Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims

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Abstract

Altered serotonin indices have been reported in the brain of suicide victims. We sought to localize the changes in presynaptic and postsynaptic serotonin receptors and identify an area of prefrontal cortex that may influence suicide risk. Quantitative autoradiography was performed in coronal sections of prefrontal cortex to determine whether serotonin 5-HT1A receptor (postsynaptic in cortex) and serotonin transporter (presynaptic) binding are different in suicide victims compared to matched controls. 5-HT1A receptor binding was higher in 85 of the 103 sampled areas in the suicide group (n = 18 pairs; P < 0.0001). The increase ranged from 17 to 30%. The increase was more pronounced in the ventrolateral prefrontal cortex. Serotonin transporter binding was found to be lower in the suicide group in all but one of the 43 sampled regions (n = 22 pairs; P < 0.0001). The reduction in binding was most pronounced in the ventrolateral prefrontal cortex, where the difference between suicides and controls ranged between 15 and 27%. Serotonin transporter and 5-HT1A binding were negatively correlated (r = -0.35 to -0.44, P = 0.04 to 0.007) within the same brain areas, suggesting common regulatory factors with opposite effects on binding to the two receptors. We conclude that suicide victims have an abnormality in the serotonin system involving predominantly the ventrolateral prefrontal cortex, and hypothesize that the serotonergic dysfunction in this brain region contributes to the risk for suicidal behavior.

Keywords: Quantitative receptor autoradiography; [3H]8-OH-DPAT; [3H]Cyanoimipramine; 5-HT1A receptor; Serotonin transporter; HPLC; 5-HIAA

1. Introduction

Suicide claims more than 30,000 lives a year in the United States [20]. There is evidence suggesting that there are alterations in the serotonergic system in the brain of suicide victims and related altered serotonin (5-hydroxytryptamine, 5-HT) system indices in patients who have made serious suicide attempts [9,10,12,13,17,48,60,68]. Such findings have led to the hypothesis that reduced serotonergic activity is associated with increased suicide risk [50,53]. This reduction in serotonergic activity correlates with suicidal acts in several diagnostic groups including major depression, schizophrenia and personality disorders. These diagnostic groups make up the vast majority of suicides not associated with alcohol or drug abuse. Thus, this serotonin deficiency was not restricted to a specific diagnosis.

Studies of the serotonergic system fall into two major domains. The first involves studies of serotonin and metabolites in postmortem brain samples from suicide completers and in cerebrospinal fluid (CSF) of suicide attempters. Levels of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), have been found in most studies to be lower in the brainstem of suicide victims compared to controls (see [2] for review), although we find no reductions in cerebral cortex [43]. Lower levels of CSF 5-HIAA have been reported in suicide attempters compared to nonattempters [10,11,51,76], and a blunted release of prolactin in response to the indirect serotonin agonist fenfluramine has been observed in suicide attempters [22,51,52].
The second domain of study involves assessment of serotonin receptors. Serotonin receptors were originally classified as 5-HT_1 and 5-HT_2 [65]. Subtypes of 5-HT_1 receptors, namely 5-HT_1A and 5-HT_1B, were soon proposed [32,64]. More recently, other 5-HT_1 receptor subtypes as well as 5-HT_1D [14,40], 5-HT_1E [28] and other [15,75] receptor subtypes have been reported. We previously found that 5-HT_2 sites, as labeled by [125I]lysergic acid diethylamide ([125I]LSD) [1] or [3H]spiroperidol [54], are increased in the brain of suicide victims, compared to controls. We also found that 5-HT_1 sites, as labeled by [3H]5-HT, are unaltered [54]. The 5-HT_1A receptor, a postsynaptic serotonin receptor in the cerebral cortex, is of particular interest because of recent reports that 5-HT_1A binding is increased in suicide victims [38,56], as well as its possible involvement in anxiety and affective disorders as suggested by the anxiolytic and antidepressant properties of partial 5-HT_1A agonists [74].

An index of the serotonin nerve terminal integrity may be obtained from binding studies of the serotonin transporter, which may be a better indicator of the abnormalities associated with suicide than cortical levels of serotonin or 5-HIAA [43]. Not all studies agree as to whether binding to the serotonin transporter on nerve terminals is reduced in cortical regions of suicide victims [2]. However, most studies of the serotonin transporter in suicide victims have employed [3H]imipramine as the ligand, which has been shown to bind to both high- and low-affinity sites [19,37], and to nonserotonergic receptors such as muscarinic cholinergic sites [24]. Only the high-affinity site represents binding to the serotonin transporter [24]. In contrast, [3H]paroxetine [34] and [3H]cyanoimipramine ([3H]CN-IMI) bind to a single class of binding site [27,35,42], the high-affinity site. Studies of [3H]imipramine in homogenates from frontal cortex are divided in that about half found a decrease in the suicide group and the remainder found no change (see [2] for review). Three studies have used [3H]paroxetine as the ligand and two of those [45,46] reported no changes in the frontal cortex (Brodman area 10) of depressed suicide victims compared to controls. The third study [36] was from our laboratory and found a 40% reduction in drug-free suicide victims.

