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Hemispheric comparisons of neuron density in the planum temporale of schizophrenia and nonpsychiatric brains

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Abstract

Postmortem and *in vivo* studies of schizophrenia frequently reveal reduced cortical volume, but the underlying cellular abnormalities are incompletely defined. One influential hypothesis, especially investigated in Brodmann's area 9 of prefrontal cortex, is that the number of neurons is normal, and the volume change is caused by reduction of the surrounding neuropil. However, studies have differed on whether the cortex has the increased neuron density that is predicted by this hypothesis. In a recent study of bilateral planum temporale (PT), we reported smaller volume and width of the outer cortex (layers I-III), especially in the left hemisphere, among subjects with schizophrenia. In the present study, we measured neuron density and size in the same PT samples, and also in prefrontal area 9 of the same brains. In the PT, separate stereological measurements were made in layers II, IIIC, and VI, whereas area 9 was sampled in layer IIIB-c. In both cortical regions, there was no significant effect of schizophrenia on neuronal density or size. There was, nevertheless, a trend-level right>left hemispheric asymmetry of neuron density in the PT, which may partially explain the previously reported left>right asymmetry of cortical width. In schizophrenia, our findings suggest that closer packing of neurons may not always explain reduced cortical volume, and subtly decreased neuron number may be a contributing factor.

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Keywords

Auditory cortex; stereology; cytoarchitectonic; cortex width; hemispheric asymmetry; neuropathology

1. Introduction

Both *post mortem* and *in vivo* measurements have found reduced volume and thickness of the cerebral cortex in schizophrenia. While deficits are present in all lobes of the brain, they are especially pronounced in some regions. One of the most affected regions is the caudal superior temporal gyrus, especially in the left hemisphere (Shenton et al., 2001; Narr et al., 2005; Smiley, 2009). The planum temporale (PT) is the caudal part of the dorsal surface of this gyrus. It extends from Heschl's gyrus (HG) to the caudal end of the lateral sulcus. The rostral part of the PT, lateral to HG, contains mainly auditory association areas, whereas the caudal PT contains mainly higher order association areas (Hackett, 2007; Smiley and Falchier, 2009).

The cellular explanations for reduced volume of cerebral cortex in schizophrenia are still obscure. A prominent hypothesis is that volume loss is caused by reduction of the cortical neuropil, without reduced neuron number. This is supported by findings of increased density of neuron cell bodies (Selemon, 2004) and decreased density of dendrites and synaptic markers [reviewed in (Dwork et al., 2009)]. However, several careful studies recently failed to confirm the increases in neuron density (Beasley et al., 2005; Cullen et al., 2006). Alternative explanations for reduced cortical volume include lower absolute numbers of neurons, or greater cortical surface area, although empirical evidence for either is lacking.

In a recent study of left and right PT in postmortem brains, we measured the volumes and widths of the upper cortex (layers I-III) and lower cortex (layers IV-VI) (Smiley et al., 2009). The schizophrenia brains had a relatively selective reduction of the upper cortex that was greater in the left hemisphere, especially in the caudal PT. These upper layer changes are consistent with previous findings of reduced cell size and synaptic density in the upper layers of auditory regions in schizophrenia (Sweet et al., 2004; Sweet et al., 2007) and suggest that upper layer cortical circuits may be especially altered.

In the present study we used stereological measurements to determine whether increased neuron density corresponds to decreased volume, as would be predicted by loss of neuropil without loss of neurons. Upper and lower layers of the rostral and caudal PT were evaluated separately, allowing comparisons of neuron densities with previous cortical width measurements from the same brains. We also evaluated prefrontal cortex to determine if changes in neuron densities might be regionally specific.

2. Methods

2.1 Diagnostic and exclusionary criteria

Tissue samples were from 11 schizophrenia and 10 non-psychiatric male brains obtained at autopsy either at the Institute for Forensic Medicine in Skopje Macedonia (matched schizophrenia and nonpsychiatric cases), or from New York State Psychiatric Hospitals (schizophrenia cases) matched with Columbia-Presbyterian Medical Center (non-psychiatric cases, Table 1). The diagnostic groups were matched as closely as possible for source (New York or Macedonia), time in formalin, age and postmortem interval. The DSM-IV clinical diagnoses were established by standardized review of medical records with the Modified Diagnostic Evaluation After Death (mDEAD) (Keilp et al., 1995), applied to all New York

cases, or by psychological autopsy interview (Kelly and Mann, 1996), applied to all Macedonian non-psychiatric cases, or by both. Information about dominant hand use was available for nearly all subjects. Brains were included only if the entire HG and PT, extending to the caudal end of the Sylvian fissure, was available from both hemispheres. Comprehensive gross and microscopic examinations, including immunohistochemistry for Alzheimer-type pathology on subjects aged 45 or older, were performed by an experienced neuropathologist as previously described (Dwork et al., 1998). Cases with clinically or pathologically diagnosed neurological disease or with incidental lesions in the region under study were excluded. Additionally, subjects were excluded if they had a history of abuse or dependence on any substance except tobacco, with the exception of one schizophrenia and one nonpsychiatric subject with a history of alcohol abuse. The study procedures were approved by the Institutional Review Boards of the New York State Psychiatric Institute, the Nathan Kline Institute, and the School of Medicine, University “Ss. Cyril & Methodius.”

2.2 Tissue processing and identification of cortical regions

Histological processing was done as previously described (Smiley et al., 2009). Briefly the PT or prefrontal cortex was fixed in formalin and blocked into 2 to 5 coronally oriented pieces. They were embedded in celloidin (nitrocellulose) and sectioned coronally at 80 μ m thickness with a sliding microtome. Consecutively numbered sections from all blocks in each hemisphere were treated as a single series of sections, and a randomly selected series of every 12th section was stained for Nissl substance with cresylecht violet (Chroma-Gesellschaft, Cellpoint Scientific, Rockville, MD).

The borders of the PT were defined as previously described (Smiley et al., 2009). The rostral border of the PT was the sulcus that formed the caudal-lateral edge of Heschl's gyrus. Its medial border was the fundus of the lateral sulcus, and its lateral border was the crest of the lateral sulcus. Its caudal border was the caudal terminus of the lateral sulcus. If there was a caudal branching of the lateral sulcus, we included only the ascending limb, following the practice of most published studies (Shapleske et al., 1999).

The PT was divided into rostral and caudal parts by a coronal plane placed at the caudal end of Heschl's sulcus, as previously described (Zetsche et al., 2001; Harasty et al., 2003; Smiley et al., 2009). In the few cases in which Heschl's sulcus extended nearly to the end of the PT, the caudal 25% of the sections through the PT were considered its caudal part, so that the caudal PT contained at least 6 sections. Neuron density measurements were from all sections in the caudal PT, but in the rostral PT were from the cortex directly lateral to primary auditory cortex (A1). Thus the rostral PT in this study differed slightly from that used in our previous study of PT width, which also included the anterior corner of the PT rostral to A1. The location of A1 was identified by its appearance in Nissl sections, and additionally by parvalbumin-immunolabeling in some brains.

Area 9 was sampled bilaterally in all cases except for one nonpsychiatric case in which only the left side was available and another in which neither side was available (Table 1). This area was identified cytoarchitectonically as previously described (Rajkowska and Goldman-Rakic, 1995a). Our regional mapping of the dorsolateral prefrontal cortex in one brain showed that area 9 was readily distinguished from area 10 rostrally, area 46 laterally, and area 8 caudally, although the borders between areas were typically seen as gradual transitions. A characteristic feature of area 9 in Nissl sections was its thin layer IV, that was bordered closely above and below by large pyramidal cells in layers IIIc and Va. Compared to area 46 laterally, area 9 had a thinner layer IV, lower neuron density that was especially apparent in layers III-V, a less organized layer VI with prominent polymorphic shaped pyramidal neurons, and a less obvious vertical organization into minicolumns. In our larger brain sample, we selected cortex with this appearance, taken from the superior frontal gyrus

2 to 3 cm caudal from the frontal pole, which was previously described as the most reliable center of area 9 (Rajkowska and Goldman-Rakic, 1995a).

