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Hippocampal CA1 Pyramidal Cell Size is Reduced in Bipolar Disorder

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SUMMARY

1. Schizophrenia and bipolar disorder are neurodevelopmental disorders with significant genetic vulnerabilities. Several trophic genes and/or proteins have been implicated in the causation for both disorders.

2. We hypothesized that these genes and/or proteins may impact neuronal growth in both disorders.

3. Hippocampal tissue sections from CA1 area of schizophrenic, bipolar, depressed, and controls subjects, matched for age, sex, PMI, drug exposure, and brain pH were prepared for cell size determination using the Stanley Medical Research Foundation postmortem brain collection.

4. Quantification of hippocampal CA1 pyramidal neuron size showed a significant 12% reduction in cell size ($p < 0.05$) in bipolar subjects vs. controls. There were nonsignificant trends for reduction in cell size in both schizophrenic and depressed subjects vs. controls.

5. These results indicate for the first time that pyramidal cell atrophy is present in hippocampus of subjects with bipolar disorder.

KEY WORDS: schizophrenia; hippocampus; bipolar disorder; pyramidal cell size; Reelin; Bcl-2; DISC1; major depression.

INTRODUCTION

Schizophrenia and mood disorders are complex neuropsychiatric disorders with genetic and environmental etiologies (Sawa and Snyder, 2005). Previous morphometric studies of the brain show evidence for abnormalities in size/volume of various brain structures in both disorders (Friedman *et al.*, 1999). These structural changes may be due to a combination of neurodevelopmental insults that may have occurred in utero and later limited neurodegeneration. Such combination of processes could affect cellular dimensions in various neuropsychiatric disorders. For example, neuronal cell size is reduced in autism (Fatemi *et al.*, 2002) and in schizophrenia, potentially due to apoptotic mechanisms (Fatemi *et al.*, 2002; Jarskog *et al.*, 2000). Thus,

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dysregulation of apoptosis could underlie both of these seemingly divergent processes (Jarskog *et al.*, 2000). Neuronal cell atrophy is a surrogate marker for apoptosis and is present in schizophrenia (Jarskog, 2006), autism (Fatemi *et al.*, 2002), bipolar disorder, and major depression (Chana *et al.*, 2003). Previous reports have also indicated decreased neuronal somal size and increased neuronal density in cortical layers IV, V, and VI of the anterior cingulate cortex (ACC) in schizophrenia, bipolar disorder, and major depression (Chana *et al.*, 2003; Torrey *et al.*, 2000). We sought to evaluate whether pyramidal somal size would be reduced in hippocampus, an important center for processing memory and synaptic plasticity, in bipolar disorder, as compared to psychiatric and normal control subjects.

MATERIALS AND METHODS

Slides of paraffin-embedded postmortem samples of anterior hippocampus were obtained from the Stanley Foundation Neuropathology Consortium (Torrey *et al.*, 2000) under approved ethical guidelines. The collection consisted of 13 subjects with schizophrenia, 14 with bipolar disorder, 14 with major depression and without psychotic features, and 14 normal controls. All groups were matched for age, sex, race, postmortem interval (PMI), and hemispheric side (Table I). Each brain hemisphere was placed in formalin immediately after removal from the body. The mean storage times in formalin were as follows: controls, 4.6 $\pm$ 3.86 months (mean $\pm$ SD), bipolars 10.1 $\pm$ 3.38 months, depressed 8.00 $\pm$ 6.64 months, and schizophrenics 10.3 $\pm$ 7.31 months. None of these values differed from control values statistically. The Stanley Foundation Neuropathology Consortium provided us with all demographic information and medical data, including lifetime use of psychotropic medications and history of drug abuse (Fatemi *et al.*, 2000) (Table I). Two psychiatrists used DSM-IV criteria to establish psychiatric diagnoses. All experiments were performed blinded to diagnosis of each subject. Each slide was 10 μm in thickness and selected slides were 40 μm apart in distance. Slides of the anterior hippocampus (at the level of the genu) were deparaffinized and stained with cresyl violet through a series of graded alcohols. Hippocampal anatomy was visualized under a 10X microscope. CA4 was identified first, as it is easily identifiable with the darkly stained granular cell layer of the dentate gyrus on its border, then CA1 was brought into the center of the microscope field. Under 20X, we employed three boxes of equal dimensions (250 μm $\times$ 250 μm), and superimposed these boxes on the cells of CA1. Cells with a clear nucleus, cell body, and cell boundaries were identified and their circumferences were measured. StereoInvestigator software (MicroBrightField, Inc., Burlington, VT) was used for cell area determinations. All area measurements were obtained in μm^2 $\pm$ SD. In general, two slides per brain were used for all cell measurements, and each slide was used for three measurements at CA1. Cell measurements for each box numbered between 8 and 10 cells per box. All cell area measurements were performed under blind conditions.

