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RESEARCH ARTICLE

Reduced Number of Oligodendrocytes in the Cingulum in Schizophrenia: A Design-Based Stereology Study

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Abstract

Schizophrenia is a severe mental disorder with a neurodevelopmental origin whose pathophysiological processes are poorly understood. Magnetic resonance imaging studies have shown structural alterations, such as volume loss in the temporal lobe, including the hippocampus and amygdala, thalamus, and frontal and temporal cortex, while other studies have found reduced oligodendrocyte numbers in the hippocampal CA4 subregion and dorsolateral prefrontal cortex. The decreased number of oligodendrocytes in CA4 was reported to be related to cognitive deficits. The hippocampus and other parts of the limbic system are connected to the white matter of the cingulum bundle, but it had remained unknown whether schizophrenia affects the number of oligodendrocytes also in the cingulum.

In a post mortem study, we applied a design-based stereological approach to investigate the number and density of oligodendrocytes in gallocyanin-stained serial sections in the cingulum of the left and right hemispheres in 12 brains from male donors with schizophrenia and 11 brains from age- and sex-matched controls without mental illness. In addition, we evaluated whether oligodendrocyte numbers and densities in the cingulum correlated with the corresponding data in the hippocampal CA4 subregion of the same brains.

In schizophrenia, both the mean volume of the left cingulum (difference, -12.7%) and the mean total oligodendrocyte number in the left cingulum (difference, -19.9%) were statistically significantly lower than in healthy controls ($p = 0.049$ and $p = 0.037$). No correlations were found for covariates (age, post mortem interval, fixation time). The individual oligodendrocyte numbers in the right cingulum correlated with those in the right hippocampal CA4, and the individual oligodendrocyte densities in the left cingulum correlated with that in the left hippocampal CA4.

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This post mortem study shows, for the first time, a statistically significant reduction in the mean volume of and the mean oligodendrocyte number in the left cingulum of patients with schizophrenia. Our results support findings of a reduction of oligodendrocytes in limbic regions in schizophrenia, reinforcing the hypothesis that deficits in myelination and trophic support of long projecting axons play a role in the functional disconnectivity of the hippocampus and prefrontal cortex in schizophrenia..

Introduction

Schizophrenia (SZ) is a severe mental disorder with a worldwide prevalence of about 1% [1]. The first acute episode of SZ usually manifests itself between the ages of 18 and 35 and is often already noticeable during school or vocational training because it causes a drop in performance and social withdrawal [2]. About half of the patients experience incomplete remission of symptoms, with residual negative symptoms (e.g., avolition, apathy, lack of drive and affective flattening) and cognitive deficits (e.g., attention, working memory, executive function, episodic memory and social cognition) [3-5]. These long-lasting symptoms cause difficulties in coping with social and professional life and result in unemployment or limited earning capacity in about 70% of patients [6]. According to the Global Burden of Disease Study, SZ is one of the 25 diseases worldwide with the highest number of disability-impaired life years in the age group of 25 to 49 years [7]. At the same time, SZ causes high costs to society and health care systems because of its early onset and often chronic course [8].

The pathophysiological mechanisms of SZ are poorly understood. The neurodevelopmental hypothesis, which is based on genome-wide association and epidemiological studies, proposes that SZ is associated with interacting genetic and environmental risk factors, such as maternal infection, obstetric and birth complications, and childhood trauma, which influence connectivity in neuronal circuits during vulnerable brain developmental periods [9]. These risk factors induce disturbances of macro- and microconnectivity in neuronal networks in the gray and white matter. Meta-analyses of Magnetic Resonance Imaging (MRI) studies have shown structural alterations, such as volume reductions in the temporal lobe, including the hippocampus and amygdala, thalamus, prefrontal including the anterior cingulate cortex and cingulum [10-12]. Voxel-Based Morphometry (VBM) and Diffusion Tensor Imaging (DTI) are two distinct methodologies for brain structural investigation, suitable for detecting different aspects of white matter alterations in neuropsychiatric disorders. In a meta-analysis of VBM and DTI studies focusing on white matter alterations in patients with SZ compared to healthy controls, VBM studies showed volume reductions in several regions including the bilateral cingulum, anterior commissure, corpus callosum, fornix, internal capsule, right anterior segment of Arcuate Fasciculus (AF), left cortico-ponto-cerebellum tract, right superior cerebellar penduculus, bilateral AF, bilateral corticospinal tract, bilateral Inferior Fronto-Occipital Fasciculus (IFOF), bilateral Inferior Longitudinal Fasciculus (ILF), bilateral inferior cerebellar penduculus, bilateral optic radiation, bilateral posterior segment of AF, bilateral first part of Superior Longitudinal Fasciculus (SLF1), bilateral central part of SLF (SLF2), bilateral final part of SFL (SLF3) and the bilateral Uncinate Fasciculus (UF) [13]. Furthermore, DTI studies showed white matter microstructure reductions in the bilateral cingulum as well, together with reductions in the anterior commissure, corpus callosum, fornix, internal capsule, left anterior segment of AF, left long segment of AF, bilateral AF, bilateral cingulum, bilateral cortico-ponto-cerebellum tract, bilateral cortico-spinal tract, bilateral IFOF, bilateral ILF, bilateral inferior cerebellar penduculus, bilateral optic radiation, bilateral posterior segment of AF, bilateral SLF1, bilateral SFL2, bilateral SLF3, bilateral superior cerebellar penduculus and the bilateral uncinate fasciculus [13]. The conjunction analysis from VBM and DTI studies found no overlapping clusters on the same white matter tract, indicating that DTI and VBM actually detect different aspects of white matter alterations [13]. Diffusion Tensor Imaging (DTI) studies revealed impaired white matter integrity, such as lower Fractional Anisotropy (FA) in white matter fiber tracts connecting these brain regions, and these findings point to deficits in myelination of fiber tracts [14,15]. A meta-analysis of DTI studies across 2937 individuals with four psychiatric disorders and healthy controls identified lower FA in patients with SZ in the anterior corona radiata, corpus callosum, fornix and cingulum [16]. A recent meta-analysis compared SZ and bipolar patients and showed disorder-specific decreased FA in the left cingulum in SZ [17].

