

# Serotonin transporter genotype modulates cognitive reappraisal of negative emotions: a functional magnetic resonance imaging study

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**A functional polymorphism within the serotonin transporter gene (5-HTTLPR) has been reported to modulate emotionality and risk for affective disorders. The short (S) allele has less functional efficacy than the long (L) allele and has been associated with enhanced emotional reactivity. One possible contributing factor to the high emotionality in S carriers may be inefficient use of cognitive strategies such as reappraisal to regulate emotional responses. The aim of the present study was to test whether the 5-HTTLPR genotype modulates the neural correlates of emotion regulation. To determine neural differences between S and L allele carriers during reappraisal of negative emotions, 15 homozygous S (S'/S') and 15 homozygous L (L'/L') carriers underwent functional magnetic resonance imaging (fMRI), while performing an instructed emotion regulation task including downregulation, upregulation and passive viewing of negative emotional pictures. Compared to L'/L' allele carriers, subjects who carry the S'/S' allele responded with lower posterior insula and prefrontal brain activation during passive perception of negative emotional information but showed greater prefrontal activation and anterior insula activation during down- and upregulation of negative emotional responses. The current results support and extend previous findings of enhanced emotionality in S carriers by providing additional evidence of 5-HTTLPR modulation of volitional emotion regulation.**

**Keywords:** cognitive reappraisal; emotion regulation; fMRI; serotonin transporter gene; 5-HTTLPR

## INTRODUCTION

Serotonin (5-HT) is critically involved in the generation and regulation of emotions (Cools *et al.*, 2008). Previous research points to diminished serotonergic functioning as a vulnerability factor for depression (Maes and Meltzer, 1995). Such serotonergic vulnerability is promoted by a genetic variation within the 5-HT transporter (5-HTT) gene (SLC6A4), which controls serotonergic neurotransmission by facilitating its reuptake from the synaptic cleft. Lesch *et al.* (1996) described a relatively common genetic polymorphism in the 5-HTT promoter region (5-HTTLPR) revealing a variable number of tandem repeats [short (S) vs long (L)] that modulate the transcriptional activity of the gene (Greenberg *et al.*, 1999). The S allele has lower transcriptional efficiency than the L allele resulting in lower serotonin uptake activity. Recently, a functional variant of a single-nucleotide polymorphism (SNP) in the L allele has been described (Hu *et al.*, 2005) revealing an Lg (as opposed to La) that is functionally equivalent to the S allele.

The S allele has been associated with increased risk for depression (Collier *et al.*, 1996; Joiner *et al.*, 2003; Lotrich

and Pollock, 2004; Gonda *et al.*, 2005; 2006), particularly following stress (Caspi *et al.*, 2003; Jacobs *et al.*, 2006; Wilhelm *et al.*, 2006; Uher and McGuffin, 2008; Karg *et al.*, in press). An explanation for this increased risk in S allele carriers is their enhanced emotional reactivity; as often evidenced by greater amygdala responses to negative emotional material (Hariri *et al.*, 2002; Bertolino *et al.*, 2005; Canli *et al.*, 2005; Hariri *et al.*, 2005; Munafò *et al.*, 2008) probably due to reduced amygdala-prefrontal coupling (Heinz *et al.*, 2005; Pezawas *et al.*, 2005). One possible contributing factor to the high emotionality in S carriers may be inefficient use of cognitive strategies such as reappraisal to regulate emotional responses. Previous neuroimaging studies have shown that cognitive reappraisal, a strategy to down- or upregulate negative emotional responses by reformulating the meaning of the initiating event (Ochsner and Gross, 2005), effectively reduces or enhances emotional experiences by top-down regulation of subcortical affective circuitry (including insula and amygdala) through engagement of the medial and lateral prefrontal cortex (PFC) and the anterior cingulate cortex (ACC) (Ochsner *et al.*, 2002, 2004; Phan *et al.*, 2005; McRae *et al.*, 2010). In fact, inefficient top-down regulation is a core vulnerability factor for mood and anxiety disorders (Gross, 2007), whereas sufficient cognitive regulation leads to enhanced control of emotion, interpersonal functioning and psychological and physical well-being (Gross and John, 2003). In support, depressed

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patients reveal inappropriate engagement of prefrontal regulatory circuitry when trying to downregulate negative emotions (Beauregard *et al.*, 2006; Johnstone *et al.*, 2007) and show enhanced engagement of limbic and medial and dorsolateral prefrontal regions during emotional upregulation through self-focused rumination (Cooney *et al.*, 2010).

Previously, most studies reporting enhanced emotionality evidenced by increased amygdala activation in S allele carriers compared to L allele carriers have used passive perception tasks and overlooked the effects of top-down control to regulate the emotional response. Recently, an association has been reported between the capacity to regulate emotions and neural responses to fearful information using a passive viewing paradigm (Drabant *et al.*, 2009), which indirectly supports the postulation that the enhanced activation of the brain's emotion circuitry in response to fearful information is due to reduced emotion regulation skills. Two recent studies have further addressed this issue using an instructed emotion regulation paradigm revealing mixed results. Schardt *et al.* (2010) showed that cognitive reappraisal to downregulate the negative emotional experiences can counteract the genotype-related effects of enhanced amygdala activation, whereas Lemogne *et al.* (2011) showed increased amygdala activation during emotional upregulation through self-referential processing for S carriers compared to L carriers. These findings suggest that not only less downregulation but also more upregulation of negative emotions may increase the risk of affective disorders in S allele carriers, particularly following negative emotional experiences. The aim of the current study was to investigate the moderating effect of 5-HTTLPR on neural activation during cognitive reappraisal of negative emotions with an instructed emotion regulation paradigm adapted from Ochsner *et al.* (2002) that allows to investigate downregulation, upregulation and passive perception of negative emotional material within one paradigm using functional magnetic resonance imaging (fMRI). To the best of our knowledge, this is the first imaging study investigating emotional reactivity and emotional down- and upregulation in the same 5-HTTLPR sample, which allows examining common and distinct neural differences between 5-HTTLPR genotypes involved in each kind of emotion regulation.

