Evidence for orbitofrontal pathology in bipolar disorder and major depression, but not in schizophrenia


Background: The orbitofrontal cortex is involved in the monitoring of reward and in judgement. Lesion studies and functional neuroimaging investigations implicate this region in affective disorders, and altered neuronal and glial cell composition have been observed in this region in subjects with major depressive disorder (MDD).

Aims: Stereologically based investigation of caudal orbitofrontal cortex (cOFC), in 60 postmortem brains from four groups of 14 subjects each with bipolar disorder (BPD), schizophrenia and MDD.

Methods: Glial cell and neuronal size and density were examined in all subjects using stereological probes such as the nucleator and the optical disector.

Results: We found statistical evidence for a neuronal size reduction in BPD in layer 1 (21%, \( p = 0.007 \)) and a trend for a reduction in layer 5 (20%, \( p = 0.05 \)). There was a significant interaction effect of brain hemisphere and group on neuronal size in layer 3 (\( p = 0.001 \)), with evidence for reduced layer 3 neuronal sizes in MDD (30%, \( p < 0.001 \)). We found no evidence for group differences in glial cell size nor for differences in glial or neuronal density.

Conclusions: These findings provide preliminary evidence that neuronal size reduction in cOFC is a component of the pathology of BPD. Overall, the data implicate this cortical region in affective disorders, but provide no evidence for neuronal or glial pathology in this region in schizophrenia.

The neuroanatomy of bipolar disorder (BPD), until recently described as an ‘uncharted wilderness’ (1), is slowly being delineated. Now, structural and functional neuroimaging investigations have shown replicable changes in certain cortical and subcortical brain regions (2–5), and these regions have become candidate regions for investigations by histological means. Coincidentally, the availability of appropriate tissues for investigation has made the cytoarchitectural exploration of this disorder a real possibility.

The orbitofrontal cortex (OFC) is one such candidate cortical region in BPD. Working in an integrated way with the amygdala (6), it plays an important role in the neural system that underlies emotional processing (7) and decision making (8), and it has been shown to demonstrate altered activation in subjects with BPD (9, 10). There is also evidence that OFC volume may be reduced in BPD (11). In MDD, functional imaging investigations have repeatedly shown altered activation (12, 13) and decreased blood flow (14) in the OFC and while few volumetric studies have specifically
assessed this region, there is evidence that the volume of this region is reduced (15). Taken as a whole, this evidence indicates that the OFC has an important role in affective disorders.

However, the OFC is not an anatomically uniform region. It is composed of different subregions, with distinct functions (8), and most studies have not attempted to specifically assess these subregions. Of those that have, there is evidence that medial (13, 15) and lateral (16) OFC are reduced in volume and activation in MDD. There is also evidence that rostral and lateral OFC activation (9) is abnormal during a manic episode and that lateral OFC is also reduced in volume in BPD (17). However, a number of other studies have examined gross volumetric measures of the whole brain and prefrontal cortices and have found no consistent differences between BPD and controls (4).

In the single previous cytoarchitectural investigation of the OFC (18) in major psychiatric disorders, a deficit of glial cells and a reduction in neuronal size was observed in MDD. This latter finding of a cortical glial cell deficit in MDD has been a fairly consistent one, observed also in the anterior cingulate cortex (ACC) (19, 20) and dorsolateral prefrontal cortex (DLPFC) (21). In contrast, while findings of cortical glial cell deficits (18, 19) in BPD have not been consistent (1, 20–22), evidence for neuronal size reductions in layer 5 of the DLPFC (18, 21) and the ACC (23) is mounting. The cytoarchitecture of the OFC has not been assessed in BPD.

Considering the role of the OFC in the processing of emotional information, and the evidence that this region is implicated in BPD (7, 9), we set out to characterize neuronal and glial cell density and neuronal size in the OFC in normal human brain, BPD, MDD and schizophrenia.

