Background: Neuroimaging studies have indicated that a number of cortical regions express altered patterns of structural covariance in schizophrenia. The relation between these alterations and specific psychotic symptoms is yet to be investigated. We used voxel-based morphometry to examine regional grey matter volumes and structural covariance associated with severity of auditory verbal hallucinations.

Methods: We applied optimized voxel-based morphometry to volumetric magnetic resonance imaging data from 26 patients with medication-resistant auditory verbal hallucinations (AVHs); statistical inferences were made at p < 0.05 after correction for multiple comparisons.

Results: Grey matter volume in the left inferior frontal gyrus was positively correlated with severity of AVHs. Hallucination severity influenced the pattern of structural covariance between this region and the left superior/middle temporal gyri, the right inferior frontal gyrus and hippocampus, and the insula bilaterally.

Limitations: The results are based on self-reported severity of auditory hallucinations. Complementing with a clinician-based instrument could have made the findings more compelling. Future studies would benefit from including a measure to control for other symptoms that may covary with AVHs and for the effects of antipsychotic medication.

Conclusion: The results revealed that overall severity of AVHs modulated cortical intercorrelations between frontotemporal regions involved in language production and verbal monitoring, supporting the critical role of this network in the pathophysiology of hallucinations.
**Instrument**

To assess the severity of AVHs, we used the Auditory Hallucinations Rating Scale (AHRS), a self-administered 7-item instrument for the measurement of specific characteristics of AVHs: frequency of the voices, reality of the voices, loudness, number of voices, length of the voice content, attentional salience and distress level. A total score is computed by adding the scores on each item (range 0–41) and is used as a typical measure of overall severity and frequency of hallucinations.

**Magnetic resonance imaging data acquisition**

We acquired high-resolution $T_1$-weighted magnetic resonance imaging (MRI) scans using a 3 T scanner (Philips Medical Systems) with a fast field echo sequence and the following parameters: 160 contiguous axial slices, repetition time 25 ms, echo time 4.6 ms, field of view 260 mm, flip angle 30º and voxel size 1 mm$^3$.

**Imaging data preprocessing**

We preprocessed structural images using optimized voxel-based morphometry implemented in SPM5 (www.filion.ucl.ac.uk/spm), running under Matlab (The MathWorks). Voxel-based morphometry is a whole-brain, unbiased, semi-automated technique for characterizing regional cerebral differences in structural MRIs. First, we segmented structural images to extract grey matter and then normalized them to an asymmetric $T_1$-weighted template in Montreal Neurological Institute (MNI) stereotactic space in a recursive fashion. Image segmentation incorporated an intensity nonuniformity correction to account for smooth intensity variations caused by gradient distortions and different positions of cranial structures within the MRI coil. We added a further step to ensure that the total amount of grey matter in each voxel was conserved before and after spatial normalization. This “modulation” step involved multiplying the spatially normalized grey matter by its relative volume before and after spatial normalization. Finally, we smoothed the resulting grey matter images with a 12-mm isotropic Gaussian kernel.

**Statistical analysis**

To identify brain regions associated with overall severity of hallucinations, we subjected the individual grey matter segments to a voxel-wise multiple regression analysis, with AHRS total score as a predictor. We made inferences after family-wise error correction for multiple comparisons across the whole brain. We modelled global grey matter volume and age as covariates of no interest to identify regionally specific associations that were not confounded by these variables. Given our a priori regions of interest (ROIs), based on previous MRI reports of associations between AVHs and grey matter volume in mainly fronto-temporal regions, we applied predefined anatomic ROI analysis, as provided by the Anatomical Automatic Labeling software (www.cyceron.fr/freeware/), including the superior temporal, middle temporal and inferior frontal gyri and the insula and hippocampus, with a significance level of $p < 0.05$, corrected for family-wise error.

