Original article

Functional MRI study of mild Alzheimer's disease using amplitude of low frequency fluctuation analysis

XI Qian, ZHAO Xiao-hu, WANG Pei-jun, GUO Qi-hao, YAN Chao-gan and HE Yong

Keywords: *Alzheimer's disease; resting-state functional MRI; amplitude of low frequency fluctuation*

Background Previous studies have shown that the functional brain activity in the resting state is impaired in Alzheimer's disease (AD) patients. However, most studies focused on the relationship between different brain areas, rather than the amplitude or strength of the regional brain activity. The purpose of this study was to explore the functional brain changes in AD patients by measuring the amplitude of the blood oxygenation level dependent (BOLD) functional MRI (fMRI) signals.

Methods Twenty mild AD patients and twenty healthy elderly subjects participated in the fMRI scan. The amplitude of low frequency fluctuation (ALFF) was calculated using REST software.

Results Compared with the healthy elderly subjects, the mild AD patients showed decreased ALFF in the right posterior cingulate cortex, right ventral medial prefrontal cortex, and in the bilateral dorsal medial prefrontal cortex. No brain region with increased ALFF was found in the AD group compared with the control group.

Conclusions The reduced activity in the posterior cingulate cortex and medial prefrontal cortex observed in the present study suggest that the functional abnormalities of those areas are at an early stage of AD. The ALFF analysis may provide a useful tool in fMRI study of AD.

Chin Med J 2012;125(5):858-862

ALzheimer's disease (AD) is a progressive neurodegenerative disorder, which is characterized by global cognitive decline, including the progressive loss of memory, reasoning, and language.[1] To date, there is still no effective treatment for AD. Considering that earlier treatment can improve disease prognosis, the early diagnosis of AD is especially important.

Structural MRI has been primarily used to differentiate AD from healthy elderly subjects, relying on volume measurements of the hippocampus and surrounding structures.[2,3] However, most patients with structural MRI abnormalities often have irreversible pathological damage to the brain. Given that functional alterations might precede structural abnormalities, the blood oxygenation level dependent (BOLD) functional MRI (fMRI) may be a technique for studying AD.[4-7] The commonly used task-based fMRI requires the patient to understand and perform specific cognitive tasks. Such a method is not appropriate for patients with cognitive impairment, and it cannot be widely used in clinics. In contrast, the resting-state fMRI does not require the subject to perform any task, which avoids any model esign, greatly simplifies the fMRI procedure, and is especially appropriate for patients who cannot complete the neuropsychological tests or perform cognitive tasks.[8-10]

The resting-state fMRI technique has also been utilized to explore the neurophysiological mechanism underlying AD.[11-13] Previous studies have shown that brain functional activity in the resting state is impaired in AD patients.[12,13] However, most studies focused on the relationship between different brain areas, e.g., functional connectivity methods based on region of interest (ROI) or independent component (ICA) analysis, rather than amplitude or strength of the regional brain activity. Because ROI identification is based on a priori hypothesis, ROI-based analysis is prone to user introduced bias. While ICA measures the BOLD signal synchrony, it is also difficult to pinpoint which area is responsible for the observed abnormality in the connectivity. An alternative way of measuring regional brain activity during resting state is to examine the amplitude of low frequency fluctuation (ALFF) of the BOLD signal.[14] Biswal et al[15] reported that the reduced low-frequency fluctuation in white matter relative to gray matter by approximately 60% suggests that ALFF is associated with field potential activity in local brain regions. In this case, the ALFF is considered to be the

DOI: 10.3760/cma.j.issn.0366-6999.2012.05.023

Department of Radiology, Tongji Hospital of Tongji University, Shanghai 200065, China (Xi Q, Zhao XH and Wang PJ)

Department of Neurology, Huashan Hospital of Fudan University, Shanghai 200040, China (Guo QH)

State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing 100875, China (Yan CG and He Y)

Correspondence to: Dr. WANG Pei-jun, Department of Radiology, Tongji Hospital of Tongji University, Shanghai 200065, China (Tel: 86-21-66111204. Fax: 86-21-56952231. Email: tongjipjwang@vip.sina.com)

This work was supported by grants from the National Natural Science Foundation of China (No. 30970818), and the National High-Tech Research and Development Program of China (No. 2008AA02Z302).

reflection of regional spontaneous neuronal activity and physiological states of the brain.[16] In the present resting-state fMRI study, a newly reported ALFF method was used to analyze the BOLD signal of the brain. It was supposed that the resting-state fMRI technique based on ALFF analysis would allow a new insight into the neurophysiology of AD.

