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Selective Pyramidal Cell Reduction of $GABA_A$ Receptor $\alpha 1$ Subunit Messenger RNA Expression in Schizophrenia

Jill R Glausier[1] and David A Lewis*[,1,2]

[1]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA

Levels of messenger RNA (mRNA) for the $\alpha 1$ subunit of the $GABA_A$ receptor, which is present in 60% of cortical $GABA_A$ receptors, have been reported to be lower in layer 3 of the prefrontal cortex (PFC) in subjects with schizophrenia. This subunit is expressed in both pyramidal cells and interneurons, and thus lower $\alpha 1$ subunit levels in each cell population would have opposite effects on net cortical excitation. We used dual-label *in situ* hybridization to quantify $GABA_A$ $\alpha 1$ subunit mRNA expression in calcium/calmodulin-dependent kinase II α (CaMKIIα)-containing pyramidal cells and glutamic acid decarboxylase 65 kDa (GAD65)-containing interneurons in layer 3 of the PFC from matched schizophrenia and healthy comparison subjects. In subjects with schizophrenia, mean $GABA_A$ $\alpha 1$ subunit mRNA expression was significantly 40% lower in pyramidal cells, but was not altered in interneurons. Lower $\alpha 1$ subunit mRNA expression in pyramidal cells was not attributable to potential confounding factors, and thus appeared to reflect the disease process of schizophrenia. These results suggest that pyramidal cell inhibition is reduced in schizophrenia, whereas inhibition of GABA neurons is maintained. The cell type specificity of these findings may reflect a compensatory response to enhance layer 3 pyramidal cell activity in the face of the diminished excitatory drive associated with the lower dendritic spine density on these neurons.
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INTRODUCTION

Prefrontal cortical (PFC) dysfunction in schizophrenia is associated with impaired local inhibitory signaling (Coyle, 2004; Lewis *et al*, 2005). For example, lower levels of the major γ-aminobutyric acid (GABA)-synthesizing enzyme, glutamic acid decarboxylase 67 kDa (GAD67), are consistently found in postmortem studies (Gonzalez-Burgos *et al*, 2010), and a single-nucleotide polymorphism in the GAD67 gene (*GAD1*) is associated with impaired cognitive performance and decreased PFC GAD67 messenger RNA (mRNA) expression in schizophrenia subjects (Straub *et al*, 2007). However, the relationship of this presynaptic deficit to the fast inhibitory neurotransmission mediated by $GABA_A$ receptors on different populations of postsynaptic cells is not well studied.

Most $GABA_A$ receptors are composed of 2α, 2β and 1γ or δ-subunit (Mohler, 2006). The $\alpha 1$ subunit is present in over 60% of cortical $GABA_A$ receptors, and its expression is enriched in both pyramidal cells and interneurons of PFC layers 3-superficial 5 (Beneyto *et al*, 2011). Some (Akbarian *et al*, 1995; Beneyto *et al*, 2011; Hashimoto *et al*, 2008a, b), but not all (Duncan *et al*, 2010), well-controlled studies have reported lower levels of $GABA_A$ $\alpha 1$ subunit mRNA in PFC gray matter from schizophrenia subjects, with this decrease most prominent in layers 3 and 4 (Beneyto *et al*, 2011).

As the effects of disease on gene expression might differ across subsets of neurons, analysis of $\alpha 1$ subunit expression in specific cell populations should have greater sensitivity for detecting disease-related differences than tissue level approaches, and thus provide greater explanatory power for determining the functional significance of disease-related differences in expression. For example, $GABA_A$ $\alpha 1$ receptors are present in both pyramidal cells and interneurons, and they are especially prominent at the powerful inhibitory inputs from parvalbumin (PV) basket neurons to the perisomatic region of pyramidal cells and between PV neurons (Klausberger *et al*, 2002; Nusser *et al*, 1996; Nyíri *et al*, 2001). Thus, lower levels of $GABA_A$ $\alpha 1$ receptors in pyramidal cells would increase net cortical excitation, whereas lower levels in interneurons would have the opposite effect. The present study was designed to discriminate between these alternatives.

