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Size Matters to Function: Brain Volume Correlates with Intrinsic Brain Activity across Healthy Individuals

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Abstract

A fundamental issue in neuroscience is to understand the structural substrates of neural activities. Intrinsic brain activity has been increasingly recognized as an important functional activity mode and is tightly linked with various cognitive functions. Structurally, cognitive functions have also shown a relation with brain volume/size. Therefore, an association between intrinsic brain activities and brain volume/size can be hypothesized, and brain volume/size may impact intrinsic brain activity in human brains. The present study aimed to explicitly investigate this brain structure-function relationship using two large independent cohorts of 176 and 236 young adults. Structural-MRI was performed to estimate the brain volume, and resting-state functional-MRI was applied to extract the amplitude of low-frequency fluctuations (ALFF), an imaging measure of intrinsic brain activity. Intriguingly, our results revealed a robust linear correlation between whole-brain size and ALFF. Moreover, specific brain lobes/regions, including the frontal lobe, the left middle frontal gyrus, anterior cingulate gyrus, Rolandic operculum, and insula, also showed a reliable, positive volume-ALFF correlation in the two cohorts. These findings offer direct, empirical evidence of a strong association between brain size/volume and intrinsic brain activity, as well as provide novel insight into the structural substrates of the intrinsic brain activity of the human brain.

Key words: brain size, brain structure-function correlation, structural MRI, resting-state fMRI
1. Introduction

One of the major challenges in neuroscience is to understand the structural substrates of functional neural activities in the human brain, especially at the large-scale systems level (Honey et al., 2010; Park and Friston, 2013). To address this issue, several investigations have been performed, with the majority of them focusing on whether and how brain structure shapes and constrains the inter-regional coupling of neural activities. As expected to some degree, white matter (WM) fiber pathways have been found to be associated with this functional coupling (Honey et al., 2009; Honey et al., 2010; van den Heuvel et al., 2008). Particularly, the structural-functional connectivity associations could be disrupted in particular brain diseases such as epilepsy (Ji et al., 2014; Zhang et al., 2011).

Notably, in addition to the inter-regional functional coupling/connectivity, there are other intriguing features of neural activities, such as the degree of intrinsic brain activity fluctuation (Garrett et al., 2010; Garrett et al., 2013). The intrinsic brain activity consumes over 95% of the brain’s energy and is likely to play a critical role in brain function (Raichle, 2006; Raichle and Mintun, 2006; Zhang and Raichle, 2010). To noninvasively characterize the degree of fluctuation of intrinsic brain activity in the human brain, a sensitive and robust resting-state functional magnetic resonance imaging (rs-fMRI) measure, called the amplitude of low frequency fluctuations (ALFF), has been proposed (Zang et al., 2007). ALFF has been widely applied in cognitive studies and found to correlate with specific types of cognitive performance (Mennes et al., 2011; Wang et al., 2013; Wei et al., 2012; Zou et al., 2013). However, to date, ALFF has rarely been applied to investigate structural substrates for the
degree of intrinsic brain activity fluctuation.

Structurally, brain size/volume is an important measure of general interests and has been repeatedly reported to correlate with cognitive/behavioral abilities, both globally and locally (Maguire et al., 2000; Rushton and Ankney, 2009; Witelson et al., 2006). Putatively, cognitive and behavioral abilities are more closely linked to the neural activities of the human brain. Therefore, the previously observed effects of brain size/volume on cognition may be related to the modulation of brain activity such as the intrinsic brain activity. In line with this premise, a number of recent studies have revealed synchronous changes in brain volume and ALFF values in specific disease processes, suggesting that the two brain measures are related (Han et al., 2012; Li et al., 2014; Liu et al., 2014). For these reasons, we hypothesize that a structure-function correlation exists between brain size/volume and ALFF values across a population and that brain size/volume impacts intrinsic neural activities of human brains. Nevertheless, direct analyses of this structure-function hypothesis remain scarce.

