Abnormalities in oligodendrocyte clusters in the inferior parietal cortex in schizophrenia are associated with insight

Natalya S. Kolomeets
Victor M. Vostrikov
Natalya A. Uranova
Laboratory of Clinical Neuropathology, Mental Health Research Center, Moscow
RUSSIA

ABSTRACT – Background and Objectives: Deficits in oligodendrocytes have been consistently reported in the brains of patients with schizophrenia and include alterations in the clustering pattern of oligodendrocytes. Recently it has been shown that oligodendrocyte progenitors proliferate in the adult mammalian brain to form oligodendrocyte clusters (OlC). We previously found a deficit of oligodendrocytes in layer 3 of the inferior parietal lobule (IPL) in subjects with schizophrenia with poor insight into disorder. We hypothesized that the number of OlC might be reduced in schizophrenia subjects with poor insight.

Methods: Nissl-stained sections from the Stanley “Parietal Collection” from male schizophrenia subjects (n = 24) that have poor, fair, or good insight into their disorder and normal matched controls (n = 24) were studied. The numerical density (Nv) of OlC was estimated in layer 3 of BA 39 and BA 40 by optical disector method.

Results: The Nv of OlC was 23% lower in BA 39 and 30% lower in BA40 in the schizophrenia group compared to the control group (p<0.01). Normal hemispheric differences in the Nv of OlC in BA 39 were absent in the schizophrenia group. The Nv of OlC was significantly decreased in BA39 in the subgroup with poor insight and in BA40 in the subgroups with fair and good insight as compared to controls. In BA40 lower Nv of OlC (~40%, p<0.01) was found in the subgroup with adolescent onset of disease as compared to controls.

Conclusions: The deficit of OlC may be associated with altered proliferation and/or maturation of oligodendrocyte progenitors in schizophrenia.

Received: 5 December 2012
Revised: 10 June 2013
Accepted: 25 June 2013
Introduction

There is increasing evidence from imaging, genetic and postmortem studies for the involvement of oligodendrocytes in the pathogenesis of schizophrenia. Morphometric studies of postmortem brains of schizophrenia subjects have consistently found a reduction in oligodendrocyte density in the grey and white matter of the prefrontal and parietal cortex that includes both perineuronal and pericapillary oligodendrocytes. We have also reported ultrastructural signs of degeneration of oligodendrocytes in the schizophrenia brain. Moreover, cell cycle abnormalities and incomplete differentiation of oligodendrocytes have also been reported in schizophrenia.

The adult mammalian brain contains a ubiquitous population of glial progenitors that can develop into mature oligodendrocytes. Oligodendrocytes are readily identified in Nissl stained sections, and they are situated as individual cells or grouped in small clusters (2-9 cells) in both the grey and white matter. Recent experimental data demonstrated that cell clusters in adult rodent and primate brain contain oligodendrocyte progenitors at different stages of maturation. Peters and Sethares also reported groups or rows (clusters) of oligodendrocytes in the frontal and visual cortices of old-aged monkeys and found a strong positive correlation between the number of oligodendrocytes per mm² and the percentage of them that were in clusters. The spatial distribution of oligodendrocytes in the white matter of prefrontal, Brodmann area 9, exhibited a less clustered arrangement in Nissl stained sections in schizophrenia. We hypothesized that the deficits of oligodendrocytes that have been reported in schizophrenia may be associated with the reduced number of OIC. We recently reported that the Nv of oligodendrocytes is reduced in the grey matter of the IPL (layer 3, BA39) in schizophrenia subjects with poor insight as compared to controls. In the present study we used the same collection of sections to determine: 1) whether there is a deficit of OIC in layer 3 of BA39 and BA40 in schizophrenia; 2) whether the Nv of OIC is associated with the Nv of oligodendrocytes in the IPL in the control and schizophrenia brains and 3) whether insight impacts the Nv of OIC.

Material and methods

Samples

Human brain specimens were donated by the Stanley Medical Research Institute’s “Parietal Collection”. The samples consisted of 48 subjects (24 controls and 24 with schizophrenia). Diagnosis was made according to DSM-IV criteria. A postmortem assessment of each person’s awareness of illness (insight) has been previously described. The mean age at the time of death was 44.3 ± 9.3 years for the control group and 39.8 ± 10.7 years for the schizophrenia group. The average postmortem interval (PMI) was 24.4 ± 10.8 hours for the control group and 29.1 ± 11.6 hours for the schizophrenia group. Fourteen cases were from the left hemisphere and 10 cases were from the right hemisphere. Complete demographic and clinical data were reported in the previous paper.

