

# Effects of *HTR1A* C(-1019)G on Amygdala Reactivity and Trait Anxiety

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**Context:** Serotonin 1A (5-hydroxytryptamine 1A [5-HT<sub>1A</sub>]) autoreceptors mediate negative feedback inhibition of serotonergic neurons and play a critical role in regulating serotonin signaling involved in shaping the functional response of major forebrain targets, such as the amygdala, supporting complex behavioral processes. A common functional variation (C[-1019]G) in the human 5-HT<sub>1A</sub> gene (*HTR1A*) represents 1 potential source of such interindividual variability. Both in vitro and in vivo, -1019G blocks transcriptional repression, leading to increased autoreceptor expression. Thus, -1019G may contribute to relatively decreased serotonin signaling at postsynaptic forebrain target sites via increased negative feedback.

**Objectives:** To evaluate the effects of *HTR1A* C(-1019)G on amygdala reactivity and to use path analyses to explore the impact of *HTR1A*-mediated variability in amygdala reactivity on individual differences in trait anxiety. We hypothesized that -1019G, which potentially results in decreased serotonin signaling, would be associated with relatively decreased amygdala reactivity and related trait anxiety.

**Design:** Imaging genetics in participants from an archival database.

**Participants:** Eighty-nine healthy adults.

**Results:** Consistent with prior findings, -1019G was associated with significantly decreased threat-related amygdala reactivity. Importantly, this effect was independent of that associated with another common functional polymorphism that affects serotonin signaling, 5-HTTLPR. While there were no direct genotype effects on trait anxiety, *HTR1A* C(-1019)G indirectly predicted 9.2% of interindividual variability in trait anxiety through its effects on amygdala reactivity.

**Conclusions:** Our findings further implicate relatively increased serotonin signaling, associated with a genetic variation that mediates increased 5-HT<sub>1A</sub> autoreceptors, in driving amygdala reactivity and trait anxiety. Moreover, they provide empirical documentation of the basic premise that genetic variation indirectly affects emergent behavioral processes related to psychiatric disease risk by biasing the response of underlying neural circuitries.

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**T**HE AMYGDALA, THROUGH ITS extensive interactions with other limbic and cortical regions, plays a central role in the generation of emotional behaviors.<sup>1</sup> Moreover, abnormal amygdala function has been implicated in psychiatric disorders, such as depression and anxiety,<sup>2</sup> often characterized by abnormal emotional responses. Converging preclinical and clinical evidence indicates that amygdala functioning is sensitive to the effects of central serotonin,<sup>3</sup> whose principle forebrain innervation is provided by the midbrain dorsal raphe nucleus. Multiple mechanisms involving de novo biosynthesis, vesicular release, active reuptake, metabolic degradation, as well as both presynaptic and postsynaptic receptors contribute to the regulation of serotonin neurotransmission and its subsequent modulation of brain function.<sup>4</sup> In general, component processes that

affect the magnitude of signaling (eg, biosynthesis, reuptake, and autoregulation) rather than localized effects on target neurons (eg, postsynaptic receptors) represent key bottlenecks in serotonin regulation of neural circuit function. Crucial among these is activation of somatodendritic serotonin 1A (5-hydroxytryptamine 1A [5-HT<sub>1A</sub>]) autoreceptors, which mediate negative feedback on dorsal raphe nucleus neurons, resulting in decreased serotonin release at postsynaptic targets in the forebrain.<sup>5</sup> Using a multimodal neuroimaging strategy, we previously reported that the density of 5-HT<sub>1A</sub> autoreceptors accounts for 30% to 44% of variability in amygdala reactivity in healthy adults,<sup>6</sup> confirming the important role of 5-HT<sub>1A</sub> autoreceptors in modulating the activity of serotonergic target regions.

As a likely consequence of the impact of the 5-HT<sub>1A</sub> autoreceptor on serotonin release, variability in 5-HT<sub>1A</sub> autorecep-

tor function has been linked to personality traits and psychiatric illnesses.<sup>7-9</sup> It has also been suggested that 5-HT<sub>1A</sub> autoreceptors constitute a critical pharmacotherapeutic target. One such example is the desensitization of these receptors after chronic administration of selective serotonin reuptake inhibitors,<sup>10</sup> which may participate in correcting pathologically decreased serotonin neurotransmission<sup>11</sup> and altered amygdala reactivity observed in patients with major depression.<sup>12</sup> Given the critical role of 5-HT<sub>1A</sub> autoreceptors in regulating serotonin signaling and its resulting influence on the functioning of major brain targets, such as the amygdala, and complex behavioral processes, it is important to identify sources of emergent variability in 5-HT<sub>1A</sub> function.

