

# A Susceptibility Gene for Affective Disorders and the Response of the Human Amygdala

Ahmad R. Hariri, PhD; Emily M. Drabant, BA; Karen E. Munoz, BA; Bhaskar S. Kolachana, PhD; Venkata S. Mattay, MD; Michael F. Egan, MD; Daniel R. Weinberger, MD

**Background:** A common regulatory variant (5-HTTLPR) in the human serotonin transporter gene (*SLC6A4*), resulting in altered transcription and transporter availability, has been associated with vulnerability for affective disorders, including anxiety and depression. A recent functional magnetic resonance imaging study suggested that this association may be mediated by 5-HTTLPR effects on the response bias of the human amygdala—a brain region critical for emotional and social behavior—to environmental threat.

**Objectives and Design:** To examine the effects of 5-HTTLPR genotype on the reactivity of the human amygdala to salient environmental cues with functional magnetic resonance imaging in a large (N=92) cohort of volunteers carefully screened for past and present medical or psychiatric illness, and to explore the effects of 5-HTTLPR genotype as well as amygdala reactivity on harm avoidance, a putative personality measure related to trait anxiety.

**Results:** We now confirm the finding of 5-HTTLPR short

allele-driven amygdala hyperreactivity in a large independent cohort of healthy subjects with no history of psychiatric illness or treatment. Furthermore, we demonstrate that these genotype effects on amygdala function are consistent with a dominant short allele effect and are equally prominent in men and women. However, neither 5-HTTLPR genotype, amygdala reactivity, nor genotype-driven variability in this reactivity was reflected in harm avoidance scores.

**Conclusions:** Our results reveal a potent modulatory effect of the 5-HTTLPR on amygdala reactivity to environmental threat. Since this genetically driven effect exists in healthy subjects, it does not, in and of itself, predict dimensions of mood or temperament. As such, the 5-HTTLPR may represent a classic susceptibility factor for affective disorders by biasing the functional reactivity of the human amygdala in the context of stressful life experiences and/or deficient cortical regulatory input.

*Arch Gen Psychiatry.* 2005;62:146-152

**M**OOD AND ANXIETY DISORDERS are serious medical conditions associated with high levels of morbidity and mortality across the life span. Even with effective treatment, these conditions often recur and result in enormous personal, family, and societal costs. Thus, substantial effort is being made to identify both biological and environmental mechanisms that contribute to the diathesis for these illnesses.<sup>1</sup>

The brain serotonin (5-hydroxytryptamine [5-HT]) system, which plays a critical role in the regulation of mood and temperament,<sup>2</sup> is a major focus of these efforts. Drugs that target serotonergic neurotransmission are efficacious for the treatment of a variety of these conditions, including depression, obsessive-compulsive disorder, anxiety, and panic.<sup>3</sup> Moreover, genetic variation in several key 5-HT subsystems, presumably resulting in altered central

serotonergic tone and neurotransmission, is associated with various aspects of mood and temperament as well as susceptibility to affective illness.<sup>4-6</sup>

The 5-HT transporter (5-HTT) plays an important role in serotonergic neurotransmission by facilitating reuptake of 5-HT from the synaptic cleft. A relatively common polymorphism (5-HTTLPR) in the promoter region of the human 5-HTT gene (*SLC6A4*) results in 2 common alleles or variants<sup>7</sup>: the so-called short (S) and long (L), comprising 14 and 16 copies of a 20 to 23 nucleotide repeat cassette, respectively. Both in vitro transfection<sup>8</sup> and in vivo imaging<sup>9</sup> studies have revealed that the 5-HTTLPR has functional effects at the level of 5-HT biology by regulating 5-HTT expression. Specifically, the S allele is associated with a nearly 50% reduction in 5-HTT availability, presumably resulting in relatively increased synaptic concentrations of 5-HT. This functional effect on 5-HT availability has encouraged clinical

**Author Affiliations:** Genes, Cognition and Psychosis Program, National Institute of Mental Health Intramural Research Program, National Institutes of Health, US Department of Health and Human Services, Bethesda, Md. Dr Hariri is currently affiliated with the Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

studies of association between 5-HTTLPR genotype and various anxiety- and depression-related phenotypes.

While there have been reports of a spectrum of behavioral and clinical associations with the less-efficient S allele, including higher levels of anxiety and risk for affective illness<sup>10</sup> as well as poorer response to selective serotonin reuptake inhibitor (SSRI)-type antidepressant drugs,<sup>11-13</sup> inconsistencies and negative reports have raised doubts about the clinical importance of this polymorphism in the risk for and expression of affective disorders.<sup>14</sup> Two recent reports, however, suggest that 5-HTTLPR genotype does play a role in mood and temperament but that the biological mechanism is complex and the behavioral effect dependent on environmental context.

