Decreased Serotonin 5-HT_{2A} Receptor-Stimulated Phosphoinositide Signaling in Fibroblasts from Melancholic Depressed Patients

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Abnormalities in serotonin (5-HT) receptors and 5-HT receptor-mediated signal transduction systems have been widely reported in mood disorders. This study was intended to evaluate 5-HT_{2A} receptor-coupled activation of phosphatidylinositol (PI) hydrolysis in subtypes of depression. Samples for fibroblast culture were obtained from patients with major depression with or without melancholia, and normal controls. Dose response curves were determined for 5-HT-induced PI hydrolysis. PI response was determined for bradykinin and 1- α -lysophosphatidic acid (LPA), alternative Gq-coupled receptor agonists. [¹²⁵I]LSD binding for 5-HT_{2A} also was conducted. Finally, Western blot analysis was performed for phospholipase C _{β 1} (PLC _{β 1}) and G $\alpha_{q/11}$ proteins. The maximum PI response observed with 5-HT was significantly lower in melancholics but not nonmelancholic patients relative to controls. Activation of PI hydrolysis by bradykinin and LPA was not reduced in melancholic vs melancholics and controls; responses to both agonists actually were increased in the melancholic group. [¹²⁵I]LSD binding, PLC _{β 1}, and G $\alpha_{q/11}$ protein levels did not differ between groups. The data raise the possibility that the reduced 5-HT_{2A} receptor-induced PI hydrolysis is intrinsic to the receptor itself or its coupling to Gq protein, and is not related to altered availability of the 5-HT_{2A} receptor, Gq or PLC.

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INTRODUCTION

Abnormalities in serotonin (5-HT) receptors and 5-HT receptor-mediated signal transduction systems have been widely reported in mood disorders (Ressler and Nemeroff 2000). Of these, the 5-HT_{2A} receptor subtype is the most widely studied. Activation of Gq-coupled 5-HT_{2A} receptors results in phospholipase C (PLC) catalyzed hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5 bisphosphate, with the formation of two second messengers: inositol(1,4,5) triphosphate (IP₃) and diacylglycerol (DAG). IP₃ plays an important role in various physiological processes by releasing calcium from intracellular stores. DAG activates protein kinase C (PKC), which, in turn, phosphorylates target proteins in the cell. Therefore, abnormalities of 5-HT_{2A} receptors have the potential to alter a diverse set of cellular processes (Aghajanian and

Sanders-Bush 2002), potentially contributing to psychiatric diseases such as major depressive disorder.

Differences in the density of 5-HT_{2A} receptors in depressives relative to normal controls have been reported in many studies; however, the results are controversial. For example, some studies of 5-HT_{2A} receptors in platelets have found significant elevations (Pandey *et al*, 1990, 1995; Hrdina *et al*, 1995, 1997; Rao *et al*, 1998; Alda and Hrdina, 2000), while others have not (McBride *et al*, 1994; Neuger *et al*, 1999; Rosel *et al*, 1999). Similar results have been shown in post-mortem brain specimens from depressed patients; in some studies, apparent 5-HT_{2A} binding was elevated (Stanley and Mann 1983; Mann *et al*, 1986; Hrdina *et al*, 1993; Arranz *et al*, 1994; Pandey *et al*, 2002a,b), but not in others (Cheetham *et al*, 1988; Rosel and Arranz, 1998; Gross-Isseroff *et al*, 1998; Rosel *et al*, 2000). Recent studies have also investigated the activity of the PLC/phosphoinositide (PI) second messenger system in mood disorders (Pacheco *et al*, 1996; Jope *et al*, 1996; Shimon *et al*, 1997; Soares and Mallinger, 1997; Pandey *et al*, 2002a,b). Pacheco *et al* (1996) found that GTP γ S-stimulated PI hydrolysis is significantly lower in the brains of depressed persons who committed suicide relative to a normal control group. They also reported slightly lower PI responses to agonists such as 5-HT and ATP. Impairments of the PI signal transduction

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system have been reported in brains from bipolar depressive subjects as well. Pandey *et al* (1999) found significantly lower PI-PLC activity and expression of the PLC isozyme PLC β_1 in the brains of teenage suicide subjects relative to normal controls. In contrast, hyperactive PI signaling has been shown in platelets from depressed patients (Karege *et al*, 1996; Alvarez *et al*, 1999; Pandey *et al*, 2001). Elevated 5-HT-stimulated intracellular calcium has also been reported in platelets from unipolar depression (Delisi *et al*, 1998) and bipolar disorder (Suzuki *et al*, 2001). Alternatively, Suzuki *et al* (2001) found no differences in the calcium response to 5-HT in platelets from patients with depressive disorder with or without melancholia in contrast to a variety of other psychiatric conditions. Together, these studies suggest that there may be an abnormality in the 5-HT_{2A} receptor in depressed patients, but the exact nature remains uncertain.

