Postmortem Studies in Schizophrenia

by Peter Powchik, Michael Davidson, Vahram Haroutunian, Stephen M. Gabriel, Dushyant P. Purohit, Daniel P. Perl, Philip D. Harvey, and Kenneth L. Davis

Abstract

The past decade has seen renewed interest in the neuropathology of schizophrenia. The advent of new postmortem techniques and functional imaging, along with a greater understanding of the neuropsychology of schizophrenia, have provided many new clues to the nature of the underlying brain dysfunction in this disorder. There has also been a greater understanding of the presence of severe cognitive dysfunction among many elderly persons with schizophrenia. In this article, a series of investigations are described that seek to answer basic questions about the neuropathology of schizophrenia, in particular as it pertains to cognitive impairment. The first study describes neuropathological findings in 100 consecutively autopsied persons with schizophrenia, the majority of whom had had detailed antemortem assessments. Results from this first study prompted the conclusion that schizophrenia is not characterized by classical, histologically identifiable neuropathology. Moreover, most cases of dementia in schizophrenia are probably not the result of neuropathologically identifiable dementing illnesses. The next four studies examined chemical markers that are altered in Alzheimer's disease and some other dementing conditions and have also been suggested to be abnormal in schizophrenia: choline acetyltransferase, catecholamines and indolamines, neuropeptides, and synaptic proteins. Schizophrenia cases as a group did not show a cholinergic deficit; nor did they differ from elderly comparison cases with respect to cortical catecholamines and indolamines. Among the schizophrenia cases, however, cognitive impairment was negatively correlated with choline acetyltransferase activity. Those with cognitive impairment showed evidence of cortical noradrenergic and serotonergic deficits. Neuropeptide deficits were also present in schizophrenia, but their pattern differed from that seen in Alzheimer's disease. Increased synaptic protein activity was found in the cingulate cortex of persons with schizophrenia, and this activity was correlated with schizophrenia symptoms. From this second series of studies, it was concluded that some biological measures in schizophrenia may be related to cognitive impairment (e.g., cortical amines), whereas others may be related to diagnosis (e.g., neuropeptide deficits). In addition, synaptic organization may correlate with schizophrenia symptoms.

Key words: Brain pathology, dementia, serotonin, norepinephrine, synaptic proteins.


Over the past three decades, our understanding of the clinical boundaries of schizophrenia, its associated cognitive impairments, and even its treatment has advanced significantly. Unfortunately, a fundamental understanding of the biological underpinnings of schizophrenia has proven elusive. The “challenge study” paradigm of the 1970s and 1980s, although still useful in advancing the understanding of some psychiatric disorders, has generally fallen out of favor as a tool to investigate the biology of schizophrenia. Replacing these methods are advances in functional imaging that can indirectly reveal differences in brain function between patients with schizophrenia and controls. Despite the knowledge that can be gained, however, functional neuroimaging remains a collection of indirect methods. To fully understand the abnormal brain structure or functional connections that are presumed to be present in and responsible for schizophrenia, more direct methods must be employed. The most direct methods use postmortem brain tissue.

As reviewed by Bogerts (1993), there were nearly 200 neuropathological studies of schizophrenia before 1957 and then, from the mid-1950s to 1990, the topic
remained conspicuously absent from the international academic venue. A major discouragement to continued neuropathological studies was the fact that classical techniques were unable to identify any consistent abnormalities in brain tissue derived from persons with schizophrenia.

The advent of powerful new neuroscience techniques sparked a renewed interest in the field (Kleinman et al. 1988; Shapiro 1993). There are even trends in findings that suggest the presence of temporal lobe (Akbarian 1993b), thalamic (Pakkenberg 1992), hippocampal (Arnold et al. 1995a), or cingulate (Benes 1993) abnormalities in schizophrenia. However, despite technical advances, consistently replicable findings with respect to the neuropathology of schizophrenia are still lacking.

Recently, several clearly definable methodological problems hampering progress in neuropathological studies of schizophrenia have been pointed out (Arnold et al. 1995b). Among those problems are tissue acquisition difficulties, which translates into a small number of cases; unreliability of clinical information; inadequate or improper comparison groups; inconsistent tissue handling; qualitative rather than quantitative neuropathological descriptions; and the generally well-recognized limitations of classical neuropathological techniques. The series of studies described here attempted to address all of these issues and shed some additional light on the neuropathology of schizophrenia.

Study Background

Our group has maintained an active role in developing a brain bank for the study of neuropsychiatric disorders since the early 1980s. In 1988, we entered into an academic association with the Pilgrim Psychiatric Center (PPC, a facility operated by the New York State Office of Mental Health) and the opportunity to develop a brain bank for schizophrenia presented itself. PPC was arguably one of the largest psychiatric hospitals in the United States, if not the world. It was originally planned to house up to 35,000 patients but at its maximum census in the early 1960s housed about 17,000 patients. In 1988, as a result of "deinstitutionalization," the patient population of PPC was approximately 2,300, with nearly 1,000 patients over 65. The striking features of the elderly population were the high prevalence of moderate to severe cognitive impairment (> 60%) and the fact that many of the cases were the high prevalence of moderate to severe cognitive impairment in particular:

- Is schizophrenia characterized by classically identifiable cortical neuropathology?
- Can the biological characteristics of postmortem brain tissue from persons with schizophrenia be related to symptoms of the illness? If so, is the pathology regional or diffuse?
- Is there a definable postmortem neuropathology and/or biology of severe cognitive dysfunction in schizophrenia?

