Decreased Oligodendrocyte and Neuron Number in Anterior Hippocampal Areas and the Entire Hippocampus in Schizophrenia: A Stereological Postmortem Study

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The hippocampus is involved in cognition as well as emotion, with deficits in both domains consistently described in schizophrenia. Moreover, the whole volumes of both the anterior and posterior region have been reported to be decreased in schizophrenia patients. While fewer oligodendrocyte numbers in the left and right cornu ammonis CA4 subregion of the posterior part of the hippocampus have been reported, the aim of this stereological study was to investigate cell numbers in either the dentate gyrus (DG) or subregions of the anterior hippocampus. In this design-based stereological study of the anterior part of the hippocampus comparing 10 patients with schizophrenia to 10 age- and gender-matched healthy controls were examined. Patients showed a decreased number of oligodendrocytes in the left CA4, fewer neurons in the left DG and smaller volumes in both the left CA4 and DG, which correlated with oligodendrocyte and neuron numbers, respectively. When exploring the total hippocampus, keeping previously published own results from the posterior part of the same brains in mind, both decreased oligodendrocyte numbers in the left CA4 and reduced volume remained significant. The decreased oligodendrocyte number speaks for a deficit in myelination and connectivity in schizophrenia which may originate from disturbed maturational processes. The reduced neuron number of the DG in the anterior hippocampus may well point to a reduced capacity of this region to produce new neurons up to adulthood. Both mechanisms may be involved in cognitive dysfunction in schizophrenia patients.

Key words: schizophrenia/hippocampus/oligodendrocytes/neurons/dentate gyrus/stereology/postmortem

Introduction

In over 50% of patients schizophrenia is linked with an unfavorable outcome due to their inability to find employment and to keep up long-term relationships.1 This unfavorable outcome is connected to the so-called negative symptoms consisting of cognitive dysfunction and residual negative symptoms such as diminished expression of affect, and avolition including anhedonia and deficits in social interaction.2 Cognitive dysfunction and residual negative symptoms are resistant to any known treatment, as demonstrated in a recent meta-analysis focusing on the second aspect.3 Besides working memory, declarative memory deficits seem to contribute profoundly to the cognitive dysfunctions in schizophrenia. Interestingly, verbal memory deficits correlate with the loss of hippocampal volume, especially on the left side.4

The human hippocampus plays an important role in cognition and the regulation of affect. While the anterior part of the hippocampus is involved in regulation of emotions, its posterior part is important for cognitive function.5 Deficits in both domains are expressed in symptoms of schizophrenia. Recently, using design-based stereology, our group conducted a postmortem investigation of the cytoarchitecture of the posterior hippocampus in schizophrenia evaluating neuronal and glial numbers. Unexpectedly, we found a significant reduction of oligodendrocyte numbers in the left and right cornu ammonis CA4 subregion.6 It can be hypothesized that decreased oligodendrocyte numbers in CA4 is not specific for the posterior part of the hippocampus. It may also be detected in the anterior part and throughout the entire
Stereology in the Hippocampus in Schizophrenia

Besides the presence of an oligodendrocyte deficit, impaired synaptic plasticity supports the hypothesis of disturbed connectivity in schizophrenia. Furthermore, a reduced number of neurons in the dentate gyrus (DG) would back the hypothesis of decreased neurogenesis in schizophrenia. Using proliferation markers, a postmortem study in the DG of schizophrenia patients showed decreased stem cell proliferation. Its impact on neuronal number and volume of this subregion, however, remained unknown so far.

The aim of the outlined design-based stereological study was to ascertain whether our recent findings on neuron and glia numbers could also be found in the anterior part of the hippocampus of the same brains postmortem and examining whether they might be a feature of the entire hippocampus. Additionally, we evaluated cell numbers in the DG to search for evidence pointing to consequences of impaired neurogenesis.

