Reciprocal Alterations in Pre- and Postsynaptic Inhibitory Markers at Chandelier Cell Inputs to Pyramidal Neurons in Schizophrenia

David W. Volk[1], Joseph N. Pierri[2], Jean-Marc Fritschy[4], Sungyoung Auh[3], Allan R. Sampson[3] and David A. Lewis[1,2]

Departments of [1]Neuroscience, [2]Psychiatry and [3]Statistics, University of Pittsburgh, PA 15260, USA and [4]Institute of Pharmacology, University of Zurich, CH-8057 Zurich, Switzerland

In the prefrontal cortex of subjects with schizophrenia, markers of the synthesis and re-uptake of GABA appear to be selectively altered in a subset of interneurons that includes chandelier cells. Determining the effect of these disturbances in presynaptic GABA markers on inhibitory signaling requires knowledge of the status of $GABA_A$ receptors at the postsynaptic targets of chandelier cells, the axon initial segments (AIS) of pyramidal neurons. Because the α_2 subunit of the $GABA_A$ receptor is preferentially localized at pyramidal neuron AIS, we quantified α_2 subunit immunoreactive AIS in tissue sections containing prefrontal cortex area 46 from 14 matched triads of subjects with schizophrenia, subjects with major depression and control subjects. Systematic, random sampling revealed that the mean number of α_2-labeled AIS per mm^2 in subjects with schizophrenia was significantly ($P = 0.007$) increased by 113% compared to control subjects and non-significantly increased compared to subjects with major depression. Furthermore, within subjects with schizophrenia, the density of α_2-labeled AIS was negatively correlated ($r = -0.49$, $P = 0.038$) with the density of chandelier axon terminals immunoreactive for the GABA membrane transporter. These data suggest that $GABA_A$ receptors are up-regulated at pyramidal neuron AIS in response to deficient GABA neurotransmission at chandelier axon terminals in schizophrenia. Thus, disturbances in inhibition at the chandelier neuron–pyramidal neuron synapse may be a critical component of prefrontal cortical dysfunction in schizophrenia.

Introduction

The most persistent and debilitating features of schizophrenia involve disturbances in cognitive abilities, such as working memory, which appear to reflect dysfunction of the dorsal prefrontal cortex (PFC) (Weinberger *et al.*, 1986; Goldman-Rakic, 1994). This dysfunction may be associated with alterations in the inhibitory circuitry of the PFC. For example, in post-mortem studies of schizophrenia, genes responsible for the synthesis of GABA, glutamic acid decarboxylase (GAD_{67}), and for the re-uptake of GABA into the nerve terminal, GABA membrane transporter (GAT-1), show decreased mRNA expression in the PFC and these alterations are restricted to a subset of inhibitory neurons (Akbarian *et al.*, 1995a; Guidotti *et al.*, 2000; Volk *et al.*, 2000, 2001). The affected subset of GABA neurons appears to include chandelier cells, since decreased expression of GAT-1 mRNA is associated with decreased GAT-1 protein immunoreactivity selectively in the axon terminals of chandelier neurons (Woo *et al.*, 1998; Pierri *et al.*, 1999) (Fig. 1*E*).

The axons of chandelier cells form vertical arrays of terminals, termed 'cartridges' (Lewis and Lund, 1990), which synapse at the axon initial segments (AIS) of pyramidal neurons (Somogyi, 1977; Freund *et al.*, 1983; Peters, 1984). The localization of these synapses near the site of action potential generation suggests that chandelier cells are positioned to regulate powerfully the output of pyramidal neurons. However, it remains to be determined whether a reduction in GAT-1 in chandelier axon cartridges is actually associated with altered inhibitory neurotransmission at the AIS of pyramidal neurons in schizophrenia.

The status of GABA activity at the AIS of pyramidal neurons may be further informed by an analysis of postsynaptic $GABA_A$ receptors at the AIS. Indeed, compensatory changes in $GABA_A$ receptors in response to alterations in extracellular GABA levels have been previously reported (Montpied *et al.*, 1991a; Mhatre and Ticku, 1994). However, previous radioligand binding and mRNA expression studies of PFC $GABA_A$ receptors in schizophrenia (Hanada *et al.*, 1987; Akbarian *et al.*, 1995b; Benes *et al.*, 1996; Huntsman *et al.*, 1998; Impagnatiello *et al.*, 1998; Dean *et al.*, 1999; Ohnuma *et al.*, 1999) have not selectively analyzed the pyramidal neuron AIS.

$GABA_A$ receptors are composed of pentamers of subunits, most commonly including at least one of each of the α, β and γ subunit classes (Sieghart *et al.*, 1999). In addition, the different $GABA_A$ receptor α subunits have distinctive subcellular distributions (Fritschy and Mohler, 1995). For example, in the superficial layers of human cerebral cortex, the α_2 subunit is prominently localized at pyramidal neuron AIS (Loup *et al.*, 1998). Indeed, although associated with only ~15% of all $GABA_A$ receptors in the cortex (Fritschy and Mohler, 1995), the α_2 subunit is found at >80% of inhibitory synapses onto pyramidal neuron AIS, at least in rat hippocampus (Nusser *et al.*, 1996; Nyíri *et al.*, 2001). Furthermore, only 0.1% of all GABA synapses are found at pyramidal AIS, whereas nearly 25% of all α_2-immunoreactive synapses are found at pyramidal AIS. Finally, $GABA_A$ receptors containing the α_2 subunit have a higher affinity for GABA and faster activation and slower de-activation times, compared to $GABA_A$ receptors containing the more commonly expressed α_1 subunit (Levitan *et al.*, 1988; Lavoie *et al.*, 1997). Thus, $GABA_A$ receptors containing the α_2 subunit appear to be anatomically positioned and functionally adapted to mediate a potent inhibitory influence on the output of pyramidal neurons and, thus, may provide critical insight into the status of inhibitory activity at pyramidal neuron AIS.

Therefore, we examined human post-mortem brain tissue containing PFC area 46 in order to determine: (i) whether the density of pyramidal neuron AIS immunoreactive for the $GABA_A$ receptor α_2 subunit (α_2-AIS) is altered in subjects with schizophrenia; (ii) whether changes in α_2-AIS density are specific to the diagnosis of schizophrenia; and (iii) whether α_2-AIS density is related to presynaptic GABA markers in chandelier axon cartridges in subjects with schizophrenia.

