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Neural and behavioral responses to threatening emotion faces in children as a function of the short allele of the serotonin transporter gene

Moriah E. Thomason[a,*], Melissa L. Henry[a], J. Paul Hamilton[a], Jutta Joormann[b], Daniel S. Pine[c], Monique Ernst[c], David Goldman[c], Karin Mogg[d], Brendan P. Bradley[d], Jennifer C. Britton[c], Kara M. Lindstrom[c], Christopher S. Monk[e], Lindsey S. Sankin[c], Hugo M.C. Louro[a], Ian H. Gotlib[a]

[a] *Department of Psychology, Stanford University, Stanford, CA, USA*
[b] *Department of Psychology, University of Miami, Miami, FL, USA*
[c] *Mood and Anxiety Disorders Program, National Institute of Mental Health, National Institutes of Health, USA*
[d] *Department of Psychology, University of Southampton, United Kingdom*
[e] *Department of Psychology, University of Michigan, Ann Arbor, MI, USA*

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ABSTRACT

Recent evidence suggests that a genetic polymorphism in the promoter region (5-HTTLPR) of the serotonin transporter gene (*SLC6A4*) mediates stress reactivity in adults. Little is known, however, about this gene-brain association in childhood and adolescence, generally conceptualized as a time of heightened stress reactivity. The present study examines the association between 5-HTTLPR allelic variation and responses to fearful and angry faces presented both sub- and supraliminally in participants, ages 9–17. Behaviorally, carriers of the 5-HTTLPR short (*s*) allele exhibited significantly greater attentional bias to subliminally presented fear faces than did their long (*l*)-allele homozygous counterparts. Moreover, *s*-allele carriers showed greater neural activations to fearful and angry faces than did *l*-allele homozygotes in various regions of association cortex previously linked to attention control in adults. These results indicate that in children and adolescents, *s*-allele carriers can be distinguished from *l*-allele homozygotes on the basis of hypervigilant behavioral and neural processing of negative material.

1. Introduction

Serotonin plays a critical role in the modulation of emotion (Lucki, 1998). A common functional variant in the human serotonin transporter gene (5-HTTLPR; *SLC6A4*) produces a short or long nucleotide repeat chain that alters serotonin availability. Compared to the long (*l*) allele, the short (*s*)-allele gene variant is associated with reduced serotonin reuptake in vitro (Heils et al., 1996; Lesch et al., 1996), which appears to have adverse behavioral consequences (Lesch et al., 1996; Mazzanti et al., 1998; Zalsman et al., 2006; Taylor et al., 2006; Kendler et al., 2005; Lohmueller et al., 2003; Caspi et al., 2003). Indeed, although results have been equivocal (Risch et al., 2009; Belsky et al., 2009), 5-HTTLPR has been found to interact with stress to predict the onset of depression (Caspi et al., 2003).

In an effort to elucidate the mechanisms that may underlie the association between the 5-HTTLPR polymorphism and depression, researchers have examined associations between 5-HTTLPR and markers of stress reactivity. For example, Gotlib et al. (2008) found that adolescent girls who were homozygous for the *s* allele secreted more cortisol over a longer period of time in response to an acute stressor than did *l*-allele carriers (Gotlib et al., 2008). Other investigators have examined the relation between 5-HTTLPR and brain function and structure. Compared with *l*-allele homozygotes, *s*-allele carriers have been found to exhibit reduced grey matter volume (Canli et al., 2005; Pezawas et al., 2005), increased amygdala activity to threatening faces (reviewed in Munafo et al., 2008), and altered functional coupling of emotion-regulation brain circuits encompassing the amygdala and associated prefrontal cortical (PFC) projection zones (Pezawas et al., 2005; Heinz et al., 2005; Canli et al., 2006). These findings of increased cortisol and increased neural responsivity to threat suggest that exacerbated stress or arousal responses to environmental threat underlie the association between the *s* allele and increased trait negative affect (Munafo et al., 2003; Schinka et al., 2004; Sen et al., 2004).

Cognitive attention theories may be relevant to our understanding of how the 5-HTTLPR gene may exert its effects, particularly implicating modulation of attention. In the clinical domain, cognitive theories linking enhanced detection of potentially threatening

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* Corresponding author at: Department of Psychology, Stanford University, Jordan Hall, Bldg. 420, Stanford, CA 94305-2130, USA. Tel.: +1 650 725 0797; fax: +1 650 725 5699.
E-mail address: moriah@stanford.edu (M.E. Thomason).