*Correspondence: Dr DA Lewis, Department of Neuroscience, Western Psychiatric Institute and Clinic, University of Pittsburgh, 3811 O'Hara Street, Biomedical Science Tower W-1654, Pittsburgh, PA, 15213, USA, Tel: +1 412 383 8548, Fax: +1 412 624 9910, E-mail: lewisda@upmc.edu
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MATERIALS AND METHODS

Human Subjects

Brain specimens from 32 subjects were recovered during autopsies conducted at the Allegheny County Medical Examiner's Office (Pittsburgh, PA) after obtaining consent from the next of kin. Inclusion criteria included ages 16–85 years, death by accident, natural causes or suicide, and death suddenly, out of the hospital, and without evidence of an agonal process. Exclusion criteria included history of intravenous drug use, hepatitis or HIV infection, neurodegenerative disorders, or mental retardation. Neuropathological examination of each brain revealed no abnormalities, except for subject 622 who had an infarction limited to the distribution of the inferior branch of the right middle cerebral artery, but PFC area 9 appeared unaffected. An independent committee of experienced research clinicians made consensus DSM-IV diagnoses for each subject, using the results of structured interviews conducted with family members and review of medical records, as previously described (Glantz and Lewis, 2000). None of the comparison subjects had any lifetime history of an Axis I diagnosis, except for one subject who had an earlier diagnosis of post-traumatic stress disorder that had been in remission for 39 years. Toxicology screens for all subjects revealed positive plasma alcohol levels ($<0.02\%$) in two comparison subjects. None of the schizophrenia subjects tested positive for drugs of abuse. The two schizophrenia subjects who died of drug overdoses were positive for propoxyphene, diazepam and acetaminophen (581) and salicylate, imipramine and phenytoin (539).

To control experimental variance and reduce biological variance between groups, each schizophrenia subject ($n=16$) was matched for sex, and as closely as possible for age, with one healthy comparison subject. Subject groups did not differ in mean age, postmortem interval (PMI), RNA integrity number (RIN), brain pH, or freezer storage time (Table 1). The schizophrenia subject in each pair has previously been shown to express lower $GABA_A$ α1 subunit mRNA in PFC area 9, relative to its matched-comparison subject (Beneyto *et al*, 2011). All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research Involving the Dead, and Institutional Review Board for Biomedical Research.

Tissue Preparation

The right hemisphere of each brain was blocked coronally, immediately frozen and stored at −80 °C (Volk *et al*, 2000). Sections (20 μm) from the anterior–posterior level, corresponding to the middle portion of the superior frontal sulcus, were cut serially on a cryostat and collected into tubes containing Trizol reagent (Invitrogen, Carlsbad, CA) for RNA isolation and RIN determination (Eggan *et al*, 2008), or mounted on SuperFrost Plus glass slides (VWR International, West Chester, PA) for *in situ* hybridization (ISH). The localization of dorsolateral PFC area 9 was determined by cytoarchitectonic criteria from the Nissl-stained sections (Rajkowska and Goldman-Rakic, 1995). Four sections from each subject were used in total for both experiments, such that there were two consecutive sets of sections approximately 300 μm apart. Each experiment used one section from each set, resulting in two sections per subject per experiment. Thus, a total of 64 slides were analyzed in the calcium/calmodulin-dependent protein kinase II α (CaMKIIα)/α1 condition. Technical issues resulted in 13 subject pairs with two slides each, and 2 subject pairs with one slide each, for a total of 56 slides analyzed in 15 subject pairs, in the glutamic acid decarboxylase 65 kDa (GAD65)/α1 condition.

Dual Label ISH

Pyramidal cells were identified using riboprobes against CaMKIIα because its mRNA expression is specific to pyramidal cells and is unaltered in schizophrenia (Albert *et al*, 2002; Jones *et al*, 1994b; Liu and Jones, 1996; Longson *et al*, 1997). Interneurons were identified using riboprobes against GAD65 because its mRNA expression is specific to interneurons and is unaltered in schizophrenia (Guidotti *et al*, 2000; Ribak, 1978). Templates for the synthesis of riboprobes against human CaMKIIα, GAD65 and $GABA_A$ α1 subunit were generated by polymerase chain reaction. Specific primer sets amplified a 366 bp fragment corresponding to bases 2787–3153 of the human *CAMK2A* gene (GenBank NM_015981), a 484 bp fragment corresponding to bases 156–640 of the human *GAD2* gene (GenBank NM_000818), and a 587 bp fragment corresponding to bases 846–1433 of the human *GABRA1* gene (GenBank NM_000806). Nucleotide sequencing revealed 99% homology for the amplified *CAMK2A* and *GAD2* fragments, and 100% homology for the amplified *GABRA1* fragment to previously reported sequences. Sense and antisense riboprobes were transcribed *in vitro* in the presence of ^{35}S-CTP (PerkinElmer, Waltham, MA) for the $GABA_A$ α1 subunit, and in the presence of digoxigenin (DIG) -11-UTP (Roche, Mannheim, Germany) for CaMKIIα and GAD65, using T7 or SP6 polymerases. The riboprobes were purified by centrifugation through RNeasy mini spin columns (Qiagen, Valencia, CA), and reduced to approximately 100 bp by alkaline hydrolysis to increase tissue penetration. Sense riboprobes showed no specific labeling for either CaMKIIα or GAD65 (Supplementary Figure 1), or for $GABA_A$ α1 (Beneyto *et al*, 2011).