Materials and Methods

Characteristics of Study Subjects

Brain specimens were obtained during autopsies conducted at the Allegheny County Coroner's Office after obtaining consent from the surviving next of kin. The 14 triads of subjects used in this study each consisted of one subject with schizophrenia matched to one control subject and one subject with major depressive disorder (MDD) for sex,

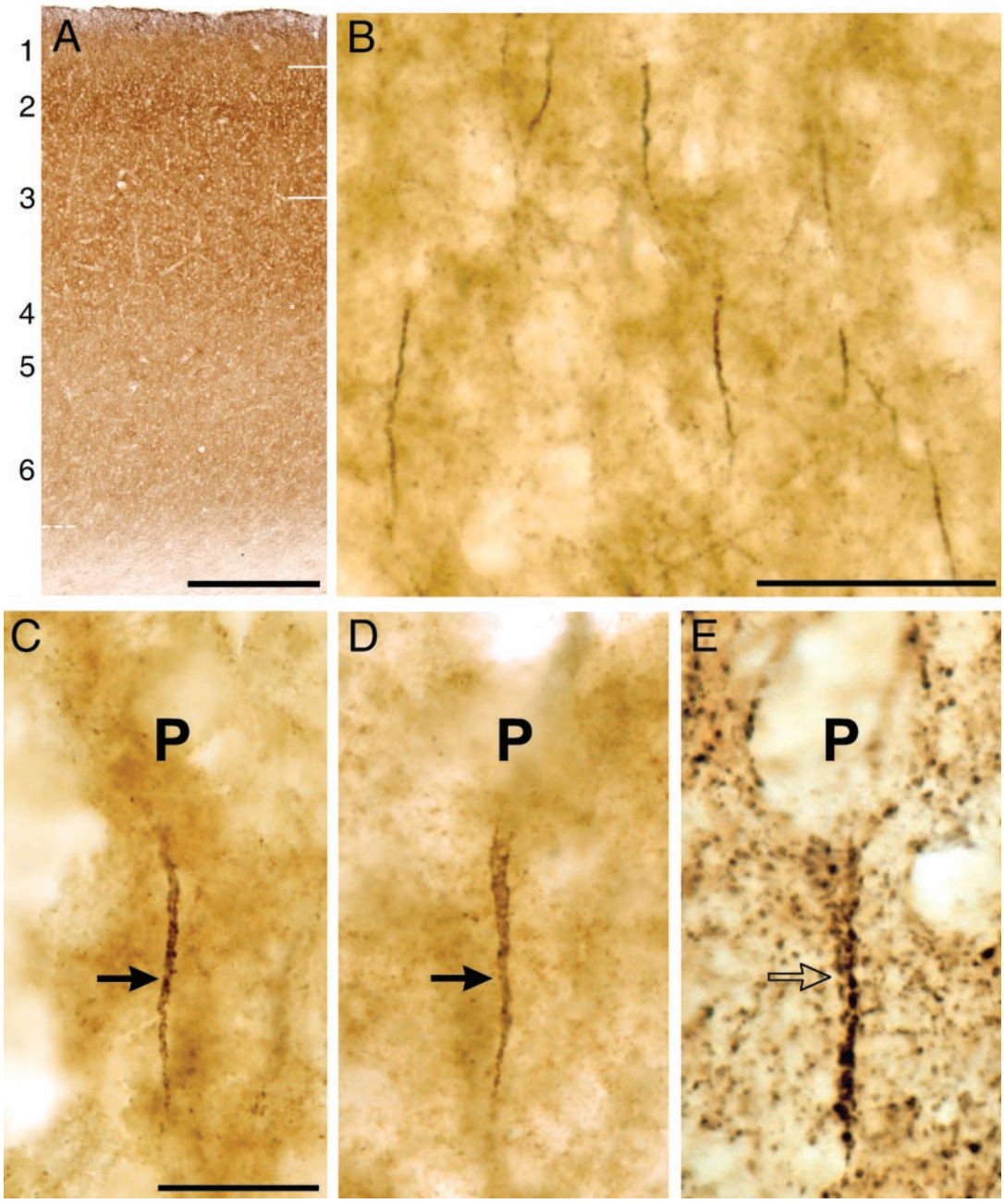

Figure 1. Immunoreactivity for the $GABA_A$ receptor α_2 subunit in human PFC area 46. (*A*) Immunoreactivity for the α_2 subunit is greatest in the superficial layers of the PFC, with the highest intensity in layer 2. The solid white lines indicate the zone sampled in this study and the dashed white line indicates the layer-6–white-matter border. Scale bar = 600 μm. (*B–D*) Immunoreactivity for pre- and postsynaptic GABA markers at the axon initial segment of pyramidal neurons. In both control (*B*,*C*) and schizophrenic (*D*) subjects, axon initial segments (solid arrows) intensely labeled with an antibody against the $GABA_A$ receptor α_2 subunit appear as vertically oriented processes below the unlabeled cell bodies of pyramidal neurons (P) and are occasionally slightly tapered from superficial to deep. (*E*) Chandelier neuron axon cartridge immunoreactive for GAT-1 (open arrow) outlines the unlabeled axon initial segment of a pyramidal neuron. Scale bar in (*B*) = 50 μm and in (*C–E*) = 20 μm.

and as closely as possible for age and post-mortem interval (PMI; Table 1). All subjects with schizophrenia, all control subjects and all but five subjects with MDD (613, 689, 693, 698 and 803; Table 1) were included in previous studies of chandelier axon cartridges immunoreactive for GAT-1 — 'GAT-1-cartridges' (Woo *et al.*, 1998; Pierri *et al.*, 1999). All subjects were under the age of 70 years and all subjects with schizophrenia were previously determined to have at least a 10% decrease in the density of GAT-1-cartridges compared to matched control subjects.

An independent panel of experienced clinicians arrived at consensus DSM-III-R diagnoses after reviewing medical records and/or the results of structured interviews conducted following written, informed consent with family members of the deceased (Glantz and Lewis, 1997). No psychiatric disorders were present in the control subjects. Six schizophrenic subjects and nine subjects with MDD had a history of an alcohol and/or substance abuse disorder (Table 1). Four schizophrenic subjects had been off antipsychotic medications prior to death (234, neuroleptic-naïve; 450, discontinued for unknown length of time; 537, 10 months; 622, 7 months; Table 1) and two subjects with MDD were on antipsychotic medications at time of death. Three schizophrenic subjects and four subjects with MDD were receiving benzodiazepines at time of death. The mean (±SD) ages of the subjects with schizophrenia and MDD at the onset of illness were 29.7 ± 9.4 and 41.6 ± 10.7 years, respectively, and the

Table 1
Control subjects, subjects with schizophrenia and subjects with MDD used in this study

