Published in final edited form as:

Psychiatry Res. 2011 September 30; 193(3): 161–167. doi:10.1016/j.psychres.2011.03.003.

Serotonin transporter genotype modulates the association between depressive symptoms and amygdala activity among psychiatrically healthy adults

Seth J. Gillihan^{a,*}, Hengyi Rao^{b,c,*}, Laretta Brennan^d, Danny JJ Wang^b, John A. Detre^b, Geena Mary V. Sankoorikal^d, Edward S. Brodtkin^d, and Martha J. Farah^a

^a Center for Cognitive Neuroscience, Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104, USA

^b Center for Functional Neuroimaging, Departments of Neurology and Psychiatry, University of Pennsylvania, Philadelphia, PA 19104, USA

^c Center for Functional Brain Imaging and Center for Studies of Psychological Application, South China Normal University, Guangzhou, China

^d Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Abstract

Recent attempts to understand the biological bases of depression vulnerability have revealed that both the short allele of the serotonin transporter-linked polymorphic region (5-HTTLPR) and activity in the amygdala are associated with depression. Other studies have reported amygdala hyperactivity associated with the 5-HTTLPR short allele, linking the genetic and neuroimaging lines of research and suggesting a mechanism whereby the short allele confers depression risk. However, fewer investigations have examined the associations between depression, 5-HTTLPR variability, and amygdala activation in a single study. The current study thus investigated whether 5-HTTLPR genotype modulates the association between depressive symptoms and amygdala activity among psychiatrically healthy adults. Regional cerebral blood flow was measured with perfusion fMRI during a task-free scan. We hypothesized differential associations between depressive symptoms and amygdala activity among individuals homozygous for the short allele and individuals homozygous for the long allele. Both whole brain analyses and region-of-interest analyses confirmed this prediction, revealing a significant negative association among the long allele group and a trend of positive association among the short allele group. These results complement existing reports of short allele related amygdala hyperactivity and suggest an

© 2011 Elsevier Ireland Ltd. All rights reserved.

Address correspondence and reprint requests to Seth J. Gillihan, Center for the Treatment and Study of Anxiety, Department of Psychiatry, University of Pennsylvania School of Medicine, 3535 Market Street, Philadelphia, PA 19104, 215-746-3327 (phone), 215-746-3311 (fax); gillihan@mail.med.upenn.edu. Danny JJ Wang is now at the Almsman-Lovelace Brain Mapping Center, Department of Neurology, UCLA, Los Angeles, CA 90095.

*These authors contributed equally to the preparation of this manuscript.

Conflict of Interest

The authors report no competing interests. Dr. Detre is an inventor on the University of Pennsylvania's patent for arterial spin labeled perfusion MRI and is entitled to institutional royalty sharing for its licensure.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

additional neurobiological mechanism whereby the 5-HTTLPR is associated with psychiatric outcomes.

Keywords

depression; mood; 5-HTTLPR; serotonin transporter; amygdala

1. Introduction

Recent attempts to understand the biological bases of depression vulnerability have focused on both genetic and neural risk factors. One of the most commonly studied genetic polymorphisms is the serotonin transporter-linked polymorphic region (5-HTTLPR). The short (S) allele of the 5-HTTLPR is associated with several psychiatric conditions, perhaps most notably depression (e.g., Lotrich and Pollock, 2004). Given its location in the promoter region upstream from the serotonin transporter gene, this polymorphism affects the efficiency of DNA transcription into messenger RNA; the S allele is associated with decreased transcription efficiency, which leads to less production of the serotonin transporter protein and subsequently to less reuptake of serotonin (Lesch et al., 1996).

Part of the behavioral mechanism by which the 5-HTTLPR exerts its effects appears to be through conferring differential reactivity to stress. For example, Caspi and colleagues (2003) demonstrated that 5-HTTLPR genotype interacts with stressful life events to predict depression outcomes; other studies have reported similar effects (e.g., Kaufman et al., 2004; Kendler et al., 2005; however, see Risch et al., 2009). More recent studies have begun to identify the effects of this genetic variability on human behavior, physiology, and neural systems. Gotlib and colleagues, for example, identified effects of the 5-HTTLPR on cortisol levels, both at rest (Chen et al., 2009) and in response to stressors (Gotlib et al., 2008); in addition, Reimold et al. (2011) reported a significant interaction between 5-HTTLPR genotype and cortisol response on serotonin transporter binding potential in the human thalamus.

An intersecting line of depression vulnerability research has focused on activity in the amygdala. There is evidence that amygdala activity is elevated during depression (Drevets et al., 2002) and during experimentally induced negative affective states (Posse et al., 2003). Other studies have shown that amygdala activity is positively associated with an improvement in depression symptoms, including in response to cognitive behavioral therapy (Siegle et al., 2006). These and similar findings have led to the hypothesis that the amygdala is part of a limbic network that is dysregulated during depression (Seminowicz et al., 2004).

