Serotonin 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} Receptor mRNA Expression in Subjects with Major Depression, Bipolar Disorder, and Schizophrenia

Antonio L. López-Figueroa, Camille S. Norton, Manuel O. López-Figueroa, Denise Armellini-Dodel, Sharon Burke, Huda Akil, Juan F. López, and Stanley J. Watson

Background: *Alterations of serotonin neurotransmission are implicated in both mood disorders and schizophrenia. Specific serotonin-receptor-based abnormalities in these psychiatric illnesses have been intensively studied; however, it has been difficult to draw any conclusions because of a lack of consensus. These inconsistencies have most likely arisen from the unavailability of selective ligands.*

Methods: *Our study used in situ hybridization to quantify 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} mRNA levels in the hippocampus (HC) and 5-HT_{1A} and 5-HT_{2A} mRNA levels in the dorsolateral prefrontal cortex (DLPFC) of subjects with a history of major depression disorder (MDD), bipolar disorder (BPD), schizophrenia, and a normal comparison group (15 subjects per group).*

Results: *In the DLPFC, there is a significant decrease in 5-HT_{1A} mRNA of subjects with MDD and in 5-HT_{2A} mRNA of subjects with BPD. Subjects with MDD have a significant decrease in 5-HT_{1A} mRNA in the HC; subjects with BPD and schizophrenia had increased 5-HT_{1B} mRNA levels and a significant decrease in 5-HT_{2A} mRNA levels in the hippocampal formation.*

Conclusions: *Alterations in 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} mRNA levels in the brains of subjects with both mood disorders and schizophrenia add further support for hypothesis of dysregulation of the serotonergic system in these psychiatric disorders.*

Key Words: Serotonin receptors, hippocampus, dorsolateral prefrontal cortex, mood disorder, in situ hybridization, mRNA

Although multiple neurotransmitters and neurohormones have been linked to the pathophysiology of mood disorders, disturbances in the serotonin (5-HT) system are most consistently associated with affective illnesses (Gold et al 1988; Melzter 1989; Nemeroff 1998). In addition, a great deal of evidence implicates the 5-HT system in other psychiatric illnesses, especially schizophrenia (Laruelle et al 1993; Scarr et al 2001). Perhaps it is not surprising that the 5-HT system is implicated in these psychiatric disorders given that it is one of the most widely distributed monoaminergic systems in the brain and thus has an impact on various brain circuits.

The 5-HT system originates in the raphe nuclei, an area that can be divided into two major parts: the cranial and caudal divisions. The cranial nucleus contains the dorsal and median raphe and projects mainly to the forebrain, whereas the caudal nucleus contains the raphe pallidus, raphe magnus, and raphe obscurus and projects to the brainstem and spinal cord (Deutch and Roth 1999). The dorsal raphe and the median raphe are among the largest regions of the raphe and innervate limbic areas such as the dorsolateral prefrontal cortex (DLPFC) and hippocampus (HC). Both of these brain regions have been implicated in the pathophysiology of mood disorders and schizophrenia (Joyce et al 1993; Laruelle et al 1993; López et al 1998, 1999; Rajkowska 2000; Rajkowska et al 2001).

To date, a majority of the postmortem studies of 5-HT receptors have concentrated on changes in receptor ligand binding, whereas fewer studies have looked at alterations in receptor mRNA expression. For example, in the prefrontal cortex of patients with schizophrenia, some studies have found an increase in 5-HT_{1A} binding (Burnet et al 1996; Hashimoto et al 1991; Simpson et al 1996), although other studies do not find any differences in binding (Dean et al 1999; Joyce et al 1993). It should be noted that whereas Burnet et al (1996) found changes in 5-HT_{1A} binding, they did not find any alterations in 5-HT_{1A} receptor mRNA levels. Except for one study from Joyce et al (1993), most studies agree that there is a decrease in 5-HT_{2A} ligand binding in different areas of the prefrontal cortex in schizophrenia (Dean and Hayes 1996; Dean et al 1998, 1999; Laruelle et al 1993).

In major depressive disorder (MDD), 5-HT_{1A} mRNA levels are lower in the HC (López et al 1998), whereas 5-HT_{2A} binding levels are higher in the prefrontal cortex of suicide victims (Arango et al 1990; Mann et al 1986; Pandey et al 2002), although these findings are variably reproducible (Arranz et al 1994; Stockmeier et al 1997). Few studies have concentrated on bipolar disorder (BPD), likely because of the limited number of available BPD postmortem brains (Dean et al 2001). Therefore, authors often extrapolate their findings in major depression to bipolar illness.

Based on ligand binding studies, the role of 5-HT receptors in psychiatric disorders remains equivocal. The lack of consensus in the literature may be due to the relatively recent explosion in the discovery and characterization of several new 5-HT receptor subtypes (Barnes and Sharp 1999). More selective ligands were not available when these earlier studies were conducted, making it difficult to assess the possible contribution of the various 5-HT receptor subtypes in these disorders.

This study compared the differential expression of 5-HT_{1A}, 5-HT_{1B} (formerly known as 5-$HT_{1D\beta}$; Hartig et al 1996; Weinshank et al 1992), and 5-HT_{2A} receptor mRNA in the brains of subjects affected with MDD, BPD, schizophrenia, and in a normal comparison group to further clarify the involvement of 5-HT receptor subtypes in severe mental illnesses. We hypothesize the serotonergic system plays a role in the pathophysiology of both mood disorders and schizophrenia that can be detected at the level of transcript expression of serotonin receptors.

From the Mental Health Research Institute and Department of Psychiatry, University of Michigan, Ann Arbor, Michigan.

Address reprint requests to Stanley J. Watson M.D., Ph.D., Mental Health Research Institute, University of Michigan, 205 Zina Pitcher Place, Ann Arbor MI 48109-0720.

Received January 16, 2003; revised September 11, 2003; accepted September 25, 2003.

0006-3223/04/$30.00
doi:10.1016/j.biopsych.2003.09.017

Methods and Materials

Subjects

Sixty subjects from the Stanley Foundation Neuropathology Consortium were analyzed in this project. Postmortem brains comprised four groups of 15 subjects each, with diagnoses of schizophrenia, bipolar disorder, major depression disorder without psychotic features, and a normal comparison group. For a detailed description of subject selection, diagnostic methods, and tissue handling, see Torrey et al (2000).

Samples were matched for age (overall mean ± SEM: 45.4 ± 1.7), gender, ethnicity, side of brain, brain pH (6.20 ± .03), and postmortem interval (PMI; 29.4 ± 1.5); there were no significant group differences. Demographic and clinical characteristics for individual subjects were available for analysis. In the clinical groups, there were brains from suicides (4 schizophrenia, 7 MDD, and 9 BPD).

Fresh frozen cryostat-sectioned samples (14 μm) were mounted onto slides and stored at −80°C from the DLPFC and the medial temporal lobe containing the hippocampus.

