Neuron Number and Size in Prefrontal Cortex of Children With Autism

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Context Autism often involves early brain overgrowth, including the prefrontal cortex (PFC). Although prefrontal abnormality has been theorized to underlie some autistic symptoms, the cellular defects that cause abnormal overgrowth remain unknown.

Objective To investigate whether early brain overgrowth in children with autism involves excess neuron numbers in the PFC.

Design, Setting, and Cases Postmortem prefrontal tissue from 7 autistic and 6 control male children aged 2 to 16 years was examined by expert anatomists who were blinded to diagnostic status. Number and size of neurons were quantified using stereological methods within the dorsolateral (DL-PFC) and mesial (M-PFC) subdivisions of the PFC. Cases were from the eastern and southeastern United States and died between 2000 and 2006.

Main Outcome Measures Mean neuron number and size in the DL-PFC and M-PFC were compared between autistic and control postmortem cases. Correlations of neuron number with deviation in brain weight from normative values for age were also performed.

Results Children with autism had 67% more neurons in the PFC (mean, 1.94 billion; 95% CI, 1.57-2.31) compared with control children (1.16 billion; 95% CI, 0.90-1.42; $P = .002$), including 79% more in DL-PFC (1.57 billion; 95% CI, 1.20-1.94 in autism cases vs 0.88 billion; 95% CI, 0.66-1.10 in controls; $P = .003$) and 29% more in M-PFC (0.36 billion; 95% CI, 0.33-0.40 in autism cases vs 0.28 billion; 95% CI, 0.23-0.34 in controls; $P = .009$). Brain weight in the autistic cases differed from normative mean weight for age by a mean of 17.6% (95% CI, 10.2%-25.0%; $P = .001$), while brains in controls differed by a mean of 0.2% (95% CI, −8.7% to 9.1%; $P = .96$). Plots of counts by weight showed autistic children had both greater total prefrontal neuron counts and brain weight for age than control children.

Conclusion In this small preliminary study, brain overgrowth in males with autism involved an abnormal excess number of neurons in the PFC.

For editorial comment see p 2031.

Author Audio Interview available at www.jama.com.

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about one-third of all of the cortex, in autistic children compared with controls. Additionally, to determine whether excess prefrontal neuron counts in autism co-occur with abnormally enlarged brains, we compared brain weight in the autistic children with age-based normative weights and with brain weight in the controls.

METHODS

Brains were obtained from the National Institute of Child Health and Human Development (NICHD), University of Maryland Brain and Tissue Bank, the Autism Tissue Program at the Harvard Brain Tissue Resource Center, and the New York State Institute for Basic Research in Developmental Disabilities. Young postmortem cases are scarce and especially so with regard to tissue suitable for modern stereological study of the entire dorsolateral (DL-PFC) and mesial (M-PFC) subdivisions of the PFC. Such unbiased cell counting procedures are necessary to ensure valid cell counts, which cannot be obtained via density estimates from small blocks of cortical tissue.\textsuperscript{18,19} Brains were obtained from 7 autistic and 6 control male children aged 2 to 16 years, representing all young control male cases available at the time of the study and nearly all known young autism cases that had had the whole PFC uniformly sectioned. Cases were not selected for any reason such as autopsy brain weight, postmortem interval (PMI), or cause of death, except that the PFC met requirements for performing valid stereological procedures.

Cases were from the eastern and southeastern United States and dates of death ranged from 2000 to 2006. Perinatal and postnatal medical conditions were obtained by the tissue banks from next of kin. Cause of death, PMI, and neuropathology were obtained from coroner’s reports. Race of each postmortem case was determined by the tissue banks. Race and ethnicity categories were based on National Institutes of Health (NIH) requirements. Research procedures were approved by the institutional review board of the University of California, San Diego. Informed consent or waiver of consent was not required because all cases were deceased and de-identified and anonymized by the tissue banks.

