Article

Neural Mechanisms Underlying 5-HTTLPR-Related Sensitivity to Acute Stress

Emily M. Drabant, Ph.D.

Wiveka Ramel, Ph.D.

Michael D. Edge, M.S.

Luke W. Hyde, M.S.

Janice R. Kuo, Ph.D.

Philippe R. Goldin, Ph.D.

Ahmad R. Hariri, Ph.D.

James J. Gross, Ph.D.

Objective: Many studies have shown that 5-HTTLPR genotype interacts with exposure to stress in conferring risk for psychopathology. However, the specific neural mechanisms through which this gene-by-environment interaction confers risk remain largely unknown, and no study to date has directly examined the modulatory effects of 5-HTTLPR on corticolimbic circuit responses during exposure to acute stress.

Method: An acute laboratory stressor was administered to 51 healthy women during blood-oxygen-level-dependent functional magnetic resonance imaging. In this task, participants were threatened with electric shocks of uncertain intensity, which were unpredictably delivered to the wrist after a long anticipatory cue period of unpredictable duration.

Results: Relative to women carrying the L allele, those with the SS genotype showed enhanced activation during threat anticipation in a network of regions, including the amygdala, hippocampus, anterior insula, thalamus, pulvinar, caudate, precuneus, anterior cingulate cortex, and medial prefrontal cortex. Individuals with the SS genotype also displayed enhanced positive coupling between medial prefrontal cortex activation and anxiety experience, whereas enhanced negative coupling between insula activation and perceived success at regulating anxiety was observed in individuals carrying the L allele.

Conclusions: These findings suggest that during stress exposure, neural systems that enhance fear and arousal, modulate attention toward threat, and perseverate on emotional salience of the threat may be engaged preferentially in individuals with the SS genotype. This may be one mechanism underlying the risk for psychopathology conferred by the S allele upon exposure to life stressors.

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Diathesis-stress models suggest that psychopathology arises through the interplay of intrinsic biological factors, such as genetic variation, and extrinsic environmental factors, such as exposure to stressors (1). Capitalizing on this framework, numerous gene-by-environment interaction studies have demonstrated that specific genetic variants interact with stress exposure to confer vulnerability to mood and anxiety disorders (e.g., references 2–5).

One of the best known demonstrations of gene-by-environment effects involves a functional promoter polymorphism (5-HTTLPR) in the serotonin transporter gene (*SLC6A4*) in which the short (S) allele interacts with exposure to stressful life events to predict risk for mood-related psychopathology (6, 7). This specific gene-by-environment interaction effect on risk for psychopathology has been widely replicated in human studies of both depression and anxiety (8, 9) and supported by nonhuman primate and rodent models (10–12), although there have been some unsuccessful attempts at replication (13). One recent large-scale meta-analysis provided strong support for this gene-by-environment interaction effect (7).

Imaging genetics studies, which leverage functional neuroimaging to uncover the neurobiological correlates of specific genetic variants (14), have shown a robust association between the S allele and increased amygdala reactivity during implicit processing of picture stimuli containing negative facial expressions (15) but no changes in response to positive facial expressions (16, 17). While these studies clearly implicate neurobiological pathways involved in mediating sensitivity to environmental stress, the specific neural mechanisms through which this gene-by-environment interaction confers risk remain largely unknown. Indeed, few studies to date have directly examined the modulatory effects of the 5-HTTLPR polymorphism on corticolimbic circuit responses during exposure to acute stress (18, 19).

To identify specific neurobiological mechanisms mediating 5-HTTLPR-by-stress effects on risk for psychopathology, it is critical to devise experimental paradigms that capture the unpredictability and aversiveness of environmental stressors in the laboratory. Such paradigms are valuable because they can overcome many limitations

of currently utilized measures of stressful life events based on self-report, including memory biases and limitations associated with retrospective assessment, self-presentation biases, mood-state effects, and substantial variability across instruments (20). Acute laboratory stressors also permit control over the timing and magnitude of stressors and examination of momentary changes in neurobiology to delineate the immediate effect of stressors on neurobiological systems.

