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Relationship between somatostatin and death receptor expression in the orbital frontal cortex in schizophrenia: a postmortem brain mRNA study

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BACKGROUND: Recently, we provided evidence showing reductions in GAD67 and Dlx mRNAs in the orbital frontal cortex (OFC) in schizophrenia. It is unknown whether these reductions relate mainly to somatostatin (SST) or parvalbumin (PV) mRNA expression changes, and/or whether these reductions are related to decreased SST mRNA+ interneuron density.

AIMS: To determine whether inhibitory interneuron deficits in the OFC from people with schizophrenia are greatest for SST or PV mRNAs, and whether any such deficits relate to mRNAs encoding cell death signalling molecules.

METHODS: Inhibitory interneuron mRNAs (SST; PV; *in situ* hybridization, quantitative PCR (qPCR)) and death signaling mRNAs [FAS receptor (FASR); TNFSF13: qPCR] were measured in control and schizophrenia subjects (38/38). SST mRNA+ interneuron-like cells were quantified in layer II in the gyrus rectus. Gray matter SST and PV mRNAs were correlated with interstitial white matter neuron (IWMN) density (GAD65/67; NeuN) and death signaling mRNAs.

RESULTS: SST mRNA was reduced in OFC layers I–VI in schizophrenia (both *in situ* and qPCR), with greatest deficit in layer II (67%). Layer II SST mRNA+ neuron density was reduced in schizophrenia (~29%). PV mRNA was reduced in layers III (18%) and IV (31%) with no significant diagnostic difference in PV mRNA measured by qPCR. FASR mRNA was increased in schizophrenia (34%). SST, but not PV, expression correlated negatively with FASR and TNFSF13 expressions and with IWMN density.

CONCLUSIONS: Our study demonstrates that SST interneurons are predominantly linked to the inhibitory interneuron pathology in the OFC in schizophrenia and that increased death receptor signaling mRNAs relate to prominent laminar deficits in SST mRNA in the OFC in schizophrenia. We suggest that SST interneurons may be more vulnerable to increased death receptor signaling than PV interneurons.

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INTRODUCTION

The orbital frontal cortex (OFC) is located on the ventral surface of the frontal lobe and is considered to be a major node of the social brain network.¹ Abnormalities, including volume deficits, in the OFC in schizophrenia have been demonstrated in neuropsychological,² functional,³ and structural^{4,5} magnetic resonance imaging studies. People with OFC lesions show abnormal behaviors, such as social–emotional deficits, which are also common to people with schizophrenia.⁶

Our recent work has demonstrated that the inhibitory interneuron deficits observed in the dorso-lateral prefrontal cortex (DLPFC)^{7,8} extend to and are greater in magnitude in the OFC^{9,10} in schizophrenia. Among the interneuron mRNAs quantified in the prefrontal cortex, we found the greatest deficit in somatostatin (SST) mRNA,^{8,11} which is consistent with reports demonstrating SST mRNA deficits in schizophrenia,^{12–14} whereas others find parvalbumin (PV) expression to be predominantly reduced in schizophrenia.^{15–17} Despite evidence suggesting that SST interneurons are the most affected in schizophrenia,^{8,11,14} much of the research focus has been on developing animal models for schizophrenia that are consistent with deficits in predominantly PV interneurons.^{18,19} Further, the nature and magnitude of deficit

for the two prominent inhibitory interneuron mRNAs (SST and PV) are still unclear in the OFC in schizophrenia.

It remains controversial whether reductions in inhibitory interneuron mRNAs (particularly SST and PV) represent reductions in interneuron numbers in people with schizophrenia. Although there are reports demonstrating a reduction in non-pyramidal/interneuron-like cells,^{16,20–22} others report an increase or no change in interneuron density in schizophrenia.^{23–26} Although some^{16,20,22,27} propose that non-pyramidal/interneuron-like cell density is reduced in schizophrenia, others²⁸ interpret decreased interneuron mRNA+ neurons as a reduction in mRNA per cell, with no change in cell density.^{13,28–30}

One possible mechanism by which cortical neurons may decrease in number or density is through activation of apoptotic pathways. The possible role of apoptosis in the pathophysiology of schizophrenia remains a topic of interest for schizophrenia researchers, with neurodevelopmental insults and neuronal loss being linked to apoptosis.^{20,31–33} Cortical volume loss coupled with possible reduction in inhibitory interneurons in people with schizophrenia raises the question as to whether interneurons are undergoing cell death? A recent study by our group, for the first time, demonstrated elevated death receptor signaling mRNAs

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[FAS receptor (FASR); TNFSF13] in the DLPFC in schizophrenia and showed that this relates to reduced inhibitory interneuron mRNAs (SST; PV).³⁴ TNFSF13 is a ligand for tumor necrosis factor receptor family members.³⁴ Among the different receptor subtypes in this family (FAS, DR3, DR4, DR5),^{35,36} FASR^{36,37} is widely expressed in the central nervous system, whereas the other three receptors have a limited expression.³⁸ TNFSF13-FASR signaling leads to activation of caspases (8, 10, 3, 6, and 9) resulting in cellular apoptosis by DNA degradation and membrane blebbing.^{34,39}

In our present study, we test for layer-specific changes in SST and PV mRNAs in the OFC in people with schizophrenia compared with controls. We also determine the relative expression of death receptor signaling markers (FASR; TNFSF13) in schizophrenia and control subjects and explore how these relate to changes in SST and PV mRNAs in the OFC.

