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Age-Related Effect of Serotonin Transporter Genotype on Amygdala and Prefrontal Cortex Function in Adolescence

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Abstract: The S and L_G alleles of the serotonin transporter-linked polymorphic region (5-HTTLPR) lower serotonin transporter expression. These low-expressing alleles are linked to increased risk for depression and brain activation patterns found in depression (increased amygdala activation and decreased amygdala–prefrontal cortex connectivity). Paradoxically, serotonin transporter blockade relieves depression symptoms. Rodent models suggest that decreased serotonin transporter in early life produces depression that emerges in adolescence, whereas decreased serotonin transporter that occurs later in development ameliorates depression. However, no brain imaging research has yet investigated the moderating influence of human development on the link between 5-HTTLPR and effect-related brain function. We investigated the age-related effect of 5-HTTLPR on amygdala activation and amygdala–prefrontal cortex connectivity using a well-replicated probe, an emotional face task, in children and adolescents aged 9–19 years. A significant genotype-by-age interaction predicted amygdala activation, such that the low-expressing genotype (S/S and S/L_G) group showed a greater increase in amygdala activation with age compared to the higher expressing (L_A/L_A and S/L_A) group. Additionally, compared to the higher expressing group, the low-expressing genotype group exhibited decreased connectivity between the right amygdala and ventromedial prefrontal cortex with age. Findings indicate that low-expressing genotypes may not result in the corticolimbic profile associated with depression risk until later adolescence. *Hum Brain Mapp 35:646–658, 2014.*

Key words: functional MRI; 5-HTTLPR; development; affect; emotion; connectivity; imaging genetics

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INTRODUCTION

Serotonin transporter regulates the amount and duration of synaptic serotonin in structures involved in processing emotion, including the amygdala [Hariri and Holmes, 2006]. The L_A allele of the serotonin transporter-linked polymorphic region variant (5-HTTLPR; [Lesch et al., 1996]) in the promoter region of the serotonin transporter gene (*SLC6A4*) results in increased transcriptional efficiency and serotonin transporter expression relative to the S and L_G alleles in *in vitro* studies (A to G SNP in L allele, rs25531; e.g., Hu et al. [2006]). In *in vivo* studies on adults, 5-HTTLPR does not appear to affect serotonin transporter expression in brain tissue [Murthy et al., 2010; Parsey et al., 2006], which suggests that effects of genotype on brain function are likely due to neural changes earlier in development [Murthy et al., 2010].

In adults, 5-HTTLPR affects emotional behavior as well as corticolimbic brain circuits underlying emotion. Adults with the low-expressing alleles, S and L_G, and a history of stressful life events during childhood and adolescence are more likely to have depression ([Caspi et al., 2003; Karg et al., 2011; but see Risch et al. [2009]). The low-expressing alleles are also linked to greater amygdala activation [Hariri et al., 2002] and weaker functional connectivity of the amygdala with ventromedial prefrontal cortex when presented with emotional face stimuli [Pezawas et al., 2005], both brain profiles that have been associated with depression [Murray et al., 2011]. The S and L_G alleles that result in less-serotonin transporter expression are linked to poorer affective outcomes in humans as well as animal models [Champoux et al., 2002; Munafo et al., 2008], whereas, paradoxically, serotonin transporter blockade with selective serotonin reuptake inhibitors relieves affective symptoms [Berton and Nestler, 2006].

Examining the developmental effect of serotonin transporter may help to reconcile this paradox. After mice are treated with serotonin transporter blockers in early life, a procedure that mimics the increased synaptic serotonin experienced by individuals with the low-expressing genotypes [Ansorge et al., 2004], depression-like behaviors begin to manifest in adolescence and persist through adulthood [Ansorge et al., 2008; Lisboa et al., 2007]. This effect in rodent models mirrors the sharp increase in depression prevalence during adolescence in humans [Hankin et al., 1998]. Conversely, treating mice with serotonin transporter blockers in adulthood does not increase depression-like behaviors [Ansorge et al., 2008]. Taken together, these studies suggest that development moderates the effects of serotonin transporter availability on brain function. Decreased availability very early in development, as occurs in humans with the low-expressing genotypes, increases risk for depression that emerges in adolescence, whereas decreased availability later in development, as occurs as a result of SSRI treatment, reduces depression symptoms. However, no brain imaging research has yet investigated the moderating influence of human development on the serotonin-brain function association.

We examined the age-related effects of 5-HTTLPR on amygdala activation and amygdala–prefrontal cortex connectivity using a well-replicated probe, emotional face presentation [e.g., Hariri et al., 2002], in a child and adolescent sample. We hypothesized that the low-expressing genotype (S/S and S/L_G) group relative to the higher expressing genotype (L_A/L_A and S/L_A) group would exhibit both increased amygdala activation and decreased amygdala–prefrontal connectivity with age.

METHODS

Participants

Data from 48 typically developing children and adolescents, aged 9–19 years, were included in this study. Of a total 65 participants, data from 17 participants were excluded from the analyses due to movement greater than 2.5 mm translation or 2.5° rotation, an incomplete scan due to discomfort in the MRI, or poor coverage of the regions of interest during MRI acquisition. Two participants with amygdala and/or ventromedial prefrontal activation more than 2.75 standard deviations away from the mean were excluded as outliers.

