Genetic Variation in Serotonin Transporter Alters Resting Brain Function in Healthy Individuals

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Background: Perfusion functional magnetic resonance imaging (fMRI) was used to investigate the effect of genetic variation of the human serotonin transporter (5-HTT) gene (5-HTTLPR, SLC6A4) on resting brain function of healthy individuals.

Methods: Twenty-six healthy subjects, half homozygous for the 5-HTTLPR short allele (s/s group) and half homozygous for the long allele (l/l group), underwent perfusion functional and structural magnetic resonance imaging during a resting state. The two genotype groups had no psychiatric illness and were similar in age, gender, and personality scores.

Results: Compared with the l/l group, the s/s group showed significantly increased resting cerebral blood flow (CBF) in the amygdala and decreased CBF in the ventromedial prefrontal cortex. The effect of functional modulation in these regions by 5-HTTLPR genotype cannot be accounted for by variations in brain anatomy, personality, or self-reported mood.

Conclusions: The 5-HTTLPR genotype alters resting brain function in emotion-related regions in healthy individuals, including the amygdala and ventromedial prefrontal cortex. Such alterations suggest a broad role of the 5-HTT gene in brain function that may be associated with the genetic susceptibility for mood disorders such as depression.

Key Words: Amygdala, ASL perfusion fMRI, cerebral blood flow, depression, ventromedial orbitofrontal cortex

Recent advances in integrating noninvasive functional neuroimaging with genetics have enabled investigators to explore the associations between specific genes and the neural pathways that mediate individual differences in both normal and abnormal human behaviors, particularly those related to negative affect (for a review, see Hariri *et al.* 2006; Hariri and Holmes 2006; Hariri and Weinberger 2003; Wurtman 2005). Previous studies (Ansorge *et al.* 2004; Graspar *et al.* 2003; Lesch *et al.* 1996; Lotrich and Pollock 2004) have demonstrated the critical role of the serotonin neurotransmitter system in the development of emotional circuitry and the onset of mood disorders. Specifically, a polymorphism in the human serotonin transporter (5-HTT) gene (5-HTTLPR or SLC6A4) associated with 5-HTT protein expression and function has been shown to modulate the influence of stressful life events on depression (Caspi *et al.* 2003; Kendler *et al.* 2005) and the responses of the amygdala to negative stimuli. Evidence from several independent groups (Bertolino *et al.* 2005; Canli *et al.* 2005; Furmark *et al.* 2004; Hariri *et al.* 2002, 2005; Heinz *et al.* 2005) utilizing blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) consistently indicates increased activation in the amygdala in response to negative stimuli in healthy individuals who carry the short allele (s) versus healthy individuals carrying the homozygous long alleles (l). Such reactivity to negatively valenced stimuli is an important aspect of psychological and neural function, one that BOLD fMRI is well suited to measure.

However, whether and how the 5-HTTLPR genotype affects the brain's ongoing activity between the occurrences of negative external stimuli, arguably comprising the majority of its functioning, is still unknown. The present study therefore was designed to investigate the possibility of genetically driven differences in brain function during a resting baseline condition as a function of serotonin transporter genotype. Such information may be critical in interpreting the different manifestations of BOLD responses to aversive stimuli in long and short allele carrier groups. For example, Canli *et al.* (2005) have shown that apparent genotypic differences in response to negatively valenced pictures may actually result from different responses to the affectively neutral pictures of the baseline condition. Valid and reliable inferences of resting amygdala activity cannot be derived from BOLD fMRI studies per se, as BOLD fMRI measures only relative changes in neural activity. For this reason, we used arterial spin labeled (ASL) perfusion fMRI to measure resting brain function in two homozygous (s/s and l/l) groups. Using magnetically labeled arterial blood water as an endogenous tracer (Detre *et al.* 1992), ASL perfusion fMRI has been reported to provide reliable quantification of absolute cerebral blood flow (CBF) (in milliliters of blood per 100 g of tissue per minute), excellent reproducibility over long time periods, and reduced across-subject variability (Aguirre *et al.* 2002; Parkes *et al.* 2004; Wang *et al.* 2003). These features suggest that ASL perfusion fMRI provides a sensitive technique for reliable visualization of brain function during the resting state as well as during task performance.