2.3 Estimation of neuron densities

Neuron density was evaluated bilaterally, with 7 separate estimates from each hemisphere. The rostral and caudal PT were each evaluated in layers II, IIIc and VI, and area 9 was evaluated in layers IIIb-c. In the PT, lines were drawn through the middle of each of the three measured layers, extending the full width of the PT, and the optical disectors were distributed along each line at regular intervals with a systematically random starting point (Figure 1). Samples were taken from the middle of each layer in order to avoid variability associated with uncertain identification of laminar borders. Layer IIIc was selected on the basis of previous studies that found changes especially in this layer (Selemon et al., 1995; Sweet et al., 2004), while inclusion of layers II and VI allowed within brain comparisons of upper, middle and lower layers. Additionally, the middle of these selected layers could be unambiguously identified even in tangentially cut sections. Other layers were not included because of the large investment of time required to obtain each separate estimate of neuron density.

In the caudal PT, every available Nissl stained section was sampled ($n = 8.2 \pm 1.6$ sections per hemisphere, mean $\pm$ S.D.). In the rostral PT, the sections lateral to A1 were sampled, using either all Nissl stained sections or in a few cases every 2nd section, so that 9.9 ± 1.9 sections were measured per hemisphere. In prefrontal area 9, measurements were taken from 5 to 7 evenly spaced sections, using every 2nd or 3rd 0.96 mm spaced Nissl stained section. Following Selemon et al. (Selemon et al., 1995), measurements were taken from cortex on the dorsal surface or lateral wall of the first gyrus adjacent to the midsagittal fissure, avoiding cortex that was markedly distorted by gyral or sulcal folding (Figure 3A). Optical disectors were distributed in a grid pattern over the lower half of the area between the layer I-II border and the layer II-IV border (Figure 3B). This compartment corresponded well with cortical layers IIIb-c.

All tissue sections were first digitally imaged by making photomontages with StereoInvestigator software (MicroBrightfield, www.mbfbioscience.com). The photomontages resolution of 146 pixels per millimeter was more than adequate to identify cortical layers. Using Object-Image software (<http://simon.bio.uva.nl/>), the pixel coordinates of the photomontages were aligned to X-Y coordinates of a motorized microscope, and regions of interest delineated on the photomontages were sampled with a systematic random pattern of optical disectors. The density of sampling sites in each layer of each hemisphere was sufficient to consistently give coefficients of error (CE) of less than 10%, as calculated according to West and Gundersen (West and Gundersen, 1990). For individual estimates of neuron density, we used 120 ± 30 disectors in prefrontal cortex (CE = 0.04 ± 0.02 , mean $\pm$ S.D.), 238 ± 64 disectors in the rostral PT (CE = 0.03 ± 0.01), and 175 ± 25 disectors in the caudal PT (CE = 0.04 ± 0.01).

At each disector site, the user manually focused on the tissue surface, and a z-stack of digital images was acquired using a 100x oil immersion objective with a 1.3 numerical aperture, and a Pulnix TM-72EX video camera linked to a Scion LG3 frame grabber board in a Macintosh computer. In the PT, z-stacks had 2 μ m spacing between sections, the upper guard zone was 10 μ m, with disector dimensions of X=45 μ m, Y=45 μ m (22.5 μ m in layer II), and Z=16 μ m, and cells were counted if the nucleolus came into focus within the counting box.

Given approximately 0.7 μ m depth of focus of the objective, occasional small nucleoli (1.3 μ m or less) may have been overlooked if they were precisely localized between consecutive 2 μ m optical slices. To estimate the effect of this optical blind spot, we used

continuous focus optical disectors in the PT to resample nucleoli in layer II, where cells are the smallest. The mean nucleolus diameter, from 60 cells (from 6 brains) was $1.49 \pm 0.35 \mu\text{m}$ (mean $\pm$ S.D.). Assuming a normal distribution, it can be estimated that 30% of the nucleoli in this layer are smaller than $1.3 \mu\text{m}$, and these will have an average nucleolar diameter of about $1.1 \mu\text{m}$. Therefore, it is expected that they will not be visible when their center falls within the $0.2 \mu\text{m}$ center of the blind spot. As this blind spot center is $1/10^{\text{th}}$ of the depth of each optical slice, about 3% of nucleoli may have been overlooked in layer II, and less in lower layers that have larger cells with larger nucleoli.

Measurements in area 9 used a slightly different counting box: the spacing between z-stack images was $3 \mu\text{m}$ and the disector dimensions were $X=30 \mu\text{m}$, $Y=30 \mu\text{m}$ and $Z=24 \mu\text{m}$. Cells were counted if the nucleus came into focus while focusing through the counting frame. Counts were done at the microscope, and for every sampled neuron an image focused on the nucleolus was saved for cell size measurements.

Neurons were identified by the presence of well stained Nissl substance in the cytoplasm, a thinner nuclear membrane, and a nucleolus, whereas glia typically had poorly stained cytoplasm, thicker nuclear membranes and inhomogeneous chromatin. Cell counting was done blind to diagnosis, by a single user (KF) in prefrontal cortex, and by two users (KF and DP) in the PT, where final results were the average of the two measurements.

2.4 Estimation of neuron size

Neurons size was measured with the nucleator method (Gundersen, 1988). For each cell, the average radius was determined by measuring the length of 3 isotropic random lines from a point in the nucleolus to the cell periphery. In the PT, measurements were done on saved Z-stacks, and in area 9 on separate images focused on the nucleolus. Measurements were made on every second cell counted in the neuron density measurements. The measurement coefficients of error (Dorph-Petersen et al., 2004), were 0.08 ± 0.01 (mean $\pm$ S.D.) in the area 9 measures, and 0.06 ± 0.02 in the PT measures, and exceeded 0.1 in only 4 of the 333 separate neuron size estimates. As originally described (Gundersen, 1988) these size measurements might be biased if there is a systematic anisotropy in the shape and orientation of the sampled neurons. We are not aware of evidence for a systematic anisotropy in the regions sampled that would affect our group or hemispheric comparisons.

2.5 Statistical methods

Schizophrenia and nonpsychiatric subjects were selected to be matched as closely as possible for age, postmortem interval, and time in formalin (Table 1) and t-tests showed that there were no significant group differences in these parameters (age, $p = 0.64$; postmortem interval $p = 0.92$; time in formalin, $p = 0.20$; two-tailed t-tests). However, brain weight was unexpectedly larger in the schizophrenia sample at trend level significance ($p = 0.07$, two-tailed t-test), and there was an unexpected inverse correlation between brain weight and whole cortical width. Therefore, brain weight was included as a covariate in all analyses. Group comparisons were evaluated with a mixed model analysis of co-variance (ANCOVA). Between-subject comparisons included diagnosis (schizophrenia and nonpsychiatric) and within-subject comparisons included layer (II, IIC and VI) and hemisphere (left and right). Partial correlation analyses, using brain weight as a covariate, were used to compare upper and lower cortex widths with neuron densities. To simplify the correlations in the upper layers, the average of the densities in layers II and IIC were compared to the upper layer cortical widths. All statistical analyses were done with SPSS software (version 15.0).

Some results are presented as percent changes, which were calculated for group differences as $= 100 \times (\text{schizophrenia-control})/\text{control}$, or for hemispheric comparisons as $= 100 \times (\text{right-left})/\text{left}$.

3. Results

3.1 Rostral PT neuron density

Neuron density measurements in the rostral PT were taken from the cortex directly lateral to the cytoarchitectonically identified primary auditory cortex, extending from Heschl's sulcus to the lateral edge of the Sylvian fissure (Figure 1). Separate estimates in each hemisphere were from layers II, IIIc and VI. Three way ANCOVA (layer $\times$ hemisphere $\times$ group) showed no significant group differences ($F(1,18) = 0.08$, $p = 0.78$) group $\times$ layer difference ($F(1,18) = 0.47$, $p = 0.63$) or group $\times$ hemispheric differences ($F(1,18) = 0.09$, $p = 0.77$). There was a trend-level hemispheric asymmetry ($F(1,18) = 3.24$, $p = 0.09$) due to slightly greater density on the right in most layers in both diagnostic groups. As expected, there were large laminar differences in neuron density (Figure 2).