In this study, we administered an acute laboratory stressor to healthy adult women during blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI). Focusing on a healthy population allowed us to investigate the mechanism of risk without the confounding variable of current or past psychopathology, which makes it difficult to infer cause from consequence. In this paradigm, electric shocks of uncertain intensity were threatened and unpredictably delivered to the wrist after a long anticipatory cue period of unpredictable duration, allowing for robust responses to be generated (21). We obtained ratings of global anxiety experience and anxiety regulation success at the end of the task. Two hypotheses were examined. First, based on existing epidemiological and neuroimaging research (8), we hypothesized that there would be an interaction between the 5-HTTLPR genotype and stress, such that in these healthy women, the short allele would be associated with greater amygdala reactivity as well as greater activation in the medial prefrontal cortex during stress exposure (22). Second, based on the brain regions identified in these analyses as well as extensive literature on neural responses to threat (23–25), we hypothesized that genotype would moderate the relationship between corticolimbic BOLD responses and reports of anxiety and regulation success. Our specific focus was brain regions implicated in triggering central and peripheral responses to threat (amygdala) (23), interoceptive processing of threat (insula) (24), and cognitive appraisal of threat (medial prefrontal cortex) (25), and we hypothesized that S-allele carriers would have greater brain-behavior coupling with anxiety experience but lesser coupling with regulation success.

Method

Participants

Fifty-one right-handed healthy women (mean age: 22 years [SD=2.4]) participated in the study (21). All potential participants were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (26). Eligible participants did not meet criteria for any psychiatric disorder within the past year, nor did they meet criteria for lifetime generalized anxiety disorder, posttraumatic stress disorder, bipolar disorder, obsessive-compulsive disorder, or psychotic disorder, and they were not currently receiving treatment with any psychotropic medication. Only Caucasian individuals were studied in order to minimize the effects of genetic background and racial heterogeneity, and women were studied to maximize the homogeneity of affective responses (27, 28). The study was approved by the institutional review board at Stanford University, and all participants gave informed consent and received monetary compensation for their participation.

Procedure

The procedure for the stress task has been reported previously (21). Briefly, the stress-exposure task consisted of three conditions presented in a pseudorandom order: safe trials (12 trials), medium-shock trials (13 trials), and strong-shock trials (13 trials). During safe trials, no shocks were administered. During shock trials, electric shocks were delivered to the left wrist above the median nerve using a Grass SD-9 stimulator (Grass Technologies, West Warwick, R.I.).

In order to maximally induce stress and prevent habituation, instructions for the medium- and strong-shock trials featured three levels of unpredictability. Event unpredictability was implemented by indicating that shocks would be delivered during 85% of the trials. Temporal unpredictability was implemented by indicating that trials would last between 0 and 20 seconds and could occur at any time (in actuality, trials ranged between 7 seconds and 11 seconds; average duration: 9 seconds) and would be terminated with a shock in 85% of the trials (two "quick" trials terminated with a shock at 3 seconds in order to maintain that shocks could occur at any time). Intensity unpredictability was implemented by leaving the exact strength of the shock unspecified within a 20% window. In the medium-shock trials, the range of the shock strength was 40%–60% of the maximum voltage (in actuality, these shocks were always given at 55% of the maximum voltage), and in strong-shock trials the range was 70%–90% of the maximum voltage (in actuality, these shocks were always given at 85% of the maximum voltage). These levels of unpredictability have been shown to potentiate emotional reactivity (29) as well as autonomic (30) and neural (31) responsiveness. At the end of each shock trial, participants provided anxiety ratings, using a scale of 1 (not at all) to 5 (very much). Each trial was followed by an interstimulus interval that varied from 3 to 6 seconds. At the end of the task, participants rated the overall anxiety they experienced as a result of the shocks as well as the success they had in reducing their anxiety during the shock trials. Analyses focused on these global ratings. The ratings for three participants were not obtained because of software malfunction. Our group has previously shown that this task increases anxiety experience, skin conductance response, and brain activity in a distributed corticolimbic network (21).

Brain responses of interest were responses during the anticipatory cue period, up to but not including the shock. This allowed us to focus our investigation on psychological factors associated with stress exposure rather than on physiological factors associated with receipt of physical stimulation. In order to examine potentially small effects of genotype, the present investigation focused on the most extreme contrast of safe trials relative to shock trials (medium and strong anticipation periods grouped together), rather than comparing medium-shock trials with strong-shock trials, since our previous investigation demonstrated limited regional differences between these trials (21).

5-HTTLPR Genotyping

Triallelic 5-HTTLPR genotyping was carried out according to standard procedures described previously (32), which included genotyping for the presence of the A4G single nucleotide polymorphism within the L allele (33).

Control Variables

In order to ensure that genotype differences were not confounded with differential histories of life stress, we assessed exposure to life stressors. We chose an interview-based method (Early Trauma Inventory) to assess early life stress exposure (occurring

up to age 18 years) in the following five domains: general trauma and general disasters and physical, emotional, and sexual abuse (34). Interviewers were blind to participant genotype. We also assessed exposure to recent life stress (occurring within the last year) using the Life Events Scale for Students (35).