MATERIALS AND METHODS

Human postmortem brain tissue samples

OFC tissue from people with schizophrenia or schizoaffective disorder ($n=38$) and control subjects ($n=38$) was obtained from the New South Wales Tissue Resource Centre (Sydney, Australia; Human Research Ethics Committee 07261; see Table 1).⁴⁰ Groups were matched for age, brain pH and postmortem interval. Tissue sections (14 μ m) were sliced from fresh frozen blocks (gyrus rectus, Brodmann area 11).

In situ hybridization

OFC tissue sections (two per case per probe) were thawed, fixed, acetylated, delipidated, and dehydrated as described earlier.¹⁰ Hybridization buffer-containing ³⁵S-uracil triphosphate-labeled SST (specific activity = 1.81×10^9 c.p.m./ μ g) or PV (specific activity = 2.12×10^9 c.p.m./ μ g) riboprobe (5 ng/ml; see Supplementary Information for details) was incubated on each section overnight at 55 °C in humidified chambers. As a control, 5 ng/ml of ³⁵S-UTP-labeled SST or PV sense riboprobe was applied to additional sections. Following post-hybridization RNase digestion, slides were washed and exposed to BioMax MR (Kodak, Rochester, NY, USA) autoradiographic film (SST—9 weeks; PV—5 weeks) with ¹⁴C standards (American Radiolabeled Chemicals, St Louis, MO, USA). To determine SST mRNA localization at the cellular level, all slides were dipped in NTB2

emulsion (Kodak), dried, and developed using D-19 developer (Kodak) under strict dark conditions (no white light). Developed slides were Nissl counterstained with thionin.

Image analysis for SST and PV mRNA expressions in OFC

Optical density measurements on all autoradiograms were conducted blind to diagnosis. The cortical area (gray/white matter) within the gyrus rectus closest to the olfactory sulcus was sampled (Figure 1).⁹ For each section, three randomly placed lines were drawn perpendicular to the pial surface. Optical density measurements were recorded along the length of the lines traversing the entire cortical gray matter (olfactory sulcus). Optical density measurements were also performed in the gyrus rectus white matter for SST mRNA level. All the autoradiograms were calibrated for optical density using a radioactive ¹⁴C standard (μ Ci/g). Data for individual cortical lamina in the OFC were obtained using the percentage of the total cortical width occupied by each layer.^{9,10}

SST mRNA positive (+) cell density was quantified using two-dimensional cell counting (Figure 2) in layer II of gyrus rectus in areas adjacent to both the olfactory and rostral sulci (see Supplementary Information for details).

RNA extraction and quantitative reverse transcription PCR

RNA extraction was carried out using TRIzol Reagent (Invitrogen Life Sciences, Melbourne, VIC, Australia). cDNA was generated using a SuperScript First-Strand Synthesis kit (Invitrogen, Carlsbad, CA, USA) as described in Weickert et al.⁴⁰

Expression levels of inhibitory interneuron mRNAs (SST; PV) and apoptosis-related transcripts (FASR; TNFSF13) were determined by quantitative PCR (qPCR) in this study using published methods⁴⁰ (see Supplementary Table S1). The averaged raw data were normalized to the geomean of expression levels of four housekeeper genes (β -actin, TATA box-binding protein, ubiquitin C, and GAPDH; Supplementary Figure S2).

Statistical analysis

All statistical analyses were performed using Statistica (version 7.1; StatSoft, Tulsa, OK, USA). Tests for normality (Kolmogorov–Smirnov and Lilliefors) were conducted on raw data for all measures conducted in the OFC. Any non-normal raw data (k -s.d. $P < 0.05$) were log transformed and outliers within each diagnostic group removed. The number of excluded group outliers was between 1 and 3 individuals per group per measure.

Two-way analysis of variance was performed with diagnosis and hemisphere, or diagnosis and gender as between-groups independent variables. Pearson's correlations were performed to determine whether mRNA expression of markers quantified was related to demographic variables or other OFC measures (white matter—NeuN, GAD65/67; gray matter—GAD67).⁹ Daily or lifetime doses of antipsychotic medication were correlated with measures performed.

For any measure having a significant correlation with demographic variables, analysis of covariance was used to test for group differences. Analysis of variances/analysis of covariances with $P < 0.05$ significance were further analyzed by Fisher least significant difference *post hoc* analysis to assess group differences.

RESULTS

SST mRNA expression is reduced in the OFC gray matter but elevated in the white matter in schizophrenia

From qualitative observation of the autoradiographic films, SST mRNA expression appeared to be punctate throughout the cortex and highest in layers II–III and V. The deep layer VI boundary and the superficial white matter boundary appeared diffuse without a distinct demarcation at the border between the gray matter and white matter (Figure 3a, b). The SST laminar mRNA expression pattern was consistent with earlier reports.^{13,41,42} The SST sense control produced no hybridization signal indicating riboprobe specificity for SST (Supplementary Figure S1A).

The mean SST mRNA expression level was significantly reduced across all cortical layers I–VI (Table 2A; Figure 3c) in people with schizophrenia relative to the controls. The magnitude of SST mRNA reduction was greatest in layer II (67%; $F(1,65)=160.31$, $P=3.31 \times 10^{-19}$; Table 2A) in schizophrenia. Consistent with this,

Table 1. Summary of demographics for control and schizophrenia groups (mean \pm s.d.)

