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Research Report

Cerebral and functional adaptation with chronic hypoxia exposure: A multi-modal MRI study

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ABSTRACT

The current study obtained multi-modal MRI data from 28 immigrant high altitude (HA) young adults who were born and grew up at Qinghai-Tibetan plateau matched with 28 matched sea level (SL) controls. We compared their regional gray matter volumes (VBM) and white matter quality (DAI FA values) as well as resting state brain activity (Regional Homogeneity (ReHo) of BOLD-fMRI). We found that HA residents showed decreased gray matter volume at bilateral anterior insula, bilateral prefrontal cortex, the left precentral, the left cingulate and the right lingual cortex; accompanied by changed FA and ReHo values in relevant and other regions. The resting state activity at the hippocampus and the right insula were increasing with SL relocation. The HA subjects performed worse on a series of working memory tasks, with the ReHo values of several regions as significant predictors of their performance. This study demonstrated the cerebral and functional modifications with chronic high altitude hypoxia.

1. Introduction

In recent years more and more people are travelling or working at high altitude (HA, (West and Readhead, 2004)). New comers to HA usually experience series of symptoms of mountain sickness, such as headache, lightheadedness, fatigue, insomnia or anorexia (Hackett and Roach, 2001; Basnyat and Murdoch, 2003), even with pulmonary (Hultgren, 1996) or cerebral edema (Hackett and Roach, 2004). In contrast, indigenous high altitude residents have been found to have several adaptive mechanisms in peripheral physiology to increase pulmonary ventilation and facilitate blood oxygen transportation (Chiodi, 1957; Hultgren and Grover, 1968; Frisancho et al., 1973; Frisancho, 1975; Moore et al., 1998; Beall, 2000; Moore, 2000; Beall et al., 2002; Wu and Kayser, 2006; Beall, 2007). These mechanisms will help to maintain oxygen supply to the brain, but it is not yet clear whether they are sufficient to compensate for the hypoxic risk to the central neural system.

Neuroimaging studies on indigenous high altitude residents have reported conflicting results. The earliest neuroimaging study found reduced $rCMR_{(glc)}$ in the brain of the Andean dwellers (Quechua) (Hochachka et al., 1994), especially at the frontal and parietal cortex, the angular gyrus and the

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thalamus, thus hypometabolism was proposed as a defense against chronic hypoxia. However when a similar study was conducted on Tibet dwellers (Sherpa), such differences did not show up (Hochachka et al., 1996). Impaired cerebral autoregulation was also repeatedly observed in Himalaya dwellers (Jansen et al., 2000) and Sherpa (Jansen et al., 2007), suggesting the adaptation capability of human brain was limited, even after the natural selection of millions of years.

The current study examined the brain morphological and functional modifications of young adult HA immigrant residents compared to age-matched controls who had been sea level (SL) residents. Using immigrant residents as subjects allows us to exclude the factor of natural selection (Moore, 2001) and focus on the development problems. We acquired multi-modal MRI data from the subjects, including anatomical high-resolution T1-weighted MRI which were used for Voxel Based Morphology (VBM) analysis (Ashburner and Friston, 2000), in order to explore possible regional changes in the volume of gray matter, white matter and cerebrospinal fluid (CSF); and diffusion tensor imaging (DTI) to explore changes in white matter quality (Basser et al., 1994); besides the structural modification, we also examined changes in the baseline function with resting state fMRI (Gusnard and Raichle, 2001; Morcom and Fletcher, 2007). Based on previous studies on indigenous high altitude residents, laboratory rodents under prolonged hypoxia, as well as reports from acute hypoxia (Mórocz et al., 2001; Hackett and Roach, 2004), we expected changes in the gray matter of frontal cortex, the motor cortex, as well as hippocampus, possibly with changes on the corpus callosum. Since working memory decline was frequently reported with high altitude hypoxia exposure (Virues-Ortega et al., 2004; West, 2004), we also administered a series of working memory behavioral tests to examine the possible changes in working memory.

