Objective: Studies comparing people suffering from depression who committed suicide with control subjects have yielded inconsistent results regarding serotonin (5-HT) involvement in pathology, possibly owing to procedural factors. Our objective was to investigate which 5-HT receptor subtypes might be associated with depression and suicide, whether receptor differences vary across brain regions and whether they are moderated by sex.

Methods: We assessed messenger ribonucleic acid (mRNA) expression of several 5-HT receptor subtypes and that of p11, a protein involved in the functional expression of 5-HT 1B, in several stress-relevant brain regions. Tissue was obtained soon after death, and RNA integrity and pH was confirmed to be appropriate. Brain tissue from suicide subjects suffering from depression and from control subjects who had died from other causes (10 men and 10 women in each condition) was obtained within 6.5 hours postmortem. Quantitative polymerase chain reaction analyses determined mRNA expression of 5-HT receptor subtypes and p11 within the frontopolar cortex, orbitofrontal cortex, hippocampus, amygdala and paraventricular nucleus. The 5-HT transporter (5-HTT) was also assessed in the raphe nucleus.

Results: Differences of 5-HT1A, 5-HT1B and p11 mRNA expression between people who committed suicide and control subjects were relatively widespread, whereas 5-HT 2A and 5-HT2C variations were restricted to the frontopolar cortex and amygdala. Within the dorsal raphe nucleus, neither 5-HT 1A nor 5-HTT mRNA expression differed between those who committed suicide and control subjects.

Conclusion: Several 5-HT receptor subtypes are associated with depression and suicide, but these receptor differences vary across brain regions and are moderated by sex.

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Medical subject headings: suicide; depression; receptors, serotonin.


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Introduction

Imaging and receptor binding studies have identified a diverse set of morphological and neurochemical disturbances that may contribute to the emergence of major depressive illness and suicide. Of these, particular attention has been devoted to serotonergic changes, owing in part to the positive effects of agents that act on serotonin (5-HT) in managing depressive illness. In contrast to the pharmacologic evidence implicating 5-HT functioning in depression and the evidence derived from imaging studies showing altered 5-HT receptor binding within the frontal cortex or hippocampus, there have also been reports indicating that depression with suicide was not associated with altered 5-HT receptors. Moreover, DNA microarray analyses did not detect molecular genetic differences within the dorsolateral and ventral prefrontal cortex (PFC) of subjects who committed suicide and control subjects.

In contrast to these negative reports, differences between suicide and control subjects have been observed with respect to 5-HT receptor subtypes, the 5-HT transporter (5-HTT) and genes coding for tryptophan hydroxylase-2. For instance, among subjects suffering from depression who committed suicide, reduced 5-HT1A (auto)receptors were found in the PFC and the gene for this receptor were elevated in the hip-pocampus of suicide subjects who suffered from depression. For several 5-HT receptor subtypes (5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C) in brain regions that have been implicated in depression (the frontopolar cortex [FPC], orbitofrontal cortex [OFC] and hippocampus), stressor reactivity and anxiety (the amygdala) and hypothalamic-pituitary-adrenal (HPA) functioning (the paraventricular nucleus [PVN] of the hypothalamus). As well, we assessed mRNA coding for p11, a protein involved in the functional expression of 5-HT2A receptors (also known as calpactin light chain, calpactin I and S100A10). Its link to depression comes from findings that in p11 knockout mice the behavioural profile observed was reminiscent of that seen in human depression and that the expression of this protein was diminished in the brains of people suffering from depression. Thus, in addition to assessing several 5-HT receptor types, we also determined whether the relation between p11 and 5-HT2A would be evident across brain regions and whether p11 was related to other 5-HT receptor subtypes. Although several other 5-HT receptor subtypes, including 5-HT1A, 5-HT2A, have been implicated in depression, the present report was restricted to those most commonly assessed.

Methods

Subjects

Brains from those who committed suicide (10 men and 10 women) and from control subjects (10 men and 10 women) who died from causes not directly involving any diseases of the central nervous system were obtained at autopsy at the Department of Forensic Medicine of the Semmelweis University Medical School, Budapest; at the Department of Neuropathology, National Institute of Psychiatry and Neurology, Budapest; and at the Department of Pathology of Saint George Hospital, Székesfehérvár, Hungary. The complete complement of tissue samples was available for the FPC, the OFC and the hippocampus. However, for the PVN and the amygdala, tissue was available from fewer female suicide subjects (4 and 4, respectively). The raphe nucleus was obtained from an independent sample of 12 suicide subjects (9 men and 3 women) and an equal number of control subjects (9 men and 3 women). The brains were microdissected and stored in the Human Brain Tissue Bank, Budapest. Of the 40 brains represented in the present analysis, 10 brains from subjects who had committed suicide and 10 brains from...
control subjects had also been used in an earlier study assessing corticotropin-releasing hormone (CRH) and γ-aminobutyric acid, subunit expression.7