**Methods**

**Subjects and tissue**

Human brain specimens from the caudal orbitofrontal cortex (cOFC; Brodmann’s area) (24) were obtained from the Stanley Foundation Brain Consortium (25). The sample consisted of 60 subjects (15 normal controls, 15 schizophrenics, 15 BPD and 15 MDD). Diagnoses were made according to Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria. Detailed case summaries were provided on demographic, clinical and histological information (see Table 1 for group summary details). All brains underwent clinical neuropathological examination and none demonstrated evidence of neurodegenerative changes or other pathological lesions. mRNA levels of the housekeeping gene glyceraldehyde phosphate dehydrogenase were measured in the Stanley Foundation Brain Consortium laboratory by the reverse transcription-polymerase

<table>
<thead>
<tr>
<th>Table 1. Group summaries of demographic, histological and clinical information on the brains donated by the Stanley Foundation Brain Consortium</th>
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<tbody>
<tr>
<td><strong>Variable</strong></td>
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<tr>
<td><strong>Demographics</strong></td>
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<tr>
<td>Age at death in years (mean ± SD)</td>
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<tr>
<td>Gender (male, female)</td>
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<td><strong>Histological</strong></td>
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<tr>
<td>Fixation time in days (mean ± SD)</td>
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<td>Postmortem interval in hours (mean ± SD)</td>
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<td>Brain hemisphere (right:left)</td>
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<td>pH (mean ± SD)</td>
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<tr>
<td><strong>Clinical</strong></td>
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<tr>
<td>Cause of death</td>
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<tr>
<td>Duration of disorder in years (mean ± SD)</td>
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<tr>
<td>Fluphenazine mg equivalents (minimum; median; maximum)</td>
</tr>
<tr>
<td>Past alcohol/drug abuse or dependence (no:yes)</td>
</tr>
<tr>
<td>Current alcohol/drug abuse or dependence (no:yes)</td>
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<tr>
<td>Treated with antidepressants at death (no:yes)</td>
</tr>
<tr>
<td>Lithium treatment at death (no:yes:mood stabilizer)</td>
</tr>
<tr>
<td>Family history of disorder (none:SCZ:BP:MDD:unknown)</td>
</tr>
<tr>
<td>Death by suicide (no:yes)</td>
</tr>
</tbody>
</table>

SCZ = schizophrenia; BPD = bipolar disorder; MDD = major depressive disorder. Fluphenazine mg equivalents is lifetime neuroleptic dose in fluphenazine milligram equivalent dose. Cause of death is categorized under the following headings: cardiovascular = a; road traffic accident = b; suicide = c; alcohol intoxication = d; pneumonia = e; subdural haematoma = f; malnutrition = g; accidental drowning = h. Note that one subject with BPD had a family history of BPD and of MDD.
chain reaction and were excellent-to-good in all groups, demonstrating good tissue preservation. Three cases, one from each disorder group, were excluded from the investigation due to the presence of postmortem artefactual changes.

Tissue was available from only one hemisphere of each brain, with roughly equal numbers sampled in a random manner from each side of the brain (Table 1). Hemispheres were fixed in 10% phosphate-buffered formalin and then cut in coronal sections of roughly 1 cm thickness. From these slices a block was taken from the cOFC rostral to the tip of the genu of the corpus callosum and processed to paraffin wax. From these coronal blocks a series of 40 sections of 25 μm thickness were taken and from these four sections were systematically randomly sampled for analysis. All sections were then stained with cresyl violet according to standard methods.

Identification of the caudal orbitofrontal cortex

The cOFC was first identified upon macroscopic examination of the tissue block and then by microscopic examination of the tissue sections taken from this block. The region covers the lateral wall of the caudal orbitofrontal sulcus (see Fig. 1). Macroscopic and microscopic criteria used to identify this region have been described previously (18), and we based our identification on these criteria (see Fig. 2). Based on the sulco-gyral pattern we determined that our tissue sections were more rostral than those previously investigated by Rajkowska et al. (18), but still within the caudal orbitofrontal region.

3-D cell counting and neuronal size estimates

In this investigation neurons were identified by the presence of a cresyl violet-stained cytoplasm, a
single nucleolus, and their generally larger shape and non-spherical outline. Glia were identified by the absence of stained cytoplasm, the presence of a thicker nuclear membrane and more heterogeneous chromatin within the nucleus (see Fig. 3). Following mounting and staining of tissue sections, thickness of the tissue sections was assessed. This reduced from 25 μm to a mean of 14.5 μm (SD 2.3 μm). We used optical disectors of a constant depth of 10 μm and our guard volumes above and below the disector averaged at 2.25 μm. The dimensions of the dissectors used, in the x- and y-axis, were 103 × 75 μm for neurons and 51.5 × 75 μm for glia respectively. There was one disector per field.

