Neuron density and serotonin receptor binding in prefrontal cortex in suicide

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Abstract
Although serotonin receptor and cytoarchitectonic alterations are reported in prefrontal cortex (PFC) in suicide and depression, no study has considered binding relative to neuron density. Therefore, we measured neuron density and serotonin transporter (SERT), 5-HT1A and 5-HT2A binding in matched suicides and controls. Suicides and normal controls (n = 15 matched pairs) were psychiatrically characterized. Neuron density and binding were determined in dorsal [Brodman area (BA) 9] and ventral (BA 47) PFC by stereology and quantitative autoradiography in near-adjacent sections. Binding index was defined as the ratio of receptor binding to neuron density. Suicides had lower neuron density in the gyrus of both areas. The binding index was lower for SERT in BA 47 but not in BA9; the 5-HT1A binding index was higher in BA 9 but not in BA 47, while the 5-HT2A binding index was not different between groups. SERT binding was lower in suicides in BA 47 but not BA 9, while 5-HT1A binding was higher in BA 9 but not BA 47. SERT binding negatively correlated with 5-HT1A binding in BA 47 in suicides. Neuron density decreased with age. The 5-HT1A binding index was higher in females than males. We found lower neuron density and lower SERT binding index in both PFC regions in suicides. More 5-HT1A binding with less SERT binding and the negative correlation in depressed suicides suggests post-synaptic receptor up-regulation, and it is independent of the difference in neuron density. Thus, abnormalities in both cortical neurons and in their serotonergic innervation are present in suicides and future studies will need to determine whether cortical changes reflect the trophic effect of altered serotonin innervation.

Key words: Autoradiography, human, receptor binding, serotonin, stereology.

Introduction
Previous studies have shown a link between serotonin (5-hydroxytryptamine, 5-HT) and suicidal behaviour involving the prefrontal cortex (PFC). A relationship between lower serotonergic activity and suicide has been demonstrated because lower 5-hydroxyindoleacetic acid (5-HIAA), the principal metabolite of serotonin, is not only found in the cerebrospinal fluid (CSF) of serious suicide attempters but also predicts risk of future suicide (see Mann & Currier, 2007 for review), and low serotonin and/or 5-HIAA are found in brainstem of suicides (Carroll, 1968; Lloyd et al. 1974; Pare et al. 1969; Shaw et al. 1967). These findings are also supported by alterations in serotonin receptors and transporter binding in the PFC and other brain regions measured in some post-mortem studies of suicides (Arango et al. 1997; Laruelle et al. 1993; Pandey et al. 2002; Stockmeier, 2003), but not all (Stockmeier et al. 1997, 2009). Consistent with low serotonin transporter (SERT) binding in the PFC, Austin and colleagues (2002) observed localized deficits in SERT immunoreactivity in axons in the PFC of depressed suicides, suggesting less serotonergic innervation. More recently, regional brain metabolic responses to evoked serotonin release and other serotonin turnover studies measured in vivo using positron emission tomography have detected related PFC abnormalities in suicidal behaviour (Oquendo et al. 2005; Smith et al. 2009). Importantly, these findings are argued to be independent of other factors such as
depressive illness and schizophrenia and indicate abnormal serotonin input into the ventral PFC (Arango et al. 1997; Mann et al. 2000).

In addition to alterations in serotonin indices in the PFC of suicides and suicide attempters, other studies have suggested that there are underlying morphometric changes and signal transduction abnormalities in the cells of the PFC, at least in mood-disordered subjects, that involve neurons and glia (Ongur et al. 1998; Rajkowska, 2002). One set of findings report decreases in the size and density of pyramidal neurons in anatomically restricted locations and in select cortical layers of the PFC (Rajkowska et al. 1999, 2005), but not in primary sensorimotor cortex (Bouras et al. 2001; Ongur et al. 1998) of major depressive disorder (MDD) and bipolar disorder (BD) patients ( Cotter et al. 2002). It is also reported that serotonin indices in target cortical neurons are abnormal, encompassing alterations in both protein and transcript as well as parts of signal transduction cascades (Hsiung et al. 2003; Pandey et al. 2001, 2006; Stockmeier, 2003).