Triad	Control subjects					Subjects with schizophrenia						Subjects with major depression (MDD)					
	Case	Sex/age	PMI[a]	Storage time[b]	Cause of death[c]	Case	Diagnoses and medications	Sex/age	PMI[a]	Storage time[b]	Cause of death[c]	Case	Sex/age	PMI[a]	Storage time[b]	Cause of death[c]	
1	250	F/47	5.3	110	ASCVD	398	Schizoaffective disorder[d,e]	F/41	10.3	88	Pulmonary embolism	210	MDD	F/50	4.7	117	Suicide by drowning
2	270	M/62	3.3	107	ASCVD	131	Chronic undifferentiated schizophrenia[d,i]	M/62	3.9	134	Pneumonia	249	MDD+P	M/57	4.3	111	ASCVD
3	451	M/48	12.0	70	ASCVD	317	Chronic undifferentiated schizophrenia[d]	M/48	8.3	101	Pneumonia	511	MDD	M/43	17.9	56	ASCVD
4	178	M/48	7.8	121	ASCVD	377	Chronic undifferentiated schizophrenia[d,f]	M/52	10.0	92	GI bleeding	505	MDD[f]	M/57	12.8	57	Suicide by gun shot
5	420	F/67	19.5	81	Accidental CO poisoning	333	Chronic undifferentiated schizophrenia[d]	F/66	17.9	99	ASCVD	803	MDD	F/65	18.0	11	Trauma
6	344	M/50	6.8	98	ASCVD	422	Chronic paranoid schizophrenia[d,e]	M/54	11.0	81	ASCVD	698	MDD+P[d]	M/59	13.0	29	Suicide by hanging
7	449	F/47	4.3	70	Accidental CO poisoning	517	Chronic disorganized schizophrenia[d,f]	F/48	3.7	56	Intracerebral hemorrhage	248	MDD[e,l]	F/48	6.3	111	Suicide by hanging
8	412	M/42	14.2	84	Aortic stenosis	466	Chronic undifferentiated schizophrenia[d]	M/48	19.0	68	ASCVD	421	MDD[i]	M/44	16.0	81	ASCVD
9	592	M/41	22.1	44	ASCVD	450	Chronic undifferentiated schizophrenia[g,k]	M/48	22.0	70	Suicide by jumping	689	MDD+P[d,e,i]	M/45	24.4	30	Suicide by acid ingestion
10	681	M/51	11.6	32	Cardiomyopathy	234	Chronic paranoid schizophrenia	M/51	12.8	113	Cardiomyopathy	602	MDD[f]	M/56	11.8	43	Suicide by gun shot
11	567	F/46	15.0	48	Mitral valve prolapse	537	Schizoaffective disorder	F/37	14.5	53	Suicide by hanging	693	MDD+P[e,j]	F/42	12.6	30	Suicide by propoxyphene overdose
12	568	F/60	9.5	48	ASCVD	559	Schizoaffective disorder[d,f]	F/61	16.8	50	ASCVD	565	MDD[h,k]	F/62	12.4	49	Suicide by gun shot
13	620	M/64	17.3	40	Accidental drowning	566	Chronic undifferentiated schizophrenia[d,e,i]	M/63	18.3	49	ASCVD	613	MDD+P[i]	M/59	15.6	41	Suicide by gun shot
14	551	M/61	16.4	51	Cardiac tamponade	622	Chronic undifferentiated schizophrenia	M/58	18.9	39	Right MCA infarction	619	MDD[e,k]	M/55	18.8	40	Suicide by gun shot
Mean		52	11.8	72				53	13.4	78				53	13.7	58	
SD		9	5.9	29				8	5.8	28				8	5.7	34	

[a]PMI indicates post-mortem interval in hours. [b]Storage time is in months. [c]ASCVD, atherosclerotic coronary vascular disease. [d]Antipsychotic medication current at time of death. [e]Benzodiazepine medication current at time of death. [f]Alcohol dependence, current at time of death. [g]Alcohol dependence, in remission at time of death. [h]Alcohol abuse, current at time of death. [i]Alcohol abuse, in remission at time of death. [j]Other substance dependence, current at time of death. [k]Other substance dependence, in remission at time of death. [l]Other substance abuse, current at time of death. +P, presence of psychotic features in subjects with MDD.

average durations of illness were 23.3 ± 8.4 and 11.7 ± 9.3 years, respectively. All procedures were approved by the University of Pittsburgh's Institutional Review Board for Biomedical Research.

Tissue Processing

Upon retrieval, brain specimens were blocked coronally, placed into 4% paraformaldehyde for 48 h, cryoprotected and then stored at –30°C. Subject groups did not significantly differ in mean storage time [$F(2,42) = 2.36$, $P = 0.11$; Table 1]. Blocks containing the left middle frontal gyrus were sectioned coronally at 40 μm on a calibrated cryostat. Nissl-stained sections were used to identify PFC area 46 in each subject using cytoarchitectonic criteria (Daviss and Lewis, 1995; Rajkowska and Goldman-Rakic, 1995). Neuropathological examination of each brain revealed no abnormalities in the region of interest and Alzheimer's disease was ruled out in each subject using clinical and neuropathological criteria.

Four tissue sections containing PFC area 46, in serial order and ~400 μm apart, from each subject were processed in a randomized block design (i.e. with one section from each subject in a triad always processed together, and with different combinations of triads in each run). Using an antibody raised in guinea pig against the cDNA-derived N-terminal sequence (amino acid residues 1–9) of the human $GABA_A$ receptor α_2 subunit (Marksitzer *et al.*, 1993), sections were processed using the avidin–biotin procedure, followed by diaminobenzidine and hydrogen peroxide as previously described (Woo *et al.*, 1998). Reaction product was intensified through serial immersions in aqueous osmium tetroxide and thiocarbohydrizide, followed by silver nitrate and gold chloride (Pucak *et al.*, 1996).

The specificity of the antibody for the $GABA_A$ receptor α_2 subunit was previously demonstrated by Western blot experiments whereby immunoprecipitation of $GABA_A$ receptor α_2 subunit by the antibody revealed a single band whose signal intensity was reduced in a dose-dependent manner following preadsorption with increasing concentrations of $GABA_A$ receptor α_2 subunit peptide (Marksitzer *et al.*, 1993). Specificity was also confirmed by anatomical observations described in the Results section.

Quantification

All quantification was conducted by one rater (D.W.V.), who was blinded to diagnosis and subject number. Using the Stereo Investigator fractionator program (MicroBrightField Inc., Colchester, VT), a region of the middle frontal gyrus containing area 46 and of uniform cortical depth

was sampled in each subject. Using a 5× objective, a contour containing layers 2–3a, defined as 25% of the total cortical width immediately below the layer 1–2 border, was drawn on each section (mean ± SD total contour area per subject: 10.7 ± 2.6 mm^2). This zone was chosen because it contains ~80% of the total number of α_2-AIS in the PFC and in order to maintain consistency with a previous study on the density of GAT-1-cartridges in this laminar location in the same cohort of subjects (Pierri *et al.*, 1999). Between 40 and 50 counting frames, 70 × 70 μm, were systematically and randomly placed within the contour for each tissue section and two sides of each counting frame were identified as exclusion boundaries. Using a 100× oil-immersion objective (NA 1.4), all α_2-AIS (see Results for criteria) within each counting frame were identified on a video monitor at a final magnification of 2450×. The total number of α_2-AIS counted in each subject ranged from 4 to 616 and the mean (±SD) coefficient of error for α_2-AIS counts per sampling frame in each subject was 0.13 ± 0.08. Assessments of intra-rater reliability in identifying α_2-AIS throughout the course of the quantification procedure revealed an intraclass correlation coefficient of 0.99 (95% CI = 0.59–1.0).

In a separate analysis using the Neurolucida program (MicroBrightField Inc., Colchester, VT), mean length of α_2-AIS was also determined in each schizophrenic and control subject. In one random tissue section from each subject, the length of every detectable α_2-AIS was measured in a series of 100 μm wide cortical traverses. The average (±SD) number of α_2-AIS measured in each subject was 54 ± 21.

Statistical Analysis

Measures of the density of α_2-AIS per mm^2 in each of the four tissue sections for each subject were treated as four correlated observations. A multivariate analysis of covariance (MANCOVA) model assuming a compound symmetric covariance structure (Neter *et al.*, 1996) was employed to test for a main effect of diagnosis, with triad and immunohistochemistry run included as blocking factors and tissue storage time as a covariate. In this model, the inclusion of triad accounted for matching of subjects for sex, age and PMI. Because regression analyses revealed a potential effect of PMI on α_2-AIS density, two additional MANCOVA models were run. One model included both diagnosis and triad factors, with PMI and tissue storage time as covariates and run as a blocking factor; the other model included a diagnosis factor with sex, age, PMI and tissue storage time as covariates, and run as a blocking factor. As all three models yielded similar results, only the results of the primary model are reported. Comparisons between any two of the diagnostic groups were also conducted using the primary model. In addition, the effects of sex, psychotropic medication at time of death and/or alcoholism on α_2-AIS density in subjects with schizophrenia were assessed using ANCOVAs with the difference in α_2-AIS density within matched pairs of schizophrenic and control subjects as the dependent variable and PMI as a covariate. Finally, a one-tailed Pearson correlation was used to test the hypothesis that GAT-1-cartridge density was inversely related to α_2-AIS density in subjects with schizophrenia. Analyses were implemented in SAS PROC Mixed (Littell *et al.*, 1996) and all statistical tests were conducted with $\alpha = 0.05$.

