Differential Effects of 5-HTTLPR Genotypes on the Behavioral and Neural Responses to Tryptophan Depletion in Patients With Major Depression and Controls

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Context: Tryptophan depletion (TD) is a model used to study the contribution of reduced serotonin transmission to the pathogenesis of major depressive disorder (MDD). Recent studies have not sufficiently addressed the relative contribution of a functional-length triallelic polymorphism in the promoter of the serotonin transporter, 5-HTTLPR, to the behavioral and neural responses to TD in individuals with remitted MDD (rMDD) and controls.

Objective: To determine the role of 5-HTTLPR on the behavioral and neural responses to TD in medication-free patients with rMDD and individually matched controls.

Design: Participants were stratified according to diagnosis and 5-HTTLPR genotypes and underwent TD on one test day and sham depletion on the other test day in a prospective, double-blind, randomized order.

Setting: Outpatient clinic.

Participants: Twenty-seven medication-free patients with rMDD (18 women and 9 men) and 26 controls (17 women and 9 men).

Interventions: Tryptophan depletion was induced by administration of capsules containing an amino acid mixture without tryptophan. Sham depletion used identical capsules containing lactose. Fludeoxyglucose F 18 positron emission tomography was performed 6 hours after TD. Magnetic resonance images were obtained for each participant.

Main Outcome Measures: Quantitative positron emission tomography of regional cerebral metabolic rates for glucose and measures of depression using the Hamilton Depression Rating Scale.

Results: Behavioral responses to TD are affected by 5-HTTLPR in patients with rMDD and controls. A direct effect of 5-HTTLPR on the regulation of regional cerebral metabolic rates for glucose was identified in patients with rMDD for the amygdala, hippocampus, and subgenual anterior cingulate cortex.

Conclusions: Variations in 5-HTTLPR modulate the sensitivity of patients with rMDD and controls to the behavioral effects of TD. In patients with rMDD, variations in triallelic 5-HTTLPR have a direct effect on regulation of regional cerebral metabolic rates for glucose in a corticolumbic circuit that has been implicated in rMDD.

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flow and rCMRGlu in these and other areas, including the amygdala and hippocampus, have also been described in medicated patients with rMDD during TD.4,5 and in patients with MDD during spontaneous episodes of MDD.6 Although there is growing consensus that this corticostriatal circuit is involved in rMDD, not all regions are reported in all studies, and there is considerable variability in the direction of cerebral blood flow and rCMRGlu changes.

On the basis of the evidence that serotonin plays an important role in the pathogenesis of rMDD, an obvious next step of investigation is to determine whether serotonin-related genes account for the robust differential neural and behavioral responses to TD in patients with rMDD and controls. One major focus of research has been the serotonin transporter (for a review see Owens and Nemeroff7) responsible for the reuptake of released serotonin from the synaptic cleft into nerve terminals,8 and it is the target of antidepressant drug therapy.9-11 A relatively common polymorphism, a 44–base pair (bp) deletion/insertion in the transcriptional control region approximately 1 kPa upstream of the transcription initiation site, designated 5-HTTLPR,13 has been studied intensively, and originally 2 variants, long (L) and short (S) allelic variants, were described. Recently, functional variants were identified in the L allele and designated as LLA and LLA;9 The LLA and S alleles have comparable levels of serotonin transporter expression, and both are lower than that of the LLA allele. This finding suggests that 5-HTTLPR is truly a triallelic functional polymorphism. This may explain some of the conflicting results in the literature about the functional significance of the biallelic polymorphism related to serotonin transporter expression14-18 or its role in the pathogenesis of MDD.19,20

In a previous study,21 an association between the biallelic 5-HTTLPR and the behavioral response to TD was reported. To date, the relative contribution of the triallelic 5-HTTLPR polymorphism to the behavioral responses to TD and to the regulation of rCMRGlu in the corticostriatal circuit that has been implicated in rMDD has not been studied. For the present study, we recruited unmedicated patients with rMDD to avoid the confounding effects of acute illness and current or recent antidepressant drug treatment. The present study sought to extend previous research by aiming to substantiate previous studies on the behavioral and neural effects of TD in patients with rMDD and controls and to examine the role of the triallelic 5-HTTLPR polymorphism to moderate the effects on TD in patients with rMDD and controls.

