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Increased Interstitial White Matter Neuron Density in the DLPFC of People with Schizophrenia

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Abstract

Background—Interstitial white matter neurons (IWMNs) may reflect immature neurons which migrate tangentially to the neocortex from the ganglionic eminence to form cortical interneurons. Alterations of interneuron markers have been detected in gray matter of dorsolateral prefrontal cortex (DLPFC) in schizophrenia and IWMNs are also reported to be altered in schizophrenia. In this study we asked if a potential link exists between these two pathological findings.

Methods—From a cohort of 29 schizophrenia subjects and 37 controls, IWMN densities were determined in the DLPFC by counting NeuN and somatostatin (SST) positive cells. Double label immunofluorescence was carried out to determine the overlap between SST+/NeuN+ and SST+/neuropeptide Y (NPY)+ neurons.

Results—We found that density of NeuN+ IWMNs in superficial white matter is significantly increased in schizophrenia subjects compared with controls. There was a significant negative correlation between SST mRNA expression in gray matter and NeuN+ IWMN density. In schizophrenic patients with increased NeuN IWMN density, the density of SST expressing neurons in the white matter was also higher compared to controls. A subpopulation of SST immunopositive cells also show co-expression of NPY.

Conclusions—Our study confirmed previous results that the density of NeuN+ IWMNs is increased in superficial white matter in schizophrenia. We, for the first time, provide evidence that increased IWMN density correlated with a gray matter interneuron deficit, suggesting that migration of interneurons from white matter to the cortex may be deficient in some patients with schizophrenia, consistent with an interneuron deficit in schizophrenia.

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Keywords

Interstitial white matter neuron; Schizophrenia; Somatostatin; Neuropeptide Y; NeuN; Interneuron

Introduction

Schizophrenia is a devastating mental illness hypothesized to have a neurodevelopmental origin (1-3). Dorsolateral prefrontal cortex (DLPFC) has been implicated as a major anatomic site of dysfunction in schizophrenia based on a range of clinical, cognitive and neurobiological findings (4,5) and one consistent cortical pathology in schizophrenia involves deficits of GABAergic interneurons (6).

Alterations of inhibitory interneuron markers have been reported in DLPFC in schizophrenia subjects. In particular, post-mortem studies demonstrate reduced mRNA and protein expression of the 67kDa isoform of glutamic acid decarboxylase (GAD67) and a reduction in parvalbumin mRNA (7-13). Recently, reductions in the density of somatostatin mRNA expressing neurons and neuropeptide Y mRNA has also implicated Martinotti and bitufted cells in the disease (8,9,14). Our previous study also found reduced SST mRNA expression in the gray matter of DLPFC of schizophrenia (Fung et al, 2010). Taken together, these studies suggest reduced gene expression and possibly reduced density (15-17) of GABAergic interneurons in the gray matter in DLPFC of schizophrenia patients.

Interstitial white matter neurons (IWMNs) are differentiated neurons found among the fibers and glia of adult white matter, subjacent to the cortex (18-20) and thought to be remnants of the subplate, a transient layer found below the cortical plate during early development (18,21). It is possible that IWMNs might also represent GABAergic neurons that tangentially migrate from the ganglionic eminence to the cortex in development (22-24). In recent years, alterations of IWMN density have been found in schizophrenia patients. To date, five out of six studies on the frontal cortex in schizophrenia do report increases in subcortical white matter neuron density in schizophrenia (25-29), making it among the most replicated postmortem findings to date. One study that failed to find an increase in subcortical neuron density used a neuronal marker that is expressed in only a subset of IWMNs [MAP2 (30)]. In schizophrenia, the increased density of subcortical neurons was greater in patients with more negative symptoms suggesting that this pathology may contribute to clinical heterogeneity (29).

Here, we sought to determine if there is an increase in density of IWMN in the DLPFC of patients with schizophrenia using the largest number of schizophrenia subjects studied to date (in this regard) with the marker of IWMNs that shows the most consistent reduction (NeuN). NeuN is a robust marker of mature neurons (31,32), that reliably detects a large number of subcortical neurons compared with MAP2 [not detected uniformly in all mature neurons (33)] and NADPH [shown to label only a small subset of subplate neurons (19)]. We hypothesized that deficits in cortical interneurons and increased IWMNs may be related. Thus, in the present study, we tested if SST mRNA levels which we recently found to be the most deficient grey matter interneuron marker in this same schizophrenia cohort, was inversely correlated with NeuN+ IWMN density.