	Whole cohort	
	Control group ($n=38$)	Schizophrenia group ($n=38$)
Age (years)	52.55 \pm 14.51	52.24 \pm 14.52
Brain pH	6.68 \pm 0.27	6.61 \pm 0.30
PMI (hours)	26.43 \pm 11.69	28.21 \pm 13.57
RIN	7.59 \pm 0.83	7.51 \pm 0.84
Freezer storage time (months)	69.57 \pm 42.73	79.80 \pm 36.84
Gender	10F; 28M	13F; 25M
Hemisphere	24R; 13L; 1NK	18R; 20L
Age of onset (years)		23.77 \pm 6.18
Duration of illness (years)		27.09 \pm 13.99
Atypical/typical		4PAT; 22PT; 1AT; 7T; 1T&AT; 3NK
Antidepressants		19Y; 16N; 3NK
Syndrome		7Neg; 24P; 7NK

Abbreviations: AT, atypical; F, female; L, left; M, male; n , number; N, no; Neg, negative; NK, not known; P, positive; PAT, predominantly atypical; PMI, postmortem interval; PT, predominantly typical; R, right; RIN, RNA integrity number; T, typical; Y, yes.
Presented as mean values \pm s.d.

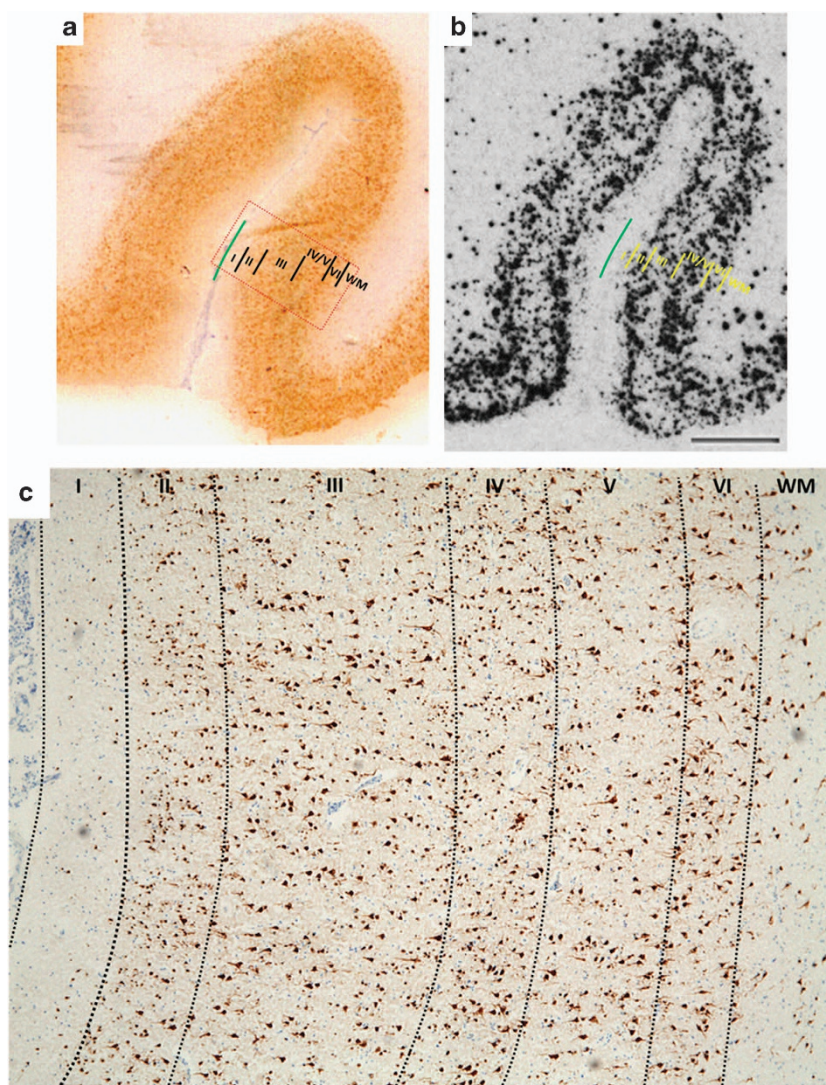


Figure 1. Representative images showing cortical lamina by (a) immunostaining (NeuN) and (b) *in situ* hybridization (somatostatin) in the orbital frontal cortex. (c) Orbitofrontal cortical lamina identification (layers I–VI) based on NeuN immunostaining (magnification $\times 4$). Red rectangular box in a represents the cortical area shown in c.

SST mRNA+ cell density was significantly reduced in the gyrus rectus layer II in people with schizophrenia compared with controls subjects (mean \pm s.d.: 16.54 ± 11.04 neurons/mm² versus 23.44 ± 11.74 neurons/mm²; $t = 2.46$, $df = 64$, $P = 0.02$; Figure 4a). Mean SST mRNA level for layers I–VI (38%; $F(1,68) = 29.63$, $P = 7.76 \times 10^{-7}$) and by qPCR (17%; $t = 2.04$, $df = 71$, $P = 0.04$; Figure 4c) were also reduced in people with schizophrenia compared with controls. qPCR SST mRNA measurements in the OFC (Figure 4c) were consistent with the SST *in situ* hybridization mRNA measurements and positively correlated in people with schizophrenia ($r = 0.77$; $P = 5.0 \times 10^{-8}$).