Following fixation using 4% paraformaldehyde in 0.1 M phosphate-buffered saline, sections were hybridized with ^{35}S-labeled α1 subunit riboprobe (1×10^7 cpm/ml) and DIG-labeled CaMKIIα riboprobe (100 ng), or ^{35}S-labeled α1 subunit riboprobe (1×10^7 cpm/ml) and DIG-labeled GAD65 riboprobe (250 ng) in a standard hybridization buffer at 56 °C for 16–20 h. After hybridization, sections were washed in a 50% formamide solution at 63 °C, treated with RNase A at 37 °C, washed in 0.1× SSC (150 mM sodium chloride, 15 mM sodium citrate) at 66 °C and air-dried. Sections were then incubated in blocking solution (3% BSA in 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Triton X-100) for 30 min, followed by anti-DIG antibody conjugated with alkaline phosphatase diluted 1 : 500 in 1% BSA, 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Triton X-100 (Roche) for 3 h at 4 °C. After washing and air-drying, sections were exposed to BioMax MR film (Eastman Kodak, Rochester, NY) for 3 days to confirm ^{35}S α1 subunit signal. Upon confirmation, slides were coated with NTB2 emulsion (Kodak), exposed for 16 days at 4 °C, developed with D-19 (Kodak), and washed. The slides were then

Table 1 Comparison and Schizophrenia Subject Characteristics

Comparison subjects									Schizophrenia subjects								
Pair	Case	Sex/ race	Age (years)	PMI	Storage time (months)	RIN	pH	Cause of death	Case	DSM IV diagnosis	Sex/ race	Age (years)	PMI	Storage time (months)	RIN	pH	Cause of death
1	592	M/B	41	22.1	160.5	9.0	6.7	ASCVD	533	Chronic undifferentiated schizophrenia	M/W	40	29.1	170.3	8.4	6.8	Accidental asphyxiation
2	567	F/W	46	15.0	164.5	8.9	6.7	Mitral valve prolapse	537	Schizoaffective disorder	F/W	37	14.5	169.6	8.6	6.7	Suicide by hanging
3	604	M/W	39	19.3	158.2	8.6	7.1	Hypoplastic coronary artery	581	Chronic paranoid schizophrenia; ADC; OAC	M/W	46	28.1	162.7	7.9	7.2	Accidental combined drug overdose
4	1406	M/B	27	14.6	29.3	8.3	6.4	Peritonitis	547	Schizoaffective disorder	M/B	27	16.5	168.2	7.4	7.0	Heat stroke
5	685	M/W	56	14.5	147.6	8.1	6.6	Hypoplastic coronary artery	622	Chronic undifferentiated schizophrenia	M/W	58	18.9	155.3	7.4	6.8	Right MCA infarction
6	822	M/B	28	25.3	124.1	8.5	7.0	ASCVD	787	Schizoaffective disorder; ODC	M/B	27	19.2	130.3	8.4	6.7	Suicide by gun shot
7	727	M/B	19	7.0	141.1	9.2	7.2	Trauma	829	Schizoaffective disorder; ADC; OAR	M/W	25	5.0	122.0	9.3	6.8	Suicide by salicylate overdose
8	871	M/W	28	16.5	113.5	8.5	7.1	Trauma	878	Disorganized schizophrenia; ADC	M/W	33	10.8	112.5	8.9	6.7	Myocardial fibrosis
9	700	M/W	42	26.1	145.2	8.7	7.0	ASCVD	539	Schizoaffective disorder; ADR	M/W	50	40.5	169.4	8.1	7.1	Suicide by combined drug overdose
10	988	M/W	82	22.5	92.1	8.4	6.2	Trauma	621	Chronic undifferentiated schizophrenia	M/W	83	16.0	155.6	8.7	7.3	Accidental asphyxiation
11	852	M/W	54	8.0	121.0	9.1	6.8	Cardiac tamponade	781	Schizoaffective disorder; ADR	M/B	52	8.0	136.0	7.7	6.7	Peritonitis
12	987	F/W	65	21.5	92.1	9.1	6.8	ASCVD	802	Schizoaffective disorder; ADC; ODR	F/W	63	29.0	127.3	9.2	6.4	Right ventricular dysplasia
13	818	F/W	67	24.0	125.2	8.4	7.1	Anaphylactic reaction	917	Chronic undifferentiated schizophrenia	F/W	71	23.8	105.2	7.0	6.8	ASCVD
14	857	M/W	48	16.6	115.3	8.9	6.7	ASCVD	930	Disorganized schizophrenia; ADR; OAR	M/W	47	15.3	101.8	8.2	6.2	ASCVD
15	739	M/W	40	15.8	140.2	8.4	6.9	ASCVD	933	Disorganized schizophrenia	M/W	44	8.3	101.2	8.1	5.9	Myocarditis
16[a]	546[a]	F/W	37	23.5	168.5	8.6	6.7	ASCVD	587[a]	Chronic undifferentiated schizophrenia; AAR	F/B	38	17.8	161.3	9.0	7.0	Myocardial hypertrophy
		Mean	44.9	18.3	127.4	8.7	6.8				Mean	46.3	18.8	140.5	8.3	6.8	
		SD	16.7	5.8	35.3	0.3	0.3				SD	16.3	9.4	26.4	0.7	0.4	
		Mean[a]	45.5	17.9	124.7	8.7	6.8				Mean[a]	46.9	18.9	139.2	8.2	6.7	
		SD[a]	17.2	5.8	34.7	0.3	0.3				SD[a]	16.7	9.7	26.7	0.7	0.4	

