

# Altered Editing of Serotonin 2C Receptor Pre-mRNA in the Prefrontal Cortex of Depressed Suicide Victims

## Clinical Study

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### Summary

Five adenosines within the coding sequence of the serotonin 2C receptor (5-HT2C) pre-mRNA are converted to inosines by RNA editing (named A, B, C' (E), C, and D sites). In human prefrontal cortex (PFC), the most abundant 5-HT2C mRNA sequences result from editing at the A site, or from the editing combinations AC'C, ABCD, and ABD. In suicide victims with a history of major depression, C' site editing is significantly increased, D site editing is significantly decreased, and the C site shows a trend toward increased editing. Treatment of mice with the antidepressant drug fluoxetine (Prozac™) causes changes in C', C, and D site editing that are exactly opposite to those seen in suicide victims. Thus, one outcome of fluoxetine treatment may be to reverse the abnormalities in 5-HT2C pre-mRNA editing seen in depressed suicide victims.

### Introduction

The 5-HT2C receptor is widely distributed in the central nervous system and is thought to play a role in regulating mood, appetite, and sexual behavior (Molineaux et al., 1989; Roth et al., 1998). In both rodents and humans, 5-HT2C pre-mRNA is a substrate for deaminating editing enzymes that attack five closely spaced adenosine residues located within sequences encoding the putative second intracellular domain of the receptor, a region thought to be of general importance for G protein coupling (Burns et al., 1997; Moro et al., 1993; Schöneberg et al., 1995). These editing sites are named (from 5' to 3') A, B, C' (E), C, and D (Burns et al., 1997; Niswender et al., 1999). Two adenosine deaminases that act on RNA (ADAR1 and ADAR2) are thought to convert the adenosine (A) residues of these sites to inosines (I) (Burns et al., 1997; Liu et al., 1999), and results of two studies on knockout mice suggest a major role of ADAR1 in the editing of the A and B sites and of ADAR2 in the editing of the C and D sites in vivo (Wang et al., 2000a; Higuchi et al., 2000). Because inosine prefers to base pair with cytidine, sense-strand cDNA copies contain guanosines at the position of the inosine. In 5-HT2C mRNA, such A to G conversions alter the coding poten-

tials of three triplet codons. Editing at the A and A/B site(s) converts an isoleucine to a valine (I/V), and editing at the B site generates a methionine at this site. Editing at the C site converts an asparagine to a serine (N/S) and editing of the C' site generates an aspartate. The combined editing at the C' and C sites generates a glycine (I/G). Editing at the D site converts the isoleucine to valine (I/V).

The 5-HT2C receptor is presently the only GPCR known to be edited. In the rodent and human brain, a mixture of differently edited and non-edited 5-HT2C receptor mRNAs are expressed (Burns et al., 1997; Niswender et al., 1999), and the significance of this editing in vivo is still unknown. However, studies on transfected cells that express individual 5-HT2C receptor isoforms indicate that certain editing combinations alter the ability of the receptor to activate phospholipase C (PLC). For example, receptor isoforms resulting from the editing combinations A(B)CD, A(B)C'C, and A(B)C'CD (the VSV, VGI, and VGV isoforms, respectively) show significantly less PLC activation than non-edited 5-HT2C receptors (Burns et al., 1997; Niswender et al., 1999; Fitzgerald et al., 1999; Wang et al., 2000b). Altogether, these studies suggest that, regardless of the final editing combination, editing at the C' and C sites most efficiently downregulates the activity of 5-HT2C receptors (Wang et al., 2000b).

In this report, we examine the expression of 5-HT2C mRNA in the human dorsal prefrontal cortex (PFC). Moreover, because lower serotonergic activity is thought to be a major contributing factor to suicide risk (Mann, 2000), we further tested whether the expression of distinctly edited 5-HT2C receptor mRNAs differs in tissues obtained from depressed suicide victims compared to healthy controls. Our results indicate significantly different 5-HT2C pre-mRNA editing site preferences in brains of suicide victims. In addition, the changes in editing site preferences detected in suicide victims with a history of major depression are opposite from changes in corresponding editing site preferences detected in mice treated chronically with the antidepressant drug fluoxetine (Prozac). Altogether, these results suggest a role for 5-HT2C pre-mRNA editing in depression. They further suggest that fluoxetine treatment may reverse the abnormalities in 5-HT2C pre-mRNA editing detected in suicide victims with major depression.

### Results

#### Editing of the 5-HT2C Pre-mRNA in the Dorsal PFC (Brodman Area 9) of Controls and Depressed Suicide Victims

Theoretically, editing of the 5-HT2C pre-mRNA could generate 32 different mRNA isoforms ( $2^5 = 32$ ; see Table 1) if all sites are edited independently. Nucleotide sequence analysis of 477 cDNA clones generated from prefrontal cortical RNA extracted from tissues of 11 different subjects revealed a total of 23 mRNA isoforms (22 of which result from RNA editing) that code for 15

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Table 1. 5-HT2C mRNA Sequences Resulting from Pre-mRNA Editing in the Human Prefrontal Cortex

Abundant Isoforms <sup>a</sup>	Rare Isoforms <sup>b</sup>	Non-Expressed Sequences
Control Brains (n = 5)		
>25%:	ABC'CD	BC'CD
AC'C	ABC'C	BC'C
>10%:	ABC'D	BC'D
ABCD, A	ABC	BCD
≥5% to <10%:	ABC'	BC'
AD, NE, ABD, ACD, AC'CD	AC'D	BC
	AB	C'CD
	AC'	C'C
	AC	C'D
Suicides (n = 6)		
>48%:	BD	
AC'C	B	
>10%:	C'	
ABCD	CD	
≥4% to <10%:	C	
ABD, A	D	

<sup>a</sup> Sequences detected in every subject of each of the two diagnostic groups.