In the present study, we sought to evaluate this hypothesis. Specifically, linear correlations between brain volume and ALFF were explicitly tested in two large, independent cohorts of healthy young adults. For each individual, structural MRI was performed to measure brain volume, and rs-fMRI was applied to extract the ALFF measures. The correlational analyses were performed at whole-brain, lobar and regional levels.

2. Materials and Methods

2.1. Participants and data acquisition
Two independent cohorts of healthy young adults were employed in this study.

2.1.1. Principal cohort

Participants: There were 176 subjects in total (19-30 years of age; 100/76 male/female ratio). All subjects were right-handed and had no history of major neurological or psychiatric disorder. Written informed consent was obtained from each participant, and the protocol for this study was approved by the Institutional Review Board of the Beijing Normal University Imaging Center for Brain Research.

MRI acquisition: For all subjects, both structural and rs-fMRI data were acquired using the same SIEMENS TRIO 3T scanner in the Imaging Center for Brain Research, Beijing Normal University. Participants rested in a supine position with their head snugly fixed by foam pads to minimize head movement. Structural MRI data were acquired using an MPRAGE sequence with the following parameters: 128 sagittal slices, repetition time (TR) = 2530 ms, echo time (TE) = 3.39 ms, inversion time (TI) = 1100 ms, slice thickness = 1.33 mm, field of view (FOV) = 256 × 256 mm², and voxel size = 1.33 × 1.00 × 1.00 mm³. Rs-fMRI data were obtained using an echo-planar imaging (EPI) sequence with the following parameters: 33 axial slices, TR = 2 s, TE = 30 ms, slice thickness = 3.5 mm, gap = 0.7 mm, FOV = 200 × 200 mm², and voxel size = 3.1 × 3.1 × 4.2 mm³, with 200 volumes in total. During the resting-state scanning, participants were instructed to close their eyes, to remain still and calm, to not think systematically about anything and to not fall asleep.

2.1.2. Replication cohort
A public dataset was used as a replication cohort. It is a sub-dataset, “SWU4”, of the public “Consortium for Reliability and Reproducibility” (CORR) data (Zuo et al., 2014). There were 235 subjects in this cohort (17-27 years of age; 121/114 male/female ratio). The MRI data were acquired using another SIEMENS TRIO 3T scanner. The sequences were the same as those used to acquire the principal dataset, but the sequence parameters differed slightly.

2.2. Image processing

All data from both cohorts were processed using the procedures described below.

2.2.1. Structural MRI

The unified segmentation algorithm was applied to the structural MRI images using Statistical Parametric Mapping (SPM8) (Ashburner and Friston, 2000). For each individual, this process ended up with results of three categories: 1) grey matter (GM), WM, and cerebrospinal fluid (CSF) probability/density maps in the T1 native space; 2) spatial transformations from the MNI space to the T1 native space. 3) GM, WM and CSF probability/density or volume maps in the MNI space. The first two were used in our subsequent analyses. To implement the lobar/regional-level analyses described below, the T1 native space GM images were further parcellated using the automated anatomical labeling (AAL) template (Tzourio-Mazoyer et al., 2002). Specifically, for each participant, the inverse of the transformation from the T1 native space to MNI space was applied to the original AAL template in MNI space, yielding an individual-specific AAL template in the T1 native space. To extract the lobar sections, the AAL regions were merged for each individual (the frontal lobe: AAL 01-34; the occipital lobe: AAL 43-56; the parietal lobe: AAL 35-36 and AAL 57-70; the temporal lobe: AAL 79-90; and
subcortical structures: AAL 37-42 and AAL 71-78).