### METHODS

#### PARTICIPANTS

Participants were entered into the study after full explanation of the purpose and procedures of the study and after written consent had been obtained as approved by the National Institute of Mental Health institutional review board. The study used a double-blind, randomized, placebo-controlled design, and at least 6 days between each test day was established to avoid carryover effects. Participants first underwent history, physical examination, and DNA extraction from blood samples to determine the triallelic 5-HTTLPR polymorphism, and then they were called back for the TD brain imaging studies after the comparison groups were constructed (Table 1). The triallelic classification was then reclassified into a biallelic model by level of transporter expression as follows: LLA/LLA and S/S alleles were classified as S/S; LLA/LS and LLA/LC as L/S; and LLA/LC as L/L.13

Twenty-seven medication-free patients with rMDD (6 were African American, 1 was Asian, 2 were Hispanic, and 18 were white) who met DSM-IV, nonpatient version, criteria for MDD in full remission and had experienced at least 2 past major depressive episodes on the basis of the Structured Clinical Interview for DSM-IV and 26 controls (6 were African American and 20 were white) were included in the study (Table 1). Duration of the depressive illness and number of episodes were estimated from the Past History of MDD addendum to the Structured Clinical Interview for DSM-IV. Remission was defined as at least 6 months without an antidepressant agent and had 24-item Hamilton Depression Rating Scale (HDRS) scores in the nondepressed range (<8).24 Information about family history of mental illness (Axis I diagnoses) was obtained from the study participants for all first-degree relatives using the Family Interview for Genetic Studies.25 Controls had no personal or family (first-degree relatives) history of psychiatric disorders. No use of medication was allowed during the study. Treatment of the most recent episode of MDD included administration of selective sero-

### Table 1. Clinical and Demographic Characteristics of 27 Unmedicated Patients With rMDD and 26 Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients With rMDD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L/L (n=8)</td>
<td>L/S (n=12)</td>
</tr>
<tr>
<td>Sex, No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Age, mean (SEM), y</td>
<td>37.0 (4.3)</td>
<td>41.0 (1.9)</td>
</tr>
<tr>
<td>Age at onset, mean (SD), y</td>
<td>22.2 (6.6)</td>
<td>23.3 (6.1)</td>
</tr>
<tr>
<td>Previous episodes, mean (SD), No.</td>
<td>3.4 (3.2)</td>
<td>3.6 (2.6)</td>
</tr>
<tr>
<td>Remission time, mean (SD), mo</td>
<td>44.7 (63.4)</td>
<td>46.0 (39.9)</td>
</tr>
<tr>
<td>Unmedicated duration, mean (SD), mo</td>
<td>48.0 (70.3)</td>
<td>36.8 (32.5)</td>
</tr>
<tr>
<td>Family history of MDD in first-degree relatives, No.</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

Abbreviations: L, long allelic variant; L/L includes individuals with LLA/LLA alleles; L/S includes individuals with LLA/LS and LLA/LC alleles; MDD, major depressive disorder; NA, not applicable; rMDD, remitted MDD; S, short allelic variant; S/S includes individuals with S/S, LLA/LS, and LLA/LC alleles.
Oligonucleotide primers and dye-labeled probes were designed to optimize allele discrimination using a software program (Primer Express; Applied Biosystems Inc, Foster City, Calif). Genotyping was accomplished in 2 stages: stage 1 discriminated S vs L alleles and stage 2 discriminated L4 vs L5 alleles. We used 4 fluorogenic probes, 2 for each stage, labeled at the 5’ end with either FAM or VIC (Applied Biosystems Inc). The L amplicon was 182 bp and the S amplicon was 138 bp. For stage 1, the allele-discriminating probe was capable of hybridizing only once to the 43-bp L insertion, and an internal control probe hybridized to a sequence located in the same amplicon but specific to a divergent repeat found only once in the index menstrual cycle.

2-STAGE 5’ NUCLEASE GENOTYPING OF 5-HTTLPR, INCLUDING S, L4, AND L5 ALLELES

Polymerase chain reaction was performed in a 25-µL volume: 25 to 50 ng of DNA, 120 nmol of allele-discriminating probe, 60 nmol of internal control probe, polymerase chain reaction primers (200 nmol each), dimethylsulfoxide (4% by volume), and 1× MasterMix (Applied Biosystems Inc). Amplification conditions were 2 minutes at 50°C, 10 minutes at 95°C, and then 40 cycles at 90°C for 15 seconds and at 62.5°C for 90 seconds. Genotypes were generated using sequence detection system software (PRISM 7700; Applied Biosystems Inc). On each plate, previously sequenced standards were introduced: stage 1 standards were S/L, S/L, and L/L, and stage 2 standards were L4/L4, L4/L5, and L5/L5. Certain samples were analyzed using real-time amplification curves, which are helpful when DNA concentrations are not optimal; otherwise, allele-specific signals were measured after DNA amplification.