<sup>b</sup> These sequences represent <1% of all 477 sequences analyzed and <2% in each of the two groups.

different receptor isoforms (Table 1). However, 15 of these mRNA isoforms were detected in only a small number of subjects (≤4) and they represent less than 1% of all sequences. Eight mRNA isoforms (coding for seven protein isoforms) were abundantly detected (see Table 1). In controls, these mRNAs represent more than 80% of all 5-HT2C mRNA isoforms (of 207 sequences analyzed) and they were consistently detected in all subjects. The isoforms resulting from ABCD, AC'C, and A site(s) editing were most frequently detected. In 270 sequences obtained from a matched group of suicide victims, only 4 of the 8 abundant sequences (those resulting from editing at the AC'C, ABCD, A, and ABD site(s)) were found in all samples where they represented more than 73% of all sequences. However, whereas transcripts edited at the AC'CD, ACD, and AD sites as well as non-edited (NE) transcripts were consistently found in all controls, they were detected in some, but not all, suicide victims (see below).

Finally, a total of nine possible editing combinations (that would encode nine additional protein isoforms) were never observed and, as evident from the results shown in Table 1, these editing site combinations occur only together with A site editing.

In addition to differences in the complexity of 5-HT2C mRNA isoform expression between controls and suicide victims, significant differences were found in the percentages of expression of isoforms that are common to both diagnostic groups. Table 2 lists the percentages of the eight major prefrontal cortical 5-HT2C mRNA isoforms for each of the five controls and six suicide victims. Although the suicide victims varied with regard to their psychiatric diagnosis (four suffered from a mood disorder (MD), one suffered from a single episode of depression (SED), and one was not diagnosed with an Axis I psychiatric illness (no 5)), the mean percentage of the AC'C-edited isoform was 2-fold higher in the suicide group compared to controls ( $p < 0.05$ ; one-way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple

Comparisons Test). In addition, the expression of the A-edited isoform was decreased more than 2-fold ( $p < 0.004$ ) in this group. The mean percentages of the other two 5-HT2C mRNA isoforms that are common to both groups (ABCD, ABD) were similar in both groups (Table 2).

However, as shown in Table 2, only five of the six suicide victims exhibited a similarly altered 5-HT2C mRNA isoform expression pattern. These are the four subjects that suffered from a mood disorder and the 18-year-old hispanic male with no documented history of an Axis I psychiatric illness. In contrast, the sixth suicide victim, a 61-year-old white male with a history of single major depressive episode (onset at the age of 60 years) exhibited similar percentages of AC'C, ABCD, and A site(s)-edited 5-HT2C mRNA isoforms compared with controls. Moreover, although non-edited transcripts were consistently found in controls ( $11.7\% \pm 1.5\%$ ), they were rarely detected in the first five suicide victims. In the sixth suicide victim, however, the percentage of non-edited mRNA isoforms was atypically high (27% of 59 sequences analyzed).

Differences were also found for the frequencies of successful editing at each of the five editing sites (Table 3). In this analysis, the percentage of successful editing was determined by dividing the number of transcripts edited at A, B, C', C, or D sites (regardless of the final editing combination) by the total number of sequences analyzed for each subject. Table 3 illustrates lower percentages of successful editing for the C' and C site and higher percentages for D site editing in controls compared with suicide victims. Consistent with the results shown in Table 2, the largest differences were found between controls and five of the six suicide victims (i.e., the first five suicide victims listed in Table 3), and this is particularly evident for their frequencies of C' and C site editing. Due to the high percentage of non-edited 5-HT2C mRNA found in the sixth suicide victim (SED), however, the percentage of successful editing is either lower (A site) or within the range of controls (B, C', C, and D sites).

In summary, five of the six suicide victims studied here suffered from a mood disorder. Among those, four had a major depressive episode and one had a single episode of a late-onset depression at the time of death. As summarized in Figure 1, the group of four subjects with major depression differs from controls by exhibiting a significantly increased expression of the AC'C-edited mRNA ( $p < 0.01$  (ANOVA)) and a significantly decreased expression of the A-edited isoform ( $p < 0.008$ ) (Figure 1A). This is due to significantly increased editing at the C' site ( $p < 0.05$ ) and a significantly decreased editing at the D site ( $p < 0.05$ ). Editing at the C site is also increased. This increase, however, does not reach statistical significance ( $p = 0.08$ ). The percentages of A and B site editing did not significantly differ between the two diagnostic groups (Figure 1B). Whereas the results of one additional suicide victim with no documented Axis I diagnosis (50 sequences analyzed) are similar to those obtained from suicides with a history of major depression, the results of another suicide victim with a history of a single episode of late-onset depression differ substantially (see Discussion).