It is not known whether these receptor binding and signalling pathway changes are due to altered numbers of neurons that express these proteins. We therefore sought to elucidate the relationship between neuron density and serotonin indices in the PFC using quantitative autoradiographic measurement of SERT, 5-HT1A and 5-HT2A receptor binding and relating it to the neuron density in the same region, in intercalated tissue sections. By taking neuron density into account in the measurement of receptor binding, an index of the receptor binding per neuron is derived. This index may have greater relevance for functional capacity at the neuron level, in that the measurement of receptor binding will take the density of neurons in the same region into account and indicate whether changes in receptor binding reflect up- or down-regulatory responses at the neuron level.

Methods

The procedures used for brain tissue collection, toxicology, psychological autopsy, receptor autoradiography and stereology have been published elsewhere (Boldrini et al. 2009; Mann et al. 2000; Underwood et al. 2007) and are only summarized here.

Normal non-psychiatric controls were matched with suicide cases on a case-by-case basis by age (±5 yr) and sex; whenever possible, matching was also performed for post-mortem interval (PMI) and race. Fifteen pairs were used and the demographic information and diagnoses are listed in Table 1. The mean age (± standard error) of controls was 46 ± 5 yr, and the mean age of suicides was 45 ± 5 yr. Written informed consent was obtained from next-of-kin.

Collection of brain samples

All study procedures, including tissue collection, were approved by the Institutional Review Board of the University of Pittsburgh. Brain tissue samples were obtained from the Coroner’s office of Allegheny County, PA. Upon removal of the brain from the cranium, the dura mater was stripped, the brainstem was separated by a transverse cut just anterior to the superior colliculus and the cerebellum was removed. The brain was bisected and the right hemicerebrum was cut into approximately 2-cm-thick sections in the coronal plane. The tissue slabs were placed on a glass plate, frozen in liquid Freon, placed in pre-labelled plastic bags and refrigerated at −80 °C. Cerebellar tissue was collected and used for brain toxicological analyses. The remaining tissue was placed in formalin for neuropathological examination, consisting of gross and microscopic examination.

Toxicological screening

Blood, urine, bile and vitreous humor were screened for drugs. In brain, over 50 drugs were screened for and quantified, if detected. The screen included neurolitics, antidepressants (tricyclic and non-tricyclic), psychomotor stimulants, recreational street drugs and other miscellaneous drugs including alcohol. Brain material was not processed further until the toxicology results were found to be negative and the interviews with key informants completed, at which point, the cases and controls were matched and studied neurochemically.

Brain sectioning

The coronal sections from the forebrain were cut on a Leica Cryopolycut at 20 μm for autoradiography. An intercalated set of sections was cut at 50 μm, stained for Nissl and used to measure neuron density (see below).

Quantitative receptor autoradiography

Receptor autoradiography assays were performed as previously described (Arango et al. 1995, 2002) on tissue sections at a pregenual level containing multiple prefrontal cortical Brodmann areas. For the present study, Brodmann area (BA) 9 and BA 47 were selected as regions representative of dorsolateral and ventral areas of PFC (Fig. 1).
Sections were pre-incubated in selected buffers to remove endogenous ligands and then incubated with radioligand under optimal conditions. Non-specific binding for each receptor subtype was determined by incubation of adjacent sections with a selective displacer. Sections were washed in incubation buffer at 4°C, briefly dipped in water, rapidly dried, and transferred to a vacuum desiccator until ready to expose (1–2 d) to film. Dried slides were arranged in an X-ray film cassette with ³H-containing polymer standards (American Radiolabeled Chemicals, USA).

We sought to determine receptor binding and neuronal density from adjacent or near-adjacent sections and hence, the region for stereology sampling of neuron density on the Nissl-stained section was manually mapped onto the autoradiograms for receptor measurement (Fig. 1). The autoradiograms were sampled using a computer-based image analysis system (MCID, Imaging Research Inc., Canada) as previously described (Arango et al. 1993, 1995, 2001).

