Prefrontal Cortical Dendritic Spine Pathology in Schizophrenia and Bipolar Disorder

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IMPORTANCE Prior studies have demonstrated reduced dendritic spine density in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia. However, it remains unclear how generalizable this finding is in schizophrenia and if it is seen in bipolar disorder, a historically distinct psychiatric condition.

OBJECTIVE To assess whether spine loss is present in the DLPFC of individuals with schizophrenia and individuals with bipolar disorder.

DESIGN, SETTING, AND PARTICIPANTS This study used postmortem human brain tissue from individuals with schizophrenia (n = 14), individuals with bipolar disorder (n = 9), and unaffected control participants (n = 19). Tissue samples containing the DLPFC (Brodmann area 46) were Golgi-stained, and basilar dendrites of pyramidal cells in the deep half of layer III were reconstructed.

MAIN OUTCOMES AND MEASURES The number of spines per dendrite, spine density, and dendrite length were compared across groups. We also assessed for the potential effects of clinical and demographic variables on dendritic parameters.

RESULTS The mean (SD) spine density was significantly reduced (ie, by 10.5%) in individuals with bipolar disorder (0.28 [0.04] spines/μm) compared with control participants (0.31 [0.05] spines/μm) (P = .02). In individuals with schizophrenia, the mean (SD) spine density was also reduced (by 6.5%; 0.29 [0.03] spines/μm) but just missed significance when compared with control participants (P = .06). There was a significant reduction in the mean (SD) number of spines per dendrite in both individuals with schizophrenia (72.8 [24.9] spines per dendrite) and individuals with bipolar disorder (68.9 [12.9] spines per dendrite) compared with controls (92.8 [31.1] spines per dendrite) (individuals with schizophrenia vs controls: 21.6% reduction [P = .003]; individuals with bipolar disorder vs controls: 25.8% reduction [P = .005]). In addition, both individuals with schizophrenia and individuals with bipolar disorder had a reduced mean (SD) dendrite length (246.5 [67.4] and 245.6 [29.8] μm, respectively) compared with controls (301.8 [75.1] μm) (individuals with schizophrenia vs controls: 18.3% reduction [P = .005]; individuals with bipolar disorder vs controls: 18.6% reduction [P = .005]).

CONCLUSIONS AND RELEVANCE Dendritic spine loss in the DLPFC was seen in both individuals with schizophrenia and individuals with bipolar disorder, suggesting that the 2 disorders may share some common pathophysiological features.
Most excitatory synapses in the brain occur on dendritic spines. Thus, spines play a crucial role in myriad brain functions. Two previous studies observed spine loss on pyramidal cells in the dorsolateral prefrontal cortices (DLPFCs) from individuals with schizophrenia (SZ). The first study found reduced total spine density in layer III, whereas the second study found lower basal dendrite spine density in the deep half of layer III. The DLPFC plays a key role in working memory, which is commonly impaired in SZ. Indeed, functional imaging studies implicate the DLPFC in the working memory deficits in SZ.

Using similar methods, this study sought to replicate the findings of Glantz and Lewis to determine the generalizability of spine pathology in SZ. To determine if spine pathology occurs in a psychiatric disorder historically distinct from SZ, a cohort of individuals with bipolar disorder (BP) was included. Although SZ and BP often differ clinically, they share many features. In both disorders, patients can develop psychosis and exhibit cognitive deficits. Imaging studies reveal similar brain areas are affected in both disorders. Genome-wide association studies have revealed multiple shared risk genes. Although spine density has not previously been studied in BP, reduced spine density in the subiculum was observed in a mood disorder cohort. Therefore, spine loss might be observed in the DLPFCs from individuals with BP. Similar to Glantz and Lewis, we expected that reduced spine density would be observed among individuals with SZ. However, given the differences between the disorders, we hypothesized that any spine loss observed in BP would be, at most, intermediate between individuals with SZ and control participants.