Materials and Methods

Human Postmortem Brains

Postmortem brains were obtained from the Düsseldorf Brain Collection and the study has been approved by the Ethics Committee of Magdeburg University. Patients fulfilled ICD-9 criteria for schizophrenia. They had been treated with antipsychotics during most of their illness. Exclusion criteria were alcohol or drug abuse and other neuropsychiatric disorders. Brains from 10 patients with schizophrenia (mean age 55.1 ± 7.7 y [mean ± SD], 5 males, 5 females, postmortem interval [PMI] 42.0 ± 17.2 h, mean disease duration: 23.0 ± 7.9 y) and 10 age- and gender-matched healthy controls without a history of a neuropsychiatric disorder, alcohol or drug abuse, dementia, neurological illness, trauma or chronic terminal disease (mean age 50.2 ± 10.1 y, 5 males, 5 females, PMI 39.4 ± 19.4 h) were in toto uniformly fixed and 10% phosphate buffered paraformaldehyde for about 7 months (pH 7.0, t = 15–20°C; for further details see Schmitt et al).

The entire middle block of the brains containing the hippocampus was embedded in paraffin and cut serially into 20-μm whole-brain coronal sections. Every 50th section was Nissl (cresyl violet) and myelin (luxol fast-blue) stained, with an intersectional distance of 1 mm. Within the hippocampal formation, we evaluated the anterior part spanning from the anterior portion to the lateral geniculate nucleus. Due to partial tissue deterioration in the anterior hippocampus we opted to include control N126 in our analysis instead of control N23 from the posterior hippocampal area1 (table 1). Therefore, except one control we analyzed the same cases as published previously by our group, allowing to summarize the results from the anterior and posterior part of the hippocampus of the same brains to obtain volumes and cell numbers of the total hippocampus. Subregions to be investigated separately were the DG, CA1, 2–3 and 4 (deep polymorph layer of the DG), and subiculum. CA2 and CA3 were lumped together according to the literature as these small regions are difficult to separate on the microscopic level. We further differentiated between neurons, oligodendroglia, and astroglia based on histological and morphological criteria in our Nissl stained sections (for further details Schmitt et al).

Stereological Analyses

All stereological analyses were performed with the raters (K.W. for the anterior hippocampus and V.N. for the DG) blind to diagnosis, using a stereologic workstation, consisting of a modified light microscope (BX50; Olympus). Boundaries of hippocampal subfields were traced on video images displayed on the computer screen and total volumes were calculated according to Cavalieri’s principle using on average 9.4 ± 2.0 sections in controls and 8.1 ± 2.0 sections in patients with schizophrenia. Total number of cells was estimated using the optional

Table 1. List of Healthy Controls and Patients With Schizophrenia Investigated in the Present Study of the Anterior Hippocampus

<table>
<thead>
<tr>
<th>Schizophrenia Patients</th>
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<th>Healthy Controls</th>
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<tbody>
<tr>
<td>No.</td>
<td>S</td>
<td>A</td>
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<tr>
<td>P3</td>
<td>M</td>
<td>48</td>
</tr>
<tr>
<td>P9</td>
<td>M</td>
<td>65</td>
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<tr>
<td>P10</td>
<td>M</td>
<td>46</td>
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<tr>
<td>P14</td>
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<td>51</td>
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<td>P20</td>
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<td>P13</td>
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<td>60</td>
</tr>
<tr>
<td>P18</td>
<td>F</td>
<td>63</td>
</tr>
<tr>
<td>P23</td>
<td>F</td>
<td>52</td>
</tr>
</tbody>
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Note: A, age at death (y); D, duration of disease; S, gender (M = male, F = female).
fractionator as described before. The predicted coefficient of error (CE) of the total number of neurons, oligodendrocytes and astrocytes was calculated and details of the stereological counting procedures are described in Table 2. For the intra-rater reliability the intra-class correlation coefficients were 0.909 for neurons, 0.889 for oligodendrocytes, 0.586 for astrocytes, and 0.853 for volume of subregions.

**Statistical Analysis**

Significance level was \( \alpha = .05 \). All tests were 2-tailed. Statistical analyses were performed with IBM SPSS statistics 22. Outcome measures were oligodendrocyte, astrocyte and neuron numbers as well as structure volumes in the anterior and in the total hippocampus (CA1, CA2/3, CA4, DG, and subiculum), with diagnostic group (control subjects, schizophrenia patients) as independent factor. Means, SDs and standard errors of the mean were calculated for all outcome measures separating left and right hemispheres. Neurons, oligodendrocyte and astrocyte cell numbers such as structure volumes were correlated with age at death, PMI and disease duration by means of bivariate product moment correlations for the total sample and—in case of significance—separately for schizophrenia patients and controls.