Abbreviations: ADC, alcohol dependence, current at time of death; ADR, alcohol dependence, in remission at time of death; AAR, alcohol abuse, in remission at time of death; ODC, other substance dependence, current at time of death; ODR, other Substance dependence, in remission at time of death; OAC, other substance abuse, current at time of death; OAR, other substance abuse, in remission at time of death; M, male; F, female; B, black; W, white; PMI, postmortem interval; RIN, RNA integrity number; ASCVD, arteriosclerotic cardiovascular disease; MCA, middle coronary artery.
[a]Subject pair excluded from GAD65/α1 analysis.

incubated with detection buffer (100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM $MgCl_2$; Roche) for 5 min at room temperature, then in 0.34 mg/ml nitroblue tetrazolium chloride and 0.18 mg/ml 5-bromo-4-chloro-3-indolyl phosphate (color substrate solution) for 62–96 h at 4 °C. The color reaction was stopped with RNase-free water, the slides were air-dried, liquid overlay (VWR) was applied, and allowed to dry for 48 h, and then slides were coverslipped using Permount (Fisher Scientific).

Quantification of GABA$_A$ α1 Subunit mRNA Expression in Pyramidal Cells and Interneurons

Counting of ^{35}S α1 subunit silver grains on coded slides was performed using a Microcomputer Imaging Device system (MCID; InterFocus Imaging, Linton, England). On each tissue section, five 1 mm-wide cortical traverses extending from the pial surface to the white matter were placed where area 9 was cut perpendicular to the cortical surface. Layer deep 3, defined as extending from 35–50% of the distance from the pial surface to the layer 6-white matter border (Glantz and Lewis, 2000), was sampled because tissue levels of α1 subunit mRNA are significantly lower in schizophrenia (Beneyto *et al*, 2011) and because PV cells are a predominant proportion of GABA neurons in primate PFC in this laminar location (Conde *et al*, 1994; Gabbott and Bacon, 1996). Four sampling frames (170 × 120 μm) were placed in layer deep 3, such that the edges of the frame were equidistant from the border of the traverse and the edge of the next sampling frame, and the top or bottom of the frames was equidistant from the layer deep 3 upper or lower border (Morris *et al*, 2008). In a bright-field image of the sampling frame, circles with a 30 μm diameter (708.4 μm^2 area) for pyramidal cells, or 22 μm diameter (380.3 μm^2 area) for interneurons (Hashimoto *et al*, 2003; Pierri *et al*, 2001; Rajkowska *et al*, 1998; Volk *et al*, 2000) were centered over each purple DIG-labeled neuron not touching the frame exclusion lines. In a dark-field image of the same sampling frame, the number of grains within each circle was calculated by the MCID system. Background grain signal was determined in each sampling frame by first free drawing around the largest area devoid of grain clusters. Next, the average number of background grains per 708.4 μm^2 for pyramidal cells, or 380.3 μm^2 for interneurons was calculated for each tissue section. This background measure was subtracted from each neuronal measure, resulting in the background-corrected number of α1 subunit grains per pyramidal cell or interneuron. These values were averaged across neurons for a subject, resulting in a single measure for each subject of the number of α1 subunit grains per pyramidal cell and interneuron. Total numbers of pyramidal cells (comparison: 1934; schizophrenia: 2070; $t_{(1,15)} = -0.926$, $p = 0.369$) and interneurons (comparison: 932; schizophrenia: 1033; $t_{(1,14)} = -1.774$, $p = 0.098$) sampled did not differ between subject groups.

