Published in final edited form as:

Biol Psychiatry. 2007 March 1; 61(5): 640–652.

Parvalbumin Neurons in the Entorhinal Cortex of Subjects Diagnosed With Bipolar Disorder or Schizophrenia

Harry Pantazopoulos, Nicholas Lange, Ross J. Baldessarini, and Sabina Berretta

Translational Neuroscience Laboratory (HP, SB), Neurostatistics Laboratory (NL), and Neuropsychopharmacology Laboratory (RJB), McLean Hospital, Belmont; Department of Psychiatry (NL, RJB, SB) and Neuroscience Program (RJB), Harvard Medical School; Department of Biostatistics (NL), Harvard School of Public Health, Boston, Massachusetts.

Abstract

Background—Growing evidence indicates that the entorhinal cortex (ECx) might be affected in schizophrenia (SZ) and bipolar disorder (BD). To test whether distinct interneuronal subpopulations might be altered, numbers of parvalbumin-immunoreactive (PVB-IR) neurons were measured in the ECx of BD and SZ subjects. These neurons play a pivotal role within ECx intrinsic circuits.

Methods—Numbers, numerical density, and soma size of PVB-IR neurons were measured in the ECx of normal control ($n = 16$), BD ($n = 10$), and SZ ($n = 10$) subjects. The volume of the ECx was measured in Nissl-stained sections.

Results—In BD, decreases of total numbers ($p = .02$) and numerical densities ($p = .01$) of PVB-IR neurons were detected in the ECx. Within distinct subregions, reductions were detected in the superficial layers of the lateral ($p = .02$), intermediate ($p = .04$), and caudal ($p = .01$) ECx. In SZ, total numbers and numerical densities were not altered. A reduction of soma size was present in the intermediate ECx ($p = .01$). Volume was unaffected in either disorder.

Conclusions—In BD, a decrease of PVB-IR neurons may alter intrinsic inhibitory networks within the superficial layers of the ECx. The likely consequence is a disruption of integration and transfer of information from the cerebral cortex to the hippocampus.

Keywords

Bipolar disorder; entorhinal cortex; immunocytochemistry; parvalbumin; postmortem; schizophrenia

The entorhinal cortex (ECx) is a key component of the medial temporal lobe, a system of interconnected cortical and subcortical brain regions involved in complex functions such as long-term memory, sensory representation, and spatial orientation (Squire et al 2004). These functions are carried out through massive reciprocal connections with unimodal and polymodal association cortical areas, the hippocampus, and subcortical regions such as the amygdala. Highly processed information is relayed from the ECx to the hippocampus. Outgoing information flow from the hippocampal formation/subicular complex returns to the ECx, which in turn relays it to the amygdala, nucleus accumbens, and several cortical regions, such as the medial and lateral prefrontal and orbito-frontal cortices and the cingulate gyrus (Insausti et al

Address reprint requests to Sabina Berretta, M.D., MRC 3 - McLean Hospital, 115 Mill Street, Belmont, MA 02478; E-mail: s.berretta@mclean.harvard.edu.

This work was supported by National Institutes of Health (NIH) grants MH063215, MH06628, and NARSAD Young Investigator Award (to SB); the Andrew P Merrill Foundation to HP; NIH grants NS37483 and Conte Center Grant MH60450 (P.I. Coyle J.T., M.D.) (to NL), as well as a grant from the Bruce J. Anderson Foundation and by the McLean Private Donors Psychopharmacology and Bipolar Disorder Research Fund (to RJB).

1987a, 1987b;Sewards and Sewards 2003;Witter et al 2000). Thus, the ECx plays a pivotal role in mediating the flow of information in and out of the hippocampal formation. Growing evidence suggests that the ECx represents more than a simple interface between cortical and subcortical regions and the hippocampus and is actively involved in functions such as memory processing (de Curtis and Paré 2004; Lavenex and Amaral 2000;Vinogradova 2001).

Clinical, imaging, and postmortem evidence indicates that the neural circuits in which the ECx is embedded are involved in the pathogenesis of major psychiatric illnesses. Cortical regions such as the medial and lateral prefrontal cortex and the anterior cingulate gyrus are affected in schizophrenia (SZ) and bipolar 6 disorder (BD) (Bartha et al 1997;Benes et al 2000;Broadbelt et al 2002;Guidotti et al 2000;Rajkowska et al 2001;Thomas et al 2003;Woo et al 2004). Within the medial temporal lobe, several interconnected regions are thought to play a particularly important role in the pathophysiology of these disorders (Arnold 1997;Benes and Berretta 2000;Blumberg et al 2003;Cirillo and Seidman 2003;Goldsmith et al 1997;Heckers et al 1998;Kurachi 2003;Shenton et al 2001;Strakowski et al 2005;Torrey 2002). Of relevance is a large body of evidence pointing to anatomical, neurochemical, and functional abnormalities in the hippocampus of SZ and BD (Altshuler et al 1998;Benes and Berretta 2001;Benes et al 1997;Bogerts 1984;Heckers et al 2002;Konradi et al 2004;Pantazopoulos et al 2004;Zhang and Reynolds 2002).

Given its powerful interactions with these regions, it is not surprising that the ECx has also been found to be affected. In SZ, volume reductions of the parahippocampal gyrus (Bogerts et al 1985;Joyal et al 2002;McDonald et al 2000;Turetsky et al 2003) or selectively of the ECx (Falkai et al 1988) have been reported. Cytoarchitectural anomalies and abnormal distributions of glutamatergic fibers suggest abnormal development of the ECx (Akil and Lewis 1997;Arnold et al 1991;Falkai et al 2000;Jakob and Beckmann 1986, 1994;Kovalenko et al 2003; but see also Krimer et al 1997;Longson et al 1996). Decreases of synaptic proteins and abnormalities relative to specific neurotransmitter systems have also been detected in the SZ ECx (Bachus et al 1997;Benes 1995;Benes and Berretta 2001;Eastwood et al 1995, 1997;Hemby et al 2002;Mizukami et al 2002;Wolf et al 1995). Surprisingly, very few postmortem microscopic studies have thus far focused on the involvement of the ECx in BD (Law et al 2004;Webster et al 2002). Recently, results from imaging studies indicate that abnormalities relative to the volume of the temporal lobe might also be present in BD (Soares 2003;Wilke et al 2004).

Abnormalities of γ -aminobutyric acid (GABA)ergic transmission in SZ and BD seem to be among the most consistent findings in postmortem studies (Akbarian et al 1995;Bachus et al 1997;Beasley and Reynolds 1997;Benes 1995;Benes et al 1991, 1992, 1996, 1997, 1998;Danos et al 1998;Ishikawa et al 2004a, 2004b, 2005;Reynolds et al 1990;Simpson et al 1989;Todtenkopf and Benes 1998;Woo et al 1998, 2004), and strongly suggest the involvement of interneurons in multiple limbic regions. As a first step in testing the hypothesis that specific subpopulations of ECx GABAergic interneurons may be affected in BD and/or SZ, we chose to measure total numbers (Tn), numerical densities (Nd), and somata size of neurons expressing the calcium-binding protein parvalbumin (PVB) (Miettinen et al 1996;Wouterlood et al 1995;Wouterlood 2002). Abnormalities relative to PVB-immunoreactive (IR) neurons have been found in other regions, such as the hippocampus and the prefrontal cortex (Lewis et al 2001;Zhang and Reynolds 2002), and recent reports have highlighted the relevance of PVB as a marker for GABAergic abnormalities in major psychoses (Knable et al 2004;Reynolds et al 2004;Torrey et al 2005).

In the present study, PVB-IR neurons were counted in the ECx of normal control, BD, and SZ subjects. We hypothesized that SZ and BD would show reduced PVB-IR neuron Tn and Nd in specific ECx subdivisions.

Methods and Materials

Human Subjects

Postmortem tissue blocks containing the whole ECx from 10 SZ, 10 BD, and 16 normal control donors (one hemisphere/ subject) were obtained from the Harvard Brain Tissue Resource Center, Belmont, Massachusetts (Table 1). Diagnoses were made by retrospective review of medical records and an extensive questionnaire about social and medical history provided by family members of the donor. In combination, these records provided information on each subject starting from the onset of the disease or earlier. Two psychiatrists reviewed all records and applied the criteria of Feighner et al (1972) for the diagnosis of SZ and DSM-III-R for the diagnosis of BD. We also analyzed medical records for estimates of lifetime exposure to various classes of psychotropic and neurotropic drugs, converted estimated daily milligram doses of antipsychotic drugs to the approximate equivalent of chlorpromazine (CPZ-eq) as a standard comparator (Baldessarini and Tarazi 1995) and reported these values as well as actual doses of lithium salts as lifetime grams per patient. The use of other classes of psychotropic drugs was reported as present or absent, because dosing data were not adequate to support more quantitative estimates. We also made a qualitative assessment of treatment-adherence as “good” or “poor,” on the basis of taking prescribed psychotropic medicines more or less than approximately half of the time, as indicated by the extensive antemortem clinical records (Table 2).

Several regions from each brain were examined by a neuropathologist. Brains with evidence for gross and/or macroscopic changes consistent with Alzheimer’s disease, cerebrovascular accident, ethanol and drug abuse, or other potentially confounding factors were excluded from the study.

