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Serotonin transporter genotype modulates amygdala activity during mood regulation

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Recent studies have implicated the short allele of the serotonin transporter-linked polymorphic region (5-HTTLPR) in depression vulnerability, particularly in the context of stress. Several neuroimaging studies have shown that 5-HTTLPR genotype predicts amygdala reactivity to negatively valenced stimuli, suggesting a mechanism whereby the short allele confers depression risk. The current study investigated whether 5-HTTLPR genotype similarly affects neural activity during an induced sad mood and during recovery from sad mood. Participants were 15 homozygous short (S) and 15 homozygous long (L) individuals. Regional cerebral blood flow was measured with perfusion functional magnetic resonance imaging during four scanning blocks: baseline, sad mood, mood recovery and following return to baseline. Comparing mood recovery to baseline, both whole brain analyses and template-based region-of-interest analyses revealed greater amygdala activity for the S *vs* the L-group. There were no significant amygdala differences found during the induced sad mood. These results demonstrate the effect of the S allele on amygdala activity during intentional mood regulation and suggest that amygdala hyperactivity during recovery from a sad mood may be one mechanism by which the S allele confers depression risk.

Keywords: mood; genetics; amygdala; depression; cognitive neuroscience

INTRODUCTION

One of the most widely studied polymorphisms in psychiatric behavioral genetics is the serotonin transporter-linked polymorphic region (5-HTTLPR), of which the 'short' (S) allele is 43 base pairs shorter than the 'long' (L) allele (Wendland *et al.*, 2006). Given its location in the promoter region upstream from the serotonin transporter genetic locus, 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). The S allele has been associated with a number of psychiatric conditions, including anxiety and neuroticism (Lesch *et al.*, 1996), psychosis (Shcherbatykh *et al.*, 2000) and perhaps most notably, depression (Lotrich and Pollock, 2004).

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Although a large volume of research has linked the 5-HTTLPR to depression, scientists are just beginning to discover possible mechanisms by which the 5-HTTLPR confers risk for depression. Several recent studies have revealed particular environmental inputs that interact with the S allele to lead to depression. The first of these studies showed that stressful life events (e.g. illness, financial problems) were more depressogenic for carriers of the S allele than they were for individuals homozygous for the L allele (Caspi *et al.*, 2003). Similar interactions have been reported among other adult samples (Kendler *et al.*, 2005) and among children (Kaufman *et al.*, 2004).

Recent studies in this area have begun to identify the more proximal effects of this genetic variability on human behavior, physiology and neural systems. Gotlib and colleagues, for example, have identified effects of the 5-HTTLPR on cortisol levels, both upon awakening (Chen *et al.*, 2009) and in response to a stressor (Gotlib *et al.*, 2008). Recent neuroimaging studies have begun to delineate the neurobiological pathways whereby the 5-HTTLPR increases risk for depression. A handful of studies have shown that certain brain areas—especially the amygdala—are selectively affected by 5-HTTLPR variability. The first such study (Hariri *et al.*, 2002) found that carriers of the S allele showed greater right amygdala reactivity in response to fearful or angry faces [stimuli known to reliably produce amygdala activity; Morris *et al.* (1996)]. Independent research labs have

replicated the greater amygdala reactivity among carriers of the S allele (e.g. Canli *et al*., 2005; Furmark *et al*., 2004). Other groups have reported greater *resting* amygdala activity associated with the S allele (Canli *et al*., 2006; Rao *et al*., 2007). A meta-analysis of these reports concluded that the average effect size of 5-HTTLPR genotype on amygdala activation is $d = 0.54$ (Munafò *et al*., 2008), which by convention falls in the 'medium' range (Cohen, 1992). The effect of 5-HTTLPR genotype on amygdala activity is intriguing given the evidence that amygdala activity is elevated during depression (Drevets *et al*., 2002) and experimentally induced negative affective states (Posse *et al*., 2003), and that the amygdala had been hypothesized to be part of a limbic network that is dysregulated during depression (Seminowicz *et al*., 2004).

A recent review (Hariri and Holmes, 2006) summarized the importance of these findings and presented an integrated model of the effects of 5-HTTLPR variability on neural emotion regulation networks. The authors presented evidence that the inhibitory feedback circuits in prefrontal cortex are less effective in carriers of the S allele, resulting in dysregulated limbic emotion centers [see also Heinz *et al*. (2005) and Pezawas *et al*. (2005)]. The result is a cascade of behavioral and neuroendocrine effects that may lead to clinical syndromes including mood and anxiety disorders.

One question that is not answered by the existing work in this area is whether these findings for greater amygdala reactivity among carriers of the S allele extend to depression-related stimuli. As Hariri and Holmes (2006; see their Table 2) demonstrated, most of the studies that have addressed emotion-related effects of the 5-HTTLPR on amygdala activity use stimuli that may or may not be associated with depressed mood. Examples of stimuli used in these studies included emotional faces (Hariri *et al*., 2002), negative words (Canli *et al*., 2005), and a public speaking paradigm (Furmark *et al*., 2004). These stimuli do not bear a substantial resemblance to the events that have been reported to interact with 5-HTTLPR genotype to predict depression onset, such as financial hardship (Kendler *et al*., 1998). The current study set out to explore the response of the brain under conditions that likely are more similar to the events commonly associated with depression risk.

One of the most common factors that precedes a depression onset is the experience of loss (Kendler *et al*., 2003), including loss of relationships (Monroe *et al*., 1999), employment and financial resources (Kendler *et al*., 1995) and the death of a loved one (Kendler *et al*., 2002); therefore, manipulations that induce the thoughts and feelings associated with loss may be well-suited to reveal depression diatheses. Moreover, because low mood is a hallmark of depression, evoking sad mood states is likely to get closer to the mechanisms by which the 5-HTTLPR affects depression risk compared to, for example, briefly viewing fearful faces. The current study tested whether the 5-HTTLPR influences neural activity during a sad mood induced by the imagined loss of a loved one.