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cues in the environment to anxiety have been influential. Thus, individuals who prioritize attention allocation (Mathews, 1990) towards negative or threatening material are more susceptible to mood and anxiety disorders (Williams et al., 1988; Eysenck, 1992). Indeed, heightened attention to negative stimuli has been observed across emotional disorders: in children and adolescents who are diagnosed with anxiety disorders (Monk et al., 2006, 2008a; Waters et al., 2004), who are at risk for depression (Joormann et al., 2007; Monk et al., 2008b), and/or who have high levels of trait anxiety (Telzer et al., 2008). By altering serotonin levels, the 5-HTTLPR gene may change the sensitivity to processing negative information on the environment. This formulation is supported by recent research indicating that children with the *s* allele of the 5-HTTLPR gene have faster responses to angry faces (Pérez-Edgar et al., 2010). It is possible, therefore, that individuals with the 5-HTTLPR *s* allele have a greater tendency to direct processing resources toward danger-relevant stimuli. If persistent hypervigilant orienting toward negative material in the environment does characterize *s*-allele carriers, then it is important to elucidate patterns of neural processing that are associated with this allocation difference, and to learn whether these behaviors are observable from a young age, before significant or chronic life stressors have been likely to exert their effects.

The present functional magnetic resonance imaging (fMRI) study was designed to examine whether 5-HTTLPR genotype affects the neural substrates of spatial orienting of attention in a sample of unselected, healthy children and adolescents. We focused on this age range because childhood and early adolescence are times when individuals may experience their first onset of depression and other disorders, as well as a time of heightened stress reactivity (Dahl and Gunnar, 2009). Consequently, we were interested in whether at a young age, this gene would affect cognitive biases, presumably training the brain for environmental processing that will shape later life experiences. In particular, we expected differences in regions of the parietal cortex and lateral frontal cortex that interact with sensory brain structures to control human visual attention (Corbetta et al., 2000; Hopfinger et al., 2000), and that are postulated to control enhanced processing of emotionally salient material (reviewed in Pourtois and Vuilleumier, 2006). During fMRI image acquisition, healthy children and adolescents completed the dot-probe task, one of the most widely used tasks in the assessment of behavioral and neural aspects of attentional biases (Monk et al., 2006, 2008a; Waters et al., 2004; Telzer et al., 2008; Vasey and MacLeod, 2001). We assess responses to both subliminal and supraliminal stimulus presentation durations because whereas briefly presented stimuli have been found to be associated with biases in anxiety, depression has been found to be characterized by biases for stimuli that are presented for longer durations (Joormann et al., 2007). Moreover, neural responses have been shown to differ with presentation rate (Monk et al., 2006, 2008a).

We hypothesized that carriers of the *s* allele would exhibit behavioral and neural responses reflecting a cognitive system that prioritizes processing of negative emotional material. Specifically, we predicted that *s*-allele carriers would demonstrate (1) enhanced neural processing (i.e., greater BOLD signal response) during the presentation of fear and angry (compared with neutral) faces than would *l*-allele homozygotes; and (2) faster behavioral responses to dot probes when they replace fear and angry (compared with neutral) faces.

2. Materials and methods

2.1. Participants

Participants were 51 adolescents (24 females) between the ages of 9 and 17 years ($M = 13.12$, SD = 2.75). Participants were recruited through local advertisements and parent networks and scanned either at the National Institute of Mental Health (NIMH) functional MRI Facility (fMRIF) ($n = 17$) or at Stanford University's Richard M. Lucas Center for Imaging ($n = 34$). All participants had no reported history of brain injury, no behavioral indications of possible mental impairment, no past or present Axis I disorder, and were fluent in English. Three participants were left-handed, and one participant had questionable lifetime diagnosis of Attention/Deficit Hyperactivity Disorder. At both sites, the participants were compensated for their time. Parents and adolescents gave informed consent and assent, respectively, as approved by the NIMH and Stanford Institutional Review Boards.

2.2. Measures

Trained interviewers assessed the diagnostic status of the adolescents by administering the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL), which has been shown to generate reliable and valid psychiatric diagnoses (Kaufman et al., 1997). Any adolescent who received a current or past Axis-I diagnosis was eliminated from the study. To assess inter-rater reliability, for Stanford participants an independent trained rater evaluated 30% of all K-SAD-PL interviews by randomly selecting audiotapes. In all cases, these diagnoses matched the diagnoses made by the original interviewer, $\kappa = 1.00$, indicating excellent inter-rater reliability. Similarly, at the NIMH site, all raters had to complete standardized training and then demonstrate acceptable reliability in interviews conducted with 10 individuals.