Tissue Processing and Immunocytochemistry

Tissue blocks were dissected from fresh brains and post-fixed in .1 mol/L phosphate buffer (PB; pH 7.4) containing 4% paraformaldehyde and .1 mol/L Na azide at 4°C for 3 weeks. These blocks were then placed in cryoprotectant solution (30% glycerol, 30% ethylene glycol, and .1% Na azide in .1 mol/L PB) at 4°C for 3 weeks. The blocks were then embedded in agar and pre-sliced in 2-mm slabs with an Antithetic Tissue Slicer. Each slab was exhaustively sectioned with a freezing microtome (American Optical 860, Buffalo, New York). Sections were stored in the same cryoprotectant solution at –20°C. Using systematic random sampling criteria (Coggeshall and Lekan 1996), serial sections through the ECx were distributed in 22 compartments (40-µm-thick sections) plus two compartments (80-3m-thick sections). Sections within one compartment (10–12) have a section separation (i.e., distance between one section and the next one) equal to 1.04 mm. Eighty-µm-thick sections were used for Nissl staining (cresyl violet), whereas adjacent 40-µm-thick sections were used for immunocytochemistry (ICC). All sections within each compartment were selected for staining, thus respecting the “equal opportunity” rule (Coggeshall and Lekan 1996).

PVB was detected with standard immunocytochemical methods. Sections from a 40-µm compartment from each brain were randomly assigned to a six-well staining dish. Sections were washed in .1 mol/L PB .5% Triton-X, boiled for 3 min in PB with 1:100 Antigen Unmasking Solution (Vector Labs, Burlingame, California), and incubated with a monoclonal anti-PVB antibody for 48 hours at 4°C (1:10,000; P3088, lot #10K4846; clone PARV-19, ascites fluids; Sigma-Aldrich, St. Louis, Missouri). Immunoblot characterization showed a single band corresponding to 12 kD (information kindly provided by Sigma-Aldrich). This antibody has been found to react with PVB (12 kDa) from human. It does not react with other members of the EF-hand family of calcium binding proteins. Sections were incubated with biotinylated secondary serum horse anti mouse (1:500; Vector Labs), followed by streptavidin

(1:5,000; Vector Labs). A solution of diaminobenzidine (.02%; Sigma-Aldrich), nickel sulphate (.08%), and hydrogen peroxide (.006%) was used for detection of the antigen/antibody complex. All steps were followed by rinses with PB-Triton. Immunostained sections were mounted on gelatin-coated glass slides, coverslipped, and coded for quantitative analysis blinded to diagnosis. Sections from all brains included in the study were processed simultaneously within the same ICC session to avoid procedural differences. Care was taken that each six-well staining dish contained sections from SZ, BD, and normal control subjects and was carried through each immuno-cytochemistry step for the same duration of time to avoid sequence effects. Identical protocols for PVB ICC with the omission of the first or secondary antibodies did not yield detectable signal. The patterns of PVB-IR in the ECx as well as the amygdala were identical to those described by other laboratories (Mikkonen et al 1997; Sorvari et al 1995).

Data Collection

Computer-assisted light microscopy, complete with stereology quantification software (Bioquant Nova Prime v6.0, R&M Biometrics, Nashville, Tennessee; and StereoInvestigator, Micro-BrightField, Williston, Vermont), was used for data collection.

For volume measurements, the ECx was traced in Nissl-stained serial sections encompassing the entire structure (10–12 sections/ subject) and sampled with the systematic random sampling criteria described (see Methods). Included in the region identified as ECx are the rostral (ECx-R), lateral (ECx-L), intermediate (ECx-I), caudal (ECx-C), olfactory, and medial intermediate subdivisions of the ECx. For measurement of PVB-IR neuron soma size, the surface area of IR neurons was measured on immunostained sections by outlining the perimeter of each IR somata.

For estimates of Tn and Nd (cells/mm³) of ECx PVB-IR neurons, the entire ECx was outlined in a complete set of serial PVB-immunostained sections from each subject (10–12 sections/ subject) with a 1.6× objective. Adjacent Nissl-stained sections, from which volume estimates were derived, were used for confirmation of the ECx borders. In each section, the ECx was thoroughly scanned within its full x-, y-, and z-axes with a 40× objective. All detectable PVB-IR neuron somata were counted.

As a second step, Nd of PVB-IR neurons were estimated within ECx subregions (ECx-R, ECx-L, ECx-I, and ECx-C; Figure 1). This step was intended to investigate whether potential differences between diagnosis groups might originate from distinct ECx subdivisions and/or layers. Nd of PVB-IR neurons were measured in a sub-sample of three sections/subject, chosen to represent the fully formed rostral, intermediate, and caudal portions of the ECx (Figure 1). The olfactory, medial intermediate, and caudal limiting ECx subdivisions were not considered for data collection because preliminary studies had shown that they contain only very sparse PVB-IR neurons. Again, the borders of each region were outlined with a 1.6× objective. Each subdivision and layer was systematically scanned through the full x-, y-, and z-axis with a 40× objective in order to determine the location of each PVB-IR neuron with respect to these subdivisions and layers.

PVB-immunostained and adjacent Nissl-stained sections were used to identify ECx subdivisions according to specific cytoarchitectonic criteria and nomenclature described by Insausti et al (1995; Mikkonen et al 1997; Tunon et al 1992) (Figure 1). Within these subdivisions, six cortical layers were identified, with a 4× objective, on the basis of the distribution of PVB immunoreactivity (Mikkonen et al 1997) and confirmed on adjacent Nissl-stained sections according to the criteria of Insausti et al (1995) (Figures 1 and 2). Particular care was taken to match the rostro-caudal level of each section across all subjects (see legend in Figure 1). The most rostral section contained the ECx-R and the rostral portion of the ECx-

L. The middle section contained the ECx-I and the caudal portion of the ECx-L. The third and most caudal section contained the ECx-C and was located rostrally to the transitional zone that defines the distinct features of the caudal limiting subdivision of the ECx.

Quality of PVB-IR was tested in a blinded pilot study. Four sections from each ECx were subjectively rated on a scale from 1 to 5, with intensity of immunoreactivity, immunolabeling penetration along the z axis, and immunoreactivity distribution pattern as the main criteria. The quality of PVB-immunostaining was entirely consistent both within and between groups and optimal for readily and unambiguously recognizing IR neurons throughout the thickness of each section.

Statistical Analyses

Nissl-stained sections, including the whole rostro-caudal extent of the ECx, were used for calculation of the ECx volume (v). Tracings of the ECx boundaries were used for calculation of the surface area (a) of the ECx in each section. Volume of the ECx was calculated according to the Cavalieri principle (Cavalieri 1966) as $v = z \times i \times \Sigma a$, where z is the thickness of the section (80 μ m) and i is the section interval (13; i.e., number of serial sections between each section and the following one within a compartment). Total number of neurons was calculated as $Tn = i \times \Sigma n$, where Σn = sum of the neurons counted. In this case, only i and not $i \times z$ was used, because PVB-IR neurons were counted through the thickness of each section (z, 40 μ m). Numerical density of PVB-IR neurons over the entire ECx was calculated as $Nd = 3Tn/v$ and expressed a number of PVB-IR neurons/mm³. To calculate Nd of PVB-IR neurons over distinct ECx subregions and layers, the fraction of volume of each subregion (vol s) contained within the section examined (rostral, middle, or caudal) was calculated as $v = z \times a_s$, where a_s is the surface area of the subregion examined within a section. The Nd was again expressed as PVB-IR neurons/mm³ and calculated as $Nd = \Sigma n/v_s$ where Σn is the sum of the number of neurons vs, counted in a subregion. Layers 2 and 3 were grouped into superficial layers, and Layers 4, 5, and 6 into deep layers for comparisons. Because the data were not normally distributed, a logarithmic transformation was applied to all original values.

Volume of the ECx and Tn, Nd, and somata size of PVB-IR neurons were considered for analysis with a stepwise linear regression process. An initial baseline model that included diagnostic group as the main comparison and age, gender, interaction of age and gender, hemisphere, and interaction of gender and hemisphere as covariates was adopted in consideration of following data. In the cohort used for this study, women were older than men ($p = .03$), and the left hemisphere was more frequently represented in men than in women. The left ECx was larger than the right ($p = .04$). Differences in size between men and women and the interaction of hemisphere and gender on the volume of ECx were not significant. The PVB-IR neurons Tn and Nd were significantly higher in the left hemisphere ($p = .01$ and $p = .03$, respectively) and in men ($p = .03$ and $p = .04$, respectively).

The effects of postmortem time interval (PMI; hours), brain weight, age at illness onset, years of illness, cause of death, and lifetime as well as final 6 months' (Table 3) exposure to antipsychotic drugs and lithium treatment were tested systematically within the framework of the baseline stepwise regression model just described for each of the outcome variables and included in the model if they significantly improved the model goodness-of-fit. Cause of death was categorized as acute (e.g., myocardial infarction) or chronic (e.g., cancer). In addition to drug exposure levels, we evaluated the potential effects of treatment-adherence to pharmacological treatment and exposure to drugs other than antipsychotic or lithium (Table 2) using analysis of variance in a separate analysis. The PVB-IR soma size was considered both as an outcome measure and as covariate because of its potential to affect Tn and Nd estimates (Guillery and Herrup 1997). The possible confounding effect of cell size on Tn were also tested by applying the same statistical analyses to Abercrombie corrected values

(Abercrombie corrected $T_n = T_n \times (z/cs \times z)$; where cs 3 average cell size measured) (Abercrombie 1946).

Statistical analyses were performed with JMP v5.0.1a (SAS Institute, Cary, North Carolina).