³H]Cyanomipramine binding was used for measuring SERT and performed using modifications of the
method of Kovachich et al. (1988); total binding was determined by incubation with 0.4 nM [3H]CN-IMI, non-specific binding was determined using 10 μM sertraline (Arango et al. 1995). [3H]8-OH-DPAT was used to label 5-HT1A receptors (2 nM). Non-specific binding was determined with 1 μM 5-HT (Arango et al. 1995). We used 2 nM [3H]ketanserin for defining 5-HT2A receptors and incubated with 1 μM prazosin and 1 μM tetrabenazine to block α1 and tetrabenazine sites, respectively; non-specific binding was determined with 1 μM mianserin (Glennon, 1990). [3H]Ketanserin was used, rather than [125I]LSD because it is more selective for the 5-HT2A receptor (Glennon, 1990).

**Stereology**

Slides were initially viewed at approximately 16x total magnification under a Leica Wild M3Z stereoscope and individual areas were demarcated on each slide of each case using the Brodmann area map of Rajkowska and Goldman-Rakic (Rajkowska & Goldman-Rakic, 1995a, b) to map BA 9 and that of other investigators (Amunts et al. 1999; Petrides & Pandya, 2002; Zald & Kim, 1996) to map BA 47. The human BA 45 is characterized by clusters of large and deeply stained pyramidal neurons in layer III (Amunts et al. 1999). In BA 47, these neurons are replaced by smaller neurons and layer IV is less developed than in BA 45, but the infragranular layers V and VI are more prominent (Beck, 1949; Petrides & Pandya, 2002; Rajkowska, 2000a). The assumption was made that neuron density and receptor binding were representative of the region of interest throughout the rostrocaudal extent of the respective Brodmann area. However, since neuron density is greater in the fold of the sulcus than the crest of the gyrus and because there is a different laminar distribution of receptor binding in the gyrus and sulcus, they were sampled and analysed separately.

Neuron counting was performed using either a Leitz Diaplan or Leitz Ergolux light microscope. Both
microscopes had motorized stages (LUDL Electronic Products Ltd, USA) controlled by the stereology software (StereoInvestigator, MicroBrightfield Inc., USA). The microscope image was captured on the Diaplan by an Optronics charge-coupled device (CCD) camera and on the Ergolux by a Sony PowerHAD (DXC-970MD) CCD camera and displayed on a computer monitor.

Outlines of the regions of interest were traced using a pointing device and StereoInvestigator software. The outline traced for neuron counting approximated the contour sampled for receptor binding and no attempt was made to delineate or represent cortical layers in the sample in order to have the measurement of neuron density reflect the measurement of receptor binding from the same underlying population of cells. The optical dissector method was used to measure the density of neurons (neurons/mm\(^3\)). The parameters for the optical dissector were 400 × 400 μm for the scan grid size, 3 μm guard distance from top of section to start of the counting frame, 15 μm counting frame height, 25 μm mounted thickness, and 150 × 150 μm for the counting frame dimensions. Mounted thickness of the sections was determined by focusing on the top and bottom of each section and reading the distance moved by the stage (Heidenhain MT12 linear encoder). A minimum of 150 sampling sites was collected for each brain region and the coefficient of error in each case was less than 10%.

Neurons were identified by shape, and when evident, by dendritic processes or nucleoli. Neuron ‘tops’ were counted (i.e. only those neurons with tops in focus within the set counting frame height were counted). The amount of receptor binding in a region of interest was done by sampling the region in three separate autoradiograms and averaging the values to produce a single value of receptor binding in that region for that individual. In order to associate the density of receptor binding with the density of neurons, neuron density was measured in Nissl-stained sections that were near-adjacent to the sections used for receptor binding. The three Nissl-stained sections closest to the sections used for receptor binding were analysed for neuron density in each case and the results averaged to produce the mean neuron density in that region for that individual. In this way, neuron density and receptor binding were sampled and thereby determined in as comparable a way as possible in the same anatomical region of interest.