In Situ Hybridization

Hybridization for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} was done using specific human cRNA probes. Robert J. Lefkowitz (Duke University Medical Center, Durham, NC; Burnet et al 1995; Fargin et al 1988) generously provided 5-HT_{1A} cDNA (National Center for Biotechnology GenBank accession number: M83181, nucleotide coding region: 948–1691). Brian F. O'Dowd (University of Toronto, Ontario, Canada; Jin et al 1992) kindly provided 5-HT_{1B} cDNA (D10995, 865–1390). Mark W. Hamblin (University of Washington, Seattle) generously provided 5-HT_{2A} cDNA (XM_007123, 300 BP 5′ untranscripted region (UTR) + 1–575). These probes have no regions of significant homology with any genes in GenBank. Riboprobes were generated by incubating linearized plasmids for 2 hours at 37°C in a solution containing 5 × transcription buffer, [^{35}S] uridine triphosphate (UTP) and cytidine triphosphate (CTP), 150 μmol/L adenosine triphosphate (ATP) and guanosine triphosphate (GTP), 12.5 mmol/L dithiothreitol, 20 units of RNase inhibitor and 6 units of either T3 or T7 RNA polymerase. At the end of the transcription reaction, 1 μL of DNase I was added for 15 min at room temperature. The labeled probe was then separated from free nucleotides on a Sephadex G50/50 column equilibrated in Tris buffer (100 mmol/L Tris-HCl, 12.5 mmol/L ethylenediamine tetraacetate, pH 8.0, 150 mmol/L NaCl, and .2% SDS). Dithiothreitol was added to a final concentration of .01 mol/L.

The in situ hybridization (ISH) technique used in these experiments has been described previously (López-Figueroa et al 2000; Norton et al 2002; Watson et al 1988). Brain sections were removed from −80°C storage and fixed for 1 hour in 4% formaldehyde, briefly washed twice in 2 × standard saline citrate (SSC) (.3 mmol/L NaCl, .03 mmol/L sodium citrate, pH 7.2) and incubated in a solution containing acetic anhydride (.25%) in triethanolamine (.1 mol/L, pH 8) for 10 min at room temperature. Sections were rinsed in distilled water and dehydrated through graded alcohols. The slides were air dried, and then the sections were covered with [^{35}S]labeled cRNA probes in a hybridization buffer containing 50% formamide, 10% dextran sulfate, 3 × SSC, 50 mmol/L sodium phosphate buffer (pH 7.4), 1 × Denhardt's solution, .1 mg/mL yeast tRNA, and 10 mmol/L dithiothreitol, yielding a final concentration of 3×10^6 cpm/220 μL. The sections were placed in a covered box with filter paper saturated with 50% formamide buffer and hybridized overnight at 55°C.

The next day, following hybridization, the coverslips were removed and the sections were rinsed twice at room temperature in 2 × SSC for 5 min, then incubated for 1 hour in RNase A (200 μg/mL in 10 mmol/L Tris-HCl buffer containing .5 mol/L NaCl, pH 8.0) at 37°C. The sections were washed in increasingly stringent solutions of SSC, 2 ×, 1 ×, and .5 ×, at room temperature for 5 min each, followed by a wash for 1 hour in .1 × SSC at 65°C. After rinsing in distilled water, the sections were dehydrated through graded alcohols, air dried, and exposed to Kodak XAR film (Eastman Kodak, Rochester, New York) for up to 4 weeks.

Control ISH studies were performed by pretreating tissue for 1 hour with RNase A (200 μg/mL) before hybridization or by using sense riboprobes generated from the same plasmid insert. The signal observed in these control studies did not differ from background.

All 60 subjects were treated simultaneously with each probe for each anatomic area. All ISH experiments and image analysis were performed blind to the diagnosis of the subjects.

Image Analysis

Densitometric analysis of autoradiographs was performed using the MCID (MicroComputer Imaging Device) image analysis system (Imaging Research, Ontario, Canada) after capturing the images with a charge couple devices (CCD) camera (TM-745, Pulnix, Cohasset, Massachusetts). Images were digitized with constant exposure time, gain, and offset.

The DLPFC was analyzed (Brodmann's areas 9 and 46) by densitometry of isodense bands obtained from each probe, labeled from pial surface to white matter as B1 to B4 for 5-HT_{1A} and B1 to B3 for 5-HT_{2A}. Bands were identified using MCID tools, where a standard template was placed over a subsection containing all the bands. Within this template the bands were establish by visual determination and a computerized-assisted pattern. Data are expressed as integrated optical densities, which represent the mean optical density values minus background in a selected area. In the DLPFC, two samples were taken for each slide, and background was calculated as the mean of all pixels with the lowest optical density within white matter, an area devoid of signal.

The HC regions analyzed in this study were Ammon's horn CA1, CA2, and CA4 and dentate gyrus (DG). These regions were identified based on gross morphologic patterns. Because of the complex architecture along the anterior–posterior axis of the human hippocampal formation and given that all sections did not belong to the same anterior–posterior level of the temporal axis, the extension of each area differed slightly between subjects. Only identification of areas CA1, CA2, CA4, and DG were made for each subject, but unambiguous identification of the CA3 region was not possible for all subjects; therefore, this region was not included in subsequent statistical analyses. The results of analysis are expressed as integrated optical densities (IOD) multiplied by area (DxA), which represent the mean optical values multiplied by the area of signal above background. In the HC, background was calculated as the mean of all pixels with the lowest optical density within an area devoid of signal. Signal was defined as 3.5 × standard deviation over background.

Statistical Analysis

Data are expressed as mean ± SEM. For each study, three slides per subject were analyzed with 15 subjects per group. Statistical analysis (StatView; SAS Institute, Cary, North Carolina) was performed for each probe and each region by two-way analysis of

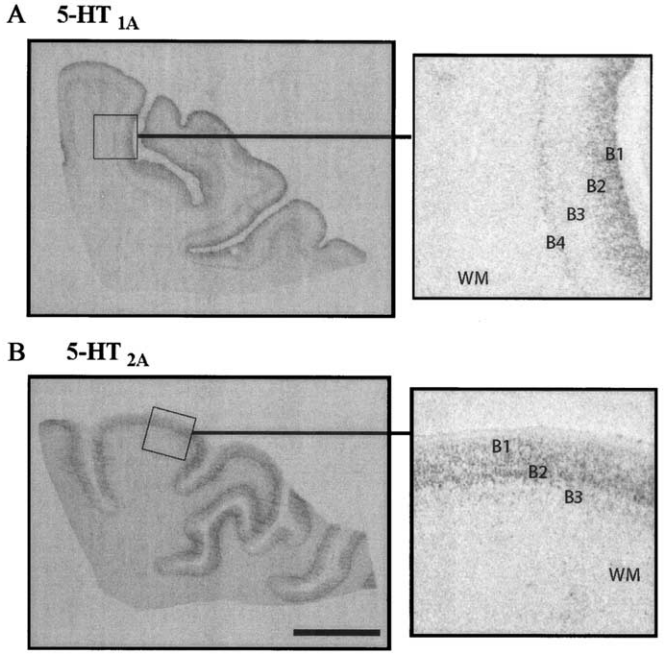

Figure 1. Digitized photomicrographs of in situ hybridization experiments comparing serotonin (5-HT) receptor mRNA levels in the dorsolateral prefrontal cortex. (**A**) 5-HT_{1A} mRNA expression; (**B**) 5-HT_{2A} mRNA expression. Scale bar = 5 mm. Enlargement: 12×. B, band; WM, white matter.

variance with two independent variables, diagnosis, and isodense band (e.g., B1), in the DLPFC or diagnosis and area (e.g., CA1) in the HC. Post hoc analyses were done by Scheffé test for equal *N*s. Values of $p < .05$ were considered statistically significant. There was no association between mRNA expression levels for any probe and age, age of disease onset, postmortem interval, or brain pH. There were no mRNA expression differences comparing deaths by suicide with other causes within diagnostic groups.

Results

Anatomic Distribution of Serotonin Receptor 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} mRNAs in Control Subjects

5-HT_{1A} mRNA. Densitometric analysis of the distribution of 5-HT_{1A} mRNA in the human DLPFC shows a pattern of four bands (Figure 1A). In the control group, labeling was more intense in B1, lower in B2 (approximately half of B1) and B4 (approximately a quarter of B1), and the lowest was B3 (approximately a 10th of B1, Figure 2A).

