Lack of Gender Influence on Cortical and Subcortical Gray Matter Development in Childhood-Onset Schizophrenia

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Abstract:

Background: Progressive cortical gray matter (GM) abnormalities are an established feature of schizophrenia and are more pronounced in rare, severe, and treatment refractory childhood-onset schizophrenia (COS) cases. The effect of sex on brain development in schizophrenia is poorly understood and studies to date have produced inconsistent results. Methods: Using the largest to date longitudinal sample of COS cases (n = 104, scans = 249, Male/Female [M/F] = 57/47), we compared COS sex differences with sex differences in a sample of matched typically developing children (n = 104, scans = 244, M/F = 57/47), to determine whether or not sex had differential effects on cortical and subcortical brain development in COS. Results: Our results showed no significant differential sex effects in COS for either GM cortical thickness or subcortical volume development (sex × diagnosis × age interaction; false discovery rate q = 0.05). Conclusion: Sex appears to play a similar role in cortical and subcortical GM development in COS as it does in normally developing children.

Key words: schizophrenia/adolescent/sex differences/cortical/subcortical/gray matter/gender/childhood-onset

Introduction

Childhood-onset schizophrenia (COS), characterized by the onset of psychosis before age 13 years, is a rare, severe, and treatment refractory form of the adult illness that is neurobiologically continuous with the adult counterpart. The progressive cortical gray matter (GM) abnormalities that are now an established feature of schizophrenia are more pronounced in COS during adolescence and become more circumscribed to the prefrontal and temporal cortices as children mature, merging into the pattern seen typically in adult onset cases.

What influences the cortical brain development in schizophrenia continues to be explored. The pattern of GM trajectory in COS appears to be an exaggeration of the normal cortical GM maturation, supporting the excessive pruning hypothesis in schizophrenia. The prefrontal and temporal cortical deficits appear to be endophenotypic in nature at least in early ages because they are shared by healthy siblings of COS patients, in whom the cortical thickness is also positively correlated with the overall functional outcome.

The role of sex in brain development in schizophrenia is unclear. The incidence of schizophrenia is different in males and females with males showing higher incidence. Similarly, the illness seems to be more severe in males, with an earlier age of onset of psychosis, while the overall prognosis appears to be better in females. Such differences in clinical phenomenology suggest that the underlying brain pathology in schizophrenia could also be different in males and females. However, this is generally not observed from studies to date. There have been several studies reporting sex influences in brain development in adult onset schizophrenia, although they have resulted in inconsistent findings. These include studies showing larger ventricles, larger intracranial volumes, and greater orbital cortex to amygdala ratios in male patients, while others showing smaller total, regional GM, or more specifically left temporal, orbitofrontal, or left inferior parietal volumes in male patients. Results are more inconsistent with GM volumes in female patients with schizophrenia with some studies showing reduced volumes, while others showing either no change or increased volumes compared with controls. The majority of these studies either have a cross-sectional design or are limited to small sample sizes, which could partially explain the inconsistencies.

Only 2 studies have addressed this question in early onset schizophrenia on smaller samples. Our earlier...
longitudinal study ($n = 60$; age $= 8–18$ years) of COS, using lobar volumes, did not find any significant sex $\times$ diagnosis interaction with brain development,$^{23}$ although more recently a smaller longitudinal study$^{24}$ on early onset schizophrenia cases ($n = 21$; average age $= 12–18$ years) found greater frontal GM loss in males compared with controls, while females had no significant time $\times$ diagnosis interaction.

Previous studies of subcortical structures in adult schizophrenia have focused mainly on the thalamus, hippocampus, amygdala, and basal ganglia/striatum areas and few have examined sex differences in these structures and their possible influence. Many studies on the thalamus have included sex in their analysis, but the results are not consistent with most showing either no or an inconsistent influence of sex.$^{25,26,27,28,29,25,29}$ Analyses on the hippocampus also show conflicting findings with several studies reporting sex effects$^{30,31,32,30,32,31}$ but just as many suggesting no influence of sex$^{30,33,34}$ on the hippocampal volumes. Similarly, several studies analyzing the amygdala have shown mixed results,$^{13,30,32,35,36}$ while basal ganglia/ striatum analyses largely show no effect of sex.$^{20,33,37}$ The effects of sex on these structures are largely unexamined for the COS population.

Here, we examine the effects of sex on cortical and subcortical brain development in COS using the largest to date longitudinal sample of COS cases ($n = 104$; 249 scans) and compared them with a matched sample of typically developing children ($n = 104$; 244 scans) using fully automated and validated methods. We hypothesized that sex would have a significantly different influence on COS GM trajectories compared with that seen in typically developing children.