A third line of research represents a potential link between the effect of 5-HTTLPR variability on depression and the association between amygdala activity and depression. The first study of this type (Hariri et al., 2002) found that S allele carriers showed greater amygdala reactivity to fearful or angry faces (stimuli that reliably produce amygdala activity [Morris et al., 1996]). Independent research laboratories have replicated the greater amygdala reactivity among S allele carriers (e.g., Furmark et al., 2004; Canli et al., 2005). Other groups have reported greater *resting* amygdala activity associated with the S allele (Canli et al., 2006; Rao et al., 2007) including among a sample of depressed individuals (Brockmann et al., in press), as well as greater amygdala activity during intentional mood regulation in S allele homozygotes (Gillihan et al., 2010; but see Viviani et al., 2010). Particularly relevant to the current study, Canli and colleagues (2006) reported a significant gene-by-environment interaction of 5-HTTLPR genotype and life stress on amygdala activity. A meta-analysis (Munafò et al., 2008) of these reports concluded that the average

effect size of 5-HTTLPR genotype on amygdala activation is $d = 0.63$ or a separation between the groups of nearly two-thirds of a standard deviation, which by convention falls in the “medium” range (Cohen, 1992).

A review of these findings (Hariri and Holmes, 2006) presented an integrated model of the effects of 5-HTTLPR variability on neural emotion regulation networks. The authors presented evidence that inhibitory feedback circuits in prefrontal cortex are less effective in S allele carriers, leading to poorly regulated activity in limbic emotion centers (see also Heinz et al., 2005; Pezawas et al., 2006). These genotype-driven differences in amygdala activity are presumed to play a role in depression vulnerability through a cascade of behavioral and neuroendocrine effects, particularly in response to stressful life events.

While the studies reviewed above provide clues as to the associations between 5-HTTLPR genotype, depression, and amygdala activity, fewer studies to date have provided direct evidence about the complete triadic relationship between these variables; the majority of empirical studies in this area have focused on subsets of these variables. However, a small and growing line of research has investigated associations between 5-HTTLPR genotype, depression, and amygdala activity. One such study in this area found that among depressed individuals, S allele carriers showed greater amygdala reactivity to emotional facial expressions (Dannlowski et al., 2008). Friedel and colleagues (2009) reported that major depressive disorder moderates the association between 5-HTTLPR genotype and functional coupling of medial prefrontal cortex (mPFC) activity and amygdala activity. Additional research on the role of 5-HTTLPR variability on neural activity during induced sadness has confirmed the relative hyperreactivity of the amygdala among carriers of the S allele (e.g., Fortier et al., 2010; Furman et al., in press).

In light of the compelling evidence of associations between 5-HTTLPR genotype, depression, and amygdala activity, we used an existing dataset to examine the effect of 5-HTTLPR genotype (SS vs. LL) on the correlation between depressive symptoms and baseline (task-free) amygdala activity among non-depressed individuals (as measured by perfusion functional magnetic resonance imaging). The data were taken from a larger study in which we found significant effects of 5-HTTLPR genotype on neural activity at baseline (Rao et al., 2007) and during the intentional regulation of an inducted sad mood (Gillihan et al., 2010). Given the hypothesized inadequate regulation of limbic emotion centers (Hariri and Holmes, 2006) and the previous report of a Gene x Stress interaction on resting amygdala activity (Canli et al., 2006), we hypothesized that individuals with the S genotype would exhibit a positive association between depressive symptoms and amygdala activity, whereas L allele carriers would show no association between depressive symptoms and amygdala activity. This prediction follows from studies showing that inhibitory prefrontal circuits are less effective in S carriers, which we expect will lead to an increase in amygdala activity with increased depression symptoms in these individuals. The more effective inhibitory prefrontal circuits in L carriers are hypothesized here to prevent an increase in amygdala activity as depressive symptoms increase, which thereby may prevent a temporary depressive mood from developing into an episode of frank depression.

2. Material and Methods

2.1. Participants and Design Overview

Participants were 15 homozygous S and 15 homozygous L individuals (14 female; all of European descent; mean age = 20.3 years, range = 18–29 years; see Table 1). Written informed consent was obtained in accordance with the Institutional Review Board of the University of Pennsylvania. We screened 275 participants for 5-HTTLPR genotype. Participants also completed behavioral measures of depression symptoms and personality

(see below). Each potentially eligible self-identified “Caucasian” who was homozygous for 5-HTTLPR genotype (SS or LL) was invited to participate in the neuroimaging portion of the research study until the target sample size was obtained; individuals not of European descent were excluded due to concerns about ethnic stratification—that is, differences in allelic frequencies that are a function of ancestry (Malhotra and Goldman, 1999). We limited our investigation to homozygotes in order to isolate the effects of each allele. During a second testing session participants underwent a psychiatric interview (SCID) to screen out participants with a current psychiatric diagnosis. All participants in the fMRI segment of the study were free of any known neurological illness or current psychiatric diagnosis; this exclusion factor allowed us to examine the effects of 5-HTTLPR genotype on mood-brain relationships outside the context of major mental disorders. None of the participants reported being a cigarette smoker and there were no significant differences in amount of caffeine consumed prior to the testing session ($p = 0.28$). The final testing session comprised an fMRI scan.