In addition to the generalizability of spine loss in SZ, the contribution of antipsychotic medication to spine pathology was unclear. In the current study, the potential confounding effects of antipsychotic medication was addressed by comparing individuals with SZ who were taking antipsychotics in the last year of life and those who were not and by assessing the effects of long-term haloperidol and clozapine administration in the rat medial prefrontal cortex (mPFC). We hypothesized that antipsychotic medications would have a minimal effect on spines in the DLPFCs of individuals with SZ and the mPFCs of rats.

Methods

Participants

Formalin-fixed, postmortem human brain tissue samples containing DLPFC (Brodmann area 46) were obtained from the Harvard Brain Tissue Resource Center (HBTRC, at McLean Hospital in Belmont, Massachusetts). The cohort included individuals with SZ (n = 20), individuals with BP (n = 18), and control participants (n = 20). Diagnoses were made using Feighner criteria for SZ and the Diagnostic and Statistical Manual of Mental Disorders (Third Edition Revised) for BP, and they were based on medical records and the questionnaires completed by the donors’ families. In addition to the information required for diagnosis, individuals were assessed for suicide risk, a history of alcohol use or dependence, substance abuse or dependence, a history of cannabis use, antipsychotic medication treatment during the last year of life, and treatment with lithium or valproic acid at the time of death. Each brain was examined by a neuropathologist for gross and microscopic changes consistent with Alzheimer dementia, other neurodegenerative disorders, cerebrovascular disease, tumors, and trauma and/or alcohol or drug abuse and was excluded from the current study if such changes were present. All postmortem human brain tissue was obtained from the HBTRC. The HBTRC obtains institutional review board approval from McLean Hospital to collect and maintain the brain tissue and distribute the brain tissue to investigators. In addition to the brain tissue, the HBTRC provides de-identified clinical information to investigators. As a result, institutional review board approval was not required for this study.

Tissue Processing

Tissue samples were dissected to produce 2 tissue blocks measuring 1.5 cm² × 0.5 cm and 1.5 cm² × 0.2 to 0.3 cm, respectively. The first block was stained using the Golgi-Kopsch method. In brief, tissue blocks were shaken in a 4% potassium dichromate in 5% paraformaldehyde solution at room temperature in the dark for 96 hours. The potassium dichromate/paraformaldehyde solution was replaced every 36 hours, and acid-cleaned glassware was used. Tissue blocks were washed in increasing concentrations of silver nitrate (0.25%, 0.5%, 0.75%, and 1%) and then shaken in 1% silver nitrate in acid-cleaned glassware at room temperature in the dark for 1 week. Stained tissue blocks were sectioned using a vibrating microtome (Vibratome) at 100 μm, mounted on gelatin-coated slides, and briefly air-dried. Tissue sections were then dehydrated with a graded series of ethanol, cleared with xylene, and coverslipped with Permount (Fisher Scientific). The second block was sectioned using a vibrating microtome at 40 μm, mounted on gelatin-coated slides, and air-dried. The tissue sections were then stained for cytoplasmic ribonucleic acid (Nissl substance) using thionin and coverslipped.

Pyramidal Cell Reconstruction

Tissue samples from 4 individuals with SZ and 6 individuals with BP could not be analyzed owing to widespread pretreatment and/or poor impregnation of staining reagents into pyramidal cells. All analyses were conducted by a single investigator blinded to participant number and diagnosis. Nissl-stained sections were used to confirm localization to DLPFC (Brodmann area 46) using cytoarchitectonic criteria and to ascertain the borders of layer III as a percentage of cortical thickness. Fifteen Golgi-stained pyramidal cells were selected for reconstruction per individual using the following criteria derived from Glantz and Lewis: (1) somata are located in the bottom half of layer III and in the middle of the section thickness; (2) the pyramidal cell is fully impregnated; (3) somata or dendrites are not obscured by large (>5 μm) staining opacities; (4) no morphological changes were associated with the postmortem interval; and (5) 3 or more basal dendrites are present, each branching at least once. For each selected pyramidal cell, the apparently longest basilar dendrite was se-
selected visually and reconstructed using Neurolucida version 11 (MicroBrightfield Bioscience) with a Zeiss Axioskop 2 Plus light microscope (Carl Zeiss) and a ×100 oil immersion objective (numerical aperture = 1.4, working distance = 0.17 mm). Reconstructions were done on live images captured using a CX9000 digital camera (MicroBrightfield Bioscience) at a final resolution of 1600 × 1200. Each dendrite terminus was classified as ending naturally or artificially at the cut surface of the section.