**Methods**

**Subjects**

COS patients were recruited nationwide as part of an ongoing study at the National Institute of Mental Health (NIMH) in Bethesda, MD, and a majority were diagnosed after a complete medical washout during inpatient observation. One hundred and four patients met the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria for schizophrenia with the onset of psychosis before the 13th birthday.$^{38}$ Exclusionary criteria included substance abuse, history of significant medical or neurological illness, or intelligence quotient (IQ) below 70 prior to onset of psychotic symptoms. Patient selection details are further described elsewhere.$^{39}$ Anatomical magnetic resonance imaging (MRI) scans were collected at each patient visit, which occurred longitudinally at approximately 2-year intervals. The control group was chosen from a larger study of brain development in normal individuals.$^{40}$ The normal volunteer (NV) group that is used in the present study includes 104 of these subjects who were individually matched with the COS participants for sex, age, and scan dates. The research protocol was approved by the institutional review board of the NIMH with informed consents and assents obtained from all participants.

**Cortical Thickness MRI Acquisition and Image Analysis**

All scans were acquired using the exact same GE 1.5 Tesla Sigma MRI scanner, and the sequence was consistent throughout the study. Protocols for head placement were standardized using previously described procedures.$^{41}$ Three-dimensional ($256 \times 256 \times 124$ resolution) T1-weighted fast spoiled gradient (SPGR) echo MRI volumes were collected longitudinally with contiguous 1.5-mm axial slices and 2.0-mm coronal slices. Imaging parameters were echo time of 5 ms, repetition time of 24 ms, flip angle of 45°, acquisition matrix of 256 $\times$ 192, number of excitations equal 1, and a 24-cm field of view. The resolution parameters were maintained throughout the study to ensure consistency between scans.

For GM cortical thickness analysis, a fully automated cortical surface extraction pipeline was used for image processing$^{40}$ and raw scans were masked using the Brain Extraction Tool method.$^{42}$ MRIs were registered into standardized stereo-taxic space (MNI-ICBM152 non-linear sixth generation symmetric target) using a 9-parameter linear transformation and corrected for non-uniformity artifacts.$^{43}$ After registering and correcting for non-uniformity artifacts, the volumes were segmented with an advanced neural net classifier into white matter (WM), GM, cerebrospinal fluid, and background.$^{44}$ GM and WM surfaces were fitted with a surface deformation algorithm using the Constrained Laplacian Anatomic Segmentation Using Proximities surface extraction procedure, which first determines the WM surface and then expands outward to find the GM-cerebrospinal fluid intersection.$^{45}$ Cortical thickness measurement was given by the root mean square thickness between corresponding nodes in native space on the surface mesh at 40 962 cortical points. Thickness measurements were aligned using surface registration to maximize thickness value that corresponds in terms of gyral patterns between subjects. Using a 30-mm surface-based blurring kernel (which has been shown to maximize statistical power by minimizing false positives) reduces noise in thickness measurement and has been previously validated.$^{46}$ The results of the study were projected onto a symmetrical left and right hemispheric template where positive values indicated thicker GM at a given point and negative values indicated thinner GM.

For the analysis of subcortical structures, image files in DICOM format were transferred to a Linux workstation for analysis. Subcortical volumes were measured
automatically with the FreeSurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu). A trained psychiatrist reviewed individual scans and none had to be excluded from analysis due to significant artifact or motion disturbance. The automated procedures for subcortical volumetric measurements of different brain structures have been described previously. This procedure automatically provides segments and labels for many brain structures and assigns a neuroanatomic label to each voxel in an MRI volume on the basis of probabilistic information estimated automatically from a manually labeled training set. Briefly, this processing includes motion correction and averaging of multiple volumetric T1-weighted images (when more than 1 is available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical WM and deep GM volumetric structures (including hippocampus, amygdala, caudate, putamen, and ventricles), intensity normalization, tessellation of the GM–WM boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. The segmentation uses 3 pieces of information to disambiguate labels: (1) the prior probability of a given tissue class at a specific atlas location, (2) the likelihood of the image intensity given that tissue class, and (3) the probability of the local spatial configuration of labels given the tissue class. This technique has previously been shown to be comparable in accuracy with manual labeling and has been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths. However, all segmentations were visually inspected for accuracy prior to inclusion in the group analysis. Total subcortical volumes (hippocampus, caudate, amygdala, thalamus, putamen, and pallidum) were calculated as the sum of the respective left and right volumes for each specific structure per participant.

