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Bipolar disorder type 1 and schizophrenia are accompanied by decreased density of parvalbumin- and somatostatin-positive interneurons in the parahippocampal region

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Abstract

GABAergic interneurons synchronize network activities and monitor information flow. Post-mortem studies have reported decreased densities of cortical inter-neurons in schizophrenia (SZ) and bipolar disorder (BPD). The entorhinal cortex (EC) and the adjacent subicular regions are a hub for integration of hippocampal and cortical information, a process that is disrupted in SZ. Here we contrast and compare the density of interneuron populations in the caudal EC and

subicular regions in BPD type I (BPD-I), SZ, and normal control (NC) subjects. Postmortem human parahippocampal specimens of 13 BPD-I, 11 SZ and 17 NC subjects were used to examine the numerical density of parvalbumin-, somatostatin- or calbindin-positive interneurons. We observed a reduction in the numerical density of parvalbumin- and somatostatin-positive interneurons in the caudal EC and parasubiculum in BPD-I and SZ, but no change in the subiculum. Calbindin-positive interneuron densities were normal in all brain areas examined. The profile of decreased density was strikingly similar in BPD-I and SZ. Our results demonstrate a specific reduction of parvalbumin- and somatostatin-positive interneurons in the parahippocampal region in BPD-I and SZ, likely disrupting synchronization and integration of cortico-hippocampal circuits.

Keywords

Bipolar disorder; Schizophrenia; Parahippocampal gyrus; Entorhinal cortex; Subiculum; Somatostatin; Parvalbumin; Interneurons

Introduction

One of the most consistent observations in bipolar disorder (BPD) and schizophrenia (SZ) is a disturbance of GABA-ergic neurons and markers in neocortical regions and hippocampus [8, 10]. The GABA synthesizing enzymes GAD65 and GAD67 are markedly decreased in prefrontal cortex (PFC) and hippocampus [1, 29, 45, 50, 56], as are GABA neuron subtype-specific markers such as parvalbumin (PV) and somatostatin (SOM), [5, 19, 25, 35, 58]. The entorhinal cortex (EC), a crucial element in cortico-hippocampal communication, has a decreased density of PV neurons in BPD [42] as well as abnormal cytoarchitecture with disturbed neuronal migration in SZ [3, 31]. Imaging studies point to abnormal cortico-hippocampal integration in SZ [28, 51]. We examined the density of three inter-neuron populations, defined by the markers PV, SOM and calbindin (CB), in the EC, parasubiculum, presubiculum and subiculum, linked structures that connect hippocampus and cortical areas.

The subiculum is innervated by CA1 pyramidal axons from the hippocampus [41] and projects to the deep layers of the EC as well as the parasubiculum [54]. The parasubiculum is innervated by projections from subiculum and CA1 [49]. Parasubiculum and presubiculum project to the superficial layers of the EC [52]. Output of the EC is layer-specific: the superficial layers of the EC project to the dentate gyrus of the hippocampus, whereas the deep layers of the EC project to cortical regions [52].

The information flow through the EC is monitored by local GABA interneurons that synchronize network activity [11]. Interneurons can be classified histologically based on the presence of calcium-binding proteins such as PV and CB, or neuropeptides such as SOM [12, 38].

PV-positive interneurons terminate on the initial axon segments of pyramidal cells [18], are 'fast-spiking', and are connected to each other via chemical synapses as well as electrical gap junctions [22]. In SZ and BPD, a reduction in cortical PV mRNA levels and neurons has

been reported [5, 26, 32, 35, 42, 47, 58]. This reduction has been postulated to cause gamma oscillation deficits and a disruption of synchronization of neural activity, and could explain some symptoms observed in BPD and SZ [4, 55].