These discordant results may be related to a variety of issues, including differences based on diagnostic subtypes, drug treatment at the time of death, or dissimilarities between depressive and controls in circulating neurotransmitters and hormones. Other factors such as marked differences between depressives and normals in the cause of death and post-mortem intervals complicate interpretation of post-mortem brain studies. The aim of the current study was to probe for differences in the 5-HT_{2A} receptor-mediated signal transduction pathway, using an *ex vivo* fibroblast cell culture model system. Previous studies in our laboratory have demonstrated significant differences in the adenylyl cyclase signaling pathway in melancholic subtype of depression *vs* controls and depressives with the atypical subtype (Shelton *et al*, 1996, 1999; Manier *et al*, 2000). Since these results were found only in a subset of depressives, the differences could be masked in the total depressive patient population, underscoring the value of diagnostic subclassification. We therefore compared 5-HT_{2A} receptor-mediated signaling in cultured fibroblasts from melancholic subtype of depression *vs* controls and nonmelancholic depressives. Fibroblasts and neurons share many of the same receptors and intracellular signaling molecules (Manier *et al*, 2000), including the 5-HT_{2A} receptor-Gq-PLC-PKC-CREB pathway. Furthermore, fibroblasts are relatively simple to obtain and maintain in culture in a controlled, reproducible environment, thus eliminating potential differences in drug treatment, transmitter or hormone exposure, or agonist states.

MATERIALS, PATIENTS, AND METHODS

Human Subjects

All procedures involving human subjects were conducted after written informed consent was obtained. Participants include patients with major depression, with or without melancholic subtype, and normal volunteers. Subjects were evaluated using the Structured Clinical Interview for DSM-IV (First *et al*, 1996) and the 17-item Hamilton Rating Scale for Depression (HAM-D) (Hamilton 1960). A second diagnostic interview was conducted by a senior psychiatrist. Diagnostic assignment, including subtyping, was made based on a consensus between the two interviewers. All the patients had a principal diagnosis of major depression;

secondary diagnoses were allowed. However, no patient had a current or past history of psychotic disorder. Normal volunteer controls were free of Axis I mental disorder by DSM-IV (American Psychiatric Association 1994).

Collecting and Culturing of Human Fibroblasts

Fibroblasts were cultured from skin biopsies, as described previously (Shelton *et al*, 1996, 1999). All samples have been subcultured for at least five passages in order to minimize any effect from exposure *in vivo* to factors such as hormones, transmitters, drugs, or other factors. Fibroblast cultures were grown at 37°C in a humidified atmosphere containing 5% CO₂. Growth medium consisted of Dulbecco's modified Eagle's medium, containing 10% fetal bovine serum (FBS) or 10% dialyzed FBS that lacks 5-HT, and an antibiotic/antimycotic solution at final concentrations of 30 U penicillin, 30 μ g streptomycin, and 70 ng amphotericin B/ml. The medium was changed three times per week and the cultures were inspected daily. After reaching confluency (4×10^6 cells/75 cm²), the samples were subcultured by exposure to 0.05% trypsin/0.02% EDTA solution.

Saucier *et al* (1998) reported that fibroblasts, including NIH-3T3 cells, lack 5-HT receptors since no specific binding or response to 5-HT was detected. However, when these cells are cultivated in medium containing 10% dialyzed FBS, which lacks 5-HT, the cells became responsive to 5-HT. In the current study, 5-HT-induced PI hydrolysis was measured in fibroblasts grown in medium supplemented with 10% normal FBS (5-HT containing) or dialyzed FBS (5-HT free). The cells were gradually changed to dialyzed serum over a 3-week period, since more rapid changes resulted in cell death. We replicated the results of Saucier *et al*; 5-HT responses were only obtained in cells that were maintained in dialyzed FBS, therefore only dialyzed serum was used for all further experiments.