Results

Immunoreactivity for the GABA$_A$ Receptor α_2 Subunit in Human PFC

As shown in Figure 1*A*, immunoreactivity for the GABA$_A$ receptor α_2 subunit in human PFC was greatest in the supragranular layers, particularly in layer 2. This pattern directly parallels previous reports of α_2-immunoreactivity in the medial PFC of rat (Dunn *et al.*, 1996) and of α_2 mRNA expression in human PFC (Akbarian *et al.*, 1995b). At higher magnification, α_2 subunit-immunoreactivity was prominently found in pyramidal neuron AIS — α_2-AIS (Nusser *et al.*, 1996; Loup *et al.*, 1998; Nyíri *et al.*, 2001) — identified as intensely immunoreactive, discrete, non-branching, vertically oriented processes located below unlabeled cell bodies, ranging in length from 8 to 40 μm and usually slightly tapered from superficial to deep (Figure 1*B–D*).

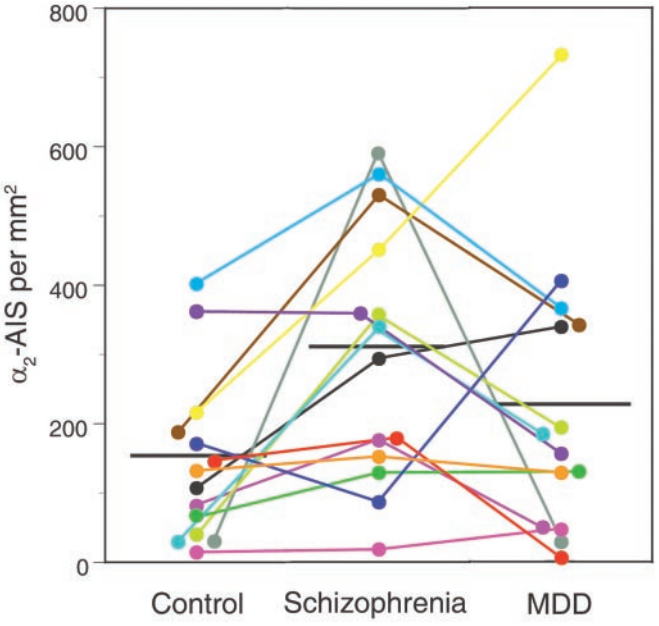

Figure 2. Scatterplots showing the number of α_2-AIS per mm^2 in each subject. Solid horizontal lines indicate the mean value for each group. Subjects in matched triads are indicated by the same color and connected by lines.

α_2-AIS Density in Schizophrenia and Major Depression

Statistical analysis using the primary MANCOVA model revealed a significant main effect of diagnosis [$F(2,25) = 4.88$, $P = 0.016$] on α_2-AIS density. The mean (±SD) number of α_2-AIS per mm^2 was significantly [$F(1,12) = 10.60$, $P = 0.007$] increased by 113% in subjects with schizophrenia (302 ± 184) compared to control subjects (141 ± 119). Furthermore, in 12 of the 14 matched pairs of schizophrenic and control subjects, α_2-AIS density was higher in the schizophrenic subject (Fig. 2). As illustrated in Figure 3, the mean increase in α_2-AIS density in individual subjects with schizophrenia compared to their matched controls did not significantly differ when the subjects with schizophrenia were subdivided into groups based on gender [$F(1,11) = 0.007$, $P = 0.934$], treatment with psychotropic medications at time of death [$F(1,11) = 0.201$, $P = 0.663$], or history of an alcohol-related disorder [$F(1,11) = 0.002$, $P = 0.968$]. In addition, mean α_2-AIS density was virtually identical in the three subjects with schizoaffective disorder (295 ± 203) and the 11 subjects with 'pure' schizophrenia (304 ± 189; Fig. 4).

In contrast to the group differences in α_2-AIS density, mean α_2-AIS length (in μm) was similar in both schizophrenic and control subjects (14.8 ± 5.6 and 14.7 ± 5.2, respectively). However, α_2-AIS length was positively correlated with α_2-AIS density in subjects with schizophrenia ($r = 0.548$, $P = 0.042$), but not in control subjects ($r = 0.124$, $P = 0.674$).

The mean density of α_2-AIS was also increased in the subjects with schizophrenia by 37% compared to subjects with MDD (Fig. 2). Although this difference was not statistically significant [$F(1,12) = 1.68$, $P = 0.22$], 10 of the 14 subjects with schizophrenia had an increased density of α_2-AIS compared to the matched subject with MDD. Furthermore, one subject with MDD (689; Table 1) appeared to be an outlier in that the density of α_2-AIS in this subject (α_2-AIS/mm^2 = 731) was 2.6 SD above the mean α_2-AIS density for all subjects with MDD (Fig. 2). Consequently, this outlier subject was included in the main statistical analysis, but excluded from subsequent analyses in which diagnosis groups were subdivided into smaller groups on

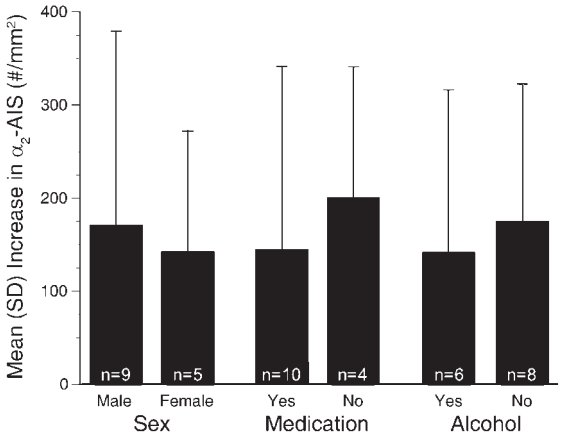

Figure 3. Bar graphs showing the mean (±SD) increase in number of α_2-AIS per mm^2 in individual schizophrenic subjects compared to matched control subjects when schizophrenic subjects are divided into groups based upon sex, psychotropic medication at time of death, or history of an alcohol-related disorder.