METHODS

Subjects

Twenty mild AD patients and twenty healthy elderly subjects participated in the study. The mild AD patients were recruited from a memory clinic at the Department of Neurology. Healthy elderly subjects were recruited from the nearby community. All subjects or their legal representatives gave written consent for participation in the study, which was approved by the Medical Research Ethics Committee of Tongji Hospital. Examinations for each subject included medical history, neurological examination, informant interview, structural MRI, and a neuropsychological assessment that included Mini-Mental State Exam (MMSE), clinical dementia rating (CDR), activity of daily living scale, Hachinski ischemic scale, and the Hamilton rating scale for depression. Patients with stroke, psychiatric diseases, drug abuse, moderate to serious hypertension and systematic diseases were ruled out. The diagnosis of AD was made on the basis of the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria.[17] All mild AD patients had a CDR scale score of 1.0. No memory complaints or neurological deficiencies were observed in the healthy elderly subjects. The demographics and neuropsychological findings of the mild AD patients and healthy elderly subjects are shown in Table 1.

Data acquisition

Functional MR images were obtained on a 1.5-T MR scanner (Marconi EDGE ECLIPSE). During scanning, all subjects were instructed to keep their eyes closed and to refrain from initiating goal-directed, attention-demanding activity. A T2 weighted, gradient-recalled echo-planar imaging sequence was obtained for functional images: echo time 40 ms, repetition time 2000 ms, slice thickness 6 mm, gap 1 mm, flip angle 90°, field of view 24 cm, and resolution 64×64 matrix.

Data preprocessing

Data of fMRI were preprocessed using Statistical Parametric Mapping (SPM2, *http://www.fil.ion.ucl.ac uk/spm/*). The first 10 volumes of the functional images were discarded to equilibrate the signal and to allow participants' adaptation to the scanning noise. For each participant, functional images were realigned using least-squares minimization without higher-order corrections for spin history and normalized to the Montreal Neurological Institute template. Images were re-sampled to 3 mm^3 and smoothed with a 4 mm full-width at half maximum.

ALFF calculating

REST package (REST, *http://resting-fmri. sourceforge. net*) was used to calculate the ALFF with a voxel-based approach. The time courses were first converted to the frequency domain using a Fast Fourier Transform (FFT), and the power spectrum was obtained. The power spectrum obtained by FFT was square-rooted and then averaged across 0.01–0.08 Hz at each voxel. This averaged square root was taken as the ALFF. To reduce the global effects of variability across the participants, the ALFF of each voxel was divided by the global mean ALFF value within the whole-brain mask obtained previously. The global mean ALFF was calculated only within the brain, with the background and other tissues outside the brain removed.

Statistical analysis

Two-sample *t*-test was used to assess the differences in age, years of education, and MMSE scores between the two groups using SPSS 13.0 (SPSS Inc., USA). To investigate the ALFF difference between the two groups, a two-sample *t*-test was executed on the individual normalized ALFF maps. The resulting statistical map was set at a combined threshold of $P<0.01$ and a minimum cluster size of 500 mm^3, which resulted in a corrected threshold of $P<0.05$.

RESULTS

Subjects' demographic information

The demographics of mild AD patients and healthy elderly subjects, including age, sex, and education years, were matched. There was a statistically significant difference in MMSE scores between AD patients and healthy elderly subjects (t=2.86, $P<0.01$).

Brain areas showed decreased ALFF in mild AD patients

Compared with the healthy elderly subjects, the mild AD patients showed significantly decreased ALFF in the right posterior cingulate cortex, right ventral medial prefrontal cortex and bilateral dorsal medial prefrontal cortex (Table 2, Figure 1). No brain region with increased ALFF was found in the AD group.