**ASSESSMENTS OF PLASMA TOTAL AND FREE TRYPTOPHAN CONCENTRATIONS**

Plasma total and free tryptophan levels were measured on each study day at baseline and then 5, 7, and 24 hours after intake of the capsules. Collected blood samples were immediately centrifuged and stored at −80°C. Before analysis, plasma was deproteinated for total tryptophan measurement or filtered for free tryptophan measurement before it was subjected to isocratic reversed-phase high-performance liquid chromatography with fluorimetric detection (Waters Corp; Milford, Mass) (excitation and emission wavelengths, 300 and 350 nm) as described previously.

**IMAGE ACQUISITION AND ANALYSIS**

Magnetic resonance images were obtained for each participant using a commercially available scanner (3.0T Signa; General Electric Medical Systems, Waukesha, Wis) and a 3-dimensional magnetization-prepared rapid-acquisition gradient-echo sequence (echo time, 2.98 milliseconds; repetition time, 7.5 milliseconds; inversion time, 725 milliseconds; and voxel size, 0.9 × 0.9 × 1.2 mm) to provide an anatomical framework for analysis, partial volume correction of the PET images, and morphologic characterization so that individuals with anatomical abnormalities could be excluded.

Because the biochemical and behavioral effects of TD peak 5 to 7 hours after administration of the amino acid mixture, fludeoxyglucose F 18 was infused approximately 6 hours after administration of the capsules containing either lactose or amino acids. The rCMRGlu was measured noninvasively by combining left ventricular chamber time activity curve data with venous blood samples to give the input function needed to calculate the metabolic rate. The left ventricular input function was obtained from dynamic PET of the heart, with venous blood samples obtained concurrently during imaging after injection of 4.5 mCi (166500 Bq) of fludeoxyglucose F 18. Image data from the heart were acquired using a GE Advance PET scanner (General Electric Medical Systems) in 2-dimensional mode for 35 minutes (10 × 30-second frames and 10 × 3-minute frames). This was followed by a 10-minute emission and an 8-minute transmission brain scan 45 minutes after tracer injection. Cardiac sections were reconstructed, and 5 left ventricular sections were identified for region of interest (ROI) placement. The 0- to 5-minute frames were averaged to allow location of the left ventricular blood pool, and the 25- to 35-minute frames allowed identification of myocardial fludeoxyglucose F 18 uptake. Circular ROIs 2 cm in diameter over the left ventricular blood pool were positioned on each of the difference images (left ventricular image blood pool minus myocardial fludeoxyglucose F 18 uptake) such that spillover from the myocardium was minimized. An average left ventricular time activity curve was obtained from the time activity curves derived from the ROI in each of the 3 sections. The time activity curve was extended in time to include the period of brain imaging by using venous blood sample values. The average of the venous blood samples
obtained at approximately 25, 30, 35, and 50 minutes and the average of the left ventricular concentration during the 25- to 35-minute period were divided. This ratio was then used to scale the 50-minute venous sample concentration, which was then appended to the left ventricular curve, completing the input function used to generate parametric images of rCMRGlu.56

The effects of TD on rCMRGlu were assessed using magnetic resonance imaging–based ROI analysis and image-processing software (MEDx; Medical Numerics Inc, Sterling, Va). Whole-brain fludeoxyglucose F 18 uptake was measured using a magnetic resonance imaging–based template. No significant main effects or interactions were found for whole-brain CMRGlu. The ROI selection was theory and data driven.

The ideal model would have included all regions shown in the accumulated literature, but the model was intentionally limited to regions that are known to share extensive, reciprocal anatomical connections with each other; that consistently have been shown in previous studies to be involved in the pathogenesis of MDD; and that have been identified in previous TD studies in patients with rMDD taking59,60 and not taking61 antidepressant drugs to be involved in the neural response to TD. The ROIs included the amygdala, hippocampus, orbitofrontal cortex, subgenual anterior cingulate, pregenual anterior cingulate, and ventral striatum. These regions were placed on each patient’s registered magnetic resonance image. A binary mask of the gray matter was then used to ensure that only gray matter pixels were included in the analysis. Regions were then transferred to the coregistered PET images, and the rCMRGlu was obtained for each ROI.