We found that the HA subjects had globally decreased volume at CSF and gray matter, as well as regionally decreased gray matter volumes, accompanied by regional increases as well as decreases in terms of fractional anisotropy (FA) from DTI and regional homogeneity (ReHo) from resting state fMRI. HA subjects showed recovery at a few regional MRI measurements with SL relocation time. The HA subjects also had poorer working memory capacity, which was correlated with their ReHo values at certain regions. In conclusion, prolonged chronic hypoxia exposure indeed impacts cerebral morphology and functionality.

2. Results

2.1. *Physical and physiological assessments*

There were no significant differences in hemoglobin levels, circulating red blood cell count, blood pressure, and pulse rate between HA residents and SL controls. Body height of both males ($p<0.01$) and females ($p<0.05$) in HA residents were significantly larger than that of SL controls. HA females had a higher diastolic pressure ($p<0.01$), HA males had a lower inspiratory reserve volume ($p<0.05$). No significant differences in cardiovascular functions were found between HA residents and SL subjects (see supplementary material).

2.2. *MRI measurements*

No subject from either group showed visible abnormalities on T1-weighted structural images. With the gender factor controlled, the HA subjects showed lower volume of gray matter and CSF than SL controls (Table 1).

2.2.1. *VBM*

VBM analysis showed that the HA residents had decreased gray matter volume compared to the SL controls at bilateral anterior insula, bilateral prefrontal cortex, the left precentral, the left cingulate and the right lingual cortex (two-sample *t*-test, $|t|>2.70$, $p<0.01$, FWE corrected) (Fig. 1, Table 2).

2.2.2. *DTI*

Voxel-by-voxel analysis revealed a significant decrease of the FA value in the right posterior cingulum and the right precentral cortex ($|t|>2.72$, $p<0.01$, FWE corrected) in HA subjects compared with SL controls (Fig. 1, Table 2). ROI analysis revealed significant increase of FA values at both the right and left anterior limb of internal capsule (ALIC) and a significant decrease of FA value at the right posterior cingulum in HA residents compared with SL controls. No significant differences were detected in other areas.

2.2.3. *ReHo*

Two-sample *t*-test found significantly increased ReHo values at the left middle frontal cortex, the right inferior frontal cortex, the right posterior insula, the left hippocampus, the right lingual cortex, and the dorsal middle pon, and decreased ReHo values at the right cingulate cortex, the right superior frontal cortex and both sides of the precuneus in HA residents compared with that of SL controls (Fig. 1, Table 2, $|t|>2.67$, $p<0.01$, FWE corrected).

2.3. *Behavioral experiments of working memory*

HA subjects showed longer reaction time in the verbal and spatial working memory tasks, they also reproduced fewer items in the Rey–Osterrieth Complex Figure Test (ROCF) either immediately after presentation or 20 min later; they also performed worse on the count forward and count backward tasks (Table 3).

2.4. *Correlation and regression analysis*

Correlation analysis revealed significant correlations between the SL relocation time and the gray matter volume at the left prefrontal cortex, and the ReHo value at the left hippocampus, as well as the ReHo value at the right insula (see Fig. 2). There were also significant correlations between the performances on the *count backward* task and the ReHo values from the left and the right sides of the lingual cortex, the pon, the right inferior frontal cortex, as well as the right middle frontal cortex (see Fig. 3), the latter four of which were found to be significant predictors of the performance (see Table 4).

3. Discussion

With multimodal MRI techniques, the current study found that childhood exposure to high altitude hypoxia was

Table 1 – Average volume (mm^3) of gray matter, white matter and CSF in the two groups (with standard deviation (SD) reported in parentheses), separately reported in females and males. ANOVA analysis revealed that there were significant differences among the two genders in each measurement ($p < 0.01$), but only marginal significant differences on the volumes of the gray matter ($p = 0.072$) and the CSF ($p = 0.058$), with no significant difference on the volumes of the white matter and the whole brain ($p > 0.9$); there were no significant interactions among the two factors also ($p > 0.1$).

