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Review

Dendritic spine alterations in schizophrenia

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HIGHLIGHTS

- Schizophrenia is a neurodevelopment disorder with multiple contributing genes.
- Dendritic impairments, including spine loss, are present in schizophrenia.
- Identification of conserved underlying molecular pathologies is ongoing.

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ABSTRACT

Schizophrenia is a chronic illness affecting approximately 0.5–1% of the world's population. The etiology of schizophrenia is complex, including multiple genes, and contributing environmental effects that adversely impact neurodevelopment. Nevertheless, a final common result, present in many subjects with schizophrenia, is impairment of pyramidal neuron dendritic morphology in multiple regions of the cerebral cortex. In this review, we summarize the evidence of reduced dendritic spine density and other dendritic abnormalities in schizophrenia, evaluate current data that informs the neurodevelopment timing of these impairments, and discuss what is known about possible upstream sources of dendritic spine loss in this illness.

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Abbreviations: BA, Brodmann area; SZ, schizophrenia; C, control; HMW, high molecular weight MAP2 isoforms (MAP2A, MAP2B); MAP2-IR, MAP2 immunoreactivity; CA1, CA2, CA3, CA4, cornu ammonis areas 1–4.

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1. Introduction

Schizophrenia is a chronic illness associated with lifelong debilitation, and reduced quality of life that affects approximately 0.5–1% of the world's population [1]. Schizophrenia is characterized by a variety of symptoms, which can be categorized into positive, negative, and cognitive symptom domains [2,3]. Positive or psychotic symptoms describe the presence of perceptions or behaviors involving distortions of reality, including disorganized thought processes, delusions, and hallucinations. Hallucinations may occur in any sensory modality, but are typically auditory and verbal in nature. Negative symptoms refer to the absence of behaviors which should be present, including alogia (absence of speech), avolition (absence of motivation), anhedonia (inability to experience pleasure), and flattened affect (inability to express emotion). Cognitive symptoms include impairments in memory performance, executive function, attention, as well as poor language and reading ability [4], and impaired social cognition [5].

The age of onset of schizophrenia, defined by the first episode of psychotic symptoms, is typically during the late adolescent to early adult years; generally between 18 and 30 years of age [2]. This might lead to a view of schizophrenia as a disorder of late neurodevelopment. However, prior to the first episode of schizophrenia, there is also evidence for cognitive deficits, and other abnormalities of movement and behavior, suggesting earlier time points in neurodevelopment are affected [6].

A definite cause of schizophrenia has yet to be identified. However, evidence suggests a strong role of genetics in the etiology of schizophrenia. Monozygotic twins demonstrate 45–60% concordance for schizophrenia and the estimate of heritability is about 80% [7]. A number of potential schizophrenia susceptibility genes have been identified, including both common and rare structural variants [8]. These susceptibility loci overlap with those for other disorders of neurodevelopment, including intellectual disability and autism [9]. Importantly, for attempts to model underlying mechanisms, no simple mendelian form of schizophrenia has yet to be identified [8]. The fact that the concordance rate between monozygotic twins is not 100% further suggests that environmental influences contribute to the likelihood of developing schizophrenia. Many environmental risk factors for developing schizophrenia have been identified [10], and include both early neurodevelopmental insults such as prenatal exposure to maternal infection [11] and obstetric complications [12], as well as later insults such as cannabis use in adolescence [13].

Thus, the clinical syndrome of schizophrenia is likely the result of a number of genetic contributions that interact with any of several environmental events to adversely impact the course of neurodevelopment. While this multiplicity of etiologies is daunting, it does not preclude the development of understanding of disease if there are any conserved “downstream” pathologies that

could instead serve as a focus for investigation [1]. This review will focus on one such conserved neuropathology of schizophrenia, reduced dendritic spine density in neocortex. Specifically, we will review the evidence of reduced dendritic spine density and other dendritic abnormalities in schizophrenia, evaluate when in neurodevelopment individuals with schizophrenia may separate from typically developing individuals on this parameter, and discuss what is known about possible upstream sources of dendritic spine loss in this illness.