The regions of interests (ROIs) in the present study include the amygdala and ventromedial prefrontal cortex (VMPFC). There is considerable evidence showing 5-HTTLPR genotype effects on both amygdala structure and function (for a review, see Hariri *et al.* 2006; Hariri and Holmes 2006; Hariri and Weinberger 2003). Abnormally elevated resting amygdala blood flow and metabolism in depressed patients relative to control subjects have been consistently reported, and increased resting

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activity has showed positive correlations with depression severity ratings (Abercrombie *et al.* 1996; Drevets *et al.* 1992, 1995; for a review, see Drevets 1999, 2000, 2003; Mayberg 2003). Based on these literatures and the increased likelihood of developing mood disorders in s carriers (Caspi *et al.* 2003; Kendler *et al.* 2005), we predicted that the 5-HTTLPR short allele would be associated with enhanced baseline amygdala activity in healthy individuals. For the VMPFC, two studies have demonstrated the effect of 5-HTTLPR genotype on the functional coupling and structural covariance between this area and the amygdala (Heinz *et al.* 2005; Pezawas *et al.* 2005). Although the observations of VMPFC changes in depression have been inconsistent in the literature (for a review, see Drevets 1999, 2000, 2003), there is evidence suggesting reduced activation in the posterior part of this area (Drevets *et al.* 1997). Furthermore, lateral and orbital prefrontal cortex (PFC) activation has been reported to correlate inversely with depression severity and amygdala metabolism (Drevets *et al.* 1992, 1995), suggesting a compensatory role of this area in amygdala-driven emotional responses (Drevets 1999). Based on these literatures and assuming an absence of mood differences between the s/s versus l/l group in the present study, we predicted that the effect of 5-HTTLPR genotype on resting activity of VMPFC may be reversed; specifically, lower VMPFC activity may be associated with the short allele to compensate for the enhanced amygdala activity in the s/s group.

To explore the possible relationship between the effects of 5-HTTLPR genotype on resting brain function and the structure of the amygdala and the VMPFC, perfusion fMRI was combined with optimized voxel-based morphometry (VBM), a quantitative morphometrical analysis of structural magnetic resonance imaging (MRI), to compare the gray matter volume between groups (Ashburner and Friston 2000; Good *et al.* 2001). We predicted that the genotype-specific resting functional difference in amygdala and VMPFC activation would not be accounted for by variations in the anatomy of these regions.

Methods and Materials

Participants

From a sample of 276 screened, healthy subjects, we recruited 30 subjects for the scanning (14 female subjects; all Caucasian; mean age 20.3 years, range 18 to 29 years). All subjects were neurologically intact with no reported history of head trauma and no current psychiatric diagnosis. Written informed consent was obtained in accordance with the Institutional Review Board of the University of Pennsylvania. Four subjects were excluded from the study due to problems in perfusion quantification. The remaining 26 subjects were divided based on the results of their prescreening genotype analysis, resulting in 13 participants homozygous for the short allele (s/s group) and 13 participants homozygous for the long allele (l/l group). The two genetic groups were similar in age and gender (both p's $> .2$). The Beck Depression Inventory II (Beck *et al.* 1996) and the NEO Five-Factor Inventory (Costa and McCrae 1992) were used as behavioral measures of self-reported depressive symptoms and the personality dimensions of each subject, respectively.

5-HTTLPR DNA Extraction and Genotyping

Each participant provided two buccal cell samples, scraping one Whatman Sterile Omni swab (Fisher Scientific, Inc., Pittsburgh, Pennsylvania) firmly against the inside of each cheek for 30 seconds. Swabs were air-dried for 2 hours. Genomic 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 the16 repeat l allele and a 375 base pair fragment for the 14 repeat s allele (Gelernter *et al.* 1997). Polymerase chain reaction (PCR) was then carried out on a Reaction Module (BioRad iCycler, #170-872, BioRad, BioRad Laboratories, Philadelphia, Pennsylvania), and the products were separated on a 2.5% agarose gel (Agarose SFR, Amresco Inc., Solon, Ohio) supplemented with ethidium bromide (.01%, Fisher Scientific) and visualized under ultraviolet light. Reliability analyses with a subset of 20 samples yielded 100% reliability.