Binding index was calculated by dividing the receptor binding values (fmol/mg tissue) for each particular region by the neuron density (neurons/mm\(^3\)) for that region. This results in a value indexing the amount of receptor binding per neuron. This value is only an index since the receptor ligands used were labelled with tritium (\(^3\)H) and therefore only reflect binding in the upper 5 μm of the tissue section (Alexander et al. 1981) and the measures of neuron density, while quantitative, are from near-adjacent tissue sections.

**Data analysis**

Primary analyses examined neuron density and the receptor binding index. Because the cell density and binding index data had very skewed distributions with outliers, all four outcome variables were transformed using the 10-based logarithm function. Then four different mixed-effect models were fit, one for each outcome variable, with fixed effects used to model the effects of suicide, Brodmann area, topology (gyrus/sulcus), age, sex and PMI, and random intercept was used for each subject from each pair (nested effect). Interaction terms between suicide, and Brodmann area were tested and removed when not significant. The overall effect of all terms involving the suicide indicator was tested using likelihood ratio test between the models with and without those terms, with parameters estimated through the maximum likelihood method. To adjust for multiple testing due to the existence of four outcome variables, the Bonferroni correction was used. Residuals for all four models were graphed and inspected for deviations from the model assumptions, possible outliers were removed from the model and the parameters re-calculated and compared to the original ones to detect influential observations. No outlier had a significant effect on any of the parameters.

Because serotonin receptor binding in depressed suicides and controls has been examined previously by our group (Arango et al. 1990, 1995; Mann et al. 2000), statistical tests of these variables were considered secondary and as such, this post-hoc testing was performed with paired t-tests.

All tests were two-tailed and data expressed as mean and standard deviation unless indicated otherwise.

**Results**

In both suicides and controls, the average neuron density (taking sulcus and gyrus together) in BA 9 was lower than in BA 47 (controls, BA 47: 40 258 ± 22 569 neurons/mm\(^3\); BA 9: 37 135 ± 16 935 neurons/mm\(^3\); suicides, BA 47: 33 966 ± 15 850 neurons/mm\(^3\); BA 9: 31 647 ± 15 175 neurons/mm\(^3\); adjusted test statistic from the mixed-effect model analysis...
Neuron density was lower in suicides compared to controls in both BA 9 and BA 47 in the gyrus, although not in the sulcus (gyrus: t = -2.6, d.f. = 12, p = 0.025; sulcus: t = -1.2, d.f. = 12, p = 0.283). Combined test statistic for suicide main effect and interaction term: $\chi^2 = 10.6$, d.f. = 2, Bonferroni adjusted p value = 0.020.

Neuron density in the gyrus (BA 47, controls: $39.615 \pm 5443$; suicides: $29.768 \pm 3470$ neurons/mm$^3$; BA 9, controls: $34.963 \pm 4439$; suicides: $28.798 \pm 3372$ neurons/mm$^3$) was 25% and 18% lower in BA 47 and BA 9 in suicides compared to controls (Fig. 2a).

For the SERT binding index (Fig. 3a), the interaction term between brain region and suicide was significant indicating that suicides had lower SERT binding index than controls in BA 47, but in BA9, where controls had less binding levels, the difference between the two groups was not statistically significant (BA 45: $t = -2.4$, d.f. = 12, $p = 0.032$; BA 9: $t = -1.2$, d.f. = 12, $p = 0.259$; $\chi^2 = 15.0$, d.f. = 2, Bonferroni adjusted $p$ value = 0.002). 5-HT$_{1A}$ receptor binding index (Fig. 3b) was higher in suicides at a trend level after adjusting for multiple comparisons ($t = 2.91$, d.f. = 13, adjusted $p = 0.068$). 5-HT$_{1A}$ receptor binding index (Fig. 3c) did not differ between groups ($\chi^2 = 2.2$, d.f. = 1, adjusted $p = 0.538$).