A common sequence variation in the human 5-HT<sub>1A</sub> gene (*HTR1A* [OMIM 109760]) represents 1 potential source of such interindividual variability. Recently, a relatively common single nucleotide polymorphism in the promoter region of *HTR1A*, C(-1019)G, was demonstrated to affect transcriptional regulation of the gene through altered binding of the transcription factors human NUDR (nuclear DEAF-1-related protein)/DEAF-1 (deformed epidermal autoregulatory factor 1) and hairy/enhancer-of-split-5 (Hes5). Specifically, the -1019G allele abolishes repression of the promoter by NUDR/DEAF-1 and partially impairs Hes5-mediated repression and, as a consequence, is associated with increased *HTR1A* protein and binding.<sup>13</sup> Consistent with this finding, *in vivo* human positron emission tomography has revealed increased 5-HT<sub>1A</sub> autoreceptor density in both healthy adults and patients with depression carrying the -1019G allele.<sup>14</sup> However, a similar effect was not observed in an earlier positron emission tomography study.<sup>15</sup> Regardless, the *in vitro* effects of the *HTR1A* -1019G allele and the more general association documented between increased 5-HT<sub>1A</sub> autoreceptor density and decreased amygdala reactivity<sup>4</sup> suggest that this common functional genetic variation may contribute significantly to the emergence of interindividual variability in amygdala reactivity.

In the current study, we used imaging genetics, a strategy previously implemented to identify the neurobiological impact of common functional variation in other serotonin-related genes,<sup>16-21</sup> to evaluate the effects of the *HTR1A* C(-1019)G variant on amygdala reactivity in response to an archival functional magnetic resonance imaging (fMRI) challenge paradigm in 89 healthy adults. We hypothesized that compared with the *HTR1A* -1019C allele, the -1019G allele, which abolishes NUDR/DEAF-1 and impairs Hes5-mediated transcriptional repression leading to increased 5-HT<sub>1A</sub> autoreceptor density, would be associated with relatively lower amygdala reactivity, putatively reflecting increased negative feedback and consequently decreased serotonin signaling. In addition, because prior studies have linked individual differences in anxiety with amygdala function,<sup>22-25</sup> we used path analyses to explore the association between *HTR1A*-mediated variability in amygdala reactivity and individual differences in trait anxiety.

Our *a priori* focus on 5-HT<sub>1A</sub> autoreceptors and not postsynaptic 5-HT<sub>1A</sub> heteroreceptors is driven by 2 major findings. The first is our earlier discovery that 5-HT<sub>1A</sub> autoreceptors account for a greater proportion of variability in amygdala reactivity than local postsynaptic het-

eroreceptors.<sup>6</sup> The second is recent *in vitro* data that illustrate cell-specific effects of the -1019G allele on transcriptional repression. Specifically, the -1019G allele leads to consistently increased expression of 5-HT<sub>1A</sub> autoreceptors but does not consistently alter and sometimes even decreases expression of postsynaptic receptors.<sup>26</sup> This *in vitro* finding is supported by the *in vivo* study documenting increased density of autoreceptors but not postsynaptic cortical or limbic receptors in -1019G allele carriers.<sup>14</sup> Collectively, these results suggest that 5-HT<sub>1A</sub> autoreceptors, not heteroreceptors, account for most of the 5-HT<sub>1A</sub>-mediated variability in amygdala reactivity and that the -1019G allele may specifically affect the regulated expression of 5-HT<sub>1A</sub> autoreceptors.

## METHODS

### PARTICIPANTS

A total of 103 participants were recruited from the Adult Health and Behavior project, an archival database encompassing detailed measures of behavioral and biological traits among a community sample of 1379 nonpatient, middle-aged volunteers. Written informed consent according to the guidelines of the University of Pittsburgh's institutional review board was provided by all subjects before their participation in our neuroimaging subcomponent of the Adult Health and Behavior project. All participants included in our analyses were in good general health and free of the following: (1) medical diagnoses of cancer, stroke, diabetes requiring insulin treatment, chronic kidney or liver disease, or a lifetime history of psychotic symptoms; (2) use of psychotropic, glucocorticoid, or cardiovascular (eg, antihypertensive or antiarrhythmic) medication; (3) conditions that affect cerebral blood flow and metabolism (eg, hypertension); and (4) any current *DSM-IV* Axis I disorder as assessed by the nonpatient version of the Structured Clinical Interview for *DSM-IV*.<sup>27</sup>

Both the Adult Health and Behavior project and our smaller neuroimaging study have been developed for the explicit purpose of facilitating hypothesis-driven investigations of variables that possibly mediate interindividual variability in behavioral traits representing potential predictive markers of physical and mental health. In fact, combinations of neuroimaging, behavioral, and molecular genetics data from a number of our 103 participants (range, 31-89) have been used in several prior studies that examined biological pathways that mediate interindividual variability in behaviorally relevant brain function.<sup>28-33</sup> In the current study, overlapping *HTR1A* C(-1019)G genotype and threat-related amygdala reactivity data were available in 89 adults of European ancestry.