Participants were recruited through flyers posted at local community organizations. The University of Michigan Institutional Review Board approved the procedures. Participants age 18 and older signed informed consent documents; minor participants gave assent and their parents gave written consent. The Peabody Picture Vocabulary Test [Dunn and Dunn, 1997] and the Ravens Standard Progressive Matrices [Raven, 1960] were administered to measure cognitive functioning. Exclusion criteria consisted of orthodontic braces, other conditions contraindicated for MRI, and history of seizures or neurological disorders. Additionally, participants were screened for psychological disorders, including anxiety, depression, attention deficit/hyperactivity disorder, and autism, with parent report (Child Behavior Checklist; [Achenbach and Edelbrock, 1981; Social Responsiveness Scale; Constantino et al., 2003; Social Communication Questionnaire; Rutter et al., 2003] and self-report (Child Depression Inventory [Kovacs, 1992; Multidimensional Anxiety Scale for Children; March et al., 1997; Spence Children's Anxiety Scales; Spence, 1997; Obsessive Compulsive Inventory—Revised; Foa et al., 2010] measures. Prior studies used parts of this dataset [Weng et al., 2010, 2011; Wiggins et al., 2011, 2012].

Genetic Analyses

The Oragene DNA kit (DNA Genotek; Kanata, Canada) was used to obtain a saliva sample from each participant. Using previously published procedures [Wiggins et al., 2012], S versus L genotype of 5-HTTLPR was determined via PCR and agarose genotyping; Sanger sequencing was used to determine the A to G SNP in the L allele (rs25531;

[Hu et al., 2006]) and to confirm PCR genotyping. As previous studies have repeatedly implicated the low-expressing alleles (S and L_G) as being vulnerable to amygdala overactivation and other poor affective outcomes [Belsky et al., 2009], for subsequent statistical analyses, participants were divided into two groups: low-expressing genotypes (S/S and S/L_G) versus higher expressing genotypes (L_A/L_A and S/L_A). (There were no participants in this cohort with the relatively rare genotypes L_G/L_G and L_A/L_G). Grouping the alleles by expression level is a common way to provide insight into functional brain differences [e.g., Praschak-Rieder et al., 2007]. Hardy–Weinberg equilibrium was tested with the alleles based on the insertion/deletion polymorphism. Genotype frequencies were not in Hardy–Weinberg equilibrium when all ethnic/racial groups were included ($N = 48$, $\chi^2 = 4.07$, df = 1, $P = 0.044$); however, when including only Caucasians ($N = 41$), genotype frequencies were in Hardy–Weinberg equilibrium ($\chi^2 = 1.90$, df = 1, $P = 0.168$). Because of this, post hoc analyses were performed to address potential effects of differing ancestry.

fMRI Data Acquisition

MRI data were acquired using a 3T GE Signa scanner. Participants wore glasses with built-in mirrors (VisuaStim XGA, Resonance Technologies) to view the faces stimuli projected onto a screen behind them. Participants made responses during the task via a button box attached to their right hand and linked with an IFIS system (MRI Devices, Milwaukee, WI). High-resolution spoiled gradient (SPGR) images were acquired, which consisted of 110 sagittal slices 1.4 mm thick (flip angle = 15°, FOV = 26 cm). Using a reverse spiral sequence [Glover and Law, 2001], T_2*-weighted blood oxygen level-dependent (BOLD) images were acquired during the emotional faces task. The BOLD images were composed of 40 adjacent 3-mm axial slices acquired parallel to the intercommissural line (TR = 2,000 ms, TE = 30 ms, flip angle = 90°, FOV = 22 cm, and matrix = 64 × 64).

Emotional Faces Task

We used a face task known to reliably activate the amygdala [Weng et al., 2011]. During image acquisition, participants were instructed to identify the gender of emotional faces from NimStim [Tottenham et al., 2009]. Thirty actors of various races and genders modeled each of the emotions (happy, sad, fearful, and neutral), and no picture (actor representing a particular emotion) was repeated. There were 30 trials of each emotion for a total of 120 trials presented in a different randomized order for each participant across two 6-min runs.

Each trial consisted of a fixation cross presented for 500 ms, followed by a face for 250 ms. A blank screen ensued for 1,500 ms. Any time during the face presentation or the subsequent blank screen, participants used a button press with their right hand to indicate whether the face was male or female. The combination of a short presentation time for the face (250 ms) with a task to do immediately afterward (i.e., identify gender) minimized group differences in attention to the faces. Intertrial intervals were jittered between 0 and 6,000 ms at intervals of 2,000 ms. The blank screen displayed between trials served as baseline. *E*-prime (Psychological Software Tools, Pittsburgh, PA) was used for stimulus presentations and recorded responses.

Participants were instructed to identify the gender of the face as quickly and accurately as possible. One participant's behavioral responses were lost due to technical failure. Before the MRI scan, participants practiced the task with different faces in a mock scanner to ensure that they were comfortable with the task and testing conditions.

FMRI Data Analysis

Data preprocessing

The fMRI data were preprocessed with the standard procedure from the University of Michigan Functional MRI Center. This process includes removing outliers ("white pixel" artifacts) from the raw *k*-space data, reconstructing the *k*-space data to image space, applying a field map correction to reduce artifacts from susceptibility regions, and correcting for both slice timing and head motion. Details on these steps are available in multiple works using this preprocessing stream [e.g., Monk et al., 2010; Weng et al., 2011]. In addition to realigning functional images to the 10th image, we further addressed potential effects of head motion by examining whether genotype groups differed in average head motion and whether head motion correlated with age. As previous studies have done [Bunge et al., 2002; Rubia et al., 1999], an index score was created by taking the grand mean of head movement measured in each of six rigid body movement modes (three translations, three rotations). We used a *t* test to compare this head motion score between genotype groups and a Pearson's correlation to examine the relationship between head motion and age.

Additional preprocessing of the data was accomplished in-house using the SPM5 MATLAB toolbox (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk). High-resolution T1 anatomical images were coregistered to the functional images. The functional images were subsequently smoothed using an isotropic 8-mm full width at half maximum Gaussian kernel. Images were normalized to the Montreal Neurological Image (MNI) space by estimating the transformation matrix for the SPGR image to SPM's template MNI image and then applying that transformation to the functional images.