Table 2. Percentages of Major 5-HT2C mRNA Isoforms in Controls and Suicide Victims

	Editing Combinations							
	AC'C	ABCD	A	AD	NE	ABD	ACD	AC'CD
Control 1	22	33	7	4	4	7	11	7
Control 2	32	10	14	7	3	7	3	11
Control 3	38.5	5.8	15.4	9.6	7.7	7.7	3.8	1.7
Control 4	31.4	5.9	11.8	9.8	4	7.8	5.9	4
Control 5	8.2	6.1	10.2	10.2	16.3	4.1	6.1	2
Suicide/MD 1 <sup>a</sup>	61.1	18.5	5.6	3.7	3.7	3.7	0	0
Suicide/MD 2 <sup>c</sup>	51.9	14.8	3.7	0	0	1.7	3.7	3.7
Suicide/MD 3 <sup>a,b</sup>	76.7	6.6	1.7	0	0	3.3	0	0
Suicide/MD 4 <sup>a</sup>	32	10	6	4	8	18	0	2
Suicide 5 <sup>a</sup>	46	14	4	4	0	10	0	0
Suicide/SED 6 <sup>d</sup>	20.7	10.3	8.6	1.7	27	10.3	8.6	0

MD: mood disorder, SED: single episode of late-onset depression, NE: non-edited.

<sup>a</sup>No documented antidepressant medication during lifetime;

<sup>b</sup>diazepam (5 mg/day) during the 3 months prior to suicide.

<sup>c</sup>Low-dose SSRI-treatment (sertraline; 50 mg/day) within 3 months of suicide that terminated before suicide; no antidepressant treatment at any other time of life.

<sup>d</sup>Medium-dose SSRI-treatment (sertraline; 100 mg/day) combined first with the tricyclic antidepressant nortriptyline (dose not documented) ending 3 weeks before suicide, followed by a combination treatment with the monoamine-reuptake blocker venlafaxine (280 mg/day), a benzodiazepine (alprazolam; 2 mg/day), and a neuroleptic drug (perphenazine; 20 mg/day) that terminated 2 days before suicide; no antidepressant medication at any other time of life.

### Cytoplasmic 5-HT2C mRNA Levels

It is possible that differences in 5-HT2C pre-mRNA editing are due to differences in the ratios of deaminating enzyme and the RNA substrate. We therefore tested whether the levels of 5-HT2C mRNA expressed in the human PFC differ between the groups of controls and suicides. Because conventional measurements of cortical 5-HT2C mRNA levels (Northern blotting or RNase protection assays) are not sensitive enough to reliably detect the very low abundant mRNA (see Julius et al., 1988), we performed exponential RT-PCR experiments to compare with Southern blots the lowest number of PCR cycles needed to detect 5-HT2C amplification products (see Experimental Procedures). As shown in Figure 2, whereas 0.1 ng and 10 pg of plasmid DNA encoding the 5-HT2C receptor led to detectable 5-HT2C amplification products after only 10 and 15 cycles of PCR, respectively, prefrontal cortical cDNA templates of all controls and the first five suicide victims analyzed in Tables 2 and 3 required 20 cycles of PCR to yield spurious amounts of 5-HT2C cDNA. Moreover, in all of these samples, the PCR reaction was saturated after 30

cycles. These data suggest that the levels of 5-HT2C mRNA do not differ between the two groups (or that differences in editing lead to different mRNA expression levels).

Another relevant question is whether the samples differ in their ratios of full-length and truncated 5-HT2C mRNA. The truncated 5-HT2C mRNA results from cleavage of an alternative 5' splice site located 11 nucleotides upstream of the first editing site (i.e., alternative splicing deletes the editing sequence; Canton et al., 1996). However, none of the prefrontal cortical RNAs, regardless of whether they are derived from controls or suicide victims, contained the truncated 5-HT2C mRNA that is, for example, abundantly expressed in the choroid plexus. Representative results of a 35 cycle RT-PCR experiment are shown in Figure 3.

### 5-HT2C Pre-mRNA Editing in the Forebrain of Fluoxetine-Treated Mice

It is possible that the different editing site preferences detected in depressed suicide victims are a consequence of long-term antidepressant drug treatment. However, as summarized in Table 2, four of six suicide victims had no documented history of antidepressant treatment during their lifetime, one suicide victim took a low dose of the serotonin-selective reuptake inhibitor (SSRI) sertraline during the last three months prior to suicide, and the subject with the late onset depression took a number of powerful antidepressants combined with a neuroleptic drug and a benzodiazepine during his last 3 months of life (see Table 2). Because it is often difficult to test the impact of various drug treatment histories on long-term alterations of neurotransmitter receptor expression and function with studies on post-mortem tissues, we examined the editing of 5-HT2C pre-mRNA in forebrain neocortical tissues of drug-naive 129Sv mice and compared these results to corresponding ones obtained from mice treated for either 3 or 28 days with the widely prescribed antidepressant drug of

Table 3. Percentages of Successful Editing at the Five Editing Sites

	Editing Sites				
	A	B	C'	C	D
Control 1	96.5	44.5	29.3	77.8	63.0
Control 2	96.5	25.0	46.4	64.3	42.9
Control 3	83.0	17.3	38.5	55.8	32.7
Control 4	86.3	23.1	45.1	56.7	49.0
Control 5	59.2	22.5	22.5	34.7	53.1
Suicide/MD 1	93.0	20.0	63.0	83.0	28.0
Suicide/MD 2	93.0	30.0	63.0	89.0	30.0
Suicide/MD 3	93.0	13.3	83.3	93.0	13.3
Suicide/MD 4	80.0	50.0	42.0	56.0	38.0
Suicide 5	90.0	42.0	54.0	70.0	34.0
Suicide/SED 6	69.5	28.8	25.4	44.1	35.6

MD: mood disorder, SED: single episode of late-onset depression.