3.2 Caudal PT neuron density

A second set of neuron density measurements included the caudal PT, behind HG. These measurements also sampled layers II, IIIc and VI, from the fundus of the lateral sulcus to the lateral edge of the Sylvian fissure (Figures 1 and 2). Three way ANCOVA (layer $\times$ hemisphere $\times$ group) showed no significant group differences ($F(1,18) = 0.07$, $p = 0.79$) group $\times$ layer difference ($F(1,18) = 0.18$, $p = 0.84$) group $\times$ hemispheric differences ($F(1,18) = 0.79$, $p = 0.39$) or hemispheric asymmetry ($F(1,18) = 0.11$, $p = 0.75$). However, as described below, pre-planned hemispheric comparisons in only the non-psychiatric brains showed a trend-level hemispheric asymmetry.

3.3 Prefrontal area 9 neuron density

A third set of neuron density measurements evaluated bilateral area 9 of prefrontal cortex (Figures 2 and 3). BA9 was available from nine of the nonpsychiatric subjects, and in one of these only from the left hemisphere (Table 1). A single laminar compartment, corresponding to layers IIIb-c, was measured in this area. Two way ANCOVA (group $\times$ hemisphere) showed no significant group effect ($F(1,16) = 0.02$, $p = 0.88$) group $\times$ hemisphere effect ($F(1,16) = 0.17$, $p = 0.90$) or hemispheric asymmetry ($F(1,17) = 0.09$, $p = 0.23$).

3.4 Comparison of neuron density and cortical width

The above comparisons showed little evidence for a relationship between diagnostic groups and neuron density. This result is somewhat unexpected, because we had previously found thinner cortex in the upper layers of the left PT in the schizophrenia sample (Smiley et al., 2009), and an inverse correlation between cortex width and neuron density is predicted on the basis of previous studies (Selemon et al., 1998; Cullen et al., 2006). Theoretically, if cortical thinning is caused exclusively by decreased cortical neuropil, then the percent increase of neuron density should closely match the percent decrease in width. However, in these brains, the left upper layers were 6% (rostral PT) and 8% (caudal PT) thinner in the schizophrenia sample, but the neuron densities (average of layers II and III) were only 0.5 and 1.5% greater, respectively. Another possibility is that there is a subtle decrease in neuron number in the superficial layers. We did not estimate neuron number in the present study, due to the difficulty of defining a reference volume. It is instructive to consider an alternative analysis, that calculates the neuron number per surface area of cortex by multiplying the upper layer widths by the average of the layer II/III neuron densities. In the left caudal PT, which had the largest group difference in cortical width, the difference

between the nonpsychiatric ($85,198 \pm 13,817$ neurons/mm² surface area) and schizophrenia ($78,256 \pm 7,778$) brains was not significant ($p = 0.17$, t-test). Power analysis (Faul et al., 2007) indicates that samples sizes of 36 per group would be need to obtain a power of 0.8 to demonstrate this differences at the error probability $\alpha = 0.05$. As considered below (see Discussion) there are potential theoretical objections to using cortical width as a reference volume for estimation of neuron number.

3.5 Nonpsychiatric hemispheric asymmetry of neuron density

Previous measurements of cortical width showed a significant hemispheric asymmetry in the caudal PT of the nonpsychiatric sample. The caudal left PT was thicker than the right by 7% in the upper layers and 4% in the lower layers. Measurements of neuron densities in the caudal PT of the nonpsychiatric sample showed a trend-level asymmetry (ANCOVA, 3 layers x 2 hemispheres, $F(1,17) = 3.5$, $p = 0.08$). In each layer the mean density was 4-10% higher in the right hemisphere (9.6% layer II, 4.5% layer IIIc, and 7.1% layer VI; Figure 2), consistent with the hypothesis that the right cortex is thinner because it has comparatively higher neuron density. This hypothesis predicts that the width asymmetry will correlate with the asymmetry of neuron densities. Consistent with this prediction, the layer VI neuron density asymmetry correlated with the lower layer width asymmetry ($r = -0.64$, $p = 0.03$). However, there was not a similar correlation of asymmetries in the upper layers ($r = 0.09$, $p = 0.66$), suggesting that neuron density may only partially explain the cortical width asymmetry.

3.6 Effects of tissue source and storage time on neuron density

Both the schizophrenia and nonpsychiatric samples each contained 7 brains from Macedonia, and 3 or 4 brains from New York (Table 1). Separation of these subgroups showed that the New York brains had higher densities, possibly due to their longer fixation times (Figure 4). Analyses (layer x hemisphere x group ANCOVA) of only the Macedonia subgroup showed group comparisons similar to the total sample. In the rostral PT there were not significant differences between diagnostic groups ($F(1,11) = 0.06$, $p = 0.81$), group x layer difference ($F(1,11) = 0.03$, $p = 0.97$), or group x hemispheric differences ($F(1,11) = 0.001$, $p = 0.98$), and in the caudal PT there were not significant group differences ($F(1,11) = 1.17$, $p = 0.30$) group x layer difference ($F(1,11) = 0.11$, $p = 0.89$) or group x hemispheric differences ($F(1,11) = 0.153$, $p = 0.70$).

3.7 Neuron soma size

We used the nucleator method to measure soma size of every second neuron sampled in the above neuron density measurements (Figures 1C and 5). In each region sampled, ANCOVA analysis did not show significant group, hemisphere, or group X hemisphere differences, and in the rostral and caudal PT there were no group x layer differences (all p values > 0.10).

For considerations of cortical shrinkage, it is of interest to know what percentage of the cortex is occupied by neuron cell bodies. This can be approximated in our preparation by using the average neuron densities and cell body volumes from layers II, IIIc and VI in the PT. Modeling cell bodies as spheres, the mean radius of $5.36 \mu\text{m}$ indicates a volume of $0.64 \times 10^{-6} \text{ mm}^3$. Multiplied by the average density of 59,600 neurons per mm^3 , the cell bodies occupy 0.038 mm^3 in each cubic mm of cortex, or about 3.8% of the cortical volume.

4. Discussion

4.1 Findings and limitations

The main finding of this study is that neuron densities and their respective hemispheric asymmetries in the PT were not significantly altered in schizophrenia brains, even in layers

of cortex that displayed selective thinning. Parallel neuron density measurements in area 9 of prefrontal cortex also showed no clear difference between schizophrenia and nonpsychiatric brains.

As is common with postmortem studies, our sample sizes were small, and it is possible that we overlooked group differences because of sampling biases. As reviewed in the following sections, some studies have found clearly increased neuron density in schizophrenia cortex, whereas other studies reported unchanged or even slightly decreased density. A possible explanation of these differences is that there are as of yet unidentified subtypes of schizophrenia with different cellular changes, similar to the previously identified clinical and pharmacological subtypes [e.g. (Scarr et al., 2009)]. In the present study nearly all of the schizophrenia subjects were severely affected patients who underwent long term hospitalization and treatment with typical antipsychotic medication. Longitudinal in vivo studies have reported progressive decrease in gray matter volume in schizophrenia, especially after treatment with typical antipsychotics (Lieberman et al., 2005; van Haren et al., 2007). Thus it might have been expected that our sample of schizophrenia brains would show especially pronounced cortical abnormalities.

Only male brains were used in the current study, in order to avoid possible variation based on sex. Most studies report that males and females have similar volume reductions of auditory areas in schizophrenia. However, there are reports in several brain areas of gender-specific changes of anatomical asymmetry in schizophrenia. In some studies, males had more pronounced loss of asymmetry than females [e.g., (Falkai et al., 1995; Rojas et al., 1997; Bryant et al., 1999; Frederikse et al., 2000; Vogeley et al., 2000)] whereas others found greater changes in females (Highley et al., 1999a) or asymmetry changes that were in opposite directions in males and females [e.g., (Highley et al., 1998; Highley et al., 1999b; McDonald et al., 2000; Highley et al., 2003)].

Our selection of areas in the PT for neuron density measurements was based on practical and historical considerations. The rostral PT lateral to the primary auditory cortex is mainly occupied by first and second order auditory association cortex (Hackett et al., 2001; Smiley, 2009). This portion of the PT is essentially identical to the region used in previous studies of cytoarchitectonic changes in schizophrenia (Sweet et al., 2004; Beasley et al., 2005; Konopaske et al., 2006) and in several studies of normal cytoarchitectonic asymmetries (Anderson et al., 1999; Harasty et al., 2003; Chance et al., 2006). The caudal PT contains mainly higher order association cortex, and its functions include phonetic processing, multisensory integration and spatial orientation (Vigneau et al., 2006; Smiley and Falchier, 2009).