To ensure that participants did not differ in current levels of depressive symptomatology, all participants completed the short form (10-item) of the Children's Depression Inventory (CDI-S), a self-report measure of depressive symptoms developed for children and adolescents between the ages of 8 and 17 (Kovacs, 1985). The CDI-S has been demonstrated to have acceptable internal consistency ($\alpha = .80$) and to correlate highly with the full CDI ($r = .89$) (Kovacs, 1992). In addition, to assess levels of anxiety, participants also completed the 41-item version of the Screen for Child Anxiety Related Emotional Disorders (SCARED). The SCARED, too, has been demonstrated to have good internal consistency ($\alpha = .74$–.93) and test–retest reliability (correlation coefficients = .7–.9) (Birmaher et al., 1997).

2.3. Procedure

The study consisted of two separate sessions. In the first session, all parent–child dyads participated in diagnostic interviews to assess DSM-IV current and lifetime diagnostic status using the K-SADS-PL (Geller et al., 1996, 2001). During this session, adolescents also provided saliva samples for genetic testing and viewed a video or visited a mock scanner to prepare them for the MRI scan session. In the second session, brain-imaging data were acquired from the adolescents using a whole-brain MRI scanner.

2.4. Stimuli

The dot-probe task has been previously used in adolescents with generalized anxiety disorder (Monk et al., 2006). The task involved viewing a central fixation point, then viewing two faces presented side-by-side, and finally indicating the location of an asterisk (probe) via a left or right button press (Fig. 1). Thus, participants were not instructed to deliberately process the face stimuli, but were instructed simply to press a button indicating the side on which the asterisk appeared following the pair of faces, or following faces + scrambled images (subliminal trials). Faces from the NIMSTIM set (Tottenham et al., 2009) were presented in pairs of neutral/neutral, neutral/angry (as seen in Fig. 1), and neutral/fearful expressions; each pair contained a single actor with two different expressions, except neutral trials, in which the same picture appeared on the right and left. Trials were balanced to have the emotion and target equally presented on the left and right. In addition, an equal

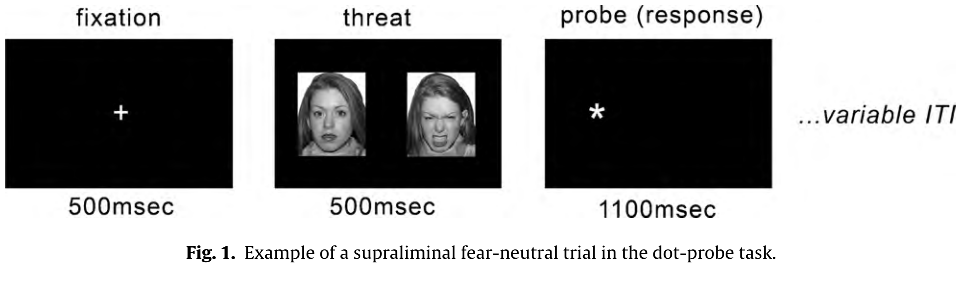

Fig. 1. Example of a supraliminal fear-neutral trial in the dot-probe task.

number of congruent (asterisk appears on the same side as the expressive emotion face) and incongruent (asterisk appears on the same side as the neutral face) trials were presented.

The task included an additional manipulation of face-stimuli presentation duration, supraliminal and subliminal, in order to assess automatic processing of emotional material. Participants were not told that the faces were being presented subliminally. Instead, they were simply taught that the task required them to press a button when a probe asterisk appeared regardless of what preceded it: a pair of faces or a pair of scrambled pictures. For subliminal trials, the timing of events was: fixation: 500 ms; face-pair: 17 ms; mask (scrambled image): 68 ms; probe: 1100 ms; blank screen: 415 ms (with timing informed by previous designs, Mogg and Bradley, 2002). For supraliminal trials, the timing of events was: fixation: 500 ms; face-pair: 500 ms; probe: 1100 ms. Although for both subliminal and supraliminal trials the total trial length was held constant (2100 ms), the structure of the two trial types were different due to the two face exposure durations; consequently, supra- and subliminal trials were analyzed independently. In this rapid-event-related design, trials were separated by a jittered (variable length) ITI that was between 750 and 1250 ms (average ITI = 1000 ms). The ITI was jittered to vary the timing of presentations for psychological purposes, so that subjects could not completely anticipate the timing of stimulus presentations, rather than for purely hemodynamic purposes. To improve hemodynamic response estimation, blank or "null" trials were included to help deconvolve the BOLD signal. Each of four functional runs consisted of 96 trials (16 of which were blank/null trials) in random order. Runs lasted 5 min and 18 s, for a total functional scan duration of approximately 21 min.

Participants were given a dot-probe practice task outside of the scanner until they understood how to perform the task, and performed 12 practice trials in the scanner immediately preceding the first run of the task. During scanning, visual stimuli were generated using Eprime (http://www.pstnet.com) on a PC computer, and were presented onto a screen viewed by the participant inside the fMRI machine. Participants used a button box interfaced to the Eprime computer to make their responses.