All control and suicide subjects were white, from Hungary and of comparable age (Table 1). Causes of death by suicide are shown in Table 1. Death in all instances was sudden and did not involve a prolonged agonal state. Medical, psychiatric and drug histories of the suicide subjects were obtained through chart review coupled with interviews with the attending physician or psychiatrist and family members. The interviews were semistructured and included questions pertaining to family history of psychiatric illness, recent major stressors or life events encountered and previous incidents of major depression or suicide attempts, or both. In each instance, a psychiatric diagnosis of major depressive disorder was on record. The postmortem psychological autopsy and the diagnoses were conducted by an experienced psychiatrist on the basis of DSM-IV criteria.2 Insofar as could be determined, the participants had not used antidepressant medication for at least 2 months before death and did not have a history of either drug or alcohol abuse. Toxicological analyses were used to determine the presence of alcohol or illicit drugs in blood samples. The presence of a positive toxicological screen was used as an exclusion criterion in cases of death by hanging or jumping from a height. In contrast to suicide subjects, interviews with family members coupled with examination of medical records indicated that control subjects had never been treated for depression or any other psychiatric disorder and did not have a history of drug or alcohol abuse during the last 10 years.

Tissue harvesting occurred after written informed consent was obtained from next of kin, which also included the request to consult the medical chart and to conduct neurochemical or biochemical analyses, or both. The local Ethics Committee (Semmelweis) also approved tissue harvesting. Likewise, the local Ethics Committee of the Department of Psychology approved analyses of tissue samples at Carleton University, Ottawa.

Tissue collection, dissection and storage

With the exception of 1 sample, brain tissue was obtained less than 6.5 hours after death. Table 1 shows the time from death to the time that tissue samples were frozen. Overall, tissue samples from those who died by suicide were obtained significantly later than from control subjects (F_{1,39} = 3.85, p < 0.01). However, as will be described shortly, mRNA expression for each of the 5-HT family members within each of the brain regions was not significantly correlated with the time at which the tissue samples were harvested. The absence of a correlation is not surprising, given that samples were obtained soon after death and within a restricted time window.

The brain samples used in the present investigation were obtained very soon after death, and thus a comment is warranted. Ethical rules for dissecting human brains vary across countries, and in some of the European countries, as in Hungary, once death is confirmed by 3 physicians or pathologists, the brain removal may proceed. Ordinarily, those who died by suicide or who died in a motor vehicle collision are defined as “medicolegal cases,” and pathological sectioning is obligatory. These brains may be removed from the skull as soon as 1–2 hours postmortem, frozen and stored until the pathological sectioning is carried out. The dissection (microdissection) of the brain can be performed after a pathological diagnosis has been obtained (including tests for HIV, tuberculosis, syphilis, hepatitis, alcohol and other drugs).

After removal from the skull, the brains were cut into 6 major pieces (4 cortical lobes, basal ganglia–diencephalon and lower brain stem–cerebellum), rapidly frozen on dry ice and stored at −70°C until microdissection (2.4–2 mo later). At the time of the dissection, the brain samples were sliced into coronal sections that were 1.0–1.5 mm thick at a temperature of 0–10°C. Two prefrontal cortical areas were cut out of the sections by a fine microdissecting (Graefe’s) knife, namely, the FPC (Brodmann’s area 10), which was dissected at the most polar portion of the frontal lobe below the intermediate frontal sulcus, and the OFC. The OFC included the anterior orbital gyrus and the anterior parts of the medial and lateral orbital gyri. These corresponded to Brodmann’s area 11 and the ventral part of the Brodmann’s area 12. The tissue samples did not contain any parts of the gyrus rectus, the posterior orbital gyrus or the neighbouring Brodmann’s areas 45 and 47. Cortical samples were always taken from the right hemisphere. In addition to these cortical regions, samples from the hippocampus were taken from 2 consecutive coronal sections of the frontal portion of the temporal lobe immediately posterior to the amygdala. Two side-by-side tissue pellets were punched from each hippocampal section with a 1.5-mm needle on each side. The tissue pellets included the dentate gyrus, all the hippocampal areas (CA1–CA3) and a portion of the subiculum, but not the presubiculum or the parahippocampal gyrus. Amygdala samples were removed from coronal sections throughout the rostral pole of the temporal lobe, at the middle portion of the amygdala just ventral to the section profile of the optic tract. For microdissection, a punch needle with 1.5-mm inside diameter was used. From 2 consecutive sections, punches were taken from each side of the amygdala. The tissue pellets included the central, medial

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<th>Table 1: Characteristics of tissue samples</th>
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<td>Group; mean (and SD)</td>
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<td>Characteristic</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Hanging</td>
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<td>Drug overdose</td>
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<td>Time to tissue harvesting, h</td>
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<td>ΔSD = standard deviation; M = male; F = female; — = no data; MI = myocardial infarction.</td>
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and basal, but not the lateral, amygdaloid nuclei. Finally, microdissection samples were obtained from the PVN of the hypothalamus. A punch needle with 1.0-mm inside diameter was used on the 2 sides of the hypothalamus between the third ventricle and the fornix. The microdissected tissue pellets contained both the parvo- and magnocellular subdivisions of the nucleus.