Our neuron measurements did not include estimates of total neuron number, which require identification of the boundaries of cytoarchitectonic areas. Preliminary attempts to delineate areas in our Nissl stained material revealed that the boundaries were difficult to resolve in some brains, resulting in volume measurements that had high variance and questionable reliability. Instead, we confined our study to more reliable measurements of neuron density and cortical width which have a more realistic chance of detecting the subtle anatomical changes typically reported in the schizophrenia cerebral cortex.

4.2 Neuron density in schizophrenia cerebral cortex

Numerous in vivo imaging studies have reported cortical volume decreases of about 5-10% in various areas including prefrontal cortex and the superior temporal gyrus (Wright et al., 2000; Shenton et al., 2001; Smiley, 2009). As estimated in the present study, neuron cell bodies occupy less than 5% of the cortical volume, so their shrinkage alone is unlikely to affect cortical volume noticeably. Therefore, it is expected that a decrease of cortical volume

will correspond to a nearly proportional loss of the surrounding neuropil. If there is no loss of neurons, then neuron density will increase proportionally. For example, a 10% cortical volume decrease would reflect an approximately 10% decrease in neuropil volume, and without neuron loss an increase of approximately 11% in neuron density (e.g., 100 neurons/(1 - 0.10) mm³ = 111 neurons/mm³). Alternatively, the same neuropil decrease along with a 10% decrease in neuron number would result in essentially unchanged neuron density. Of course, other scenarios are possible: for example a 10% volume loss with a 5% neuron loss would predict about 5% greater neuron density.

Table 2 summarizes the published literature on Nissl-stained neuron density and number in the neocortex. Not included here are additional studies that measured neuron density in the hippocampus [for reviews see (Dwork, 1997; Harrison, 1999; Walker et al., 2002)] or that measured the density of subpopulations of total neurons [e.g., (Benes et al., 2001; Dorph-Petersen et al., 2009), for review see (Dwork et al., 2009)]. Most studies measured neuron density without estimating neuron number. In prefrontal areas 9 and 46, Selemon et al. (Selemon et al., 1995) found 17-21% increases in neuron density, and more recently replicated this finding in a separate sample of brains where neuron density in area 9 was 14% greater in schizophrenia (Selemon et al., 2003). This difference appeared to be regionally specific: in area 44 the density was non-significantly lower in schizophrenia. Pakkenberg (Pakkenberg, 1993) also found evidence for increased density, but it was not stated if the samples were matched, and importantly for density measurements, the brains had long postmortem intervals (this would not affect total numbers of neurons, and Pakkenberg (Pakkenberg, 1993) found no deficit in schizophrenia). In contrast, several other studies of area 9 and other cortical areas have generally found unchanged or slightly reduced neuron density, similar to the results obtained in the present study (Table 2).

Estimates of total neuron number are less common, because it is necessary to define a reference volume, which is problematic in cortical areas whose boundaries are not easily visualized. A few studies reported non-significant decreases in neuron number of 6-7% in areas 24 and 32 of cingulate cortex (Stark et al., 2004) and 10% in whole prefrontal cortex (Thune et al., 2001), whereas another (Ongur et al., 1998) reported a non-significant increase of 7% in subgenual area 24 and parietal area 3b. A recent study found a 25% decrease of neurons in primary visual cortex, but there were no reductions of cortical thickness or neuron density, suggesting that the difference was caused by reduced surface area (Dorph-Petersen et al., 2007). Together, these few studies provide tentative evidence for the possibility of modestly decreased neuron number in the cerebral cortex in schizophrenia.

In auditory cortex, we found largely unchanged neuron density even in the upper layers of the left PT that were 6-8% thinner. If cortex width were to be used as a proxy for volume, then the 8% thinner upper layers in our caudal left PT, combined with about 2% average greater neuron density in layers II-III, indicates a 6% decrease of neuron number. This result is similar to a recent study of the rostral PT by Beasley et al. (Beasley et al., 2005) that used 2-dimensional counting methods. While that study reported non-significant changes, the layer I-III width was about 8% thinner in schizophrenia, and the average neuron density in those layers was about 3% greater. Another study in primary auditory cortex found an increase in the density of morphologically identified layer III pyramidal cells, but it was not clear to what extent this neuron subset corresponds to the total neuron density (Dorph-Petersen et al., 2009).

The combination of reduced width and unchanged neuron density suggests lower neuron number, but theoretical objections can be raised to this calculation. First, it can be argued that, even though the cortex is thinner, it may have increased surface area and thus unchanged or

even increased volume. This argument is difficult to refute, because definitive surface area boundaries are not available in the PT. It can only be argued that *in vivo* studies generally have not demonstrated the combination of decreased thickness and increased surface area. In contrast, a study of early onset schizophrenia concluded that volume reduction was caused by both thinner cortex and reduced surface area (Voets et al., 2008). If correct, this suggests that calculations based on thickness alone actually underestimate the deficit of neuron number in the PT. Second, it could be suggested that our study, as well as that of Beasley et al. (2005), did not clearly identify cytoarchitectonic boundaries, and the differences between diagnostic groups may reflect greater inclusion in the schizophrenia sample of some cytoarchitectonic area with thinner layers. Arguing against this scenario, we observed similar results in both the rostral and caudal PT, and the study of Beasley et al. was confined to the rostral PT. Third, it might be argued that selectively reduced width of the upper layers is caused by differential gyrification. Sulci have disproportionately thin lower layers, so the relatively thinner upper layers in schizophrenia could be caused a lower density of sulci. In the PT we addressed this by deleting sulci from our width measurements, and this did not alter the finding of thinner upper layers in schizophrenia (Smiley et al., 2009).

Possible decreases in neuron number might be caused by disrupted neurogenesis or by neuron loss. Findings of increased neuron density in the subcortical white matter suggest that developing neurons do not reach their final cortical positions (Akbarian et al., 1993; Connor et al., 2009), but this alteration of neuron density is not universally replicated [reviewed in (Dwork et al., 2009)]. In the adult schizophrenia cortex, there is some evidence for neuronal apoptosis (Jarskog et al., 2005; Glantz et al., 2006). Additionally, there are reports of reduced numbers of GABA neurons (Benes et al., 2001; Beasley et al., 2002; Cotter et al., 2002a; Chance et al., 2005) but other studies did not find this deficit (Daviss and Lewis, 1995; Woo et al., 1997; Tooney and Chahl, 2004).

It should be noted as well that some studies have found reduced density of glial cells in the cerebral cortex from individuals with schizophrenia (Benes et al., 1986; Cotter et al., 2001b; Cotter et al., 2002b; Stark et al., 2004; Beasley et al., 2005), although this finding is not universally reported (Ongur et al., 1998; Selemon et al., 1998; Cullen et al., 2006). We did not measure glial cell density, but our findings of unchanged neuron density are not inconsistent with glial changes: several studies reported reduced glial cell density in the presence of unchanged neuron density (Cotter et al., 2001b; Cotter et al., 2002b; Stark et al., 2004; Beasley et al., 2005). Similarly, our measurements of neuron densities may be insensitive to disruptions of the laminar and minicolumnar organization of cortex indicated by some studies (Benes, 1987; Beasley et al., 2005; Casanova et al., 2005; Casanova et al., 2008; Chance et al., 2008).

In summary, there are theoretical challenges to demonstrating decreased neuron number by combining cortical width and neuron density. Additionally, our findings must be considered with caution in light of the small samples and small effect sizes. In particular, there is a disparity between studies that have found increased neuron density in schizophrenia cortex and those that have not (Table 2), and one possible explanation is that there is some schizophrenia subtype with increased neuron density that is under-represented in our sample. Nevertheless, our observation of cortical thinning without increased neuron density in this sample suggests that decreased neuron number may also contribute to the widespread cortical thinning consistently reported by *in vivo* studies (Kuperberg et al., 2003; White et al., 2003; Narr et al., 2005; Wang et al., 2007; Nesvag et al., 2008).