The volume for the whole-brain, lobes, or AAL regions was uniformly calculated in the T1 native space. Specifically, a voxel with a GM probability/density greater than 0.4 was defined as a GM voxel. Similarly, the WM or CSF voxels were defined using the WM or CSF probability/density. Here, the intracranial volume (ICV) was applied as a measure of whole-brain volume, as done previously (Im et al., 2008; Takao et al., 2011; Toro et al., 2008; Yan et al., 2011). The ICV value was computed as the total volume of all GM, WM, and CSF voxels. For the lobes and AAL regions, the volume was calculated as the total volume of all GM voxels within the corresponding regions on the T1 native space image.

2.2.2. rs-fMRI

The rs-fMRI data processing was performed using the Data Processing Assistant for Resting-State fMRI (DPARSF) (Yan and Zang, 2010), which is a pipeline toolkit that uses SPM8 and the Resting-State fMRI Data Analysis Toolkit (REST) (Song et al., 2011). The pipeline procedure included the following steps: 1) discarding the first 10 volumes; 2) slice timing; 3) head motion correction; 4) regressing out of nuisance variables (15 in total), including 6 head motion parameters and their derivatives, the average CSF and WM signal, and the linear term; and 5) smoothing with a 6 mm FWHM Gaussian kernel.

Notably, we controlled for the effect of frame-wise micro-motion by including the derivatives of the 6 head motion parameters as nuisance variables (Power et al., 2012; Van Dijk et al., 2012). However, the scrubbing method was not applied because the removal of time points could disturb the temporal structure of the signal, thereby confounding frequency-based
analyses such as ALFF (Yan et al., 2013). Participants were excluded from our final analysis if their maximum head motion translation was larger than 2 mm or their maximum rotation was larger than 2 degrees. Accordingly, 19 participants (3 from the principal cohort and 16 from the replication cohort) were excluded.

For each individual, a whole-brain ALFF map in the EPI native space was calculated. Specifically, the rs-fMRI time series for each voxel was first transformed to a frequency domain with a fast Fourier transform. The square root was calculated for each frequency of the entire power spectrum. The ALFF value was defined as the average square root across the low frequency band of 0.01-0.08 Hz; rs-fMRI signal fluctuations within this band are widely believed to represent spontaneous/intrinsic neuronal activity (Biswal et al., 1995; Fox and Raichle, 2007; Zang et al., 2007; Zuo et al., 2010). Because the neural activity that is observed through fMRI is putatively meaningful mainly within GM tissue, we confined our ALFF calculation to GM voxels.

For each individual, the GM mask as well as the lobar/AAL-regional masks in the T1 native space was resampled into the EPI native space, by co-registering the T1 image to the EPI image. The whole-brain, lobar, and AAL-regional ALFF values were then calculated as the average ALFF value of all GM voxels within the corresponding regions on the EPI native space image.

As described above, the present study quantified both volume and ALFF values at the whole brain, lobar and AAL-regional levels in subject’s native space, but not at the voxel level in the MNI space. The motivation for such a strategy is to control for the partial volume effect (PVE)
contamination to subsequent volume-ALFF correlational results. In the MNI space, the voxel-wise ALFF values were substantially affected by the PVE around the boundary voxels (Qing et al., 2014). This might ultimately introduce artificial volume-ALFF correlations for a voxel-wise analysis in the MNI space.

2.3. Correlation analysis
Using general linear models (GLMs), we performed a correlation analysis between ALFF and volume across participants for 1) the whole brain, 2) the lobes, and 3) the AAL regions. In the linear models, age and gender were included as covariates. To evaluate whether the ALFF-volume correlation was modulated by gender, we first included a “gender × brain volume” interaction term in the model. This interaction term was removed from the model in cases in which it was not significant (Engqvist, 2005).