PI Hydrolysis Assay

Cultured cells were prelabeled with [³H]myo-inositol (NEN Life Science Products, Boston, MA 02118) for 16–20 h. The formation of [³H]inositol monophosphate ([³H]-IP) in the presence of 10 mM lithium and 10 μ M pargyline was used as an index of PLC activation. [³H]-IP was isolated by extraction and purified by ion exchange chromatography as described previously (Barker *et al*, 1994). Concentration-response curves were generated by incubation with 5-HT (100 fmol–10 μ M) and were fit using Graph Pad Prism, a computer graphics/analysis program (Graph Pad, San Diego, CA) to calculate EC₅₀ and maximum effect. In addition, PI hydrolysis was determined for bradykinin (10 μ M) and 1- α -lysophosphatidic acid (LPA) (10 μ M).

[¹²⁵I]LSD Binding to 5-HT_{2A} Receptors

For each determination, twenty 75 mm plates were combined. Cells were homogenized in 5 ml hypotonic medium (50 mM Tris/10 mM MgCl₂), by using a polytron for 2 s and centrifuged at 13 K rpm for 20 min at 4°C. The pellet was resuspended in 50 mM Tris buffer (pH 7.5) and used for the determination of the 5-HT_{2A} receptor level. The receptor binding assay was carried out using a single concentration

of [¹²⁵I]LSD (NEN Life Science Products, Boston, MA 02118), each data point replicated 6–10 times. A 250 µl aliquot of membrane suspension (~25 µg protein), 25 µl [¹²⁵I]LSD (3 nM), and 25 µl incubation buffer (50 mM Tris) or ketanserin (1 µM) were incubated 1 h at 37°C. The assay was terminated by filtration through Whatman GF/C filters. The filters were washed for 30 s with 50 mM Tris buffer (pH 7.7) containing 0.01% bovine serum albumin, dried and counted in γ counter. Specific binding was defined as total binding minus binding in the presence of 1 µM ketanserin.

Western Blots of PLC β_1 Isozyme and G $\alpha_{q/11}$ Protein

Cells were homogenized in 5 ml hypotonic medium (50 mM Tris/10 mM MgCl₂), using a polytron for 2 s and centrifuged at 13 K rpm for 20 min at 4°C. The pellet was suspended in homogenizing buffer containing 10 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 1% Na orthovanadate, 1.5 mM pepstatin, 2 mM leupeptin, and 0.2 U/ml aprotinin. Equal volumes of membrane fraction (120 µl) were subjected to 10% polyacrylamide gel by using a gel electrophoresis apparatus. The proteins were subsequently transferred electrophoretically to nitrocellulose membrane using a protein transfer unit. Membranes were washed for 10 min and blocked with 3% milk powder in PBS. Then the blots were incubated with primary monoclonal antibodies (anti-PLC β_1 and anti-G $\alpha_{q/11}$, Santa Cruz Biotechnology, Santa Cruz, CA 95060), diluted at 1:200 in the blocking solution for 90 min at room temperature. The membranes were then thoroughly washed and incubated with horseradish peroxidase-linked secondary antibody (anti-rabbit IgG, 1:3000 in blocking solution) for 60 min at room temperature. To reduce the interblot variability, membranes were stripped and reprobed with β -actin antibody (Sigma-Aldrich, St Louis, MO 63195) at a concentration of 1:1000 for 90 min at room temperature and then incubated with anti-mouse IgG as a secondary antibody (Upstate Biotechnology, Lake Placid, NY 12946) at a concentration 1:2000 for 90 min at room temperature. Bands on the autoradiogram were quantified using Image J image analysis system (NIH), and the optical density of each sample was corrected by dividing by the optical density of the corresponding β -actin band.

Statistical Analysis

Differences between the three groups were tested using a one-way analysis of variance (ANOVA) for independent groups, with *post hoc* Tukey HSD test. Two-group comparisons were conducted with Student's *t*-tests.