In the HC, 5-HT_{1A} mRNA was present in all the regions analyzed (CA1, CA2, CA4, and DG), with the highest levels in CA1, followed by CA2 and DG (with approximately half of CA1's intensity) and the lowest expression in CA4 (approximately a 10th of CA1, Figure 3A–4A).

5-HT_{1B} mRNA. In the DLPFC, the expression of 5-HT_{1B} mRNA was barely detectable above the background and therefore was not quantified.

In the HC, although the expression of 5-HT_{1B} mRNA was low, the signal was consistently above background, and therefore quantification was possible. The 5-HT_{1B} transcription levels were higher in DG and CA1 and lower in CA2 and CA4 (approximately a third of DG and CA1, Figure 3B–4B).

5-HT_{2A} mRNA. In the DLPFC, 5-HT_{2A} mRNA shows a different laminar pattern than 5-HT_{1A} mRNA. The highest expression is

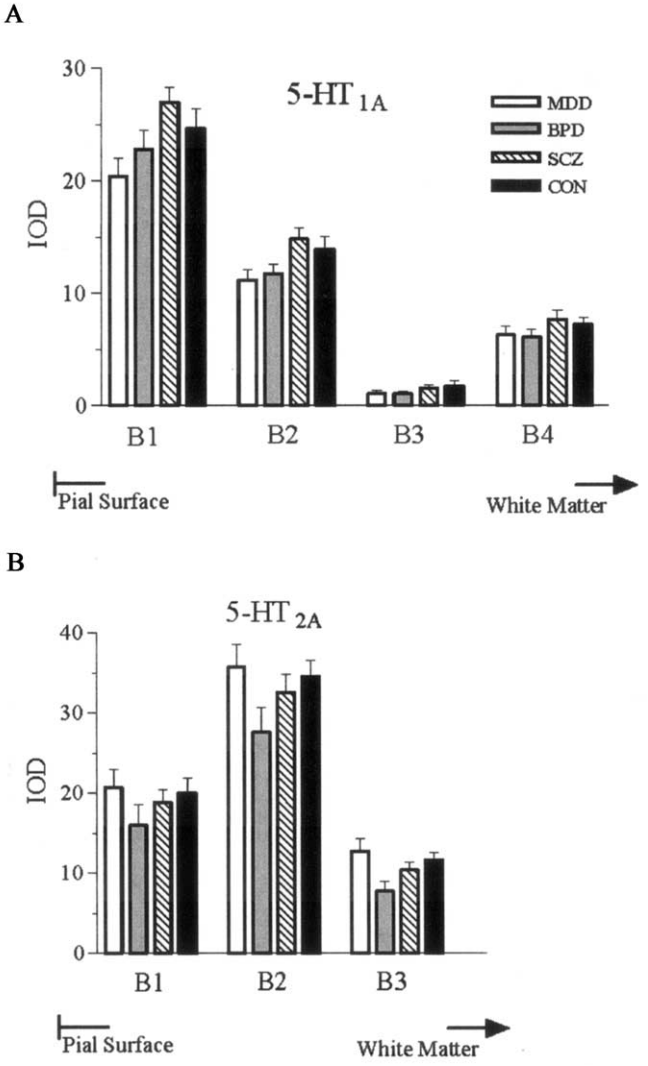

Figure 2. Serotonin (5-HT) receptor mRNA levels in the dorsolateral prefrontal cortex (DLPFC) from major depression disorder (MDD), bipolar disorder (BPD), schizophrenia (SCZ), and normal comparison group (CON) tissue. (**A**) Analysis of 5-HT_{1A} mRNA levels. (**B**) Analysis of 5-HT_{2A} mRNA levels. Data are expressed as the mean ± SEM (n = 15 per group). IOD, integrated optical density.

localized in B2, with an intermediate intensity of expression in B1 (approximately 60% of B2), and with the lowest intensity in B3 (approximately 35% of B2, Figure 1B–2B).

In the HC, 5-HT_{2A} mRNA expression was higher in CA1, lower in CA2 and DG (approximately half of CA1), and the lowest was in CA4 (approximately a 10th of CA1, Figure 3C–4C).

Serotonin Receptors 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} mRNAs in Psychiatric Patients

Dorsolateral Prefrontal Cortex. In the DLPFC, 5-HT_{1A} and 5-HT_{2A} mRNAs were measured in all isodense bands studied (Figure 1). The morphologic pattern of laminar distribution of each probe was similar in all four groups; however, the quantitative analysis showed differences in the intensity of expression between groups. The expression of 5-HT_{1B} mRNA was barely detectable and therefore was not quantified.

5-HT_{1A} mRNA expression was significantly different according to diagnosis ($F = 7.25$, $df = 3208$, $p < .0001$). Post hoc

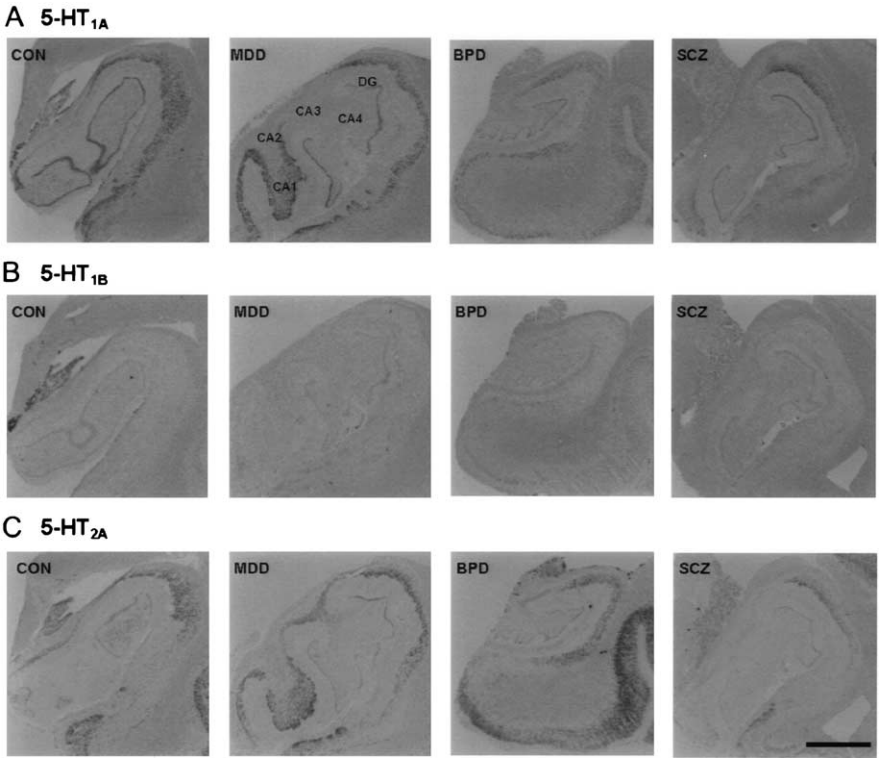

Figure 3. Digitized photomicrographs of in situ hybridization experiments comparing serotonin (5-HT) receptor mRNA levels in the hippocampus (HC). Tissue is from subjects with major depression disorder (MDD), bipolar disorder (BPD), and schizophrenia (schizophrenia) and from a normal comparison group (CON). (**A**) Expression of 5-HT_{1A} mRNA levels. (**B**) Expression of 5-HT_{1B} mRNA levels. (**C**) Expression of 5-HT_{2A} mRNA levels. CA, Ammon's Horn areas; DG, dentate gyrus. Scale bar = 5 mm.

analysis showed that the MDD group was significantly lower than the control group ($p = .036$) and the schizophrenia group ($p = .007$, Figure 2A).