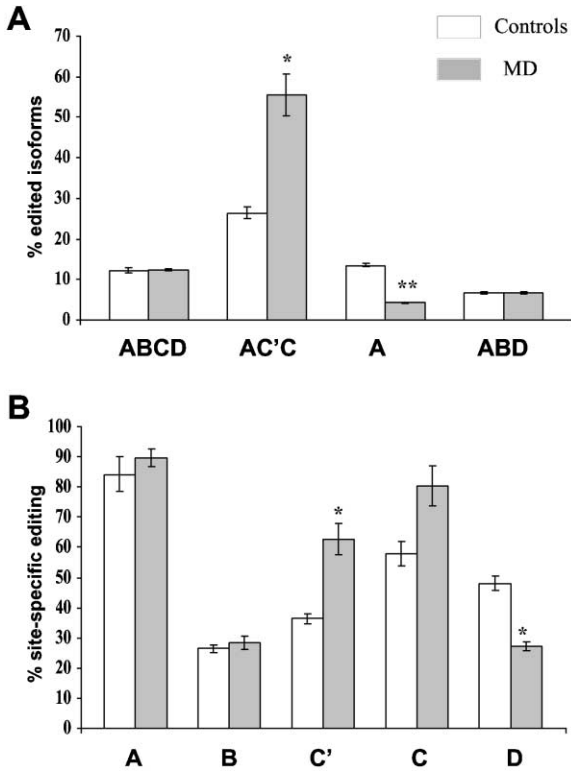


Figure 1. Expression of 5-HT2C mRNA Isoforms in the Dorsal Prefrontal Cortex of Controls and Suicide Victims with a History of a Mood Disorder

(A) shows comparison of the percentages of major edited 5-HT2C mRNA isoforms. Mean  $\pm$  SEM of determinations made in groups of five controls and the four suicide victims marked "MD" in Table 2. Controls: white bars, suicides: gray bars. There are no significant differences between subjects of the same group. \* $p < 0.01$ ; \*\* $p < 0.008$ ; controls compared to depressed suicides (ANOVA). (B) shows percentage of successful editing at the five editing sites. Mean  $\pm$  SEM of editing frequencies of controls (white bars) and depressed suicide victims (gray bars). \* $p < 0.05$  (controls compared to depressed suicides).

the SSRI class, fluoxetine (Prozac<sup>TM</sup>). Nucleotide sequence analysis of 674 cDNA clones generated from forebrain neocortical RNA extracted from both drug-naive and fluoxetine-treated mice revealed that the majority of 5-HT2C mRNAs are edited. The isoforms resulting from editing of the ABD and ABCD sites are most frequently detected. In nontreated and fluoxetine-treated mice, similar percentages of the ABCD-edited 5-HT2C mRNA isoform were found. However, as shown in Figure 4A, compared to nontreated mice, mice treated with fluoxetine for 28 days express a larger percentage of ABD-edited mRNA isoform ( $p = 0.068$ ; one-way analysis of variance followed by Tukey-Kramer Multiple Comparisons Test). Figure 4B further illustrates that fluoxetine treatment leads to changes in editing site preferences. Mice treated with fluoxetine for 3 days exhibit a significant decrease in C' site editing ( $p < 0.05$ ) and none of the 5-HT2C mRNA sequences expressed in mice treated with this drug for 28 days are edited at this site. Moreover, a prolonged fluoxetine treatment (28 days) leads to a significant increase in D site editing ( $p < 0.05$ ).

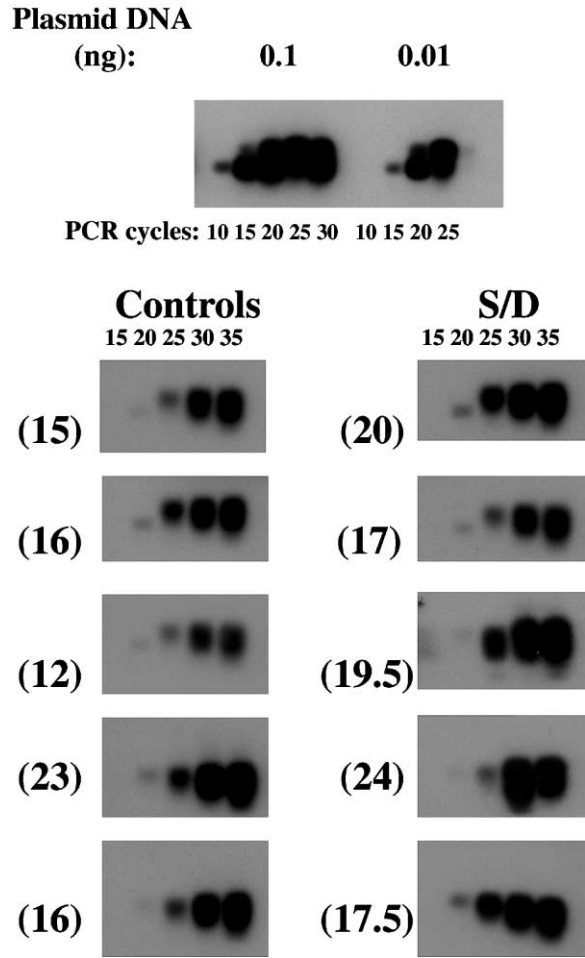


Figure 2. Expression Levels of 5-HT2C mRNA in the Prefrontal Cortex

Southern blot of exponential RT-PCR amplification of cDNA encoding a 270 nt long sequence of 5-HT2C mRNA sequence containing the edited region is shown. Aliquots of the PCR reactions were removed after 10, 15, 20, 25, 30, and 35 cycles of amplification. For comparison, PCR amplifications of 0.1 and 0.01 ng of plasmid DNA encoding the full-length 5-HT2C receptor are shown on top. Blots were exposed to film for 3 hr. For each tissue sample, the PMI is indicated in parenthesis. C: controls, S/D: suicides.