Sections were viewed first using a 10x objective using a Leica DMLB microscope (Leica, Milton Keynes, UK), a Hitachi 3CCD colour camera (HVC20: Hitachi, London, UK), and a Marzhauser 100 × 100 x-, y-motorized stage (Marzhauser Wetzlar, Wetzlar-Steindorf, Germany) attached to a Heidenhain z-axis depth gauge which is accurate to < 1 μm. Using the software Image-Pro Plus 4.0/Stereology (Media Cybernetics, MD, USA), we obtained a series of contiguous colour images of the OFC from pia to the grey/white matter border and extending from near the depths of the sulcus to near the top of the gyrus. From these images a single composite image was formed, and on this we traced the borders of cortical layers 1–6, and also measured the thickness of each cortical layer. The software then selected points randomly within each cortical layer for density and size estimations. These were carried out using a Leica 100X (Fluotar; numerical aperture 1.3) oil immersion objective lens. Cell density estimations were made with the aid of the same image analysis software according to the stereological optical disector method (26).

A sampling strategy was optimized prior to the investigation so that an equal proportion of sampled neurons was obtained from each of the four sections used in each case. This involved estimating the number of fields required to give a total of approximately 100 sampled neurons per layer per case. For neuronal and glial estimations an average of 28 fields were counted per case for layers other than 2 and 4, and in layers 2 and 4 an average of 20 fields per case were counted. An average of 96 neurons and 174 glia were counted in each layer of each case (except for layer 1 neurons from which an average of 53 neurons per case were sampled). Values for the coefficient of error (27) of the neuronal and glial density estimates in the different cortical layers were < 5% for layers 2–6 and < 6% for layer 1.

The neuronal size of all disector sampled neurons was estimated using the stereological estimator of number weighted volume, the nucleator (28). Thus, we calculated the size (expressed in μm³) of almost 100 neurons from each individual layer of each subject. In the same way, glial nuclear size was assessed. Neuronal and glial cell densities are expressed as cells × 10³/mm³.

Statistical analysis
We analysed the thickness of layer, neuronal and glial cell density, and neuronal and glial cell size. The objective of each of these main statistical analyses was to compare the outcomes in each of the three patient groups with that of the controls. All five outcome variables were recorded for each of the six cortical layers and all group comparisons were carried out within layers. To account for the multiple layer-wise comparisons we compared the p-values of the group tests with the Bonferroni-adjusted significance level of 0.05/6 = 0.0083.

All group comparisons were adjusted for gender differences as the Stanley Foundation sample consisted of two subsamples drawn in each group; a subsample of size 9 from the male patient population and one of size 6 from the female population. The demographic and histological variables listed in Table 1 were considered potential confounders of group differences if they differed between the psychiatric groups and the

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**Fig. 3.** Nissl-stained layer 1 neurons (solid arrows) and glial cells (dotted arrows). Scale bar, 10 μm.
control group according to t-tests or chi-squared tests at the 10% significance level. Comparisons of cell sizes and cell densities between groups (see below) were also adjusted for section thickness to rule out any potential confounding due to tissue shrinkage (data not shown).

In addition, for each outcome variable a forward selection procedure with 10% inclusion threshold was employed to identify further demographic and histological variables which could be shown empirically to be predictors. Group comparisons of outcomes were then adjusted for potential confounders and empirical predictors identified in this way.

**Layer thickness.** As mentioned above the analysis of layer thickness was carried out in two stages. In the first stage a forward selection procedure was employed on the complete data set of layer-wise cortical widths (averaged over the four slides) to identify variables which could be shown empirically to predict layer thickness. For this purpose a linear mixed model was employed. The initial model for layer thickness contained random effects for subjects to account for the correlations between layer-wise observations and a layer factor. This model was then used to test potential predictive effects of age, tissue pH and hemisphere.

In the second stage, after potential predictors to be adjusted for had been identified, group differences in layer thickness were assessed for each layer separately. Standard multiple regression models sufficed for this purpose. All layer thickness analyses were carried out in SPSS 11 (SPSS Inc., Chicago, IL, USA).

