Cortical Pyramidal Neurons Show a Selective Loss of New Synapses in Chronic Schizophrenia

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Forty-five years ago, the eminent neurologist Frederick Plum, M.D., opined that schizophrenia "was the graveyard of neuropathology" (1). This pessimistic view reflected the inability of investigators to identify consistent pathology in the brains of individuals with schizophrenia. This failure "to see pathology" in schizophrenia largely stemmed from limitations of tissue stains, which were successfully employed in neuropathology to reveal neuronal loss, astrocytosis, senile plaques, and Lewy bodies in neurodegenerative diseases. However, these stains cannot reveal synaptic structures, the microscopic wiring of the nervous system that is situated in the space between neurons and that is known as "neuropil." Nevertheless, CT studies (2) at the time and subsequent MRI studies (3) in schizophrenia revealed consistent, significantly reduced cortical volume and increased ventricular size. Concerted efforts were made to identify the missing neurons that could account for this cortical atrophy in schizophrenia. However, no neuronal loss commensurate with the degree of cortical atrophy could be found in the brains of individuals with schizophrenia with rigorous stereology. However, using standard cell stains, Selemon et al. (4) reported that the reduced size of the neuronal cell bodies and increased neuronal "packing" density resulting from loss of neuropil accounted for the cortical atrophy in schizophrenia.

The millennium heralded two major advances that dramatically improved our ability to decipher the neuropathology of schizophrenia. First, a powerful confocal microscopic imaging method was developed that used laser illumination to visualize the localization of fluorescent-labeled immune markers with high resolution. Thus, it became possible to study the components of synaptic structures that were, up to that time, inaccessible with standard light microscopy (5). Second, advances in molecular genetics permitted the identification of risk genes that provide both targets as well as context for interpreting synaptic pathology. Thus, a recent genome-wide association study (GWAS) sufficiently powered (>100,000 subjects) to achieve a rigorous statistical threshold of 5×10^{-8} to compensate for the multiple comparisons inherent in a GWAS found 108 sites on the genome associated with significant risk for schizophrenia (6). These sites do not involve highly penetrant dominant or recessive mutations but rather subtle genetic variants, often located in noncoding regions of the genes, such as promoters.

The present study from MacDonald et al. (7) involved a reanalysis of a cohort of postmortem brain samples from schizophrenic patients and carefully matched comparison subjects that showed a 19% reduction of dendritic spine density on pyramidal neurons in deep layer 3 of the primary auditory cortex in schizophrenia (8). Dendritic spines are the protrusions on neuronal dendrites where excitatory glutamatergic terminals form synapses. MacDonald et al. sought to determine whether mature synapses or recently formed synapses were reduced in schizophrenia. As synapses evolve from tentative, immature connections to stable, mature ones, the structure of the spine expands from a small, wispy protrusion to ultimately assume a mushroom-like structure. To objectively measure spine size, the authors devised a clever immunocytochemical-fluorescent method to visualize the dendritic spines. Two proteins are enriched in the spines: spinophilin and filamentous-actin (F-actin). To minimize artifacts, spine volume was ascertained only on the profiles where the fluorescent labels of both proteins overlapped. The authors found that the overall reduction in spine density could be accounted for by significant reductions in the smallest, least mature spines with no loss in the larger, mature spines.

Reduced spine density, which corresponds with reduced excitatory synapses on the principal projecting neurons (i.e., the pyramidal neurons) of the cerebral cortex, might be caused by reduced formation of synapses or increased removal or "pruning" of synapses.