Antipsychotic Administration in Rats
Twenty-four adult, male Sprague Dawley rats receiving haloperidol 1 mg/kg/d, clozapine 25 mg/kg/d, or sterile saline (8 per group) intraperitoneally for 28 days22-23 were euthanized 24 hours after the last injection, and the frontal cortex was dissected out. The frontal cortex was then stained using the Rapid Golgi Kit (FD NeuroTechnologies). Stained tissue blocks were sectioned using a vibrating microtome (Vibratome) at 100 μm, mounted on gelatin-coated slides, and coverslipped. The longest basilar dendrite on 8 pyramidal cells with their somata localized in the middle layers (III-V) of the mPFC was reconstructed in a manner similar to that of the postmortem human brain tissue.

Statistical Analyses
Analysis of patient data by 1-way analysis of variance revealed no differences in mean age, postmortem interval, or pH across groups (P > .05). However, storage time in formalin did differ across groups (P < .01) and was included as a covariate in dendritic parameter analyses. To ensure that an optimal statistical model was used for each dendritic parameter, each of the following factors were systematically assessed alone and in combination with other factors using analysis of covariance models with diagnosis and storage time in formalin included in each model. These included age, sex, postmortem interval, pH, hemisphere, suicide risk, history of alcohol abuse or dependence, history of substance abuse or dependence, history of cannabis use, treatment with antipsychotic medication in the last year of life, treatment with lithium at the time of death, and treatment with valproic acid at the time of death. An optimized model was selected for each dendritic parameter using the corrected Akaike Information Criterion24-25 to identify the simplest best-fitting model in each case. The corrected Akaike Information Criterion, which is the Akaike Information Criterion corrected for small samples, resolves the bias-variance tradeoff in model selection by determining which covariates to include (to remove bias) and which to exclude (to minimize variance). A test-wise false-positive error rate was set at 0.05, thus controlling for potential experiment-wise errors. For any parameter having a significant analysis of covariance effect for diagnosis (P < .05), post hoc pairwise comparisons were conducted using the Dunnett method to control for multiple comparisons. Because we expected to replicate the findings of Glantz and Lewis,3 post hoc pairwise comparisons (using the Dunnett method) for spine density and dendrite length were conducted using 1-tailed tests; all other analyses were conducted using 2-tailed tests.

There were patients in the SZ (n = 2), BP (n = 3), and control (n = 1) groups who had incomplete substance abuse histories in the medical records and thus were not included in the dendritic parameter analyses. Table 1 and eTable 1 in the Supplement summarize the clinical and demographic data of the individuals with SZ (n = 14) or BP (n = 9) and the control participants (n = 19) included in the analyses.

The relationship between the number of spines per dendrite, spine density (ie, the number of spines per micrometer dendrite), dendrite length, and clinical variables were assessed in SZ and BP groups. The following clinical variables were analyzed: history of alcohol abuse or dependence (yes/no), history of cannabis use (yes/no), history of other substance abuse or dependence (yes/no), suicide risk (yes/no), antipsychotic medication treatment in the last year of life (yes/no), lithium treatment at the time of death (yes/no), and valproic acid treatment at the time of death (yes/no). Clinical variables were analyzed with t tests assuming unequal variances, and P values were corrected using the false discovery rate to control for multiple tests. Statistical analyses were conducted using Stata version 12 (StataCorp), and false discovery rate calculations were conducted using QVALUE version 1.26

Results

DLPFC Layer III Borders
The upper border position for DLPFC layer III as a mean (SD) percentage of the distance from the pia to white matter was 19.3% (1.9%) for control participants, 20% (2.0%) for individuals with SZ, and 19.2% (2.6%) for individuals with BP. The lower border position was 54.7% (2.6%) for control participants, 55.1% (3.0%) for individuals with SZ, and 55.5% (4.0%) for individuals with BP. Analyses of the upper and lower border positions by 1-way analysis of variance revealed no differences across groups (P > .05). The mean (SD) gray matter thickness was 2690.2 (719.5) μm for control participants, 2550.8 (343.1) μm for individuals with SZ, and 2286.0 (435.8) μm for individuals with BP; analysis of covariance revealed no differences between groups (P > .05). The mean (SD) final section thickness was 74.7 (14.3) μm for control participants, 72.6 (12.1) μm for individuals with SZ, and 69.9 (10.1) μm for individuals with BP, and 1-way analysis of variance revealed no differences across groups (P > .05).