In the first, Hariri et al<sup>15</sup> used a functional magnetic resonance imaging (fMRI) paradigm to measure the physiologic response of the human amygdala during the perceptual processing of fearful and threatening faces. This fMRI paradigm has been shown to be a reliable measure of the experience of potential danger in the environment, consistent with the role of the amygdala in fear conditioning in animals.<sup>16</sup> In this study, S allele carriers exhibited a significantly greater amygdala response than L allele homozygotes in 2 small independent cohorts of healthy volunteers. These results suggest that 5-HTTLPR S allele-driven variation in amygdala 5-HT signaling creates a bias toward a heightened brain response to environmental threat, but that this relative hyperresponsivity alone does not predict individual differences in mood and temperament.

In the second report, Caspi et al<sup>6</sup> found that the 5-HTTLPR S allele significantly increased risk for depressive symptoms, diagnosable depression, and suicidality in a large epidemiological cohort in New Zealand, but only in the context of stressful life events. In contrast, such stressful life events had no significant effect on these indices of mood in L allele homozygotes. Their results suggest that the 5-HTTLPR contributes to susceptibility for mood disorders, but that this effect is dependent on individual experience and exposure to environmental events, presumably because variation in the gene colors the brain's response to such experiences.

While the studies of Hariri et al<sup>15</sup> and Caspi et al<sup>6</sup> provide independent evidence for the importance of the 5-HTTLPR in the neural architecture of mood and temperament as well as the risk for developing affective disorders, a direct link between amygdala excitability and susceptibility to mood disorders has not been established. In this study, we sought to replicate our initial finding of 5-HTTLPR S allele-driven amygdala hyperreactivity in a large independent cohort of volunteers, as well as to explore the influence of both sex and S allele load on amygdala function and, in turn, dimensions of temperament associated with depression and anxiety.

## METHODS

### SUBJECTS

From September 2000 to July 2003, 163 subjects recruited as normal volunteers were scanned with blood oxygen level-dependent (BOLD) fMRI as part of a large ongoing study ex-

ploring the genetics of cognition and emotion. All subjects gave written informed consent and participated in the study according to the guidelines of the National Institute of Mental Health institutional review board. All subjects underwent extensive historical reviews and in-depth clinical interviews, including the Structured Clinical Interview for *DSM-III-R* for lifetime psychiatric diagnosis<sup>17,18</sup> and physical examination. Thirty-nine subjects were excluded for preexisting neurological, psychiatric, and/or substance abuse problems and history of other medical problems and/or treatment relevant to cerebral metabolism and blood flow; for screening details, see Egan et al.<sup>19</sup> To control for confounding effects of population substructure and ethnic heterogeneity, we limited our fMRI data analyses to 102 European American subjects from the larger sample of 124 healthy volunteers. There was minor overlap (n=19) between the current cohort of 92 subjects included in the final fMRI data analysis (which will be discussed) and those from our original study. This was done to maximize the samples of homozygote individuals.

### MOOD AND PERSONALITY ASSESSMENT

The harm avoidance (HA) subset of the Tridimensional Personality Questionnaire<sup>20</sup> was administered as a putative index of behaviors, such as fear and anxiety, related to amygdala function and influenced by serotonergic neurotransmission. Analysis of variance was used to identify genotype, sex, and genotype-by-sex effects on total HA scores as well as scores from 2 anxiety-related HA subscales, anticipatory worry (HA1) and fear of uncertainty (HA2), which have been previously associated with the 5-HTTLPR.<sup>21</sup> Harm avoidance scores were available in 83 of the 92 subjects included in the final BOLD fMRI analysis.

### AMYGDALA REACTIVITY TASK

During fMRI scanning, subjects completed a simple perceptual task involving the matching of fearful and angry facial expressions known to robustly engage the amygdala.<sup>22-24</sup> In this task, 2 blocks of an emotional task were interleaved with 3 blocks of a sensorimotor control task. During the emotional task, subjects viewed a trio of faces and selected the 1 of 2 faces (bottom) that was identical to the target face (top). Each emotional block consisted of 6 images, 3 of each sex and target affect (angry or afraid), all derived from a standard set of pictures of facial affect, presented sequentially for 5 seconds. During the sensorimotor control block, subjects viewed a trio of geometric shapes (circles and vertical and horizontal ellipses) and selected the 1 of 2 shapes (bottom) that matched the target shape (top). Each control block consisted of 6 different images presented sequentially for 5 seconds.

### fMRI ACQUISITION PARAMETERS

Each subject was scanned using a 3-T GE Signa scanner with a real-time functional imaging upgrade (Milwaukee, Wis). An automated shim procedure was applied to minimize possible magnetic field inhomogeneities. Blood oxygenation level-dependent functional images were acquired with a gradient echo-planar imaging sequence and covered 24 axial slices (4-mm thick, 1-mm gap) that began at the cerebral vertex and encompassed the entire cerebrum and the majority of the cerebellum (repetition time [TR], 2000 msec; echo time [TE], 28 msec; field of view [FOV], 24 cm; matrix, 64 × 64 pixels). These parameters were selected to optimize signal across the entire volume of acquisition, including the medial temporal lobes.