Statistical Analysis

Two analysis of covariance (ANCOVA) models were performed. The first model assessed the effect of diagnostic group on number of α1 subunit grains per neuron, using subject pair as a blocking factor, and PMI, RIN, pH, and tissue storage time as covariates because they may affect mRNA integrity, quantity, and preservation (Beneyto *et al*, 2009). A second unpaired ANCOVA model was performed to validate the first model, using diagnostic group as the main effect, and sex, age, PMI, pH, RIN, and storage time as covariates. These ANCOVAs revealed age as the only covariate with a significant effect. Thus, the reported unpaired ANCOVAs include only age as a covariate. As age was a pairing factor, the paired ANCOVAs include only pair as a blocking factor.

The influences of potential confounding factors on the number of α1 subunit grains per neuron in subjects diagnosed with schizophrenia were assessed with ANCOVA models, using each confounding variable (schizoaffective disorder; suicide; antidepressants, benzodiazepines, or sodium valproate, or antipsychotics at time of death; diagnosis of substance abuse or dependence ATOD) as the main effect, and sex, age, pH, RIN, PMI, and tissue storage time as covariates.

RESULTS

In CaMKIIα-labeled tissue, both large, DIG-labeled, pyramidal neurons with α1 subunit grain clusters, and fewer, smaller, single-labeled grain clusters, likely to be interneurons, were observed (Figure 1a). Mean (SD) α1 subunit grains per pyramidal cell were significantly 40% lower (paired: $F_{(1,15)} = 19.06$, $p = 0.001$; unpaired: $F_{(1,32)} = 9.774$, $p = 0.004$) in schizophrenia subjects (82.2 ± 44.2) than in comparison subjects (136.9 ± 59.1). In 15/16 subject pairs, the schizophrenia subject exhibited fewer α1 subunit grains per pyramidal cell than its matched comparison subject (Figure 1c).

In GAD65-labeled tissue, smaller DIG-labeled interneurons with α1 subunit grain clusters, and more numerous, larger, single-labeled grain clusters, likely to be pyramidal cells, were observed (Figure 1b). Mean α1 subunit grains per interneuron did not differ (paired: $F_{(1,14)} = 1.794$, $p = 0.202$; unpaired: $F_{(1,27)} = 1.515$, $p = 0.229$) between schizophrenia (72.7 ± 20.7) and comparison (84.8 ± 32.3) subjects (Figure 1d). The 14% difference in the group means was largely driven by one comparison subject with α1 subunit mRNA levels nearly three SD greater than the group mean. Excluding that subject pair confirmed that the group means for interneuron α1 subunit mRNA levels (schizophrenia subjects: 73.6 ± 21.1; comparison subjects 78.5 ± 22.2) were nearly identical (paired: $F_{(1,13)} = 0.699$, $p = 0.418$; unpaired: $F_{(1,25)} = 0.324$, $p = 0.574$).

In the schizophrenia subjects, α1 subunit grains per pyramidal cell (all $F_{(1,8)} < 2.909$, all $p > 0.127$) (or per interneuron (all $F_{(1,6)} < 2.238$, all $p > 0.185$)) did not differ as a function of sex; schizoaffective diagnosis; a diagnosis of substance abuse or dependence ATOD; benzodiazepines, sodium valproate or antipsychotics use ATOD; or suicide (Figure 2). Eight schizophrenia subjects had a current or past diagnosis of alcohol abuse or dependence, and seven had no lifetime history of any substance-use diagnosis. The density of α1 subunit grains per pyramidal cell was 43 and 45% lower, respectively, in these schizophrenia subjects relative to their comparison subjects, further confirming that the group differences are associated with a diagnosis of