The above studies suggest that there are serotonin receptor alterations in the brain of suicide victims. Most studies examined homogenates from prefrontal cortex and provide little anatomical information as to the extent and localization of serotonin receptor changes in suicide. We therefore sought to localize where, within the prefrontal cortex of suicide victims, presynaptic (serotonin transporter) and postsynaptic (5-HT_1A) serotonin receptor changes are present, and whether these changes in pre- and postsynaptic binding sites are colocated.

We used the selective ligands [3H]8-hydroxy-2-(di-n-propyl-amino)-tetralin ([3H]8-OH-DPAT) [32] and [3H]CN-IMI [42] to perform quantitative receptor autoradiography of 5-HT_1A and serotonin transporter binding, respectively, in adjacent sections of tissue taken from the prefrontal cortex of suicide victims and matched controls. This approach allowed mapping of binding sites across multiple regions of the prefrontal cortex and the comparison of the precise localization of changes associated with suicide.

2. Materials and methods

Tissue was provided by local Coroner or Medical Examiner offices in accordance with city or county regulations and the protocol was approved by the Institutional Review Board for Biomedical Research. An essential aspect of the methods involved performing the assays for the two binding site populations (5-HT_1A and serotonin transporter) on adjacent tissue sections cut from the same block of tissue from each case, in order to permit maximal comparability for assessing the degree of colocalization of changes in binding to the two sites.

We employed a matched-pairs design. The cause of death was determined by the Coroner or Medical Examiner. Each suicide case (n = 22) was matched with a control subject (n = 22) on the basis of postmortem interval (PMI, ± 5 h), age (± 5 years), sex, season of death, side of brain and, whenever possible, race. The two groups were tested for differences in age, sex and race. The results are described below. The brain samples were coded and assayed together by personnel blind to the cause of death. Individuals with a history of cerebral trauma, central nervous system disease, chronic alcoholism, illicit or therapeutic drug use or AIDS were excluded. Body fluids (blood, bile, aqueous humor and urine) underwent toxicological screening for cocaine, opiates, alcohol and other acidic and basic drugs.

2.1. Collection, dissection and storage of brain samples

Brains were collected and bisected at autopsy. The right hemispheres were cut coronally into 1.5-cm-thick sections using a Lipshaw macrotom (Lipshaw Corp., Detroit, MI). Blocks were placed on a glass slide, immersed in freon (-20°C) and stored at -80°C until sectioning. Samples from the left hemisphere were saved for neuropathological and toxicological analyses. After a control and suicide were matched, tissue was sectioned at 20 µm with a large body Lipshaw cryotome (1800-N Research model, Lipshaw Corp., Detroit, MI, -14 to -18°C). Coronal sections from the entire hemicerebrum were taken from a level just anterior to the genu of the corpus callosum. This level was chosen because it contains prefrontal cortical areas believed to be involved in higher cognitive functions, affect and emotion, as well as in the temporal organization of behavior [30]. Sections (n = 100) were collected serially and thaw-mounted onto 3" × 5" gelatin-subbed, acid-
cleaned glass slides. An intercalating set (every 200 µm) was stained for Nissl substance. Six sections per brain were used for each receptor assay. This collection protocol allowed for the study of multiple receptor populations in essentially identical brain cortical areas. Tissue sections were desiccated and stored in sealed boxes (at −20°C for 24 h, and −80°C thereafter).

2.2. *In vitro* receptor autoradiography

**Association / Dissociation / Competition Experiments**

To obtain optimal assay conditions, association, dissociation, and competition curves were obtained (in quadruplicate) using slide-mounted rat brain sections prior to beginning the human experiments. Sections were wiped from the slide with filter discs (Whatman GF/B) and counted in a liquid scintillation spectrophotometer (Packard Instrument Co., Meriden, CT). Binding of [3H]8-OH-DPAT reached equilibrium in 60 min (t_{1/2} = 7 min), and the best specific/nonspecific ratio was achieved following a 10 min wash. Competition curves with 12 concentrations of serotonin (100 pM to 100 µM) determined the IC_{50} to be 3.16 nM. Binding of [3H]CN-IMI reached equilibrium at 24 h (t_{1/2} = 4 h) and the IC_{50} for sertraline was 36 nM. The optimum wash time was determined to be 60 min.