The cingulum is a white matter bundle located between the cingulate cortex and the dorsal surface of the corpus callosum that reaches up to the temporal lobe [18]. It connects the anterior thalamic nucleus, cingulate gyrus and parahippocampal region, and plays a role in emotional and social cognition, both of which are impaired in SZ [19-21]. In SZ, speed of processing, attention, executive function and social cognition were found to correlate with disrupted white matter integrity in the cingulum bundle, particularly in the left hemisphere [22-26]. Moreover, patients with delusions of control have lower FA in the cingulum than patients without such delusions [27]. A 7 T magnetization transfer study confirmed aberrant myelination of the cingulum in patients with such delusions (Schneiderian delusions) [28]. Lower functional connectivity during rest and working memory tasks was detected in the cingulum around the cingulate cortex and hippocampus of patients with SZ and correlated with cognitive performance [29].

The cingulum contains many oligodendrocytes, which generate myelin sheaths around neuronal axons. They provide trophic support to the axons [30] and improve axonal conduction velocity [31], thus contributing to proper functional connectivity. Within the limbic system, design-based stereological studies revealed reduced mean oligodendrocyte numbers in the hippocampal CA4 subregion in SZ [32,33], a finding that was replicated in an independent sample [34]. The reduced oligodendrocyte number in the CA4 subregion was more pronounced in patients with definite cognitive deficits [35,36]. In SZ, the number of oligodendrocytes was also reported to be decreased in the white matter directly subjacent to the Dorsolateral Prefrontal Cortex (DLPFC) [37]. In at least a subgroup of patients with SZ, the number of oligodendrocytes in the CA4 subregion and DLPFC may be lower because of deficits in oligodendrocyte maturation and differentiation of oligodendrocyte precursor cells, and these changes may be related to cognitive deficits [38]. However, a more widespread reduction of oligodendrocytes in limbic regions may underlie the impaired connectivity in SZ, and to date, it remains unknown whether patients with SZ have a loss of oligodendrocytes also in the white matter of the cingulum.

In view of these changes in white matter integrity in the cingulum and its association with cognitive deficits predominantly in the left hemisphere, we tested (using design-based stereology) the hypothesis that mean oligodendrocyte numbers and densities are decreased in the cingulum in SZ, and, if so, that the reductions are more pronounced in the left hemisphere.

Materials and Methods

Post mortem brains

The autopsied brain tissue examined in this study was provided by the Heinsen Collection (University of Wuerzburg, Wuerzburg, Germany), which is managed by H.H. The further use of the brains for scientific purposes was approved by the ethics committee of the University of Wuerzburg.

This study evaluated both hemispheres of 12 post mortem brains from multi-episode male patients with SZ and poor outcome (participant codes, S1-S12; age range, 22 to 63 years; mean age, 50.5 years; mean Post Mortem Interval [PMI], 38.1 hours). As a control group, we used 11 post mortem brains of age-matched men who had not had a mental disorder (participant codes, C2-C13; age range, 36 to 65 years; mean age, 54.5 years; mean PMI, 23.5 hours). Participants' clinical characteristics, i.e., age at death, age at disease onset, cause of death, PMI, fixation duration and clinical diagnosis (for patients with SZ only) are listed in table 1. All patients with SZ fulfilled the diagnostic criteria of the Diagnostic Statistical Manual, 4th revision, and International Statistical Classification of Diseases and Related Health Problems, 10th revision. They had been treated in German clinical facilities (state psychiatric hospitals and local district hospitals) and had all received treatment with "typical" antipsychotics for decades. However, we were unable to calculate lifetime medication exposure because most of the patients were not hospitalized for the entire duration of their disease, so we did not have documentation on their entire medication history. Both groups had a comparable, white European ethnic background.