Editing at the C site is also decreased. This decrease, however, does not reach statistical significance ( $p = 0.08$ ).

In summary, the changes in C', C, and D site editing resulting from long-term fluoxetine treatment are the opposite of those detected in the suicide victims with a history of major depression. Similar to the approach taken in Figure 2, we also tested with exponential RT-PCR experiments whether a 3 or 28 day treatment with Prozac<sup>TM</sup> alters cytoplasmic expression levels of 5-HT2C mRNA. No differences were found between nontreated and fluoxetine-treated mice (not shown).

### Discussion

Three abundant 5-HT2C mRNA isoforms expressed in the PFC differ in their relative expression in brains of

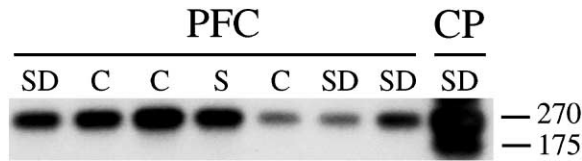


Figure 3. Expression of Full-Length and Truncated 5-HT2C mRNA in the Prefrontal Cortex (PFC) and the Choroid Plexus (CP)

Shown is a Southern blot of RT-PCR amplification of cDNA encoding a 270 nt long sequence of the full-length 5-HT2C receptor and a 175 nt long fragment of the alternatively spliced (5-HT2Ctr) mRNA. An overexposed film is shown here to illustrate that, in contrast to the abundant expression of 5-HT2Ctr in the CP, this mRNA variant is not detected in the PFC. SD: suicide/depression, C: controls, S: suicide.

controls and depressed suicide victims. In suicides, a significantly increased expression of the AC'C-edited receptor mRNA (encoding the VGI isoform) is accompanied by a significantly decreased expression of the A-edited mRNA. Moreover, non-edited mRNA is commonly found in controls but this mRNA isoform is only rarely detected in suicides that suffered from major depression. These differences in editing pattern are due to significantly increased C' site editing, a trend toward significantly increased C site editing, and a significantly

decreased editing of the D site. Our data suggest that, despite the differences in the editing site preferences, 5-HT2C mRNA levels do not differ between the two diagnostic groups. Whether the activities of editing enzymes differ in the PFC of these groups remains to be determined.

Our sequence analysis was performed on PCR-amplified cDNAs, an experimental condition that could potentially distort the relative ratios of differently edited isoforms. However, we found that, for each individual subject, the analysis of a *minimum* of ~30 cDNA clones derived from a single bacterial transformation provides a reliable estimate of the relative ratios of major edited isoforms that is closely replicable in several independent experiments. This indicates that, similar to the study of Sommer et al. (1991), the PCR amplification is not subject to larger variations from experiment to experiment but rather revealing the relative abundance of major edited isoforms present in the input samples. However, the complexity of expression of differently edited 5-HT2C mRNA isoforms necessitated the analysis of a large number of RNA sequences for each subject and thus, in practical terms, limited the number of subjects studied in this report. Two recent studies took less laborious approaches to characterize the editing of the 5-HT2C pre-mRNA in the PFC of controls and schizo-

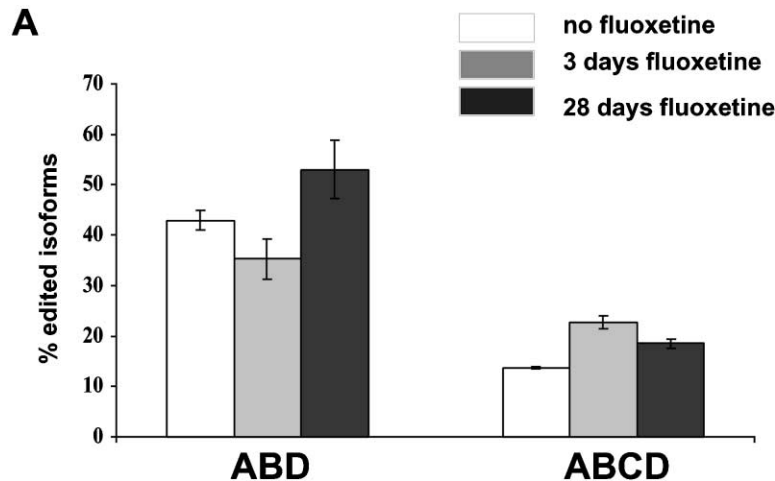
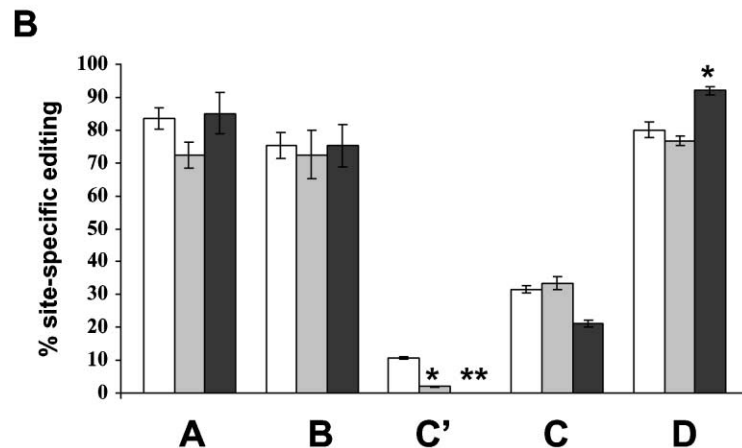


