Published in final edited form as:

Genes Brain Behav. 2010 March 1; 9(2): 224–233. doi:10.1111/j.1601-183X.2009.00550.x.

Prefrontal Morphology, 5-HTTLPR Polymorphism, and Biased Attention for Emotional Stimuli

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Abstract

Biased attention for emotional stimuli has been associated with vulnerability to psychopathology. This study examines the neural substrates of biased attention. Twenty-three adult women completed high-resolution structural imaging followed by a standard behavioral measure of biased attention (i.e., spatial cueing task). Participants were also genotyped for the serotonin transporter-linked promoter region (5-HTTLPR) gene. Results indicated that lateral prefrontal cortex morphology was inversely associated with maintained attention for positive and negative stimuli, but only among short 5-HTTLPR allele carriers. No such associations were observed for the medial prefrontal cortex or the amygdala. Results from this study suggest that brain regions involved in cognitive control of emotion are also associated with attentional biases for emotion stimuli among short 5-HTTLPR allele carriers.

Keywords

structural MRI; genetic association; depression vulnerability; information processing

Prominent cognitive theories of depression (Beck, 1976, Teasdale, 1988) posit that biased processing of emotion stimuli is an important marker of depression vulnerability. Similarly, other theorists have asserted that the ability to allocate attention to emotion cues in the environment is a crucial element of adaptive self-regulation (Posner & Rothbart, 2000). Although it is adaptive for salient stimuli to capture attention, successful behavioral regulation requires flexibility and control over attention. This includes strategic filtering, timely disengagement, and being appropriately vigilant for meaningful emotion cues.

To understand factors that contribute to biased processing of emotion stimuli, we sought to identify neural substrates involved in processing of emotion stimuli. Our analyses will focus two cortical regions, lateral and medial prefrontal cortices, and one subcortical region, the amygdala (Johnstone *et al.*, 2007). The lateral prefrontal region includes primarily ventral lateral regions (Brodmann areas 45, 46, and 47) and the rostral middle frontal, pars

triangularis, and pars orbitalis regions (Brodmann areas 10, 11, 25, and 32). The medial prefrontal region includes the medial orbital frontal and rostral anterior cingulate regions. We identify these cortical and white matter regions in Figure 1.

The lateral prefrontal cortex (LPFC) has previously been implicated in cognitive regulation of emotional information (Ochsner *et al.*, 2002, Ochsner & Gross, 2005). It is involved in cognitive control in general, especially when competing responses have to be inhibited or new information is selected (e.g., Aron & Poldrack, 2005, Nee *et al.*, 2007). The ventral lateral PFC (vLPFC), in particular, is thought to modulate emotion responses through an attentional biasing mechanism that acts on subcortical regions, such as the amygdala (Wager *et al.*, 2008).

The medial PFC also has an important role in regulation of emotion information, particularly for the assessment of emotion experience and how it relates to the self (Beer & Ochsner, 2006, Craik *et al.*, 1999, Kelley *et al.*, 2002). For instance, medial PFC is recruited when comparing the desirability of self to others (e.g., Ochsner *et al.*, 2005), or when a negative emotional experience is anticipated (e.g., Ploghaus *et al.*, 1999). It is also involved in evaluating emotion stimuli, particularly when evaluating one's own emotion (Lee & Siegle, 2009).

The amygdala is a subcortical region that has repeatedly been implicated in detecting, attending to, and encoding into memory emotional information (Phelps & LeDoux, 2005, Whalen & Phelps, 2009). The amygdala produces rapid physiological and behavioral arousal in response to salient, emotion stimuli (Adolphs, 2008). In addition to influencing the activity of hypothalamic and brainstem autonomic centers, the amygdala also initiates activity in the ventromedial and orbitofrontal cortices (Whalen & Davis, 2001). A recent meta-analysis documented that the amygdala is consistently activated during emotion processing tasks (Kober *et al.*, 2008).

A recent study (Wager *et al.*, 2008) demonstrated that the ventral lateral PFC acts upon the amygdala to moderate emotional experience during cognitive reappraisal of emotion information (i.e., self-reported failure to effectively reappraise emotion stimuli). As expected, several medial prefrontal cortical regions, including the medial frontal pole, ventromedial PFC, and rostral medial PFC, also contributed to the experience of negative emotion. Thus, coordinated activity among the lateral PFC, medial PFC, and the amygdala were critically involved in the cognitive regulation and experience of emotion stimuli.