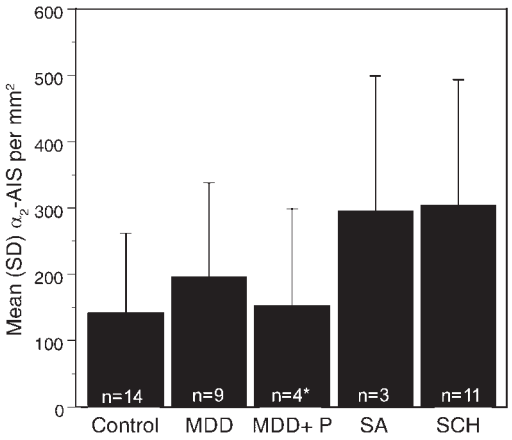

Figure 4. Bar graphs showing the mean (±SD) number of α_2-AIS per mm^2 in control subjects, subjects with major depressive disorder grouped by the absence (MDD) or presence (MDD + P) of psychotic features, subjects with schizoaffective disorder (SA) and subjects with 'pure' schizophrenia (SCH). *One subject with MDD, 689, was determined to be an outlier (see Results) and, consequently, was not included in this figure.

the basis of demographic variables. Importantly, no demographic variable, such as psychotropic medication at time of death, history of alcoholism, presence of psychotic features, tissue storage time, or PMI, appeared to contribute to the increased α_2-AIS density in this subject, or any other subject. When triad 9, which included subject 689, was excluded from analysis, the mean density of α_2-AIS in subjects with schizophrenia was increased by 59% compared to subjects with MDD [182 ± 138, $F(1,11) = 2.86$, $P = 0.12$]. Furthermore, mean α_2-AIS density did not significantly differ between control subjects and subjects with MDD [$F(1,11) = 0.53$, $P = 0.48$]. Finally, mean α_2-AIS density in the four subjects with MDD with psychotic features did not differ from either the MDD subjects without psychotic features or the control subjects (Fig. 4).

Reciprocal Changes in α_2-AIS and GAT-1-Cartridge Density in Schizophrenia

The density of GAT-1-cartridges in area 46 was previously determined for all subjects with schizophrenia, all control subjects and nine subjects with MDD examined in the present

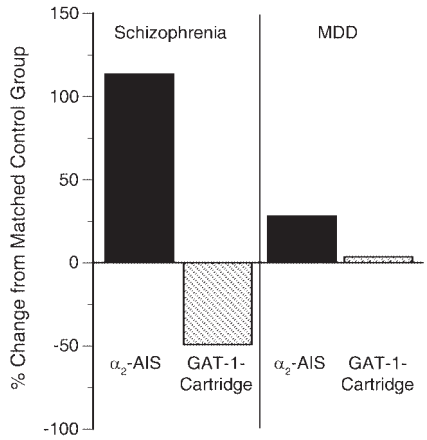

Figure 5. Bar graph showing the percentage change in the mean densities of α_2-AIS (solid bars) and GAT-1-cartridges (cross-hatched bars) in schizophrenic subjects (left) and in subjects with MDD (right) compared to the matched control subjects. The percentage changes in α_2-AIS and GAT-1-cartridge densities in MDD were calculated from the nine subjects with MDD and the nine matched control subjects for whom GAT-1-cartridge densities were available.

study (Woo *et al.*, 1998; Pierri *et al.*, 1999). Whereas the mean α_2-AIS density in the subjects with schizophrenia was approximately twice that in the matched control subjects, the mean GAT-1-cartridge density in the same schizophrenic subjects was less than half that in the matched control subjects (Fig. 5). Furthermore, a significant inverse relationship ($r = -0.49$, $P = 0.038$) between the densities of α_2-AIS and GAT-1-cartridges was present in the subjects with schizophrenia. In contrast, in the nine subjects with MDD included in the previous GAT-1-cartridge study, neither the mean density of α_2-AIS nor of GAT-1-cartridges differed significantly from the nine matched control subjects (Fig. 5).

Discussion

In this study, α_2-AIS density in the superficial PFC layers of subjects with schizophrenia was significantly increased compared to control subjects and was also increased, though non-significantly, compared to subjects with MDD. Furthermore, α_2-AIS density was inversely related to GAT-1-cartridge density in subjects with schizophrenia. These data suggest that presynaptic alterations in chandelier neurons are associated with postsynaptic changes in $GABA_A$ receptors at the AIS of pyramidal neurons in schizophrenia.

Specificity of Findings for Schizophrenia

The increase in α_2-AIS density appears to be relatively specific to the diagnosis of schizophrenia, or at least not a common feature of MDD, even when that disorder is accompanied by psychosis. Consistent with these findings, previous studies of the frontal cortex in subjects with MDD have failed to find differences in the concentration, synthesis, or re-uptake of GABA (Cheetham *et al.*, 1988; Korpi *et al.*, 1988; Sundman *et al.*, 1997). Thus, alterations in PFC GABA neurotransmission at the chandelier cell–pyramidal neuron synapse appear to be a characteristic of schizophrenia that is not associated with depression or psychosis, *per se.*

Several lines of evidence suggest that treatment with antipsychotic medications and benzodiazepines or a history of alcoholism do not account for the changes in PFC GABA markers in subjects with schizophrenia. First, α_2-AIS density was

increased in subjects with schizophrenia, regardless of the use of psychotropic medications at time of death or history of an alcohol-related disorder. Consistent with these observations, studies of haloperidol-treated monkeys revealed no changes in the expression of GAD_{67} or GAT-1 mRNAs or the density of GAT-1-cartridges in the PFC (Pierri *et al.*, 1999; Volk *et al.*, 2000, 2001). In addition, pharmacological manipulations in rats have found that: (i) antipsychotic medications result in decreased muscimol binding to $GABA_A$ receptors (Johnson *et al.*, 1994; Farnbach-Pralong *et al.*, 1998); (ii) benzodiazepine treatment causes no changes in cortical $GABA_A$ receptor α_2 subunit mRNA or protein expression levels (O'Donovan *et al.*, 1992; Holt *et al.*, 1996, 1997; Impagnatiello *et al.*, 1996; Chen *et al.*, 1999; Tietz *et al.*, 1999); and (iii) ethanol results in either decreases or no change in cortical $GABA_A$ receptor α_2 mRNA expression (Montpied *et al.*, 1991b; Mhatre *et al.*, 1993; Chen *et al.*, 1998)

Methodological Considerations

The stereological principle of systematic, random sampling was employed in this study to reduce sampling bias; however, the lack of clear boundaries of PFC area 46 precluded the determination of the total number of α_2-AIS in this region. Importantly, measures of α_2-AIS density were not confounded by differences in α_2-AIS length between the schizophrenic and control groups. Furthermore, given that α_2-AIS and GAT-1-cartridge densities were changed in opposite directions in nearby tissue sections from the same subjects with schizophrenia, neither the method of quantification nor potential differences in cortical volume across subject groups appear to have introduced a systematic confound.

We interpret the greater density of α_2-AIS in schizophrenia as reflecting an increase in the amount of α_2 subunit and the total density of $GABA_A$ receptors, per AIS, resulting in a greater number of AIS with detectable levels of immunoreactivity. Unfortunately, direct quantification of $GABA_A$ receptor subunits in pyramidal neuron AIS would require the use of procedures — for example immunogold-labeling and electron microscopy or immunofluorescence at the light microscopic level (Sutoo *et al.*, 1998) — that are technically challenging in post-mortem human brain. However, it seems unlikely that the increased α_2-AIS density reflects an increase in pyramidal neuron density. First, in previous studies, PFC neuron density was reported to be either unchanged (Akbarian *et al.*, 1995a), or, at ~20%, not increased enough (Selemon *et al.*, 1995, 1998) in schizophrenia to account for the present findings. Second, in the same subjects examined in the present study, the density of GAT-1-cartridges was actually reduced by >50%, which further argues against an increase in pyramidal neuron density. Finally, re-analysis of data from a previous study (Pierri *et al.*, 2001) revealed no difference in deep layer 3 pyramidal neuron density between the same cohort of subjects with schizophrenia (48.4 ± 7.7 cells/0.001 mm^3) and control subjects (49.1 ± 8.7 cells/0.001 mm^3) examined in the present study.

