Schizophrenia and the Parvalbumin-Containing Class of Cortical Local Circuit Neurons

Tsung-Ung Woo, M.D., Ph.D., Jed L. Miller, B.S., and David A. Lewis, M.D.

Objective: *The purpose of this study was to test the hypothesis that abnormalities in the parvalbumin-containing subclass of local circuit neurons contribute to altered γ-aminobutyric acid (GABA) neurotransmission in the prefrontal cortex of schizophrenic subjects.* *Method:* *Profile counts and somal size measures were made of parvalbumin-immunoreactive neurons in areas 9, 46, and 17 from 15 matched pairs of schizophrenic and normal comparison subjects.* *Results:* *No differences in relative density, laminar distribution, or somal size of labeled neurons were found in any region.* *Conclusions:* *These findings suggest that altered GABA neurotransmission in schizophrenia is due to either abnormalities in other subpopulations of prefrontal cortical GABA neurons or abnormalities in the parvalbumin-containing subclass that could not be detected in the present study.*

(Am J Psychiatry 1997; 154:1013–1015)

Alterations in γ-aminobutyric acid (GABA) neurotransmission are thought to contribute to dysfunction of the prefrontal cortex and other brain regions in patients with schizophrenia (1–4). For example, expression of the mRNA for glutamic acid decarboxylase, the synthesizing enzyme for GABA, is decreased in the prefrontal cortex of schizophrenic subjects (5), and the activity of this enzyme is reduced (6, 7). In addition, $GABA_A$ receptor binding has been reported to be increased (8), perhaps as a compensatory response to diminished GABA neurotransmission.

GABA-containing neurons can be categorized into subtypes on the basis of morphological and biochemical features. The calcium-binding proteins calbindin, calretinin, and parvalbumin are present in separate subpopulations of local circuit neurons (9), which together constitute 90% of GABA neurons (10). It is of interest that parvalbumin is expressed in two morphological subtypes, wide arbor and chandelier cells (11). These subtypes form symmetric, inhibitory synapses with the cell body and axon initial segment, respectively, of pyramidal neurons, the major class of cortical projection neurons. Because these two classes of local circuit neurons provide potent inhibitory regulation of pyramidal neuron activity, they play a critical role in the flow of information processing within the prefrontal cortex. Consequently, we used immunocytochemical techniques to determine whether the size, relative density, or laminar distribution of parvalbumin-immunoreactive neurons was altered in the prefrontal cortex of schizophrenic subjects.

Received Sept. 27, 1996; revision received Feb. 12, 1997; accepted Feb. 27, 1997. From the Departments of Psychiatry and Neuroscience, University of Pittsburgh. Address reprint requests to Dr. Lewis, W1650, Biomedical Science Tower, Western Psychiatric Institute and Clinic, University of Pittsburgh, 3811 O'Hara St., Pittsburgh, PA 15213.

Supported by NIMH grants MH-45156 and MH-43784 and NIMH Independent Scientist Award MH-00519.