Based on this review, we hypothesized that lateral prefrontal cortex morphology may be an important neural substrate that underlies biased attention for emotional stimuli. To examine this possibility, we examined whether grey and white matter morphology in the lateral PFC is associated with individual differences in biased attention for negative and positive stimuli. To test for specificity, we also examined whether medial PFC and amygdala volume, regions that are hypothesized to be involved in the experience rather than control of emotion stimuli, were not associated with biased attention for emotion stimuli.

Participants in the current study were also genotyped for the serotonin transporter-linked polymorphic region (5-HTTLPR) gene because this polymorphism has been associated with morphology of the ventral lateral PFC (Canli *et al.*, 2005), as well as less functional coupling between regions of the prefrontal cortex and the amygdala (Pezawas *et al.*, 2005). 5-HTTLPR genotype predicts differential activation in limbic (including the amygdala), striatal, and cortical brain regions in response to negative, positive, and neutral word stimuli (Canli *et al.*, 2005). Given these findings, and evidence that brain morphology and function are associated (Brodmann *et al.*, 2009), we reasoned that lateral PFC morphology may be

particularly relevant to the processing of emotion stimuli for carriers of the low expressing 5-HTTLPR allele.

Methods

Participants

Participants were twenty-three female adults recruited from the Austin, Texas community (see Table 1 for demographic information). We recruited women only for this study in order to obtain a homogenous group of participants and because women are at significantly higher risk for depression than men (Kessler et al., 2003). Participants were recruited using flyers posted in the community and with ads posted on websites. Inclusion criteria included normal or corrected to normal vision, fluency in the English language, and age between 18 and 30. Exclusion criteria included elevated symptoms of depression (i.e., CESD > 16), current medication use, and any medical or physical conditions that would preclude participation in an imaging study (e.g., orthodontic braces). Participants are a subset from a previous report examining associations between diffusion tensor imaging and the 5-HTTLPR polymorphism who also completed the exogenous cueing task (Pacheco *et al.*, 2009).

Assessments

Center for Epidemiological Studies Depression Scale (CESD; Radloff, 1977)—

This measure was used to exclude individuals with higher levels of depressive symptoms. The CESD consists of 20 items assessing a wide range of depressive symptoms and the frequency with which they are experienced. Participants use a 4-point scale to indicate the frequency with which they have experienced a given symptom in the past week. Participant scores range from 0 to 60, with the threshold of 16 or greater representing a putative indication of depression (Radloff, 1977). Previous research indicates acceptable levels of internal consistency (alpha coefficient = 0.87) and test-retest reliability ($r = 0.51$) (Hann *et al.*, 1999).

Imaging—The primary indices of gray matter (GM) and white matter (WM) volume were measured from T1-weighted MR images of the brain. Two high resolution SPGR, T1-weighted scans, were collected from each participant using 3 Tesla GE MRI equipped with an 8 channel phased array head coil (GE Medical Systems, Milwaukee Wisconsin) using the following image parameters: TR = 6000 ms, TE = 1.2 ms, flip angle = 11°, slice thickness = 1.3 mm, 176 slices, FOV = 256×256 mm. Slices were collected in the sagittal plane and these scans were set up to optimize for high contrast between GM and WM, and GM and cerebrospinal fluid (CSF). The two T1 scans were first motion corrected, averaged, and resampled into 1mm³ voxels to create a single high signal, high contrast volume.

The primary gray matter analysis procedures have been described in detail and validated in a number of publications. The technical details can be found in those reports (Dale *et al.*, 1999, Fischl & Dale, 2000, Fischl *et al.*, 2001, Fischl *et al.*, 2002, Fischl *et al.*, 2004a, Fischl *et al.*, 1999, Salat *et al.*, 2004). For this study, T1 images were analyzed using computerized reconstruction of the cortical surface (<http://surfer.nmr.mgh.harvard.edu>) to produce cortical thickness and volume measurements for predefined parcellated and segmented units. This analysis involves a multistep procedure that includes intensity normalization, stripping of non-brain tissue, and gray and white matter segmentation.