Exclusion criteria for both groups were neurological diseases that would have required treatment or resulted in cognitive impairment (e.g., apoplexy with aphasia), known history of seizure disorders (e.g., epilepsy), severe head injury with loss of consciousness, diabetes mellitus with poor glycemic control (free plasma glucose > 200 mg/dl), and alcohol and drug intoxications. On the other hand, head trauma could not be excluded in participants who had died by suicide. Brains were examined for neuropathologies to rule out tumors,

Table 1: Clinical characteristics of male patients with schizophrenia and controls.

No.	A	O	Cause of death	PMI	Fix	Diagnosis	
	y	y		h	d	DSM-IV	ICD-10
S1	22	19	Suicide by jumping from a high building	88	130	295.30	F20.00
S2	36	28	Suicide by strangulation	< 72	115	295.30	F20.00
S3	46	24	Systemic hypothermia	< 24	327	295.30	F20.01
S4	50	17	Peritonitis	< 24	203	295.30	F20.00
S5	50	22	Suicide by strangulation	18	170	295.30	F20.00
S6	51	17	Septicemia	33	127	295.60	F20.50
S7	54	20	Septicemia	27	250	295.60	F20.50
S8	55	22	Right-sided heart failure	25	84	295.30	F20.00
S9	57	37	Septicemia	76	163	295.30	F20.00
S10	60	24	Pulmonary embolism	< 48	311	295.30	F20.01
S11	62	19	Aspiration	7	171	295.30	F20.00
S12	63	22	Acute myocardial infarction	15	338	295.60	F20.50
C2	36	-	Gunshot	24	143	-	-
C3	47	-	Acute myocardial infarction	< 24	133	-	-
C5	50	-	Avalanche accident	23	498	-	-
C6	51	-	Septicemia	7	285	-	-
C7	54	-	Acute myocardial infarction	18	168	-	-
C8	56	-	Acute myocardial infarction	60	3570	-	-
C9	58	-	Acute myocardial infarction	28	126	-	-
C10	60	-	Gastrointestinal hemorrhage	18	101	-	-
C11	60	-	Gastrointestinal hemorrhage	27	302	-	-
C12	62	-	Acute myocardial infarction	< 24	3696	-	-
C13	65	-	Bronchopneumonia	6	2289	-	-

S: Patient with schizophrenia; C: Age-matched control; A: Age at death; O: Age at disease onset; PMI: Postmortem Interval (i.e., the time between death and autopsy); Fix: Fixation time.

infarcts, heterotopias, evidence of autolysis, staining artifacts and gliosis.

Tissue processing

All tissues were fixed and processed in the same way at the Morphological Brain Research Unit (University of Wuerzburg, Wuerzburg, Germany). For detailed information, Kreczmanski P, et al. [39], who investigated the same post mortem brains in previous studies. To prepare the histological specimens, the brainstem, including the cerebellum, was first separated at the level of the rostral pons, and the hemispheres were separated medio-sagittally. The separated hemispheres were then fixed in 4% formalin solution for at least three months. After being pretreated with glycerol-dimethylsulfoxidformalin and embedded in gelatin, the hemispheres were deep frozen in isopentane at -60 °C. Series of frontal/coronal sections with a thickness of 600 to 700 µm were prepared with a cryomicrotome (Jung, Nussloch, Germany; Heinsen H. [40] for further details). One control brain (participant C7) was embedded in celloidin [41] and sectioned into a series of 440-µm thick coronal sections with a sled microtome (Polycut, Cambridge Instruments, UK). Every second to third section of each brain was stained with gallocyanin, a Nissl stain [40], and the remaining sections were placed in formalin and stored for further use. One section of the brain of each participant aged over 40 years was not stained with gallocyanin and was processed through the central parts of the entorhinal and transentorhinal cortex by the Gallyas method to detect neurofibrillary changes [42]. In addition, each section was coded and tumors, infarcts, heterotopia, signs of autolysis, staining artifacts and gliosis were excluded. This procedure took place under the same conditions for all brains (except for sections from control group participant C7, which were embedded in celloidin instead of gelatin). The mean (SD) numbers of whole-brain sections used were as follows: patients

with SZ, 20.75 (0.92); controls, 21.55 (1.62). The whole cingulum was analyzed in both hemispheres (Figure 1).

Design-based stereological analyses

We used histological and morphological criteria to identify oligodendrocytes as small, dark cells [34] in photomicrographs of histologically stained cells using a 40x objective (Figure 2). Mature oligodendroglia showed no staining in the cytoplasm and no nucleolus but did show pronounced nuclear staining with scattered chromatin. We also assessed immature oligodendrocytes which are larger and more pale than mature ones [43]. Oligodendrocytes could be distinguished from astrocytes because the latter show less dense staining [34] (Figure 2(a,b)). All stereological examinations were performed with a light microscope (Zeiss Axio Imager.M2 Microscope; Carl Zeiss Microscopy GmbH, Jena, Germany); Zeiss Objectives (1,25×; 40×); a stage controller; and software (Stereo Investigator 2018.2.2 64 bit; MBF Bioscience, Williston, VT, USA). The delineation of the cingulum (Figure 1) was performed using a 1.25x objective. The volume of the cingulum was determined according to the Cavalieri principle [44], and the total number of oligodendrocytes was determined using the optical fractionator method [45]. We used the prediction methods described by Schmitz [46] and Schmitz and Hof [47] to calculate the predicted coefficient of error in the estimates of total numbers of oligodendrocytes. In all examinations of the sections, the investigator was blinded to participant group. The details of the stereological procedures for counting oligodendrocytes are listed in table 2.