RESULTS

Participant Characteristics

The participants included patients with a diagnosis of major depression, melancholic subtype ($n=8$, seven females), major depression without melancholia ($n=10$, eight females), and normal volunteers ($n=10$, nine females); mean age 49.1 years, (SD = 16.5), 41.9 years (SD = 18.1), and 36.9 years (SD = 7.9) (ANOVA, $F=1.538$, $df=2,25$, $p=NS$), respectively. The mean HAM-D score was 24.5 (SD = 2.9)

for melancholics and 22.7 (SD = 4.2) for nonmelancholics ($t=1.023$, $df=16$, $p=NS$). Only one patient, a 69-year-old nonmelancholic depressed man, was on an antidepressant, venlafaxine at 150 mg/day for 6 weeks, at the time of the biopsy. Excluding him from the analyses did not change the outcomes reported below.

5-HT Stimulated PI Hydrolysis

5-HT dose response curves for activation of PI hydrolysis were determined in fibroblasts from normal controls, melancholics, and nonmelancholic patients (Figure 1). 5-HT increased PI hydrolysis with nearly identical $-\log EC_{50}$ values (6.5 for normal subjects, 6.8 for melancholics, and 6.4 for nonmelancholics). The 5-HT response was eliminated by pretreatment with 100 nM MDL-100907, a 5-HT_{2A} receptor selective antagonist, suggesting that 5-HT-stimulated PI hydrolysis is mediated by 5-HT_{2A} receptors in these cells (data not shown). To determine if the decrease in PI hydrolysis is specific for the 5-HT_{2A} receptor or if it reflects a more general alteration in lipid labeling or intracellular signaling, the method was modified to eliminate potential differences in cell number or radiolabeling by expressing the data as a ratio of [³H]PI over [³H] total phospholipids, thus correcting for differences in labeling in the lipid fraction. The maximum signal was significantly lower in melancholic patients vs controls or nonmelancholics (ANOVA: $F=14.209$, $df=2,15$, $p<0.001$; Tukey HSD: melancholics vs nonmelancholics and controls, $p=0.001$) (Figure 2). In contrast, the maximum response did not differ between nonmelancholic subjects and normal controls (Tukey HSD, $p=NS$). These data suggest that the 5-HT_{2A} receptor signal is blunted in fibroblasts from subjects diagnosed with melancholic depression, but not in patients with nonmelancholic depression.

An examination of the basal constitutive activity of the 5-HT_{2A} receptor revealed a small [³H]-IP signal that was reproducible in both normal control samples and melancholic patients (Figure 2). An unexpected increase in

Figure 1 Phosphatidylinositol hydrolysis ([³H]IP) in fibroblasts from representative control, nonmelancholic (nonMEL), and melancholic depressed (MEL) subjects. 5-HT produced a concentration-dependent increase in PI hydrolysis in fibroblasts from normal, nonmelancholic, and melancholic subjects. While maximum signal was significantly lower in melancholic patients, no differences were observed in nonmelancholics compared with controls.

Figure 2 Basal and 5HT-stimulated phosphatidylinositol hydrolysis ($[^3\text{H}]\text{PI}$) in fibroblasts from control, nonmelancholic depressed (nonMEL), and melancholic depressed (MEL) subjects (mean $\pm$ SD). The basal signal was greater in nonmelancholics in contrast to controls (* $p=0.002$) and melancholics (** $p<0.03$). Maximum signal was significantly lower in melancholic patients vs both controls and nonmelancholics (*** $p=0.001$).

the basal $[^3\text{H}]\text{-IP}$ formation was found in nonmelancholic patients relative to the other two groups (ANOVA: $F=6.941$, $df=2,57$, $p=0.002$; *post hoc* Tukey HSD: non-melancholics vs controls, $p=0.002$; nonmelancholics vs melancholics, ** $p<0.03$; controls vs melancholics, $p=NS$) (Figure 2). This difference was eliminated by the addition of clozapine (100 nM), a 5-HT_{2A} receptor inverse agonist (data not shown), suggesting that the 5-HT_{2A} receptor has constitutive activity in fibroblasts from nonmelancholic patients.

Bradykinin and LPA Stimulated PI Hydrolysis

To test whether or not the altered PI response was specific to the 5-HT_{2A} receptors or, alternatively, related to the postreceptor mechanisms, we compared and examined the effects of bradykinin (10 μM) and LPA (10 μM), two other Gq-coupled receptor agonists, on PI hydrolysis (Figure 3). Unlike the response seen with 5-HT, the maximum bradykinin and LPA signals are actually increased in melancholic patients vs normal controls (bradykinin: $t=-3.733$, $df=5$, $p<0.02$; LPA: $t=-7.712$, $df=6$, $p<0.001$). This indicates that the decreased 5-HT signal is not related to alterations in postreceptor components of the signaling machinery.