Imaging Data Acquisition

A continuous ASL technique was conducted on a Siemens 3.0T Trio whole-body scanner (Siemens AG, Erlangen, Germany), using a standard transmit/receive head coil for perfusion fMRI scans (Wang *et al.* 2005b). Interleaved images with and without labeling were acquired using a gradient echo-planar imaging (EPI) sequence. Acquisition parameters consisted of the following: field of view (FOV) = 22 cm, matrix = 64 × 64, repetition time (TR) = 3 sec, echo time (TE) = 17 msec, label time = 1.6 sec, delay time = .8 sec, flip angle = 90°. The resting perfusion scanning protocol lasted 6 minutes during which subjects were instructed to "lie still and let their minds go blank, but keep their eyes open and stay awake." Fourteen slices (8 mm thickness with 2 mm gap) were acquired in sequential order from inferior to superior. Before the functional scan, high-resolution anatomic images were obtained by a 3D Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence with TR = 1620 msec, time to inversion (TI) = 950 msec, TE = 3 msec, flip angle = 15°, 160 contiguous slices, 1 × 1 × 1 mm resolution. Subjective mood ratings of anxiety and sadness on a scale from 0 to 100 (where 0 is maximal sadness or anxiety) were reported by each subject before and after each functional scan.

Functional Imaging Data Analysis

Functional and structural MRI data processing and analyses were carried out primarily with the Statistical Parametric Mapping software (SPM99 and SPM2, Wellcome Department of Cognitive Neurology, London, United Kingdom implemented in Matlab 6, Math Works, Natick, Massachusetts), with some additional modifications for perfusion analysis (http://cfn.upenn.edu/perfusion/software.htm).

For each subject, functional images were realigned to correct for head motion and coregistered with the anatomical image. Perfusion weighted image series were then generated by pairwise subtraction of the label and control images, followed by conversion to absolute CBF image series based on a single compartment continuous arterial spin labeling (CASL) perfusion model (Wang *et al.* 2005b). For each individual subject, one mean resting CBF image was generated, normalized to the Montreal Neurological Institute (MNI) template, smoothed, and then entered into the whole brain voxel-wise analysis using the general linear model (GLM). The GLM analysis was a two-sample t test with global CBF, age, and gender as three nuisance covariates to account for the variance associated with these variables. Activation clusters were identified for the whole brain at a significance level of uncorrected $p < .005$ and cluster size larger than 100 voxels. A small volume correction (SVC) based on

the a priori determined regions of interest was conducted on the activation results.

The ROIs in amygdala were determined a priori from an automated anatomical labeling ROI library (Tzourio-Mazoyer *et al.* 2002) in the SPM Marsbar toolbox (Brett *et al.* 2002). The ROI in VMPFC was the same as that used in a previous study (Heinz *et al.* 2005), which defined a 36 mm diameter sphere centered between the genu of the corpus callosum and the anterior pole. For each ROI and each subject, the quantitative global and regional CBF value was read out and calculated. Global corrected (relative) CBF values were calculated by normalizing the global CBF to 60 mL/100 g per minute. Using the SPSS 12 software package (SPSS Inc., Chicago, Illinois), two-sample *t* tests were performed to explore the difference between the two genotype groups. Multivariate regression analyses were performed first using genotype values as a predictor variable with global CBF and gender as covariates to explain the total variance of the absolute CBF values in the amygdala ROI. In addition, to explore whether the baseline CBF differences could be accounted for by variations in behavioral measurements, scores of personality and subjective mood ratings were included in multivariate regression analyses.