The ability to *regulate* a sad mood also has been suggested to play a key role in depression risk (Gilboa and Gotlib, 1997); without the ability to recover from low moods, episodes of normal sadness potentially could lead to a bout of depression (see Segal *et al*., 2006). Therefore the current study also examined whether 5-HTTLPR genotype modulates neural activity during sad mood regulation. The conscious regulation of negative emotion is significantly associated with amygdala activity (Schaefer *et al*., 2002), and decreases in amygdala activity predict decreases in negative affective responses (Phelps *et al*., 2004).

Given the time course of the events of interest—moods lasting several minutes rather than more ephemeral emotional responses measured on the order of seconds—the current study used perfusion functional magnetic resonance imaging (fMRI), which is better suited to capture longer-term brain changes than is blood oxygen level-dependent (BOLD) fMRI (Wang *et al*., 2005). Our primary region of interest (ROI) was the amygdala, based on existing studies reviewed above. The subgenual anterior cingulate cortex (subACC) was included as a secondary ROI, for several reasons. First, activity in subACC varies as a function of brief, non-pathological mood states (Damasio *et al*., 2000) as well as with frank depression (Seminowicz *et al*., 2004). Furthermore, there is evidence suggesting that electrical stimulation of this region may relieve treatment-refractory depression (Mayberg *et al*., 2005), and that 5-HTTLPR genotype predicts the degree to which subACC and amygdala activity are 'functionally coupled' (Pezawas *et al*., 2005). Hariri and Holmes (2006) incorporate subACC into a neural emotion regulation circuit that may be dysregulated among carriers of the S allele. Importantly, the cingulate cortex has the densest concentration of serotonin transporter sites in the human cortex (Gurevich and Joyce, 1996). Although fewer studies have tested for main effects of 5-HTTLPR genotype on subACC activity, existing data suggest that carriers of the S allele will show higher subACC activation during sad mood and during sad mood regulation, including at least one study that found higher ACC activation for S allele carriers during masked viewing of emotional faces (Dannlowski *et al*., 2008). For these reasons we tested for effects of 5-HTTLPR genotype on subACC activation as well as amygdala activation during sad mood and recovery from sad mood.

Hypotheses

On the basis of the studies reviewed here, we made two primary hypotheses: 1(a) S *vs* L participants will show greater amygdala reactivity during a sad mood; and 1(b) S *vs* L participants will show greater amygdala activity during recovery from a sad mood. We made the following secondary hypotheses for subACC activity: 2(a) S *vs* L participants will show greater subACC reactivity during a sad mood; and 2(b) S *vs* L participants will show greater subACC activity during recovery from a sad mood.

METHODS

Participants and design overview

Participants were 15 homozygous S and 15 homozygous L individuals. Written informed consent was obtained in accordance with the Institutional Review Board of the University of Pennsylvania. In order to obtain our final sample of 30 participants (14 female; all Caucasian; mean age 20.3 years, range 18–29 years), we screened 275 participants for 5-HTTLPR genotype and for self-reported racial background. Participants also completed behavioral measures of depression symptoms and personality (see below). Homozygotes who self-identified as Caucasian were invited to participate in the neuroimaging segment of the research study; non-Caucasian participants were excluded due to concerns about ethnic stratification—that is, differences in allelic frequencies that are a function of ancestry (Malhotra and Goldman, 1999). 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. The final testing session comprised an fMRI scan that included four blocks: baseline (no task), induced sad mood, mood regulation, and a second task-free scan following return to baseline mood (see Figure 1). Mood ratings (sadness and anxiety) were obtained before and after each scan block. Anxiety ratings were obtained to ensure that possible differences in anxiety between the genotype groups were not responsible for any observed neural differences between the groups, given evidence that the S allele is associated with anxiety-related traits (Lesch *et al.*, 1996).

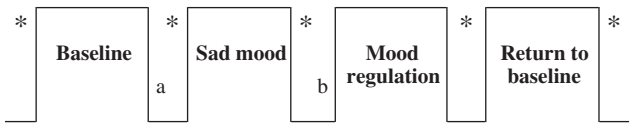
Behavioral measures and tasks

Behavioral measures included the Beck Depression Inventory (BDI; 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 (SCID; First *et al.*, 1996) to assess current and lifetime psychiatric diagnoses.

Mood induction procedure. Participants were asked to imagine vividly the death of a living, healthy loved one. The experimenter read a brief script that asked the participants to imagine specific aspects of losing their loved one, and to 'allow yourself to really experience any sad feelings that you have' as they imagined the death scenario. Participants squeezed a rubber bulb that triggered a tone to alert the experimenter when they had reached their saddest mood; the experimenter then obtained anxiety and sadness ratings. The sad mood scan began immediately thereafter.

Mood regulation instructions. At the end of the sad mood scan, participants were instructed to 'use whatever technique you can think of to get out of this sad mood'. The experimenter briefly discussed possible options for alleviating the sad mood, including imaging their loved one 'healthy, happy, and safe', or turning their minds to 'more pleasant scenes or memories'.

Mood ratings. The experimenter asked participants to rate their moods from 0 to 100 (lower indicates worse mood) for both anxiety and sadness at five points during the fMRI session (see Figure 1).



*Mood ratings; [a]mood induction instruction; [b]mood regulation instruction

Fig. 1 fMRI study design. The sad mood scan began once participants indicated that they had reached their saddest mood; the mood regulation scan began immediately following the mood regulation instructions.

Genetic analyses

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 s. Swabs were air dried for 2 h. Genomic deoxyribonucleic acid (DNA) was prepared from buccal cells using the Qiagen QIAamp® Blood Mini Kit. 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) supplemented with Ethidium Bromide (0.01%, Fisher Scientific) and visualized under ultraviolet light.