Table I. Demographic Information on Brain Specimens Obtained from the Stanley Foundation Neuropathology Consortium (Range Shown in Parentheses)

Variable	Control	Schizophrenia	Bipolar disorder	Major depression
Age (years)	48.6 (29–68)	45.7 (25–62)	41.8 (25–61)	45.2 (30–56)
Gender	8 M, 6 F	8 M, 5 F	8 M, 6 F	8 M, 6 F
Race	13 C, 1 AA	11 C, 2 As	12 C, 2 AA	14 C
PMI (h)	24.6 (8–42)	32.0 (12–61)	33.5 (13–62)	28.1 (7–47)
pH	6.3 (5.8–6.6)	6.2 (5.8–6.6)	6.2 (5.8–6.5)	6.2 (5.8–6.5)
Side of brain	6 R, 8 L	5 R, 8 L	8 R, 6 L	5 R, 9 L
Formalin storage time (months)	4.6 (1–10)	10.3 (3–31)	10.1 (2–14)	8.0 (1–15)
Suicide as mode of death	0	3	8	7
Substance abuse severity[a]	13 none; 1 low	10 none; 1 low; 1 high; 1 highest	6 none; 5 low; 1 high; 2 highest	10 none; 1 lowest; 1 high; 2 highest
Lifetime quantity of fluphenazine or equivalent use of antipsychotics (mg)	0	50,307 (0–200,000)	22,945 (0–60,000)	0

Note. M = Male; F = Female; C = Caucasian; AA = African-American; As = Asian; R = Right; L = Left; PMI = postmortem interval.
[a]Substance abuse: none = little or none; lowest = social user; low = moderate use in past; high = moderate use in present; highest = heavy use in present.

Statistical Analysis

Statistical analysis of data was conducted using SPSS v.12.0 (Chicago, IL) software. Several confounders (i.e., age, sex, race, PMI, pH of brain, side of brain, brain weight, mode of death, formalin storage time, age of onset of disease, psychosis, history of substance use, severity of substance use and severity of alcohol use) were tested for differences across groups and for their effects on the cell size (Table II). One-way analyses of variance (ANOVA), chi-square tests, or Pearson's correlations were calculated for the confounder effect analyses. Group difference involving the cell size was examined using ANOVA. In case of significance, posthoc Tukey's HSD test was performed to examine differences among the three diagnostic groups.

RESULTS

Significant between-group differences were found in four confounder variables (Table II): (1) Age of onset of disorder (depression mean age = 33.14 vs. bipolar mean age = 21.79, Tukey's HSD test $p < 0.05$); (2) Psychosis (schizophrenia = 100%, bipolar = 71%, major depression = 0%, and control = 0%, $\chi^2 = 43.26$, $p < 0.001$); (3) Severity of substance abuse (bipolar mean = 2.07 vs. control mean = 0.14, Tukey's test $p < 0.05$); and finally (4) Formalin time (schizophrenic mean months = 10.3 vs. control mean = 4.57, Tukey's test $p < 0.05$). None of these confounds had any significant effects on cell size (Table II). The modes of death included suicide, cardiac causes, pneumonia, motor vehicle accident, accidental fall, drowning, and pulmonary embolism. The number