### GENOTYPING

High-molecular weight DNA was isolated from EDTA-anticoagulated whole blood samples obtained from all participants using a salting-out procedure. Each sample was genotyped using polymerase chain reaction amplification and fluorescence polarization primers. Primers were designed to produce a 272-base pair (bp) fragment containing the *HTR1A* C(-1019)G single-nucleotide polymorphism (rs6295; primers available from the corresponding author upon request). Polymerase chain reaction was carried out for 35 cycles at an annealing temperature of 55°C in a reaction mixture containing 1.5-mM of Mg<sup>++</sup>. Resulting products were cleaned by 1.5 hours of incubation with ExoSAP (USB Corporation, Cleveland, Ohio). Genotyping of the C→G transversion was performed using the LJL Ana-

lystHT (Molecular Devices, Sunnyvale, California). In addition to *HTRIA* C(-1019)G, 5-HTTLPR, MAOA 30-bp variable number of tandem repeats, and *TPH2* G(-844)T were all genotyped using published protocols.<sup>16,20,28</sup> All of these genotypes were scored by 2 independent readers using sequence-verified standards, and all call rates were greater than 95%. No additional polymorphisms in *HTRIA* were examined in our study.

We used the program STRUCTURE<sup>34</sup> to evaluate the presence of genetic substructure in the sample. Fifteen ancestry informative markers (rs1022106, rs1335995, rs1439564, rs1502812, rs1860300, rs548146, rs705388, rs715994, rs720517, rs722743, rs730899, rs734204, rs9059966, rs1328994, and rs1485405), which are unlikely to be related to phenotypes of interest, were genotyped for this analysis. We ran STRUCTURE, assuming a model with admixture and correlated allele frequencies, individual  $\alpha$  parameters, and independent  $F_{ST}$  for all subpopulations. We tested models with 1, 2, 3, and 4 subpopulations using a burn-in of 40 000 followed by 80 000 repetitions and compared the likelihoods of models fitting the data.

### TRAIT ANXIETY ASSESSMENT

The Spielberger State-Trait Anxiety Inventory (STAI) is a self-report scale indexing the frequency with which individuals perceive encountered situations to be threatening and respond to such situations with subjective feelings of apprehension and tension.<sup>35</sup> The STAI has been used extensively as a clinical and research instrument, including as an endophenotype in genetic association studies of candidate genes for neuropsychiatric disorders. This inventory consists of 2 scales, 1 assessing the general tendency to be anxious as a personality trait (STAI-Trait) and 1 measuring the degree of anxiety at a particular moment as a situation-dependent state (STAI-State). In this study, only the STAI-Trait version of the scale was administered, as trait scores better reflect dispositional anxiety.<sup>35</sup>

### AMYGDALA REACTIVITY PARADIGM

The experimental fMRI paradigm consisted of 4 blocks of a face-processing task interleaved with 5 blocks of a sensorimotor control task.<sup>28,31,32,36</sup> Participant performance (accuracy and reaction time) was monitored during all scans. During the face-processing task, participants viewed a trio of faces (expressing either anger or fear) and selected 1 of 2 faces (bottom) that was identical to a target face (top). Angry and fearful facial expressions can represent honest indicators of an ecologically valid threat, especially that related to conspecific challengers.<sup>37</sup> Within this context, we interpret the amygdala activation elicited by our task as being threat-related. Each face-processing block consisted of 6 images, balanced for sex and target affect (angry or fearful), all of which were derived from a standard set of pictures of facial affect.<sup>38</sup> During the sensorimotor control blocks, participants viewed a trio of simple geometric shapes (circles and vertical and horizontal ellipses) and selected 1 of 2 shapes (bottom) that were identical to a target shape (top). Each sensorimotor control block consisted of 6 different shape trios. All blocks were preceded by a brief instruction ("Match faces" or "Match shapes") that lasted 2 seconds. In the face-processing blocks, each of the 6 face trios was presented for 4 seconds with a variable interstimulus interval of 2 to 6 seconds (mean, 4 seconds), for a total block length of 48 seconds. In the sensorimotor control blocks, each of the 6 shape trios was presented for 4 seconds with a fixed interstimulus interval of 2 seconds, for a total block length of 36 seconds. Total task time was 390 seconds. As we were not interested in neural networks associated with face-specific processing per se, but rather in eliciting

a maximal amygdala response across all participants that we could then investigate for genotype effects, we chose not to use neutral faces as control stimuli because neutral faces can be subjectively experienced as affectively laden or ambiguous and thus engage the amygdala.<sup>22,39</sup>

