Synaptic changes in the brain of subjects with schizophrenia

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Abstract

Clinical, epidemiological, neuroimaging and postmortem data all suggest schizophrenia is a neurodevelopmental disorder, and that synaptic disturbances might play a critical role in developing the disease. In 1982, Feinberg proposed that the schizophrenia might arise as a result of abnormal synaptic pruning. His hypothesis has survived 40 years of accumulated data, and we review the critical findings related to synaptic dysfunction of schizophrenia. While it is clear that synaptic disturbances are integral and important for understanding the pathophysiology of schizophrenia, it has also become obvious that synaptic disturbances cannot be studied and understood as an independent disease hallmark, but only as a part of a complex network of homeostatic events. Development, glial–neural interaction, changes in energy homeostasis, diverse genetic predisposition, neuroimmune processes and environmental influences all can tip the delicate homeostatic balance of the synaptic morphology and connectivity in a uniquely individual fashion, thus contributing to the emergence of the various symptoms of this devastating disorder. Finally, we argue that based on a predominant change in gene expression pattern we can broadly sub-stratify schizophrenia into “synaptic”, “oligodendroglial”, “metabolic” and “inflammatory” subclasses.

1. Introduction

Schizophrenia is a devastating mental disorder with a complex etiology that arises as an interaction between genetic and environmental factors (Carpenter and Buchanan, 1994; Lewis and Levitt, 2002; Marenco and Weinberger, 2000). Clinical, epidemiological, neuroimaging and postmortem data all suggest schizophrenia is a neurodevelopmental disorder, and that normal brain development and function is impaired long before the onset of the first full-blown clinical symptoms.

Huttenlocher (1979) quantified the density of synaptic profiles in layer III of the frontal gyrus in 21 brains ranging from newborn to 90 years in age, and came to conclusion that the synaptic density in the neonatal brain was already comparable to those seen in adulthood. Furthermore, he reported that in infancy there was a sharp increase in the number of synapses to about 150% of that seen in adulthood, followed by a progressive decline in synaptic density between the ages of 2 and 16. Based on findings of progressive postnatal “synaptic pruning” and the clinical presentation of the disease, Feinberg (1982) (Keshavan et al., 1994) formulated a radically new theory on the cause of schizophrenia, proposing that the disease presentation might arise from abnormal synaptic pruning in the affected individuals. While Feinberg hypothesized that altered cortical pruning is critical for developing the disease, he was uncommitted about the precise mechanism by which the pathophysiology might occur, stating that “… as a result of some abnormality in this process, too many, too few, or the wrong synapses are eliminated. Regrettably, we have no basis to choose among these abnormalities”. The initial Feinberg hypothesis was further expanded by Hoffman and Dobscha (1989), who proposed that hyperpruning of collateral axons in the prefrontal cortex (PFC) leads to the clinical manifestation of schizophrenia, and by Jernigan et al. (1991), who proposed that defective pruning of certain brain structures underlies schizophrenia. Almost 30 years later, these initial hypotheses are still very intriguing and are supported by multiple lines of indirect evidence.

2. Pruning in the central nervous system

After the initial Huttenlocher reports (Huttenlocher, 1979; Huttenlocher et al., 1982; Huttenlocher and de Courten, 1987), synaptic pruning in the CNS attracted a considerable research interest. It has been established that synaptic pruning is (1) present in virtually all brain regions, including the prefrontal, visual, motor and associative cortices, hippocampus and cerebellum (Bourgeois and Rakic, 1993; Eckenhoff and Rakic, 1991; Innocenti, 1995; Rakic...
et al., 1994; Takacs and Hamori, 1994); (2) proceeds coarsely from caudal to frontal regions (Rakic et al., 1994); (3) is present in more than one mammalian species (human, monkey, cat, rat) (Bourgeois and Rakic, 1993; Eckenhoff and Rakic, 1991; Innocenti, 1995; Rakic et al., 1994; Takacs and Hamori, 1994), although with a different time-course, and (4) represents an experience-dependent process (Roe et al., 1990; Stryker and Harris, 1986). Importantly, these changes could not be explained by enlargement of brain volume over development (Rakic et al., 1994).