The DRN was removed from the ventral portion of the periaqueductal grey matter with a punch needle having a 0.5-mm inside diameter. The micropunches were located on the 2 sides of the midline between the medial longitudinal fascicule and the aqueduct throughout the posterior half of the midbrain, as caudal as the beginning of the fourth ventricle.

**Tissue analyses**

We undertook a reverse transcription–quantitative polymerase chain reaction analysis (RT-QPCR) of 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C and p11 mRNA expression.

The samples were stored in airtight containers at −70°C over a period spanning 2000–2005. After they were thawed, guanidinium thiocyanate-phenol-chloroform extraction (Trizol) was used to isolate total cellular RNA from cellular protein and genomic DNA, as described by the manufacturer’s protocol. The samples were verified as free of contaminating DNA because no signal originated from genomic DNA among the no-reverse-transcription control subject samples. We checked isolated RNA for purity by ensuring that the optical density 260/280 ratio was greater than 1.8. An analysis of the RNA quality using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, Calif.) showed little degradation of the 18- and 20-second bands of the mRNA. No high-molecular-weight nucleic acid was detected in any sample, further indicating that contaminating genomic DNA was undetectable. With regard to the RNA integrity number (RIN) for control subjects and those who committed suicide, we found 2 samples with low values. When these samples were removed, the mean RIN values for control and suicide subjects were 5.67 (standard deviation [SD] 0.30) and 5.78 (SD 0.15), respectively, and all scores exceeded 5. It will be noted that the outcomes were the same regardless of whether these 2 samples were or were not included in the analyses. The correlation between the RIN and the cycle threshold (Ct) of synaptophysin was −0.31 for control subjects and and −0.13 for suicide subjects, suggesting that RNA integrity was not related to the ability to detect mRNA. As well, Brainbank Budapest, as a member of the European Brain Bank Consortium (BrainNet II Europe) had 2 member institutions (Imperial College, London, and Universitat de Barcelona) perform DNA stability tests on 50 of its tissue samples (in 16 brains), including 5 brains represented in the present study. Of these, 48 samples were rated “good quality,” and only 2 samples (from a single brain not represented in this study) were unacceptable. Finally, analyses of pH from FPC samples of 20 male and 20 female brains indicated that the pH was in a reasonable range and did not differ between those who died by suicide and control subjects, with a mean of 6.58 (standard error of the mean [SEM] 0.06) for suicide subjects and a mean of 6.45 (SEM 0.06) for control subjects. The values in the present investigation fell between those reported by Li and colleagues and Torrey and colleagues. Importantly as well, the pH values were very similar in the suicide and the control populations. Given the acceptable pH values and the fact that the RIN was reduced to the CT values, we included all the samples in the analyses.

We prepared samples for QPCR analyses by reverse transcribing with an anchored oligo(dT) primer 5.0 μg of total RNA, using iScript II reverse transcriptase (Invitrogen; Burlington, Ontario). Aliquots of this reaction were then used in simultaneous QPCR reactions. For QPCR, SYBR Green I detection was used according to the manufacturer’s protocol (Stratagene Brilliant QPCR Kit, Stratagene, Cedar Creek, Tex.). A Stratagene MX-4000 Real Time thermocycler was used to collect the data. All PCR primer pairs used generated amplifications between 114 and 194 base pairs. No primer dimers were formed. This was verified in 2 ways, by running products in agarose gels and by performing a melt curve after the PCR run. Products all had melting temperature (Tm) values greater than 80°C. Amplicon identity was checked by restriction analysis. All primers were designed with the use of GeneRunner Software (Hastings Software, V. 3.05, Hudson, New York, 1994) and we assessed PCR primer efficiency empirically by determining the relation between the Ct and a dilution series of reverse-transcribed RNA. The slope of this relation was used to calculate efficiency with the Stratagene MX-4000 software. Efficiency for all primer pairs was greater than 93%. The primer sequences were as follows: synaptophysin sense, CAG ACA GGG AAC ACA TGC AAG G; antisense, GCC CCA GCC TGT CTC CTT AAA C; 5-HT1A sense, ACA GGT ACT GGG CCA TCA CG; antisense, GCC GGA TAG AGA TGA GGA AGC; 5-HT2A sense, TGC TCC C; antisense, TCA CCG ACC TGC TTT TGT CC; antisense, TCCAGGGCGATGACAGACAG; 5-HT2C sense, TCG TCA TCA TGG CAG TCT GCC; antisense, AAA CCT TGC TGC GCA GAG GC; 5-HT2C sense, TCC CTA GAC ATG CCT C; antisense, CGC AGA GGT AGA TGA TGA AGC; 5-HT5B sense, TCA CCG ACC TGC TTT TGT CC; antisense, GCT TGT GAA GCC CAC TTT GC.