2.5. Genetic analysis

DNA through saliva samples were analyzed using the Oragene Kit (DNA Genotek, Inc., Ottawa, Ontario, Canada), an all-in-one system for the collection, preservation, transportation and purification of DNA from saliva. This procedure is minimally invasive. DNA extracted by this method is of high quality and allows for genotyping with a high success rate (Rylander-Rudqvist et al., 2006). To examine the 5-HTTLPR polymorphism, oligonucleotide primers flanking the 5-HTT-linked polymorphic region (Heils et al., 1996) and corresponding to the nucleotide positions −1416 to −1397 (stpr5, 5′-GGC GTT GCC GCT CTG AAT GC) and −910 to −888 (stpr3, 5′-GAG GGA CTG AGC TGG ACA ACC AC) of the 5-HTT gene 5′-flanking regulatory region were used to generate 484 bp or 528 bp fragments. The PCR products were electrophoresed through 5% polyacrylamide gel (acrylamide/bis-acrylamide ratio 19:1) at 60 V for 60 min.

Following this genotyping procedure, two groups of children were identified: those possessing at least one copy of the 5-HTTLPR *s* allele ($n = 31$) and those carrying two *l* alleles ($n = 20$). Classifying participants based on the triallelic classification of 5-HTTLPR (i.e., considering the A-G single nucleotide substitution in the *l* allele, Neumeister et al., 2006; Wendland et al., 2006) resulted in 10 participants with two l_A alleles and 41 participants with at least one l_G or s-allele. Because of both the small number of l_A participants in the sample and the considerably greater body of research the bi-allelic classification, we conducted analyses using the biallelic (*ss*/*sl* vs. *ll*) classification that yielded more balanced numbers in each gene group.

2.6. Behavioral analysis

Attentional bias scores were calculated from the latency data for each type of emotional face (fear and angry) and for exposure duration (subliminal [17 ms] and supraliminal −[500 ms]) as described by Joormann et al. (2007). Briefly, the bias score was calculated by subtracting the mean reaction time for identifying probes appearing on the same side as the emotion face from the mean reaction time for identifying probes appearing on the opposite side as the emotion face, after excluding error trials. Thus, positive scores indicate greater attentional capture by the emotional face, and negative scores reflect the tendency to avoid the emotional face. Attentional bias scores were analyzed using a three-way (genotype group [*l*-allele homozygotes, *s*-allele carriers], emotion [fear, angry], presentation duration [subliminal, supraliminal]) analysis of variance (ANOVA). Alpha = .05 was used to test significant main effects and interactions. Bias scores were also submitted to one sample *t*-tests in order to determine if bias scores were significantly different than zero.

2.7. fMRI data acquisition

Magnetic resonance imaging was performed on a 3.0 T GE whole-body scanner at both sites. A purpose-built single channel T/R head was used at Stanford and an 8-channel head coil was used at NIH. To reduce motion-related artifacts during scanning, participants were stabilized by clamps and a bite bar formed with dental impression wax at Stanford (made of Impression Compound Type I, Kerr Corporation, Romulus, MI) and with expandable cushions surrounding the head at NIH. Senior physicists at each of the two sites optimized institution-specific scanning parameters; therefore, the scan parameters were consistent at both sites unless described otherwise.

High-resolution T2-weighted fast spin-echo structural images (TR = 3000 ms; TE = 68 ms) were acquired for anatomical reference. A T2*-sensitive gradient echo spiral in/out pulse sequence (Glover and Law, 2001) was used for all functional imaging at Stanford and NIH used a gradient echo single-shot bottom-up interleaved sequence (TR = 2100 ms; TE = 30 ms; flip angle = 77° at Stanford, 78° at NIH; FOV = 22 cm; 64 × 64; 29 axial slices with 4 mm slice thickness and no skip). An automated high-order shimming procedure, based on spiral acquisitions, was used to reduce B0 heterogeneity (Kim et al., 2002). High-resolution volume scans (140 slices at Stanford, 144 slices at NIH; 1 mm slice thickness) were collected for every participant using a spoiled grass gradient recalled (SPGR) sequence for T1 contrast (TR = 3000 ms at Stanford, 700 ms at NIH; TE = 68 ms at Stanford, minimum at NIH; TI = 500 ms; flip angle = 11°; FOV = 25 cm at Stanford, 22 cm at NIH; 256 × 256). During the functional scans, heart-rate and respiration waveforms were recorded.