### BOLD fMRI ACQUISITION PARAMETERS

Each participant underwent scanning with a Siemens 3-T MAGNETOM Allegra (Siemens AG, Erlangen, Germany), which was developed specifically for advanced brain imaging applications and is characterized by increased T2\* sensitivity and fast gradients (slew rate, 400 T/m/s), which minimize echo-spacing, thereby reducing echoplanar imaging geometric distortions and improving image quality. Blood oxygenation level-dependent (BOLD) functional images were acquired with a gradient-echo echoplanar imaging sequence (repetition time/echo time = 2000/25 milliseconds, field of view = 20 cm, matrix = 64 × 64), which covered 34 interleaved axial slices (3-mm slice thickness) aligned with the AC-PC plane and encompassing the entire cerebrum and most of the cerebellum. All scanning parameters were selected to optimize the quality of the BOLD signal while maintaining a sufficient number of slices to acquire whole-brain data. Before collecting fMRI data for each participant, we acquired a reference echoplanar imaging scan, which we visually inspected for artifacts (eg, ghosting) and good signal across the entire volume of acquisition, including the amygdala and ventral striatum. Additionally, an autoshimming procedure was conducted before the acquisition of BOLD data in each participant to minimize field inhomogeneities. The fMRI data from all 89 participants included in this study were cleared of such problems.

### IMAGE PROCESSING AND ANALYSIS

Whole-brain image analysis was completed using the general linear model of SPM2 (Wellcome Department of Imaging Neuroscience, London, England). Images for each participant 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 difference in gyral anatomy with a gaussian filter set at 6 mm full-width at half-maximum. Voxelwise signal intensities were ratio-normalized to the whole-brain global mean. These preprocessed data sets were analyzed using second-level random-effects models that accounted for both scan-to-scan and participant-to-participant variability to determine task-specific regional responses.

After preprocessing, linear contrasts using canonical hemodynamic response functions were used to estimate condition-specific (ie, faces > shapes) BOLD activation for each individual and scan. These individual contrast images (ie, weighted sum of the beta images) were then used in second-level random-effects models to determine (1) mean condition-specific amygdala reactivity using 1-sample *t* tests, (2) main effects of *HTRIA* genotype on amygdala reactivity, and (3) the association between amygdala reactivity and STAI-Trait score using multiple regression (with 5-HTTLPR genotype as a covariate).

Our amygdala region of interest was constructed using the Talairach Daemon option of the WFU PickAtlas Tool, version 1.04 (Wake Forest University School of Medicine, Winston-Salem, North Carolina). Exploratory analyses of genotype effects were conducted in prefrontal regions, namely, the orbitofrontal cortex (Brodmann area [BA] 11), ventrolateral prefrontal cortex (BA 47), and dorsolateral prefrontal cortex (BA 9/44/45), exhibiting a main effect of task. These regions

**Table. Demographic and Performance Variables as a Function of *HTR1A* Genotype**

Characteristic	Mean (SD) by <i>HTR1A</i> C(-1019)G Genotype			P Value
	C/C (n=25)	C/G (n=36)	G/G (n=28)	
Sex, M/F, No.	13/12	18/18	14/14	.99
Age, y	44.1 (7.02)	45.5 (6.00)	47.5 (6.45)	.33
History of a mood or anxiety disorder, No.	4	5	5	.54
Accuracy for faces, % correct	99.5 (1.96)	99.6 (1.28)	99.3 (3.03)	.89
Reaction time for faces, ms	1409 (332)	1319 (246)	1321 (247)	.39
STAI-Trait score	29.3 (7.54)	27.0 (7.56)	27.6 (5.09)	.10

Abbreviation: STAI-Trait, State-Trait Anxiety Inventory–Trait.

of interest were also defined using PickAtlas. A threshold at a voxel level of  $P < .05$  was used for all analyses; analyses were false discovery rate–corrected for multiple comparisons within an inclusive mask of activations of interest; and an extent threshold of at least 10 contiguous voxels was also used. Because of our a priori, directionally specific hypotheses and our use of a rigorous random-effects model, these statistical thresholds effectively control for false-positives arising from multiple comparisons. Moreover, these statistical thresholds have recently been demonstrated to effectively limit false-positive associations in imaging genetics studies below 5% (0.2%-4.1%) and are, in fact, conservative.<sup>40</sup> All neuroimaging data are reported using the coordinate system of Talairach and Tournoux.