**Table. Sample Demographics and Behavioral Performance\***

| 5-HTTLPR Genotype                              | L Homozygotes   | S Carriers†     |
|--|-----------------|-----------------|
| No.  | 27              | 65              |
| Sex, M/F                                       | 13/14           | 32/33           |
| Age  | 30.04 ± 1.45    | 30.89 ± 1.08    |
| IQ   | 105.69 ± 1.53   | 106.35 ± 1.17   |
| Harm avoidance total score                     | 9.6 ± 1.14      | 10.22 ± 0.68    |
| Harm avoidance 1 score:<br>anticipatory worry  | 2.52 ± 0.38     | 2.71 ± 0.26     |
| Harm avoidance 2 score:<br>fear of uncertainty | 3.12 ± 0.34     | 3.47 ± 0.19     |
| Accuracy, % correct                            | 98.4 ± 0.66     | 97.27 ± 0.93    |
| Reaction time, msec                            | 1729.15 ± 84.39 | 1822.23 ± 56.01 |

\*Data are presented as mean ± SEM unless otherwise indicated.  
†No significant differences in any measures between L/S and S/S.

## fMRI DATA ANALYSIS

Blood oxygen level–dependent fMRI data analysis was completed using the general linear model of SPM99 (<http://www.fil.ion.ucl.ac.uk/spm>) and analysis of variance in SPSS software, version 11.5 (SPSS Inc, Chicago, Ill). Briefly, images for each subject were realigned to the first volume in the time series to correct for head motion, spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12-parameter affine model, and smoothed to minimize noise and residual differences in gyral anatomy with a Gaussian filter, set at 8-mm full width at half maximum. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Ten data sets were excluded from the sample of 102 European American subjects for image-related artifacts (eg, excessive head motion, signal loss/susceptibility in medial temporal lobes).

Predetermined condition effects for the entire functional brain volume were calculated using a *t* statistic, producing a statistical image for the contrast of the emotional task vs the sensorimotor control for each subject. These individual contrast images were then used in a second-level random-effects model, which accounts for both scan-to-scan and subject-to-subject variability, to identify significant ( $P < .05$ , corrected for multiple comparisons across all suprathreshold brain voxels) activations (percentage of BOLD signal change between emotional and control tasks) in the amygdala. Mean percentage of BOLD signal-change values were then extracted from these functionally defined amygdala clusters for each subject. Analysis of variance was then carried out on these single-subject values for both the left and right amygdala to identify genotype, sex, and genotype-by-sex effects. Multiple regression analyses were employed to examine the relationship between genotype, amygdala reactivity, and HA scores.

## GENOTYPING

DNA isolation and analysis were conducted on blood samples using standard procedures. Oligonucleotide primers flanking the 5-HTTLPR and corresponding to the nucleotide positions –1416 to –1397 (stpr5, 5′-GGCGTTGCCGCTCTGAATGC) and –910 to –888 (stpr3, 5′-GAGGGACTGAGCTGGACAAC-CAC) of the 5-HTT gene 5′-flanking regulatory region were used to generate 484–base pair and 528–base pair fragments. Polymerase chain reaction amplification and gel separation were accomplished using published methods.<sup>7</sup> The individuals in the 5-HTTLPR genotype groups were also genotyped with a panel of 100 unlinked single nucleotide polymorphism loci and

showed no significant variation in frequency at any of these single nucleotide polymorphisms, including several that have been associated with variation in cortical function (eg, catechol *O*-methyltransferase, brain-derived neurotrophic factor, and apolipoprotein E ε4) (available on request).

## RESULTS

### SAMPLE DEMOGRAPHICS

The 5-HTTLPR allele and genotype frequencies were consistent with expected values in European American populations and were in Hardy-Weinberg equilibrium (**Table**). Neither allele nor genotype frequencies differed between men and women. A 2-factor (5-HTTLPR genotype and sex) analysis of variance revealed no significant genotype, sex, or genotype-by-sex effects on IQ. This was true using either a 2-genotype (L/L and S carrier) or a 3-genotype (L/L, L/S, and S/S) classification scheme in either cohort. There were also no significant 5-HTTLPR genotype, sex, or genotype-by-sex effects on fMRI task performance.