CB-immunoreactive interneurons terminate on distal pyramidal cell dendrites and are non-fast-spiking [17, 57]. Studies of CB in BPD or SZ are inconsistent. In SZ, levels of CB mRNA and density of neurons have been reported as increased [15, 19], unchanged [46], and decreased [6, 13, 43], whereas studies in BPD reported non-significant reductions [6, 13, 43].

Neurons containing the neuropeptide SOM synapse onto dendrites of pyramidal cells [37]. SOM-positive cells are low-threshold-spiking and are connected via electrical gap junctions in addition to chemical synapses [7]. SOM mRNA levels and cell densities are reduced in SZ [19, 21, 24, 34, 39] as well as BPD [33, 35], with one report of upregulations in mRNA levels [40].

Here we examine PV, SST and CB interneuron populations in EC and subicular regions in BPD type 1 (BPD-I) and SZ, two disorders accompanied by psychosis [14], to test the hypothesis that a similar pattern of interneuron population changes may be shared by major psychoses.

Materials and methods

Sample collection

Brains were collected at the Harvard Brain Tissue Resource Center (HBTRC; McLean Hospital, Belmont, MA, USA). The HBTRC is funded by NIH and follows all regulations implemented by the Office for Human Research Protections.

For all the subjects included in this study, two psychiatrists established DSM-IV diagnoses based on the review of a questionnaire filled out by legal next of kin and a review of all available medical records. Normal control (NC) cases had sufficient information from next of kin and medical records to rule out major medical, neurologic, and psychiatric conditions. All brains underwent a neuro-pathological examination and cases with histopathological abnormalities were excluded from this study.

Three diagnostic groups, comprised of 17 NC cases, 13 BPD-I cases and 11 SZ cases were matched for gender, age, post-mortem interval, and hemisphere (Table 1). Because PV staining was crucial for the delineation of brain regions, SOM- and CB samples could only be used if PV staining as well as SOM or CB staining were of good quality in adjacent slices.

Tissue collection and processing

Tissue was immersion-fixed in 4.0% paraformaldehyde (0.1 M phosphate buffer (PBS), pH 7.4) at 4.0°C for 3 weeks and placed in cryoprotectant (0.1 M PBS, pH 7.4/0.1% sodium azide/30.0% ethylene glycol/30.0% glycerol), immersed in agar and cut into 2.5 mm thick coronal slabs using an antithetic tissue slicer. Sections were cut on a sliding microtome

(American Optical Company, Buffalo, NY, USA), with a thickness of 100 μm for Nissl stain, or 50 μm for immunocytochemistry. Sections were mounted on gelatin-coated glass slides and consecutive slices within a tissue block were stained with 0.1% cresyl violet (Nissl stain) or used for PV, SOM and CB immunocytochemistry.

Immunocytochemical procedure

Sections were rinsed in 0.01 M phosphate buffered saline with 0.5% Triton-X100 (PBS-TX) for 3×5 min, boiled in Vector Antigen Unmasking Solution (1:100 in 0.1 M PBS; Vector laboratories, Burlingame, CA, USA) for 3 min, rinsed in PBS-TX for 3×10 min, incubated in 0.03% hydrogen peroxide solution in 0.01 M PBS for 30 min, rinsed in PBS-TX for 3×5 min, incubated in 2% bovine serum albumin (BSA; Fraction V; Sigma, MO, USA) in 0.01 M PBS for 1 h and rinsed in PBS-TX for 3×5 min. Sections were incubated with primary antibody in 0.01 M PBS with 1% BSA for 72 h at 4°C . Sections were rinsed in PBS-TX 3×5 min and incubated in biotinylated secondary antibody in 0.01 M PBS with 1% BSA for 2 h. After 3×5 min rinses in PBS-TX, sections were incubate with streptavidin horseradish peroxidase (HRP) conjugate (1:5,000; Zymed-Invitrogen; Carlsbad, CA, USA) in 0.01 M PBS with 1% BSA, rinsed 3×5 min, and incubated for 2–10 min in DAB solution (Sigma) in 0.1 M PBS with 0.003% hydrogen peroxide and nickel sulfate. Sections were transferred to PBS-TX and washed 3×5 min. Unless noted otherwise, all steps were carried out at room temperature [35].