Neuroimaging measures and analyses

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: field of view = 22 cm, matrix = 64 × 64, retention time (TR) = 3 s, echo time (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, inversion time (TI) = 950 ms, TE = 3 ms, flip angle = 15°, 160 contiguous slices, $1 \times 1 \times 1\ mm^3$ resolution.

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, 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 cerebral blood flow [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, normalized to the Montreal Neurological Institute (MNI) template, smoothed, and then entered into the whole brain voxel-wise analyses.

The ROIs in the left and right 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); this procedure uses templates based on structural parameters to identify the ROI in each participant. The ROIs in the subACC were determined to be the ventral portion of the ACC from the AAL library, which were defined as the ACC region below the corpus callosum. 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.

We present within-genotype effects of sad mood and sad mood regulation on neural activity to identify candidate brain regions for future investigations and for inclusion in reviews and meta-analyses in this area. For example, to identify brain areas more active during sadness relative to baseline for each genotype, we carried out the contrast (sadness–baseline) separately for the S and the L group. In order to address our primary hypotheses we first examined genotype effects by testing for interactions between scan condition and genotype. For example, to identify brain areas that were more active in the L *vs* the S group during the sad mood scan, we carried out the interaction contrast, $[(\text{sadness} - \text{baseline})]_{\text{L group}} - [(\text{sadness} - \text{baseline})]_{\text{S group}}$. Baseline is defined as the average CBF values for the first and last (task free) scans, which produced a more stable estimate of baseline neural activity; repeated measures *t*-tests confirmed that there were no significant differences between self-reported mood for the two scans for either genotype ($P = 0.16$–0.47) nor any significant differences in activation in the ROIs ($P = 0.22$–0.56).

Whole brain analyses. Individual CBF images were entered into exploratory whole brain voxel-wise regression analysis. The genotype information (coded as 0 or 2 S alleles) was entered in the model as a covariate of interest. Three additional nuisance covariates (global CBF, age, gender) were included in the model to account for any variance associated with these variables. Activation clusters were identified for the whole brain at a significance level of $P < 0.005$ (uncorrected) and cluster size larger than 50 voxels; similar significance criteria have been used in existing studies in this area (e.g. Canli *et al*., 2006).

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). A small volume-based correction (SVC) on the a priori determined ROIs was conducted on the activation results from the whole brain analyses; the SVC is based on the appropriate correction threshold given the shape and size of the ROI (Brett, 2006). We also conducted template-based ROI analyses as a more sensitive test of the a priori ROIs (amygdala, subACC). Analyses of covariance (ANCOVAs; $\alpha = 0.05$, two-tailed) were performed to test for an effect of 5-HTTLPR genotype on CBF values (i) during sad mood and (ii) during recovery from the sad mood, covarying for baseline CBF in the ROIs. Previous studies have reported greater baseline amygdala activation associated with the S allele (Canli *et al*., 2006), including among the current sample of participants (Rao *et al*., 2007). Therefore it is essential to control for baseline CBF in the current analyses.

Behavioral statistical analyses

Tests for behavioral differences between groups on demographics, personality, and depression variables were done using independent samples *t*-tests. Tests for group differences on mood ratings comprised analyses of covariance (ANCOVA; $\alpha = 0.05$, two-tailed) with mood ratings as the outcome of interest, group as a predictor variable, and baseline mood ratings as covariates.

RESULTS

Behavioral

The two genetic groups were very similar on age, gender, depression symptoms, and personality dimensions (see Table 1). As can be seen in Figure 2, both groups showed a significant decrease in mood during the sad mood induction ($P < 0.0001$), which was maintained throughout the sad mood scan; after the mood regulation scan, both groups showed a significant increase in mood ($P < 0.0001$). There was no significant effect of genotype on subjective sadness response or on return to baseline mood ($P > 0.45$), as evidenced by the nearly parallel slopes of the lines between data points in Figure 2. Furthermore there was no significant

effect of baseline anxiety on response to the sad mood induction or significant genotype effects on any ratings of anxiety ($P = 0.25$–0.91). Moreover, inclusion of anxiety ratings as a covariate in the models did not significantly alter any of the reported results; therefore anxiety ratings were dropped from all subsequent analyses. These results indicate that any significant differences in neural activity are unlikely to be due to subjective differences in anxiety, sadness response, or recovery from the sad mood.

Table 1 Demographic, depression symptom and personality scores by genotype

Variable	L group mean (SD)	S group mean (SD)	*P*-value
Age	20.6 (2.6)	20.0 (1.4)	0.44
Female/male	6/9	8/7	0.46
BDI	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

BDI, Beck Depression Inventory.

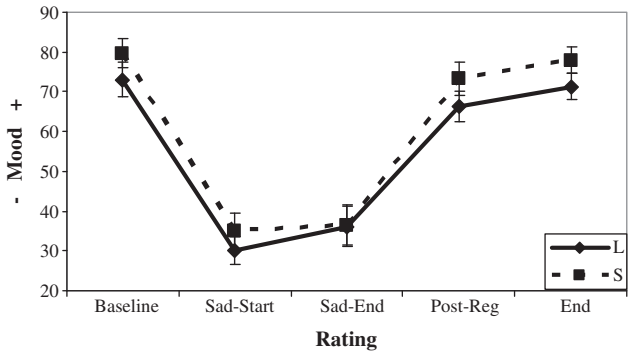


Fig. 2 Sadness ratings during fMRI scan by genotype. Reg, mood regulation.