Table 1. Demographics and neuropsychological findings of healthy elderly subjects and mild AD patients

Groups	Age (years)	Male/Female (*n*/*n*)	Education (years)	MMSE	CDR
Healthy elderly	64.72±5.62	10/10	12.21±2.47	28.23±1.77*	0
Mild AD patients	68.83±8.65	9/11	12.09±4.41	20.57±2.32*	1

MMSE: Mini-Mental State Exam. CDR: Clinical dementia rating. Values are means±SD. *$P<0.01$. There were no significant differences ($P>0.05$) in age, sex and education years between the two groups.

Table 2. Brain areas of decreased ALFF in the mild AD patients compared with the controls

Related areas		Brodmann area	Talairach coordinates			*t* values	*P* values	Volume (mm^3)
R	PCC	23, 31	8	–56	26	9.946	0.00055	923
R	vMPFC	11, 25, 32	3	50	–13	4.654	0.0062	584
B	dMPFC	9, 10, 32	0	52	26	3.787	0.00057	1249

R: right. B: bilateral. *T* and *P* values from a *t*-test of the peak voxel (showing greatest statistical difference within a cluster), which corresponds to a corrected *P* <0.01. PCC: posterior cingulate cortex. vMPFC: ventral medial prefrontal cortex. dMPFC: dorsal medial prefrontal cortex.

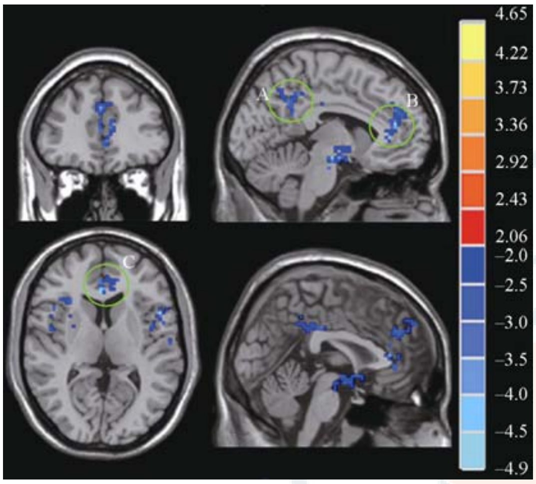

Figure 1. ALFF differences between mild AD patients and healthy elderly subjects. The blue color indicates that the mild AD patients show decreased ALFF compared with the healthy elderly subjects. Threshold was set at *P* <0.01 (corrected). The left hemisphere of the brain corresponds to the left side of the image. A: PCC; B: dMPFC; C: right vMPFC.

DISCUSSION

Using resting-state fMRI based on ALFF analysis, we found abnormal functional activity in mild AD patients. AD patients showed decreased ALFF compared to controls in the posterior cingulate cortex and medial prefrontal cortex indicating functional deficiency.

ALFF method with fMRI

When attempting to interpret the ALFF differences that we found between AD patients and control subjects, one primary question that arose was the nature of the ALFF determined with the resting-state fMRI. Unlike previous resting-state fMRI studies, such as the above mentioned functional connectivity methods based on ROI or ICA analysis, the purpose of this study was to investigate the localization of cerebral functional deficits in AD. Some investigators have attributed the low frequency fluctuation (LFF) to spontaneous neuronal activity (SNA).[18] Electroneurophysiological studies have shown that SNA is of great physiological importance, and that many brain regions generate their own cyclical patterns that interact with those of other interconnecting regions.[19] Brain diseases may exhibit abnormal local SNA and/or inter-regional SNA synchronization. More direct evidence came from a study that simultaneously monitored the local field potential (LFP) and the BOLD signal in the primary visual cortex of anesthetized monkeys.[20] It found that the impulse response function computed from LFP and BOLD under the condition of no stimulation could efficiently predict the response under conditions of stimulation. This finding implies that the LFF of the BOLD signal in the resting-state fMRI should have the same underlying electrophysiological mechanism as the task-induced fMRI BOLD signal. It could be considered that ALFF reflects the extent of SNA in the resting state.