Comparisons between schizophrenia patients and controls were performed using multivariate analysis of covariance (MANCOVA) with diagnosis and gender as between-subject factors, hemisphere as within-subject factor and with covariate age at death. Analyses were adjusted for PMI and disease duration if they showed a significant impact in the initial correlative calculations (compare Schmitt et al). In case of significant interactions between diagnosis and hemisphere, subsequent group comparisons were performed separately for the left and right hemisphere.

Due to the explorative character of the study results are presented without error probability correction. If a Bonferroni adjustment of the type I error probability was applied, no significant differences between schizophrenia patients and controls would remain. However, with an adjustment of the error probability the power of detecting existing mean differences would be too low.

**Results**

In the anterior hippocampus, MANCOVA showed significant effects of diagnosis \( \times \) hemisphere \( (F_{(1,15)} = 19.4, P = .0005) \) on the mean number of oligodendrocytes in CA4, on mean number of neurons in CA4 \( (F_{(1,15)} = 6.2, P = .025) \) and in the DG \( (F_{(1,15)} = 6.1, P = .026) \) and on volume of the substructures CA4 \( (F_{(1,15)} = 6.6, P = .021) \) and DG \( (F_{(1,15)} = 6.05, P = .027) \). Using subsequent univariate tests, we detected a significant reduction in oligodendrocyte numbers in CA4 on the left hemisphere \( (−32.2\%, \quad F_{(1,15)} = 11.3, P = .004) \) in schizophrenia patients \((0.56 ± 0.25 million [m]) compared to healthy controls \((0.83 ± 0.32 m)\) as well as a significant reduction in the number of neurons in the DG on the left side \( (−29\%, \quad F_{(1,15)} = 10.22, P = .006) \) in the patient group \((4.26 ± 1.21 \text{ m}) \) compared to controls \((6.0 ± 1.6 \text{ m}) \). In schizophrenia patients the volume of the substructure was decreased in the left CA4 \( (−35.9\%, \quad F_{(1,15)} = 6.37, P = .023) \) and left DG \( (−16.6\%, \quad F_{(1,15)} = 4.58, P = .049) \) in schizophrenia patients \((46.9 ± 16.9 \text{ mm}^3, \quad \text{DG: } 19.1 ± 5.9 \text{ mm}^3) \) compared to healthy controls \((46.5 ± 20.2 \text{ mm}^3, \quad \text{DG: } 23.4 ± 5.6 \text{ mm}^3) \).

No significant differences between patients and non-psychiatric controls were found with respect to the mean numbers of neurons and oligodendrocytes in the other subregions.

<table>
<thead>
<tr>
<th>Table 2. Details of the Stereologic Counting Procedures</th>
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<tr>
<td>Subiculum</td>
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<td>Obj.</td>
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<tr>
<td>B (μm²)</td>
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<tr>
<td>H (μm)</td>
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<tr>
<td>D (μm)</td>
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<tr>
<td>ΣCS</td>
</tr>
<tr>
<td>ΣQ⁺ neurons</td>
</tr>
<tr>
<td>ΣQ⁺ astrocytes</td>
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<tr>
<td>ΣQ⁺ oligodendrocytes</td>
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<tr>
<td>CEpred (neurons)</td>
</tr>
<tr>
<td>CEpred (astrocytes)</td>
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<tr>
<td>CEpred (oligodendrocytes)</td>
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</tbody>
</table>