Structural Imaging Data Analysis

Structural images of all 26 subjects were analyzed using the optimized VBM protocol as described in previous studies (Canli *et al.* 2005; Good *et al.* 2001). The spatially normalized segments of each subject's gray matter images were modulated, smoothed, and entered into the whole brain GLM analysis, using a two-sample *t* test with total gray matter volume, age, and gender as three nuisance covariates. Gray matter volumes in each ROI were calculated with and without the correction of total gray matter volume. Two-sample *t* tests were performed on these values to explore the difference between the two genotype groups. To explore whether the resting CBF differences in amygdala can be accounted for by changes in amygdala volume, additional multivariate regression analyses were performed by including the amygdala or VMPFC gray matter volume, gender, and global CBF as four covariates along with short and long genotype values to explain the total variance of the absolute CBF values in the amygdala and VMPFC, respectively.

Results

Study demographics and the scores of behavioral measurements of 26 subjects are listed in Table 1. No significant differences were observed between the two genotype groups (all *p*'s $>$ .2). The self-reported mood ratings of anxiety (61.2 for l/l group vs. 72.3 for s/s group) and sadness (71.9 for l/l group vs. 81.2 for s/s group) also showed no significant differences between the two groups (both *p*'s $>$ .1).

Table 1. Demographics and Scores of Behavioral Measurement of the 26 Subjects

Variable	l/l Group (Mean ± SD)	s/s Group (Mean ± SD)	*p* Value
Age	20.8 ± 2.8	20.0 ± 1.3	.38
Female/Male	5/8	8/5	.26
BDI	5.9 ± 5.4	6.7 ± 6.1	.74
Neuroticism	31.6 ± 6.9	31.5 ± 6.7	.98
Extraversion	40.7 ± 5.3	40.8 ± 7.1	.95

BDI, Beck Depression Inventory.

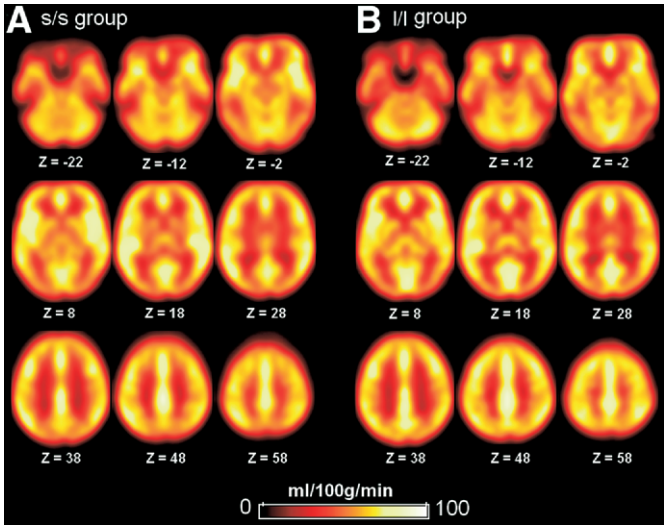

Figure 1. Quantitative resting CBF image for the s/s group **(A)** and l/l group **(B)**. CBF, cerebral blood flow; s, short allele; l, long allele.

The quantitative resting CBF images averaged from the s/s and l/l groups are shown in Figure 1. Resting CBF images showed perfusion signals in all brain regions with good sensitivity and illustrated clear contrast between gray and white matter in perfusion intensity. No difference was found between global CBF intensities across the two groups (60.6 and 61.0 mL/100 g per minute for l/l and s/s groups, respectively, $p = .9$). Mean CBF images showed enhanced CBF in basal ganglia and inferior temporal regions and reduced CBF in the anterior ventromedial prefrontal regions for the s/s group compared with the l/l group (see slice $Z = -12$ in Figure 1A and 1B, respectively).

The results from the analysis of voxel-wise general linear modeling are listed in Table 2 and illustrated in Figure 2. Comparing the s/s group with the l/l group, significantly greater CBF was found in a large cluster including bilateral amygdala regions and extending to the striatum, insula, parahippocampal gyrus, temporal pole, and posterior and lateral orbitofrontal cortex, while significantly less CBF was found in the anterior VMPFC (including orbitofrontal and gyrus rectus) and left parietal lobe. The areas in which the CBF differences survived small volume correction ($p < .05$) were the amygdala and VMPFC (Table 2).

