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Prefrontal cortical dendritic spine pathology in schizophrenia and bipolar disorder

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Abstract

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 a historically distinct psychiatric condition, bipolar disorder.

Objective—To assess whether spine loss is present in the DLPFC of subjects with schizophrenia and bipolar disorder.

Design, Setting, and Participants—This study utilized postmortem human brain tissue from subjects with schizophrenia (n=14), bipolar disorder (n=9) and unaffected control subjects (n=19). Tissue samples containing the DLPFC (BA 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—Spine density was significantly reduced in bipolar disorder subjects. In schizophrenia subjects, spine density was also reduced, but just missed significance. Relative to control subjects, there was a significant reduction in the number of spines per dendrite in both schizophrenia and bipolar disorder subjects. In addition, both schizophrenia and bipolar disorder subjects had reduced dendrite length.

Conclusions and Relevance—Dendritic spine loss in the DLPFC was seen in both schizophrenia and bipolar disorder subjects suggesting that the two disorders may share some common pathophysiological features.

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Introduction

Most excitatory synapses in the brain occur on dendritic spines. Thus, spines play a crucial role in a myriad brain functions[1]. Two studies observed spine loss on pyramidal cells in the dorsolateral prefrontal cortex (DLPFC) from schizophrenia (SZ) subjects. The first found reduced total spine density in layer III, whereas the second found lower basilar dendrite spine density in the deep half of layer III[2,3]. The DLPFC plays a key role in working memory[4,5] which is commonly impaired in SZ[6–8]. Indeed, functional imaging studies implicate the DLPFC in the working memory deficits in SZ[9–12].

Using similar methods, this study sought to replicate the findings of Glantz and Lewis (2000)[3] 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 bipolar disorder (BP) subjects was included. Although SZ and BP often differ clinically, they share many features. In both disorders, patients can develop psychosis and exhibit cognitive deficits[13]. Imaging studies reveal similar brain areas are affected in both disorders[14]. Genome-wide association studies (GWAS) have revealed multiple shared risk genes[15,16]. Although spine density has not been studied in BP previously, reduced spine density in the subiculum was observed in a mood disorder cohort[17]. Therefore, spine loss might be observed in the DLPFC from BP subjects. Similar to Glantz and Lewis (2000)[3], we expected that reduced spine density would be observed among subjects with SZ. However, given the differences between the disorders, we hypothesized that any spine loss observed in BP would be, at most, intermediate between SZ and controls.

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 SZ subjects who were on and off antipsychotics in the last year of life and by assessing the effects of chronic haloperidol and clozapine administration in the rat medial prefontal cortex (mPFC). We hypothesized that antipsychotic medications would have a minimal impact on spines in the DLPFC from SZ subjects and the mPFC of rats.

Methods

Subjects

Formalin-fixed, postmortem human brain tissue samples containing DLPFC (BA 46) were obtained from the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA). The cohort included subjects with SZ (n=20), BP (n=18), and control subjects (n=20). Diagnoses were made using Feighner criteria for SZ[18], and DSM-III-R (American Psychiatric Association 1987) for BP, and were based on medical records and a questionnaire completed by the donor's family. In addition to the information required for diagnosis, subjects were assessed for suicide, a history of alcohol abuse 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's dementia, other neurodegenerative disorders, cerebrovascular

disease, tumors, trauma and/or alcohol or drug abuse and was excluded from the current study if such changes were present.

Tissue Processing

Tissue samples were dissected to produce two tissue blocks measuring 1.5 cm^2 × 0.5 cm and 1.5 cm^2 × 0.2–0.3 cm, respectively. The first block was stained using the Golgi-Kopsch method[19]. 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 utilized. 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, St. Louis, MO) 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 cover-slipped.