Methods

The Population. Brain tissue samples used in the series of studies presented here were derived from a population of long-term psychiatric inpatients who have been previously described (Harvey et al. 1992; Davidson et al. 1995). Subjects were patients at PPC, a large hospital that cares primarily, though not exclusively, for persistently ill
geriatric patients with schizophrenia. The series of patients described here represents 100 autopsies consecutively obtained from 1989 to 1995. Of these 100 cases, 69 had postmortem diagnostic assessments and 31 were diagnosed retrospectively by chart review. Of the 69 cases that had postmortem diagnostic assessments, 63 had full symptom and cognitive assessments as well. The 31 cases assessed retrospectively did not differ in terms of age, gender distribution, or length of illness.

Subjects ranged in age from 52 to 101 years (mean = 78.5, standard deviation [SD] = 10.5). All cases met DSM-III-R (American Psychiatric Association 1987) criteria for chronic schizophrenia. To generate a lifetime diagnosis of DSM-III-R schizophrenia, an experienced psychiatrist reviewed all available medical records, interviewed collateral informants (most often clinical staff), and used data from interviews with the patients themselves when they were able to contribute information pertinent to presenting symptoms and course of illness. For the subjects who had a postmortem diagnostic assessment, a similar procedure was followed (other than the patient interview) within several weeks of the patient’s death. In these cases, the main caregiver and the treating psychiatrist were interviewed, and an extensive review of medical records was performed.

Psychiatric subjects without schizophrenia (n = 47) were patients at PPC who did not meet DSM-III-R criteria for schizophrenia or a developmental disorder but who manifested psychiatric symptoms during life that resulted in long-term hospitalization. These subjects ranged in age from 53 to 106 years (mean = 76.9, SD = 11.4).

AD cases were those that met criteria for AD established by the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) (Mirra et al. 1991) and who had no other significant neuropathological findings. The age of subjects with AD (n = 135) from whom samples were derived averaged 83.3 years (SD = 15.4, range 56–100 years). All subjects with AD were assessed while still alive by trained clinical raters.

Specimens from nonpsychiatric, non-AD elderly comparison cases (n = 50) were defined as those derived from subjects who had no chart history of neurological or psychiatric disease, who manifested no cognitive deficits or questionable ones as defined by the Clinical Dementia Rating Scale (CDR; Hughes et al. 1982), and whose brains at autopsy showed no neuropathological lesions other than those that might be expected given the subjects’ age (e.g., age-related density of senile plaques). The mean age of this sample was 78.5 years (SD = 13.5, range 53–96 years). Tissue samples from several additional elderly cases with multi-infarct dementia (MID) were used in the neuropeptide (n = 4), neurotransmitter (n = 2), and synaptic protein studies (n = 3). Characteristics of subjects in all studies are shown in table 1.

The Assessment. Assessment instruments included the Positive and Negative Syndrome Scale (PANSS; Kay et al. 1987), the CERAD battery of cognitive assessments (Morris et al. 1989), and the CDR scale of Hughes et al. (1982). Interrater agreement for the PANSS was high, with intraclass correlation coefficients for its 30 items ranging from 0.86 to 1.00 (with all p values < 0.001 based on 54 interviews conducted by two raters). A detailed description of reliability in the use of these scales and the entire CERAD cognitive battery has been previously published (Harvey et al. 1992).

Specimen Retrieval and Preparation. At autopsy, the brain was divided at the mid-sagittal plane. The right half was fixed in 4 percent formalin for use in neuropathological evaluations. Some fresh specimens and all brain stems and cerebella were available for neuropathological examination. The left half of the brain was coronally sectioned into 0.5- to 0.8-mm slabs and flash-frozen in liquid nitrogen for storage at –80 °C. These frozen sections were used for all assays other than classical neuropathology.

Neuropathological Methods. A detailed description of the neuropathological methods has been previously published (Purohit et al. 1993). Specimens were examined using the standardized protocol of the Mount Sinai-Bronx Veterans Affairs Medical Center Alzheimer’s Disease Research Center, which was, in turn, adopted from procedures developed by CERAD (Mirra et al. 1991). Paraffin tissue blocks were prepared from five neocortical areas (Brodmann areas 9, 45/47, 21/22, 39, and 17), rostral and caudal hippocampus, the nucleus basalis of Meynert, amygdala, midbrain, pons, medulla, and cerebellum. Sections were stained with hematoxylin and eosin, thioflavine S, and modified Bielschowsky’s stains. Immunohistological staining with ubiquitin was done to identify Lewy body formation.