2.4. Frequency sub-band
Within the 0.01-0.08 Hz low frequency band, two sub-bands have been suggested as meaningful in rs-fMRI: slow-4 (0.01–0.027 Hz) and slow-5 (0.027–0.073 Hz) (Buzsaki and Draguhn, 2004; Zuo et al., 2010). An ALFF value was calculated for each of these two sub-bands (abbreviated as ALFF$_{S4}$ and ALFF$_{S5}$). Particularly, ALFF$_{S4}$ and ALFF$_{S5}$ have demonstrated differential reliability and spatial patterns (Zuo et al., 2010). To further investigate whether the relationship between volume and ALFF showed any frequency specificity, we re-performed all of the correlational analyses described above using both ALFF$_{S4}$ and ALFF$_{S5}$ and then evaluated the similarity/dissimilarity of the correlational results between the entire low frequency band (0.01-0.08 Hz) and the two sub-bands. Similar
correlational results between these two ALFF measures indicate a lack of frequency specificity for the relationship between volume and ALFF, whereas dissimilar results indicate the opposite.

3. Results

3.1. The whole-brain level

In the principal cohort, the “gender × ICV” interaction effect was not significant ($F = 0.02$, $p = 0.88$) (Figure 1). After controlling for age and gender, a significant positive correlation across all subjects was observed between ICV and whole-brain ALFF ($R = 0.17$, $p = 0.03$). Within the male and female sub-groups, the ICV-ALFF correlation did not reach significance but did display a positive trend (male, $R = 0.16$, $p = 0.11$; female, $R = 0.18$, $p = 0.14$). These whole-brain level results for the replication dataset were largely similar: a non-significant “gender × ICV” interaction ($F < 10^{-3}$, $p = 0.99$); a significant ICV-ALFF correlation across all subjects ($R = 0.16$, $p = 0.02$); and ICV-ALFF correlational trends within each gender group (male, $R = 0.13$, $p = 0.17$; female, $R = 0.19$, $p = 0.06$) were observed (Figure 1).

In addition, we evaluated the correlations between the total GM volume and whole-brain ALFF. The results were quite consistent with the ones using the ICV measure: 1) “gender × total GM” interaction, the principal cohort: $F = 0.33$, $p = 0.56$; the replication cohort: $F = 0.03$, $p = 0.86$; 2) total GM-ALFF correlation, the principal cohort: $R = 0.14$, $p = 0.07$; the replication cohort: $R = 0.11$, $p = 0.10$. 
Figure 1. The correlations between intracranial volume (ICV) and whole-brain ALFF. Scatter plots are displayed for both the principal and replication cohorts.

3.2. The lobar level

For each lobar section, the “gender × volume” interaction effect was not significant in either cohort. In the principal cohort, a significant positive volume-ALFF correlation was found for the frontal and temporal lobes, as well as for the subcortical structures (frontal lobe: $R = 0.20$, $p = 0.009$; temporal lobe: $R = 0.19$, $p = 0.01$; subcortical structures: $R = 0.23$, $p = 0.002$).

Using the replication dataset, we observed a significant correlation for the frontal lobe ($R = 0.22$, $p = 0.001$) but not for the temporal lobe ($R = 0.09$, $p = 0.19$) or for the subcortical
structures ($R = 0.06, p = 0.38$). Significant correlations for the occipital and parietal lobes were not found in either cohort. All lobar results are summarized in Table I.

Table I. The correlations between lobar volume and ALFF

<table>
<thead>
<tr>
<th></th>
<th>Frontal</th>
<th>Occipital</th>
<th>Parietal</th>
<th>Temporal</th>
<th>Subcortical</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALFF</td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>p</td>
<td>R</td>
</tr>
<tr>
<td>Principal</td>
<td>0.20</td>
<td>0.009**</td>
<td>0.11</td>
<td>0.15</td>
<td>0.19</td>
</tr>
<tr>
<td>Replication</td>
<td>0.22</td>
<td>0.001**</td>
<td>0.08</td>
<td>0.68</td>
<td>0.10</td>
</tr>
<tr>
<td>ALFF$_{S4}$</td>
<td>Principal</td>
<td>0.22</td>
<td>0.003**</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>Replication</td>
<td>0.21</td>
<td>0.002**</td>
<td>0.03</td>
<td>0.70</td>
<td>0.11</td>
</tr>
<tr>
<td>ALFF$_{S5}$</td>
<td>Principal</td>
<td>0.12</td>
<td>0.11</td>
<td>0.09</td>
<td>0.21</td>
</tr>
<tr>
<td>Replication</td>
<td>0.18</td>
<td>0.008**</td>
<td>0.04</td>
<td>0.52</td>
<td>0.09</td>
</tr>
</tbody>
</table>