Characteristics and meaning of the decreased ALFF brain regions in AD

Compared with the healthy elderly subjects, the patients with mild AD showed significantly decreased ALFF in the right posterior cingulate cortex (PCC). The PCC is the central region primarily affected by AD-associated alterations such as hypometabolism or an elevated atrophy rate, which contains episodic memory-related neural substrates and participates in short-term memory. Reduced activity in the PCC was observed in mild AD patients in the present study, which is consistent with previous AD reports.[5,21]

In our current study, in addition to the PCC, there was also decreased ALFF in the right ventral medial prefrontal cortex (vMPFC) and bilateral dorsal medial prefrontal cortex (dMPFC). MPFC is assumed to play a general role in emotional processing, such as attention to emotion and identification or regulation of emotion, and it guides motivational behavior by modulating or appraising autonomic emotional responses.[22] The vMPFC is closely related to the amygdala, striatum, hypothalamus, periaqueductal gray and brainstem nuclei, indicating that these areas might play a role in integrating the visceral emotional information with the endogenous and exogenous environmental information.[23] The dMPFC is required to produce spontaneous self-awareness and awareness activities.[24]

The medial temporal lobe (MTL), including the hippocampus (HC), parahippocampal cortex (PHC), and entorhinal cortex (EC), is critical for memory function. And memory damage is the earliest and most prominent cognitive impairment in AD patients.[11,25] In our study, we did not find a significant ALFF difference in the MTL between mild AD patients and controls. In previous memory task fMRI studies increased recruitment of the frontal and parietal lobes was detected in healthy elderly subjects, and increased activation in the prefrontal and left frontal-parietal lobes was observed in AD patients.[26,27] This difference was considered as a kind of compensatory process.[28] It has been thought that AD patients are able to accomplish episodic memory tasks due to compensatory neuronal activity.[29] Therefore, we conclude that no significant change in ALFF in the MTL in mild AD patients suggests that mild AD patients can recruit network resources to maintain memory function and the integrity of the MTL.

Several methodological issues concerning the use of ALFF should be considered when interpreting these results. As in all resting-state fMRI studies, we reduced but could not eliminate the effects of physiological noise, such as cardiac pulsation, by modeling low-frequency (0.01–0.08 Hz) fluctuations of the BOLD signal, into which cardiac and respiratory noises are aliased because of the relatively low sampling rate (TR=2 seconds) for multi-slice acquisitions.[23,30] Future studies should record simultaneous cardiac rate to deal with this potential problem.

In conclusion, this research applied the resting-state fMRI method to collect data and the ALFF method to analyze data. The decreased intrinsic activities in the PCC and MPFC were found to be references for distinct brain activity signatures in the mild AD patients. These abnormal activities may implicate the underlying neurophysiological mechanism in mild AD. This study provides a new method and hypothesis to study the etiology of AD, and it confirms the possibility of applying ALFF for preclinical and clinical AD studies. However, our findings of the alteration in resting-state functional activity still need to be supported by behavioral tasks. Future studies should examine the mechanism of abnormal neural activities in these brain regions in AD individuals.