Methods and Materials

Cohort and tissue preparation

DLPFC tissue from schizophrenia patients (n=29) and controls (n=37) was obtained from the New South Wales Tissue Resource Centre (Sydney, Australia; HREC 07261) as

described previously (34). Groups were matched for age, sex, race, brain pH, and post mortem interval (PMI) (defined as time between death and brain freezing). Schizophrenia patients all met the DSM-IV criteria for schizophrenia. Middle frontal gyrus was identified as the region of gray matter between the superior and inferior frontal sulci and was trimmed from frozen coronal slabs. Tissue blocks (stored frozen at -80°C) were cut into $14\mu\text{m}$ slices on a cryostat. BA46 was determined following the cytoarchitectural criteria described by (35), and the closest white matter to this was sampled.

Immunohistochemistry

For DAB immunohistochemistry, fresh frozen tissue sections were thawed at room temperature (RT) for 20 min and fixed in 4% paraformaldehyde in phosphate buffered saline [(PBS) 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4 , 2 mM KH_2PO_4 , pH 7.4] for 10 min at 4°C . Slides were washed in PBS ($3\times 15\text{min}$), treated for 20 min at RT with methanol + 3% H_2O_2 (3:1) solution, washed and incubated with 10% normal goat serum in diluent [0.05% bovine serum albumin (BSA), 0.3% triton X-100 in PBS] for 1 hr at RT. Slides were incubated with anti-NeuN primary antibody (1:1000 in diluent, Millipore MAB377) overnight at 4°C , washed, followed by goat anti-mouse IgG biotinylated secondary antibody (1:500, Vector Laboratories, Cat # BA-9200) for 1 hr at RT. Slides were washed, incubated at RT (1 hr) in avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector Laboratories) and treated with 3,3'-diaminobenzidine (DAB; Sigma) (12 mM final concentration in PBS with 0.003% H_2O_2) for 5–7 min. Slides were Nissl stained (1.5 min exposure to 0.02% thionin) and coverslipped.

For double-label immunohistochemistry, slides were blocked with 10% donkey serum in diluent. Slides were incubated with first primary antibody (rabbit anti-SST, 1:200, Santa Cruz Biotechnology, cat # sc-13099), 4°C overnight, washed and incubated with secondary antibody (1:500, Invitrogen, cat # A21207) at RT (1 hr) and washed prior to reblocking in 10% donkey serum and incubation with second primary antibody (NeuN, 1:1000, Millipore MAB377 or NPY, 1:300, Abnova, cat # H00004852-M03) at 4°C overnight, washed and incubated with secondary antibody (1:500, Invitrogen, cat # A21202) at RT (1 hr). Slides were washed with DAPI (1:1000) in PBS for 5 min, and with 5mM CuSO_4 in 50mM $\text{CH}_3\text{COONH}_4$ (pH 5.0) for 20 min, for the diminution of lipofuscin autofluorescence then coverslipped.

SST mRNA expression in the grey matter

qPCR for SST mRNA expression in grey matter was performed using the TaqMan Gene Expression Assay Hs00356144_m1 (ABI) and total RNA isolated from DLFPC as described. Quantity values were normalised by the geometric mean of four housekeeping genes: ubiquitin C, actin β , glyceraldehyde-3-phosphate dehydrogenase, TATA box binding protein (34).