ALFF: values for the entire low frequency band (0.01-0.08 Hz). ALFF$_{S4}$ and ALFF$_{S5}$: values for the slow-4 (0.027-0.073 Hz) and slow-5 (0.01-0.027 Hz) sub-bands, respectively. *, $p < 0.05$; **, $p < 0.01$.

3.3. The regional level

After FDR correction, the “gender × volume” interaction effect was not significant for any AAL region in either cohort. In the principal cohort, 13 regions exhibited a significant positive volume-ALFF correlation ($q < 0.05$) after controlling for age and gender. These regions were mainly located around the bilateral anterior cingulate gyrus, Rolandic operculum, putamen, left middle frontal gyrus, insula, lingual gyrus, fusiform gyrus, supramarginal gyrus, right superior occipital gyrus and olfactory cortex (Figure 2). Only the left paracentral lobule showed a significant negative correlation between volume and ALFF.

In the replication cohort, five regions exhibited a significant positive volume-ALFF
correlation, including the left middle frontal gyrus, anterior cingulate gyrus, Rolandic operculum, olfactory cortex, and insula. With the exception of the left olfactory cortex, all of these regions also showed significant positive correlations in the principal cohort. The left caudate showed a significant negative correlation in the replication cohort (Figure 2).

To quantitatively evaluate the reproducibility of the regional results, we compared the spatial similarity of the volume-ALFF correlational maps between the principal and replication datasets. The two correlational maps were largely similar, and there was a significant between-cohort correlation of the Z scores for the observed volume-ALFF correlations across all AAL regions (Figure 2, R = 0.61, p < 10^{-6}).

**Figure 2.** The volume-ALFF correlations for AAL regions. A) Rose plots of the volume-ALFF correlations are shown for both the principal and replication cohorts. The length of each bar represents the absolute value of the R value for each AAL region. The scatter plot displays the between-cohort correlations of the Z-scores of the
volume-ALFF correlations across all of the AAL regions. Each blue circle represents an AAL region. B) The regional volume-ALFF correlational brain maps are shown, both before and after thresholding (FDR corrected p < 0.05).

3.4. The lack of frequency specificity

The correlational results for both ALFF<sub>S4</sub> and ALFF<sub>S5</sub> were similar to the results for the entire low frequency band, which were described above. For example, the whole-brain ALFF<sub>S4</sub> significantly correlated with ICV in both cohorts (the principal cohort, R = 0.17, p = 0.03; the replication cohort, R = 0.16, p = 0.02). There was also a significant correlation between the whole-brain ALFF<sub>S5</sub> and ICV in the replication cohort (R = 0.15, p = 0.03) and a trend in the principal cohort (R = 0.13, p = 0.08).

As shown in Table I, the lobar sections that were identified as significant for both ALFF<sub>S4</sub> and ALFF<sub>S5</sub> were largely consistent with those that were identified for the entire low frequency band.

At the regional level, the significant or trend regions for the slow-5 and slow-4 bands largely overlapped with those for the entire low frequency band. Quantitatively, the correlational values for both the slow-4 and slow-5 bands were significantly correlated with those for the entire low frequency band (Figure 3). These results indicate that the correlational maps for the slow-4, slow-5, and entire low frequency bands exhibited similar spatial patterns.
Figure 3. The similarity of the regional volume-ALFF correlations for the entire low frequency band and for the slow-4 and slow-5 sub-bands. The scatter plots show the between-band correlations of the Z-scores of volume-ALFF correlations across all of the AAL regions. Each blue circle represents an AAL region.