#### ADDITIONAL DATA ANALYSES

A path model was used to examine the association between *HTR1A* genotype, amygdala reactivity, and trait anxiety. The cluster selected for the path analysis exhibited overlapping effects of genotype and trait anxiety and was identified by applying a mask created from the activation cluster correlated with trait anxiety to a subsequent regression analysis between amygdala reactivity and *HTR1A* genotype. This 2-step approach revealed a single activation cluster in the right amygdala exhibiting effects of both trait anxiety and genotype. Extracted activation values from the maximally activated voxel in the amygdala cluster showing overlapping effects of *HTR1A* genotype and STAI-Trait score were fitted using Mplus 4.0,<sup>41</sup> which can test indirect effects through 2 complementary methods.<sup>42,43</sup> First, Mplus 4.0 uses a product of coefficients test (also called the Sobel method<sup>42</sup>) to quantify the magnitude of the indirect effects with more power than some other widely used methods.<sup>44</sup> Second, Mplus 4.0 constructs unbiased confidence intervals using bootstrapping methods, which do not assume normality of the distribution of indirect effects and can represent more powerful tests in smaller samples such as ours.<sup>43</sup> While not the focus of our indirect effects analysis, path models generated in Mplus 4.0 can be tested for fit of the hypothesized model to the observed data. In our analyses, fit of a path model was considered acceptable if it had a nonsignificant  $\chi^2$  fit statistic, a root-mean-square error of approximation (RMSEA) smaller than 0.08, and a standardized root-mean-square residual (SRMR) close to 0.<sup>45</sup> Consistent with our general proposal that genetic effects on behavior are mediated through their effects on brain function,<sup>30,46</sup> we predicted that the link between *HTR1A* genotype and trait anxiety would be mediated through amygdala reactivity (ie, *HTR1A* genotype predicts amygdala reactivity which, in turn, predicts trait anxiety). However, to explore possible direct links between *HTR1A* genotype and trait anxiety, we also modeled a direct path between these 2 variables. As this direct path was nonsignificant and the overall model fit decreased, this direct path was dropped from the final model.

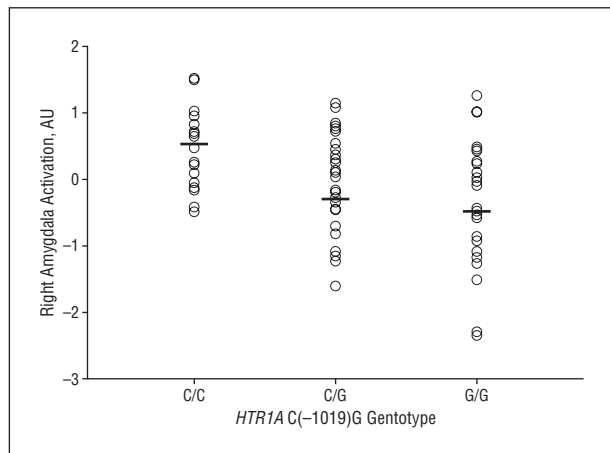
## RESULTS

### SAMPLE DEMOGRAPHICS

The distribution of our observed genotype frequencies (**Table**) from the total cohort of 89 participants ( $C/C = 25$ ,  $C/G = 36$ ,  $G/G = 28$  participants) was consistent with prior reports and did not deviate from Hardy-Weinberg equilibrium ( $\chi^2 = 0.38$ ,  $P = .83$ ). STRUCTURE analyses revealed no evidence of genetic substructure in our sample (log probability of data for  $k = 1$ ,  $k = 2$ ,  $k = 3$ , and  $k = 4$  subpopulations in STRUCTURE were  $-1292.6$ ,  $-1304.6$ ,  $-1287.6$ , and  $-1296.3$ , respectively) and thus, no further adjustments were made to control for type I or type II errors attributable to genetic stratification. Moreover, *HTR1A* genotype groups did not differ (all  $\chi^2$ 's  $< 4.47$ , all  $P$ 's  $> .11$ ) in the distribution of multiple additional functional serotonin-related polymorphisms (ie, 5-HTTLPR, MAOA 30-bp variable number of tandem repeats, and TPH2 G[-844]T) previously linked with variability in amygdala reactivity.<sup>16,20,28</sup> This was true for either the 2- ( $C/C$  vs  $G$  carriers) or 3- ( $C/C$ ,  $C/G$ , and  $G/G$ ) genotype group classification schemes. Finally, genotype groups (using either the 2- or 3-genotype classification schemes) did not differ with respect to age, sex distribution, history of mood or anxiety disorders, task performance, or STAI-Trait score, which was normally distributed in our sample (Table). Although the distribution of 5-HTTLPR across *HTR1A* genotype groups was random, we nevertheless entered 5-HTTLPR genotype as a covariate in our neuroimaging data analysis because of its well-documented effect on amygdala reactivity,<sup>47</sup> which may be mediated through altered 5-HT<sub>1A</sub> autoreceptor density.<sup>15</sup>

### *HTR1A* C(-1019)G EFFECTS ON AMYGDALA REACTIVITY

The main effects of task contrast (faces  $>$  shapes) were associated with significant bilateral amygdala reactivity across all participants. Regression analyses, corrected for effects of 5-HTTLPR, revealed a significant effect of *HTR1A* genotype on bilateral amygdala reactivity (**Figure 1**). This pattern was confirmed using analysis of covariance on the extracted maximal voxel amygdala activation values (right hemisphere:  $F_{2,86} = 3.66$ ,  $P = .03$ ; left hemi-