Alternatively, an increased amount of α_2 subunit at the AIS could be coupled with reductions in other subunits reported to be located at pyramidal neuron AIS, such as the α_1 and α_3 subunits (Nusser *et al.*, 1996; Loup *et al.*, 1998), without changes in the total number of $GABA_A$ receptors. Such 'subunit switching' between the α_2 and α_1 subunits occurs in rat hypothalamic oxytocin neurons during pregnancy (Brussaard and Herbison, 2000). Unfortunately, quantification of α_1-labeled AIS in schizophrenia is not possible, since the much greater density of α_1 subunit at other locations (Nusser *et al.*, 1996) prohibits the resolution of α_1-labeled AIS by light microscopy. In addition, we were not able to detect α_3-immunoreactivity in pyramidal neuron AIS in our human tissue samples. Yet, even if 'subunit switching', without a change in total receptor number, occurs in schizophrenia, an increased proportion of $GABA_A$ receptors containing the α_2 subunit would still likely confer a greater inhibitory response to GABA at pyramidal neuron AIS (Levitan *et al.*, 1988; Lavoie *et al.*, 1997). Thus, the increase in α_2-AIS density, whether reflecting an increased total density of $GABA_A$ receptors or an increased proportion of $GABA_A$ receptors containing the α_2 subunit, suggests that postsynaptic $GABA_A$ receptors at pyramidal neuron AIS are functionally up-regulated in schizophrenia.

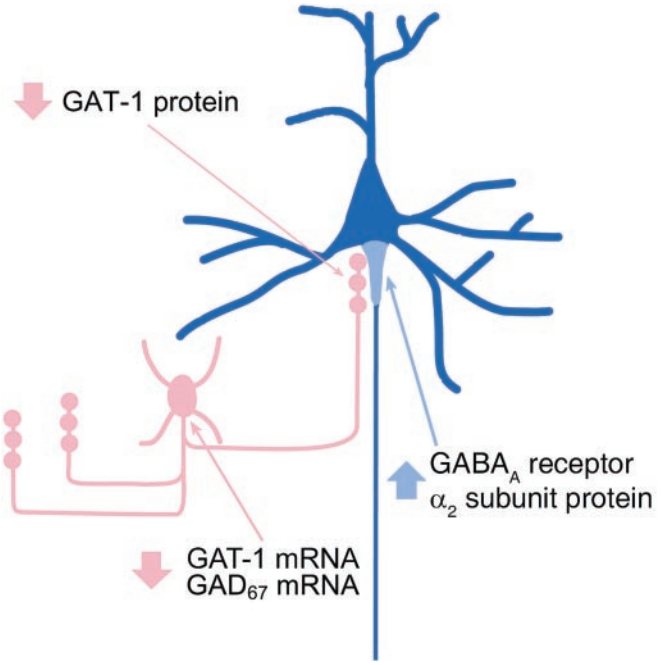

Figure 6. Alterations in GABA neurotransmission at the chandelier neuron–pyramidal neuron synapse in schizophrenia. In a subset of GABA neurons that appears to include chandelier neurons (pink), the mRNA expression for GAD_{67} and GAT-1 are both reduced below a detectable level (Volk *et al.*, 2000, 2001) and the protein level of GAT-1 is selectively reduced in chandelier neuron axon terminals (Woo *et al.*, 1998; Pierri *et al.*, 1999), which synapse at the AIS of pyramidal neurons. In postsynaptic pyramidal neurons (blue), immunoreactivity for the $GABA_A$ receptor α_2 subunit is increased at the axon initial segment (light blue), suggesting that $GABA_A$ receptors are up-regulated in response to a reduction in inhibitory neurotransmission in chandelier neuron axon terminals.

Pathophysiological Significance

The inverse relationship between the densities of α_2-AIS and GAT-1-cartridges in the same subjects with schizophrenia suggests that $GABA_A$ receptors are up-regulated at pyramidal neuron AIS in response to deficient GABA activity in chandelier axon terminals in schizophrenia (Fig. 6). Although experimental models of a selective decrease in GABA release at chandelier axon terminals are not available, previous studies (Mhatre and Ticku, 1994) have demonstrated that $GABA_A$ receptor antagonists produce increased α_2 subunit mRNA expression in embryonic chick neurons. Furthermore, in subjects with temporal lobe epilepsy, a loss of GABA neurons is associated with a substantial increase in α_2 subunit immunoreactivity in pyramidal cells (Loup *et al.*, 2000).

The mRNA expression for presynaptic inhibitory markers, including GAD_{67} and GAT-1, is decreased in the PFC of subjects

with schizophrenia (Akbarian *et al.*, 1995a; Ohnuma *et al.*, 1999; Guidotti *et al.*, 2000; Volk *et al.*, 2000, 2001). In contrast, mRNA levels of the major postsynaptic $GABA_A$ receptor subunits appear to be unchanged in schizophrenia (Akbarian *et al.*, 1995b), although some studies have reported increases in the α_1 subunit (Impagnatiello *et al.*, 1998; Ohnuma *et al.*, 1999) and a reduction in the short isoform of the γ_2 subunit (Huntsman *et al.*, 1998). Of particular interest, α_2 subunit mRNA expression was unchanged in PFC area 46 of subjects with schizophrenia (Akbarian *et al.*, 1995b). Although other explanations are possible, the increase in α_2-AIS density may reflect a locally regulated increase in α_2 subunit at pyramidal neuron AIS. However, alterations in α_2-immunoreactivity at locations other than the AIS, such as pyramidal neuron soma (Nyíri *et al.*, 2001), cannot be excluded. Furthermore, radiolabeled ligand binding studies of PFC $GABA_A$ receptors have revealed increases in muscimol binding (Hanada *et al.*, 1987; Benes *et al.*, 1996; Dean *et al.*, 1999) that were most prominent at pyramidal neuron soma (Benes *et al.*, 1996). Together, these data suggest that $GABA_A$ receptors located at the soma and AIS of pyramidal neurons are locally up-regulated in schizophrenia in response to a reduction in proximal inhibitory input.

The pathophysiological mechanism that selectively initiates disturbances in a subset of GABA neurons that appears to include chandelier neurons, but not in the majority of PFC GABA neurons (Woo *et al.*, 1998; Volk *et al.*, 2000, 2001), remains unclear. One possibility is that reduced inhibitory neurotransmission in chandelier neurons reflects an abnormality intrinsic to this cell class. Alternatively, inhibitory neurotransmission may be reduced in chandelier neurons in response to an alteration in their excitatory inputs. For example, morphological alterations in layer 3 pyramidal neurons in PFC area 46 (Garey *et al.*, 1998; Rajkowska *et al.*, 1998; Glantz and Lewis, 2000; Pierri *et al.*, 2001) and reduced neuronal number in the mediodorsal thalamic nucleus (Pakkenberg, 1990; Popken *et al.*, 2000; Young *et al.*, 2000; Byne *et al.*, 2002) have been reported in subjects with schizophrenia. Interestingly, in monkey PFC area 46, the dendrites of parvalbumin-containing cells, which include chandelier neurons, receive synaptic inputs from local pyramidal neurons (Melchitzky *et al.*, 2001) and from the mediodorsal thalamic nucleus (Melchitzky *et al.*, 1999), whereas other classes of GABA cells do not (Melchitzky and Lewis, 2000). Further studies of the mechanism(s) responsible for altered chandelier-cell–pyramidal-neuron inhibitory neurotransmission may lead to novel therapeutic strategies for remediating prefrontal cortical dysfunction in schizophrenia.