2. Dendritic alterations in schizophrenia

2.1. Reduced dendritic spine density

Reduced density of dendritic spines is one of the most consistently observed neuropathologic alterations in postmortem brain tissue studies of individuals with schizophrenia. Dendritic spine density has been evaluated in 7 separate studies (Table 1) [14–20]. Multiple areas within frontal and temporal neocortex have been evaluated, as have primary visual cortex and the subiculum within the hippocampal formation. Significantly, reduced spine density was found in schizophrenia subjects relative to control subjects in most comparisons. In two other studies of layer 3 pyramidal neurons, one in primary visual cortex and one in prefrontal cortex, spine density was modestly reduced in schizophrenia, approaching but not reaching significance [15,20]. Only in a single study, confined to layers 5 and 6 of Brodmann area (BA) 46, was there no reduction in spine density [16]. The median reported decrease in spine density was 23% (range 6.5–66%). Reduced spine density has been found using Golgi-impregnated tissue to yield an estimate of density of spines per dendritic length [14,15,18,20], using antibody to label the dendritic spine protein, spinophilin [17], and using dual labeling with antibody to spinophilin and the F-actin binding toxin, phalloidin [19]. These latter, immunohistochemical approaches provide an estimate of the density of spines per volume of gray matter rather than per dendritic length. In the one study to estimate spine number, which reflects total number of spines in a region of interest independently of changes in volume of the surrounding gray matter, in deep layer 3 of primary auditory cortex reductions of 19% were found in schizophrenia [19]. Two studies evaluated whether reduced spine density in deep layer 3 of BA 46 was specific for schizophrenia. One found evidence for spine density reductions in bipolar illness, while the other found no evidence of reduction in a group consisting predominantly of major depression with psychotic features [15,20]. Finally, two studies examined whether spine density reductions might result from antipsychotic exposure in an experimental animal. In a non-human primate model a slight, but not significant, increase in spine density after more than one year of haloperidol exposure was reported [17].

Table 1
Studies of dendritic spine density in schizophrenia.

| References | Brain regions | N of subjects SZ/C | Method | Finding(s) |
|-----------------------|----------------------------|-----------------------|--|--|
| Garey et al. [14] | BA 10, BA 11, BA 45 | 13/11 | Golgi | 66% decrease in layer 3 |
| Garey et al. [14] | BA 38, BA 20, BA 21, BA 22 | 13/11 | Golgi | 59% decrease in layer 3 |
| Glantz and Lewis [15] | BA 46 | 15/15 | Golgi | 23% decrease in deep layer 3. No significant reduction in superficial layer 3 |
| Glantz and Lewis [15] | BA 17 | 13/15 | Golgi | 14% reduction (not significant) in layer 3 |
| Rosoklija et al. [18] | Subiculum | 13/8 | Golgi | 35% decrease |
| Kolluri et al. [16] | BA 46 | 14/15 | Golgi | No difference in layers 5 and 6. |
| Sweet et al. [17] | BA 41 | 15/15 | Anti-spinophilin antibody | 27% decrease in deep layer 3 |
| Sweet et al. [17] | BA 42 | 15/15 | Anti-spinophilin antibody | 22% decrease in deep layer 3 |
| Shelton et al. [19] | BA 41 | 20/20 | Dual label with anti-spinophilin antibody and phalloidin | 20% decrease in spine density and spine number in deep layer 3 |
| Konopaske et al. [20] | BA 46 | 14/19 | Golgi | 6.5% decrease in spine density and 21.6% decrease in number of spines per dendrite in deep layer 3 |

Table 2
Studies of dendritic length and number in schizophrenia.

| References | Brain region | N of subjects SZ/C | Method | Finding(s) |
|-----------------------|--------------|-----------------------|--------|--|
| Glantz and Lewis [15] | BA 46 | 15/15 | Golgi | 14% decrease in basilar dendrite length in deep layer 3, but not superficial layer 3, in schizophrenia |
| Glantz and Lewis [15] | BA 17 | 13/15 | Golgi | No difference in basilar dendrite length in layer 3 in schizophrenia |
| Kalus et al. [23] | BA 11 | 5/5 | Golgi | 29% decrease in basilar dendrite length in layer 3 |
| Broadbelt et al. [22] | BA 32 | 11/11 | Golgi | Layer 3–17% decrease in primary and 15% decrease in secondary dendrite numbers in schizophrenia; layer 5–29% decrease in primary and 46% decrease in secondary dendrite numbers in schizophrenia |
| Black et al. [21] | BA 10 | 15/18 | Golgi | 40% decrease in basilar dendrite field size in layer 5 |
| Kolluri et al. [16] | BA 46 | 14/15 | Golgi | No difference in basilar dendrite length in layers 5 or 6 |
| Konopaske et al. [20] | BA 46 | 14/19 | Golgi | 18.3% decrease in basilar dendrite length in deep layer 3 |