$[^{125}\text{I}]\text{LSD}$ Binding Studies

Preliminary radioligand binding experiments suggested a low density of 5-HT_{2A} receptors in the cultured fibroblasts; this combined with the low yield of membrane protein from the fibroblasts cultured in dialyzed serum forced us to limit the binding experiments to a single concentration of $[^{125}\text{I}]\text{LSD}$. $[^{125}\text{I}]\text{LSD}$ binding was performed in fibroblasts from normal, nonmelancholic, and melancholic patients that were used previously in the PI hydrolysis experiments; for each patient, twenty 75 mm plates were prepared and the specific binding of 3 nM of $[^{125}\text{I}]\text{LSD}$ determined as described in methods, $[^{125}\text{I}]\text{LSD}$ binding was not significantly

Figure 3 Phosphatidylinositol ($[^3\text{H}]\text{PI}$) hydrolysis with Gq-coupled receptor agonists in fibroblasts from control and melancholic depressed (MEL) subjects (mean $\pm$ SEM). The maximum bradykinin (10 μM) and LPA (10 μM) signal is greater in melancholic patients vs normal controls. $n=10$ for normal controls and $n=8$ for melancholic subjects. * $p<0.05$ vs controls.

Figure 4 5-HT receptor density in fibroblasts from normal, nonmelancholic depressed (nonMEL) and melancholic depressed (MEL) subjects (mean $\pm$ SEM). Specific $[^{125}\text{I}]\text{LSD}$ binding (3 nM) was determined in membrane preparations as described in Materials and Methods. The values are reported as fmol/mg protein. The density of binding sites was not different between the three groups ($n=6$ for each group).

cantly different between the three groups ($F[2,15]=2.06$, $p<0.2$) (Figure 4). Although binding in the melancholic group was numerically higher than normal controls, this difference did not reach statistical significance.

Immunolabeling of PLC β_1 Isozyme and G $\alpha_{q/11}$ Protein

As an additional test of possible postreceptor alterations, we evaluated whether the low 5-HT-stimulated PI hydrolysis in fibroblasts from melancholics is due to altered expression of the PLC β_1 isozyme or G α_q protein. Fibroblasts membranes were isolated and proteins determined by Western blot analyses using β -actin as an internal control. The level of PLC β_1 isozyme and G α_q protein were not different in melancholic compared with normals and nonmelancholic patients ($n=9$ for normals, $n=7$ for nonmelancholics, and

$n=8$ for melancholics). These results confirm that the decreased 5-HT signal is not related to a reduction in level of two key downstream mediators, PLC β_1 isozyme and G $_q$ protein.

DISCUSSION

In the present study, the activity of the 5-HT-stimulated phospholipase C signaling pathway was investigated in fibroblasts from melancholic and nonmelancholic depressed patients and normal controls. Concentration response experiments revealed that PI hydrolysis was stimulated by 5-HT with a similar EC₅₀ in the three groups, suggesting that these responses were the result of 5-HT interaction with receptors exhibiting similar binding characteristics and a similar degree of receptor reserve. MDL-100907, a highly specific 5-HT_{2A} receptor antagonist, blocked 5-HT-stimulated PI hydrolysis, indicating that the effect was mediated via 5-HT_{2A} receptors. The maximum 5-HT-stimulated PI hydrolysis response was markedly reduced in fibroblasts from melancholic subjects *vs* normal controls; however, this difference was not found in fibroblasts from nonmelancholic subjects. The maximum response of two other G $_q$ -coupled receptors, bradykinin and LPA, was increased in melancholic subjects rather than decreased. The elevation of PI hydrolysis after activation by bradykinin and LPA is interesting in itself; the cause for this increase is unknown, and it needs to be explored further. However, the fact that the response via these two G $_q$ -coupled receptor agonists is increased rather than reduced indicates that the effect seen with the activation of the 5-HT_{2A} receptor is likely to be inherent in the receptor itself or its coupling to G $_q$ protein. Furthermore, measurement of the radiolabeled lipid fraction showed that there were no differences in the level of substrates or lipid labeling between normals and melancholics. Thus, we conclude that the decrease in 5-HT-mediated PI hydrolysis is specific to the 5-HT_{2A} receptor itself, rather than an alteration in the overall function of the cells. Since the blunting of the 5-HT_{2A} receptor signal was maintained in subcultured cells, this change is not state dependent (ie secondary to drug treatment, hormone levels, etc.), but seems to be related to an underlying pathophysiological state.