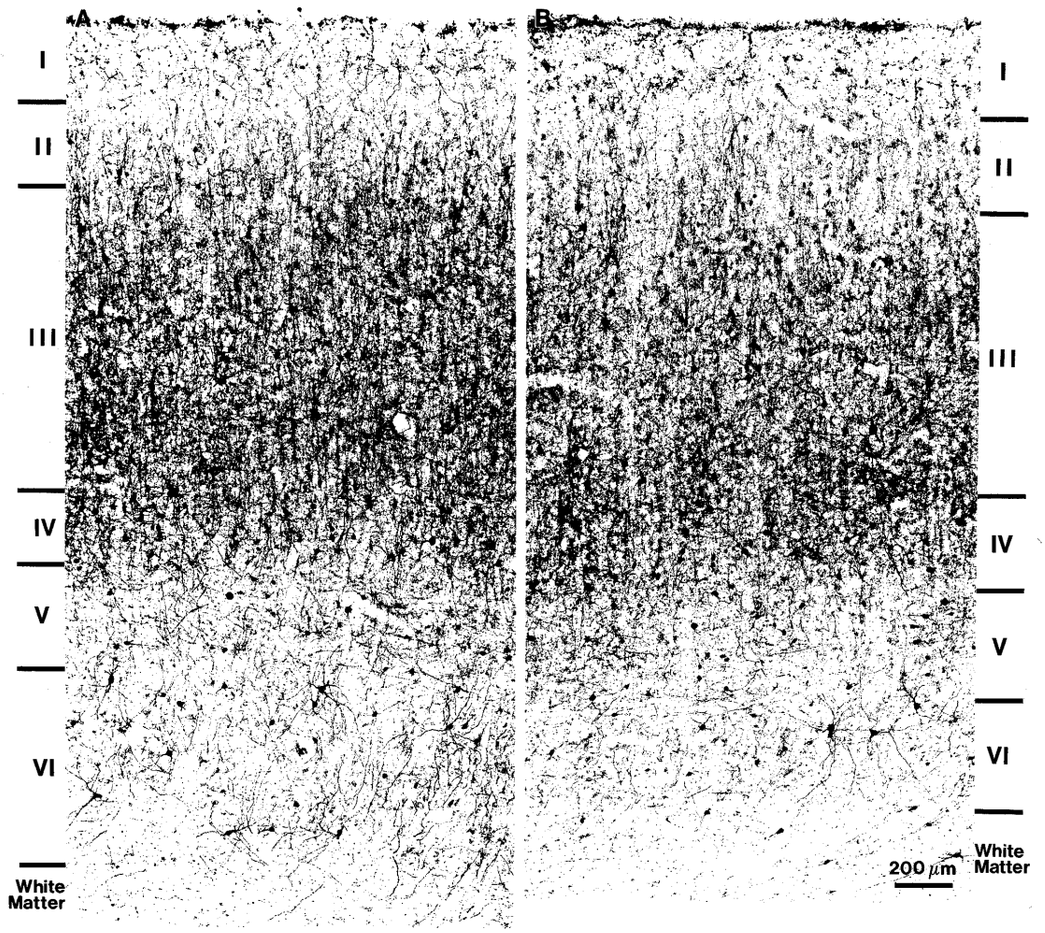
METHOD

Brain tissue was obtained from 15 matched pairs (10 male, five female) of schizophrenic and normal comparison subjects. The schizophrenic subjects did not differ in mean age (53.6 years, SD=13.0) or postmortem interval (11.7 hours, SD=5.6) from the comparison subjects (53.9 years [SD=13.8], and 11.3 hours [SD=5.4], respectively). Clinical information was obtained, and consensus diagnoses were made for each case, as previously described (12). Written informed consent was obtained from the next of kin.

The left hemisphere of each brain was blocked coronally, immersed in cold, phosphate-buffered 4% paraformaldehyde for 48 hours, and stored in an antifreeze solution at –30°C (12). Mean storage time did not differ between the schizophrenic (45.9 months, SD=18.7) and comparison (44.2 months, SD=16.9) subjects. Sections (40 μm) were cut on a cryostat from the relevant tissue blocks, and Nissl-stained sections were examined to identify the location of prefrontal cortex areas 9 and 46, and occipital area 17 (primary visual cortex). The latter region was examined in order to determine the regional specificity of observations made in the prefrontal cortex. Tissue sections that contained the cytoarchitectonic regions of interest were processed for parvalbumin immunoreactivity by using a rabbit antiserum (provided by Dr. K. Baimbridge, University of British Columbia, Vancouver), a standard Vectastain kit (Vector, Burlingame, Calif.), and diaminobenzidine (9). The specificity of the parvalbumin antiserum has been previously demonstrated (9). Sections from each case within a pair were processed together.