	HA		SL	
	Female	Male	Female	Male
Gray matter	668.79 (88.131)	714.17 (56.002)	675.05 (85.488)	796.97 (32.1)
White matter	381.36 (40.123)	409.04 (48.08)	375.92 (28.426)	412.7 (25.29)
CSF	292.09 (57.417)	368.39 (57.044)	263.13 (77.883)	318.2 (50.512)
Total	1342.2 (91.173)	1491.6 (114.66)	1314.1 (78.463)	1527.9 (31.8)

associated with structural and functional modifications in the brain, including decreased regional gray matter volumes, changes in regional white matter FA values, and changes in regional neural activity at resting state. Among HA subjects, the gray matter volume at the left prefrontal cortex and the ReHo value at the hippocampus and the right insula were increasing with SL relocation time. The HA subjects performed poorer on a series of working memory tasks. We further identified the ReHo values at the right lingual cortex, the pon, the right inferior frontal cortex, as well as the right middle frontal cortex were significant predictors of the performance in a working memory test (count backward).

Abnormality at the hippocampus, the cingulate cortex and the motor cortex were previously also reported on indigenous residents and laboratory rodents under prolonged hypoxia exposure. Indigenous residents were reported to have

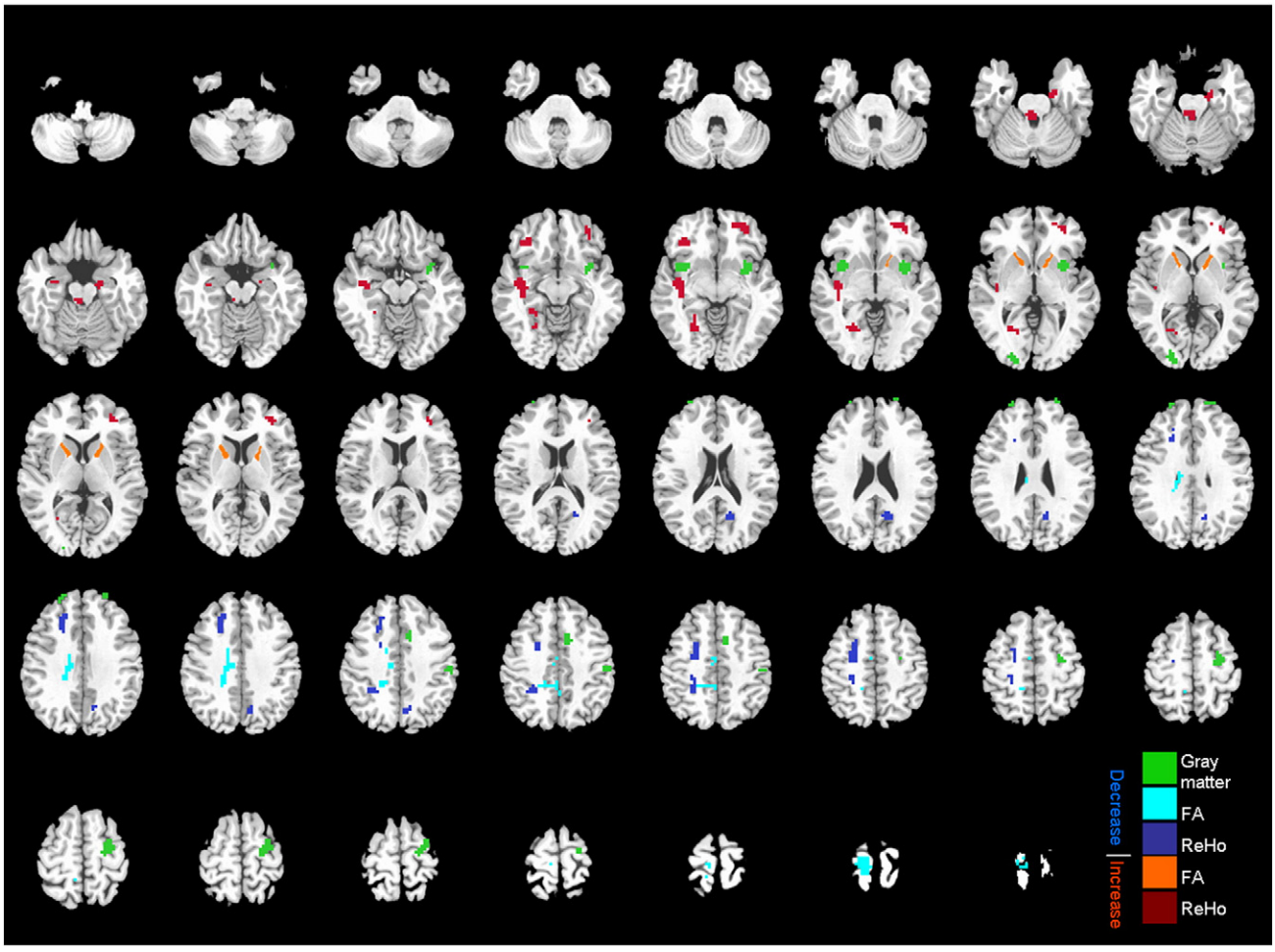

Fig. 1 – Statistical maps showing significantly different regions with different MRI measurements, labeled with different colors as shown in the legend. Green: regions with decreased gray matter volume among HA subjects; Cyan: regions with decreased FA values from the voxel-by-voxel analysis. Blue: regions with decreased ReHo values. Orange: regions with increased FA values in the ROI analysis. Red: regions with increased ReHo values.

Table 2 – Regional information of significant differences between the HA group and the SL group on gray matter volume (|t|>2.70, $p<0.01$, FWE corrected), FA values from voxelwise analysis (|t|>2.72, $p<0.01$, FWE corrected) and ROI analysis ($p<0.01$) as well as the ReHo values (|t|>2.67, $p<0.01$, FWE corrected). Coordinate and t value are from the voxel with the peak t value. Negative t value means decrease in HA subjects.

Measurements	Area	Volume (SD)	BA[a]		Talairach (peak)			t value
					x	y	z	
Gray matter volume	Precentral cortex	2006	6	L	22	16	63	−3.54
	Insula	1556	13	L	36	−2	−5	−4.95
		811	13	R	−36	−5	−3	−4.34
	Lingual cortex	579	17	R	−19	90	5	−4.04