Table II. Group Differences in Confounding Variables and Their Effects on Cell Size

Variable	Group difference	Effect on cell size
Age	$F = 0.86, p = 0.468$	$r = 0.119, p = 0.388$
Sex	$\chi^2 = 0.08, p = 0.994$	$F = 4.17, p = 0.046$[f]
Age of onset	$F = 4.76, p = 0.014$	$r = -0.106, p = 0.510$
PMI	$F = 1.34, p = 0.272$	$r = -0.178, p = 0.194$
pH	$F = 0.68, p = 0.567$	$r = 0.099, p = 0.478$
Brain weight	$F = 0.51, p = 0.680$	$r = 0.230, p = 0.091$
Side	$\chi^2 = 1.54, p = 0.673$	$F = 1.01, p = 0.321$
Suicide	$\chi^2 = 3.50, p = 0.174$[a]	$F = 0.44, p = 0.511$
Storage time (months) in formalin	$F = 3.16, p = 0.032$[b]	$r = 0.046, p = 0.741$
Psychosis	$\chi^2 = 43.26, p = 0.000$[c]	$F = 1.00, p = 0.322$
Substance abuse history	$\chi^2 = 6.45, p = 0.109$	$F = 1.46, p = 0.243$
Severity of substance abuse	$F = 3.38, p = 0.025$[d]	$r = -0.237, p = 0.085$
Severity of alcohol abuse	$F = 2.63, p = 0.061$[e]	$r = -0.136, p = 0.348$

[a] If controls included, $\chi^2 = 13.05, p = 0.005$.
[b] Posthoc Tukey's HSD test, normal < schizophrenia, $p < 0.05$.
[c] Psychosis in schizophrenia = 100%, bipolar = 71%, MD = 0%, and Control = 0%.
[d] Posthoc Tukey's HSD test, normal < bipolar, $p < 0.05$.
[e] Posthoc Tukey's HSD test, normal < bipolar, $p < 0.05$.
[f] Male > female.

of subjects who died by suicide included three in schizophrenia, eight in bipolar disorder, and seven in major depression (Table I). There were no significant effects of suicide on cell size determination. There was only one significant confounder effect of sex on cell size, i.e., male subjects had a significantly larger cell size than female subjects (male mean cell size (μm^2) = 1811.49 vs. female mean cell size (μm^2) = 1692.66, ANOVA $p < 0.05$). However, this effect was seen in all males with all psychiatric diagnoses vs. healthy controls. Cell size was not different between male and female bipolar patients. Finally, there was a marginally significant group effect on pyramidal cell size (ANOVA $p = 0.066$). Posthoc test revealed that the pyramidal cell size was determined to be smaller by 12% ($p < 0.05$) in bipolar subjects (mean = 1640.11) as compared to controls (mean = 1856.36) (Fig. 1).

DISCUSSION

The present results show a significant decrease in pyramidal somal size in bipolar disorder vs. controls. There were nonsignificant decreases in pyramidal somal size in schizophrenia and major depression. Reduced somal size and increased neuronal density in cortical layers V and VI of the anterior cingulate cortex (ACC) have been recently reported in schizophrenia, major depression, and bipolar disorder (Chana *et al.*, 2003). Additionally, abnormalities of cortical neuronal organization and reductions in neuronal somal size have been reported in schizophrenia (Guidotti *et al.*, 2000). Reduction in size of pyramidal neurons in the posterior hippocampus has also been reported in schizophrenia but not in bipolar disorder (Benes *et al.*, 1991). Reductions in levels of neurotrophic agents, such as Reelin, Bcl-2, and DISC1, and increases in proapoptotic factors, such as P53, may be