### 5-HTTLPR EFFECT ON HARM AVOIDANCE

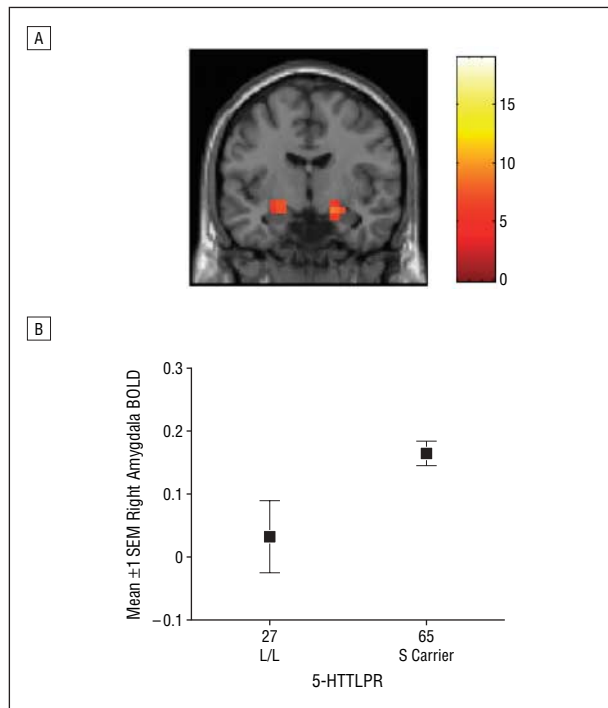
As expected,<sup>25</sup> there was a main effect of sex on total HA ( $F_{1,82} = 11.27$ ,  $P = .001$ ), with higher scores in women (mean ± SEM, 11.90 ± 0.70) than in men (mean ± SEM, 8.21 ± 0.85). While there was no sex difference in HA1, women (mean ± SEM, 4.00 ± 0.18) also exhibited significantly higher mean scores on HA2 ( $F_{1,82} = 16.73$ ,  $P < .001$ ) in comparison with men (mean ± SEM, 2.74 ± 0.25). However, using either a 2-genotype (L/L and S carrier) or a 3-genotype (L/L, L/S, and S/S) classification scheme, there were no significant genotype or genotype-by-sex effects on any HA scores (ie, total, HA1, or HA2).

### 5-HTTLPR EFFECT ON AMYGDALA REACTIVITY

Consistent with our previous work, perceptual processing of fearful and threatening facial expressions was associated with bilateral amygdala activity in all subjects (**Figure 1A**). Analysis of variance in the select sample of 92 European American subjects revealed that the activity of the right amygdala was significantly greater in S allele carriers in comparison with L allele homozygotes (**Figure 1B**). There was no significant effect of 5-HTTLPR genotype on left amygdala activity ( $F_{1,90} = 0.23$ ,  $P = .63$ ). Additional analyses indicated that the 5-HTTLPR effect on right amygdala activity was independent of both sex and S allele load (**Figure 2**). Similar effects were identified with post hoc comparisons in our entire sample of 124 subjects, including both European American and non-European American subjects.

### AMYGDALA REACTIVITY AND HARM AVOIDANCE

Exploratory analyses in 83 subjects with overlapping amygdala BOLD and HA data revealed no significant relationships between either left or right amygdala activity and total HA (**Figure 3**). There was also no signifi-

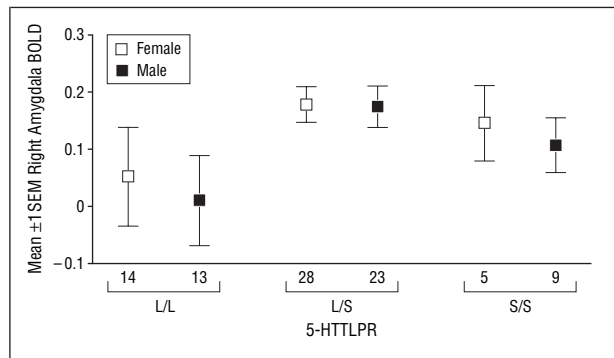


**Figure 1.** 5-HTTLPR effects on amygdala reactivity to environmental threat. A, Statistical parametric map of amygdala activation during the perceptual processing of fearful and threatening facial expressions. Activations are shown overlaid onto an averaged structural magnetic resonance image in the coronal plane passing through the amygdala. Color bar represents z scores for activations. Talairach coordinates and voxel level statistics ( $P < .05$ , corrected for multiple comparisons across all suprathreshold brain voxels) for the maximal voxel in the right and left amygdala are  $x = 18.8$  mm,  $y = -8.4$  mm,  $z = -12.2$  mm; cluster size, 11 voxels; z score = 7.80;  $P < .001$ ; and  $x = -18.8$  mm,  $y = -11.3$  mm,  $z = -12.1$  mm; cluster size, 7 voxels; z score, 6.62;  $P < .001$ , respectively. B, 5-HTTLPR effect on right amygdala activation. Line graphs represent the mean  $\pm$  SEM blood oxygen level-dependent (BOLD) signal change in the entire right amygdala functional cluster identified in part A. There was a main effect of 5-HTTLPR genotype on right amygdala activation, with S allele carriers having significantly greater activity than L allele homozygotes ( $F_{1,33} = 4.72$ ,  $P = .04$ ). As Levene test revealed that the variances between genotype groups was not equal ( $L/L > S$  carrier;  $F_{1,30} = 9.66$ ,  $P = .003$ ), we employed the Brown-Forsythe equality of means test, where variances are not assumed to be equal, to interpret the analysis of variance results.

cant genotype-by-BOLD interaction effect on total HA. Significant amygdala BOLD or genotype-by-BOLD interaction effects were also absent with respect to HA1 and HA2 subscales.