	Cingulate cortex	532	32	L	10	−9	42	−3.33
	Prefrontal cortex	502	10	R	−24	−59	20	−3.52
		485	9	L	23	−60	26	−3.77
FA value	Cingulum	2237	23	R	−14.0	19.4	32.0	−6.05
	Precentral gyrus	1085	6	R	−9.5	29.2	66.0	−7.62
	Anterior limb of internal capsule			R	ROI analysis			2.782
				L				2.874
ReHo value	Middle frontal cortex	2970	11	L	31.5	−40.5	11.5	4.90
	Cingulate cortex	1296	24	R	−19.5	−1.5	41.5	−3.96
	Superior frontal cortex	1215	46	R	−19.5	−31.5	35.5	−4.13
	Inferior frontal cortex	810	47	R	−34.5	−28.5	−3.5	4.37
	Insula	2727	13	R	−40.5	16.5	−3.5	4.62
	PCC/Precuneus	1107	31	L	13.5	55.5	23.5	−3.60
		918	31	R	−25.5	40.5	41.5	−3.73
	Hippocampus	918		L	16.5	13.5	−18.5	3.58
	Lingual cortex	1836	19	R	−22.5	58.5	−0.5	3.53
	Pon	1053			−1.5	31.5	−18.5	4.62

[a] BA: Brodmann area.

changed metabolic rate in these regions (Hochachka et al., 1994, 1996), laboratory rodents were observed to have increased cerebral microvessel density (LaManna et al., 1992; Boero et al., 1999; Kanaan et al., 2006). In the current study, HA subjects did not show gray matter volume change at the hippocampus, yet they showed increased resting state ReHo value instead. Recently resting state BOLD-fMRI has been investigated intensively and applied widely. It has been considered as a baseline condition of the brain (Gusnard and Raichle, 2001; Morcom and Fletcher, 2007). Regional homogeneity (ReHo) measurement was designed to reflect the similarity of the time series of a given voxel to those of its neighboring voxels. It is supposed that when a functional brain area was active, voxels within the area were more temporally homogeneous (Zang et al., 2004). ReHo has been widely applied to investigate many mental disorders (He et al., 2007; Bai et al., 2008; Liu et al., 2008; Yuan et al., 2008; Tao et al., 2009; Yao et al., 2009). Increase in the ReHo value might be associated with the possible increase of microvessal density in relevant regions, as a mechanism to increase the blood supply to cope with chronic hypoxia. The hippocampus, the cingulate cortex and the motor cortex are critical brain regions for survival, with their well-known function in spatial cognition (Arleo and Gerstner, 2000), cognitive control (MacDonald et al., 2000) and motor control. Increased ReHo values in these regions might indicate compensatory mechanisms to main functioning when they suffered neuron loss due to hypoxia.