All neuropathological assessments were performed blind to clinical information. This assessment included quantitative estimates (using a 4-point scale: absent, sparse, moderate, and severe) for the density of senile plaque, neurofibrillary tangles (NFTs), and other AD-related histological changes, such as amyloid angiopathy, neocortical neuronal loss, neuropil degeneration and gliosis, hippocampal degeneration (including neuronal loss, granulovacuolar degeneration, and Hirano bodies), and degeneration of the subcortical and brain stem nuclei. A further assessment of the senile plaque density in the neocortex was carried out using the following quantitative...
Table 1. Subject characteristics in each experiment

<table>
<thead>
<tr>
<th>Neuropathology</th>
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<th>AD</th>
<th>Non-MID elderly</th>
<th>Psychiatric disorders (not schizophrenia)</th>
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<tr>
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<td>100</td>
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<td>50</td>
<td>47</td>
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<td>83.3 ± 11.5</td>
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<td>76.9 ± 11.4</td>
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<td>51:84</td>
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<td>29:18</td>
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<td>3.72 ± 1.5 (3-5)</td>
<td>0.15 ± 0.23 (0-0.5)</td>
<td>2.7 ± 1.3 (0-5)</td>
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<table>
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<th>MID elderly</th>
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<td>135</td>
<td>20</td>
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</tr>
<tr>
<td>Age, mean ± SD (range)</td>
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<td>83.3 ± 11.5</td>
<td>78.5 ± 12.1</td>
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<tr>
<td>(56-100)</td>
<td>(56-100)</td>
<td>(55-96)</td>
<td></td>
<td></td>
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<tr>
<td>Gender, male:female</td>
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<td>51:84</td>
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<tr>
<td>CDR</td>
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<td>3.72 ± 1.5 (3-5)</td>
<td>0.28 ± 0.08 (0-0.5)</td>
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<th>MID elderly</th>
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<td>Age, mean ± SD (range)</td>
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<td>82.9 ± 10.4</td>
<td>92 ± 10</td>
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<td>(70-96)</td>
<td>(85-99)</td>
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<td>CDR</td>
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<td>4.7 ± 0.7 (4-5)</td>
<td>0.23 ± 0.25 (0-0.5)</td>
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<th>MID elderly</th>
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<td>3</td>
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<td>80.0 ± 10.2</td>
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<td>(65-99)</td>
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<td>16:8</td>
<td>5:8</td>
<td>3:0</td>
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<td>CDR</td>
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<td>4.2 ± 0.9 (3-5)</td>
<td>0.23 ± 0.25 (0-0.5)</td>
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<th>MID elderly</th>
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<td>13</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Age, mean ± SD (range)</td>
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<td>79.1 ± 7.5</td>
<td>85.3 ± 4.9</td>
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<td>(58-88)</td>
<td>(71-88)</td>
<td>(78-92)</td>
<td></td>
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<tr>
<td>Gender, male:female</td>
<td>12:7</td>
<td>9:4</td>
<td>4:0</td>
<td>4:0</td>
</tr>
<tr>
<td>CDR</td>
<td>2.2 ± 1.2 (0.5-4)</td>
<td>4.4 ± 0.7 (3-5)</td>
<td>0.1 ± 0.2 (0-0.5)</td>
<td>2.6 ± 1.5 (2-5)</td>
</tr>
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</table>

Note.—AD = Alzheimer's disease; MID = multi-infarct dementia; SD = standard deviation; CDR = Clinical Dementia Rating scale (Hughes et al. 1982).

Methods: The neocortical senile plaques were counted in five areas (selected from high-senile plaque-density areas identified at low-power scanning) in each thioflavine-S-stained slide of the neocortex, using medium-power (X250) magnification, giving a calculated visual field of 0.5 mm². The plaques that showed neuritic changes and/or amyloid cores were counted for this assessment. From these results, a mean neocortical senile plaque count per square area was calculated for each case.

Neuropathological examination also included documentation of infarctions, neoplasms, cerebrovascular changes, and other morphological abnormalities. Other neurodegenerative disorders were also screened for (e.g., Parkinson's disease, diffuse Lewy body disease, Jakob-Creutzfeldt disease, and Pick's disease). A neuropathological diagnosis of AD was made using the CERAD diagnostic criteria (Mirra et al. 1991).

Biological Assays.

Choline Acetyltransferase (ChAT). Inferior parietal cortex from 95 persons with schizophrenia (from the 100 total cases), 135 persons with neuropathologically confirmed AD, and 20 normal controls was used to estimate ChAT. Before assay, frozen tissue slabs containing the inferior parietal lobe were warmed to -20 °C and then dissected using a scroll saw. The dissected inferior parietal lobe was immersed in liquid nitrogen, crushed, and pulverized in a liquid nitrogen-cooled mortar and pestle. The powdered homogenate was aliquoted into 50–100 mg portions and kept at -80 °C until assay. The procedures used for the ChAT activity assay were identical to those previously described by Haroutunian et al. (1994).

Other Neurotransmitters. Brain tissue samples were stored at -80 °C until assay by high-performance liquid chromatography with electrochemical detection.
Postmortem Studies