Primers that amplify synaptophysin mRNA were used as a control to normalize the data because this mRNA species is a stably expressed housekeeping gene even under extreme perturbations (static epilepsy). For the OFC and FPC, 2 housekeeping genes were used, namely, synaptophysin and cyclophilin. The correlation of their Cts was found to be high (r = 0.90 and 0.93, n = 40), supporting the reliability of using synaptophysin as a single housekeeping gene for the remaining brain regions. Predictably, given the high correlations between synaptophysin and cyclophilin, the analyses of variance (ANOVA) or 5-HT receptor subtypes in the PFC and OFC were comparable. Although there was intersubject variability in the C for synaptophysin, the average C for the suicide and control tissues were comparable across brain regions and did not differ between control subjects and suicide victims. To control for individual variability that ordinarily exists within the assay, the expression of each species was
normalized (denoted as Cₘ) by subtracting its Cₜ from the synaptophysin Cₚ. Because synaptophysin is usually a more abundant gene, this gives a negative Cₘ. Thus more negative Cₘs mean less expression in comparison with a Cₚ that is closer to 0 (equal synaptophysin expression). Thus the difference between Cₘs for an mRNA species represents the fold change (as a power of 2) in abundance. A difference of 1 cycle represents doubling, a difference of 2 cycles reflects quadrupling, and so on. For the QPCR analysis, aliquots of the same RT reaction were set up from the same PCR reaction master mix run in parallel. The QPCR analyses for the various 5-HT family members were run concurrently so that the panel of mRNA species was always run simultaneously for each subject. The abundance of 5-HT was low in each of the terminal family members were run concurrently so that the panel of mRNA species was always run simultaneously for each subject. The abundance of 5-HTTT was low in each of the terminal regions, and hence was not analyzed further because potential differences would not have been considered meaningful.

The synaptophysin Cₚs were unrelated to the postmortem harvest time (across brain regions, these correlations ranged from −0.01 to 0.09 for FPC, −0.26 to 0.04 for OFC, 0.06 to 0.36 for amygdala, −0.21 to 0.18 for hippocampus and −0.14 to 0.23 for PVN). Similarly, the subject’s age was not correlated with 5-HT receptor variations, with the exception of the OFC. Correlations across brain regions ranged as follows: −0.2 to 0.19 for FPC, −0.10 to 0.34 for amygdala, −0.21 to 0.23 for hippocampus and −0.12 to 0.02 for PVN. Within the OFC, the age at which death occurred was related to 5-HT₁₆ (r = 0.60, p < 0.001), 5-HT₁₉ (r = 0.43, p < 0.001) and p11 (r = 0.61, p < 0.001).

Statistical analyses

The Cₘ values for each of the 5-HT receptor subtypes, as well as p11, were analyzed by 2 (cause of death, suicide v. control subject) × 2 (sex) ANOVAs. Follow-up comparisons of the simple effects of significant interactions were analyzed by t tests with a Bonferroni correction to maintain the α level at 0.05. For each analysis, η² is provided as an index of the variance accounted for. In the case of the amygdala and the dorso-lateral raphe, where there were fewer female suicide samples, we conducted separate analyses. In the first, the data for men and women were pooled, and hence, effects related to sex or the sex × suicide interaction were not determined; in the second, only the data from men were included in the analysis. In each instance, the data analyses yielded consistent results.

We conducted Pearson product correlations independently for male and female tissue samples to determine the relations between specific 5-HT family members. As already indicated, correlations were also conducted to determine whether the subject’s age was related to mRNA expression and whether postmortem time to harvest tissue was correlated with mRNA expression. Although time of tissue harvesting was unrelated to mRNA expression, as already indicated, subject age was related to several measures within the OFC. For these analyses, age was included as a covariate in the ANOVA of the mRNA expression. Finally, analyses of tissue from subjects who died through drug overdose could not be distinguished from those who had died by hanging or jumping from a height. To be sure, the number of subjects was small, which likely precluded detection of significant effects, but insofar as this factor was concerned, the amount of variance accounted for (η²) was small.

Results

Owing to the multiple brain regions and receptor transcripts determined, the complete data set is somewhat complex. Accordingly, we provide an overview of the findings in Table 2.