In line with previous findings, it was hypothesized that healthy homozygous S and/or L<sub>G</sub> carriers (hereafter referred to as S'/S') will show enhanced amygdala activation during passive perception of negative emotional material compared to L<sub>A</sub> homozygotes (hereafter referred to as L'/L'). Further, we expected a downregulation deficit for S'/S' carriers evidenced by less attenuation of limbic regions (such as insula and/or amygdala) reflecting less efficient downregulation of negative emotions. Based on previous results (Drabant *et al.*, 2009) showing a negative association between habitual reappraisal use and activity in prefrontal brain regions integral to effortful emotional control, we may also expect greater activity for S'/S' carriers in those prefrontal regions

during downregulation of negative emotions. Further, we hypothesized that S'/S' carriers will also differ from L'/L' carriers in emotion regulation by enhanced upregulation of negative emotions; indicated by enhanced engagement of limbic and medial/dorsolateral prefrontal brain regions resembling findings in depressed patients (Cooney *et al.*, 2010) and individuals with a cognitive risk factor for depression (Ray *et al.*, 2005).

## PARTICIPANTS AND METHODS

Students from Maastricht University completed a standardized questionnaire package concerning general information (health, personal or family history of medical or psychiatric complaints, smoking and drinking habits, caffeine consumption, weight and height, use of psychoactive drugs) and concerning relevant symptoms and psychopathology. Exclusion criteria were health or medical complaints; personal or family history of psychiatric illness; history of medical illness; medication use; metabolic, hormonal or intestinal diseases; irregular diet; deviant eating habits or excessive alcohol or drug use. Following this first selection, 90 participants attended a buccal sample extraction session to genotype for 5-HTTLPR (SS, 26%; LS, 46%; LL, 28%). Since brain 5-HT vulnerabilities for affective changes are mainly found in homozygous S compared to homozygous L allele carriers (Caspi *et al.*, 2003; 2010; Wilhelm *et al.*, 2006; Zalsman *et al.*, 2006) only homozygous S allele (S/S, S/L<sub>G</sub>, L<sub>G</sub>/L<sub>G</sub>; classified as S'/S') and homozygous L allele (L<sub>A</sub>/L<sub>A</sub>; classified as L'/L') were then invited to participate in the experimental study. After invitation, a number of eight S'/S' and 10 L'/L' refrained from further participation because of shortage of time or other non-specific reasons.

A total of 15 healthy S'/S' allele carriers and 15 healthy L'/L' allele carriers completed the experiment. Both groups included Caucasian participants with European background and revealed normal body mass indexes (BMI in kg/m<sup>2</sup> between 20 and 25; mean 22 ± 2) (see for demographic characteristics Table 1). All subjects passed an MRI safety screening and were requested not to use alcohol or any kind of drugs 24 h prior to and during the course of the MRI scan. The study was approved by the local Ethics Committee and the procedures followed were in accordance with the Declaration of Helsinki of 1975 as revised in 1983. All participants gave their informed consent to participate in the experiment and were paid 30€ for participation.

**Table 1** Demographic characteristics

	L'/L'	S'/S'
N	15	15
Age	20.9 (1.5)	20 (1.6)
BDI	3.1 (2.6)	4.4 (4.1)

Values represent mean (SD).

### Experimental task and stimulation protocol

A similar emotion regulation paradigm as that of Ochsner (2002) was employed. During the task, participants were instructed to use cognitive reappraisal to down- or upregulate their emotional responses to negative emotional pictures (reappraisal conditions) and passively view negative or neutral pictures (look conditions) while undergoing fMRI. Before scanning, participants were trained in cognitive reappraisal. For the downregulation condition, participants were asked to imagine that the situation depicted had a positive outcome or view the situation as unreal, whereas for the upregulation condition, participants were asked to imagine themselves or a loved one experiencing the situation depicted and imagine a bad outcome. For the look condition, participants were asked to passively view the pictures without using any reappraisal strategy. The training was succeeded when participants reported to use the strategies as instructed and reported being able to perform the task.

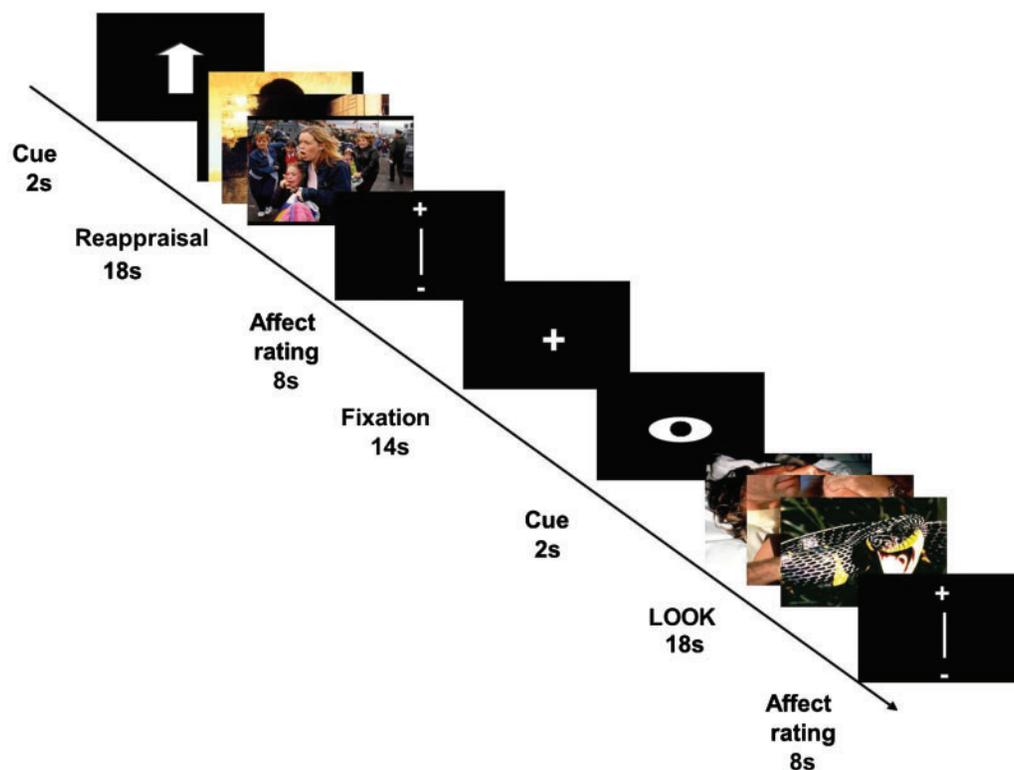
Participants were scanned while performing the following protocol (Figure 1). They viewed 18-s blocks of aversive pictures; each picture was presented for 6 s consecutively without an inter-stimulus interval. Prior to each block of pictures, the instruction cue to look, downregulate or upregulate appeared at the center of a black screen for 2 s. Immediately following each block, a visual analogue scale (VAS) scale appeared on the screen for 8 s asking participants to rate the intensity of their negative affect. This was