5-HT_{2A} mRNA expression showed a significant main effect due to diagnosis ($F = 4.84$, $df = 3154$, $p < .003$). Post hoc analysis revealed that the BPD was significantly lower than other groups: control ($p = .034$) and MDD ($p = .007$, Figure 2B).

In all the 5-HT receptor transcripts, there were no interactions between diagnoses and bands analyzed in the DLPFC. Neither 5-HT_{1A} ($F = 1.265$, $df = 3208$, $p < .25$) nor 5-HT_{2A} ($F = .925$, $df = 3252$, $p < .52$), showed significant differences.

Hippocampus. In the HC, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} mRNAs were identified in all the areas studied. The morphologic pattern of regional distribution of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} mRNA was similar in all four groups (Figure 3); however, the quantitative analysis showed differences in the intensity of expression between groups. Although the expression of 5-HT_{1B} mRNA was low, the signal was consistently above background, and therefore quantification was possible (Figure 3).

We found that 5-HT_{1A} mRNA expression significantly differed across diagnosis ($F = 3.021$, $df = 3175$, $p < .031$), and post hoc analysis showed that this main effect was because the subjects with MDD had significantly lower expression compared with the normal comparison group ($p = .018$, Figure 4A).

There was a significant main effect for diagnosis on 5-HT_{1B} mRNA expression ($F = 6.98$, $df = 3200$, $p < .0002$). Post hoc analysis showed that the BPD group differed significantly from the control ($p = .031$) and MDD groups ($p = .006$). There was a trend for the schizophrenia group to differ from the control ($p = .077$) and was significantly different from the MDD group ($p = .017$). Levels of 5-HT_{1B} mRNA were higher in the BPD and the schizophrenia groups compared with the other groups (Figure 4B).

We also found that 5-HT_{2A} mRNA expression showed a significant main effect of diagnosis ($F = 5.358$, $df = 3204$, $p < .014$). Post hoc analysis revealed that the 5-HT_{2A} mRNA levels were significantly lower in the schizophrenia ($p = .003$) and BPD ($p = .043$) groups compared with the control group (Figure 4C).

In all the 5-HT receptor transcripts, there were no interactions between diagnoses and areas analyzed in the HC. 5-HT_{1A} ($F = .871$, $df = 3175$, $p < .55$), 5-HT_{1B} ($F = 1.024$, $df = 3200$, $p < .42$), and 5-HT_{2A} ($F = .871$, $df = 3204$, $p < .55$) did not show significant differences.

Discussion

Distribution of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} in the Dorsolateral Prefrontal Cortex and Hippocampus

The expression pattern of each mRNA followed a different pattern of hybridization within the DLPFC and HC. In the DLPFC the "densitometric" patterns are referred to as bands to differentiate these patterns from the already characterized and well-described anatomic cortical layers. The bands might not correspond exactly with the number, extension, and distribution of the classical layers. As described subsequently, the results are consistent with previous descriptions, but we note that the bands correspond to more than one layer. Our results shows overall differences between groups along all bands, not changes in particular layers.

The overall expression patterns of the 5-HT_{1A} mRNA in the human hippocampus and prefrontal cortex presented herein are consistent with studies in the literature (Burnet et al 1995; Pasqualetti et al 1996). The distribution of 5-HT_{1A} mRNA was previously found primarily in pyramidal neurons of layers II and III, the external granular and external pyramidal layers, respectively (Burnet et al 1995; Pasqualetti et al 1996). Our densitometric bands seem to be in agreement with those results; we localized band B1 expression on layer II and B2 on layer III (Figures 1A, 2A). Although those studies described the cell type in which the signal was found, because of the limitations in

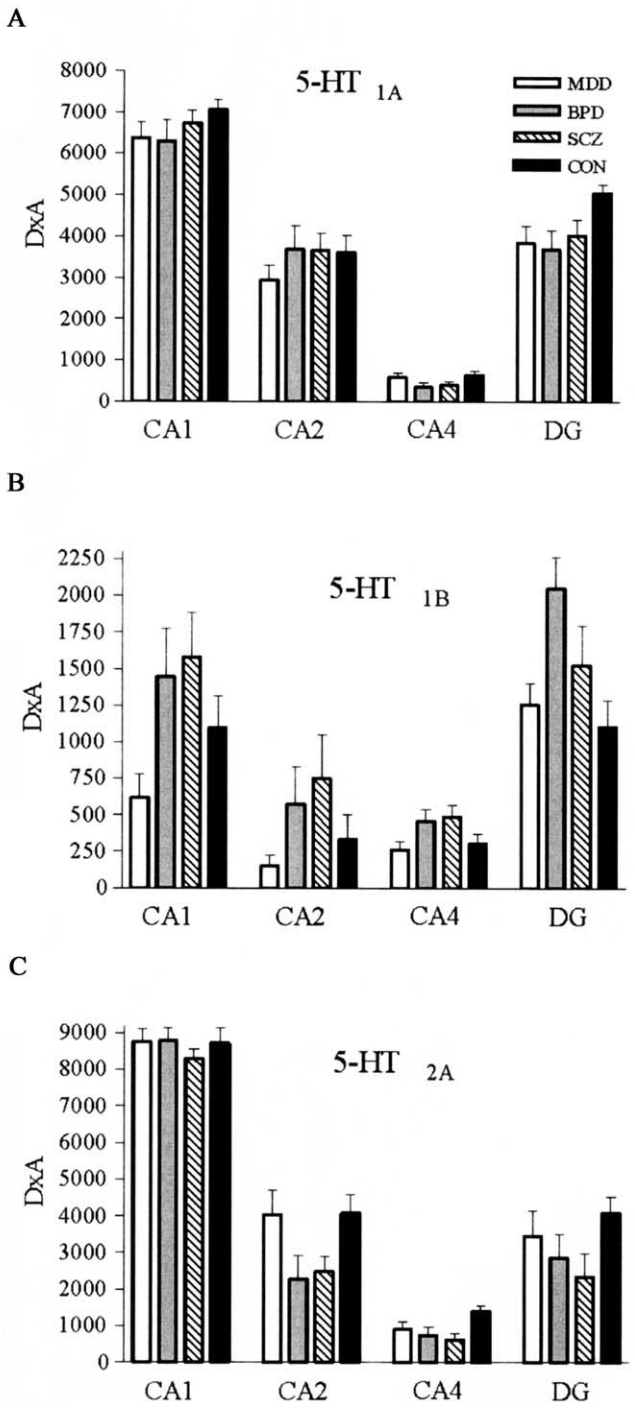

Figure 4. Bar graphs illustrating the serotonin (5-HT) receptor transcript levels in hippocampal regions from major depression disorder (MDD), bipolar disorder (BPD), schizophrenia (SCZ), and normal comparison (CON) tissues. (**A**) Analysis of 5-HT_{1A} mRNA levels. (**B**) Analysis of 5-HT_{1B} mRNA levels. (**C**) Analysis of 5-HT_{2A} mRNA levels. Data are expressed as the mean ± SEM (n = 15 per group). Density multiplied by Area (DxA) was the imaging measure used.

resolution of the autoradiograms we cannot confirm the cell types. In the HC, 5-HT_{1A} mRNA is present in all subregions, with levels more abundant in area CA1 (Figures 3A, 4A). The same previous studies had described messenger RNA to be present in the pyramidal cells of Ammon's Horn and granular cells of the DG, whereas glial cells lack expression. The patterns of mRNA expression noted in the aforementioned studies and in the overall pattern of the current study parallel patterns of ligand binding (Joyce et al 1993; Pazos et al 1987a; Pompeiano et al 1992).