Contrast images

Individual-level analyses were performed in SPM5. For each participant, face conditions were modeled with SPM5's canonical hemodynamic response function (HRF) as well as the temporal derivative of the HRF [Friston et al., 1997]. Trials where participants incorrectly identified the gender of the face were excluded from analyses. Images were generated for each participant for the contrast of all faces versus baseline by estimating the contrast value at every voxel. These images, which convey how much activation differed between the two conditions (seeing faces versus a blank baseline screen) at every voxel in the brain for that individual, were then used in group-level analyses.

Connectivity images

A psychophysiological interaction analysis was performed to generate functional connectivity images [Friston et al., 1997; Gitelman et al., 2003]. The seed and contrast for the psychophysiological interaction were set at the peak amygdala activation difference from the first hypothesis (average of four voxels surrounding $xyz = 22, -8, -16$; all faces vs. baseline), following previous work [Monk et al., 2010].

Group-level analyses

Group-level analyses were performed with SPM8, unless otherwise indicated. As a preliminary step, we examined whether there were overall group differences between the low-expressing genotype (S/S and S/L_G) group relative to the higher expressing (L_A/L_A and S/L_A) genotype group, regardless of age. A voxel-wise independent sample's t test was first performed with the all faces versus baseline contrast images. A small volume correction was then performed with the right amygdala, as defined by the Wake Forest Pickatlas [Maldjian et al., 2002]. The right amygdala was chosen as the mask, because genetic effects on brain function have previously been found in the right amygdala [Hariri et al., 2002, 2005]. Significance thresholds were corrected for multiple comparisons within the right amygdala using family-wise error (FWE) correction [Worsley et al., 1996].

To address our first hypothesis, increased amygdala activation with age in the low-expressing genotype group relative to the higher expressing group, we created a genotype-by-age interaction model for all the faces versus baseline contrast images, using voxel-wise multiple regression. For this model, three regressors were entered—genotype, age, and the interaction of genotype-by-age—predicting the activation to all faces versus baseline. To test whether there was an interaction in the right amygdala, the locus of genetic effects in prior research [Hariri et al., 2002, 2005], a small volume correction with the right amygdala was performed with the image mapping the betas of the interaction term. This small volume correction restricted the search for voxels with a significant interaction beta to the right amygdala and also applied a FWE correction based on the number of voxels (158) within the right amygdala.

Post hoc analyses were also performed to further characterize the interaction by testing whether the simple slopes for the low and higher expressing groups differed from zero. Activation values from a 4-mm sphere around the peak voxel of the interaction from the first hypothesis ($xyz = 22, -8, -16$) were extracted and averaged. These data were then exported to SPSS, where two regressions were run—one for individuals with low-expressing genotypes, and one for higher expressing genotypes—in which age predicted extracted activation values. The betas for age in both regressions were each tested against zero to examine whether individuals with the low and higher expressing genotypes, separately, increased or decreased in amygdala activation with age.

As a preliminary step for the connectivity analyses, just as for the amygdala activation, an independent sample's t test was performed to examine group differences in amygdala–prefrontal connectivity. The images used in this model were generated for each individual by the psychophysiological interaction analysis with the contrast of all faces versus baseline. The laterality of the connectivity effects in the literature is not clear, as previous work used a bilateral amygdala seed [Pezawas et al., 2005]; because of this, the present connectivity analyses were exploratory in terms of laterality and used the left and right ventromedial prefrontal cortex. The ventromedial prefrontal cortical masks in the small volume corrections were composed of the intersection of the medial orbitofrontal cortex, medial frontal gyrus, and cingulate region for the left and right hemispheres as defined by the Wake Forest Pickatlas [Maldjian et al., 2002]. These masks represent the ventromedial prefrontal cortical region that, in adults, has previously demonstrated altered connectivity with amygdala, depending on 5-HTTLPR genotype [Pezawas et al., 2005]. (See Supporting Information Fig. S1 for a visual representation of the left and right ventromedial prefrontal cortical masks.)

To address our second hypothesis, decreased amygdala–prefrontal connectivity with age in the low-expressing group relative to the higher expressing group, we again created a genotype-by-age interaction model, but for the connectivity images. The left and right ventromedial prefrontal cortex masks were again used for small volume corrections.

Post hoc analyses were again performed to better understand the interaction, as described for the first hypothesis, but with connectivity values extracted from the peak voxel of the interaction for the second hypothesis ($xyz = -8, 40, -14$). Simple slopes for each genotype group were tested against zero to determine whether the low-expressing group increased or decreased in connectivity with age and whether the same occurred for the higher expressing group.

TABLE I. Subject characteristics

	Low-expressing genotypes			Higher expressing genotypes				
	S/S	S/L_G	L_G/L_G	L_A/L_A	S/L_A	L_A/L_G		
Number of participants	15	2	0	16	15	0		
Race/ethnicity								
Asian	2	0	0	0	0	0		
African American	1	0	0	1	1	0		
Hispanic/Latino	1	0	0	1	0	0		
Caucasian	11	2	0	14	14	0		
							χ^2 (df = 1)	*P*
Gender (M:F)		13:4			25:6		0.115	0.735
Handedness (R:L)		15:2			28:1		1.17	0.280
							t_{46}	*P*
Age, mean (SD)		15.2 (1.94)			14.4 (3.15)		0.993	0.326
PPVT		116 (12.6)			114 (12.8)		0.567	0.574
Ravens SPM		106 (10.6)			104 (12.5)		0.584	0.562
SCAS		15.6 (8.32)			14.9 (8.55)		0.268	0.790
MASC		33.0 (10.6)			29.2 (14.6)		0.920	0.363
OCI-R		11.5 (8.88)			9.55 (9.14)		0.704	0.485
CDI		5.53 (3.94)			4.84 (5.76)		0.440	0.662
CBCL–total		46.2 (8.63)			43.3 (7.64)		1.21	0.234
CBCL–internal		47.4 (9.71)			45.8 (8.20)		0.585	0.561
CBCL–external		47.3 (6.56)			42.7 (8.01)		2.02	0.050
SCQ		3.12 (2.55)			2.94 (3.33)		0.196	0.845
SRS		44.5 (8.17)			42.3 (6.73)		1.04	0.455
Task accuracy		96.9% (3.21)			97.5% (1.97)		0.825	0.414
Task RT (ms)		673 (119)			763 (135)		2.27	0.028