followed by a fixation period that lasted 14 s. Participants underwent three functional and two anatomical runs. Every functional run contained 12 experimental blocks (four per condition) and lasted about 12 min. The order of blocks was pseudo-randomized within runs and the order of runs was counterbalanced across subjects. The dependent variables were mean percentage signal change of blood oxygenation level-dependent (BOLD) response in activated brain regions and subjective negative affect.

The stimuli set comprised 108 high-arousing, negatively valenced photos and 36 neutral photos taken from the International Affective Picture Database (IAPS) (Lang et al., 2001). Each photo was shown only once for a given participant, and negative pictures were randomly assigned to the negative picture conditions. Negative pictures were more negatively valenced and higher in arousal than neutral pictures (based on norm ratings on a 1–9 point scale). The average ( $\pm$ SD) valence and arousal ratings were: valence: neutral =  $5.25 \pm 0.27$ ; negative =  $2.08 \pm 0.4$ ; arousal: neutral =  $2.86 \pm 0.47$ ; negative =  $6.1 \pm 0.75$ .

### Genotyping and genetic analyses

Buccal cell samples for 5-HTTLPR genotype determination were obtained using sterile swabs (Omni Swabs, Whatman's, Hertogenbosch, The Netherlands). Genomic DNA was isolated from buccal swabs using QIamp DNA Mini Kits from Qiagen (Westburg, Leusden, The Netherlands) and



**Fig. 1** Exemplar segment of the experimental paradigm. Pictures were presented in 18-s blocks, preceded by an instruction cue to passively look at the picture or cognitively re-appraise (downregulate or upregulate) the evoked emotional response. The experimental block was followed by a subjective affect rating.

5-HTTLPR genotyping was performed using the polymerase chain reaction (PCR) protocol according to Glatz *et al.* (2003). In compliance with previous work (e.g. Neumeister *et al.*, 2006; Zalsman *et al.*, 2006; Walderhaug *et al.* 2007; Markus and Firk 2009; Markus and De Raedt 2011), triallelic variants were reclassified into a biallelic model as follows: S/S, S/L<sub>G</sub> and L<sub>G</sub>/L<sub>G</sub> were classified as S'/S' and L<sub>A</sub>/L<sub>A</sub> as L'/L'. Hardy–Weinberg equilibrium was determined on the original 5-HTTLPR database ( $N=90$ ) using  $\chi^2$  tests, revealing that the genotype frequencies of S'/S' ( $N=24$ ), S/L ( $N=40$ ) and L'/L' ( $N=26$ ) did not depart significantly from Hardy–Weinberg equilibrium ( $\chi^2_{(1)} = 1.45$ ,  $P = 0.23$ ).

### Functional image acquisition and preprocessing

Images were acquired with a 3T Siemens Magnetom Allegra Head-only Scanner at the Maastricht Brain Imaging Centre (MBIC) using a birdcage volume coil. Gradient-echo planar (EPI) imaging volumes were acquired (31 slices, TR = 2000 ms). The relevant imaging factors included oblique axial imaging with a negative (i.e. backward) tilt angle of 30, a voxel size of  $2 \times 2 \times 2.5$  mm with a gap size of 1 mm and a short echo time of 25 ms. The voxel matrix size was  $128 \times 128$  mm, and the field of view (FoV) was  $256 \times 256$  mm. Acquisition of images yielded 384 volumes per run. Two high-resolution whole-brain anatomical T1-weighted scans were acquired: MPRAGE sequence (TR = 2250 ms, TE = 2.6 ms, flip angle = 9 degrees,  $1 \times 1 \times 1$  mm).

All processing and analysis of the fMRI data was performed using Brainvoyager QX (Brain Innovations, Maastricht, the Netherlands). The first two volumes of the T2\*-weighted functional images were discarded due to magnetic saturation effects. Pre-processing comprised slice scan timing correction (using sinc interpolation), motion correction (using a 3D rigid-body transformation of each volume to the first volume of each run and using trilinear/sinc interpolation) and high-pass filtering to remove low-frequency noise (up to three cycles in the single run time-course). Individual functional data were smoothed using a 6-mm full-width-at-half-maximum isotropic Gaussian Kernel. For each participant, the two anatomical scans obtained throughout the experiment were averaged using a 3D rigid-body alignment to obtain a high-resolution and high-contrast anatomical scan. The skull was removed by an automatic skull stripping procedure. Functional data were averaged for each participant per condition and aligned with the mean anatomical scan. The mean anatomical scan and the functional data were then spatially normalized using talairach transformation procedures. For group analysis, the normalized individual functional data were averaged, accounting for both scan-to-scan and participant-to-participant variability.

### Statistical analysis

#### Behavioral data

Affect ratings were analyzed by means of repeated measures analysis of variance (ANOVA) by using the General Linear Model (GLM; SPSS 15.0 for Windows) with *Genotype* (S'/S' vs L'/L') as between-subjects factor and *Condition* (down-regulation vs upregulation vs neutral look vs negative look) as within-subjects factor.

#### Functional data

A whole-brain, voxel-wise random effects (RFX) ANOVA was used to test for differences in BOLD signal between conditions. BOLD time courses of individual voxels were regressed onto a pre-specified model into a GLM. GLM predictors were based on 18-s blocks, convolved with a hemodynamic response gamma function (Boynton *et al.*, 1996). Contrasts between conditions were assessed with *t*-statistics for each voxel. To correct for multiple comparisons, a minimum cluster size of nine contiguous voxels was adopted yielding a whole-brain corrected statistical threshold of  $P < 0.05$  as decided by a Monte Carlo simulation (cluster threshold estimator plug in for Brain Voyager QX).