**Cell size.** We employed regression modelling with robust standard errors to compare cell sizes between groups. Standard errors were constructed so that the inferences were robust against correlations between repeated observations on the primary sampling units. Cell size was recorded for approximately 31,980 neurons and 62,640 glial cells from 60 cases with the cases constituting the (independent) primary sampling units. Because of their positively skewed distributions we summarized the size data by their medians (Table 3) and analysed them on the log-scale where empirical distributions were well approximated by normal distributions.

As mentioned above analysis of the cell sizes was carried out in two stages. In the first forward selection procedure the complete data set was employed. The regression models included layer as a factor and the level chosen for inclusion of further variables was again 10%. In the second stage groups were compared within each layer after adjusting for relevant histological and demographic variables. The robust model fitting was carried out in Stata 7 (29).

In addition, we employed robust regression modelling to test for an interaction effect between group membership and brain hemisphere on cell size in each layer as hemisphere specific effects have been implicated in some of psychiatric disorders (1). More precisely, we added further terms to the model which represented hemisphere specific cell size ratios and tested their significance. When evidence for such an interaction was found cell sizes were compared between groups for each brain hemisphere separately. In that case a further adjustment for two hemisphere-wise tests was necessary [i.e. group tests were judged significant at the experiment-wise 5% level if the p-value was below 0.05/(6 × 2) = 0.0042].

**Cell density.** Poisson modelling was employed to compare cell densities between groups. In the forward selection procedure all layer-wise cell counts were modelled simultaneously employing a log-link Poisson model which used the volume of the case’s disector fields as an offset. A dispersion parameter was introduced to account for spatial clustering within sections. In addition, a random effect for subject was included to account for dependencies between layer-wise densities and layer was included as a factor. The random effects Poisson models were fitted using the procedure GLMM in the statistical package Genstat 5 (30) which employs Schall’s method (31) to fit a generalized linear mixed model.

Having identified demographic and histological variables for which to adjust cell densities, separate log-link Poisson models, with layer-wise field volumes as offsets and an overdispersion parameter were fitted to the cell count data in each layer. For each cell type and layer a model contained the adjustment variables and dummy variables whose parameters represented the density ratios between each of the three psychiatric groups and the control group. Accumulated analysis of deviance (32) using the experimental method was employed to test for differences between the patient groups and the control group. This Poisson modelling was again carried out using Genstat 5 (30).

As for cell sizes, we employed the Poisson models to test for an interaction effect between group membership and brain hemisphere on cell density in each layer. We found no statistical evidence for the existence of such interactions for either neuronal or glial densities (see Results) and therefore excluded interaction terms from the final models.
Results

Demographics, histological and clinical data for the Stanley sample are shown in Table 1. Tissue sections from three cases (one case from each disorder group) were noted to show postmortem artefact and were excluded from the study prior to the analysis. At the 10% level mean fixation times for schizophrenics (p = 0.003) and those with BPD (p = 0.001) were significantly longer than for controls. Mean postmortem intervals for schizophrenics (p = 0.038) and those with BPD (p = 0.085) were also significantly higher than for controls. No significant group differences were detected for the remaining demographic and histological variables in Table 1. Thus all modelling of all outcome variables was adjusted for gender, fixation time and postmortem interval.

Layer thickness

Layer thickness summary is presented in Table 2. The forward selection procedure identified no further empirical predictors of total layer thickness at the 10% test level. Hence no further adjustments besides gender, fixation time and postmortem interval were made for total layer thickness. The formal comparison of layer thickness between the control and patient groups showed no statistically significant group differences after adjustment (all p-values >0.1).

Cell size

A summary of the neuronal size is presented in Table 3. As for layer thickness all modelling of cell sizes was adjusted for gender, fixation time and postmortem interval. In addition, an adjustment was made for section thickness. At the 10% level tissue age at death was found to be predictive of neuronal cell size (t(56) = -2.26, p = 0.028; estimated decrease in cell size of 6% per 10 additional years, 95% CI 0.7–11%) and tissue pH of glial cell size (t(55) = -3.46, p = 0.001; estimated decrease in cell size of 3.5% per 0.1 increase in pH, 95% CI 1.5–5.5%). Cell size comparisons were therefore adjusted accordingly.