All autism diagnostic classifications (Table 1) were based on the results of postmortem administration of the Autism Diagnostic Interview-Revised (ADI-R) to a parent or legal guardian of the deceased by a psychologist, which is the standard method for autism postmortem research. The ADI-R is a standardized parent interview used for determining developmental history and behavior for the purposes of diagnosing autism. Questions are designed to elicit relevant information through queries closely associated with diagnostic criteria set forth in the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition; DSM-IV). The administration, scoring, and diagnostic determination are the same as when it is administered to the parent or legal guardian of a living individual. The psychologists who determined the intellectual ability level of each autistic child was blinded to knowledge of the neuropathology and neuron counts. Nonintellectual disability was defined as IQ of 71 or greater on standardized IQ tests or evidence from the ADI-R narrative of understanding of most words and sentences, communicative use of words and language, and some initiation of appropriate activities such as looking at books, using computers, showing some interest in mother, or playing games. Intellectual disability was defined as IQ equal to 70 or lower or little to no understanding or use of words, lack of appropriate activities, presence of self-injurious behavior, and/or nonresponsiveness to others.

All control cases were from the NICHD Brain and Tissue Bank. This tissue bank determined control cases to be free of mental illness, intellectual disability, and neurological disorder, including autism, based on information gathered in a detailed questionnaire at the time of death from next of kin. Cases with a history of chemotherapy or radiation treatment or being resuscitated following ischemic hypoxia were excluded as controls by the tissue banks.

### Age-Based Normative Mean Brain Weights

Brain weight in normal male individuals increases with age.\textsuperscript{5} To test whether brain weight in the autistic children in the present study exceeded normal mean weight for age, the brain weight of each case was compared with the normative mean weight for age and expressed as a percent difference from that normative mean. To determine whether control children had brain weights expected for typical individuals, each weight was likewise compared to the normative mean

### Table 1. Diagnostic Characteristics of Study Cases With Autism\textsuperscript{a}

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y</th>
<th>ADI-Social\textsuperscript{b}</th>
<th>ADI-Communication\textsuperscript{c}</th>
<th>ADI-Restrictive and Repetitive\textsuperscript{d}</th>
<th>Intellectual Disability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>14</td>
<td>9</td>
<td>6</td>
<td>No\textsuperscript{e}</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>20</td>
<td>8</td>
<td>3</td>
<td>No\textsuperscript{f}</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>22</td>
<td>14</td>
<td>8</td>
<td>Yes\textsuperscript{g}</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>14</td>
<td>10</td>
<td>3</td>
<td>Yes\textsuperscript{g}</td>
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<tr>
<td>5</td>
<td>7</td>
<td>29</td>
<td>14</td>
<td>3</td>
<td>Yes\textsuperscript{g}</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>19</td>
<td>7</td>
<td>4</td>
<td>No\textsuperscript{h}</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>29</td>
<td>14</td>
<td>7</td>
<td>Yes\textsuperscript{h}</td>
</tr>
</tbody>
</table>

Abbreviation: ADI-R, Autism Diagnostic Interview-Revised.\textsuperscript{a} All cases were male. All cases met or exceeded cutoffs for a diagnostic classification of autism using the ADI-R instrument.\textsuperscript{b} Qualitative abnormalities in reciprocal social interaction (cutoff, 10; maximum score, 30).\textsuperscript{c} Qualitative abnormalities in communication (cutoff, 7; maximum nonverbal communication score, 14).\textsuperscript{d} Restricted, repetitive, and stereotyped patterns of behavior (cutoff, 3; maximum score, 12).\textsuperscript{e} Intellectual disability status was determined using available standardized IQ scores (which have an intellectual threshold for intellectual disability on a standardized measure of intelligence of 70 or lower; a mean score of 100).\textsuperscript{f} In the absence of a standardized IQ score, determination of intellectual disability was made based on review of specific questions and responses about verbal communication, expression abilities, and adaptive behavior skills on the ADI-R Narrative.
weight for age and expressed as a percent difference. The resulting age-based percent differences in brain weight within and between study groups were then compared. The age-based normative brain weights for males (eTable 2, available at http://www.jama.com) are based on approximately 11,000 cases reported in 10 normative brain weight studies.