**General procedure**

Dry sections from one matched pair were surrounded with dental wax (Miles Inc., South Bend, IN) and incubated with 8–10 ml of ligand in a humidity chamber. Washes were done in large plexiglass jars, thus ensuring the presence of excess buffer to remove nonspecific binding. All buffers were isosmotic to preserve the integrity of the tissue. Following the washes, the slides were dipped in distilled H_{2}O (4°C), the tissue was dried with cold, filtered air and vacuum desiccated overnight at 4°C. Dried sections were taken from each of the individual isodensity bands, as well as across the full width of the cortical gray matter, from both the gyrus and sulcus. Measurements of binding were taken from each of the individual isodensity bands, as well as across the full width of the cortical gray matter, from both the gyrus and sulcus. Measurements were corrected daily for radioactive decay. Quantitation of autoradiograms

Autoradiograms were analyzed using a PC-based image analysis system (Imaging Research, Inc., St. Catherine, Ont., Canada). Films were digitized, shade corrected and calibrated against tritium standards (ARC, St. Louis, MO). Histological examination of Nissl-stained sections was used in the determination of the boundaries of cortical layers and their correspondence to isodensity bands in the autoradiograms. Brodmann areas 8, 9, 46, 45, 47, 11, 12, 32 and 24 were identified using gyral and sulcal landmarks, cytoarchitecture and a standardized coronal atlas (Robert Perry and Edward Bird, personal communication). Images from sections incubated in the presence of the radioligand alone (total binding) were acquired first. Images produced from incubation in the presence of displacer (nonspecific binding) were then acquired and digitally subtracted from the total binding. We thus measured specific binding, in fmol/mg tissue, for each receptor. Measurements of binding were taken from each of the individual isodensity bands, as well as across the full width of the cortical gray matter, from both the gyrus and sulcus. Measurements were taken from all areas available in the three resulting autoradiograms per brain. Multiple readings from each region were averaged. In instances where gray matter was discontinuous, measurements excluded missing tissue. Binding to white matter was taken as the average of multiple measurements throughout the sections. Standards were corrected daily for radioactive decay.

2.3. High pressure liquid chromatography (HPLC) analysis of serotonin, 5-HIAA, 5-hydroxytryptophan (5-HTP) and 1-tryptophan (1-try) levels

A modification of the method of Korpi and colleagues [41] was used for determination of the levels of serotonin, its precursors (5-HTP, 1-try) and principal metabolite (5-HIAA). Tissue was sonicated in 0.1 M perchloric acid (4°C) containing N-methyl-5-hydroxytryptamine (NMET, 0.1 µM) as the internal standard. An aliquot of the homogenate was used for protein analysis [49], and the remaining homogenate was centrifuged at 10,000 × g (8 min, 4°C). Supernatants were diluted 2 fold with cold 0.1 M perchloric acid and injected into the HPLC column.
The HPLC consisted of a Waters M-501 high pressure pump, a WISP auto sampler, a Rainin Dynamax 5 μM C18 reverse-phase analytical and guard columns and a Waters 460 electrochemical detector. The mobile phase consisted of 75 mM sodium phosphate buffer (pH 3.0) containing 7.5% acetonitrile (v/v), 0.9 mM 1-octane sulfonic acid and 50 μM disodium EDTA. The flow rate was 1.0 ml/min. Working electrode potential was kept at +0.8V against an Ag/AgCl reference electrode. Typical retention times (K*) for 5-HTP, 5-HIAA, serotonin, L-try and NMET were 5.9 min, 11.4 min, 20.5 min, 24 min and 25.2 min, respectively. All data were expressed as pmol per mg protein.

Data were acquired and analyzed using a Waters Base-line 810 software package (Dynamic Solutions, Millipore, Milford, MA). Both external and internal standards were used for quantitation and data were calculated based on sample peak area relative to internal standard peak area. The standard curves were based on external standards which encompassed the concentrations of the unknowns or samples.

2.4. Statistical methods

We employed a multivariate testing procedure, namely a forward, stepwise moving, logistic multivariate regression model. This procedure was used not only to pick out the significant variables that explain the logistic model, but also to identify the distinctive profiles of the dichotomous dependent variable. This analysis was used to separately study differences in group, sex and dichotomized age, for binding to the 5-HT1A and the serotonin transporter receptors. Post-regression analyses were carried out, using paired t-tests for the significant variables. The descriptive statistics on these variables and correlations were included to support and complement the analysis. SPSSWINDOWS (6.0) was used to analyze the data.

Binding to 5-HT1A sites in each brain was examined in

![Fig. 1. Pseudocolor images of subtracted autoradiograms representing total specific 5-HT1A (A, left panel) and serotonin transporter (B, right panel) binding sites in adjacent coronal sections of the prefrontal cortex of the human. Note that: (1) the distribution of 5-HT1A receptors is laminar and most of the binding is localized in a band overlaying cortical layer II in all cortical areas; (2) Binding to the serotonin transporter is higher in the outer cortical layers and densest in the medial prefrontal cortex; (3) binding to the gray matter is much greater than to the white matter for both pre- and postsynaptic sites.](image)
103 bands involving nine Brodmann areas (sulcus and gyrus) and 18 pairs were included in the analysis. The natural logarithms of these 103 measures were taken to reduce skewness, and constituted the variables to be tested for $5\text{-HT}_{1A}$ differences. Binding to the serotonin transporter was studied in 43 bands, using data on 22 matched pairs of subjects. The binding levels in these 43 brain regions were not transformed as there was little skewness.