In a rodent model of 28 day exposure to haloperidol or clozapine no significant effect on spine density was seen [20].

2.2. Reduced dendritic arborization

Six studies have evaluated pyramidal neuron dendrite length, field size, and/or dendrite number in schizophrenia (Table 2) [15–16,20–23]. The regions studied have included 3 prefrontal cortical areas (BA 10, BA 11, and BA 46), anterior cingulate cortex (BA 32), and primary visual cortex (BA 17). Reduced length of basilar dendrites and reduced dendritic number have been found in layer 3 in BA 10, BA 11, BA 46, and BA 32 [15,20–23]. Of note, the reduction in dendritic length found in deep layer 3 of BA 46 was also seen in groups diagnosed with other psychiatric disorders, predominantly bipolar illness and major depression with psychotic features [15,20]. In contrast, reductions in dendritic length were not found in layer 3 of primary visual cortex in schizophrenia subjects, but were seen in the subjects with other psychiatric disorders [15]. Kolluri et al. [16] found no difference in length of basal dendrites of pyramidal neurons in layer 5 and layer 6 in BA 46, but other studies found decreases in layer 5 neuron dendritic field size [21] and dendritic number [22].

2.3. Dendrite development and spine plasticity

MAP2 is located within cell bodies and dendrites, where it serves to bind, stabilize, and space microtubule bundles [24,25]. As a result, MAP2 is an important regulator of dendritic development and plasticity [26–31]. As a core constituent of the post-synaptic density in spines [32], MAP2 also binds filamentous actin [33]. Because dendritic spine plasticity depends on actin and microtubule dynamics; it is thus, not surprising that spine plasticity is also effected by MAP2 [34–38].

Thus, it is notable that reduced MAP2 immunoreactivity (IR) has been reported across multiple brain regions in 6 studies of subjects with schizophrenia [19,39–43] although some conflicting reports exist [44–46] (Table 3). Reduced MAP2-IR does not correlate with common confounds such as antipsychotic exposure and postmortem interval [19,42], and is not associated with mood disorders [42]. MAP2 mRNA expression and MAP2-IR are not altered by antipsychotic exposure, although MAP2 phosphorylation is increased [19,47,48]. Because of the role of MAP2 in dendrite development and spine plasticity, reductions in MAP2-IR in schizophrenia may contribute to abnormalities in these structures observed in the neocortex of subjects with schizophrenia [19].

3. Neurodevelopment timeline of reduced dendritic spine density in schizophrenia

Although the onset of schizophrenia symptoms typically occurs during late adolescence or young adulthood, there are also several lines of evidence suggesting that perinatal insults contribute

to schizophrenia risk [1]. As a result it has been hypothesized that reduced dendritic spine density in schizophrenia results from either a failure to elaborate normal numbers of dendritic spines in early development, or from more rapid elimination of dendritic spines in adolescent development, or both [49–51]. The findings of reduced density of dendritic spines in subjects with schizophrenia, identified in brain samples obtained at the time of death (i.e., typically in midlife or later) cannot distinguish between these hypotheses. However, other albeit indirect, lines of evidence suggest that greater adolescent dendritic spine elimination is the more likely source of the deficit in schizophrenia.

The understanding of normative human gray matter development has been recently informed by longitudinal studies using MRI to assess gray matter density in normally developing children, adolescents, and young adults. Throughout the late teen years and early twenties, certain brain regions continue to mature via the refinement of synaptic connections and myelination of fiber tracts [52]. In humans, prefrontal and superior temporal cortices are among the last brain regions to mature, and their development is not complete until the third decade of life [53,54]. Protracted maturation in these regions may leave them vulnerable to disruptive insults during development, and in support of this, these cortical regions are heavily implicated in the neuropathology of schizophrenia, including the presence of dendritic spine reductions described above.