The modification in the insula may be related to its function in respiratory modulation. Under high altitude hypoxia condition, because oxygen pressure was low, in order to obtain sufficient oxygen for the human body, people need to inhale more deeply and more rapidly, as obviously observed among travelers to high altitude; similar behaviors is also observed when common people are doing exercise, during which the body demands more oxygen than usual. Insula has been reported to modulate inspiration and articulation (Shelley and Trimble, 2004; Nagai et al., 2007), there was also a report on the correlation between the aerobic capacity (assessed with blood-lactate concentration) and gray matter density at the insula (Peters et al., 2009). Yet native HA residents do not demonstrate such “short-of-breath” behavior in high altitude; indigenous residents were reported to have larger lung capacity (Frisancho, 1975), yet the HA subjects in the current study were not indigenous residents, and they did not show significant difference with the SL control on most of the measurements of lung capacity. They might have developed some modulation mechanism in the brain to compensate for insufficient oxygen, yet the exact mechanism is not clear yet.

White matter edema was frequently reported with acute high altitude hypoxia exposure (Hackett et al., 1998; Mórocz et al., 2001; Hackett and Roach, 2004). Laboratory rodents were observed with decreased myelination in the corpus callosum (Kanaan et al., 2006). In the current study with DTI, long-term HA residents did not show white matter volume changes. But they had decreased FA value at the right posterior cingulum and increased FA value at both sides of the ALIC. FA values from DTI have been shown to reflect neural axons of the white matter in the brain (Gulani and Sundgren, 2006). Abnormality in the FA value has been associated with various kinds of mental disorders (for review see (Lim and Helpern, 2002)). Abnormality in the regional FA values might indicate abnormality in white matter quality, pointing to possible impairment or compensation.

Table 3 – Working memory test scores from the two groups.

Group	Verbal* (RT)		Spatial* (RT)		SRTT (RT)		Recall immediately*		Recall in 20 min*		Count backward		Count forward**	
	HA	SL	HA	SL	HA	SL	HA	SL	HA	SL	HA	SL	HA	SL
Mean	70.15	73.58	73.22	75.36	1691.06	1625.14	30.12	32.76	30.19	33.00	205.36	210.53	89.90	106.26
SD	7.02	3.01	3.82	2.58	355.57	237.35	4.57	2.56	5.00	2.84	21.85	18.97	24.25	4.21
t-test	$t(35.5)=-2.33^{\$}$		$t(45.9)=-2.38^{\$}$		$t(37.0)=0.62^{\$}$		$t(38.3)=-2.61^{\$}$		$t(38.7)=-2.52^{\$}$		$t(55)=-0.93$		$t(27.6)=-3.45^{\$}$	

Note. *$p<0.05$, **$p<0.01$, $ degree of freedom adjusted for inequality of variance.
SSRT: the Serial Reaction Time Task;
Recall immediately, Recall in 20 min: the response accuracy in the ROCF task at the immediate recall and delayed recall (recall 20 min later) conditions.
RT: reaction time.
There were no gender differences on any of the measurements ($p>0.2$).

The correlation between SL relocation time and the regional MRI measurement values might indicate recovery with SL normoxia of the cerebral impairment after chronic hypoxia exposure, although it still requires longer-term observation before a definite conclusion can be made. It has been reported that blood volume and erythropoietic activity changes with intermittent exposure to high altitude (Neubauer, 2001; Schmidt, 2002); the observed changes in regional ReHo values with hypoxia might be related to the vascular modifications. Previous reports have indicated the sensitivity of the microvessel density at the frontal cortex and the hippocampus (Kanaan et al., 2006). It is also perceivable to have hyperactivity at the insula with changed oxygen concentration after coming to the SL given its function in modulating inspiration (Shelley and Trimble, 2004; Nagai et al., 2007). The correlation between the working memory performance and the regional ReHo values indicate that the cerebral modifications indeed could affect their cognitive capability, although not as much as that in acute hypoxia (Virues-Ortega et al., 2004).