Dendritic Parameter Analyses
The Golgi-Kopsch staining method can produce very good staining of DLPFC deep layer III pyramidal cell basilar dendrites and spines in postmortem human brain tissue with prolonged storage in formalin (Figure 1). However, several individuals with SZ and several individuals with BP had poor staining and were excluded. To elucidate potential sources of suboptimal staining, clinical and demographic variables were assessed between the included and excluded individuals. Continuous variables were assessed using t tests assuming unequal variances, and categorical variables were assessed using χ² tests. No parameter was significantly different between groups; however, excluded individuals tended to have a lower
mean (SD) tissue pH than did included individuals (6.2 [0.4] vs 6.4 [0.6]) (P = .10). The pH of staining solutions is an important parameter in the Golgi-Kopsch staining method, and a low initial tissue pH might have produced poor staining in select individuals.

The analysis of covariance model used for each dendritic parameter is given in Table 2 in the Supplement. Individuals with BP had a significant 10.5% reduction in spine density relative to control participants (P = .02, 1-tailed). Spine density was reduced by 6.5% in individuals with SZ, which barely missed significance (P = .06, 1-tailed). Significant reductions in the number of spines per dendrite were observed in both individuals with SZ (21.6%; P = .003) and individuals with BP (25.8%; P = .005). Reduced dendrite length was observed in both individuals with SZ (18.3%; P = .005, 1-tailed) and individuals with BP (18.6%; P = .05, 1-tailed). Spine density per dendritic segment did not differ across groups for any segment (P > .05; Figure 2). The mean pyramidal cell somal area did not differ among groups, and there were no significant differences across groups for the other dendritic parameters (P > .05; Table 2).

Effects of Clinical Variables
There was no significant effect of any clinical variables on the number of spines per dendrite in either individuals with SZ or individuals with BP. Among SZ individuals, a history of alcohol abuse or dependence and a history of cannabis use were associated with increased spine density. Individuals with SZ who were taking lithium had longer dendrites relative to individuals with SZ not treated with lithium (Figure 3).

Antipsychotic-Administered Rats
A 28-day administration of haloperidol or clozapine had no significant effect on the number of spines per dendrite, spine density, or dendrite length among pyramidal cells in the mPFCs of rats (eFigure in the Supplement).

Discussion
Spine density was significantly reduced in individuals with BP. Spine density was also reduced in individuals with SZ but just missed significance. Both the number of spines per
dendrite and dendrite length were significantly reduced in individuals with SZ and individuals with BP. These findings are significant for the following 2 reasons: (1) spines appear similarly affected in both disorders and (2) the magnitude of spine density reduction in SZ in the current study (6.5%) is much less than previously reported (23%), suggesting variability in spine pathology in SZ. In their review, Glausier and Lewis discussed data on spine pathology in SZ, cortical spine density in the context of development, and possible mechanisms that might contribute to reduced spine density. The mechanisms discussed include dysregulation of the actin cytoskeleton, decreased presynaptic activity and/or deafferentation, and impaired energy metabolism, among others. Our discussion focuses on synaptic activity and the regulation of the actin cytoskeleton, given their potential relevance to both SZ and BP.

The actin cytoskeleton is highly dynamic and modulated by synaptic activity. Through its effects on actin, synaptic activity regulates the formation, morphology, and maintenance of spines. Several studies indicate that activity at N-methyl-D-aspartate (NMDA)-type glutamate receptors are particularly important in the regulation spines via the actin cytoskeleton. Multiple mechanisms could alter NMDA receptor function, including deafferentation, decreased presynaptic release, altered glutamate cycling and metabolism, altered astrocyte activity, and alterations in NMDA receptors or their downstream signaling partners.