Pyramidal Cell Reconstruction

Tissue for 4 SZ and 6 BP subjects could not be analyzed due to widespread precipitation and/or poor impregnation of staining reagents into pyramidal cells. All analyses were conducted by a single investigator (G.T.K.) blinded to subject number and diagnosis. Nissl-stained sections were used to confirm localization to DLPFC (BA 46) using cytoarchitectonic criteria[20] and to ascertain the borders of layer III as a percentage of cortical thickness. Fifteen Golgi-stained pyramidal cells were selected for reconstruction per subject using the following criteria derived from Glantz and Lewis (2000)[3]: 1) Somata 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 associated with postmortem interval (PMI)[21]; and 5) The presence of ≥3 basilar dendrites each branching at least once. For each selected pyramidal cell, the apparently longest basilar dendrite was selected visually and reconstructed using Neurolucida (version 11, MicroBrightfield Bioscience, Williston, VT) with a Zeiss Axioskop 2 Plus light microscope (Carl Zeiss, Germany) and a ×100 oil immersion objective (NA=1.4, working distance=0.17 mm). Reconstructions were done on live images captured using an CX9000 digital camera (MicroBrightfield Bioscience, Williston, VT) 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

24 adult, male Sprague Dawley rats receiving haloperidol 1mg/kg/day, clozapine 25mg/kg/day, or sterile saline (n=8 per group) i.p. for 28 days[22,23] were euthanized 24 hours after the last injection, and the frontal cortex dissected out. The frontal cortex was then stained using the Rapid Golgi Kit (FD NeuroTechnologies, Columbia, MD). Stained tissue blocks were sectioned using a vibrating microtome (Vibratome, St. Louis, MO) at 100 μm, mounted on

gelatin-coated slides and cover-slipped. The longest basilar dendrite on 8 pyramidal cells, with their somata localized in the middle layers (III-V) of the mPFC, was reconstructed in manner similar to that of the postmortem human brain tissue.

Statistical Analyses

Analysis of subject data by one-way ANOVA revealed no differences in mean age, PMI, or pH across groups ($p>0.05$). However, storage time in formalin did differ across groups ($p<0.01$) and was included as a covariate in dendritic parameter analyses. To ensure that an optimal statistical model was utilized for each dendritic parameter, each of the following factors were systematically assessed alone and in combination with other factors using ANCOVA models with diagnosis and storage time in formalin included in each model. These included: age, sex, PMI, pH, hemisphere, suicide, 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's Information Criterion (AIC_C)[24,25] to identify the simplest, best-fitting model in each case. The AIC_C, which is the AIC 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 ANCOVA effect for diagnosis ($p<0.05$), *post-hoc* pairwise comparisons were conducted using Dunnett's method to control for multiple comparisons. Since we expected to replicate the findings of Glantz and Lewis (2000)[3], *post-hoc* pairwise comparisons (again using Dunnett's method) for spine density and dendrite length were conducted using one-tailed tests, all other analyses were conducted using two-tailed tests.

There were subjects in the SZ (n=2), BP (n=3) and control (n=1) groups who had incomplete substance abuse histories in the medical records, 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 SZ (n=14), BP (n=9) and control (n=19) subjects included in the analyses.

The relationship between the number of spines per dendrite, spine density (i.e., the number of spines per μm 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 (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 (v. 12, College Station, TX), and false discovery rate calculations were conducted using QVALUE (v. 1)[26].

Results

DLPFC Layer III Borders

The upper border position for DLPFC layer III, as a percentage of the distance from the pia to white matter, was 19.3±1.9% for control, 20±2% for SZ, and 19.2±2.6% for BP subjects. The lower border position was 54.7±2.6% for control, 55.1±3% for SZ, and 55.5±4% for BP subjects. Analyses of the upper and lower border positions by one-way ANOVA revealed no differences across groups ($p>0.05$). Grey matter thickness was 2690.2±719.5 μm for control, 2550.8±343.1 μm for SZ and 2286±435.8 μm for BP subjects, analysis by ANCOVA revealed no differences between groups ($p>0.05$). Final section thickness was 74.7±14.3 μm for control, 72.6±12.1 μm for SZ and 69.9±10.1 μm for BP subjects, and one-way ANOVA revealed no differences across groups ($p>0.05$).

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 having prolonged storage in formalin (see Figure 1). However, several SZ and BP subjects had poor staining and were excluded. To elucidate potential sources of suboptimal staining, clinical and demographic variables were assessed between the included and excluded subjects. Continuous variables were assessed using *t*-tests assuming unequal variances and categorical variables were assessed using chi squared tests. No parameter was significantly different between groups, however pH trended toward significance. Excluded subjects tended to have lower tissue pH 6.2±0.4 vs. 6.4±0.6 ($p=0.1$). pH of staining solutions is an important parameter in the Golgi-Kopsch staining method[19] and a low initial tissue pH might have produced poor staining in select subjects.