Note: Two individuals were missing handedness data. One person was missing reaction time (RT) data due to computer failure. Means and standard deviations (in parentheses) reported for age, cognitive functioning, and symptom measures. PPVT, Peabody Picture Vocabulary Test; Ravens SPM, Ravens Standard Progressive Matrices; SCAS, Spence Children's Anxiety Scale; MASC, Multidimensional Anxiety Scale for Children; OCI-R, Obsessive Compulsive Inventory—Revised; CDI, Children's Depression Inventory; CBCL, Child Behavior Checklist; CBCL–internal, internalizing subscale; CBCL–external, externalizing subscale; SCQ, Social Communication Questionnaire—Lifetime; SRS, Social Responsiveness Scale; task accuracy, accuracy of identifying gender in fMRI task; task RT, reaction time to identify gender in fMRI task. Likelihood ratio used for chi-square tests.

RESULTS

To ensure that genotype was not acting as a proxy for psychopathology, we screened for psychopathology using both parent report and self-report measures (see Methods section for a full list of measures). All participants scored below the clinical cutoff on each measure. The low and higher expressing genotype groups also did not differ on any of the symptom measure or cognitive functioning scores (Table I). Furthermore, low and higher expressing genotype groups did not differ in average head motion ($t_{46} = 0.258$, $P = 0.797$). Age and head motion were not correlated ($r = 0.041$, $P = 0.780$).

Accuracy for identifying the gender of the faces stimuli was high (mean = 97.3%, SD = 2.46%), and the genotype groups did not differ on accuracy (Table I). As age was correlated with accuracy ($r = 0.324$, $P = 0.025$), trials in which participants incorrectly identified gender of the face were removed from subsequent fMRI analyses. Relative to the higher expressing group, reaction time (RT) during the gender identification task for the low-expressing group was significantly shorter (Table I). Also, age was significantly correlated with RT ($r = -0.416$, $P = 0.004$). However, the interaction of genotype by age did not significantly predict RT ($\beta = 0.354$, $t_{43} = 1.144$, $P = 0.259$). Additional analyses were performed to address potential RT effects.

Across all participants, the amygdala was significantly activated in the contrast of all faces versus baseline ($xyz = 24, -2, -14$, $t_{47} = 10.55$, $P = 0.0000000000036$, corrected for multiple comparisons within the right amygdala). Similarly, the ventromedial prefrontal cortex was significantly connected with the right amygdala seed across all participants ($xyz = -4, 26, -14$, $t_{47} = 3.70$, $P = 0.007$, corrected for multiple comparisons within the left ventromedial prefrontal cortex; $xyz = 2, 26, -12$, $t_{47} = 3.22$, $P = 0.021$, corrected for multiple comparisons within the right ventromedial prefrontal cortex).

As a preliminary step, we first examined whether the low-expressing group differed from the higher expressing group in amygdala activation, regardless of age. The group difference was not significant ($xyz = 28, -8, -16$, t_{46}

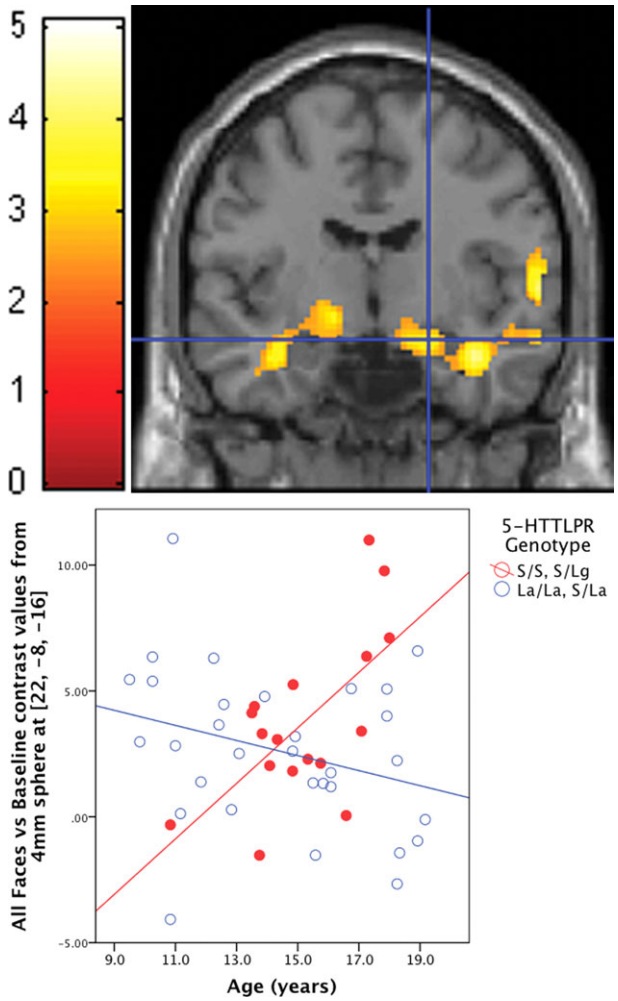

Figure 1.