#### COMMENT

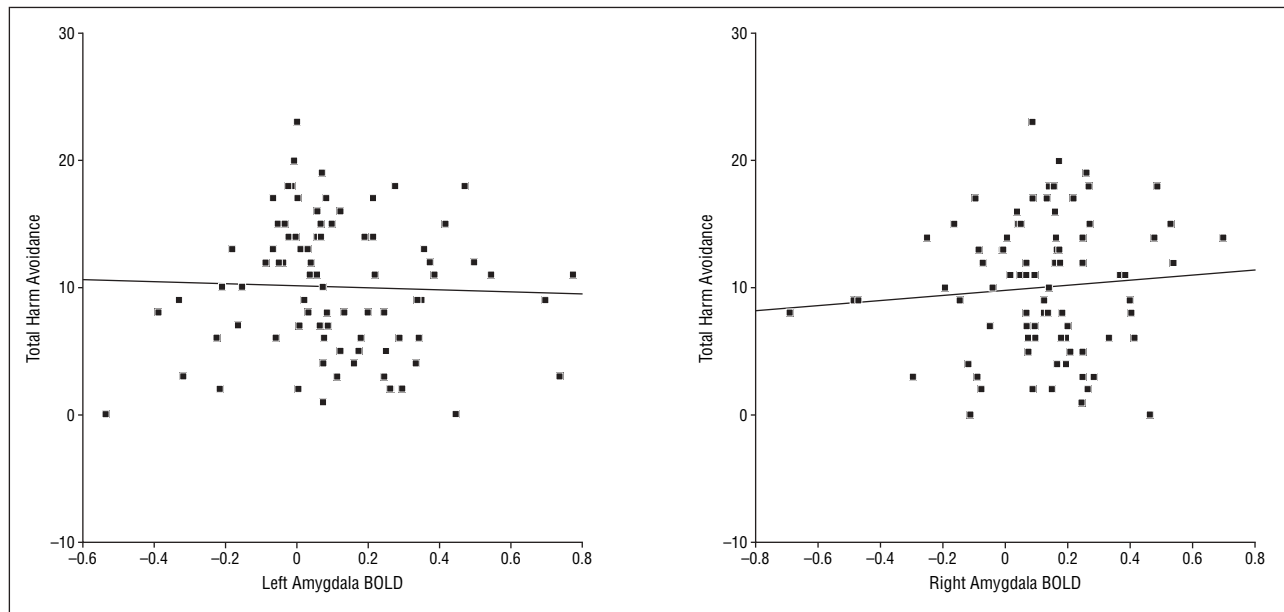
Our results reveal that the 5-HTTLPR S allele has a robust effect on human amygdala function independent of both sex and S allele load. Importantly, the absence of group differences in age, sex, IQ, and ethnicity indicates that our observed effects are not likely a reflection of systematic variation in such nongenotype factors. Rather, our data suggest that heritable variation in 5-HT signaling associated with the 5-HTTLPR results in relatively heightened amygdala responsivity to salient environmental cues. That these results emerged in a sample of ethnically matched normal volunteers carefully screened to exclude any lifetime history of psychiatric illness or treatment argues that they represent geneti-



**Figure 2.** Sex and S allele load effects on right amygdala activation. Line graphs represent the mean  $\pm$  SEM blood oxygen level-dependent (BOLD) signal change in the entire right amygdala functional cluster identified in Figure 1A as a function of both sex and S allele load. There was no main effect of sex ( $F_{1,90} = 0.26$ ,  $P = .61$ ) or a genotype-by-sex interaction ( $F_{2,86} = 0.08$ ,  $P = .92$ ) on amygdala activity. While there was a significant main effect (Brown-Forsythe  $F_{2,48} = 3.99$ ,  $P = .02$ ) of genotype when accounting for S allele load (ie, L/L, L/S, and S/S), post hoc comparisons indicated that there was no difference between L/S and S/S groups ( $P = .38$ ).

cally determined biological traits not related to manifest psychiatric illness. The fact that the groups did not differ in allele frequencies at 100 unlinked single nucleotide polymorphisms across the genome suggests that the groups did not differ in terms of genetic admixture.<sup>26</sup> However, our demonstration of an effect of the 5-HTTLPR S allele on amygdala response probably does not capture all the genetic variance associated with this gene. Recent studies have identified another potentially functional variant in the 5-HTTLPR that may further parse the S allele effect.<sup>27,28</sup> This is a minor additional genetic effect, however, that suggests some further refinement of the physiologic response based on 5-HTTLPR genotype may be possible. Of course, it is likely that many additional functional variants, both within and beyond the 5-HT system, will additively or epistatically affect amygdala physiology and further contribute to observed individual differences in reactivity.

While it is likely that constitutive variation in 5-HT signaling affects the biology of distributed brain systems beyond the amygdala, we have focused on the effects of the 5-HTTLPR on amygdala function because this region plays a central role in the generation of behavioral arousal and orientation as well as specific emotional states such as fear. Consistent with our previous study,<sup>15</sup> 5-HTTLPR S allele carriers exhibited significantly increased right amygdala activation in response to our fMRI challenge paradigm. Two recent functional imaging studies have reported similar S allele-driven amygdala hyperreactivity in both healthy volunteers<sup>29</sup> and patients with social phobia.<sup>30</sup> In addition, our current data reveal that 5-HTTLPR S allele-driven amygdala hyperresponsivity is equally pronounced in both sexes and independent of S allele load. The equivalent effect of 1 or 2 S alleles on amygdala function is consistent with the original observations of Lesch et al<sup>8</sup> on the influence of the 5-HTTLPR on in vitro gene transcription efficiency and subsequent 5-HTT availability. The absence of sex differences suggests that the increased prevalence of mood disorders in women may be related to factors other than the direct risk effect of the 5-HTTLPR S allele.