Statistical analyses

The dependent variables were cingulate volume and the number and density of oligodendrocytes in both hemispheres. The independent factor was diagnosis (patients with SZ, controls), and the intervening variables were age, PMI and fixation time. All Spearman correlations between dependent variables and age, PMI and fixation time were non-significantly different from zero, so they were not considered as covariates in the main analysis. To adequately test the hypothesis of a hemisphere-specific deficit, analyses

Figure 1 Representative section from the cingulum at the level of the anterior cingulate cortex of the right hemisphere from a patient with schizophrenia. The dorsal cingulum is shown in purple, and the subgenual cingulum in yellow.
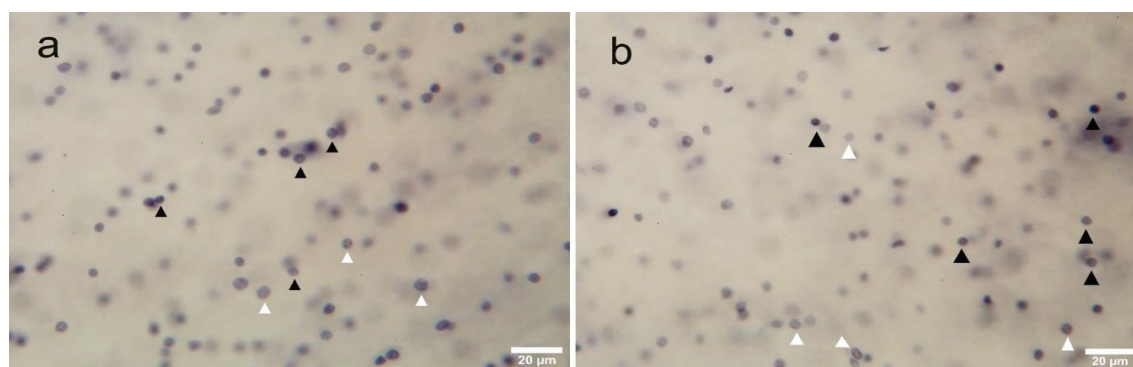

Figure 2 Photomicrograph from the cingulum of a patient with schizophrenia (a) and a control (b) captured using a 40x objective showing oligodendrocytes (black arrowheads) and astrocytes (white arrowheads).

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Table 2: Details of the stereological procedures used for oligodendrocyte counting.

Objective used	40x
B μm²	900
H μm	20
D μm	925
ΣCS	487
ΣQ-	627
Cepred	0.041

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 mean sum of unbiased virtual counting spaces used in one hemisphere of one participant; ΣQ- mean number of counted oligodendrocytes in one hemisphere of one participant; CEpred, mean predicted coefficient of error of estimated cell numbers.

of variance with factor diagnosis were calculated for all dependent variables separately for the left and right hemisphere. Additionally, repeated measures analyses of variance were calculated with the between-subject factor diagnosis and within-subject factor hemisphere. We also investigated whether the volume and oligodendrocyte numbers and densities in the cingulum were correlated with the corresponding values in the hippocampal subregion CA4 of the same sample [34]. The significance level of α = 0.05 was used for all analyses. All tests were two-tailed. SPSS version 28 (IBM, Armonk, NY, USA) was used for statistical analyses.

Results

The mean cingulum volume was statistically significantly lower in patients with SZ than in controls in the left hemisphere (mean [SD]: SZ, 2205 [399] mm³; controls, 2525 [328] mm³; $F_{(1, 21)}$ = 4.382; p = 0.049) but not in the right hemisphere (mean [SD]; SZ, 2173 [669] mm³; controls, 2571 [384] mm³; $F_{(1, 21)}$ = 2.98; p = 0.099) (Figure 3a).

The mean total number of oligodendrocytes in the cingulum was lower in SZ patients (mean [SD]; left hemisphere, 160.12 [51.21] million; right hemisphere, 143.81 [62.33] million) than in controls (mean [SD]; left hemisphere, 199.83 [30.77] million; right hemisphere, 189.21 [40.13] million). Oligodendrocyte numbers were statistically significantly lower in the left hemisphere (-19.9%; $F_{(1, 21)}$ = 4.96; p = 0.037) but not in the right hemisphere (-24%; $F_{(1, 21)}$ = 4.222; p = 0.053) (Figure 3b). The mean number of