With respect to the 5-HT_{1B} receptor transcripts, mRNA was identified in areas CA1, CA2, and CA4 and in the DG (Figures 3B, 4B). To our knowledge, the homogenous distribution of the 5-HT_{1B} receptor mRNA throughout all hippocampal subfields has not been reported in human postmortem tissue. Jin et al (1992) demonstrated a general distribution of this receptor mRNA in the HC, but the distribution was not described in the subregions. Because of the weak signal observed in our study, these results need to be interpreted cautiously, and more detailed studies are required to determine the precise cellular localization of this receptor mRNA.

5-HT_{2A} mRNA is present in cortical layers III and layer V (internal pyramidal layer), with a more intense expression pattern seen in layer V (Burnet et al 1995; Pasqualetti et al 1996), and in agreement with those results, we localized the densitometric band B1 on layer II, band B2 (higher mRNA expression) lengthwise on layers III to V, and B3 on layer VI (Figures 1B, 2B). In the prefrontal cortex, those previous studies described pyramidal neurons to express high mRNA levels, whereas in interneurons, expression was lower. In addition, in the HC, 5-HT_{2A} mRNA is described to be present primarily in the pyramidal cells of CA1, whereas glial cells have not been noted to express the mRNA for this receptor. Studies analyzing 5-HT_{2A} binding have shown similar distribution patterns in cortex and HC (Pazos et al 1987b; Joyce et al 1993; Pompeiano et al 1994).

Schizophrenia

Schizophrenia is a psychiatric disorder that has been extensively investigated, and the most widely studied 5-HT receptor subtype has been the 5-HT_{2A} receptor. Most studies agree that there is a decrease in 5-HT_{2A} binding, at least in the prefrontal cortex (Arora and Meltzer 1991; Burnet et al 1996; Dean and Hayes 1996; Dean et al 1999; Laruelle et al 1993; Mita et al 1986; but also see Joyce et al 1993; Reynolds et al 1983; Whitaker et al 1981). With respect to the 5-HT_{1A} receptor, binding is unaltered in some studies (Dean et al 1999; Joyce et al 1993) and increased in others (Burnet et al 1996; Hashimoto et al 1991; Simpson et al 1996; Sumiyoshi et al 1996).

Our study did not show any changes in the expression of 5-HT_{1A}, 5-HT_{1B}, or 5-HT_{2A} receptor mRNA in the DLPFC. To our knowledge, only one other study has looked at 5-HT_{1A} and 5-HT_{2A} mRNA levels in the brains of patients suffering from schizophrenia (Burnet et al 1996). Similar to our findings, Burnet and colleges did not find alterations in 5-HT_{1A} mRNA levels in the DLPFC. In contrast to the findings presented here, Burnet et al (1996) determined that 5-HT_{2A} mRNA levels were lower in the prefrontal cortex, compared with control subjects.

Although we did not detect changes in 5-HT_{2A} mRNA receptor expression in the DLPFC, we did find that levels are lower in the HC in patients with schizophrenia compared with control subjects. Burnet and colleagues did not find any changes in the expression of 5-HT_{2A} in the HC. The only other studies looking at 5-HT_{2A} in the HC include two binding studies, one of which found an increase in 5-HT_{2A} binding (Joyce et al 1993) whereas the other found no differences (Burnet et al 1996). In contrast to the findings with 5-HT_{2A}, 5-HT_{1B} mRNA is higher in the HC in schizophrenia patients compared with control subjects. To our knowledge, our study is the first to report a disturbance of

5-HT_{1B} mRNA expression in the HC of patients with schizophrenia. Down-regulated expression of 5-HT postsynaptic receptors such as 5-HT_{2A} could be a compensatory response to altered hippocampal 5-HT transporter activity in schizophrenia (Dean et al 1999; Scarr et al 2001). This hypothesis does not explain the up-regulation of the postsynaptic 5-HT_{1B} receptor, however.

We were surprised that contrary to what has been reported in several studies (Arora and Meltzer 1991; Burnet et al 1996; Dean and Hayes 1996; Dean et al 1998, 1999; Hashimoto et al 1991; Laruelle et al 1993; Mita et al 1986; Simpson et al 1996; Sumiyoshi et al 1996), we did not find any changes in 5-HT receptor expression in the DLPFC. Likewise, it is unclear why there are certain inconsistencies in the HC between our study and that of Burnet et al (1996). Possible explanations include the following: the tissue used in our study was younger (mean age was 45) compared with most studies in the literature, in which subjects older than 65 years at the time of the death were included. In schizophrenia, positive symptoms (hallucinations, delusions) are more prominent at younger ages, whereas negative symptoms (emotional flatness, anhedonia) and dementia are more prominent in older life stages. Hippocampal malfunction is thought to contribute to the positive symptoms (Silbersweig et al 1995), whereas changes in the DLPFC may be more related to the negative symptoms. Alterations in 5-HT receptors in the DLPFC may occur during the latter stages of the disease. Thus, our findings in the HC may be representative of changes that occur earlier on in the disease. Another possible difference is that Burnet and colleges analyzed two areas of HC separately (CA1 and DG), whereas we analyzed HC including CA2 and CA4 together with CA1 and DG.

Although this is a human study, it is interesting to use the results from animal studies to speculate on how changes in 5-HT receptor expression may alter the neurochemical interactions in the HC and PFC. 5-HT_{2A} receptors are known to inhibit norepinephrine release (Done and Sharp 1994) and to increase GABA release (Farber et al 1998). Additionally, 5-HT interacts with acetylcholine (ACh) to modulate glutamatergic transmission in the hippocampal formation, where 5-HT_{1B} has an inhibitory effect on cholinergic neurons (Izumi et al 1994). Interestingly, cholinergic receptors are heavily concentrated on GABAergic interneurons (Van der Zee et al 1993), and 5-HT_{1B} heteroreceptors are located on GABA terminals. A down-regulation of 5-HT_{2A} and an up-regulation of 5-HT_{1B} receptor could lead to an overall decreased GABAergic activity (Moret and Briley 2000). A reduction in GABAergic activity could then lead to a disinhibition of glutamatergic efferents, similar to what it has been proposed in the N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis (Olney et al 1999, 2002).

A possible scenario based on the interactions described here taken together with the results of this study might be that a decrease in 5-HT_{2A} and an increase in 5-HT_{1B} could result in enhanced hippocampal glutamatergic output, secondary to altered serotoninergic receptors expression. Based on these interactions, 5-HT_{1B} and 5-HT_{2A} receptors may play an important role in altered hippocampal neuronal transmission in schizophrenia patients.

Bipolar Disorder

Levels of 5-HT_{2A} transcripts are significantly lower in the DLPFC of patients with BPD compared with matched control subjects. The diminution of 5-HT receptor mRNA expression found in BPD may be due to two basic mechanisms: a reduction in mRNA synthesis in each neuron or a decrease in the number of cells containing the mRNA. The latter situation is likely the decrease in the density of the prefrontal cortex of patients with BPD resulting from the loss of both large and small neurons (Rajkowska 2000; Rajkowska et al 2001). In contrast, MDD patients have a relative depletion of large neurons and an increase in the density of small neurons, suggesting cell atrophy as opposed to cell loss (Manji et al 2000; Rajkowska 2000; Rajkowska et al 2001).