The ANCOVA model utilized for each dendritic parameter is given in eTable 2 in the Supplement. BP subjects had a significant 10.5% reduction in spine density relative to controls ($p=0.024$, one-tailed). Spine density was reduced by 6.5% in SZ subjects, which barely missed significance ($p=0.061$, one-tailed). Significant reductions in the number of spines per dendrite were observed in both SZ (21.6%, $p=0.003$) and BP (25.8%, $p=0.005$) subjects. Reduced dendrite length was observed in both SZ (18.3%, $p=0.005$, one-tailed) and BP (18.6%, $p=0.05$, one-tailed) subjects. Spine density per dendritic segment did not differ across groups for any segment ($p>0.05$, see Figure 2). Mean pyramidal cell somal area did not differ among groups, and there were no significant differences across groups for the other dendritic parameters ($p>0.05$, see Table 2).

Effects of Clinical Variables

There was no significant effect of any clinical variables on the number of spines per dendrite in either SZ or BP subjects. Among SZ subjects, a history of alcohol abuse or dependence and a history of cannabis use were associated with increased spine density. SZ subjects on lithium had longer dendrites relative to SZ subjects not treated with lithium (see 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 mPFC of rats (see eFigure 1 in the Supplement).

Discussion

Spine density was significantly reduced in BP subjects. Spine density was also reduced in SZ subjects, but it just missed significance. Both the number of spines per dendrite and dendrite length were significantly reduced in both SZ and BP. These findings are significant for two 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%)[3] suggesting variability in spine pathology in SZ. In their excellent recent review, Glausier and Lewis (2013)[27], discussed data on spine pathology in SZ, cortical spine density in the context of development, and possible mechanisms which 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 will focus 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 is modulated by synaptic activity. Through its effects on actin, synaptic activity regulates the formation, morphology, and maintenance of spines[28]. Several studies indicate that activity at NMDA-type glutamate receptors are particularly important in the regulation spines via the actin cytoskeleton[29–32]. Multiple mechanisms could alter NMDA receptor function including deafferentation[33], decreased presynaptic release[34], altered glutamate cycling and metabolism[35], altered astrocyte activity[36], and alterations in NMDA receptors or their downstream signaling partners[37,38]. 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 appears to be altered NMDA receptors and their signaling partners. A review of postmortem data from studies conducted in the DLPFC from subjects with SZ and BP reveals alterations in molecules involved in NMDA receptor signaling. mRNA expression for the NMDA subunit NR1 was reduced in both SZ and BP, and the NR2A subunit was reduced in SZ[39]. mRNA expression of the NR3A subunit was increased in SZ, but decreased in BP[40]. NF-L is a post-synaptic density molecule involved in NMDA receptor signaling which binds the NR1 subunit and PSD-95, a molecule involved NMDA receptor clustering. SAP102 also participates in the clustering of NMDA receptors. NF-L protein expression was reduced in SZ[41,42] and SAP102 mRNA expression was reduced in BP[39]. The NR2B subunit and PSD-95 were found to have increased phosphorylation[43] and several molecules involved in the trafficking of the NR2B subunit had altered expression in SZ[44]. 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 have reported reduced somal size of layer III pyramidal cells in the DLPFC from SZ subjects[45–47]. In contrast to these studies, but in agreement with Glantz and Lewis (2000)[3] no change in somal area was found across groups in the current study. This may

reflect differences in staining methods[48]. Studies which found reduced somal size in SZ used Nissl staining, whereas Glantz and Lewis (2000)[3] and the current study utilized 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^9-tetrahydrocannabinol (THC) reduced spine density in the rat hippocampus[49,50]. Moreover, cannabis use is associated with an increased risk of SZ in genetically predisposed individuals[51–53] and with increased psychosis in SZ patients[54]. Cannabis appears to accelerate and alcohol might delay or have no effect on psychosis onset[55,56]. Alcohol use negatively impacts working memory function, but chronic cannabis might improve cognition in SZ patients[57,58]. Additional research is required to elucidate the potential impact of these substances on spines in SZ subjects.