4. Discussion

In the current study, we utilized an rs-fMRI measure of intrinsic brain activity in the human brain (i.e., ALFF) to explore the relationship between brain size and intrinsic brain activity in two large independent cohorts. Intriguingly, positive linear correlations were reliably observed between whole-brain volume and ALFF. Moreover, specific brain lobes/regions, including the frontal lobe, left middle frontal gyrus, anterior cingulate gyrus, Rolandic operculum, and insula, also showed a robust positive volume-ALFF correlation in the two cohorts. Further analyses indicated that ALFF frequency had only a limited effect (i.e., a very slight trend) on the relationships we observed between volume and ALFF. Our findings provide direct empirical evidence of a strong association between brain size/volume and
intrinsic brain activity, suggesting that brain size serves as a structural substrate for the intrinsic brain activity of the human brain.

The relation between whole-brain size and behavioral/cognitive abilities has long been a topic of general interest (Rushton and Ankney, 2009). Although some negative findings have been reported, the majority of previous investigations have provided data in favor of such a relationship (Adamson et al., 2010; Paradiso et al., 1997; Rushton and Ankney, 2009; Witelson et al., 2006). Intriguingly, whole-brain size has also been related with various structural aspects of the human brain, which might contribute to the relationships between whole-brain size and behavior. These structural aspects included micro-properties, such as neuronal numbers (Larsen et al., 2006; Samuelsen et al., 2003) and WM fiber integrity (Takao et al., 2011), as well as macro-properties, such as GM tissue morphology (Im et al., 2008; Luders et al., 2002; Toro et al., 2008) and whole-brain WM network patterns (Yan et al., 2011). The advance of the present study is the demonstration of a relationship between whole-brain size and intrinsic brain activity, which supports the idea that whole-brain size has a substantial impact on the function of the human brain. Given that cognitive/behavioral abilities are more closely related to the brain activity of the human brain, it is possible that previously observed correlations between brain size and cognition (Adamson et al., 2010; Paradiso et al., 1997; Rushton and Ankney, 2009; Witelson et al., 2006) are mediated by intrinsic brain activity. It is also possible that intrinsic brain activity influences cognition by modulating functional activity during cognitive tasks.

The most reliable local volume-ALFF correlation was observed in the frontal lobe, which is
involved in higher order cognitive functions, such as decision-making, reasoning, and cognitive control (Stuss and Knight, 2013). More specifically, a set of frontal regions, including the anterior cingulate cortex, middle frontal gyrus, Rolandic operculum, and insula, exhibited robust regional-level volume-ALFF correlations in both of the two cohorts. Notably, these regions largely overlap with the cingulo-opercular system, a critical part of the cognitive control network (Dosenbach et al., 2007; Sadaghiani and D'Esposito, 2014; Taylor et al., 2009). The cingulo-opercular system is activated across a diverse range of control-related tasks and likely relates to intrinsically maintained “tonic alertness” (Sadaghiani and D'Esposito, 2015). Intriguingly, previous studies have revealed that ALFF values within the cingulo-opercular system can successfully predict functional activity and performance during attention and executive control-related tasks (Mennes et al., 2011; Zou et al., 2013). By identifying ALFF-behavior correlations within a specific functional system, these studies support that ALFF may act as an intermediate in the volume-behavior relationship.