Greater amygdala activation with age in the low-expressing genotype group. For the contrast of all faces (happy, sad, fearful, and neutral) versus baseline (blank screen), there was a significant genotype-by-age interaction in the right amygdala (xyz = 22, −8, −16, cluster size = 88 voxels, t_{44} = 3.38, P = 0.012, corrected for multiple comparisons within the right amygdala), depicted in the coronal section of the brain (upper). For this and the subsequent brain image, the threshold was set at $P < 0.01$ and $k > 100$ contiguous voxels for illustration purposes. Crosshairs are set at the peak voxel (xyz = 22, −8, −16). To depict activation levels in each individual, values from a 4-mm sphere around the peak voxel (xyz = 22, −8, −16) were extracted and plotted (lower). Contrast values for all faces versus baseline are on the Y axis. The scatterplot shows the relationship between age and amygdala activation to all faces versus baseline contrast in the low and higher expressing genotype groups. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

= 2.50, P = 0.082, corrected for multiple comparisons within the right amygdala). However, the pattern was consistent with previous studies in adults and children [e.g., Hariri et al., 2002; Lau et al., 2009], greater amygdala response to the faces compared to baseline in the low expressing compared to the higher expressing genotype group.

Consistent with the first hypothesis, there was a significant genotype-by-age interaction predicting amygdala activation (xyz = 22, −8, −16, cluster size = 88 voxels, t_{44} = 3.38, P = 0.012, corrected for multiple comparisons within the right amygdala; Fig. 1). Specifically, the low-expressing genotype group showed a greater increase in amygdala activation with age compared to the higher expressing group. Post hoc analyses to further characterize the interaction indicated that, whereas the decrease in amygdala activation with age for the higher expressing group was not significant (simple slope = −0.299, t_{29} = 1.69, P = 0.102), the low-expressing group showed a significant increase in amygdala activation with age (simple slope = 0.637, t_{15} = 3.202, P = 0.006).

As a preliminary step for the connectivity data, we examined genotype group differences regardless of age in amygdala–prefrontal connectivity calculated from the psychophysiological interaction with all faces versus baseline. The genotype groups did not differ significantly in connectivity between the amygdala and ventromedial prefrontal cortex (xyz = −2, 32, −12, t_{46} = 1.93, P = 0.261, corrected for multiple comparisons within the left ventromedial prefrontal cortex; xyz = 2, 32, −12, t_{46} = 2.12, P = 0.197, corrected for multiple comparisons within the right ventromedial prefrontal cortex).

Consistent with our second hypothesis, we found that the effect of genotype on amygdala–prefrontal connectivity depended on age. A genotype-by-age interaction was detected in the left ventromedial prefrontal cortex (xyz = −8, 40, −14, cluster size = 159 voxels, t_{44} = 3.12, P = 0.030, corrected for multiple comparisons within the left ventromedial prefrontal cortex; Fig. 2). Specifically, compared to the higher expressing group, the low-expressing genotype group showed steeper decreases with increasing age in connectivity values between the right amygdala and left ventromedial prefrontal cortex. Post hoc analyses indicated that whereas there was little change in connectivity with age in the higher expressing genotype group (simple slope = 0.172, t_{29} = 0.943, P = 0.353), individuals with the low-expressing genotype evidenced decreases in connectivity values with age (simple slope = −0.573, t_{15} = 2.71, P = 0.016). The genotype-by-age interaction predicting right amygdala to right ventromedial prefrontal cortex connectivity was a trend (xyz = 6, 32, −12, cluster size = 139 voxels, t_{44} = 2.85, P = 0.051, corrected for multiple comparisons within the right ventromedial prefrontal cortex).

Additional Analyses

Other factors may have influenced our findings, such as population stratification, gender differences, and allele

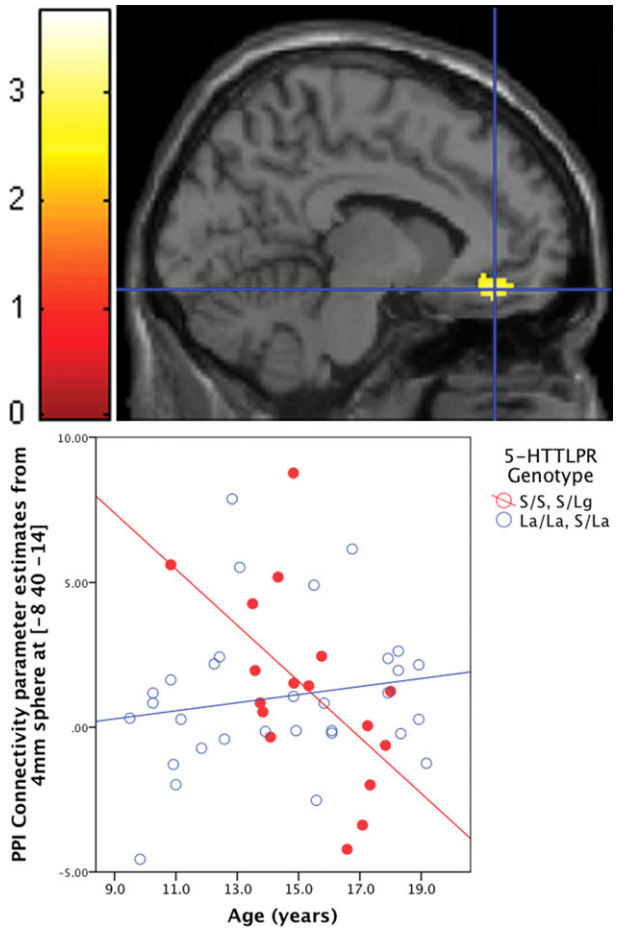

Figure 2.