SST mRNA expression was detectable in the white matter in most sections and was significantly increased (17%; $F(1,62) = 13.33$, $P = 5.39 \times 10^{-4}$; Figure 4b; Table 2B) in people with schizophrenia relative to control subjects.

PV mRNA expression is reduced in cortical layers III and IV in schizophrenia

PV mRNA expression appeared to be highest in the middle cortical layers (layers III–V; Figure 3f). Unlike SST *in situ* mRNA expression,

the gray matter and white matter boundaries were clearly demarcated, with no signal above background evident in the white matter in OFC (Figure 3d, e). Absence of signal in the PV sense control (Supplementary Figure S1B) and laminar expression pattern, consistent with previous reports,^{26,29} indicated specificity of the PV riboprobe used.

PV mRNA was significantly reduced in layers III (18%; $t = 2.59$, $df = 64$, $P = 0.01$) and IV (31%; $F(1,64) = 18.73$, $P = 2.52 \times 10^{-5}$) in people with schizophrenia compared with controls. PV mRNA measured by qPCR did not show a diagnostic difference ($t = -0.37$; $df = 68$; $P = 0.71$; Figure 4d) between schizophrenia (mean = 1.56; s.d. = 0.60) and control (mean = 1.52; s.d. = 0.47) subjects.

FASR mRNA expression is elevated in the OFC in schizophrenia

FASR mRNA expression was significantly increased in people with schizophrenia compared with control subjects (31%; mean \pm s.d.: 1.67 ± 1.01 versus 1.28 ± 0.58 ; $t = -2.10$, $df = 71$, $P = 0.04$; Figure 4e). FASR mRNA expression positively correlated with TNFSF13 mRNA expression and interstitial white matter neuron NeuN density⁹ in the whole cohort (Table 3E).

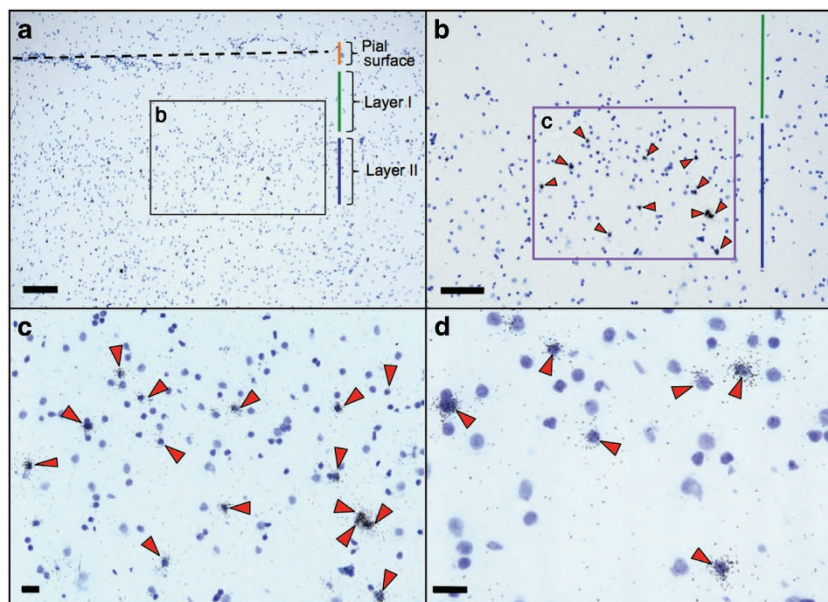


Figure 2. Somatostatin (SST) mRNA+ cell density quantification in orbital frontal cortex (OFC) layer II. Representative photomicrographs showing (a) OFC layer II (magnification $\times 4$; scale bar = 200 μm ; dotted line represents olfactory sulcus; quantification was performed in the gyrus rectus gray matter layer II), (b) orientation of the quantification frame (purple framed box; magnification $\times 10$; scale bar = 100 μm), (c) SST mRNA+ neurons in the quantification frame (magnification $\times 20$; scale bar = 20 μm), and (d) $\times 40$ magnified view (scale bar = 20 μm) showing SST mRNA+ neurons. Orange line indicates pial surface region (a), green line indicates OFC layer I region (a, b) and blue line indicates OFC layer II region (a, b). Red arrowheads (b–d) indicate SST mRNA+ neurons in OFC layer II.