With regard to the FPC, Figure 1 shows the mRNA expression for each of the 5-HT receptors within the FPC. Within this region, 5-HT₁₆ mRNA expression was significantly higher among women than among men (F₁,₂₉ = 31.16, p < 0.01, η² = 0.41) and higher in suicide than in control subjects (F₁,₂₉ = 7.05, p = 0.01, η² = 0.09). Although the interaction between these variables was not significant, the increase of 5-HT₁₆ in the suicide group was only significant in men. In the case of 5-HT₁₉, mRNA expression varied as a function of the cause of death × sex interaction (F₁,₂₉ = 6.31, p = 0.01, η² = 0.10). Follow-up tests indicated that 5-HT₁₉ mRNA expression in the brains of male control subjects was lower than in female samples. As in the case of 5-HT₁₆, the 5-HT₁₉ mRNA expression was elevated among male suicide subjects relative to control subjects, but a similar effect was not apparent among female subjects. The analysis of 5-HT₂X expression yielded a significant cause of death × sex interaction, F₁,₃₆ = 5.68, p = 0.02, η² = 0.13. The follow up tests confirmed that among men 5-HT₂X mRNA expression did not differ as a function of the cause of death, whereas among women 5-HT₂X expression was lower in suicide subjects than in control subjects. Finally, mRNA expression of 5-HT₁₅ receptors was lower in suicide subjects than in control subjects (F₁,₂₉ = 8.06, p < 0.05, η² = 0.10) as was p11 expression (F₁,₂₉ = 6.74, p = 0.01, η² = 0.15).

With regard to the OFC, given its presumed involvement in cognitive processes and modulation of emotional behaviours, we were particularly interested to establish whether 5-HT variations would be apparent in the brains of those who died by suicide. In contrast to the FPC, the mRNA expression of 5-HT₁₆ within the OFC was lower in the suicide group than in control subjects (F₁,₂₉ = 6.37, p < 0.001, η² = 0.31) (Fig. 2). The expression of 5-HT₁₉ was relatively variable and did not differ between male and female samples or as a function of suicide subject compared with control subject. The expression of 5-HT₂X was likewise found not to differ as a function of either sex interaction, F₁,₂₉ = 5.68, p = 0.01, η² = 0.13.

Table 2: Summary of 5-HT-related mRNA expression changes in brain tissue of suicide group

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Brain region</th>
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<tr>
<td></td>
<td>FPC</td>
</tr>
<tr>
<td>5-HT₁₆</td>
<td>↑male</td>
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<tr>
<td>5-HT₁₅</td>
<td>↑male</td>
</tr>
<tr>
<td>5-HT₁₉</td>
<td>↓ female</td>
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<tr>
<td>5-HT₂X</td>
<td>↓ male</td>
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<tr>
<td>p11</td>
<td>↓</td>
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</tbody>
</table>

mRNA = messenger ribonucleic acid; FPC = frontopolar cortex; OFC = orbitofrontal cortex; Hippo = hippocampus; PVN = paraventricular nucleus; — = no effect.

*Female amygdala and PVN tissue samples were limited. Thus the summary reflects only analyses of male tissue samples.
the cause of death or of sex, although there was a modest trend for lower 5-HT<sub>2C</sub> expression in those who committed suicide when compared with control subjects ($p = 0.11$).

The expression of 5-HT<sub>1A</sub> receptors varied as a function of the cause of death $\times$ sex interaction ($F_{1,29} = 4.54, p < 0.05, \eta^2 = 0.09$). The follow-up tests indicated that mRNA expression did not differ between male suicide subjects and control subjects ($F_{1,16} = 2.97, p = 0.09, \eta^2 = 0.14$). The mRNA expression of p11, however, was significantly diminished in the amygdala of suicide subjects ($F_{1,16} = 5.74, p = 0.05, \eta^2 = 0.18$).

With regard to the PVN of the hypothalamus, the number of samples from women was relatively small (5 control and 4 suicide subjects), and we therefore conducted separate analyses that included or did not include samples from women. Because the number of female and male samples were roughly the same, and because the statistical outcomes were comparable irrespective of whether sex was included as an independent variable, the data are presented with sex included as a variable. As depicted in Figure 5, both 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> mRNA expression was significantly higher in suicide samples than in control subject samples ($F_{1,29} = 5.62$ and 9.76, $p < 0.05$ and 0.02, $\eta^2 = 0.17$ and 0.27, respectively). In contrast to the mRNA variations for these receptor subtypes, mRNA expression for 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub> and p11 did not reach significance.