Given the a priori interest in the amygdala, a secondary region of interest (ROI) analysis was performed within the amygdala using an anatomical mask. The anatomical mask was constructed by using a Brainvoyager definition of the SPM anatomy toolbox (Amunts *et al.*, 2005; Eickhoff *et al.*, 2005). To correct for multiple comparisons, a minimum cluster size of six contiguous voxels was adopted yielding a within-ROI corrected statistical threshold of  $P < 0.05$ .

## RESULTS

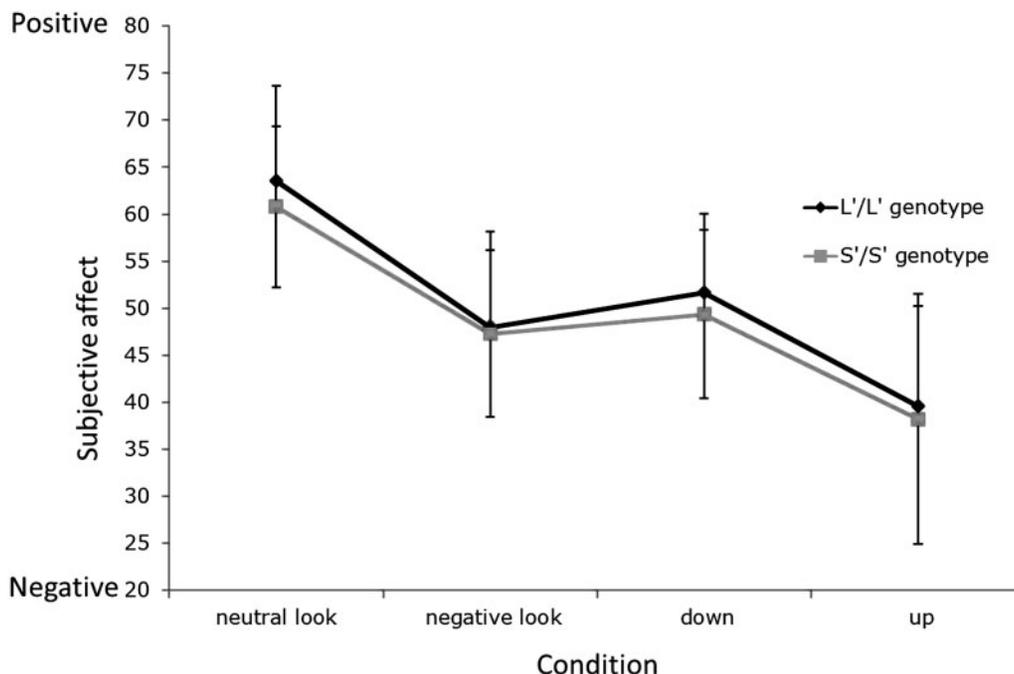
#### Behavioral data

Repeated measures ANOVA on affect ratings only revealed a significant main effect of *Condition* [ $F(3,26) = 29.91$ ,  $P < 0.001$ ]. *Post hoc t*-tests showed that negative affect was greater for the negative look condition than the neutral look condition [ $t(29) = 7.29$ ,  $P < 0.001$ ]. When downregulating negative emotions, participants reported less negative affect compared to the negative look condition [ $t(29) = 3.23$ ,  $P = 0.003$ ], whereas upregulating increased negative affect compared to the negative look condition [ $t(29) = 7.71$ ,  $P < 0.001$ ]. Although the genotype  $\times$  condition interaction was not significant [ $F(3,26) < 1$ , *ns*] separate analyses revealed a significant downregulating effect only for the L'/L' genotype group [ $t(14) = 3.28$ ,  $P = .006$ ] but not for the S'/S' genotype group [ $t(14) = 1.49$ ,  $P = 0.16$ ]. The subjective affect ratings are visualized in Figure 2.

#### Functional data

##### Group differences in emotion perception

To identify differences in neural activation between genotype groups, between-group *t*-tests for the negative look vs neutral look were conducted (Table 2). Differential activations



**Fig. 2** Mean (SE) subjective affect ratings yielded a main effect of condition ( $P < 0.001$ ) revealing stronger positive affect for the neutral look condition than the negative look condition ( $P < 0.001$ ), an increase in positive affect when downregulating negative emotions ( $P < 0.01$ ), and a decrease in positive affect when upregulating negative emotions ( $P < 0.001$ ). Although the genotype by condition interaction was not significant, only in the L'/L' genotype group positive affect increased during downregulation of negative emotions ( $P < 0.01$ ), but not in the S'/S' genotype group ( $P > 0.05$ ).

**Table 2** Significant activations between S'/S' and L'/L' genotype groups for the negative look versus neutral contrast

Region	BA	Talairach coordinates			k	t
		x	y	z		
<b>L'/L' &gt; S'/S'</b>						
R anterior cingulate (dACC)	32/8	10	24	38	825	2.89
L middle frontal gyrus (dlPFC)	8	-20	26	38	444	2.81
R anterior cingulate (vACC)	32/10	15	47	1	160	2.85
L superior temporal gyrus	38	-36	3	-21	84	2.79
L posterior insula	13	-33	-25	18	99	2.77
L cerebellum		-35	-65	-37	107	2.89
<b>S'/S' &gt; L'/L'</b>						
R cerebellum		23	-31	-45	235	3.07

Notes: dACC, dorsal anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; vACC, ventral anterior cingulate cortex; t, t-value; k, cluster size; BA, Brodmann area; R, right; L, left. Talairach coordinates reported in RAI (x=right, y=anterior, z=inferior) of peak voxel activations. Cluster threshold >9 voxels; voxelwise  $P < .01$ , clusterwise  $P < 0.5$  corrected.