Results

ECx Volume

The volume (mean $\pm$ SD) of the ECx was not found to be altered in BD ($315.59 \pm 82.7 \text{ mm}^3$) or SZ ($318.35 \pm 148.9 \text{ mm}^3$), in comparison with normal control subjects ($304.04 \pm 134.4 \text{ mm}^3$) (Figure 3). The values measured in our study are consistent with those reported by other research groups (Bussiere et al 2002; Krimer et al 1997). Brain weight was found to be a significant covariate ($p = .01$) and was included in the baseline regression model. None of the other covariates were found to have significant effects.

PVB-I R Neurons

Effect of Confounding Variables—Differences between hemispheres (left > right) were retained in diagnostic groups, although they were not always significant. None of the covariates tested within the baseline stepwise regression model (i.e., PMI, brain weight, age of onset, cause of death, years of illness, and lifetime as well as last 6 months' antipsychotic and lithium exposure) had significant effects on T_n and N_d of PVB-IR neurons in the entire ECx and N_d of PVB-IR neurons in the ECx subregions considered. Furthermore, correlations between CPZ-eq dose (within the last 6 months or throughout the illness) and the main outcome variables were not significant. Similarly, no significant correlations were detected between lithium treatment (within the last 6 months or throughout the illness) and the main outcome variables. Therefore, the effect of diagnostic group on T_n and N_d of PVB-IR neurons in the entire ECx and N_d of PVB-IR neurons in each of the ECx subregions considered was tested with the baseline model only.

In contrast to T_n and N_d , somata size was affected significantly by lifetime grams of antipsychotic drug exposure. For instance, within the baseline linear model used for this analysis, the effect of CPZ-eq dose on PVB-IR neuron size in the entire ECx was significant ($p = .01$). This covariate was included in the final model testing the effect of diagnostic group on somata size.

Soma Size of PVB-IR Neurons—The BD subjects showed no significant changes of PVB-IR soma size in the entire ECx or in any of its subdivisions. In SZ subjects, somata size was not altered significantly when considered within the entire ECx ($p = .1$), but it was significantly smaller in the ECx-I ($p = .009$). No changes were detected in the other subdivisions (Figure 4). As previously noted, lifetime grams of CPZ-eq antipsychotic exposure was included in the model because it showed a significant effect on cell size. This variable was positively and significantly correlated with cell size throughout the ECx ($p = .001$) as well as in several of the ECx subdivisions (ECx-R superficial layers, $p = .05$; ECx-R deep layers, $p = .03$; ECx-L deep layers, $p = .01$; ECx-I superficial layers, $p = .01$; ECx-C superficial layers, $p = .03$; ECx-C deep layers, $p = .03$).

T_n of PVB-IR Neurons Within the Entire ECx—The BD subjects showed a significant reduction of total numbers of PVB-IR neurons ($p = .02$). In contrast, no significant change was detected in the SZ group (Figure 5). Despite a lack of correlation of this measure with pharmacological treatment, the mean ($\pm$ SEM) T_n of PVB-IR neurons ranked subjects with good adherence ($5.71 \pm .4$) > subjects with poor adherence ($5.09 \pm .4$) > non-treated patients

(one SZ and three BD; $3.93 \pm .5$), suggesting a subtle effect of drug exposure (analysis of variance; $p < .05$).

Given the cell size decrease detected in SZ, Abercrombie correction for cell size was applied. The effect of cell size was also tested by including it as covariate in the stepwise model. Both of these strategies showed that results relative to Tn were not affected by cell size.

Nd of PVB-IR Neurons Within the Entire ECx—In BD, PVB-IR neuron Nd is significantly reduced ($p = .01$) (Figure 5). Although the SZ group also seemed to show a reduction (Figure 5), the difference was not significant when tested for the effect of group only and became even less so when the other covariates included in the baseline model were considered ($p = .9$).

Nd of PVB-IR Neurons Within ECx Subdivisions and Layers—In BD, significant decreases of PVB-IR neuron Nd were detected in the superficial layers of the ECx-L ($p = .02$), ECx-I ($p = .04$), and ECx-C ($p = .01$) (Figures 6 and 7; Table 4). Again, the SZ group did not show significant changes in Nd of PVB-IR neurons. Although most SZ subjects showed low PVB-IR neuron Nd in the superficial layers of ECx-L, three subjects exhibited high values (Figure 6). Review of the medical records did not provide distinguishing features that might define them as a distinct subpopulation. The standard confounding variables tested in this analysis, pharmacological treatment in particular, as well as other factors such as nicotine exposure and electroshock treatment did not seem likely to explain the segregation of these three subjects. Notably, the exclusion of these three subjects did not change the outcome of the linear model because of the strong effect of age and gender.

Discussion

These results indicate that the Tn and Nd of PVB-IR neurons are decreased in the ECx of BD patients. To our knowledge, this is the first evidence for a disruption of inhibitory intrinsic PVB-IR neurons in the ECx of BD subjects. No significant changes were detected in SZ subjects, with the exception of a cell size decrease restricted to the ECx-I.

Experimental Design Considerations

With this study, standard stereological methods were used for estimating the volume of the ECx. For the purpose of assessing Tn and Nd of PVB-IR neurons within the entire ECx, this method was adapted to the practical requirements of immunocytochemical procedures and distribution patterns of the neurons to be counted (Benes and Lange 2001; Guillery and Herrup 1997). Sections were selected using a systematic random sampling scheme and the whole ECx was sampled. The PVB-IR neurons were counted exhaustively within each ECx section. These neurons are sparsely represented and unevenly distributed. Exhaustive counts throughout each section increase the sensitivity in the x-y plane, thus reducing the risk of underestimating poorly represented neuronal populations (Benes and Lange 2001). Furthermore, relatively thin sections (40 μm) were used to maximize the antibody penetration. These sections were found to collapse to a final estimated thickness of 5–6 μm . The ratio between the thickness of the optical disector and that of neurons to be counted is not appropriate for the optical disector method, whereas nucleoli or even nuclei are not detectable in PVB immunostained sections (see Figure 2). Thicker sections could not be used because antibody penetration would be insufficient. The potential bias in estimating the “true” cell numbers related to the “split cell error” would introduce a difference between diagnostic groups only if there were a difference of PVB-IR neuron size (Guillery and Herrup 1997). Our results rule out this difference in BD. In SZ, correction for cell size did not alter results relative to Tn and Nd.

Decreases of PVB-IR neurons could result from several, non-reciprocally exclusive factors, such as downregulation of protein expression, developmental abnormalities, or actual cell death. Temporary changes in expression of calcium-binding proteins have been reported in experimental animal models (e.g., Johansen et al 1990; Magloczky and Freund 1995). Given the pivotal role that PVB plays in regulating several Ca^{++} -dependent aspects of neuronal physiology, it is reasonable to posit that its downregulation would result in profound functional abnormalities of the neurons expressing it as well as of the neural networks in which these neurons are embedded (Caillard et al 2000; Chard et al 1993; Lee et al 2000; Schwaller et al 2002; Vreugdenhil et al 2003). Developmental abnormalities affecting the ECx (Arnold et al 1991; Falkai et al 2000; Jakob and Beckmann 1986, 1994; Kovalenko et al 2003; Longson et al 1996) might contribute to lower PVB-IR neuron Tn and Nd. The late postnatal maturation of PVB-IR neurons in several brain regions supports this hypothesis (Berdel and Morys 2000; Lopez-Tellez et al 2004; Reynolds and Beasley 2001). Notably, the human ECx follows a protracted postnatal development, and ECx PVB-IR neurons are not detectable until the 5th month after birth (Grateron et al 2002, 2003). Finally, the possibility that cell death might be a contributing factor cannot be excluded. Existing evidence pointing to neuronal loss in BD (Manaye et al 2005; Rajkowska 2002) is consistent with this possibility. Regardless of its causes, a decrease of PVB-IR somata might be reasonably interpreted as reflecting altered functioning of this particular interneuronal population (Eyles et al 2002). Thus, these findings will be, from here on, referred to as a “decrease” of PVB-IR neurons to denote a decrease of function and/or number of cells.

Reduction of PVB-IR Soma Size in SZ Subjects

In SZ, decreases of PVB-IR neurons have been reported in several brain regions, (Beasley et al 2002; Danos et al 1998; Lewis et al 2001; Reynolds et al 2002; Zhang and Reynolds 2002). The present results suggest that changes of PVB-IR neurons in the ECx of SZ might be subtle and restricted to the ECx-I. Reduction of cell size could be explained by an overall shrinkage of PVB-IR neurons or by selective loss of the larger IR neurons. At the moment, we cannot discern between these two possibilities. Significant positive correlations of cell size with CPZ-eq exposure, detected in several ECx subregions, might suggest a corrective effect of antipsychotic treatment. This possibility is consistent with a postulated neuroprotective effect of antipsychotic drugs. However, such effect was not detected in other studies in which neuronal size was found to be reduced in SZ subjects (Arnold et al 1995; Pierri et al 2001; Sweet et al 2004).

The discrepancy between findings in BD and SZ might point at intriguing differences between these two diseases. We speculate that overlapping genetic vulnerabilities, possibly affecting PVB expression, might result in divergent phenotypes, depending on the timing, duration, and nature of concurrent developmental factors impacting on similar neural circuits as well as on other gene clusters concurrently affected.