2.8. fMRI analysis

fMRI data were preprocessed using Analysis for Functional Neuroimages (AFNI, http://afni.nimh.nih.gov/afni) (Cox, 1996) and custom MATLAB routines. Preprocessing included slice-timing correction, realignment, smoothing (4 mm), and bandpass filtering ($.011 < f < .15$). Four runs of the experiment were concatenated into one long run, for which task vectors specific to each randomized run were generated from participants' behavioral files. Once convolved with a canonical hemodynamic response function (HRF) the extracted task vectors for each emotion condition (fear, angry, neutral) within each of supra- and subliminal, were then used to model BOLD response to each condition of interest. We created contrasts for fear > neutral and angry > neutral and submitted these to between-group (*l*-allele homozygotes vs. *s*-allele carriers) *t*-tests to assess neural response to emotion-face vs. neutral-face baseline for each of four conditions: (1) fear > neutral subliminal; (2) angry > neutral subliminal; (3) fear > neutral supraliminal; and (4) angry > neutral supraliminal. Results were spatially constrained to a grey matter mask image and transformed to Talairach space for reporting. Results are reported for $p < .01$, corrected. Multiple testing correction was performed using the AFNI subroutine.

Multiple testing correction was performed using the AFNI subroutine, AlphaSim, which used 10,000 Monte Carlo simulation to estimate the number of contiguous voxels one would expect to observe in a significant cluster, given the *p* threshold used and number of comparisons made. The correction is based on the principal that true regions of activation will tend to occur over contiguous voxels; whereas, noise has much less tendency to form clusters of activated voxels (Ward, 2000). For the present study data, AlphaSim was applied using a grey matter mask, which contains 23,866, 3.75 (Lesch et al., 1996) voxels, and a 4 mm FWHM smoothing kernel. Using these parameters, a cluster size threshold of 9 voxels was necessary to achieve $p < .01$ correction.

3. Results

3.1. Group characteristics

The two genotype groups (*l*-allele homozygotes, *s*-allele carriers) did not differ significantly with respect to age, $t(49) = 1.18$, $p = .25$, CDI-S scores, $t(47) = .27$, $p = .79$, or SCARED scores, $t(46) = .78$, $p = .43$. In addition, the observed frequency of the short and long alleles were in Hardy–Weinberg equilibrium, $\chi^2 = 4.62$, $p = .1$.

3.2. Dot-probe behavioral data

Four participants were excluded from analysis because of errors in data collection. For all participants, trials with errors were discarded. The mean percentage of data loss to participant errors was low (less than 5%) and did not differ between gene groups, $t(45) = 1.16$, $p = .25$.

The ANOVA conducted on the behavioral data did not yield significant main effects for gene group, $F(1,49) = .19$, $p = .67$, for face emotion, $F(1,49) = .90$, $p = .35$, or for the duration of presentation, $F(1,49) = 3.21$, $p = .08$. Moreover, none of the two-way interactions or the three-way interaction of gene group, face emotion, or presentation duration were significant, all *p*s > .05. Because we hypothesized that *s*-allele carriers would demonstrate greater attentional bias to fear and angry emotional faces than would *l*-allele homozygotes, we conducted separate *t*-tests on participants' bias scores for each of the four conditions: subliminal fear, sub-

Table 1
Means and attentional bias scores for each gene group.

		ll carriers (*N* = 20)	*s*-allele carriers (*N* = 31)	Between groups statistic
	Age	12.6 (2.7)	13.5 (2.8)	$t(49) = 1.18$, $p = .25$
Average reaction times across trials	Subliminal fear	554 (127)	557 (120)	$t(49) = .11$, $p = .92$
	Supraliminal fear	532 (116)	537 (123)	$t(49) = .15$, $p = .88$
	Subliminal angry	546 (122)	557 (126)	$t(49) = .31$, $p = .76$
	Supraliminal angry	527 (120)	537 (117)	$t(49) = .29$, $p = .77$
	Subliminal neutral	551 (126)	555 (118)	$t(49) = .12$, $p = .91$
	Supraliminal neutral	532 (116)	541 (131)	$t(49) = .24$, $p = .81$
Attentional bias scores	Subliminal fear	−4.05 (28)	12.14 (22)**	**$t(49) = 2.28$, $p = .027$**
	Supraliminal fear	5.89 (45)	4.95 (37)	$t(49) = .08$, $p = .94$
	Subliminal angry	2.12 (38)	−0.47 (23)	$t(49) = .30$, $p = .76$
	Supraliminal angry	18.56 (32)*	15.35 (45)	$t(49) = .28$, $p = .78$

Means and standard deviations are provided for each comparison. Significant between group differences in attentional bias were observed for the subliminal fear condition (p value shown in bold text). Asterisks are used to denote the significance of one-sample t-tests (**at the .01 level or *at the .05 level) aimed at answering whether within-group means are significantly different than zero.

liminal angry, supraliminal fear, supraliminal angry to examine whether the gene groups differed significantly in any of these four conditions. These analyses indicated that the gene groups differed significantly in their attentional bias only in the subliminal fear condition, $t(49) = 2.28$, $p = .03$. The group difference in response to subliminal fear resulted from a significant positive mean attentional bias score $t(30) = 2.97$, $p < .01$ within *s*-allele carriers and a non-significant negative attentional bias score in *l*-allele homozygotes, reflecting greater attentional capture by subliminally presented fear faces in *s*-allele carriers; see Table 1.