Anatomic Delineations of Prefrontal Subdivisions

We analyzed the DL-PFC and the M-PFC, 2 of the 3 major prefrontal subdivisions (Figure 1; eAppendix); orbital PFC was not measured. Anatomists identified all anatomical boundaries of DL-PFC and M-PFC, blinded to diagnostic membership, age of case, and the purpose, literature, and theories associated with this study to ensure unbiased anatomic decisions. Anatomic delineation of the DL-PFC and M-PFC regions throughout their rostrocaudal extent was based on previous definitions6,20 and on overall gross anatomy, including tracking of sulci. Reliable boundaries were further refined based on cytoarchitectonic criteria,21,22 including layer 4 granularity and the presence of Betz cells; the overall cortical width and layer 6/white-matter transition; and density and clarity of cortical columns were also used in some cases (eAppendix).

Stereology Procedures

Brains were serially sectioned and prepared for stereologic analysis (eAppendix; eTable 1). Quantifications of neuron number and mean cell volume within the DL-PFC and M-PFC were carried out blind to diagnostic membership, age of case, and the purpose, literature, and theories behind this study to ensure unbiased anatomic measurement. The sum of these 2 subdivisions gives the combined prefrontal neuron counts. Microglia and satellite oligodendrocytes (small nonmyelinat-
MCV estimates were done on tissue sectioned in the coronal plane, rather than random planes, a small orientation bias could be present to the same degree for both autism and control cases.

**Distinguishing Neurons From Microglia and Satellite Oligodendrocytes**

Detailed morphological examination and characterization in 3D was performed in this study to distinguish neurons from microglia and satellite oligodendrocytes on Nissl-stained sections. Cell size alone was not sufficient because neurons possess much more cytoplasm and have distinct characteristics in their nucleus and processes. We estimate that, when randomly sampled, difficulty in judging whether a cell is a neuron, microglia, or satellite oligodendrocyte occurred in approximately 1 of 25 cells (which would contribute an error of <0.4% in neuron counts).

**Statistical Analyses**

Analyses were made using SPSS version 18.0.0 to test for differences in percent difference from brain weight of age norms, neuron counts, microglia counts, neuron volume), as the dependent variables. With the neuron count models, diagnosis remained in the model as a significant factor; but age, PMI, and the interaction term were not significant. A second full model with PMI, diagnosis, and their interaction was evaluated. Diagnosis remained in the model as a significant factor, PMI and the interaction term were not significant. Pearson correlations were used to examine the relationships between neuron counts for DL-PFC and M-PFC, prefrontal neuron counts and brain weight deviance, and each of these variables and age. Using a best fit line of the relationship between neuron counts and brain weight deviance in control children, neuron counts in each autistic child were used to predict the brain weight deviation from age-based norms. Independent samples t test with equal group variances were performed to test for differences in group means. Group variances were tested initially, and none were found to be significantly different. All tests of statistical significance were 2-sided. A P value of less than .05 was considered significant.

### RESULTS

#### Diagnostic Characteristics

All autistic cases met criteria for autistic disorder on the 3 subscales of the Autism Diagnostic Interview-Revised (ADI-R) diagnostic assessment (Table 1). Scores on social and communication ADI-R scales ranged from less severe to more severe impairment. Intellectual ability ranged from having normal language and/or daily functional abilities to having little or no language comprehension and production and very impaired functional abilities (Table 1). No autism case had a diagnosis of Asperger syndrome or pervasive development disorder-not otherwise specified. One of the autistic children had received an autism diagnosis via the Autism Diagnostic Observation Schedule that had been administered when the child was still living (case 7, Table 1).

#### Clinical Characteristics

Except for 1 child in the control group, children in the autism and control groups were born full term and perinatal courses were unremarkable (Table 2). One 7-year-old in the autism group had a history of seizures and was being treated with medication. He had been diagnosed with a heart murmur at birth and had a fever 3 days after birth that required hospitalization. One 7-year-old in the control group received medication for hyperactivity. An 8-year-old in the autism group had rhabdomyosarcoma, received treatment including chemotherapy, and died of the...
Brain size.

with increases in neuronal numbers or cases are not known to be associated and the causes of death among these 13 tal, medication, and medical historiesatory of any case. The prenatal, perinatal, that was not in the medical history of any case. The prenatal, perinatal, medication, and medical histories and the causes of death among these 13 cases are not known to be associated with increases in neuronal numbers or brain size.