Correlations with age, PMI and freezer storage time were calculated using the Pearson's coefficient of correlation. Missing cells were replaced by their corresponding subgroup means for each of the receptor populations. Results are reported as mean ± standard error of the mean. All $P$ values are two-tailed. Alpha was preset at $P < 0.05$.

2.5. Subjects

Demographic variables from suicide and control subjects were similar. The mean age of the suicide group was $41.5 ± 4.14$ y ($14–78$) compared to $41.3 ± 4.4$ years ($15–79$) for the controls ($t = 0.23$, $21$ df, $P = 0.98$). The PMI of the suicide group was $14.2 ± 0.3$ h ($4–22$ h) compared to $13.9 ± 0.2$ h ($4–22$) for the controls ($t = 0.83$, $21$ df, $P = 0.78$). The ratio of men to women was $14:8$ in each group. The mean freezer storage time (time from tissue collection to assay) for samples assayed for $5\text{-HT}_{1A}$ binding in the suicide group was $518.8 ± 55.5$ days compared to $525.5 ± 47.5$ days for the control group ($t = 0.14$, $17$ df, $P = 0.82$). Likewise, the storage time for samples assayed for the serotonin transporter was not different in the two groups ($622.6 ± 40.1$ d vs. $587.5 ± 31.2$ days; $t = 0.82$, $21$ df, $P = 0.42$). Causes of death in the suicide group were: hanging (10), fall from height (6), firearm (4), subway (1) and ingestion of lye (1). In the controls, causes of death were: fall (3), motor vehicle accident (8), firearm (3), cardiac failure (3), lacerations (2), explosion (1), aneurism (1) and respiratory failure (1). These criteria for matching pairs enabled us to limit possible variance due to PMI, sex, storage and age, that may affect receptor binding. Assaying the matched pairs together reduced interassay variance effects.

2.6. Drugs and reagents

$[^3\text{H}]8\text{-OH-DPAT}$ ($125–162$ Ci/mmol) and $[^3\text{H}]\text{CN-IMI}$ ($78.8–83.6$ Ci/mmol) were purchased from New England Nuclear (Boston, MA), stored in ethanol at $-80°C$ in the dark and diluted in buffer immediately prior to assay. Sertraline-HCl was generously supplied by Pfizer Pharmaceuticals (Groton, CT). All other chemicals were purchased from Sigma Chemical Co (St. Louis, MO).

3. Results

Gyral and sulcal cortex exhibit different cyto- and myeloarchitectonic characteristics [79], with further differences between the sulcal wall and the fundus. Therefore, we examined binding to gyri and sulci separately in order to take into account potential anatomical differences.

Serotonin transporter and $5\text{-HT}_{1A}$ binding data were analyzed separately for differences in each of the variables of group (suicide and control), sex and age.

3.1. $[^3\text{H}]8\text{-OH-DPAT}$ binding to serotonin $5\text{-HT}_{1A}$ receptors

Five isodensity bands were observed in all cortical areas studied in suicides and controls (Fig. 1A, Fig. 2), which corresponded to histologically identified cortical layers as follows: band I (cortical layer I), band II (cortical layer II), band III (upper layer III), band III–IV (inner layer III and layer IV) and band V–VI (layers V and VI).

Group differences

The logistic procedure separated the two groups using binding data from four regions. The data from these re-
Model coefficients for 5-HT1A receptor binding in prefrontal cortex: significant variables identified by the logistic regression analysis in a group of suicide victims and controls

<table>
<thead>
<tr>
<th>Variable: Group</th>
<th>Value of coefficient (transformed)</th>
<th>Sig. of Wald’s stat. (P)</th>
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<tr>
<td>LN 111AS1</td>
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<td>LN121AS56</td>
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<td>LN321AGGR</td>
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<td>0.0195</td>
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<td>LN451ASGR</td>
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</thead>
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<td>LN321AG56</td>
<td>-29.4346</td>
<td>0.0615</td>
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<tr>
<td>LN471AG34</td>
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<td>LN471AS34</td>
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<table>
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<th>Variable: Age</th>
<th>Value of coefficient (transformed)</th>
<th>Sig. of Wald’s stat. (P)</th>
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</tr>
<tr>
<td>Constant</td>
<td>3.1024</td>
<td>0.3988</td>
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</table>

The variable names include the following: LN = log; The first two digits indicate the Brodmann area; 1A identifies the 5-HT1A receptor, followed by S (sulcus) or G (gyrus); the next one or two characters indicate the cortical laminae corresponding to a given isodensity band or to the entire gray matter (GR).