Imaging

Imaging was performed on a General Electric 3-T Signa magnet (General Electric Medical Systems, Milwaukee). BOLD signal was acquired using a T_2-weighted gradient echo spiral-in/out pulse sequence (36) and a custom-built quadrature "dome" elliptical birdcage head coil. Head movement was minimized using a bite bar and head padding. A total of 446 functional volumes were obtained during the functional run from 22 sequential axial slices (TR=1,500 msec, TE=30 msec, flip angle=70°, field of view=22 cm, matrix=64×64, single-shot, in-plane resolution=3.438 mm^2, slice thickness=4.5 mm). Three-dimensional high-resolution anatomical scans were acquired using fast spin-echo spoiled gradient recall (0.859×1.2 mm; field of view=22 cm, frequency encoding=256).

fMRI Data Analysis

Functional data were analyzed using Analysis of Functional NeuroImages (AFNI) software (37). Preprocessing included coregistration, motion correction, 4 mm^3-isotropic Gaussian spatial smoothing, high-pass filtering (0.011 Hz), and linear detrending. Only volumes that demonstrated less than 1 mm of motion in the x, y, and z coordinates were included. Three participants exhibited volumes with motion above this threshold and were removed from all subsequent analyses, leaving 48 participants in the analysis. There was no evidence of stimulus-correlated motion when conducting correlations of condition-specific reference functions and x, y, or z motion-correction parameters (all p values >0.05).

A multiple regression model implemented using AFNI 3dDeconvolve software included baseline parameters to remove polynomial trendlines to the fifth order as well as individual motion-related variance in the BOLD signal in six orientations. The model also included regressors for each separate condition coding for the anticipation, shock, and rating periods, all of which were convolved with the gamma variate model of the hemodynamic response function. Contrasts were computed by weighting the appropriate columns in the design matrix. Statistical maps were resampled to 3.438 mm^3 and converted to Talairach atlas space (38), and second-level statistical parametric maps were produced according to a random-effects analysis to enhance the generalizability of the results.

To correct for multiple comparisons, AlphaSim, a Monte Carlo simulation bootstrapping program in the AFNI library, was employed to estimate a joint probability distribution specifying a voxelwise threshold and a minimum cluster-volume threshold to establish a cluster-wise p value that protects against false positive detection of activation clusters (39). A voxelwise threshold p value of 0.005 (t=2.946) resulted in a minimum cluster volume threshold of 257 mm^3 (six voxels) to protect against false positive detection of clusters of activation at a p value <0.05. All clusters cited in this study survived this correction.

The relationship between triallelic 5-HTTLPR genotype and BOLD responses was investigated using a whole-brain regression analysis to examine linear dose-response (individuals with the LL genotype: –1, individuals with the LS genotype: 0, individuals with the SS genotype: 1) effect on the contrast of safe trials relative to trials of shock anticipation. Because a regression analysis is uninformative with respect to the role of the intermediate group, follow-up analyses were conducted to examine the relationship between the three genotype groups in all functional clusters using analysis of variance (ANOVA) with post hoc comparisons. Based on these results, a post hoc whole-brain independent-sample t test analysis was conducted using AFNI software comparing individuals with the SS genotype and L-allele carriers.

In order to investigate the relationship between 5-HTTLPR genotype, BOLD activation, and behavior, we used a linear regression model. Mean beta weights were extracted from the functional clusters identified in the group maps (comparing SS- and L-allele carriers) for each subject. Interactions were analyzed, probed, and graphed according to guidelines by Preacher et al. (40), using Predictive Analytics SoftWare, version 18 (SPSS, Inc., Chicago), on the three brain regions of interest: amygdala, insula, and medial prefrontal cortex. Brain activation values and genotype as well as the brain-genotype interaction term were entered into a regression predicting behavioral ratings (41).

Results

Sample

The genotype distribution in our final cohort of 48 women was consistent with prior reports and did not deviate from Hardy-Weinberg equilibrium (11 with the LL genotype [all L_AL_A]; 22 with the LS genotype [L_AS=20, L_AL_G=2]; and 15 with the SS genotype [L_GS=5, SS=10]). The genotype groups did not differ with respect to age or IQ. There were no significant differences between the three genotype groups on any index of life stress, including childhood stress (Early Trauma Inventory) and stress within the last year (Life Events Scale for Students).