The brain specimens were coded, and all cytoarchitectural assessments were done blind to diagnosis. Tissue was available from one hemisphere of each brain. The angular gyrus (BA 39) and the supramarginal gyrus (BA 40) were identified according to macroscopic landmarks. Ten serial sections through the IPL (one section every 17th) were mounted on slides and Nissl-stained.
Stereological analysis

The sublayers of layer 3 (a, b and c) in BA 39 and BA 40 were readily identified. The Nv of OIC was estimated in BA 39 and BA 40 in each sublayer of layer 3 using an optical disector method. The sections were viewed on a Carl Zeiss Axio Imager M1 microscope with AxioVision microscope software. Section thickness was measured on slides and ranged from 14-16 μm. For each brain, the mean oligodendrocyte cluster density was corrected by a z-axis shrinkage factor. Original section thickness (60 μm) was divided by the average final thickness of the sections in each brain (mean ± S.D : 15.0 ± 1.2 μm for the control group and 14.9 ± 2.3 μm for the schizophrenia group).

Prior to the actual analysis, the optimal parameters for counting box size were determined. Disector dimensions were x = 55 μm, y = 55 μm, and z = 10 μm, with an upper and lower guard zones = 4 μm. Sections were examined using a 100 × 1.4 oil immersion objective. Oligodendrocytes were identified by the presence of a small round or oval nucleus, with relatively dense nuclear staining (more chromophilic than astroglial nuclei) and a narrow unstained rim of cytoplasm. The OIC was identified as pairs and groups of 3-9 oligodendrocytes closely apposed to each other and seen in 3D space (Fig. 1). 100 fields were counted per each sublayer of layer 3 per case.

Figure 1. A photomicrograph of Nissl stained section showing a cluster of 8 oligodendrocytes. Scale bar = 10 μm.
Statistical analyses

Statistical analysis was performed using Statistica (Version 7). The data were examined using the Kolmogorov-Smirnov test for normality. Correlation analysis was performed to assess correlations between the parameter measured and age, PMI, pH, refrigerator interval, brain weight, lifetime antipsychotics, age at onset and duration of disease. Comparisons between diagnostic groups were made using a two-way MANOVA with the Nv of OIC in three sublayers of layer 3 as the dependent variables, and diagnosis and hemispheres as the independent variables. A one-way MANOVA was used to compare the control group and three schizophrenia insight subgroups (poor, fair and good insight). MANOVA was followed by post hoc Duncan’s test.

Results

Numerical density of OIC

We found a decrease in the Nv of OIC in all sublayers of layer 3 in both BA 39 [22-25%, F(1,46) ≥ 7.5; p ≤ 0.01] and BA 40 [25-38%, F(1,46) ≥ 10.9; p<0.01] in the schizophrenia group as compared to the control group (Fig. 2). In BA 39, but not in BA 40, the Nv of OIC in the left hemisphere was greater than in the right hemisphere in all three sublayers in the control group (22-27%, p<0.05). The interhemispheric asymmetry was not apparent in the schizophrenia group in either BA 39 or BA 40 (Fig. 3). Previously, we estimated the Nv of oligodendrocytes using the same IPL sections7. A pearson’s correlation analysis demonstrated a significant positive correlation (r ≥ 0.6, p ≤ 0.002) between the Nv of OIC and the Nv of oligodendrocytes in all sublayers of BA40 in the control group but not in the schizophrenia group (Table 1).

Table 1
Correlations between Nv of oligodendrocyte clusters and Nv of oligodendrocytes for three sublayers of layer 3 in the control and schizophrenia groups

<table>
<thead>
<tr>
<th>Sublayer</th>
<th>BA 39</th>
<th></th>
<th></th>
<th>BA 40</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group (n = 24)</td>
<td>Schizophrenia group (n = 24)</td>
<td>Control group (n = 24)</td>
<td>Schizophrenia group (n = 24)</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>r = 0.17, p = 0.4</td>
<td>r = 0.14, p = 0.5</td>
<td>r = 0.6, p = 0.002*</td>
<td>r = 0.39, p = 0.06</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>r = 0.23, p = 0.3</td>
<td>r = 0.01, p = 0.96</td>
<td>r = 0.64, p = 0.0001*</td>
<td>r = 0.29, p = 0.17</td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>r = 0.03, p = 0.9</td>
<td>r = -0.2, p = 0.3</td>
<td>r = 0.66, p = 0.0001*</td>
<td>r = 0.48, p = 0.017*</td>
<td></td>
</tr>
</tbody>
</table>

r and p values for Pearson correlations.
* - significant correlations.
Figure 2. The numerical density of oligodendrocyte clusters in three sublayers of layer 3 in BA39 and BA40 in the control group and in the schizophrenia group (SCH). Data are given as mean ± standard deviation. p-values: ** p<0.01, *** p<0.001.