Consistent with binding index results, SERT binding was 34% lower in the suicide group in BA 47 ($p < 0.05$, Fig. 2b), but was not different between groups in BA 9. Conversely, 5-HT$_{1A}$ binding (Fig. 2c) was 20% higher in suicides in BA 9 ($t = -2.033$, d.f. = 28, $p = 0.05$) but was not different between groups in BA 47 ($p > 0.05$). 5-HT$_{1A}$ binding was not significantly different between controls and suicides ($p > 0.05$ all regions, Fig. 2d). SERT binding negatively correlated with 5-HT$_{1A}$ binding in BA 47 in suicides ($r = -0.513$, $p = 0.05$) but not in controls.

**Sex, age, PMI and pH**

5-HT$_{1A}$ binding was 35% higher in females than males in BA 9 ($t = -3.070$, d.f. = 28, $p = 0.005$) and 25% more in BA 47 ($t = -2.881$, d.f. = 28, $p = 0.008$). The 5-HT$_{1A}$ binding index was also greater in females than males ($t = 2.290$, d.f. = 13, $p = 0.039$). The 5-HT$_{1A}$ binding index for suicides remained significantly different from controls even when accounting for sex (General Linear Model × group, with age and sex as covariates: $F = 7.081$, $p = 0.013$). Neuron density decreased with age ($t = -2.33$, d.f. = 12, $p = 0.038$); but age as a covariate did not account for the observed group differences between controls and suicides (General Linear

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**Fig. 2.** (a) Cortical neuron density, (b) [$^3$H]cyanoimipramine binding to the serotonin transporter (SERT), (c) [$^3$H]8-OH-DPAT binding to the 5-HT$_{1A}$ receptor and (d) [$^3$H]ketanserin binding to the 5-HT$_{1A}$ receptor in Brodmann area (BA) 9 and BA 47 of prefrontal cortex of suicides and controls. Note (a) the decrease in neuron density, (b) the decrease in SERT binding and (c) the increase in 5-HT$_{1A}$ binding in suicides ($p < 0.05$).
Model \(r\) group, with age and sex as covariates, BA 47: \(F = 4.219, p = 0.015\); BA 9: \(F = 2.876, p = 0.05\). None of the receptor binding or neuron density outcome variables correlated with PMI or tissue pH in either the controls or the suicides or with all cases combined (\(p > 0.05\) for all correlations; data not shown). However, the PMI for controls was shorter than for suicides (controls: \(13 \pm 5\) h; suicides \(18 \pm 6\) h; \(t = -2.884, \text{d.f.} = 14, p = 0.012\)).

Discussion

In the present study, we found lower neuron density, lower SERT binding index and higher 5-HT\textsubscript{1A} receptor binding index in dorsal and ventral PFC in depressed suicides. No differences were observed in 5-HT\textsubscript{2A} binding or in 5-HT\textsubscript{2A} binding index.

**Neuron density**

Our finding of less neuron density in the PFC is the first such report in depressed suicides, but is consistent with previous reports of lower neuron density in the PFC and orbitofrontal cortex in mood disorders (Rajkowska, 2000\textsuperscript{b}; Rajkowska et al. 1999). The majority (12/15) of the suicides studied here had a diagnosis of mood disorder. Rajkowska and colleagues (1999) reported lower density of large neurons and greater density of smaller neurons, indicating a pathological change that affects larger and smaller neurons differently. Subsequent studies found that neuronal changes were anatomically restricted to PFC (Rajkowska et al. 1999) and orbitofrontal cortex, but were not in primary sensorimotor cortex (Bouras et al. 2001; Ongur et al. 1998), and may only be in select cortical layers (Benes et al. 2001; Rajkowska et al. 2001) in MDD and BD (Cotter et al. 2002). However, the findings in this study suggest that the lower neuron density is more widespread in PFC in suicides with MDD and includes dorsal and ventral regions. The involvement of PFC, but not sensorimotor cortex, is not only consistent with the notion of specialization of the PFC in mood regulation, but also suggests a primary pathology within the PFC as suggested by functional imaging (Mayberg, 2007; Milak et al. 2005). Differences in neuron number, density and morphometry are also reported for mood disorders in other brain regions such as the thalamus (Young et al. 2000, 2004) and the hypothalamus (Raadsheer et al. 1994).