Decreased amygdala to ventromedial prefrontal cortex connectivity with age in the low-expressing genotype group. There was a significant genotype-by-age interaction predicting connectivity between the right amygdala and left ventromedial prefrontal cortex ($xyz = -8, 40, -14$, cluster size = 159 voxels, $t_{44} = 3.12$, $P = 0.036$, corrected for multiple comparisons within the left ventromedial prefrontal cortex), depicted in the sagittal plane (upper). Crosshairs are set at the peak voxel ($xyz = -8, 40, -14$). To depict connectivity strength in each individual, PPI parameter estimates from a 4-mm sphere around the peak voxel ($xyz = -8, 40, -14$) were extracted and plotted (lower). Unstandardized parameter estimates for the psychophysiological interaction are on the *Y* axis. The scatterplot demonstrates the relationship between age and amygdala–prefrontal connectivity to all faces versus baseline in the low and higher expressing genotype groups. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

grouping. As such, we conducted additional analyses to assess the potential impact of these factors. Because of the reduced power to detect effects (due to reduced degrees of freedom), we used a threshold of $P < 0.05$ without FWE correction.

As genotype frequencies vary by ancestry (e.g., higher S allele frequencies in Asian samples; [Ha et al., 2005; Kato et al., 2005; Kim et al., 2007]) and can contribute to spurious associations [Pritchard and Rosenberg, 1999], we conducted additional analyses to determine whether our effects were primarily driven by population stratification. We excluded seven individuals who were non-Caucasian and repeated the group-level analyses addressing our two hypotheses.

Supporting our first hypothesis, in Caucasian participants only, a genotype-by-age interaction was detected in the right amygdale, such that youth with low-expressing genotypes demonstrated greater increases in amygdala activation with age compared to youth with higher expressing genotypes ($xyz = 18, -8, -16$, $t_{37} = 2.93$, $P = 0.003$).

Supporting our second hypothesis, Caucasian participants with the low-expressing genotype showed decreased connectivity values with age compared to Caucasian participants with the higher expressing genotypes ($xyz = -8, 40, -14$, $t_{37} = 2.96$, $P = 0.003$). Additionally, the genotype-by-age interaction predicting amygdala to right ventromedial prefrontal cortex connectivity was significant at this more lenient threshold ($xyz = 6, 32, -12$, $t_{37} = 2.84$, $P = 0.004$). To summarize, our findings including only Caucasian participants mirrored the original findings with participants of all ancestries.

Although genotype groups did not significantly differ with regard to gender (Table I), our sample was predominantly male. As such, we conducted additional analyses excluding female participants to examine whether gender primarily drove our findings. Consistent with the first hypothesis, the genotype-by-age interaction was significant in the right amygdala ($xyz = 20, -6, -16$, $t_{34} = 2.84$, $P = 0.0038$), indicating that the low-expressing genotype was associated with greater increases in amygdala activation with age compared to the higher expressing genotype. Similarly, the second hypothesis was confirmed with male participants only; in both the left and right ventromedial prefrontal cortex, there were significant genotype-by-age interactions (left: $xyz = -8, 38, -14$, $t_{34} = 3.32$, $P = 0.0011$; right, $xyz = 6, 32, -12$, $t_{34} = 3.92$, $P = 0.00020$). Overall, excluding females from the analyses did not alter the pattern of findings.

Although the interaction of genotype by age did not significantly predict RT to identify gender in the faces task, older participants tended to respond more quickly than younger participants, and the low-expressing genotype group had shorter RTs than the higher expressing genotype group (see beginning of Results). We conducted additional analyses covarying RT to assess whether our results were driven by differences in latency to identify the gender of the face. Mean RT was imputed for one participant whose RTs were lost due to computer failure. In line with the first hypothesis, the genotype-by-age interaction significantly predicted activation in the right amygdala when variance associated with RT was removed ($xyz = 22, -8,$

−16, $t_{43} = 3.25$, $P = 0.001$). In addition, consistent with the second hypothesis, the genotype-by-age interaction significantly predicted connectivity in the ventromedial prefrontal cortex when covarying RT (left: $xyz = -8, 40, -14$, $t_{43} = 3.03$, $P = 0.002$; right: $xyz = 6, 32, -12$, $t_{43} = 2.52$, $P = 0.008$). Thus, both hypotheses were still confirmed when variance associated with RT to identify the gender of the face was removed.

We also repeated the analyses with an alternative genotype grouping, S/S versus heterozygotes (S/L_A and S/L_G) versus L_A/L_A (as in Wiggins et al. [2012]), to investigate whether the patterns still persisted when participants were split into these three groups. Following statistical procedures from Wiggins et al. [2012]), the three levels of genotype were dummy-coded. The dummy-coded genotype variables and age were entered into the model as well as the two dummy-coded genotype-by-age interaction variables. An F test of the change in model fit after including the two dummy-coded interaction variables indicated the overall interaction between genotype and age [Allison, 1977; Irwin and McClellan, 2001].

With three levels of genotype (S/S; heterozygotes S/L_A and S/L_G; and L_A/L_A), the same pattern of findings was still apparent. Confirming the first hypothesis, individuals with S/S had the greatest increases in amygdala activation with age compared to the L_A/L_A and heterozygous groups ($xyz = 22, -8, -16$, $F_{2,42} = 6.28$, $P = 0.004$). Similarly, confirming the second hypothesis, compared to both the L_A/L_A and heterozygous groups, individuals with the S/S genotype had the greatest decreases in amygdala to left and right ventromedial prefrontal cortex connectivity with age ($xyz = -8, 40, -14$, $F_{2,42} = 4.10$, $P = 0.023$; $xyz = 6, 32, -12$, $F_{2,42} = 3.78$, $P = 0.031$). To summarize, regardless of whether participants were divided into three or two genotype groups, the main hypotheses were confirmed.