Next, for ROIs showing a significant association with AVHs, we explored patterns of structural covariance with our a priori ROIs as a function of hallucination severity (as indexed by AHRS total score) using the Pearson product-moment correlation coefficient. To this end, we extracted regional grey matter volume from each ROI using coordinates taken from the literature. The coordinates were $-18.4, 11.08, -14.57$ (left) and $-57, -35, 8$ (right) for the superior temporal gyrus; $-38.64, -6.23, 6.45$ for the middle temporal gyrus; $37.92, 35.48, 13.88$ for the inferior frontal gyrus; $-43, 12, -10$ (left) and $45, 16, -9$ (right) for the insula; and $18.56, -4.29, -20.73$ for the hippocampus. We set the level of significance at $p < 0.05$, 2-tailed. Figure 1 depicts the peak coordinates reported by studies on structural correlates of hallucinations from which our ROIs were defined.

**Results**

Our sample comprised 26 patients with schizophrenia who were experiencing treatment-resistant auditory hallucinations. Patient characteristics are summarized in Table 1. Severity of auditory verbal hallucinations, as indexed by...
the AHRs questionnaire, was positively correlated with grey matter volume in the left inferior frontal gyrus (MNI coordinates −32, 28, −24; cluster size 29 voxels; t score 4.20; z score 3.56; corrected for family-wise error; Fig. 2). No other regions survived the correction for multiple comparisons.

There were no significant sex differences in AHRs total score ($F_{1,14} = 0.013$, $p = 0.91$) nor in grey matter volume of the left inferior frontal gyrus region that was correlated with AHRs total score ($F_{1,14} = 2.083$, $p = 0.16$). Given the lack of significant sex differences, we report the results for men and women combined.

We analyzed patterns of structural covariance as a function of hallucination severity between volume in the left inferior frontal gyrus and the a priori ROIs. This revealed that overall hallucination severity was positively associated with structural covariance between the left and the contralateral inferior frontal ($r = 0.405$, $p = 0.040$), left superior temporal ($r = 0.485$, $p = 0.012$) and middle temporal gyri ($r = 0.489$, $p = 0.011$); bilateral insula (left $r = 0.410$, $p = 0.038$; right $r = 0.418$, $p = 0.034$); and right hippocampus ($r = 0.526$, $p = 0.006$). In sum, patients with greater hallucination severity showed increased structural covariance between these regions.

**Discussion**

We investigated whether patterns of covariance between regional volumes in the schizophrenic human brain would be modulated by overall frequency and severity of AVHs. First, we identified a positive correlation between grey matter volume in the left inferior frontal gyrus and hallucination severity. Functional neuroimaging studies have indicated enhanced left inferior frontal gyrus activation during hallucinations. Prolonged use of a structure may result in volumetric increases, although it is also possible that alterations in grey matter volume may underlie changes in a region’s functional activity. The left inferior frontal gyrus region receives convergent input from temporal lobe regions and plays a central role in speech processing, which is an intrinsic process in AVHs as they consist of spoken language. Thus, it would not be surprising that, among a sample of hallucinators, greater severity correlated with increased grey matter volume therein. This finding is consistent with studies that have explored “state” hallucinations, identifying the aforementioned increases in activation. Of note, our findings cannot be explained by age or individual differences in overall brain size because we included these factors as confounding variables in the statistical analyses.

Grey matter volume in temporal regions did not appear to be associated with hallucination severity, as measured by the AHRs. Although temporal lobe abnormalities have been repeatedly reported in schizophrenia, this has mainly been found compared with healthy controls. The absence of correlations between AVH severity and the volume of regions other than the inferior frontal gyrus in the present study may be related to the nature of the patient sample, which was restricted to patients with medication-resistant AVHs.

The analysis of structural covariance revealed that hallucination severity influenced cortical intercorrelations between grey matter volume of the left inferior frontal gyrus and a number of hallucination-related regions (i.e., left middle temporal, superior temporal and contralateral inferior frontal