Table 2
Post-hoc paired t-tests for 5-HT1A receptor binding in Brodmann areas 45 and 46

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± S.E.M.</th>
<th>t</th>
<th>P (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brodmann area 45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Band II, Gyrus</td>
<td>C: 3.59 ± 0.10</td>
<td>2.27</td>
<td>0.037</td>
</tr>
<tr>
<td>Band III–IV, Gyrus</td>
<td>C: 1.48 ± 0.11</td>
<td>S: 1.76 ± 0.10</td>
<td>3</td>
</tr>
<tr>
<td>Band V–VI, Gyrus</td>
<td>C: 2.11 ± 0.09</td>
<td>S: 2.30 ± 0.09</td>
<td>2.7</td>
</tr>
<tr>
<td>Gyral gray</td>
<td>C: 2.55 ± 0.08</td>
<td>S: 2.77 ± 0.07</td>
<td>3.17</td>
</tr>
<tr>
<td>Band II, Sulcus</td>
<td>C: 3.57 ± 0.07</td>
<td>S: 3.76 ± 0.07</td>
<td>3.71</td>
</tr>
<tr>
<td>Band III–IV, Sulcus</td>
<td>C: 1.56 ± 0.08</td>
<td>S: 1.81 ± 0.08</td>
<td>3.41</td>
</tr>
<tr>
<td>Sulcal gray</td>
<td>C: 2.52 ± 0.06</td>
<td>S: 2.77 ± 0.06</td>
<td>4.46</td>
</tr>
</tbody>
</table>

| Brodmann area 46 |
| Band II, Gyrus | C: 3.55 ± 0.09 | S: 3.64 ± 0.08 | 2.37 | 0.030 |
| Band II, Sulcus | C: 3.46 ± 0.10 | S: 3.67 ± 0.08 | 3.67 | 0.002 |
| Band III–IV, Sulcus | C: 1.50 ± 0.11 | S: 1.72 ± 0.12 | 3.02 | 0.008 |

Sex differences

The procedure identified the two sexes with only one mismatch out of the 36 cases, using receptor binding results (natural log of fmol/mg tissue) from three regions: band V–VI of gyral area 32, (2.35 ± 0.06 for males vs. 2.41 ± 0.05 for females); band III–IV for both sulcal and gyral area 47 (1.83 ± 0.06 for males vs. 2.24 ± 0.05 for females and 1.89 ± 0.07 for males vs. 2.04 ± 0.07 for females, respectively). The accuracy of classification was 97%. See Table 1 for details on these variables. Binding was greater in females than males in 98/103 brain regions.
Fig. 3. Sex differences in $[^{3}H]8$-OH-DPAT binding in cortical layers in the Sulcus of Brodmann area 46. Bar graph comparing the specific binding of $[^{3}H]8$-OH-DPAT to 5-HT$_{1A}$ sites between males and females once again utilizing the lateral prefrontal cortex (sulcus of Brodmann area 46) as a representative area. Specific binding is expressed in fmol/mg tissue ± S.E.M. for 26 males (light stipple) and 10 females (dark stipple). Data for each subject was obtained as described in Figs. 2 and 3.

$P < 0.0001$). Females had higher binding than males with the difference ranging from 1 to 40%. Fig. 3 shows the untransformed data for males and females in sulcal area 46.

**Age differences**

The age of each individual was transformed into a dichotomous variable using the median age of 32.5 y. Receptor binding (natural log of fmol/mg tissue) in two regions were identified by the model as significant. These regions are: sulcal band III–IV of area 12 (2.15 ± 0.09 for above-median group vs. 2.06 ± 0.08 for below median group); band V–VI of sulcal area 45 (1.92 ± 0.08 for above median group vs. 2.17 ± 0.07 for below median group). The accuracy of classification of this dichotomous variable was 77%, with ten misclassifications. 71/103 brain regions had lower binding in the older group. Table 1 presents details on the logistic analysis.

3.2. $[^{3}H]CN$-IMI binding to the serotonin transporter

Binding to the serotonin transporter was mostly bilaminar (Fig. 1B). Therefore, we measured specific binding over cortical layers I, II and upper III (outer band), and over inner III, IV, V and VI (inner band), as well as over the entire thickness of the gray matter. The outer cortical layers had higher levels of binding. For consistency with $[^{3}H]8$-OH-DPAT binding, both sulcal and gyral regions were measured separately, but did not differ ($P > 0.05$). The bilaminar pattern was most striking in the anterior cingulate gyrus, where the outer band had more than twice
the level of binding of the inner band. The bilaminar pattern of receptor distribution was preserved in suicide victims. Levels of binding differed greatly across cortical areas within the prefrontal cortex, and was more than four times higher in the anterior cingulate gyrus than in the association cortex of area 9. As with 5-HT1A binding, the pattern of differential binding to the serotonin transporter across Brodmann areas in prefrontal cortex was preserved in suicide victims.

The multivariate statistical procedure used for serotonin binding analyses is the same as above. The variables were not transformed because skewness in the receptor binding levels was negligible. As pointed out earlier, the logistic analysis was performed three times to study group, sex and age differences using binding levels (fmol/mg tissue) in all 43 regions (bands). The results are presented separately for each of the three variables.

**Group differences**

The control and suicide groups were identified by the model using the binding data from only one region, namely, inner band of gyral area 46 (5.96 ± 0.43 for the control group vs. 4.72 ± 0.42 for the suicide group). There were thirteen mismatches out of a total 44 observations, yielding an accuracy of 71%. Table 3 presents the model parameters. Serotonin transporter binding was lower in the suicide group in 42 of the 43 brain regions (Wilcoxon Matched-Pairs Signed-Ranks Test, P < 0.0001, see Fig. 4). The exception was in the inner band of gyral area 32. The decrease in binding was more pronounced in Brodmann areas 45 and 46, the difference ranging between 16 and 27%. In the other cortical regions, the difference ranged between zero and 21%.