Decreases of PVB-IR Neurons in the ECx of BD Subjects

In BD, decreases of PVB-IR neurons were found to be significant in the superficial layers of the ECx-L, ECx-I, and ECx-C. The ECx superficial layers receive most of the cortical inputs directed to the hippocampus, whereas deeper layers are the target of the majority of hippocampal/subicular inputs (Burwell and Amaral 1998; Kohler 1985; Suzuki and Amaral 1994; Witter et al 2000). The superficial layers of the ECx-L and ECx-I but not the deeper layers or the medial ECx have also been found to receive the bulk of the inputs from the amygdala (Saunders and Rosene 1988), further emphasizing differences in connectivity within ECx subdivisions. These connections suggest a predominant role for the superficial layers of the ECx in the integration and transfer of highly processed sensory information to the hippocampus (Lavenex and Amaral 2000; Witter et al 2000). The present results suggest that the ECx “input”

station, which mediates the flow of information to the hippocampal formation, is altered in BD as a consequence of decreases of PVB-IR neurons in the superficial layers of the ECx.

Parvalbumin-immunoreactive neurons correspond to fast spiking inhibitory neurons (Jones and Buhl 1993) and form basket- or cartridge-like clusters of synaptic terminals around the somata and proximal axonal segments of projection neurons (Mikkonen et al 1997; Wouterlood et al 1995). These features enable them to exert a powerful control over the activity of output neurons. Furthermore, their dendritic arborization spans across ECx layers (Mikkonen et al 1997), suggesting that these neurons might be involved in the integration of input and output streams of information processed within the ECx. Such integration has been suggested to mediate functions such as memory processing and novelty detection (Witter et al 2000). Together, these considerations suggest that a decrease of PVB-IR neurons might result in altered information processing within ECx intrinsic networks and disrupted outflow of information to the hippocampal formation.

Finally, it is important to consider that the cingulate cortex (area 25) and the orbito-frontal cortex send heavy projections primarily to the superficial layers of the ECx-L and have been implicated in BD (Bakchine et al 1989; Benes et al 2001; Blumberg et al 1999; Cotter et al 2005; Drevets 1999; Eastwood and Harrison 2001; Starkstein et al 1991). We suggest that altered outflow of emotional information from these cortical regions might be further distorted by abnormal activity in this region. These considerations are consistent with the hypothesis that the pathophysiology of BD might emerge from interactions of abnormalities affecting interconnected networks of cortico-limbic regions, including several prefrontal cortex regions, the amygdala, ECx, and hippocampal formation (Benes and Berretta 2001; Blumberg et al 2002; Phillips et al 2003; Rajkowska 2002). It follows that changes related to PVB-IR neurons in the ECx are not in themselves likely to account for the full clinical manifestation of this disease. The spread of values for Tn and Nd of these neurons in normal control subjects supports this idea. As part of pathological changes affecting cortico-limbic networks, a disruption of ECx intrinsic networks and outflow of information to the hippocampal formation might contribute to particular clinical features attributed to BD, including impairment of sensory gating, visuo-spatial processing, visually dependent sustained attention, and verbal memory (Baker et al 1990; Chang et al 2004; Clark et al 2005; El-Badri et al 2001; Kieseppa et al 2005; Olincy and Martin 2005; Saccuzzo and Braff 1986).

Acknowledgements

We thank the Harvard Brain Tissue Resource Center, funded by NIH R24MH068855 and directed by Francine M. Benes, M.D., Ph.D., for providing the tissue used in these investigations.

References

- Abercrombie M. Estimation of nuclear population from microtome sections. *Anat Rec* 1946;94:239 – 247.
- Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr, Jones EG. Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch Gen Psychiatry* 1995;52:258 –266. [PubMed: 7702443]
- Akil M, Lewis DA. Cytoarchitecture of the entorhinal cortex in schizophrenia. *Am J Psychiatry* 1997;154:1010 –1012. [PubMed: 9210754]
- Altshuler LL, Bartzokis G, Grieder T, Curran J, Mintz J. Amygdala enlargement in bipolar disorder and hippocampal reduction in schizophrenia: an MRI study demonstrating neuroanatomic specificity. *Arch Gen Psychiatry* 1998;55:663– 664. [PubMed: 9672058]
- Arnold SE. The medial temporal lobe in schizophrenia. *J Neuropsychiatry Clin Neurosci* 1997;9:460 – 470. [PubMed: 9276847]

- Arnold SE, Franz BR, Gur RC, Gur RE, Shapiro RM, Moberg PJ, Trojanowski JQ. Smaller neuron size in schizophrenia in hippocampal subfields that mediate cortical-hippocampal interactions. *Am J Psychiatry* 1995;152:738–748. [PubMed: 7726314]
- Arnold SE, Hyman BT, Van Hoesen GW, Damasio AR. Some cytoarchitectural abnormalities of the entorhinal cortex in schizophrenia. *Arch Gen Psychiatry* 1991;48:625–632. [PubMed: 2069493]
- Bachus SE, Hyde TM, Herman MM, Egan MF, Kleinman JE. Abnormal cholecystokinin mRNA levels in entorhinal cortex of schizophrenics. *J Psychiatr Res* 1997;31:233–256. [PubMed: 9278188]
- Bakchine S, Lacomblez L, Benoit N, Parisot D, Chain F, Lhermitte F. Manic-like state after bilateral orbitofrontal and right temporoparietal injury: Efficacy of clonidine. *Neurology* 1989;39:777–781. [PubMed: 2725871]
- Baker NJ, Staunton M, Adler LE, Gerhardt GA, Drebing C, Waldo M, et al. Sensory gating deficits in psychiatric inpatients: relation to catecholamine metabolites in different diagnostic groups. *Biol Psychiatry* 1990;27:519–528. [PubMed: 2310807]
- Baldessarini, RJ.; Tarazi, FI. Pharmacotherapy of psychosis and mania. In: Brunton, LL.; Lazo, JS.; Parker, KL., editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11. New York: McGraw-Hill Press; 1995. p. 461-500.
- Bartha R, Williamson PC, Drost DJ, Malla A, Carr TJ, Cortese L, et al. Measurement of glutamate and glutamine in the medial prefrontal cortex of never-treated schizophrenic patients and healthy controls by proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 1997;54:959–965. [PubMed: 9337777]
- Beasley C, Zhang Z, Patten I, Reynolds G. Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biol Psychiatry* 2002;52:708. [PubMed: 12372661]
- Beasley CL, Reynolds GP. Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophr Res* 1997;24:349–355. [PubMed: 9134596]
- Benes FM. Altered glutamatergic and GABAergic mechanisms in the cingulate cortex of the schizophrenic brain. *Arch Gen Psychiatry* 1995;52:1015–1018. [PubMed: 7492253]
- Benes FM, Berretta S. Amygdalo-Entorhinal Inputs to the Hippocampal Formation in Relation to Schizophrenia. *Ann N Y Acad Sci* 2000;911:293–304. [PubMed: 10911881]
- Benes FM, Berretta S. Gabaergic interneurons. Implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* 2001;25:1–27. [PubMed: 11377916]
- Benes FM, Kwok EW, Vincent SL, Todtenkopf MS. A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressive. *Biol Psychiatry* 1998;44:88–97. [PubMed: 9646890]
- Benes FM, Lange N. Two-dimensional versus three-dimensional cell counting: A practical perspective. *Trends Neurosci* 2001;24:11–17. [PubMed: 11163882]
- Benes FM, McSparren J, Bird ED, SanGiovanni JP, Vincent SL. Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. *Arch Gen Psychiatry* 1991;48:996–1001. [PubMed: 1747023]
- Benes FM, Todtenkopf MS, Logiotatos P, Williams M. Glutamate decarboxylase(65)-immunoreactive terminals in cingulate and prefrontal cortices of schizophrenic and bipolar brain. *J Chem Neuroanat* 2000;20:259–269. [PubMed: 11207424]
- Benes FM, Vincent SL, Alsterberg G, Bird ED, SanGiovanni JP. Increased GABAA receptor binding in superficial layers of cingulate cortex in schizophrenics. *J Neurosci* 1992;12:924–929. [PubMed: 1372045]
- Benes FM, Vincent SL, Marie A, Khan Y. Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. *Neuroscience* 1996;75:1021–1031. [PubMed: 8938738]
- Benes FM, Vincent SL, Todtenkopf M. The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. *Biol Psychiatry* 2001;50:395–406. [PubMed: 11566156]
- Benes FM, Wickramasinghe R, Vincent SL, Khan Y, Todtenkopf M. Uncoupling of GABA-A and benzodiazepine receptor binding activity in the hippocampal formation of schizophrenic brain. *Brain Res* 1997;755:121–129. [PubMed: 9163547]
- Berdel B, Morys J. Expression of calbindin-D28k and parvalbumin during development of rat's basolateral amygdaloid complex. *Int J Dev Neurosci* 2000;18:501–513. [PubMed: 10884595]