REFERENCES

1. Blennow K, de Leon MJ, Zetterberg H. Alzheimer’s disease. Lancet 2006; 368: 387-403.
2. Bozzali M, Filippi M, Magnani G, Cercignani M, Franceschi M, Schiatti E, et al. The contribution of voxel-based morphometry in staging patients with mild cognitive impairment. Neurology 2006; 67: 453-460.
3. Whitwell JL, Przybelski SA, Weigand SD, Knopman DS, Boeve BF, Petersen RC, et al. 3D maps from multiple MRI illustrate changing atrophy patterns as subjects progress from mild cognitive impairment to Alzheimer's disease. Brain 2007; 130: 1777-1786.
4. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 2007; 8: 700-711.
5. Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 2004; 101: 4637-4642.
6. Vannini P, Almkvist O, Dierks T, Lehmann C, Wahlund LO. Reduced neuronal efficacy in progressive mild cognitive impairment: a prospective fMRI study on visuospatial processing. Psychiatry Res 2007; 156: 43-57
7. Gotman J, Kobayashi E, Bagshaw AP, Benar CG, Dubeau F. Combining EEG and fMRI: A multimodal tool for epilepsy research. J Magn Reson Imaging 2006; 23: 906-920.
8. Lui S, Deng W, Huang X, Jiang L, Ma X, Chen H, et al. Association of cerebral deficits with clinical symptoms in antipsychotic-naive firstepisode schizophrenia: an optimized voxel-based morphometry and resting state functional connectivity study. Am J Psychiatry 2009; 166: 196-205.
9. Wang W, Wang YR, Qin W, Yuan K, Tian J, Li Q, et al. Changes in functional connectivity of ventral anterior cingulate cortex in heroin abusers. Chin Med J 2010; 123: 1582-1588.
10. Peng DH, Jiang KD, Fang YR, Xu YF, Shen T, Long XY, et al. Decreased regional homogeneity in major depression as revealed by resting-state functional magnetic resonance imaging. Chin Med J 2011; 124: 369-373.
11. Remondes M, Schuman EM. Role for a cortical input to hippocampal area CA1 in the consolidation of a long-term memory. Nature 2004; 431: 699-703.
12. Dickerson BC, Salat DH, Greve DN, Chua EF, Rand-Giovannetti E, Rentz DM, et al. Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. Neurology 2005; 65: 404-411.
13. Bai F, Zhang Z, Yu H, Shi Y, Yuan Y, Zhu W, et al. Default-mode network activity distinguishes amnestic type mild cognitive impairment from healthy aging: A combined structural and resting-state functional MRI study. Neurosci Let 2008; 438: 111-115.
14. Zang YF, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev 2007; 29: 83-91.
15. Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echoplanar MRI. Magn Reson Med 1995; 34: 537-541.
16. Yang H, Long XY, Yang YH, Yan H, Zhu CZ, Zhou XP, et al. Amplitude of low frequency fluctuation within visual areas revealed by resting-state functional MRI. Neuroimage 2007; 36: 144-152.
17. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s disease. Neurology 1984; 34: 285-297.
18. Pelled G, Goelman G. Different physiological MRI noise between cortical layers. Magn Reson Med 2004; 52: 913-916.
19. McCormick DA. Spontaneous activity: signal or noise? Science 1999; 285: 541-543.
20. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. Nature 2001; 412: 150-157.
21. Sorg C, Riedl V, Mühlau M, Calhoun VD, Eichele T, Läer L, et al. Selective changes of resting-state networks in individuals at risk for Alzheimer’s disease. Proc Natl Acad Sci U S A 2007; 104: 18760-18765.
22. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception: I. The neural basis of normal emotion perception. Biol Psychiatry 2003; 54: 504-514.
23. Gusnard DA, Raichle ME. Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2001; 2: 685-694.
24. Gusnard DA, Akbudak E, Shulman GL, Raichle ME. Medial prefrontal cortex and self-referential mental activity: Relation to a default mode of brain function. Proc Natl Acad Sci U S A 2001; 98: 4259-4264.
25. De Jager CA, Hogervorst E, Combrinck M, Budge MM.

Sensitivity and specificity of neuropsychological tests for mild cognitive impairment, vascular cognitive impairment and Alzheimer's disease. Psychol Med 2003; 33: 1039-1050.
26. Grady CL, Bernstein LJ, Beig S, Siegenthaler AL. The effects of encoding task on age-related differences in the functional neuroanatomy of face memory. Psychol Aging 2002; 17: 7-23.
27. Gould RL, Arroyo B, Brown RG, Owen AM, Bullmore ET, Howard RJ. Brain mechanisms of successful compensation during learning in Alzheimer disease. Neurology 2006; 67: 1011-1017.
28. Prvulovie D, Hubl D, Sack AT, Melillo L, Maurer K, Frölich L, et al. Functional imaging of visuospatial processing in Alzheimer's disease. Neuroimage 2002; 17: 1403-1414.
29. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci 2008; 1124: 1-38.
30. Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, et al. Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. Am J Neuroradiol 2001; 22: 1326-1333.

(Received August 20, 2011)
Edited by WANG De