The distance between the GM/WM boundary and the outer cortical surface was used to calculate cortical thickness at each point across the cortical mantle (Salat *et al.*, 2004). Following cortical reconstruction and segmentation an algorithm was implemented that automatically assigned a neuroanatomical label to each voxel in an MR volume based on a

probabilistic atlas of class statistics derived from a manually labeled training set (Fischl *et al.*, 2002, Fischl *et al.*, 2004b). This technique has been shown to be comparable in accuracy and reliability to manual labeling in normal brains (Fischl *et al.*, 2002). Volumetric measurements were automatically calculated from neural structures with this procedure and labeled as they correspond to a predefined atlas (Desikan *et al.*, 2006).

Regional WM volumes were obtained as described in Salat *et al.* (2009). The cortical parcellation was used to generate a Voronoi diagram based on the distance to the closest cortical parcellation label, using a distance constraint of 5-mm to ensure exclusion of centrum semiovale and periventricular regions. Thus, a complete labeling of the cerebral WM was created with each Voronoi polygon adopting the label of the parcellation unit producing 'cortically associated' gyral WM labels. The current analysis focused on WM regions associated with the lateral and medial PFC regions.

Genotyping—Genomic DNA were isolated from buccal cells using a modification of published methods (Freeman *et al.*, 1997, Lench *et al.*, 1988, Meulenbelt *et al.*, 1995, Spitz *et al.*, 1996). The cheeks and gums are rubbed for 20 s with three sterile, cotton-tipped wooden swabs. The swabs are placed in a 50-ml capped polypropylene tube containing lysis buffer (500 μ l of 1 M Tris-HCl; 200 mM disodium ethylene diaminetetracetic acid (EDTA), pH 8.0; 500 μ l of 10% sodium dodecyl sulfate; and 100 μ l of 5 M sodium chloride). Participants then rinse out the mouth vigorously with 10 ml of distilled water for 20 seconds and this was added to the 50-ml tube. The tubes were stored at 4°C until the DNA was extracted.

To extract the DNA, proteinase K (0.2mg/ml) was added to the tubes and the tubes were incubated at 65°C for 60 min. The swabs were removed and residual lysis buffer was extracted by centrifugation (using a 3-ml syringe barrel and sterile 15ml tube) for 5 minutes at 1000 $\times$ g. The residual fluid was added back to the original sample. An equal volume of isopropyl alcohol was then added to each tube, the contents were mixed, and the DNA was collected by centrifugation at 3500 $\times$ g for 10 min. The DNA pellet was rinsed once with 1ml of 50% isopropyl alcohol and allowed to air dry. The pellet was resuspended in 1 ml of 10 μ M TRIS-HCl, 10mM EDTA buffer (pH 8.0) and place in a 1.8-ml cryovial.

The 5-HTT gene (SLC6A4), which maps to 17q11.1-17q12, contains a 44 bp deletion in the 5' regulatory region of the gene (Heils *et al.*, 1996). The VNTR in the promoter appears to be associated with variations in transcriptional activity: the long (L) variant has approximately three times the basal activity of the shorter (S) promoter with the deletion (Lesch *et al.*, 1996). The assay is a modification of the method of Lesch and colleagues (Lesch *et al.*, 1996). The primer sequences are: forward, 5'-GGCGTTGCCGCTCTGAATGC-3' (fluorescently labeled), and reverse, 5'-GAGGGACTGAGCTGGACAACCAC-3'. These primer sequences yield products of 484 or 528 bp. Two investigators scored allele sizes independently and any inconsistencies were reviewed and rerun (less than 20% of sample). Using this approach, allele frequencies were SS: $n = 5$ (29.7%), SL: $n = 10$ (43%), LL: $n = 8$ (34%). Genotype distribution was in Hardy-Weinberg equilibrium, $\chi^2 = 0.31$, $p = .58$. Consistent with previous imaging research indicating a dominant S allele effect (Hariri *et al.*, 2005) and to maximize statistical power, the S and SL alleles were combined to form an S-carrier group ($n = 15$) and were compared to the LL homozygotes ($n = 8$).