Figure 3. Hemispheric differences in the numerical density of oligodendrocyte clusters in three sublayers of layer 3 in BA39 in the control group are absent in the schizophrenia group. Data are given as mean ± standard deviation. p-values: * p<0.05, ** p<0.01.
Potential confounding factors

We found no effects of the confounding factors (age, PMI, refrigerator interval, brain weight, brain pH, lifetime antipsychotics) on the parameter measured in both areas studied.

Effects of insight

There was a significant effect of insight on the Nv of OIC in both BA39 and BA 40. In BA 39 the subgroup of subjects having poor insight had a significantly lower Nv of OIC in all three sublayers of layer 3 [23-33%, F(3,44) ≥ 3.0, p<0.05] compared to the control group (Fig. 4). There were no significant differences between the three insight subgroups. In BA40 a decrease in the Nv of OIC was found in all sublayers of layer 3 in the subgroups with fair insight (p<0.001) and good insight (p<0.05) compared to the control group [F (3,44) ≥ 5.8, 46-65%, 30-47% respectively]. The subgroup with fair insight differed significantly from the subgroup with poor insight (p<0.05 for all sublayers).

Effects of age at onset of disease

The duration of disease was not correlated with the Nv of OIC in BA 39 and BA 40 (for all p ≥ 0.2; r ≤ 0.27, Spearman correlation). However, there was a significant positive correlation between the Nv of OIC and age at onset of disease (r ≥ 0.4, p ≤ 0.05, Spearman correlation) in BA 40. Therefore we compared the subgroup of schizophrenia cases with adolescent onset of disease (9-18 y.o., n = 13) to the subgroup with adult onset of disease (19-34 y.o., n = 11) and to the control group. MANOVA showed a significant effect of age at onset of disease only in BA 40. The parameter was decreased significantly only in the subgroup with adolescent onset of disease [31-46% for all sublayers of layer 3, F (2,45) ≥ 6.4,
p<0.01] compared to the control group (Fig. 5). The subgroup with adult onset of disease did not differ significantly from the control group or from the subgroup with adolescent onset of disease. In BA 39 the Nv of OIC was reduced significantly in both subgroups as compared to the control group [22-27% for all sublayers, F (2,45) ≥ 3.7, p<0.05] (Fig. 5).

![Graph of Figure 5](image)

**Figure 5.** The numerical density of oligodendrocyte clusters in the control group and in schizophrenia (SCH) subgroups with adolescent and adult onset of disease in BA39 and in BA40. Data are given as mean ± standard deviation. p-values: * p<0.05, ** p<0.01.

**Discussion**

The present study is a continuation of our previous study using the same collection of IPL sections. We reported a reduced Nv of oligodendrocytes in layer 3 of BA39 but not of BA40 in schizophrenia. Here we demonstrate for the first time a deficit in the clustering of oligodendrocytes in layer 3 in BA39/40 of the IPL in the schizophrenia group compared to the control group. A reduction in the number of OIC in the grey matter of the IPL in schizophrenia corroborates the data of Hof et al. who showed that the spatial distribution of oligodendrocytes in the white matter of prefrontal cortex exhibited a less clustered arrangement in schizophrenia compared to healthy controls.

We also demonstrated that left>right hemispheric asymmetry of the Nv of OIC in BA39 in the control group was absent in the schizophrenia group. This result is in accordance with the altered asymmetry of oligodendrocyte density that we previously reported in BA39 in schizophrenia. This data also supports the MRI reports that show a reversal of
the left > right IPL asymmetry in first episode of schizophrenia and chronic schizophrenia that appears to be localized to the angular gyrus (see for review). Thus, our results support the hypothesis that schizophrenia is characterized by abnormal hemispheric asymmetry. Finally, we show that there is a strong positive correlation between the Nv of oligodendrocytes and the Nv of OlC in all sublayers of layer 3 in BA40 in the control group but not in the schizophrenia group. These data indicate that the Nv of OlC is associated with the Nv of oligodendrocytes in BA40 in the control but not in schizophrenia brains.

Pairs and groups of oligodendrocytes have been described in the primate cortex previously, and strong positive correlations have been shown between number of oligodendrocytes and the percent of them that are in pairs and groups. It is believed that these groups are derived from the division of oligodendrocytes progenitors which replenish the oligodendrocyte population. Recently a population of residual, mitotically competent oligodendrocyte progenitor cells that express NG2-antigen have been found in the adult brain of rodent, primate, and human. These cells are able to renew the population of mature myelinating oligodendrocytes. Clonal analysis combined with BdU injection revealed that mitotic division of the NG2 cells resulted in the formation of cell groups (~4 cells) that contained oligodendrocyte precursors. These cells shared a common lineage with oligodendrocytes and resemble oligodendrocytes morphologically making it difficult to distinguish them from oligodendrocytes. Taken together, this accumulated experimental data suggests that OlC in the human IPL may represent sites of oligodendrocyte precursor proliferation and differentiation.