The majority of the volume-ALFF correlations we observed were positive. Compatibly, previously observed ALFF-behavior and volume-behavior correlations have also been largely positive (Maguire et al., 2000; Mennes et al., 2011; Rushton and Ankney, 2009; Wang et al., 2013; Wei et al., 2012). Interestingly, for the left paracentral lobule and the caudate, the present study also found a significant negative correlation between volume and ALFF. Nevertheless, these negative correlations were not reproducible across the two cohorts; thus, the validity of these correlations is unclear. If negative volume-ALFF correlations do exist, they may involve very complicated mechanisms that warrant specific investigation in the future.
Notably, our currently observed structure-function relation is based on two macro imaging measures, i.e., volume and ALFF. The two measures are supposed to reflect particular biological aspects at the neural or microscopic level. For example, the macro-level GM volume relates to neural dendritic density, cellular size, neural complexity, and the number of cells (Laughlin and Sejnowski, 2003; Samuelsen et al., 2003; Larsen et al., 2006; Huang et al., 2010). On the other hand, the functional activity measure ALFF was derived from the BOLD signals of fMRI, which are largely determined by the blood flow, blood volume, and oxygen consumption rate, and related to neural activities measured by local field potential (Logothetis et al., 2001; Goensel and Logothetis, 2008) or optogenetics (Lee et al., 2010; Gerits et al., 2012). The moment to moment fluctuation of BOLD signals, which is what the ALFF measure represents, has been suggested to reflect several properties of neural processing, such as the dynamic ranges, optimizing, or wandering process, which all facilitate the neural adaptability (Garrett et al., 2013).

Obviously, both volume and ALFF are complicated imaging measures that are associated with multiple biological components. At this point, it is difficult to sort out specific underlying biological components that contribute to the currently observed volume-ALFF correlation. It is possible that the increased neural number, size, or dendritic density for a larger brain leads to more complex and flexible local neural systems, which in turn increases the variation of neural activities, as reflected by a greater value of ALFF. On the other hand, the human brain tends to be organized by minimizing the wiring cost, and macroscopic fMRI measures were also found to follow such a principle (Alexander-Bloch et al., 2013; Bullmore and Sporns, 2012; Salvador et al., 2005). Given the less wiring cost, smaller brains might have specific
optimizing or functional activity model, leading to a smaller overall ALFF. It should be noted that the observed correlational R values are modest, suggesting that only a small amount of ALFF variance among subjects can be explained by the volume difference. It is likely that there are a number of biological factors that influence the ALFF together, and the brain volume is one of them. These speculations, however, need to be evaluated with animal models in the future.

Methodologically, to avoid potential PVE-related artifacts, the present study chose to calculate the ALFF and volume in the native space. For such an analysis, a GM probability/density threshold of 0.4 was applied when defining GM voxels. This threshold choice might have an impact on the inclusion of PVE contamination across individuals, ultimately affecting the volume-ALFF correlations. To evaluate the potential effect of this choice on our current findings, we re-run all analyses under 6 different thresholds from 0.4 to 0.9. The results showed that, when the threshold is increased (i.e., more “PVE voxels” were excluded), the ALFF-volume correlational results were largely remained. For example, at an extreme threshold of 0.9, the main results were almost the same: 1) For the whole-brain ICV-ALFF correlations: the principal cohort, R = 0.17, p = 0.03; the replication cohort, R = 0.16, p = 0.02; 2) For the significant lobar sections: the principal cohort, frontal lobe, R = 0.20, p = 0.008, temporal lobe, R=0.19, p=0.01, and subcortical structures, R = 0.23, p = 0.002; the replication cohort, frontal lobe, R = 0.23, p = 0.0006; 3) At the regional level, the ALFF-volume effect size and spatial patterns were also quite stable against the threshold choice (Figure 4). These additional results further supported the validity of our currently observed volume-ALFF correlations. However, it would be important to further verify our
current findings with higher-resolution fMRI datasets (e.g. human connectome project) in the future, which have a less degree of PVE in nature.

**Figure 4.** The evaluation of the GMD-threshold effect on the volume-ALFF correlation at the AAL-regional level.

The volume-ALFF correlation for each AAL region is largely remained for different GMD thresholds (A and D).

The spatial patterns of the ALFF-volume correlation map are very similar between every two GMD-thresholds (B and E). The scatter plots (C and F) illustrate a high spatial correlation of the ALFF-volume correlation results between the GMD threshold of 0.4 and 0.9.