With regard to the dorsal raphe nucleus, in contrast to most of the other brain regions examined, mRNA expression of 5-HTT, 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, and p11 were relatively variable and did not vary as a function of suicide. The number of female samples was small (3 suicide and 3 control subjects), and the meaningfulness of the data might therefore be questionable. However, the absence of an effect was notable regardless of whether only male samples were assessed or whether the data from male and female samples

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**Fig. 1:** Mean (and standard error of the mean) messenger ribonucleic acid (mRNA) expression of 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>1B</sub> and p11 in the frontopolar cortex of suicide victims who suffered from depression and of control subjects. Data were presented as normalized cycle thresholds ($C_{t}$), wherein the expression of each species was normalized by subtracting its cycle threshold ($C_{t}$) from the synaptophysin $C_{t}$. Thus a negative $C_{tn}$ indicates that the mRNA species was less abundant than that of synaptophysin. A change of 1 $C_{tn}$ is equivalent to a 2-fold difference in the abundance of that species. *$p < 0.05$ relative to control subjects of the same sex.*
were pooled. It was found that among women there was a trend for higher mRNA levels (especially for 5-HT$_{1A}$ and 5-HTT, but a greater sample size will clearly be needed before adequate conclusions can be drawn.

**Correlations between 5-HT receptors and p11**

Within the FPC, OFC and hippocampus the correlations between p11 and 5-HT$_{1A}$ were significant among both men and

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**Fig. 2:** Mean (and standard error of the mean) messenger ribonucleic acid expression of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_{1B}$ and p11 in the orbital frontal cortex of suicide victims who suffered from depression and of control subjects. The left panel shows the data for men, and the right panel presents the data for women. Data are presented as normalized cycle thresholds (Ct) wherein the expression of each species was normalized by subtracting its cycle threshold (Ct) from the synaptophysin Ct. *p < 0.05 relative to control subjects of the same sex.

**Fig. 3:** Mean (and standard error of the mean) messenger ribonucleic acid expression of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_{1B}$ and p11 in the hippocampus of suicide victims who suffered from depression and of control subjects. The left panel shows the data for men, and the right panel presents the data for women. Data are presented as normalized cycle thresholds (Ct) wherein the expression of each species was normalized by subtracting its cycle threshold (Ct) from the synaptophysin Ct. *p < 0.05 relative to control subjects of the same sex.

**Fig. 4:** Mean (and standard error of the mean) messenger ribonucleic acid expression of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_{1B}$ and p11 in the amygdala of suicide victims who suffered from depression and of control subjects. Because there were only a few samples from women, only the data from men are presented. Data are presented as normalized cycle thresholds (Ct) wherein the expression of each species was normalized by subtracting its cycle threshold (Ct) from the synaptophysin Ct. *p < 0.05 relative to control subjects of the same sex.

**Fig. 5:** Mean (and standard error of the mean) messenger ribonucleic acid expression of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_{1B}$ and p11 in the paraventricular nucleus of the hypothalamus of suicide victims who suffered from depression and control subjects. The left panel shows the data for men, and the right panel presents the data for women. Data are presented as normalized cycle thresholds (Ct) wherein the expression of each species was normalized by subtracting its cycle threshold (Ct) from the synaptophysin Ct. *p < 0.05 relative to control subjects of the same sex.
women and among suicide and control subjects (r values ranged from 0.59 to 0.96). These correlations were less pronounced in the amygdala (r = 0.60 in control subjects, and only 0.40 among suicide subjects) and the PVN (r = 0.81 among suicide subjects but only 0.11 in control subjects). In the OFC and PVN, the correlations between 5-HT₁A and 5-HT₁B were relatively pronounced (r ranged from 0.55 to 0.71), but analyses in other brain regions did not reveal similar effects.

**Discussion**

The confluence of evidence implicating 5-HT processes in depression has come from pharmacologic studies, positron emission tomography analyses, and assessments of specific 5-HT–related gene polymorphisms. As well, relevant data have come from studies that assessed postmortem 5-HT receptor binding or mRNA expression in specific brain regions of subjects who committed suicide relative to a comparison sample that died suddenly. Nevertheless, as indicated earlier, the frequent inconsistencies reported across laboratories or as a function of techniques used to assess the 5-HT variations have been disquieting. In addition to including a range of brain regions associated with depression and stressor reactivity, in the present investigation, we revisited the issue of 5-HT receptor changes associated with suicide and depression, but we used tissue collected soon after death and included both men and women in our study. Moreover, the viability of the RNA was confirmed to be adequate in both an in-house analysis and in an analysis conducted by personnel from 2 members of the Brain Bank Consortium (BrainNet II Europe), namely, the Imperial College, London, and the Universitat de Barcelona. In addition, the pH levels of samples were acceptable and were comparable in suicide and control subjects. Of course, the data in the present investigation were restricted to mRNA expression, and ultimately, the findings will need to be confirmed with respect to protein content.