Spatial cueing task—This task is based upon the Attention Network Task developed by Posner (1980) and modified by others to incorporate emotional cues (Koster *et al.*, 2005). Each trial sequence (shown in Figure 2) began with a fixation cross presented in the center of visual field for 500ms followed by a face cue presented in left or right visual field for

1000ms. After cue offset, a probe (either * or **) appeared on left or right side of visual field for 250ms. Participants were to identify probe type by pushing a corresponding response button as quickly and accurately as possible. After the participant responded, the screen was blank for 500ms before the next trial began. Importantly, for 75% percent of trials probes appeared on the same side of visual field as the cue (a valid trial) and for 25% percent of trials probes appeared on the opposite side of visual field as the cue (an invalid trial). Both valid and invalid trials had a 50% chance of having either the single or double asterisk probe. Because some trials (i.e., invalid cues) measure shifting of attention, a probe with one or two dots is used to ensure that participants fixate on the target prior to indicating response (see Figure 2).

Cue stimuli were images from the “NimStim” collection developed by the Research Network on Early Experience and Brain Development (Tottenham et al., in press). We selected human faces because facial expressions receive special processing priority (Farah *et al.*, 1998), they have been used extensively in behavioral and imaging studies, and are arguably more ecologically valid than written words. We selected 27 faces from each of the following categories: happy, sad, and neutral. All stimuli were back projected into a screen mounted in the MRI bore and viewed through a mirror mounted on the head coil above the participant. Visual angle was approximately 16.5° from center of fixation cross to center of facial cue. Functional imaging data were collected during this task and will be presented in a separate report.

There were 324 trials presented in 4 blocks with the 6 conditions (3 cue categories $\times$ 2 cue validity conditions) distributed equally across each block. Each block consisted of 81 trials: 20 valid trials for each stimulus category and 7 invalid trials per category. Order of cue and target presentation was randomized. Participants were given short breaks between blocks and the entire task took approximately 30 minutes to complete.

We were primarily interested in measuring biased attention with the spatial cueing task. As suggested by Mogg et al. (2008), a general measure of attentional bias can be derived from the spatial cueing task by subtracting the cue validity effect for emotion and non-emotion cues using the following formula:

$$\text{Attentional bias score} = (\text{RT invalid emotion cue trials} - \text{RT valid emotion cue trials}) - (\text{RT invalid neutral cue trials} - \text{RT valid neutral cue trials}) \quad (1)$$

If the cue validity effect is stronger for emotion cues than neutral cues, we would observe a positive biased attention score for emotion stimuli. This would indicate attention was maintained by emotion cues to a greater extent than neutral cues. That is, shifting attention away from the invalid cue location takes longer for emotion cues than neutral cues (Mogg et al., 2008). In contrast, negative values indicate maintained attention for neutral cues relative to emotion cues. Mogg et al. (2008) demonstrated this approach is an ideal measure of attentional bias, as it does not confound attentional cueing and response slowing effects and this index is comparable to attentional bias scores obtained with other tasks (e.g., dot-probe task).

Procedure

Participants contacted the Mood Disorders Laboratory at the University of Texas at Austin expressing an interest in study participation. Participants were screened for current medication, current depressive symptoms, and contraindications for participation in an imaging study (e.g., orthodontic braces). Participants who qualified were scheduled for an appointment where they provided informed consent, completed the CESD, and provided

buccal cells via a cheek swab/mouthwash procedure for genotyping. Next, participants completed structural imaging scans followed by the spatial cueing task (and other tasks not included in this report). Upon completion of study procedures, participants were debriefed and paid \$65 for participation. The Internal Review Board at the University of Texas at Austin approved all study procedures.

Results

Sample Characteristics

Descriptive statistics are presented in Table 1 stratified by 5-HTTLPR allele status. There were no significant differences as a function of 5-HTTLPR allele grouping for age, $F(1, 22) = 0.52, p = .47$, ethnicity, $\chi^2(1, N = 23) = 0.18, p = .67$, race, $\chi^2(1, N = 23) = 0.18, p = .38$, depressive symptoms, $F(1, 23) = 2.52, p = .12$, and surface area of the inner cranial vault, $F(1, 23) = 0.03, p = .88$. Further, surface area of inner cranial vault, a standard measure of head size, was not significantly correlated with biased attention for happy ($r = -.17, p = .44$) or sad ($r = -.18, p = .41$) stimuli; thus, uncorrected structural volumes were used in analyses.