In summary, the current study showed that chronic hypoxia exposure in early childhood impacted brain morphology and functionality, including lost regional gray matter, changed white fiber microstructure, and changed regional neural activity at resting state. SL relocation might lead to recovery from the impairment. The cerebral modifications might contribute to the observed decline of cognitive capability with chronic hypoxia accompanying the early development.

4. Experimental procedures

4.1. *Subjects*

Participants in the current study consisted of 28 HA immigrant residents and 28 matched SL (< 400 m) residents with12 males and 16 females in each group (Table 1 in supplementary material). HA residents were born and raised in Qinghai-Tibetan Plateau at the altitude of 2616–4200 m and currently relocated at SL (< 400 m). The average age of the high altitude group was 20.19 (standard deviation (SD) = 1.52, range 17–24), and the average age of the sea level group was 20.33 (SD = 1.37, range 18–24); there were no significant difference between the two groups on age ($t=-0.38$, $p=0.7$) or gender ratio (significance of Chi-Square test = 0.89). All subjects were from the Han race to avoid possible ethnic and ancestral differences. Subjects were examined physiologically and were excluded if they had: (1) chronic mountain sickness (CMS), (2) a documented neurological disorder, or (3) a past history of head injury with loss of consciousness. The experimental protocol was approved by the Research Ethics Review Board of the Institute of Psychology, Chinese Academy of Sciences. Written informed consent was obtained from each participant.

4.2. *Physical and physiological assessments*

Before MRI scanning, subjects underwent physical and physiological examinations, including body height and body weight, hematological measurements, blood pressure, pulse rate, and pulmonary function (see supplementary material). Hematological

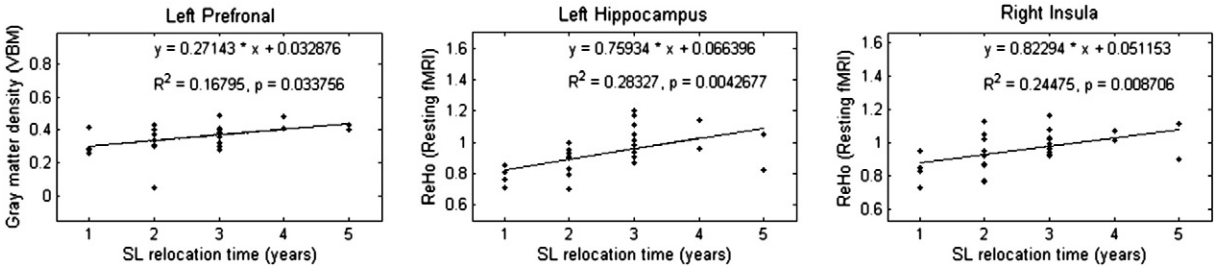

Fig. 2 – Significant correlations between the SL relocation time and the MRI measurements.

measurements were tested using a hematology analyzer (Sysmex XE-2100, TOA Medical Electronics, Kobe, Japan).

4.3. *MRI data acquisition and analysis*

Structural and functional images were acquired on a GE 3.0 T Signa Excite Gemse MRI system (GE Medical, Milwaukee, WI, USA) at Huaxi Magnetic Resonance Research Center (West China Hospital, Chengdu, China).

A 3D structural MRI was acquired from each subject using a T1-weighted MPRAGE sequence (TR/TE = 8.5 ms/3.4 ms, TI = 400 ms, FOV = 28 cm, flip angle = 12º), yielding 156 contiguous axial slices (1 mm thick) covering the whole brain. The data were analyzed following the protocol proposed by (Good et al., 2001) with VBM2 toolbox (Department of Psychiatry, University of Jena) and SPM2 (Wellcome Department of Imaging Neuroscience, London) on MATLAB 6.5 (MathWorks, Natick, MA) platform. We created customized templates of gray matter, white matter and CSF with the subject pool of the current study. Using these study-specific templates, the 3D images for each individual were spatially normalized to Montreal Neurological Institute (MNI) space and segmented, and then smoothed using a Gaussian kernel of 10 mm full-width at half-maximum (FWHM). The data were also standardized with the total volume of gray matter, white matter, CSF as well as the whole brain before conducting independent sample *t*-test to examine between-group differences. The statistical parametric map was generated with threshold at $|t| > 2.70$, $p < 0.01$ (FWE corrected), and transformed to Talairach and Tournoux space.