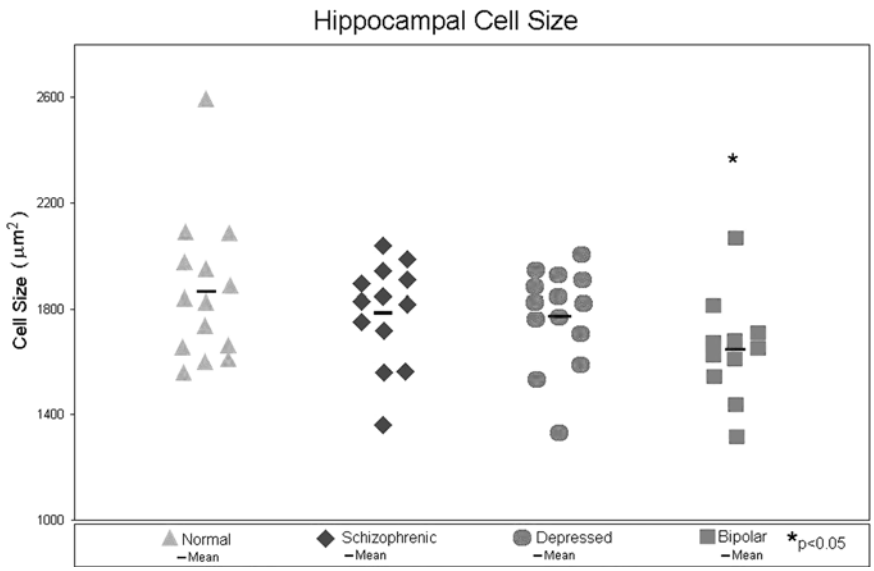

Fig. 1. A scatterplot of the mean cell area per subject.

involved in the genesis of these abnormalities (Guidotti *et al.*, 2000; Fatemi *et al.*, 2000; Fatemi, 2001, 2005; Jarskog and Gilmore, 2000; Burdick *et al.*, 2005).

The limitations of this study included: (1) Use of a two-dimensional cell size measurement vs. a three-dimensional method of volume estimation; and (2) The lack of absolute certainty that formalin exposure times did not affect cell size measurements. However, while bipolar brains spent 10.1 months in formalin vs. 8 months for depression and 10.3 months for schizophrenics, only the schizophrenic subjects showed a significant effect. Also, while bipolar subjects showed the smallest cell size, one would have expected the other two groups to show significantly smaller cell sizes than controls if tissue shrinkage caused these observations in bipolar slides. This obviously is not the case, indicating lack of an association between formalin exposure times and cell size.

Reelin glycoprotein is an important protein involved in dendrite development, neural stem cell migration, learning, memory, and LTP (Fatemi, 2005). Decreases in Reelin protein levels have been identified in schizophrenia and bipolar disorder (Fatemi *et al.*, 2000; Guidotti *et al.*, 2000; Fatemi, 2001). Reelin protein is reduced in the CA1–CA4 areas of the hippocampus and dentate gyrus in schizophrenia, bipolar disorder, and major depression vs. controls (Fatemi *et al.*, 2000). Reelin has clearly defined neurodevelopmental roles during brain development in man and mouse (Fatemi, 2001, 2005). It is possible that graded deficits in level of Reelin may cause variable levels of brain synaptic and behavioral abnormalities. Reports also indicate that Reelin deficiency may be associated with decreased synaptic transmission and reduced learning and memory in psychiatric patients (Fatemi, 2005). Cell atrophy in CA1 of hippocampus in bipolar disorder may also be related to decreased Reelin in this area (Fatemi *et al.*, 2000).