oligodendrocytes did not differ between the two hemispheres ($F_{(1, 21)}$ = 2.89, p = 0.104). We observed an effect of the factor hemisphere on oligodendrocyte densities in that mean densities were larger in the left hemisphere (mean [SD]; SZ, 72072 [15986] cells/mm³; controls, 79241 [7031] cells/mm³) than in the right hemisphere (mean [SD]; SZ, 65024 [17884] cells/mm³; controls, 73273 [8623] cells/mm³; $F_{(1, 21)}$ = 6.552; p = 0.018). However, we found no statistically significant differences in mean oligodendrocyte densities between the patients with SZ and controls in the left ($F_{(1, 21)}$ = 1.874, p = 0.186) and right ($F_{(1, 21)}$ = 1.925, p = 0.180) hemispheres (Figure 3c). The results
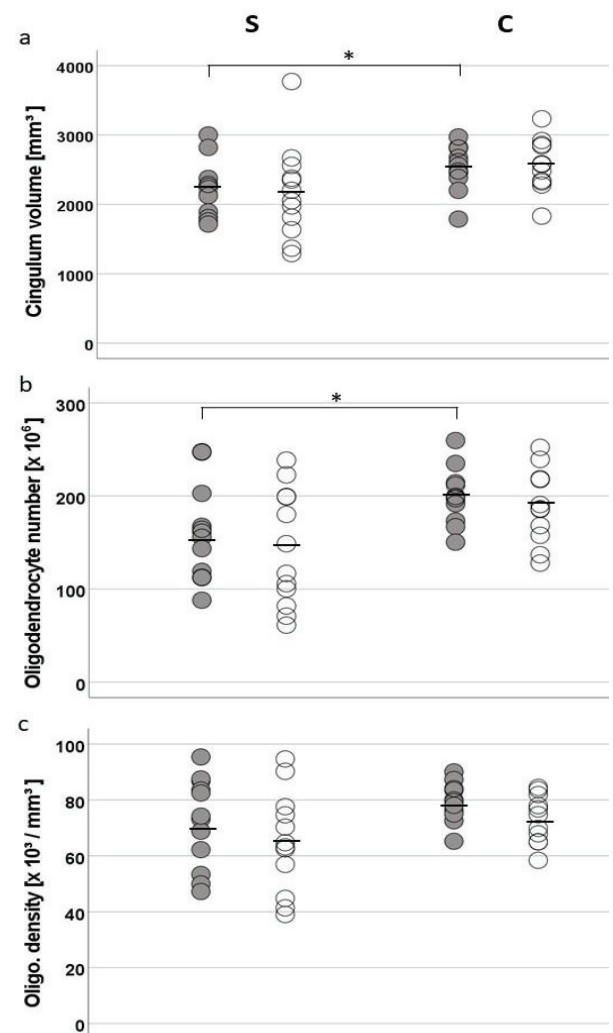

Figure 3 Mean and individual values standard error of the mean of cingulum volume (a), oligodendrocyte number (b) and oligodendrocyte density (c) in the cingulum in post mortem brains from male patients with schizophrenia (S) and age- and sex-matched controls (C). Closed dots represent data from the left hemispheres, and open dots data from the right hemispheres. *p < 0.05.

from the brain of control participant C7 (which was embedded in celloidin) did not systematically deviate from those of the gelatin-embedded brains.

In patients with SZ and controls, we found no statistically significant correlations of any dependent variable with age (all $p < 0.346$, $p > 0.257$), PMI (all $\rho < 0.446$, $p > 0.169$) or duration of fixation (all $p < 0.503$, $p > 0.095$) (Figure 4(a-i)). In the whole sample, we found a statistically significant correlation between the individual oligodendrocyte numbers in the right cingulum and right CA4 ($r = 0.530$, $p = 0.011$; figure 5(a-d)) and between the individual oligodendrocyte densities in the left cingulum and left CA4 ($r = 0.429$, $p = 0.047$; figure 5(e,f)).

Discussion

The present study detected a statistically significantly lower mean volume of the left cingulum and a statistically significant reduction in the mean oligodendrocyte number in the left cingulum in male patients with SZ compared with age- and sex-matched controls. In contrast, mean oligodendrocyte densities were not different between the groups, which may be due to a bias in cell density measurements caused by tissue shrinkage after fixation and staining procedures. This issue is addressed by design-based

stereological methods [44,47,48]. In principle, cell numbers can change without a corresponding change in the density of the same cells [49] and cell density measurements cannot serve as a surrogate of cell number measurements because cell densities may change without alterations in the number of the same cells [47].