STATISTICAL ANALYSIS

A full-factorial repeated-measures multivariate analysis of variance was used to examine the whole-brain CMRGlu and rCMRGlu for 6 ROIs (amygdala, hippocampus, subgenual and pregenual anterior cingulate cortices, orbitofrontal cortex, and ventral striatum). The model included group (rMDD vs control), sex, and genotype (L/L, L/S, and S/S) as between-subject factors and treatment (TD vs SD) and brain side (left vs right) as within-subject factors. Multivariate tests were followed by separate analyses of variance for each ROI using the same factors as the multivariate analysis of variance.

Behavioral measures were examined by means of repeated-measures analysis of variance, where group and genotype were between-subject factors and treatment and time (baseline, 7 hours, and 24 hours) were within-subjects factors. The Shapiro-Wilks test was used to evaluate the normality of the distribution of each outcome variable, and the Mauchly test was used to examine sphericity. When problems with sphericity arose, the Greenhouse-Geisser adjustment was used. Significant interactions were followed by Bonferroni-corrected simple effects tests. Significance was interpreted at P<.05, 2-tailed, and corrected P values are reported. Data are reported as mean ± SEM.

RESULTS

BIOCHEMICAL EFFECTS

As predicted, TD lowered the plasma levels of total tryptophan (treatment × time interaction: F 2,237,15 =12.27; P<.001 and F 2,9,60.49 =5.79; P=.01, respectively) in patients with rMDD, irrespective of genotype. No sadness was evoked in control subjects during TD. An additional effect of the 5-HTTLPR genotype, however, was found for the anxiety response to TD (treatment × genotype interaction: F 2,47 =6.01; P=.005). Among patients with rMDD, anxiety increased in all 3 genotype groups during TD (S/S: t11 =8.27, P=.01; L/S: t11 =6.72, P<.001; and L/L: t11 =5.85, P<.001). In contrast, controls showed increased anxiety scores only in the S/S (t6 =2.32; P=.05) and L/S (t11 =3.36; P=.005) subgroups but not in the L/L subgroup (t6 =0.70; P=.91).

Behavioral effects of TD were transient, and all the patients who experienced depressive or anxiety symptoms during TD reported feeling back to baseline at the follow-up interview 24 hours after TD. There were no sex effects on any of the behavioral outcome measures.

EFFECTS OF TD ON rCMRGlu

Relative to SD, TD was associated with higher rCMRGlu in patients with rMDD compared with controls in the orbitofrontal cortex (F 2,47 =6.89; P=.01), the subgenual anterior cingulate (F 1,47 =4.93; P=.03), and the pregenual anterior cingulate (F 1,47 =3.98; P=.05). No significant differences were found in the other a priori–selected ROIs of the circuit.

BEHAVIORAL RESULTS

At baseline, no significant between-group (rMDD vs control) differences in HDRS total scores were found. Tryptophan depletion, but not SD, induced profound behavioral effects that differed significantly between groups. Patients with rMDD showed greater mood changes than controls 7 hours after ingestion of the amino acids (treatment × genotype × time: F 2,56,10 =26.85; P<.001).

There was an association between 5-HTTLPR genotypes and depressive response to TD in the rMDD and control samples (treatment × group × genotype: F 2,47 =3.48; P=.04). This association was accounted for by a nearly significant treatment × group × genotype × time interaction (F 2,56,10 =2.95; P=.05). Among patients with rMDD, TD induced significant increases in HDRS total scores at 7 hours for all the genotype groups (Figure 1A). Compared with baseline, increases in depression scores were more prominent in carriers of the L/L (t11 =9.17) and L/S (t11 =9.42) genotypes than in S/S carriers (t6 =4.46; P<.001 for all). Among controls, TD induced an increase in HDRS total scores from baseline to 7 hours after ingestion of the amino acid mixture in S/S (t11 =2.14; P=.11) and L/S (t11 =4.15; P<.001) carriers (Figure 1B).

In contrast, L/L carriers remained unaffected by TD.