5-HTTLPR and Neural Responses to Stress

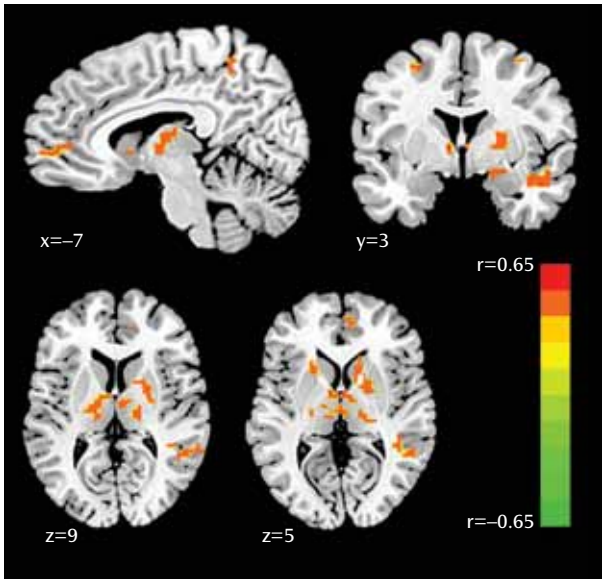
Regression analysis indicated that as the number of S alleles increased, activation increased in the amygdala, thalamus, putamen, caudate, middle temporal gyrus, middle frontal gyrus, fusiform gyrus, and precuneus (Figure 1 [also see Table 1 in the data supplement accompanying the online edition of this article]). No regions showed the opposite effect (i.e., L alleles correlating with increased activation). We extracted and graphed the mean beta weights from the functional clusters identified in this analysis for each participant. This revealed a predominant pattern in which similar activation levels were observed in LL and LS individuals, while increased activation was observed in SS individuals (Figure 2). We thus conducted an ANOVA along with post hoc comparisons to examine the genotype groups in a pairwise fashion across all significant clusters. Results indicated that significantly different activation was not present in the majority of the clusters in LL and LS individuals (see Table 2 in the data supplement). Based on these results, individuals with the LL or LS genotype were grouped with the L carriers for the remaining analyses. A second post hoc whole-brain analysis was conducted comparing individuals with the SS genotype and L-allele carriers, since this model seemed to fit the data more accurately. The findings were similar to those for the linear regressor but uncovered a number of additional regions, including the hippocampus, anterior insula, pulvinar, anterior cingulate cortex, and medial prefrontal cortex, which showed a significant effect of genotype (Table 1, Figure 3 [also see Figure 1 in the data supplement]). Sub-

TABLE 1. Functional Regions of Interest in a Whole-Brain t Test Among Healthy Women (SS > L)[a]

Region	Hemisphere	Brodmann's Area	Talairach Coordinates (x, y, z)[b]	Voxels	t
Thalamus (ventral anterior nucleus) [c]	Left		–10, –6, 8	247	5.47
Putamen	Left		–21, 11, 1		
Pulvinar	Left		–10, –27, 1		
Pulvinar	Right		17, –30, 1		
Precuneus[c]	Right	7	7, –48, 53	192	5.76
Superior parietal lobule	Right	7	28, –51, 63		
Postcentral gyrus	Right	3	28, –20, 50		
Superior frontal gyrus	Right	6	24, 7, 53		
Precentral gyrus	Right	4	14, –30, 63		
Putamen[c]	Right		21, 4, 5	116	4.75
Caudate	Right		21, –10, 22		
Middle temporal gyrus	Right	39	55, –51, 12	75	4.82
Insula	Right	41	34, –31, 15	64	4.31
Cingulate gyrus	Right	24	10, –10, 36	52	4.90
Fusiform gyrus	Left		–28, –61, –12	44	4.48
Supramarginal gyrus	Left	40	–55, –51, 32	43	4.46
Supramarginal gyrus	Right	39	41, –55, 29	36	4.22
Superior temporal gyrus	Right	38	52, 1, –9	33	5.55
Precuneus	Right	31	10, –61, 22	30	4.55
Middle frontal gyrus	Right	9	34, 31, 32	26	4.06
Medial frontal gyrus	Right	10	10, 56, 1	20	4.25
Middle temporal gyrus	Left	21	–55, –31, –2	18	4.27
Insula	Left	13	–31, –20, 15	17	3.81
Precuneus	Left	7	–10, –51, 50	15	3.61
Middle temporal gyrus	Left	21	–52, –3, –9	14	4.21
Middle temporal gyrus	Right	37	48, –48, –6	13	4.26
Precuneus	Left	7	–10, –65, 50	13	4.79
Posterior cingulate	Left	23	–3, –55, 15	12	3.66
Superior frontal gyrus	Left	9	–14, 35, 36	12	4.21
Medial frontal gyrus	Left	9	–14, 28, 32	10	3.44
Superior parietal lobule	Right	7	34, –48, 63	10	3.60
Amygdala	Right		17, –3, –12	9	3.52
Superior temporal gyrus	Right		41, –41, 1	9	3.88
Postcentral gyrus	Left	40	–58, –24, 15	9	4.09
Culmen	Right		28, –48, –23	8	4.10
Hippocampus	Right		28, –27, –9	8	4.62
Putamen	Left		–17, 1, 15	8	3.71
Medial frontal gyrus	Left	10	0, 62, 8	7	3.11
Middle frontal gyrus	Left	10	–34, 49, 19	7	3.67
Putamen	Left		–24, –6, 22	7	4.34
Superior frontal gyrus	Right	9	14, 49, 32	7	3.84
Cingulate gyrus	Left	24	–10, –6, 36	7	3.75
Precuneus	Left	7	0, –72, 46	7	4.38
Superior temporal gyrus	Left	22	–52, 7, –6	6	3.88
Lingual gyrus	Left	18	–17, –82, –6	6	3.55
Middle temporal gyrus	Left	22	–48, –41, 8	6	4.12
Insula	Right	13	48, –13, 12	6	3.58
Medial frontal gyrus	Left	10	–3, 56, 19	6	3.27
Inferior parietal lobule	Right	13	45, –41, 22	6	3.52
Precentral gyrus	Left	6	–31, –10, 53	6	3.31