**Figure 3.** Relationship between amygdala reactivity and harm avoidance. Plots illustrate the absence of significant correlations between either left ( $r = -0.032$ ,  $P = .77$ ) or right ( $r = 0.086$ ,  $P = .44$ ) amygdala activity and total harm avoidance scores in a sample of 83 subjects with overlapping amygdala blood oxygen level-dependent (BOLD) and harm avoidance data.

Amygdala hyperreactivity to environmental threat in 5-HTTLPR S allele carriers, who presumably have relatively higher levels of synaptic 5-HT, may at first appear counterintuitive to the reported anxiolytic and antidepressant effects of SSRIs, which are associated with a net increase in 5-HT neurotransmission. However, recent studies have revealed that the clinical effects of SSRIs are complex, temporally graded, and dependent on alterations in several 5-HT subsystems. For example, Blier and de Montigny<sup>3</sup> have argued that the anxiolytic effects of SSRIs are mediated through long-term down-regulation of presynaptic 5-HT<sub>1A</sub> autoreceptors, resulting in normalization of 5-HT tone, and not simply through increased synaptic 5-HT resulting from 5-HTT blockade. Importantly, the SSRI-mediated 5-HT<sub>1A</sub> down-regulation occurs approximately 2 to 3 weeks after initiation of treatment, a time course parallel to that of the drugs' anxiolytic effects. Thus, constitutive variation in 5-HT availability affecting amygdala reactivity is unlikely to be directly related to the SSRI-mediated anxiolytic response. Moreover, it is conceivable that congenital increases in synaptic 5-HT may also translate into down-regulation of the postsynaptic signaling apparatus, rendering S allele carriers relatively desensitized to 5-HT. In fact, several studies have demonstrated that 5-HTTLPR S allele carriers respond poorly to SSRIs and/or require higher doses than L allele homozygotes,<sup>13</sup> further supporting the hypothesis that 5-HTT availability may dictate the relative effectiveness of SSRIs to increase synaptic 5-HT, leading to negative feedback on 5-HT<sub>1A</sub> autoreceptors, postsynaptic adaptations, and long-term therapeutic effect.

It is important to emphasize that the 5-HTTLPR S allele effect on amygdala function exists in a sample of healthy volunteers with no history of affective or other psychiatric disorders. This is consistent with a recent fMRI study reporting that while amygdala hyperexcitability re-

flects a stable, heritable trait associated with inhibited behavior, it does not by itself predict the development of affective disorders.<sup>31</sup> Two recent studies suggest that the existence of significant stressors in the environment of individuals carrying the 5-HTTLPR S allele may be necessary to further tip the balance toward the development of pathologic features and illness.<sup>6,32</sup> Similarly, abnormal social behavior<sup>33</sup> and 5-HT metabolism<sup>34</sup> have been reported in rhesus macaques with the 5-HTTLPR S allele homologue, but only in peer-reared and thus environmentally stressed individuals.

The presence, and perhaps even necessity, of such environmental stressors acting on an extended neural circuitry in facilitating 5-HTTLPR S allele influences on behavior is underscored by the absence of significant genotype or genotype-by-sex effects on HA as well as correlations between amygdala reactivity and HA in our healthy subjects. This suggests that 5-HTTLPR-driven variation in the responsiveness of the amygdala, while robust and consistent, does not uniformly result in altered mood and temperament, per se. However, the current study design limits our ability to fully explore the complex interplay of genes, brain, and behavior. For example, our selection of a unique cohort of volunteers rigorously screened for medical and psychiatric health may have reduced the phenotypic variability necessary for detection of genetically driven variance in behavioral traits likely to exist along a continuum. Moreover, 2 recent meta-analyses have indicated that the behavioral instrument used in our study (ie, Tridimensional Personality Questionnaire-HA) is not sensitive to the 5-HTTLPR, even in much larger samples, and that the neuroticism scale based on the 5-factor model of personality is more strongly associated with the 5-HTTLPR and possibly its effects on amygdala reactivity.<sup>35,36</sup> Finally, our current fMRI task is designed to elicit a maximal response from the amygdala (by comparing its response to biologically salient vs

nonsalient stimuli) and cannot discriminate between differential responses to specific classes of emotional stimuli (eg, angry, fearful, happy, and neutral faces). 5-HTTLPR effects on behavior may be more closely related to such differential amygdala reactivity to affect-specific stimuli previously linked with behavioral variance and psychiatric illness.<sup>37</sup> Future attempts to explore the links between the 5-HTTLPR, amygdala reactivity, and dimensional measures of mood and temperament will benefit from extending the phenotypic variability of the sample, employing more varied behavioral instruments and applying fMRI paradigms that allow for differential amygdala responses to specific classes of emotional stimuli.