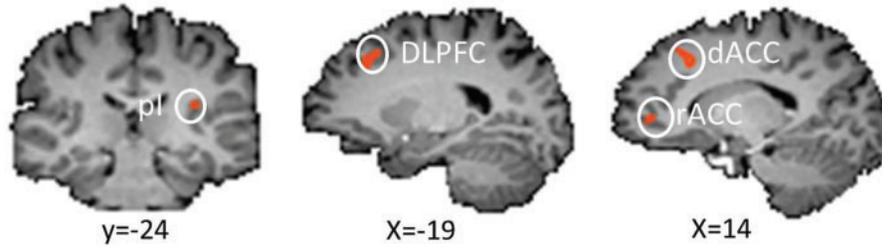
between genotype groups were found in the right dorsal ACC, the left dorsolateral PFC (dlPFC), the rostral ACC and the left posterior insula (Figure 3). The L'/L' genotype group demonstrated greater activations when looking at negative pictures compared to S'/S' genotypes in all of these regions. *Post hoc* analysis showed that these effects were mainly due to a decrease in brain activation for the

S'/S' genotype group when looking at negative compared to neutral pictures (Figure 4).

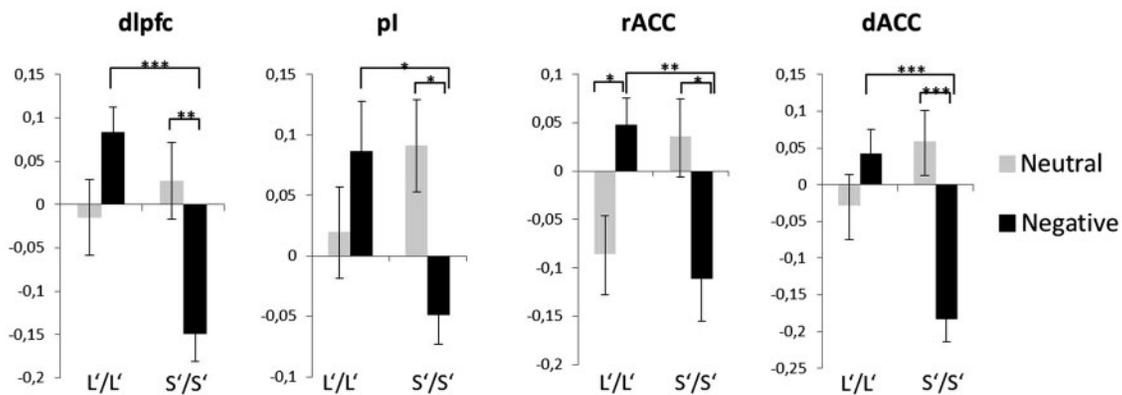
The ROI analyses of the amygdala showed that no differences between genotype groups during emotion perception survived the significance threshold of  $P < 0.05$ . Therefore, genotype groups were collapsed to assess overall effects of emotion perception on amygdala activation, revealing greater right amygdala activity in response to negative compared to neutral photos (Table 3).

**Group differences in emotion regulation**

To identify differences in neural activation between genotype groups, between-group t-tests for the downregulate vs negative look contrast and upregulate vs negative look contrast were conducted (Tables 4 and 5). On the downregulate vs negative look contrast, whole brain analyses revealed greater activations for the S'/S' genotype group in the left anterior PFC and the left anterior insula than the L'/L' genotype group (Figures 5 and 6). On the upregulate vs negative look contrast, whole brain analyses revealed between-group differences in the bilateral dlPFC, right anterior PFC, left dorsal ACC, right lateral orbitofrontal cortex (OFC), right subgenual ACC and bilateral anterior insula. The S'/S' genotype group recruited all of these brain regions to a greater extent than the L'/L' genotype group (Figures 7 and 8). *Post hoc* t-tests showed that genotype-related differences during upregulation have been influenced by group



**Fig. 3** L'/L' carriers showed greater BOLD responses than S'/S' carriers for look negative vs look neutral pictures in the following brain areas: posterior insula (pl), dorsolateral prefrontal cortex (DLPFC), dorsal anterior cingulate cortex (dACC) and rostral anterior cingulate cortex (rACC).



**Fig. 4** Bar plots represent mean % signal change (SE). \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

**Table 3** Amygdala activations collapsed across genotypes

Region	Talairach coordinates					
	BA	x	y	z	k	t
Negative look > neutral look						
R amygdala		18	-8	-8	17	2.78
Downregulate > negative look						
No significant activation clusters						
Upregulate > negative look						
R amygdala		26	-8	-10	345	2.94
L amygdala		-11	-9	-11	616	3.35

Notes: *t*, *t*-value; *k*, cluster size; BA, Brodmann area; R, right; L, left. Talairach coordinates reported in RAI (*x*=right, *y*=anterior, *z*=inferior) of peak voxel activations. Cluster threshold >5 voxels; voxelwise *P* < .01, clusterwise *P* < 0.5 corrected.

**Table 4** Significant activations between S'/S' and L'/L' genotype groups for the downregulate versus negative look contrast

Region	Talairach coordinates					
	BA	x	y	z	k	t
L'/L' > S'/S'						
No differences						
S'/S' > L'/L'						
L Superior Frontal Gyrus (aPFC)	9/10	-31	51	28	545	2.91
L Anterior Insula	13	-36	2	2	282	2.93

Notes: aPFC, anterior prefrontal cortex; *t*, *t*-value; *k*, cluster size; BA, Brodmann area; R, right; L, left. Talairach coordinates reported in RAI (*x*=right, *y*=anterior, *z*=inferior) of peak voxel activations. Cluster threshold >9 voxels; voxelwise *P* < .01, clusterwise *P* < 0.5 corrected.

differences during the negative look condition in some activated brain areas (Figure 8).

The ROI analyses of the amygdala yielded no differences between genotype-groups during emotional up- and downregulation of emotions. To assess overall effects of emotion regulation on amygdala activation, genotype groups were collapsed. While the downregulate vs negative look contrast revealed no differences in amygdala activity, the upregulate vs negative look contrast yielded bilateral amygdala

activation with greater responses during upregulation compared to looking at negative photos (Table 3).