Imaging studies of ultra-high risk adolescent subjects transitioning to schizophrenia reveal an accelerated gray matter volume reduction in prefrontal cortex in comparison to the gray matter volume reductions seen in ultra-high risk subjects, who do not develop psychosis [55]. Similarly, individuals experiencing their first psychotic episode, and ultra-high risk individuals who later develop psychosis show accelerated reductions in superior temporal gyrus gray matter volume that correlate with severity of delusions [56]. Together with other findings, the emerging picture is one in which gray matter volume deficits are not prominent at the end of childhood development in those individuals who will later develop schizophrenia. Instead, these deficits result from the concurrent acceleration of normal adolescent volume reductions and onset of schizophrenia symptoms [55].

A key adolescent neurodevelopmental process is the net loss, “pruning” of synapses in the cortex, where synapse density peaks prior to adolescence, then declines during adolescence before reaching stable adult levels [57–60]. Excitatory synapses onto dendritic spines, especially within layer 3, undergo the most dramatic elimination during the adolescent developmental period [57,61]. Thus, reductions in layer 3 dendritic spines are presumed to contribute at least a portion of the normal adolescent gray matter volume reductions observed in longitudinal imaging studies [55]. By extension, a likely interpretation of the imaging findings of accelerated adolescent gray matter reduction in schizophrenia subjects, in combination with the post-mortem tissue observations of reduced spine density in schizophrenia subjects is that processes that increase net elimination of dendritic spines contribute to the

Table 3
Studies of microtubule-associated protein 2 immunoreactivity in schizophrenia.

| Study | N of subjects SZ/C | Region | Antibody name | Antibody phospho- dependence | Antibody isoform specificity | Finding(s) |
|---------------------------|-----------------------|--|--|-------------------------------------|------------------------------------|--|
| Arnold et al. [39] | 6/12 | Hippocampus and associated areas ^a | AP14 AP18 M12, T24, T34, T39, T42 | Independent Dependent Unknown | HMW All Unknown | Decreased MAP2-IR in the subiculum of 83% of SZ subjects and entorhinal cortex of 67% of SZ subjects |
| Bouras et al. [44] | 44/55 | BA 24 | AP14 AP18 | Independent Dependent | HMW All | No difference in MAP2-IR using quantitative immunodot analysis |
| Cotter et al. [45] | 8/11 | Hippocampus | 972 305 | Independent Dependent | All All | Increase in non-phosphorylated MAP2-IR in left subiculum and left CA1 in SZ subjects |
| Cotter et al. [46] | 12/13 | Hippocampus | B9 972 | Independent Independent | All All | Increase in dendritic arborization in hippocampal regions CA1, CA2, CA3, and CA4 in SZ subjects |
| Jones et al. [40] | 7/7 | BA 9 BA 32 | Anti-MAP2 ^b | Unknown | Unknown | MAP2 area fraction is reduced by 40% in layer 3 and 45% in layer 5 of BA 9 in SZ subjects. MAP2 area fraction is reduced by 32% in layer 3 and 44% in layer 5 of BA 32 in SZ subjects |
| Rosoklija et al. [42] | 64/47 | Hippocampus and associated areas | Anti-MAP2 | Independent | HMW | Decreased MAP2-IR in subiculum of 20% of SZ subjects |
| Rioux et al. [41] | 11/7 | Olfactory bulb | AP18 Clone C | Dependent Independent | All All | Significant reduction in MAP2-IR in SZ subjects |
| Somenarain and Jones [43] | 8/8 | BA 9 | Anti-MAP2 | Independent | HMW | MAP2 area fraction is reduced by 39% in layer 3 and 38% in layer 5 in SZ subjects |
| Shelton et al. [19] | 20/20 | BA 41 | SMI-52 | Independent | All | MAP2-IR is reduced by 70% in BA41 deep layer 3. MAP2-IR below lowest value observed in any control subject in 60% of SZ subjects |

^a Associated areas refers to subiculum, parahippocampal cortex, and entorhinal cortex.^b Anti-MAP2 here refers to the binding target of the antibody and is not meant to suggest that all antibodies termed anti-MAP2 are identical.

early disease course in subjects with schizophrenia. An important corollary of this interpretation would be that interventions at or around the time of transition to syndromal psychosis may be able to prevent the progression of spine loss.