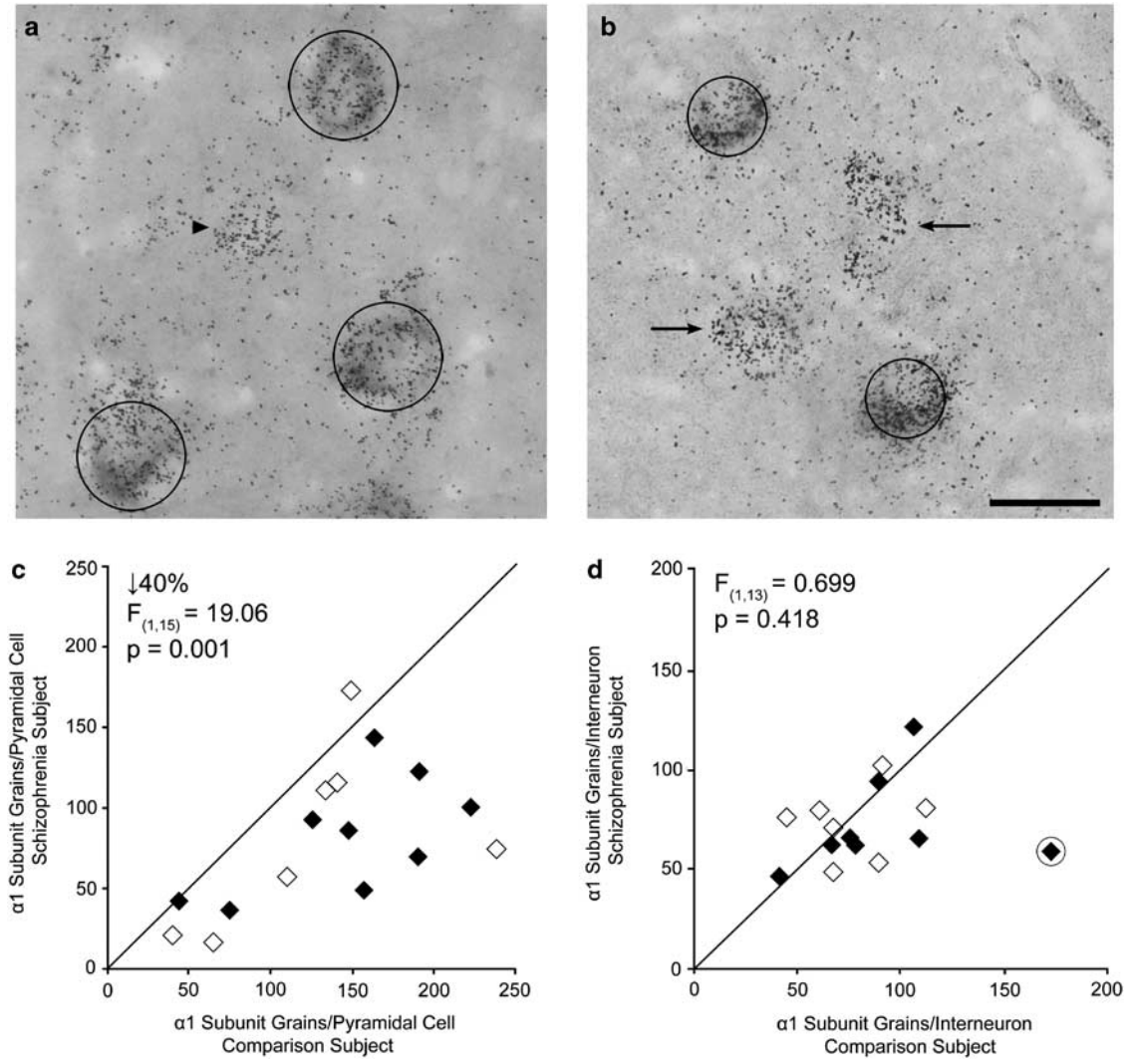

Figure 1 Representative micrographs of calcium/calmodulin-dependent kinase II α (CaMKIIα)/α1 subunit labeling (a), and glutamic acid decarboxylase 65 kDa (GAD65)/α1 subunit labeling (b). Pyramidal cells and interneurons were identified by the purple digoxigenin (DIG) reaction product, and circles of 30 μm (a) or 22 μm (b) diameter were centered over the DIG label. $GABA_A$ α1 subunit silver grains were quantified within each circle. Note the presence of single $GABA_A$ α1-labeled grain clusters in (a; arrowhead, presumed interneuron) and (b; arrows, presumed pyramidal cells). Comparison of $GABA_A$ α1 subunit messenger RNA (mRNA) levels in pyramidal cells (c) and interneurons (d) in matched pairs of comparison subjects, and subjects with schizophrenia (filled) or schizoaffective disorder (open). Markers below the diagonal unity line indicate lower mean α1 subunit grains per neuron in the schizophrenia subject relative to its comparison subject. In (d), the outlier pair is circled. Scale bar is 30 μm.