Neuropathological Characteristics

Gross examination of the brain showed no abnormalities in most autistic and control cases, according to medical examiner or neuropathology reports (eTable 3). In frontal lobes, neuropathology reports stated the presence of a single focal dysplasia associated with cortical thickening in 1 autistic case and a single ectopia in white matter and distortion of the normal radial orientation of neurons in superior-posterior cortex in another (Table 3). In the cerebellum, flocculonodular lobe dysplasia was reported in 4 of the 7 autistic cases (eTable 3). Pathologies of the cerebrum consistent with acute hypoxic ischemia were reported for 2 autistic cases and 2 control cases.

Brain Weight

The mean brain weight of the autistic children (1484 g; 95% CI, 1324-1644 g) was 2.4% greater than the mean brain weight reported for autistic 2- to 16-year-olds in the literature (N=18; 1449 g; 95% CI, 1324-1644 g) (eTable 4). This difference was not significant (t1,23=-0.5, P=.62).

Brain weight in the autistic sample deviated from normative mean weight for age by 17.6% (95% CI, 10.2%-25.0%; t6=5.807; P=.001), while control brains (1299 g; 95% CI, 1155-1442 g) deviated from age-based norms by 0.2% (95% CI, −8.7 to 9.1; t6=0.051; P=.96) (Figure 2, Table 4; for age-based norms, Table 3). This autistic vs control group difference was significant (group comparison, P = .003; Table 4).

Prefrontal Neuron Counts

Statistically significant differences in neuron counts in the PFC were found in the autistic children compared with controls (Table 4); counts for each autistic and control case in each region are shown in eTable 5. There were 79% more neurons in DL-PFC in the autistic cases compared with the control cases (Figure 3A) and 29% more in M-PFC (Figure 3B). The mean DL-PFC count in the autistic children was 1.57 billion neurons (95% CI, 1.20-1.94) compared with a mean of 0.88 billion neurons (95% CI, 0.66-1.10) in control children (P=.003). The mean M-PFC count in the autistic group was 0.36 billion neurons (95% CI, 0.33-
0.40) compared with a mean of 0.28 billion neurons (95% CI, 0.23-0.34) in controls (P = .009). Together, these 2 subdivisions gave a total combined prefrontal neuron count that was 67% greater in the autistic children (mean, 1.94 billion; 95% CI, 1.57-2.31) compared with controls (mean, 1.16 billion; 95% CI, 0.90-1.42; P = .002; Figure 3C). Significant group differences remained after controlling for PMI and age; ANCOVA model results are given in Table 5. Neither age nor PMI was a significant covariate in the models; however, diagnosis was sig-

Table 3. Neuropathology Characteristics and Postmortem Information on Autistic and Control Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y</th>
<th>Cause of Death</th>
<th>Postmortem Interval, h</th>
<th>Hemisphere</th>
<th>Brain Weight, g</th>
<th>% Difference From Normative Mean Brain Weight for Age</th>
<th>Reported Frontal Cortex Neuropathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism 1</td>
<td>2</td>
<td>Drowning</td>
<td>4</td>
<td>Right</td>
<td>1328</td>
<td>1069</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Drowning</td>
<td>12.5</td>
<td>Left</td>
<td>1389</td>
<td>1196</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Drowning</td>
<td>15</td>
<td>Left</td>
<td>1330</td>
<td>1196</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Drowning</td>
<td>No records</td>
<td>Right</td>
<td>1280</td>
<td>1196</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>Drowning</td>
<td>25</td>
<td>Right</td>
<td>1610</td>
<td>1361</td>
<td>18</td>
</tr>
<tr>
<td>Control 6</td>
<td>8</td>
<td>Rhabdomyosarcoma</td>
<td>22.2</td>
<td>Right</td>
<td>1570</td>
<td>1361</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>Undetermined</td>
<td>47.9</td>
<td>Right</td>
<td>1880</td>
<td>1434</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>Drowning</td>
<td>24</td>
<td>Left</td>
<td>1240</td>
<td>1069</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>Respiratory insufficiency</td>
<td>14</td>
<td>Left</td>
<td>997</td>
<td>1069</td>
<td>−7</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>Drowning</td>
<td>12</td>
<td>Right</td>
<td>1240</td>
<td>1361</td>
<td>−9</td>
</tr>
<tr>
<td>11</td>
<td>13</td>
<td>Asphyxia by hanging</td>
<td>5</td>
<td>Right</td>
<td>1420</td>
<td>1434</td>
<td>−1</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
<td>Electrocution</td>
<td>20</td>
<td>Right</td>
<td>1464</td>
<td>1434</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>16</td>
<td>Multiple injuries</td>
<td>16</td>
<td>Right</td>
<td>1440</td>
<td>1434</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