- Blumberg HP, Charney DS, Krystal JH. Frontotemporal neural systems in bipolar disorder. *Semin Clin Neuropsychiatry* 2002;7:243–254. [PubMed: 12382207]
- Blumberg HP, Kaufman J, Martin A, Whiteman R, Zhang JH, Gore JC, et al. Amygdala and hippocampal volumes in adolescents and adults with bipolar disorder. *Arch Gen Psychiatry* 2003;60:1201–1208. [PubMed: 14662552]
- Blumberg HP, Stern E, Ricketts S, Martinez D, de Asis J, White T, et al. Rostral and orbital prefrontal cortex dysfunction in the manic state of bipolar disorder. *Am J Psychiatry* 1999;156:1986–1988. [PubMed: 10588416]
- Bogerts B. Neuropathology of schizophrenias. *Fortschr Neurol Psychiatr* 1984;52:428–437. [PubMed: 6519627]
- Bogerts B, Meertz E, Schonfeldt-Bausch R. Basal ganglia and limbic system pathology in schizophrenia. A morphometric study of brain volume and shrinkage. *Arch Gen Psychiatry* 1985;42:784–791. [PubMed: 4015323]
- Broadbelt K, Byne W, Jones LB. Evidence for a decrease in basilar dendrites of pyramidal cells in schizophrenic medial prefrontal cortex. *Schizophr Res* 2002;58:75–81. [PubMed: 12363393]
- Burwell RD, Amaral DG. Perirhinal and postrhinal cortices of the rat: Interconnectivity and connections with the entorhinal cortex. *J Comp Neurol* 1998;391:293–321. [PubMed: 9492202]
- Bussiere T, Friend PD, Sadeghi N, Wicinski B, Lin GI, Bouras C, et al. Stereologic assessment of the total cortical volume occupied by amyloid deposits and its relationship with cognitive status in aging and Alzheimer's disease. *Neuroscience* 2002;112:75–91. [PubMed: 12044473]
- Caillard O, Moreno H, Schwaller B, Llano I, Celio MR, Marty A. Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. *Proc Natl Acad Sci U S A* 2000;97:13372–13377. [PubMed: 11069288]
- Cavalieri, B. *Geometria degli Indivisibili*. Unione Tipografico-Editrice Torinese; 1966. [original work —*Geometria Indivisibilium Continuorum*. Torino: Bononi, Typis Clementis Feronji—published 1635]
- Chang K, Adelman NE, Dienes K, Simeonova DI, Menon V, Reiss A. Anomalous prefrontal-subcortical activation in familial pediatric bipolar disorder: a functional magnetic resonance imaging investigation. *Arch Gen Psychiatry* 2004;61:781–792. [PubMed: 15289277]
- Chard PS, Bleakman D, Christakos S, Fullmer CS, Miller RJ. Calcium buffering properties of calbindin D28k and parvalbumin in rat sensory neurones. *J Physiol* 1993;472:341–357. [PubMed: 8145149]
- Cirillo MA, Seidman LJ. Verbal declarative memory dysfunction in schizophrenia: from clinical assessment to genetics and brain mechanisms. *Neuropsychol Rev* 2003;13:43–77. [PubMed: 12887039]
- Clark L, Kempton MJ, Scarna A, Grasby PM, Goodwin GM. Sustained attention-deficit confirmed in euthymic bipolar disorder but not in first-degree relatives of bipolar patients or euthymic unipolar depression. *Biol Psychiatry* 2005;57:183–187. [PubMed: 15652878]
- Coggeshall RE, Lekan HA. Methods for determining number of cells and synapses: A case for more uniform standard of review. *J Comp Neurol* 1996;364:6–15. [PubMed: 8789272]
- Cotter D, Hudson L, Landau S. Evidence for orbitofrontal pathology in bipolar disorder and major depression, but not in schizophrenia. *Bipolar Disord* 2005;7:358–369. [PubMed: 16026489]
- Danos P, Baumann B, Bernstein HG, Franz M, Stauch R, Northoff G, et al. Schizophrenia and anteroventral thalamic nucleus: Selective decrease of parvalbumin-immunoreactive thalamocortical projection neurons. *Psychiatry Res* 1998;82:1–10. [PubMed: 9645546]
- de Curtis M, Pare D. The rhinal cortices: A wall of inhibition between the neocortex and the hippocampus. *Prog Neurobiol* 2004;74:101–110. [PubMed: 15518955]
- Drevets WC. Prefrontal cortical-amygdalar metabolism in major depression. *Ann N Y Acad Sci* 1999;877:614–637. [PubMed: 10415674]
- Eastwood SL, Burnet PW, Harrison PJ. Altered synaptophysin expression as a marker of synaptic pathology in schizophrenia. *Neuroscience* 1995;66:309–319. [PubMed: 7477874]
- Eastwood SL, Harrison PJ. Synaptic pathology in the anterior cingulate cortex in schizophrenia and mood disorders. A review and a Western blot study of synaptophysin, GAP-43 and the complexins. *Brain Res Bull* 2001;55:569–578. [PubMed: 11576753]
- Eastwood SL, Kerwin RW, Harrison PJ. Immunohistochemical evidence for a loss of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate-preferring on-N-methyl-D-aspartate glutamate receptors

- within the medial temporal lobe in schizophrenia. *Biol Psychiatry* 1997;41:636 – 643. [PubMed: 9066986]
- El-Badri SM, Ashton CH, Moore PB, Marsh VR, Ferrier IN. Electrophysiological and cognitive function in young euthymic patients with bipolar affective disorder. *Bipolar Disord* 2001;3:79 – 87. [PubMed: 11333067]
- Eyles DW, McGrath JJ, Reynolds GP. Neuronal calcium-binding proteins and schizophrenia. *Schizophr Res* 2002;57:27–34. [PubMed: 12165373]
- Falkai P, Bogerts B, Rozumek M. Limbic pathology in schizophrenia: The entorhinal region- a morphometric study. *Biol Psychiatry* 1988;24:515–521. [PubMed: 3167140]
- Falkai P, Schneider-Axmann T, Honer WG. Entorhinal cortex pre-alpha cell clusters in schizophrenia: Quantitative evidence of a developmental abnormality. *Biol Psychiatry* 2000;47:937–943. [PubMed: 10838061]
- Feighner JP, Robins E, Guze SB. Diagnostic criteria for use in psychiatric research. *Arch Gen Psychiatry* 1972;26:57– 63. [PubMed: 5009428]
- Goldsmith SK, Shapiro RM, Joyce JN. Disrupted pattern of D2 dopamine receptors in the temporal lobe in schizophrenia. A postmortem study. *Arch Gen Psychiatry* 1997;54:649 – 658. [PubMed: 9236549]
- Grateron L, Cebada-Sanchez S, Marcos P, Mohedano-Moriano A, Insausti AM, Munoz M, et al. Postnatal development of calcium-binding proteins immunoreactivity (parvalbumin, calbindin, calretinin) in the human entorhinal cortex. *J Chem Neuroanat* 2003;26:311–316. [PubMed: 14729133]
- Grateron, L.; Insausti, AM.; Garci-Bragado, F., et al. Postnatal development of the human entorhinal cortex. In: Witter, MP.; Wouterlood, FG., editors. *The Parahippocampal Region Organization and role in cognitive function*. New York: Oxford University Press; 2002.
- Guidotti A, Pesold C, Costa E. Newneurochemical markers for psychosis: A working hypothesis of their operation . *Neuro-chem Res* 2000;25:1207–1218. In Process Citation
- Guillery RW, Herrup K. Quantification without pontification: Choosing a method for counting objects in sectioned tissues. *J Comp Neurol* 1997;386:2–7. [PubMed: 9303520]
- Heckers S, Rauch SL, Goff D, Savage CR, Schacter DL, Fischman AJ, Alpert NM. Impaired recruitment of the hippocampus during conscious recollection in schizophrenia. *Nature Neuroscience* 1998;1:318 –323.
- Heckers S, Weiss AP, Alpert NM, Schacter DL. Hippocampal and brain stem activation during word retrieval after repeated and semantic encoding. *Cereb Cortex* 2002;12:900 –907. [PubMed: 12183389]
- Hemby SE, Ginsberg SD, Brunk B, Arnold SE, Trojanowski JQ, Eberwine JH. Gene expression profile for schizophrenia: discrete neuron transcription patterns in the entorhinal cortex. *Arch Gen Psychiatry* 2002;59:631–640. [PubMed: 12090816]
- Insausti R, Amaral DG, Cowan WM. The entorhinal cortex of the monkey: II. Cortical afferents. *J Comp Neurol* 1987a;264:356 –395. [PubMed: 2445796]
- Insausti R, Amaral DG, Cowan WM. The entorhinal cortex of the monkey: III. Subcortical afferents. *J Comp Neurol* 1987b;264:396 – 408. [PubMed: 3680636]
- Insausti R, Tunon T, Sobreviela T, Insausti AM, Gonzalo LM. The human entorhinal cortex: A cytoarchitectonic analysis. *J Comp Neurol* 1995;355:171–198. [PubMed: 7541808]
- Ishikawa M, Mizukami K, Iwakiri M, Asada T. Immunohistochemical and immunoblot analysis of gamma-aminobutyric acid B receptor in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Neurosci Lett* 2005;383:272–277. [PubMed: 15955420]
- Ishikawa M, Mizukami K, Iwakiri M, Hidaka S, Asada T. GABAA receptor gamma subunits in the prefrontal cortex of patients with schizophrenia and bipolar disorder. *Neuroreport* 2004a;15:1809 – 1812. [PubMed: 15257153]
- Ishikawa M, Mizukami K, Iwakiri M, Hidaka S, Asada T. Immunohistochemical and immunoblot study of GABA(A) alpha1 and beta2/3 subunits in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Neurosci Res* 2004b;50:77– 84. [PubMed: 15288501]
- Jakob H, Beckmann H. Prenatal developmental disturbances in the limbic allocortex in schizophrenics. *J Neural Transm* 1986;65:303–326. [PubMed: 3711886]