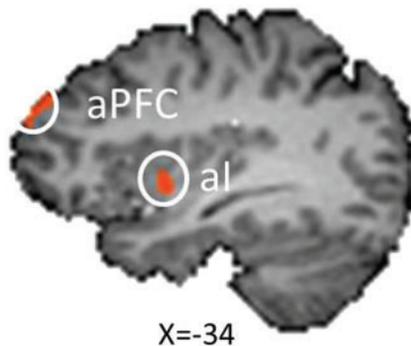
**DISCUSSION**

The aim of the present study was to examine functional differences in neural systems supporting emotion perception and regulation (including up- and downregulation of negative emotions) in individuals with different 5-HTTLPR genotypes. During emotion perception, L'/L' allele carriers

**Table 5** Significant activations between *S'/S'* and *L'/L'* genotype groups for the up-regulate versus negative look contrast

Region	BA	Talairach coordinates			<i>k</i>	<i>t</i>
		<i>x</i>	<i>y</i>	<i>z</i>		
<i>L'/L' &gt; S'/S'</i>						
No differences						
<i>S'/S' &gt; L'/L'</i>						
L middle frontal gyrus (dlPFC)	8	-22	28	34	79	2.82
R middle frontal gyrus (dlPFC)	8	30	31	42	88	2.75
L anterior cingulate (dACC)	32	-13	9	31	399	3.09
R superior frontal gyrus (aPFC)	10	20	50	25	132	2.77
R inferior frontal gyrus (sgACC)	25/47	16	16	-15	121	2.78
R inferior frontal gyrus (IOFC)	47	46	32	-5	117	2.79
L anterior insula	13	-35	0	-7	121	2.82
R anterior insula	13	36	7	-4	343	2.97

Notes: dlPFC, dorsolateral prefrontal cortex; dACC, dorsal anterior cingulate cortex; aPFC, anterior prefrontal cortex; sgACC, subgenual anterior cingulate cortex; IOFC, lateral orbitofrontal cortex; *t*, *t*-value; *k*, cluster size; BA, Brodmann area; R, right; L, left. Talairach coordinates reported in RAI (*x* = right, *y* = anterior, *z* = inferior) of peak voxel activations. Cluster threshold > 9 voxels; voxelwise *P* < 0.01, clusterwise *P* < 0.5 corrected.



**Fig. 5** *S'/S'* carriers showed greater BOLD responses than *L'/L'* carriers for down-regulation vs look negative pictures in the following brain areas: anterior prefrontal cortex (aPFC) and anterior insula (al).

showed greater activity in limbic brain areas such as the posterior insula implicated in emotional awareness (Straube and Miltner, 2011) and in brain areas integral to effortful emotional control (e.g. dlPFC, Ochsner and Gross, 2005; Phillips *et al.*, 2008) and automatic regulation of emotions (e.g. ACC, Phillips *et al.* 2008), whereas during emotional up- and downregulation *S'/S'* carriers showed greater activations in emotional appraisal systems (e.g. anterior insula) suggesting enhanced emotional reactivity and regions critically involved in top-down control of emotions (e.g. aPFC, dlPFC, Ochsner *et al.*, 2004).

### Behavioral differences in emotion perception and regulation

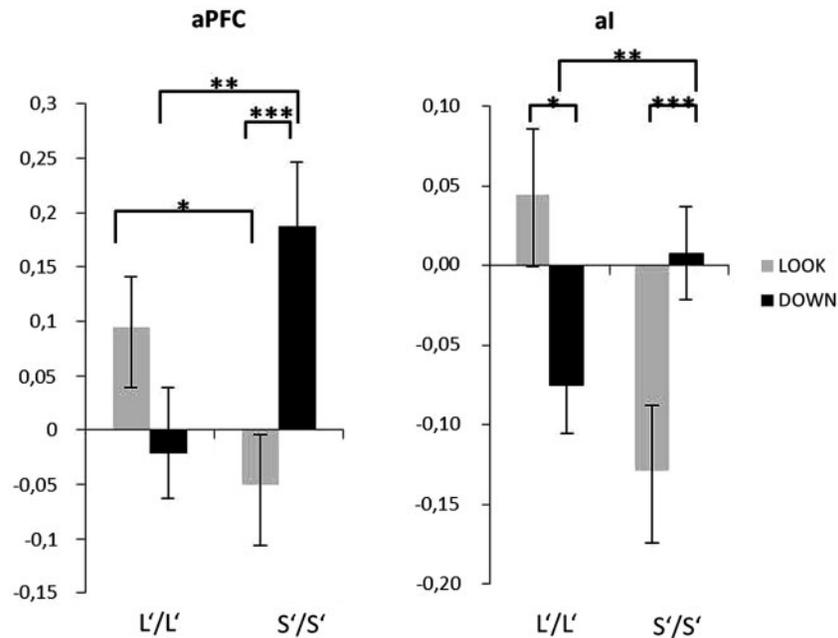
Behaviorally, manipulation of emotion regulation appeared to be successful and was similar between genotype groups.

All participants, regardless of 5-HTTLPR, reported more negative affect when viewing negative compared to neutral pictures and this negative affect further increased after up-regulation. Interestingly, downregulation only in the *L'/L'* allele group decreased negative mood, which may suggest that *S'/S'* carriers downregulate their emotions less effectively. However, this finding should be interpreted with caution since this apparent genotype effect was not preceded by a significant genotype by condition interaction.

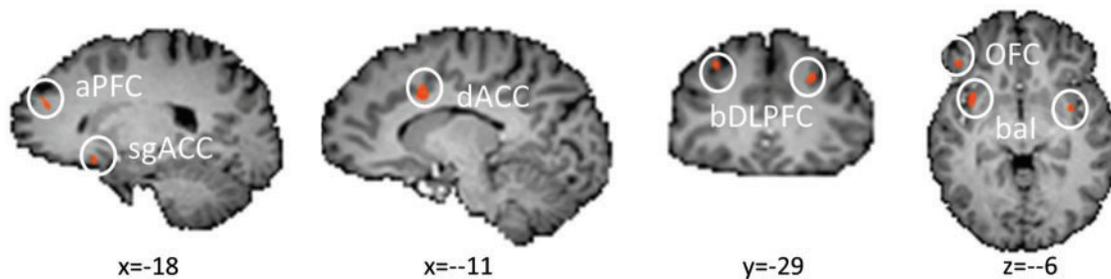
### Neural differences in emotion perception