aAdapted from Redcay and Courchesne. 

bDetermined by coroner report and subsequent neuropathology reports.

cInterpolated from brain volume from magnetic resonance imaging; differs from the 1130 g reported by the Autism Tissue Program.

dCoroner and/or neuropathology reports not available through the Autism Tissue Program.

eDetermined by neuropathology report only.

fFresh brain weight at autopsy; differs from weight of 1990 g reported by the Autism Tissue Program. Parenchyma weight was estimated to be 1751 g, interpolated from in vivo magnetic resonance imaging brain volume at age 13 years.

gDetermined by coroner report only.
significant for DL-PFC and M-PFC regions and the total combined prefrontal regions.

Figure 1 shows that these global increases in prefrontal neuron numbers were not apparent either at low or high magnification, and thus undetectable by neuropathology visual inspection and neuron density measurements without formal quantitative stereological procedures.

Prefrontal Neuron Counts and Brain Weight

Figure 4 plots the total prefrontal neuron counts as a function of percent difference of brain weight from age-based norms. The control group had a strong, significant positive linear correlation between counts and weight deviations \((r = 0.949; P = .004)\). Six of the 7 cases in the autistic group had neuron counts that met or exceeded the regression line of those in the control group, indicating that they had as many or more neurons than would be predicted from their large brain weights. The exception was a 7-year-old in the autistic group (Figure 4) who had a history of severe seizures. For the 6 cases in the autistic group without a confounding seizure disorder, the mean brain weight deviation predicted from their actual total prefrontal neuron counts was 29.4% beyond age-based norms.

Neuron Volume and Glia Counts

There were no significant differences in DL-PFC or M-PFC neuron sizes between groups. There were also no significant differences in glia counts for DL-PFC or M-PFC regions between groups (Table 4).

COMMENT

In this small, preliminary study, male children with autism had a mean 67% more prefrontal neurons than those in the control group. The excess was greater within DL-PFC than in M-PFC, a difference that parallels MRI volumetric data showing greater deviation in DL-PFC than M-PFC in living autistic toddlers. MRI studies show that enlargement is not restricted to DL-PFC and M-PFC; whether increased neuron counts in autism extend beyond these 2 major prefrontal subdivisions to include other cortical areas remains to be determined.

The autistic group also had larger than average brain weight. In 6 of the 7 cases, neuron numbers equaled or exceeded predictions based on brain weight compared with controls. These data indicate that a pathological increase in neuron numbers may be a key contributor to brain overgrowth in autism. However, our data also illustrated a strong positive correlation between total neuron numbers and brain weight in the control cases that was not found in the autistic cases. Thus, the autistic brains exhibited a substantial disturbance in the normal linear relationship between neuron quantity and overall brain weight. Neuron counts in the autistic children should have been accompanied by brain weights considerably larger than was observed, reaching 29.4% enlargement rather than the observed 17.6% enlargement. Thus, the size of the autistic brain, overall though it is, might actually underestimate the pathology of excess neuron numbers.