Antibodies and dilutions: the PV antibody was diluted 1:10,000 (monoclonal mouse anti frog muscle parvalbumin, clone PARV-19; Sigma); the SOM antibody was diluted 1:500 (monoclonal rat anti synthetic cyclic somatostatin peptide corresponding to amino acids 1–14; Millipore, Billerica, MA, USA); the CB antibody was diluted 1:8,000 (monoclonal mouse anti-calbindin D-28K, clone CB-955; Sigma). Secondary antibodies were biotinylated, goat anti-mouse IgG for PV and CB (Vector laboratories), and goat anti-rat IgG for SOM. Secondary antibodies were diluted 1:500.

For each immunocytochemical labeling, all sections were processed simultaneously to avoid procedural differences. Each staining dish contained sections from SZ, BPD-I and NC cases and all dishes were treated for the same duration.

Cell counts

All cases were coded and data collection was performed without the knowledge of diagnosis. Morphometric analysis was carried out using a Zeiss Axioskop 2 Plus microscope (Germany) equipped with a LEP MAC 5000 automated stage (Ludl Electronic Products, Hawthorne, NY, USA). The microscope was interfaced with the Stereo Investigator stereological software (v 6.55, Microbrightfield, Colchester, VT, USA) via an Optronics DEI-750 video camera (Goleta, CA, USA). All regions were outlined using a $1.25\times$ objective lens and cells were marked and counted with a $20\times$ objective lens. All immunopositive cells were counted within each region on each slice. Three guidelines were applied to include a cell into the neuron count: size and shape of the body, presence of processes, and staining intensity. To be consistent across all samples, only slides with a particular PV staining pattern (as seen in Fig. 1b) were included in the analysis, amounting

to one to three slices per case at 2.5 mm intervals, which were averaged for each case. Average number of slices per case was 1.8 for PV, 1.5 for SOM and 1.6 for CB. All data are presented as cells/mm² in sections of 50 µm thickness. The entire dataset was counted by the same investigator who was blinded to the diagnosis. Cases were randomized for the counting.

Delineation of brain regions

Interneuron numerical density in subicular regions and the EC was examined in the caudal part of the rostral–caudal axis [30]. In this area, the highest density of PV-positive neurons is observed in the EC in a very distinctive macroscopic pattern [42]. This pattern was used to ensure that all cases are counted in the same anatomical region.

To determine the borders between CA1/subiculum, subiculum/presubiculum, presubiculum/parasubiculum, parasubiculum/EC, and superficial layers EC/deep layers EC, we used a combination of structural markers and neuronal density, primarily based on Nissl stain and PV staining of the neuropil, with emphasis on reproducibility of markers across all cases (Fig. 1).

The border between CA1 and subiculum was determined in the Nissl-stained samples by the widening of the pyramidal cell layer in the subiculum and the loss of the stratum radiatum (Fig. 1a) [16]. Similar in structure to the hippocampus, the subiculum has a three-layered cortex (Fig. 2a). Darker stained neuropil patches were particularly visible in the PV and CB staining. These patches also contained groups of small CB-positive neurons (Fig. 2b). The border between the subiculum and the presubiculum was determined by the narrowing of the pyramidal cell layer and the increased density of small pyramidal cells in the superficial layer of the presubiculum (Lamina principalis externa), which frequently formed cellular islands that were densely innervated by PV-positive and CB-positive fibers (Figs. 1b, d, 3a), [16]. In preparations 5 mm caudal to the example used in Fig. 3a, the overlapping pattern between the dense cellular islands in the Nissl stain and the dark neuropil staining in the PV (Fig. 3b) as well as CB immunocytochemistries (not shown) was evident. Inter-neurons in the lamina principalis externa were generally smaller than interneurons in the lamina principalis interna (Fig. 3c). Due to the somewhat irregular borders between the two laminae and the changing pattern between adjacent slices (compare Fig. 1b–d), neuron counts were not separated for the two laminae.