Table 2
Regions of significant differences between 5-HTTLPR polymorphism groups for both subliminal and supraliminal contracts.

	BA	*x*	*y*	*z*	Volume (voxels)	T score	Fig. 2 label
Subliminal fear > neutral							
S-carriers > LL							
Parietal							
Precuneus	R7	23	−70	36	18	3.97	C
Precuneus	R3I	16	−50	35	10	3.89	D
Precuneus	L7	−24	−61	38	24	2.56	E
Posterior cingulate	L23	−6	−31	26	13	3.48	B
Occipital							
Middle	L18	−13	−88	17	40	3.27	A
LL > S-carriers							
For this contrast, no clusters survived threshold							
Suhliminal angry > neutral							
S-carriers > LL							
Limbic							
Cingulate	R24	12	1	32	27	3.69	G
Cingulate	R23	7	−24	28	11	2.24	F
LL > S-carriers							
For this contrast, no chusters survived threshold							
Supraliminal fear > neutral							
S-carriers > LL							
Parietal							
Inferior	R40	56	−37	42	16	3.26	1
LL > S-carriers							
Temporal							
Superior	R21	55	−24	−1	12	3.14	H
Supraliminal angry > neutral							
S-carriers > LL							
Frontal							
Inferior	R44/45	57	10	22	13	3.10	K
Superior	R1O	25	49	19	11	3.91	L
Inferior	L44	−48	12	11	10	4.20	
Parietal							
Inferior	L40	−40	−32	41	120	2.78	O
Inferior	R7	32	−53	45	47	3.42	N
Interior	R40	51	−36	40	37	2.82	M
Limbic							
Insula	L13	−35	−1	5	9	2.79	J
LL > S-carriers							
For this contrast, no clusters survived threshold							

Coordinates are given in Talairach and Tournoux convention. BA = Brodmann's area. Results provided for $p < .01$, corrected. LL = *ll* allele homozygotes; S-carriers = carriers of at least one s-allele; R = right; L = left.

3.3. fMRI results

3.3.1. Fear > neutral subliminal

Between-groups comparisons for fear > neutral subliminal revealed that *s*-allele carriers had significantly ($p < .01$, corrected) greater BOLD activation than did *l*-allele-homozygotes in regions of the parietal and occipital cortices, in particular, medial as well as lateral aspects of the precuneus, and the posterior cingulate. The reverse contrast at the same threshold (*l*-allele homozygotes > *s*-allele carriers) did not yield any significant clusters; see Table 2.

3.3.2. Angry > neutral subliminal

The between-groups comparison for angry > neutral subliminal contrast images resulted in greater activation in the *s*-allele carriers than in the *l*-allele homozygotes participants in the cingulate gyrus, compared to no significant clusters of greater response in *l*-allele homozygotes.

3.3.3. Fear > neutral supraliminal

Between-groups analysis of fear > neutral supraliminal trials showed that *s*-allele carriers exhibited significantly greater ($p < .01$, corrected) activation in an inferior parietal region encompassed by Brodmann's area 40. For the reverse contrast, there was a cluster in the superior temporal gyrus that showed greater BOLD response to fear > neutral supraliminal in *l*-allele homozygotes than in *s*-allele carriers; see Table 2.

3.3.4. Angry > neutral supraliminal

The between-groups comparison of angry > neutral supraliminal statistical maps revealed numerous regions, including frontal, parietal, and paralimbic (insula cortex) regions, in which response was significantly greater ($p < .01$, corrected) in *s*-allele carriers than in *l*-allele homozygotes. In contrast, for the same statistical threshold, there were no brain regions in which *l*-allele homozygotes exhibited greater response in this contrast than did the *s*-allele carriers; see Table 2.