2.2. Behavioral Measures and Tasks

Behavioral measures included the Beck Depression Inventory, 2nd edition (BDI-II; Beck et al., 1996); the NEO-Five-Factor Inventory (NEO-FFI; Costa and McCrae, 1992), a self-report inventory used to assess the major personality dimensions; and the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I; First et al., 1996) to assess current and lifetime psychiatric diagnoses.

Mood ratings—The experimenter asked participants to rate their moods from 0–100 (lower indicates worse mood) for both anxiety and sadness prior to and following the fMRI scan blocks.

2.3. 5-HTTLPR DNA Extraction and Genotyping

Each participant provided two buccal cell samples, scraping one Whatman® Sterile Omni swab (Fisher Scientific) against the inside of each cheek for 30 seconds. Swabs were air dried for 2 hours. Genomic deoxyribonucleic acid (DNA) was prepared from buccal cells using the Qiagen QIAamp® Blood Mini Kit (Qiagen, Inc, Valencia, California). Forward (5'-ATG CCA GCA CCT AAC CCC TAA TGT-3') and reverse (5'-GG ACC GCA AGG TGG GCG GGA-3') primers were used to amplify a fragment from the serotonin transporter promoter region. These primers amplify a 419 base pair fragment for the 16-repeat L allele and a 375 base pair fragment for the 14-repeat S allele (Gelernter et al., 1997). Polymerase chain reaction (PCR) was carried out on a Reaction Module (BioRad iCycler, #170–872), and the products were separated on a 2.5% agarose gel (Agarose SFR, Amresco Inc., Solon, Ohio) supplemented with Ethidium Bromide (0.01%, Fisher Scientific) and visualized under ultraviolet light.

2.4. Neuroimaging Measures and Analyses

2.4.1. Image Acquisition—A continuous ASL technique was conducted on a Siemens 3.0 Tesla Trio whole-body scanner (Siemens AG, Erlangen, Germany), using a standard Transmit/Receive head coil for perfusion fMRI scans. Interleaved images with and without labeling were acquired using a gradient echo-planar imaging (EPI) sequence. Acquisition parameters were: FOV = 22 cm, matrix = 64×64, TR = 3 s, TE = 17 ms, label time = 1.6 s, delay time = 0.8 s, flip angle = 90°. The resting perfusion scanning protocol lasted 6 min just before which participants received the following instruction: “Lie still and let your mind go blank, but keep your eyes open and stay awake.” Fourteen slices (8 mm thickness with 2 mm gap) were acquired from inferior to superior in sequential order. Before the functional scan, high-resolution anatomical images were obtained by a 3D MPRAGE sequence with

TR = 1620 ms, TI = 950 ms, TE = 3 ms, flip angle = 15°, 160 contiguous slices, 1×1×1 mm resolution.

2.4.2. Functional Imaging Data Analysis—Functional and structural MRI data processing and analyses were carried out primarily with the Statistical Parametric Mapping software (SPM5, Wellcome Department of Cognitive Neurology, UK) implemented in Matlab 11 (Math Works, Natick, MA), with some additional modifications for perfusion analysis (<http://cfn.upenn.edu/perfusion/software.htm>).

For each participant, functional images were first realigned to correct for head motion, and then coregistered with the anatomical image. Perfusion weighted image series were then generated by pair-wise subtraction of the label and control images, followed by conversion to absolute CBF image series based on a single compartment CASL perfusion model (Wang et al., 2005). One mean CBF image was generated for each scan block of each individual participant and normalized to a $2 \times 2 \times 2 \text{ mm}^3$ Montreal Neurological Institute (MNI) template using bilinear interpolation. These normalized CBF images were smoothed using a Gaussian filter with a full-width at half-maximum (FWHM) of 10 mm, and then entered into the whole brain voxel-wise analyses.

The ROI in amygdala was determined a priori from an automated anatomical labeling (AAL) ROI library (Tzourio-Mazoyer et al., 2002) in the SPM Marsbar toolbox (Brett et al., 2002); this procedure uses templates based on structural parameters to identify the ROI in each participant. For each participant, the quantitative global CBF value was calculated by SPM scripts and the quantitative CBF values in the ROIs were read out by the SPM Marsbar toolbox. The globally corrected (relative) CBF values were calculated by normalizing the global CBF to 60 ml/100 g/min.