Figure 4. Expression of 5-HT2C mRNA Isoforms in the Forebrain Neocortex of 129Sv Mice

(A) shows comparison of the percentages of major edited 5-HT2C mRNA isoforms expressed in nontreated and fluoxetine-treated mice. Mean  $\pm$  SEM of determinations made in four animals per treatment group. For each animal, 51 to 73 5-HT2C cDNA clones obtained from a single bacterial transformation were analyzed. (B) Percentage of successful editing at the five editing sites. Mean  $\pm$  SEM of editing frequencies of nontreated and fluoxetine-treated mice. (\* $p < 0.05$  and \*\* $p < 0.01$ .) White bars: controls, light gray bars: 3 day fluoxetine treatment, dark gray bars: 28 day fluoxetine treatment.



phrenic patients. One involved a primer-extension analysis of the editing region in 5-HT<sub>2C</sub> mRNAs, an approach that allowed a comparison between groups composed of much larger numbers of subjects (Niswender et al., 2001) at the expense of conducting a less sensitive and incomplete analysis of the five editing sites found in 5-HT<sub>2C</sub> pre-mRNA. The other study (Sodhi et al., 2001) used a very small number of nucleotide sequences (~10 per individual) to characterize 5-HTC pre-mRNA editing. It is thus likely that the different results obtained in these two studies relate to the insufficiency of both approaches to characterize the editing of 5-HT<sub>2C</sub> pre-mRNA in the PFC.

Previous studies that characterized the functional properties of recombinant non-edited, partially edited, and fully edited isoforms expressed singly in transfected cells have shown that editing combinations ABCD and ABC'CD differ from non-edited and other partially edited isoforms in their reduced ability to activate G protein in response to agonist stimulation (Burns et al., 1997; Niswender et al., 1999; Fitzgerald et al., 1999). In earlier studies, this compromised ability to activate G protein was found to be largest only for the fully edited isoform (ABC'CD) (Niswender et al., 1999), which is rarely found in the PFC (see Table 1). However, as recently shown (Wang et al., 2000b), editing at the C'C sites (as it occurs in the AC'C-edited isoform) is sufficient to achieve maximum downregulation of receptor activity. This phenomenon is most apparent when EC<sub>50</sub> concentrations of weak and partial agonists are compared for their ability to stimulate PLC upon binding to non-edited and C'C sites-edited receptor isoforms whose responses to *maximum* stimulation with serotonin (5-HT) do not differ (Niswender et al., 1999; Wang et al., 2000b). The present data therefore suggest an overall decreased 5-HT<sub>2C</sub> receptor activity in the PFC of suicide victims with a history of major depression. However, it is evident from the results shown in Table 1 that a variety of differently edited 5-HT<sub>2C</sub> receptor isoforms is expressed in the PFC. Thus, the net outcome of activating this mixed population of receptors *in vivo* is not known. It is also clear that editing of 5-HT<sub>2C</sub> pre-mRNA does not lead to drastic changes in receptor function. The rather subtle functional changes that are reflected in the increased EC<sub>50</sub> concentration of full or partial 5-HT<sub>2C</sub> receptor agonists support the recent hypothesis that this editing, like the majority of site-specific A to I editing of neuronal transcripts that have thus far been identified, enable a "fine-tuning" of the activity of distinct physiological processes (see Seeburg, 2000). Hence, one would predict that differences in 5-HT<sub>2C</sub> receptor activity that result from the alterations in editing described here would become most apparent when serotonergic neurotransmission is reduced. In this regard, it is of interest to note that a study on nondepressed and depressed suicide victims found a widespread reduction in the expression of serotonin transporter radioligand binding sites only in the PFC of depressed suicide victims (Mann et al., 2000). Thus, although the *in vivo* significance of the altered editing described here is still unclear and although the actual synaptic concentration of 5-HT in the PFC is also not known, the present data suggest that the increased expression of the AC'C-edited isoform, which occurs together with a decreased expression of

the fully functional non-edited and A site-edited mRNA, has functional consequences under conditions of a compromised serotonergic neurotransmission.