Results from the ROI analyses that confirmed the GLM results are shown in Figure 3. After controlling for individual global CBF variance, the relative CBF for the s/s group compared with the l/l group was significantly higher in bilateral amygdala and lower in VMPFC (left amygdala: 61.9 vs. 54.8 mL/100 g per minute, $p = .04$; right amygdala: 58.9 vs. 51.5 mL/100 g per minute, $p = .03$; VMPFC: 51.9 vs. 62.6 mL/100 g per minute, $p = .001$; Figure 3A). The magnitudes of CBF changes were 13.4%, 14.3%, and −17.1%, respectively. The multivariate regression analysis using 5-HTTLPR genotype in addition to global CBF and gender as covariates revealed that genotype accounted for 17.2% ($p = 0.005$), 17.1% ($p = .01$), and 36.8% ($p < .001$) of the total variance in absolute baseline CBF in left amygdala, right amygdala, and VMPFC, respectively (Figure 3B). The effect of genotype on the resting CBF in these regions remained significant when the scores of personality and subjective mood ratings were included in the regression models (all *p*'s $< .05$, Table 3).

To explore the possible association between the influence of 5-HTTLPR genotype on brain function and anatomy, additional

Table 2. Resting CBF Differences Between the Two Genotype Groups

Brain Regions	MNI Coordinates X	Y	Z	T Score	Peak *p* Score	BA
Resting: s/s > l/l						
Left Amygdala	−20	−2	−26	4.74	$p^a < .001$ $p^b = .002$	–
Right Amygdala	30	−2	−26	3.55	$p^a < .001$ $p^b = .01$	–
Resting: l/l > s/s						
Bilateral Ventromedial Prefrontal Cortex	4	56	−20	4.98	$p^a < .001$ $p^b = .005$	BA 10/11
	−8	56	−18	4.96	$p^a < .001$ $p^b = .005$	
Left Parietal Cortex	−28	−40	44	3.88	$p^a < .001$	BA 40/7

CBF, cerebral blood flow; MNI, Montreal Neurological Institute; BA, Brodmann area; l, long allele; s, short allele.
[a]Uncorrected *p*.
[b]Small volume corrected *p*.

morphometrical analyses were performed on the high-resolution structural images. No significant difference was found in the volume of amygdala or VMPFC between the two groups (all *p*'s > .05). The effect of genotype on resting CBF in amygdala and VMPFC remained significant with the volume of amygdala and VMPFC included in the respective regression models (all *p*'s < .05; Table 3).

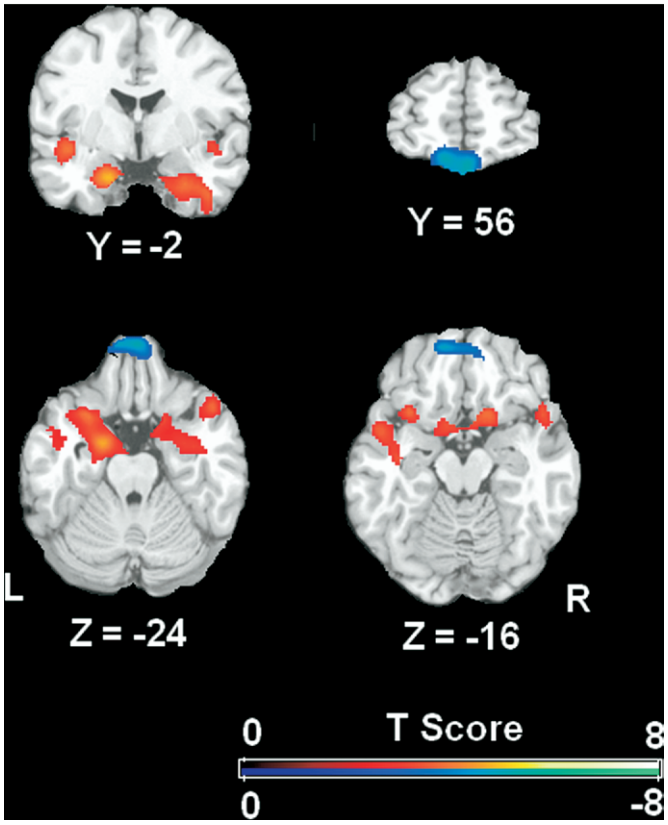

Figure 2. SPM maps from the GLM analysis showing the higher resting CBF in bilateral amygdala and temporal regions and lower resting CBF in ventromedial prefrontal regions for s/s group versus l/l group. GLM, general linear model; CBF, cerebral blood flow; s, short allele; l, long allele.