For negatively compared to neutrally valenced emotional images, *S'/S'* and *L'/L'* genotypes showed different patterns of neural activation. *L'/L'* carriers showed greater activations in the right dorsal and ventral/rostral ACC, the left dlPFC and the left posterior insula. In contrast to our expectations, less activation of the posterior insula, which has been implicated in increased attention to one's own emotion (Straube and Miltner, 2011), may indicate reduced emotional awareness in *S'/S'* carriers when passively viewing negative emotional pictures. Interestingly, *S'/S'* carriers also recruited prefrontal brain areas to a lesser extent than *L'/L'* carriers. Recently, reduced activation of the dlPFC in response to maternal criticism in remitted MDD was found suggesting that diminished prefrontal activation in response to a challenge might be a sign of vulnerability to depression (Hooley *et al.*, 2009). The dlPFC is critically involved in cognitive control processes facilitating goal-directed behavior (Davidson *et al.*, 1999; Mansouri *et al.*, 2009) and is recruited during the generation, maintenance and selection of control strategies that are needed to up- or downregulate emotional experiences and behavior (Ochsner and Gross, 2005). Furthermore, less activation was observed for *S'/S'* carriers in the ACC. The ACC is an important part of the limbic system involved in the generation and regulation of affective states (Critchley *et al.*, 2005) (Bush *et al.*, 2000; McCormick *et al.*, 2006; Mohanty *et al.*, 2007) and has been indicated to play a crucial role in the neurobiology of affective disorders (Davidson *et al.*, 2002). Previous genetic imaging studies revealed inconsistent findings with respect to ACC activations in 5-HTTLPR genotypes. Dannlowski *et al.* (2008) reported increased activation of the ACC (supragenual and perigenual) in response to masked facial emotions in S allele carriers, whereas Shah *et al.* (2009) reported reduced ventral ACC activation in S allele carriers. Philips *et al.* (2008) suggested that the dorsal ACC is involved in automatic and voluntary emotion regulation and the rostral ACC may be particularly involved in the automatic redirection of attention away from emotion.

Thus, greater activations of the dorsal and rostral ACC in *L'/L'* genotypes during this contrast might implicate that the 5-HTTLPR modulates ACC activity as an attempt to automatically regulate negative emotions. However, the differential activation of the posterior insula suggests that *L'/L'* carriers may also be more aware of their own emotions.



**Fig. 6** Bar plots represent mean % signal change (SE). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Fig. 7**  $S'/S'$  carriers showed greater BOLD responses than  $L'/L'$  carriers for upregulation vs look negative pictures in the following brain areas: anterior prefrontal cortex (aPFC), subgenual anterior cingulate cortex (sgACC), dorsal anterior cingulate cortex (dACC), bilateral dorsolateral prefrontal cortex (bDLPFC), orbitofrontal cortex (OFC) and bilateral anterior insula (bal).

A tentative explanation to integrate these findings might be that emotional awareness is required to efficiently regulate emotions, suggesting that when passively viewing negative pictures,  $S'/S'$  carriers have reduced emotional awareness and automatic regulation of negative emotions. This is also indirectly supported by findings of increased prefrontal activity during emotion perception in individuals with greater reappraisal use in everyday life (Drabant *et al.*, 2009).  $L'/L'$  carriers might also use reappraisal strategies more often in their daily lives which might result in spontaneous use of regulation strategies to decrease the emotional impact of negative emotions. However, with the present design it was not possible to test the hypothesis that  $L'/L'$  carriers were more likely to use cognitive strategies during passive viewing and therefore merits further research.

#### Neural differences in emotion regulation

Cognitive reappraisal of negative emotional stimuli (reducing negative affect by reformulation) has been associated

with activity in a network of prefrontal cortical regions that support the selection and application of reappraisal strategies and decreases or increases activity in appraisal systems such as the amygdala or insula to achieve the goal of reappraisal (Ochsner and Gross, 2005; 2008). However, the specific regions that have been indicated differ between studies (e.g. Ochsner *et al.*, 2004; Phan *et al.*, 2005; Kalisch *et al.*, 2006; Kim and Hamann, 2007). In the current study, reappraisal (downregulation) of negative emotions yielded differential activations between genotype groups in the left anterior PFC and in the anterior insula. Greater activation of the anterior insula, important for emotional appraisal, suggests that downregulation of negative emotions is less effective in  $S'/S'$  carriers, which may also be supported by the behavioral data. Thus, also when instructed to downregulate negative emotions, our findings suggest that  $S'/S'$  carriers still show enhanced emotional reactivity. This is in contrast to the findings by Schardt *et al.* (2010), who did not find