Our results are in agreement with reported reductions in mean oligodendrocyte numbers in other brain regions, such as the CA4 subregion of the hippocampus, in patients with SZ in the same sample [34] and in an independent cohort [32,33], as well as in the DLPFC of another series [37]. In a previous post mortem analysis, we found a positive relationship between the oligodendrocyte number in the hippocampal subregions CA4 and CA2/3 and the volume of the hypothalamus as predictors for a diagnosis of SZ [50]. Our results support the notion that an oligodendrocyte pathology may contribute to cognitive deficits in limbic circuits in SZ. We have investigated the volume and total number of oligodendrocytes in the gray matter of the hippocampus CA4 within the same brains and found no volume reduction, whereas in the left cingulum, a volume reduction of 12.7% on average was detected. The total number of oligodendrocytes was reduced
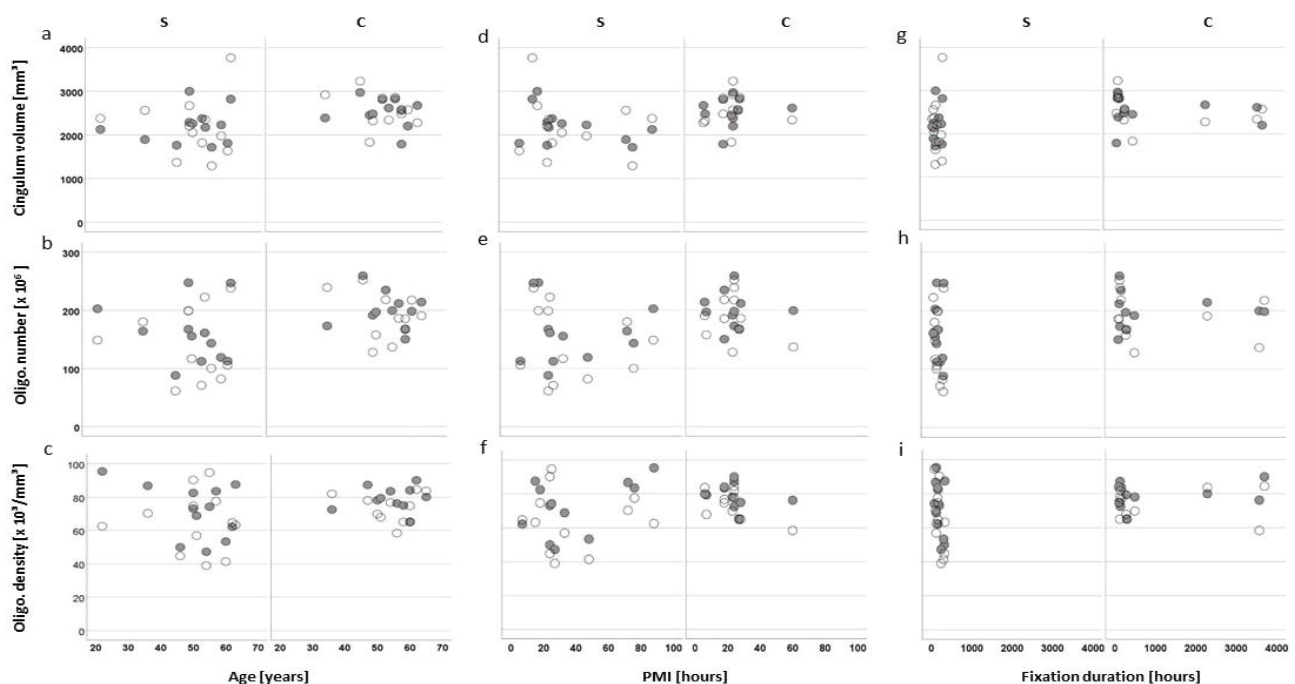