2.4.2.1. Whole brain analyses: Individual CBF images were entered into three exploratory whole brain voxel-wise multiple regression analyses. First, in order to evaluate the hypothesis of differential associations between depressive symptoms and amygdala activity among the S and L genotype groups, a multiple regression analysis with genotype, BDI scores, and their interaction term was conducted. In this analysis, the BDI scores of the two genotype groups were orthogonalized by mean central correction and entered in the model as two independent covariates of interest. The genotype information (coded as number of S alleles) was entered in the model as another covariate. Three nuisance covariates (global CBF, age, gender) were also included in the model to account for any variance associated with these variables. The genotype x BDI interaction was defined by the contrast between the correlations with the two BDI covariates. Since we were primarily concerned with amygdala, a threshold of whole brain uncorrected $p < 0.005$ and small volume corrected (SVC) $p < 0.05$ using the a priori defined amygdala ROI (Tzourio-Mazoyer et al., 2002) was applied to this analysis.

Moreover, in order to confirm the results from the above interaction analysis, two additional multiple regression analysis with BDI scores as the covariate of interest and global CBF, age, gender as nuisance covariates were conducted on the S and L groups separately. Activation clusters were also identified for the whole brain at a significance level of $p < 0.005$ (uncorrected) and cluster size larger than 30 voxels, and small volume correction (SVC) was applied to the amygdala activations.

2.4.2.2. ROIs: ROI analyses comprise many fewer statistical comparisons relative to whole brain analyses, and therefore present less risk of a Type I error; in addition, ROI analyses can increase the signal-to-noise ratio, assuming that the voxels in the region respond similarly as part of a functional unit (Huettel et al., 2004). We conducted template-based

ROI analyses of the a priori ROIs in L and R amygdala. In order to address our primary hypotheses we carried out regression analyses using a model that included 5-HTTLPR genotype, depression, and their interaction as predictor variables and left (L) and right (R) amygdala perfusion as outcome variables.

2.5. Behavioral Statistical Analyses

Tests for behavioral differences between groups on age, personality, and depression were done using independent samples *t*-tests; a chi-square test was used to determine whether the male:female ratio differed by genotype group.

3. Results

3.1. Behavioral

The two genetic groups were very similar on age, gender, depression symptoms, and personality dimensions (see Table 1). These results indicate that any significant differences in neural activity are unlikely to be due to these variables; moreover, inclusion of these variables in the neuroimaging analyses did not substantially alter any of the reported effects.

3.2. Neuroimaging

3.2.1. Whole Brain Analyses (Table 2)—Consistent with our hypothesis, whole brain multiple regression analyses with the interaction term revealed a significantly greater correlation between BDI scores and right amygdala activity in the S group than L group (small volume corrected $p < 0.05$, Table 2). This analysis also detected a significant interaction in the left occipital cortex (uncorrected $p < 0.001$, Table 2). When examining the two groups separately, there was a significant negative correlation between BDI scores and right amygdala activity (small volume corrected $p < 0.05$) in the L group, while no significant correlation was found between BDI scores and amygdala activity in the S group. However, when the threshold was lowered to uncorrected $p < 0.05$, there was a trend toward a positive correlation between depressive symptoms and right amygdala activity in the S group. Among the L group BDI also was negatively correlated with activity in left hippocampus and positively correlated with activity in right orbitofrontal cortex. The S group showed additional correlations between BDI and left fusiform gyrus (negative) and left orbitofrontal cortex, right caudate, and left occipital lobe (positive).

3.2.2. Template-based ROI Analyses—The regression model that included 5-HTTLPR genotype, depression scores, and their interaction in predicting amygdala activity was significant for right amygdala, $F(3, 26) = 3.47$, $p = 0.03$, but not for left amygdala, $p = 0.64$. Consistent with our hypothesis, there was a significant interaction between 5-HTTLPR genotype and depressive symptoms (BDI-II) on amygdala activity in right amygdala, $p = 0.007$. Follow-up correlation analyses revealed that the interaction was driven by a significant negative correlation between depression scores and amygdala activity for the L group, $r = -0.54$, $p = 0.03$, and a nearly significant positive correlation between depression and amygdala activity for the S group, $r = 0.46$, $p = 0.08$ (see Figure 2).

4. Discussion

The current study found that depression symptoms and amygdala activity among a sample of non-depressed individuals had opposite patterns of association based on 5-HTTLPR genotype; depression symptoms were associated with less amygdala activity among carriers of the L allele and with marginally greater amygdala activity among S allele carriers. This interaction effect obtained for right amygdala only; there was no differential pattern of association between depression and amygdala perfusion for left amygdala. Although the

current results are consistent with earlier studies (e.g., Pezawas et al., 2005; Friedel et al., 2009; Brockmann et al., in press) and with the study hypothesis, they are surprising in that it appears that the interaction effect was driven in large part by the significant negative association between depression symptoms and amygdala activity in L carriers (see Table 2 and Figure 2). That is, these individuals tended to have greater amygdala activity at lower levels of depressive symptoms. This association is somewhat puzzling, given that depression has been shown to be associated with *increased* amygdala activity (e.g., Drevets et al., 2002); however, it is important to note that no subjects in the present study met criteria for a current depressive episode.