3. Structural brain changes in schizophrenia

Defining neuropathology of schizophrenia has been challenging, with findings that often did not reproduce across various cohorts. However, most of the studies to date suggest that the disease is characterized by (1) a mild enlargement of ventricles; (2) decreased cortical thickness; (3) altered neuronal density and decreased neuron size in limbic, temporal, and frontal regions; (4) abnormal dendritic spine densities in the cortex; (5) altered cortical cytoarchitecture, perhaps related to abnormal neuronal migration, differentiation, and/or cell pruning; and (6) molecular changes that encompass altered expression of genes related to synaptic function, energy metabolism, immune system activation, and oligodendrocyte transcripts (reviewed by Harrison, 1999; Harrison and Weinberger, 2005; Hof and Schmitz, 2009; Iritani, 2007; Mirnics et al., 2006)). However, it is important to point out that most of the postmortem studies to date did not uncover progressive neurodegenerative disease lesions or ongoing astrocytosis that would indicate post-maturational neural injury (Arnold and Trojanowski, 1996). At least theoretically, all these changes can be directly related to altered synaptic pruning in schizophrenia, arising from a complex interplay of genetic predisposition and adverse environmental influences (Horvath and Mirnics, 2009).

Johnstone et al. (1976), using computerized tomography (CT) noted a significant ventricular enlargement in a group of chronic schizophrenics. These data were later replicated in both the lateral and 3rd ventricles by multiple groups of investigators with a prevalence rates ranging from 18 to 40% (Maser and Keith, 1983; Okasha and Madkour, 1982; Weinberger et al., 1979). Furthermore, based on a comprehensive meta-analysis study of 39 datasets Van Horn and McManus (1992) concluded that “there is a difference in ventricle:brain ratio between schizophrenics and controls which would seem to be an indisputable characteristic of schizophrenia”. However, the findings also suggested that the changes were too small to be of practical significance for establishing a patient diagnosis. Follow-up studies also suggested that ventricular enlargement might be correlated with disease symptoms, where patients with ventricular enlargement showed predominantly negative symptoms and impaired functioning, while patients without ventricular enlargement tended to have a preponderance of positive symptoms and a normal sensorium (Andreasen et al., 1982). Furthermore, ventricular enlargement is present in unmedicated, first-break patients, suggesting that the findings are not a result of disease progression or medication effects (Niemann et al., 2000; Vita et al., 2006). The ventricular enlargement is accompanied by an overall loss of brain tissue averaging ~3% (Lawrie and Abukmeil, 1998), albeit the degree of ventricular enlargement and the decrease in the brain volume do not appear to be correlated. Magnetic resonance imaging (MRI) studies suggest that the temporal lobes and medial temporal structures are the most affected (Mata et al., 2009, Prasad et al., 2009). All these structural changes can arise as a result of various factors and cellular deficits, however, in the absence of marked neuronal loss in schizophrenia, the findings are consistent with the idea that reduction in dendrites and synapses might be an important contributor to the cortical volume reduction (McGlashan and Hoffman, 2000).

4. Synapse-related neuroanatomical and molecular changes

To date, multiple studies have examined cellular and synaptic morphology in postmortem tissue of subjects with schizophrenia. Golgi-impregnation studies revealed a region- and disease-specific decrease in dendritic spine density in dorsolateral prefrontal cortex layer 3 pyramidal cells in subjects with schizophrenia (Glantz and Lewis, 2000). Importantly, these synapse-related changes do not appear to be a result of chronic antipsychotic medication: (1) monkeys receiving chronic antipsychotic medication do not show reduced expression of synaptic markers, (2) schizophrenic subjects not receiving antipsychotic medications at the time of death show synaptic alterations, and (3) antipsychotic medication treated subjects with diagnoses other than schizophrenia show no apparent synapse-related pathology. Furthermore, ultrastructural studies suggest that striatal spines in schizophrenics are reduced in size by ~30% when compared to the control population, raising the possibility that this change might be directly related to aberrant synaptic conductance and/or efficacy in the subcortical gray matter (Roberts et al., 1996). Kung et al. (1998) also pointed out that the density and/or proportion of symmetric synaptic profiles was primarily affected in the caudate nucleus (and not the putamen) arguing for an imbalance in inhibitory synaptic transmission between these two structures. In addition, the density of perforated synaptic profiles, cortical afferents thought to be involved in synaptic turnover and cognition, was lower in the striatum of the schizophrenic subjects compared to the control groups. Finally, the same study found that density of axodendritic synaptic profiles, particularly of the asymmetric type, was decreased in the caudate, but not the putamen.