Interestingly, in parallel to what was observed in the HC of the schizophrenic group, we found higher levels of 5-HT_{1B} transcript and lower levels of 5-HT_{2A} mRNA expression in the BPD group compared with control subjects. There is no evidence supporting histologic alterations of neuronal cells in the hippocampal formation (Rajkowska 2000), although an alteration in nonpyramidal cells in area CA2 has been reported (Benes et al 1998). The alterations in the nonpyramidal cells in CA2 most likely do not account for the observed changes in 5-HT receptor transcripts, because the changes were found in every hippocampal subregion.

Our results suggest that BPD and schizophrenia share a common disturbance in a specific area of the brain, the hippocampal formation. These findings support the clinical hypothesis that BPD shares some features with schizophrenia that could be related to a hippocampal malfunction, including psychotic episodes (Silbersweig et al 1995). It is important to note that BPD has a unique pathology; 5-HT receptor mRNA changes in the DLPFC of BPD group were not found in the schizophrenic group. In light of these findings, it appears that the 5-HT system plays an important role in the pathophysiology of BPD (Prange et al 1974; Price et al 1990). Moreover, several authors (Bunney and Davis 1965; Prange et al 1974; Shiah and Yatham 2000; Vawter et al 2000) have postulated a "permissive" hypothesis of BPD. This hypothesis suggests that MDD is characterized by low 5-HT, norepinephrine, and dopamine function, whereas BPD is characterized by low 5-HT and high norepinephrine and dopamine function. Moreover, alterations in other neurotransmitters (e.g., low activities of GABA and ACh) have also been postulated to exist in BPD. Therefore, the interrelationship between 5-HT and these other neurotransmitter systems may play an important role in the pathophysiology of BPD.

Major Depression Disorder

Our results show a reduction of 5-HT_{1A} mRNA expression in the HC and in the DLPFC of MDD patients compared with control subjects. The decrease of 5-HT_{1A} mRNA in the HC of the MDD group is similar to findings reported by López et al (1998) and comparable to ligand binding studies (Cheetham et al 1990; Lowther et al 1997). As presented here, 5-HT_{1A} mRNA is decreased in the DLPFC, whereas other authors using binding techniques have found no differences (Arranz et al 1994; Lowther et al 1997; Matsubara et al 1991; Stockmeier 1997) or increased binding (Arango et al 1995). Although these previous studies have been done in postmortem tissue, only one has studied the involvement of 5-HT_{1A} mRNA in the HC of MDD patients; other studies have reported binding differences in suicide victims that include but are not restricted to patients suffering from MDD (Cheetham et al 1990; Lowther et al 1997).

It has been noted that this decrease could be related to a down-regulation of the limbic circuitry under stress conditions because cortisol hypersecretion has been associated with MDD (Chalmers et al 1993, 1994; López et al 1997, 1998, 1999). Previous studies in our lab with tissue from the same set of patients showed a decreased expression of mineralocorticoid

receptor (MR) and glucocorticoid receptor (GR) in the DLPFC of MDD patient. Alteration of 5-HT_{1A} and MR and GR in mood disorders could be understood as mechanisms by which stress may induce or maintain depressive episodes (López et al 1998). The human postmortem finding correlates with animal models of hypercortisolemia, in which 5-HT_{1A} mRNA expression in the HC is downregulated by chronic stress and by exogenous glucocorticoid administration (Chalmers et al 1993; Meador-Woodruff et al 1990; Meijer and de Kloet 1998; Mendelson and McEwen 1991, 1992). These studies established a regression of the chronic-stress-induced process by removing the adrenal glands, recovering the previous levels of 5-HT_{1A} expression. Therefore, certain mechanisms, including serotonin receptor 5-HT_{1A} and corticoid system, may modulate the response to stress.

Considerations

Some technical considerations to think about are the few studies of the 5-HT system done in BPD postmortem tissue. Most of these human postmortem studies were performed in the context of depression, and the numbers of BPD subjects were either small (Dean et al 2001) or pooled together in a suicidal group (Arango et al 1990; Mann et al 1986). The Stanley Foundation Neuropathology Consortium allows for investigation of possible differences within mood disorders (Knable et al 2001, 2002). On the other hand, relatively small sample sizes and a high proportion of suicide cases in mood disorder postmortem studies make it difficult to generalize findings.

Other considerations are inconsistent findings in the literature that may be due to multiple factors, such as the heterogeneity of the disease, interspecies differences (Bruinvels et al 1994), low mRNA expression, the use of small samples, differences in brain regions, unavailability of selective ligands, as well as different histories of medication or illicit drug exposure. Consequently, gene expression may be altered in patients with mood disorders or schizophrenia as a response to medication. For example, in schizophrenia, atypical neuroleptic drugs, such as clozapine and risperidone, have proven to relieve positive and negative symptoms, possibly through an antagonist action on 5-HT_{2A} and 5-HT_{2C} receptors (Sawa and Snyder 2002). In our subjects with schizophrenia, nine were taking classical neuroleptics, seven were taking atypical neuroleptics, four were taking antidepressants, two were taking lithium, and five were taking anxiolytics. Given the possibility that specific mRNA levels are altered in patients with schizophrenia, a response to medication should also be considered. In mood disorders, antidepressant medications increase 5-HT activity in depressive states and may thereby contribute to normalization of serotonergic functions. Drug treatment with lithium, neuroleptics, and antidepressants may play an important role in neuronal survival (Chen et al 2000; Price et al 1990; Sawa and Snyder 2002). In our study, subsets of subjects were taking these drugs at the time of death; consequently, the effects of medication cannot be ruled out. In animal studies, tricyclic antidepressant administration decreases 5-HT_{2A} binding in cortex (Peroutka and Snyder 1980), but one study has reported increases in 5-HT_{2A} mRNA following fluoxetine and electroconvulsive treatment (Butler et al 1993). No consistent changes in postsynaptic 5-HT_{1A} or 5-HT_{1B} levels have been reported after chronic administration of psychotropic medications. Therefore, the direction of receptor mRNA changes found in our studies is not consistent with simply a medication effect. Under these circumstances, the effects of medication cannot be ruled out until specific studies for each drug can be performed on more restricted and controlled cohorts.

In conclusion, our results show alteration of 5-HT receptor mRNA expression in patients with major psychiatric disorders. This study supports the hypothesis that the serotonergic system, perhaps in combination with other neurotransmitters and specific neuronal networks, has an important role in the pathophysiology of both mood disorders and schizophrenia.

We acknowledge the support of Drs. James H Meador-Woodruff, Charles Neal Jr., Marco Cecchi, Mohamed Kabbaj, Mónica Beneyto Santonja, Inés Morano, Lucio Díaz-Flores Feo, Tomás González Hernández, and Elena Vigo Moreno. Postmortem brains were donated by the Stanley Medical Research Institute's Brain Collection, courtesy of Drs. Michael Knable, E. Fuller Torrey, Maree J. Webster, Serge Weis, and Robert H. Yolken.

Arango V, Ernsberger P, Marzuk PM, Chen JS, Tierney H, Stanley M, et al (1990): Autoradiographic demonstration of increased serotonin 5-HT2 and beta-adrenergic receptor binding sites in the brain of suicide victims. *Arch Gen Psychiatry* 47:1038–1047.

Arango V, Underwood MD, Gubbi AV, Mann JJ (1995): Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res* 688:121–133.

Arora RC, Meltzer HY (1991): Serotonin2 (5-HT2) receptor binding in the frontal cortex of schizophrenic patients. *J Neural Transm Gen Sect* 85:19–29.