Data Reduction

Trials with incorrect responses or reaction times that were at least 2.5 standard deviations above participants' mean for each run were considered outliers and deleted (3.5% of all trials).

Main results

Hierarchical multiple regression examined associations between structural volumes in regions of interest and biased attention for happy and sad cue stimuli among the 5-HTTLPR groups. For lateral PFC, left and right hemisphere white and grey matter volumes for rostral middle frontal, pars triangularis, and pars orbitalis were combined. These are parcellated separately for the Desikan *et al.* (2006) atlas, but there is no indication from the literature on emotional control and lateral PFC that these regions are functionally differentiated. For medial PFC, we combined white and grey matter volume for medial orbitofrontal and rostral anterior cingulate cortex regions. For each analysis, independent variables were brain region volume, 5-HTTLPR status (S carrier, LL homozygote), and brain region $\times$ 5-HTTLPR status interaction. In the absence of a significant interaction, analyses were performed with the interaction term removed to test for presence of main effects. Results were empirically examined for influential cases and, in cases where influential cases were identified, analyses were re-run with influential cases removed. Results are reported with and without influential cases.

Lateral PFC

For biased attention for happy cue stimuli, analyses indicated a significant lateral PFC $\times$ 5-HTTLPR allele status interaction ($B = -.004, SE = .002, t = -2.82, p = .01$), a non-significant lateral PFC volume main effect ($B = .001, SE = .001, t = 1.20, p = .24$), and a significant 5-HTTLPR allele status effect ($B = 315.03, SE = 113.59, t = 2.77, p = .01$). Follow-up of the interaction indicated a significant negative association between biased attention and lateral PFC volume among short 5-HTTLPR allele carriers ($r = -.63, p = .01$) and a non-significant positive association among long 5-HTTLPR homozygotes ($r = .38, p = .31$). The difference between these correlations was significant ($z = 2.35, p = .02$). This model explained 32% of the variance in bias for happy cue stimuli.

For biased attention for sad cue stimuli, analyses indicated a non-significant interaction between lateral PFC volume $\times$ 5-HTTLPR allele status ($B = -.002, SE = .001, t = -1.57, p$

= .13). The main effects for 5-HTTLPR allele status ($B = -.001$, $SE = .001$, $t = -1.66$, $p = .12$) and 5-HTTLPR allele status were also non-significant ($B = 3.85$, $SE = 11.67$, $t = 0.33$, $p = .74$). However, regression diagnostic plots indicated an influential observation (case #29; Cook's Distance = 1.04, which greatly exceeds the recommended cut-off of $4/n$). With this participant removed, a significant interaction was observed ($B = -.002$, $SE = .001$, $t = -2.85$, $p = .01$). Follow-up analyses with outlier removed indicated a negative correlation between bias for sad cue stimuli and lPFC volume among short 5-HTTLPR carriers and a positive correlation among long 5-HTTLPR allele homozygotes ($r = -.63$, $p = .008$; $r = .47$, $p = .20$, respectively). The difference between these correlations was statistically significant ($z = 2.56$, $p = .01$). This model explained 34% of the variance in bias for sad stimuli (see Figure 3).¹

Medial PFC

For biased attention for happy cue stimuli, analyses indicated non-significant effects for mPFC $\times$ 5-HTTLPR allele status interaction ($B = -.01$, $SE = .007$, $t = -1.73$, $p = .10$), mPFC volume ($B = -.001$, $SE = .003$, $t = 0.46$, $p = .64$), and 5-HTTLPR allele status ($B = -4.27$, $SE = 18.82$, $t = -0.23$, $p = .82$). A similar pattern emerged for biased attention for sad cue stimuli. Non-significant effects for mPFC $\times$ 5-HTTLPR allele status interaction ($B = .002$, $SE = .004$, $t = 0.36$, $p = .73$), mPFC volume ($B = -.003$, $SE = .002$, $t = -1.39$, $p = .18$), and 5-HTTLPR allele status ($B = 1.73$, $SE = 11.78$, $t = 0.15$, $p = .89$). mPFC volume was not significantly related to biased attention for happy or sad emotion cues.