The parasubiculum was defined as the area between presubiculum and EC [16] (Fig. 4). The parasubiculum has a neocortical architecture with six layers, in which PV staining of the neuropil was lighter (Figs. 1b, 4a). Density of PV- and CB-positive neurons was higher in the superficial layers, and patches of lightly stained CB-positive neurons were interspersed with darker stained neurons (Fig. 4b).

In the six-layered EC [53], layers I, II, and III were delineated as superficial layers, and layers IV, V, and VI as deep layers [16]. Both layers were counted separately. Layer III, which contains medium-sized pyramidal cells [16], had strongly stained neuropil in the PV immunocytochemistry [48], (Fig. 5a); layer IV, the lamina dissecans, is marked by an absence of neurons [20]. The border between the superficial and deep layers of the EC was

drawn based on darker PV neuropil staining in layer III, and the absence of cells in layer IV (Nissl stain). CB-positive neurons in the deep layers were smaller and lighter stained, while in the superficial layer darkly stained neurons with rich arborization were interspersed with lighter stained neurons (Fig. 5b). The PV staining observed in the EC was comparable to the literature [44].

The contours for SOM (Fig. 1c) and CB (Fig. 1c) staining were based predominantly on the contours of PV (Fig. 1b) staining and Nissl stain (Fig. 1a), which were from adjacent sections and adjusted for minor differences in cytoarchitecture. Cases in which regions could not be clearly delineated or immunostaining was insufficient were excluded from the analysis.

Statistical analysis

Study subjects were initially closely matched for demographic factors, but exclusion of poorly stained or otherwise damaged sections introduced some variability (Table 1). Although no group had statistically significant differences in demographic data, analyses of covariance (ANCOVA) were applied to all results, with diagnosis as the independent variable and age, gender and PMI as covariates. Initial analysis included all three groups (NC, BPD-I and SZ), followed by post-hoc ANCOVAs with diagnosis, age, gender and PMI as covariates.

Results

Pattern of PV-, SOM-, and CB-positive neurons in parahippocampal regions in NC cases

CB-positive neurons had the highest density in all regions examined (Fig. 6a, b, c). This was in large part due to CB-clusters scattered throughout all regions, predominantly in the superficial layers and frequently only lightly stained (Figs. 2, 3, 4, 5, 6). The density of PV-positive cells was higher than the density of SOM-positive cells in all brain regions except for the parasubiculum and the deep layers of the EC (Fig. 6a, b).

The subiculum and deep layers of the EC were consistently among the areas with the lowest density of PV-, SOM-, and CB-positive neurons, while the superficial EC had the highest density in all immunocytochemical stainings (Fig. 6).

Density of PV-positive cells in subicular regions and EC of subjects with BPD-I, SZ, or NC

ANCOVA of the subiculum and the presubiculum with all three diagnostic groups showed no difference in PV cell density (subiculum: $F_{5,34} = 1.6$, $p = 0.218$; presubiculum: $F_{5,35} = 0.4$, $p = 0.685$; age, gender, PMI as covariates). PV cell density in the parasubiculum was significantly different between the diagnostic groups ($F_{5,35} = 7.2$, $p = 0.002$; age, gender, PMI as covariates). Post-hoc ANCOVAs showed a reduction in cell density in BPD-I as well as SZ (Fig. 6a). The superficial layers, deep layers and combined layers ('all') of the EC had significant differences in PV cell density between the diagnostic groups (superficial EC: $F_{5,34} = 4.2$, $p = 0.024$; deep EC: $F_{5,34} = 4.4$, $p = 0.020$; all EC: $F_{5,34} = 4.6$, $p = 0.017$; age, gender, PMI as covariates). PV cell density in the superficial layers of the EC was significantly reduced in SZ, with a trend in BPD-I, whereas in the deep layers of the EC

BPD-I subjects showed a significant reduction. Both disorders showed a reduction when data for all layers were combined (Fig. 6a).