DISCUSSION

To our knowledge, this is the first study to investigate the effect of 5-HTTLPR on corticolimbic function across age in children and adolescents. We found that children and adolescents with the low-expressing (S/S and S/L_G) genotypes demonstrated a greater increase in amygdala activation with age compared to those with the higher expressing (L_A/L_A and S/L_A) genotypes when viewing faces versus baseline. We further investigated this genetic effect on the development of amygdala activation by examining amygdala connectivity with the ventromedial prefrontal cortex using a psychophysiological interaction analysis comparing the faces condition to baseline. We found that children and adolescents with the low-expressing genotypes showed sharper decreases in connectivity values with age between the amygdala and ventromedial prefrontal cortex compared to those with the higher expressing genotypes. The overall developmental pattern we observed demonstrated that the genetic effects on brain function that have been documented in adults [Hariri et al., 2002; Pezawas et al., 2005] may not occur until later in adolescence.

The neurophysiological profile of depression includes amygdala overactivation [e.g., Drevets et al., 1992; Sheline et al., 2001; Surguladze et al., 2005; Monk et al., 2008] and amygdala–prefrontal underconnectivity [e.g., Almeida et al., 2009; Carballedo et al., 2011]. Adults with the low-expressing 5-HTTLPR genotypes evidence these brain activation patterns that are associated with depression [Champoux et al., 2002; Munafo et al., 2008]. Our findings add a developmental perspective to the literature on 5-HTTLPR and depression; that individuals with the low-expressing genotypes may not display this neurophysiological profile associated with depression until adolescence. The age-related effect documented in the present study helps to explain why the lower expressing genotypes, which result in reduced serotonin transporter, are associated with poorer affective phenotypes [Karg et al., 2011; Munafo et al., 2008], but pharmacologic serotonin transporter blockade, which also results in reduced serotonin transporter, reduces affective symptoms [Berton and Nestler, 2006]. Our findings support the view that the effects of serotonin transporter on affect depend on when in development serotonin transporter levels are altered. If serotonin transporter is decreased very early in development (e.g., perinatally), due to having a low-expressing 5-HTTLPR genotype, individuals exhibit a poorer affective phenotype starting in adolescence. However, if serotonin transporter is decreased later in development, due to selective serotonin reuptake inhibitors, this alleviates affective symptoms. More work is necessary to directly evaluate this view.

Only three previous studies examined 5-HTTLPR and corticolimbic function in adolescent humans [Battaglia et al., 2011; Lau et al., 2009; Thomason et al., 2010], two of which documented heightened amygdala activation in adolescent S allele carriers [Battaglia et al., 2011; Lau et al., 2009], consistent with adult studies. However, unlike the present work, these studies did not examine age-related changes across childhood and adolescence nor did they examine functional connectivity between the prefrontal cortex and the amygdala. The present findings help to bridge the gap between youth and adult 5-HTTLPR studies by examining incrementally the age-related changes in brain function.

A previous study from our laboratory examined the effects of 5-HTTLPR across age, but on a different set of brain structures, the default network that has also been linked to psychopathology [Wiggins et al., 2012]. The findings from the present study are consistent with the previous study, which found that individuals with the low-expressing genotype of 5-HTTLPR failed to develop default network connectivity as strong as those with the higher expressing genotypes through adolescence [Wiggins et al., 2012]. Both studies suggest that low-expressing genotypes do not result in the brain phenotype associated with psychopathology until later adolescence.

There are two main possibilities to explain the increasing amygdala activation and decreasing ventromedial prefrontal cortex connectivity across childhood and adolescence in individuals with the low-expressing genotypes. First, in addition to its function as a neurotransmitter, serotonin acts perinatally as a neurotrophic growth factor, affecting neuron differentiation and synaptogenesis [Lauder, 1990; Lauder and Krebs, 1978]. Exposure to increased serotonin early in development in individuals with the low-expressing genotypes may affect brain growth trajectories. Such perinatal neural differences may compound over time and become apparent in corticolimbic function during adolescence, a time of significant corticolimbic maturational changes [Somerville et al., 2010]. Moreover, serotonin transporter-binding potential does not differ between genotype groups in adults [Murthy et al., 2010; Parsey et al., 2006], which is consistent with the view that 5-HTTLPR alters brain phenotypes via neurotrophic means in development and not via direct influence on binding potential. The finding that SSRIs effectively treat depression in adolescents [Bujoreanu et al., 2011] is also in line with the view that the age-related changes observed in the present study are due to compounding differences in growth trajectories from early exposure to altered levels of serotonin as a growth factor. It is possible that serotonin's role differs across development, such that SSRIs administered to adolescents with depression reduce symptoms because of their influence on serotonin as a neurotransmitter. On the other hand, perinatal alterations to serotonin levels due to genotype produce depression emerging in adolescence because of serotonin's neurotrophic properties.

Second, genetic vulnerabilities may manifest in functional brain differences during adolescence because of a stress-by-genotype interaction [Belsky et al., 2009; Casey et al., 2010; Caspi and Moffitt, 2006]. Although "storm and stress" does not occur for every adolescent, adolescence is often experienced as a stressful transition period [Spear, 2000], with the introduction of new environmental pressures [Eccles et al., 1993]. Thus, in a stress-by-genotype interaction framework, the onset of stress in adolescence may lead to amygdala hyperreactivity and altered connectivity in individuals with the low-expressing genotypes compared to higher expressing genotypes, as we found in this study. Future research is necessary to better understand the mechanisms underlying the developmental aspect of 5-HTTLPR.