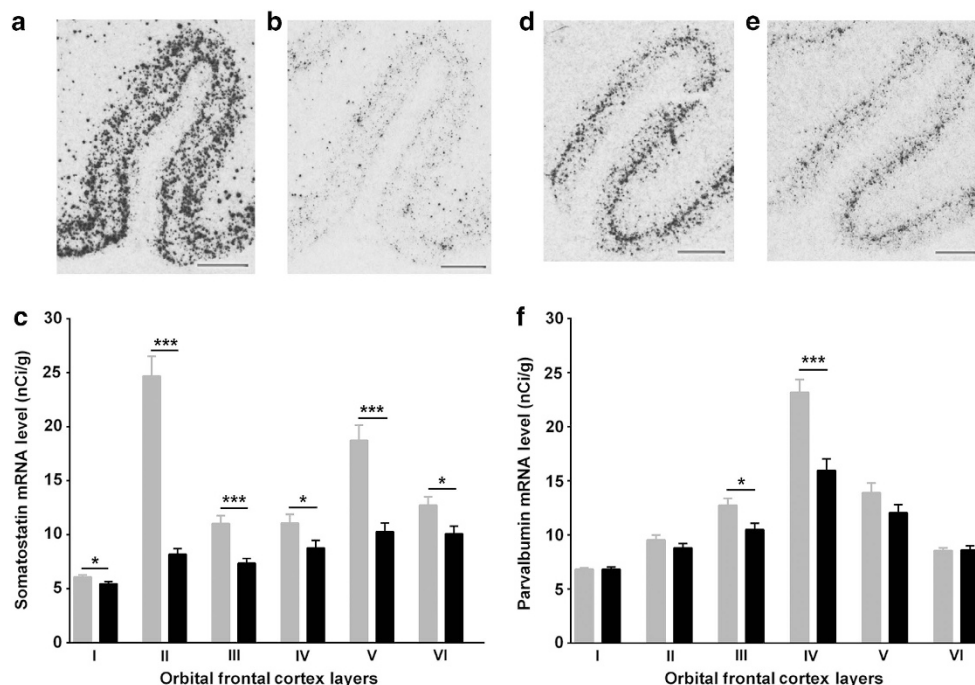


Figure 3. Representative *in situ* hybridization images showing somatostatin (SST: a, b) and parvalbumin (PV: d, e) mRNA distributions in human orbital frontal cortex in control (a, d) and schizophrenia (b, e) subjects, respectively. Laminar-specific SST (c) and PV (f) mRNA levels (nCi/g) in the gray matter in control (gray) and schizophrenia (black) subjects (* $P < 0.05$; *** $P < 0.001$). Scale bar = 2 mm; error bars represent s.e.m.

There was no difference in TNFSF13 mRNA expression in people with schizophrenia compared with control subjects overall (mean \pm s.d.: 0.47 ± 0.190 versus 0.45 ± 0.14 ; $t = -0.515$, $df = 72$, $P = 0.61$; Figure 4f).

Relationship between interneuron markers and cell death signaling mRNAs in the OFC

Next, we examined the relationship between SST mRNA expression (*in situ* hybridization—layer II and qPCR; Table 3A, B) and

Table 2. Analyses of SST mRNA level (nCi/g) in the OFC gray matter (A) and white matter (B) and PV mRNA level (nCi/g) in the OFC gray matter (C)

	% Change	Mean control	s.d. control	Mean schizophrenia	s.d. schizophrenia	t-value	F	df	P-value	Covariates
(A) SST—layers										
I	–10	6.07	1.22	5.45	1.17	—	5.09	66	0.03*	pH, age at death
II	–67	24.69	11.10	8.20	2.97	—	160.31	65	3.31×10^{-19}***	pH, age at death, RIN
III	–60	11.03	7.61	4.39	2.85	—	16.55	65	1.31×10^{-4}***	pH, age at death, RIN
IV	–21	11.08	4.86	8.78	4.13	—	4.63	68	0.04*	pH, age at death
V	–45	18.75	8.58	10.29	4.60	—	24.99	66	4.51×10^{-6}***	pH, age at death, RIN
VI	–21	12.73	4.67	10.09	4.11	—	6.20	65	0.02*	pH, age at death, RIN
(B) White matter										
	17	6.50	1.06	7.62	1.88	—	13.33	62	5.39×10^{-4}***	pH, age at death
(C) PV—layers										
I	0	6.83	0.75	6.83	1.21	—	0.55	64	0.46	PMI
II	–8	9.54	2.68	8.81	2.28	0.60	—	65	0.55	—
III	–18	12.74	3.69	10.50	3.31	2.59	—	64	0.01*	—
IV	–31	23.18	6.74	15.97	6.14	—	18.73	63	5.48×10^{-5}***	pH
V	–13	13.91	5.06	12.06	4.36	—	3.93	64	0.05	Freezer (months)
VI	1	8.55	1.50	8.62	2.16	—	0.03	63	0.86	Freezer (months)

Abbreviations: OFC, orbital frontal cortex; PMI, postmortem interval; PV, parvalbumin; RIN, RNA integrity number; SST, somatostatin.

* $P < 0.05$; *** $P < 0.001$.

other OFC mRNA measures (gray matter—FASR, TNFSF13, and GAD67). We also correlated superficial white matter neuron density measurements (NeuN; GAD65/67)⁹ performed in our earlier study with gray matter mRNA measurements from our current study to examine how increased white matter neuron density may be related to these measures (SST mRNA; SST mRNA+ cell density; PV mRNA; FASR mRNA) using the same postmortem brain tissue cohort.⁹ SST mRNA expression measured by qPCR negatively correlated with FASR and TNFSF13 mRNAs (Table 3A). SST layer II mRNA levels negatively correlated with FASR mRNA, TNFSF13 mRNA, and superficial white matter neuron density (NeuN; GAD65/67)⁹ in the whole cohort (Table 3B). FASR mRNA expression positively correlated with TNFSF13 mRNA and NeuN superficial white matter neuron density,⁹ but inversely with GAD67 (layers I–VI)⁹ mRNA levels in the OFC (Table 3E).

In contrast to SST mRNA, PV mRNA expression (qPCR—Table 3D; *in situ*—Table 3E, F) did not correlate with death receptor signaling mRNAs (FASR; TNFSF13) or superficial white matter neuron density (Table 3D).