- Jakob H, Beckmann H. Circumscribed malformation and nerve cell alterations in the entorhinal cortex of schizophrenics. Pathogenetic and clinical aspects. *J Neural Transm Gen Sect* 1994;98:83–106. [PubMed: 7734114]
- Johansen FF, N Tn, Zimmer J, Baimbridge KG, Diemer NH. Short-term changes of parvalbumin and calbindin immunoreactivity in the rat hippocampus following cerebral ischemia. *Neurosci Lett* 1990;120:171–174. [PubMed: 2293104]
- Jones RSG, Buhl EH. Basket-like interneurons in layer II of the entorhinal cortex exhibit powerful NMDA-mediated synaptic excitation. *Neurosci Lett* 1993;149:35–39. [PubMed: 8469376]
- Joyal CC, Laakso MP, Tiihonen J, et al. A volumetric MRI study of the entorhinal cortex in first episode neuroleptic-naïve schizophrenia. *Biol Psychiatry* 2002;51:1005–1007. [PubMed: 12062885]
- Kieseppa T, Tuulio-Henriksson A, Haukka J, Van Erp T, Glahn D, Cannon TD, et al. Memory and verbal learning functions in twins with bipolar-I disorder, and the role of information-processing speed. *Psychol Med* 2005;35:205–215. [PubMed: 15841678]
- Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF. Molecular abnormalities of the hippocampus in severe psychiatric illness: Postmortem findings from the Stanley Neuropathology Consortium. *Mol Psychiatry* 2004;9(544):609 – 620. [PubMed: 14708030]
- Kohler C. Intrinsic projections of the retrohippocampal region in the rat brain. I. The subicular complex. *J Comp Neurol* 1985;236:504 –522. [PubMed: 3902916]
- Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry* 2004;61:300 –308. [PubMed: 14993118]
- Kovalenko S, Bergmann A, Schneider-Axmann T, Ovary I, Majtenyi K, Havas L, et al. Regio entorhinalis in schizophrenia: More evidence for migrational disturbances and suggestions for a new biological hypothesis. *Pharmacopsychiatry* 2003;36(suppl 3):S158 –S161. [PubMed: 14677073]
- Krimer LS, Herman MM, Saunders RC, Boyd JC, Hyde TM, Carter JM, et al. A qualitative and quantitative analysis of the entorhinal cortex in schizophrenia. *Cereb Cortex* 1997;7:732–739. [PubMed: 9408037]
- Kurachi M. Pathogenesis of schizophrenia: Part I. Symptomatology, cognitive characteristics and brain morphology. *Psychiatry Clin Neurosci* 2003;57:3– 8. [PubMed: 12519448]
- Lavenex P, Amaral DG. Hippocampal-neocortical interaction: A hierarchy of associativity. *Hippocampus* 2000;10:420 – 430. [PubMed: 10985281]
- Law AJ, Weickert CS, Hyde TM, Kleinman JE, Harrison PJ. Reduced spinophilin but not microtubule-associated protein 2 expression in the hippocampal formation in schizophrenia and mood disorders: Molecular evidence for a pathology of dendritic spines. *Am J Psychiatry* 2004;161:1848–1855. [PubMed: 15465982]
- Lee SH, Rosenmund C, Schwaller B, Neher E. Differences in Ca²⁺ buffering properties between excitatory and inhibitory hippocampal neurons from the rat. *J Physiol* 2000;525:405– 418. [PubMed: 10835043]
- Lewis DA, Cruz DA, Melchitzky DS, Pierri JN. Lamina-specific deficits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: Evidence for fewer projections from the thalamus. *Am J Psychiatry* 2001;158:1411–1422. [PubMed: 11532725]
- Longson D, Deakin JF, Benes FM. Increased density of entorhinal glutamate-immunoreactive vertical fibers in schizophrenia. *J Neural Transm* 1996;103:503–507. [PubMed: 9617791]
- Lopez-Tellez JF, Vela J, del Rio JC, Ramos B, Baglietto-Vargas D, et al. Postnatal development of the alpha1 containing GABAA receptor subunit in rat hippocampus. *Brain Res Dev Brain Res* 2004;148:129 –141.
- Magloczky Z, Freund TF. Delayed cell death in the contralateral hippocampus following kainate injection into the CA3 subfield. *Neuroscience* 1995;66:847– 860. [PubMed: 7651613]
- Manaye KF, Lei DL, Tizabi Y, Davila-Garcia MI, Mouton PR, Kelly PH. Selective neuron loss in the paraventricular nucleus of hypothalamus in patients suffering from major depression and bipolar disorder. *J Neuropathol Exp Neurol* 2005;64:224 –249. [PubMed: 15804054]
- McDonald B, Highley JR, Walker MA, Herron BM, Cooper SJ, Esiri MM, Crow TJ. Anomalous asymmetry of fusiform and parahippocampal gyrus gray matter in schizophrenia: A postmortem study. *Am J Psychiatry* 2000;157:40 – 47. [PubMed: 10618011]

- Miettinen M, Koivisto E, Riekkinen P, Miettinen R. Coexistence of parvalbumin and GABA in nonpyramidal neurons of the rat entorhinal cortex. *Brain Res* 1996;706:113–122. [PubMed: 8720498]
- Mikkonen M, Soininen H, Pitkanen A. Distribution of parvalbumin-, calretinin-, and calbindin-D28k-immunoreactive neurons and fibers in the human entorhinal cortex. *J Comp Neurol* 1997;388:64 – 88. [PubMed: 9364239]
- Mizukami K, Ishikawa M, Hidaka S, Iwakiri M, Sasaki M, Iritani S. Immunohistochemical localization of GABAB receptor in the entorhinal cortex and inferior temporal cortex of schizophrenic brain. *Prog Neuropsychopharmacol Biol Psychiatry* 2002;26:393–396. [PubMed: 11817519]
- Olinicy A, Martin L. Diminished suppression of the P50 auditory evoked potential in bipolar disorder subjects with a history of psychosis. *Am J Psychiatry* 2005;162:43– 49. [PubMed: 15625200]
- Pantazopoulos H, Stone D, Walsh J, Benes FM. Differences in the cellular distribution of D1 receptor mRNA in the hippocampus of bipolars and schizophrenics. *Synapse* 2004;54:147–155. [PubMed: 15452863]
- Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biol Psychiatry* 2003;54:515–528. [PubMed: 12946880]
- Pierri JN, Volk CL, Auh S, Sampson A, Lewis DA. Decreased somal size of deep layer 3 pyramidal neurons in the prefrontal cortex of subjects with schizophrenia. *Arch Gen Psychiatry* 2001;58:466 – 473. [PubMed: 11343526]
- Rajkowska G. Cell pathology in bipolar disorder. *Bipolar Disord* 2002;4:105–116. [PubMed: 12071508]
- Rajkowska G, Halaris A, Selemon LD. Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol Psychiatry* 2001;49:741–752. [PubMed: 11331082]
- Reynolds GP, Abdul-Monim Z, Neill JC, Zhang ZJ. Calcium binding protein markers of GABA deficits in schizophrenia–postmortem studies and animal models. *Neurotox Res* 2004;6:57– 61. [PubMed: 15184106]
- Reynolds GP, Beasley CL. GABAergic neuronal subtypes in the human frontal cortex - development and deficits in schizophrenia. *J Chem Neuroanat* 2001;22:95–100. [PubMed: 11470557]
- Reynolds GP, Beasley CL, Zhang ZJ. Understanding the neurotransmitter pathology of schizophrenia: selective deficits of subtypes of cortical GABAergic neurons. *J Neural Transm* 2002;109:881– 889. [PubMed: 12111475]
- Reynolds GP, Czudek C, Andrews HB. Deficit and hemispheric asymmetry of GABA uptake sites in the hippocampus in schizophrenia. *Biol Psychiatry* 1990;27:1038 –1044. [PubMed: 2159350]
- Saccuzzo DP, Braff DL. Information-processing abnormalities: Trait and state-dependent components. *Schizophr Bull* 1986;12:447– 459. [PubMed: 3764361]
- Saunders RC, Rosene DL. A comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: I. Convergence in the entorhinal, perirhinal, and entorhinal cortices. *J Comp Neurol* 1988;271:153–184. [PubMed: 2454246]
- Schwaller B, Meyer M, Schiffmann S. ‘New’ functions for ‘old’ proteins: the role of the calcium-binding proteins calbindin D-28k, calretinin and parvalbumin, in cerebellar physiology. *Studies with knockout mice. Cerebellum* 2002;1:241–258. [PubMed: 12879963]
- Sewards TV, Sewards MA. Input and output stations of the entorhinal cortex: superficial vs. deep layers or lateral vs. medial divisions? *Brain Res Brain Res Rev* 2003;42:243–251. [PubMed: 12791442]
- Shenton ME, Dickey CC, Frumin M, McCarley RW. A review of MRI findings in schizophrenia. *Schizophr Res* 2001;49:1–52. [PubMed: 11343862]
- Simpson MDC, Slater P, Slater JF, Deakin JFW, Royston MC, Skan WJ. Reduced GABA uptake sites in the temporal lobe in schizophrenia. *Neuroscience Lett* 1989;107:211–215.
- Soares JC. Contributions from brain imaging to the elucidation of pathophysiology of bipolar disorder. *Int J Neuropsychopharmacol* 2003;6:171–180. [PubMed: 12890310]
- Sorvari H, Soininen H, Paljarvi L, Karkola K, Pitkanen A. Distribution of parvalbumin-immunoreactive cells and fibers in the human amygdaloid complex. *J Comp Neurol* 1995;360:185–212. [PubMed: 8522643]
- Squire LR, Stark CE, Clark RE. The medial temporal lobe. *Annu Rev Neurosci* 2004;27:279 –306. [PubMed: 15217334]