based on the procedure of Maruyama et al. (1980). Cortical tissue samples (Brodmann areas 8, 32, 44, 22, 36, and 7) from 19 elderly people with chronic schizophrenia without evidence of neuropathology (12 of whom were cognitively impaired: CDR score \( \geq 1 \)), 10 persons with neuropathologically confirmed AD, and 9 elderly normal controls were used. Tissue samples were extracted into perchloric acid containing dihydroxybenzylamine (DHBA) as internal standard ethylenediamine tetraacetic acid (EDTA), sodium metabisulfite, and cysteine hydrochloride using a Fisher Sonic Dismembranator 300 with attached microtip (at 35% power). After centrifugation (6,000 rpm \( \times 10 \) min), the supernatant was filtered in microfilterfuge tubes (Rainin Instruments). Standard mixtures of norepinephrine (NE), 3-methoxy-4-hydroxyphenolglycol (MHPG), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were diluted in 0.1 M perchloric acid also containing DHBA as internal standard and run in parallel with the brain tissue samples. Samples (50–100 mL) were injected using a Waters 712 refrigerated autosampler connected to a Shimadzu 6,000 pump, and detection was made with an LC–4C detector and CC–4C cell (Bioanalytical Systems Inc., West Lafayette, IN). A Phase 11 analytical column (C18 250 \( \times \) 4.6 mm, 5 \( \mu m \), BAS) was protected by a C18 guard column (15 \( \times \) 4.6 mm, 5 \( \mu m \), Brownlee). The mobile phase consisted of 0.15 M monochloracetic acid, 0.12 M sodium hydroxide, disodium EDTA (0.25 mg/L), sodium octylsulfate (300 mg/L), and methanol (8%) at pH 2.7. Concentrations of amines and metabolites in the samples were calculated: \((\text{Ratio sample: internal standard } [\text{IS}] \times \text{standard concentration} \times 2 \text{ mL/(Ratio standard:[IS]} \times \text{weight of frozen tissue})\) 

Synaptic Proteins (SP). The methods and results of the SP experiments are reported in detail by Gabriel et al. (1997) and summarized here. In the first series of experiments, presynaptic protein immunoreactivities from cerebral cortical areas were assessed using an antibody capture immunoassay in 19 elderly patients with schizophrenia, 24 patients with AD, and 16 elderly controls. Immunoreactivities to brain SPs were estimated by antibody-capture, enzyme-linked immunosorbant assay (ELISA) using the previously described monoclonal antisera: EP10 and SP4, which recognize synaptophysin (Honer et al. 1989, 1992, 1993), and SP6 and SP14, which recognize syntaxin and SNAP–25 immunoreactivity, respectively (Honer et al. 1993). All three proteins recognized by these antibodies are associated with the presynaptic membrane and involved in vesicle docking and neurotransmitter release (Sollner et al. 1993; Bajjalieh and Scheller 1995; Littleton and Bellen 1995). Brain tissue (cortical tissue corresponding to Brodmann areas 24, 8, 20, and 7) for these studies was derived from the total pool of schizophrenia, AD, and normal elderly brain samples and included tissue from 19 persons with schizophrenia, 24 persons with neuropathologically confirmed AD, and 16 elderly controls. The demographic details of these cases are published elsewhere (Gabriel et al. 1997) and summarized in table 1 here. Thirteen of the 16 elderly controls were cognitively normal and had no discernible neuropathology; the other 3 were cognitively impaired and on neuropathological examination showed evidence of vascular disease and multiple infarcts. These cases were included in the study because the infarcts were distal to the cortical regions sampled and abnormalities had not been detected in earlier neurochemical studies of these cases (Gabriel et al. 1993, 1996).

Immunoreactivity data were combined with subject information and analyzed for differences between diagnostic groups using analysis of variance (ANOVA), for repeated measures. Although these proteins are distinct, each is intimately associated with presynaptic elements, and therefore their immunoreactivities were treated as repeated measures, while each cortical region was analyzed separately. Differences between groups were examined using Tukey’s honestly significant difference (HSD) test for uneven sample sizes and a \( p \) value of less than or equal to 0.05 was considered significant.

In a second experiment, brain tissue from 28 schizophrenia subjects who had antemortem assessments of psychopathology was analyzed for levels of immunoreactivity of the presynaptic proteins recognized by SP4 (synaptophysin) and SP14 (SNAP–25) using methods identical to those described above. Part of that antemortem assessment was the PANSS. Pearson correlations between SP4 and SP14 immunoreactivity and PANSS positive and PANSS negative subscale scores were calculated.

Neuropeptides. The details of these procedures have been previously described (Gabriel et al. 1996). Concentrations of the neuropeptides galanin, somatostatin, neuropeptide Y, corticotropin-releasing hormone (CRH), vasointestinal peptide (VIP), and cholecystokinin (CCK) were determined in six cortical regions from 19 persons with schizophrenia, 13 with AD, and 8 elderly subjects (4 considered “normal elderly” and 4 with MID). Neuropeptide concentrations were quantified in six Brodmann areas corresponding to the superior frontal gyrus, cingulate gyrus, superior temporal gyrus, and parahippocampal gyrus, as well as the superior parietal gyrus. One-way ANOVAs were performed for each peptide with diagnosis as the grouping variable.
Results

Neuropathological Findings. Table 2 gives the breakdown of neuropathological findings in brain tissue derived from the 100 consecutive autopsies of persons with schizophrenia. Eighty-seven of the 100 cases showed no neuropathological evidence of a dementing illness, but nonetheless had a mean CDR score of 2.35 which is indicative of more than moderate dementia in this group.

No difference was found between the density of age-related neocortical senile plaque formation in subjects with schizophrenia and the elderly comparison group (364.9 ± 427 vs. 192.6 ± 315.4, NS by Student's t test). The distribution of senile plaques in the three diagnostic groups according to age is shown in table 3. A two-factor ANOVA (age × diagnosis) showed a strong age effect on the density of plaques (F_{AGE} = 4.0, df = 6,176, p < 0.05), but no effect of diagnostic group (F_{DIAGNOSIS} = 1.8, df = 2,176, p > 0.5) and no interaction between age and diagnosis (F_{AGE × DIAGNOSIS} = 0.9, df = 12,176, p > 0.05). Among the subjects with schizophrenia, there was no correlation between plaque density and cognitive impairment (see figure 1).