The deficit of OlC that we find in the present study may be important for the etiology of schizophrenia because: 1) a correlation analysis did not reveal any effects of potential confounding factors (age, PMI, refrigerator interval, brain weight, brain pH, life time antipsychotic) on the Nv of OlC; 2) a decrease in the Nv of OlC was not due to the changes in laminar thickness, because Smiley et al. using the same IPL collection as used here did not detect any significant changes in laminar thickness or volume, or in neuronal size and density in BA39/40 in schizophrenia; 3) we found an effect of insight and of age at onset of disease on the Nv of OlC. Consistent with our previous data we find here that only the subgroup with poor insight showed a significant decrease ~25% in the Nv of OlC in BA39 compared to the control group. In contrast, in BA40 the Nv of OlC was decreased non-significantly in the subgroup with poor insight but was significantly lower in the subgroups with fair and good insight compared to the control group. A strong effect of the age at onset on the Nv of OlC may be a possible reason for this region discrepancy. In BA40 a highly significant decrease in the Nv of OlC was revealed only in the subgroup with early onset of disease (age 9-18 years). This effect can mask the effect of insight in BA40: the subgroup with good insight (9 cases) contained 4 subjects with the earliest onset of disease (9-14 years) and 2 subjects with onset of disease at 16 and 17 years. The subgroup with poor insight (10 cases) contained 6 subjects with age at onset of disease >18 years.

A prominent deficit of OlC in the subgroup with the early onset of disease may be a very important finding because early onset schizophrenia is a particularly severe form of schizophrenia and adolescence coincides with a key time point in myelination. The result is consistent with data from imaging studies in childhood onset schizophrenia that have reported a decrease in the volume and fractional anisotropy of white matter in the
parietal regions\textsuperscript{32}. Imaging studies have also linked insight in schizophrenia with impaired functioning\textsuperscript{33} and reduced grey matter volume of the IPL in the schizophrenia patients\textsuperscript{24}. Since IPL abnormalities have been implicated in both insight and onset of schizophrenia, it is likely that the reduced number of OIC detected in the present study may be directly associated with the disease. Moreover, our analysis did not reveal any effects of neuroleptic medication on the Nv of OIC. In fact neuroleptics have been shown to stimulate proliferation and development of oligodendrocyte progenitors\textsuperscript{34-36} rather than decrease them. However, oligodendrocyte precursors are sensitive to environmental stress signals such as oxidative stress, glutamate-relative excitotoxicity\textsuperscript{37} and persistent viral infection\textsuperscript{38} all of which have been implicated in the etiology of schizophrenia. Mature oligodendrocytes are much less sensitive to these stress signals.

As with most postmortem studies the present study has some limitations. First, Nissl staining is not appropriate method to count oligodendroglial progenitor cells. Immunohistochemical identification should be used to analyze abnormalities in oligodendroglial progenitors. Second, the sample sizes for the insight and onset subgroups are quite small. And so to more fully define the effects of insight and onset the results will have to be confirmed in a larger sample.

We hypothesize that the replenishing of the oligodendrocyte population that is necessary throughout life for normal neural function may be altered in schizophrenia. This suggestion supports our previous data showing that the normal age-related increase in the number of oligodendrocytes in the prefrontal cortex was absent in schizophrenia\textsuperscript{39}. These abnormalities may contribute to more profound abnormalities in the brain of patients with schizophrenia including abnormal expression of oligodendrocyte- and myelin-related genes, of genes that govern oligodendrocyte development and function, and of genes involved in the cell cycles.

**Authors’ contributions**

Dr. Kolomeets designed the study, carried out data collection and interpretation and wrote the manuscript. Dr. Vostrikov contributed to the study design, data collection and interpretation, preparation of the manuscript. Dr. Uranova designed the study, performed statistical analysis, wrote and revised the manuscript.

**Funding source**

This study was supported by the Stanley Medical Research Institute. Grant number: 07R-1787.

**Acknowledgments**

The authors would like to thank the Stanley Medical Research Institute for support this work. Postmortem brain sections were donated by Dr. M.J Webster from the Stanley “Parietal Collection”. We express our gratitude to Dr. E. Fuller Torrey for examination of clinical records and editing the manuscript and Dr. M.J. Webster for editing the manuscript.

**References**

1. Konrad A, Winterer G. Disturbed structural connectivity in schizophrenia primary factor in pathology or epiphenomenon? Schizophr Bull 2008; 34(1): 72-92.


39. Vostrikov VM, Uranova NA. Age-related increase in the number of oligodendrocytes is dysregulated in schizophrenia and mood disorders. Schizophr Res Treatment 2011; 2011: 174689.