Amygdala Volume

For biased attention for happy cue stimuli, analyses indicated non-significant effects for amygdala volume $\times$ 5-HTTLPR allele status interaction ($B = -.05$, $SE = .04$, $t = -1.12$, $p = .28$), amygdala volume ($B = -.01$, $SE = .02$, $t = -0.61$, $p = .55$), and 5-HTTLPR allele status ($B = -6.98$, $SE = 19.19$, $t = -0.36$, $p = .72$). A similar pattern emerged for biased attention for sad cue stimuli. Non-significant effects for amygdala $\times$ 5-HTTLPR allele status interaction ($B = -.01$, $SE = .03$, $t = -0.38$, $p = .71$), amygdala volume ($B = -.02$, $SE = .02$, $t = -1.60$, $p = .12$), and 5-HTTLPR allele status ($B = -2.74$, $SE = 11.90$, $t = -0.23$, $p = .81$). Amygdala volume was not significantly related to attentional biases for happy or sad emotion cues.

5-HTTLPR associations with brain morphology

We next examined whether 5-HTTLPR allele status was associated with vlPFC, mPFC, and amygdala volume. There were no significant differences between 5-HTTLPR groups (S-carriers, LL homozygotes) for any regions: vlPFC, $F(1, 22) = 0.37$, $p = .54$, mPFC, $F(1, 22) = 0.01$, $p = .91$, and amygdala, $F(1, 22) = 1.05$, $p = .32$ (see Table 1).

Behavioral Results for Cue Validity Task

A 2 (cue validity: valid, invalid) $\times$ 3 (cue valence: sad, happy, neutral) $\times$ 2 (5-HTTLPR allele status; S carrier, LL) repeated measures analysis of variance tested for differences in reaction time to identify target. Analyses indicated a significant cue validity effect, $F(1, 22) = 4.99$, $p = .04$, that was moderated by a significant cue validity $\times$ cue valence interaction, $F(2, 44) = 3.26$, $p = .05$. The main effects for cue valence, $F(2, 44) = 0.23$, $p = .79$, 5-HTTLPR allele group, $F(1, 22) = 0.54$, $p = .47$, cue validity $\times$ 5-HTTLPR allele group, $F(1, 22) = 0.34$, $p = .57$, cue valence $\times$ 5-HTTLPR allele group, $F(2, 44) = 0.13$, $p = .87$, and the three way interaction, $F(2, 44) = 0.07$, $p = .93$, were not significant.

¹Results for left and right hemisphere vlPFC, mPFC, and amygdala volumes were very similar when analyzed separately; thus, left and right volumes were combined and analyzed as a single variable for each region.

To decompose the significant cue validity $\times$ cue valence interaction, we first computed a cue validity score (i.e., reaction time following invalid cues – reaction time following valid cues) for each cue valence and then compared reaction times across cue valences. Results indicated no differences for cue validity scores for happy and sad cues ($t(23) = 0.47, p = .64$); however, cue validity score was significantly longer for happy and sad cues compared to neutral cues ($t(23) = 2.67, p = .01$ and $t(23) = 2.17, p = .04$, respectively). Thus, participants had greater maintained attention for happy and sad cues compared to neutral cues (see Figure 4).

Discussion

This overarching goal of the current study was to identify morphological underpinnings of biased attention for emotion cues. We expected individual differences in lateral prefrontal regions (i.e., areas involved in cognitive control of emotion) to be associated with biased attention for emotion stimuli. Medial prefrontal and amygdala volumes, regions involved in the experience (but not control) of emotion, were not expected to be associated with biased attention for emotion cues. Further, we hypothesized that the 5-HTTLPR polymorphism may moderate these associations, given that the 5-HTTLPR polymorphism modulates a cortical-limbic circuit implicated in the regulation of emotion (Pezawas et al., 2005).

Using high-resolution structural brain imaging, morphology of the lateral prefrontal cortex was strongly associated with biased attention for emotion stimuli among short 5-HTTLPR allele carriers. Although there were no 5-HTTLPR allele group differences for lateral prefrontal volume, the morphology of this region was strongly associated with biased attention for positive and negative stimuli among short 5-HTTLPR allele carriers—smaller volumes were associated with greater biased attention. Attentional biases were not significantly associated with morphology of the medial prefrontal region and the amygdala, suggesting the effects were specific to the lateral prefrontal region. Importantly, these effects were observed among a sample of healthy, non-depressed, non-medicated women. Therefore, the results from the current study are not a symptomatic outcome of altered mood state.