Note: Obj, objective used; B and H, base and height of the unbiased virtual counting spaces; D, distance between the unbiased virtual counting spaces in mutually orthogonal directions x and y; ΣCS, average sum of unbiased virtual counting spaces used in one hemisphere of one proband; ΣQ⁺ neurons, average number of counted neurons in one hemisphere of one proband; ΣQ⁺ astrocytes, average number of counted astrocytes in one hemisphere of one proband; CEpred, average predicted coefficient of error of estimated cell numbers; Oligodendrocytes, oligodendrocytes; DG, dentate gyrus; CA, Cornu Ammonis.
Fig. 1. (I) Left column (a)–(e): Number of oligodendrocytes ($\times 10^5$) in schizophrenia (SZ) and control subjects in anterior hippocampus dentate gyrus (DG, [a]), CA 4 (b), CA 2/3 (c), CA 1 (d), and subiculum (Sub, [e]). Bars represent mean oligodendrocyte number ± standard error of the mean. Light grey bars: left hemisphere, dark grey bars: right hemisphere. Decreased oligodendrocyte number in SZ compared to controls was observed in CA 4, left side ($P = .004$). (II) Medium column (f)–(j): Number of neurons ($\times 10^5$) in SZ and control subjects in anterior hippocampus DG (f), CA 4 (g), CA 2/3 (h), CA 1 (i), and Sub (j). Light grey bars: left hemisphere, dark grey bars: right hemisphere. Bars represent mean ± standard error of the mean. Decreased neuron number in SZ compared to controls was observed in DG, left side ([f], $P = .006$). (III) Right column (k)–(o): Structure volume (mm$^3$) in SZ and control subjects in anterior hippocampus in DG (k), CA 4 (l), CA 2/3 (m), CA 1 (n), and Sub (o). Light grey bars: left hemisphere, dark grey bars: right hemisphere. Bars represent mean ± standard error of the mean. Decreased structure volume in SZ compared to controls was observed in DG, left side ([k], $P < .05$) and in CA 4, left side ([l], $P = .02$). *$P < .05$, **$P < .01$. 

* $P < .05$, ** $P < .01$. 

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investigated subregions of the anterior section of the hippocampus. Additionally, astrocyte numbers did not differ between patients and controls.

In our calculation of data from the total hippocampus, including findings for the anterior region and previously published results from the posterior part of the same brains, MANCOVA revealed effects of diagnosis × hemisphere on oligodendrocyte numbers in CA4 \((F_{(1,14)} = 11.9, P = .004\), on number of neurons in CA4 \((F_{(1,14)} = 6.6, P = .022\), and on volume of the substructure in CA4 \((F_{(1,14)} = 6.2, P = .026\) and DG \((F_{(1,13)} = 8.6, P = .011\)). Effects of diagnosis could be shown on the number of oligodendrocytes in CA4 \((F_{(1,14)} = 9.7, P = .008\) and on number of neurons in the subiculum \((F_{(1,13)} = 5.1, P = .043\)). Univariate ANCOVA revealed reduced oligodendrocyte numbers in the left CA4 \((-31.8\%, F_{(1,14)} = 17.51, P = .001\) of schizophrenia patients \((1.07 \pm 0.33 \text{ m})\) compared to healthy controls \((1.58 \pm 0.42 \text{ m})\). Additionally, the number of neurons was decreased in the left subiculum \((-24.2\%, F_{(1,13)} = 7.94, P = .015\) of schizophrenia patients \((7.77 \pm 2.24 \text{ m})\) compared to healthy controls \((10.26 \pm 2.11 \text{ m})\) while there was only a trend for reduced neuron numbers in the left DG \((F_{(1,13)} = 3.7, P = .077\); patients: \(9.50 \pm 3.22 \text{ m}\), controls: \(12.46 \pm 3.48 \text{ m}\); figure 2). It has to be noted, that in the posterior DG region of the hippocampus, no significant alterations in neuron numbers have been detected in the patient group. Compared to controls \((107.7 \pm 29.3 \text{ mm}^3)\), schizophrenia patients \((82.8 \pm 28.0 \text{ mm}^3)\) showed a significant reduction in the volume of the left CA4 \((-23.1\%, F_{(1,14)} = 5.85, P = .030\); figure 2). In all other subregions of the total hippocampus no differences were detected. As in the anterior part of the hippocampus, the number of astrocytes did not differ between patients and controls.

Interestingly, in the anterior hippocampus, the number of oligodendrocytes correlated with the volume of the CA4 substructure in the left \((r = .668, P = .001\) and right \((r = .715, P < .0005)\) hemisphere. In the left \((r = .774, P < .0005)\) and right \((r = .884, P < .0005)\) DG, neuron numbers correlated with the volume of this substructure. In the total hippocampus, the volume of the left \((r = .748, P < .0005)\) and right \((r = .724, P < .0005)\) CA4 correlated with oligodendrocyte numbers.