Notes

These studies were supported by NIH grants MH 43784, MH 45156 and MH 64320 and by a Scottish Rite Schizophrenia Fellowship Award.

Address correspondence to David A. Lewis, University of Pittsburgh, 3811 O'Hara Street, W1650 BST, Pittsburgh, PA 15213, USA. Email: lewisda@msx.upmc.edu.

References

Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr, Jones EG (1995a) Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatry 52:258–266.

Akbarian S, Huntsman MS, Kim JJ, Tafazzoli A, Potkin SG, Bunney WE Jr, Jones EG (1995b) $GABA_A$ receptor subunit gene expression in human prefrontal cortex: comparison of schizophrenics and controls. Cereb Cortex 5:550–560.

Benes FM, Snyder-Marie A, Vincent S, Khan Y (1996) Up-regulation of GABA-A receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. Neuroscience 75:1021–1031.

Brussaard AB, Herbison AE (2000) Long-term plasticity of postsynaptic $GABA_A$-receptor function in the adult brain: insights from the oxytocin neurone. Trends Neurosci 23:190–195.

Byne W, Buchsbaum MS, Mattiace LA, Hazlett EA, Kemether E, Elhakem SL, Purohit DP, Haroutunian V, Jones L (2002) Postmortem assessment of thalamic nuclear volumes in subjects with schizophrenia. Am J Psychiatry 159:59–65.

Cheetham SC, Crompton MR, Katona CL, Parker SJ, Horton RW (1988) Brain $GABA_A$/benzodiazepine binding sites and glutamic acid decarboxylase activity in depressed suicide victims. Brain Res 460:114–123.

Chen F, Rezvani A, Jarrott B, Lawrence AJ (1998) Distribution of $GABA_A$ receptors in the limbic system of alcohol-preferring and non-preferring rats: *in situ* hybridisation histochemistry and receptor autoradiography. Neurochem Int 32:143–151.

Chen S, Huang X, Zeng XJ, Sieghart W, Tietz EI (1999) Benzodiazepine-mediated regulation of α_1, α_2, β_{1-3}, and γ_2 $GABA_A$ receptor subunit proteins in the rat brain hippocampus and cortex. Neuroscience 93:33–44.

Daviss SR, Lewis DA (1995) Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. Psychiatry Res 59:81–96.

Dean B, Hussain T, Hayes W, Scarr E, Kitsoulis S, Hill C, Opeskin K, Vopolov DL (1999) Changes in serotonin2A and $GABA_A$ receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. J Neurochem 72:1593–1599.

Dunn E, Fritschy J-M, Carter DB, Merchant KM (1996) Differential distribution of γ-aminobutyric $acid_A$ receptor subunit (α_1, α_2, α_3, α_5, and β_{2+3}) immunoreactivity in the medial prefrontal cortex of the rat. Neurosci Lett 210:213–217.

Farnbach-Pralong D, Bradbury PR, Spurlock G, McGuffin P (1998) Clozapine and olanzapine treatment decreases rat cortical and limbic $GABA_A$ receptors. Eur J Pharmacol 349:R7–R8.

Freund TF, Martin KAC, Smith AD, Somogyi P (1983) Glutamate decarboxylase-immunoreactive terminals of Golgi-impregnated axoaxonic cells and of presumed basket cells in synaptic contact with pyramidal neurons of the cat's visual cortex. J Comp Neurol 221:263–278.

Fritschy J-M, Mohler H (1995) $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154–194.

Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, Barnes TRE, Hirsch SR (1998) Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. J Neurol Neurosurg Psychiatry 65:446–453.

Glantz LA, Lewis DA (1997) Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia: regional and diagnostic specificity. Arch Gen Psychiatry 54:943–952.

Glantz LA, Lewis DA (2000) Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Arch Gen Psychiatry 57:65–73.

Goldman-Rakic PS (1994) Working memory dysfunction in schizophrenia. J Neuropsychiatry 6:348–357.

Guidotti A, Auta J, Davis JM, Gerevini VD, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D, Costa E (2000) Decrease in reelin and glutamic acid decarboxylase$_{67}$ (GAD_{67}) expression in schizophrenia and bipolar disorder. Arch Gen Psychiatry 57:1061–1069.

Hanada S, Mita T, Nishino N, Tanaka C (1987) [^{3}H]Muscimol binding sites increased in autopsied brains of chronic schizophrenics. Life Sci 40:239–266.

Holt RA, Bateson AN, Martin IL (1996) Chronic treatment with diazepam or abecarnil differentially affects the expression of $GABA_A$ receptor subunit mRNAs in the rat cortex. Neuropharmacology 35:1457–1463.

Holt RA, Bateson AN, Martin IL (1997) Chronic zolpidem treatment alters $GABA_A$ receptor mRNA levels in rat cortex. Eur J Pharmacol 329:129–132.

Huntsman MM, Tran BV, Potkin SG, Bunney WE, Jones EG (1998) Altered ratios of alternatively spliced long and short gamma 2 subunit mRNAs of the gamma-amino butyrate type A receptor in prefrontal cortex of schizophrenics. Proc Natl Acad Sci USA 95:15066–15071.

Impagnatiello F, Pesold C, Longone P, Caruncho H, Fritschy J-M, Costa E, Guidotti A (1996) Modifications of γ-aminobutyric $acid_A$ receptor subunit expression in rat neocortex during tolerance to diazepam. Mol Pharmacol 49:822–831.

Impagnatiello F, Guidotti AR, Pesold C, Dwivedi Y, Caruncho H, Pisu MG, Uzunov DP, Smalheiser NR, Davis JM, Pandey GN, Pappas GD, Teuting P, Sharma RP, Costa E (1998) A decrease of reelin expression as a putative vulnerability factor in schizophrenia. Proc Natl Acad Sci USA 95:15718–15723.

Johnson AE, Liminga U, Liden A, Lindefors N, Gunne LM, Wiesel F-A (1994) Chronic treatment with a classical neuroleptic alters excitatory amino acid and GABAergic neurotransmission in specific regions of the rat brain. Neuroscience 63:1003–1020.

Korpi ER, Kleinman JE, Wyatt RJ (1988) GABA concentrations in forebrain areas of suicide victims. Biol Psychiatry 23:109–114.

Lavoie AM, Tingey JJ, Harrison NL, Pritchett DB, Twyman RE (1997) Activation and deactivation rates of recombinant $GABA_A$ receptor channels are dependent on α-subunit isoform. Biophys J 73: 2518–2526.

Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Kohler M, Fujita N, Rodriguez HF, Stephenson A, Darlison MG, Barnard EA, Seeburg PH (1988) Structural and functional basis for $GABA_A$ receptor heterogeneity. Nature 335:76–79.

Lewis DA, Lund JS (1990) Heterogeneity of chandelier neurons in monkey neocortex: corticotropin-releasing factor and parvalbumin immunoreactive populations. J Comp Neurol 293:599–615.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. Cary, NC: SAS Institute Inc.

Loup F, Weinmann O, Yonekawa Y, Aguzzi A, Wieser H-G, Fritschy J-M (1998) A highly sensitive immunofluorescence procedure for analyzing the subcellular distribution of $GABA_A$ receptor subunits in the human brain. J Histochem Cytochem 46:1129–1139.