Figure 4 Individual values showing no statistically significant correlation with age (a, b, c), post mortem interval (d, e, f) or duration of fixation (g, h, i) in patients with schizophrenia and controls. Closed dots represent data from the left hemispheres, and open dots data from the right hemispheres. S, patients with schizophrenia; C, controls.
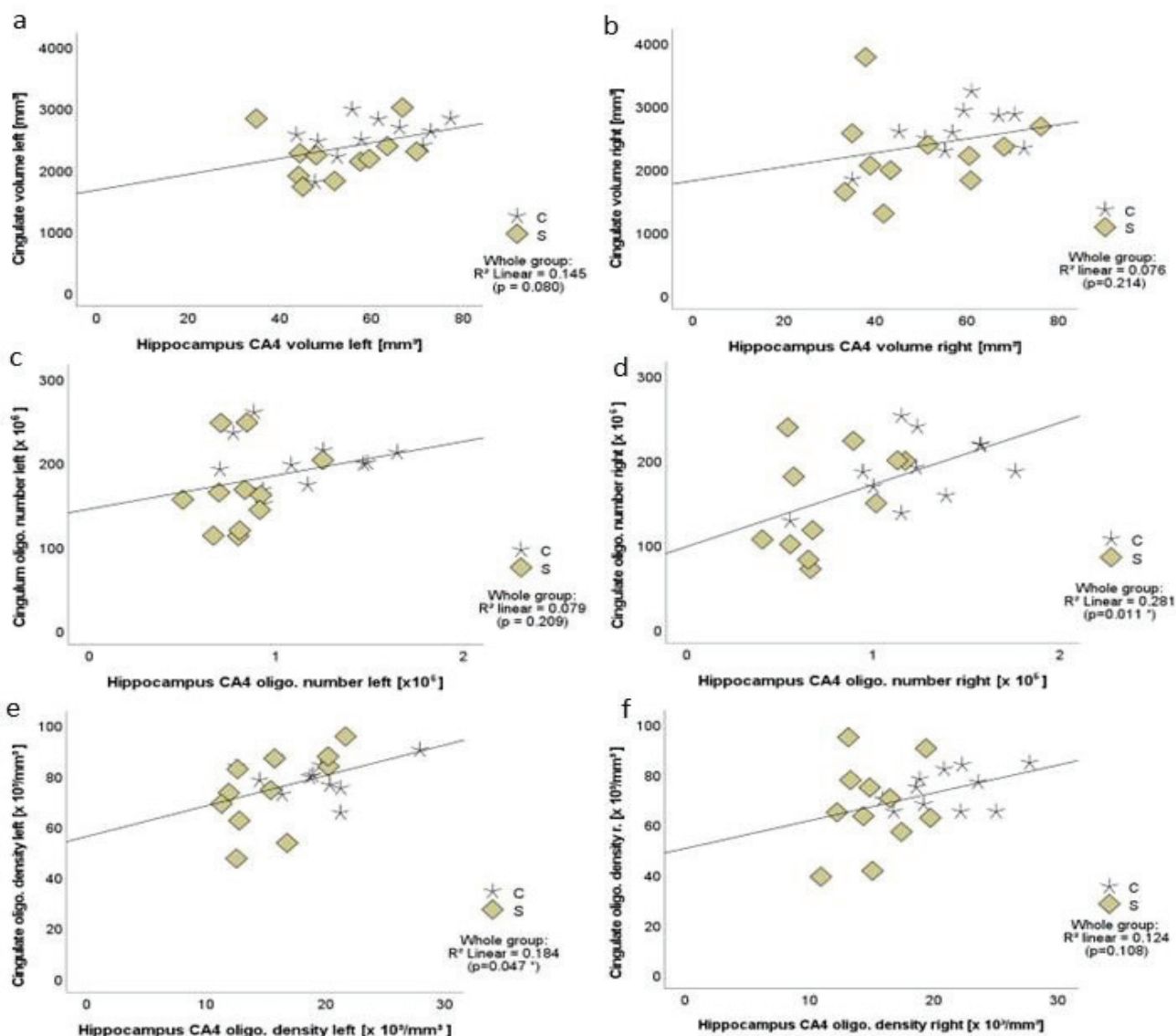
Figure 5 Individual values for patients with schizophrenia (*n* = 12) and controls (*n* = 11) showing the correlation of cingulum volume (a,b) and oligodendrocyte number (c,d) and density (e,f) in the cingulum with the corresponding values in the hippocampal CA4 subregion in the whole group of participants. S, patients with schizophrenia; C, controls.

in the CA4 subregion in the left hemisphere by averaged 28%, in the right hemisphere by averaged 39%, in the left cingulum by averaged 19.9% and in the right cingulum by averaged 24% (the reduction in the right cingulum was not statistically significant). Collectively these data indicate a larger effect of diagnosis on the volume and a slightly smaller effect on the total number of oligodendrocytes in the left cingulum in SZ. Furthermore, we found a statistically significant correlation between the individual total number of oligodendrocytes in the right cingulum and right CA4 and between the oligodendrocyte density in the left CA4 and left cingulum. Therefore, we hypothesize a more widespread process in the

brain. A lateralization of brain abnormalities has often been described in SZ. We found a statistically significant reduction of the volume and total number of oligodendrocytes only in the left cingulum. This pronounced reduction in our multi-episode patients with poor outcome reflects the finding from a recent DTI study, that poor outcome patients had a specific reduction of fractional anisotropy in the left cingulum compared to good outcome patients [51].

Previously, we hypothesized that an oligodendrocyte deficit underlies the cognitive deficits in SZ [36,38]. An earlier study found that a reduced oligodendrocyte number in the hippocampus was

associated with impaired cognition [35]. However, in the present study we did not have information on disturbed cognitive abilities of the patients with SZ. The cingulum is involved in emotional expression, attention, motivation and working memory [19,21,52], and all of these functions are impaired in SZ [3,20]. DTI studies found reduced FA (as a marker of white matter fiber density, axonal diameter and myelination) in the cingulum of patients with SZ [16,17]. Moreover, in SZ reduced performance in cognitive domains such as processing speed, attention, executive function and social cognition correlates with impaired white matter integrity and connectivity in the cingulum [22-27]. Previous DTI studies detected stronger findings in the left cingulum, and our post mortem results also point to significant changes in volume of the left cingulum and an oligodendrocyte pathology predominantly in the left hemisphere.

The present study is the first to detect an oligodendrocyte deficit in the white matter region of the cingulum in SZ. Oligodendrocytes play an important role in the myelination of neuronal axons and thus in neuronal network connectivity based on myelination [53,54]. Therefore our findings suggest a deficit in myelination and trophic support of long projecting axons in the limbic white matter and reinforce the hypothesis that the disconnectivity of multiple brain regions within neuronal networks based on oligodendrocyte pathology plays a major role in SZ. Myelination is an important process during postnatal brain development. White matter pathways develop in the early years of childhood in particular, but they continue to be myelinated into puberty and young adulthood. The late myelinating regions include the associative cortical areas such as the cingulate and prefrontal cortex [55]. A disturbed myelination process during this critical window of brain development may have implications for the onset of SZ in young adulthood [9].