Larger neurons in the PFC are more likely to be excitatory while smaller interneurons tend to be GABAAergic and inhibitory. Our study did not measure neuron size or differentiate large from small neurons and therefore cannot contribute to the understanding of whether there is a selective loss of a particular class or size of neurons. Reductions in glia number and/or density are also reported in the PFC and orbital cortex.
whether the reduction in glia density is associated with suicide, as opposed to MDD, is not clear (Hercher et al. 2009; Rajkowska et al. 1999).

Lower neuron or glia density in the PFC and other regions are reported in other psychiatric disorders including schizophrenia and BD. Reduced glia density is reported in BD (Ongur et al. 1998). In BD there is a reduction in large cortical neurons without any concomitant increase in the size of smaller neurons suggesting neuron loss (Rajkowska et al. 2001). Both decrease and increase in the density of neurons in the PFC is reported in schizophrenia (Beasley & Reynolds, 1997; Benes et al. 1991; Daviss & Lewis, 1995; Rajkowska et al. 1998; Sellem et al. 1995), raising a question about the diagnostic specificity of the cytoarchitectonic alterations observed.

Other studies have found reduced innervation of the PFC in mood disorders arising from neurons in several subcortical brain regions including serotonergic neurons in the dorsal raphe nucleus (Arango et al. 1995; Austin et al. 2002), noradrenergic neurons in the locus coeruleus (Arango et al. 1996) or GABAergic neurons in the dorsal thalamus (Gos et al. 2009). Taken together, the observation of neuropathological findings in the PFC and in subcortical structures providing afferent innervation to the PFC would suggest that there are both cortical and subcortical alterations that contribute to abnormal prefrontal cortical neurotransmission as part of the pathogenesis of mood disorders or suicide.

Receptor binding index

Our present finding of lower neuronal density, and other reports of change in size and number of neurons and glia in the PFC in MDD (Arango et al. 2002; Rajkowska, 2000b), could affect the interpretation of PFC receptor binding alterations in MDD since neurons and glia are the target cells of the serotonin system. We propose that normalizing the raw serotonin receptor binding measurement made from the quantitative autoradiography by the neuron density measurements made from adjacent tissue sections, provides an index of serotonin input at a neuronal level, and this is the first time such data are reported in this manner for suicide and depression. Our findings indicate less SERT binding overall, but given the lower neuronal density we also find less SERT binding at a neuronal level, indicating less serotonin input per neuron. Concurrently, we found higher 5-HT1A post-synaptic receptor binding index in BA 9 in suicides, suggesting an up-regulatory effect at a neuronal level. The inverse correlation between SERT and 5-HT1A binding is further support for receptor up-regulation, particularly since this relationship was only detected in suicides. The 5-HT1A receptor binding, or the receptor binding index, in suicides in BA 9 or BA 47 was comparable to non-psychiatric sudden death controls. Our finding was obtained using a 5-HT1A antagonist ligand and adult cases and perhaps that explains why it differs from our previous reports of greater 5-HT1A binding using the agonist ligand LSD (Arango et al. 1990) and from findings reported in youth suicide (Pandey et al. 2002), also using LSD as a ligand. However, ligand alone cannot explain the discrepancies, since other groups reported an increase in frontal cortex of suicides using the antagonist [3H]ketanserin (Gross-Isseroff et al. 1990; Hrdina et al. 1993; Turecki et al. 1999) or [3H]spiroperidol (Mann et al. 1986; Stanley & Mann, 1983). Since the three [3H]ketanserin reports mentioned above used subjects that were younger than ours, while groups, also using [3H]ketanserin in older subjects did not get an increase (Lowther et al. 1994; Oquendo et al. 2006; Stockmeier et al. 1997), it is possible that the increase in 5-HT1A binding may be more pronounced in young individuals. This appears true in alcoholic suicides, where there was more 5-HT1A antagonist binding in the <25 yr age group (Underwood et al. 2004). In this paper we only have one suicide-control pair aged <25 yr.