Density of SOM-positive cells in subicular regions and EC of subjects with BPD-I, SZ, or NC

ANCOVA of the subiculum for all three diagnostic groups showed no difference in SOM cell density ($F_{5,32} = 2.2$, $p = 0.122$; age, gender, PMI as covariates). SOM cell density in the presubiculum, parasubiculum, superficial layers, deep layers and combined layers ('all') of the EC was significantly different between the diagnostic groups (presubiculum: $F_{5,32} = 3.8$, $p = 0.032$; parasubiculum: $F_{5,32} = 7.6$ $p = 0.002$; superficial EC: $F_{5,32} = 7.4$ $p = 0.002$; deep EC: $F_{5,31} = 3.8$ $p = 0.035$; all EC $F_{5,31} = 7.3$ $p = 0.003$; age, gender, PMI as covariates). Post-hoc ANCOVAs showed a reduction in SOM cell density in BPD-I and SZ in parasubiculum, superficial layers of EC and combined layers of EC, while in presubiculum SZ subjects only, and in the deep layers of EC BPD-I subjects only showed a significant reduction (Fig. 6b). Interestingly, the density of SOM-positive neurons declined with age, a situation that was factored into our analysis (Fig. 6d). PV and CB densities were not affected by age, gender or PMI, and SOM densities were not affected by gender or PMI.

Density of CB-positive cells in subicular regions and EC of subjects with BPD-I, SZ, or NC

The density of CB neurons was similar in all diagnostic groups across all brain regions examined (Fig. 6c).

Discussion

In both psychiatric disorders, we observed a gradient of reduced neuron density, with no changes in the subiculum and significant reductions in the EC. The pattern of reduced density was similar for PV- and SOM stains, pointing to a pathological abnormality in psychotic disorders that affects both interneuron populations and is region-specific. The brain areas examined are tightly interconnected with each other, as well as with the hippocampus and the PFC [23, 41, 49, 52, 54]. Despite this interconnection, the subiculum showed normal interneuron density whereas hippocampus and EC had lower PV- and SOM densities in BPD-I and SZ [34, 35, 42]. Unlike PV and SOM-positive neurons, CB neurons had a normal density in all brain regions examined in both disorders, indicating that the reduction in inter-neuron density is not only specific for brain region, but also specific for particular interneuron populations.

BPD-I and SZ had similar patterns of reduced PV and SOM cell density, and no reduction in CB cell density, adding to the notion that these two disorders share significant pathological roots [9, 14]. Two exceptions to these similarities were observed: (1) the SOM staining in the presubiculum showed reduced neuron density exclusively in SZ, and (2) PV- and SOM-neuron density was reduced in the deep layers of the EC in BPD-I, but did not reach significance in SZ.

The decrease in PV-immunopositive neurons in the EC of BPD-I subjects is consistent with a previous report by Pantazopoulos et al. [42]. These authors observed a trend toward a decrease of PV neurons in the caudal EC of subjects with SZ, while the present study found

a significant reduction [42]. Specifics of the subject cohort and the area of caudal EC investigated may account for this slight discrepancy between these reports.

The presubiculum connects to PFC and hippocampus [23, 52]. Loss of SOM-positive neurons in this area reduces synchronized network activity [11] and could be a central contributor to the disrupted cortico-hippocampal integration observed in SZ [28, 51]. However, interneuron deficits in the deep layers of EC, predominantly observed in BPD-I, could equally affect communication between EC and PFC and disrupt cortico-hippocampal integration in BPD-I [2, 52].