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**Table 1: Characteristics of 26 patients with schizophrenia and treatment-resistant auditory verbal hallucinations**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>36 (12)</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>13:13</td>
</tr>
<tr>
<td>Education, yr</td>
<td>14 (2)</td>
</tr>
<tr>
<td>Duration of illness, mo</td>
<td>163 (137)</td>
</tr>
<tr>
<td>Medication dosage, CPZ b</td>
<td>484 (378)</td>
</tr>
<tr>
<td>Global grey matter volume, mm³</td>
<td>0.8006 (0.1012)</td>
</tr>
<tr>
<td>AHRs total score</td>
<td>26.23 (6.79)</td>
</tr>
<tr>
<td>Frequency</td>
<td>6.15 (2.87)</td>
</tr>
<tr>
<td>Reality</td>
<td>3.81 (1.50)</td>
</tr>
<tr>
<td>Loudness</td>
<td>2.77 (0.91)</td>
</tr>
<tr>
<td>Number</td>
<td>3.04 (1.91)</td>
</tr>
<tr>
<td>Length</td>
<td>3.12 (1.14)</td>
</tr>
<tr>
<td>Attention to voices</td>
<td>3.88 (1.77)</td>
</tr>
<tr>
<td>Arousal</td>
<td>3.46 (1.17)</td>
</tr>
</tbody>
</table>

AHRS = Auditory Hallucinations Rating Scale; CPZ b = chlorpromazine equivalent units; SD = standard deviation.

*Unless otherwise indicated.
gyri; hippocampus; and insula). Although we defined our ROIs using coordinates from previous studies, future research using another method (e.g., Brodmann-based anatomic mask or using coordinates from prior functionally defined regions) could further validate these findings. Alter
ted associations between left frontal and temporal structures have been found in schizophrenia patients compared with healthy controls.\(^2\) Furthermore, structural covariance between fronto-temporal regions, indicating common volumetric variations, is abnormal in schizophrenia.\(^24\) Our results extend these prior findings by relating them to a specific psychotic symptom and add further support to the notion that language processing and verbal monitoring neurocircuitry are critical in the generation of hallucinated speech.\(^2,5\) In addition, these results demonstrate that AHRS severity affects volumetric correlations with medial structures such as the insular cortex and the hippocampus, consistent with the idea that these areas play a part in the genesis and modulation of the perceptual/affective content of the hallucinations.\(^4,21\) Finally, increased fractional anisotropy of fronto-temporal bundles in patients with schizophrenia and AVHs compared with patients without hallucinations has also been reported.\(^25,26\) Taken together, these results highlight the critical involvement of aberrant fronto-temporal interactions in the hallucinatory experience.

**Limitations**

The present work was based on self-reported severity of auditory hallucinations. Potential drawbacks of relying on self-reported measures could have been minimized with the inclusion of a clinician-based instrument. Nevertheless, the AHRS is a widely used tool for the rating of AVHs and in studies on the efficacy of AVH treatments.\(^4\) Future studies could benefit from including a measure to control for factors such as other symptoms that may covary with AVHs and effects of medications, which have been suggested to have neurotrophic properties that could affect cortical thickness in treated patients compared with untreated ones.\(^7\) The present investigation did not control for medication effects as we studied a homogeneous sample of chronic patients with severe medication-resistant hallucinations. Given that regional volume alterations have also been found in voxel-based morphometry studies of patients with an at-risk mental state for psychosis,\(^29,30\) such a population might represent an excellent opportunity to explore neuroanatomical abnormalities linked to specific symptoms free from potential influences of undergoing chronic antipsychotic treatment.

**Conclusion**

To our knowledge, ours is the first study to use voxel-based morphometry to assess structural covariance dependent on severity of AVHs. The main finding was that hallucination severity modulated cortical intercorrelations of regional volumes within a speech processing and verbal monitoring network of fronto-temporal regions, supporting the critical role of their interplay in the neurobiology of hallucinations.

**Acknowledgments:** This study was supported by a European Science Foundation EURYI grant (NWO No. 044035001) to Prof. André Aleman. We thank Hanneke Westenbroek, MD, and Richard Bruggeman, MD, PhD, for help with inclusion of patients; the staff and patients of the department for patients with a psychotic disorder of the University Medical Center of Groningen, and Anita Kuiper for assistance with MRI scanning.

**Competing interests:** None declared.

**Contributors:** Mses. Modinos and Vercammen and Dr. Aleman designed the study. Ms. Vercammen and Dr. Knegttering acquired the data, which Ms. Modinos and Drs. Mechelli and McGuire analyzed. Ms. Modinos wrote the article. All authors reviewed the article and approved the final version for publication.

**References**