3.3.5. Consistencies across contrasts

In sum, there was consistency in the whole-brain between-groups comparisons: *s*-allele carriers exhibited greater activation than did *l*-allele homozygotes in numerous parietal and paralimbic regions; see Fig. 2 and Table 2. There was only one region (superior

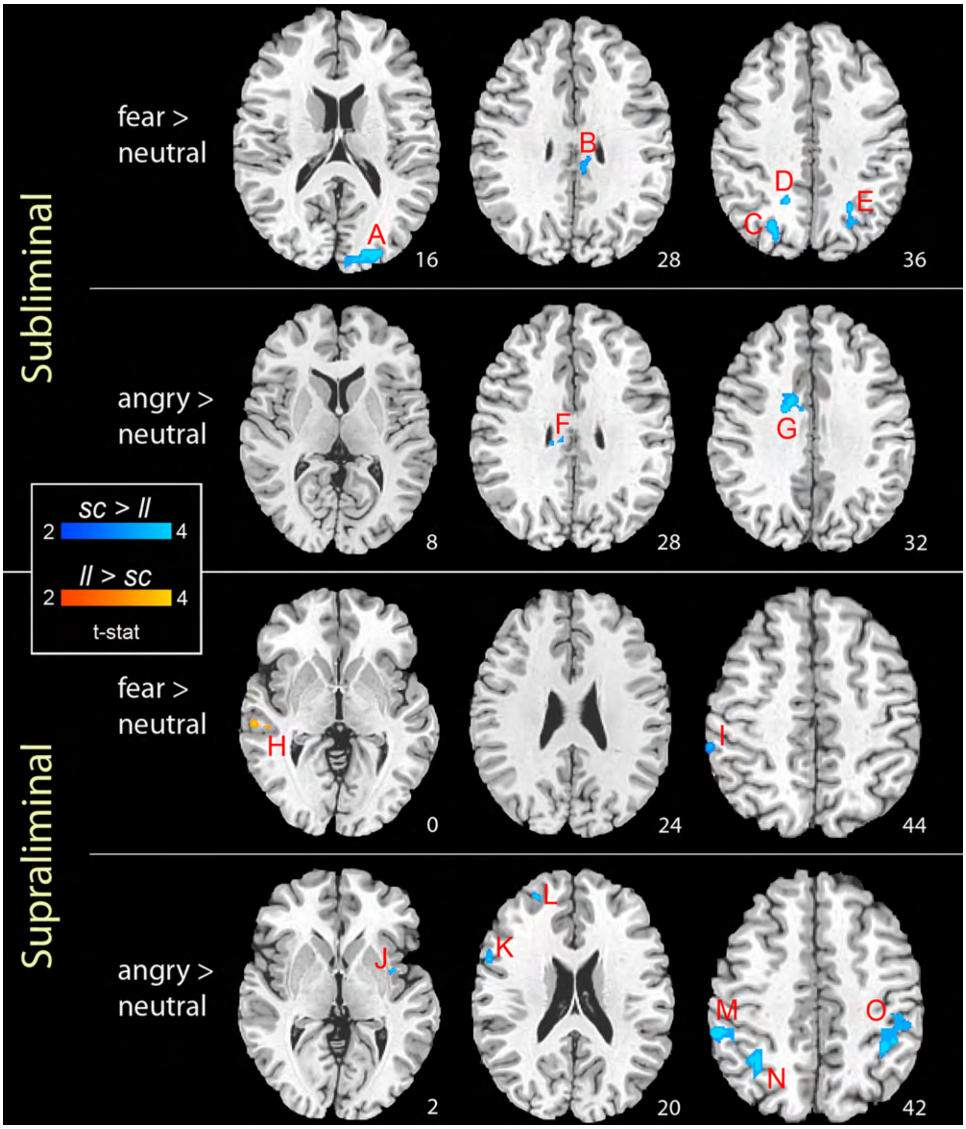

Fig. 2. Between-group whole brain *t*-tests ($p < .01$ corrected) show that, overall, carriers of the 5-HTTLPR *s*-allele demonstrate greater neural response to emotion faces across conditions than *l*-allele homozygotes. Regions where neural response was greater in *s*-allele carriers are shown in blue; regions where neural response was greater in *l*-allele homozygotes are shown in orange. Letters correspond to clusters provided in Table 2. *sc* = *s*-allele carrier; *ll* = l-allele homozygotes.

temporal cortex) in which *l*-allele homozygotes exhibited significantly ($p < .01$; cluster min, or $k \geq 9$) greater BOLD response than did *s*-allele carriers.

4. Discussion

Recent studies suggest that attentional biases to threat play a causal role in the development of anxiety in both children (Brotman et al., 2007; Eldar et al., 2008; Roy et al., 2008) and adults (Macleod et al., 1986). There is also mounting evidence in children and adolescents that common variations in the serotonin transporter gene are associated with biases to threat (Pérez-Edgar et al., 2010; Gibb et al., 2009). Recent work has added to this formulation, demonstrating that cortisol responses (indicative of biological stress reactivity) are greater in children who are homozygous for 5-HTTLPR *s*-allele than for *l*-allele carriers (Gotlib et al., 2008). The neural mechanisms underlying these relations, however, are not yet well understood. We examined whether 5-HTTLPR polymorphism is associated with behavioral biases in processing of emotional material and investigated the neural bases of these responses. Importantly, we recruited a sample of healthy adolescents with no current psychopathology or history of any disorder.