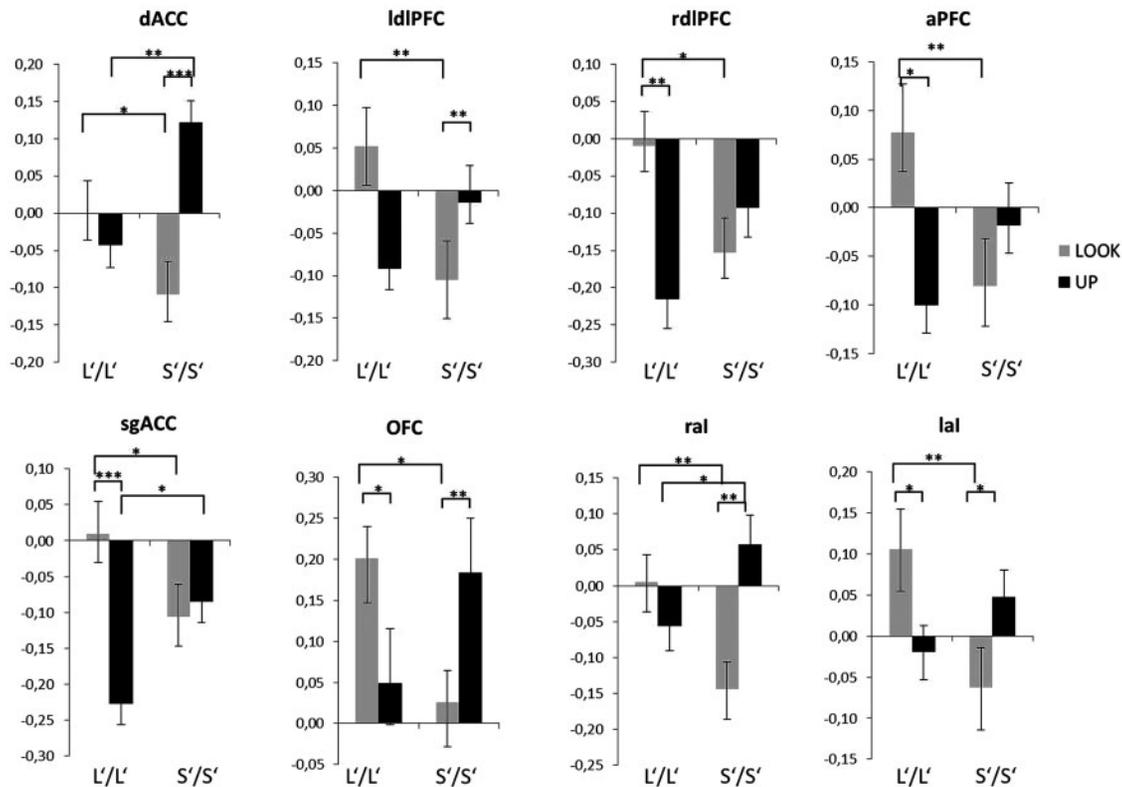


Fig. 8 Bar plots represent mean % signal change (SE). \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ .

genotype-related differential activations during volitional emotion regulation in limbic brain regions.

Further, greater activation of the left anterior PFC, implicated in self-focused regulation of emotions (Ochsner *et al.*, 2004) was seen in S'/S' carriers. One may speculate that S'/S' carriers require more PFC engagement to achieve downregulation of negative emotions, which may have been partly a compensatory response triggered by increased activation of the anterior insula. S'/S' carriers may automatically appraise stimuli as more threatening (Hariri *et al.*, 2002; Pezawas *et al.*, 2005) and may therefore require robust engagement of these systems to attenuate the emotional response. This also converges with findings from studies with depressed individuals (Beauregard *et al.*, 2006; Johnstone *et al.*, 2007) or anxious individuals (Campbell-Sills *et al.*, 2011), suggesting that PFC hyperactivity during downregulation of negative emotion may be a shared risk factor for affective disorders.

As indicated in the introduction, maladaptive emotion regulation may also include enhanced upregulation of negative emotions. As expected, the current study indeed also revealed strong differences in upregulation between genotype groups including brain areas involved in emotional reactivity and emotion regulation. S'/S' carriers showed increased prefrontal activations (right anterior PFC, bilateral dlPFC, right lateral OFC), ACC (left dorsal and right subgenual ACC) and limbic activations (bilateral anterior insula).

Greater activation of the anterior insula suggests that upregulation of negative emotions is more effective in S'/S' carriers. The anterior insula is integral to the subjective awareness of emotion (Craig, 2009). Previously, activation of the anterior insula was observed in association with emotions such as anger, sadness or fear (Damasio *et al.*, 2000), suggesting enhanced emotional processing in S'/S' carriers compared to L'/L' carriers during upregulation of negative emotions. Furthermore, greater activation of the ACC was found in S'/S' carriers. Craig (2009) suggests that the insula and the ACC are complementary limbic regions, with the insula being the site for awareness of feelings and the ACC being the site for initiating emotional behavior based on joint activation of the anterior insula and the ACC in subjects experiencing emotional feelings. Olsson and Ochsner (2008) suggest that the anterior insula and ACC facilitate emotional understanding, which in turn might provide a substrate for empathy (Lamm *et al.*, 2007). In support of this idea, empathic feelings were associated with increased activation of the bilateral anterior insula (Singer *et al.*, 2004), whereas reduced empathy was associated with reduced activity in the anterior insula (Sterzer *et al.*, 2007). Further, heightened anxiety has also been associated with increased activity of the anterior insula (Paulus and Stein, 2006). Thus, the enhanced activation of the anterior insula and ACC during upregulation in S'/S' carriers might play a role in the heightened emotionality of S carriers and may, on the

one hand, be a substrate for increased vulnerability to negative emotional experiences (e.g. stressful life events) that makes them more vulnerable for depression and other affective disorders and, on the other hand, may be adaptive by providing the basis for high empathic behavior. Recently, Homberg and Lesch (2011) urged for a conceptual change in the current deficit-oriented connotation of the 5-HTTLPR variants by postulating that the positive effects of the S allele may counterbalance or offset the negative consequences of the anxiety-related traits, which would explain why the S allele was maintained throughout evolution.

### Limitations

Contrary to earlier reports (Hariri *et al.*, 2002; 2005; Munafo *et al.*, 2008), no differences in amygdala activity were found between genotype groups. One explanation for that may be that such small effects are subtracted out due to the neutral condition that also included social pictures and not only inanimate objects. Furthermore, previous reports on genotype-related differential activation of the amygdala employed passive viewing paradigms including fearful facial expression, which might activate the amygdala more strongly. Additionally, the current study might be underpowered to detect such small effects, which could have resulted in type II errors (Munafo *et al.*, 2008). Nevertheless, the present findings of differential activations between genotype groups in the insula and the prefrontal cortex, which may play an even more important role in emotion processing than the amygdala (Pessoa and Adolphs, 2010) and extend previous findings of a strong genetic influence on brain activations during emotion perception and regulation.

### CONCLUSION

The present findings reveal 5-HTTLPR-related differential brain activation during emotion perception and regulation. Compared to L'/L' allele carriers, subjects who carry the S'/S' allele showed less activation in the posterior insula and in prefrontal brain areas during passive perception of negative emotional information but showed greater anterior insula activation and prefrontal activation during down- and up-regulation of negative emotional responses. These results support previous findings of enhanced emotionality in S'/S' carriers by providing additional evidence for differential neural activation in S'/S' carriers during volitional emotion regulation. Specifically, the findings of exaggerated prefrontal and limbic activations to negative stimuli in S'/S' carriers during cognitively increasing emotional responses resembles findings in depressed patients (Cooney *et al.*, 2010) and might contribute to the challenge of explaining the increased vulnerability of 5-HTTLPR-S carriers to affective disorders.

### Conflict of Interest

None declared.

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