4. Possible causes of dendritic spine loss in schizophrenia

The recent advent of two-photon in vivo imaging has contributed substantially to the understanding of the age-dependent formation and elimination of dendritic spines. These studies have demonstrated that dendritic spines are dynamic, not fixed, structures. At all ages studied some proportion of spines are generated, some remain stable, and some retract. These proportions depend on the cortical region and laminar location of the neurons studied, as well as the age and sensory experience of the animal [62]. However, in keeping with observations of total spine numbers, net spine gain outpaces spine elimination until adolescence, at which point elimination predominates until a period of equilibrium without net changes in spine numbers is reached and persists for most of adulthood [62]. Thus, a compelling hypothesis for the lower dendritic spine density in subjects with schizophrenia is that there is a shift in the molecular factors underlying this balance, leading to a greater net elimination of spines during adolescence.

One critical process underlying spine enlargement and stabilization, or conversely shrinkage and retraction, is the stabilization or destabilization, respectively, of F-actin [63]. Many mediators of the regulation of this process within dendritic spines are known, although the signaling cascades are complex and overlapping [64–66]. Nevertheless, this has led to several tests to determine whether expression of molecules that promote F-actin stabilization, spine growth, and spine stabilization is reduced in the cortex of individuals with schizophrenia. Conversely, some tests of whether expression of molecules that promote F-actin destabilization, spine retraction, and spine elimination are increased in schizophrenia have also been reported.

Hill et al. [67] examined mRNA expression of kalirin-7, Cdc42, RhoA, Rac1, and drebrin in dorsolateral prefrontal cortex of subjects with schizophrenia. All showed reduced expression, but only kalirin-7 and Cdc42 remained significantly decreased when corrected for multiple comparisons. The expression levels of Cdc42, which has been shown to promote dendritic spine outgrowth [68], and kalirin-7, which promotes spine outgrowth and mediates long term potentiation-induced spine growth [69], were each correlated with prior spine density measurements in the same subjects. However, prior observations of spine reductions in these subjects were selective for layer 3, whereas kalirin-7 and Cdc42 mRNA reductions were not limited to this cortical layer; suggesting that reduced expression of spine growth mediators is necessary, but not sufficient, for reducing spine density. In a follow-up study examining the Cdc42 signaling pathway in greater detail, only a trend toward reduced Cdc42 mRNA expression was found, but increased mRNA expression of the layer 2 and 3 selective Cdc42 effector protein 3 (Cdc42EP3) was detected [70]. Importantly, expression of Cdc42, kalirin-7, and Cdc42EP3 mRNAs was not altered in monkeys exposed long term to antipsychotic medications [67,70].

Subsequently, Rubio et al. assessed protein levels of kalirin-7, Cdc42, PAK1 (a downstream target of Cdc42 and Rac1) and phospho-PAK1 in dorsolateral prefrontal cortex and anterior cingulate cortex of schizophrenia and control subjects [71]. They found reduced kalirin-7 levels in schizophrenia subjects in both regions. In contrast, levels of Cdc42 were only decreased in anterior cingulate cortex. Examination of PAK1 revealed increased total levels in dorsolateral prefrontal cortex, but reductions in PAK1 phosphorylated at threonine 423 in both regions. The reductions in phosphorylated PAK1 were not associated with alterations in downstream signaling that would reduce F-actin stability via the LIMK1-cofilin pathway, as assessed by levels of total and phosphorylated LIMK1 and cofilin in these subjects. Somewhat counter-intuitively, however, in anterior cingulate cortex Rex et al. detected increased levels of phosphorylated myosin light chain, an alteration likely to pro-

molecule, such as kalirin-9, can also be influenced by the age-dependent molecular context in which they occur.