Arranz B, Eriksson A, Mellerup E, Plenge P, Marcusson J (1994): Brain 5-HT1A, 5-HT1D, and 5-HT2 receptors in suicide victims. *Biol Psychiatry* 35:457–463.

Barnes NM, Sharp T (1999): A review of central 5-HT receptors and their function. *Neuropharmacology* 38:1083–1152.

Benes FM, Kwok EW, Vincent SL, Todtenkopf MS (1998): A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. *Biol Psychiatry* 44:88–97.

Bruinvels AT, Landwehrmeyer B, Gustafson EL, Durkin MM, Mengod G, Branchek TA, et al (1994): Localization of 5-HT1B, 5-HT1D alpha, 5-HT1E and 5-HT1F receptor messenger RNA in rodent and primate brain. *Neuropharmacology* 33:367–86.

Bunney WE Jr, Davis JM (1965): Norepinephrine in depressive reactions. A review. *Arch Gen Psychiatry* 13:483–494.

Burnet PW, Eastwood SL, Harrison PJ (1996): 5-HT_{1A} and 5-HT_{2A} receptor mRNAs and binding site densities are differentially altered in schizophrenia. *Neuropsychopharmacology* 15:442–455.

Burnet PW, Eastwood SL, Lacey K, Harrison PJ (1995): The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. *Brain Res* 676:157–168.

Butler MO, Morinobu S, Duman RS (1993): Chronic electroconvulsive seizures increase the expression of serotonin2 receptor mRNA in rat frontal cortex. *J Neurochem* 61:1270–1276.

Chalmers DT, Kwak SP, Mansour A, Akil H, Watson SJ (1993): Corticosteroids regulate brain hippocampal 5-HT1A receptor mRNA expression. *J Neurosci* 13:914–923.

Chalmers DT, López JF, Vazquez DM, Akil H, Watson SJ (1994): Regulation of hippocampal 5-HT1A receptor gene expression by dexamethasone. *Neuropsychopharmacology* 10:215–222.

Cheetham SC, Crompton MR, Katona CL, Horton RW (1990): Brain 5-HT1 binding sites in depressed suicides. *Psychopharmacology* 102:544–548.

Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK (2000): Enhancement of hippocampal neurogenesis by lithium. *J Neurochem* 75:1729–1734.

Dean B, Hayes W (1996): Decreased frontal cortical serotonin2A receptors in schizophrenia. *Schizophr Res* 21:133–139.

Dean B, Hayes W, Hill C, Copolov D (1998): Decreased serotonin2A receptors in Brodmann's area 9 from schizophrenic subjects. A pathological or pharmacological phenomenon? *Mol Chem Neuropathol* 34:133–145.

Dean B, Hussain T, Hayes W, Scarr E, Kitsoulis S, Hill C, et al (1999): Changes in serotonin2A and GABA(A) receptors in schizophrenia: Studies on the human dorsolateral prefrontal cortex. *J Neurochem* 72:1593–1599.

Dean B, Pavey G, McLeod M, Opeskin K, Keks N, Copolov D (2001): A change in the density of[(3)H] [(3)H]flumazenil, but not [(3)H] [(3)H]muscimol binding, in Brodmann's area 9 from subjects with bipolar disorder. *J Affect Disord* 66:147–158.

Dean B, Tomaskovic-Crook E, Opeskin K, Keks N, Copolov D (1999): No change in the density of the serotonin1A receptor, the serotonin4 receptor or the serotonin transporter in the dorsolateral prefrontal cortex from subjects with schizophrenia. *Neurochem Int* 34:109–115.

Deutch A, Roth R (1999): Neurotransmitters. In: Zigmond M, editor. *Fundamental Neuroscience.* San Diego: Academic Press, 208–212.

Done CJ, Sharp T (1994): Biochemical evidence for the regulation of central noradrenergic activity by 5-HT1A and 5-HT2 receptors: Microdialysis studies in the awake and anaesthetized rat. *Neuropharmacology* 33:411–421.

Farber NB, Hanslick J, Kirby C, McWilliams L, Olney JW (1998): Serotonergic agents that activate 5HT2A receptors prevent NMDA antagonist neurotoxicity. *Neuropsychopharmacology* 18:57–62.

Fargin A, Raymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ (1988): The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. *Nature* 335:358–360.

Gold PW, Goodwin FK, Chrousos GP (1988): Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress. *N Eng J Med* 319:413–420.

Hartig PR, Hoyer D, Humphrey PP, Martin GR (1996): Alignment of receptor nomenclature with the human genome: Classification of 5-HT1B and 5-HT1D receptor subtypes. *Trends Pharmacol Sci* 17:103–105.

Hashimoto T, Nishino N, Nakai H, Tanaka C (1991): Increase in serotonin 5-HT_{1A} receptors in prefrontal and temporal cortices of brains from patients with chronic schizophrenia. *Life Sci* 48:355–363.

Izumi J, Washizuka M, Miura N, Hiraga Y, Ikeda Y (1994): Hippocampal serotonin 5-HT1A receptor enhances acetylcholine release in conscious rats. *J Neurochem* 62:1804–1808.

Jin H, Oksenberg D, Ashkenazi A, Peroutka SJ, Duncan AM, Rozmahel R, et al (1992): Characterization of the human 5-hydroxytryptamine1B receptor. *J Biol Chem* 267:5735–5738.

Joyce JN, Shane A, Lexow N, Winokur A, Casanova MF, Kleinman JE (1993): Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics. *Neuropsychopharmacology* 8:315–336.

Knable MB, Barci BM, Bartko JJ, Webster MJ, Torrey EF (2002): Molecular abnormalities in the major psychiatric illnesses: Classification and Regression Tree (CRT) analysis of post-mortem prefrontal markers. *Mol Psychiatry* 7:392–404.

Knable MB, Torrey EF, Webster MJ, Bartko JJ (2001): Multivariate analysis of prefrontal cortical data from the Stanley Foundation Neuropathology Consortium. *Brain Res Bull* 55:651–659.

Laruelle M, Abi-Dargham A, Casanova MF, Toti R, Weinberger DR, Kleinman JE (1993): Selective abnormalities of prefrontal serotonergic receptors in schizophrenia. A postmortem study. *Arch Gen Psychiatry* 50:810–818.

López JF, Akil H, Watson SJ (1999): Neural circuits mediating stress [review]. *Biol Psychiatry* 46:1461–1471.

López JF, Chalmers DT, Little KY, Watson SJ (1998): A.E. Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: Implications for the neurobiology of depression. *Biol Psychiatry* 43:547–573.

López JF, Vazquez DM, Chalmers DT, Watson SJ (1997): Regulation of 5-HT receptors and the hypothalamic-pituitary-adrenal axis. Implications for the neurobiology of suicide. *Ann N Y Acad Sci* 836:106–134.

López-Figueroa MO, Day HE, Lee S, Rivier C, Akil H, Watson SJ (2000): Temporal and anatomical distribution of nitric oxide synthase mRNA expression and nitric oxide production during central nervous system inflammation. *Brain Res* 852:239–246.

Lowther S, De Paermentier F, Cheetham SC, Crompton MR, Katona CL, Horton RW (1997): 5-HT1A receptor binding sites in post-mortem brain samples from depressed suicides and controls. *J Affect Disord* 42:199–207.

Manji HK, Moore GJ, Rajkowska G, Chen G (2000): Neuroplasticity and cellular resilience in mood disorders. *Mol Psychiatry* 5:578–593.