Bcl-2 is an important antiapoptotic regulatory protein that exerts a powerful neuroprotective effect on neurons (Jarskog and Gilmore, 2000; Zhong *et al.*, 1993; Chen *et al.*, 2001). Bcl-2 shows developmental upregulation in human frontal cortex across the lifespan, increasing from early childhood into adulthood (Jarskog and Gilmore, 2000). Cells that overexpress Bcl-2 demonstrate considerable resistance to a variety of proapoptotic insults (Chen *et al.*, 2001). Importantly, Bcl-2 also has a neurotrophic property that appears to be independent of its antiapoptotic function, i.e., it can promote dendritic branching and produce regeneration of damaged central nervous system (CNS) neurons (Chen *et al.*, 2001). Levels of Bcl-2 are reduced in schizophrenia, bipolar disorder, and autism (Araghi-Niknam and Fatemi, 2003; Jarskog *et al.*, 2000; Jarskog, 2006). The deficit has several potential pathophysiological implications: Firstly, Bcl-2 is a potent inhibitor of apoptosis, and a reduction of this protein would suggest that brain neurons (like those in the hippocampal CA1 area) in bipolar disorder and schizophrenia are more vulnerable to proapoptotic stimuli, whether those stimuli are products of normal physiology and aging (Kamiya *et al.*, 2005) or are due to a pathological process. Secondly, because Bcl-2 protein has neurotrophic properties that are independent of apoptosis (Chen *et al.*, 2001), a limited reduction of Bcl-2 could promote neuronal atrophy and result in reduced axodendritic branching, without overt effects on cell death. These data might suggest that pyramidal cells of CA1 in hippocampus in subjects with bipolar disorder experience more neuronal atrophy than those of schizophrenic and major depression subjects, leading to greater deficit in pyramidal somal size. However, little is known of the effects of early developmental insults on long-term effects on Bcl-2 expression in bipolar disorder, schizophrenia, or major depression. Additionally, there may be differences in mechanisms of apoptosis operating in bipolar disorder vs. schizophrenia. For example, the Bax/Bcl-2 ratio was higher, only in schizophrenia and not bipolar disorder (Jarskog *et al.*, 2004) indicating increased apoptotic activity in temporal cortex. However, support for increased vulnerability towards insults in hippocampus of bipolar subjects is lacking currently.

Finally, the DISC1 gene (disrupted in schizophrenia) is a susceptibility gene in patients with schizophrenia and mood disorders (Harrison and Weinberger, 2005; Kamiya *et al.*, 2005; Burdick *et al.*, 2005; Thomson *et al.*, 2005). DISC1 is expressed in regions of the brain implicated in the genesis of psychiatric symptoms (Kamiya *et al.*, 2005). Reduced expression of DISC1 in mice disturbs proper neuronal migration and arborization of dendritic neuronal processes in the developing cerebral cortex (Kamiya *et al.*, 2005). How DISC1 influences mental function is unknown but is beginning to be clarified. In schizophrenic patients, DISC1 variants are linked to specific neurocognitive impairments (Burdick *et al.*, 2005). A single-neuropeptide polymorphism in DISC1 that leads to an amino acid change (Ser704Cys) is associated with schizophrenia and correlates with variations in hippocampal size and function during cognitive tasks in normal subjects as well as cognitive variations in aged normal subjects (Thomson *et al.*, 2005). This evidence suggests that DISC1 can reconcile developmental disturbances in brain structure with aberrations in neurotransmitter-mediated information processing. It is possible that DISC1 gene expression and its protein level change could be related to the CA1 pyramidal cell atrophy in bipolar disorder, but its mechanistic role needs further exploration.

In conclusion, our data indicate for the first time that CA1 hippocampal pyramidal cells are smaller in bipolar disorder, possibly as a result of decreased levels of Reelin, Bcl-2, and potentially DISC1. Future studies should ascertain the causative factors for this reduction in cell size.

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REFERENCES

Araghi-Niknam, M., and Fatemi, S. H. (2003). Levels of Bcl-2 and P53 are altered in superior frontal and cerebellar cortices of autistic subjects. *Cell. Mol. Neurobiol.* **23:**945–952.

Benes, F. M., Sorensen, I., and Bird, E. D. (1991). Reduced neuronal size in posterior hippocampus of schizophrenic patients. *Schizophr. Bull.* **17:**597–608.

Burdick, K. E., Hodgkinson, C. A., Szeszko, P. R., Lencz, T., Ekholm, J. M., Kane, J. M., Goldman, D., and Malhotra, A. K. (2005). DISC1 and neurocognitive function in schizophrenia. *Neuroreport* **16:**1399–1402.