Like any experimental approach to examine the biological underpinnings of psychiatric disorders, postmortem studies have limitations. For example, we cannot answer the question if the reduced density of immuno-positive PV and SOM neurons is due to a lower density of neurons or due to the loss of GABA-specific markers synthesized by these neurons. Total cell counts within cortical areas capture predominantly the vast number of pyramidal neurons which are unchanged in BPD and SZ [27, 35], making it possible that a loss of the small population of interneurons remains undetected. This has been demonstrated in previous studies in which we counted subtype-specific interneurons throughout the entire hippocampus and compared them to stereological estimates of all neurons [34, 35]. While we found a large reduction of PV- and SOM-positive interneurons in BPD-I and SZ, the number of missing cells was smaller than the non-significant reduction in total neuron number estimates.

A second question inherent in post-mortem studies is the effect of drug treatment. Although we have carried out extensive studies in rats on the effect of chronic treatment with antipsychotic drugs or mood stabilizers [35, 36], the treatment durations are not comparable to the many years of treatment of patients. Thus, while there was no change of GABA markers with any treatment in rats, we cannot rule out a treatment effect in the present study.

Finally, density estimates are biased and affected by factors such as differences in tissue shrinkage. Unfortunately, extensive pilot testing showed that we could not use the optical disector or fractionator to quantify immunocytochemically stained neurons for unbiased sampling in the *z* axis. Three factors prevented us from doing this: (1) the relatively thin (50 μ m) sections, chosen to maximize antibody penetration, collapsed to less than 10 μ m, making it impossible to place a sufficiently large disector box; (2) precipitation of the immunoreaction product resulted in different somal shapes of immunoreactive cells; and (3) the intensity of the immunocytochemical stains made it often impossible to identify a unique structure for a disector count. However, we found that reduced cell density was specific to some brain regions and some interneuron subtypes within the same histological preparation, which substantially alleviates concerns about shrinkage artifacts.

The present study adds to the growing literature on pathological mechanisms in BPD. The patients in the study were diagnosed with BPD-I and all had presented with psychosis. The pattern of interneuron deficits in these patients was remarkably similar to the pattern seen in SZ, both in the brain areas affected as well as the interneuron subtypes affected. The overlap between both disorders suggests that measurable pathophysiological abnormalities map onto

the dimension of psychosis rather than the categorical distinction of schizophrenia versus bipolar disorder.

Our findings also add a number of new aspects to the increasing literature on interneuron pathology in the cortex in SZ. We show that parahippocampal regions have reduced interneuron density and that the reduction does not affect all interneuron populations. The observed abnormalities indicate de-synchronization of network activity and abnormal cortico-hippocampal integration as observed in SZ and BPD-I.

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Abbreviations

BPD	Bipolar disorder
SZ	Schizophrenia
NC	Normal control
EC	Entorhinal cortex
PFC	Prefrontal cortex
PV	Parvalbumin
SOM	Somatostatin
CB	Calbindin

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Fig. 1.

Overview of subiculum, presubiculum, parasubiculum and EC in a 42-year-old female NC subject. Regional borders are marked by *arrowheads*, *boxes* demarcate areas shown in subsequent figures. Neurons were counted within the entire region between *two arrowheads*.

a Nissl stain; **b** PV immunocytochemistry, **c** SOM immunocytochemistry, **d** CB immunocytochemistry. Note the characteristic pattern in the PV stain. In the Nissl stain, a widening of the pyramidal cell layer between CA1 and subiculum defined the medial subicular border, whereas the narrowing of the pyramidal layer between subiculum and presubiculum defined the lateral subicular border

Fig. 2.
a Comparison of Nissl stain, PV immunocytochemistry, SOM immunocytochemistry and CB immunocytochemistry in the subiculum. The pyramidal cell layer (II) is located between two distinct layers. *Stars* denote the same blood vessel in all tissue slices. **b** Representative neurons. Note the different populations of CB-positive neurons