In addition, our current ALFF measure is based on BOLD signals of rs-fMRI, the scale of which is arbitrary. It is possible that there exist individual differences of the BOLD signal scale between individuals, which might have an influence on our currently observed volume-ALFF correlation across individuals. To evaluate this, we calculated the mean BOLD signal intensity (MSI) of each voxel for each subject, and then included the corresponding MSI measure as a covariate in the statistical model when correlating the volume with the
ALFF at the whole-brain, lobar, or regional level. The results showed that the volume-ALFF correlations were highly consistent with the original ones without controlling for the MSI: 1) For whole-brain ICV-ALFF correlations: in the principal cohort, $R = 0.14$, $p = 0.07$; in the replication cohort, $R = 0.16$, $p = 0.02$; 2) For significant lobar sections: in the principal cohort, frontal lobe, $R = 0.17$, $p = 0.03$ and subcortical structures, $R = 0.23$, $p = 0.002$; in the replication cohort, frontal lobe, $R = 0.17$, $p = 0.01$; 3) At the regional level, the spatial correlation between the volume-ALFF correlations before/after controlling for the MSI: in the principal cohort, $R = 0.94$; in the replication cohort, $R = 0.92$. These additional results suggest that individual differences of the BOLD signal scales have a very limited effect on our current findings.

Finally, a few limitations and future works should be noted. First, the current study did not include cognitive/behavioral data. It is important to combine volumetric measures, ALFF measures, and cognitive/behavioral scores together and to explicitly evaluate a possible mediating relationship between the three factors. Second, to control for the confounding effects of human brain development and aging, we chose two healthy cohorts with a narrow age range (i.e., young adults). It is unclear whether the volume-ALFF relationship we observed in these cohorts can be generalized to other age ranges; therefore, caution should be exercised when extrapolating our findings to other ages. Particularly, future investigations are highly desired to explore the volume-ALFF associations in various brain diseases such as Alzheimer disease (AD) and epilepsy. Third, the present study performed the volume-ALFF correlations at the whole-brain, lobar, and regional level, but not including the voxel level. The reason is due to the technical difficulties of controlling for the PVE-related artifacts for
such a voxel-level analysis. This issue needs to be addressed in the future. Next, there are other informative fMRI measures reflecting different aspects of the variability/fluctuation of resting brain activities (Garrett et al., 2013). For example, the standard derivation (i.e., variance) was applied to measure the degree of fluctuation of rs-fMRI signals in time domain (Garrett et al., 2010), the scale-free properties are able to represent the spatiotemporal structures of rs-fMRI signals (He, 2011; He et al., 2010), and the entropy can characterize the rs-fMRI signal irregularity (Yang et al., 2013; Wang et al., 2014). It would be intriguing to study relationships between the brain volume and these rs-fMRI measures in the future, which will provide more valuable information for comprehensively understanding the volume-fluctuation coupling. Last but not least, it has been suggested that the resting-state fMRI signal fluctuation, i.e., ALFF, is sensitive to non-neural vascular influences (Di et al., 2013; Kannurpatti et al., 2011). Therefore, a vascular effect may contribute to our currently observed volume-ALFF correlations; this requires future validations by employing data with quantitative measurements of vascular effects.

5. Conclusion

The present study demonstrated a robust linear correlation between brain volume and ALFF in healthy young people, suggesting a structure-to-function association between brain size and intrinsic brain activity. This finding highlights a substantial impact of brain size on human brain functional activity and provides novel insight into the structural substrates that underlie the intrinsic brain activity of the human brain.

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6. References


Dosenbach, N.U., Fair, D.A., Miezin, F.M., Cohen, A.L., Wenger, K.K., Dosenbach, R.A.,


Fox, M.D., Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 8, 700-711.


He, B.J., Zempel, J.M., Snyder, A.Z., Raichle, M.E., 2010. The temporal structures and


cognition. Science 342, 1238411.


One 9, e89948.


Figure 1
Figure 2
Figure 3
Figure 4