- Starkstein SE, Fedoroff P, Berthier ML, Robinson RG. Manic-depressive and pure manic states after brain lesions. *Biol Psychiatry* 1991;29:149–158. [PubMed: 1995084]
- Strakowski SM, Delbello MP, Adler CM. The functional neuroanatomy of bipolar disorder: A review of neuroimaging findings. *Mol Psychiatry* 2005;10:105–116. [PubMed: 15340357]
- Suzuki WA, Amaral DG. Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. *J Neurosci* 1994;14:1856–1877. [PubMed: 8126576]
- Sweet RA, Bergen SE, Sun Z, Sampson AR, Pierri JN, Lewis DA. Pyramidal cell size reduction in schizophrenia: Evidence for involvement of auditory feedforward circuits. *Biol Psychiatry* 2004;55:1128–1137. [PubMed: 15184031]
- Thomas EA, Dean B, Scarr E, Copolov D, Sutcliffe JG. Differences in neuroanatomical sites of apoD elevation discriminate between schizophrenia and bipolar disorder. *Mol Psychiatry* 2003;8:167–175. [PubMed: 12610649]
- Todtenkopf MS, Benes FM. Distribution of glutamate decarboxylase 65 immunoreactive puncta on pyramidal and nonpyramidal neurons in hippocampus of schizophrenic brain. *Synapse* 1998;29:323–332. [PubMed: 9661250]
- Torrey EF. Studies of individuals with schizophrenia never treated with antipsychotic medications: A review. *Schizophr Res* 2002;58:101–115. [PubMed: 12409150]
- Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB. Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biol Psychiatry* 2005;57:252–260. [PubMed: 15691526]
- Tunon T, Insausti R, Ferrer I, Sobreviela T, Soriano E. Parvalbumin and calbindin D-28K in the human entorhinal cortex. An immunohistochemical study. *Brain Res* 1992;589:24–32. [PubMed: 1422819]
- Turetsky BI, Moberg PJ, Roalf DR, Arnold SE, Gur RE. Decrements in volume of anterior ventromedial temporal lobe and olfactory dysfunction in schizophrenia. *Arch Gen Psychiatry* 2003;60:1193–1200. [PubMed: 14662551]
- Vinogradova OS. Hippocampus as comparator: Role of the two input and two output systems of the hippocampus in selection and registration of information. *Hippocampus* 2001;11:578–598. [PubMed: 11732710]
- Vreugdenhil M, Jefferys JG, Celio MR, Schwaller B. Parvalbumin-deficiency facilitates repetitive IPSCs and gamma oscillations in the hippocampus. *J Neurophysiol* 2003;89:1414–1422. [PubMed: 12626620]
- Webster MJ, Knable MB, O'Grady J, Orthmann J, Weickert CS. Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. *Mol Psychiatry* 2002;7(924):985–994. [PubMed: 12399952]
- Wilke M, Kowatch RA, DelBello MP, Mills NP, Holland SK. Voxel-based morphometry in adolescents with bipolar disorder: First results. *Psychiatry Res* 2004;131:57–69. [PubMed: 15246455]
- Witter MP, Wouterlood FG, Naber PA, Van Haeften T. Anatomical organization of the parahippocampal-hippocampal network. *Ann N Y Acad Sci* 2000;911:1–24. [PubMed: 10911864]
- Wolf SS, Hyde TM, Saunders RC, Herman MM, Weinberger DR, Kleinman JE. Autoradiographic characterization of neurotensin receptors in the entorhinal cortex of schizophrenic patients and control subjects. *J Neural Transm Gen Sect* 1995;102:55–65. [PubMed: 8785024]
- Woo TU, Walsh JP, Benes FM. Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Arch Gen Psychiatry* 2004;61:649–657. [PubMed: 15237077]
- Woo TU, Whitehead RE, Melchitzky DS, Lewis DA. A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. *Proc Natl Acad Sci U S A* 1998;95:5341–5346. [PubMed: 9560277]
- Wouterlood F, Hartig W, Bruckner G, Witter M. Parvalbumin-immunoreactive neurons in the entorhinal cortex of the rat: Localization, morphology, connectivity and ultrastructure. *J Neurocytol* 1995;24:135–153. [PubMed: 7745443]

- Wouterlood, FG. Spotlight on the neurons (I): Cell types, local connectivity, microcircuits, and distribution of markers. In: Witter, M.; Wouterlood, F., editors. *The Parahippocampal Region: Organization and Role in Cognitive Function*. New York: Oxford University Press; 2002. p. 61-88.
- Zhang ZJ, Reynolds GP. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophr Res* 2002;55:1–10. [PubMed: 11955958]

Figure 1.

Diagrams representing typical rostro-caudal levels chosen for analysis of parvalbumin-immunoreactive (PVB-IR) somata numerical densities (Nd) within entorhinal cortex (ECx) main subdivisions and layers. The following specific criteria were used to choose distinct and equivalent rostro-caudal levels from each subject and to identify distinct ECx subregions. Within the most rostral section, the ECx-rostral (R) and the rostral portion of the lateral (ECx-L) were considered for analysis. Other landmark regions were the fully formed olfactory ECx (ECx-O) and the rostral basolateral amygdala (A). This section was rostral to hippocampal formation. The ECx-R is situated between the olfactory ECx and rostral portion of the ECx-L, within the rostral portion of the gyrus ambiens. In Nissl-stained sections, the ECx-R was clearly distinguishable from ECx-L because of its irregular distribution of small darkly stained cells in layer II. The rostral portion of the ECx-L is contained between the ECx-R and the perirhinal cortex (PRh). Within a rostro-caudal gradient, it is the first region to show rounded cell island clusters in layer II. The presence of these cell islands also distinguishes it from the adjacent perirhinal cortex. In the middle section, PVB-IR neurons were counted in the intermediate (ECx-I) and caudal portion of the ECx-L. The ECx-I subdivision is located between the medial intermediate ECx (ECx-MI) and the caudal portion of the ECx-L. In Nissl sections, it was clearly distinguishable from the ECx-L on the basis of its wart-like verrucae in layer I; its large, widely dispersed cell islands in layer II; and less densely populated layer III. In contrast, the caudal portion of the ECx-L consists of smaller and more elongated

layer II cell islands, a more densely packed layer III, and a thinner cellsparse layer IV. Other landmark regions were the caudal portion of the basolateral A and the most rostral tip of the hippocampus (HP). The third and most caudal section contained the caudal (ECx-C) and was located rostrally to the transitional zone that defines the distinct features of the caudal limiting subdivision of the ECx. The ECx-C subdivision has the highest numbers of PVB-IR neurons. Layer I of the ECx-C is wider than in other subdivisions, and layer II consists of well-separated, oval-shaped cell islands. The gyrus intralimbicus of the uncus, rostral part of the dentate gyrus (DG), and uncal notch (UN) were detectable in this section. cs, collateral sulcus; CA3 (IG), cornus ammonis, sector 3 (intralimbic gyrus); hf, hippocampal fissure; sub, subiculum.

Figure 2.

Photomicrographs showing parvalbumin-immunoreactive (PVB-IR) immunostaining in the lateral entorhinal cortex (ECx-L) from a normal control subject. **(A)** Six cortical layers were identified, using a $4\times$ objective, on the basis of the distribution of PVB-IR immunoreactivity according to Mikkonen et al (1997) and confirmed on adjacent Nisslstained sections according to the criteria of Insausti et al (1995) (see also Figure 1). Layer III is easily distinguishable on the basis of its strong PVB-IR neuropil and high numbers of PVB-IR somata. Layers IV and V contained only very sparse PVB-IR somata. Layer VI contained higher numbers of PVB-IR somata comparable to those detectable in layer III and was defined by adjacent Nissl sections. Scale bar, 600 μm . **(B)** Examples of multipolar PVB-IR neurons in layer II and layer III from a normal control case. Note the denser PVB-IR neuropil in layer III. Scale bar, 75 μm .

Figure 3.

The volume of the entorhinal cortex (ECx) is not altered in bipolar disorder (BD) and schizophrenia (SZ). Scattergram showing volumetric values (mm³; logarithmic transformation) for the ECx of normal control, SZ, and BD subjects. No statistically significant changes were detected in any of the diagnostic groups.

Figure 4.

Size of parvalbumin-immunoreactive (PVB-IR) neurons somata in the entorhinal cortex (ECx). In bipolar disorder (BD), no changes relative to the size of PVB-IR neuron somata were detected in any of the ECx subdivisions investigated. In schizophrenia, a subtle but significant ($p < .01$) reduction was found in the ECx-I. Significance values are derived from the baseline stepwise linear model adopted in this study. ECx-R, rostral ECx; ECx-L, lateral ECx, ECx-I, intermediate ECx; ECx-C, caudal ECx.

Figure 5.

Total number (Tn) and numerical density (Nd) of parvalbuminimmunoreactive (PVB-IR) neurons are significantly decreased in the entorhinal cortex (ECx) of bipolar disorder (BD). **(A)** A significant decrease of Tn of PVB-IR neurons (logarithmic transformation; $p = .02$) was detected in BD but not in schizophrenia (SZ) patients. **(B)** Numerical density of PVB-IR neurons in the ECx was decreased significantly in BD as compared with control subjects ($p = .01$). In contrast, changes in SZ were not statistically significant. Values are expressed as the logarithmic transformation of Nd PVB-IR neurons/mm³ of ECx. Significance values are derived from the baseline stepwise linear model adopted in this study. Scatterplots show the mean (black line) and 95% confidence intervals (grey lines).