Consistent with our earlier study that demonstrated a negative correlation between brain pH and death receptor mRNAs,³⁴ we found a significant negative correlation between TNFSF13 and FASR mRNA expressions and tissue pH (r value between –0.4 and –0.5).

Statistical analysis revealed no significant relationship between measures quantified in the current study and antipsychotic medication, hemisphere, or gender ($P > 0.05$).

DISCUSSION

Main findings

The present study demonstrates that laminar SST mRNA (layers I–VI) deficits are more pronounced in magnitude and extent than PV mRNA (layers III and IV) deficits in the OFC in schizophrenia. These observations are consistent with our earlier observations demonstrating reduced inhibitory interneuron mRNAs (Dlx1; GAD67; SST) in the OFC in three separate cohorts of people with schizophrenia.^{9–11} We provide evidence to show that the cell death receptor mRNA encoding FASR is elevated in the OFC in

people with schizophrenia and, in particular, relates more strongly to SST mRNA than PV mRNA.

Interpretation of findings

Inhibitory interneuron pathology in the DLPFC in schizophrenia is well established, particularly involving SST and PV interneuron mRNAs.^{7,8,13,14} Data from independent postmortem cohorts suggest that the inhibitory interneuron subtype that is most affected in the prefrontal cortex in schizophrenia is SST,^{8,11,14} although this deficit also often includes PV mRNA.^{8,14,29} Unlike SST expression that was reduced both by *in situ* hybridization and qPCR measurements, layer-specific (layers III and IV) reduction for PV was not evident in qPCR results in the current study. These results suggest that SST mRNA deficit may be a major contributor to the cortical inhibitory interneuron pathology found in the OFC of patients with schizophrenia.^{9,10} Our laminar SST mRNA measurements showed greatest reduction in layer II in people with schizophrenia, consistent with previous studies showing reduced gamma-aminobutyric acid (GABA)ergic neuron density in layer II in other cortical areas in schizophrenia.^{15,43,44} We therefore asked whether this reduction in SST mRNA translates into reduced SST mRNA+ cell density. Indeed, our results confirmed reduced SST inhibitory interneuron cell density in the OFC layer II in schizophrenia. PFC layer II non-pyramidal/interneuron-like neurons are known to be susceptible to cell damage or death^{45,46} and are among the last to differentiate in cortical development in humans.⁴⁷ The deficit found in SST layer II interneuron density suggests that there could be a reduction in the number of inhibitory neurons in this layer.

Reduced cortical volume and neuronal loss have been associated with apoptosis^{20,33,48} potentially as a result of increased oxidative stress and excessive glutamatergic activity.^{49,50} In animal models of schizophrenia, NMDA receptor blockade (phencyclidine; ketamine; MK-801) has been shown to result in loss of inhibitory interneurons by apoptosis^{48,51} and behavioral deficits similar to schizophrenia in humans.^{51,52} A recent study from our laboratory demonstrated that increased death receptor signaling mRNAs (FASR; TNFSF13) were inversely related to both SST and PV mRNAs,³⁴ known to be reduced in the DLPFC.^{8,11,14,30} In the OFC,