Spinophilin, an F-actin binding protein, is a mediator of long term depression, a mechanism that induces spine retraction and loss [82,83]. Thus, spinophilin knockout results in excess dendritic spine numbers during development [82]. Spinophilin recruits the RhoA GEF, Lfc to spines [83] and targets protein phosphatase 1 to glutamate receptors [82], and to actin [84]. No change in spinophilin protein expression in prefrontal and auditory cortex [79,85] and both increased [86] and unchanged [87] expression of spinophilin mRNA in prefrontal cortex have been found in subjects with schizophrenia. However, because spinophilin is predominantly expressed in dendritic spines in cerebral cortex [88], spine loss may mask any increase in spinophilin levels within spines when gray matter homogenates are studied. Resolution of this confound awaits study using approaches that can detect spinophilin levels within remaining dendritic spines, in essence accounting for reductions in spine number [89].

5. Conclusions and future directions

Current evidence suggests that alterations of dendritic morphology, including reduced dendritic spine density are a common pathological feature, present in multiple brain regions in many subjects with schizophrenia. Moreover, available studies indicate that this pathology is not likely to be present solely as a result of co-morbidities associated with schizophrenia, or a result of the treatments provided for these patients. These conclusions should be tempered, however, by what are still relatively small numbers of individuals with and without disease who have been studied for these parameters.

Important future directions to follow up on these correlative observations from diseased tissue would include efforts to identify potential upstream molecular sources of these dendritic impairments and evaluate them in mechanistic models to establish causality and create opportunities for testing interventions. One approach is to identify protein changes that are conserved across large number of diseased individuals, possibly indicating a common pathway, i.e., one downstream of multiple genetic and environmental contributors, that generates dendritic pathology (Fig. 1). One example of this might be the observations regarding MAP2-IR which is substantially reduced (appearing qualitatively absent) in up to 60–80% of individuals with schizophrenia, and which multiple lines of evidence indicate could be proximate to spine pathology. Identifying the process indexed by the loss of MAP2-IR, and establishing a mechanism linking it to spine loss would have high potential for rapid translational impact. MAP2 has substantial homology of sequence, regulation, and function with MAP tau [24]. Thus, the study of MAP2 in schizophrenia could potentially take advantage of the substantially greater knowledge of tau biology, including the rapidly emerging field of tau therapeutics [90].

A related approach would be to identify protein changes that are conserved across large numbers of diseased individuals, but which, in some cases are also genetically linked to disease risk. For example, increased kalirin-9 protein levels in auditory cortex were present in a majority (77%) of schizophrenia cases, and genetic evidence additionally suggests a role for kalirin-9 in disease. Rare mutations in the kalirin-9 sequence have been associated with risk for schizophrenia, possibly by altering activity of kalirin's RhoA GDP/GTP exchange factor function [80]. Using this approach, it would be possible to model both common disease related increases in kalirin-9 levels and rare disease-associated kalirin mutations, providing the potential to reveal both proximate and early causal steps in pathogenesis. Because most evidence suggests that cortical gray matter volume reductions within indi-

viduals with schizophrenia emerge as an acceleration of the normal developmental trajectory during adolescence and early adulthood, the assessment of such models should evaluate this developmental phase closely.

Finally, additional efforts are necessary to understand the larger network of changes in dendritic spine proteins linked to spine loss in disease. Because post-synaptic glutamate signaling reflects the coordinated activity of many proteins [66], understanding the genesis of altered spine density can benefit from concurrent analysis of the components and structure of this larger network [91]. Recently, the emergence of quantitative proteomics approaches suitable for the study of synaptic proteins in postmortem human tissue has enabled this approach [92,93]. Analysis of protein networks within dendritic spines has the potential to reveal unanticipated evidence of pathologic or compensatory processes, such as increased co-expression of proteins involved in glutamate signaling to the cytoskeleton in those individuals with dendritic spine reductions [94]. In addition, because most current medications target proteins, study of the larger protein network has high potential for translation into potential drug-able targets. This potential can be enhanced by combining such approaches with bioinformatic analyses in which known cellular compartments, functional pathways, and pharmacologic targets can be overlaid on the network structure to generate hypotheses regarding potential interventions to reverse or prevent spine loss.

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