Because cortical neurons are not generated in postnatal life, this pathological increase in neuron numbers in autistic children indicates prenatal causes, including unchecked proliferation, reduced apoptosis, or both. Proliferation of cortical neurons is exponential between 10 and 20 weeks gestation and normally results in a net overabundance of neurons by as much as 100%. In animal models, dysregulation of genetic mechanisms are known that cause an even greater neuron overabundance and lead to increased head, brain, and cortical size, as is found in young chil-

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**Table 4. Group Analyses of Brain Weight, Neuron Count and Size, and Glia Count**

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Control (n = 6)</th>
<th>Autism (n = 7)</th>
<th>t Value (df = 11)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmortem brain weight, g</td>
<td>1299 (179)</td>
<td>1484 (216)</td>
<td>1.66</td>
<td>.12</td>
<td></td>
</tr>
<tr>
<td>Brain weight % difference from normative mean for age</td>
<td>0.2 (8.5)</td>
<td>17.6 (8.0)</td>
<td>3.81</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>DL-PFC neuron count, billions</td>
<td>0.88 (0.21)</td>
<td>1.57 (0.40)</td>
<td>3.81</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>M-PFC neuron count, billions</td>
<td>0.28 (0.05)</td>
<td>0.36 (0.04)</td>
<td>3.20</td>
<td>.009</td>
<td></td>
</tr>
<tr>
<td>Total PFC neuron count, billions&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16 (0.24)</td>
<td>1.94 (0.40)</td>
<td>4.12</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>DL-PFC neuron size, (\mu^3)</td>
<td>1337.12 (483.94)</td>
<td>1169.90 (244.81)</td>
<td>3.81</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>M-PFC neuron size, (\mu^3)</td>
<td>1256.84 (352.76)</td>
<td>1127.81 (364.66)</td>
<td>3.81</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>DL-PFC glia count, billions&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36 (0.36)</td>
<td>0.26 (0.28)</td>
<td>-0.51</td>
<td>.62</td>
<td></td>
</tr>
<tr>
<td>M-PFC glia count, billions&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14 (0.13)</td>
<td>0.12 (0.20)</td>
<td>-0.28</td>
<td>.78</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Abbreviations: DL-PFC, dorsolateral prefrontal cortex; M-PFC, mesial prefrontal cortex; PFC, prefrontal cortex.

<sup>b</sup>Total combined prefrontal neuron count equals DL-PFC count plus M-PFC count.

<sup>c</sup>Glia counts were not available for 1 case in the control group, and 3 standard deviations of the mean and was removed as an outlier.
children with autism. Functional analyses of genes located within copy number variation regions in autism also raise the possibility of dysregulation of proliferation during development. Apoptotic mechanisms during the third trimester and early postnatal life normally remove subplate neurons, which comprise about half the neurons produced in the second trimester. A failure of that key early developmental process could also create a pathological excess of cortical neurons. A failure of subplate apoptosis might additionally indicate abnormal development of the subplate itself. The subplate plays a critical role in the maturation of layer 4 inhibitory functioning as well as in the early stages of thalamocortical and corticocortical connectivity development. Reduced inhibitory functioning and defects of functional and structural connectivity are characteristic of autism, but the causes have remained elusive. The possibility of abnormal development of the subplate in autism merits investigation.

Future studies of neuron numbers and underlying molecular and genetic mechanisms in autism face many limitations, as encountered in the present study. For example, the sample of postmortem tissue from children with autism—all that were available at the time of the study—was small. Despite the small sample size, evidence of excess neuron numbers in our autistic cases was statistically robust and occurred in cases with varying characteristics, such as autism with intellectual disability.

Table 5. Analysis of Covariance Tests

<table>
<thead>
<tr>
<th>controlling for PMI</th>
<th>Group</th>
<th>Age, y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t Value</td>
<td>P Value</td>
</tr>
<tr>
<td>DL-PFC neuron count</td>
<td>3.19</td>
<td>.01</td>
</tr>
<tr>
<td>M-PFC neuron count</td>
<td>2.69</td>
<td>.03</td>
</tr>
<tr>
<td>Total PFC neuron count</td>
<td>3.39</td>
<td>.008</td>
</tr>
<tr>
<td>DL-PFC neuron size</td>
<td>-.83</td>
<td>.43</td>
</tr>
<tr>
<td>M-PFC neuron size</td>
<td>-.75</td>
<td>.47</td>
</tr>
<tr>
<td>DL-PFC glia count</td>
<td>-.13</td>
<td>.9</td>
</tr>
<tr>
<td>M-PFC glia count</td>
<td>0.41</td>
<td>.69</td>
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</tbody>
</table>

Abbreviations: DL-PFC, dorsolateral prefrontal cortex; M-PFC, mesial prefrontal cortex; PMI, postmortem interval.