**Statistical Analysis**

**Demographic Data.** Group differences were tested with ANOVA for continuous measures and chi-square tests of independence for categorical measures. ANOVA assumptions of homogeneity of variance and normality were assessed, and data were visually screened for outliers.

**Image Analyses: GM Cortical Thickness.** Diagnostic differences in developmental GM sex differences across the cortical surface were analyzed using a linear mixed-effects regression model at each of the 40,962 GM points per hemisphere. At each point, the dependent variable was GM thickness. Fixed effects included the group (COS and NV), sex (male and female), age (centered at sample average age [17.27]), and all 2- and 3-way interactions. We included a random intercept per person to account for within-subject dependence. Type I error was controlled per hemisphere using the false discovery rate procedure with q set at 0.05. Our main hypothesis of different developmental sex differences in COS was tested using the sex × diagnosis × age interaction and the sex × diagnosis interaction. Because there was insufficient data to detect curvilinear terms (cubic or quadratic), our models of GM development were linear.

**Subcortical Structures.** To examine diagnostic differences in developmental GM sex differences of total, left, and right subcortical volume (hippocampus, caudate, amygdala, thalamus, putamen, and pallidum) measures, we used the same mixed-effect regression model as above. Specifically, fixed effects included group (COS and NV), sex (male and female), age (centered at sample average age [17.27] and age if appropriate), and all higher order interactions. Random effects included an intercept per person (to account for within person dependence). Hypotheses for shape of developmental curves were tested with F statistics to determine the order (cubic, quadratic, or linear) of growth. Quadratic development was evident for the hippocampus, caudate, and amygdala. All other subcortical regions were modeled with linear trajectories.

**Results**

Sample demographics are in table 1.

The COS and healthy control groups were comparable with respect to age, sex, and race. In contrast, the COS group had a significantly lower Full Scale IQ (Mean = 16.1, df = 172, P < .001), socio-economic status (Mean = 5.23, df = 200, P < .001), and were more non-right handed (X² = 13.7, df = 3, P = .003). When IQ and handedness were included as covariates, they did not notably change the cortical or subcortical GM sex × diagnosis × age or sex × diagnosis interaction results. Consequently, unadjusted findings are reported below (table 2).

**GM Cortical Thickness**

Current results are consistent with previous reports of typically developing males and females sex differences in mid-adolescence (e.g., thicker temporal cortices). Also, a significant diagnosis × age normalizing effect was evident in the parietal regions of the cortex, which is consistent with our previous findings.

In terms of differential sex effects between the COS and healthy control group, there were no significant sex × diagnosis × age interaction coefficients. This indicates
that the trajectory difference between the COS males and females and the trajectory difference between healthy control males and females was not significantly different across the cortical surface. The subsequent sex × diagnosis analysis indicated that sex differences in cortical thickness at the average age were not significantly different between COS and healthy controls in most brain regions except for 2 small regions; in the left frontal medial gyrus