We found that the *s*-allele was associated with significantly greater attentional bias to subliminally presented fear faces. This behavioral observation supports the formulation that attentional mechanisms are altered in *s*-allele carriers. In the present study, we show for the first time that even when emotional information is presented too briefly to reach awareness, biased information processing is associated with variation in 5-HTTLPR. Therefore, individual differences in the processing of rapidly presented emotional stimuli that do not reach awareness may underlie the association between the *s*-allele and the emergence of difficulties in emotion regulation. Our observation that differences in early attentional biases are related to the serotonin transporter gene, as well as recent work by other groups also examining behavioral consequences of this gene in children (Pérez-Edgar et al., 2010; Gibb et al., 2009), suggest that early automatic orienting toward or away from threatening or appetitive stimuli is affected by the 5-HTTLPR gene. This might be compared, for example, to the finding that aggressive children show a hostile attribution bias (e.g., see Dodge et al., 1986).

We found that healthy children and adolescents who carry the 5-HTTLPR *s*-allele showed greater neural responses in parietal, frontal and limbic regions than did youth who were homozygous for the *l*-allele. There was only one region (superior temporal cortex) in which *l*-allele homozygotes exhibited greater BOLD response to the emotion face > neutral face contrast than did *s*-allele carriers.

In the present study, we describe genotype-associated differences in neural processing that reflect differences in perceptual processing that have previously been reported (Pérez-Edgar et al., 2010). In a review of dot-probe neuroimaging studies, Pourtois and Vuilleumier (2006) reviewed dot-probe studies and showed that the attentional bias towards fearful faces was modulated by posterior parietal and intraparietal control over extrastriate regions. Dot-probe studies that used either event-related potentials (ERPs) and/or fMRI methods and found that the temporal dynamics of ERP responses indicated a positive relation between parietal control regions and sensory cortices (Pourtois and Vuilleumier, 2006). The differences in behavior observed between the 5-HTTLPR groups in our study and in past investigations may result from upregulation of perceptual responses to these emotional faces by regions that control attentional allotment to afferent sensory pathways. It is as if a gain mechanism in the processing of negative emotion faces is ‘boosted’ in carriers of the *s* allele, a neurobiological finding supported by the behavior observed in the subliminal neutral condition: that *s*-allele carriers were more likely to respond faster when targets and negative-emotion faces appeared in the same visual field.

It is important to consider the findings in light of several limitations of this study. First, because of the small number of l_A participants, we conducted analyses using a bi-allelic (ss/sl vs. ll) classification that yielded more balanced numbers in each gene group. Because the l_G allele shows transporter expression closer to the *s* allele, the effect of classifying l_G with l_A may have reduced our ability to detect differences between gene groups. On the other hand, we enrolled participants with a wide range of ages (9–17); consequently, there was likely considerable variability due to staging in neural development. Second, the combination of a small sample size and multiple testing may have led to some of the findings by chance, and independent replication is needed. Third, we conducted our scans at two centers. We did examine the effect of site on the neural results and found that scan site did not moderate the obtained gene group differences. Finally, we conducted this study with diagnostically healthy individuals who volunteered to participate in this research. It is possible that this “self-selecting” class of participants represents a relatively narrow range of individuals by virtue of volunteering to participate in neuroimaging research studies. Also, by having studied healthy individuals there was little variability in anxiety propensity; consequently, the findings cannot be related to behavior. Thus, implications of these findings for the development of affective clinical symptoms is not yet clear.

Studying a sample of children and adolescents, we have shown that neural networks that support visual selective attention operate distinctly in different 5-HTTLPR gene variants, even in the early years of life, before the experience of major chronic stress and in the absence of a history of psychopathology. We posit that the *s*-allele carrier gain in perceptual response to threatening stimuli in childhood and adolescence is part of a tendency to exhibit maladaptive behaviors in response to threatening stimuli, and to experience anxiety, depression, and other mood disorders. We expect that this early life feature will persist into adulthood and be formative in development. Our work fits with a growing body of literature that suggests that these genotype-associated differences in neural function and structure mediate individuals’ capacity to deal with stress (Hariri and Holmes, 2006). Future studies might examine how the general attentional system responsible for allocating resources is altered under stress conditions, and investigate whether and how this attentional style interacts with 5-HTTLPR genotype. Future studies should also examine how this cognitive system may be similarly altered in individuals who carry other risk factors for the development of mood and anxiety disorders.

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