda CL, Cendes F, Damasceno BP (2010). Learning, retrieval, and recognition are compromised in aMCI and mild AD: are distinct episodic memory processes mediated by the same anatomical structures? *Journal of the International Neuropsychological Society* **16**, 205–209.

Balthazar ML, Yasuda CL, Pereira FR, Pedro T, Damasceno BP, Cendes F (2009). Differences in grey and white matter atrophy in amnestic mild cognitive impairment and mild Alzheimer's disease. *European Journal of Neurology* **16**, 468–474.

Bell-McGinty S, Lopez OL, Meltzer CC, Scanlon JM, Whyte EM, Dekosky ST, Becker JT (2005). Differential cortical atrophy in subgroups of mild cognitive impairment. *Archives of Neurology* **62**, 1393–1397.

Bourgeat P, Chetelat G, Villemagne VL, Fripp J, Raniga P, Pike K, Acosta O, Szoeke C, Ourselin S, Ames D, Ellis KA, Martins RN, Masters CL, Rowe CC, Salvado O (2010). β-Amyloid burden in the temporal neocortex is related to hippocampal atrophy in elderly subjects without dementia. *Neurology* **74**, 121–127.

Brambati SM, Belleville S, Kergoat MJ, Chayer C, Gauthier S, Joubert S (2009). Single- and multiple-domain amnestic mild cognitive impairment: two sides of the same coin? *Dementia and Geriatric Cognitive Disorders* **28**, 541–549.

Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, Andrews-Hanna JR, Sperling RA, Johnson KA (2009). Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *Journal of Neuroscience* **29**, 1860–1873.

Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA (2005). Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *Journal of Neuroscience* **25**, 7709–7717.

Bullmore E, Sporns O (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature Reviews Neuroscience* **10**, 186–198.

Cavanna AE (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* **129**, 564–583.

Chen G, Chen G, Xie C, Ward BD, Li W, Antuono P, Li SJ (2012). A method to determine the necessity for global signal regression in resting-state fMRI studies. *Magnetic Resonance in Medicine* **68**, 1828–1835.

Delbeuck X, Collette F, Van der Linden A (2007). Is Alzheimer's disease a disconnection syndrome? Evidence from a crossmodal audio-visual illusory experiment. *Neuropsychologia* **45**, 3315–3323.

Dickerson BC, Salat DH, Greve DN, Chua EF, Rand-Giovannetti E, Rentz DM, Bertram L, Mullin K, Tanzi RE, Blacker D, Albert MS, Sperling RA (2005). Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. *Neurology* **65**, 404–411.

Dorfel D, Werner A, Schaefer M, von Kummer R, Karl A (2009). Distinct brain networks in recognition memory share a defined region in the precuneus. *European Journal of Neuroscience* **30**, 1947–1959.

Dosenbach NU, Fair DA, Miezin FM, Cohen AL, Wenger KK, Dosenbach RA, Fox MD, Snyder AZ, Vincent JL, Raichle ME, Schlaggar BL, Petersen SE (2007). Distinct brain networks for adaptive and stable task control in humans. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 11073–11078.

Duyn JH, Moonen CT (1993). Fast proton spectroscopic imaging of human brain using multiple spin-echoes. *Magnetic Resonance in Medicine* **30**, 409–414.

Ersche KD, Jones PS, Williams GB, Turton AJ, Robbins TW, Bullmore ET (2012). Abnormal brain structure implicated in stimulant drug addiction. *Science* **335**, 601–604.

Ferreira LK, Diniz BS, Forlenza OV, Busatto GF, Zanetti MV (2011). Neurostructural predictors of Alzheimer's disease: a meta-analysis of VBM studies. *Neurobiology of Aging* **32**, 1733–1741.

Fox MD, Raichle ME (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature Reviews Neuroscience* **8**, 700–711.

Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de Leon M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B (2006). Mild cognitive impairment. *Lancet* **367**, 1262–1270.

Gili T, Cercignani M, Serra L, Perri R, Giove F, Maraviglia B, Caltagirone C, Bozzali M (2011). Regional brain atrophy and functional disconnection across Alzheimer's disease evolution. *Journal of Neurology, Neurosurgery and Psychiatry* **82**, 58–66.

Greicius MD, Srivastava G, Reiss AL, Menon V (2004). Default-mode network activity distinguishes Alzheimer's

disease from healthy aging: evidence from functional MRI. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 4637–4642.

Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ, Sporns O (2008). Mapping the structural core of human cerebral cortex. *PLoS Biology* **6**, e159.

Hamalainen A, Tervo S, Grau-Olivares M, Niskanen E, Pennanen C, Huuskonen J, Kivipelto M, Hanninen T, Tapiola M, Vanhanen M, Hallikainen M, Helkala EL, Nissinen A, Vanninen R, Soininen H (2007). Voxel-based morphometry to detect brain atrophy in progressive mild cognitive impairment. *NeuroImage* **37**, 1122–1131.