Table 1. Sample Demographics

<table>
<thead>
<tr>
<th></th>
<th>COS (N = 104)</th>
<th>NV (N = 104)</th>
<th>Statistic (df)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>47 F; 57 M</td>
<td>47 F; 57 M</td>
<td>$X^2 = 1.00$ (1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean age overall (SD)</td>
<td>17.3 (4.4)</td>
<td>17.23 (4.3)</td>
<td>$t = 0.391$ (493)</td>
<td>0.69</td>
</tr>
<tr>
<td>Race</td>
<td>5 A; 30 B; 8 H; 9 O; 52 W</td>
<td>4 A; 22 B; 4 H; 5 O; 69 W</td>
<td>$X^2 = 6.20$ (4)</td>
<td>0.184</td>
</tr>
<tr>
<td>IQ, mean (SD)</td>
<td>73.9 (17.6)</td>
<td>111.0 (12.6)</td>
<td>$t = 16.1$ (172)</td>
<td>0.01</td>
</tr>
<tr>
<td>Socio-economic status, Mean (SD)</td>
<td>61.4 (28.9)</td>
<td>43.1 (20.0)</td>
<td>$t = 5.23$ (200)</td>
<td>0.01</td>
</tr>
<tr>
<td>Handedness</td>
<td>12 L; 12 M; 74 R</td>
<td>9 L; 3 M; 92 R</td>
<td>$X^2 = 13.7$ (3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Race</td>
<td>5 A; 30 B; 8 H; 9 O; 52 W</td>
<td>4 A; 22 B; 4 H; 5 O; 69 W</td>
<td>$X^2 = 6.20$ (4)</td>
<td>0.184</td>
</tr>
<tr>
<td>Mean age scan 1 (SD)</td>
<td>14.5 (3.1) (N = 104)</td>
<td>14.6 (3.1) (N = 104)</td>
<td>$t = 0.045$ (206)</td>
<td>0.964</td>
</tr>
<tr>
<td>Mean age scan 2 (SD)</td>
<td>16.8 (2.8) (N = 70)</td>
<td>17.0 (3.0) (N = 70)</td>
<td>$t = 0.279$ (138)</td>
<td>0.781</td>
</tr>
<tr>
<td>Mean age scan 3 (SD)</td>
<td>19.6 (2.9) (N = 42)</td>
<td>19.6 (3.0) (N = 42)</td>
<td>$t = 0.087$ (82)</td>
<td>0.931</td>
</tr>
<tr>
<td>Mean age scan 4 (SD)</td>
<td>21.9 (3.1) (N = 19)</td>
<td>22.1 (3.5) (N = 15)</td>
<td>$t = 0.174$ (32)</td>
<td>0.863</td>
</tr>
<tr>
<td>Mean age scan 5 (SD)</td>
<td>24.7 (2.6) (N = 8)</td>
<td>24.5 (2.1) (N = 10)</td>
<td>$t = 0.154$ (16)</td>
<td>0.879</td>
</tr>
<tr>
<td>Mean age scan 6 (SD)</td>
<td>27.3 (2.6) (N = 6)</td>
<td>29.6 (3.5) (N = 3)</td>
<td>$t = 1.12$ (7)</td>
<td>0.299</td>
</tr>
</tbody>
</table>

Note: COS, childhood-onset schizophrenia; NV, normal volunteer; SD, standard deviation; t, independent samples t-test; $X^2$, P value for 1-way ANOVA; Race: A, Asian; B, Black/African-American; H, Hispanic; O, other/mixed race; W, white; Handedness: L, left; M, mixed; R, right.

Table 2. Trajectory Profiles for Subcortical Structures

<table>
<thead>
<tr>
<th>Region</th>
<th>NV Male Total Value (SE)</th>
<th>NV Female Total Value (SE)</th>
<th>Height P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Trajectory P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV Sex Differences in Subcortical Trajectories</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9516.86 (124.1)</td>
<td>8755.93 (101.5)</td>
<td>&lt;.01</td>
<td>.538</td>
</tr>
<tr>
<td>Caudate</td>
<td>7855.62 (128.7)</td>
<td>7290.36 (154.2)</td>
<td>.005</td>
<td>.454</td>
</tr>
<tr>
<td>Amygdala</td>
<td>3440.61 (43.0)</td>
<td>3077.62 (53.8)</td>
<td>&lt;.01</td>
<td>.290</td>
</tr>
<tr>
<td>Thalamus</td>
<td>15 994.30 (175.7)</td>
<td>14 674.79 (202.0)</td>
<td>&lt;.01</td>
<td>.645</td>
</tr>
<tr>
<td>Putamen</td>
<td>12 546.4 (157.5)</td>
<td>11 361.7 (180.3)</td>
<td>&lt;.01</td>
<td>.651</td>
</tr>
<tr>
<td>Pallidum</td>
<td>3452.25 (47.4)</td>
<td>3185.77 (55.4)</td>
<td>&lt;.01</td>
<td>.622</td>
</tr>
<tr>
<td>Sex × Dx Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region Total</td>
<td>t</td>
<td>df</td>
<td>$P$ Value</td>
<td></td>
</tr>
<tr>
<td>Hippocampus&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−0.41</td>
<td>226</td>
<td>.68</td>
<td></td>
</tr>
<tr>
<td>Caudate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−0.37</td>
<td>216</td>
<td>.71</td>
<td></td>
</tr>
<tr>
<td>Amygdala&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−0.98</td>
<td>230</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>−1.25</td>
<td>195</td>
<td>.21</td>
<td></td>
</tr>
<tr>
<td>Putamen</td>
<td>−0.81</td>
<td>193</td>
<td>.41</td>
<td></td>
</tr>
<tr>
<td>Pallidum</td>
<td>−1.38</td>
<td>197</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>Sex × Dx Trajectory Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region total</td>
<td>t</td>
<td>df</td>
<td>$P$ Value</td>
<td></td>
</tr>
<tr>
<td>Hippocampus&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−0.48</td>
<td>355</td>
<td>.37</td>
<td></td>
</tr>
<tr>
<td>Caudate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01</td>
<td>326</td>
<td>.36</td>
<td></td>
</tr>
<tr>
<td>Amygdala&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07</td>
<td>396</td>
<td>.93</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>−1.21</td>
<td>346</td>
<td>.22</td>
<td></td>
</tr>
<tr>
<td>Putamen</td>
<td>0.65</td>
<td>326</td>
<td>.51</td>
<td></td>
</tr>
<tr>
<td>Pallidum</td>
<td>0.12</td>
<td>401</td>
<td>.90</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Males greater volumes than females.
<sup>b</sup>Quadratic curve, the rest were linear models.
where COS males had thicker GM compared with COS females, but normal females were thicker compared with normal males; and in the right occipital cuneus where the difference in cortical thickness between COS males and COS females was greater than the difference between normal males and females.