Figure 6.

In bipolar disorder (BD), a significant decrease of numerical density (Nd) of parvalbumin-immunoreactive (PVB-IR) was detected in the superficial layers of the lateral entorhinal cortex (ECx-L). Within the ECx-L, reductions of PVB-IR neurons Nd were restricted to the superficial layers ($p = .02$) in BD. No significant changes were detected in schizophrenic subjects (SZ). The deep layers of the ECx-L and the ECx-rostral ECx-(R) did not show changes in either group. Note that the ECx-R has overall lower Nd, with several subjects within each group showing values close to zero. Values are expressed as the logarithmic transformation of Nd PVB-IR neurons/mm³. Scatterplots show the mean (black line) and 95% confidence intervals (grey lines). Significance values are derived from the baseline stepwise linear model adopted in this study. C, control subjects.

Figure 7.

Lower numerical densities (Nd) of parvalbumin-immunoreactive (PVB-IR) neurons in the superficial layers of the intermediate entorhinal cortex (ECx-I) and caudal ECx (ECx-C) of bipolar disorder (BD). In BD, significant reductions of PVB-IR neurons were detected in the superficial layers of the lateral ECx (ECx-L) ($p = .04$) and ECx-C ($p = .01$). No changes were detected in the deep layers of these regions. In schizophrenic subjects (SZ), no significant changes were detected in any of the subregions and layers examined. The superficial layers of the ECx-C approached significance in SZ ($p = .8$; **). Values are expressed as the logarithmic transformation of Nd PVB-IR neurons/mm³. Scatterplots show the mean (black line) and 95% confidence intervals (grey lines). Significance values are derived from the baseline stepwise linear model adopted in this study. Other abbreviations as in Figure 6.

Table 1
Sample Demographic and Descriptive Characteristics

Code	Age (yrs)	Gender	PMI (hours)	Cause of Death	Years III
Schizophrenia					
4	60	Female	19.6	Cancer (colon)	22
7	82	Female	23.9	Cancer	Unknown
13	61	Female	14.0	Pneumonia	41
15	73	Female	24	Lung cancer	51
18	48	Female	33.8	Cardiac arrest	31
20	49	Male	19.0	Suicide by hanging	30
22	72	Female	21.8	Cancer	48
32	32	Male	7.8	Cardiac arrest/pancreatitis	16
38	55	Male	21.4	Cardiac arrest	20
42	92	Female	17.8	Cardiac arrest	68
—	62.4 ± 17.8 ^a	3M / 7F	20.3 ± 6.8 ^a	—	36.3 ± 17.1
Bipolar Disorder					
3	74	Male	24.8	Pneumonia (renal failure)	22
12	42	Male	23.2	Cardiac arrest	10
17	76	Female	22.8	Cardiopulmonary failure	9
26	73	Female	20.8	Sepsis (renal failure)	53
29	74	Male	14.2	Pneumonia	56
30	66	Male	17.4	Pneumonia	31
34	25	Male	12.5	Pulmonary edema	10
37	73	Male	7.18	Pneumonia	43
44	86	Female	11.3	Unknown	Unknown
46	75	Female	36.0	Cardiac arrest	48
—	66.4 ± 18.4 ^a	6M / 4F	19.0 ± 8.3 ^a	—	31.3 ± 19.3
Control Subjects					
2	70	Male	23.2	Cardiac arrest	—
5	68	Female	14.8	Cardiac arrest	—
8	78	Female	23.9	Cancer	—
11	37	Male	18.8	Electrocution	—
14	58	Female	17.8	Carbon monoxide	—
16	72	Male	28.2	Cardiac arrest	—
19	52	Male	32.1	Cardiac arrest	—
21	53	Female	24	Melanoma	—
23	62	Male	29.2	Cardiac arrest	—
25	71	Male	24	Cardiac arrest	—
28	74	Male	15.8	Cardiac arrest	—
31	65	Male	17.3	Cardiac arrest	—
33	36	Male	24.5	Cardiac arrest	—
35	74	Female	12.1	Breast Cancer	—
36	70	Male	17.3	Aortic aneurysm	—
43	85	Male	20.3	Cancer	—
—	64.1 ± 13.1 ^a	11M; 5F	21.5 ± 5.6 ^a	—	—

PMI, postmortem interval (hours).

^aMean ± SD.

Table 2
Lifetime Exposure to Psychotropic or Neurotropic Drugs

Code	Dx	CPZ- eq	Lithium	VPA	CBZ	GPN	TCAs	SRI	BZD	Anti- ACh	Adherence
4	SZ	365	0	no	no	yes	no	yes	yes	no	poor
7	SZ	—	—	—	—	—	—	—	—	—	unknown
13	SZ	3550	0	no	no	no	no	yes	no	yes	unknown
15	SZ	4978	0	no	no	no	no	yes	no	no	poor
18	SZ	772	3287	yes	no	no	yes	no	no	yes	poor
20	SZ	789	0	yes	no	no	no	no	no	yes	good
22	SZ	420	0	no	no	no	no	no	no	yes	poor
32	SZ	671	0	no	no	no	no	no	no	no	poor
38	SZ	793	0	no	no	no	yes	no	no	no	good
42	SZ	0	0	no	no	no	no	no	no	no	not treated
3	BD	103	3945	yes	yes	no	no	no	no	no	good
12	BD	0	0	no	no	no	no	no	no	no	not treated
17	BD	78	1321	no	no	no	no	no	yes	no	poor
26	BD	202.7	4273	no	yes	no	no	no	no	no	good
29	BD	2842	2192	yes	no	no	no	no	yes	no	good
30	BD	731	1096	no	no	no	no	no	yes	no	unknown
34	BD	3289	3945	no	no	yes	no	yes	no	no	good
37	BD	3.6	0	no	no	yes	no	no	yes	no	good
44	BD	0	0	no	no	no	no	no	no	no	not treated
46	BD	0	0	no	no	no	no	no	no	no	not treated

Data are in estimated total lifetime grams of each type of drug per patient; adherence is estimated from clinical records. Dx, diagnosis; CPZ-eq, chlorpromazine-equivalent dose (mg/day); VPA, valproate; CBZ, carbamazepine; GPN, gabapentin; TCAs, tricyclic antidepressants; SRI, serotonin-reuptake inhibitor; SZ, schizophrenia; BZD, benzodiazepines; Anti-ACh, anticholinergic; BD, bipolar disorder.

Table 3
Exposure to Psychotropic or Neurotropic Drugs During the Last 6 Months of Life

Code	Dx	CPZ-eq	Lithium
4	SZ	11.25	0
7	SZ	0	0
13	SZ	45	0
15	SZ	67.5	0
18	SZ	18	0
20	SZ	36	0
22	SZ	18	0
32	SZ	20.25	0
38	SZ	59.76	0
42	SZ	0	0
3	BD	40.5	108
12	BD	0	0
17	BD	9	27
26	BD	18	0
29	BD	63	27
30	BD	0	54
34	BD	0	216
37	BD	13.5	0
44	BD	0	0
46	BD	0	0

Data are in estimated total grams of each type of drug per patient during the last 6 months of life. Dx, diagnosis; CPZ-eq, chlorpromazine-equivalent dose (mg/day); VPA, valproate; CBZ, carbamazepine; GPN, gabapentin; TCAs, tricyclic antidepressants; SRI, serotonin-reuptake inhibitor; SZ, schizophrenia; BZD, benzodiazepines; Anti-ACh, anticholinergic; BD, bipolar disorder.

Table 4

Numerical Densities of PVB-IR Neurons in the Superficial and Deep Layers of Distinct ECx Subregions in Normal Control, BD, and SZ Subjects

ECx Subregions		Control Subjects	Bipolar Disorder			Schizophrenia		
Division	Layer	Mean $\pm$ SD	Mean $\pm$ SD	<i>t</i>	<i>p</i>	Mean $\pm$ SD	<i>t</i>	<i>p</i>
ECx-L	II–III	3.9 $\pm$ 1.2	3.1 $\pm$ .6	-2.4	.02	3.7 $\pm$ 1.3	1.6	ns
ECx-L	IV/V–VI	2.8 $\pm$ 1.2	2.6 $\pm$ .7	-.6	ns	2.8 $\pm$ .9	.5	ns
ECx-R	II–III	2.3 $\pm$ 1.5	2.1 $\pm$ 1.1	-.2	ns	1.0 $\pm$ 1.4	-.9	ns
ECx-R	IV/V–VI	1.8 $\pm$ 1.4	1.0 $\pm$ 1.2	-1.2	ns	.9 $\pm$ 1.3	-.3	ns
ECx-I	II–III	3.5 $\pm$ 1.2	2.0 $\pm$ 1.7	-2.1	.04	3.0 $\pm$ 1.6	1.1	ns
ECx-I	IV/V–VI	1.7 $\pm$ 1.5	1.8 $\pm$ 1.3	-.1	ns	1.9 $\pm$ 1.6	.4	ns
ECx-C	II–III	4.9 $\pm$ 1.3	3.8 $\pm$ 1.4	-2.6	.01	4.6 $\pm$ .9	1.8	.08
ECx-C	IV/V–VI	3.6 $\pm$ 1.5	3.0 $\pm$ .9	-1.4	ns	3.3 $\pm$ .9	.7	ns

P values not significant at $\alpha=.05$. PVB-IR, parvalbumin-immunoreactive; ECx, entorhinal cortex; BD, bipolar disorder; SZ, schizophrenia; L, lateral; R, rostral; I, intermediate; C, caudal.