While Golgi-studies and cellular density measurements suggest that the reduced synaptic marker expressions are a result of structural deficits related to the neuropil, these studies cannot exclude the possibility that many of the observed deficits are of functional nature. The anatomical substrate might be preserved (e.g., presynaptic terminal), and the studies reporting deficient presynaptic mRNA/protein levels (revealed by bulk tissue assessment methods or immunohistochemistry) often cannot distinguish between the “reduced expression in a synaptic terminal” and “reduced number of synaptic terminals” scenarios. Regardless, it is obvious that both mechanisms (reduced number of synapses or synapses expressing sub-threshold levels of presynaptic genes) are likely to have a significant impact on brain function. While the causality of the schizophrenia-related presynaptic deficits is somewhat open to interpretation, the research data suggest that multiple key transcripts and proteins of the presynaptic secretory machinery are reduced in schizophrenia. For example, quantitative Western blot analysis of human postmortem hippocampus from the brains of schizophrenics and age-matched controls revealed reduced levels of synapsin I in the diseased subjects (Browning et al., 1993). Similarly, synaptophysin immunostaining revealed a reduction in protein levels in prefrontal, but not visual cortex of diseased subjects (Glantz and Lewis, 1997). Furthermore, prefrontal reduction of synaptophysin and SNAP-25 has been also reported on a different cohort of subjects, suggesting that ‘hypofrontality’ of schizophrenia arises from abnormalities of synaptic number or structural integrity in prefrontal cortex (Karson et al., 1999). Synaptophysin
immunoreactivity was also significantly reduced in both the inner and outer molecular layers of the dentate gyrus, but not in the hilus (Chambers et al., 2005), arguing that the synaptophysin defect affects multiple cortical regions in the schizophrenic brain. Lower phosphorylated Syntaxin 1 (pSTX1) levels has been also reported in the prefrontal cortex of schizophrenia, and reduced pSTX1 levels were associated with reduced binding of STX1 to SNAP-25 and MUNC18 and decreased SNARE complex formation (Castillo et al., in press). In addition, data-driven gene expression profiling methods also identified a robust, subjects-specific reduction in presynaptic secretory machinery transcripts in the prefrontal cortex (synapsin II, N-ethylmaleimide sensitive factor, synaptotagmin, synaptophysin and synaptobrevin), which could not be attributed to chronic neuroleptic treatment (Minnics et al., 2000, 2001a). Furthermore, in a recent study of genomic convergence analysis of cerebellar cortices shotgun cDNA sequencing identified twenty three genes with altered expression and involvement in presynaptic vesicular transport (Mudge et al., 2008).

The vast majority of the published studies suggest that schizophrenia is characterized by reduced expression of presynaptic transcripts/proteins, however, there have been also a few cohort-specific reports suggesting that presynaptic gene expression might actually increase in some brain regions (Gabriel et al., 1997; Sokolov et al., 2000). The disease process of schizophrenia is also likely to affect postsynaptic elements. Postsynaptic receptor expression changes have been reported in the monoamine, glutamatergic and GABAergic systems, yet these are more suggestive of functional deficits, and not anatomical deficits of postsynaptic structures. Perhaps most interestingly, several studies suggest that postsynaptic density proteins (e.g., PSD93, PSD95, neurofilament-light and SAP102) are also affected by the disease process (Hahn et al., 2006; Kristiansen et al., in press; Meador-Woodruff et al., 2003; Ohnuma et al., 2000). However, the reduced expression of these binding partners of NMDA receptor subunits and mediators of synaptic plasticity has been less consistently replicated across various studies.

5. Which synapses are affected in schizophrenia?

Theoretically, any brain disease process can affect inhibitory or excitatory synapses, or both simultaneously. Furthermore, the functional outcome would also depend on the exact cell type affected and the brain region where the alteration would occur. In schizophrenia, synapse-related deficits appear to be widespread, affecting multiple cortical and subcortical regions, involving both glutamatergic projection neurons and multiple classes of GABAergic inhibitory neurons (Hashimoto et al., 2008a,b; Lewis et al., 2005; Lewis and Hashimoto, 2007). However, it appears that the disease process has a differential effect on projection neurons and interneurons: synaptic deficits in projection neurons appear to be both microanatomical and functional, while synaptic deficits in the interneurons are likely to be predominantly functional. Prefrontal cortical projection neurons in schizophrenia show both reduced size (Pierrì et al., 2003; Rajkowska et al., 1998; Sweet et al., 2003) and decreased dendritic spine density (Glantz and Lewis, 2000; Hill et al., 2006). In a study of Golgi-impregnated pyramidal neurons of the dorsolateral prefrontal cortex (Glantz and Lewis 2000) found that deep layer III projection neurons had a dendritic spine decrease of ~20%. In this study, dendritic spine alterations were not observed in superficial cortical layers, other cortical areas, or psychiatry control group treated with antipsychotic medication. On the other hand, perhaps the most intriguing functional synaptic abnormality in schizophrenia is observed in the cortical parvalbumin-containing interneuron subclass, the chandelier cells. Chandelier cells, via their characteristic axon terminals (cartridges) provide potent axon initial segment inhibition to the cortical projection neurons. In the prefrontal cortex of subjects with schizophrenia, GAT1 immunoreactivity appears to be preferentially reduced in the chandelier cell cartridges in a treatment-independent fashion (Lewis, in press). However, at the present it is believed that reduced GAT1 immunoreactivity is part of molecular reorganization at the axon initial segment, and not a result of chandelier cartridge pruning (Pierrì et al., 1999). Still, at the current time we have to consider the possibility that interneuron dendritic trees or axon terminals also undergo structural remodeling as part of the diseases process.