The individual item analysis on the HDRS revealed that lowering of serotonin function induced sadness and anxiety (treatment × time × group interaction: F 2,22.37,15 =12.27; P<.001 and F 2,9,60.49 =5.79; P=.01, respectively) in patients with rMDD, irrespective of genotype. No sadness was evoked in control subjects during TD. An additional effect of the 5-HTTLPR genotype, however, was found for the anxiety response to TD (treatment × genotype × time interaction: F 2,47 =6.01; P=.005). Among patients with rMDD, anxiety increased in all 3 genotype groups during TD (S/S: t11 =8.27, P=.02; L/S: t11 =6.72, P<.001; and L/L: t11 =5.85, P<.001). In contrast, controls showed increased anxiety scores only in the S/S (t6 =2.32; P=.05) and L/S (t11 =3.36; P=.005) subgroups but not in the L/L subgroup (t6 =0.70; P=.91).

Behavioral effects of TD were transient, and all the patients who experienced depressive or anxiety symptoms during TD reported feeling back to baseline at the follow-up interview 24 hours after TD. There were no sex effects on any of the behavioral outcome measures.
In patients with rMDD, the triallelic 5-HTTLPR polymorphism exerted significant effects on the rCMRGlu responses to TD (Figure 2A). The treatment × group × genotype interaction was significant for the hippocampus (F2,47 =3.79; P =.03) and subgenual anterior cingulate (F2,47 =3.25; P =.048) and showed a trend for the amygdala (F2,47 =2.68; P =.08). A significant effect of brain side was found for the amygdala (F1,47 =15.21; P <.001). During TD, relative to SD, patients with rMDD who were L/S carriers showed decreased rCMRGlu in the left amygdala (t11=3.57; P <.001) but not in the right amygdala and in the hippocampus (t11=1.99; P =.05). Patients with rMDD who carried the L/L genotype showed increased rCMRGlu during TD relative to SD in the left amygdala (t7=3.40; P <.001) but not the right amygdala, the hippocampus (t7=2.52; P =.02), and the subgenual anterior cingulate cortex (t7=2.55; P =.01). Patients with rMDD who carried the S/S genotype showed decreased rCMRGlu during TD vs SD in the hippocampus (t6=2.05; P =.046).

In patients with rMDD, genotype did not affect the rCMRGlu response to depletion condition in the pregenual anterior cingulate cortex, the orbitofrontal cortex, or the ventral striatum. Tryptophan depletion did not induce any significant changes in rCMRGlu relative to SD in the controls for any genotype group (Figure 2B). Sex did not affect the outcome.

COMMENT

The results of the present study agree with those of previous studies3,10-12 showing that TD induces a transient return of depressive symptoms in patients with rMDD. Moreover, TD induces abnormal regulation of rCMRGlu in patients with rMDD but not in controls in areas of a corticolimbic circuit, suggesting a trait abnormality that is characteristic of MDD.3 In addition, we show differential effects of the triallelic 5-HTTLPR polymorphism on the behavioral and neural responses to TD in controls and patients with rMDD. We found that patients...
with rMDD who carry at least 1 copy of the $L_A$ allele show a transient return of depressive symptoms during TD. In contrast, control subjects who carry at least 1 copy of the $S$ or the $L_G$ allele, both of which are associated with lower serotonin transporter expression than $L_A$, show an increase in depression ratings during TD. Patients with rMDD showed a return of depression and anxiety symptoms, whereas in controls, we observed increased anxiety but no sadness during TD. Control subjects were capable of maintaining their mood in a nondepressed range and maintained brain activity in a way that did not significantly vary from baseline measures.

The present results provide novel evidence that the triallelic 5-HTTLPR polymorphism directly affects the regulation of rCMRGlu in the amygdala, hippocampus, and subgenual anterior cingulate in rMDD, whereas no such effect was found in controls. Patients with $L/L$ rMDD showed increased rCMRGlu during TD in the left amygdala, hippocampus, and subgenual anterior cingulate cortex. This suggests a common final pathway of TD-induced amygdaloid-hippocampal complex and subgenual anterior cingulate cortex hyperreactivity in $L/L$ patients in a circuit that plays an important role in MDD. Patients with $L/S$ rMDD who did not differ from $L/L$ carriers in their phenotypic response to TD showed decreased rCMRGlu in the left amygdala and hippocampus. This suggests that carriers of this genotype may represent a distinct and not necessarily intermediate group between $S/S$ and $L/L$ carriers with rMDD.