The localization of 5-HT1A and 5-HT1A receptors on glia in the PFC could be considered problematic to the interpretation of the results in the present study because the density of glial cells was not measured. However, 5-HT1A and 5-HT1A receptor number on glia relative to neurons is minimal (Azmitia et al. 1996; Burnet et al. 1995; Deecher et al. 1993; Miner et al. 2003). From another perspective, differences in the size and number of target neurons can contribute to differences in binding and may provide an explanation for differences between investigators (Lewis, 2002). The same may apply to in-vivo studies of neuroreceptors. However, lower neuronal density does not explain previous post-mortem and in-vivo reports of lower SERT (Arango et al. 1995, 2002; Austin et al. 2002; Mann et al. 2000; Parsey et al. 2006a,b) and higher 5-HT1A binding in mood disorders (Arango et al. 1995; Parsey et al. 2006c).

SERT binding is a measure of serotonin nerve terminals and serotonergic innervation, and the neuron density ‘corrected’ data suggest that, in the PFC, there is less serotonergic innervation per neuron. The combination of less serotonin innervation per neuron and fewer target neurons is consistent with
many reports of less SERT binding per milligram of tissue or protein in PFC and other brain regions associated with suicide (Arango et al. 1995, 2002; Austin et al. 2002; Laruelle et al. 1993; Leake et al. 1991; Mann et al. 2000), and less PFC transporter binding with in-vivo brain imaging (Parsey et al. 2006a, b). Note that we have previously reported that lower transporter binding in dorsal lateral PFC (BA 9 and BA 47) is associated with MDD and lower binding in suicide is found only in ventral PFC (BA 47, Arango et al. 2002). The region-specific alterations associated with MDD are anatomically more widespread, consistent with the complex psychopathology of this disorder. In the present study, we observed that neuron density, SERT binding and SERT binding index were lower in both dorsal and ventral PFC, but that the magnitude of this decrease is greater in ventral than dorsal PFC. A loss of neurons, or lower density of those neurons may reflect the chronic, recurrent course of the illness, and reports of less PFC grey matter and glucose utilization reported in MDD imaging studies (Soares & Mann, 1997a, b). We find that despite less density of neurons, the transporter binding per neuron is still low, emphasizing the deficient serotonin input to PFC. Our results are consistent with a report of serotonin axonal loss in PFC (Austin et al. 2002) and less serotonin release per terminal and compensatory accelerated internalization of the transporter (Lau et al. 2008; Parsey et al. 2006b; Zhao et al. 2009). Fewer SERT-labelled axons or terminals could be the result of fewer axons or conceivably to reduced trafficking of SERT from the soma to the terminal, and the present study cannot distinguish between these possibilities.

We found the 5-HT\textsubscript{1A} receptor binding index was higher in depressed suicides, a potential up-regulatory effect consistent with, but not direct evidence for, less serotonin input which is indicated by less SERT binding per neuron. Greater 5-HT\textsubscript{1A} binding alone (without correcting for neuron density) was detected only in the sulcus of BA 9 in suicides. However, once the binding was ‘corrected’ for neuron density, higher binding index was found in both the gyrus and sulcus of the dorsal PFC. The increase in the binding index in the gyrus of the ventral PFC was of smaller magnitude than in the dorsal PFC and did not reach statistical significance. This suggests not only that it is important to know the cytoarchitecture when receptor binding is being measured, but also that there can be a quantitatively different effect in dorsal compared with ventral PFC for reasons that we do not understand at this time. Similarly, we knew a priori that there are differences in neuron density and in the laminar organization of receptor binding in the sulcus and the gyrus, most likely reflecting morphological consequences of the anatomical constraints imposed by the gyrification of the cerebral cortex during development. We therefore separately defined, sampled and analysed the gyri and sulci of BA 9 and BA 47. The findings here of differences in neuron density, receptor binding and binding index depending on region topology further raises the possibility of functional differences within the broad definitions of Brodmann regions. Such anatomical and functional differences between sulci and gyri are reported in the cerebral (Toro & Burnod, 2005) as well as the cerebellar (Nishiyama & Linden, 2004) cortex.