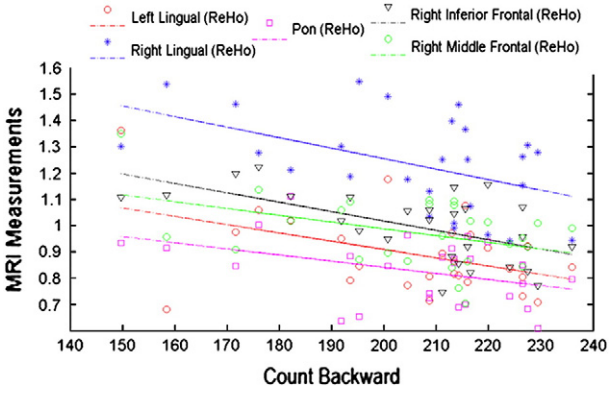

Fig. 3 – Significant correlations between the performance score of a working memory task (count backward) and MRI measurements.

A DTI pulse sequence with single shot diffusion-weighted echo planar imaging (TR/TE = 10000/70.8 ms, FOV = 24 cm) was applied sequentially in 16 different directions. We acquired 42 contiguous 3-mm thick slices (no gap) covering the whole brain. DTI data were preprocessed with AFNI (http://afni.nimh.nih.gov/) with similar procedure as those on the resting state BOLD-fMRI date as described below, then analyzed with the FDT toolbox of FSL (FMRIB Software Library (FSL), version 5.00, UK; www.fmrib.ox.ac.uk/fsl) and the DTI module of Analyze software (Version 4.0, Mayo Clinic, http://www.mayo.edu/bir/Software/Analyze/ Analyze.html). FA maps were spatially normalized to MNI space and smoothed with a Gaussian filter with FWHM of 4 mm. Random effect two sample *t*-tests were conducted to compare FA values both on the voxel-by-voxel level and the region of interest (ROI) level. ROIs for FA comparison were manually outlined on low b-value (b0) images by an operator who was blind to subjects and research purpose. (see supplementary material for ROI definition).

A 7-min resting state BOLD fMRI was obtained with an EPI sequence (TR/TE = 2000/30 ms, FOV = 24 × 24 cm^2, flip angle = 90°). Totally 200 volumes of 28 contiguous axial slices at 5 mm thickness (without gaps) covering the whole brain were acquired from each subject (subjects were instructed to remain awake with their eyes closed). We did the following pre-processing with AFNI on each time series: discarding the first 10 volumes for scanner calibration, slice timing, motion correction, removal of linear drift, bandpass filtering (0.01–0.08 Hz), smoothing with a Gaussian filter of 6 mm FWHM, and normalizing to Talairach and Tournoux space (Talairach and Tournoux, 1988); as regression analysis was conducted with the following regressors: the estimated profiles of head motion (three for translation and three for rotation), the average time series from white matter and CSF, therefore to remove identifiable variance in the BOLD signal (Fox et al.,

Table 4 – Regression models of the working memory tests.

	Subtraction		
	Beta	*T*	Sig
Left lingual (ReHo)	0.036	0.338	0.739
Right lingual (ReHo)	−0.774	−6.500	0.000
Pon (ReHo)	−0.242	−2.142	0.045
Right inferior frontal (ReHo)	−0.406	−3.569	0.002
Right middle frontal (ReHo)	−0.413	−3.415	0.003
Regression model	$R^2 = 0.803$, Sig = 1.85E-06		

2005). The residuals from the regression were then used for the following analysis separately. None of the subjects' head motion exceeded 1 voxel, so no subject was excluded. Regional homogeneity was analyzed according to the protocol of Zang et al. (2004). The regional homogeneity of each voxel was calculated as Kendall's coefficient of concordance (KCC) of its time series with the time series of its surrounding 26 voxels. The coefficients were further transformed to Z scores. Random-effects two-sample *t*-test was carried out on ReHo Z maps in a voxel-wise way to explore the differences between the HA residents and the control group. Statistical parametric maps were generated with threshold at $|t|>2.67$, $p<0.01$ (FWE corrected).