To develop insight into possible mechanisms accounting for the reduced immature spine density, the authors measured a peptide fragment that contained an amino acid sequence found in a family of proteins known as calcium channel beta subunits, of which *CACNB4* is expressed in the temporal cortex. *CACNB4* had previously been identified as a risk gene for schizophrenia in the GWAS (6). These *CACNB* subunits modulate calcium flux through voltage-dependent calcium channels, a critical signal driving synaptic plasticity mediated by excitatory neurotransmission. To determine the effect of *CACNB4* on spine density, the authors grew fetal rat cortical neurons in tissue culture. After 12 days in culture, the *CACNB4* gene was inserted (transfected) into the cultured neurons in order to overexpress its protein. After 3 additional days of culture, the neurons overexpressing *CACNB4* exhibited reduced spine density, with immature spines bearing

the brunt of the loss. Closing the loop, the authors showed that although the absolute amount of the *CACNB4* measured in the patients' cortex did not differ from that of comparison subjects, there was a significant inverse correlation between the immature spine density and *CACNB4* levels that was not observed with the mature spines in the schizophrenic subjects.

Reduced spine density, which corresponds with reduced excitatory synapses on the principal projecting neurons (i.e., the pyramidal neurons) of the cerebral cortex, might be caused by reduced formation of synapses or increased removal or "pruning" of synapses. Synaptic pruning peaks in late adolescence, the age when individuals at risk for schizophrenia develop psychosis (9). At least a dozen of the risk genes for schizophrenia found in the GWAS directly affect *N*-methyl-D-aspartate (NMDA) receptor function or its downstream mediators. The NMDA receptor is a voltage-dependent calcium channel that is critical for neuroplasticity and spine formation and maintenance. Reduced NMDA receptor function caused by silencing the risk gene encoding serine racemase, the enzyme that synthesizes the NMDA receptor co-agonist D-serine, or by overexpressing the calcium channel inhibitory subunit *CACNB4*, as shown in the present study, reduces spine density to a degree similar to that seen in schizophrenia (9, 10). In light of their finding of a selective loss of immature spines, MacDonald et al. (7) come down on the side of impaired formation of new synapses, and not pruning of established synapses, as the primary defect in schizophrenia. Impaired formation of new synapses is certainly consistent with ongoing NMDA receptor hypofunction and the cognitive deficits of schizophrenia (10).

Discounting excessive pruning as a contributor to the pathophysiology of schizophrenia may be premature. First, the genetic risk factors of schizophrenia are so complex, with 108 sites on the genome associated with risk (6), that the "either/or" parsing of causality ignores the possibility that two or more processes affecting spine formation or stability could occur in the same subject. Notably, the gene encoding the innate immune protein, complement component 4 (C4), towers over all the other risk genes in the Manhattan plot from the GWAS of schizophrenia (11). A Manhattan plot presents the levels of significance of all genes on the genome on the abscissa so that the varying heights look like the Manhattan skyline. The risk variant of C4, which in the brain targets synapses for elimination, is associated with increased synaptic pruning in schizophrenia. Given the salience of this pruning gene to schizophrenia risk, it is surprising that its effects were not observed, at least in a subset of patients, in this study. Second, the brain tissue for this study came from subjects with an average age of 46 years and duration of psychosis of 20 years. As Krystal and Anticevic (12) have recently emphasized, schizophrenia is not a static condition but evolves from the high-risk state to the chronic state with striking changes in brain structure, function, and chemistry. Divining a pathologic process occurring at the transition to psychosis (i.e., increased pruning) from the pathology in a chronic state is like predicting the first chapter of a novel based on reading only the last chapter. Thus, it is possible that evidence of excessive pruning in the second decade of life was obscured by pathologic changes transpiring over the subsequent two decades.

In summary, a recurrent finding with immunocytochemical staining and with the Golgi technique (a method that randomly impregnates entire neurons with silver) is a significant reduction in spine density and in dendritic complexity of pyramidal neurons in the cerebral cortex in schizophrenia (13, 14). In aggregate, the total loss of excitatory synapses on pyramidal neurons as assessed by multiplying spine density by total dendritic length appears to be in the range of 20%–30% or more in schizophrenia, indicating a remarkable loss of synaptic connectivity in the cerebral cortex. Thus, the neuropathology of schizophrenia is hardly subtle when studied with the right tools.

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Dr. Coyle has served as a paid consultant to AbbVie, Forum Pharmaceuticals, and Novartis and holds a patent on the clinical use of D-serine that is owned by McLean Hospital. Dr. Freedman has reviewed this editorial and found no evidence of influence from these relationships.

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