Consistent with earlier reports, in the current investigation, 5-HT₁A and 5-HT₁B mRNA expression was elevated in the FPC of male suicide samples, whereas this difference was not significant in samples from women. It will be recalled that inconsistent results have been reported concerning 5-HT₁A variations in frontal cortical regions of suicide subjects, compared with control subjects, and it is possible that sex differences might account for the contradictory results that have been reported.

In animal studies, chronic stressors reduced 5-HT₁B binding and altered 5-HT₁A functioning has been implicated as a factor associated with depressive illness, although correspondence was not found between 5-HT₁A receptor gene polymorphisms and suicidality. In the present investigation, 5-HT₁B mRNA receptor expression was markedly reduced in the FPC of suicide subjects, as it was in the OFC and the hippocampus. Further, consistent with the perspective that p11 modulates 5-HT₁A functioning, we observed that p11 was reduced in the FPC and that the abundance of 5-HT₁A and p11 transcripts in both control and suicide subjects was highly correlated.

The function of p11 is to act as a calcium sensor that localizes other proteins with membrane phospholipids; it is part of a protein complex that includes annexin 2. In the absence of p11 (e.g., in p11 knockout mice) the localization of 5-HT₁A at the cell surface is markedly reduced. These findings suggest tightly coupled transcriptional regulation, but it is not clear how this might occur because little is known about the regulation of 5-HT receptors at the transcriptional level. A common promoter region is unlikely because the receptor genes are not located on the same chromosomes. The p11 gene is on chromosome 1, whereas the 5-HT₁A gene has been localized to chromosome 6 (see NLM GenBank www.ncbi.nlm.nih.gov/Genbank/). Given that suicide has been associated with various processes other than those related to 5-HT functioning, including brain-derived neurotrophic factor, as well as GABA and CRH functioning, it is uncertain whether one of these factors or some other 5-HT receptor contributes to the coincident changes of 5-HT₁A and p11 expression.

The OFC has been implicated as part of the neural circuitry involved in depression. Indeed, among suicide attempters, α-[11C]methyl-L-tryptophan trapping was reduced (relative to control subjects) in the OFC and PFC, suggesting low rates of 5-HT synthesis in these regions. Like other PFC regions, the OFC has been implicated in cognitive processing and possibly contributes to emotional rather than cognitive perspective taking as well as impulsive-aggressive behaviours. Further, 5-HT and dopamine in the medial PFC and OFC have been shown to differentially modulate impulsive decision making and 5-HT₁A and 5-HT₂A antagonists have been shown to differentially influence impulsive responding. In light of these considerations, it may not be entirely surprising that 5-HT receptor differences between suicide and control subjects in the OFC did not parallel those evident the FPC. In this regard, within the OFC, the mRNA expression of 5-HT₁A was lower in suicide than in control subjects (recall that 5-HT₁A expression was elevated in the FPC of suicide subjects), whereas 5-HT₁B and 5-HT₂C mRNA expression was unaffected in this region. Contrary to these between-region differences, the changes of both 5-HT₁A and p11 mRNA expression in the OFC and the FPC were comparable to one another. This raises the possibility that there is a shift in the balance of 5-HT signaling between those areas that modulate impulsivity, which may result in impaired assessment of behavioural consequences (suicidality).

Although the hippocampus has frequently been implicated as an essential conduit in the neuronal processing associated with depressive illness, analyses of the 5-HT receptor changes that occur in the hippocampus have not provided an encouraging or consistent profile of variations. For example, although several studies indicated that neither 5-HT₁A nor 5-HT₁A receptor binding differed in the hippocampus of suicide and control subjects, other investigators found that 5-HT₁A receptor binding was reduced in the hippocampus of people suffering from depression. As well, Rosel and colleagues reported that hippocampal 5-HT₁A binding sites were reduced in cases of violent suicide but that this was coupled with higher binding affinity between the receptor and the G protein and therefore with increased concentrations
of the second messenger, IP3. Further, it was reported that variations of the 5-HT₁A receptor gene at the A-161T locus were associated with impulsive-aggressive behaviours and were more prominent in suicide than in control subjects. In effect, it seems that the A-161T locus, which potentially influences 5-HT₁A transcription, might contribute to suicide predisposition by influencing impulsive-aggressive behaviours. Adding still more complexity to the mix was the finding that 5-HT₁A mRNA expression within the hippocampus was elevated in teenage suicide subjects relative to a comparison group. The present findings, unfortunately, do little to resolve the paradoxical findings, except to add that within the hippocampus neither 5-HT₁A nor 5-HT₂A mRNA receptor expression differed between suicide and control subjects. This was the case among both men and women and was observed in the context of other 5-HT receptors (notably, 5-HT₁B and p11) being altered within the hippocampus and in other brain regions.