**Table 3**

Model coefficients for serotonin transporter receptor binding in prefrontal cortex: significant variables identified by the logistic regression analysis in a group of suicide victims and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value of coefficient</th>
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<table>
<thead>
<tr>
<th>Variable</th>
<th>Value of coefficient</th>
<th>Sig. of Wald’s stat. (P)</th>
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<td>Constant</td>
<td>2.7435</td>
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* The variable names are constructed as follows: the first two digits indicate the Brodmann area, U identifies the serotonin transporter (uptake) site, followed by S (sulcus) or G (gyrus); the next two characters indicate the inner (IN) cortical laminae corresponding to an isodensity band.

Post-hoc paired t-tests showed that probabilities associated with the mean values for the serotonin transporter binding were significant in six bands in areas 45 and 46. The mean values for the suicide group was lower than the control group in all the six significant regions. Table 4 present details of these results.

**Sex differences**

The inner layers of sulcal area 32 (8.56 ± 0.61 for males vs. 5.52 ± 0.51 for females) had significantly different binding. Male and female subjects were identified with a mismatch of twelve subjects, yielding a classification.
Table 4
Post-hoc paired t-tests for serotonin transporter receptor binding in Brodmann areas 45 and 46

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± S.E.M. *</th>
<th>t</th>
<th>P (2-tailed)</th>
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<tr>
<td>Brodmann area 45</td>
<td></td>
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<tr>
<td>Sulcal gray</td>
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<td></td>
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<tr>
<td>C</td>
<td>11.39 ± 1.20</td>
<td>2.72</td>
<td>0.013</td>
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<tr>
<td>S</td>
<td>8.36 ± 0.91</td>
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<tr>
<td>Inner band, Sulcus</td>
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<tr>
<td>C</td>
<td>8.60 ± 0.82</td>
<td>2.59</td>
<td>0.017</td>
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<tr>
<td>S</td>
<td>6.43 ± 0.65</td>
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<td>Outer band, Sulcus</td>
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<tr>
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<td>15.19 ± 1.69</td>
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<tr>
<td>S</td>
<td>11.16 ± 1.29</td>
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<tr>
<td>Brodmann area 46</td>
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<tr>
<td>Gyral gray</td>
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<td></td>
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<tr>
<td>C</td>
<td>7.01 ± 0.54</td>
<td>2.23</td>
<td>0.037</td>
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<tr>
<td>S</td>
<td>5.56 ± 0.52</td>
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<tr>
<td>Inner band, Gyrus</td>
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<td></td>
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<tr>
<td>C</td>
<td>5.96 ± 0.43</td>
<td>2.20</td>
<td>0.039</td>
</tr>
<tr>
<td>S</td>
<td>4.71 ± 0.42</td>
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<tr>
<td>Outer band, Gyrus</td>
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<tr>
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<td>2.22</td>
<td>0.037</td>
</tr>
<tr>
<td>S</td>
<td>6.34 ± 0.62</td>
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</table>

* fmol/mg tissue, obtained from 22 pairs of suicides and controls.

Accuracy of 72.7%. Table 3 describes the details. Females had lower binding than males in all cortical brain regions examined (but not in white matter, Fig. 5) and the percentage difference ranged from 15 to 38% (P < 0.0001).

3.3. Correlations between 5-HT1A and serotonin transporter binding

Correlations were studied within Brodmann areas 45 and 46 for the two binding sites. It should be noted that the isodensity bands for the two sites are quite different and so an exact match from the same cortical layers was not done. Instead we compared sulcal or gyral subregions from the same Brodmann areas. In Brodmann area 45, correlation between the log transformed binding value for 5-HT1A in gyrual outer layer III and the binding for serotonin transporter in the inner gyral layers was \( r = -0.341 \) (P = 0.04), while that for gyral layers III-IV for 5-HT1A with the inner laminae for serotonin transporter binding was \( r = -0.394 \) (P = 0.019). The binding in the sulcal regions for these two receptors had five significant correlations ranging from \( r = -0.353 \) to \( r = -0.436 \) with probabilities between 0.037 and 0.009. The regions involved were the sulcal layer II, III-IV and the gray matter for 5-HT1A and the inner laminae of gyrus and sulcus for the transporter. Area 46 had three significant correlations involving the inner and outer sulcal layers for the serotonin transporter and the sulcal gray for 5-HT1A, the r values ranging from \( -0.372 \) to \( -0.445 \) with probabilities from 0.028 to 0.007. Other brain regions had weaker, but nevertheless, negative correlations.