Joubert S, Brambati SM, Ansado J, Barbeau EJ, Felician O, Didic M, Lacombe J, Goldstein R, Chayer C, Kergoat MJ (2010). The cognitive and neural expression of semantic memory impairment in mild cognitive impairment and early Alzheimer's disease. *Neuropsychologia* **48**, 978–988.

Karas G, Sluimer J, Goekoop R, van der Flier W, Rombouts SA, Vrenken H, Scheltens P, Fox N, Barkhof F (2008). Amnestic mild cognitive impairment: structural MR imaging findings predictive of conversion to Alzheimer disease. *American Journal of Neuroradiology* **29**, 944–949.

Karas GB, Scheltens P, Rombouts SA, Visser PJ, van Schijndel RA, Fox NC, Barkhof F (2004). Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. *NeuroImage* **23**, 708–716.

Kong L, Chen K, Tang Y, Wu F, Driesen N, Womer F, Fan G, Ren L, Jiang W, Cao Y, Blumberg HP, Xu K, Wang F (2013). Functional connectivity between the amygdala and prefrontal cortex in medication-naive individuals with major depressive disorder. *Journal of Psychiatry and Neuroscience* **38**, 417–422.

Laird AR, Eickhoff SB, Li K, Robin DA, Glahn DC, Fox PT (2009). Investigating the functional heterogeneity of the default mode network using coordinate-based meta-analytic modeling. *Journal of Neuroscience* **29**, 14496–14505.

Liu H, Qin W, Li W, Fan L, Wang J, Jiang T, Yu C (2013). Connectivity-based parcellation of the human frontal pole with diffusion tensor imaging. *Journal of Neuroscience* **33**, 6782–6790.

Lui S, Deng W, Huang X, Jiang L, Ma X, Chen H, Zhang T, Li X, Li D, Zou L, Tang H, Zhou XJ, Mechelli A, Collier DA, Sweeney JA, Li T, Gong Q (2009). Association of cerebral deficits with clinical symptoms in antipsychotic-naive first-episode schizophrenia: an optimized voxel-based morphometry and resting state functional connectivity study. *American Journal of Psychiatry* **166**, 196–205.

Machulda MM, Senjem ML, Weigand SD, Smith GE, Ivnik RJ, Boeve BF, Knopman DS, Petersen RC, Jack CR (2009). Functional magnetic resonance imaging changes in amnestic and nonamnestic mild cognitive impairment during encoding and recognition tasks. *Journal of the International Neuropsychological Society* **15**, 372–382.

Markesbery WR (2010). Neuropathologic alterations in mild cognitive impairment: a review. *Journal of Alzheimer's Disease* **19**, 221–228.

Mason MF, Norton MI, Van Horn JD, Wegner DM, Grafton ST, Macrae CN (2007). Wandering minds: the default network and stimulus-independent thought. *Science* **315**, 393–395.

Mesulam M (2009). Defining neurocognitive networks in the BOLD new world of computed connectivity. *Neuron* **62**, 1–3.

Miller SL, Fenstermacher E, Bates J, Blacker D, Sperling RA, Dickerson BC (2008). Hippocampal activation in adults with mild cognitive impairment predicts subsequent cognitive decline. *Journal of Neurology, Neurosurgery and Psychiatry* **79**, 630–635.

Morbelli S, Piccardo A, Villavecchia G, Dessi B, Brugnolo A, Piccini A, Caroli A, Frisoni G, Rodriguez G, Nobili F (2010). Mapping brain morphological and functional conversion patterns in amnestic MCI: a voxel-based MRI and FDG-PET study. *European Journal of Nuclear Medicine and Molecular Imaging* **37**, 36–45.

Palop JJ, Chin J, Mucke L (2006). A network dysfunction perspective on neurodegenerative diseases. *Nature* **443**, 768–773.

Pessoa L (2008). On the relationship between emotion and cognition. *Nature Reviews Neuroscience* **9**, 148–158.

Petersen RC (2004). Mild cognitive impairment as a diagnostic entity. *Journal of Internal Medicine* **256**, 183–194.

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999). Mild cognitive impairment: clinical characterization and outcome. *Archives of Neurology* **56**, 303–308.

Qi Z, Wu X, Wang Z, Zhang N, Dong H, Yao L, Li K (2010). Impairment and compensation coexist in amnestic MCI default mode network. *NeuroImage* **50**, 48–55.

Ringman JM, Diaz-Olavarrieta C, Rodriguez Y, Chavez M, Fairbanks L, Paz F, Varpetian A, Maldonado HC, Macias-Islas MA, Murrell J, Ghetti B, Kawas C (2005). Neuropsychological function in nondemented carriers of presenilin-1 mutations. *Neurology* **65**, 552–558.