Despite these limitations, our results suggest that individual differences in complex, emergent phenomena, such as harm avoidance, as well as disease vulnerability will likely reflect the effects of genetic variation acting in concert with specific environmental factors on a distributed brain system involved in not only mediating physiologic and behavioral arousal (eg, amygdala) but also regulating and integrating this arousal in the service of adaptive responses to environmental challenges (eg, prefrontal cortex).<sup>38-40</sup> For example, the experience of environmental insult before the maturation of relatively late-developing prefrontal regulatory circuits<sup>41</sup> may result in further biased amygdala drive in S allele carriers. Such relative hyperamygdala and hypoprefrontal activity has been documented in affective disorders<sup>42,43</sup> and thus may reflect a critical predictive biological marker.

Along these lines, 2 recent imaging genomics studies have reported increased prefrontal responsivity during the monitoring of performance errors<sup>44</sup> and increased functional coupling of the amygdala and prefrontal cortex during affect processing<sup>29</sup> in healthy S allele carriers. Thus, intact dynamic interactions of the amygdala and prefrontal cortex may be critical for normal behavioral responses in individuals possessing the 5-HTTLPR S allele. As the impact of genetically driven variation in dopamine availability (eg, catechol O-methyltransferase) on prefrontal function has been well documented,<sup>19,45</sup> it will be of increasing importance to model the influence of heritable variation in both amygdala and prefrontal activity in exploring the influence of genes on behavior. We have begun to examine the direct and interactive effects of several functional polymorphisms (eg, 5-HTTLPR and catechol O-methyltransferase) on the dynamics of the amygdala and prefrontal cortex during the generation, integration, and regulation of affect. Furthermore, we are now exploring the impact of early environmental stress on such genetically driven variation in brain function contributing to the etiology of mood and other affectively laden disorders in large longitudinal studies of children and adolescents.

**Submitted for Publication:** June 16, 2004; final revision received August 25, 2004; accepted September 9, 2004.

**Correspondence:** Daniel R. Weinberger, MD, Genes, Cognition and Psychosis Program, National Institute of Mental Health, National Institutes of Health, Room 4S-235, 10 Center Dr, Bethesda, MD 20892 (weinberd@intr.nimh.nih.gov).

1. Charney DS, Babich KS. Foundation for the NIMH strategic plan for mood disorders research. *Biol Psychiatry*. 2002;52:455-456.
2. Lucki I. The spectrum of behaviors influenced by serotonin. *Biol Psychiatry*. 1998;44:151-162.
3. Blier P, de Montigny C. Serotonin and drug-induced therapeutic responses in major depression, obsessive-compulsive and panic disorders. *Neuro-psychopharmacology*. 1999;21(suppl 2):91S-98S.
4. Murphy DL, Andrews AM, Wichems CH, Li Q, Tohda M, Greenberg B. Brain serotonin neurotransmission: an overview and update with an emphasis on serotonin subsystem heterogeneity, multiple receptors, interactions with other neurotransmitter systems, and consequent implications for understanding the actions of serotonergic drugs. *J Clin Psychiatry*. 1998;59(suppl 15):4-12.
5. Reif A, Lesch KP. Toward a molecular architecture of personality. *Behav Brain Res*. 2003;139:1-20.
6. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 2003;301:386-389.
7. Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP. Allelic variation of human serotonin transporter gene expression. *J Neurochem*. 1996;66:2621-2624.
8. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996;274:1527-1531.
9. Heinz A, Jones DW, Mazzanti C, Goldman D, Ragan P, Hommer D, Linnoila M, Weinberger DR. A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. *Biol Psychiatry*. 2000;47:643-649.
10. Lesch KP, Mossner R. Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? *Biol Psychiatry*. 1998;44:179-192.
11. Zanardi R, Serretti A, Rossini D, Franchini L, Cusin C, Lattuada E, Dotoli D, Smeraldi E. Factors affecting fluvoxamine antidepressant activity: influence of pindolol and 5-HTTLPR in delusional and nondelusional depression. *Biol Psychiatry*. 2001;50:323-330.
12. Yu YW, Tsai SJ, Chen TJ, Lin CH, Hong CJ. Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. *Mol Psychiatry*. 2002;7:1115-1119.
13. Lotrich FE, Pollock BG, Ferrell RE. Polymorphism of the serotonin transporter: implications for the use of selective serotonin reuptake inhibitors. *Am J Pharmacogenomics*. 2001;1:153-164.
14. Glatt CE, Freimer NB. Association analysis of candidate genes for neuropsychiatric disease: the perpetual campaign. *Trends Genet*. 2002;18:307-312.
15. Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR. Serotonin transporter genetic variation and the response of the human amygdala. *Science*. 2002;297:400-403.
16. LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci*. 2000;23:155-184.
17. Williams JB, Gibbon M, First MB, Spitzer RL, Davies M, Borus J, Howes MJ, Kane J, Pope HG Jr, Rounsaville B. The Structured Clinical Interview for DSM-III-R (SCID), II: multisite test-retest reliability. *Arch Gen Psychiatry*. 1992;49:630-636.
18. Spitzer RL, Williams JB, Gibbon M, First MB. The Structured Clinical Interview for DSM-III-R (SCID), I: history, rationale, and description. *Arch Gen Psychiatry*. 1992;49:624-629.
19. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A*. 2001;98:6917-6922.
20. Cloninger CR. A systematic method for clinical description and classification of personality variants: a proposal. *Arch Gen Psychiatry*. 1987;44:573-588.
21. Mazzanti CM, Lappalainen J, Long JC, Bengel D, Naukkarinen H, Eggert M, Virkkunen M, Linnoila M, Goldman D. Role of the serotonin transporter promoter polymorphism in anxiety-related traits. *Arch Gen Psychiatry*. 1998;55:936-940.
22. Hariri AR, Mattay VS, Tessitore A, Fera F, Smith WG, Weinberger DR. Dextroamphetamine modulates the response of the human amygdala. *Neuro-psychopharmacology*. 2002;27:1036-1040.
23. Hariri AR, Bookheimer SY, Mazziotta JC. Modulating emotional responses: effects of a neocortical network on the limbic system. *Neuroreport*. 2000;11:43-48.
24. Tessitore A, Hariri AR, Fera F, Smith WG, Chase TN, Hyde TM, Weinberger DR, Mattay VS. Dopamine modulates the response of the human amygdala: a study in Parkinson's disease. *J Neurosci*. 2002;22:9099-9103.