In Situ Hybridization

$14\mu\text{m}$ thick tissue sections (two/case/probe) were used for *in situ* hybridization. Slides were thawed, fixed, acetylated, delipidated, and dehydrated as previously described (37). ^{35}S -UTP labeled antisense riboprobes to base pairs 207–446 of the human somatostatin mRNA (NM_001048.3) were prepared, with specific activity of $2.48 \times 10^9\text{cpm}/\mu\text{g}$ (Riboprobe in vitro Transcription System, Promega, Madison, WI, USA). 200 μL of hybridization buffer containing ^{35}S -UTP labeled riboprobe (5 ng/mL) was incubated on each section overnight at 55°C in humidified chambers. As a control, 5ng/ μL of ^{35}S -UTP labeled sense riboprobe was applied to additional sections. Slides were washed and exposed to BioMax autoradiographic film (Kodak, Rochester, NY) for 6 weeks with ^{14}C standards (American Radiolabeled Chemicals, Inc. St. Louis, MO). In order to determine SST mRNA localisation at the cellular

level, slides were dipped in emulsion (NTB-2, Kodak), dried and developed after being in the dark for 3.5 months. Slides were developed using D-19 developer (Kodak) and Nissl counterstained (with thionine).

Sampling

Images of NeuN immunostaining along the entire length of one middle frontal gyrus/section were captured at 10x magnification and stitched together (Supplement: Figure S1) using the Virtual Slice facility in the Stereo Investigator Software (MBF Biosciences). Boundaries between the gray matter and white matter were identified by a sharp change in NeuN density and delineated on the stitched sections (see Supplement: Figure S1). The sampled area of white matter was selected from the middle bank of the sulcus to avoid the crown at the surface and the curve at the deep end of the sulcus, allowing for consistent identification of the gray/white matter border. The sampling area was divided into two compartments, one superficial and one deep. A 700 μ m margin deep to the gray/white border on each side of the gyrus was designated as 'superficial' white matter. The area between these two superficial compartments on each side was deemed 'deep' white matter. Sampling boxes of 470 $\times$ 470 μ m were placed within the designated compartments from an arbitrary starting point near the crown with one side of the counting box placed parallel to the gray/white matter border. Distances with which to move counting boxes within their compartment were determined using a random number table. In the majority of cases (87%), 18-20 boxes were placed in each compartment. All NeuN+ IWMNs within boxes were counted, except those touching the right and bottom lines of the counting box. Each slide was independently sampled and counted by two researchers (Cronbach's $\alpha=0.99$) and this process was repeated in another set of slides from the entire cohort. The final NeuN+ density was calculated as the average of ~20 boxes of the two compartments from the two slides per case (~80 counting boxes/case). Researchers were blind to cases and controls throughout the experimental and quantification procedure. IWMN cross-sectional area was measured for NeuN immunostained IWMNs ($n=10$ randomly sampled in superficial white matter per subject, $n=660$ neurons in total, <8% measurement variance) using Image J (38).

The same counting strategy was applied to calculating the density of SST mRNA+ cells (Supplement: Figure S2) in a subset of 18 subjects (Supplement: Table S1), those patients with schizophrenia with the highest density of NeuN+ IWMNs (>1.5 SD from mean) and matched controls. We hypothesized that the schizophrenia cases with highest IWMN density would be the most likely to display an increase in SST+ cells in the white matter. The boundary was drawn in reference to the adjacent NeuN immunostained slide of the same case. From the dipped sections, superficial white matter SST mRNA+ cells were identified as having robust silver grain clusters and were counted/box. SST immunopositive cells that were co-labelled for NPY immunoreactivity were similarly counted in the white matter of the 18 subjects.

Statistics

Outliers within a group (no more than 1 control/schizophrenia case for each dependant variable) were removed. Student's *t*-tests were conducted to compare IWMN density, SST mRNA density and NPY+/SST+ cell ratio between patients with schizophrenia and controls. Two-way ANOVA was performed with diagnosis and hemisphere as between-group independent variable. Pearson correlation analysis was performed to determine the relationship between IWMNs and gray matter SST expression. All statistical analyses were performed using Statistica 7.