The brain samples analyzed in this study were well matched with regard to age, sex, ethnic background, and postmortem interval. Although each diagnostic group was composed of subjects with diverse ethnic backgrounds and although both groups were not matched for other parameters such as health, nutrition, socioeconomic status, jobs, etc., the frequency distribution of major edited 5-HT<sub>2C</sub> mRNA isoforms as well as the percentages of editing of each of the five editing sites did not significantly differ between subjects of the control group or the group of suicides with a history of major depression. Suicide, however, often occurs in the context of a psychiatric illness and is associated most often with major depression (Mann, 2000). Therefore, postmortem studies on brains of suicide victims often leave unresolved whether any of the observed biochemical abnormalities are due to the presence of major depression or whether they reflect abnormalities that characterize suicidal behavior. In the present study, only five of six suicide victims suffered from depression. The remaining subjects committed suicide at the age of 18 in the context of an antisocial personality disorder, a condition characterized by an elevated suicide rate and impulsive aggression. The latter trait is associated with impaired serotonergic function and a predisposition to suicidal behavior (Mann, 2000) and the results obtained from this suicide victim are very similar to those found for the suicide victims that suffered from major depression. Thus, to further evaluate the role of major depression in the abnormalities described here, future studies should test for differences between nondepressed suicides and suicide victims with a history of major depression.

Differences in 5-HT<sub>2C</sub> pre-mRNA editing site preferences could also exist between different diagnostic subclassifications or different courses of depressive illness. The results obtained from an additional 61-year-old suicide victim who suffered from a single (documented) episode of depression are not only different from controls but also from the other suicide victims. In fact, this subject expressed an abnormally high percentage of non-edited 5-HT<sub>2C</sub> mRNA. This preliminary observation suggests that, in addition to testing for differences in 5-HT<sub>2C</sub> pre-mRNA editing between nondepressed and depressed suicide victims, more information is needed to evaluate whether different courses of depressive illness (age of onset, number of depressive episodes) are associated with different 5-HT<sub>2C</sub> pre-mRNA editing site preferences.

Finally, a major concern in all studies that attempt to correlate mood and schizophrenic disorders with changes in the expression and/or function of neurotransmitter receptors is whether the observed molecular abnormalities are related to pharmacological interventions that (directly or indirectly) target these receptors. Clearly, the question whether the altered editing seen in brains of depressed suicide victims is a consequence of antidepressant drug treatment that is known to alter serotonergic neurotransmission is of particular relevance. In the present study, however, four of six suicide victims had no lifetime history of antidepressant treatment. One other subject took a low dose of sertraline

for a period of 3 months and terminated the drug prior to suicide. The sixth subject (who suffered from a single, late-onset depressive episode) was treated with a complex combination of antidepressant, neuroleptic, and anxiolytic drugs for the last 3 months of his life and this treatment terminated only 2 days before suicide. Because it is difficult to test the impact of various drug treatment histories on long-term alterations of neurotransmitter receptor expression and function with studies on postmortem tissues, we have begun to test for changes in 5-HT<sub>2C</sub> pre-mRNA editing in the forebrain neocortex of mice treated chronically with antidepressant drugs. Interestingly, our studies on mice treated chronically with the most widely prescribed antidepressant SSRI fluoxetine revealed drug-induced alterations in the editing of the 5-HT<sub>2C</sub> receptor that are opposite from those detected in suicide victims with a history of major depression. These results suggest that one therapeutic outcome of fluoxetine treatment is a reversal of the abnormalities in 5-HT<sub>2C</sub> pre-mRNA editing described here. This is not inconsistent with the results obtained from a suicide victim who was treated with the less potent SSRI sertraline because this subject took a low dose (50 mg/day) that is typically administered only at the very beginning of the treatment and thus may have been too low to affect the 5-HT<sub>2C</sub> pre-mRNA pattern. Moreover, the subject terminated the medication before suicide. However, it remains possible that the very distinct editing pattern of the other subject who suffered from a late-onset depression resulted from his complex psychopharmacological treatment history and future studies should investigate whether, in addition to SSRIs, other types of antidepressant drugs or even anxiolytic, sedative, or neuroleptic drugs decrease site-specific editing of 5-HT<sub>2C</sub> pre-mRNA in the PFC.

In any case, the data of both the human and mouse studies suggest that the editing of 5-HT<sub>2C</sub> pre-mRNA is regulated in a serotonin-dependent manner. This can now be tested with mice depleted of the neurotransmitter serotonin.

### Conclusions

The present study describes alterations in 5-HT<sub>2C</sub> pre-mRNA editing site preferences in the dorsal PFC of depressed suicide victims. Previous studies have identified abnormally increased alternative splicing activities in brains of patients with schizophrenia which alter the expression of the dopamine D<sub>3</sub> (Schmauss, 1996) and GABA<sub>A</sub> receptors (Huntsman et al., 1998). In all cases presently identified, the affected mRNAs encode neurotransmitter receptors that are targets (directly or indirectly) for drugs with antipsychotic potencies: neuroleptic drugs (D<sub>3</sub> receptor), benzodiazepines (GABA<sub>A</sub> receptors), and antidepressants (5-HT<sub>2C</sub> receptor). Furthermore, in all cases, altered alternative splicing or editing activities lead to the expression of mRNAs encoding either nonfunctional (D<sub>3</sub>) or less functional (GABA<sub>A</sub>, 5-HT<sub>2C</sub>) receptors. This suggests that the posttranscriptional regulation of gene expression plays a role in modulating the expression of distinct neurotransmitter receptors in brains of patients with major psychiatric disorders.