schizophrenia and not an effect of substance abuse. Antidepressants ATOD did have a significant effect on the number of α1 subunit grains per pyramidal cell in schizophrenia subjects ($F_{(1,8)} = 8.091$, $p = 0.022$); however, in schizophrenia subjects not on antidepressants ATOD, the density of α1 subunit grains per pyramidal was still significantly 32% lower than in comparison subjects ($t_{(1,10)} = 3.520$, $p = 0.006$). Age was significantly correlated with α1 subunit mRNA expression in pyramidal cells ($r = 0.404$, $F_{(1,30)} = 5.863$, $p = 0.022$) and interneurons ($r = 0.451$, $F_{(1,26)} = 6.619$, $p = 0.016$).

DISCUSSION

In schizophrenia subjects, α1 subunit mRNA expression was significantly 40% lower in layer deep 3 pyramidal cells, but was not altered in interneurons. The selective reduction of α1 subunit mRNA in pyramidal cells appears to be a consequence of the illness, and not of factors commonly associated with the illness. Expression of α1 subunit mRNA in pyramidal cells did not differ as a function of antipsychotic medication use ATOD, consistent with earlier findings that cortical α1 subunit mRNA levels are not altered in monkeys chronically treated with haloperidol or olanzapine (Beneyto *et al*, 2011). Similarly, pyramidal cell levels of α1 mRNA were significantly lower in schizophrenia subjects independent of antidepressant use ATOD, consistent with previous findings that cortical α1 subunit mRNA levels are not altered in mice chronically treated with selective serotonin reuptake inhibitors (Surget *et al*, 2009).

Lower pyramidal cell α1 subunit transcript levels could indicate: (1) downregulation of postsynaptic receptors in response to increased presynaptic GABA, (2) fewer inputs

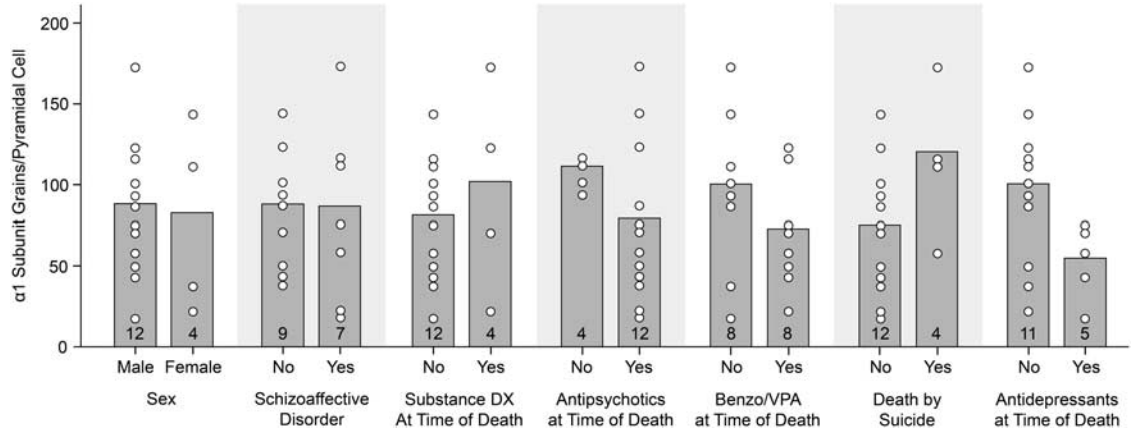

Figure 2 The effect of potential confounding factors on pyramidal cell GABA$_A$ α1 subunit mRNA levels in subjects with schizophrenia. Sex, diagnosis of schizoaffective disorder, substance abuse, or dependence at time of death (ATOD); antipsychotic, benzodiazepine and/or valproic acid medications ATOD; or death by suicide did not significantly affect pyramidal cell α1 subunit messenger RNA (mRNA) levels (all $p \geqslant 0.127$). Antidepressants ATOD did significantly affect pyramidal cell α1 subunit mRNA levels ($p = 0.022$). Numbers at the bottom of bars indicate number of schizophrenia subjects per group. DX, diagnosis; Benzo, benzodiazepine; VPA, valproic acid.