Although the negative correlation between depression symptoms and amygdala activity in the homozygous L group is surprising, similarly inverted relationships have been found for L carriers in at least two other studies examining other variables. First, Canli et al. (2006) showed not only that life stress is positively associated with amygdala activity in S carriers but that life stress is *negatively* correlated with amygdala activity in the L group (see their Fig. 2, p. 16035). Given the strong association between life stress and depression, it is plausible that the negative correlations reported for the L groups in the current study and in Canli et al. represent two manifestations of the same underlying effect. Similarly, Dannlowski et al. (2008) also found in a group of depressed individuals that emotional faces (contrasted with neutral faces) provoked a relative *increase* in amygdala activity in S allele carriers whereas the L allele was associated with a relative *decrease* in amygdala activity. Thus the present findings along with those of Canli et al. and Dannlowski et al. suggest the need to more closely examine the effects of the L allele on depression- and amygdala-related variables.

The present results were found using perfusion ASL fMRI, which provides a measure of absolute CBF that can be quantified in a well-characterized physiological unit of ml/100g/min (Detre et al., 1992). Because CBF is regionally coupled to brain metabolism (which requires the delivery of oxygen and nutrients), it provides a surrogate marker for mapping regional brain activity (Raichle, 1998). Previous work has shown that approximately 70% of the observed variance in CBF data is due to stable trait-like effects (Hermes et al., 2009), which suggests that the present results were driven in large part by stable differences in amygdala activity as a function of the interaction of 5-HTTLPR genotype and depressive symptoms and not to extraneous variables (e.g., differential reactivity to the scanning environment). The results also seem to be driven by enhanced regional CBF in the amygdala and not by more global vascular changes, given that the analyses used normalized measures of amygdala CBF that controlled for global CBF.

The right-lateralized specificity of the reported effect is consistent with the original report by Hariri and colleagues (2002) which found differential activity for right amygdala only, although the authors interpreted their results as deriving from the special role of the right hemisphere in facial processing. Based on the current results, it is possible that this right lateralization is a more general effect since the results in the current study were found during a task-free scan. A meta-analysis of 5-HTTLPR effects on amygdala activation (Munafò et al., 2008) noted that the effect size estimate (based on a subset of studies that reported right and left amygdala activity separately) was larger for right versus left amygdala ($d = 0.68$ vs. 0.36 , respectively), although this difference was not statistically significant.

These results complement existing reports in this area, including results from an earlier report from additional scans of the same participants that found greater amygdala activity among carriers of the S allele during the conscious regulation of an induced negative mood (Gillihan et al., 2010; for a review see Hariri and Holmes, 2006). A plausible explanation of these findings is that in non-depressed individuals with the S allele, negative affective states

and amygdala activity are part of a positive feedback loop that leads to persistence and aggravation of depressive states; in contrast, in individuals with the L allele, neural regulatory regions work to dampen amygdala activity as depressive symptoms increase, thereby maintaining affective homeostasis through negative feedback.

This explanation is consistent with reports of altered associations among S allele carriers in the functional coupling of the amygdala with prefrontal regulatory regions (e.g., Pezawas et al., 2005). However, this interpretation may not account for the negative correlation (vs. no association) between amygdala activity and depressive symptoms found among L carriers. It is possible that the negative correlation is caused by the L carriers' temporarily "over-regulating" amygdala activity; longitudinal studies of 5-HTTLPR genotype, depressive symptoms, and neural activity could help to determine whether the reported differential correlations predict different depression outcomes. While the observed effects are consistent with this interpretation, the cross-sectional design of the current study cannot provide definitive evidence for the direction or the cause of the observed effects.

A study by Schardt et al. (2009) has potentially important implications vis-à-vis the results reported here. The authors reported that the amygdala hyperreactivity associated with the S allele could be attenuated by the intentional regulation of negative emotion. Thus clinical techniques that teach individuals to regulate their emotions, such as those practiced in cognitive-behavioral therapy, may be effective in lowering the risk for psychopathology associated with the S allele. Further research is necessary to investigate this hypothesis.

The a priori hypotheses about the amygdala and the significant results for both the whole brain and ROI analyses, together with previous positive findings in these regions as a function of the 5-HTTLPR, minimize concerns of a Type I error with regard to the amygdala. Other brain regions whose activity was found to vary as a function of 5-HTTLPR genotype and depression symptoms are listed in Table 2 for consideration in future studies; it is noteworthy that in the whole brain analyses, depression symptoms and 5-HTTLPR genotype significantly predicted only right amygdala activation at a significance of $p < 0.05$, corrected. For this reason, considerable caution should be exercised in the level of confidence that is placed in these findings with regard to regions other than the amygdala, particularly in light of the absence of a priori hypotheses about these other regions.