ChAT. Among the three groups used for these studies, one-way ANOVAs revealed significant differences in age (F_{AGE} = 8.6, df = 2,247, p < 0.001), PMI (F_{PMI} = 74.1, df = 2,247, p < 0.001), and CDR score (F_{CDR} = 17.8, df = 2,210, p < 0.001). Covarying for these three in a one-way analysis of covariance (ANCOVA) with diagnostic group as the grouping variable revealed a significant group effect on ChAT activity (F_{DIAGNOSIS} = 18.25, df = 2,206, p < 0.0001). Post-hoc tests (Tukey's HSD for unequal ns) showed that the significant group differences were attributable to differences between the AD group and the schizophrenia and elderly comparison groups. There was no difference in ChAT activity between the latter two groups (p > 0.06). When subjects with schizophrenia and long PMIs (> 8 hours) were excluded from the analysis, significant differences in parietal cortex ChAT activity remained between groups (F_{DIAGNOSIS} = 20.9, df = 2,176, p < 0.001).

In this study, subjects with schizophrenia were significantly cognitively impaired (mean CDR = 2.18 ± 0.12). Despite the lack of diminished cortical ChAT activity in these subjects, there was a significant correlation of ChAT activity with their CDR scores (r = -0.29, p < 0.005), which was essentially identical to that among AD subjects (r = -0.36, p < 0.0003). These data suggest that although ChAT activity is not diminished in subjects with schizophrenia compared with normal controls, its activity may nonetheless contribute to cognitive functioning.

Other Neurotransmitters. Repeated measures ANOVAs with diagnosis as the grouping variable and cortical region as the repeated measure were conducted for each of the neurotransmitters and metabolites. No differences between subjects with schizophrenia and normal subjects were found in any of the neurotransmitters or their metabolites in any of the regions, although there was a nonsignificant trend toward lower DA, HVA, and...
DOPAC in subjects with schizophrenia compared with controls. Post-hoc tests showed that subjects with AD had marked deficits in 5-HT compared with normal subjects ($p < 0.006$ by Tukey’s HSD test for unequal ns) but not compared with schizophrenia subjects ($p = NS$ by Tukey’s HSD test for unequal ns), and deficits in 5-HIAA compared with normal subjects ($p < 0.003$ by Tukey’s HSD test for unequal ns) and schizophrenia subjects ($p < 0.005$ by Tukey’s HSD test for unequal ns). Subjects with schizophrenia were no different from normal ones on these measures.

To investigate the relationship between neurochemical measures and cognition, subjects with schizophrenia were then divided according to cognitive status and compared with each other. Subjects with schizophrenia who had significant cognitive impairment (i.e., those with a CDR score $\geq 1$) showed marked cortical deficits of 5-HT ($F_{CDR} = 5.6$, $df = 1,12$, $p < 0.04$ by repeated measures ANOVA) and 5-HIAA ($F_{CDR} = 12.6$, $df = 1,11$, $p < 0.005$). Deficits of 5-HIAA were most prominent in all Brodmann areas tested, whereas deficits of 5-HT were most prominent in Brodmann areas 32 (cingulate cortex).
and 22 (temporal cortex). Deficits of NE, but not MHPG were found in Brodmann areas 8, 32, 44, and 7 (all ps < 0.04 by Scheffé’s test). These relationships are shown in figures 2 and 3.

SPs. A significant diagnostic group effect was found in the cingulate cortex \( F_{\text{DIAGNOSIS}} = 5.07, df = 2,51, p < 0.005 \) with immunoreactivities in the anterior cingulate cortex (Brodmann area 24) of subjects with schizophrenia being elevated compared with both control and AD cases \( (p < 0.02 \) by Tukey’s HSD for unequal ns) (figure 4). Subjects with schizophrenia did not differ from elderly controls in the three other cortical regions, although significant group effects were found in the superior frontal gyrus (Brodmann area 8; \( F_{\text{DIAGNOSIS}} = 10.8, df = 2,40, p < 0.0001 \), inferior temporal gyrus (Brodmann area 20; \( F_{\text{DIAGNOSIS}} = 3.3, df = 2,30, p < 0.05 \)).

Although there was no significant correlation between PMI and presynaptic protein immunoreactivity, and additional repeated measures ANOVA was performed on a subset of nine schizophrenia cases with the lowest PMI matched to within 100 minutes of nine controls \( (\text{PMI}_{\text{SCHIZOPHRENIA}} = 441 \pm 206 \text{ min}, \text{PMI}_{\text{CONTROL}} = 393 \pm 180 \text{ min}) \). As in the larger sample, a significant effect of diagnostic group was found in the cingulate cortex \( F_{\text{DIAGNOSIS}} = 7.5, df = 1,11, p < 0.02 \). In this smaller subset of cases with similar PMIs, a significant group effect on presynaptic protein immunoreactivity was also found in the inferior temporal gyrus \( F_{\text{DIAGNOSIS}} = 11.42, df = 1,11, p < 0.006 \). There were no differences between