Fig. 3.

a Comparison of Nissl stain, PV immunocytochemistry, SOM immunocytochemistry and CB immunocytochemistry in the presubiculum, a transition zone between 3-layered hippocampus and 6-layered neocortex. The presubiculum was divided into lamina principalis externa and interna. Stars denote the same blood vessels in all tissue slices. **b** A lower magnification of an adjacent slice of the same subject shows the overlap between cell-dense areas in the Nissl stain and darkly stained neuropil in the PV immunocytochemistry. Arrowheads denote boundaries of the presubiculum. **c** Representative neurons from each lamina

Fig. 4.

a Comparison of Nissl stain, PV immunocytochemistry, SOM immunocytochemistry and CB immunocytochemistry in the parasubiculum. To improve contrast of layers II–VI, layer I has been overexposed in the Nissl stain. Note: *the top* torn part of the SOM sample was slightly rotated for better fit in the allocated space (compare to unaltered Fig. 1). **b** Representative neurons from layers II/III and IV–VI

Fig. 5.

a Comparison of Nissl stain, PV immunocytochemistry, SOM immunocytochemistry and CB immunocytochemistry in the EC. The border between layers III and IV was defined by the contrast in neuropil staining in the PV samples and the neuron-free layer IV in the Nissl stain, and is marked by *arrowheads*. **b** Representative neurons from layers I–III and IV–VI

Fig. 6.

Box- and Whisker plots of interneuron counts/mm² in BPD-I, SZ and NC subjects. **a** PV-positive interneuron density, BPD = 13, SZ = 11, NC = 17. **b** SOM-positive interneuron density, BPD = 13, SZ = 9, NC = 16; **c** CB-positive interneuron density, BPD = 9, SZ = 9, NC = 16. **d** Decrease in SOM neuron count with increasing age in subiculum, parasubiculum and EC in NC subjects. R^2 values, F ratios and p values are presented in the *right upper corner* of each plot. Significant correlations were also seen in presubiculum and in the superficial as well as deep layers of the EC. *Boxes* in (a–c) cover the 25th to 75th quartiles; *lower dashed line* starts at the minimum sample number, *upper dashed line* ends at

the maximum sample number. Outliers are shown in *diamond shape*. The *horizontal line* shows the median sample number. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ of two-group ANCOVAs with diagnosis as independent variable and age, gender and PMI as covariates (see “Results” for data)

Table 1

Demographic data of study subjects

Diagnosis	Hemisphere	Gender	PMI	Age	Fresh brain weight (g)	Cause of death	Included in PV	Included in SOM	Included in CB
BPD	R	M	32.9	83	1,220	Congestive heart failure	x	x	
BPD	L	M	30.8	40	1,385	Suicide	x	x	
BPD	L	F	13.4	62	1,210	Congestive heart failure	x	x	x
BPD	R	F	21.9	40	1,340	Sepsis	x	x	x
BPD	L	M	22.0	38	1,290	Co poisoning	x	x	x
BPD	R	F	33.3	77	1,400	Pneumonia	x	x	
BPD	L	F	24.2	23	1,340	Suicide	x	x	x
BPD	L	F	17.2	52	1,140	Liver failure	x	x	x
BPD	R	F	24.8	78	1,180	Dehydration	x	x	x
BPD	L	F	16.3	47	1,000	Major systems failure	x	x	x
BPD	L	F	35.1	51	1,260	Ischemic heart disease	x	x	
BPD	L	F	22.6	79	1,085	Ovarian cancer	x	x	x
BPD	R	M	17.9	18	1,490	Motor vehicle accident	x	x	x
SZ	L	F	23.0	49	1,240	Pulmonary embolism	x		
SZ	R	F	22.0	44	1,265	Cardiopulmonary arrest	x	x	x
SZ	R	F	18.7	56	1,185	Cardiopulmonary arrest	x	x	x
SZ	R	M	33.3	41	1,610	Cardiopulmonary arrest	x	x	
SZ	L	F	28.6	74	1,325	Pneumonia	x	x	x
SZ	L	F	15.7	85	1,200	Cardiopulmonary arrest	x	x	x
SZ	R	M	14.8	52	1,280	Cardiopulmonary arrest	x	x	x
SZ	R	M	25.3	62	1,340	Sepsis	x	x	x
SZ	R	M	18.0	36	1,480	Suicide (OD)	x		x
SZ	L	M	32.4	58	1,160	Chronic obstruction pulmonary disease	x	x	x
SZ	R	M	21.4	68	1,255	Cardiopulmonary arrest	x	x	x
NC	R	M	21.5	22	1,360	Myocardial infarct	x	x	x
NC	R	F	23.9	68	1,390	Chronic obstruction pulmonary disease	x	x	x
NC	L	F	12.5	60	1,160	Breast cancer	x	x	x
NC	L	M	24.6	77	1,190	Cardiopulmonary arrest	x		x