Results

Density of NeuN-stained IWMNs in the DLPFC of people with schizophrenia

NeuN+ cells were identified in the white matter of all controls and all schizophrenia patients and many of these neurons display processes orientated parallel to the pial surface (Figure 1). Mean density ($\pm$ SD) of NeuN+ IWMNs in superficial white matter was significantly higher (20%) in subjects with schizophrenia ($49.4 \pm 12.4/\text{mm}^2$) compared to controls ($41.5 \pm 10.6/\text{mm}^2$) ($t = -2.77$, $df = 63$, $p = 0.004$, one tailed; Figure 2A). In contrast, mean density of NeuN+ IWMNs in deep white matter revealed no significant difference in subjects with schizophrenia ($12.6 \pm 2.9/\text{mm}^2$) compared to controls ($12.1 \pm 3.6/\text{mm}^2$) ($t = -0.63$, $df = 62$, $p = 0.53$; Figure 2B). Daily, lifetime or last recorded dose of antipsychotic medication were not found to correlate with IWMN density (Supplement: Table S2) and IWMN density was not different between those subjects taking antidepressants versus not (superficial $t = 0.68$, $df = 27$, $p = 0.50$; deep $t = 0.43$, $df = 27$, $p = 0.67$). NeuN+ superficial IWMN cross sectional area was not significantly different between controls (89.8 ± 15.5) and people with schizophrenia (85.9 ± 19.9 ; $t = 0.90$, $df = 64$, $p = 0.37$).

We identified a subset of patients ($n = 9$) with particularly high NeuN+ IWMN density (>1.5 SD). Interestingly, 5/9 schizophrenics (56%) with the highest density of superficial IWMNs tended to be those with a predominantly negative symptom profile, while only 2/18 of the individuals (11%) with predominantly negative symptoms made up the remainder of patients (chi squared = 7.034, $df = 1$, $p = 0.008$) (Table 1). Subjects within the subset were not significantly different from remaining cases in age of onset, age at death, duration of illness or lifetime antipsychotic dose ($t = 0.58$ to 0.03 , all $p > 0.56$), but there was a trend to reduced PH, PMI and RIN in the subset ($t = -1.7$ to -1.8 , $p = 0.08$ to 0.1).

The effect of hemisphere on IWMNs

We next examined the effect of hemisphere on density of NeuN+ IWMNs in both superficial and deep white matter in control versus schizophrenia cases. For the superficial white matter, the interaction effect of diagnosis and hemisphere was not significant ($F = 0.075$, $df = 61$, $p = 0.78$); however, there tended to be more IWMNs in the left hemisphere compared to the right hemisphere (main effect of hemisphere, $F = 3.37$, $df = 61$, $p = 0.07$) (Figure 2C). For the NeuN+ density in the deep white matter, the interaction effect between diagnosis and hemisphere was at the level of statistical significance ($F = 3.9$, $df = 60$, $p = 0.05$). We found that density of NeuN+ deep IWMNs was significantly (28%) higher in left hemisphere compared to right hemisphere in controls ($p = 0.008$), while the NeuN+ IWMN density was not significantly different in left hemisphere compared to right hemisphere in subjects with schizophrenia ($p = 0.87$) (Figure 2D).

SST mRNA of gray matter and IWMNs in the superficial white matter

Next we asked if density of NeuN+ IWMNs may be linked to any pathology of inhibitory interneurons in the overlying gray matter. Previously, we found by quantitative PCR that SST mRNA expressed in the gray matter showed the greatest disease-related deficit in the middle frontal gyrus of our cohort of patients with schizophrenia (39). Thus, we tested if SST mRNA levels (from the same cohort) correlated with the density of NeuN+ IWMNs. In the whole cohort, SST mRNA in gray matter correlated significantly and negatively with the density of NeuN+ IWMNs in the superficial compartment ($r = -0.31$, $p = 0.015$; Figure 3). This demonstrates that when a greater density of neurons was found in the white matter, less SST mRNA was detected in the overlying gray matter.

SST mRNA expression in white matter

We next performed SST in situ hybridization to determine if cells underlying the cortex expressed SST mRNA. Autoradiographic film images (Figures 4A and 4B) showed intense signals in the location of layer II and layer IV of gray matter, and the signal is punctate with sparse intense signal below the gray matter (arrows in Figure 4A, 4B), of which there was no obvious difference in schizophrenia subjects and controls. By microscopy, the SST mRNA+ cells in the white matter were easily distinguished by dense clusters of silver grains over nuclei compared to the low background of silver grains in the white matter (Figures 4C and 4D; Supplement: Figure S2). SST non-expressing cells contained only background levels of silver grains.