4.4. *Working memory behavioral experiments*

Subjects went through a series of behavioral tasks for assessment of working memory capacity, the procedures of which are explained below.

4.4.1. *Spatial and verbal "two-back" working memory*

In the verbal working memory task, the letters: A, B, C, D, E, F, G, or H was presented in a programmed sequence for 2 s. Subjects were instructed to determine whether each presented letter was the same as the one presented two stimuli previously. In the spatial working memory task, a square was presented for 2 s in one of eight different locations around an imaginary square centered on a fixation cross. Subjects were instructed to decide whether each square was in the same position as the square shown two stimuli previously. In each test, there were 84 trials, with a ratio of targets to distracters of approximately 1/4. Reaction time was measured for each trial and averaged across trials.

4.4.2. *Serial Reaction Time Task (SRTT)*

We adapted the SRTT from previous literature (Nissen and Bullemer, 1987; Gomez Beldarrain et al., 2002). Subjects were seated in front of a computer screen with their middle and index fingers of their left and right hands resting on a keyboard over keys 1, 2, 3, and 4, respectively. An asterisk was presented in any one of four positions horizontally located on the screen aligned above the response keys. Subjects were instructed to press the key that was spatially aligned with the asterisk as fast as possible with the corresponding finger. The asterisk did not disappear until the correct button was pressed, after which the next stimulus appeared after a delay of 500 ms. Subjects were told that there would be a fixed sequence of the spatial location of the asterisk that would be repeated several times, but they had to find out by themselves. The test consisted of six blocks with 120 trials in each block. In blocks 1 and 6 the asterisk positions randomly presented at each of the four positions; in blocks 2–5, a fixed sequence (3–4–1–4–3–4–2–4–1–3–4–2) was repeated 10 times. Reaction time was measured for each presentation. None of the subjects had detected the sequence.

4.4.3. *Rey–Osterrieth Complex Figure Test (ROCF).*

The Rey–Osterrieth Complex Figure (ROCF) (Rey, 1941; Watanabe et al., 2005) was used to assess the short and long-term visual memory and visuo-constructional ability. Subjects were presented with a complex figure from the ROCF set (maximum score=36) on a card measuring 21×29.7 cm and they were asked to copy it. The ROCF was drawn with felt pens in four colors, which were changed in a fixed order to enable the examiner to track the drawing sequence. Immediately following completion of the copy trial, the figure was removed and subjects were asked to reproduce the figure from memory (testing for immediate recall). Approximately 20 min after the last exposure to the figure, subjects were asked to reproduce the figure from memory again (a test for delayed recall). Two scores were derived from the immediate recall and delayed recall. Accuracy of reproduction was recorded according to the instruction.

4.4.4. *Count forward and count backward*

As parts of the Webster's intelligence tests (Wechsler, 1971), subjects were instructed to count increasingly at the step of 1 from 1 to 100 (i.e., 1, 2, 3, …, 100) as well as in the reverse sequence, constituting the *count forward* and *count backward* tasks. A normalized score was obtained based on the ratio of errors according to the scale manual.

4.5. *Correlation and regression analysis*

After indentifying the significantly different regions with the above MRI measurements, the significant regions were taken as masks; we extracted the average measurements from each region on each subject, and explored all possible significant cross-subject correlations between the regional MRI measurements and the demographic factors (SL relocation time, altitude of residence, generations of HA residence), as well as performance in the working memory tasks.

There were significant correlations between the SL relocation time and the gray matter volume at the left prefrontal cortex, and the ReHo value at the left hippocampus, as well as the ReHo value at the right insula (see Fig. 2). Since there were only three types of generations of residence (2nd, 3rd or 4th), correlation or regression analysis were not justified, so we conducted one-way ANOVA analysis, in which we found significant differences between the generations of residence on the gray matter volume at the right insula, the ReHo value of the right hippocampus, as well as the ReHo value at the right insula (see supplementary material).

There were also significant correlations between the performances on the *count backward* task and the ReHo values from the left and the right sides of the lingual cortex, the pon, the right inferior frontal cortex, as well as the right middle frontal cortex (see Fig. 3), the latter four of which were found to be significant predictors of the performance (see Table 3).

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