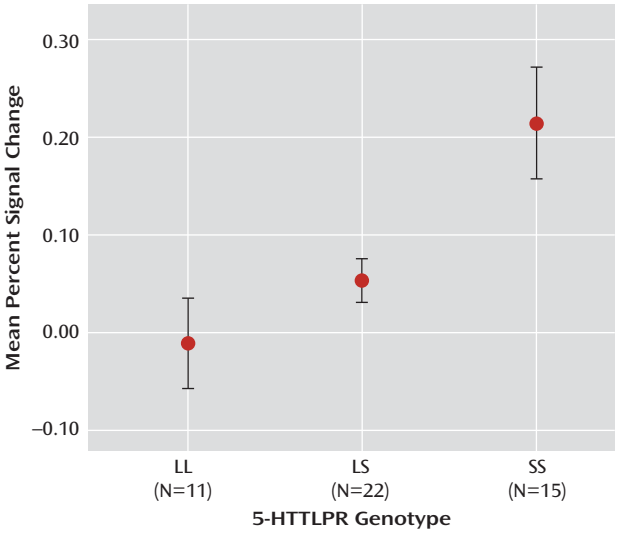
[a] All clusters survived a corrected p value <0.05.
[b] Coordinates indicate the peak of the cluster.
[c] Region was identified as a supracluster, and the subcluster peaks are listed directly below it.

FIGURE 1. Regions Showing an Effect of 5-HTTLPR Genotype in a Whole Brain Regression (SS > LS > LL)[a]



[a] The images are depicted in neurological convention (left=left), with a corrected p value <0.05 (t>2.95); the color bar indicates r statistic. (Coordinates for all regions showing an effect of genotype are listed in Table 1.)

FIGURE 2. Mean Blood-Oxygen-Level-Dependent Responses Across All Clusters Showing an Effect of 5-HTTLPR Genotype in a Whole-Brain Regression Analysis[a]



[a] Functional regions of interest were identified at a corrected p value <0.05, and then extracted values were averaged across all significant clusters within each participant for illustration purposes. Follow-up post hoc comparisons of individuals with the LL genotype relative to individuals with the LS genotype in each cluster revealed that these groups did not significantly differ across the majority of clusters. Error bars indicate a standard error of 2.

sequent analyses of brain-behavior relationships focused on functional clusters identified in the analysis comparing individuals with the SS genotype with L-allele carriers.