Chana, G., Landau, S., Beasley, C., Everall, I. P., and Cotter, D. (2003). Two-dimensional assessment of cytoarchitecture in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia: Evidence for decreased neuronal somal size and increased neuronal density. *Biol. Psychiatry* **53:**1086–1098.

Chen, D. F., Schneider, G. E., Martinou, J. C., and Tonegawa, S. (2001). Bcl-2 promotes regeneration of severed axons in mammalian CNS. *Nature* **385:**434–439.

Fatemi, S. H. (2001). Reelin mutations in mouse and man: From reeler mouse to schizophrenia, mood disorders, autism and lissencephaly. *Mol. Psychiatry* **6:**129–133.

Fatemi, S. H. (2005). Reelin glycoprotein: Structure, biology and roles in health and disease. *Mol. Psychiatry* **10:**251–257.

Fatemi, S. H., Earle, J. A., and McMenomy, T. (2000). Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. *Mol. Psychiatry* **5:**654–663, 571.

Fatemi, S. H., Halt, A. R., Realmuto, G., Earle, J., Kist, D. A., Thuras, P., and Merz, A. (2002). Purkinje cell size is reduced in cerebellum of patients with autism. *Cell. Mol. Neurobiol.* **22:**171–175.

Friedman, L., Findling, R. L., Kenny, J. T., Swales, T. P., Stuve, T. A., Jesberger, J. A., Lewin, J. S., and Schulz, S. C. (1999). An MRI study of adolescent patients with either schizophrenia or bipolar disorder as compared to healthy control subjects. *Biol. Psychiatry* **46:**78–88.

Guidotti, A., Auta, J., Davis, J. M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D. R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., and Costa, E. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch. Gen. Psychiatry* **57**:1061–1069.

Jarskog, L. F., and Gilmore, J. H. (2000). Developmental expression of Bcl-2 protein in human cortex. *Brain Res. Dev. Brain Res.* **119:**225–230.

Jarskog, L. F., Gilmore, J. H., Selinger, E. S., and Lieberman, J. A. (2000). Cortical bcl-2 protein expression and apoptotic regulation in schizophrenia. *Biol. Psychiatry* **48**:641–650.

Jarskog, L. F., Selinger, E. S., Lieberman, J. A., and Gilmore, J. M (2004). Apoptotic proteins in the temporal cortex in schizophrenia: High Bax/Bcl2 ratio without caspase-3 activation. *Am. J. Pscyhiatry* **161:**109–115.

Jarskog, L. F. (2006). Apoptosis in schizophrenia: Pathophysiologic and therapeutic considerations. *Curr. Opin. Psychiatry* **19:**307–312.

Kamiya, A., Kubo, K., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., Sawamura, N., Park, U., Kudo, C., Okawa, M., Ross, C. A., Hatten, M. E., Nakajima, K., and Sawa, A. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nat. Cell. Biol.* **7:**1067–1078.

Sawa, A., and Snyder, S. H. (2005). Genetics. Two genes link two distinct psychoses. *Science* **310:**1187–1191.

Thomson, P. A., Harris, S. E., Starr, J. M., Whalley, L. J., Porteous, D. J., and Deary, I. J. (2005). Association between genotype at an exonic SNP in DISC1 and normal cognitive aging. *Neurosci. Lett.* **389:**41–45.

Torrey, E. F., Webster, M., Knable, M., Johnston, N., and Yolken, R. H. (2000). The stanley foundation brain collection and neuropathology consortium. *Schizophr. Res.* **44:**151–155.

Zhong, L. T., Sarafian, T., Kane, D. J., Charles, A. C., Mah, S. P., Edwards, R. H., and Bredesen, D. E. (1993). Bcl-2 inhibits death of central neural cells induced by multiple agents. *Proc. Natl. Acad. Sci. U.S.A.* **90:**4533–4537.