Tests for interaction effects of brain hemisphere and group differences on neuronal sizes showed evidence for the existence of hemisphere-specific effects for layer 3 (p = 0.0013). Interaction effects on neuronal sizes in all other layers were not significant (all p-values >0.1) nor was there any evidence for interaction effects on glial sizes in any layer (all p-values >0.05). Group comparisons of neuronal and glial cell sizes were therefore carried out on the basis of all cases, irrespective of whether their tissue was from the right or left hemisphere, except for neuronal sizes in layer 3.

Predicted cell sizes and 95% CI for each group and layer after adjusting for relevant covariates are illustrated in Fig. 4. Results comparing the psychiatric group with the control group are presented in Table 4. We detected decreased neuronal sizes in BPD relative to controls in layer 1 (p = 0.007; estimated decrease 21%, 95% CI 6–33%), and a trend for reduction in layer 5 (p = 0.05; estimated decrease 20%, 95% CI 0–36%). Layer 3 hemisphere-specific group comparisons showed a significant neuronal size reduction for patients with MDD in the right hemisphere (p < 0.001; estimated decrease 30%, 95% CI 21–38%) but not in the left hemisphere (p = 0.82). We did not detect any group differences in glial sizes for any of the layers (all p-values >0.2).

Cell density

A summary of neuronal and glial densities is presented in Table 3. As for cell size all modelling of densities was adjusted for section thickness, gender, fixation time and postmortem interval. At the 10% level none of the variables (tissue pH, age and hemisphere) were found to be empirical predictors of neuronal density and no further adjustments were made. In contrast, age at death was found to be an empirical predictor of glial density (Wald test: chi-square(1) = 2.9, p = 0.09; estimated increase in density of 4.7% per 10 year increase in age, 95% CI −0.8 to 10.5%) and the layer-wise group comparisons of glial density were also adjusted for age.

Layer-wise tests for interactions between brain hemisphere and group membership showed no
significant interaction effects on neuronal or glial
densities (all p-values >0.1). Group comparisons
of densities were therefore carried out on the basis
of all cases, irrespective of whether their tissue was
from the right or the left hemisphere. We did not
detect any statistically significant differences in
neuronal or glial cell densities for any of the layers
(all p-values >0.05).

Discussion
We found evidence for reductions in neuronal size
in layers 1 and a trend for a reduction in layer 5 of
the cOFC in subjects with BPD. The OFC has been
implicated in the regulation of affective states and
in BPD from functional imaging and human lesion
studies (7–10, 33), however there have been no
neuroanatomical investigations of this region in
BPD. Our data provide preliminary cytoarchitec-
tural evidence implicating this region in BPD.

There are several methodological advantages of
this study. These include the pragmatic application
of stereologically derived methodology, the
assessment of all cortical layers, and the presence
of three psychiatric groups with good sample size,
clinical details and careful pathological character-
ization. There are also a number of potential
confounders. For example, reduced neuronal size
could be secondary to pharmacological treatments
or group differences in tissue fixation time, tissue
pH or postmortem interval. In keeping with the
literature on effects of aging on the brain (34), we
found that neuronal size reduced and glial density
increased with age. We corrected for all of these
potential confounders in our analysis. Another
methodological consideration involves our use of
the nucleator to assess neuronal size in coronal
sections rather than isotropic uniform random
sections. While the latter method would have
ensured completely unbiased estimates of neuronal
size it would not have allowed us to obtain layer-
specific data. The orientation of the tissue we
sampled was consistently in the coronal plane and
this would have minimized any potential for biased
cell density and cell size values. Any minimal case-
to-case variation in coronal sectioning was random.
and thus would not have resulted in biased estimations. Additionally, because we did not assess the total volume of cOFC, a difficult task involving uncertain boundary definitions, our data are limited to density comparisons and cannot exclude the possibility that total cell counts differed between groups. However, our main findings of reduced neuronal size in BPD are not influenced by this potential confounder and are robust. These stereologically derived methods, used by us previously (20), are deviations from the traditional stereological approaches but approaches necessitated by the requirement to assess laminar-specific cell density and size information and to examine a cytoarchitectural region with poorly delineated boundaries.