6. Relationship of synaptic changes to other molecular deficits

In addition to altered expression of multiple single-genes, the molecular pathophysiology of schizophrenia encompasses synaptic, oligodendrogial, mitochondrial and immune system disturbances (Minnics et al., 2006). The exact relationship between these disturbances is poorly understood, but it is very likely that the deficits are intertwined, perhaps even causally related (Minnics et al., 2001a). For example, synaptic activity requires energy, and reduction in neuropil might lead to reduced energy requirements (Middleton et al., 2002). As a result, adaptation takes place, and the transcripts/proteins of the energy metabolism pathway are downregulated. However, the opposite might also hold true if a neuronal metabolism is impaired, the affected cells might not be able to support an extensive arborization. Similarly, in an attempt to compensate for inefficient presynaptic release during development, postsynaptic changes may follow, including (but not limited to) down-regulation of regulator of G-protein signaling 4 (RGS4) (Minnics et al., 2001b). Such a reduction will give rise to increased duration of signaling through a number of different G-protein coupled receptors, attempting to compensate for reduced input from the presynaptic structures. Unfortunately, such adaptational events may not be effective, and they can even have a further detrimental effect on the function of the postsynaptic cell, resulting in further desynchronization and accelerated pruning (Minnics et al., 2001a).

7. Developmental time course of synaptic deficits in schizophrenia

Unfortunately, human postmortem studies offer very limited insight into the time course by which the synaptic alterations develop in schizophrenia. As synapse development and pruning are dynamic processes following a complex trajectory, genetic–environmental influences can alter synapse development through a wide time window that encompasses prenatal development to the onset of the disease (Lewis and Levitt, 2002). Importantly, the clinical symptoms of the disease might develop many years after the adverse genetic or environmental influences. Within this context, there are two possible mechanisms by which this can occur: less synapse production or overpruning. If synapse developmental trajectory is suppressed at the time of insult, fewer synapses are generated, but the reduced number of synapses is still sufficient to maintain almost normal function during the “pre-pruning” period. However, in late adolescence/early adulthood, when normal developmental pruning occurs, the number of synapses fall below a “psychosis threshold”, arising to symptoms of the disease. Alternatively, one can envision that synapse generation during development is unimpared, but the pruning mechanisms are accelerated, perhaps due to eliminating and increased number of inefficiently functioning synapses. Thus, the synapse loss due to overpruning would also result in reaching “psychosis threshold”, once again leading to the clinical manifestations of the disease.
8. Synaptic schizophrenia?

Schizophrenia is a heterogeneous disorder that encompasses different phenotypic manifestations of the disease. Genetic, gene expression and animal model studies unequivocally suggest that the molecular pathophysiology of the disease is complex, perhaps even unique to each patient. While synaptic alterations can be clearly demonstrated in the brain of some subjects with schizophrenia, they are not present in each and every one of the studied postmortem brains. Based on gene expression data in the literature, we argue that schizophrenia can be broadly subclassified at a molecular level as “synaptic” [Mirnics et al., 2001a], “oligodendroglial” [Hakak et al., 2001], “metabolic” [Middleton et al., 2002] or “inflammatory” [Arion et al., 2007]. From these categories, it appears that “synaptic” and “oligodendroglial” gene expression phenotypes are the most distinct, non-overlapping, and are not found within the same brains. Yet, this classification does not imply homogeneity within the molecular sub-phenotype. Subjects in the “synaptic schizophrenia” subclass could be affected by any number or combination of the genetic or environmental insults and that pattern likely varies between individuals. Simply put, different subjects with schizophrenia within this sub-class have different, subject-specific synaptic deficits, but they all converge at a functional level to affect synaptic transmission between neurons.

In summary, anatomical and functional synaptic disturbances are most likely a strong contributing factor to the development, pathology and possibly symptomatology of schizophrenia. However, the synaptic disturbances might be unique to each patient. The genetic predisposition to the disease will be different, it might affect different cell types (interneurons and projection neurons) or different cellular compartments (axonal arborization and dendritic tree). Importantly, synaptic disturbances in schizophrenia cannot be studied and understood as an independent disease hallmark, but only as a part of a complex network of events. Development, glial–neural interaction, changes in energy homeostasis, diverse genetic predisposition, neuroimmune processes and environmental influences all can tip the delicate homeostatic balance of the synaptic morphology and connectivity in a uniquely individual fashion, thus contributing to the emergence of the various symptoms of this devastating disorder.

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