from PV basket cell axons, or (3) a homeostatic synaptic plasticity response to maintain excitatory–inhibitory (E/I) balance in the face of reduced excitatory drive to pyramidal cells. The first interpretation seems unlikely, as cortical GABA levels appear to be lower in schizophrenia due to reduced expression of the predominant synthesizing enzyme, GAD67 (Gonzalez-Burgos *et al*, 2010). The second interpretation is supported by the finding that the density of PV-immunoreactive boutons (putative basket cell axon terminals) is significantly lower selectively in PFC layers 3–4 in schizophrenia (Lewis *et al*, 2001). Additionally, genetic reductions of GAD67 in PV interneurons during development results in diminished PV basket cell axonal arborization and synapse formation (Chattopadhyaya *et al*, 2007). Thus, the undetectable levels of GAD67 mRNA in ~45% of PFC PV interneurons in schizophrenia (Hashimoto *et al*, 2003), if present early in life, could lead to fewer PV basket cell inputs, and thus fewer synapses needing GABA$_A$ α1 receptors in postsynaptic cells. However, diminished PV basket cell axonal arbors would be expected to affect all postsynaptic cells, including other PV basket cells (Melchitzky *et al*, 1999), and we did not find lower α1 subunit mRNA in layer 3 interneurons, ~50% of which express PV (Conde *et al*, 1994; Gabbott and Bacon, 1996). Furthermore, the lower density of PV-immunoreactive boutons in PFC layers 3–4 in schizophrenia (Lewis *et al*, 2001) may reflect lower levels of PV protein/axon terminal, rather than fewer axon terminals, an interpretation supported by the lower levels of PV mRNA in these same layers in schizophrenia (Hashimoto *et al*, 2003).

The existing data may be most consistent with a compensatory, pyramidal cell-specific downregulation of GABA$_A$ α1 receptors to maintain E/I balance in the face of fewer excitatory inputs to layer 3 pyramidal cells. The density of dendritic spines, which reflect the number of glutamatergic synapses to pyramidal cells (Gray, 1959; Harris and Kater, 1994), is significantly decreased in schizophrenia (Glantz and Lewis, 2000; Sweet *et al*, 2009), and this deficit is most pronounced in layer 3 (Kolluri *et al*, 2005). Excitatory spine deficits may represent an upstream pathology which induces homeostatic synaptic plasticity responses of lower presynaptic GABA production in PV interneurons (Hashimoto *et al*, 2003) and fewer postsynaptic GABA$_A$ α1 receptors in pyramidal cells, while maintaining GABA$_A$ α1 receptor levels in, and the inhibition of, interneurons. Consistent with this interpretation, experimental reductions in network activity produce fewer inputs to pyramidal cells from PV interneurons, but not other interneurons (Bartley *et al*, 2008), and an activity-dependent downregulation of GAD67 (Jones *et al*, 1994a).

Although reductions in inhibitory input from PV basket cells to pyramidal cells may restore E/I balance, the new level of balance may not be conducive to tasks requiring cognitive control. That is, strong inhibition from PV basket cells to layer 3 pyramidal cells via α1-containing GABA$_A$ receptors is critical for the neural network oscillations at γ frequency (30–80 Hz) (Gonzalez-Burgos *et al*, 2010; Sohal *et al*, 2009; Wulff *et al*, 2009) associated with cognitive control tasks. Thus, pre- and postsynaptic reductions in PV basket cell inhibition of layer 3 pyramidal cells, while partially restoring E/I balance, may contribute to the impaired γ-oscillations and poorer performance seen in schizophrenia subjects under tasks conditions requiring high levels of cognitive control (Cho *et al*, 2006; Minzenberg *et al*, 2010).

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DISCLOSURE

Dr Glausier has no conflicts of interest to declare. Dr Lewis currently receives investigator-initiated research support from the BMS Foundation, Bristol-Myers Squibb, Curridium Ltd and Pfizer, and in 2008–2010, served as a

consultant in the areas of target identification and validation and new compound development to AstraZeneca, BioLine RX, Bristol-Myers Squibb, Merck, Neurogen, and SK Life Science.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)