These partially unpredicted study results raise the question about the underlying biology that explains the robust differential effects of 5-HTTLPR genotypes on behavioral and neural responses to TD in patients with rMDD and controls. Given the central role of the serotonin transporter for regulation of the extracellular concentrations of serotonin, as well as evidence that the triallelic 5-HTTLPR polymorphism is functional in people, one can expect profound changes in serotonin homeostasis in carriers of different 5-HTTLPR genotypes. In addition, because serotonin dysfunction is an important pathophysiologic factor in MDD, it is reasonable to consider other regulatory components of serotonin transmission besides the serotonin transporter in a model to explain what is going on at the synaptic level. This provides an opportunity to generate models of interactions of multiple interactive components, including the serotonin transporter and presynaptic serotonin type 1A and serotonin type 1B receptors, all of which affect the regulation of synaptic availability of serotonin.

We propose that in healthy $S/S$ carriers, compensatory changes that occur in response to elevated synaptic concentrations of serotonin lead to decreased postsynaptic serotonin type 1A receptors that may mediate the anxiety responses during TD, similar to the consequences of reduced serotonin type 1A receptor expres-

Figure 2. Effects of the triallelic polymorphism in the promoter of the serotonin transporter, 5-HTTLPR, genotype on regional cerebral metabolic rates for glucose (rCMRGlu) in unmedicated patients with remitted major depressive disorder (rMDD) (A) and control subjects (B) during tryptophan depletion (TD) and sham depletion. The rCMRGlu data are presented as TD−sham depletion mean values. AC indicates anterior cingulate; $L$, long allelic variant; $S$, short allelic variant; *, $P<.001$; and †, $P<.05$. Error bars represent SEM. $L_A/S$, $L_A/L_G$, and $S/S$ were classified as $S/S$; $L_A/S$ and $L_A/L_G$ as $L/S$; and $L_A/L_A$ as $L/L$. 

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sion in MDD (Figure 3A). Reduction in presynaptic serotonin type 1A receptors permits firing of serotonin neurons despite high synaptic serotonin concentrations, which may prevent development of the more pronounced symptoms seen in patients with rMDD during TD. Further adjustment of serotonin release is mediated by serotonin type 1B receptors, which adjust the release amount according to the level of synaptic serotonin. Although unknown, we speculate that the serotonin type 1B receptor–mediated process of regulation of the amount of released serotonin into the synaptic cleft is normal and responsive to the amount of extracellular serotonin in healthy people. In contrast, L/L carriers have lower synaptic serotonin concentrations but elevated postsynaptic serotonin receptor expression relative to healthy S/S carriers (Figure 3B). This, and an increased serotonin type 1A autoreceptor–mediated inhibitory threshold, makes neuronal firing more likely and allows the system to quickly adjust to a state of short-term disrupted serotonin transmission. Low synaptic serotonin concentrations permit serotonin type 1B receptor–mediated enhanced serotonin release during TD. This maintains optimal functioning of critical circuits necessary for normal affective functioning and prevents symptom provocation. Important differences between depressed patients and controls that may account for the profound between-group differences in behavioral and neural responses to TD are the widespread reduction in postsynaptic serotonin type 1A receptors and reductions in serotonin transporter expression reported in patients with MDD. No association, however, was found between the triallelic 5-HTTLPR polymorphism and serotonin transporter expression in patients with MDD and controls. The serotonin type 1B receptor has also been implicated in the pathogenesis of MDD, which may result in loss of responsiveness to extracellular serotonin concentrations so that levels of serotonin released remain constant. We propose that patients with rMDD who carry the L/L genotype have lower postsynaptic serotonin type 1A receptors but increased presynaptic serotonin type 1A receptors, resulting in a decreased threshold that makes firing less likely (Figure 3C). Also, impaired serotonin type 1B receptor–mediated serotonin release makes these individuals more vulnerable to a transient state of serotonin deficiency during TD. We had predicted that S/S rMDD carriers with lower postsynaptic serotonin type 1A receptors and possibly also serotonin type 1B receptors but higher synaptic serotonin availability were particularly vulnerable to the effects of TD (Figure 3D). Instead, these individuals were capable of maintaining normal function of the corticolimbic circuit during TD, as suggested by the absence of changes in brain activity compared with SD, and did not show robust depressive symptom recurrence during TD. Animal data suggest that
Nevertheless, we cannot exclude the possibility that
we designed this study to determine the effects of
relative contributions of the many genes that have been
implicated in the pathogenesis of MDD. We designed this study to determine the effects of 5-HTTLPR variants on the behavioral and neural responses to TD in patients with rMDD and controls. Nevertheless, we cannot exclude the possibility that or gene-gene interactions may have affected the results. Animal models using behavioral phenotyping of mutant mice on multiple genetic backgrounds and simulated dynamic models of epistatic interactions among regulatory components of transmitter systems may guide in the design of future studies aimed at selecting candidate genes in humans that will allow us to characterize genetically driven variations in brain function and behavior in mood disorders and to identify vulnerable people before illness onset.51