Potential confounding effects of antipsychotic medications on dendritic parameters were assessed by comparing subjects with SZ off of antipsychotic medications for at least 1 year prior to death with those on antipsychotics (all BP subjects were on antipsychotics). The effects of chronic 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 administration in rats (e.g. six months) might have produced an effect. Nevertheless, antipsychotic medications do not appear to be a significant factor in the observed spine pathology. The effects of mood stabilizers were also assessed by comparing SZ and BP subjects who were on and off lithium and valproic acid at the time of death. In SZ, lithium treatment was associated with longer basilar dendrites. Indeed, lithium prevents reductions in hippocampal dendrite length due to chronic stress in rats[59]. Given the small groups sizes no firm conclusions can be drawn without further research, but lithium treatment might be neuroprotective in SZ patients.

Historically, SZ and BP have been viewed as distinct entities. However, as stated previously, they share many features[13–17] suggesting pathophysiological commonalities exist. Unlike GWAS studies, which include thousands of subjects, 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 two disorders which could lead to the development of novel biomarkers and therapeutics.

The current study has detected spine losses on pyramidal cell dendrites in deep layer III of the DLPFC in both SZ and BP subjects. 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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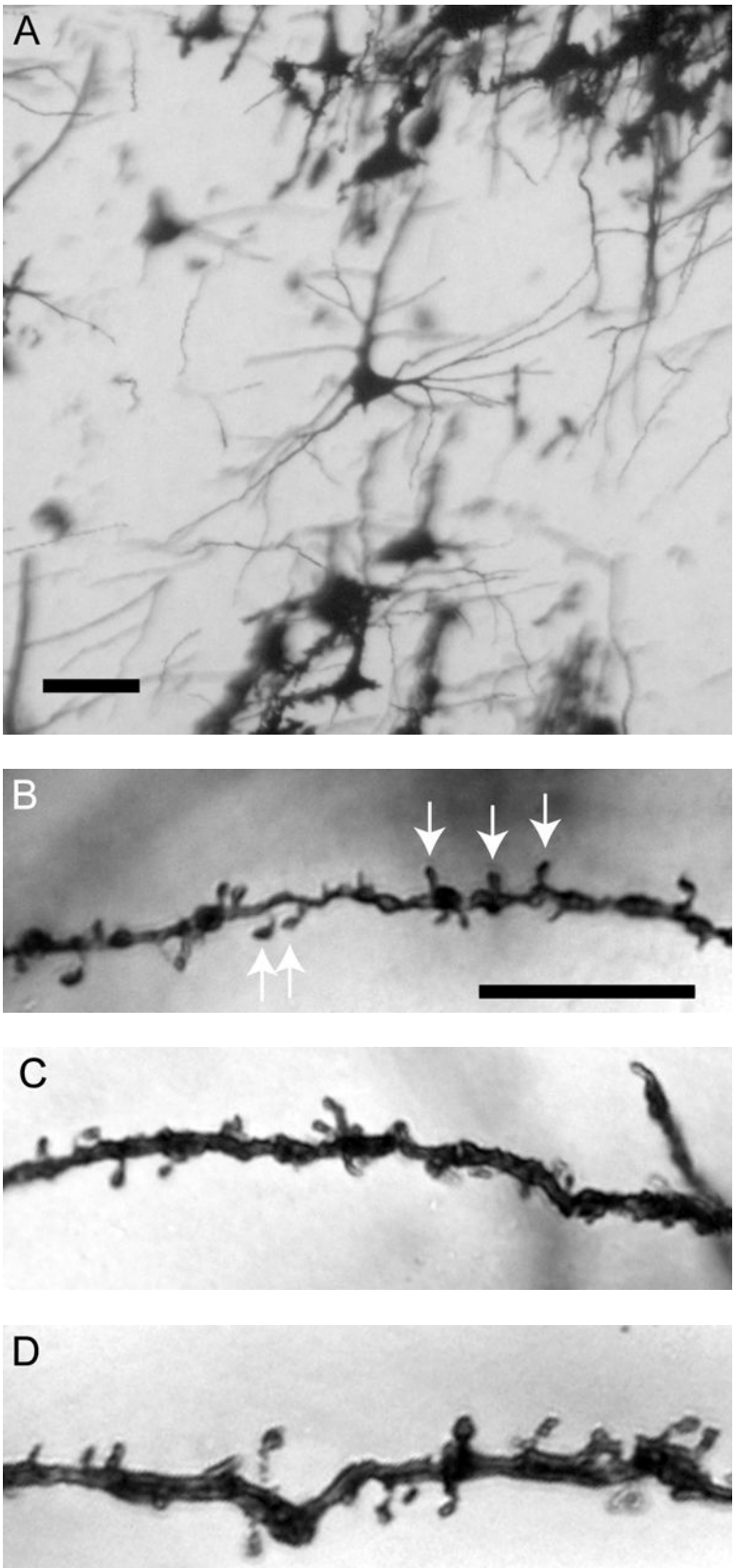
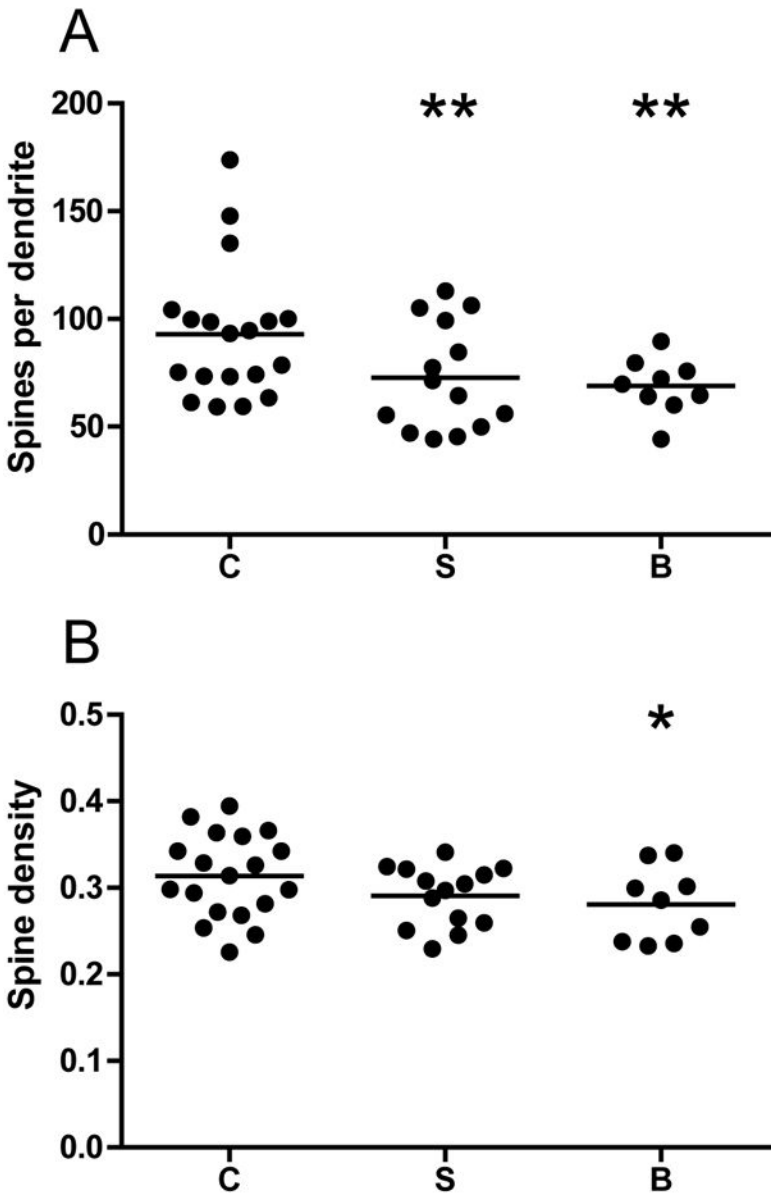


Figure 1.

Brightfield photomicrographs of Golgi-stained pyramidal cells having their somata localized in the deep half of layer III. Low power image from a control subject (A). High power images of basilar dendrites from a control subject (B), schizophrenia subject (C) and a bipolar disorder subject (D). Scale bar=50 μm for panel A and 10 μm for panel B.