### Experimental Procedures

#### Brain Tissues

Prefrontal cortical brain samples were obtained from autopsy material derived from the brain tissue collection of the New York State Psychiatric Institute and Columbia University. Dorsal prefrontal cortical tissues (Brodman area 9) from five controls (males, mean age: 40.8 ± 10.1 years (mean ± SD); age range: 18 to 63 years; postmortem interval (PMI): 19.1 ± 1.5 hr; ethnic background: white (n = 2), black (n = 1), hispanic (n = 2)) were studied in parallel to a matched group of six suicide victims (males, mean age: 44.2 ± 8.9 years; age range: 18 to 64 years; PMI: 16.6 ± 1.9 hr; ethnic background: white (n = 3), black (n = 1), hispanic (n = 2)). All subjects died without a prolonged agonal period. Cause of death was determined by the coroner or medical examiner. Postmortem toxicological analysis, performed on all cases, ruled out recent consumption of alcohol and, with one exception, psychotropic medication or other substances of abuse. (One suicide victim consumed cannabis prior to death.) The classification of suicide victims required evidence of intent and a self-inflicted fatal act. Cases of uncertain causes of death were excluded. Psychological autopsies (performed on all cases) and reviews of hospital records revealed that four of the six suicide victims suffered from major depression (one of whom was also diagnosed with a schizoid personality disorder), and one suffered from a single episode of late-onset depression. The remaining suicide victim died at the age of 18 with no Axis I psychiatric diagnosis but exhibited symptoms of an antisocial personality. These diagnoses, verified by a psychiatrist, were made according to DSM-III criteria and validated for Axis I and Axis II disorders using the Structural Clinical Interview I and II for diagnosis, the Suicide History Form (Columbia University, New York) for past suicidal acts, the Brown Goodwin Aggression History Scale for lifetime aggression, and a checklist for demographic data and other clinical details. All control subjects had no history of psychiatric disorders and died from causes other than suicide.

In addition, one tissue sample of the choroid plexus of white male suicide victim (age: 26 years; PMI: 30 hr) with a history of major depression was obtained.

#### RNA Extraction, Generation of 5-HT<sub>2C</sub> cDNA, Nucleotide Sequence Analysis, Exponential RT-PCR

RNA was extracted from 0.4 g of fresh-frozen autopsy material using guanidine/cesium chloride ultracentrifugation. Ten micrograms of total RNA was used as a template for first-strand cDNA synthesis that was primed with an oligo-dT primer. 5-HT<sub>2C</sub> cDNA was amplified by polymerase chain reaction (PCR) specified by the primers 5'-CTG GCC ACT ACC TAG ATA-3' and 5'-GTC GTT GAG CAC GCA CGT-3'. The amplified sequence comprises nucleotides 355 to 635 of the human 5-HT<sub>2C</sub> mRNA (Saltzman et al., 1991) and contains the edited region.

5-HT<sub>2C</sub> cDNA was cloned into the plasmid vector pCRII (Invitrogen, Carlsbad, CA). Recombinant plasmids were transformed into bacteria and plasmid DNA was recovered from single bacterial colonies for cDNA sequence analysis using the dideoxy-chain-termination method in conjunction with <sup>35</sup>S-dATP and the primer 5'-GTA GCA CAG CGT CCA TCA-3'. For two subjects per diagnostic group, 27 to 30 5-HT<sub>2C</sub> nucleotide sequences were analyzed and for all other subjects per group, 50 to 59 sequences were obtained. There was no significant change in the frequency distribution of major edited isoforms determined in 27 or 59 sequences. For each subject, all sequences were derived from plasmid DNAs recovered from a single bacterial transformation.

For exponential RT-PCR experiments, equal amounts of oligo-dT-primed cDNA generated from 10 μg of total RNA of ten subjects was added to a mastermix of a PCR buffer containing the four deoxynucleotide triphosphates, the 5' and 3' PCR primers described above, and Taq polymerase (Stratagene; La Jolla, CA). The PCR reaction was terminated after 10, 15, 20, 25, 30, and 35 cycles of amplification by removing an aliquot of the PCR reaction at each time point. All aliquots were loaded onto a single 1% agarose gel and transferred to a membrane that was probed with a <sup>32</sup>P-radiolabeled cDNA encoding a HindIII restriction fragment of the 5' portion of the open reading frame of the human 5-HT<sub>2C</sub> cDNA.

#### Drug Treatment of 129Sv Mice and Analysis of 5-HT<sub>2C</sub> mRNA

All procedures involving animals were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Columbia University. Fluoxetine HCl was administered to two groups of adult male 129Sv mice (postnatal age P60 to P90) with the drinking water. One group, composed of four group-housed animals, received fluoxetine treatment for 3 days. The other group, also composed of four group-housed animals, received fluoxetine treatment for a period of 28 days. Four additional mice received regular drinking water (controls). During the first week of fluoxetine treatment, mice drank 5 ml of water/24 hr and thus consumed 5 mg/kg of the drug. In the second and third weeks of treatment, their water intake increased to 7 ml/24 hr (7 mg/kg of the drug) and this was accompanied by a 10% to 12% increase in body weight and an increased spontaneous locomotor activity. During the fourth week of treatment, we increased the drug dosage to 10 mg/kg/24 hr. At the end of the treatment, animals were killed by decapitation and their forebrain neocortex was dissected from both hemispheres. RNA extraction, PCR amplification (using the mouse-specific primer pair: 5'-TATTTGTGCCCGTCTG-3' and 5'-GAGCACGCAGGTAGTATT-3'), cloning, and nucleotide sequencing (using the mouse specific primer 5'-TATTTGTGCCCGTCTG-3') were performed as described above. For each of the 12 mice, 51 to 73 cDNAs recovered from a single bacterial transformation were sequenced.

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