Neuroimaging

Sadness: whole brain analyses (Table 2). During the sad mood scan relative to baseline the S group showed greater activity only in ventral PFC (Table 2, panel A.i), while the L group showed greater activity in various regions of bilateral PFC and insula (Table 2, panel A.ii). Contrary to hypotheses 1(a) and 2(a), the interaction analyses revealed no significant differences between genotype groups in amygdala or subACC activity during the sad mood scan relative to baseline (Table 2, panel B.1).

Sadness: template-based ROI analyses.

Amygdala. Contrary to our hypothesis 1(a), there was no significant effect of 5-HTTLPR genotype on either left (S group = 64.3 ml/100 g per minute, L group = 60.3 ml/100 g per minute, $F[1, 27] = 1.19$, $P = 0.28$) or right (S group = 55.5 ml/100 g per minute, L group = 53.5 ml/100 g per minute, $F[1, 27] = 0.71$, $P = 0.41$) amygdala activity during the sad mood (see Figure 3) in an ANCOVA model controlling for baseline neural activity in the respective regions.

SubACC. Likewise and contrary to hypothesis 2(a), there was no significant effect of genotype on subACC activity during the induced sad mood (S group = 78.1 ml/100 g per min, L group = 76.3 ml/100 g per minute, $F[1, 27] = 0.49$, $P = 0.49$).

Table 2 Neural activations: sadness—baseline contrast

Brain regions	Side	BA	MNI coordinates			*t*-score	Peak *P* value	Cluster size
			X	*Y*	*Z*			
(A) Within groups								
(i) Short allele group								
Ventral PFC	L	11	−24	42	−8	3.39	0.001	62
(ii) Long allele group								
VLPFC	R	47	50	38	−12	4.37	<0.001	232
Superior frontal gyrus	R	6	6	10	60	4.26	<0.001	274
VLPFC	L	47	−46	28	−18	4.21	<0.001	159
Precentral gyrus	R	6	62	4	8	3.48	<0.001	115
Anterior cingulate	R/L	24	6	24	22	3.70	<0.001	86
		32	2	18	38	3.69	<0.001	198
Insula	L	13	−44	6	0	3.91	<0.001	172
Insula	R	13	38	6	−4	3.88	<0.001	109
(B) Group interaction								
(i) Short > long								
Precuneus*	R	39	38	−74	32	3.96	<0.001	425
(ii) Long > short								
VLPFC	R	47	54	42	−12	3.44	<0.001	58
Precentral gyrus	R	6	62	4	8	3.51	<0.001	146
Posterior cingulate	R/L	23	4	−32	24	3.25	<0.001	127

BA, Brodmann's area; R, right; L, left. *$P < 0.05$, whole-brain corrected.

Regulation: whole brain analyses (Table 3). Comparing the mood regulation condition to baseline, whole brain analyses within genotype group showed significant CBF increases among the S group in prefrontal and temporal regions and in right amygdala, while the L group showed only bilateral postcentral gyrus activation and no significant amygdala CBF changes (Table 3, Panels A.1 and A.2, respectively). In support of hypothesis 1(b), interaction analyses revealed significantly greater amygdala activation in the S *vs* the L group (see Figure 4), as well as greater activity in parietal and cingulate regions (Table 3, panel B.1). The reverse contrast (L > S) found greater right-sided temporal and frontal activity (Table 3, panel B.2).

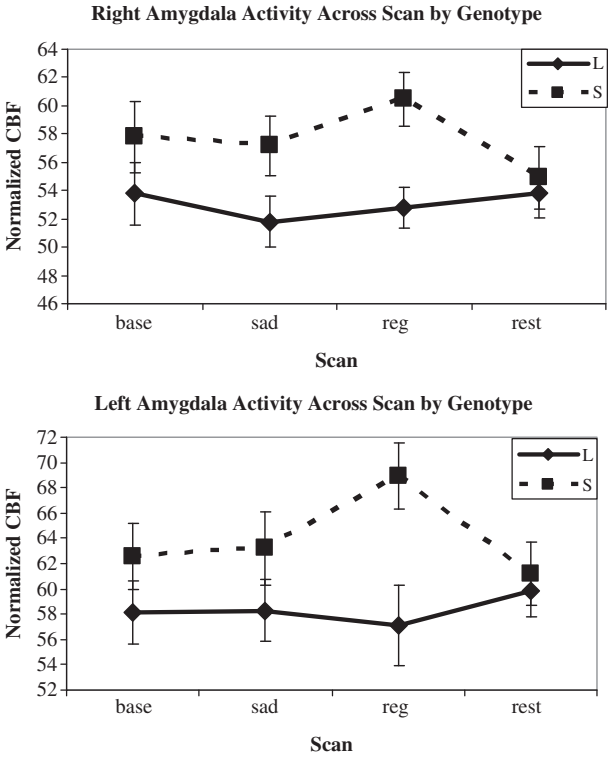


Fig. 3 Template-based ROI analyses for right (top) and left (bottom) amygdala activity during the four scan blocks by genotype group. Base, baseline; Reg, mood regulation.

Regulation: template-based ROI analyses.

Amygdala. ROI analyses of quantitative CBF (based on ANCOVA models that controlled for baseline CBF) using an anatomically-defined amygdala template revealed significantly greater amygdala activity for the S *vs* the L group during the mood regulation scan (see Figure 3) in left amygdala (S group = 66.9 ml/100 g per minute, L group = 59.4 ml/100 g per minute, $F[1, 27] = 4.69$, $P = 0.03$, $d = 0.63$), and a trend toward greater activity among the S group in right amygdala (S group = 57.2 ml/100 g per minute; L group = 52.0 ml/100 g per minute, $F[1, 27] = 2.92$, $P = 0.10$, $d = 0.55$). These results provide further confirmation of hypothesis 1(b) that the S group would demonstrate greater amygdala activity during regulation of a sad mood than would the L group.