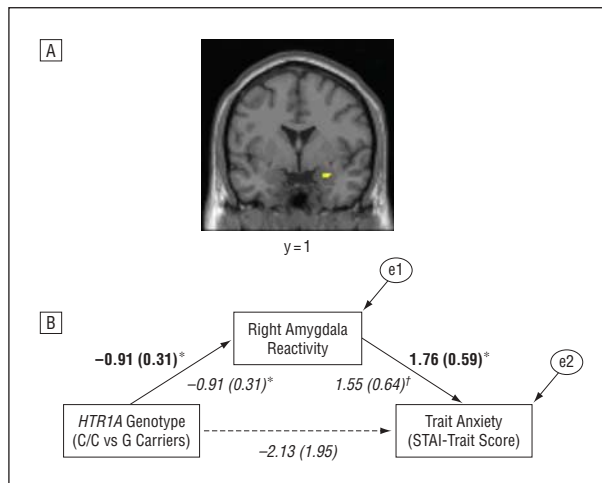


**Figure 1.** Single-subject mean ( $\pm$  standard error of the mean) activation values from the maximal voxel in a right amygdala cluster exhibiting an allele-load independent decrease in reactivity associated with *HTR1A* -1019G ( $x=20$  mm,  $y=-3$  mm,  $z=-20$  mm; cluster size=11 voxels;  $z=2.59$ ,  $P<.05$ , false discovery rate (FDR)-corrected). Nearly identical effects were identified in a left amygdala cluster ( $x=-22$  mm,  $y=-5$  mm,  $z=-20$  mm; cluster size=20 voxels;  $z=2.39$ ,  $P<.05$ , FDR-corrected). AU indicates arbitrary units.

sphere:  $F_{2,86}=3.21$ ,  $P=.045$ ). Post hoc analyses revealed significant differences between individuals with the C/C genotype and those with either C/G (right hemisphere:  $t_{59}=2.11$ ,  $P=.04$ ; left hemisphere:  $t_{57}=2.06$ ,  $P=.045$ ) or G/G (right hemisphere:  $t_{47}=2.59$ ,  $P=.01$ ; left hemisphere:  $t_{51}=2.45$ ,  $P=.02$ ). There was no significant difference between C/G and G/G genotypes (right hemisphere:  $t_{62}=0.95$ ,  $P=.35$ ; left hemisphere:  $t_{62}=0.73$ ,  $P=.47$ ). As these results indicated that effects of C(-1019)G on amygdala reactivity were independent of -1019G allele load, all subsequent analyses were conducted using a simplified 2-genotype classification scheme (C/C homozygotes vs G carriers). Statistical parametric analyses using this 2-genotype classification confirmed the results of the analysis of covariance by identifying significant differences between C/C homozygotes and G carriers (right hemisphere: 36 voxels,  $z=2.86$ ,  $P<.05$ ; left hemisphere: 27 voxels,  $z=2.58$ ,  $P<.05$ ). Finally, exploratory analyses of *HTR1A* genotype effects on task-related activation in prefrontal regions of interest did not reveal any statistically significant effects.

#### INDIRECT PREDICTION OF TRAIT ANXIETY THROUGH AMYGDALA REACTIVITY BY *HTR1A* C(-1019)G

Mean single-subject activation values from the maximal voxel in the right amygdala cluster exhibiting a correlation with both *HTR1A* genotype and STAI-Trait score (**Figure 2A**) were extracted for use in our path models. Analyses in Mplus 4.0 using these extracted values revealed no significant direct path between *HTR1A* genotype and STAI-Trait score in the model ( $B=-2.13$ ,  $SE=1.95$ ,  $P>.25$ ) and thus this path was dropped. In contrast, analyses in Mplus 4.0 revealed significant direct paths from *HTR1A* genotype to amygdala reactivity ( $B=0.91$ ,  $SE=0.31$ ,  $P<.01$ ) and from amygdala reactivity to STAI-Trait score ( $B=1.76$ ,  $SE=0.59$ ,  $P<.01$ ) (Figure 2B). Moreover, the in-



**Figure 2.** A, Statistical parametric map illustrating the right amygdala cluster correlated with both *HTR1A* genotype and State-Trait Anxiety Inventory-Trait (STAI-Trait) scores (cluster size=13 voxels;  $z=2.86$ ,  $P=.02$ , false discovery rate-corrected). Single-subject activation values from the maximal voxel in this cluster ( $x=26$ ,  $y=1$ ,  $z=-15$ ) were entered into our path analyses. B, Path model showing indirect effects of *HTR1A* genotype on trait anxiety (STAI-Trait). Lines are labeled with unstandardized path coefficients and SEs in parentheses. Coefficients in bold represent values from the trimmed model, while italicized coefficients represent values from the full model with all paths included. Indirect effects of *HTR1A* genotype on trait anxiety were significant ( $\alpha\beta=-1.60$ ,  $SE=0.73$ ,  $P<.05$ ), while direct effects were nonsignificant and dropped from the model. e1 and e2 represent the residual variance not explained by model variables. \* $P<.01$ , † $P<.05$ .