Mann JJ, Stanley M, McBride PA, McEwen BS (1986): Increased serotonin2 and beta-adrenergic receptor binding in the frontal cortices of suicide victims. *Arch Gen Psychiatry* 43:954–959.

Matsubara S, Arora RC, Meltzer HY (1991): Serotonergic measures in suicide brain: 5-HT1A binding sites in frontal cortex of suicide victims. *J Neural Transm Gen Sect* 85:181–194.

Meador-Woodruff JH, Greden JF, Grunhaus L, Haskett RF (1990): Severity of depression and hypothalamic-pituitary-adrenal axis dysregulation: Identification of contributing factors. *Acta Psychiatr Scand* 81:364–371.

Meijer OC, de Kloet ER (1998): Corticosterone and serotonergic neurotransmission in the hippocampus: Functional implications of central corticosteroid receptor diversity. *Crit Rev Neurobiol* 12:1–20.

Melzter H (1989): Serotonergic dysfunction in depression. *Br J Psychiatry* 155:25–31.

Mendelson SD, McEwen BS (1991): Autoradiographic analyses of the effects of restraint-induced stress on 5-HT1A, 5-HT1C and 5-HT2 receptors in the dorsal hippocampus of male and female rats. *Neuroendocrinology* 54:454–461.

Mendelson SD, McEwen BS (1992): Autoradiographic analyses of the effects of adrenalectomy and corticosterone on 5-HT1A and 5-HT1B receptors in the dorsal hippocampus and cortex of the rat. *Neuroendocrinology* 55:444–450.

Mita T, Hanada S, Nishino N, Kuno T, Nakai H, Yamadori T, et al (1986): Decreased serotonin S2 and increased dopamine D2 receptors in chronic schizophrenics. *Biol Psychiatry* 21:1407–1414.

Moret C, Briley M (2000): The possible role of 5-HT(1B/D) receptors in psychiatric disorders and their potential as a target for therapy [review]. *Eur J Pharmacol* 404:1–12.

Nemeroff CB (1998): The neurobiology of depression. *Sci Am* 278:42–49.

Norton CS, Neal CR, Kumar S, Akil H, Watson SJ (2002): Nociceptin/orphanin FQ and opioid receptor-like receptor mRNA expression in dopamine systems. *J Comp Neurol* 444:358–368.

Olney JW, Newcomer JW, Farber NB (1999): NMDA receptor hypofunction model of schizophrenia [review]. *J Psychiatr Res* 33:523–533.

Olney JW, Newcomer JW, Farber NB (2002): Is NMDA receptor hypofunction in schizophrenia associated with a primary hyperglutamatergic state? *Arch Gen Psychiatry* 59:466–468.

Pandey GN, Dwivedi Y, Rizavi HS, Ren X, Pandey SC, Pesold C, et al (2002): Higher expression of serotonin 5-HT(2A) receptors in the postmortem brains of teenage suicide victims. *Am J Psychiatry* 159:419–29.

Pasqualetti M, Nardi I, Ladinsky H, Marazziti D, Cassano GB (1996): Comparative anatomical distribution of serotonin 1A, 1D alpha and 2A receptor mRNAs in human brain postmortem. *Brain Res Mol Brain Res* 39:223–233.

Pazos A, Probst A, Palacios JM (1987a): Serotonin receptors in the human brain—III. Autoradiographic mapping of serotonin-1 receptors. *Neuroscience* 21:97–122.

Pazos A, Probst A, Palacios JM (1987b): Serotonin receptors in the human brain–IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience* 21:123–139.

Peroutka SJ, Snyder SH (1980): Regulation of serotonin2 (5-HT2) receptors labeled with [3H]spiroperidol by chronic treatment with the antidepressant amitriptyline. *J Pharmacol Exp Ther* 215:582–587.

Pompeiano M, Palacios JM, Mengod G (1992): Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: Correlation with receptor binding. *J Neurosci* 12:440–453.

Pompeiano M, Palacios JM, Mengod G (1994): Distribution of the serotonin 5-HT2 receptor family mRNAs: Comparison between 5-HT2A and 5-HT2C receptors. *Brain Res Mol Brain Res* 23:163–178.

Prange AJ Jr, Wilson IC, Lynn CW, Alltop LB, Stikeleather RA (1974): L-tryptophan in mania. Contribution to a permissive hypothesis of affective disorders. *Arch Gen Psychiatry* 30:56–62.

Price LH, Charney DS, Delgado PL, Heninger GR (1990): Lithium and serotonin function: Implications for the serotonin hypothesis of depression. *Psychopharmacology* 100:3–12.

Rajkowska G (2000): Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol Psychiatry* 48:766–777.

Rajkowska G, Halaris A, Selemon LD (2001): Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol Psychiatry* 49:741–752.

Reynolds GP, Rossor MN, Iversen LL (1983): Preliminary studies of human cortical 5-HT2 receptors and their involvement in schizophrenia and neuroleptic drug action. *J Neural Transm Suppl* 18:273–277.

Sawa A, Snyder SH (2002): Schizophrenia: Diverse approaches to a complex disease. *Science* 296:692–695.

Scarr E, Copolov DL, Dean B (2001): A proposed pathological model in the

hippocampus of subjects with schizophrenia. *Clin Exp Pharmacol Physiol* 28:70–73.
Shiah IS, Yatham LN (2000): Serotonin in mania and in the mechanism of action of mood stabilizers: A review of clinical studies. *Bipolar Disord* 2:77–92.
Silbersweig DA, Stern E, Frith C, Cahill C, Holmes A, Grootoonk S, et al (1995): A functional neuroanatomy of hallucinations in schizophrenia. *Nature* 378:176–179.
Simpson MD, Lubman DI, Slater P, Deakin JF (1996): Autoradiography with [3H]8-OH-DPAT reveals increases in 5-HT(1A) receptors in ventral prefrontal cortex in schizophrenia. *Biol Psychiatry* 39:919–928.
Stockmeier CA (1997): Neurobiology of serotonin in depression and suicide. *Ann N Y Acad Sci* 836:220–232.
Stockmeier CA, Dilley GE, Shapiro LA, Overholser JC, Thompson PA, Meltzer HY (1997): Serotonin receptors in suicide victims with major depression. *Neuropsychopharmacology* 16:162–173.
Sumiyoshi T, Stockmeier CA, Overholser JC, Dilley GE, Meltzer HY (1996): Serotonin1A receptors are increased in postmortem prefrontal cortex in schizophrenia. *Brain Res* 708:209–214.
Torrey EF, Webster M, Knable M, Johnston N, Yolken RH (2000): The Stanley Foundation Brain Collection and Neuropathology Consortium. *Schizophr Res* 44:151–155.
Van der Zee EA, Luiten PG (1993): GABAergic neurons of the rat dorsal hippocampus express muscarinic acetylcholine receptors. *Brain Res Bull* 32:601–609.
Vawter MP, Freed WJ, Kleinman JE (2000): Neuropathology of bipolar disorder. *Biol Psychiatry* 48:486–504.
Watson SJ, Patel P, Burke S, Herman J, Schafer M, Kwak S (1988): In situ hybridization of mRNA in nervous tissue: A primer. In: Sundermann A, editor. *Short Course 1 Syllabus.* Washington, DC: Society for Neuroscience, 4–29.
Weinshank RL, Zgombick JM, Macchi MJ, Branchek TA, Hartig PR (1992): Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT1D alpha and 5-HT1D beta. *Proc Natl Acad Sci U S A* 89:3630–3634.
Whitaker PM, Crow TJ, Ferrier IN Tritiated (1981): LSD binding in frontal cortex in schizophrenia. *Arch Gen Psychiatry* 38:278–280.