SubACC. ROI analyses failed to confirm hypothesis 2(b) that the S allele would be associated with greater subACC activity during mood regulation (S group = 78.6 ml/100 g per minute, L group = 79.7 ml/100 g per minute, $F[1, 27] = 0.11$, $P = 0.74$).

DISCUSSION

The current study found that amygdala activity varied systematically as a function of 5-HTTLPR genotype during

Table 3 Neural activations: regulation—baseline contrast

Brain regions	Side	BA	MNI Coordinates			*t*-score	Peak *P* value	Cluster size
			X	*Y*	*Z*			
(A) Within groups								
(i) Short allele group								
Medial PFC	R/L	9	−4	56	18	3.92	<0.001	254
Amygdala	R	–	22	−8	20	3.88	<0.001	69
Superior temporal gyrus	R	38	46	14	−30	3.83	<0.001	104
Anterior cingulate	R/L	33	4	8	26	3.65	<0.001	159
		32	−10	32	28	3.25	<0.001	148
(ii) Long allele group								
Postcentral gyrus	L	40	−46	−36	52	4.20	<0.001	175
Postcentral gyrus	R	3	60	−16	48	3.58	<0.001	116
(B) Group interaction;								
(i) Short > long								
Amygdala*	R	–	28	−2	−18	3.42	<0.001	54
Precuneus	R	19	34	−78	34	3.40	0.001	85
Anterior cingulate	L	32	−12	34	24	3.34	0.001	82
(ii) Long > short								
Middle temporal gyrus	R	21	54	−48	6	3.91	<0.001	168
Medial frontal gyrus	R	6	10	−4	62	3.44	<0.001	74

*$P = 0.053$, small volume correction.

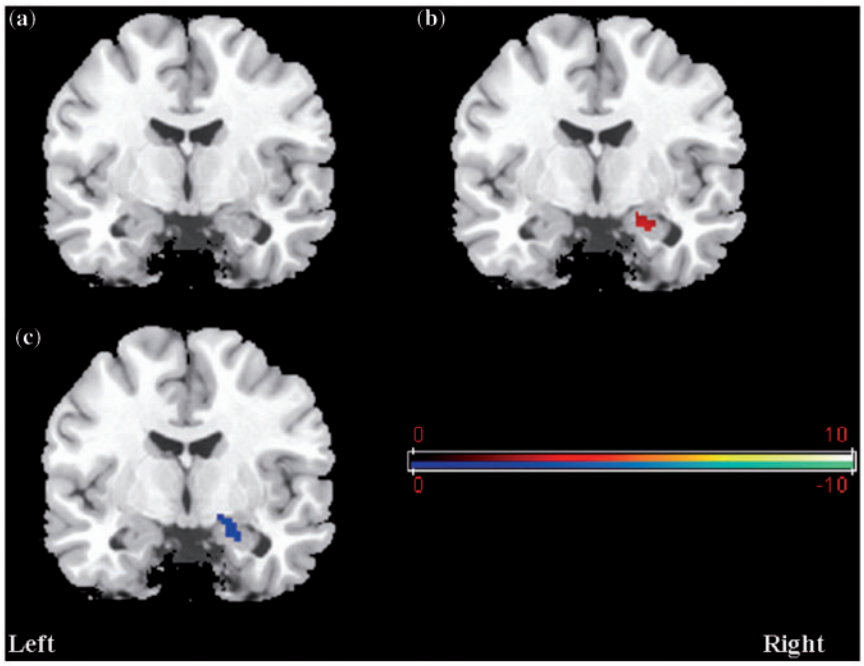


Fig. 4 Coronal slices showing regulation minus baseline contrasts for (a) L group, (b) S group, and (c) L minus S interaction. Activations in (b) and (c) are centered on the amygdala.

the intentional regulation of a sad mood, whereas no significant effect of the polymorphism emerged during the experience of sadness. It is important to note that the S allele was associated with greater amygdala activation during mood regulation even after controlling for the greater baseline amygdala activity among this group (see Figure 3). These results extend previous BOLD fMRI findings that relied on very brief presentation (a few seconds) of stimuli that are not obviously associated with depression risk (e.g. fearful faces; Hariri *et al.*, 2002), and show that amygdala differences also emerge under conditions that may have more direct relevance to depression risk. The lack of an effect on sadness-related amygdala activity was surprising and contrary to expectations. However, reports of amygdala activation during sad mood have been inconsistent (e.g. Damasio *et al.*, 2000). The apparent specificity of the 5-HTTLPR effect on amygdala activity during sad mood regulation suggests that the primary 'action' of the S allele with respect to mood (as opposed to the perception of threat as in previous studies) may occur *following* a mood insult rather than during the mood insult itself. Similarly surprising was the lack of effects of 5-HTTLPR genotype on subACC activity at any time point. These findings suggest that 5-HTTLPR genotype primarily affects functional coupling between subACC activity and other limbic structures [especially amygdala; see Pezawas *et al.* (2005)] rather than exerting a direct effect on subACC activation.

On the basis of the current results, one might speculate that depressed carriers of the S allele may be especially likely to benefit from psychotherapies [such as cognitive behavioral therapy (CBT)] that focus on developing more adaptive mood regulation behavior. Additional support for this hypothesis comes from a study (Siegle *et al.*, 2006) showing that depressed individuals who showed prolonged amygdala activity in response to negative emotional stimuli were significantly more likely to benefit from CBT.

These results also raise an important question: What drives the amygdala activation difference during mood regulation, given that subjective mood reports were not significantly different? Some researchers (Whalen *et al.*, 1998) have proposed that the amygdala hyperactivity seen in depression likely indicates greater vigilance to potentially negative stimuli in the environment; such an interpretation is in accord with the association between depressed mood and negative cognitive biases (Gotlib *et al.*, 2004). Greater amygdala activity may predispose depressed individuals to attend to negative and threat-relevant stimuli, thereby perpetuating a low mood state. Therefore we might speculate that the greater amygdala activity among S allele carriers in the current study indicates greater persistence of alertness to loss-related stimuli, such as images of the loved one's death and worries about his or her health and safety.