To our knowledge, our study is also the first to examine genetic influences on functional connectivity of the amygdala with the prefrontal cortex in children and adolescents. Our findings of both stronger amygdala activation and decreased amygdala–prefrontal connectivity values with age in the low-expressing genotype group suggest that the prefrontal cortex may be less able to suppress amygdala activation during adolescence in those with the low-expressing genotypes. In adults, the prefrontal cortex modulates amygdala activity via robust structural projections to the amygdala [Ghashghaei et al., 2007, Quirk et al., 2003]. During adolescent development, however, the prefrontal cortex undergoes a protracted maturational time course that lags behind amygdala maturation, and thus the prefrontal cortex exerts less regulatory control over the amygdala in adolescents compared to adults (see reviews: [Somerville et al., 2010; Steinberg, 2005]). This developmental discrepancy, in which the prefrontal cortex is immature relative to the amygdala during adolescence, may be more pronounced in individuals with the low-expressing genotypes, because we found that they exhibited decreased amygdala–prefrontal connectivity values in adolescence. Moreover, the amygdala and prefrontal cortex developmental discrepancy may be mediated, in part, by alterations in the overall serotonin system. Because serotonin receptor density is linked to both weaker amygdala–prefrontal connectivity and greater amygdala reactivity [Fisher et al., 2009], it suggests that differences in the serotonin system (on which 5-HTTLPR has influence) can affect the degree to which the amygdala and prefrontal cortex work and mature in concert with each other. Of note, however, one diffusion tensor-imaging study did not find a 5-HTTLPR genotype-by-age effect on fractional anisotropy of the uncinate fasciculus, the white-matter pathway that connects the amygdala and prefrontal cortex [Pacheco et al., 2009]. It is certainly possible that there are differences in functional connectivity but not white-matter integrity. Alternatively, the difference in structural and functional findings may be because Pacheco et al.'s [2009] sample consisted of an older cohort (ages 13–28 years) compared to our sample (ages 9–19 years). Future research could help to resolve this discrepancy by examining 5-HTTLPR's effect on brain development across a larger age range.

We did not find significant differences between the genotype groups on multiple symptom and behavioral measures (with the exception of a trend toward greater externalizing behavior in the low-expressing genotype group). Differences between the genotype groups on symptoms were not expected, as participants were screened for psychopathology. This screening step was important to ensure that genotype was not acting as a proxy for psychopathology in the analyses, and so alterations in brain activation patterns were not simply due to behavioral or symptom differences. The fact that we detected effects of genotype on the brain that are not evident in symptomatology or behavior has two implications. First, brain differences may be a more sensitive measure of genotype effects than behavior. The brain may be more proximally and directly affected by genetic activity [Meyer-Lindenberg, 2009], leading to potentially greater effect sizes. This would allow researchers the advantage of examining brain differences between genotype groups in smaller samples than behavioral studies. Alternatively, we may not be seeing symptom and behavioral differences in our sample of developing children and adolescents, because brain differences precede measurable differences

at the macrolevel of behavior in development. A longitudinal study will be necessary to examine whether genetically influenced brain differences give rise to behavioral differences later in life. For either possibility, understanding mechanisms at the levels of the gene, brain, and behavior across development will be important for future prevention and intervention strategies.

The primary purpose of our faces task was to reliably elicit amygdala activity in participants, and the response required from participants (identify the gender of the face) was to ensure that participants attended to the faces stimuli. The task robustly activated the amygdala across all participants, but RT was shorter in individuals with the low-expressing genotypes than the higher expressing genotypes. Although our findings with amygdala function are not driven by RT (see "Additional Analyses" section), it is possible that the shorter latency to identify gender represents increased vigilance to the emotional faces in the low-expressing genotypes. Future research is necessary to evaluate this possibility.

This study has several limitations. First, we included all ethnic groups in our sample, which can contribute to spurious associations due to population structure in genetic studies. To determine whether results were due to the presence of non-Caucasians in the analyses, we removed non-Caucasian participants and repeated the analyses. Albeit at a more lenient threshold (no correction for multiple comparisons) because of the reduced power, the result patterns were still significant with non-Caucasians excluded from the analyses. This indicates that results were not primarily driven by heterogeneity in ancestries. Nevertheless, the lack of understanding of genetic effects in ethnic groups other than Caucasians is a pervasive problem in the field that must be addressed with future work.

Second, with 17 youth in the low expressing and 31 in the higher expressing genotype groups (total $N = 48$ participants), our sample size is modest. This sample size is comparable to similar imaging genetics studies (e.g., 15 low and 15 high-expressing adults, 31 lower and 20 high-expressing children, and 13 lower and 6 high-expressing children in Roiser et al. [2009], Thomason et al. [2010], and Battaglia et al. [2011], respectively). However, our results will need to be replicated with a larger sample.

Third, we had relatively fewer participants with low-expressing genotypes that were young compared to higher expressing genotypes. In visually inspecting the age-by-genotype results (see Fig. 1), it appears possible that two low-expressing individuals below the age of 14 are driving our genotype-by-age interaction results. To address this, we removed these individuals from the dataset and repeated the analyses in SPSS with values from 4 mm spheres around the peak voxels from the original findings. Excluding these two individuals, the genotype-by-age interaction was still significant in the same loci for both amygdala activation ($t_{42} = 2.533$, $P = 0.015$) as well as amygdala–ventromedial prefrontal cortical connectivity ($t_{42} = 2.571$, $P = 0.014$). This suggests that our findings were not wholly driven by a couple of individuals.

Although replication of our findings is necessary, the present study lays the foundation for future studies to better understand the developmental effects of 5-HTTLPR. First, the increase in reproductive hormones during puberty may differentially affect emotion-related brain activation in individuals with the low-expressing genotypes versus the higher expressing genotypes [Forbes and Dahl, 2010]. Researchers may wish to tease apart the effects of age and pubertal status. Forbes et al. [2010] model one approach to accomplish this, assessing puberty with multiple measures, including Tanner stage and testosterone level. Second, future investigations may also examine more directly the possibility of a stress-by-genotype interaction underlying the developmental differences we found between the genotype groups. Recruiting a high-risk sample with pronounced stressful life events as well as assessments of the adolescents' home environment to obtain a more valid measure of stress would be important in addressing this question [Belsky and Beaver, 2010]. To conclude, the findings from our study facilitate subsequent studies to better understand the developmental aspect of 5-HTTLPR, a key polymorphism in affective disorders.

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