4.3 Neuron size in schizophrenia cerebral cortex

If schizophrenia is associated with a reduction of the neuropil, it might be expected that neuron somas would be reduced in size. In our study, neither neuron size nor neuron density

were reduced in the schizophrenia sample. Previous studies of neuron size have produced inconsistent findings. Reduced size was reported across layers of prefrontal cortex (Rajkowska et al., 1998) and in layer II of the insula (Pennington et al., 2008). However, other studies of prefrontal, cingulate, and auditory areas reported unchanged neuron size (Benes et al., 2001; Cotter et al., 2001a; Cotter et al., 2002b; Selemon et al., 2003; Beasley et al., 2005). More selective studies might reveal more consistent positive findings. For example, reduced size (Pierri et al., 2001; Sweet et al., 2004) or altered asymmetry (Cullen et al., 2006) of pyramidal-shaped cells has been reported, although this has not been observed in other studies of immunolabeled pyramidal neurons, possibly for technical reasons [for discussion see (Miguel-Hidalgo et al., 2005)].

4.3 Normal hemispheric asymmetries of neuron density

Our previous findings showed a left>right width asymmetry in the caudal PT (Smiley et al., 2009), consistent with previous *in vivo* findings (Hamilton et al., 2007). The present results showed a trend-level right>left neuron density, and at least in the lower layers there was some correlation between the width asymmetry and the neuron density asymmetry. These findings suggest that changes in neuron packing density may account in part for hemispheric differences in cortical width. This interpretation appears to be approximately consistent with previously described cytoarchitectonic asymmetries [reviewed in (Smiley, 2009)] including findings that the left PT has larger layer III pyramidal cells (Hutsler, 2003), more white matter, thicker myelin sheaths (Anderson et al., 1999), and greater spacing between minicolumns (Buxhoeveden and Casanova, 2000; Buxhoeveden et al., 2001; Chance et al., 2008). Together, these changes might be interpreted to suggest a proportionally greater volume of neuropil and possibly a greater number of synaptic connections in the left hemisphere. However, a relationship between these structural asymmetries has not been established, and it should be noted that the cortical width asymmetry is most clear in the caudal PT, whereas most of the other differences were studied in the rostral PT. There is a need for more definitive cytoarchitectonic criteria to guide investigations of PT asymmetry.

In prefrontal area 9, a previous hemispheric comparison of neuron density found a left>right asymmetry of neuron density and pyramidal cell size in nonpsychiatric brains (Cullen et al., 2006). Our results did not replicate this normal hemispheric asymmetry, but both studies were small, and that of Cullen et al. (2006) included both men and women.

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References

- Akbarian S, Bunney WE, Potkin SG, Wigal SB, Hagman JO, Sandman CA, Jones EG. Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. *Archives of General Psychiatry*. 1993; 50:169–177. [PubMed: 7679891]
- Anderson B, Southern BD, Powers RE. Anatomic asymmetries of the posterior superior temporal lobes: a postmortem study. *Neuropsychiatry, Neuropsychology and Behavioral Neurology*. 1999; 12:247–254.
- Beasley CL, Chana G, Honavar M, Landau S, Everall IP, Cotter D. Evidence for altered neuronal organisation within the planum temporale in major psychiatric disorders. *Schizophrenia Research*. 2005; 73(1):69–78. [PubMed: 15567079]

- Beasley CL, Zhang ZJ, Patten I, Reynolds GP. Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biological Psychiatry*. 2002; 52(7):708–715. [PubMed: 12372661]
- Benes FM, Bird ED. An analysis of the arrangement of neurons in the cingulate cortex of schizophrenic patients. *Archives of General Psychiatry*. 1987; 44:608–616. [PubMed: 3606326]
- Benes FM, Davidson J, Bird ED. Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. *Archives of General Psychiatry*. 1986; 43:31–35. [PubMed: 3942472]
- Benes FM, Vincent SL, Todtenkopf M. The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. *Biological Psychiatry*. 2001; 50(6): 395–406. [PubMed: 11566156]
- Bryant NL, Buchanan RW, Vladar K, Breier A, Rothman M. Gender differences in temporal lobe structures of patients with schizophrenia: a volumetric MRI study. *American Journal of Psychiatry*. 1999; 156(4):603–609. [PubMed: 10200741]
- Buxhoeveden D, Casanova M. Comparative lateralisation patterns in the language area of human, chimpanzee, and rhesus monkey brains. *Laterality*. 2000; 5(4):315–330. [PubMed: 15513150]
- Buxhoeveden DP, Switala AE, Roy E, Litaker M, Casanova MF. Morphological differences between minicolumns in human and nonhuman primate cortex. *American Journal of Physical Anthropology*. 2001; 115(4):361–371. [PubMed: 11471134]
- Casanova MF, de Zeeuw L, Switala A, Kreczmanski P, Korr H, Ulfing N, Heinsen H, Steinbusch HW, Schmitz C. Mean cell spacing abnormalities in the neocortex of patients with schizophrenia. *Psychiatry Research*. 2005; 133(1):1–12. [PubMed: 15698672]
- Casanova MF, Kreczmanski P, Trippe J 2nd, Switala A, Heinsen H, Steinbusch HW, Schmitz C. Neuronal distribution in the neocortex of schizophrenic patients. *Psychiatry Research*. 2008; 158(3):267–277. [PubMed: 18280583]
- Chance SA, Casanova MF, Switala AE, Crow TJ. Minicolumnar structure in Heschl's gyrus and planum temporale: Asymmetries in relation to sex and callosal fiber number. *Neuroscience*. 2006; 143(4):1041–1050. [PubMed: 17049176]
- Chance SA, Casanova MF, Switala AE, Crow TJ. Auditory cortex asymmetry, altered minicolumn spacing and absence of ageing effects in schizophrenia. *Brain*. 2008; 131(Pt 12):3178–3192. [PubMed: 18819990]
- Chance SA, Walker M, Crow TJ. Reduced density of calbindin-immunoreactive interneurons in the planum temporale in schizophrenia. *Brain Research*. 2005; 1046(1-2):32–37. [PubMed: 15927548]
- Connor CM, Guo Y, Akbarian S. Cingulate white matter neurons in schizophrenia and bipolar disorder. *Biological Psychiatry*. 2009; 66(5):486–493. [PubMed: 19559403]
- Cotter D, Landau S, Beasley C, Stevenson R, Chana G, MacMillan L, Everall I. The density and spatial distribution of GABAergic neurons, labelled using calcium binding proteins, in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia. *Biological Psychiatry*. 2002a; 51(5):377–386. [PubMed: 11904132]
- Cotter D, Mackay D, Chana G, Beasley C, Landau S, Everall IP. Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cerebral Cortex*. 2002b; 12(4):386–394. [PubMed: 11884354]
- Cotter D, Mackay D, Landau S, Kerwin R, Everall I. Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. *Archives of General Psychiatry*. 2001a; 58(6):545–553. [PubMed: 11386983]
- Cotter DR, Pariante CM, Everall IP. Glial cell abnormalities in major psychiatric disorders: the evidence and implications. *Brain Research Bulletin*. 2001b; 55(5):585–595. [PubMed: 11576755]
- Cullen TJ, Walker MA, Eastwood SL, Esiri MM, Harrison PJ, Crow TJ. Anomalies of asymmetry of pyramidal cell density and structure in dorsolateral prefrontal cortex in schizophrenia. *British Journal of Psychiatry*. 2006; 188:26–31. [PubMed: 16388066]
- Daviss SR, Lewis DA. Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. *Psychiatric Research*. 1995; 59:81–96.