One possible explanation for the blunted 5-HT_{2A} receptor-mediated PI hydrolysis is a decrease in the density of receptors or other critical components of the 5-HT_{2A} receptor-linked signal transduction pathway. The specific binding of [¹²⁵I]LSD did not differ between the three groups, suggesting that the reduced PI hydrolysis in melancholic participants was not dependent merely on altered receptor number. However, these data are very preliminary and certainly not definitive. Owing to the technical limitation of very low receptor protein, only a single concentration of [¹²⁵I]LSD was used; it is possible, then, that a difference would be masked if the density of receptors was decreased and the affinity for the ligand increased in parallel. Future studies in tissue subtypes with higher 5-HT_{2A} protein concentration are needed in order to determine whether density or affinity are significantly changed.

PLC and G $_q$ proteins are crucial components of the PI signaling pathway, and abnormalities in these components have been described in mood disorders in the past (Pandey *et al*, 1999, 2002a,b). However, in the current study, PI hydrolysis elicited by bradykinin and LPA actually was increased, not diminished, in melancholic patients relative to the other groups, suggesting that the abnormality in 5-HT_{2A} receptor signaling was not related to the PLC-PI pathway *per se*. In addition, the levels of PLC β_1 and G $_q$ protein were not significantly decreased in fibroblasts from melancholic compared with normal and nonmelancholic subjects. Together, these results indicate that the abnormality in 5-HT-induced PI hydrolysis is directly related to the 5-HT_{2A} receptor or its coupling to G proteins, and not other aspects of the intracellular signaling cascade.

These results must be viewed with caution, since the sample size was relatively small and a peripheral tissue was used. Replication in a larger sample size, and confirmation in human brain of the basic finding of a specific defect in melancholic *vs* nonmelancholic and controls is needed. However, it is more difficult to examine mechanism in postmortem human brain. Given that the reduction in 5-HT_{2A} receptor signal was retained after many cell passages, a genetic alteration is likely, either in the receptor itself or key regulatory factors, such as multidomain scaffolding proteins. For example, recent evidence suggests that the 5-HT_{2A} receptor (Xia *et al*, 2003) and its close relative, the 5-HT_{2C} receptor (Parker *et al*, 2003), are regulated by interaction with a multifunctional PDZ domain protein. In addition, genetic studies have suggested that polymorphisms of the 5-HT_{2A} receptor are associated with mood disorders, but the results have been mixed (Zhang *et al*, 1997; Spurlock *et al*, 1998; Turecki *et al*, 1999; Du *et al*, 1999; Hrdina and Du, 2001; Crawford *et al*, 2000; Arias *et al*, 2001). Four coding single-nucleotide polymorphisms (SNPs) that alter the amino-acid sequence of the 5-HT_{2A} receptor have been identified, but have not yet been adequately evaluated in functional studies. An interesting possibility is that the blunted 5-HT_{2A} receptor signal could be the result of different SNPs that produce a common functional deficit.

The current findings of alterations in 5-HT_{2A} receptor response only in the melancholic subtype of depression may explain some of the earlier discrepancies in the literature. Melancholia is a subtype of depressive disorder that is characterized by specific clinical characteristics, especially so-called 'endogenous' features: markedly decreased sleep and appetite, psychomotor symptoms (eg motor retardation or agitation), loss of the capacity for pleasure and an absence of mood reactivity to stimuli that would be expected to elicit a reward response. As noted by Ressler and Nemeroff (2000), monoamines, including 5-HT, are critically involved in the modulation of neurobiologic systems that regulate these processes, and alterations in 5-HT function might be expected to contribute to these types of depressive symptoms. Further experiments of both the structure of the 5-HT_{2A} receptor itself, as well as its interaction with potential intracellular binding partners, are needed to determine the mechanism of the reduced 5-HT_{2A} receptor signal. Defining the mechanism of this abnormality of signal transduction could lead to new treatment strategies for melancholia.

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