direct path from *HTR1A* genotype to STAI-Trait score through amygdala reactivity was significant ( $\alpha\beta=-1.60$ ,  $SE=0.73$ ,  $P<.05$ ). This model accounted for 9.2% of the variability in STAI-Trait scores, indicating that relatively decreased amygdala reactivity contributes to decreased trait anxiety in -1019G carriers and that the effect of *HTR1A* genotype on trait anxiety is through its effect on amygdala reactivity. The bootstrapped confidence interval for this estimate did not contain 0, further indicating a significant indirect effect. The proposed model also had an acceptable fit ( $\chi^2=1.35$ ,  $ns$ ,  $RMSEA=0.06$ ,  $SRMR=0.05$ ). In addition, the results were consistent across different models and extraction methods. The indirect effect was significant in the model containing the direct path from *HTR1A* genotype to STAI-Trait score ( $\alpha\beta=-1.41$ ,  $SE=0.70$ ,  $P<.05$ ), as well as when using a model containing the mean value extracted from the entire activation cluster rather than maximal voxel ( $\alpha\beta=-1.33$ ,  $SE=0.66$ ,  $P<.05$ ).

#### COMMENT

Consistent with our hypothesis, the *HTR1A* -1019G allele was associated with significantly decreased threat-related amygdala reactivity. This effect was independent of -1019G allele load, with both C/G and G/G genotypes exhibiting significantly reduced amygdala reactivity compared with C/C homozygotes as well as occult genetic stratification and other functional serotonin polymorphisms that affect amygdala reactivity, most notably 5-HTTLPR.<sup>16,20,28</sup> Path models revealed no significant direct genotype effect on trait anxiety. The marginal nature of this association ( $P>.25$ ) is consistent with previous studies in relatively small samples, which are

likely insufficiently powered to detect direct effects between genotype and distal behavioral phenotypes. In contrast, *HTR1A* C(-1019)G and amygdala reactivity indirectly predicted a significant proportion (9.2%) of individual differences in trait anxiety through their respective indirect and direct paths.

Our observation of decreased amygdala reactivity in carriers of -1019G is specifically consistent with the *in vitro*<sup>13</sup> and *in vivo*<sup>14</sup> effects of this allele (ie, increased 5-HT<sub>1A</sub> autoreceptor expression associated with -1019G) and with our previous study demonstrating an inverse association between 5-HT<sub>1A</sub> autoreceptor density and amygdala reactivity.<sup>6</sup> This pattern is more generally consistent with that reported for other common functional polymorphisms, namely the 5-HTTLPR short allele<sup>16,47</sup> and MAOA low-activity alleles,<sup>20</sup> which are also associated with relatively increased serotonin signaling. Collectively, these findings further implicate relatively increased serotonin signaling, regardless of the putative molecular mechanism, in driving amygdala reactivity and related behavioral processes, such as anxiety.<sup>3</sup> Not only does this parallel the effects of increased serotonin in animal models,<sup>48-52</sup> but also the findings of a recent study demonstrating that acute blockade of serotonin reuptake with intravenous citalopram results in dose-dependent potentiation of human amygdala reactivity.<sup>53</sup>

Although this convergent data strongly implicate serotonin in driving amygdala reactivity, the detailed molecular mechanisms through which such effects are mediated are not fully understood. This effect likely reflects the complex coexpression of inhibitory and excitatory postsynaptic serotonin-receptor subtypes on both glutamatergic projection neurons and GABAergic interneurons of the amygdala.<sup>54</sup> For example, serotonin-induced inhibition of glutamatergic activity in the lateral amygdala, which processes afferent sensory information, may be mediated through activation of excitatory serotonergic receptors on interneurons.<sup>55</sup> However, agonism of excitatory 5-HT<sub>2A/2C</sub> and 5-HT<sub>3</sub> postsynaptic receptors can increase the activity of both projection neurons and interneurons, and agonism of 5-HT<sub>1A</sub> postsynaptic receptors can decrease activity of interneurons.<sup>56</sup> Furthermore, while excitatory postsynaptic 5-HT<sub>2A/2C</sub> receptors have been localized to both projection and interneurons<sup>57</sup> and thus can both increase and decrease amygdala activity, a recent study suggests that 5-HT<sub>2A/2C</sub> receptors mediate the potentiation of amygdala-related conditioned fear responses following acute serotonin reuptake inhibition.<sup>48</sup> The synaptic localization of serotonin receptors may also bias the net effect of serotonin on amygdala reactivity. In other forebrain target regions, inhibitory 5-HT<sub>1A</sub> receptors are localized within the synapse while excitatory 5-HT<sub>2A/2C</sub> receptors are extrasynaptic.<sup>5</sup> Thus, a greater level of serotonin release (ie, volume transmission) may be necessary to evoke stimulation of these targets while a lesser level evokes inhibition. It is possible that the decreased serotonin release associated with -1019G is biased toward greater inhibition of amygdala target neurons (via preferential stimulation of synaptic 5-HT<sub>1A</sub>), reflected as decreased reactivity in BOLD fMRI. However, this putative mechanism is dependent on the appropriate expression of serotonin receptor subtypes, which remains largely unknown. Finally, although *in vivo* assays of 5-HT<sub>1A</sub> autoreceptor density indicate a functional effect of -1019G,<sup>14</sup>

our observed differences in amygdala reactivity may reflect early neurodevelopmental phenomena associated with altered serotonin signaling.<sup>58</sup> In fact, only transgenic inactivation of the murine 5-HT<sub>1A</sub> gene during early development and not adulthood is associated with altered anxiety-like behaviors.<sup>59</sup>