Previous cytoarchitectural investigations of BPD have shown evidence of reduced neuronal size in cortical layer 5 in the DLPFC (18, 21) and the ACC (23), and of the two studies which did not

Fig. 4. Predicted geometric mean cell sizes (in $\mu$m$^3$) and 95% CIs by layer and patient group (black = controls; green = schizophrenia; red = bipolar disorder; blue = major depression). Due to the presence of an interaction between group membership and hemisphere for neuronal sizes in layer 3, mean cell sizes are estimated separately for the right and left hemispheres. (A) Neuronal sizes are adjusted to sample average values: 61% females, fixation period = 496 days, postmortem interval = 30 h, section thickness = 14.5 $\mu$m, age at death = 45.4 years. (B) Glial cell sizes are adjusted to sample average values: 61% females, fixation period = 496 days, postmortem interval = 30 h, section thickness = 14.5 $\mu$m, pH = 6.2.
demonstrate this reduction, one did not assess the individual cortical layers (19), while the other observed a 11% reduction in layer 5 neuronal size (20). Taken together, these data suggest a layer 5 neuronal size decrement in BPD. However, in the current study only the reductions in neuron sparse layer 1 reached statistical significance and reductions in layer 5 only attained trend level. Reduced density of layer 1 medium-sized neurons in BPD has been shown previously (35) and our study, showing reduced neuronal size in layer 1 in BPD, also implicates this region. The embryonic origin of neurons from cortical layer 1 is from medial ganglionic eminence and not from the ventricular neuroepithelium (36) as is the case for the majority of neurons from the remaining cortical layers. This raises the possibility that the changes observed in this investigation in BPD may have their basis in the unique embryological origin of these cells. In keeping with our findings, cortical layer 1 in BPD has also been implicated by the recent observation of reduced density of reelin staining, possibly Cajal-Retzius, neurons in this layer in DLPPC (37). However, in our study we did not attempt to quantify subpopulations of neurons within individual cortical layers and therefore we cannot address the issue of whether the neuronal size decrement is secondary to an altered distribution and size of the Cajal-Retzius population of neurons. Consequent upon the findings of the current investigation, a further investigation of these Cajal-Retzius cells in BPD is warranted.

We also observed right hemisphere neuronal size reductions in MDD in layer 3. Such a hemisphere-specific effect on neuronal size has not been observed before and must be interpreted with some caution because in our sample we did not have the contralateral hemisphere available for comparison. While right, but not left, orbital frontal alterations in activation have been observed in mania (9) and are in keeping with an increased vulnerability to mania following right hemisphere lesions (38), in general there is little evidence for a hemisphere-specific pathology in BPD (4). However, in MDD, there is consistent evidence for left-lateralized changes in the ACC in functional neuroimaging investigations (39–41), with evidence that right prefrontal cortical activity may change in a

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Cortical layer</th>
<th>Test Estimate of effect</th>
<th>Test Estimate of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons</td>
<td>t(56) p-value</td>
<td>Est. ratio CI</td>
<td>t(55) p-value  Est. ratio CI</td>
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<td>1.18 0.24 1.13 0.92,1.38</td>
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<td>3</td>
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<td>0.99 0.75,1.31</td>
<td>1.23 0.23 1.11 0.94,1.32</td>
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<td>L: -0.69 0.49</td>
<td>0.89 0.63,1.25</td>
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</tr>
<tr>
<td>4</td>
<td>-0.12 0.91</td>
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<td>0.41 0.69 1.04 0.87,1.24</td>
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<tr>
<td>5</td>
<td>0.16 0.87</td>
<td>1.02 0.81,1.29</td>
<td>1.24 0.22 1.13 0.92,1.39</td>
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<tr>
<td>6</td>
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<td>1.04 0.82,1.31</td>
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<td>-0.21 0.83 0.98 0.82,1.17</td>
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<td>2</td>
<td>-1.11 0.27</td>
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<td>-1.62 0.11</td>
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<td>0.13 0.89</td>
<td>1.02 0.79,1.3</td>
<td>-0.14 0.89 0.98 0.78,1.24</td>
</tr>
</tbody>
</table>