Discussion

Genomic neuroimaging studies provide emerging methods to understand human emotion and behavior. The combination of neuroimaging and genetic approaches will facilitate investigations of genotypes and the associated brain mechanisms that underlie the vulnerability to mood disorders (Drevets *et al.* 2000; Hariri *et al.* 2006; Hariri and Holmes 2006; Wurtman 2005). In line with this direction, the present study integrated perfusion-based functional MRI with genomic analysis to quantify and compare the resting brain CBF in the s/s and l/l 5-HTTLPR genotype groups. The results from voxel-by-voxel comparisons and the ROI analyses consistently demonstrated that the 5-HTTLPR genotype alters resting amygdala function in healthy individuals by increasing resting CBF in bilateral amygdala areas

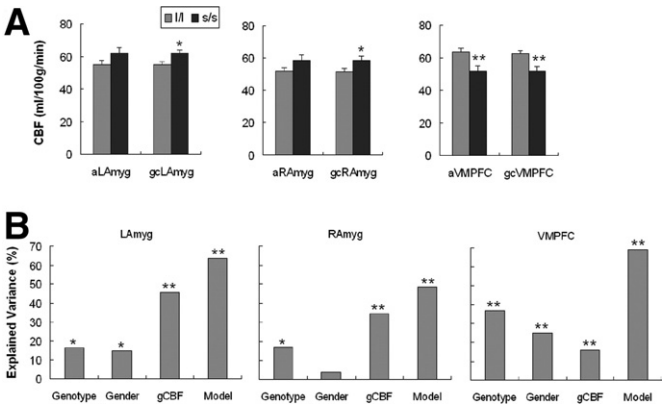

Figure 3. (A) Mean absolute and relative (global corrected) resting CBF values in left amygdala (aLAmyg, gcLAmyg), right amygdala (aRAmyg, gcRAmyg), and VMPFC (aVMPFC, gcVMPFC), respectively. After correction of global CBF variance, the resting CBF were significantly highly in bilateral amygdala but lower in VMPFC in the s/s group. **(B)** Genotype, global CBF (gCBF), and gender explained the absolute CBF in amygdala and VMPFC in multivariate regression models. Note genotype effects were significant in all these models. (*$p < .05$; **$p < .005$). CBF, cerebral blood flow; aLAmyg, absolute left amygdala; gcLAmyg, global corrected left amygdala; aRAmyg, absolute right amygdala; gcRAmyg, global corrected right amygdala; VMPFC, ventromedial prefrontal cortex; aVMPFC, absolute ventromedial prefrontal cortex; gcVMPFC, global corrected ventromedial prefrontal cortex; gCBF, global cerebral blood flow.

Table 3. The Multiple Regression Analysis of Variance of Resting CBF in Bilateral Amygdala and VMPFC Explained by the Genotype with Global CBF and Gender as Fixed Covariates and Personality Scores, Subjective Reported Mood Ratings, and Amygdala or VMPFC Volumes as Additional Covariates

	Left Amygdala		Right Amygdala		VMPFC	
Additional Covariate	Explained Variance	*p* Value	Explained Variance	*p* Value	Explained Variance	*p* Value
BDI	16.8%	.006	16.5%	.018	38.2%	<.001
Neuroticism	17.2%	.006	17.1%	.017	37.1%	<.001
Extraversion	17.3%	.006	17.2%	.017	37.6%	<.001
Anxiety	16.4%	.009	18.7%	.016	34.3%	<.001
Sadness	14.8%	.013	15.8%	.029	29.6%	<.001
Left amygdala Volume	20.4%	.003	–	–	–	–
Right Amygdala Volume	–	–	16.7%	.020	–	–
VMPFC Volume	–	–	–	–	40.6%	<.001