Figure 2. 5-HIAA and 5-HT in postmortem brain tissue

Cortical 5HIAA in Demented & Nondemented Schizophrenics

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{5-HIAA in Demented & Nondemented Schizophrenics}
\end{figure}

Cortical 5HT in Demented & Nondemented Schizophrenics

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{5HT in Demented & Nondemented Schizophrenics}
\end{figure}

5-HIAA = 5-hydroxyindoleacetic acid; CDR = Clinical Dementia Rating scale (Hughes et al. 1982); SE = standard error; 5-HT = serotonin.
Figure 4. Synaptic protein activity in cingulate cortex of subjects with schizophrenia and controls

Immunoactivity: Relative density per microgram of protein

Discussion

The series of postmortem studies presented here was designed and implemented with the intention of overcoming many, if not all, of the shortcomings of earlier studies. These neuropathological studies of schizophrenia are, to our knowledge, the largest in an antemortem-characterized cohort and thus address the issue of small sample size. Tissue handling was uniform for all studies because samples were obtained from a single brain bank. Diagnoses were made in most cases while the subjects were still alive, and retrospective diagnoses were made immediately after death on the basis of information in detailed clinical records. Quantitative measurements of plaques, tangles, and other measures of neuropathology performed by experienced research neuropathologists were the inviolate rule, not the exception, as highlighted by the use of a standardized assessment procedure developed by the CERAD group (Mirra et al. 1991). Thus, the neuropathological diagnostic assignments can be considered both valid and reliable. Finally, comparison groups were made up of other well-characterized cases whose tissues were handled identically to those from the subjects with schizophrenia.

The data thus support a series of conclusions:

Schizophrenia is not characterized by classical histologically identifiable neuropathology. This conclusion is in line with most previous reports on smaller cohorts,
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most without antemortem assessments (see Shapiro 1993). When leukotomized cases and the small number of AD and Parkinson’s cases were excluded, the subjects with schizophrenia displayed remarkably little neuropathology. Even brain weights did not differ, in contrast to the findings of other groups (e.g., Pakkenberg 1987; Bruton et al. 1990). However, the size of the sample, the well-characterized diagnoses of the cohort, and the uniformity of tissue handling and examination allow us to state with confidence that gross neuropathology and classical staining methods are insufficient to differentiate the brains of persons with schizophrenia from the brains of persons who do not have schizophrenia.

Most cases of dementia in schizophrenia are probably not the result of neuropathologically identifiable dementing illnesses. The vast majority of the cohort of patients with schizophrenia in this study had severe cognitive impairment. Only 13 of the 100 cases had evidence of neuropathology that would explain that impairment. In the 87 cases without prominent neuropathology, the mean CDR score was 2.35, indicating moderate to severe cognitive and functional impairment. This is similar to the findings of Arnold et al. (1994) and El-Mallakh et al. (1991) in 10 demented subjects with schizophrenia, but in contrast to those of Prohovnik et al. (1993). The Arnold study employed techniques quite similar to those presented here. The Prohovnik study, which reported a high incidence of AD, was heroic in its scale (more than 1,000 cases) but nonetheless relied primarily on retrospective diagnostic assessments, a dearth of cognitive information, and variable handling of tissue (which was collected over several decades).

AD clearly occurs in persons with definite schizophrenia, at a rate comparable to that seen in the general elderly population. Other neuropathology also exists in this cohort of subjects with schizophrenia (e.g., age-related plaque accumulation, mild vascular disease, and so on), but it is of a severity that would likely not result in dementia if it occurred in an average 80-year-old. However, the possibility of “normal” pathology coexisting in an already compromised brain might form the basis for severe cognitive impairment. An analogous situation exists in AD with regard to level of education. When matched for density of plaque counts, persons with higher levels of education (which correlates with future cognitive capacity) were less severely impaired during life (Stern et al. 1994). Stated differently, those persons with less cognitive capacity required less neuropathology to manifest cognitive impairment. It may be that persons with schizophrenia are more susceptible to pathological changes that persons without schizophrenia can compensate for. Arguing against this is the fact that there does not seem to be a relationship between plaque density and cognitive impairment (see figure 1).

Some biological measures in schizophrenia may be related to cognitive impairment and others to diagnosis. Cognitive impairments in patients with schizophrenia share some common biological correlates with AD. These include cortical somatostatin and serotonergic deficits. Demented subjects with schizophrenia also tend to have lower parietal lobe concentrations of SPs. They differ from patients with AD in that cortical CRH deficits are not present (except in the cingulate cortex), no cholinergic deficit is present, and brain tissue does not react with Alz-50 (Powchik et al. 1993). The biological findings presented here that might be specific to a diagnosis of schizophrenia include cortical deficits of VIP and neuropeptide Y and increased SP concentrations in the cingulate and possibly temporal cortex as well.