4.1. Limitations and Future Directions

The current study had several limitations. First, as noted above, the correlational design does not permit the establishment of causation or precedence of the observed effects (e.g., whether symptoms of depression in individuals with the L allele lead to dampening of amygdala activity to rein in negative affect). Also, the relatively modest sample size (cf. Munafò et al., 2008) points to the need for replication with larger samples. Additionally, recent research (published after the analysis of our DNA samples, which are no longer available) has revealed an A-to-G single nucleotide polymorphism (SNP) in the long allele, with a more common "Long-A" allele (high 5-HTT expressing) and less common "Long-G" allele (low 5-HTT expressing; Hu et al., 2005). Therefore our L homozygotes likely were somewhat heterogeneous in terms of low- versus high-5-HTT expression. Future studies should include a distinction between long allele variants, which could lead to finer-grained resolution of the interactive effect of the 5-HTTLPR and symptoms of psychopathology on neural response. Furthermore, it is unknown based on the current data whether heterozygotes are more similar to the S or the L group. Generalizability of these findings to 5-HTTLPR heterozygotes could be tested in future research with larger sample sizes.

Although we limited our analyses to individuals of European descent, occult stratification may have been present. Future studies could include more sensitive tests of stratification.

Finally, additional work may reveal whether the reported right lateralization of findings are reliable and specific to resting tasks or whether 5-HTTLPR variability is differentially associated with right versus left amygdala activity.

5. Conclusion

The current results demonstrate an opposite pattern of association between depression and amygdala activity based on 5-HTTLPR genotype. As such these results add another piece to the rather complex puzzle of how this genetic variability interacts with life stress to affect neural systems and influence risk for psychopathology. Additional investigations that clarify the temporal sequence of these effects may be a fruitful area of future research; these studies also may attempt to replicate and extend the intriguing finding of a negative correlation between depressive symptoms and amygdala activity among L allele carriers.

Acknowledgments

This research was made possible through the support of the National Institutes of Health, F31-MH073363 (SJG), R01-HL102119 (HR), R01-HD043078, R01-DA18913, R01-DA14129, R21-DA01586 (MJF), KO8-MH068586 (ESB), and a Burroughs Wellcome Fund Career Award in the Biomedical Sciences (ESB). We thank Wade Berrettini, MD, PhD, for his consultation on the design of the current study, Holly C. Dow and Kristin A. Kaercher for assistance with genotyping, Mallory Bowers and Megan B. Goldman for assistance with data entry, and Jonathan D. Schaefer for assistance with formatting.

References

- Beck, AT.; Steer, RA.; Brown, GK. Beck Depression Inventory Manual. 2. Psychological Corporation; San Antonio, TX: 1996.
- Brett M, Anton JL, Valabregue R, Poline JB. Region of interest analysis using an SPM toolbox. *Neuroimage*. 2002; 16:497A.
- Brockmann H, Zobel A, Schuhmacher A, Daamen M, Joe A, Biermann K, Schwab SG, Biersack H-J, Maier W, Boecker H. Influence of 5-HTTLPR polymorphism on resting state perfusion in patients with major depression. *Journal of Psychiatric Research*. in press.
- Canli T, Omura K, Haas BW, Fallgatter A, Constable RT, Lesch KP. Beyond affect: A role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. *Proceedings of the National Academy of Sciences*. 2005; 102:12224–12229.
- Canli T, Qui M, Omura K, Congdon E, Haas BW, Amin Z, et al. Neural correlates of epigenesis. *Proceedings of the National Academy of Sciences*. 2006; 103:16033–16038.
- Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, Herrmann MJ, Constable RT, Lesch KP. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*. 2003; 301:386–389. [PubMed: 12869766]
- Chen MC, Joormann J, Hallmayer J, Gotlib IH. Serotonin transporter polymorphism predicts waking cortisol in young girls. *Psychoneuroendocrinology*. 2009; 34:681–686. [PubMed: 19128885]
- Cohen J. A power primer. *Psychological Bulletin*. 1992; 112:155–159. [PubMed: 19565683]
- Costa, PT., Jr; McCrae, RR. Revised NEO Personality Inventory (NEO-PI-R) and NEO-Five-Factor Inventory (NEO-FFI) Professional Manual. Psychological Assessment Resources; Odessa, FL: 1992.
- Dannlowski U, Ohrmann P, Bauer J, Deckert J, Hohoff C, Kugel H, Arolt V, Heindel W, Kersting A, Baune BT, Suslow T. 5-HTTLPR biases amygdala activity in response to masked facial expressions in major depression. *Neuropsychopharmacology*. 2008; 33:418–424. [PubMed: 17406646]
- Detre JA, Leigh JS, Williams DS, Koretsky AP. Perfusion imaging. *Magnetic Resonance in Medicine*. 1992; 23:37–45. [PubMed: 1734182]
- Drevets WC, Price JL, Bardgett ME, Reich T, Todd RD, Raichle ME. Glucose metabolism in the amygdala in depression: Relationship to diagnostic subtype and plasma cortisol levels. *Pharmacology, Biochemistry and Behavior*. 2002; 71:431–447.

- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV). American Psychiatric Press, Inc; Washington, D.C: 1996.
- Fortier E, Noreau A, Lepore F, Boivin M, Pérusse D, Rouleau GA, Beauregard M. Early impact of 5-HTTLPR polymorphism on the neural correlates of sadness. *Neuroscience Letters*. 2010; 485:261–265. [PubMed: 20851164]
- Friedel E, Schlagenhauf F, Sterzer P, Park SQ, Birmphohl F, Ströhle A, Stoy M, Puls I, Hägele C, Wrase J, Büchel C, Heinz A. 5-HTT genotype effect on prefrontal-amygdala coupling differs between major depression and controls. *Psychopharmacology*. 2009; 205:261–271. [PubMed: 19387615]
- Furman DJ, Hamilton P, Joormann J, Gotlib IH. Altered timing of amygdala activation during sad mood elaboration as a function of 5-HTTLPR. *Social Cognitive and Affective Neuroscience*. in press.
- Furmark T, Tillfors M, Garpenstrand H, Marteinsdottir I, Långström B, Orelund L, Fredrickson M. Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neuroscience Letters*. 2004; 362:189–192. [PubMed: 15158011]
- Gelernter J, Cubells JF, Kidd JR, Pakstis AJ, Kidd KK. Population studies of polymorphisms of the serotonin transporter protein gene. *Human Genetics*. 1997; 101:243–246. [PubMed: 9402979]
- Gillihan SJ, Rao H, Wang J, Detre JA, Breland J, Sankoorikal GMV, Brodtkin ES, Farah MJ. Serotonin transporter genotype modulates amygdala activity during mood regulation. *Social Cognitive and Affective Neuroscience*. 2010; 5:1–10. [PubMed: 19858108]
- Gotlib IH, Joormann J, Minor KL, Hallmayer J. HPA axis reactivity: A mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biological Psychiatry*. 2008; 63:847–851. [PubMed: 18005940]
- Hariri AR, Holmes A. Genetics of emotion regulation: The role of the serotonin transporter in neural function. *Trends in Cognitive Sciences*. 2006; 10:182–191. [PubMed: 16530463]
- 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. [PubMed: 12130784]
- Heinz A, Braus DF, Smolka MN, Wrase J, Puls I, Hermann D, Klein S, Grüsser SM, Flor H, Schumann G, Mann K, Büchel C. Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature Neuroscience*. 2005; 8:20–21.
- Hermes M, Hagemann D, Britz P, Lieser S, Bertsch K, Naumann E, Walter C. Latent state-trait structure of cerebral blood flow in a resting state. *Biological Psychology*. 2009; 80:196–202. [PubMed: 18838099]
- Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcoholism: Clinical and Experimental Research*. 2005; 29:8–16.
- Huettel, SA.; Song, AW.; McCarthy, G. Functional magnetic resonance imaging. Sunderland, MA: Sinauer Associates, Inc; 2004.
- Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz L, Krystal JH, Gelernter J. Social supports and serotonin transporter gene moderate depression in maltreated children. *Proceedings of the National Academy of Sciences*. 2004; 101:17316–17321.
- Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: A replication. *Archives of General Psychiatry*. 2005; 62:529–535. [PubMed: 15867106]
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller 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. [PubMed: 8929413]
- Lotrich FE, Pollock BG. Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatric Genetics*. 2004; 14:121–129. [PubMed: 15318024]
- Malhotra AK, Goldman D. Benefits and pitfalls encountered in psychiatric genetic association studies. *Biological Psychiatry*. 1999; 45:544–550. [PubMed: 10088044]

- Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, Dolan RJ. A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature*. 1996; 383:812–815. [PubMed: 8893004]
- Munafò MR, Brown SM, Hariri AR. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: A meta-analysis. *Biological Psychiatry*. 2008; 63:852–857. [PubMed: 17949693]
- Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS, Egan MF, Mattay VS, Hariri AR, Weinberger DR. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: A genetic susceptibility mechanism for depression. *Nature Neuroscience*. 2005; 8:828–834.
- Posse S, Fitzgerald D, Gao K, Habel U, Rosenberg D, Moore GJ, Schneider F. Real-time fMRI of temporolimbic regions detects amygdala activation during single-trial self-induced sadness. *Neuroimage*. 2003; 18:760–768. [PubMed: 12667853]
- Raichle ME. Imaging the mind. *Seminars in Nuclear Medicine*. 1998; 28:278–289. [PubMed: 9800235]
- Rao H, Gillihan SJ, Wang J, Korczykowski M, Sankoorikal GMV, Kaercher KA, Brodtkin ES, Detre JA, Farah MJ. Genetic variation in serotonin transporter alters resting brain function in healthy individuals. *Biological Psychiatry*. 2007; 62:600–606. [PubMed: 17481593]
- Reimold M, Knobel A, Rapp MA, Batra A, Wiedemann K, Ströhle A, Zimmer A, Schönknecht P, Smolka MN, Weinberger DR, Goldman D, Machulla HJ, Bares R, Heinz A. Central serotonin transporter levels are associated with stress hormone response and anxiety. *Psychopharmacology*. 2011; 213:563–572. [PubMed: 20585760]
- Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, Merikangas KR. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: A meta-analysis. *Journal of the American Medical Association*. 2009; 301:2462–2471. [PubMed: 19531786]
- Schardt DM, Erk S, Nüsser C, Nöthen MM, Cichon S, Rietschel M, Treutlein J, Goshke T, Walter H. Volition diminishes genetically mediated amygdala hyperreactivity. *Neuroimage*. 2010; 53:943–951. [PubMed: 19969089]
- Seminowicz DA, Mayberg HS, McIntosh AR, Goldapple K, Kennedy S, Segal Z, Rafi-Tari S. Limbic-frontal circuitry in major depression: A path modeling meta-analysis. *Neuroimage*. 2004; 22:409–418. [PubMed: 15110034]
- Siegle GJ, Carter CS, Thase ME. Use of fMRI to predict recovery from unipolar depression with cognitive behavior therapy. *American Journal of Psychiatry*. 2006; 163:735–738. [PubMed: 16585452]
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002; 15:273–289. [PubMed: 11771995]
- Viviani R, Sim EJ, Lo H, Beschoner P, Osterfeld N, Maier C, Seeringer A, Godoy AL, Rosa A, Comas D, Kirchheiner J. Baseline perfusion and the serotonin transporter promoter polymorphism. *Biological Psychiatry*. 2010; 67:317–322. [PubMed: 19878929]
- Wang J, Zhang Y, Wolf RL, Roc AC, Alsop DC, Detre JA. Amplitude modulated continuous arterial spin labeling perfusion MR with single coil at 3T-feasibility. *Radiology*. 2005; 235:218–228. [PubMed: 15716390]

Figure 1. S and L groups showed differential relationships with BDI scores in amygdala. Top: Interactions between S and L groups (threshold was set as small volume corrected $p < 0.05$). Middle: Right amygdala activity negatively correlated with BDI scores in the L group (threshold was set as small volume corrected $p < 0.05$). Bottom: Right amygdala activity positively correlated with BDI scores in the S group (threshold was set as uncorrected $p < 0.05$).

Figure 2. Right amygdala perfusion as a function of depressive symptoms for a) S group and b) L group.

Table 1

Demographic, Depression Symptom, and Personality Scores by Genotype

Variable	L group mean (SD)	S group mean (SD)	<i>p</i> value
Age	20.6 (2.6)	20.0 (1.4)	0.44
Female / Male	6/9	8/7	0.46
BDI-II	7.1 (6.4)	6.5 (5.6)	0.81
Neuroticism	31.3 (6.5)	30.8 (6.5)	0.82
Extraversion	41.9 (6.0)	40.5 (7.1)	0.56

Note. BDI-II = Beck Depression Inventory, 2nd edition.

Table 2

Areas that showed significant association with BDI scores in the L/L and S/S groups. Significance levels were set as small volume corrected (SVC) $p < .05$ for amygdala activity and uncorrected $p < .001$ for other regions of interest.

Brain Regions	Coordinates [*]			Z Score	P Value		Cluster size (mm ³)
	X	Y	Z		uncorr.	SVC	
L group, negative correlation							
Right Amygdala	28	0	-18	3.63	<0.001	0.026	69
	20	2	-18	3.45	<0.001	0.041	
Left hippocampus	-28	-14	-16	3.19	0.001	-	30
L group, positive correlation							
Right Orbitofrontal	24	42	-14	3.20	0.001	-	43
S group, negative correlation							
Left Fusiform	-30	-68	-16	3.39	<0.001	-	34
S group, positive correlation							
Left Orbitofrontal	-28	40	-4	4.18	<0.001	-	65
Right Caudate	-12	30	-4	3.96	<0.001	-	37
Left Occipital	-34	-74	20	3.82	<0.001	-	51
Right Amygdala [*]	20	0	-18	1.95	0.026	0.73	6
S vs. L group							
Right Amygdala	20	0	-18	3.40	<0.001	0.046	58
Left Occipital	-30	-80	10	3.88	<0.001	-	43

* Note: Right amygdala activity survived only the threshold of uncorrected $p < 0.05$.