CBF, cerebral blood flow; VMPFC, ventromedial prefrontal cortex.

in short allele carriers. This alteration could not be accounted for by either variations in amygdala anatomy or variations in behavioral measurements. Given the higher vulnerability to environmental stress for developing depression in short allele carriers (Caspi *et al.* 2003; Kendler *et al.* 2005), the elevated resting amygdala activity in the s/s group in this study is consistent with abnormal resting and sustained amygdala activity in depression (Drevets 1999, 2000, 2003; Mayberg 2003; Siegle *et al.* 2002, 2003, 2006). Further, the current results suggest that increased resting amygdala activity may be a risk factor for developing mood disorders.

The critical role of the amygdala in processing aversive and negative emotions such as fear, in addition to processing the salience of environmental stimuli, has been widely accepted in both human and animal studies (Phan *et al.* 2002; Phelps and LeDoux 2005). Among the diverse emotion-related functions in which the amygdala may be involved, a primary function is the modulation of neural systems underlying cognitive and social behaviors in response to emotional cues (Anderson and Phelps 2000; Whalen 1998). Moreover, the amygdala is part of the limbic-paralimbic brain network that normally shows decreased hemodynamic activity during performance of attention-dependent cognitive tasks (Drevets *et al.* 2002; Drevets and Raichle 1998; Shulman *et al.* 1997). Further, the amygdala can process emotional signals automatically, irrespective of attention (Anderson *et al.* 2003; Vuilleumier *et al.* 2001) and awareness (Morris *et al.* 1998; Whalen *et al.* 1998). The heightened resting amygdala CBF in short allele carriers therefore may reflect enhanced neural excitability coupled with increased hemodynamic responses during the resting state. This automatic amygdala-mediated evaluating and processing of environmental and social cues may dynamically interact with ongoing cognitive processes in the context of subjective emotional states (Raichle *et al.* 2001; Simpson *et al.* 2001a, 2001b).

The present finding of enhanced resting amygdala activity provides a plausible explanation for the observed deactivation of amygdala with neutral stimuli in s carriers (Canli *et al.* 2005). Specifically, the deactivation in short allele carriers may be driven by the enhancement of amygdala activity during the baseline condition rather than by the reduction of amygdala activity in response to neutral stimuli. The consistently reported enhancement in neural reactivity to aversive stimuli in short allele carriers (Bertolino *et al.* 2005; Furmark *et al.* 2004; Hariri *et al.* 2002, 2005) may reflect the 5-HTTLPR genotype modulation of the neural processing of emotional stimuli rather than of nonemotional neutral stimuli. Moreover, a recent study by Canli *et al.* (2006) reported significant interactions between the 5-HTTLPR and life stress on neural activity in amygdala and other brain regions. This novel finding of a gene × environment interaction effect on neural reactivity is of particular importance given the evidence that the 5-HTTLPR and stress interact to predict depression (Caspi *et al.* 2003; Kendler *et al.* 2005) and provides additional clues to the mechanisms whereby stress leads to greater depression risk among carriers of the short allele.

It is unlikely that increased resting CBF in the amygdala associated with the short allele is simply accounted for by a heightened response to the noise and confinement associated with the magnetic resonance scanning environment. First, the self-reported anxiety or sadness scores revealed no difference in the behavioral responses to scanning for the two genotype groups. Second, previous studies have shown that the human amygdala activity may quickly inactivate during repeated exposures to the same aversive stimuli (LaBar *et al.* 1998). In the present study, the amygdala should have adapted to the potentially stressful stimuli related to the scanning environment when the resting perfusion scan began approximately 10 minutes after the subjects entered the scanner. Third, Canli *et al.* (2006), in a report published after submission of the present study, also found significantly higher resting amygdala CBF associated with the s allele using another ASL perfusion technique after BOLD scan, which replicated our finding and provided additional evidence for genotype-modulated amygdala resting activity. Finally, the control experiment of our recent perfusion fMRI study on stress (Wang *et al.* 2005a) did not observe any differences in stress responses to scanning, further reducing the possibility that our findings are due to the responses to the scanning environment.