5-HTTLPR Genotype, Neural Responses, and Behavior

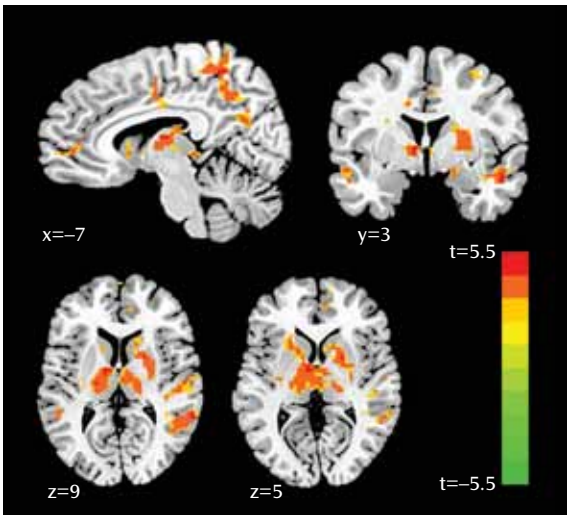
On the basis of previous literature on threat processing, we selected the amygdala, insula, and medial prefrontal cortex as target regions of interest for an investigation of the links between 5-HTTLPR genotype, neural responses, and behavior. We examined whether genotype affected the relationship between brain activation in these functional regions of interest and task-dependent ratings of anxiety and regulation success. Independent-sample t tests indicated that there was no difference between the genotype groups in ratings of anxiety or regulation success, and anxiety and regulation success were not significantly correlated.

We did not find a moderating effect of genotype on the relationship between amygdala activation and anxiety or regulation success. However, we did find evidence that 5-HTTLPR genotype significantly moderated the relationship between medial prefrontal cortex activation and anxiety (β=0.50, p=0.04). Simple-slope analyses indicated that a significant positive correlation between medial prefrontal cortex activation and anxiety was present in individuals with the SS genotype (simple slope=5.8 [SE=2.8], p<0.05), whereas a significant relationship between these variables was not observed in L-allele carriers (Figure 4). We also found evidence that 5-HTTLPR genotype significantly moderated the relationship between insula activation and regulation success (β=0.58, p=0.02). Simple-slope analyses revealed a significant negative correlation between the insula and regulation success in L-allele carriers (simple slope=10.0 [SE=3.13], p=0.003), whereas a significant relationship between these variables was not observed in individuals with the SS genotype (Figure 5). For further details, see Table 3 in the data supplement.

Discussion

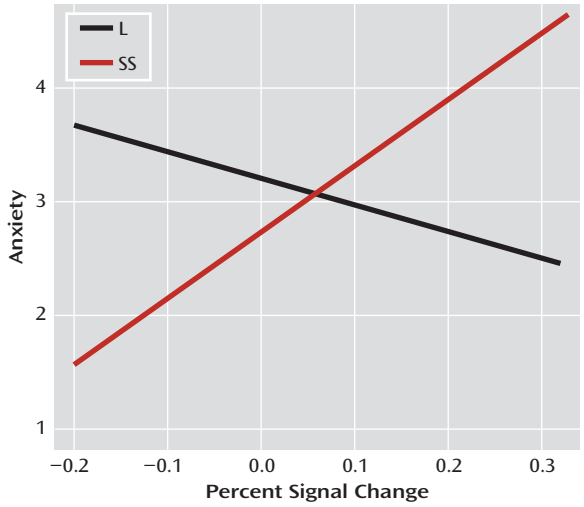
In this study, we examined neural correlates of genetic sensitivity to acute stress exposure conferred by the 5-HTTLPR short allele. Findings revealed that stress-induced activation was enhanced in the amygdala, hippocampus, anterior insula, thalamus, pulvinar, caudate, precuneus, anterior cingulate cortex, and medial prefrontal cortex in women with the homozygous SS genotype, compared with women carrying the L allele. Notably, enhanced right amygdala reactivity observed in women with the SS genotype was located in the dorsal region encompassing the central nucleus, which is known to drive behavioral and physiological arousal (42) through interaction with the thalamus and cortex. This study also demonstrated substantial modulation of the thalamus by 5-HTTLPR genotype, particularly the dorsomedial nucleus and pulvinar. These regions are known to modulate emotion and mood; the thalamus gates sensory information to the amygdala and mediates the flow of information within the limbic system (43). Moreover, the pulvinar is anatomically connected to both the anterior cingulate cortex and the amyg-

FIGURE 3. Regions Showing an Effect of 5-HTTLPR Genotype in a Whole Brain t Test (SS > L)[a]



[a] The images are depicted in neurological convention (left=left), with a corrected p value <0.05 (t>2.95); the color bar indicates t statistic. (Coordinates for all regions showing an effect of genotype are listed in Table 1.)