Loup F, Wieser H-G, Yonekawa Y, Aguzzi A, Fritschy J-M (2000) Selective alterations in $GABA_A$ receptor subtypes in human temporal lobe epilepsy. J Neurosci 20:5401–5419.

Marksitzer R, Benke D, Fritschy J-M, Trzeciak A, Bannwarth W, Mohier H (1993) $GABA_A$-receptors: drug binding profile and distribution of receptors containing the α_2-subunit *in situ*. J Receptor Res 13: 467–477.

Melchitzky DS, Sesack SR, Lewis DA (1999) Parvalbumin-immunoreactive axon terminals in monkey and human prefrontal cortex: laminar, regional and target specificity of Type I and Type II synapses. J Comp Neurol 408:11–22.

Melchitzky DS, Lewis DA (2000) Intrinsic axon terminals of layer 3 pyramidal neurons in monkey prefrontal cortex: target selectivity for a subpopulation of GABA neurons. Soc Neurosci Abstr 26:1236.

Melchitzky DS, Gonzalez-Burgos G, Barrionuevo G, Lewis DA (2001) Synaptic targets of the intrinsic axon collaterals of supragranular pyramidal neurons in monkey prefrontal cortex. J Comp Neurol 430:209–221.

Mhatre MC, Ticku MK (1994) Chronic GABA treatment downregulates the $GABA_A$ receptor α_2 and α_3 subunit mRNAs as well as polypeptide expression in primary cultured cerebral cortical neurons. Mol Brain Res 24:159–165.

Mhatre MC, Pena G, Sieghart W, Tietz EI (1993) Antibodies specific for $GABA_A$ receptor α subunits reveal that chronic alcohol treatment down-regulates α-subunit expression in rat brain regions. J Neurochem 61:1620–1625.

Montpied P, Ginns EI, Martin BM, Roca D, Farb DH, Paul SM (1991a) γ-Aminobutyric acid (GABA) induces a receptor-mediated reduction in GABAA receptor α subunit messenger RNAs in embryonic chick neurons in culture. J Biol Chem 266:6011–6014.

Montpied P, Morrow AL, Karanian JW, Ginns EI, Martin BM, Paul SM (1991b) Prolonged ethanol inhalation decreases γ-aminobutyric $acid_A$ receptor α subunit mRNAs in rat cerebral cortex. Mol Pharmacol 39:157–163.

Neter J, Kutner MH, Nachtsheim CJ, Wasserman W (1996) Applied linear statistical models. Chicago, IL: Irwin.

Nusser Z, Sieghart W, Benke D, Fritschy J-M, Somogyi P (1996) Differential synaptic localization of two major γ-aminobutyric acid type A receptor α subunits on hippocampal pyramidal cells. Proc Natl Acad Sci USA 93:11939–11944.

Nyíri G, Freund TF, Somogyi P (2001) Input-dependent synaptic targeting of α_2-subunit-containing $GABA_A$ receptors in synapses of hippocampal pyramidal cells of the rat. Eur J Neurosci 13:428–442.

O'Donovan MC, Buckland PR, Spurlock G, McGuffin P (1992) Bi-directional changes in the levels of messenger RNAs encoding γ-aminobutyric $acid_A$ receptor α subunits after flurazepam treatment. Eur J Pharmacol 226:335–341.

Ohnuma T, Augood SJ, Arai H, McKenna PJ, Emson PC (1999) Measurement of GABAergic parameters in the prefrontal cortex in schizophrenia: focus on GABA content, $GABA_A$ receptor α-1 subunit messenger RNA and human GABA transporter-1 (HGAT-1) messenger RNA expression. Neuroscience 93:441–448.

Pakkenberg B (1990) Pronounced reduction of total neuron number in mediodorsal thalamic nucleus and nucleus accumbens in schizophrenics. Arch Gen Psychiatry 47:1023–1028.

Peters A (1984) Chandelier cells. In: Cerebral cortex (Jones EG, Peters A, eds), vol. 1, pp. 361–380. New York: Plenum Press.

Pierri JN, Chaudry AS, Woo T-U, Lewis DA (1999) Alterations in chandelier neuron axon terminals in the prefrontal cortex of schizophrenic subjects. Am J Psychiatry 156:1709–1719.

Pierri JN, Volk CLE, Auh S, Sampson A, Lewis DA (2001) Decreased somal size of deep layer 3 pyramidal neurons in the prefrontal cortex in subjects with schizophrenia. Arch Gen Psychiatry 58:466–473.

Popken GJ, Bunney WE Jr, Potkin SG, Jones EG (2000) Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. Proc Natl Acad Sci USA 97:9276–9280.

Pucak ML, Levitt JB, Lund JS, Lewis DA (1996) Patterns of intrinsic and associational circuitry in monkey prefrontal cortex. J Comp Neurol 376:614–630.

Rajkowska G, Goldman-Rakic PS (1995) Cytoarchitectonic definition of prefrontal areas in the normal human cortex: I. Remapping of areas 9 and 46 using quantitative criteria. Cereb Cortex 5:307–322.

Rajkowska G, Selemon LD, Goldman-Rakic PS (1998) Neuronal and glial somal size in the prefrontal cortex: a postmortem morphometric study of schizophrenia and Huntington disease. Arch Gen Psychiatry 55:215–224.

Selemon LD, Rajkowska G, Goldman-Rakic PS (1995) Abnormally high neuronal density in the schizophrenic cortex: a morphometric analysis of prefrontal area 9 and occipital area 17. Arch Gen Psychiatry 52:805–818.

Selemon LD, Rajkowska G, Goldman-Rakic PS (1998) Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. J Comp Neurol 392:402–412.

Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Hoger H, Adamiker D (1999) Structure and subunit composition of $GABA_A$ receptors. Neurochem Int 34:379–385.

Somogyi P (1977) A specific axo-axonal interneuron in the visual cortex of the rat. Brain Res 136:345–350.

Sundman I, Allard P, Eriksson A, Marcusson J (1997) GABA uptake sites in frontal cortex from suicide victims and in aging. Neuropsychobiology 35:11–15.

Sutoo D, Akiyama K, Yabe K (1998) Quantitative mapping analyzer for determining the distribution of neurochemicals in the human brain. J Neurosci Methods 85:161–173.

Tietz EI, Huang X, Chen S, Ferencak WF III (1999) Temporal and regional regulation of α_1, β_2, and β_3, but not α_2, α_4, α_5, α_6, β_1, or γ_2 $GABA_A$ receptor subunit messenger RNAs following one-week oral flurazepam administration. Neuroscience 91:327–341.

Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA (2000) Decreased GAD_{67} mRNA expression in a subset of prefrontal cortical GABA neurons in subjects with schizophrenia. Arch Gen Psychiatry 57:237–245.

Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA (2001) GABA transporter-1 mRNA in the prefrontal cortex in schizophrenia: decreased expression in a subset of neurons. Am J Psychiatry 158:256–265.

Weinberger DR, Berman KF, Zec RF (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. Arch Gen Psychiatry 43:114–124.

Woo T-U, Whitehead RE, Melchitzky DS, Lewis DA (1998) A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci USA 95:5341–5346.

Young KA, Manaye KF, Liang C-L, Hicks PB, German DC (2000) Reduced number of mediodorsal and anterior thalamic neurons in schizophrenia. Biol Psychiatry 47:944–953.