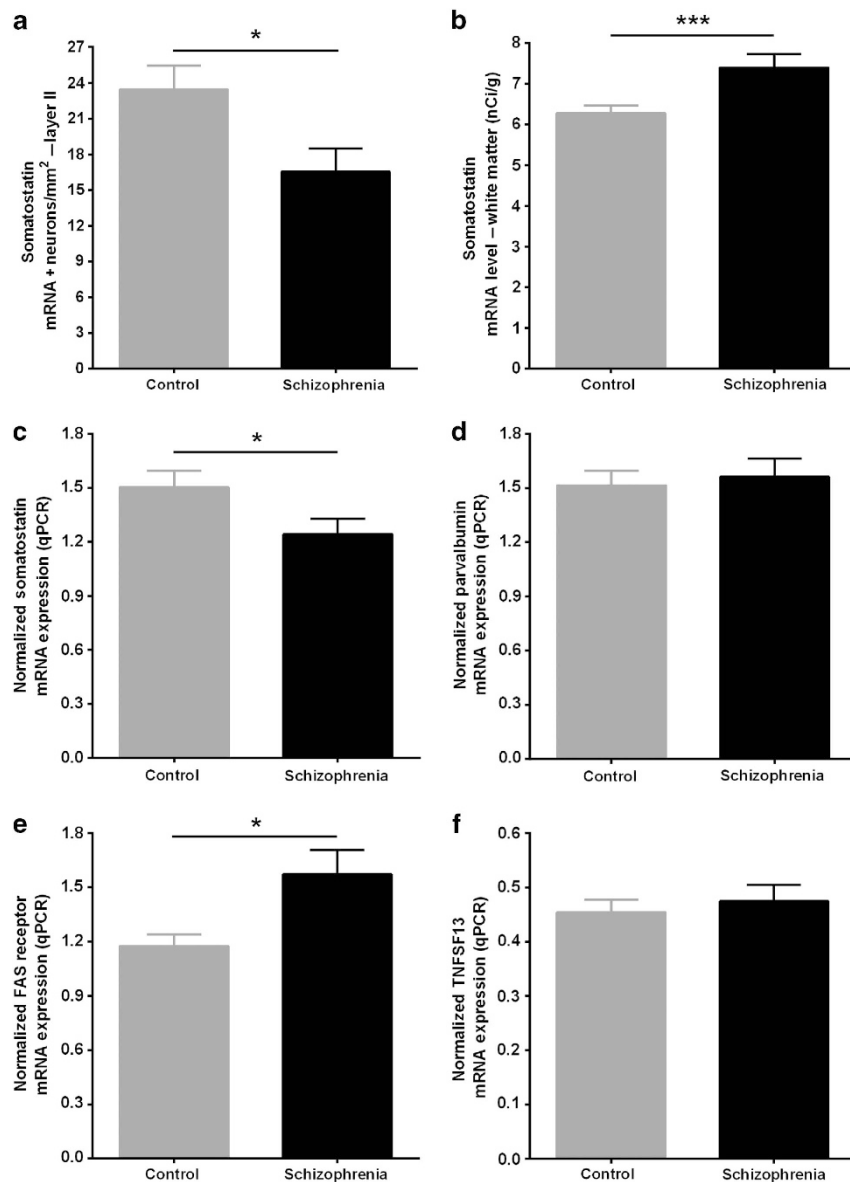


Figure 4. (a) Orbital frontal cortex (OFC) layer II somatostatin (SST) mRNA+ neuron density in OFC layer II (*in situ* hybridization). (b) SST mRNA expression in the white matter in human OFC. Quantitative PCR (qPCR) mRNA expression for (c) SST, (d) parvalbumin, (e) FAS receptor, and (f) TNFSF13 in schizophrenia (black) compared with control (gray) subjects in the OFC. Error bars represent s.e.m. * $P < 0.05$; *** $P < 0.001$.

we found an increase in the FASR mRNA expression in schizophrenia. The death receptor signaling mRNAs (FASR and TNFSF13) quantified in the OFC inversely correlated with SST mRNA, but not with PV mRNA. More specifically, SST mRNA and SST mRNA+ cell density in layer II also showed a significant inverse relationship with the death receptor signaling mRNAs in people with schizophrenia. This suggests that, in our cohort, SST interneurons are vulnerable to the elevated death receptor signaling in the OFC resulting in putative neuronal loss as evident from our reduced SST mRNA+ cell density counts for layer II in schizophrenia. The relationship between death receptor signaling mRNAs and inhibitor interneuron markers determined in the current study suggests that reduction in SST mRNA is more specifically linked to elevated death receptor signaling mRNAs in the ventral (OFC) portions of the human frontal cortex as compared with the dorsal (DLPFC).

Multiple studies have demonstrated reduced inhibitory interneuron density in the cortices of people with schizophrenia, but

without a testable hypothesis to account for this drop in neuronal density.^{15,17,20,22,27} Some studies that reported a reduction in GAD67 mRNA+ cell density interpreted the change in density to be due to reduced mRNA expression per cell (below levels of detection) with no change in cell density.^{23,28,29} Akbarian *et al.*²³ drew this inference from their findings showing a significant reduction in GAD67 mRNA+ neurons (greatest in layer II) but no change in the total number of Nissl-stained neurons, whereas Volk *et al.*²⁸ seemed to base their interpretation primarily on findings by Akbarian *et al.*²³ This interpretation was extended to subsequent studies showing reduced inhibitory interneuron density in people with schizophrenia.^{13,29,30,53,54} Interestingly, as calbindin mRNA expression^{8,11} as well as cell density⁵⁵ have been shown to be elevated in the prefrontal cortex, it is possible that reduction in SST neuron density may be compensated for by an increased calbindin neuron density resulting in subtle or no significant overall changes in small Nissl-stained neurons in people with schizophrenia as reported by Akbarian *et al.*²³

Table 3. (A) SST mRNA (qPCR), (B) SST layer II mRNA (*in situ* hybridization), (C) SST layer II mRNA+ cell density, (D) PV mRNA (qPCR), (E) FASR mRNA (qPCR), and (F) TNFSF13 (qPCR) correlation with other OFC measures