Anxiety is frequently comorbid with depression, and so neurochemical activity within the amygdala might be considered in the analysis of major depressive disorder. Although particular attention has been devoted to amygdala CRH and noradrenaline involvement in mediating anxiety, increasing evidence from both animal and human studies has also implicated amygdala 5-HT in this regard. Serotonergic changes in the amygdala have not typically been related to depression and suicide, but postmortem analyses have revealed that 5-HT utilization (reflected by 5-HIAA accumulation) was elevated in the amygdala of subjects who died by suicide relative to age-matched control subjects. Further, positron emission tomography analyses indicated lower 5-HTT binding potential in the amygdala of drug-free patients with major depressive disorder relative to comparison subjects. Likewise, 5-HT₁ receptor abundance, reflected by [3H]ketanserin binding, has been found to be elevated in the amygdala of suicide subjects relative to control subjects, although others did not detect comparable effects. In the present investigation, 5-HT₂A mRNA expression was elevated in the amygdala of suicide subjects, whereas 5-HT₁B receptor expression, and to a lesser extent 5-HT₁A and p11 mRNA expression, were reduced in this region. Whether these effects were related to anxiety or more closely aligned with depression is uncertain. Nonetheless, it does appear that suicide was associated with distinct 5-HT receptive changes, just as 5-HT receptor alterations were evident in cortical regions. Indeed, in most respects, the nature of changes in the amygdala paralleled those seen in the PFC.

Elevated HPA functioning has been associated with depressive illness, and it has been reported that 5-HT₁A receptors within the PVN moderate hypothalamic CRH and, hence, pituitary adrenocorticotropic hormone and adrenal cortisol release. Further, 5-HT₁A receptors may promote the desensitization of 5-HT₁A receptors, and the interaction between these receptors has been found to influence HPA neuroendocrine activity. As such, it was of interest to establish whether these receptor subtypes would be altered in the PVN of suicide subjects. It was indeed found that 5-HT₁A expression was elevated in the PVN of suicide subjects, as was 5-HT₁B expression, and the expression of these receptor subtypes was significantly correlated. Because treatments that affect PVN 5-HT₁A and 5-HT₁B receptors influence neuroendocrine functioning, the present data are consistent with the view that neuroendocrine variations associated with depression may be related to these 5-HT processes. This view is in keeping with the proposition that multiple 5-HT receptor variations may be associated with pathology, and ultimately, that treatment strategies may be served well by concurrent tweaking of different receptor subtypes.

It has been reported that 5-HT₁A (auto)receptors were increased in the DRN of persons who suffered from depression and committed suicide, which might influence forebrain 5-HT availability. In the present investigation, as previously observed, 5-HT₁A variations were not evident in the DRN of suicide subjects. Similarly, unanimity has not been reached concerning 5-HTT changes in the DRN of those who died by suicide. Although the findings of Caspi and colleagues have focused attention on the potential role of 5-HTT gene polymorphism in depression, several studies indicated that 5-HTT ligand binding was not altered among suicide subjects. However, it was reported that there was a marked reduction in the number of neurons that expressed 5-HTT mRNA. In the present investigation, as in these other studies, there was no evidence of altered 5-HTT or 5-HT₁ receptor mRNA expression in the DRN. Thus the role of 5-HT₁ and 5-HTT in supporting suicide and depression is uncertain.

In general, conclusions regarding neurotransmitter and receptor differences in the brains of suicide subjects, compared with control subjects, have been hampered by the diversity of procedures that exist across reports. In the present investigation, some of these problems were dealt with effectively (e.g., sex of subjects, verification of RNA quality, obtaining of tissue samples soon after death). However, as with most studies that have assessed the brains of those who died by suicide, it was not possible to relate biological factors to specific symptoms that each subject might have presented. Nevertheless, some of the differences that exist across studies could potentially reflect characteristics of the suicide subjects examined. In this regard, van Heeringen and colleagues tied frontal cortical 5-HT₁A receptors in persons who attempted suicide to elevated feelings of hopelessness and harm avoidance. Moreover, Oquendo and colleagues indicated that lifetime aggression scores were associated with elevated 5-HT₁A receptor binding in the PFC of suicide subjects. The essential point is that suicidality across people suffering from depression may involve multiple processes and that the suicidal phenotype is a complex one. Thus it should not be surprising that varied results are evident across studies, which is possibly related to the specific characteristics of those who comprised the study population. Of course, the possibility also exists that the biological effects that have been observed reflect processes related to suicide (e.g., impulsivity, disinhibition, anxiety, executive functioning) rather than depression per se. In fact, it has been reported that variations of the 5-HT₁A receptor gene (at the A-161T locus), which has been associated with impulsive-aggressive behaviours, were more prominent in suicide subjects than in control subjects. Ultimately, analysis of the processes related to depression and suicide might be
References