3.4. Correlations with postmortem interval

The effect of postmortem interval on binding in gyrus gray and sulcus gray areas was studied. PMI correlated positively with the binding to the serotonin transporter in all Brodmann areas studied. Correlation values ranged from 0.439 to 0.627 with associated probabilities between 0.008 and 0.000. The effect of PMI was most dramatic in the group with the short PMI (less than or equal to 15.9 h, the median PMI for the sample). There were no correlations between 5-HT1A and PMI. The group with PMI greater than the median (> 15.9 h) did not correlate with binding to either site. Thus the binding to the transporter seems to be temporally dependent.

Another aspect of the effect of PMI on the serotonin transporter is its special affinity for the suicide group. The binding to the transporter in all the 14 gray areas (sulcus and gyrus) showed very high correlations with PMI, ranging from 0.55 to 0.81, with probabilities from 0.024 to 0.000. Nine of these correlations were above 0.7. In addition, the binding was higher in sulci than in gyri (in 6 out of 7 Brodmann areas). In contrast, only three of the 14 correlations were significant for the control group (with probabilities 0.05 to 0.02). The correlations were negative and were not significant for 5-HT1A, ranging mostly from \(-0.25\) to \(-0.45\) for the suicide group and almost zero for controls.

3.5. Relationship between serotonin, 5-HIAA, 5-HTP and l-try and binding sites

Levels of serotonin in Brodmann area 9 were not different in suicide victims (6.9 ± 3.2 pmol/mg protein) compared to controls (6.7 ± 2.0 pmol/mg protein; P = 0.95). Levels of 5-HIAA from the same area also did not differ (13.4 ± 2.0 vs. 11.7 ± 2.1 pmol/mg protein, P = 0.52). Similarly, levels of the precursors l-try and 5-HTP did not differ between groups. No correlations were found between levels of serotonin, its precursors or metabolite and either 5-HT1A or serotonin transporter binding in the same Brodmann area.

4. Discussion

Using quantitative receptor autoradiography we found a decrease in the binding to the presynaptic serotonin transporter, and a concomitant increase in the level of postsynaptic 5-HT1A binding in the prefrontal cortex of suicide victims compared to controls. This is the first study to examine both pre- and postsynaptic serotonin receptors by quantitative receptor autoradiography in adjacent sections from the prefrontal cortex of suicide victims and controls.

Levels of serotonin and 5-HIAA appear to be lower in the brainstem of suicide victims, [2] and 5-HIAA levels are lower in CSF from suicide attempters, [51] suggesting that
reduced serotonergic function is associated with suicide and attempted suicide. This reduction appears to be independent of diagnostic group and related to a history of a serious suicidal act. We therefore hypothesized that the postsynaptic 5-HT$_{1A}$ receptor may be upregulated and the nerve terminal serotonin transporter may be reduced in the suicide group compared to controls.

The differences in binding levels for the serotonin transporter were more uniformly widespread (42/43 regions) than for the 5-HT$_{1A}$ receptor (85/103 regions). Both binding site populations in suicide showed the greatest differences from controls in the orbital and lateral prefrontal cortex, suggesting the greatest abnormality in suicides are in these regions. Furthermore, the correlations between the 5-HT$_{1A}$ and serotonin transporter binding levels were found to be negative in more than 90% of the regions studied. Thus, we find both a negative correlation in binding to the 5-HT$_{1A}$ receptor and the serotonin transporter in both groups, as well as significant changes in these pre- and postsynaptic serotonin receptors in suicide victims compared to controls in ventrolateral prefrontal cortex. We interpret these results to suggest that there is an underlying common influence on 5-HT$_{1A}$ and serotonin transporter binding, and this influence results in changes in opposite directions on the two receptor subtypes.

Earlier binding studies of 5-HT$_1$ receptors in suicide victims [23,54,58,59] used $[^3$H]5-HT in homogenates from frontal cortex and other regions. None of these studies reported any differences between groups, perhaps because such an approach fails to distinguish between serotonin receptor subtypes. $[^3$H]5-HT binds with nanomolar affinity to 5-HT$_{1A}$, 5-HT$_{1C}$, 5-HT$_{1D}$ and probably other receptor subtypes. More recent studies are in disagreement as to whether there is an increase in 5-HT$_{1A}$ and serotonin transporter binding levels were found to be negative in more than 90% of the regions studied. Thus, we find both a negative correlation in binding to the 5-HT$_{1A}$ receptor and the serotonin transporter in both groups, as well as significant changes in these pre- and postsynaptic serotonin receptors in suicide victims compared to controls in ventrolateral prefrontal cortex. We interpret these results to suggest that there is an underlying common influence on 5-HT$_{1A}$ and serotonin transporter binding, and this influence results in changes in opposite directions on the two receptor subtypes.