The density of SST mRNA+ cells within the white matter were counted in the subset of patients that showed the highest NeuN+ IWMN density (n=9) compared to their matched control (n=9). We found that within the “high IWMN density” subset the mean density of SST+ IWMNs in superficial white matter showed a trend to be 54% higher in subjects with schizophrenia ($2.60 \pm 1.23/\text{mm}^2$) compared to controls ($1.69 \pm 0.98/\text{mm}^2$) ($t = -1.74$, $df = 16$, $p = 0.10$, two tailed; $p = 0.05$ one tailed) (Supplement: Figure S3). The fairly low density of SST+ neurons compared to the total NeuN+ neurons in both patients with schizophrenia (5%) and controls (4%) suggested that the SST+ contributes only a small percentage of the total NeuN+ cells in the IWMNs. In order to confirm if the SST+ were a subset of the NeuN+ + IWMNs, we did double-label immunofluorescence study in the same subset of subjects (n=18) and found that the SST+ neurons are mostly, but not always, NeuN+ (of 362 SST+ neurons evaluated, 357 are also NeuN+), i.e. 1.4% SST+ neurons are NeuN-. Furthermore, we observed that many of the double labeled neurons only express a weak NeuN+ signal (Figure 5).

Double label shows that NPY expressing neurons are a subset of SST expressing neurons in white matter

We found that SST+ IWMNs were also immunopositive for NPY in both patients with schizophrenia and controls (Figure 6). However, only a subset of SST+ neurons in white matter are also NPY+. In contrast, we didn't detect any IWMNs that are NPY+ but SST-, suggesting that within the prefrontal cortical white matter of humans, NPY+ neurons are a subset of SST+ neurons. In the control group, 30% (45/148) of the SST+ neurons are NPY- (red only), while in schizophrenia 22% (36/167) of the SST+ neurons are NPY-. This difference was not statistically significant ($t = 1.55$, $df = 15$, $p = 0.14$).

Discussion

Abnormal density of NeuN+ IWMNs and grey matter interneuron deficit could be considered two of the more consistently replicated findings in patients with schizophrenia. For the first time, we found that increased density of NeuN+ IWMNs was associated with less SST mRNA in the overlying gray matter, uncovering a novel relationship among two major pathological findings in people with schizophrenia. Furthermore, we found a novel increase in the density of SST mRNA+ neurons in superficial white matter in schizophrenia suggesting that this subtype of interneuron contributes to the IWMN density increase.

Our study confirmed previous findings that the density of superficial IWMNs is increased in schizophrenia. We replicated these observations in three ways: 1) by showing that the overall density of NeuN+ cells is in line with that reported previously (28), 2) by finding that the magnitude of IWMN density increase is around 19% in schizophrenia, similar to a 20% increase in BA9/46 (28), and 3) by identifying a subset of patients with a more profound increase in IWMNs density. There is less agreement in changes in overall density of IWMNs

found at deeper levels below the cortex, and our study supports that the overall density in deep white matter is unaffected (28). We are also the first to report a right versus left hemisphere difference in the density of IWMNs in normal human frontal cortex, with the left having significantly higher density. Interestingly, this hemispheric asymmetry of IWMN density is not detected in deep white matter of schizophrenia subjects.

Not all previous studies report similar densities of IWMN and this may be due to methodological differences. For example, we defined a less distinct deep from superficial compartment compared to Eastwood (28) and our density of IWMNs in deep white matter is slightly higher, as would be expected. In addition to differences in definition of white matter compartments, the cortical region examined and neural marker measured may contribute to the differences reported in density of IWMNs. For example, Akbarian and colleagues (40) found the density NeuN+ IWMNs of the white matter that is close to gray matter in cingulate cortex is less than $10/\text{mm}^2$, much less than that of the DLPFC in the current study ($\sim 43/\text{mm}^2$) and Eastwood study ($\sim 45/\text{mm}^2$), suggesting that the density of white matter neurons varies considerably across cortical regions that vary in anatomical and functional features.