- Dorph-Petersen KA, Delevich KM, Marcsisin MJ, Zhang W, Sampson AR, Gundersen HJ, Lewis DA, Sweet RA. Pyramidal neuron number in layer 3 of primary auditory cortex of subjects with schizophrenia. *Brain Research*. 2009; 1285:42–57. [PubMed: 19524554]
- Dorph-Petersen KA, Pierri JN, Sun Z, Sampson AR, Lewis DA. Stereological analysis of the mediodorsal thalamic nucleus in schizophrenia: volume, neuron number, and cell types. *Journal of Comparative Neurology*. 2004; 472(4):449–462. [PubMed: 15065119]
- Dorph-Petersen KA, Pierri JN, Wu Q, Sampson AR, Lewis DA. Primary visual cortex volume and total neuron number are reduced in schizophrenia. *Journal of Comparative Neurology*. 2007; 501(2):290–301. [PubMed: 17226750]
- Dwork A, Liu D, Kaufman M, Prohovnik I. Archival, formalin-fixed tissue: its use in the study of Alzheimer's type changes. *Clinical Neuropathology*. 1998; 17:45–49. [PubMed: 9496540]
- Dwork AJ. Postmortem studies of the hippocampal formation in schizophrenia. *Schizophrenia Bulletin*. 1997; 23:385–402. [PubMed: 9327505]
- Dwork, AJ.; Smiley, JF.; Colibazzi, T.; Hoptman, MJ. Postmortem and *in vivo* structural pathology in schizophrenia. In: Charney, DS.; Nestler, EJ., editors. *Neurobiology of Mental Illness*. Oxford University Press; Oxford: 2009. p. 281-302.
- Falkai P, Bogerts B, Schneider T, Greve B, Pfeiffer U, Pilz K, Gonsiorczyk C, Majtenyi C, Ovary I. Disturbed planum temporale asymmetry in schizophrenia. A quantitative post-mortem study. *Schizophrenia Research*. 1995; 14:161–176.
- Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavioral Research Methods*. 2007; 39(2): 175–191.
- Frederikse M, Lu A, Aylward E, Barta P, Sharma T, Pearlson G. Sex differences in inferior parietal lobule volume in schizophrenia. *American Journal of Psychiatry*. 2000; 157:422–427. [PubMed: 10698819]
- Galaburda A, Sanides F. Cytoarchitectonic organization of the human auditory cortex. *Journal of Comparative Neurology*. 1980; 190:597–610. [PubMed: 6771305]
- Glantz LA, Gilmore JH, Lieberman JA, Jarskog LF. Apoptotic mechanisms and the synaptic pathology of schizophrenia. *Schizophrenia Research*. 2006; 81(1):47–63. [PubMed: 16226876]
- Gundersen HJ. The nucleator. *Journal of Microscopy*. 1988; 151(Pt 1):3–21. [PubMed: 3193456]
- Hackett, TA. Organization and correspondence of the auditory cortex of humans and nonhuman primates. In: Kaas, J., editor. *Evolution of the Nervous System*. Elsevier; Oxford: 2007. p. 109-119.
- Hackett TA, Preuss TM, Kaas JH. Architectonic identification of the core region in auditory cortex of macaques, chimpanzees, and humans. *Journal of Comparative Neurology*. 2001; 441:197–222. [PubMed: 11745645]
- Hamilton LS, Narr KL, Luders E, Szaszko PR, Thompson PM, Bilder RM, Toga AW. Asymmetries of cortical thickness: effects of handedness, sex, and schizophrenia. *Neuroreport*. 2007; 18(14):1427–1431. [PubMed: 17712268]
- Harasty J, Seldon HL, Chan P, Halliday G, Harding A. The left human speech-processing cortex is thinner but longer than the right. *Laterality*. 2003; 8(3):247–260. [PubMed: 15513225]
- Harrison PJ. The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain*. 1999; 122(Pt 4):593–624. [PubMed: 10219775]
- Highley JR, DeLisi LE, Roberts N, Webb JA, Relja M, Razi K, Crow TJ. Sex-dependent effects of schizophrenia: an MRI study of gyral folding, and cortical and white matter volume. *Psychiatry Research*. 2003; 124(1):11–23. [PubMed: 14511792]
- Highley JR, Esiri MM, McDonald B, Cortina-Borja M, Cooper SJ, Herron BM, Crow TJ. Anomalies of cerebral asymmetry in schizophrenia interact with gender and age of onset: a post-mortem study. *Schizophrenia Research*. 1998; 34:13–25. [PubMed: 9824873]
- Highley JR, Esiri MM, McDonald B, Cortina-Borja M, Herron BM, Crow TJ. The size and fiber composition of the corpus callosum with respect to gender and schizophrenia: a post-mortem study. *Brain*. 1999a; 122:199–110.
- Highley JR, McDonald B, Walker MA, Esiri MM, Crow TJ. Schizophrenia and temporal lobe asymmetry. *British Journal of Psychiatry*. 1999b; 175:127–134. [PubMed: 10627794]

- Hutsler JJ. The specialized structure of human language cortex: pyramidal cell size asymmetries within auditory and language-associated regions of the temporal lobes. *Brain and Language*. 2003; 86(2): 226–242. [PubMed: 12921766]
- Jarskog LF, Glantz LA, Gilmore JH, Lieberman JA. Apoptotic mechanisms in the pathophysiology of schizophrenia. *Progress in Neuropsychopharmacology and Biological Psychiatry*. 2005; 29(5): 846–858.
- Keilp JG, Waniak C, Goldman RG, Zemishlany Z, Alexander GE, Gibbon M, Wu A, Susser E, Prohovnik I. Reliability of post-mortem chart diagnoses of schizophrenia and dementia. *Schizophrenia Research*. 1995; 17(2):221–228. [PubMed: 8562497]
- Kelly TM, Mann JJ. Validity of DSM-III-R diagnosis by psychological autopsy: a comparison with clinician ante-mortem diagnosis. *Acta Psychiatrica Scandinavica*. 1996; 94(5):337–343. [PubMed: 9124080]
- Konopaske GT, Sweet RA, Wu Q, Sampson A, Lewis DA. Regional specificity of chandelier neuron axon terminal alterations in schizophrenia. *Neuroscience*. 2006; 138(1):189–196. [PubMed: 16337746]
- Kuperberg GR, Broome MR, McGuire PK, David AS, Eddy M, Ozawa F, Goff D, West WC, Williams SC, van der Kouwe AJ, Salat DH, Dale AM, Fischl B. Regionally localized thinning of the cerebral cortex in schizophrenia. *Archives of General Psychiatry*. 2003; 60(9):878–888. [PubMed: 12963669]
- Lieberman JA, Tollefson GD, Charles C, Zipursky R, Sharma T, Kahn RS, Keefe RS, Green AI, Gur RE, McEvoy J, Perkins D, Hamer RM, Gu H, Tohen M. Antipsychotic drug effects on brain morphology in first-episode psychosis. *Archives of General Psychiatry*. 2005; 62(4):361–370. [PubMed: 15809403]
- 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. *American Journal of Psychiatry*. 2000; 157:40–47. [PubMed: 10618011]
- Miguel-Hidalgo JJ, Dubey P, Shao Q, Stockmeier C, Rajkowska G. Unchanged packing density but altered size of neurofilament immunoreactive neurons in the prefrontal cortex in schizophrenia and major depression. *Schizophrenia Research*. 2005; 76(2-3):159–171. [PubMed: 15949649]
- Morosan P, Rademacher J, Schleicher A, Amunts K, Schormann T, Zilles K. Human primary auditory cortex: cytoarchitectonic subdivisions and mapping into a spatial reference system. *Neuroimage*. 2001; 13:684–701. [PubMed: 11305897]
- Narr KL, Bilder RM, Toga AW, Woods RP, Rex DE, Szeszko PR, Robinson D, Sevy S, Gunduz-Bruce H, Wang YP, DeLuca H, Thompson PM. Mapping cortical thickness and gray matter concentration in first episode schizophrenia. *Cerebral Cortex*. 2005; 15(6):708–719. [PubMed: 15371291]
- Nesvag R, Lawyer G, Varnas K, Fjell AM, Walhovd KB, Frigessi A, Jonsson EG, Agartz I. Regional thinning of the cerebral cortex in schizophrenia: effects of diagnosis, age and antipsychotic medication. *Schizophrenia Research*. 2008; 98(1-3):16–28. [PubMed: 17933495]
- Ongur D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Science U S A*. 1998; 95(22):13290–13295.
- Pakkenberg B. Total nerve cell number in neocortex in chronic schizophrenics and controls estimated using optical disectors. *Biological Psychiatry*. 1993; 34:768–772. [PubMed: 8292680]
- Pennington K, Dicker P, Hudson L, Cotter DR. Evidence for reduced neuronal somal size within the insular cortex in schizophrenia, but not in affective disorders. *Schizophrenia Research*. 2008
- 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. *Archives of General Psychiatry*. 2001; 58(5):466–473. [PubMed: 11343526]
- Rademacher J, Caviness VS, Steinmetz H, Galaburda AM. Topographical variation of the human primary cortices: Implications for neuroimaging, brain mapping and neurobiology. *Cerebral Cortex*. 1993; 3:313–329. [PubMed: 8400809]
- Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in normal human cortex: II. Variability in locations of areas 9 and 46 and relationship to the Talairach coordinate system. *Cerebral Cortex*. 1995a; 5:323–337. [PubMed: 7580125]

- Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex: I. Remapping of areas 9 and 46 using quantitative criteria. *Cerebral Cortex*. 1995b; 5:307–322. [PubMed: 7580124]
- Rajkowska G, Selemon LD, Goldman-Rakic PS. Neuronal and glial somal size in the prefrontal cortex: a postmortem morphometric study of schizophrenia and Huntington disease. *Archives of General Psychiatry*. 1998; 55:215–224. [PubMed: 9510215]
- Rojas DC, Teale P, Sheeder J, Simon J, Reite M. Sex-specific expression of Heschl's gyrus functional and structural abnormalities in paranoid schizophrenia. *American Journal of Psychiatry*. 1997; 154(12):1655–1662. [PubMed: 9396942]
- Scarr E, Cowie TF, Kanellakis S, Sundram S, Pantelis C, Dean B. Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. *Molecular Psychiatry*. 2009; 14(11):1017–1023. [PubMed: 18317461]
- Selemon LD. Increased cortical neuronal density in schizophrenia. *American Journal of Psychiatry*. 2004; 161(9):1564. [PubMed: 15337643]
- Selemon LD, Mrzljak J, Kleinman JE, Herman MM, Goldman-Rakic PS. Regional specificity in the neuropathologic substrates of schizophrenia: a morphometric analysis of Broca's area 44 and area 9. *Archives of General Psychiatry*. 2003; 60(1):69–77. [PubMed: 12511174]
- Selemon LD, Rajkowska G, Goldman-Rakic PS. Abnormally high neuronal density in the schizophrenic cortex. *Archives of General Psychiatry*. 1995; 52:805–818. [PubMed: 7575100]
- Selemon LD, Rajkowska G, Goldman-Rakic PS. Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional stereologic counting method. *Journal of Comparative Neurology*. 1998; 392:402–412. [PubMed: 9511926]
- Shapleske J, Rossell SL, Woodruff PWR, David AS. The planum temporale: a systematic quantitative review of its structural, functional and clinical significance. *Brain Research. Brain Research Reviews*. 1999; 29:26–49. [PubMed: 9974150]
- Shenton ME, Dickey CC, Frumin M, McCarley RW. A review of MRI findings in schizophrenia. *Schizophrenia Research*. 2001; 49(1-2):1–52. [PubMed: 11343862]
- Smiley, JF. Auditory cortex anatomy and asymmetry in schizophrenia. In: Lajtha, A., editor. *Handbook of Neurochemistry and Molecular Neurobiology*. 3rd Edition. Springer; Schizophrenia. New York: 2009. p. 353-382.
- Smiley JF, Falchier A. Multisensory connections of monkey auditory cerebral cortex. *Hearing Research*. 2009; 258:37–46. [PubMed: 19619628]
- Smiley JF, Rosoklija G, Mancevski B, Mann JJ, Dwork AJ, Javitt DC. Altered volume and hemispheric asymmetry of the superficial cortical layers in the schizophrenia planum temporale. *European Journal of Neuroscience*. 2009; 30:449–463. [PubMed: 19656176]
- Stark AK, Sanz-Arigita E, Pakkenberg B. Glial cell loss in the anterior cingulate cortex, a subregion of the prefrontal cortex, in subjects with schizophrenia. *American Journal of Psychiatry*. 2004; 161:882–888. [PubMed: 15121654]
- Sweet RA, Bergen SE, Sun Z, Marcsisin MJ, Sampson AR, Lewis DA. Anatomical evidence of impaired feedforward auditory processing in schizophrenia. *Biological Psychiatry*. 2007; 61(7):854–864. [PubMed: 17123477]
- 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. *Biological Psychiatry*. 2004; 55(12):1128–1137. [PubMed: 15184031]
- Thune JJ, Uylings HB, Pakkenberg B. No deficit in total number of neurons in the prefrontal cortex in schizophrenics. *Journal of Psychiatric Research*. 2001; 35(1):15–21. [PubMed: 11287052]
- Tooney PA, Chahl LA. Neurons expressing calcium-binding proteins in the prefrontal cortex in schizophrenia. *Progress in Neuropsychopharmacology and Biological Psychiatry*. 2004; 28(2):273–278.
- van Haren NE, HulshoffPol HE, Schnack HG, Cahn W, Mandl RC, Collins DL, Evans AC, Kahn RS. Focal gray matter changes in schizophrenia across the course of the illness: a 5-year follow-up study. *Neuropsychopharmacology*. 2007; 32(10):2057–2066. [PubMed: 17327887]

- Vigneau M, Beaucousin V, Herve PY, Duffau H, Crivello F, Houde O, Mazoyer B, Tzourio-Mazoyer N. Meta-analyzing left hemisphere language areas: phonology, semantics, and sentence processing. *Neuroimage*. 2006; 30(4):1414–1432. [PubMed: 16413796]
- Voets NL, Hough MG, Douaud G, Matthews PM, James A, Winmill L, Webster P, Smith S. Evidence for abnormalities of cortical development in adolescent-onset schizophrenia. *Neuroimage*. 2008; 43(4):665–675. [PubMed: 18793730]
- Vogeley K, Schneider-Axmann T, Pfeiffer U, Tepest R, Bayer TA, Bogerts B, Honer WG, Falkai P. Disturbed gyrification of the prefrontal region in male schizophrenic patients: a morphometric postmortem study. *American Journal of Psychiatry*. 2000; 157:34–39. [PubMed: 10618010]
- Walker MA, Highley JR, Esiri MM, McDonald B, Roberts HC, Evans SP, Crow TJ. Estimated neuronal populations and volumes of the hippocampus and its subfields in schizophrenia. *American Journal of Psychiatry*. 2002; 159(5):821–828. [PubMed: 11986137]
- Wang L, Hosakere M, Trein JC, Miller A, Ratnanather JT, Barch DM, Thompson PA, Qiu A, Gado MH, Miller MI, Csernansky JG. Abnormalities of cingulate gyrus neuroanatomy in schizophrenia. *Schizophrenia Research*. 2007; 93(1-3):66–78. [PubMed: 17433626]
- West MJ, Gundersen HJG. Unbiased stereological estimation of the number of neurons in the human hippocampus. *Journal of Comparative Neurology*. 1990; 296:1–22. [PubMed: 2358525]
- White T, Andreasen NC, Nopoulos P, Magnotta V. Gyrification abnormalities in childhood-and adolescent-onset schizophrenia. *Biological Psychiatry*. 2003; 54(4):418–426. [PubMed: 12915286]
- Woo T-U, Miller JL, Lewis DA. Schizophrenia and the parvalbumin-containing class of cortical local circuit neurons. *American Journal of Psychiatry*. 1997; 154:1013–1015. [PubMed: 9210755]
- Wright IC, Rabe-Hesketh S, Woodruff PWR, David AS, Murray RM, Bullmore ET. Meta-analysis of regional brain volumes in schizophrenia. *American Journal of Psychiatry*. 2000; 157:16–25. [PubMed: 10618008]
- Zetsche T, Meisenzahl EM, Preuss UW, Holder JJ, Kathmann N, Leinsinger G, Hahn K, Hegerl U, Moller H-J. In-vivo analysis of the human planum temporale (PT): does the definition of PT borders influence the results with regard to cerebral asymmetry and correlation with handedness? *Psychiatric Research: Neuroimaging*. 2001; 107:99–115.

Figure 1.

Illustrations of the methods used to measure neuron density in the planum temporale (PT).