The 5-HTTLPR polymorphism may moderate the association between lateral PFC morphology and biased attention for emotion stimuli because the 5-HTTLPR polymorphism impacts a cortical-limbic circuit that is critical for regulating emotional information. For instance, among healthy participants, Pezawas et al. (2005) used fMRI to assess relative activation of the pACC and amygdala in response to negative stimuli (e.g., angry and scared facial expressions) and found that short 5-HTTLPR allele carriers had less functional coupling between the pACC and the amygdala. The “uncoupling” of this emotion circuit may explain why short-allele carriers have greater amygdala responses to emotional stimuli (Bertolino et al., 2005, Hariri et al., 2005, Hariri et al., 2002). Given heightened neural reactivity, lateral prefrontal morphology may be particularly important for successful regulation of emotion stimuli among carriers of the low expressing 5-HTTLPR alleles.

Brain region volumes included both white and grey matter. White matter volume reflects the density of white matter (i.e., axonal myelin and fiber tracts) and is critical to the transmission of electrical signals that guide the performance of higher order cognitive functions (Bartzokis, 2004). As a result, decreased white matter volume in lateral prefrontal regions may reflect lower functional connectivity between cortical regions that are critical to cognitive control of emotional responses to stimuli. This may be particularly important for regulating emotion among short 5-HTTLPR allele carriers, due to enhanced reactivity (and therefore greater regulatory demand) to emotional stimuli.

So what factors might contribute to individual differences in development of the prefrontal cortex? There are many possibilities, including, genetics, nutrition, toxins, bacteria, viruses, hormones, among others (Giedd, 2004). One intriguing possibility is engagement (or lack thereof) of the lateral prefrontal cortex during important developmental periods (i.e., when arborization and pruning occurs) may have influenced white and grey matter development in those regions. There are a number of reasons why some individuals may engage this region less often (e.g., differential exposure to environmental stress, coping styles, genetic propensity, etc.), but regardless of etiology, this differential development may have better equipped some individuals for cognitive regulation of emotion. Given that the prefrontal cortex continues to develop until early adulthood (Barnea-Goraly et al., 2005), many opportunities likely exist for interactions with environmental stressors to impact the morphology of this region, particularly for short 5-HTTLPR allele carriers who have heightened sensitivity to environmental stress (Gotlib *et al.*, 2008).

It was notable that morphological associations with biased attention were, for the most part, consistent for positive and negative emotion cues. That is, neural substrates underlying attentional biases were similar even though the correlation between biased attention for positive and negative stimuli was moderate ($r = .44$). This suggests that the lateral prefrontal region plays a critical role for regulating emotional information in general (Ochsner & Gross, 2005). Most prior research has studied regulation of negative stimuli, so this possibility remains largely untested. Additional research examining regulation of positive and negative stimuli is needed to further refine current models of emotion regulation (cf. Britton *et al.*, 2006).

While these findings document relations between genetic, neural, and cognitive risk factors for depression, future work must continue to identify factors mediating these relationships. Specifically, it remains unclear how 5-HTTLPR variation influences the expression of specific neurological differences that may, in turn, produce cognitive and behavioral risk factors for depression. Individuals with a history of major depression (and suicide) have significantly reduced serotonin transporter receptor density in prefrontal cortical regions (including dramatic reductions in vlPFC); however, these differences appear to be unrelated to 5-HTTLPR variation (Arango *et al.*, 1995, Mann *et al.*, 2000). Future efforts must focus on identifying plausible mediating factors for observed relations between genes, brain structure, and attentional bias.