FIGURE 4. 5-HTTLPR Genotype Predicts Differential Correlation Between Brain Activation in the Left Medial Prefrontal Cortex and Anxiety in Response to the Stress Exposure Task[a]



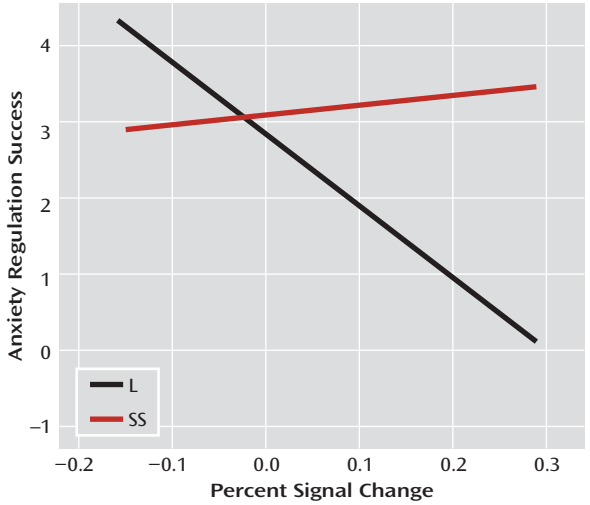
[a] The graph depicts the functional cluster (centered at x=–14, y=35, z=36) revealing positive coupling with anxiety in individuals with the SS genotype but not in L-allele carriers.

dala (44, 45) and relays emotionally salient information about the environment to the limbic system (46). Our findings are in line with previous research indicating that the thalamus contains one of the highest levels of serotonin transporters in the brain (47), and the pulvinar, specifically, is enlarged in S-allele carriers (48, 49). Enhanced activation in the anterior insula, a region implicated in interoceptive processing, awareness of negative emotions, and anticipation of pain (31, 50), provides further evidence of upregulated affective salience in individuals with the homozygous SS genotype. In addition to bottom-up affective processing, it is possible that top-down attentional mechanisms also drive sensitivity to threat. In individuals with the SS genotype, increased activation observed in the dorsal anterior cingulate and the precuneus bilaterally is consistent with biased attention toward the danger of the upcoming shock and perhaps greater responsivity in the face of uncertainty (31). Lastly, activation in the medial prefrontal cortex has been consistently shown to be necessary for the generation of conscious appraisal of threat, and the increased activation we observed in individuals with the SS genotype may reflect altered cognitive interpretation of the shock trials.

It is striking that when a task more potent than simple images is utilized, as with previous imaging genetics studies, genetic effects in a larger and more sophisticated network for processing environmental threat are unmasked. It is possible that neural alterations associated with the SS genotype result in upregulation of affective information entering the limbic system, via the thalamus and amygdala, which drives further salience and processing in a distributed cortical network. Taken together, the present findings suggest that when individuals carrying two S alleles are exposed to acute stress, neural systems that enhance fear and arousal, modulate attention toward threat, and perseverate on emotional salience of the threat are engaged. In turn, this may be a mechanism underlying risk for psychopathology conferred by the S allele upon exposure to life stressors and may specifically speak to risk for anxiety disorders, which are characterized by chronic worry and anxiety about future events.

Using a task that generates visceral emotional responses allowed us to examine the relationship between psychological responses to stress, neural activity, and genotype. Results indicated a markedly increased positive relationship between medial prefrontal cortex activation and anxiety reactivity in response to the task in women with the homozygous SS genotype. This interaction effect demonstrates that medial prefrontal cortex activation signals or triggers feelings of anxiety in individuals with the SS genotype, suggesting that they may elaborate on the nature of the threat via neural circuits different from those engaged in L-allele carriers. We also found that in L-allele carriers, there was a significant negative relationship between insula activation and regulation success in response to the task. In L-allele carriers, perhaps efforts to regulate anxiety result in an extinguished response in the insula, which is a putatively more adaptive response. Interestingly, we did not find an association between 5-HTTLPR genotype and the coupling of amygdala with anxiety or regulation success. One possible interpretation is that the amygdala functions to rapidly and often unconsciously alert higher brain systems to environmental threat, but those higher structures are required to elaborate on the threat in order to generate subjective experience of anxiety and successful

FIGURE 5. 5-HTTLPR Genotype Predicts Differential Correlation Between Brain Activation in the Left Insula and Successful Anxiety Regulation in Response to the Stress Exposure Task[a]



[a] The graph depicts the functional cluster (centered at x=–31, y=–20, z=–15) revealing negative coupling with anxiety regulation success in L-allele carriers but not in individuals with the SS genotype.

regulation of that anxiety. Thus, the interactions observed appear to indicate that in those with the SS genotype, compared with L-allele carriers, there are different circuit dynamics that translate differentially into behavior based on genotype. Notably, we found no direct associations between 5-HTTLPR genotype and behavioral phenotypes. This is a common occurrence when working with relatively small samples, possibly reflecting the minimal effect that genotype has on any distal behavioral phenotype (14). These results help us to appreciate the relevance of a network of structures beyond the amygdala demonstrating effects of 5-HTTLPR in the face of stress exposure.