The a priori hypotheses about the amygdala, coupled with previous positive findings in this region as a function of the 5-HTTLPR, minimize concerns of a Type I error with regard to the amygdala. Other brain regions where activity was found to vary as a function of 5-HTTLPR genotype and scan condition (see Tables 2 and 3) warrant brief discussion, although significant caution should be exercised in the level of confidence that is placed in these findings.

First, greater ventrolateral prefrontal cortex (VLPFC) activity was found among the L group during the sad mood scan. Several studies on the neural correlates of sadness have reported activity of VLPFC, including during the recall of sad memories (e.g. Damasio *et al*., 2000) and in response to viewing sad movies (Lévesque *et al*., 2003). Given the apparent absence of significant differences between genotype groups on subjective ratings of sadness, it is somewhat puzzling that there was significantly greater VLPFC activity seen among the L group. One possible explanation comes from studies showing that the VLPFC plays a role in the suppression of sadness-related memories (Anderson *et al*., 2004) and in suppression of negative emotional responses (Phan *et al*., 2005), such that the greater activity among the L group reflects more successful engagement of this region in dampening the activation of sadness-related neural networks.

Also intriguing was the finding of greater sadness-related posterior cingulate activity among the L group. Previous studies have found evidence for the role of the posterior cingulate in emotion-related processes, including the processing of both pleasant and unpleasant words (Maddock *et al*., 2003) and the experience of sadness (Damasio *et al*., 2000; Mayberg *et al*., 1999). Greater activation of the posterior cingulate also has been associated with depression remission (Mayberg *et al*., 1999, 2005), recall of familiar people (Maddock *et al*., 2001), and with personally salient autobiographical memory retrieval (Fink *et al*., 1996). The greater activity exhibited in posterior cingulate by the L group may be due to discrepancies in the strategies that L allele participants used to maintain their sad mood, such as greater reliance on recalling personally salient sad memories.

Finally, differences in dorsal ACC emerged during mood regulation, with greater activity among the S group. The dorsal portion of the ACC has been linked to a variety of cognitive operations, including the evaluation of one's cognitive states (Carter *et al*., 2000) and the monitoring of cognitive conflict, such as is present in Stroop-type tasks (e.g. Botvinick *et al*., 2004). Thus the greater activity among the S group may represent monitoring of their current mood state (sadness) vis-à-vis their intended mood state (return to baseline); alternatively, the ACC activity may reflect monitoring possible conflict between the recovery-focused thoughts they intend to have and ruminative thought processes related to sad or anxious thoughts about their loved one. Adequate tests of these speculations require studies designed explicitly to that end.

LIMITATIONS AND FUTURE DIRECTIONS

The current study was limited in several ways. First, the current study used a block design data analysis approach that contrasted neural activity as a function of scan block, rather than as a function of individual participants' mood ratings. This approach was chosen due to the hypotheses about group differences during sadness and mood regulation and due to the striking similarity in the mood dynamics between the two groups. Furthermore, the current study had very limited power to detect potential differences in the correlations between mood ratings and neural activity, given the relatively small sample size in each group (see Munafò *et al*., 2008). Different study paradigms with larger *N*s may be better suited to address these types of questions.

Second, the imaginal aspect of the mood induction undoubtedly diminished the magnitude of the perceived loss compared to the actual death of a loved one. Nevertheless, the mood induction replicated at least part of the loss response; while the scenario was imaginary, the sadness was real, as evidenced by the self-ratings and by the tears that several participants shed. Future studies could use still more ecologically valid mood inductions and depression-related stressors.

Third, participants had limited options for mood regulation in the scanner given that they could not engage in commonly reported mood regulation techniques that are difficult to do in an fMRI magnet [e.g. having sex, shopping, eating (Parkinson and Totterdell, 1999)]. Future neuroimaging studies could provide more options for mood regulation, in addition to obtaining precise measures of participants' mood regulation behavior during the experiment. It also will be important to determine whether the differences that emerged are specific to intentional mood regulation; for example, would similar differences in amygdala activity be observed during other psychological processes carried out following a sad mood?

Additionally, recent research (published after 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- *vs* high-5-HTT expression. Future studies should include a distinction between long allele variants, which could lead to finer-grained resolution of the effect of the 5-HTTLPR on neural response. Also, it cannot be known based on these data whether heterozygotes are more similar to the S/S group or the L/L group. Future research with larger sample sizes could include heterozygotes in order to test the generalizability of the current findings.

Finally, although we limited our analyses to Caucasian individuals, it is possible that occult stratification may have been present. Future studies could address this possibility by performing more sensitive tests of stratification.

Although the current study did not find genotype-related differences on amygdala activity during sad mood, it is possible that differences would have emerged if neural activation were assessed during the onset of sadness (given that the sad mood scan began only after participants reported having achieved a sad mood). Future studies

could extend the current paradigm to investigate neural activity during the transition from a neutral mood to sadness. Interestingly, neural differences were present between the genotype groups while there were not significant mood rating differences between the groups. One possible explanation is that the S allele confers depression risk via the neural mechanisms that S/S individuals use to recover from a sad mood, rather than through the short-term effectiveness of their efforts to recover from a sad mood; thus greater amygdala activity during mood regulation leaves S/S individuals more vulnerable to poor recovery from subsequent mood insults. Alternatively, the mood induction used in the present study was insufficiently potent to reveal behavioral differences in mood recovery; perhaps stronger or real-life stressful events would reveal significant effects of genotype on mood recovery. Additional investigations that further clarify mood regulation variability as a mechanism whereby the 5-HTTLPR genotype affects depression risk may be a fruitful area of future research.