	Schizophrenia		Whole cohort		Control	
	r	P-value	r	P-value	r	P-value
(A) SST qPCR						
FASR	−0.391	0.018*	−0.243	0.043*	0.105	0.553
TNFSF13	−0.282	0.095	−0.243	0.041*	−0.189	0.275
GAD67 (LI–VI)	0.143	0.428	0.340	0.005**	0.437	0.01*
NeuN (sIWMN)	−0.146	0.411	−0.222	0.073	−0.162	0.375
GAD65/67 (sIWMN)	−0.249	0.176	−0.204	0.112	−0.038	0.837
(B) SST LII						
FASR	−0.485	0.004**	−0.415	4.79 × 10^{−4}***	−0.215	0.223
TNFSF13	−0.382	0.026*	−0.249	0.041*	−0.156	0.379
GAD67 (LI–VI)	0.391	0.027*	0.559	1.06 × 10^{−6}***	0.620	9.17 × 10^{−5}***
NeuN (sIWMN)	−0.233	0.191	−0.457	1.32 × 10^{−4}***	−0.155	0.398
GAD65/67 (sIWMN)	−0.226	0.229	−0.356	0.005**	−0.077	0.681
Dlx1	0.085	0.631	0.308	0.009**	0.005	0.976
(C) SST LII mRNA+ cell density						
SST LII mRNA	0.475	9.14 × 10^{−4}***	0.541	5.77 × 10^{−6}***	0.519	0.002**
FASR	−0.574	5.90 × 10^{−4}***	−0.509	1.76 × 10^{−5}***	−0.263	0.147
TNFSF13	−0.452	0.011*	−0.336	0.007**	−0.187	0.306
GAD67 (L I–VI)	0.387	0.042*	0.526	1.59 × 10^{−5}***	0.575	5.82 × 10^{−4}***
NeuN (sIWMN)	−0.503	0.005**	−0.238	0.069	0.345	0.062
GAD65/67 (sIWMN)	−0.178	0.384	−0.245	0.072	−0.165	0.393
(D) PV qPCR						
FASR	0.013	0.943	−0.004	0.975	−0.071	0.698
TNFSF13	−0.251	0.139	−0.210	0.086	−0.137	0.454
GAD67 (LI–VI)	0.166	0.363	0.170	0.179	0.245	0.176
NeuN (sIWMN)	0.094	0.603	0.075	0.554	−0.052	0.782
(E) FASR qPCR						
TNFSF13	0.710	8.38 × 10^{−7}***	0.607	1.53 × 10^{−8}***	0.387	0.022*
PV LIV	−0.164	0.362	−0.118	0.357	0.271	0.147
GAD67 (LI–VI)	−0.452	0.008**	−0.341	0.005**	−0.021	0.906
NeuN (sIWMN)	0.239	0.173	0.293	0.018*	0.154	0.409
(F) TNFSF13 qPCR						
PV LIII	−0.185	0.304	−0.089	0.487	0.073	0.697
PV LIV	−0.257	0.142	−0.119	0.349	0.081	0.670
PV LI–VI	−0.272	0.114	−0.105	0.396	0.176	0.326

Abbreviations: FASR, FAS receptor; OFC, orbital frontal cortex; qPCR, quantitative PCR; PV, parvalbumin; sIWMN, superficial interstitial white matter neuron; SST, somatostatin.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Although one study found a significant reduction in grain density per cell in PV mRNA+ neurons (22%) with no change in PV mRNA+ neuron density,²⁹ the same group of investigators reported a relatively similar reduction in grain density per cell in SST mRNA+ neurons (25 and 31% layer V and layer II, respectively) and a significant reduction in SST mRNA+ neuron density (23 and 26%, respectively) in people with schizophrenia.¹³ Interestingly, reduction in both PV and SST mRNA+ neuron densities was interpreted as reduced mRNA expression beyond level of detection with no change in neuron density. Our findings in the OFC may explain these contrasting interpretations by Benes (loss of layer II non-pyramidal/interneuron-like neurons)²⁰ and Akbarian/Lewis (reduced interneuron mRNAs below levels of detection without change in neuronal density)^{23,28} more appropriately, as our findings suggest that indeed there may be a loss of SST inhibitory interneuron subtype due to elevated death receptor signaling resulting in cell death, whereas other inhibitory interneuron

subtypes may be unchanged or even increased resulting in no net change in small neuron density.

Strengths and limitations

Previous findings from our laboratory also demonstrate that the magnitude of another neuropathology, subcortical interstitial white matter neuron density increase, is more pronounced in the OFC.^{9,11} In the OFC, we find that SST layer II mRNA+ neuron density and the superficial white matter neuron measures (NeuN; GAD65/67)⁹ are inversely correlated indicating that reduced SST mRNA+ neuron density in layer II is related to increased white matter neuron density. A limitation of our study is that we are unable to discern whether these two events are causally related to one another, nor are we able to completely rule out the effects of antipsychotic medications. It was difficult to detect TNFSF13 and FASR by immunohistochemistry due to its low abundance in the human postmortem brain tissue, as evident

from the *in situ* hybridization conducted on human brain tissue. Lack of laminar mRNA expression data and protein measurement for TNFSF13/FASR/TUNEL labeling remains a limitation of our current study.

We found a significant laminar effect for PV mRNA expression (reduced in layers III and IV) in the OFC in schizophrenia, but not by qPCR. It is possible that the layer-specific effect found for PV mRNA expression in layers III and IV (mid-cortex) may be difficult to detect when using a homogenate-based mRNA measurement approach. This is consistent with our earlier observation in the DLPFC where we found that laminar-specific mRNA change (ErbB4-pan) can be diluted in homogenate-based measurement.⁴⁶

Conclusions

To conclude, we provide evidence linking inhibitory interneuron deficits and elevated cell death signaling mRNAs in schizophrenia. These findings provide some support to earlier studies that found reduced inhibitory interneuron densities.^{7,13,54} The current study also brings forth death receptor signaling, involving FASR and TNFSF13 mRNAs, as a potential mechanism by which interneurons may be compromised in schizophrenia. The possible greater pathology in SST mRNA containing neurons as compared with PV containing neurons in people with schizophrenia may be a key finding to be taken into consideration when developing relevant animal models for schizophrenia. Our study also emphasizes that it may be informative to compare the extent of neuropathological changes across multiple brain regions in people with schizophrenia.

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CONTRIBUTIONS

DJ with CSW designed the study and wrote the protocol. DJ, VSC, and JCO performed the experiments. DJ and VSC wrote the first draft. DJ, VSC, and CSW were responsible for the data analysis. DJ, VSC, JCO, and CSW reviewed and revised the manuscript draft. Each of the authors have reviewed the manuscript and have approved the final manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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