It is noteworthy that the post hoc analyses in this study clearly indicated that it was most appropriate to group individuals carrying the L allele. Consistent with previous imaging genetics studies, our a priori model was one of codominance, in which the S allele added a dose effect, and thus we conducted a whole-brain regression. However, a regression analysis is agnostic to the effect of the intermediate group, prompting us to extract the data from all of the significant clusters to explore the relationship between the three genotype groups graphically. The majority of the graphs showed a similar pattern of activation among L-allele carriers. While these results are unexpected, it is not clear whether many published studies of 5-HTTLPR genotype have explored multiple analytic models or extracted data to examine the fit of the model to the three genotype groups. Of the studies that have clearly considered the genotype groups individually, some found no differences between individuals with the LL and LS genotypes in hypothalamic-pituitary-adrenal reactivity in response to a laboratory stressor (51) or in the likelihood of developing depression in response to moderately threatening life events (52). As suggested by Gotlib et al. (51), a single laboratory stressor may be too transient to elicit significantly different physiological reactivity between LL and LS carriers. Along with the present findings, this suggests that individuals with the SS genotype may have a lower threshold for stress sensitivity than their L-allele counterparts. However, the fact that these analyses were post hoc is a limitation, and further investigation is needed.

Recently, some controversy has arisen in the literature on the 5-HTTLPR-by-stress interaction because not all studies have replicated this gene-by-environment effect (13, 53). Importantly, of those studies that did not replicate the effect, nearly all relied on retrospective questionnaire assessments of life stress exposure. Conversely, studies that utilized interview-based methods and rich multisource objective data in carefully followed epidemiological cohorts consistently found evidence of this gene-by-environment effect (7, 8, 54, 55). This further highlights the utility of employing laboratory-based stressors, particularly when examining neural mechanisms mediating this effect. Moreover, in contrast to previous imaging genetics studies that relied on presentation of pictures acting as conditioned stimuli, the task we used provides an immediate and robust psychological and bodily threat, which is a visceral model of real-world stressors. As the field moves forward, it will be important to shift toward the type of paradigms that can powerfully unmask genetic sensitivity to threat and stress, such as the one used in this study.

Our results add to the mounting evidence, accumulated in studies of both animals and humans, suggesting that the S allele renders individuals stress-sensitive by biasing neurobiological systems underlying threat reactivity and arousal.

Despite its promise, this study has several limitations. First, while we prioritized studying a relatively homogeneous sample in order to decrease error variance, this sampling decision limits the generalizability of our findings. Future studies should examine neural mechanisms of genetic sensitivity to acute stress in men and within a wider range of genetic backgrounds. Second, while focusing on a healthy population allowed us to investigate the mechanism of risk without the confound of current or past psychopathology, it will be important for future studies to longitudinally follow participants from this type of imaging genetics experiment in order to determine whether these neural biomarkers result in the onset of psychopathology in the face of exposure to life stressors. Third, our small sample size limited power to detect all effects of genotype, particularly any effect on the amygdala (15). Studies of larger samples are needed. Fourth, while research suggests that recent stressors are the most potent predictor of increased risk for psychopathology (56) and hence this anxiogenic task provides a proxy for such acute real-life stressors, this design does not allow for investigation of the effects of chronic stressors or the full range of

life stressors known to trigger psychopathology. Indeed, it may be the accumulation of chronic stress exposure over time that interacts with genotype to confer risk. Finally, our examination of brain-behavior relations is suggestive, but the directionality of these associations cannot be inferred; it could be that neural reactivity drives behavior or vice versa.

Received Nov. 29, 2010; revisions received June 30 and Oct. 31, 2011; accepted Nov. 10, 2011 (doi: 10.1176/appi.ajp.2011.10111699). From the Department of Psychology, Stanford University, Stanford, Calif.; the Neurosciences Program, Stanford University School of Medicine, Stanford, Calif.; the Department of Psychology, University of California Berkeley, Berkeley, Calif.; the Department of Psychology, University of Pittsburgh, Pittsburgh; the Department of Psychology, Ryerson University, Toronto; and the Department of Psychology and Neuroscience, Duke University, Durham, N.C. Address correspondence to Dr. Gross (gross@stanford.edu).

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