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1. Charney DS, Manji HK. Life stress, genes, and depression: multiple pathways
lead to increased risk and new opportunities for intervention. Sci STKE. 2004;
2004:re5.

2. Neumeister A. Tryptophan depletion, serotonin, and depression: where do we

3. Neumeister A, Nugent AC, Waldeck T, Geraci M, Schwartz M, Bonne O, Bain EE,
Luckenbaugh DA, Herscovitch P, Charney DS, Drevets WC. Neural and behav-
ioral responses to tryptophan depletion in unmedicated patients with remitted
major depressive disorder and controls. Arch Gen Psychiatry. 2004:61:
765-773.

4. Smith KA, Morris JS, Friston KJ, Cowen PJ, Dolan RJ. Brain mechanisms asso-
ciated with depressed relapse and associated cognitive impairment following acute

5. Bremner JD, Innis RB, Ng CK, Saft LH, Salomon RM, Bronen RA, Duncan J,
Positron emission tomography measurement of cerebral metabolic correlates
of yohimbine administration in combat-related posttraumatic stress disorder. Arch

813-829.

7. Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depres-

8. Gainetdinov RR, Caron MG. Monoamine transporters: from genes to behavior.

visualization of serotonin transporters in the human brain during fluoxetine

Deecke L, Podreka I, Brucke T. 5-HT SPECT demonstrates blockade of SHT-

Occupancy of serotonin transporters by paroxetine and citalopram during treat-
158:1843-1849.

66:2621-2624.

13. Hu X, Zhu G, Lipsky R, Goldman D. HTTLPR allele expression is codominant,
correlating with gene effects on fMRI and SPECT imaging, intermediate pheno-

14. van Dyck CH, Malison RT, Staley JK, Jacobsen LM, Seibyl JP, Lullebe M, Bald-
win RM, Inns RB, Gelenther J. Central serotonin transporter availability mea-
sured with [(123)I]I-CIT SPECT in relation to serotonin transporter genotype. Am

15. Shioke K, Ichimiya T, Suhara T, Takanou A, Sudo Y, Yasuno F, Hirano M, Shino-
bara M, Kagami M, Okubo Y, Nankai M, Kamba S. No association between geno-
type of the promoter region of serotonin transporter gene and serotonin trans-

Tauscher J, Fuchs K, Sieghart W, Hornik K, Aschauer HN, Brucke T, Kasper S.
No evidence for in vivo regulation of midbrain serotonin transporter availability
50:8-12.

17. Mann JJ, Huang YY, Underwood MD, Kassir SA, Oppenheim S, Kelly TM, Dwork
AJ, Arango V. A serotonin transporter gene promoter polymorphism (5-
HTTLPR) and prefrontal cortical binding in major depression and suicide. Arch

Weinberger DR. A relationship between serotonin transporter genotype and in vivo
protein expression and alcohol neurotoxicity. Biol Psychiatry. 2000;47:
643-649.

RM, Vallada HP, Innis RB, Gelernter J. Central serotonin transporter availability
158:1843-1849.

G, Martin J, Craig I, Maves L, Potash JN, Rutter M, Poulton R. Influence of life stress on depression: mod-

Bendy B. Serotonin-2A-receptor and -transporter polymorphisms: lack of

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41. Knobelmann DA, Hen R, Lucki I. Genetic regulation of extracellular serotonin by 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) autoreceptors in different brain regions of the mouse. *J Pharmacol Exp Ther.* 2001;298:1083-1091.
48. de Groot L, Olivier B, Westenberg HG. Extracellular serotonin in the prefrontal cortex is limited through terminal 5-HT(1B) autoreceptors: a microdialysis study in knockout mice. *Psychopharmacology (Berl).* 2002;162:419-424.