Reductions in 5-HT and NE may be related to cognitive impairment in schizophrenia. The role of 5-HT in schizophrenia has been reviewed elsewhere (Bleich et al. 1988; Roth and Meltzer 1995). Among the schizophrenia cases in this series of studies, the most robust associations between a biological measure and cognitive impairment were the 5-HT and 5-HIAA deficits in the cortex of demented subjects with schizophrenia. Loss of raphe 5-HT neurons in AD has been previously reported (Halliday et al. 1992), but the relationship to dementia in schizophrenia is not clear. For example, that loss of raphe neurons was not consistently seen in patients with AD but when it occurred, it occurred in those patients with symptoms of dementia only and was prominent in those patients with a rapid progression. The present studies did not specifically examine the raphe for numbers of serotonergic neurons, but there was no gross midbrain or brain stem pathology, and clinical features of dementia in schizophrenia are different from those seen in AD (Davidson et al. 1995, 1996). Specifically, the dementia in schizophrenia is generally not rapidly progressive and although we do not have reliable information about its rate of progression in the cases studied here (because the majority were assessed only once before death), it would be a marked coincidence if the demented cases were peculiar in that they displayed rapid progression.

Earlier studies of 5-HIAA in the cerebrospinal fluid (CSF) of patients with schizophrenia may be consistent with the current findings. For example, cerebral ventricle enlargement has been associated with decreased levels of CSF 5-HIAA (Potkin et al. 1983). Members of that same group found that CSF 5-HIAA correlates with prefrontal regional cerebral blood flow (Weinberger et al. 1988). Increased ventricular size may be related to poor outcome in schizophrenia (Keefe et al. 1996) and decreased cere-
bral blood flow with poor cognitive performance (Weinberger et al. 1988). Conversely, Csemany et al. (1990) reported that CSF 5-HIAA correlates directly with the severity of negative symptoms. This is just the opposite of what would be expected, given that cognitive performance is inversely correlated with the severity of negative symptoms (Davidson et al. 1996).

Postmortem studies of 5-HT in schizophrenia have focused primarily on receptor binding, and the results have been inconsistent (see Roth and Meltzer 1995). We know of only one study that looked specifically at cortical 5-HT in schizophrenia (Joseph et al. 1979), and no differences were found between subjects with schizophrenia and controls. Although the cases in that study were retrospectively diagnosed, the findings were similar to those presented here. Moreover, the design of that experiment did not allow for an examination of any relationship between cognitive impairment and amine levels. Bridge et al. (1985) did report lower NE levels in the hippocampus of demented as compared to nondemented patients with schizophrenia but did not study 5-HT or 5-HIAA in that series. In addition, there have been several studies of 5-HT receptors in the cortex of patients with schizophrenia (e.g., Mita et al. 1986; Joyce et al. 1994) that may be consistent with the findings presented here. For example, Joyce et al. found an increased number of 5-HT2 receptors in the hippocampus and in the cingulate and temporal cortices. Similarly, Mita et al. reported a decreased number of 5-HT2 receptors in the frontal and occipital cortex of patients with schizophrenia and also concluded that the findings were unrelated to neuroleptic treatment.

The findings of NE in these studies are congruent with those of Bridge et al. (1985) and are provocative, given the role of NE in cognitive processes and possibly schizophrenia. Dysfunction of the prefrontal cortex (PFC) has long been implicated in schizophrenia—see the discussion by Weinberger (1993). Although most research in schizophrenia has focused on DA, there is much evidence that the PFC is involved in cognitive functions relevant to schizophrenia (Goldman-Rakic 1994). The PFC displays rich catecholaminergic innervation (Lewis 1992), so dysfunction of this region is likely to involve disruption of catecholaminergic function. Noradrenergic neurons projecting to cortical regions do so through the dorsal noradrenergic bundle (DNAB). Animal models of lesions of the DNAB lead to a variety of reproducible cognitive deficits. Acquisition of new information is more strongly impaired than previously learned information, and this finding is similar to the phenomenology seen in schizophrenia (Tamlyn et al. 1992). A variety of attention deficits, including deficits in sustained attention (Cole and Robbins 1992) and shifting attention (Devauges and Sara 1990), can be measured in DNAB-lesioned animals.

In terms of NE's role in schizophrenia, there have been reports that schizophrenia symptoms are correlated with MHPG (e.g., Kaneko et al. 1992; Maas et al. 1993). Changes in CSF NE have been reported to help predict relapse in people with chronic schizophrenia (van Kammen et al. 1994). In contrast, our group did not find such a relationship between CSF NE and either symptoms or neuropsychological measures in a small sample (Kahn et al. 1994). Others have suggested that clozapine's ability to increase NE neurotransmission is related to its superior clinical efficacy (Breier 1994), but peripheral NE did not differentiate clozapine responders from nonresponders (Davidson et al. 1993).

**Synaptic organization may correlate with schizophrenia symptoms.** The immunoreactivities of the four SPs studied here were generally elevated in the cortex of patients with schizophrenia compared with controls. The only exception was in the parietal cortex. These findings disagree with a previous study of synapsin in the hippocampus of seven persons with schizophrenia (Browning et al. 1993). However, the present studies were performed on a larger sample and did not include measurements of SPs in the hippocampus.

A more recent study by Sower et al. (1995) may be congruous with the present findings. These authors report increases in growth-associated phosphoprotein (GAP–43) in the frontal and occipital cortices, but not the parietal cortex. GAP–43 is thought to be involved in synaptic reorganization. The elevation of SP concentrations reported here may represent differences in synaptic organization in patients with schizophrenia compared with normals. It is unclear whether the differences first manifest during development or maturation (see e.g., Feinberg 1982; Keshavan et al. 1994) or over the long course of the disorder. Nonetheless, it is intriguing that the severity of some of the core symptoms of schizophrenia would correlate with any postmortem biological measure.