SCZ = schizophrenia; BPD = bipolar disorder; MDD = major depressive disorder; R = right brain hemisphere; L = left brain hemisphere.
aSignificant at the experiment-wise significance level of 5%, adjusting for 6 layer-wise comparisons (single test level of 0.83%).
bSignificant at the single test significance level of 5%, not adjusted for multiple layer-wise comparisons.
cSignificant at the experiment-wise significance level of 5%, adjusting for 6 layer-wise and 2 hemisphere-wise comparisons (single test level of 0.42%).
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reciprocal manner to ACC activity (42). The extent to which cOFC demonstrates right-lateralized changes in the volume and activity of MDD is less clear and needs to be clarified in the light of the current findings.

In the current investigation we did not observe any group differences in cortical glial cell densities between groups. Stereological investigations have previously shown a deficit in these cells in MDD in the OFC (18), ACC (19, 20) the DLPFC (21) and the amygdala (22). In BPD a glial cell deficit has also been observed (22, 35) but with less consistency (1). The most likely explanation for these differences relate to the presence of different regional vulnerabilities to cortical glial cell deficit in MDD. In support of this explanation we note that our current findings in MDD are transitional between those observed by Rajkowska et al. (18) in caudal and rostral OFC in MDD. In this latter study the main findings were of glial cell deficit in caudal OFC and reduced neuronal size in rostral OFC. Glial cell deficits were not observed by Rajkowska et al. (18) in rostral OFC. Considering that we assessed cOFC at a more rostral level than the cOFC of this previous investigation, our findings are consistent with these findings.

We observed no differences in neuronal density between groups in the current investigation. This is in keeping with a previous study of this region in MDD (18). In BPD, stereological studies have shown reduced neuronal density in the DLPFC (35) but not the ACC (19, 20). While neuronal density has not been previously assessed in schizophrenia in cOFC, our negative findings for schizophrenia are in keeping with recent findings by Selemon et al. (43) which suggest that it is the DLPFC which is particularly vulnerable to altered cell density in schizophrenia. However, there is evidence that specific subpopulations of neurons are deficient in schizophrenia (44) and it is possible that our methods which did not include the analysis of these subpopulations were not sensitive to such changes. Nonetheless, as we observed no alterations in either neuronal size or in neuronal density in cOFC, it is unlikely that marked pathology is present in this region in schizophrenia.

While no cytoarchitectural investigations of cOFC in schizophrenia have been undertaken previously, neuroimaging investigations have focused on this region with mixed results. In general, the region is less affected than the DLPFC (4) in schizophrenia, with some evidence that right hemisphere (45) and females (4) may demonstrate more prominent total grey matter volume reductions while others have observed such changes only in the straight gyrus (46). These apparent inconsistencies are likely due to different definitions of OFC, different neuroimaging methods, sample sizes and clinical characteristics of the subjects. Interestingly, Gur et al. (47), who demonstrated OFC volume reductions in female schizophrenic subjects, also observed an inverse correlation between OFC volumes and severity of clinical depression. This latter study, which included data from lateral OFC, strengthens our suggestion that OFC pathology reflects a vulnerability to affective symptoms.

The basis of the observed neuronal size changes in BPD and MDD is not known. Neuronal somal size is proportionate to the extent of its dendritic and axonal tree (46) suggesting that alterations in these parameters may be also observed in this brain region in these disorders, although the primary alteration is not known. It is tantalizing that our findings may be related to those of altered volume (48, 49) and activation (50, 51) of the amygdala in BPD. Both regions are known to be closely interconnected (52, 53). Furthermore, there is evidence from surgical disconnection studies that these regions act as an integrated neural system (6), and that rats with neurotoxic OFC lesions demonstrate functional changes in amygdala activity (54). This raises the possibility that neuronal size reduction in the OFC could result in OFC dysfunction and ultimately in dysfunction of the amygdala in BPD. Against this possibility, functional imaging studies, despite showing clear OFC and amygdala changes in BPD, have not established the relationship of these changes to each other. Furthermore the direction of the changes in amygdala volume in BPD is unclear. Future studies, both neuropathological and neuroimaging, should seek to clarify this relationship. In the meantime, our findings suggest that cOFC is specifically altered in affective disorders, but not in schizophrenia.

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