In addition to the increased resting CBF observed in bilateral amygdala, reduced resting CBF was observed in the VMPFC for short allele carriers. Through the reciprocal connection with the amygdala, the VMPFC modulates amygdala activity for processing emotionally salient stimuli (Garcia *et al.* 1999; Ongur and Price 2000). Furthermore, the VMPFC is involved in the processing of both negative affect (Mayberg *et al.* 1999; Zald *et al.* 2002) and positive emotion such as reward (Kringelbach 2005) and humor (Goel and Dolan 2001). Given that the two genotype groups showed almost the same global CBF values and no differences in behavioral measurements of mood and personality, the short allele decreases in resting CBF in the VMPFC may reflect a compensatory modulation to offset the enhanced resting emotional processing in amygdala.

Although the region of interest in medial prefrontal cortex in the present study was the same as that used by Heinz *et al.* (2005), the peak location of the decreased CBF in VMPFC was about 1 cm inferior to their VMPFC location, where they showed

enhanced functional coupling between amygdala and VMPFC in s carriers compared with the l/l genotype. In addition, the location of our differential resting VMPFC activity was about 2 cm anterior and largely nonoverlapping with the area of subgenual anterior cingulate cortex (ACC) reported by previous studies in healthy and depression groups (Drevets *et al.* 1997; Pezawas *et al.* 2005; Siegle *et al.* 2006). That the genotype effect on resting CBF was present in amygdala but absent in subgenual ACC in healthy individuals suggests that depression may be more specifically associated with abnormal activity in subgenual ACC, which is supported by the observation that subgenual ACC activity predicts the response to depression therapy (Pizzagalli *et al.* 2001; Siegle *et al.* 2006). However, Pezawas *et al.* (2005) demonstrated a reduced functional connectivity between amygdala and the present VMPFC area in s carriers compared with l/l genotype. Such reduced connectivity may alter the feedback regulation of amygdala activity and contribute to the observed increase in amygdala activity during resting baseline and responding to aversive stimuli (Bertolino *et al.* 2005; Canli *et al.* 2005; Furmark *et al.* 2004; Hariri *et al.* 2002, 2005). The altered resting brain function in amygdala and VMPFC regions may therefore reflect a decreased regulatory capacity for emotional circuitry for adapting neural responses to affective stimuli. This reduced flexibility may subsequently increase the risk for developing mood disorders induced by life stress (Caspi *et al.* 2003; Kendler *et al.* 2005).

There were several limitations in the present study. First, due to the low signal-to-noise ratio for the present CBF time series, we did not perform the functional connectivity analysis on the resting perfusion data; thus, the genotype effect on the resting-state connectivity is still open to further study. Second, we included only the s and l homozygotes; the resting brain activation pattern associated with the heterozygous 5-HTTLPR genotype (s/l) is still unknown. Moreover, genotyping of the 5-HTTLPR did not include the recently reported A to G single nucleotide polymorphism (SNP) within the long allele (Beitchman *et al.* 2006; Hu *et al.* 2006) due to lack of sufficient DNA for additional genotyping. However, it is unlikely that the A to G SNP in the long allele would have a substantial impact on the results of the study, since the L_G allele (low-expressing long allele) frequency and the L_GL_G genotype frequency is relatively low in Caucasian populations (Hu *et al.* 2006). Finally, the present sample size ($n = 26$) was relatively small compared with a previous study (Pezawas *et al.* 2005; $n = 114$), which may account for our failure to observe the morphometrical difference in amygdala or subgenual ACC in this study. However, the significant genotype-dependent difference in amygdala and VMPFC resting CBF suggests a higher sensitivity of perfusion fMRI compared with the morphometrical analysis to further investigate the complex relationship between 5-HTTLPR genotype, brain structure, and neural activity.

In conclusion, we demonstrated an association of 5-HTTLPR genotype with resting amygdala and VMPFC function in the healthy human brain using perfusion-based functional MRI. Our findings add new evidence to an emerging genomic neuroimaging literature pointing to neural activity in emotional circuitry as an important mediator of the increased likelihood of developing mood disorders in short allele carriers.

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The first two authors contributed equally.

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