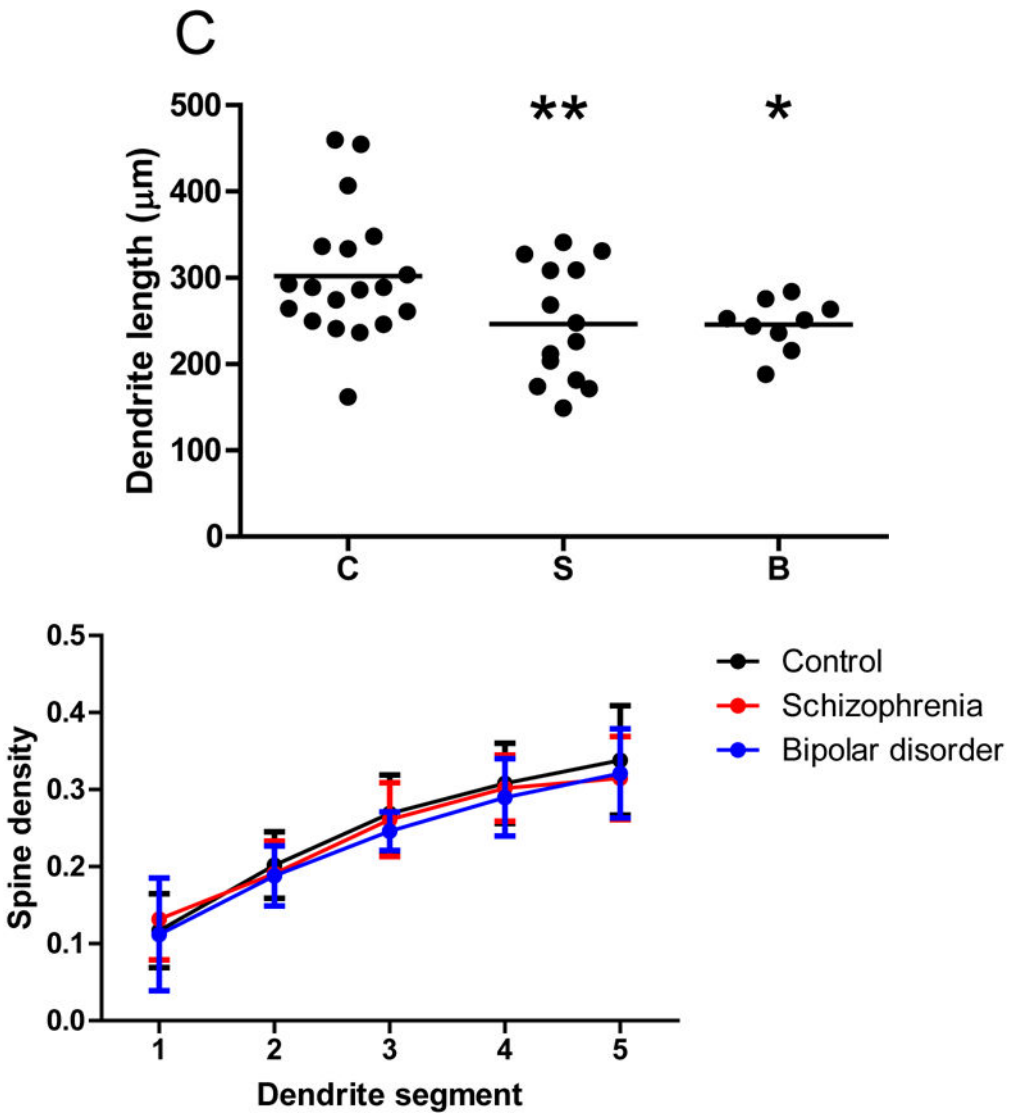