We observed a subset of patients with higher density of superficial IWMNs. This subpopulation of people with schizophrenia had a high percentage of patients with predominantly negative symptoms, which may suggest an overlap with the increased IWMN density found in the “deficit” subgroup of people schizophrenia reported by Kirkpatrick (29,41). The observed increase in SST mRNA in white matter contrasts findings from our laboratory and others which found significant reductions in gray matter SST mRNA levels in the prefrontal cortex of patients with schizophrenia (8,9,14,39). A previous report, however, found that SST mRNA in the white matter was not differentially expressed between patients and controls (14). Since we analyzed the density of SST+ neurons, it is possible that there is less SST mRNA/cell in the IWMN in schizophrenia which would lead to no apparent change in expression when analyzed at a more macroscopic level [that of autoradiographic film as measured previously (14)], or that the sparse distribution of SST mRNA+ cells hampered the ability to detect a signal change.

In addition to observing an increase in SST mRNA+ IWMNs, we see a suggestion of increased mean ratio of SST+/NPY+ to SST+/NPY- cells in schizophrenia patients compared to controls. This increased colocalisation of these markers in the disease state, may be paralleled with changes of SST+ cells, although quantification remains to be carried out. It should be noted that in a previous report, density of NPY+ IWMNs was unaltered in schizophrenia subjects (42), although a decrease of NPY+ IWMNs was noted in schizoaffective subjects suggesting that the NPY+ IWMN subpopulation may be susceptible in a particular diagnostic type of schizophrenia.

Some methodological considerations concerning measurement of 2D cell density counting are important in interpreting our results. Primarily, this method may overestimate the presence of larger cells, however we do not observe any difference in cell size between controls and schizophrenia cases, consistent with previous reports (30,40,43). Use of the 2D method is consistent with previous studies measuring IWMN density (27,28,30,40,44) and it has been suggested that 3D counting methods are not without sampling bias (45) concerning non-uniformly distributed cell populations such as IWMNs. Indeed, Connor et al (40) show a good correlation between 2D and 3D density counting.

Implication

IWMNs are assumed to be remnants of subplate neurons (18,21) and most undergo programmed cell death (46) with a small portion surviving as IWMNs into adulthood (21).

We and others have found subsets of IWMNs to express interneuron markers, such SST and NPY (21), these may also represent interneurons arrested in migration. Indeed, a previous study has shown that the 22q11.2 deletion, a genetic lesion associated with schizophrenia, disrupts normal distribution and migration of neurons during early brain development (47). Furthermore, it has been suggested that reduced expression of reelin (affecting the migration of neurons (48)) may be related to increased IWMN density (27).

We found that a small percentage of SST+ neurons are NeuN-, and some SST+ neurons have less intense NeuN signal. Since NeuN is expressed in mature postmitotic neurons (32), our finding may indicate that the SST+/NeuN- cells are immature neurons. It should be noted that some studies have suggested that newly born neurons might also exist in adult prefrontal cortex. Although Kornack and Rakic (49) found no evidence of neurogenesis in the neocortex of primates, Gould (50) found BrdU+ neurons in prefrontal cortex of adult monkey, and these new neurons may migrate through white matter to reach the cortex. Cai et al (51) found cells expressing migration markers (DCX+/PSA-NCAM+) in the white matter of adult monkeys, suggesting that these cells are in the process of migrating. These observations, together with a reduction in SST mRNA+ cells in the grey matter of patients (14) could support a role for neuronal migration in the adult cortex and suggest that our hypothesis (that negative correlation between SST mRNA in the grey matter and SST+ IWMN density may implicate a deficiency in migration of these cells into the schizophrenic cortex) may be feasible.

While we replicate many studies showing increase in IWMN density in schizophrenia, heterogeneity of the disease, the region sampled, the IWMN marker used to quantify, and demographics of the cohort as well as clinical variables differ between studies. Evidence that antipsychotic medication can alter neurogenesis and cell survival (52-54) is inconsistent between species and brain region studied (see (55)) and in our study no correlation was found between medication and IWMN density.