An exploration of all these mechanisms is beyond the scope of this discussion, but the mechanism with the most relevance to both SZ and BP are altered NMDA receptors and their signaling partners. A review of postmortem data from studies conducted in the DLPFCs from individuals with SZ and individuals with BP reveals alterations in molecules involved in NMDA-receptor signaling. The messenger RNA expression for the NMDA subunit NR1 was reduced in both SZ and BP, and the NR2A subunit was reduced in SZ. The messenger RNA expression of the NR3A subunit was increased in SZ but decreased in BP. NF-L is a postsynaptic density molecule involved in NMDA-receptor signaling, which binds the NR1 subunit and PSD-95, a molecule involved in NMDA-receptor clustering. SAP102 also participates in the clustering of NMDA receptors. The NF-L protein expression was reduced in SZ, and SAP102 messenger RNA expression was reduced in BP. The NR2B subunit and PSD-95 had increased phosphorylation, and several molecules involved in the trafficking of the NR2B subunit had altered expression in SZ. Together, these data suggest that alterations in NMDA receptors and their associated signaling partners might contribute to spine pathology in both SZ and BP.

Previous studies reported reduced somal size of layer III pyramidal cells in the DLPFCs from individuals with SZ. In contrast to these studies but in agreement with Glantz and Lewis, no change in the somal area was found across groups in the current study. This may reflect differences in staining methods. Studies that found reduced somal size in individuals with SZ used Nissl staining, whereas Glantz and Lewis and the current study used Golgi staining.

Alcohol abuse or dependence and cannabis use were associated with increased spine density in SZ but not in BP. Both chronic ethanol and Δ-hexahydrocannabinol reduced spine density in the rat hippocampus. Moreover, cannabis use was associated with an increased risk of SZ in genetically predisposed individuals and increased psychosis in patients with SZ. Cannabis use appears to accelerate psychosis onset, whereas alcohol use might delay or have no effect. Alcohol use negatively impacts working memory function, but long-term cannabis use might improve cognition in patients...
Additional research is required to elucidate the potential effect of these substances on spines in individuals with SZ.

Potential confounding effects of antipsychotic medications on dendritic parameters were assessed by comparing individuals with SZ not taking antipsychotic medications for at least 1 year prior to death with those taking antipsychotics (all individuals with BP were taking antipsychotics). The effects of long-term haloperidol and clozapine administration on pyramidal cell dendritic parameters were also assessed in the mPFC of rats. Antipsychotic medications had no effect on spines or dendrite length. The effects of antipsychotic medications cannot be ruled out entirely, and it is possible that a longer duration of administration in rats (eg, 6 months) might...
have produced an effect. Nevertheless, antipsychotic medications did not appear to be a significant factor in the observed spine pathology. The effects of mood stabilizers were also assessed by comparing individuals with SZ and individuals with BP who were taking or not taking lithium and valproic acid at the time of death. For individuals with SZ, lithium...
treatment was associated with longer basilar dendrites. Indeed, lithium prevented reductions in hippocampal dendrite length owing to chronic stress in rats. Given the small group sizes, no firm conclusions can be drawn without further research, but lithium treatment might be neuroprotective for patients with SZ.

Conclusions

Historically, SZ and BP have been viewed as distinct entities. However, as stated previously, they share many features, suggesting that pathophysiological commonalities exist. Unlike genome-wide association studies, which include thousands of individuals, postmortem studies include orders of magnitude fewer. However, rigorously designed and executed postmortem studies are necessary to understand how risk genes might contribute to the pathophysiology of these disorders. The current study suggests that spine pathology is common to both SZ and BP. Moreover, the study of the mechanisms underlying the spine pathology might reveal additional similarities and differences between the 2 disorders, which could lead to the development of novel biomarkers and therapeutics.

The current study detected spine losses on pyramidal cell dendrites in deep layer III of the DLPFCs of individuals with SZ and individuals with BP. These findings suggest that DLPFC spine loss may be a shared pathophysiological feature of both disorders. In addition, altered NMDA signaling might contribute to the observed spine pathology.

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