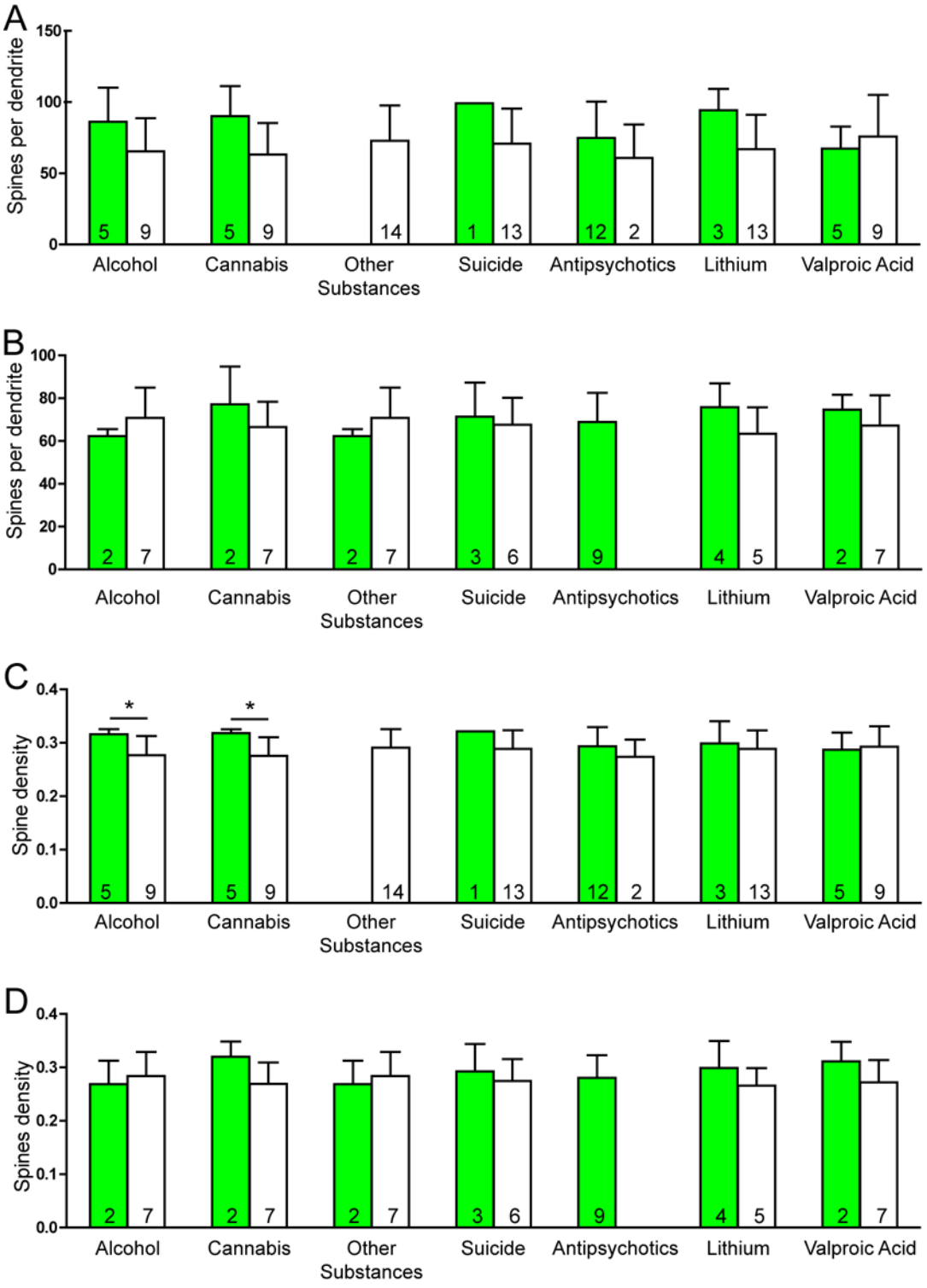