Loss of hemisphere asymmetry in the density of deep IWMNs

We show that NeuN density in deep white matter is higher in left hemisphere than right in controls, and this asymmetry is lost in schizophrenia. Hemisphere asymmetry (both neuroanatomy and function) has been widely reported in healthy brains, and a loss of brain lateralization has been reported in schizophrenia at the cytoarchitectural level in gray matter (56). Cullen and colleagues found overall neuronal density was greater in the left than right hemisphere of controls, while the reverse was seen in schizophrenia. Our data are consistent with the notion that schizophrenia is associated with reduced brain lateralization, while lending support at cellular level in white matter.

In summary, we confirmed previous results that superficial IWMN density is increased in schizophrenia subjects in a large cohort, and for the first time found a hemisphere difference in deep IWMNs between control and schizophrenia subjects. Using SST as a marker, we further provide evidence that a subset of interneurons are also increased in superficial white matter. A decrease in SST mRNA in gray matter of the same cohort leads us to hypothesis that migration of these interneurons from the white matter to the cortex may be deficient in patients with schizophrenia. While such a theory requires further experimental evidence, such as testing the expression of markers of immature and/or migrating neurons in these IWMNs, this would be consistent with one of the leading theories of schizophrenia pathology, that is that development of the inhibitory interneurons is altered in the brains of people with schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Neuronal nuclear antigen (NeuN)-positive neurons (A) below grey matter (grey matter/white matter border represented by dotted line). In superficial white matter from (B) control and (C) schizophrenia subjects. Arrows show representative NeuN immunopositive interstitial white matter neurons that were quantified. Arrowheads indicate processes aligned parallel to the pial surface. Calibration bar (A) 200 μm, (B, C) 50 μm.

Figure 2. Density of NeuN+ IWMNs (neurons/mm²) in (A) superficial white matter and (B) deep white matter in controls (squares) and schizophrenics (triangles). Hemisphere (left: black, right: gray) dependant alterations of IWMNs density in (C) superficial white matter and (D) deep white matter. ** p<0.01.

Figure 3. Superficial NeuN+ IWMNs density (neurons/mm²) negatively correlates with gray matter SST expression in controls and schizophrenia (controls, squares; schizophrenia, triangles).

Figure 4.

Representative in situ hybridization images showing SST mRNA signals in control (A, C) and schizophrenia (B, D) subjects. Autoradiographic film images (A, B) show the signal distribution in a single gyrus. Photomicrographs (C, D) show SST mRNA expression at the cellular level. SST mRNA positive cells could be distinctly determined with dense black silver grains over the nucleus (counterstained by cresyl violet). Calibration bar =25 μ m.

Figure 5. SST and NeuN expression in IWMN cells. Double label immunohistochemistry for SST (red) and NeuN (green), with DAPI nuclear counterstaining (blue). A-D shows a neuron that is SST positive but NeuN negative; E-H shows a neuron that is SST positive and also NeuN positive. Calibration bar = 10 μ m.

Figure 6. SST and NPY expression in IWMN cells. A-C Double label immunohistochemistry showing SST (red) and NPY (green), with DAPI nuclear counterstaining (blue). D shows a neuron that is SST positive and also NPY positive. Calibration bar = 10 μ m.

Table 1

Demographics of control and schizophrenia groups (presented as mean values + /- SD. M=male, F=female, L=left, R=right, N=negative, P=positive)

	whole cohort		subset with high IWMN density	
	control group (n=37)	schizophrenia group (n=29)	control group (n=9)	schizophrenia group (n=9)
Age (years)	51.1 ± 14.6	50.7 ± 13.0	50+/-16.3	51+/-16.1
Brain pH	6.66 ± 0.30	6.59 ± 0.30	6.56+/-0.34	6.44 +/- 0.35
PMI (hrs)	24.8 ± 11.0	29.0 ± 14.5	27.3+/-11.9	22.3+/-8.6
Gender	7F, 30M	9F, 20M	7M	3F, 6M
Hemisphere	23R, 14L	13R, 16L	6R, 3L	3R, 6L
Age of onset (years)		23.0 ± 5.8		23.3+/-6.34
Duration of illness (years)		27.7 ± 13.1		27.7+/-15.2
Predominant symptom type		7N, 22P		5N, 4P