Error bars indicate 95% CIs. For between-group comparisons, statistical tests were as follows: \( P = .003 \) for panel A, \( P = .009 \) for panel B, and \( P = .002 \) for panel C. Autistic case with lowest neuron count value in panels A and C had a seizure disorder, adverse perinatal medical conditions, and intellectual disability.
as with less severe and more severe autistic symptoms, and with and without intellectual disability. None of the causes of death for autistic cases in this study produce an increase in postmortem brain weight or neuron numbers. Most of the autistic and control children died of acute global ischemic hypoxia. Nearly every autistic and control case came from a full-term pregnancy. A history of medication and adverse medical conditions was not present in most of the cases, particularly for the 4 youngest autistic cases, each of whom had substantial excess neuron counts. Conversely, the lowest prefrontal neuron number in the autism group was found in a 7-year-old boy with a seizure disorder, which may explain why he had fewer counts than other autistic children. The potential effect of seizures on cellular and molecular measures in autism is important to investigate further.

Our sample of autistic children was not large enough to statistically examine brain-behavior relationships. Future studies with many more cases of autistic children might reveal important relationships between neuron counts and symptom severity or intellectual ability. Also, our sample of autistic cases had brain weights typical of brain weights in larger postmortem samples of autistic children. The 1 autistic child with a brain size within the 95% CI of controls had among the greatest prefrontal neuron counts in the study, which raises the question of whether excess prefrontal neuron counts may be present in other autistic children who have near normal or smaller brain sizes.

The small sample of young control children cannot be viewed as representative of all healthy young children, but control cases were not chosen for any reason other than age, sex, availability of the required PFC sections, absence of neurological or mental illness, and absence of treatment for cancer. Brain size in our control sample was typical for age, deviating by only 0.2% from expected mean weight for age. Also, prefrontal neuron counts in controls did not vary with age, which is concordant with literature that cortical neurons are generated prenatally, not postnaturally. It would be invaluable to study larger samples of autistic and control cases at a younger and narrower age range to confirm excess counts in autism at the youngest ages, as well as to study larger samples across a wider age range to identify patterns of age-related change in autism. It will be important to include female cases in future studies, as etiological mechanisms may be discordant between sexes. Whether female autistic patients also have excess prefrontal neuron numbers at young ages remains to be tested, but very few cases exist that have complete prefrontal sections.

To our knowledge, this study is the first direct quantitative test and confirmation of the theory that a pathologically overabundance of neurons in critical brain regions is present at a young age in autism. Because cortical neurons are generated in prenatal, not postnatal life, pathological overabundance of neurons indicates early developmental disturbances in molecular and genetic mechanisms that govern proliferation, cell cycle regulation, and apoptosis. Therefore, the finding has significance for understanding the etiological and neural development and functional origins of autism.

**Author Contributions:** Dr Courchesne had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Courchesne, Mouton, Semendeferi, Ahrens-Barbeau.

**Acquisition of data:** Courchesne, Mouton, Calhoun, Semendeferi, Ahrens-Barbeau, Barnes.

**Analysis and interpretation of data:** Courchesne, Mouton, Calhoun, Semendeferi, Ahrens-Barbeau, Hallet, Barnes, Pierce.

**Drafting of the manuscript:** Courchesne, Mouton, Barnes.

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**Statistical analysis:** Mouton, Calhoun, Hallet, Pierce.

**Obtained funding:** Courchesne, Semendeferi.

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Online-Only Material: The eAppendix, eTables 1-5, eFigure, and Author Audio Interview are available at http://www.jama.com.

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