Figure 2.

Graphs representing dendritic parameters for DLPFC deep layer III pyramidal cells. The number of spines per dendrite (A), spine density (B), dendrite length (C), and spine density per dendrite segment (D). Data were obtained from reconstructions of Golgi-stained pyramidal cells. C=control, S=schizophrenia, and B=bipolar disorder; * = p <0.05 relative to controls, ** = p <0.01 relative to controls.



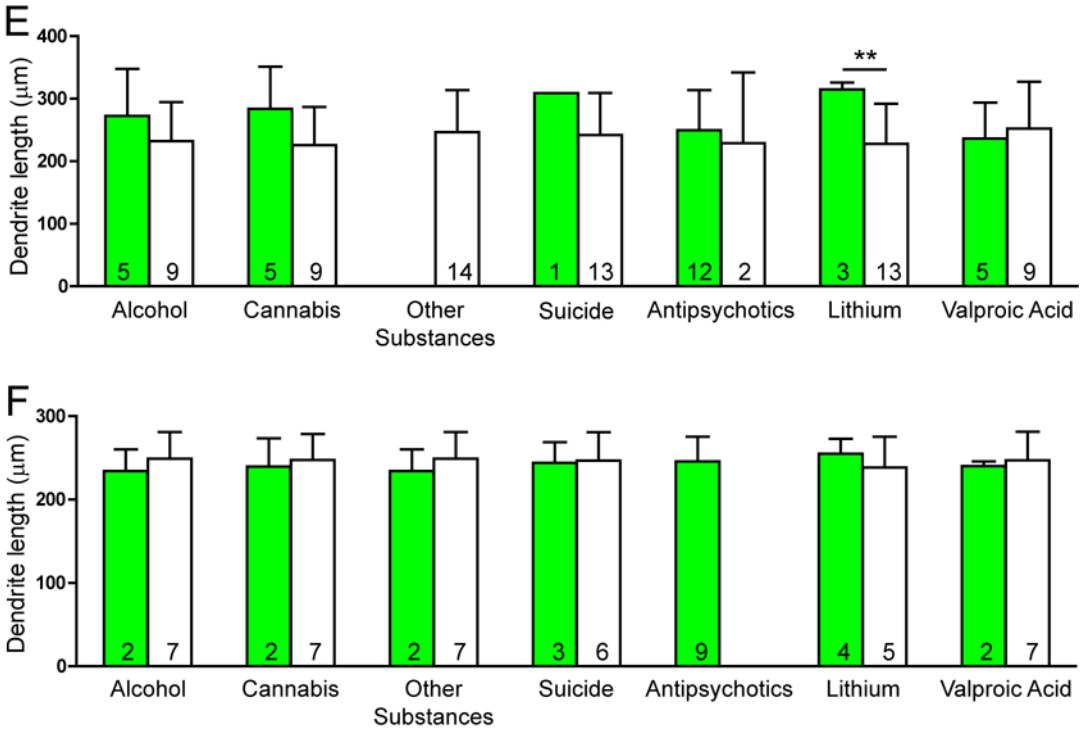


Figure 3.

Graphs representing dendritic parameters for DLPFC deep layer III pyramidal cells. The number of spines per dendrite (A), spine density (B), dendrite length (C), and spine density per dendrite segment (D). Data were obtained from reconstructions of Golgi-stained pyramidal cells. C=control, S=schizophrenia, and B=bipolar disorder; * = $p < 0.05$ relative to controls, ** = $p < 0.01$ relative to controls.

Table 1

Clinical and demographic data of subjects included in dendritic parameter analyses.

	Schizophrenia	Bipolar disorder	Control	*p*-value
Sex (M/F)	9/5	2/7	13/6	
Age	58.9±12.6	59.4±22.3	56.8±13.6	*n.s.*
PMI (hours)	24.9±8	23±6.2	22±3.8	*n.s.*
Storage time in formalin (months)	125.9±17	110.7±17.9	99±9	**<0.05**
pH	6.7±1	6.5±0.3	6.4±0.2	*n.s.*
Hemisphere (R/L)	9/5	5/4	11/8	
Suicide (Y/N)	1/13	3/6	0/19	
History of alcohol abuse or dependence (Y/N)	5/9	2/7	2/17	
History of cannabis use (Y/N)	5/9	2/7	2/17	
History of other substance abuse or dependence (Y/N)	0/14	2/7	2/17	
Antipsychotic medication (Y/N)	12/2	9/0	0/19	
Valproic acid (Y/N)	5/9	2/7	0/19	
Lithium (Y/N)	3/11	4/5	0/19	

Values are means ± S.D.

Table 2

Summary of dendritic parameter measurements.

Parameter	Schizophrenia	Bipolar disorder	Control
Spines per dendrite	72.8±24.9 **	68.9±12.9 **	92.8±31.1
Spine density (spines per μm)	0.29±0.03	0.28±0.04 *	0.31±0.05
Dendrite length (μm)	246.5±67.4 **	245.6±29.8 *	301.8±75.1
Somal area (μm^2)	375.4±92.2	363.6±45.3	378.3±63.4
Number branch segments	14.8±3.2	14.3±2.2	14.9±2.1
Max. branch order	5.3±0.5	5.1±0.4	5.3±0.4
Number artificial ends	2.2±1.2	1.8±1.2	2.2±1.2
Number natural ends	5.7±1	5.9±0.7	5.7±0.7

Values are means ± S.D.;

* =$p<0.05$ relative to controls,

** =$p<0.01$ relative to controls.