In the anterior hippocampus, ANOVA revealed effects of gender on neuron number in the right CA4 \((F_{(1,18)} = 9.92, P = .006)\) and volume of the right CA4 \((F_{(1,18)} = 12.12, P = .003)\) with females showing smaller cell number and volumes compared to males. For the entire sample, in left CA1 age correlated with the number of oligodendrocytes \((r = .598, P = .005)\), most evident in schizophrenia patients: \(r = .766, P = .010\) and in the left subiculum, age correlated with number of astrocytes \((r = .500, P = .025)\) and oligodendrocytes \((r = .501, P = .024)\), most pronounced in schizophrenia patients: \(r = .660, P = .038\), while in the right subiculum, age correlated with number of oligodendrocytes \((r = .502, P = .024)\). In the left DG the number of neurons correlated with age only in schizophrenia patients \((r = .654, P = .010)\). In the patient group, duration of disease correlated with astrocyte number in the right \((r = .760, P = .011)\) subiculum. In the anterior hippocampus, the PMI had no influence on the results. In the total hippocampus, ANOVA revealed effects of gender on astrocyte number \((F_{(1,15)} = 5.45, P = .033)\) neuron number \((F_{(1,13)} = 11.17, P = .004)\) and volume \((F_{(1,17)} = 10.78, P = .004)\) of the right CA4 with females showing smaller cell numbers and volumes compared to males. For the entire sample, age correlated with total number of astrocytes in right CA1 \((r = .462, P < .05)\) and in subiculum \((left: r = .663, P = .003, subgroup analysis significant for controls: r = .795, P = .011; right: r = .746, P < .001, controls: r = .771, P = .015, schizophrenia patients: r = .713, P = .021). PMI correlated with neuron number \((r = -.539, P = .017)\) and volume \((r = -.510, P = .026)\) in the left CA1 and with astrocyte number in left CA4 \((r = -.490, P = .033)\), and disease duration correlated with neuron number in the left \((r = .671, P = .048)\) and right \((r = .853, P = .002)\) subiculum.

**Discussion**

In our recent postmortem investigation of the posterior section of the hippocampus in schizophrenia, we found a significant bilateral reduction of oligodendrocytes in the CA4 region. In the present study, with exception of one control we examined the anterior part of the hippocampus in almost the same cases and were able to confirm a reduction of oligodendrocyte numbers, but only for the left CA4 region. Combining the results from both the anterior and posterior hippocampus, decreased oligodendrocyte numbers in the left CA4 were confirmed throughout the total formation and seem not to be specific for the anterior or posterior region. This is relevant for validation of our results, because the border between anterior and posterior area had been defined using the external landmark lateral geniculate nucleus and the volume of this region may differ between patients and controls, possibly confounding our results.