Conflict of Interest

None declared.

REFERENCES

Anderson, M.C., Ochsner, K.N., Kuhl, B., et al. (2004). Neural systems underlying the suppression of unwanted memories. *Science*, *303*, 232–5.

Beck, A.T., Steer, R.A., Brown, G.K. (1996). *Beck Depression Inventory Manual*, 2nd edn, San Antonio, TX: Psychological Corporation.

Botvinick, M.M., Cohen, J.D., Carter, C.S. (2004). Conflict monitoring and anterior cingulate cortex: an update. *Trends in Cognitive Sciences*, *8*, 539–46.

Brett, M. (2006). *Small Volume Corrections Using the Theory of Random Fields*. Retrieved February 28, 2009, from http://imaging.mrc-cbu.cam.ac.uk/imaging/SmallVolumeCorrection.

Brett, M., Anton, J.L., Valabregue, R., Poline, J.B. (2002). Region of interest analysis using an SPM toolbox [abstract/497] presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2–6, Sendai, Japan. Available on CD-ROM in *NeuroImage*, *16*(2).

Canli, T., Omura, K., Haas, B.W., Fallgatter, A., Constable, R.T., Lesch, K. P. (2005). 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*, *102*, 12224–9.

Canli, T., Qui, M., Omura, K., et al. (2006). Neural correlates of epigenesis. *Proceedings of the National Academy of Sciences*, *103*, 16033–8.

Carter, C. S., Macdonald, A. M., Botvinick, M., et al. (2000). Parsing executive processes: strategic vs. evaluative functions of the anterior cingulate cortex. *Proceedings of the National Academy of Sciences of United States of America*, *97*, 1944–8.

Caspi, A., Sugden, K., Moffitt, T.E., et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*, *301*, 386–9.

Chen, M.C., Joormann, J., Hallmayer, J., Gotlib, I.H. (2009). Serotonin transporter polymorphism predicts waking cortisol in young girls. *Psychoneuroendocrinology*, *34*, 681–6.

Cohen, J. (1992). A power primer. *Psychological Bulletin*, *112*, 155–9.

Costa, P.T. Jr., McCrae, R.R. (1992). *Revised NEO Personality Inventory (NEO-PI-R) and NEO-Five-Factor Inventory (NEO-FFI) professional manual*. Odessa, FL: Psychological Assessment Resources.

Damasio, A.R., Grabowski, T.J., Bechara, A., et al. (2000). Subcortical and cortical brain activity during the feeling of self-generated emotions. *Nature Neuroscience*, *3*, 1049–56.

Dannlowski, U., Ohrmann, P., Bauer, J., et al. (2008). 5-HTTLPR biases amygdala activity in response to masked facial expressions in major depression. *Neuropsychopharmacology*, *33*, 418–24.

Drevets, W.C., Price, J.L., Bardgett, M.E., Reich, T., Todd, R.D., Raichle, M.E. (2002). Glucose metabolism in the amygdala in depression: relationship to diagnostic subtype and plasma cortisol levels. *Pharmacology, Biochemistry and Behavior*, *71*, 431–47.

Fink, G.R., Markowitsch, H.J., Reinkemeier, M., Bruckbauer, T., Kessler, J., Heiss, W.-D. (1996). Cerebral representation of one's own past: neural networks involved in autobiographical memory. *Journal of Neuroscience*, *16*, 4275–82.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B. (1996). *Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV)*. Washington, DC: American Psychiatric Press, Inc.

Furmark, T., Tillfors, M., Garpenstrand, H., et al. (2004). Serotonin transporter polymorphism related to amygdala excitability and symptom severity in patients with social phobia. *Neuroscience Letters*, *362*, 189–92.

Gelernter, J., Cubells, J.F., Kidd, J.R., Pakstis, A.J., Kidd, K.K. (1997). Population studies of polymorphisms of the serotonin transporter protein gene. *Human Genetics*, *101*, 243–46.

Gilboa, E., Gotlib, I.H. (1997). Cognitive biases and affect persistence in previously dysphoric and never-dysphoric individuals. *Cognition and Emotion*, *11*, 517–38.

Gotlib, I.H., Joormann, J., Minor, K.L., Hallmayer, J. (2008). HPA axis reactivity: a mechanism underlying the associations among 5-HTLPR, stress, and depression. *Biological Psychiatry*, *63*, 847–51.

Gotlib, I.H., Krasnoperova, E., Yue, D. N., Joormann, J. (2004). Attentional biases for negative interpersonal stimuli in clinical depression. *Journal of Abnormal Psychology*, *113*, 127–35.

Gurevich, E.V., Joyce, J.N. (1996). Comparison of [^{3}H]paroxetine and [^{3}H]cyanoimipramine for quantitative measurement of serotonin transporter sites in human brain. *Neuropsychopharmacology*, *14*, 309–23.

Hariri, A.R., Holmes, A. (2006). Genetics of emotion regulation: the role of the serotonin transporter in neural function. *Trends in Cognitive Sciences*, *10*, 182–91.

Hariri, A.R., Mattay, V.S., Tessitore, A., et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science*, *297*, 400–3.

Hu, X., Oroszi, G., Chun, J., Smith, T.L., Goldman, D., Schuckit, M.A. (2005). 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*, *29*, 8–16.

Huettel, S.A., Song, A.W., McCarthy, G. (2004). *Functional Magnetic Resonance Imaging*. Sunderland, MA: Sinauer Associates, Inc.

Kaufman, J., Yang, B.-Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, L., Krystal, J.H., Gelernter, J. (2004). Social supports and serotonin transporter gene moderate depression in maltreated children. *Proceedings of the National Academy of Sciences*, *101*, 17316–21.