Labeled neuronal profiles in four 500-μm-wide cortical traverses, from the pial surface to the white matter border, were plotted at a magnification of 400× for each cortical region of each subject. All counts were made by one investigator (J.L.M.) without knowledge of case number or diagnosis. Recounting of a subset of the same cortical traverses at a different time revealed neuronal counts that differed by

FIGURE 1. Photomicrographs Illustrating the Distribution of Parvalbumin Immunoreactivity in Area 46 From a Normal Comparison Subject and a Matched Schizophrenic Subject[a]



[a]Panel A: 48-year-old white male normal comparison subject with a 7.4-hour postmortem interval; panel B: 52-year-old white male schizophrenic subject with a 10.0-hour postmortem interval. Note the similar patterns of distribution of labeled neurons and processes in both subjects.

less than 2%. The ratio of each laminar boundary to total cortical thickness on Nissl-stained sections was used to determine the location of laminar boundaries on the parvalbumin-labeled sections for each subject. Measurements of the somal size of parvalbumin-immunoreactive neurons were also made in area 9, as previously described (13). Paired t tests (df=14) were used to compare differences in the mean values for the density and somal size of parvalbumin-labeled neurons, and for cortical thickness, in schizophrenic and normal comparison subjects.

RESULTS

The mean relative density and laminar distribution of parvalbumin-immunoreactive neurons did not differ between schizophrenic and comparison subjects in any of the three regions examined (figure 1 and table 1). In addition, the mean somal size of the 647 parvalbumin-labeled neurons of the schizophrenic subjects (mean=137.3 μm^2, SD=63.9) did not differ from the somal size of the 664 parvalbumin-labeled neurons of the comparison subjects (mean=138.0 μm^2, SD=60.7). Finally, compared to the normal comparison group, mean cortical thickness in the schizophrenic subjects was decreased by 3.2% in area 9, 4.7% in area 46, and 5.9% in area 17, but none of these differences achieved statistical significance.

DISCUSSION

Although the profile counting procedure used in this study cannot be used to estimate absolute neuronal density or number, our findings do demonstrate that the relative density and laminar distribution of parvalbumin-immunoreactive local circuit neurons are not altered in the prefrontal cortex of schizophrenic subjects. Furthermore, the absence of a difference in somal size indicates that this factor did not confound the comparisons of neuronal density. Greater neuronal density has been observed in the prefrontal cortex of schizophrenic

TABLE 1. Relative Density and Laminar Distribution of Parvalbumin-Immunoreactive Neurons in 15 Matched Pairs of Schizophrenic and Normal Comparison Subjects

	Density (neurons/mm^2)		Percentage of Neurons Per Layer					
			I–III		IV		V–VI	
Brain Area and Subject Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Prefrontal cortex area 46								
Comparison subjects	76.8	27.9	52.8	8.1	18.1	5.0	29.2	6.8
Schizophrenic patients	75.7	21.3	52.3	8.5	17.3	5.7	30.3	9.4
Prefrontal cortex area 9								
Comparison subjects	60.3	16.4	50.7	6.8	13.4	2.8	35.8	7.8
Schizophrenic patients	61.2	14.6	49.6	9.3	14.8	4.3	35.6	10.5
Occipital area 17								
Comparison subjects	200.7	56.0	35.8	6.5	49.3	7.1	14.9	8.2
Schizophrenic patients	188.4	40.3	36.5	10.9	49.9	7.4	13.6	5.9

subjects in some (14), but not all (5), studies and has been attributed to a loss of cortical neuropil. Consequently, it is possible that the number of parvalbumin-containing neurons is actually decreased in schizophrenic subjects but that the density of these neurons appears normal because of a reduced cortical volume. However, this effect would appear to be small in the present study, since there was only a 3.2%–5.9% decrease in mean cortical thickness in the schizophrenic relative to the comparison subjects. In addition, the failure to find a difference in the density of parvalbumin-immunoreactive neurons between schizophrenic and comparison subjects does not appear to be a consequence of inadequate sample size, since the magnitude of the effect reported in a previous study of cortical local circuit neuron density (4) indicates that the power of the current study exceeds 0.95.