**Subcortical Structures**

Current results support normal males having greater volumes than females for all structures. However, no significant control group sex differences in longitudinal trajectory were evident (table 2). For all subcortical structures, COS males showed greater volumes than COS females and the difference between the longitudinal trajectories (slopes) for the COS males and females did not differ significantly from the difference between NV male and female trajectories (table 2). Also, no significant sex \( \times \) diagnosis effects were found (table 2).

**Discussion**

To date, this is the first longitudinal MRI study evaluating sex differences in GM cortical thickness and subcortical structures in COS. Sex had no influence on the development of either GM cortical thickness or subcortical structures between COS and healthy controls.

These results show that the pattern of GM development between males and females in the COS population is comparable with normally developing males and females. In healthy brain development, the influence of sex is evident in longitudinal brain analysis. Typically, developing males have greater total cerebral size and volume, most prominent in the frontal and occipital poles bilaterally which is maintained throughout life. Typically, developing females on the other hand show proportionately greater frontal GM volumes, thicker cortices in the right inferior parietal, and posterior temporal regions. Longitudinal studies also show sexually dimorphic trajectories of brain development with females peaking 4 years earlier than males for nearly all structures, and significantly in the frontal lobe, and more efficient androgen receptor functioning may be related to masculinization of developmental trajectory in cortical subregions during adolescence.

On the other hand, influences of sex on schizophrenia pathology have been unclear and studies to date have shown inconsistent findings, with some showing either larger, smaller, or no difference in volumes in schizophrenia patients of each sex. There is a notion that male patients with schizophrenia have a more severe manifestation of the illness than their female counterparts, with more severe negative symptoms and female patients showing better processing and modulation of emotion. Sex-specific structural brain abnormalities, if seen, could have explained the differential clinical disease expression. Consistent with the lack of sex influence on brain development, the COS cohort shows no sex-specific clinical difference either.

Our COS cohort had varying levels of exposure to neuroleptic and/or other psychotropic medications both at baseline and at follow-up scans, and it is possible that it could have potentially "normalized" the GM trajectories. This limitation is harder to overcome because it is not possible to have scans on medication naïve patients, but our other studies indicate a general lack of medication influence on GM development in COS, and there are no medication differences between male and female COS patients in this study. Our initial study comparing progressive cortical GM loss in COS and in children with atypical psychoses (multidimensionally impaired) found no medication effect on GM change over a 2-year period. A follow-up study, which involved dynamic mapping of cortical development before and after onset of pediatric bipolar illness, also found no medication influence, which included mood stabilizers on GM trajectories. A recent study comparing 2 differently medicated COS groups showed no differences in GM trajectories between the clozapine- and olanzapine-treated patients, suggesting that these medications have no significant differential influence on GM development in COS.

Our negative finding, however, still does not exclude the possible influence of sex in understanding the complex and rare occurrence of COS. The absence of structural differences do not rule out the role of sex hormones in the early brain development, probably more at microscopic or functional levels, which could not be detected by this study. The substantial increase in sex hormone levels during pubertal maturation during adolescence and the prolonged shaping of neural connectivity could influence and differentiate the brain. These differences would most like present themselves in structures that regulate sexual processes, such as the hippocampus and thalamus, but could possibly lead to sex differences observed in cognition and behavior. Future longitudinal imaging studies should include a measure of sex hormones to clarify the influence that it may have on sex-based differences in the development of COS. Another possibility is that the sex differences may be discovered in more subtle shape analyses of brain structures rather than the overall volume or in the connectivity between brain structures. These analyses are currently under way.

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Sex and Brain Development in Schizophrenia

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