Future work should consider testing associations between lateral prefrontal morphology and biased attention following dysphoric mood manipulations. Cognitive biases are more likely to be revealed following dysphoric mood provocations (Scher *et al.*, 2005) and increased mood-linked negative thinking predicts onset of major depressive disorder (Segal et al., 2006). Given that short 5-HTTLR allele carriers appear to be more susceptible to self-referent negative thinking following a dysphoric mood induction (Beevers *et al.*, 2009), lateral prefrontal morphology may be an important predictor of biased processing of emotional stimuli following dysphoric mood inductions. Further, individual differences in lateral prefrontal morphology may explain why carriers of the low expressing 5-HTTLPR have difficulty regulating emotional states in the context of life stress (Caspi et al., 2003).

Several limitations of this study should be noted. First, this study only included female participants. Women are twice as likely to experience major depressive disorder than men (Kessler et al., 2003), so they are an appropriate group to recruit for a depression vulnerability study. However, additional work is needed to determine whether our findings are applicable to men. As with any genetic association study, population stratification is a potential concern (Hutchison *et al.*, 2004). Population stratification occurs when cases and controls differ with respect to their ethnic background or another variable that may have led

to a pattern of non-random mating. In our study, this confound is unlikely as 5-HTTLPR allele frequencies did not differ across race or ethnicity. Third variable explanations, such as the 5-HTTLPR promoter polymorphism is in linkage disequilibrium with another functional genetic marker, should also be considered as explanations for the observed effects. Finally, future research with larger samples should examine the newly identified single nucleotide polymorphism that occurs at the sixth nucleotide (adenine to guanine; A to G) in the first of two extra 20 to 23 bp repeats in the L allele (Hu et al., 2005).

Nevertheless, we believe this study makes an important and interesting contribution to identifying neural substrates that contribute to biased processing of emotion cues. Individuals with lower grey and white matter volumes in lateral prefrontal regions who inherit the short variant of the 5-HTTLPR gene display significant attentional biases for positive and negative stimuli. This focus on emotional aspects of the environment, in turn, may increase sensitivity to life stress and place these individuals at greater risk for depression. Additional work is now needed that examines complex etiological models of depression that link these various mechanisms of risk. Studying mechanisms of risk across levels of analyses (genetic, neural, cognitive, environmental) will facilitate development of comprehensive models of depression vulnerability and further our understanding of this debilitating disorder.

Acknowledgments

Preparation of this article was facilitated by a grant (R01MH076897) from the National Institute of Mental Health to Christopher Beevers and shared equipment grants (1S10RR023457-01A1) from the National Center for Research Resources and the Department of Veteran Affairs to John McGeary.

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Figure 1.

Prefrontal regions examined in the current study. Panel A is a coronal slice with all ten cortical and associated white matter regions of the prefrontal cortex displayed. The left side of panel B is a medial sagittal slice (showing the medial orbital frontal and the rostral anterior cingulate) and the right side of panel B is a lateral sagittal slice (showing rostral middle frontal, pars triangularis, and pars orbitalis).

Figure 2. Stimuli sequence for valid and invalid trials for the spatial cueing task. Cue stimuli are not to scale for illustration purposes. Blank screen duration varies in order to optimize temporal jitter.

Figure 3.

Associations between vIPFC, mPFC, and amygdala volume and biased attention for sad and happy stimuli as a function of 5-HTTLPR allele status. The dashed and solid regression lines are for the LL and S carrier group, respectively.

Figure 4.

Box-and-whisker plot for cue validity score presented by cue valence condition and 5-HTTLPR allele status. Diamonds indicate group mean. Data points are plotted within group. Whiskers represent 5th and 9th percentile.

Table 1

Study 1 demographics presented by 5-HTTLPR allele status. There are no significant group differences for these variables.

	LL (<i>n</i> = 8)	S carrier (<i>n</i> = 15)
Age (years)	21.25 (2.19)	22.07 (2.79)
Hispanic (Yes / No)	12% / 88%	7% / 93%
Race (Caucasian / Other)	63% / 37%	53% / 47%
Household income (mean)	\$96,250	\$61,057
Depressive symptoms (CESD)	5.50 (3.66)	7.53 (4.52)
Inner cranial vault volume (mm ³)	1343619.15 (245870.15)	1334592.42 (224908.08)
vlPFC volume (mm ³)	75372.89 (12580.15)	78231.00 (10049.92)
mPFC volume (mm ³)	21995.56 (2675.05)	22113.80 (2456.37)
Amygdala volume (mm ³)	3382.44 (462.89)	3215.47 (333.00)