The findings of the present study do not exclude the possibility of other abnormalities in the parvalbumin class of local circuit neurons that might reflect altered GABA neurotransmission. For example, during adolescence, the density of parvalbumin-containing neurons in monkey prefrontal cortex remains constant, but the detectability of parvalbumin immunoreactivity in the axon terminals of chandelier neurons decreases substantially (15). Since these axon terminals cannot be identified reliably in postmortem prefrontal cortex, alterations in the parvalbumin content of these terminals in schizophrenia cannot be excluded.

Finally, a previous study failed to reveal a difference in the density of calretinin-containing prefrontal cortex local circuit neurons in schizophrenia but did show an increase in the density of neurons that express calbindin (16). Together, these findings suggest that abnormalities in GABA neurotransmission in schizophrenia may be restricted to certain subpopulations of prefrontal cortex local circuit neurons.

REFERENCES

1. Benes FM, Khan Y, Vincent SL, Wickramasinghe R: Differences in the subregional and cellular distribution of $GABA_A$ receptor binding in the hippocampal formation of schizophrenic brain. Synapse 1996; 22:338–349
2. Kiuchi Y, Kobayashi T, Takeuchi J, Shimizu H, Ogata H, Toru M: Benzodiazepine receptors increase in post-mortem brain of chronic schizophrenics. Eur Arch Psychiatry Neurol Sci 1989; 239:71–78
3. Simpson MDC, Slater P, Deakin JFW, Royston MC, Skan WJ: Reduced GABA uptake sites in the temporal lobe in schizophrenia. Neurosci Lett 1989; 107:211–215
4. Benes FM, McSparren J, Bird ED, SanGiovanni JP, Vincent SL: Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. Arch Gen Psychiatry 1991; 48:996–1001
5. Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr, Jones EG: Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatry 1995; 52:258–266
6. Bird ED, Spokes EGS, Iversen LL: Increased dopamine concentration in limbic areas of brain from patients dying with schizophrenia. Brain 1979; 102:347–360
7. Sherman AD, Davidson AT, Baruah S, Hegwood TS, Waziri R: Evidence of glutamatergic deficiency in schizophrenia. Neurosci Lett 1991; 121:77–80
8. Benes FM, Snyder-Marie A, Vincent S, Khan Y: Up-regulation of GABA-A receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. Neuroscience 1996; 75:1021–1031
9. Condé F, Lund JS, Jacobowitz DM, Baimbridge KG, Lewis DA: Local circuit neurons immunoreactive for calretinin, calbindin D-28k, or parvalbumin in monkey prefrontal cortex: distribution and morphology. J Comp Neurol 1994; 341:95–116
10. Gabbott PLA, Bacon SJ: Local circuit neurons in the medial prefrontal cortex (areas 24a, b, c, 25 and 32) in the monkey: II. Quantitative areal and laminar distributions. J Comp Neurol 1996; 364:609–636
11. Lund JS, Lewis DA: Local circuit neurons of developing and mature macaque prefrontal cortex: golgi and immunocytochemical characteristics. J Comp Neurol 1993; 328:282–312
12. Glantz LA, Lewis DA: Reduction of synaptophysin immunoreactivity in the prefrontal cortex of schizophrenic subjects: regional and diagnostic specificity. Arch Gen Psychiatry (in press)
13. Hayes TL, Lewis DA: Nonphosphorylated neurofilament protein and calbindin immunoreactivity in layer III pyramidal neurons of human neocortex. Cereb Cortex 1992; 2:56–67
14. Selemon LD, Rajkowska G, Goldman-Rakic PS: Abnormally high neuronal density in the schizophrenic cortex: a morphometric analysis of prefrontal area 9 and occipital area 17. Arch Gen Psychiatry 1995; 52:805–818
15. Condé F, Lund JS, Lewis DA: The hierarchical development of monkey visual cortical regions as revealed by the maturation of parvalbumin-immunoreactive neurons. Brain Res Dev Brain Res 1996; 96:261–276
16. Daviss SR, Lewis DA: Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. Psychiatry Res 1995; 59: 81–96