**Neuropeptide abnormalities may represent a neurodevelopmental lesion in schizophrenia.** The data presented here demonstrate widespread neuropeptide deficits in schizophrenia and may be relevant to the role of gamma-aminobutyric acid (GABAergic) neurotransmission in this disorder. Somatostatin, neuropeptide Y, VIP, CCK, and CRH have all been localized to within the cortical GABAergic interneurons (Ong and Garey 1991; Foley et al. 1992; Rogers 1992; Dennison-Cavanagh et al. 1993). The pattern of deficits seen in the present studies does not fit a disorder of multiple neuropeptides containing ascending afferents. For example, if a noradrenergic afferent were deficient, both neuropeptide Y and galanin should be affected and they are not (Gabriel et al. 1994). Similarly, the lack of a galanin and consistent VIP change agrees with the lack of cholinergic deficit found here in
patients with schizophrenia. Data from these neuropeptide studies are consistent with a deficit of GABAergic interneurons that are neuropeptide expressing in the cortex of persons with schizophrenia and consistent with Benes’ observations (Benes et al. 1987, 1991, 1992; Benes 1993).

Somatostatin and neuropeptide Y were both deficient in schizophrenia patients. Neuropeptide Y is co-localized within a larger population of somatostatin-containing neurons, the majority of which also exhibit nicotinamide-adenine dinucleotide phosphate (NADPH) diaphorase activity (Vincent et al. 1983; Unger and Lange 1992). A report of altered distribution of NADPH-staining neurons in postmortem cortices of subjects with schizophrenia (Akbarian et al. 1993a, 1993b) suggests that disturbed neurodevelopment may contribute to the disorder. It may be that the population of neurons identified by Akbarian et al. is a subpopulation of somatostatin neurons that also contain neuropeptide Y. The involvement of a specific class of neurons in schizophrenia has implications for understanding its pathogenesis, as well as developing unique pharmacotherapeutic approaches.

Overall Implications of These Studies

Differences between patients with schizophrenia, AD, and age-matched controls were not uncommon. Thus, far from being a disorder in which postmortem neuropathological findings are a rarity, schizophrenia appears to be characterized by multiple possible abnormalities. These and other results raise the question of whether all these differences are real and which, if any, have a meaningful relationship to the core pathology of schizophrenia.

The series reported here is a large one relative to other postmortem samples of schizophrenia. However, the sample is still too small to definitively determine whether factors unrelated to the schizophrenia process might really be accounting for some of the observed differences between subjects with schizophrenia and other study populations. For example, PMI, neuroleptic status, length of institutionalization, and periagonal events were variable across this population. Whereas some patients had been neuroleptic free for prolonged periods of time, others were receiving neuroleptics close to the time of their death. Whereas some patients had a PMI of very few hours, in other patients the interval was measured in days. Whereas some patients may have been virtually drug free at the time of their agonal event, others may have received multiple cardioactive drugs with central nervous system effects just before dying. Certainly, it can be reasonably argued that these differences could account for some of the postmortem findings and those of many other studies. Even when sample sizes approach 100 patients, the many subgroupings that derive from simply creating a matrix of all possible confounding variables can yield sample sizes that are still too small to answer specific questions about the power of particular combinations of confounding variables.

Superimposed on these circumstances is the heterogeneity of the symptoms patients present near the time of death. Some patients are profoundly demented, whereas others show a relatively normal Mini-Mental State Examination (Folstein et al. 1975) or a CDR score of less than 0.5. Also varying from patient to patient is the severity of positive and negative symptoms, as well as the balance between them. Thus, if one were to ask what SP abnormalities exist in patients with a postmortem interval under 3 hours, who have also been neuroleptic free for over 2 years, are nondemented, and possess severe negative symptoms, despite a sample size of over 100 patients it would be impossible to generate a cohort of patients large enough for a meaningful statistical analysis. Hence, this work points to the importance of developing multiple large brain banks with well-characterized patients and comparable techniques for brain extraction and processing. The notion that any single brain bank can provide patient samples large enough to answer all the postmortem questions that need to be addressed is at best unrealistic and at worst will mislead the field for possibly decades to come.

In part because of the complications that arise from so many potentially confounding variables, an important aspect of this research has been to answer a seemingly straightforward question: What is the neurochemical and/or neuropathological basis for the dementia of schizophrenia? Some clear answers to that question can be derived from this data base, particularly in terms of what the dementia of schizophrenia is not. Specifically, it is not a cholinergic deficit, characterized by neurofibrillary tangles and senile plaques, nor is it associated with the neuropeptidergic abnormalities of AD. It is less clear what the dementia of schizophrenia is. There are hints that it may be associated with diminished serotonergic and noradrenergic function, but this finding needs to be replicated as well as evaluated in greater detail. Hence, even to the straightforward question of what the dementia of schizophrenia is in a cohort characterized by relative homogeneity, straightforward answers still require significantly more investigation.

In conclusion, therefore, schizophrenia’s heterogeneity, which has dogged clinical studies for so long, is bound to be even more problematic in postmortem investigations. As the field advances, it is essential that this problem be dealt with by an extensive antemortem evaluation of symptoms and by large study populations. A national, if not international, effort will truly be necessary.
to coordinate this work and generate the resources necessary to apply the very promising methodologies of neurobiology that are available to study postmortem tissue.

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