Exposure to substantial stressors during adolescence may trigger a cascade of events that result in SZ in adulthood [56]. However, our post mortem study does not help to clarify these neurodevelopmental aspects because we investigated the brains from older adults, in whom the long-term disease process may have shown its effect. Animal studies have shown that juvenile social isolation, a stress paradigm during puberty, leads to SZ-related behavior deficits in prepulse inhibition of the acoustic startle response, deficits in working memory, decreased social exploration, altered

oligodendrocyte morphology, reduced myelin thickness and decreased oligodendrocyte protein expression [57,58]. Genome-wide association studies revealed that Transcription Factor 4 (TCF4) is a risk gene for SZ and is related to cognitive deficits [59,60]. In double heterozygous Tcf4 oligodendrocyte lineage transcription factor 2 (Olig2) null mutant mice, Tcf4 was shown to be the preferred heterodimerization partner for Olig2 in oligodendrocytes and required for oligodendrocyte precursor cell differentiation. Double heterozygous Tcf4/Olig2 mice have deficits in oligodendrocyte maturation and display reduced numbers of oligodendrocytes, as shown in the spinal cord [61]. Moreover, implantation of human induced pluripotent stem cell-derived oligodendrocyte progenitor cells from patients with childhood-onset SZ into myelin-deficient shiverer mice had a negative effect on the myelination properties of those cells in the mouse chimeras [62]. It has been proposed that a deficit in differentiation and maturation of oligodendrocyte precursor cells is an underlying pathophysiology of cognitive deficits and impaired frontotemporal connectivity in SZ and that this deficit could be restored by pro-myelinating drugs and aerobic exercise [38].

One limitation of our study is that the results are restricted to male subjects. Male patients with SZ tend to develop the condition earlier than females. Additionally, male patients with SZ typically experience more severe psychotic symptoms and generally have poorer outcomes, including more pronounced negative symptoms and cognitive issues [63,64]. Another constrain is that our sample was relatively small limiting its power. Therefore, the results should be confirmed in an independent, larger sample. We cannot exclude effects of the disease process itself and long-term antipsychotic treatment in our patient group, but a stereological study of the parietal cortex of monkeys after treatment with typical and second-generation antipsychotics showed no significant reduction of the number of oligodendrocytes immunostained with the oligodendrocyte enzyme-related antibody 2′,3′-cyclic-nucleotide 3′-phosphodiesterase [65]. In the hippocampus of mice, treatment with typical antipsychotics increased the number of Olig2-expressing oligodendrocytes and activated oligodendrocyte precursor cells [66]. We identified oligodendrocytes by histological gallocyanin staining, but we did not confirm the results by immunohistochemical labeling because antibodies usually do not penetrate through thick

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tissue sections like those used in the present study [48]. Future immunohistochemical studies using antibodies for oligodendrocyte precursors (e.g., breast carcinoma amplified sequence 1 (BCAS1) [67] or mature oligodendrocytes) are necessary to conclude if a deficit in maturation and differentiation of oligodendrocyte precursors is the underlying cause of the described cell loss [68].

Conclusion

In summary, we found a lower mean volume and lower mean oligodendrocyte number in the left cingulum in male patients with SZ, but we did not find changes in mean oligodendrocyte density. Missing oligodendrocytes reduce the volume of the myelin sheaths, and may result in a reduction of the volume of the cingulum. Since the cingulum is smaller, the fewer oligodendrocytes are compressed in a smaller volume and, thus, the density of these cells is not changed. This context has already been shown in a study on neuron numbers and densities from animal brains [49]. A decreased oligodendrocyte number may indicate that mature oligodendrocytes are damaged or that differentiation of oligodendrocyte precursor cells is impaired; the latter can be clarified in a future study by immunohistochemical staining of oligodendrocyte precursor cells at the early myelination stage [38]. From magnetic resonance imaging studies it is known that SZ is associated with volume loss in brain regions such as the hippocampus, insula, anterior cingular cortex, gyrus frontalis medius, temporal cortex and thalamus [12,69-72], and with deficits in structural and functional connectivity in specific networks in SZ, comprising the default-mode network comprising the cingulate gyrus, the salience network comprising the dorsal anterior cingulate cortex oder the fronto-parietal network [73-75]. Therefore it is unlikely that an oligodendrocyte loss is limited to one specific brain region. Up to now, a loss of oligodendrocytes has been detected by design-based stereology in the gray matter of the hippocampus CA4 subregion, the dorsolateral prefrontal cortex and now for the first time in the white matter of the cingulum, supporting the hypothesis of a microstructural deficit in brain circuits in SZ [68]. Additionally, future studies should investigate limbic gray and white matter regions in SZ and assess other brain regions as other axonal pathways are likely to be comparably affected. Such studies may result in new insights that can lead to innovative treatment strategies that target remyelination of oligodendrocyte precursor cells and thus improve impaired connectivity in SZ.

Declaration of Interests

Author Contributions

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

No generative AI and AI-assisted technologies have been used in the writing process.

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available for ethical reasons.

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