Rombouts SARB, Barkhof F, Goekoop R, Stam CJ, Scheltens P (2005). Altered resting state networks in mild cognitive impairment and mild Alzheimer's disease: an fMRI study. *Human Brain Mapping* **26**, 231–239.

Schmidt-Wilcke T, Poljansky S, Hierlmeier S, Hausner J, Ibach B (2009). Memory performance correlates with gray matter density in the ento-/perirhinal cortex and posterior hippocampus in patients with mild cognitive impairment and healthy controls – a voxel based morphometry study. *NeuroImage* **47**, 1914–1920.

Schneider JA, Boyle PA, Arvanitakis Z, Bienias JL, Bennett DA (2007). Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. *Annals of Neurology* **62**, 59–66.

Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD (2009). Neurodegenerative diseases target large-scale human brain networks. *Neuron* **62**, 42–52.

Serra L, Giulietti G, Cercignani M, Spano B, Torso M, Castelli D, Perri R, Fadda L, Marra C, Caltagirone C, Bozzali M (2013). Mild cognitive impairment: same identity for different entities. *Journal of Alzheimer's Disease* **33**, 1157–1165.

Smith CD (2010). Neuroimaging through the course of Alzheimer's disease. *Journal of Alzheimer's Disease* **19**, 273–290.

Smith CD (2012). Structural imaging in early pre-states of dementia. *Biochimica et Biophysica Acta* **1822**, 317–324.

Sorg C, Riedl V, Muhlau M, Calhoun VD, Eichele T, Laer L, Drzezga A, Forstl H, Kurz A, Zimmer C, Wohlschlager AM (2007). Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 18760–18765.

Sporns O, Honey CJ, Kotter R (2007). Identification and classification of hubs in brain networks. *PLoS ONE* **2**, e1049.

Spulber G, Niskanen E, Macdonald S, Kivipelto M, Padilla DF, Julkunen V, Hallikainen M, Vanninen R, Wahlund LO, Soininen H (2012). Evolution of global and local grey matter atrophy on serial MRI scans during the progression from MCI to AD. *Current Alzheimer Research* **9**, 516–524.

Venneri A, Gorgoglione G, Toraci C, Nocetti L, Panzetti P, Nichelli P (2011). Combining neuropsychological and structural neuroimaging indicators of conversion to Alzheimer's disease in amnestic mild cognitive impairment. *Current Alzheimer Research* **8**, 789–797.

Wang L, LaViolette P, O'Keefe K, Putcha D, Bakkour A, Van Dijk KRA, Pihlajamäki M, Dickerson BC, Sperling RA (2010). Intrinsic connectivity between the hippocampus and posteromedial cortex predicts memory performance in cognitively intact older individuals. *NeuroImage* **51**, 910–917.

Whitwell JL, Petersen RC, Negash S, Weigand SD, Kantarci K, Ivnik RJ, Knopman DS, Boeve BF, Smith GE, Jack CR Jr (2007a). Patterns of atrophy differ among specific subtypes of mild cognitive impairment. *Archives of Neurology* **64**, 1130–1138.

Whitwell JL, Przybelski SA, Weigand SD, Knopman DS, Boeve BF, Petersen RC, Jack CR Jr (2007b). 3D maps from multiple MRI illustrate changing atrophy patterns as subjects progress from mild cognitive impairment to Alzheimer's disease. *Brain* **130**, 1777–1786.

Wilson RS, Leurgans SE, Foroud TM, Sweet RA, Graff-Radford N, Mayeux R, Bennett DA (2010). Telephone assessment of cognitive function in the late-onset Alzheimer's disease family study. *Archives of Neurology* **67**, 855–861.

Xie C, Bai F, Yu H, Shi Y, Yuan Y, Chen G, Li W, Zhang Z, Li SJ (2012). Abnormal insula functional network is associated with episodic memory decline in amnestic mild cognitive impairment. *NeuroImage* **63**, 320–327.

Xie C, Li SJ, Shao Y, Fu L, Goveas J, Ye E, Li W, Cohen AD, Chen G, Zhang Z, Yang Z (2011). Identification of hyperactive intrinsic amygdala network connectivity associated with impulsivity in abstinent heroin addicts. *Behavioural Brain Research* **216**, 639–646.

Xu Y, Xu G, Wu G, Antuono P, Rowe DB, Li SJ (2008). The phase shift index for marking functional asynchrony in Alzheimer's disease patients using fMRI. *Magnetic Resonance Imaging* **26**, 379–392.

Yuan K, Qin W, Dong M, Liu J, Sun J, Liu P, Zhang Y, Wang W, Wang Y, Li Q, Zhao L, von Deneen KM, Liu Y, Gold MS, Tian J (2010). Gray matter deficits and resting-state abnormalities in abstinent heroin-dependent individuals. *Neuroscience Letters* **482**, 101–105.

Zar J (1996). *Biostatistical Analysis*, 3rd edn. Prentice-Hall, Inc.: Upper Saddle River, NJ.