Diagnosis	Hemisphere	Gender	PMI	Age	Fresh brain weight (g)	Cause of death	Included in PV	Included in SOM	Included in CB
NC	L	F	23.0	74	1,100	Pneumonia	x	x	x
NC	R	M	27.2	41	1,815	Cardiopulmonary arrest	x	x	x
NC	L	M	18.4	68	1,520	Heart failure	x	x	x
NC	R	F	23.1	51	1,375	Cardiopulmonary arrest	x	x	x
NC	R	F	27.5	55	1,245	Cardiopulmonary arrest	x	x	x
NC	R	F	17.4	81	1,135	Colon cancer	x	x	x
NC	L	F	20.3	42	1,480	Myocardial infarct	x	x	x
NC	R	F	18.1	36	1,390	Cardiopulmonary arrest	x	x	x
NC	R	M	14.8	30	1,570	Suicide	x	x	x
NC	L	M	13.1	52	1,450	Heart disease	x	x	x
NC	R	M	28.3	61	1,510	Myocardial infarct	x	x	x
NC	R	F	17.8	60	1,220	Cardiac dysrhythmia	x	x	x
NC	L	M	30.3	60	1,190	Cardiopulmonary arrest	x	x	x
PV									
BPD	5R/8L	4M/9F	24.0 ± 7.1	52.9 ± 21.6	1,256.9 ± 136.9	<i>n</i> = 13			
SZ	7R/4L	6M/5F	23.0 ± 6.3	56.8 ± 14.8	1,303.6 ± 134.4	<i>n</i> = 11			
NC	10R/7L	8M/9F	21.3 ± 5.4	55.2 ± 16.6	1,358.8 ± 189.2	<i>n</i> = 17			
SOM									
BPD	5R/8L	4M/9F	24.0 ± 7.1	52.9 ± 21.6	1,256.9 ± 136.9	<i>n</i> = 13			
SZ	6R/3L	5M/4F	23.6 ± 6.8	60.0 ± 14.1	1,291.1 ± 134.2	<i>n</i> = 9			
NC	10R/6L	7M/9F	21.1 ± 5.5	53.8 ± 16.2	1,369.4 ± 190.1	<i>n</i> = 16			
CB									
BPD	3R/6L	2M/7F	20.0 ± 3.9	48.6 ± 21.7	1,230.6 ± 150.1	<i>n</i> = 9			
SZ	6R/3L	5M/4F	21.9 ± 5.9	59.4 ± 15.0	1,276.7 ± 97.6	<i>n</i> = 9			
NC	10R/6L	7M/9F	20.7 ± 5.0	54.9 ± 17.1	1,369.4 ± 190.1	<i>n</i> = 16			

Cohorts for cell counts are identified in the last three columns. Mean (±SD) demographic data for cohorts are shown at the bottom of the table.

No significant differences were observed in any parameter