25. Cloninger CR, Svrakic DM, Przybeck TR. A psychobiological model of temperament and character. *Arch Gen Psychiatry*. 1993;50:975-990.
26. Pritchard JK, Donnelly P. Case-control studies of association in structured or admixed populations. *Theor Popul Biol*. 2001;60:227-237.
27. Sakai K, Nakamura M, Ueno S, Sano A, Sakai N, Shirai Y, Saito N. The silencer activity of the novel human serotonin transporter linked polymorphic regions. *Neurosci Lett*. 2002;327:13-16.
28. Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol Psychiatry*. 2000;5:32-38.
29. Heinz A, Smolka M, Braus D. Amygdala activation, prefrontal metabolism and the serotonin transporter. *Biol Psychiatry*. 2004;55(suppl 1):43.
30. Furmark T, Tillfors M, Garpenstrand H, Marteinsdottir I, Langstrom B, Orelund L, Fredrikson M. Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neurosci Lett*. 2004;362:189-192.
31. Schwartz CE, Wright CI, Shin LM, Kagan J, Rauch SL. Inhibited and uninhibited infants "grown up": adult amygdala response to novelty. *Science*. 2003;300:1952-1953.
32. Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, Plomin R, Craig IW. Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Mol Psychiatry*. 2004;9:908-915.
33. Champoux M, Bennett A, Shannon C, Higley JD, Lesch KP, Suomi SJ. Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Mol Psychiatry*. 2002;7:1058-1063.
34. Bennett AJ, Lesch KP, Heils A, Long JC, Lorenz JG, Shoaf SE, Champoux M, Suomi SJ, Linnoila MV, Higley JD. Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Mol Psychiatry*. 2002;7:118-122.
35. Sen S, Burmeister M, Ghosh D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *Am J Med Genet*. 2004;127B:85-89.
36. Schinka JA, Busch RM, Robichaux-Keene N. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Mol Psychiatry*. 2004;9:197-202.
37. Stein MB, Goldin PR, Sareen J, Zorrilla LT, Brown GG. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch Gen Psychiatry*. 2002;59:1027-1034.
38. Rosenkranz JA, Moore H, Grace AA. The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *J Neurosci*. 2003;23:11054-11064.
39. Hariri AR, Mattay VS, Tessitore A, Fera F, Weinberger DR. Neocortical modulation of the amygdala response to fearful stimuli. *Biol Psychiatry*. 2003;53:494-501.
40. Keightley ML, Winocur G, Graham SJ, Mayberg HS, Hevenor SJ, Grady CL. An fMRI study investigating cognitive modulation of brain regions associated with emotional processing of visual stimuli. *Neuropsychologia*. 2003;41:585-596.
41. Lewis DA. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. *Neuropsychopharmacology*. 1997;16:385-398.
42. Siegle GJ, Steinhauer SR, Thase ME, Stenger VA, Carter CS. Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. *Biol Psychiatry*. 2002;51:693-707.
43. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception, II: implications for major psychiatric disorders. *Biol Psychiatry*. 2003;54:515-528.
44. Fallgatter AJ, Herrmann MJ, Roemmler J, Ehliis AC, Wagener A, Heidrich A, Ortega G, Zeng Y, Lesch KP. Allelic variation of serotonin transporter function modulates the brain electrical response for error processing. *Neuropsychopharmacology*. 2004;29:1506-1511.
45. Mattay VS, Goldberg TE. Imaging genetic influences in human brain function. *Curr Opin Neurobiol*. 2004;14:239-247.