Studies of $[^3$H]imipramine in homogenates from frontal cortex have reported conflicting results. Most of these studies found a decrease in the number of binding sites in the frontal cortex of the suicide group [5,6,23,62,70,71]. Three studies found no change [7,8,58], and one study found an increase in these sites in the suicide group [57]. In looking at other brain regions, one study [62] found a 30% decrease in the hypothalamus of the suicide group, and another [46] found a decrease in the putamen of depressed suicide victims dying from an overdose. Four studies have used $[^3$H]paroxetine as the ligand and two of those [45,46] reported no changes in the prefrontal cortex (Brodmann area 10) of depressed suicide victims compared to controls. The other studies [43,44] found a 35% and a 26% reduction in the prefrontal and temporal cortex, respectively, of drug-free suicide victims compared to controls [43], and a 43% reduction in prefrontal cortex [44]. As indicated above, $[^3$H]imipramine is not a specific ligand for the transporter and therefore some of the differences in results may be related to changes in other receptors, such as the non-transporter imipramine binding site (protease-resistant, not displaced by serotonin), $\alpha_1$-adrenergic or muscarinic cholinergic sites [24]. Moreover, if, as our present study indicates, the decrease in transporter binding is generalized but greater in specific subregions of the prefrontal cortex, then the results of homogenate assays are somewhat dependent on the accuracy of dissection of specific cortical regions.

Of the six studies that have reported no significant decreases in serotonin transporter binding in suicide victims compared to controls, four had postmortem intervals greater than 35 h [2]. We found evidence of an increase in the full range of PMI’s. The other two studies reporting no reductions in binding had PMI’s shorter than 27 h. On the other hand, all the studies reporting decreases in transporter binding in the suicide group had mean postmortem intervals shorter than 22 h [2,18,38]. The cause of the increase in binding with increased PMI is unknown.
One previous study utilized \textsuperscript{3}H]imipramine by autoradiography [33] on coronal sections from one hemicerebrum and found reduced binding in the postcentral gyrus, the insular cortex and the claustrum, and increased binding in several areas of the hippocampal formation. Our study of the prefrontal cortex indicates a relatively localized decrease in the ventrolateral region. Thus the variability of results in studies of the serotonin transporter, may be due in part to the choice of ligand, the brain region examined, as well as the age and sex of the population studied, postmortem interval and the use of antemortem medication.

Binding of \textsuperscript{3}H]8-OH-DPAT had a similar cortical laminarization in both suicide victims and controls, and therefore the greater binding was not due to a change in the distribution or organization of 5-HT\textsubscript{1A} binding sites. In normal brain, binding to gyrus and sulci had similar laminar organization, although in the sulcus, cortical laminae were thinner and closer together. However, in both suicides and controls, binding varied similarly across cortical areas which emphasizes the need for precise comparison of the same brain regions between study groups. For example, binding was higher in the orbital cortex and medial cortex than in the dorsolateral cortex. Such regional comparisons in 5-HT\textsubscript{1A} binding within multiple regions at one coronal level are not addressed in previously published normative studies [63].

The suggestion that greater 5-HT\textsubscript{1A} binding represents upregulation of postsynaptic 5-HT\textsubscript{1A} serotonergic sites secondary to decreased serotonergic innervation is, for the most part, not supported by animal models where no alterations have been reported after chemical lesions of the serotonergic system [39,73]. On the other hand, we have found increased 5-HT\textsubscript{1A} binding in frontal cortex of rats after electrolytic lesions of the dorsal raphe nucleus [77] and a 20-40% increase in 5-HT\textsubscript{1A} mRNA was reported in rat 11 days after 5,7-dihydroxytryptamine lesions [16], as well as an increase in 5-HT\textsubscript{1A} immunoreactivity in the hippocampus 14 days post-lesion [61]. Our results are consistent with an upregulation hypothesis for greater 5-HT\textsubscript{1A} binding in the ventrolateral prefrontal cortex of suicide victims. Studies of the effect of serotonin depletion on serotonin transporter binding are lacking.

The functions of the prefrontal cortex (PFC) in humans are unknown, but evidence suggests that it may be involved in behavioral inhibition and the expression of emotion (see [31,47] for review). Lesions of the PFC, either as a result of trauma or stroke [25,66,67,72], are commonly associated with the development of depression or aggression. Disinhibition is also a consequence of a prefrontal lesion, suggesting that this area has an executive inhibitory action. Epilepsy involving the frontal cortex, particularly the right hemisphere, has been associated with pathological crying [67]. An early study [78] of patients with localized frontotemporal lobe contusion vs. brainstem contusion showed that 5-HIAA in the cerebrospinal fluid was reduced in the first group, compared to controls and to those patients with brainstem dysfunction, a condition which persisted for 6 months. Dysfunction of the prefrontal cortex may be the result of damaged subcortical afferent pathways including serotonergic fibers. Lesions of subcortical pathways may at least partly result in reduced serotonergic input. Alternatively, an abnormality may exist at the level of serotonin cell bodies in the raphe nuclei, which are yet to be studied directly. Regardless of the site of the lesion, the result of loss of serotonin input into the prefrontal cortex may result in both disinhibition and affective changes that lead to suicidal acts.

Future postmortem studies should address the location of altered serotonin input in terms of cell bodies, fiber pathways, nerve terminals or target cortical neurons. These studies should also confirm that the association of altered serotonin function and suicide is independent of diagnosis by conducting careful psychological autopsies.

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