Despite their consistency and convergence with those from *in vitro* and *in vivo* assays of -1019G effects on 5-HT<sub>1A</sub> autoreceptors, our current results differ from 2 studies examining the effects of *HTR1A* C(-1019)G on amygdala reactivity in patients with major depression<sup>21</sup> and panic disorder.<sup>60</sup> The -1019G allele, which is associated with relatively decreased amygdala reactivity in our sample of healthy adults, was associated with relatively increased amygdala reactivity in both patient populations. In the latter patient sample, however, this effect was limited to the left amygdala's responses to happy expressions; there was no difference in amygdala activation to fearful expressions. In addition, -1019G was associated with relatively decreased prefrontal activation to fearful expressions in these same patients. In contrast, we did not find a significant effect of *HTR1A* genotype on task-related prefrontal activation. The presence or absence of psychopathology across these samples represents an obvious potential factor driving these differing patterns. The findings in the much smaller samples of patients may reflect an interaction of *HTR1A* genotype with ongoing pathologic processes as well as other genetic and/or environmental factors that act in concert to produce psychopathology.<sup>61</sup> The divergent effects reported in patients may also reflect additional variability in serotonin signaling following chronic exposure to psychotropic medications, especially selective serotonin reuptake inhibitors. In these studies, all patients with major depression<sup>21</sup> and half the patients with a panic disorder<sup>60</sup> were treated with selective serotonin reuptake inhibitors. Prospective studies in at-risk populations as well as in medication-naïve patients at pretreatment and posttreatment are necessary to better characterize the association between the *HTR1A* C(-1019)G genotype, amygdala reactivity, the emergence of psychopathology, and therapeutic response.

Superficially, our current findings may appear contrary to reports that link -1019G with increased risk for mood and anxiety disorders<sup>62</sup> as well as increased neuroticism and harm avoidance,<sup>63</sup> all of which may be characterized by increased amygdala reactivity.<sup>2,23,64,65</sup> As emphasized in a recent review, available association studies between -1019G and psychiatric liability are far from unequivocal, and studies to date have been generally underpowered.<sup>66</sup> Furthermore, in contrast to the effects of -1019G on autoreceptor expression, several studies have documented decreased 5-HT<sub>1A</sub> autoreceptors in a range of mood and anxiety disorders.<sup>67-72</sup> Regardless, our existing data reflect only 1 factor involved in shaping both normal and pathologic emotional responses to the environment, namely limbic drive in the form of amygdala reactivity. We did not observe significant genotype effects on task-related prefrontal activation. However, the ability to examine neurobehavioral effects of serotonin signaling using BOLD fMRI is critically dependent on the challenge paradigms used. Our paradigm is focused on threat-related amygdala and

extended corticolimbic reactivity associated with bottom-up limbic drive. It is possible that more complex, top-down (eg, emotion regulation) tasks may reveal effects of the -1019G allele extending to alterations in prefrontal regulatory circuitries whose dysfunction greatly contributes to and may characterize disorders of mood and emotion.<sup>73,74</sup> Given the importance of serotonin in the development and function of corticolimbic circuitries,<sup>58</sup> it is reasonable to speculate that decreased serotonin signaling associated with the -1019G allele also reduces prefrontal activation in response to amygdala drive (possibly via decreased stimulation of excitatory postsynaptic 5-HT<sub>2A</sub> receptors located on glutamatergic pyramidal neurons). This, in turn, may lead to insufficient regulation of the amygdala and the emergence of pathologic mood and emotion.

We believe that there is clearly a place for an alternative, neurobiologically informed view in the literature. In this regard, our current findings, which are remarkably consistent with the basic biology of serotonin as well as the C(-1019)G, provide an important mechanistic platform from which existing findings can be better appreciated and future, directionally specific hypothesis-driven association studies can be planned. Indeed, such staging has proved essential for advancing our understanding of many other genetic variants (eg, COMT val158met, BDNF val66met, 5-HTTLPR). Our simple, reliable, and robust paradigm has produced findings that constitute a necessary initial step toward understanding the influence of HTR1A C(-1019)G on more complex circuitries and processes. More importantly, our current findings represent an important step in imaging genetics research by providing empirical documentation for the basic premise that genetic variation indirectly affects emergent behavioral processes by biasing the response of underlying neural circuitries.<sup>30,46</sup>

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