We found 5-HT\textsubscript{1A} receptor antagonist binding was not higher in suicides even when expressed as per neuron. Interestingly, it has been reported that up to 65–80% of cortical pyramidal neurons in the PFC express 5-HT\textsubscript{1A} receptors, and up to 80% of those neurons co-express the 5-HT\textsubscript{2A} receptor (Amargos-Bosch et al. 2004; Wedzony et al. 2008). The difference in the apparent up-regulatory response of the binding index between the 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors further suggests different mechanisms of receptor regulation. Alternatively, we found higher 5-HT\textsubscript{2A} agonist binding in suicides (Arango et al. 1990) and higher 5-HT\textsubscript{1A} binding in suicides in this and other studies (Arango et al. 1995). Agonist binding may be more sensitive to up-regulation than antagonist binding, because initially the effect may involve a change in the ratio of high-affinity to low-affinity binding, detectable by agonist ligands and not antagonist ligands. However, a recent study of individuals with MDD (not all suicides) utilized both 5-HT\textsubscript{1A} agonist and antagonist ligands for autoradiography (Stockmeier et al. 2009) and reports decreases in antagonist, but not agonist binding in the MDD group. We used the antagonist ketanserin to label the 5-HT\textsubscript{2A} receptor rather than the partial agonist LSD that we have used in previous studies because ketanserin is reported to be more selective (Glennon, 1990). Studies comparing agonists and antagonists at the 5-HT\textsubscript{1A} receptor are needed to determine whether such differences in affinity state explain differences in receptor-binding studies using different ligands. Regardless, 5-HT\textsubscript{2A} binding index was not found to be increased in this study, suggesting that neuron density cannot explain differences in receptor binding at the 5-HT\textsubscript{2A} receptor.

**Limitations**

We would have liked to have been able to differentiate effects on receptors or neurons associated with suicide from effects associated with MDD. We could not...
compare depressed suicides \((n = 12)\) with non-depressed suicide cases \((n = 3)\) due to too few of the latter cases and this needs to be the subject of a future study. Other studies have found differences in brain regions manifesting low SERT binding in suicide and MDD such that suicides have SERT binding deficiencies confined to the anterior cingulate and ventral PFC (Arango et al. 2002).

The present study did not distinguish phenotypes of the neurons counted, nor did it determine the density of glia and future work is needed to determine more precisely what type of neuron population is lost in the PFC in depressed suicides and whether there is evidence of suicide or MDD as a neurodegenerative disorder. A neuropil loss in the depressed suicides here is unlikely since we observed a decrease in neuron density. It will also be of importance to determine whether low neuron density extends beyond the PFC into other critical regions such as amygdala or ventral striatum. Similarly, it would have been preferable to have the receptor binding and neuron density measured from the exact same tissue section instead of from adjacent sections. However, technical limitations required that the receptor binding measurements and stereological measurement of neuron density be performed on different sections, resulting in there being a variable physical separation between measured sections. We believe the impact of this separation on the variability of the measures or of the binding index being representative of the receptor binding per neuron is minimal. The average diameter of cortical neurons is 25–50 μm, thus making separation distances of even hundreds of microns of tissue thickness actually only tens of neurons away.

In conclusion, our study is the first to report low neuron density in dorsal and ventral PFC in suicides, and it does not explain all the serotonin receptor and transporter binding changes found in suicides.

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Statement of Interest

None.

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