Kendler, K.S., Hettema, J.M., Butera, F., Gardner, C.O., Prescott, C.A. (2003). Life event dimensions of loss, humiliation, entrapment, and danger in the prediction of onsets of major depression and generalized anxiety. *Archives of General Psychiatry*, *60*, 789–96.

Kendler, K.S., Karkowski, L.M., Prescott, C.A. (1998). Stressful life events and major depression: risk period, long-term contextual threat, and diagnostic specificity. *Journal of Nervous and Mental Disease*, *186*, 661–9.

Kendler, K.S., Kessler, R.C., Walters, E.E., et al. (1995). Stressful life events, genetic liability, and onset of an episode of major depression in women. *American Journal of Psychiatry*, *152*, 833–42.

Kendler, K.S., Kuhn, J.W., Vittum, J., Prescott, C.A., Riley, B. (2005). 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*, *62*, 529–35.

Kendler, K.S., Sheth, K., Gardner, C.O., Prescott, C.A. (2002). Childhood parental loss and risk for first-onset of major depression and alcohol dependence: the time-decay of risk and sex differences. *Psychological Medicine*, *32*, 1187–94.

Lesch, K.-P., Bengel, D., Heils, A., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, *274*, 1527–31.

Lévesque, J., Eugène, F., Joanette, Y., et al. (2003). Neural circuitry underlying voluntary suppression of sadness. *Biological Psychiatry*, *53*, 502–10.

Lotrich, F.E., Pollock, B.G. (2004). Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatric Genetics*, *14*, 121–9.

Maddock, R.J., Garrett, A.S., Buonocore, M.H. (2001). Remembering familiar people: the posterior cingulate cortex and autobiographical memory retrieval. *Neuroscience*, *104*, 667–76.

Maddock, R.J., Garrett, A.S., Buonocore, M.H. (2003). Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. *Human Brain Mapping*, *18*, 30–41.

Malhotra, A.K., Goldman, D. (1999). Benefits and pitfalls encountered in psychiatric genetic association studies. *Biological Psychiatry*, *45*, 544–50.

Mayberg, H.S., Liotti, M., Brannan, S.K., et al. (1999). Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *American Journal of Psychiatry*, *156*, 675–82.

Mayberg, H.S., Lozano, A.M., Voon, V., et al. (2005). Deep brain stimulation for treatment-resistant depression. *Neuron*, *45*, 651–60.

Monroe, S.M., Rohde, P., Seeley, J.R., Lewinsohn, P.M. (1999). Life events and depression in adolescence: relationship loss as a prospective risk factor for first onset of major depressive disorder. *Journal of Abnormal Psychology*, *108*, 606–14.

Morris, J.S., Frith, C.D., Perrett, D.I., et al. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature*, *383*, 812–5.

Munafò, M.R., Brown, S.M., Hariri, A.R. (2008). Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biological Psychiatry*, *63*, 852–7.

Parkinson, B., Totterdell, P. (1999). Classifying affect-regulation strategies. *Cognition and Emotion*, *13*, 277–303.

Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., et al. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience*, *8*, 828–34.

Phelps, E.A., Delgado, M.R., Nearing, K.I., LeDoux, J.E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron*, *43*, 897–905.

Posse, S., Fitzgerald, D., Gao, K., et al. (2003). Real-time fMRI of temporolimbic regions detects amygdala activation during single-trial self-induced sadness. *NeuroImage*, *18*, 760–8.

Phan, K.L., Fitzgerald, D.A., Nathan, P.J., Moore, G.J., Uhde, T.W., Tancer, M.E. (2005). Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biological Psychiatry*, *57*, 210–9.

Rao, H., Gillihan, S.J., Wang, J., et al. (2007). Genetic variation in serotonin transporter alters resting brain function in healthy individuals. *Biological Psychiatry*, *62*, 600–6.

Schaefer, S.M., Jackson, D.C., Davidson, R.J., Aguirre, G.K., Kimberg, D.Y., Thompson-Schill, S. (2002). Modulation of amygdalar activity by the conscious regulation of negative emotion. *Journal of Cognitive Neuroscience*, *14*, 913–21.

Segal, Z.V., Kennedy, S., Gemar, M., Hood, K., Pedersen, R., Buis, T. (2006). Cognitive reactivity to sad mood provocation and the prediction of depressive relapse. *Archives of General Psychiatry*, *63*, 749–55.

Seminowicz, D.A., Mayberg, H.S., McIntosh, A.R., et al. (2004). Limbic-frontal circuitry in major depression: a path modeling meta-analysis. *NeuroImage*, *22*, 409–18.

Shcherbatykh, T.V., Golimbet, V.E., Orlova, V.A., et al. (2000). Polymorphisms in the human serotonin transporter gene in endogenous psychoses. *Russian Journal of Genetics*, *36*, 1445–48.

Siegle, G.J., Carter, C.S., Thase, M.E. (2006). Use of fMRI to predict recovery from unipolar depression with cognitive behavior therapy. *American Journal of Psychiatry*, *163*, 735–8.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*, *15*, 273–89.

Wang, J., Zhang, Y., Wolf, R.L., Roc, A.C., Alsop, D.C., Detre, J.A. (2005). Amplitude modulated continuous arterial spin labeling perfusion MR with single coil at 3T-feasibility. *Radiology*, *235*, 218–28.

Wendland, J.R., Martin, B.J., Kruse, M.R., Lesch, K.-P., Murphy, D.L. (2006). Simultaneous genotyping of four functional loci of human *SLC6A4*, with reappraisal of *5-HTTLPR* and rs25531. *Molecular Psychiatry*, *11*, 224–6.

Whalen, P.J., Rauch, S.L., Etcoff, N.L., McInerney, S.C., Lee, M.B., Jenike, M.A. (1998). Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *The Journal of Neuroscience*, *18*, 411–8.