A) Measurements were taken from across the width of the PT. In these measurements, lines were traced along the horizontal length of the PT at the centers of layers II, IIIc, and VI, and optical disector sampling sites were systematically spaced on these lines. This method was used to avoid the ambiguity of identifying laminar interfaces, and to reduce the variance introduced by including multiple layers in each sample. The cortex in Heschl's sulcus (HS) lateral to A1 was not included in these measurements, because it is cytoarchitecturally distinct, and has been considered a primary-like auditory area by some authors (Galaburda and Sanides, 1980; Rademacher et al., 1993; Morosan et al., 2001). **B)** For each hemisphere, a 3-dimensional reconstruction was made from video images of the block face during sectioning. Reconstructions were made from the every 12th section, using the same series of sections used for Nissl staining. The parietal operculum was removed from the reconstruction in order to view the PT surface. Separate measurements of neuron density were taken from the rostral (PT_r) and caudal (PT_c) parts of the PT. The PT_r was defined as the cortex lateral to cytoarchitecturally-defined primary auditory cortex (A1). The PT_c was defined as the cortex caudal to the end of Heschl's sulcus (HS). The location of A1 was always on the caudal part of Heschl's gyrus (HG), occupying about half of its surface. **C)** Density measurements of Nissl-stained cells were made by overlaying a counting box onto video images that were captured with a 100x objective. Neuron size was measured by the nucleator method. The cell radius was the average length of three isotropic lines (white lines) measured from a point on the nucleolus to the cell periphery (white dots). Scale bar in A = 2mm, the section spacing in the reconstruction in B is 0.96 mm, and the width of the disector box in C = 30 μ m.

Figure 2. Summary of neuron density measurements. Measurements from the planum temporale (PT) were taken from layers II, IIIc and VI in both its rostral and caudal parts. In area 9 of prefrontal cortex, measurements were taken from layer IIIb-c. The box inset at the bottom shows the numerical values plotted in the bar graph. Filled bars show nonpsychiatric values and white bars show schizophrenia values. All values are mean $\pm$ S.D.

Figure 3.

Neuron density measurements were taken from cytoarchitecturally identified Brodmann's area 9 of prefrontal cortex, as described previously (Rajkowska and Goldman-Rakic, 1995b). **A)** This image of the grossly dissected, coronally cut tissue shows the location of the cortical region that was sampled (large black arrow). Measurements were restricted to approximately flat cortical surfaces on the dorsal or lateral surface of the superior frontal gyrus. The small black arrow indicates the location of the midsagittal fissure (msf), at the medial surface of the superior frontal gyrus. **B)** In BA9, the area between the top of layer 2 and the bottom layer 3 was divided in half, and measurements were taken from the bottom half that approximately corresponded to layers IIIb-c (dashed white line). This area was sampled with a grid of disector sites placed on the tissue in a systematically random distribution.

Figure 4.

Comparison of neuron densities in brain samples separated by source. As described in Table 1, the nonpsychiatric and schizophrenia samples each contained 7 brains from Macedonia (solid lines) and 3 or 4 brains collected in New York (dashed lines). The brains from New York had higher neuron densities, possibly due to their longer fixation times. As described in the text, analysis of only the Macedonian brains revealed group comparisons similar to the total brain sample. (Values on the X-axis represent cortical layers, “L” = left and “R” = right).

Figure 5. Summary of neuron size measurements. The nucleator method was used to estimate the average radius of every second cell counted in the neuron density measurements. The box inset at the bottom shows the numerical values plotted in the bar graph. Filled bars show nonpsychiatric values and white bars show schizophrenia values. All values are mean $\pm$ S.D.

Table 1

Brain samples, demographic and descriptive data

	age (years)	PMI (h)	Formald. (months)	weight (g)	Dominant hand	Tissue Origin	LNE (years)	Diagnosis	Cause of Death
Schizophrenia group									
D37 ⁺	43	19	6	1363	R	RM	>10	PS	7
D20	53	15	16	1358	R	RM	>10	RS	1
D39	58	13	7	1621	R	RM	>10	RS	2
D53	33	20	15	1389	R	RM	>10	DS	4
D55	49	41	4	1335	R	RM	>10	PS	5
D56	73	7	7	1420	R	RM	>10	US	8
D15	48	7	6	1395	R	RM	>10	SA ⁺⁺	8
D44	37	24	111	1500	R	NY	>10	US	8
D11	79	50	96	1440	R	NY	>10	US	1
D49	42	24	180	1450	R	NY	>10	PS	8
D51	29	24	180	1540	n.a.	NY	<5	US	6
Mean $\pm$ S.D.	49 $\pm$ 16	22 $\pm$ 13	57 $\pm$ 72	1437 $\pm$ 87					
Control group									
D38 ⁺	50	7	8	1306	R	RM			3
D14	60	23	17	1277	R	RM			9
D16	60	20	7	1436	R	RM			11
D52	31	4	15	1540	R	RM			10
D54	42	24	7	1405	R	RM			13
D13	80	9	16	1400	R	RM			11
D57	39	13	8	1230	R	RM			9
D12 [*]	44	24	111	1240	R	NY			8
D50	80	48	120	n.a.	R	NY			12
D33 ^{**}	42	24	129	1360	R	NY			1
Mean $\pm$ S.D.	53 $\pm$ 17	20 $\pm$ 13	44 $\pm$ 53	1355 $\pm$ 102					

Abbreviations: Form. = formaldehyde; LNE = lifetime neuroleptic exposure; M = mixed handed; n.a. = not available; NY = New York; PMI = postmortem interval; R = right handed; RM = Republic of Macedonia;

Diagnosis: DS = disorganized schizophrenia; PS = paranoid schizophrenia; RS = residual schizophrenia; SA = schizoaffective disorder; US = undifferentiated schizophrenia;

Cause of death: 1=pneumonia; 2=aspiration; 3=accidental electrocution; 4=hypothermia; 5=suicide; pesticide poisoning; 6=suicide hanging; 7=suicide jumping; 8=cardiorespiratory arrest; 9=traffic accident; shock traumatic shock; 10=homicide; 11=cerebral contusion; 12=gastrointestinal bleeding; 13=myocarditis; 14=lung cancer and hemorrhage; 15=renal failure.

+ alcohol abuse

++ mild mental retardation

* Right prefrontal not available

** Left and right prefrontal not available.

Table 2

Literature of neuron counts in schizophrenia neocortex

Reference	Neuron density % difference by cortical region					Neuron number % difference	N Sz	N Cntr	Comment
	Frontal	Cingulate	Temporal	Parietal	Occipital				
3D counting methods									
Present Study	-1 BA9		-2 BA42		-4 BA17	--	11	9	¥
“ ”						--	11	10	¥
Dorph-Peterson (2007)						-25 BA17	10	10	
Cullen et al. 2006	-8 BA9					--	10	10	+
Stark et al. (2004)		-4 BA24				-6 BA24	12	14	
“ ”		0 BA32				-7 BA32	“ ”	“ ”	
Selemon et al. (2003)	+14 BA9					--	6	9	
“ ”	-3 BA44					--	9	14	
Cotter et al., (2001)		-2 BA24				--	15	15	¥ +
“ ” Cotter et al. (2004)			-1 BA41			--	“ ”	“ ”	¥ +
Thune (2001)	-5 PFC					-10 PFC	8	10	
Ongur et al. (1998)		0 BA24sg				+7 BA24sg	11	11	¥ +
“ ”				+7 BA3b		+7 BA3b	13	12	¥ +
Selemon et al. (1995/1998)	+17 BA9					--	16	19	
“ ”	+21 BA46					--	9	10	
“ ”					+10 BA17	--	9	10	
Pakkenberg (1993)	+13 FL		+29 TL	+6 PL	+16 OL	-5 PFC,+10 TL,-14 PL,+20 OL	8	16	++
“ ”									
2D counting methods									
Beasley et al. (2005)			-2 BA42			--	15	15	¥ +
“ ” Chana et al. (2003)		+17 BA24b				--	“ ”	“ ”	¥ +
“ ” Cotter et al. (2002)	-1 BA9					--	“ ”	“ ”	¥ +
Akbarian (1995)	+1 BA9					--	10	10	¥

Reference	Neuron density % difference by cortical region				Neuron number % difference	N Sz	N Cntr	Comment
	Frontal	Cingulate	Temporal	Parietal	Occipital			
Benes et al. (1986)	-17 BA10	-12 BA24				10	9	¶
" "	-21 BA4					8	5	

Abbreviations: BA = Brodmann's area; FL = frontal lobe; OL = occipital lobe; PFC = prefrontal cortex; PL = parietal lobe;PT=planum temporale; TL = temporal lobe.

⁺ Sz sample had longer fixation time

⁺⁺ PMI, time in formalin, and type of Sz not reported

¶ Values are average of reported L1-6 densities

‡ Values are average of left and right hemispheres, and all layers measured.