The CA4 region is also known as polymorph layer of the DG and is among the regions suggested to be involved in disturbed connectivity in schizophrenia. Axonal fibers of the fornix originate in pyramidal neurons in CA4, connecting the hippocampus with the mammillary bodies, septal nuclei, thalamus, and finally medial prefrontal cortex. In schizophrenia patients, a recent diffusion tensor imaging (DTI) study of the fornix showed reduced fractional anisotropy and radial diffusivity, reflecting disturbed myelination and axonal integrity. A decreased number of oligodendrocytes in CA4 of schizophrenia patients may be associated with abnormal myelination and disturbed maturation or proliferation of these glial cells resulting from impaired neurodevelopmental processes or myelination in young adulthood.
Fig. 2. (I) Left column (a)–(e): Number of oligodendrocytes ($\times 10^5$) in schizophrenia (SZ) and control subjects in total hippocampus (anterior + posterior): dentate gyrus (DG, [a]), CA 4 (b), CA 2/3 (c), CA 1 (d), and subiculum (Sub, [e]). Bars represent mean oligodendrocyte number ± standard error of the mean. Light grey bars: left hemisphere, dark grey bars: right hemisphere. Decreased oligodendrocyte number in SZ compared to controls was observed in CA 4, left side ($P = .001$). (II) Medium column (f)–(j): Number of neurons ($\times 10^5$) in SZ and control subjects in total (anterior + posterior) hippocampus: DG (f), CA 4 (g), CA 2/3 (h), CA 1 (i), and Sub (j). Light grey bars: left hemisphere, dark grey bars: right hemisphere. Bars represent mean ± standard error of the mean. Decreased neuron number in SZ compared to controls was observed in the Sub, left side ([j], $P = .015$). (III) Right column (k)–(o): Structure volume (mm$^3$) in SZ and control subjects in total (anterior + posterior) hippocampus in DG (k), CA 4 (l), CA 2/3 (m), CA 1 (n), and Sub (o). Light grey bars: left hemisphere, dark grey bars: right hemisphere. Bars represent mean ± standard error of the mean. Decreased structure volume in SZ compared to controls was observed in CA 4, left side ([l], $P = .03$). *$P < .05$, **$P < .01$. 
Oligodendrocyte death or dysfunction have been hypothesized to influence signal flow and processing of information. Moreover, disturbed oligodendrocyte-axonal interaction may induce trophic deficits on neurons and their axons and these alterations may contribute to cognitive deficits in schizophrenia. Oligodendrocyte gene variants have been reported to influence cognitive performance and white matter integrity in chronic schizophrenia patients. Furthermore, in an animal model, disruption of neuregulin-1 signaling induced cognitive deficits and delayed oligodendrocyte development, indicating the influence of oligodendrocyte-related genetic risk variants on cognition. In fact, DTI studies have linked deficits in verbal declarative memory with decreased left-side dominant fractional anisotropy in the hippocampus and fornix of schizophrenia patients, supporting our finding of a left-sided deficit in the oligodendrocyte population. In line with our findings, electron microscopy revealed atrophy of axons and swelling of peri-axonal oligodendrocyte processes. Moreover, in microarray studies, decreased expression of oligodendrocyte-related proteins has consistently been reported in the hippocampus of schizophrenia patients.

A structural magnetic resonance imaging study investigating subfields of the hippocampus in schizophrenia reported decreased volume of the CA4/DG region. In our postmortem study we found volumes of both left-hemispheric subregions to be decreased, correlating to either oligodendrocyte number in CA4 or neuron number in the DG and reflecting the impact of cellular deficits on brain structure. In the left DG of the anterior hippocampus, we found decreased neuron numbers, possibly resulting from decreased neurogenesis during neurodevelopment or adulthood. In postmortem studies, decreased cell proliferation has been found in the DG of schizophrenia patients, especially in the anterior section, and decreased neurogenesis may contribute to cognitive deficits in schizophrenia, ranging from working memory to pattern separation. In schizophrenia, deficits in pattern separation and declarative memory have been hypothesized to result in psychotic symptoms. They may be related to dysfunction of the DG and a failure in the glutamate-dependent plasticity of the DG-CA3 part of the trisynaptic pathway.

In the total hippocampus of schizophrenia patients, we found decreased neuron numbers only in the left subiculum. In schizophrenia patients this subregion has also been shown to be smaller. The subiculum constitutes the main output structure of the hippocampus and is connected to the neocortex, entorhinal cortex and subcortical nuclei. Decreased neuron numbers, contributing to disturbed connectivity, may be the result of deficits in the neurodevelopmental period, although a subtle neurodegenerative process cannot be excluded. However, as in the posterior section of the hippocampus, in our design-based stereological study did not reveal astrocytosis as a sign of classical neurodegeneration in schizophrenia.

As a limitation, we evaluated only the total number of neurons and we did not separate pyramidal neurons from interneurons due to overlapping morphological criteria. In an immunohistochemical study of the hippocampus in schizophrenia, somastostatin- and parvalbumin-positive interneurons have been reported to be reduced in CA4 and CA1 with no changes in overall neuron number. Additionally, effects of antipsychotic treatment cannot be excluded, because all patients had been treated with typical neuroleptics over long periods.

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In summary, using a design-based stereological approach, we found a decreased number of oligodendrocytes in the left CA4 along with reduced volume of this subregion of the anterior and total hippocampus. In
addition, we detected smaller volumes of the left DG and decreased number of neurons in this subregion of the anterior hippocampus and decreased neuron numbers in the left subiculum of the total hippocampus. Our results point to both disturbed hippocampal connectivity and impaired neurogenesis in chronic schizophrenia, possibly resulting in cognitive deficits. These findings impact on the development of future innovative treatment strategies targeting the function of cellular subfractions in the hippocampus.

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References


